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INVESTIGATION OF LEG COLOUR POLYMORPHISM IN *PTEROSTICHUS MADIDUS* (F.) IN RELATION TO CLIMATIC FACTORS

By

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2002

Thesis submitted in partial fulfilment of the requirements of The Nottingham Trent University for the degree of Doctor of Philosophy.

DECLARATIONS

1. The observations presented in this study are, except where otherwise stated, entirely the work of the author.
2. The author has not submitted any part of the thesis in partial fulfilment of any other higher degree.
3. The author has attended conferences and programmes of study relevant to the present research.
4. Due acknowledgements have been made for the assistance given during the course of this work and in the presentation of the thesis on which it is based.

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ABSTRACT

The ground beetle, *Pterostichus madidus* (F) (Coleoptera: Carabidae), which is widespread in Britain, is dimorphic for two leg colour forms, red and black. Terrell-Nield (1990a) found an association between leg colour morph proportions and temperature in England and Wales, and proposed that this species could be used as an indicator of climate change. The genetics of leg colour in *P. madidus* and the mechanism of selection were unknown.

This study investigates the association between the morph frequency distribution of *P. madidus* and environmental factors from field data collected from a number of regions of England and Wales. Using multiple regression analysis as a diagnostic tool, a positive correlation was found between the red-legged morph and a higher minimum temperature in cooler regions or periods and a lower maximum temperature in warmer regions or periods. Use of monthly climatic data identified winter and spring as the most critical periods. Regardless of the spatial or temporal resolution, the red-legged morph appears to be better adapted to a more equitable climate and is associated with urban and wooded sites; the black-legged morph is adapted to more extreme temperatures and is associated with intensive agricultural areas. These results could explain why *P.madidus* is a forest species in Europe, where it is predominantly red-legged, but has extended its range to more northern latitudes and to open country in Britain.

A method for rearing *P.madidus* larvae under laboratory conditions has been developed. Variables such as pre-reproductive mortality indicate that each developmental stage is adapted to temperatures that would be experienced in the field. A model of the thermal rate to complete development shows that, depending on the month of hatching, 8 to 10 months is required to reach full maturity, adult emergence coinciding over a relatively short period in June. The most critical period for development is from late Instar 3 to emergence. These developmental stages occur from late winter to early summer in the field. This period also produced the strongest coefficient of determination in the multiple regressions. The data sets were too small to identify any difference between the two morphs in their immature stages in terms of their development, growth and survivorship.

Breeding experiments show that the genes coding for red are dominant; this has implications for the modelling of morph frequency change over time.

This study has shown that the mechanism for selection of leg colour is related to temperature but there appear to be many ecotypes among the *P. madidus* population, each one adapted to different temperature conditions. It is not known whether the leg colour phenotypes are linked to specific ecotypes by pleiotropic genes.

The leg colour morph distribution of *P. madidus* could be a good indicator of microclimatic conditions on a small spatial scale, which should be of assistance when making decisions about land use. Due to the year-by-year variability in climate and morph proportions, a long time series of 10 to 20 years is needed to identify a correlation between directional changes in morph proportions and climatic factors.

ACKNOWLEDGEMENTS

I would like to thank:

Dr. Chris Terrell-Nield (Supervisor) for supplying the 1975/6 *P. madidus* morph data, for setting traps along Transect 2 in 1996, and for his support and guidance during the study.

Dr. Rob Beattie (2nd Supervisor) for his support and advice.

Dr Martin Luff (University of Newcastle) for supplying the Close House *P. madidus* morph data and for showing interest in this study.

Environmental Change Network for supplying *P. madidus* leg colour data for the ECN sites.

Heather Stacey (Translation Services, Edinburgh) for translating Zakhov (1990) from the Russian.

Terry Campion for making up the agar plates for the beetles, and supplying the equipment for field work.

Maggie Martin for the photographs of *P. madidus.*

Department of Life Sciences (Nottingham Trent University) for funding this study in 1998.

Thanks also to my colleagues in the research laboratory, project students and other academic and technical staff at Nottingham Trent University for their help and support.

Finally, I would like to thank my family and friends for their interest in this study over the last few years, in particular, for map-reading on some of the beetle trips or for letting me lay pitfall traps in their gardens.

Special thanks to:

My parents, Gordon and Betty Taylor
My daughters, Ellen and Delia Pudney
Terry Conn
Pam and Steve Kerr
Helen Lambert
Julia and John Marriot
Liz and John Woodyatt

CHAPTER 1: Introduction

Although there is now agreement among scientists that Earth is warming due to the rising levels of anthropogenically derived greenhouse gases in the atmosphere (Houghton *et al*, 1996), there remains considerable uncertainty about the magnitude and pattern of climate change which may ensue. There is also uncertainty about how climatic changes will affect the abundance and distribution of insects. However, many insect species are already well adapted to heterogeneous and changeable environments, as reflected in the enormous genetic and phenotypic variation of natural populations. This may facilitate their survival as climate changes. By consideration of phenotypic variation, it has been proposed that a common, easily identified ground beetle, *Pterostichus madidus*, could be used as a bio-indicator of the direction of climate change in Britain (Terrell-Nield, 1990a).

The known ecology and distribution of this beetle, the background to work investigating its potential to adapt to climatic change, the use of other polymorphic insects as bio-indicators of environmental change and the mechanisms of phenotypic variation are reviewed in this chapter and related to the overall aims of the thesis.

1.1 Ecology and life history of *Pterostichus madidus*

The black ground beetle, *Pterostichus madidus* F. (Coleoptera: Carabidae), is widespread throughout Britain. It inhabits deciduous woodlands, copses and cultivated land such as hedgerows, grassland and gardens (e.g. Greenslade, 1968; Luff *et al*, 1989; Terrell-Nield, 1990b).

The adult and larval stages are shown in Plates 1.1 to 1.5. Although unable to fly, the adults are very active on the ground surface, living under vegetation and stones. They are mainly carnivorous, having a varied diet of small arthropods and soft-bodied animals such as molluscs (Luff, 1974). The larvae burrow into the soil. Entirely carnivorous, they feed on soft-bodied animals (Luff, 1974), with a preference - probably a requirement - for live worms (see Section 5.1).

Plate 1.1 Adult *Pterostichus madidus*, black-legged female (13-17mm)

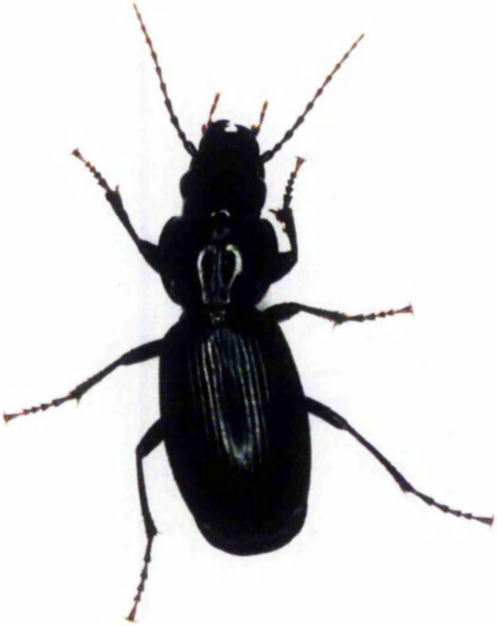

Plate 1.2 Adult *Pterostichus madidus*, red-legged female (13-17mm)

Plate 1.3 **Instar 1**

i) **Newly hatched larvae** (5-6mm)

ii) **Instar 1 larva, after feeding (10-12mm)**

Plate 1.4 Fully grown larva at Instar 2 **(18- 20mm)**

Plate 1.5 Instar 3 larva shortly after instar change **(20-22mm)**

The life history of the beetle has been described by Luff (1973). The adult female lays batches of 10 to 30 eggs at weekly to fortnightly intervals over a period of up to six weeks. Normally, 20 to 45 eggs per healthy female are laid during this period but there is considerable variability. The timing of egg-laying also varies. Some females lay their first batch in late July; others do not complete oviposition until late November. Some females also have a second maturation cycle of the ovaries.

Table 1.1 shows the time of year when stages have been found in the field, not the duration of each stage, which depends, to a large extent, on temperature. The first larval stage can be found from late summer to late autumn. The second stage occurs from early to late autumn and occasionally during early winter. The third stage normally occurs throughout winter to late spring, but has been recorded in the field as early as October.

Pupation and emergence of the adult are from May to June. July and August are periods of high activity when the adults mate, although some reproductive activity can continue into September. In autumn, the males and many of the females die, but a proportion of immature and spent females over-winter to resume reproductive activity early the following season.

Table 1.1 Occurrence of immature stages of *Pterostichus madidus* in the field and annual activity of adults.

	Instar 1	Instar 2	Instar 3	Pupa	Activity of Adults
July					reproduction
Aug					
Sept	✓				
Oct	✓				dying
Nov	✓	✓			
Dec	✓	✓	✓		
Jan	✓	✓	✓		over-wintering of proportion of females
Feb		✓	✓		
March		✓	✓		
April		✓	✓	✓	
May			✓	✓	emergence of adults and over-wintered females
June				✓	

There is, therefore, overlapping of generations amongst the females and a considerable overlap of larval stages. The longevity of the female, her iteroparous fecundity and extended period of egg-laying are adaptive strategies in response to the unpredictable variability of the weather within and between years. The survival to maturity of at least some of the offspring is thus enhanced.

1.2 Geographical range of *Pterostichus madidus*

A life cycle plasticity can enable a species to exist over a wide climatic range. Yet the distribution of *P. madidus* in continental Europe is resticted to a 10° latitudinal range from the Pyrenees to the Netherlands (Turin *et al*, 1977). Although common in woodlands in western Europe within this range, *P. madidus* has not been successful in colonising southern Denmark or Finland (Lindroth, 1986). These regions lie at latitudes higher than 55°. By contrast, in Britain, the beetle has not only been recorded at a latitude of 59° in northern Scotland, it has also extended its habitat to more open country (e.g. Eyre *et al*, 1986; Luff *et al*, 1989).

The more northern distribution of *P. madidus* in Britain may be due to the milder, more oceanic climate of this country compared with the continent. The Gulf Stream off the western coast of Scotland also keeps Britain warm relative to the same latitudes on the continent. For example, the average high and low January temperatures for Fanø (on the west coast of Denmark (55°41′N) are 3° and –2°C respectively compared with an average high and low of 6° and 2°C for Oban (56°25′N) on the west coast of Scotland (Pearce & Smith, 1984).

1.3 Climate change predictions and the response of insects

The evolutionary history of *P. madidus* - when and how it arrived in Britain - are not known. Its colonisation of this country has been highly successful, possibly due to factors such as climate, food availability, the absence of predators and less competition for resources from other insect species. However, the failure of *P. madidus* to colonise more northern latitudes on the continent seems to indicate that climate, in particular temperature, is a major factor restricting the beetle's range.

Climatic modelling at the Hadley Centre, Bracknell, predicts a reduction in cold spells in the UK and an increase in the number of hot summers, the west warming more slowly than the east due to its proximity to the Atlantic Ocean. Precipitation is expected to increase during winter, but summers may become drier (Bennetts, 1995). While the warmest summers on record have occurred since the early 1980s and there has been a trend towards milder winters (Cannell and Pitcairn, 1993), scientists[1] are also warning that Britain could become colder if global warming affects the circulation of the Gulf Stream.

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[1] Scott Polar Institute, Cambridge, reported in The Guardian, 24 June 1996.

The accuracy of climate change models remains uncertain, therefore, particularly at the regional level. This is partly due to the limited spatial and temporal resolution of General Circulation Models (Goodess & Palutikof, 1992). More fundamentally, there is still a lack of understanding of all the physical and chemical processes influencing feedback mechanisms (Bennetts, 1995). Climatic cyclicity as well as natural variability also mask general trends.

How insects will respond to climatic change is equally uncertain. There may be a rapid turnover of species with widespread extinctions but there is little evidence of this from fossil records (Coope 1995). Moreover, Coope has found that fossils of Coleoptera from the Quaternary period (the past 2.4 million years), when frequent and sometimes rapid climate change occurred in Britain, show little evidence of morphological adaptation. He proposes that the main response of insect species to climatic change has been to change their geographic range, rather than adapt to their environment by Darwinian evolution. A behavioural response of tracking climate is also thought to preserve physiological constancy, including the same temperature requirements.

1.4 Investigations into the response of *Pterostichus madidus* to climate change

Although migration is likely to be an important response to long term directional change, many insect species are already pre-adapted to rapid environmental fluctuations due to their life cycle plasticity and genetic variation. These adaptive strategies, which allow an insect species to inhabit a wider geographical range, could also ensure survival at the initial stages of larger climatic events. Such strategies would be of particular importance to flightless insects, such as *P. madidus*, which lack the mechanism for rapid migration over large distances.

In the context of climate change, Butterfield (1996) has considered the flexible life history of *P. madidus* and Terrell-Nield (1990a) has investigated its phenotypic variation. Both investigators have concentrated on the adult stage of the beetle.

1.4.1 Life cycle plasticity of *Pterostichus madidus*

Butterfield (1996) has examined whether the biennial life cycle, adopted by a proportion of the females of *P. madidus* and other carabid species, will allow adaptation to climatic change. A strategy of delaying breeding to the following year overcomes the problem of slower larval development and later adult emergence at lower temperatures.

On an altitudinal transect with an annual mean temperature reduction of 0.43°C per 100m, Butterfield has shown that *P. madius* females at 305m altitude entered the breeding season with a 33% reduction in mandible tip length. She proposes that mandible wear reduces the female's chance of survival, hence reproductive success, the following season. Therefore, despite the potential to switch to a biennial life cycle, the ability of *P. madidus* to survive at lower temperatures could be constrained by its morphology.

1.4.2 Polymorphism in *Pterostichus madidus*

Terrell-Nield (1990a) has considered the phenotypic variability of *P. madidus* and proposes that polymorphism in this beetle may have allowed it to extend its range in Britain to regions experiencing a wider range of mean annual temperatures, i.e. higher summer maximum and lower winter minimum temperatures.

Polymorphism has been defined by E. B. Ford (1940) as "the occurrence together in the same locality of two or more discontinuous forms of the same species in such proportions that the rarest of them cannot be maintained merely by recurrent mutation". In a continually fluctuating environment, both forms can be maintained because they have different advantages.

Like most carabid beetles, *Pterostichus madidus* is predominantly black, the black coloration produced by the pigment, melanin. However, the beetle is dimorphic for two leg colour forms, red and black (Plates 1.1 and 1.2). The red appearance is due to lack of melanin.

Terrell-Nield's work has shown that the distribution of the two leg-colour morphs is related to annual climatic variables for agroclimatic areas. Using pitfall trapping data obtained between 1975 and 1989 from 76 woodland and hedgerow sites throughout England and Wales, the frequency of the red-legged morph increased northwards and decreased eastwards (Fig 1.1), correlating negatively with mean minimum temperature and positively with rainfall. A positive and highly significant correlation was also found between the black-legged morph and Conrad's Index of Continentality (described by Tout, 1976). This takes into account latitude and the annual range of mean temperature, i.e. the black-legged morph frequency increased with annual temperature range and decreased with increasing latitude.

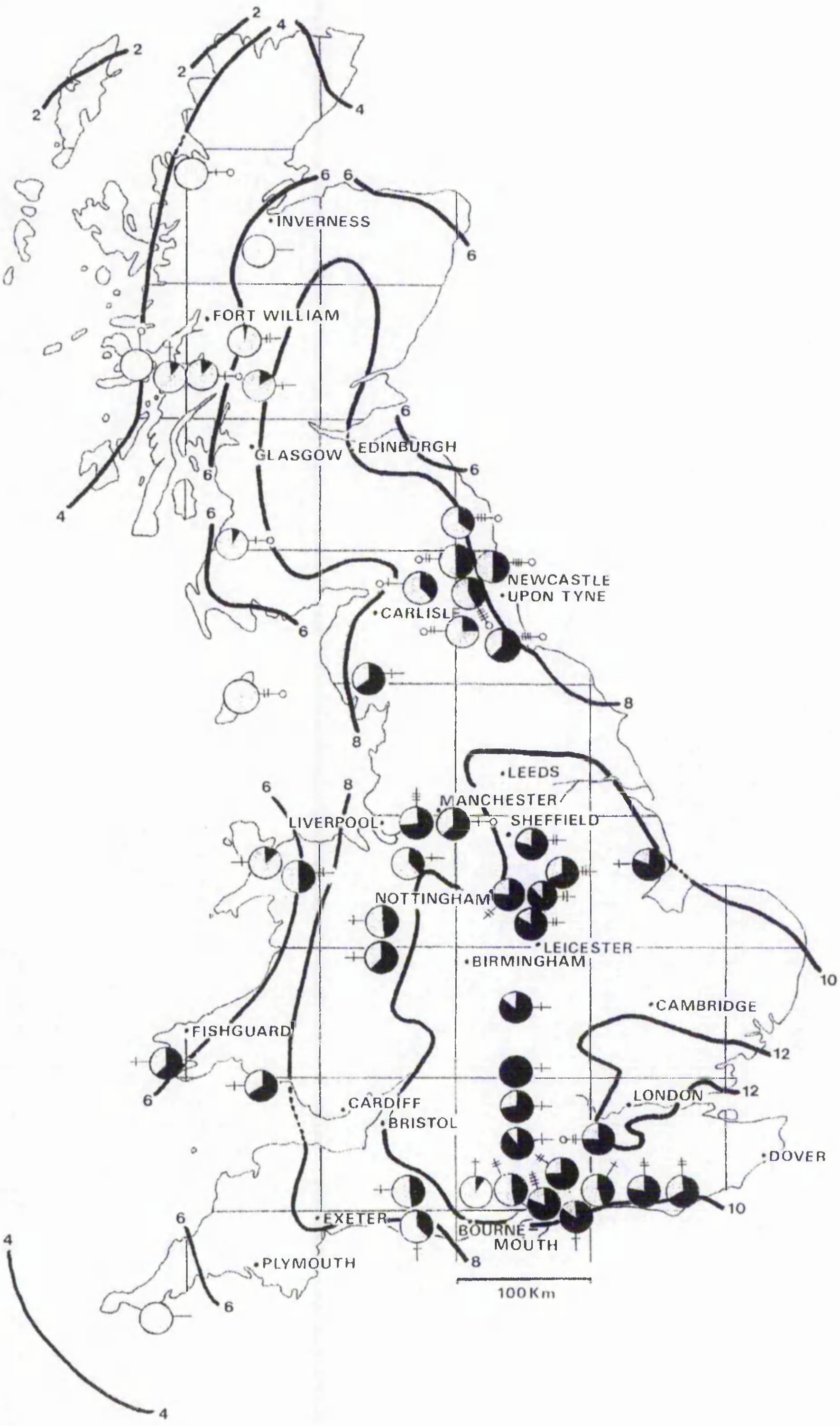


Fig 1.1 **Frequency of *Pterostichus madidus* morphs in Great Britain. ● = 100% black legs; O = 100% red legs; O—(= data from other workers; —(= < 50 animals caught; +—(= 51-100; ++—(= 101-500; +++—(= 501-1000; ++++—(= >1000. Isoclines represent Conrad's Index of Continentality.** (From Terrell-Nield, 1990a).

The interpretation is less straightforward when data from nearby trapping sites are analysed. As shown in Fig 1.2, steep clines from high red leg morph frequency to almost exclusively black-legged morphs occurred over short distances of 10km along a north-south transect sampled in 1975 (Nottinghamshire to Southampton), and an east-west transect sampled in 1976 (East Sussex to West Dorset). These gradients may indicate that the relative fitness of the two morphs is sensitive to small changes in climatic variables. However, the annual weather data used by Terrell-Nield lacked the spatial and temporal resolution to investigate this hypothesis.

The red-legged morph has been described by Lindroth (1986) as the most usual form on the European continent. Terrell-Nield suggests that the black leg coloration is a mutation which has been selected for under the climatic conditions of Britain, and allowed *P. madidus* to extend its range to the less maritime climatic conditions inland and eastwards in this country. Fairhurst (1969) has observed experimentally a trend towards a narrower and lower range of optimal temperature and a preference for higher humidity in the red-legged adult. In France and south-west Germany, the red-legged forms of other carabids dimorphic for leg colour (e.g. *Chysocarabus auronitens* F., *Carabus monilis* F., *C. arvensis* Hbst.) show a preference for cool, moist, afforested areas compared to the drier plains where the black-legged form is more frequent (Blanc *et al*, 1978; M. Baehr, reported in Terrell-Nield, 1990a).

Terrell-Nield has invoked thermal melanism as an explanation, the greater area of black enabling the black-legged morph to absorb and radiate heat more rapidly, so allow longer daytime and earlier annual activity as well as activity in more open habitats. Under more continental conditions, an ability to sustain activity at a wider temperature range would be expected to improve reproductive success.

However, as Terrell-Nield also pointed out, leg colour may have no adaptive value, but is a chance phenotypic expression of the pleiotropic effects of closely linked loci, - what Ford (1940) called a super-gene, - which also codes for enzymes related to temperature. The dominance relationship is not known, although the melanic forms of polymorphic Lepidoptera species are usually dominant (Kettlewell, 1961). If a pleiotropic gene is involved, it should not be assumed that an allele dominant for one trait will be dominant for another.

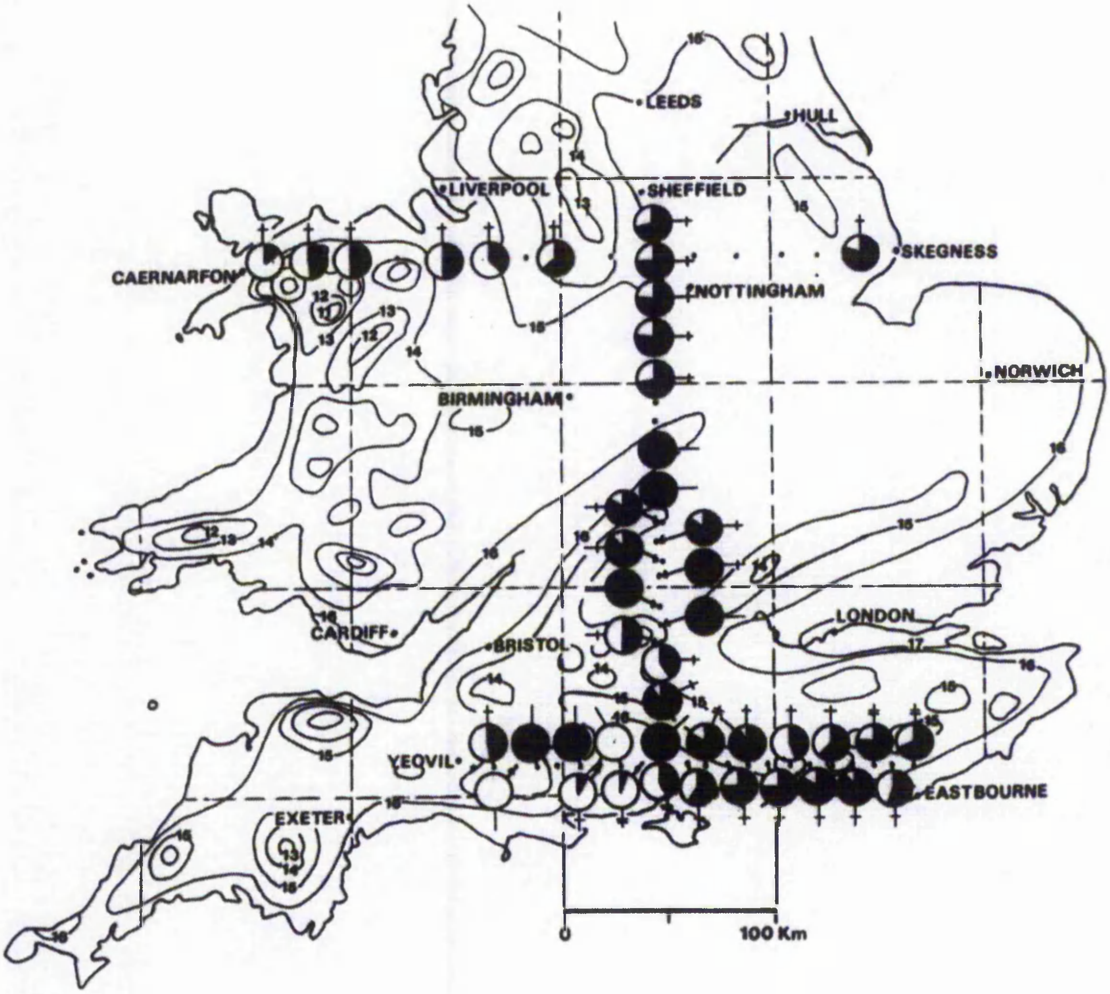


Fig 1.2 **Distribution of *Pterostichus madidus* leg colour morphs From Hampshire to Nottinghamshire, as determined by transect trapping. The map also shows mean August temperatures (°C). —(= 25-50 animals caught; +—(= 51-100; ++—(= 101-500. (Adapted from Terrell-Nield, 1990a)**

Control of the amount of melanin produced can also have an environmental component although the phenotypes are not, usually, discontinuous forms. This has been demonstrated for the alpine butterfly, *Colias* (Peridae) and for some aphid species. Cooler temperatures during pupation result in darker adults in the montane populations of *C. eriphyle*. Larvae of *C. eurytheme* reared under shorter photoperiods also result in darker adults (Hoffman, 1978). In a multivoltine aphid species, *Drepanosiphum platanoides*, the spring and autumn generations are more melanised than those that appear in the summer (Dixon, 1972).

In these insects, increased melanism is an adaptation to cooler temperatures because darker bodies are more efficient at absorbing low-level solar radiation (Dixon, 1972; Roland, 1982). However, an environmentally induced polymorphism - termed "seasonal polyphenism" (Shapiro, 1976) - can only evolve when the inducer is a reliable cue to future conditions, as is the case for insect species inhabiting climatic regions with sharply defined seasons, or for multivoltine species with seasonal generations. It is less likely to have evolved in *P. madidus*, a univoltine species subjected to the unpredictable conditions of a temperate climate.

1.5 Use of colour polymorphism in insects as a bio-indicator of environmental change

The investigation of environmental change by the study of the discontinuous colour forms in an insect species is not new. Unfortunately, simple cause and effect relationships cannot be assumed, even in the now classic work on the cryptic colour forms of the peppered moth, *Biston betularia* L. (Lepidoptera). Extensive studies on the two-spot ladybird, *Adalia bipunctata* L. (Coleoptera, Coccinellidae), have also found a complex relationship between colour morphs and the biotic and abiotic factors selecting for these morphs. The studies on *B. betularia* and *A. bipunctata* are reviewed and related to the work undertaken on *Pterostichus madidus* and other arthropods which show colour polymorphism.

1.5.1 The peppered moth, *Biston betularia* (L.) (Lepidoptera: Geometridae)

Biston betularia shows three colour forms, a pale and speckled *typica* form, the black, *carbonaria* form and an intermediate melanic phenotype, *insularia*. The gene for the *carbonaria* form is completely dominant whereas the intermediate form is due to the presence of partially dominant melanic alleles at the *carbonaria* locus collectively known as *insularia* (Clarke & Shepherd, 1964; Lees & Creed, 1977; Steward, 1977). It is thought that *insularia* became established in regions where the *carbonaria* allele was absent (Merrell, 1994).

Kettlewell and numerous other workers over the past five decades argued that these colour morphs are an example of adaptation to a changing environment, the darker forms having a selective advantage in industrial regions subject to high levels of sulphur dioxide pollution.

The usual interpretation is as follows. The peppered moths, which are active at night, rest during the day on the pale-coloured, lichen-covered bark of tree trunks. Before the Industrial Revolution, the most frequent form was *typica* because it was less conspicuous to bird predators that hunt by sight. As sulphur dioxide and soot from air pollution killed the lichens and darkened the bark of tree trunks, the darker phenotypes replaced the typical form, which had now become more visible to predators. Since the Clean Air Acts of 1956 and 1968 and subsequent recovery of lichen growth, the frequency of melanic forms in industrial areas declined because they are no longer at a selective advantage (Cook *et al*, 1970; Clarke *et al*, 1985; Cook *et al*, 1986).

These observations were supported by experimental evidence of selective predation by birds according to how conspicuous the moth is against its background (Kettlewell, 1955; 1956). However, if bird predation was the only directional force, there were a number of discrepancies in the observed frequencies.

The most important of these are:

i) A fixation of the dominant gene for melanism would be predicted for the heavily polluted region of Manchester (Haldane, 1956). In fact, polymorphism was maintained with a *carbonaria* frequency of 95% (Cook & Mani, 1980).

ii) A higher than expected frequency of *carbonaria* was found in rural regions of East Anglia (Lees & Creed, 1975).

iii) After the enactment of anti-pollution legislation, the rate of decline of the melanic form was more rapid than expected in the Liverpool-Manchester conurbation (Clarke *et al*, 1985), as well as London and urban East Anglia (Cooke *et al*, 1986).

iv) Along a Liverpool-Manchester-Wales transect, inexplicably large changes in morph frequency occurred over very short distances, with *carbonaria* occurring at higher frequencies further into north Wales than predicted (Bishop, 1972).

A number of explanations have been given. Lees & Creed (1975) suggest heterozygous advantage, and laboratory breeding experiments have indicated that the melanic forms are more viable than the typical forms (e.g. Creed *et al*, 1980). Non-visual selection favouring the *carbonaria* would account for ii) - the higher than expected frequency of melanism in unpolluted areas, but does not explain i) - the maintenance of the recessive typical form in polluted regions. In fact, Merrell (1981) re-examined pre- and post-1940 breeding data and found that the higher melanic ratio was due to the poor viability of just a few typical homozygous broods. He concluded that the data sets were too small to support a hypothesis of heterozygous advantage.

Gene flow between areas of high and low frequency may explain both i) and ii). The males are estimated to migrate up to 2.5 km per day on emergence (Bishop, 1972; Mani, 1980). Although inclusion of male migration in selective-frequency models improved the predictions (Mani, 1980, 1982; Cook & Mani, 1980), Brakefield & Liebert (1990) have found evidence of a more complicated migration behaviour than was assumed in the development of these models. Furthermore, the larvae, which are suspended on silk threads, are passively dispersed by air currents (Kettlewell, 1973; Liebert & Brakefield, 1987).

A weak frequency-dependent selection by birds has also been proposed to explain the maintenance of the less frequent, disadvantaged form (Cook & Mani, 1980). If moths at low density experience a low rate of predation, the disadvantaged morph, regardless of colour, would survive. The predation experiments conducted by Kettlewell in the 1950s involved higher densities of moths than are found in the natural population (Bishop *et al*, 1978), producing over-estimations of the extent of predation by birds.

Finally, Mikkola (1984), Howlett & Majerus (1987) and Liebert & Brakefield (1987) have all found that the moths do not normally rest on tree trunks, but on small, shaded branches high in the canopy or the main branches of trunks. Even Kettlewell (1958) admitted that the normal resting-place of the moth is "beneath the larger boughs of trees, less commonly on the trunks". Howlett & Majerus (1987) pointed out that resting in shaded places would give the melanic form a greater advantage than the pale, typical form even in unpolluted rural areas, perhaps explaining the higher than expected frequency in North Wales and rural East Anglia.

Liebert & Brakefield (1987), who developed a technique for observing the resting behaviour of live females in the wild, found, in the absence of pollution, a considerable heterogeneity in resting backgrounds due to the variety of epiphytic flora growing on trees. They suggest that, in regions of declining air pollution, foliose lichens may re-establish rapidly on the younger branches in the upper tree canopy, so favouring *typica*. This may produce an abrupt change in relative fitness and a rapid decline in *carbonaria*, which would account for (iii) above.

However, the importance of lichens in the evolution of melanism has itself been questioned. Recent work in Michigan, America, has found a decrease in the *carbonaria* frequency correlating with a decline in atmospheric sulphur dioxide and suspended particulates, but no accompanying change in the lichen flora which is thought to be a pre-requisite of *typica* recovery (Grant *et al*, 1996).

Furthermore, there may be evidence of physiological differences between the different forms affecting behaviour. For example, Mikkola (1984) noted the higher nocturnal activity of the *carbonaria* moth at low temperatures and suggested that these forms would fly further on cool nights. In the absence of solar radiation, behavioural thermoregulation is unlikely. Kettlewell (1961) reported a quicker feeding rate in the typical larvae compared with *carbonaria* leading to earlier pupation, and suggested that the faster development of *typica* may be to avoid an early onset of winter. These observations suggest a slightly different metabolism of the colour forms, the *carbonaria* form apparently better adapted to lower temperatures.

This re-visiting of the original assumptions is not intended to undermine the qualitative conclusions of Kettlewell. However, the work on *Biston betularia* has revealed an enormous complexity of interacting biotic and abiotic factors influencing selection. Factors such as migration and dispersal affecting gene flow, changes in predator/prey behaviour in response to changing conditions, lichen diversity and distribution, complicate attempts to quantify morph fitness, hence frequency at a given pollution level. As proposed for *Pterostichus madidus*, there may also be non-visual factors associated with the genes controlling melanism, which may be influential at some or all of the life stages of the peppered moth.

The danger is that, without a quantitative understanding of the relative importance of the mechanisms affecting fitness, any reasonable-sounding factor can be invoked to explain away awkward data. Consequently, complex mechanistic models, such as those developed by Mani (1980, 1982), will have a strong empirical element and be of limited predictive value.

1.5.2 The two-spot ladybird, *Adalia bipunctata* (L.) (Coleoptera: Coccinellidae)

It was thought that *Adalia bipunctata*, like the peppered moth, could be used as a bio-indicator of atmospheric pollution - in this instance, smoke (particulate) pollution. However, attempts to find a simple relationship between atmospheric variables and the frequency distribution of the colour morphs of *A. bipunctata* have been even more problematic.

Most work has been on the adults. These often have two generations per year with possibly some overlap. In some years and in some parts of the geographical range, only one annual generation occurs (Muggleton, 1978), whereas three generations per year are reported in Berlin (Creed, 1975).

In Britain, the adults hibernate from autumn to April on trees, in crevices of walls, and in buildings (Creed, 1975; Brakefield, 1984a). The adults become active in spring and, after feeding, mate. The larvae feed voraciously on aphids for about 10 to 15 days when they pupate (Harde, 1984). Emergence of the new generation is usually towards the end of June and a second generation is often produced later in the summer (Majerus, 1994).

The two-spot ladybird is typically red-coloured with two black spots on the centre of each elytron (Fig 1.3.iii). In European populations, two other colour forms commonly occur: black with three red spots on each elytron, var. *sexpustulata* (Fig 1.3.ii) and black with two red spots on each elytron, var. *quadrimaculata* (Fig 1.3.i). The three forms are controlled by three alleles at a single locus, the melanic forms dominant to the red form (Lus, 1932).

Fig 1.3 **The pattern of the three most common forms of *Adalia bipunctata*.** (From Majerus, 1994).

i) *typical* **ii) *quadrimaculata*** **iii) *typica***

The red pigmentation is due to carotenoids; the black pigments are melanin (Britton *et al*, 1977). The coloration is aposematic, i.e. warningly coloured. The typical form of *A. bipunctata* is known to be mildly distasteful to many predators (Frazer & Rothschild, 1960), but both forms contain similar levels of the alkaloid chemical defense, adaline (Pasteels, 1973; Marples, 1993). Although *A. bipunctata* can form the diet of some birds, there is no evidence that the morphs are preyed upon selectively (Muggleton, 1978; Brakefield, 1984a).

Consequently, frequency data was presumed to be unaffected by predator-prey relationships, and abiotic factors were thought to explain the distribution. The associations found between melanic morphs and atmospheric variables are described below and summarised in Table 1.2.

i) Association with atmospheric pollution

Many investigators have found a positive correlation between smoke pollution in industrial areas of Europe and high melanic morph frequencies of *A. bipunctata* (e.g Creed, 1971, 1975; Muggleton *et al* 1975; Scali & Creed, 1975; Brakefield, 1984a; Zakhorov & Sergievsky, 1983; Mikkola & Albrecht, 1988 – see Table 1.2).

In Britain, the decline in the melanic frequency since the enactment of air pollution legislation preceded that in *Biston betularia* by approximately 10 years (Brakefield & Lees, 1987), indicating a direct or indirect association between *A. bipunctata* melanics with particulate (smoke) pollution rather than gaseous (sulphur dioxide) pollution. After legislation, atmospheric pollution levels for smoke fell faster than those for sulphur dioxide. A lag period for epiphyte recovery would also delay the response of the peppered moth to reduced pollution levels.

Although Creed (1971, 1975) and Scali & Creed (1975) proposed that smoke had a direct effect on morph frequency, with the melanic morph somehow more tolerant of the toxic constituents in the pollution, no mechanism has been found.

There are also a number of inconsistencies between countries and regions. For example, there was no association between melanics and smoke pollution in western Norway (Bengtson & Hagen, 1977). The increase in melanism with increasing smoke pollution did not occur, as expected, in cities such as London and Moscow (Zakhorov, 1990), but has occurred in parts of rural Britain (Majerus, 1994).

Table 1.2 Association between melanic forms of *Adalia bipunctata* in Europe and various atmospheric, climatic, geographical and seasonal factors.

+ positive association; - negative association; 0 no association; bracketed:- associations assumed, environmental variables not measured.

	smoke pollution levels	sunshine hours	clear days	mean annual temp	annual temp range	spring temp	summer temp	autumn temp	rainfall	relative humidity	"oceanity index"	distance inland from sea	spring	summer	autumn	winter
ITALY (Scali & Creed, 1975)	+	(+)		+					-			-				
WESTERN NORWAY (Bengtson & Hagen, 1977)	0		-	+	-				+		+					
HELSINKI, GULF OF FINLAND (Mikkola & Albrecht, 1988)	(+)	**(-) city** **(+) coast**		**(+) city**	**(+) coast**	**(-) coast**	**(+) coast**	**(+) coast**				-				
LENINGRAD, RUSSIA (Zakhorov & Sergievsky, 1983)	(+)	**(-) city** **(+) coast**		**(+) city**	**(+) coast**	**(-) coast**	**(+) coast**	**(+) coast**				-				
THE NETHERLANDS (Brakefield, 1984a, 1984b, 1985a)	+	**- spring**		(+)	-					-	-	+	+	**+ (early)** **- (late)**	-	+
BERLIN, EAST GERMANY (Timofeeff-Ressovsky, 1940; Creed, 1975)	+			+										+	+	-
BRITAIN (Creed, 1975; Muggleton *et al* 1975; Brakefield & Wilmer, 1987)	+	-		+												

ii) Association with climatic factors

Investigators also found various associations between melanic frequencies of *Adalia bipunctata* and climatic factors (see Table 1.2).

In north-west Italy, Scali & Creed (1975) found a higher melanic frequency in coastal areas, which are not only more polluted but also warmer, with higher sunshine levels and lower rainfall than inland, high altitude sites (see Fig 1.4). In western Norway, the melanics are similarly associated with a more maritime, humid climate which has a narrower annual range in temperature as measured by an "Index of Oceanity" (Bengtson & Hagen, 1977). A positive association with mean annual temperature was also found, but there was a weak negative correlation with the number of clear days. The association with rainfall is also opposite to that found for Italy, presumably because rainfall does not increase with distance inland for the Norwegian sites sampled (see Fig 1.5).

An association with both maritime conditions and smoke pollution was found in and around Helsinki (Mikkola & Albrecht, 1988) and Leningrad (Zakhorov & Sergievsky, 1983) - see Fig 1.6. Mikkola & Albrecht argue that both these cities experience higher average temperatures and lower insolation relative to the surrounding rural regions. However, they point out that the more coastal regions, where the melanic frequency remains relatively high, would experience higher temperatures and insolation in summer and lower temperatures in spring and late summer due to the cooling effect of the sea. This suggests melanics have an adaptation to a wider temperature range and conflicts with results for Norway.

In Britain, Muggleton *et al* (1975) obtained a positive correlation between the black morph frequency of *A. bipunctata* and the low sunshine levels of smoke-polluted cities. A positive association with lower insolation was also found in the Netherlands and northern Belgium for the months of April to June but not winter (Brakefield, 1984a). In fact, Brakefield obtained a stronger and negative correlation with annual mean relative humidity and the index of oceanity used by Bengtson & Hagen (1977). Although inconsistent with the results for Norway, the oceanity index obtained for the Netherlands was a lower order of magnitude probably due to a much lower annual rainfall (not reported). However, as is apparent from Fig 1.7, the melanic frequency of the Netherlands increases inland southwards and eastwards, suggesting an association with a higher annual mean temperature (not reported).

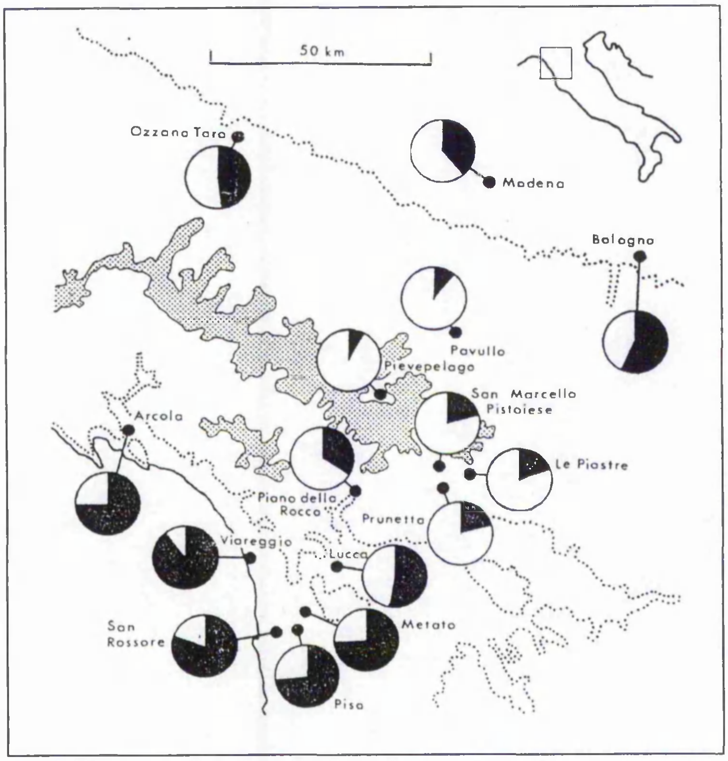


Fig 1.4 **Map showing location of sampling sites and frequency of melanic morphs (black segments) of *Adalia bipunctata* in Italy; relationship of this area to Italy as a whole is shown top right. Dotted contour is at 100m above sea level; stippled area is over 1000m.** (From Scali & Creed, 1975).

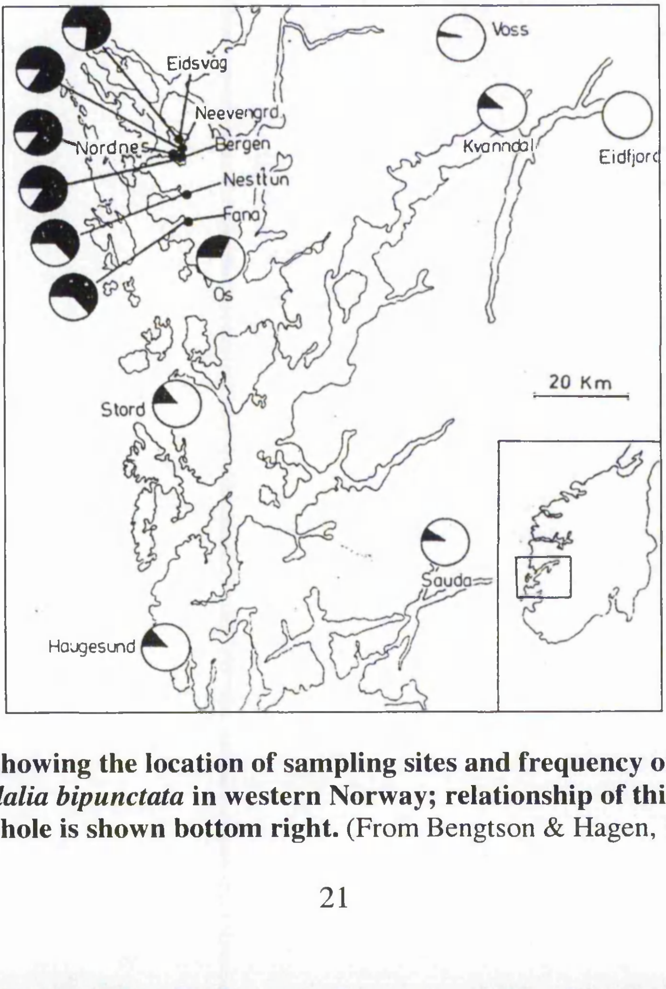


Fig 1.5 **Map showing the location of sampling sites and frequency of melanic morphs of *Adalia bipunctata* in western Norway; relationship of this area to Norway as a whole is shown bottom right.** (From Bengtson & Hagen, 1977).

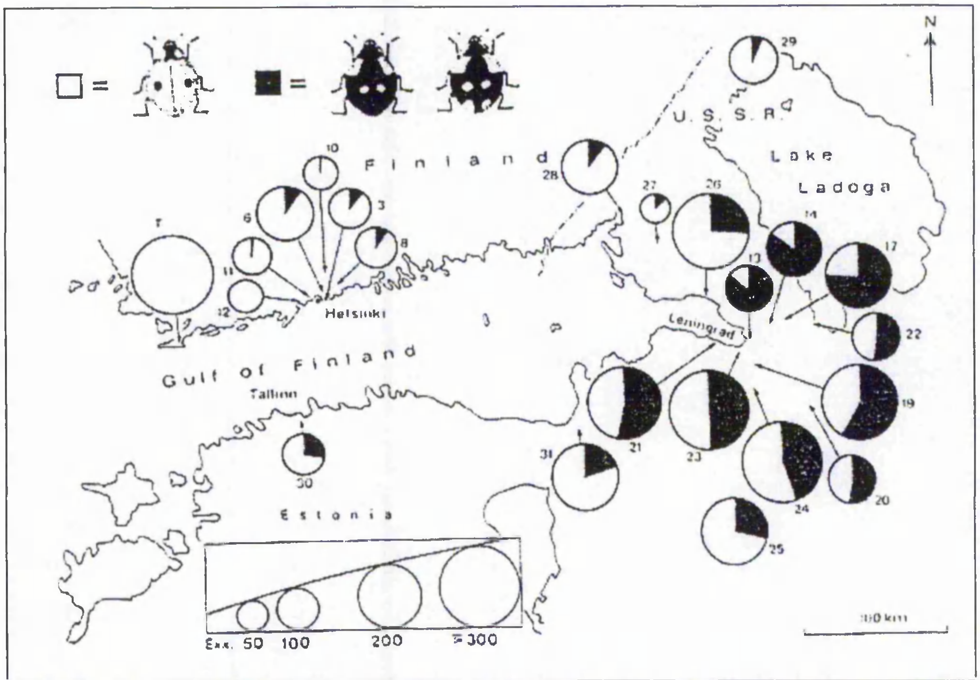


Fig 1.6 **The melanism of *Adalia bipunctata* from selected localities around the Gulf of Finland. The black sector of the diagrams shows the melanic frequency.** (From Mikkola & Albrecht, 1988).

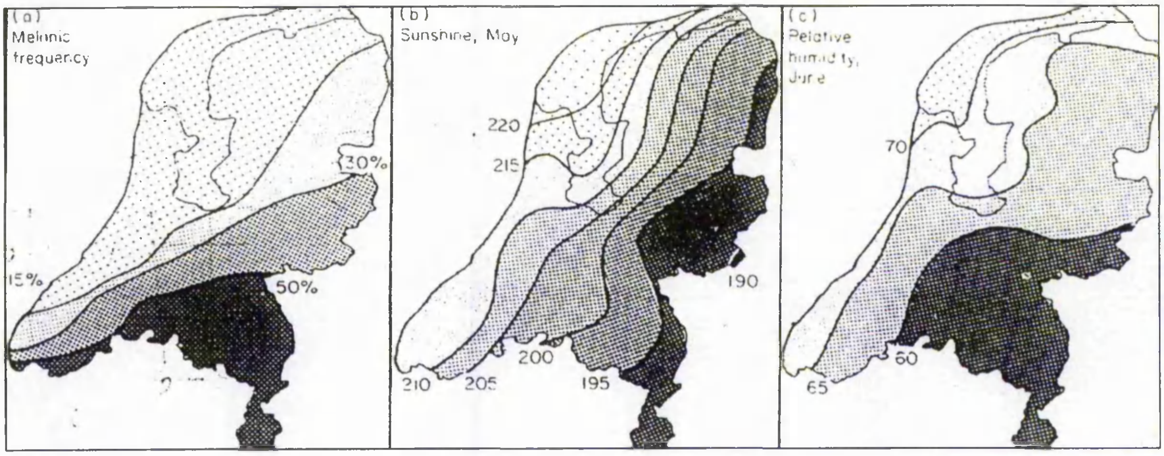


Fig 1.7 **Contour maps for melanic frequency in *Adalia bipunctata* and for hours of sunshine and % relative humidity in the Netherlands.** (From Brakefield, 1984a).

There are also seasonal inconsistencies between the geographical regions (see Table 1.2). In Birmingham (Britain) and Berlin (Germany), where there is a positive association between the melanic morphs and a higher mean temperature (Creed, 1975), a greater selection against the melanics in winter and *typica* in summer was found for Berlin compared with Birmingham. In Berlin, there is a wider range between the winter minimum and summer maximum temperatures, with January mean minima and July mean maxima of -3°C and 24°C respectively compared with 2°C (January) and 20°C (July) for Birmingham (Pearce & Smith, 1984). Timofeeff-Ressovsky (1940) also found a seasonal change in morph frequency in Berlin, with an increase in melanic frequency in autumn compared with spring.

However, in The Netherlands, there was an increase in melanic frequency over the spring-summer reproductive period and a selection against melanics in late summer or early autumn (Brakefield, 1985a). In addition, near Utrecht, where winter temperatures for that year (1980-81) averaged 4°C below normal becoming comparable with temperatures experienced in Berlin, an intense selection occurred during December and January favouring the melanics.

Overall, therefore, conflicting results have been obtained from different countries. In fact, as shown in Table 1.2, the only common factor is a positive association between the melanics and higher annual mean temperatures. However, the climatic range for *A. bipunctata* is enormous, from predominantly Mediterranean conditions in Italy to an alpine/tundra climate in Norway. Independently of colour polymorphism, the beetle is clearly well adapted to this range, presumably due to its generational plasticity during the summer - generation time decreasing with increasing temperature - and its ability to withstand cold conditions in winter. Since there is no overall gradient in morph frequency across Europe as a whole, the changes in melanic frequency must be localised responses within each country caused by the differential effects of one or more environmental factor on morph fitness.

iii) Thermal melanism: a unifying theory?

Creed (1975) suggested that the "heat island" effect of cities, as described by Chandler (1962), could explain the steep gradient of high melanic frequency in urban areas to relatively low frequencies in the surrounding rural areas. Urban temperatures are generally milder and - particularly in winter – warmer, due to pollution increasing cloud cover and the heating of buildings. This mesoclimate is not unlike the milder maritime climates experienced in coastal areas, which also tend to be characterised by higher melanic frequencies. However, the direct effect of ambient temperature on morph fitness has not been considered by other workers.

An alternative explanation was given by Lusis (1961), who proposed that the association between melanism and smoke pollution arose from the indirect effect of particulates reducing solar radiation reaching the ground. Subsequently, thermal melanism, which rests on the theory that darker bodies are more efficient at absorbing solar radiation, was invoked by most workers to explain the spatial and temporal changes in morph frequency.

The mechanism of thermal melanism has been demonstrated experimentally. Benham *et al* (1974), using chilled *A. bipunctata* under a tungsten lamp, found that the black forms were more active than the red at low temperatures (5°C and 7.5°C) due to differential absorption of light radiation. Brakefield & Wilmer (1985), using tungsten light illumination at 20-25°C, obtained evidence that the melanic forms gained a larger temperature excess over the ambient air temperature than the non-melanic form. They also gained and lost heat at a faster rate. Freshly killed ladybirds were used, so interpretation of results was not complicated by possible intrinsic differences in the metabolic rate between morphs.

These findings appeared to explain not only the association between melanics and low sunshine levels in Britain and the Netherlands but also the seasonal changes in morph frequency associated with spring. Spring is a period of mating and oviposition when the adults are more likely to be exposed to direct solar radiation at low temperatures. Brakefield (1984a, 1984b, 1985a) argues that thermal melanism would lead to an earlier and more intense activity of the post-hibernating melanics, hence the observed earlier eclosion of the pupae. This would increase the chance of a second generation. The selection against the melanics (The Netherlands) or the typical (Berlin) later in the year was less easy to interpret.

As Brakefield points out, heat stress selecting against the melanics is unlikely. For example, the hotter, drier and sunnier conditions in Italy favoured the melanics (Scali & Creed, 1977). However, a faster developmental rate in the melanic due to its thermal advantages would increase the chance of a third generation of this form in Berlin, hence its higher frequency later in the season. In the cooler temperature conditions of the Netherlands - although there may be a similar temporal separation between the morphs, with the second generation of the typicals emerging later in the summer - the melanic may not gain the advantage of an extra generational cycle. As a result, *typica* will appear to be "selected for".

The interpretation of changes in morph frequency over the season is complicated further when predator/prey relationships are considered. The main prey species, aphids, peak in numbers at different months in different years (Dixon, 1973). If a temporal separation in morph development occurs due to thermal melanism or any other factor, synchronisation of larval hatching and eclosion with prey availability may favour the melanics in one year or one generational cycle, and *typica* in another.

Finally, attempts to explain the differential survival of the over-wintering morphs by thermal melanism have been contradictory. On the one hand, Lusis (1961) suggests that a higher activity reduces fat stores, which would have a metabolic cost on the melanics, perhaps explaining their poorer survival in Berlin. On the other hand, Brakefield (1985a) proposes that the higher survival rate of melanics during the coldest spells in Utrecht was due to a few sunny days giving the melanics a thermal advantage.

As for the peppered moth, the danger is that inconsistent results, if not actually ignored, are explained away by the favoured theory without any rigorous testing under normal field conditions. For example, Majerus (1994) has written:

“The lack of accord between the different studies does not undermine the thermal melanism hypothesis, for most of them have provided evidence that high melanic frequencies occur in regions with lower sunshine levels, *or* lower temperatures" (my italics).

This is clearly not true for Italy (Scali & Creed, 1975) and Finland (Mikkola & Albrecht, 1988) where a higher melanic frequency occurs in regions with higher insolation *and* higher mean temperatures. De Jong *et al* (1996) have developed a thermal balance model, which predicts a temperature excess in the ladybird melanics in the presence of a strong source of radiation. On the basis of model predictions and laboratory experiments, which measured the melanic and non-melanic *A. bipunctata* activity under different temperature, light and wind speed conditions, the authors conclude that the thermal advantages for the melanics would be at low ambient temperatures and high intensities of radiation. However, there is no consistent evidence from the field that the melanics are selected for under these climatic conditions (see Table 1.2). This is peculiar if thermal melanism is the only mechanism influencing morph fitness.

iv) Biotic factors

Two biotic factors – non-random mating between morphs, and selective predation - have been put forward to explain some of the inconsistencies.

Majerus, O'Donald & Weir (1982) have demonstrated that a proportion of the melanic and non-melanic female ladybirds has a genetically determined preference for melanic males. The advantage would be greatest once the melanics are rare, because of the high density of females favouring melanic males. However, even these results are not consistent between populations. Experiments performed by Tomlinson (1996), using *A. bipunctata* samples from a Welsh population where the melanic frequency is high, found no evidence of a mating advantage for melanic males but an inexplicable over-representation of melanic females in the matings.

Despite lack of evidence, Brakefield (1985b) has suggested that melanism in the two spot ladybird evolved in a form of Müllerian mimicry due to selective bird predation. A bird's previous experience with more distasteful but similarly marked insects influences its choice of prey, and this behaviour can be adaptive (Marples & Brakefield, 1995). Both the melanic and the non-melanic colour forms of *A. bipunctata* mimic noxious insects but there may be spatial and temporal differences as to which morph is perceived as noxious. However, Majerus (1994) points out, in Britain at least, there is no regional association between the melanics of the two-spot ladybird and similarly patterned black-with-red ladybird species such as the pine or kidney-spot ladybirds.

v) Non-visual differences in fitness

Thermal melanism does not explain the consistently positive association between the melanics and higher annual mean temperatures (see Table 1.2). As for the peppered moth, there could be slight differences in the physiology of the colour morphs that are temperature related. The typical form of *A. bipunctata* is characterised by a higher quantity of the pigmentation, carotenoids, which are associated with a gene cluster, the non-visual effects of which are not known (Brakefield, 1988). There is a diversity of non-visual effects associated with genes controlling melanism (reviewed by Brakefield, 1988). Is it possible that pleiotropic effects are producing slight differences in the metabolism of the morphs onto which thermal melanism is superimposed?

This hypothesis assumes stability in the gene clusters. Majerus (1994) has suggested that the inconsistencies between regions could be due to a volatile super-gene, i.e. recombination of the pigmentation alleles may be occurring, which would, of course, increase the genetic variability within the morph populations in a way that cannot be predicted by visual inspection alone.

In summary, despite all the work done on the two-spot ladybird, our understanding of the mechanisms influencing changes in morph frequency in this species remains limited. There are clearly several biotic and abiotic factors that are having direct or indirect effects. The relative importance of these appears to vary with geographical region. Finally, two major investigators of the two-spot ladybird, Brakefield and Majerus, have independently argued for the need to understand the underlying genetics of the super-gene coding for the colour forms. Could a similarly complicated picture emerge for *Pterostichus madidus*?

1.6 Non-selective factors influencing frequency distribution of colour polymorphism

It has been assumed that selective processes are influencing *Biston betularia* and *Adalia bipunctata* morph frequencies. However, polymorphism can be maintained within populations by non-selective processes providing none of the morphs has a selective advantage over the others. The non-selective processes affecting morph frequencies are gene flow and genetic drift (see Endler, 1977).

Gene flow - the spread of a genotype from its point of origin - has a directional effect on morph frequency. Seasonal migration is a special case of gene flow, and is important for some species. Populations can also undergo bottlenecks due to gene flow barriers - whether spatial, such as waterways - or temporal, for example, when the population of one year is drastically reduced in size due to predation or adverse weather.

Genetic drift is non-directional and stochastic, tending to produce localised variations in morph freqencies. It can be caused by continuously low population numbers i.e. the smaller the reproductive population, the greater the potential for random fluctuations in gene frequency. It can also arise through founder effects, whereby the genotypic frequency of the population reflects that of the first colonisers. This process occurs in isolated populations as well as habitats subject to repeated extinction-recolonisation cycles.

The following sections examine attempts to quantify the balance between selective and non-selective processes influencing morph frequency variation in other arthropod species.

1.6.1 The spider, *Enoplognatha ovata*: Selection, gene flow or genetic drift?

Enoplognatha ovata (Clerck) (Araneae: Theriidae) shows three main colour forms, creamy yellow (var. *lineata*), creamy yellow with two dorsolateral stripes (var. *ovata*) and creamy yellow with a solid carmine shield on the dorsal surface (var. *redimata*). From a nation-wide survey in Britain, Oxford (1985) found a weak southeast-northwest cline in England and an east-west cline in Scotland along which the *redimata* morph frequency declined. Oxford suggests that climatic factors are influencing morph frequency indirectly, but the mechanism of interaction is not known.

Oxford does not consider whether gene flow may the dominant mechanism affecting the *redimata* frequencies. At the egg-laying stage, the spiders can be found inside the rolled up leaves of soft fruit (e.g. blackberry, raspberry). It is possible that the *redimata* was introduced into Britain from the continent via the soft fruit industry and the clines observed are due to the allelic spread of this form throughout the country from their points of introduction. The correlation of *redimata* frequency with the north-south and east-west climatic gradients of Britain would, therefore, be coincidental.

Oxford also found considerable local variation in morph frequency and suggests this may be due to genetic drift, presumably because the population on each plant is small and isolated. However, as Oxford points out, it is too easy to invoke stochastic processes when other explanations are not apparent.

1.6.2 The walking-stick *Timema cristinae*: Selection or gene flow?

By investigating a sedentary insect species in relatively patchy habitats, Sandoval (1994) has tried to distinguish between the relative influence of selection and gene flow on the colour morph frequencies in the herbivorous insect, the walking-stick *Timeme cristinae* (Phasmatodeae: Timemideae).

The two morphs of this species are found on those plants where they are most cryptic. From perturbation experiments, Sandoval observed that selection for the cryptic form outweighed gene flow when the area of the food plant (patch) was (1) very large (2) larger than other patches or (3) isolated from other patches. She argues that selection would lead to fixation of the favoured gene, but in a spatially heterogeneous habitat where patches are small relative to gene flow distance, polymorphism is maintained. Gene flow between adjacent but heterogeneous patches can, therefore, make the identification of the adaptive function of different morphs difficult.

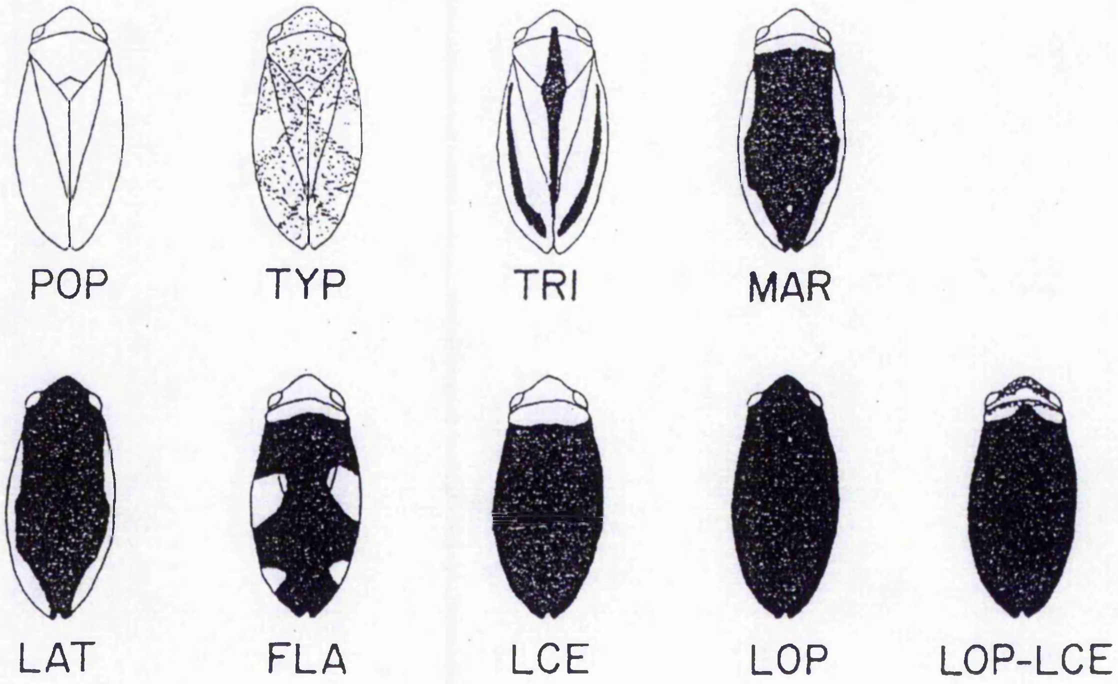
1.6.3 The spittlebug *Philaenus spumarius*: Selection, gene flow or genetic drift?

Work on another sedentary herbivore, the meadow spittlebug *Philaenus spumarius* (Homoptera: Aphrophoridae) has also attempted to identify the relative importance of selective and non-selective factors. *P. spumarius*, has several melanic forms in the female (Fig 1.8). Though a weak flier, it is able to migrate some distance by air currents and by floating on water (Halkka & Halkka, 1990)

Brakefield (1990a) sampled populations of the spittlebug on 26 islands in the Isles of Scilly archipelago. The size of the islands varies from 0.2 to 662 ha. The phenotypic and genetic characteristics of populations on the larger islands were similar to populations in southern England but a high genotypic and phenotypic diversity was found between the smaller islands, including close neighbours. Brakefield suggests that the large island populations are influenced by selective factors whereas the greater diversity of the smaller islands is a consequence of random genetic drift associated with bottle-necks in population sizes and founder effects caused by catastrophic events such as flooding or summer droughts. He also proposes that the significant variation between neighbouring small island populations indicates a low gene flow rate.

To investigate the length of time for morph frequencies to re-establish after perturbation, an exchange experiment was conducted by Halkka & Halkka (1990). Isolated islands in the Gulf of Finland have specific morph frequencies of *P. spumarius*. Adult individuals were exchanged between two islands, which were rich in *leucocephalus* (LCE) and *leucopthalmus* (LOP) respectively. After 19 years (equivalent to 19 generations), the original morph frequency of the two islands was restored, indicating that selective factors are influential in maintaining an island specific polymorphism.

Fig 1.8 ***Philaneus spumarius* colour forms and their standard abbreviations. POP = *populi*, TYP = *typicus*, TRI = *trilineatus*, MAR = *marginellus*, LAT = *lateralis*, FLA = *flavicollis*, LCE = *leucocephalus*, LOP = *leucophthalmus*.** (From Thompson, 1984).



These selective factors are not fully understood. On a large geographic scale, all the *P. spumarius* melanics except *marginellus* (MAR) have been weakly correlated with lower temperatures in both Europe and North America, whereas the *marginellus* had the opposite association, its frequency increasing southwards (Thompson, 1988). Thompson suggested that the former association was due to thermal melanism (see also Berry & Willmer, 1986) whereas the latter was due to aposematic or apostatic selective factors. This does not explain the positive association of the *marginellus* form with temperature, however.

Halkka & Halkka (1990) noted that some *P. spumarius* morphs were found disproportionately on certain types of plants. It is not known whether this is due to physiological, edaphic or microclimatic factors. There is no evidence that colour has a cryptic adaptive value. Nevertheless, the morph frequency clines may be indirectly affected by the geographic range of the preferred plants. Multiniche selection has also been suggested as an important process, allowing species survival in disturbed environments undergoing floral succession.

Finally, Halkka & Halkka hypothesise that some clinal variability may be due to tightly linked ecophysiologically reacting genes in the neighbourhood of the pigmentation locus. As suggested by Brakefield (1988) for *Adalia bipunctata* and Terrell-Nield (1990a) for *Pterostichus madidus*, they propose that the colour alleles are acting as "markers" revealing the distribution of these "ecophysiological alleles" in *P. spumarius*.

1.7 Examples of stability in colour morph frequency distribution.

Colour morphs do not always show clinal variability over time and space. The melanic and typical morphs of the ten spot ladybird, *Adalia decempunctata*, show little temporal or spatial variation in Britain and The Netherlands (Brakefield, 1985a; Brakefield & Lees, 1987). Similarly, Honek & Furlan (1995) found a geographic and temporal stability in the melanic and non-melanic morph frequency of the beetle *Agriotes ustulatus*, even though the two regions sampled, northern Italy and the Czech Republic have a dramatically different climate. This does assume, of course, that climate is the only factor.

The arboreal habit of the ten spot ladybird and the long subterranean development of *Agriotes ustulatus* (two years) are invoked to explain the stability. It is assumed that thermal melanism cannot exert a selective pressure because the usual habitat of these species precludes direct insolation. However, it is possible that a "super-gene" producing linked physiological effects has simply not evolved in these species.

1.8 Summary of selective and non-selective factors affecting colour morph frequencies.

Although the frequency distribution of colour morphs in many arthropod species can show spatial and temporal variation, it is evident that several selective and non-selective processes can be occurring which have no direct relationship with the adaptive value of colour. These processes are summarised in Table 1.3. They are by no means comprehensive and do not include possible physiological effects if a pleiotropic gene is involved. As yet, there is no experimental evidence of gene linkage in these species.

Table 1.3 Factors influencing the frequency distribution of colour morphs.

FACTOR	MECHANISM	EFFECTS	SPECIES	REFERENCE
SELECTIVE: VISUAL	cryptic	selective predation	*Biston betularia* *Timema cristinae*	e.g. Kettlewell (1955; 1956) Sandoval (1990)
	thermal: more efficient absorption of solar radiation	higher activity; earlier development; temporal niches	*Adalia bipunctata* *Philaenus spumarius*	Brakefield & Wilmer (1985) Berry & Wilmer (1986)
	aposematic or apostatic: warning coloration	selective predation	*Adalia bipunctata* *Philaenus spumarius*	Brakefield (1985b) Thompson (1988)
SELECTIVE: NON-VISUAL	non-random mating	melanic advantage	*Adalia bipunctata*	e.g. Howlett & Majerus (1982)
	habitat preference	spatial niches	*Philaenus spumarius* *Timema cristinae*	Halkka & Halkka (1990) Sandoval (1994)
NON-SELECTIVE FACTORS	gene flow: migration	gene mixing between areas	*Biston betularia*	e.g. Mani (1980)
	gene flow: dispersal	gene mixing between areas; directional spread from point of origin	*Biston betularia* *Timema cristinae* possibly: *Enoplognatha ovata*	e.g. Mani (1980) Sandoval (1994) Oxford (1985)
	genetic drift: bottleneck and founder effects	random (possibly localised effects)	*Philaenus spumarius* *Enoplognatha ovata*	e.g. Brakefield (1990a) Oxford (1985)

1.9 Selective and non-selective factors in relation to *Pterostichus madidus* morph frequencies

Since the publication of Terrell-Nield's 1990a paper, there has been considerable interest in the potential of *P. madidus* to act as a bio-indicator of climate. The leg colour morphs are now monitored as part of the Environmental Change Network (ECN) at the Institutes of Terrestrial Ecology in the U.K (Sykes & Lane, 1996). However, the mechanisms affecting morph frequency distribution in *P. madidus* are not known. Table 1.4 summarises the possible factors, which are discussed below.

Table 1.4 Selective and non-selective factors which may be influencing the frequency distribution of leg colour morphs in *Pterostichus madidus*.

FACTOR	COMMENTS	EVIDENCE
cryptic	• Both forms cryptic. • Selective predation unlikely.	
aposematic	• No chemical defences reported. • Red leg coloration is not conspicuous.	
thermal (temperature-related)	• Rarely in direct insolation. • Differential effect of reflected radiation and/or a physiological difference in metabolism of morphs possible. • may produce slight differences in spatial and temporal niches. • Advantage to blacklegged morph at lower temperatures?	• Higher activity found in black-legged morph at lower and higher temperatures and lower humidity. (Fairhurst, 1969). • Higher frequency of redlegged morph with distance into woods (Terrell-Nield, 1990a). • Association between redlegged morph and smaller temperature range (Terrell-Nield, 1990a).
reproduction: e.g. choice of mate, fecundity, timing of oviposition	not known	
growth, developmental rate, survival	not known	
migration, dispersal, gene flow	• Flightless but high surface activity during reproductive season. • Gene mixing: slow over large distances; rapid between adjacent habitats.	
genetic drift	• Repeated extinctions/recolonisations not reported. • Probably important at edge of range. • Bottleneck effects possible.	

Cryptic and warning coloration

The adaptive value of the black coloration of *P. madidus* is probably cryptic, the insect becoming virtually invisible on dark soil surfaces. It is not known whether the black coloration is aposematic although chemical defence mechanisms have been reported for carabid beetles (Evans, 1975). The dull red-leg colour, which is on the ventral surface of the beetle, is hard to distinguish from black in the shaded places where this species is normally found. Selective predation on the two leg colour forms is therefore unlikely.

Thermal melanism

The woodland and vegetated ground surface habitat of *P. madidus* means that it is not normally exposed to sunlight although, at low temperatures, sunlit patches may be sought. The leg colour differences constitute a small proportion of the total surface area of the animal, but the surface to volume ratio of the legs is high and any differential heating from external sources would be expected to affect locomotor activity. A weaker source of radiated heat, which may be important for a ground active beetle, is reflected from the ground and vegetated surfaces but, at longer wavelengths, the absorptivity and emissivity of biological materials is very high (95-99%), and colour is considered to be unimportant (Porter & Gates, 1969).

Spatial and temporal niches

Terrell-Nield (1990a) found some evidence that the red-legged morph frequency increased with distance into a wood and suggests this is due to the milder temperature and higher humidity conditions of woodland interiors. It is not known whether there are slight differences in the developmental rates between the morphs producing seasonal changes in frequency.

Morph fitness

It is not known whether there are differences in the fecundity or viability of the morphs. Attempts to rear *P. madidus* under laboratory conditions have not been successful beyond the third instar stage (Luff, 1973; Terrell-Nield, pers. comm.).

Sexual selection

Populations of red and black-legged morphs freely mix, but it is not known whether mating is random.

Gene flow, genetic drift, founder and bottle-neck effects

Although flightless, the *P. madidus* adult has been reported to cover 100m in a night (Terrell-Nield, 1990a). Even so, only around 10km could be covered in a season (3-4 months) and genetic mixing between spatially separated habitats is likely to be slow. The movement of *P. madidus* is also restricted by physical barriers, waterways, mountains, wide roads etc.

By contrast, rapid genetic mixing within and between adjacent habitats is very probable because of the high activity of the beetle during its reproductive season. This would tend to weaken clines between adjacent habitats, even when these are deemed to favour the morphs differentially.

Over a larger geographic range and on islands, it could be argued that the spatial variation in morph frequency is due to founder effects, i.e. the frequency reflects the original colonisers, modified only by genetic mixing from adjacent populations. The large-scale clines may be due to the slow diffusion of alleles from the founder populations. Physical barriers creating bottleneck effects may be producing the steep clines over relatively short distances.

In addition, genetic drift may occur in small isolated populations towards the edge of the beetle's range or after localised catastrophic events such as flooding, change of land use etc.

All in all, given the uncertainty about the factors contributing to the morph frequency variation in the different regions of the UK, it is not clear whether *Pterostichus madidus* can side step some of the interpretative difficulties found for other arthropod species.

1.10 Summary and overall aims of the research

Colour polymorphism occurs in many insect species. In particular, melanic forms are common. It is believed that selective factors are the main influence on highly variable morph frequencies but investigations have been dogged by the assumption that the visible indicator of variation, colour, is the direct cause of the variation. Up to the mid 1980s, variation in frequency distribution was usually explained in terms of the cryptic, aposematic or thermal adaptive value of morph colours, but there were a number of inconsistencies, which could not be explained by the adaptive value of colour alone.

By the late 1980s, investigators were, separately, suggesting that the pleiotropic effects of closely linked genes with no pigmentation function may be producing ecophysiological differences between morphs (e.g. Brakefield, 1988; Halkka & Halkka, 1990; Terrell-Nield, 1990a). There have been no investigations of a "super gene" and more recent investigations have tended to concentrate on the non-selective processes affecting morph frequency. These were found to dominate in environments subject to repeated catastrophic events. In more stable habitats, selective factors continue to be invoked to explain morph frequency distributions.

P. madidus, like *B.betularia* and *A. bipunctata*, shows considerable geographical variation in morph frequency and selective factors are believed to be the main influence. Large-scale frequency distribution of the leg colour morphs has been associated with climatic factors and it is thought that the beetle could be used as a bio-indicator of the direction of climatic change in Britain. However, the genetics of leg colour in *P. madidus* is not known, knowledge of the development of *P. madidus* is limited, and the underlying mechanisms which may be influencing morph frequencies are not understood.

The overall aims of this research are therefore:

- to estimate the random and systematic error on morph frequency data in order to assess its level of precision (Chapter 2);
- to extend Terrell-Nield's study by investigating changes in morph frequency over time (Chapter 3);
- to investigate associations between environmental factors and morph frequencies on a smaller spatial scale than that used in the Terrell-Nield study in order to identify the most important factors affecting variations in morph frequency (Chapter 4);
- to understand the genetics of leg colour polymorphism through breeding experiments (Chapter 5);
- to investigate the growth, development and survival of *P. madidus* in its immature stages in order to improve our understanding of the whole ecology of the beetle and identify whether there may be a physiological difference between the morphs in the larval, pupal or adult stages (Chapter 6).

Models are developed, firstly, for predicting morph frequencies in the different geographical regions of Britain and, secondly, for predicting *P. madidus* development in the field under different climatic conditions. The research is, therefore, divided between field and laboratory work (Sections B and C respectively). The discussion in Chapter 7 (Section D) attempts to draw together the results for both field and laboratory investigations. Conclusions and suggestions for further work are also given in this chapter.

SECTION B

Field work

CHAPTER 2: Estimation of error on morph frequency data

2.1 Aims

i) To estimate the standard deviation, standard error and confidence limits on morph frequencies obtained during one season from seven sites in a woodland area.

ii) To investigate the relationship between morph frequency measurements and location of pitfall traps within a sampling area, and time of trapping within one year.

iii) To relate the size of random and systematic errors to sampling technique.

2.2 Introduction

Much of the analysis in Chapters 3 and 4 is based on morph frequency data obtained from only one sampling point at one period of time in the season. This is common practice (e.g. Terrell-Nield, 1990a). Taking repeated measurements at several sampling points within a habitat is rarely practical for large scale, countrywide monitoring of morph frequencies. Even when several data sets over time or space are available, if the number of animals sampled per site or trapping period is low, the sub-samples are pooled (e.g. Oxford, 1985). Consequently, the variation in morph frequency between different plots or different trapping dates within one site (systematic error) is not always known. A further consideration is the minimum number of animals required to minimise random error (noise). Although sample numbers of 30 (Scali & Creed, 1975) or 40 (e.g. Oxford, 1985; Brakefield, 1990b) are usually regarded as adequate representations of the actual frequency in the field, numbers as low as 20 have been quoted when one morph is apparently absent (e.g. Bengtson & Hagen, 1977; Terrell-Nield, 1990a).

However, for various reasons, morph frequency stability within a site or season cannot be assumed. For example, Oxford (1985) found a high variability in sub-samples with respect to morph frequencies of the spider, *Enoplognatha ovata* (Clerck). Brakefield (1984a) identified seasonal differences in the activity of the two-spot ladybird morphs from his analysis of sequential sampling. Terrell-Nield (1990a) found an increase in the red-legged morph frequency of *Pterostichus madidus* with increasing distance into a Nottinghamshire woodland, the frequency ranging from 0 to 29% with a mean of 15%.

On the other hand, Doberski & Gazzy (2000) did not detect a significant variation in *P. madidus* morph frequency between different plots characterised by different tree species within two sites in Thetford Forest (East Anglia), although the overall morph frequency was significantly different between the two sites. Doberski also found stability over the season, with frequencies fluctuating within 5% of the mean.

This chapter, therefore, examines the spatial and temporal variation in *P. madidus* morph frequencies within a typical woodland area in order to estimate the random and systematic errors that might be occurring.

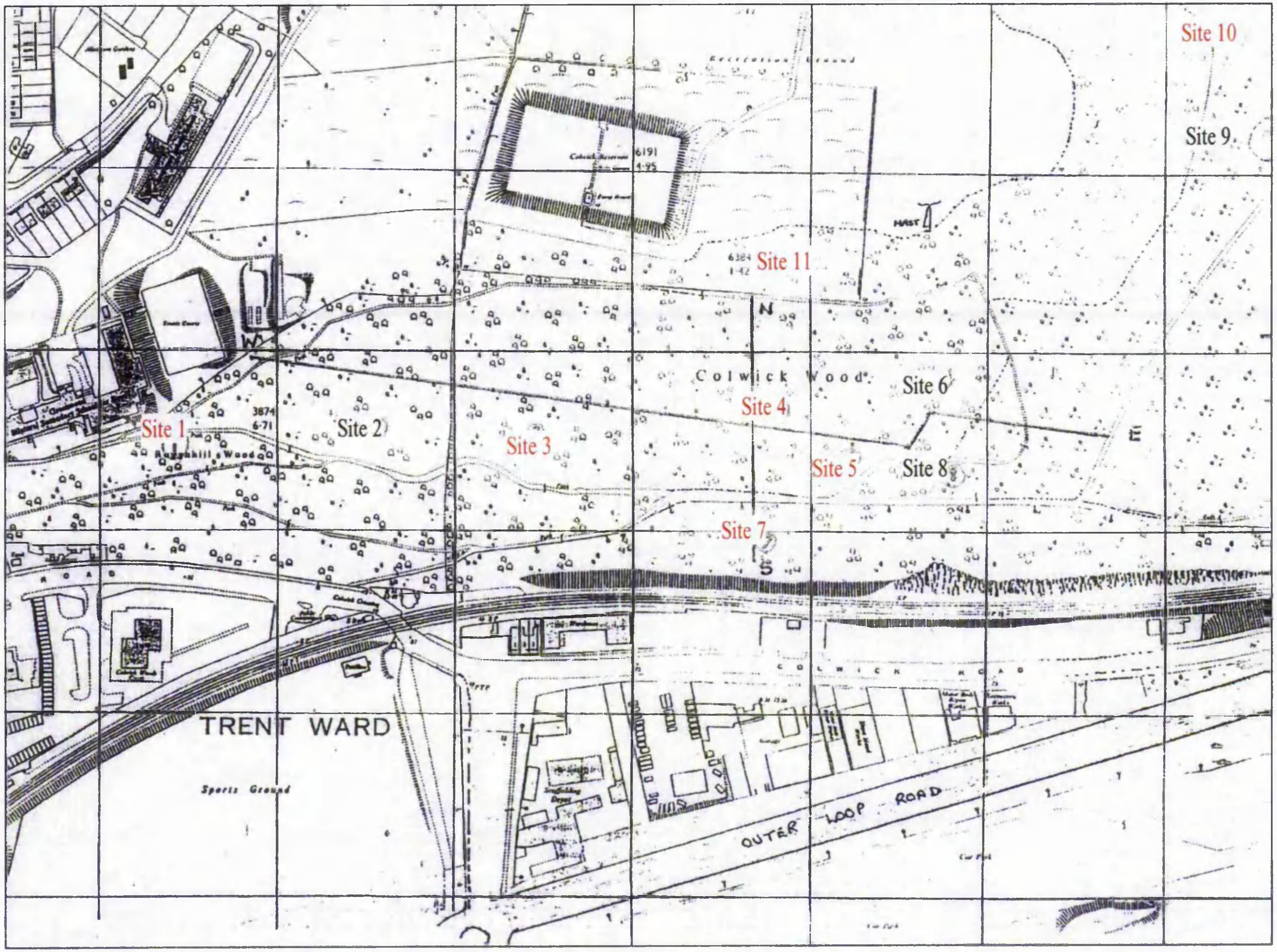
2.3 Site description (Fig 2.1)

The site is a deciduous woodland area of around 12 hectares, Colwick and Roughill Wood (Grid ref: SK 597398), which lies between densely populated residential and industrial conurbations in the south-east region of the City of Nottingham. The two woods were once divided along the line of an old field boundary. This is still visible in Fig 2.1. The woodland is situated on a steep, south-west facing escarpment which lies 700m north of the river Trent and rises from 23m to 96m above sea level, one of the highest points of the city.

The history of Colwick and Roughill Wood has been described by Lynn (1990). Originally an ancient woodland of some 6 hectares, it is recorded in the Domesday Book as an underwood (a coppiced wood). The woodland area increased to around 35 hectares by the sixteenth century, as abandoned agricultural land reverted to hawthorn scrub. By the nineteenth century, the wood was reduced to the size and shape it is today, partly due to tree felling to make way for the railway. During the nineteenth century, it was subdivided between a number of private owners and companies, each of whom managed their portion in different ways. The Nottingham City Council purchased the wood in 1925. Apart from planting some silver birch and oak trees and replacing the scrub valley with a grassed area, the woodland is largely unmanaged. It is now an amenity wood, popular with local residents.

Given this history, the woodland has developed into a mosaic of mature, widely spaced trees, such as beech, ash and oak, regenerating areas of birch and sycamore, and scrub regions of elder or hawthorn. The ground layer is typically ground ivy and bramble. Ancient woodland species such as bluebells are also present. Towards the edge of the wood and close to pathways, grasses and aspect species such as Dog's Mercury are present.

<u>Fig 2.1</u> Colwick and Roughill Wood in the city of Nottingham, showing position of sites 1 to 10 sampled in 1989 by S. Lynn and (in red) sites sampled in this study. Each square = 1 ha. (Adapted from Lynn, 1990).
(Note: A vegetation survey was conducted by S. Lynn along the N-S and E-W transect lines shown on map).



2.4 Method

2.4.1 **Sampling** (see Fig 2.1 and Table A.1 in Appendix A)

Sampling of Colwick and Roughill Wood was originally undertaken in May 1989 by S. Lynn of Nottingham Trent University. Seven of the ten sites monitored by Lynn were re-set in 1995. The site numbers shown in Fig 2.1 are those given by Lynn (1990). Table A.1 gives a brief description of each site. (Note: Results for Site 11, which was not established until 1997, are not presented in this chapter). Site 7 was established at the beginning of June 1995. Site 3 was closed half way through August because of repeated vandalism and Sites 1 and 10 were closed at the end of August. With these exceptions, the sites were sampled weekly from the beginning of May until the end of August. After this, the traps were left undisturbed for three weeks before sampling at the end of week 3 in September. The traps were then left undisturbed for five weeks from week 4 of September until the end of October, before the final sampling.

The standard method for pitfall trapping was followed (e.g. Terrell-Nield, 1990a). At each site, a 10 x 2 array of pitfall traps was set, with a distance of one metre between each trap. 50% ethylene glycol was used as a preservative. The pitfall trapping method allows the relative activity of *Pterostichus madidus* to be estimated. However, because catch size is influenced by a wide range of factors (e.g. Luff 1975), population size cannot be estimated.

2.4.2 Analysis

i) Test for normal distribution

The overall distribution of morph frequencies from 49 samples was checked by obtaining a normal probability plot generated by Minitab 11, which measured its straightness by the correlation coefficient. For the probability plot, normal scores were obtained for each value. These are the expected values of the order statistics (actual values in order of smallest to largest value) for a standard normal curve. The expected value of the smallest data value (termed first order statistic) is calculated first. Then the expected value for the second order statistic is calculated and so on.

ii) Calculation of error on the morph frequency data.

The frequencies for each site and each sampling date were averaged and the averages were compared with the actual frequencies. The standard deviation, standard error and confidence limits were found for each averaged value using Excel 97.

iii) Analysis of frequency variation within and between samples (sites or trapping periods)

Because frequency values were obtained, non-parametric tests were used. These do not assume normal distribution.

Variability within sites and trapping periods: Chi-squared tests were performed to determine whether the variation in morph frequency for (1) each site over time and (2) each sampling period over space is significant, using the equation:

$$\text{Chi-squared} = \frac{\sum(O - E)^2}{E} \qquad \text{(eq. 2.1)}$$

where O is the observed value

E is the expected value

Contingency tables were produced to obtain the expected value, E, where:

$$E = \frac{(\text{Row total}) \times (\text{Column total})}{\text{Grand total}} \qquad \text{(eq.2.2)}$$

Variability between sites and trapping periods: The Kruskal-Wallis H test (for more than two samples) or Mann-Whitney U test (for two samples) were performed using Minitab 11 to assess whether the variations in spatial and temporal morph frequencies are significant. The Kruskal-Wallis and Mann-Whitney tests are non-parametric equivalents of One Way Analysis of Variance and t-tests respectively, and test for differences between the medians.

2.5 Results

In order to obtain an average of at least 40 individuals per site and trapping period, data has been amalgamated over 4 weeks for May and June, and 2 weeks for July and August. There were no separate data sets for the September and October period.

Using the pooled data, Table 2.1 shows the actual numbers of red and black-legged morphs trapped at each site from May to October. The sites are arranged in order of their distance into the wood. For Site 10, the numbers given for weeks 3 and 4 of August include the data for weeks 1 and 2 when numbers of *Pterostichus madidus* were low (< 10) due to disturbance of some traps by an animal. Overall red-legged morph frequencies for each site and trapping period are presented in the final row and column of Table 2.1.

Table 2.2.i presents the red-legged morph frequencies for each site over time (columns 2 to 8) and each trapping period over space (rows 2 to 9), as calculated from the data in Table 2.1. Actual and averaged frequencies are given in bold for all the sites (columns 9 and 10) and trapping periods (rows 10 and 11). The average number of *P. madidus* trapped per site and trapping date is shown in the final row and column respectively. The standard deviation, standard error and confidence limits for the average frequencies are given in rows 12 to 14 and columns 11 to 13. Table 2.2.ii gives the statistics for all the frequency measurements.

2.5.1 Spatial and temporal activity of *P. madidus* in Colwick and Roughill Wood.

P. madidus was at highest activity at Site 5 (penultimate row in Table 2.1). This was a relatively open area of mature trees in the centre of the wood, where dense bramble ground cover may have provided protection from larger predatory mammals and birds (see Table A.1). The least favoured sites were Sites 1 and 10 which, by their proximity to the wood edge (Site 1) and firebreak (Site 10), may be more exposed.

The highest activity of *P. madidus* occurred during the last two weeks of July, with relatively high activity during the earlier part of July and throughout August (see column 8 in Table 2.1). A sharp drop in activity occurred from September onwards as temperatures fell, with low activity from late September.

2.5.2 Range and distribution of red-legged morph frequencies.

Using all the sampling data from Table 2.1, 49 measurements in total, the actual red-legged morph frequency in Colwick and Roughill Wood was found to be 32.9% (see Table 2.2. ii). The average of the 49 site frequencies presented in Table 2.2.i fell within 1% of the true frequency for the wood, whether or not the more variable autumn frequencies were excluded from the data (rows 1 and 2 of Table 2.2.ii).

Table 2.1 **Number of red and black-legged *Pterostichus madidus* morphs trapped at each site on each trapping date in Colwick and Roughill Wood, showing actual red-legged morph frequency for pooled data for each site and trapping date.**
(B = black-legged morph; R = red-legged morph; T = total).

Trapping period	Site number (distance in metres from the nearest woodland edge in brackets)																					*Average per trapping period*			Total per trapping period			
	1 (8m)			8 (13m)			7 (18m)			10 (20m)			3 (28m)			5 (40m)			4 (53m)									
	B	R	T	B	R	T	B	R	T	B	R	T	B	R	T	B	R	T	B	R	T	*B*	*R*	*T*	B	R	T	%R
May (4 wks)	48	13	61	81	34	115				19	9	28	35	19	54	97	47	144	40	21	61	*53*	*24*	*77*	320	143	463	30.9
June (4 wks)	23	10	33	65	41	106	24	14	38	40	15	55	55	30	85	68	30	98	35	12	47	*44*	*22*	*66*	310	152	462	32.9
July (wks 1+2)	24	9	33	98	44	142	24	14	38	20	7	27	64	40	104	158	73	231	121	55	176	*73*	*35*	*107*	509	242	751	32.2
July (wks 3+4)	56	29	85	233	113	346	74	49	123	39	16	55	192	114	306	381	164	545	134	67	201	*158*	*79*	*237*	1109	552	1661	33.2
August (wks 1+2)	40	12	52	88	39	127	67	29	96				33	23	56	107	50	157	124	79	203	*77*	*39*	*115*	459	232	691	33.6
August (wks 3+4)	81	39	120	113	68	181	74	41	115	39	22	61				121	41	162	120	62	182	*91*	*46*	*137*	548	273	821	33.3
Sept (wks 1+2+3)	26	11	37	37	22	59	32	11	43	12	3	15				43	22	65	16	4	20	*28*	*12*	*40*	166	73	239	30.5
Sept (wk 4), Oct (4 wks)				14	5	19	26	18	44							44	30	74	17	5	22	*25*	*15*	*40*	101	58	159	36.5
Total per site	298	123	421	729	366	1095	321	176	497	169	72	241	379	226	605	1019	457	1476	607	305	912				**GRAND TOTAL**			
Average per site	*43*	*18*	*60*	*91*	*46*	*137*	*46*	*25*	*71*	*28*	*12*	*40*	*76*	*45*	*121*	*127*	*57*	*185*	*76*	*38*	*114*				3522	1725	5247	
%R	29.2			33.4			35.4			29.9			37.4			31.0			33.4						32.9			

Table 2.2 **Red-legged morph frequencies (%) in Colwick and Roughill Wood.**

(SD = standard deviation; SE = standard error; CL = confidence limits on the averaged means).

i) Red-legged morph frequencies for each site and trapping date, showing actual and averaged mean frequencies and mean number of *P. madidus* trapped per site and trapping date.

Trapping	Site number (distance in metres into wood in brackets)							*actual*	**average**			95%	*mean no.*
period	1 (8m)	8 (13m)	7 (18m)	10 (20m	3 (28m)	5 (40m)	4 (53m)	*%R*	%R	SD	SE	CL	*per site*
May (4 wks)	21.3	29.6		32.1	35.2	32.6	34.4	*30.9*	**30.9**	4.64	1.89	4.87	*77*
June (4 wks)	30.3	38.7	36.8	27.3	35.3	30.6	25.5	*32.9*	**32.1**	4.60	1.88	4.60	*66*
July (wks 1+2)	27.3	31.0	36.8	25.9	38.5	31.6	31.3	*32.2*	**31.8**	4.24	1.73	4.24	*107*
July (wks 3+4)	34.1	32.7	39.8	29.1	37.3	30.1	33.3	*33.2*	**33.8**	3.50	1.43	3.50	*237*
August (wks 1+2)	23.1	30.7	30.2		41.1	31.8	38.9	*33.6*	**32.6**	5.95	2.66	6.84	*115*
August (wks 3+4)	32.5	37.6	35.7	36.1		25.3	34.1	*33.3*	**33.5**	4.00	1.79	4.60	*137*
Sept (wks 1+2+3)	28.1	37.3	25.6	20.0		33.8	20.0	*30.3*	**27.5**	6.49	3.22	8.28	*40*
Sept (wk 4) + Oct (4 wks)		26.3	40.9			40.5	22.7	*36.5*	**32.6**	8.20	3.23	10.27	*40*
actual %R	*29.2*	*33.4*	*35.4*	*29.9*	*37.4*	*31.0*	*33.4*						
average %R	**28.1**	**33.0**	**35.1**	**28.4**	**37.5**	**32.1**	**30.0**						
SD	4.35	4.14	5.03	5.02	2.19	3.99	6.14						
SE	1.64	1.47	1.90	2.05	0.98	1.41	2.17						
95% CL	4.03	3.47	4.66	5.27	2.72	3.34	5.14						
mean no. per trapping period	60	137	71	40	121	185	114						

ii) Statistics for all measurements.

	actual	**average**			95%
	%R	%R	SD	SE	CL
All data (n=49)	*32.9*	**31.9**	5.51	0.79	1.58
All data to end of August (n=39)	*32.9*	**32.5**	4.68	0.74	1.50

Using these 49 calculated frequencies, the histogram in Fig 2.2 shows an approximately normal distribution, with slight skewing at the lower frequencies. The frequencies ranged from 20 to 41%, with half the measurements lying within ± 4% of the mean. The normal probability plot in Fig 2.3 gives evidence of a close fit of the data to a straight line ($r = 0.991$; $P < 0.01$), indicating that the samples are from a normal population.

An approximately normal distribution also appears to be the case for individual sites and trapping period when actual frequencies are compared with average frequencies. With the exception of Site 4, average frequencies of each site (row 11 of Table 2.2.i) fell within ± 1.5% of the true frequency (row 10). The average frequency for Site 4 was under-estimated by 3.4% mainly due to two low frequency values for the September and October trapping periods. The average frequency for each trapping period fell within ± 1% of the true frequency until the September/October trapping periods when there was an under-estimation of 3 to 4% (see columns 9 and 10 of Table 2.2.i).

2.5.3 Number of individuals per sample, standard deviation, standard error and 95% confidence limits.

Fig 2.4 shows a high variability in the red-legged morph frequency when the number of *P. madidus* trapped fell below 50, with individual frequencies up to +8% and –15% of the actual mean. For $n < 35$, most frequencies were less than the mean, suggesting that the red-legged morph is less active than the black-legged morph when the activity of both morphs is low.

Not surprisingly, the standard deviation on the mean frequency was sensitive to the mean number of individuals trapped. A weak, negative logarithmic relationship is shown in Fig 2.5. The standard deviation is ± 4 to 5 of the mean when the number of individuals per sample exceeds 60, falling to ± 3 to 4 for sample numbers higher than 150. However, the standard deviation rises to around ± 7 when the sample falls to 40 individuals. Fig 2.5 also shows that samples collected from several sites on one occasion only (filled circles) have higher standard deviations than samples collected from one site only on several occasions (open circles).

Fig 2.2 Histogram of red-legged morph frequencies obtained from Roughill and Colwick Wood, using data for all sites and trapping dates (49 measurements in total).

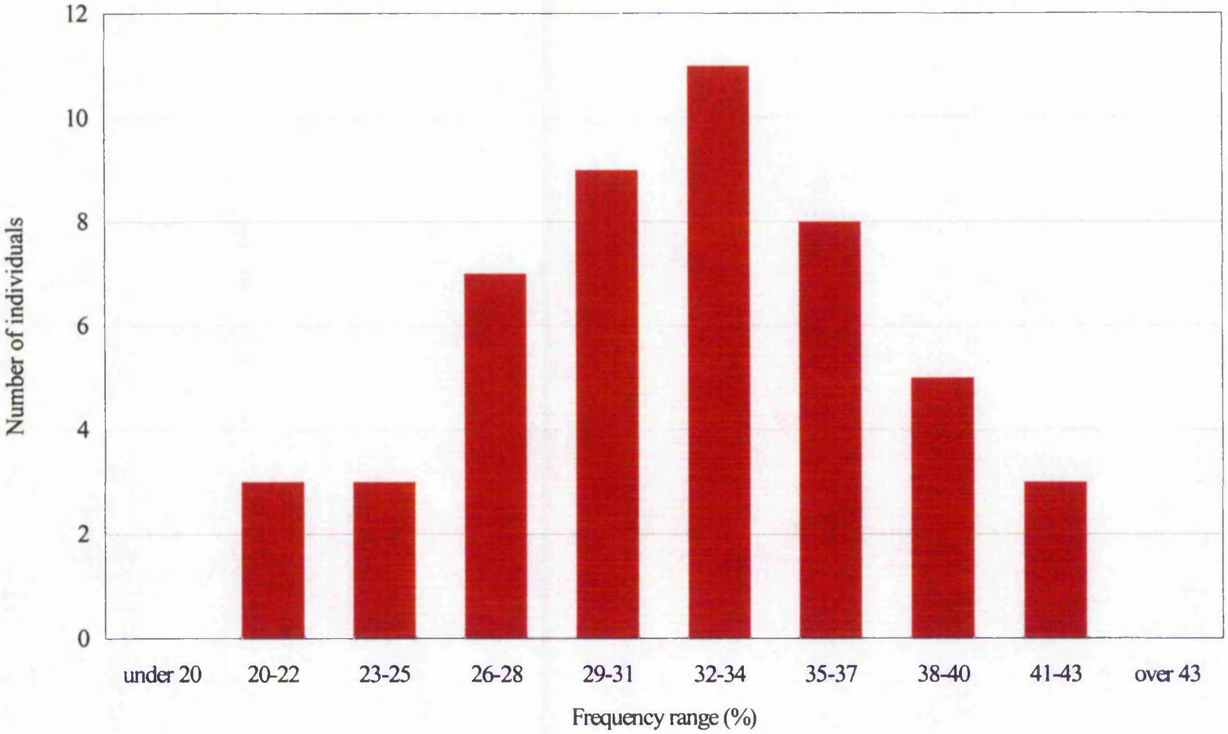
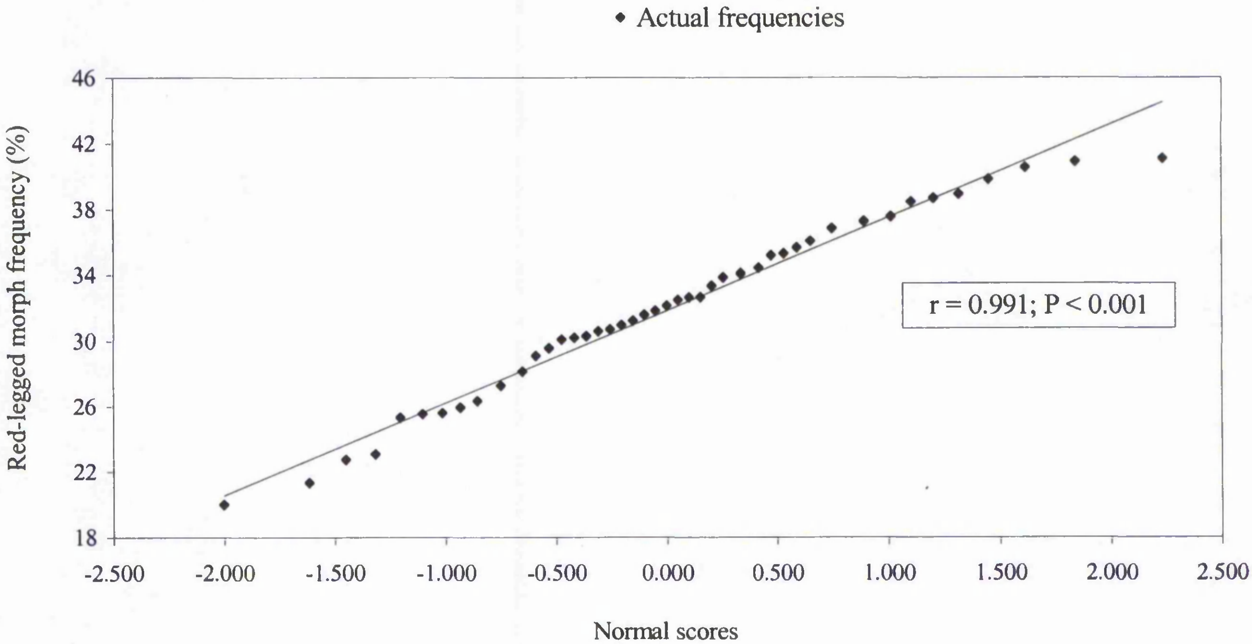


Fig 2.3 Normal probability plot of red-legged morph frequencies obtained from Roughill and Colwick Wood, using data for all sites and trapping dates (49 measurements in total). Correlation with straight line is shown.



The minimum number of individuals per sample depends on the level of precision required. This can be estimated from a pre-determined standard for the confidence limits. Southwood (1978) suggests that a half-width confidence limit of ± 10% of the mean is satisfactory. Since the statistics on black-legged morph frequencies produce the same confidence limits, the probability of occurrence for both morphs needs to be considered. The required number of individuals (N) is given by the formula (in Southwood, 1978):

$$N = \frac{t^2pq}{D^2} \qquad \text{(eq 2.3)}$$

where t is the Student's t at a probability of 0.05 and depends on the number of samples

p is the probability of occurrence (= 0.329)

q is 1-p (= 0.671)

D is the predetermined half-width of the confidence limits (= 0.1)

Using the value of 2.0 for $t_{0.05}$ (a reasonable approximation for more than 40 samples), the number of individuals required at these morph proportions would be 88. The relationship in equation 2.3 is shown in Fig C.1 of Appendix C. The equation predicts that more individuals per sample are required as the two morphs approach a 1:1 ratio.

The confidence limits and standard error can also be used as predetermined standards to estimate the number of samples that should be taken. A standard error of 5% of the mean is considered satisfactory (Karandinos, 1976; Southwood, 1978). When based on frequency measurements, the mean for both morphs needs to be taken into account. Since the average of both means is 50 (whatever the frequency), the predetermined standard is ± 2.5 for a standard error at 5% of the mean, and ± 5 for the half-width of the 95% confidence limits at 10% of the mean. As shown in Fig 2.6, neither the standard error nor the confidence limits exceed these predetermined levels when the number of samples taken over time or space is 7.

2.5.4 Spatial and temporal variation in red-legged morph frequency.

Results for the statistical analyses are summarised in Tables 2.3 and 2.4. Figs 2.7 and 2.8 show the variation in red-legged morph frequency at each site for each trapping date (Fig 2.7) and the variation at each trapping date for each site (Fig 2.8). The averaged frequency with one standard deviation is also given (red bar). Sites are arranged by increasing distance into the wood.

Fig 2.4 Relationship between numbers of *P. madidus* trapped and % red-legged morph frequency expressed as deviation from the average frequency (49 samples in total).

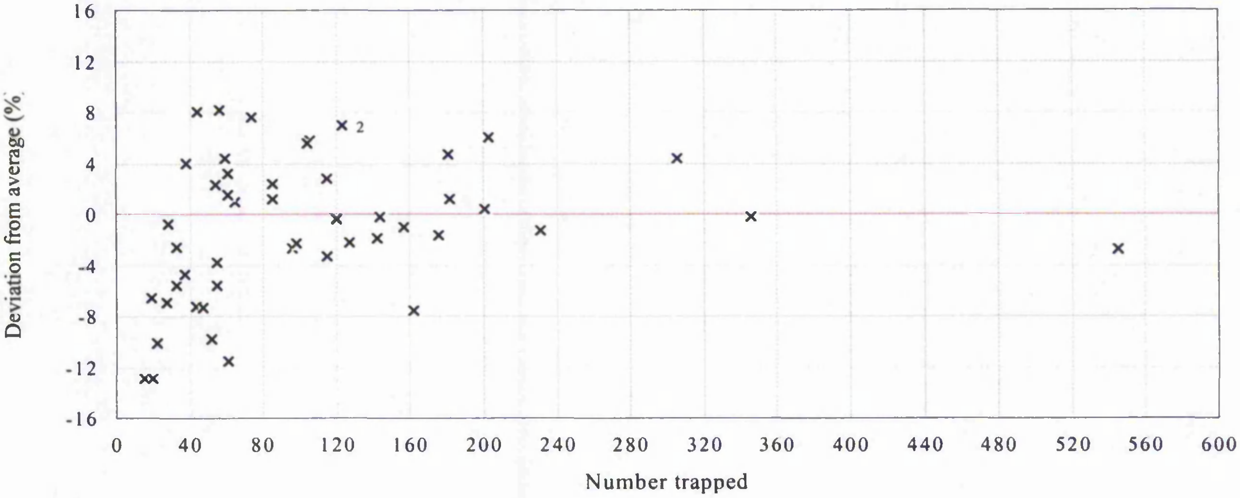


Fig 2.5 Association between standard deviation and mean number of *P. madidus* trapped at each site over time (open circles) and at each trapping date over space (filled circles). (Trendline shows logarithmic relationship using all values).

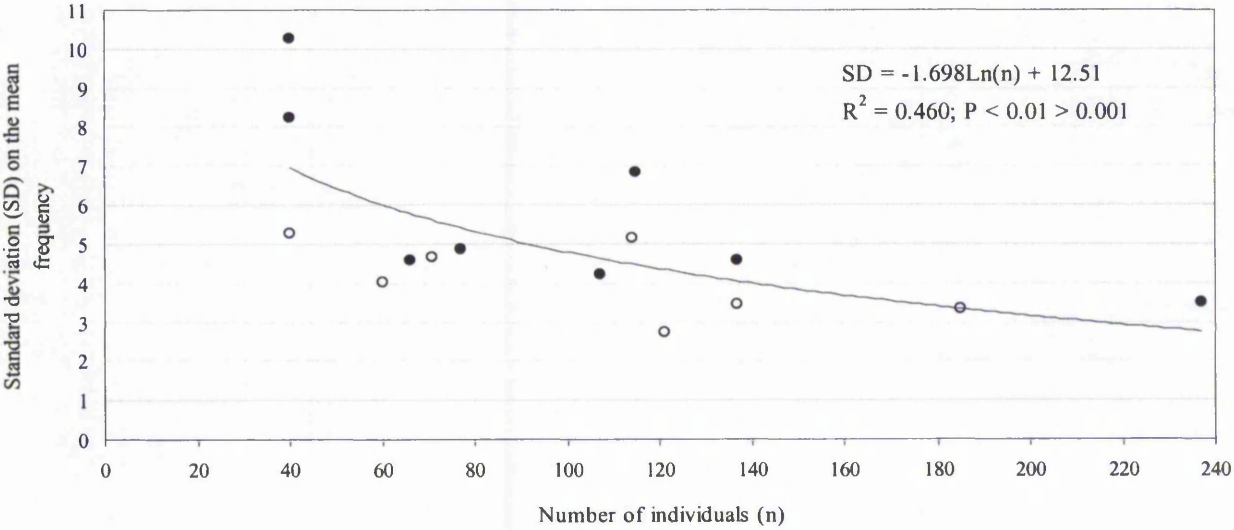
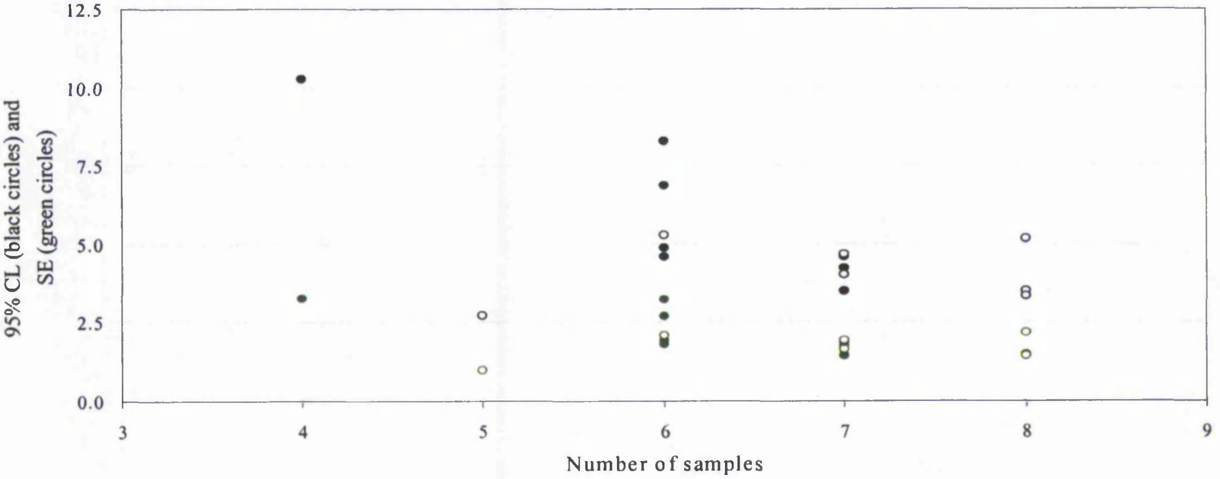


Fig 2.6 Association of 95% confidence limits (CL) and 1 standard error (SE) with number of samples taken for each site (open circles) and each trapping date (filled circles).



i) Variability within samples (sites or trapping period)

Temporal variation within each site: Chi-squared analysis found no significant difference in the morph proportions within each site over the period of trapping (Table 2.3.i). With the exception of Site 3, which exhibited an increase in the red-legged morph frequency for the short period this site was monitored, the temporal frequency variation at each site appears to be random (see Fig 2.7).

Spatial variation within each trapping period: Chi-squared analysis found no significant difference in the morph proportions of each site for each trapping date (Table 2.3.ii). However, Fig 2.8 indicates an increase in red-legged morph frequency with increasing distance into the wood for May and the first two weeks of August. As shown in Fig 2.9, the relationship is logarithmic and was found to be significant for May ($r^2 = 0.74$; $P = 0.03$) and almost significant for weeks 1 and 2 of August ($r^2 = 0.62$; $P = 0.06$). There is probably no correlation at distances over 30m into the wood.

ii) Variability between samples (sites or trapping periods)

Morph frequency variation between sites: The Kruskal-Wallis test identified a significant difference between the median morph frequencies of the sites (P = 0.03, see Table 2.4.i). Obviously, there is a significant difference between samples with the largest negative and positive z values[1] (Sites 1 and 3 respectively). Using the equivalent of the Kruskal-Wallis test for two samples (the Mann-Whitney U-test), a significant difference was also found between Sites 10 and 3 (P = 0.02), Sites 1 and 7 (P = 0.04) but not Sites 7 and 10 (P = 0.07). The tendency towards lower morph frequencies at Sites 1 and 10 and higher morph frequencies at Sites 3 and 7 is also evident from the means shown in Fig 2.7.

Morph frequency variation between trapping dates: The Kruskal-Wallis test found no difference in the median morph frequencies of the trapping periods (P = 0.64 – see Table 2.4.ii). Although the final trapping period was excluded from this analysis because the sample number in this group was less than 5, the mean frequencies shown in Fig 2.8 similarly give no evidence of any difference between the trapping dates.

In summary, these results suggest spatial heterogeneity in the wood but, between the sites, a stable morph frequency over the season, i.e. the morphs maintain their relative proportions over time but, under certain environmental conditions, show a difference in their relative activity or distribution over space.

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[1] z value indicates how the mean rank for group differs from the mean rank for all observations.

Fig 2.7 Variation in red-legged morph frequency at each site over time, showing (in red) the standard deviation on the average frequency. Distance into the wood in metres is given in brackets after each site number.

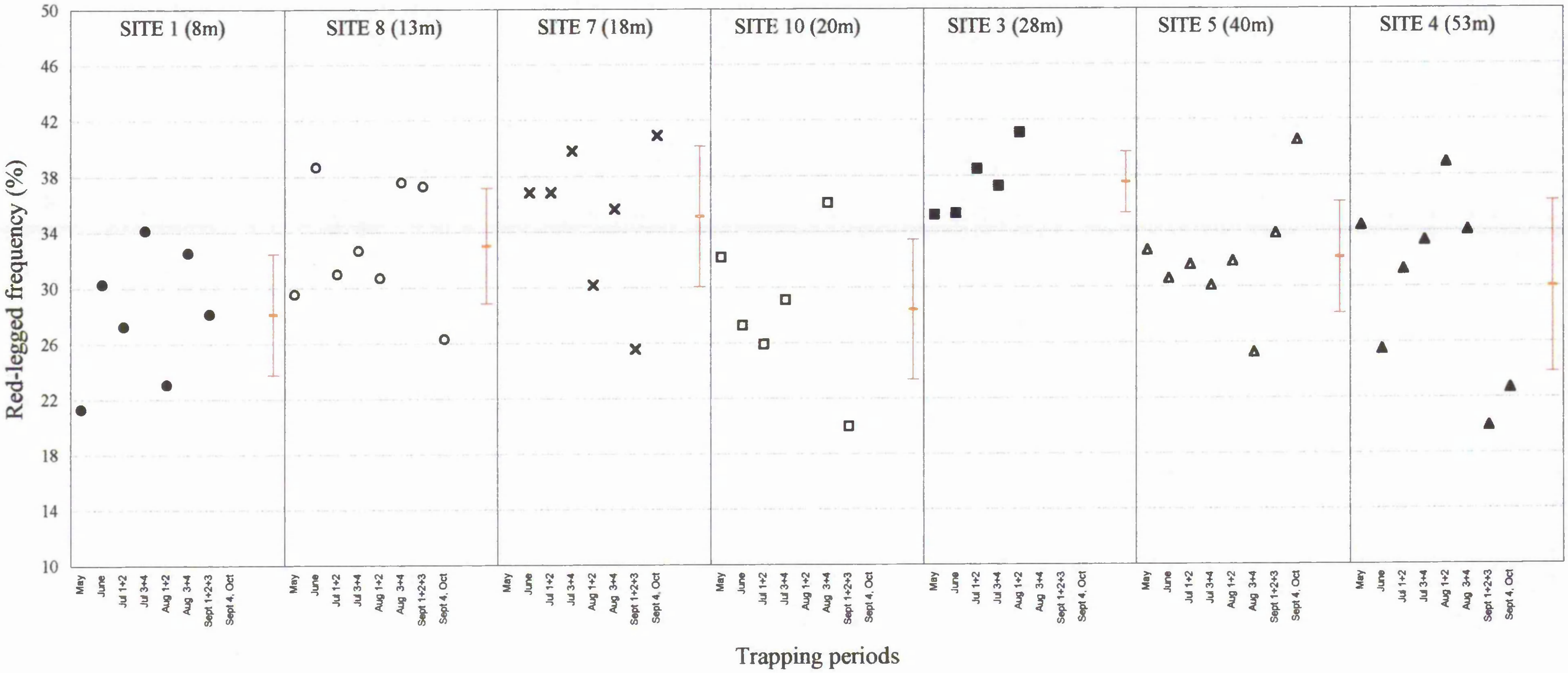


Fig 2.8 Variation in red-legged morph frequency for each trapping period over space, showing (in red) the standard deviation on the average frequency.

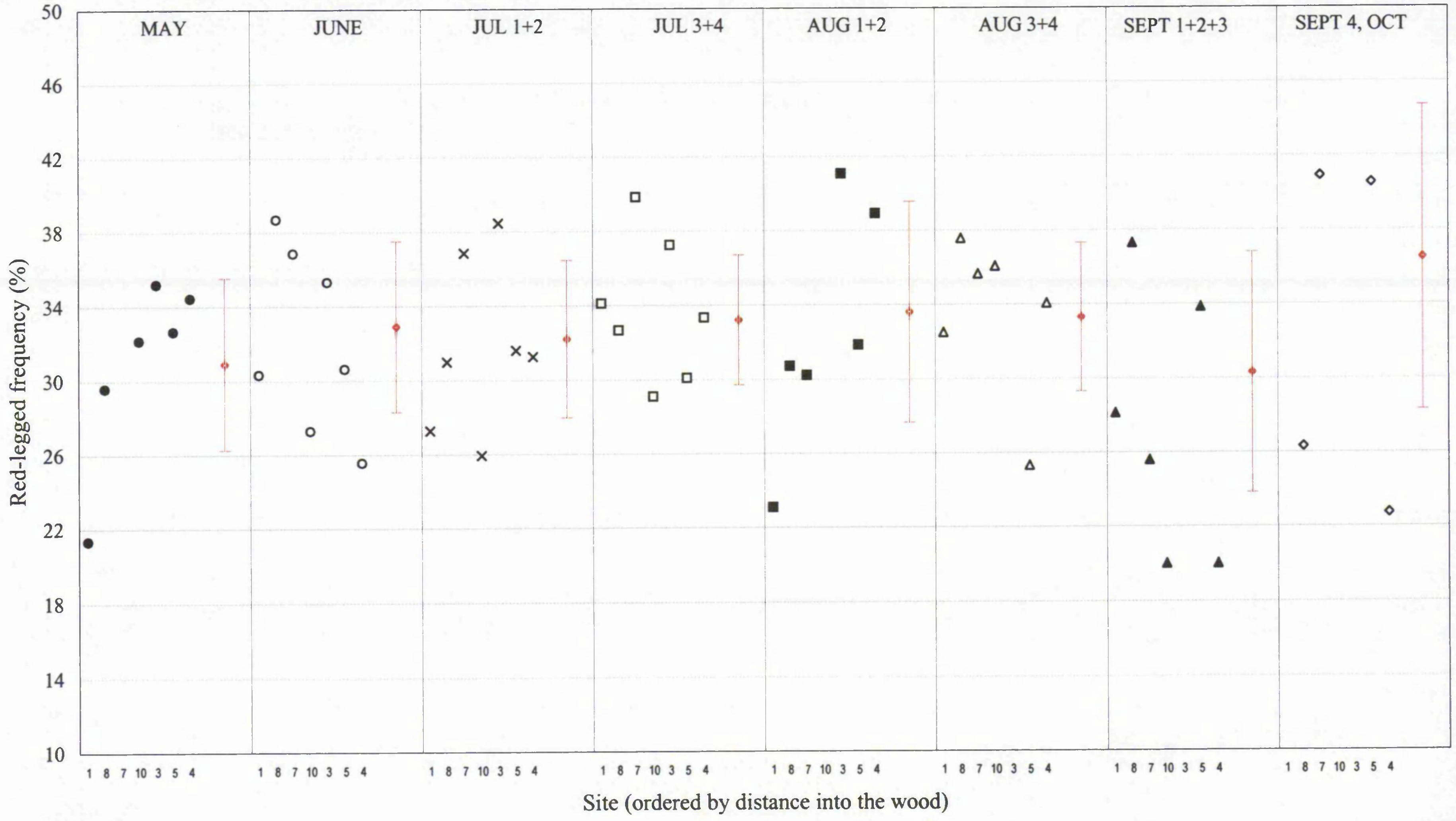
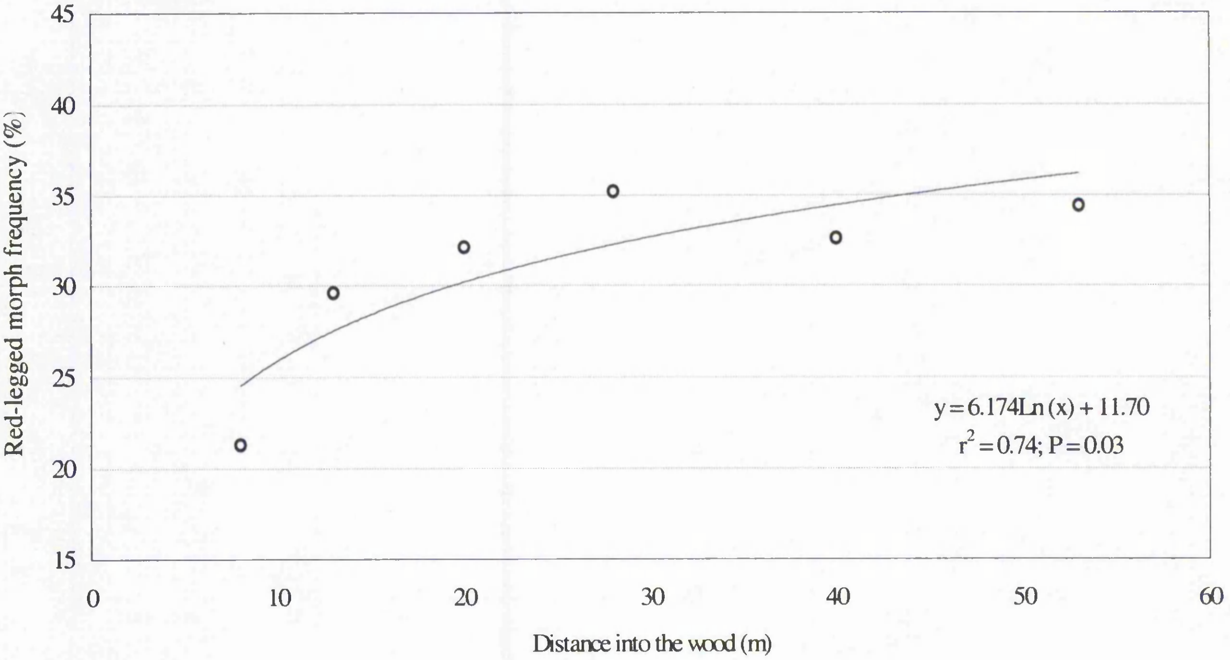


Fig 2.9 Correlation of % red-legged morph frequency and distance into Roughill and Colwick Wood in 1995.

i) MAY



ii) AUGUST, weeks 1+2

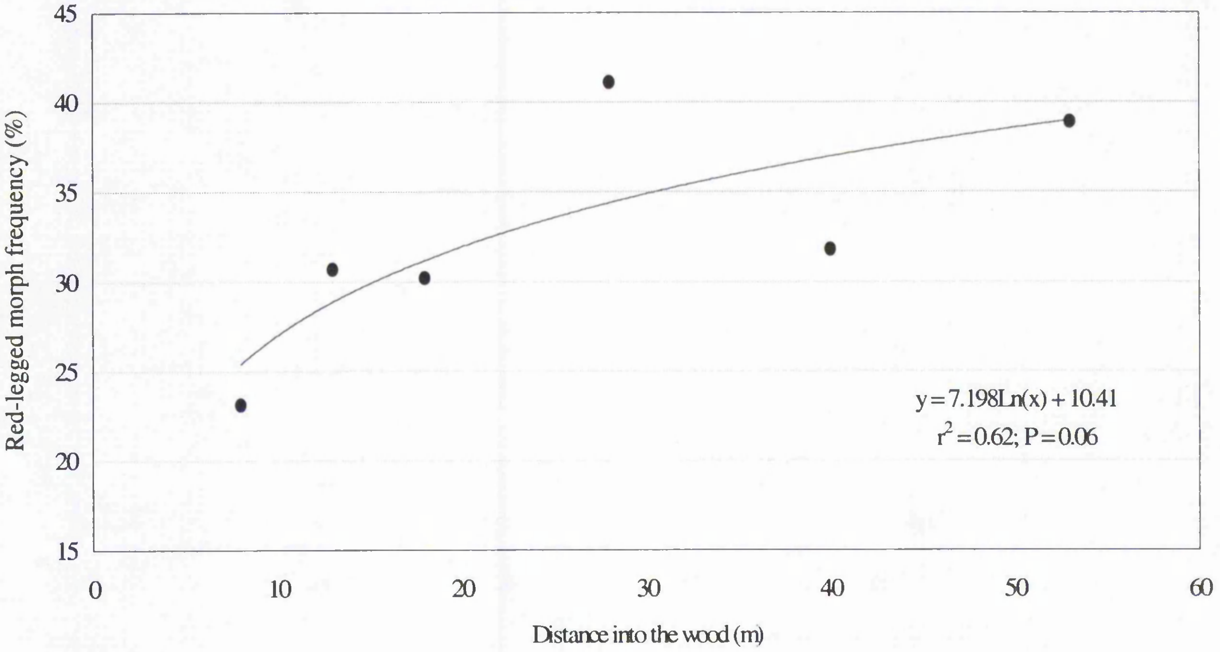


Table 2.3 **Results of chi-squared analysis on variation in morph proportions at each site and trapping date.**

i) Temporal variation within each site

SITE	1	8	7	10	3	5	4
no. of trapping dates	5	6	6	4	3	6	6
total *P. madidus*	421	1095	497	72	605	1476	912
$\Sigma\chi^2$	4.49	5.20	4.66	2.28	0.65	6.34	7.25
d.f	6	7	7	5	4	7	7
P	0.61	0.64	0.70	0.81	0.96	0.50	0.40

ii) Spatial variation within each trapping date

TRAPPING DATE	May	June	July 1+2	July 3+4	Aug 1+2	Aug 3+4	Sept 1+2+3	Sept 1, Oct
no. of sites	4	5	5	5	4	4	4	2
total *P. madidus*	463	462	751	1661	691	821	239	159
$\Sigma\chi^2$	3.77	4.37	3.30	7.58	7.74	6.73	3.94	3.54
d.f	5	6	6	6	5	5	5	3
P	0.58	0.63	0.77	0.27	0.17	0.24	0.56	0.32

Table 2.4 **Results of Kruskal-Wallis test for differences in % red-legged morph frequencies between sites and trapping dates.** (n = number of samples).

i) Between sites

SITE	1	8	7	10	3	5	4
n	7	8	7	6	5	8	8
median	28.1	31.9	36.8	28.2	37.3	31.7	32.3
ave rank	15.4	27.6	33.1	16.3	40.3	24	21.6
z value	-1.91	0.55	1.63	-1.59	2.53	-0.22	-0.73
H = 14.10; d.f. = 6; p = 0.029							

ii) Between trapping dates

TRAPPING DATE	May	June	July 1+2	July 3+4	Aug 1+2	Aug 3+4	Sept 1+2+3
n	6	7	7	7	6	6	6
median	32.4	30.6	31.3	33.3	31.3	34.9	26.9
ave rank	21.2	22.9	22.1	27.3	24.5	27.9	14.6
z value	-0.37	-0.03	-0.19	0.94	0.3	0.98	-1.69
H = 4.8; d.f. = 6; p = 0.639							

2.6 Discussion

The 49 red-legged morph frequency measurements obtained from Roughill and Colwick Wood in 1995 show an approximately normal distribution around a mean of 33%. Therefore, the standard deviations, standard errors and confidence limits obtained should be reliable.

The logarithmic relationship found between standard deviation and the mean number of *Pterostichus madidus* per sample meant that a high standard deviation (±7) is produced when the mean number of individuals per site or trapping period is 40. This decreases to ± 4 to 5 for numbers over 60 but further increases in the sample number will obviously bring diminishing returns on the reduction in standard deviation. Therefore equation 2.3, which predicts that the number of individuals required per sample for a 2:1 ratio, - as found in Roughill and Colwick Wood - is 88, is rather cautious. These figures are higher than minimum sample numbers accepted by investigators of *Adalia bipunctata*, although the melanic morphs of this species are usually at low frequency. According to the equation, a sample number of 15 is acceptable when one morph is rare (less than 5%). However, this does not take into account non-random patchiness in morph distributions.

To obtain a level of precision on the standard error and confidence limits recommended by Southwood (1978), there should be at least 7 samples taken over time or space. Unfortunately, for countrywide sampling, it is usually not possible to collect more than one sample. If only one sample is taken, how certain can we be that this is representative of the population?

It is known that for normal distribution, approximately 67% of the measurements in the population lie within one standard deviation from the mean and approximately 95% of measurements lie within two standard deviations from the mean (reported in Chalmers & Parker, 1986). If the sample contains sufficient numbers of individuals and a standard deviation of 4.5 can be assumed, there is a 2 in 3 chance that the frequency lies within ± 4.5 of the true % frequency and only a 1 in 20 chance that it deviates more than ± 9 of the frequency. The direction of deviation would not be known, of course.

The above analysis has assumed that error on the frequency measurements is random and a red-legged morph frequency of 32.9% is present throughout the wood over the entire season. Chi-squared analysis did not identify significant differences in morph proportions (1) between sites monitored on one occasion only and (2) between sequential measurements for one site.

There was, however, some non-random variability, which produced significantly different morph frequencies for some sites, and a directional trend in morph frequency with distance into the wood for some periods of the year.

It seems the position of the site in relation to the wood edge is important. The edge of a stand is known to experience greater insolation and exposure to wind (Geiger, 1966). Sunlight can enter the wood horizontally as well as vertically because of the low angle of solar radiation at temperate latitudes as well as the south-west aspect of Roughill and Colwick Wood. As described by Geiger (1966) and Unsworth & Montieth (1990), higher light levels result in higher air and soil temperatures, a greater range in air temperatures and a higher evapotranspiration rate which increases the soil temperature range; wind exposure at the wood edge tends to reduce temperatures.

A larger temperature range, particularly when associated with a lower minimum temperature, has been found to favour the black-legged morph (Terrell-Nield, 1990a). Both Sites 1 and 10 had lower median red-legged frequencies. Site 1 is within 10m of the edge of the wood. The north-east aspect of Site 10, although 20m from the wood edge, would expose it to colder winds. In addition, the close proximity of the site to a wide firebreak would subject it to higher light levels, as indicated by the grassy ground cover (see Table A.1). Beer's Law (reported in Unsworth & Monteith, 1990) predicts an exponential decay in solar radiation through a wood due to interception by the leaf canopy. Consequently, at a certain distance inside the wood, edge effects become insignificant. This appears to be the case in this investigation at distances over 30m into the wood.

The expected increase in red-legged morph frequency with distance into the wood occurred only in May and the first half of August. Terrell-Nield (1990a) also found a higher abundance of red-legged morphs inside Cheveral Wood (Nottinghamshire), using data collected in May. There may be a later emergence of the red-legged morph towards the edge of the wood, due to lower soil temperatures at more exposed sites until late spring. This hypothesis could explain the lack of correlation in June. At the other temperature extreme, under the exceptionally hot, dry conditions during the early part of August for the year of trapping, a higher proportion of the red-legged morph may have retreated into the cooler interior of the wood. However, it is not possible to distinguish between differences in development, dispersal or survival of the two morphs from frequency values obtained from pitfall trapping.

In summary, within a fairly heterogeneous habitat, it is reasonable to assume a normal distribution of morph frequencies with a standard deviation of ± 4 to 5 providing a sufficient number of individuals is trapped. Fig C.1 gives an approximation of the numbers required for a given frequency and justifies estimations of frequency based on low numbers at sites where one morph appears to be in high frequency. There is also some systematic variation in the morph frequencies, when overall frequencies between sites and trapping periods are compared. This can be explained by the position of the site in relation to the wood edge. Factors such as site altitude, aspect, tree density and canopy are also likely to affect micro-climate.

These observations suggest, when comparing red-legged frequencies from different years or different regions, the location of the trapping site may be important. The choice of woodland edges is useful for rapid sampling over a wide area, as occurred with much of the transect trapping by Terrell-Nield (1990a) and in this investigation. It also allows better comparison with frequency data from copses and hedgerows where edge effects are unavoidable.

CHAPTER 3: Investigation of morph frequency change in the UK over time

3.1 Aims

i) To compare 1975/6 morph frequencies along the north-south and south coast transects established by C. Terrell-Nield (Nottingham Trent University) with morph frequencies obtained in 1995, 1996 or 1998 along the same transects.

ii) To investigate morph frequency change over 3 or more years during the late 1980s or 1990s, using countrywide data collected by various investigators.

iii) To investigate whether there are differences in the male/female ratio of the two morphs and, if so, whether the sex ratio affected overall morph frequencies.

iv) To compare the direction of morph frequency changes over time with the seasonal temperature records for Central England for the same period.

v) Assuming leg colour is inherited and Mendelian genetics is operating (1) to compute the relative fitness of the two morphs for selection to produce the frequency changes found in Aims i) and ii), and (2) to compute the number of generations required to produce a significant change in morph frequency.

3.2 Introduction

Little is known about changes in the leg colour morph frequency of *Pterostichus madidus* over time. From pitfall trapping of two sites in a Corsican pine plantation in Thetford Forest, East Anglia, Doberski & Gazzy (2000) found a fluctuation in the red-legged frequency of only 5% around a mean of 90% over a six-year period (1989 to 1994). The frequency remained stable even though tree thinning halfway through this period produced a more open site. Doberski & Gazzy concluded that climatic factors did not appear to influence the relative fitness of the two morphs.

By contrast, work on both *Biston betularia* and *Adalia bipunctata* has shown evidence of a consistent reduction in melanic morph frequencies over time coinciding with a reduction in atmospheric pollution.

Comparing data for 1983 and 1984 with data from 1952 to 1970, Cook *et al* (1986) estimated a disadvantage to the *carbonaria* form of the peppered moth of about 12 per cent compared with 20 years earlier. Using data for consecutive years over an 18-year period, Brakefield & Lees (1987) estimated a 1.93 per cent per year decline in the melanic frequency of *A. bipunctata* in Birmingham, U.K., corresponding to a selective disadvantage for the melanics of about 10 per cent for each year. Although the melanic frequency fell from 46% to 10% over the 18 year period (see Fig 3 of Brakefield & Lees, 1987) it is, nevertheless, predicted to fall by less than 6% in 3 years. Given the typical standard error on the data (see Section 2.4.3), such a reduction would not be perceptible. It is therefore not surprising that Doberski & Gazzy (2000) found no observable change in morph frequency after the Corsican pine plantation was thinned, even if this had affected the microclimate sufficiently to alter the balance between the two morphs.

This chapter examines the evidence for temporal changes in morph proportions from data collected by various investigators. The direction of morph frequency change over time is compared with the seasonal temperature record for Central England for the same period. The overall change in the relative fitness of the two morphs and the number of years required for morph frequency changes to become perceptible are computed.

3.3 Method

3.3.1 Transect trapping (Fig 3.1)

Data on morph frequency were collected by re-establishing the two transects sampled by Terrell-Nield in 1975 (Transect 1) and 1976 (Transect 2) (see also Section 1.3.2 and Fig 1.2). Transect 1 was monitored in 1995 and 1996. Transect 2 was monitored in 1996, with some sites trapped again in 1998.

The sites were set at approximately 10km intervals along both transects. Tables A.2 (Transect 1) and A.3.i (Transect 2) in Appendix A give the grid reference, habitat and altitude of each site for each year of trapping. For Transect 2, which lies close to the south coast, the distance from the sea is also given. Fig 3.1 shows the location of the sites along both transects.

Following the pitfall trapping method described in Section 2.4.1, a grid of ten traps, with ethylene glycol as preservative, was laid at each site in hedgerows, copses, woodland edges or waste ground, and left for two weeks. For woodland sites, traps were laid within 10m of the wood or copse edge. At sites coded '*We*' in Table A.1, the traps were laid literally at the edge of a wood to which there was no access. A woodstrip (*Ws*) is defined as a double row of mature trees bordering a road and acting as a shelter belt.

i) Transect 1 (Table A.2, Appendix A)

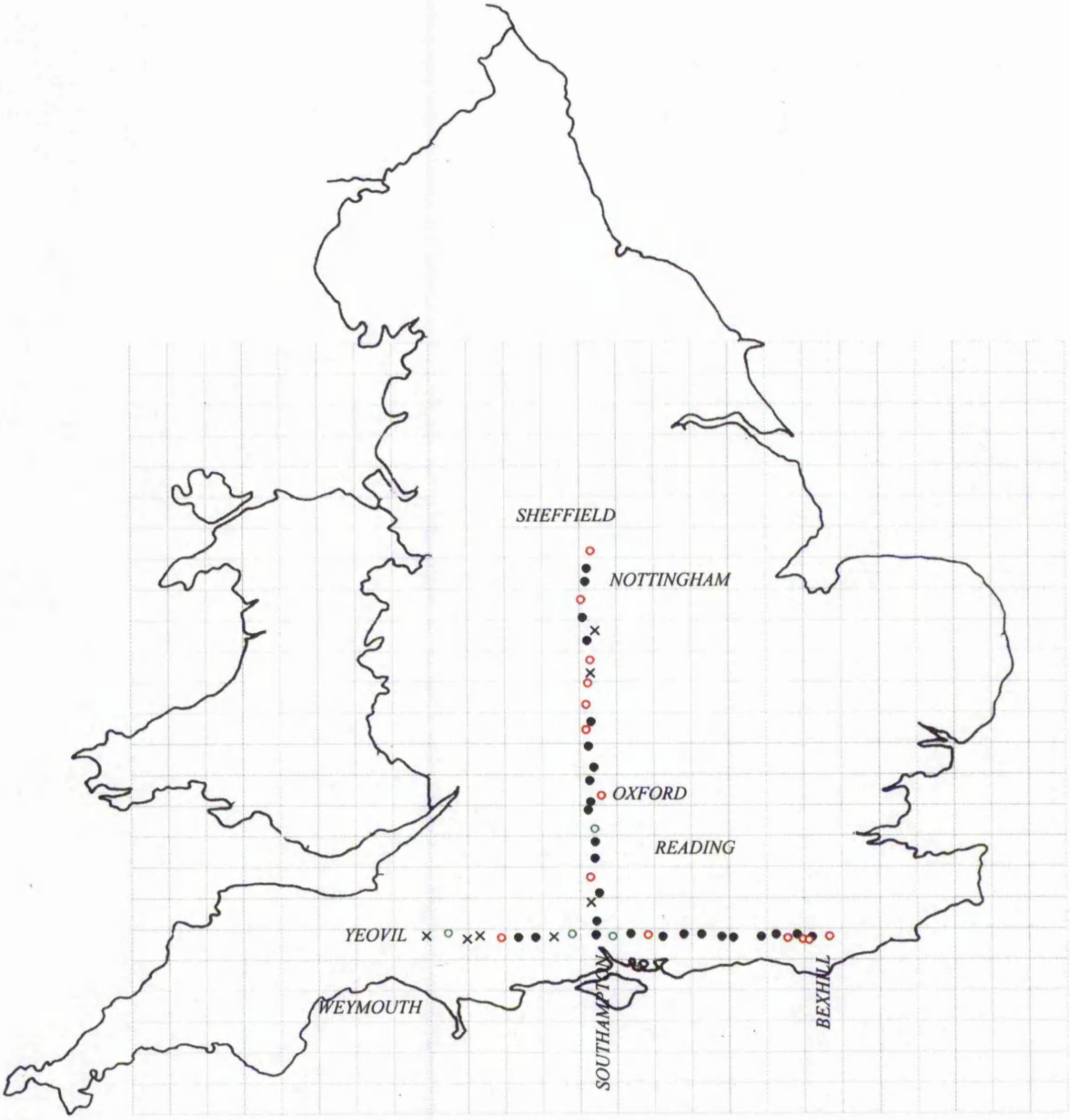
Transect 1 runs north to south from the north Nottinghamshire/Derbyshire border to Southampton in Hampshire. Sites 2 to 25 were first established in July 1975. The most southern site (Site 1) forms part of Transect 2 and was sampled for the first time in 1976 (see Fig 3.1).

The 25 sites were re-established in August 1995. In addition, Site 26, the most northern site was established. Where monitoring of the original site was not possible due to inaccessibility or changes in land use, the nearest wooded area or hedgerow was sampled. Eight sites in total were re-located (Sites 3, 4, 9, 11, 13, 14, 16, 17 and 21). In another four instances (Sites 1, 6, 12 and 26) a different area of the same wood was sampled. As shown in Table A.2, all new locations were within 3km north or south, and east or west of the original sites.

Sites where *Pterostichus madidus* was absent or in low numbers in 1995 were relocated in 1996. Seven sites (Sites 2, 8, 12, 13, 17, 21 and 23) were moved to wooded areas within 4km north or south and 7km east or west of the 1975 or 1995 sites. Site 3 was also relocated, but to the original wooded area of 1975 for which access had now been found. At Priors Hardwick (Site 15), a different and drier area of the same wood was sampled. At Site 18 (Wibtoft), traps were laid in a more central stretch of the same woodstrip.

Fig 3.1 Sampling UK sites along Transect 1 and Transect 2.
Grids = 20km x 20km. n = number of *Pterostichus madidus* .

● n > 15, both sampling periods
○ n > 15, 1995/6/8 only
○ n > 15, 1975/6 only
× n < 15, both sampling periods



Other adaptations to the method were intended to assess the consistency in the morph frequency over time and space as follows:

- Selected sites in the north Midlands (Sites 22, 23, 24 and 26) were sampled over more than one two-weekly period from mid-July to the end of August.
- Two sets of grids were laid in different parts of the wood at Greenham Common (Site 6) and West End at Southampton (Site 1).
- An additional site in the city of Oxford was established (Site 11a).

ii) Transect 2 (Table A.3.i, Appendix A)

Transect 2 runs east to west close to the south coast from East Sussex to Somerset. Originally trapped in July 1976, it was re-established in August 1996. Site 10 at West End in Southampton is Site 1 of Transect 1 (Fig 3.1). Only three sites (Sites 5, 18 and 19) were moved to a different wood to that sampled in 1976. These were within 1km north or south and 4km east or west of the original sites. Other changes involved no more than a slight relocation within the same wooded or waste ground area.

To overcome the problem of low numbers of *P. madidus* at some sites, and to check for consistency between years, 10 of the 22 sites were trapped again in August 1998. Six of these were moved to different wooded areas within 3km north or east of the original sites (see Table A.3.i). In addition:

- The transect was extended eastwards to Battle (Site 23).
- Three sites intermediate to Sites 20, 21 and 22 were established (termed 21a, 21c, 22a). These are shown in italics in Table A.3.i.

3.3.2 Monitoring of sites over three or more consecutive years since 1989 (Fig 3.2)

There were two sources of data:

- University of Newcastle upon Tyne (data from M.L.Luff) - one site in north-east England.
- Environmental Change Network (data obtained from Data Manager, A. M. J. Lane) – five sites in southern England and two sites in southern Scotland.

Fig 3.2 shows the location of the six sites in relation to each other. Table A.4 in Appendix A gives their grid reference, habitat description and period of monitoring.

i) Close House Field Station sampled by M.L.Luff and his team at Newcastle University.

Close House in south Northumberland is an experimental station of the University of Newcastle upon Tyne. At about 20m altitude, it lies 1km north of the river Tyne in a rural area between the villages of Heddon on the Wall and Wylam. The grid reference for the site given in Table A.4 has been rounded to the nearest kilometre for both eastings and northings.

Live trapping was conducted, using ten 2m length gutter traps in cultivated and semi-cultivated plots between a small wood and an arable field (for details see Luff, 1975 and 1990). Sampling took place from April to October over a 14-year period from 1981 to 1995.

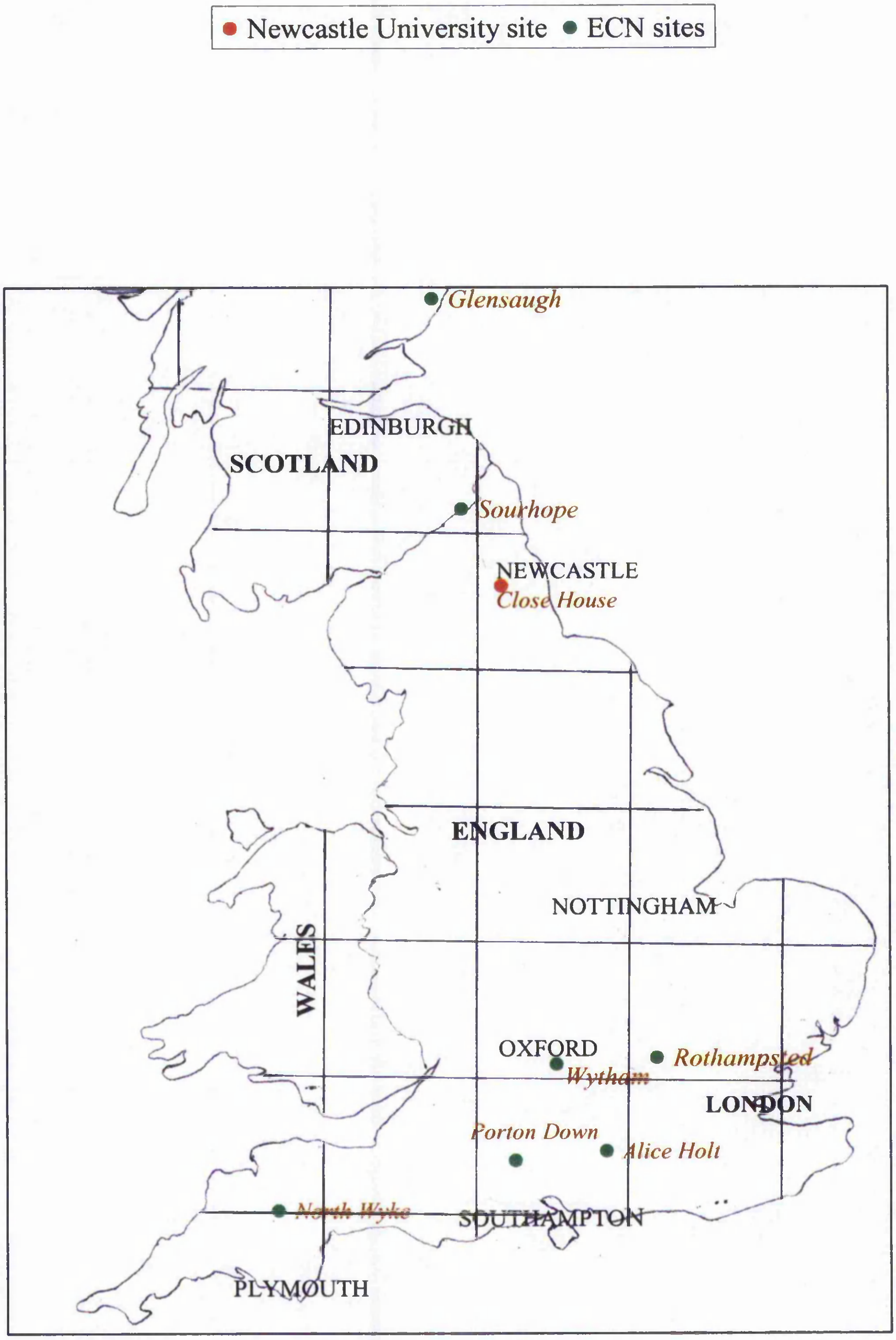
iii) Environmental Change Network (ECN)

Sykes & Lane (1996) give the history and an overall description of the ten terrestrial Network sites at each of the research institutes where Carabidae are monitored. *Pterostichus madidus* is present at seven of these sites. The sex ratio of each morph is recorded at four of the sites. At each site, a pitfall trapping system has been established along three transects within a 100m x 100m Target Sampling Site, with 10 pitfall traps per transect and 10m spacing between each trap (Sykes & Lane, 1996). The grid references in Table A.4 and grid points in Fig 3.2 are for the 1km centroid location of each ECN site, not the individual transects.

Using antifreeze as the preservative, the traps were set out on the first Wednesday in May and replaced fortnightly until the end of October, giving 13 sample periods in total for each year.

The three 100m transects at each ECN site are intended to represent different habitats. As shown in Table A.4, the transects at the Scottish institutes are characterised by mineral grassland and dry and wet peat areas. At Wytham (Oxfordshire) and Rothampsted (Hertfordshire), the contrast is mainly between woodland and more open grassed areas. Different types of grassland are represented at Porton Down in Wiltshire. Although the transects at North Wyke in Devon are close to or within a grazed pasture, they differ by their proximity to more sheltered sites (woodland and scrub) and the river Taw. At Alice Holt (Surrey), the three transects are within the same extensive deciduous wood (Alice Holt Forest), which covers an area of about 12km^2.

<u>Fig 3.2</u> Position of sites monitored by Newcastle University and Environmental Change Network (ECN) for a minimum of three consecutive years between 1989 and 1997. Grids = 100km x 100km.



3.3.3 Comparison of morph frequency with temperature data for Central England.

Changes in morph frequencies over time were compared with annual and seasonal mean temperatures for Central England - a triangular area of the UK enclosed by Preston in the north, London in the south-east and Bristol in the south-west. Monthly temperature data for Central England were obtained from BADC (British Atmospheric Data Centre).

Annual and seasonal averages have been calculated from monthly data, with the seasons defined as follows:

Spring: March, April, May
Summer: June, July, August
Autumn: September, October, November
Winter: December, January, February

The monthly series for Central England dates back to 1659. Initially compiled up to 1973 by Manley (1974), it was later updated by Parker *et al* (1992), who calculated the daily series from which monthly averages are obtained. Both the monthly and daily series are now kept up to date by the Climate Data Monitoring section of the Hadley Centre, Meteorological Office. Since 1974, the data have been adjusted to allow for warming due to increasing urbanisation.

3.3.4 Statistical analysis

The statistical packages used were Excel 97 or Minitab 13.

i) Transects 1 and 2

A chi-squared analysis (2 x 2 contingency test) was performed on the results to establish whether there was a significant difference in the morph proportions (1) between adjacent sites and (2) between sampling periods. If the numbers of *P. madidus* were low for one or more years, data from adjacent sites within ± 10% of the frequency range were pooled. The correlation of red-legged morph frequency with (1) type of habitat and (2) male to female ratios was also investigated.

ii) Sites monitored since 1989

A similar analysis was performed for the countrywide sites monitored since 1989. The red-legged morph frequency trends (1) between adjacent sites (or transects) and (2) over consecutive years were investigated. Where possible, the association between morph frequencies with type of habitat and male to female ratios was also analysed.

3.3.5 Calculation of relative fitness and rate of morph frequency change using different values for relative fitness

i) Relative fitness

Relative fitness (*w*) measures the relative number of offspring produced by each parental genotype in each generation. A value of 1 is assigned to the genotype that produces the most offspring. Although normally applied to genotypes, the calculation of the relative fitness of phenotypes can be estimated from equation 3.1, if the fitness of the dominant homozygote and heterozygote is assumed to be the same (Cook *et al*, 1986).

$$\log w = \frac{\log(B_0R_0) - \log(B_nR_n)}{n} \qquad \text{eq 3.1}$$

where w is the relative fitness of the less fit phenotype

B is the frequency of the black-legged phenotype

R is the frequency of the red-legged phenotype

o denotes initial frequency

n is number of generations after selection

ii) Model for estimating rate of morph frequency change

The table method given by Russell (1996) for calculating the change in allelic frequency due to natural selection has been adapted into a spreadsheet which estimates morph frequency change per generation for a given relative fitness and initial allelic frequency.

This method can be used to calculate morph phenotypic frequency changes, whether the trait is dominant, co-dominant, recessive or over-dominant (i.e. superiority in the heterozygote). It assumes (1) random mating between the morphs and (2) the genotypes are in Hardy-Weinberg proportions with initial frequencies of p^2, pq and q^2, where p is the dominant allele and q is the recessive allele and $p + q = 1$. Hence, p^2 is the dominant homozygote (A_1A_1), pq is the heterozygote (A_1A_2) and q^2 is the recessive homozygote (A_2A_2) - (see Table C.1 and Fig C.2 in Appendix C). Assuming Mendelian genetics, the frequency of the recessive allele (q) can be estimated from the frequency of the recessive phenotype (q^2).

The method is shown in Table C.2 in Appendix C. The spreadsheet model can be found in file 'genetic1.xls' on the CD-ROM supplied.

To use the table method, an initial frequency for q^2 is required (column 6, row 1 or generation 0) from which q is calculated (column 3 – see Table C.2). p, given in column 2, is $1 - q$.

Columns 4, 5 and 6 calculate the initial frequency of the genotypes: p^2, pq, q^2. The morph proportions are obtained from these and shown in columns 7 and 8; The dominant form is $p^2 + pq$ and the recessive form is q^2.

Column 9 is the total proportion and should equal 1. Columns 10, 11 and 12 require inputs for the relative fitness of the three genotypes (w_{11}, w_{12} and w_{22}). The contribution of each genotype to the next generation is the initial frequency of the genotype multiplied by its fitness (e.g. p^2 x w_{11}). The results of these calculations are given in Columns 13, 14 and 15.

Since the contributions of the three genotypes do not add up to 1, the relative contribution of each genotype to the next generation (i.e. their relative frequencies) needs to be calculated. This is done by dividing each genotype by the mean fitness of the population ($\overline{w}$) where:

$$\overline{w} = \frac{(p^2 w_{11} + 2pqw_{12} + q^2 w_{22})}{3} \qquad \text{(eq. 3.2)}$$

The mean fitness ($\overline{w}$) is shown in column 16 of Table C.2. The relative frequencies of each genotype, A_1A_1, A_1A_2 and A_2A_2 are shown in columns 17, 18 and 19 respectively. The new allelic frequency for generation 1 (column 1, row 2) is then calculated from the genotype frequencies of columns 17, 18 and 19 as follows :

$$p = \text{(frequency of } A_1A_1) + (0.5 \text{ x frequency of } A_1A_2);$$
$$q = \text{(frequency of } A_2A_2) + (0.5 \text{ x frequency of } A_1A_2) \qquad \text{(eq. 3.3)}$$

In the spreadsheet model (genetic1.xls) the above procedure can be repeated for as many generations as required. The value for the relative fitness of the genotypes can be changed at any stage over time.

3.4 Results

Full results are presented in Appendix B. The tables give grid references, habitat type and total numbers of *Pterostichus madidus* trapped at each site. A red-legged morph frequency has been obtained for sites with 12 or more *P. madidus* beetles. Where male/female data are available, male frequencies for each morph were found for sites with 40 or more *P. madidus*.

Tables B.1.i and B.2.i give the results for Transects 1 and 2 respectively, with male/female data in Tables B.1.ii (Transect 1) and B.2.ii (Transect 2). The sex of *P. madidus* was not recorded in 1975/76, because numbers were too low. Tables B.5 and B.6 give the results for Close House and the ECN sites respectively, with male and female frequencies where known.

3.4.1 Transect 1 (Southampton to north Nottinghamshire)

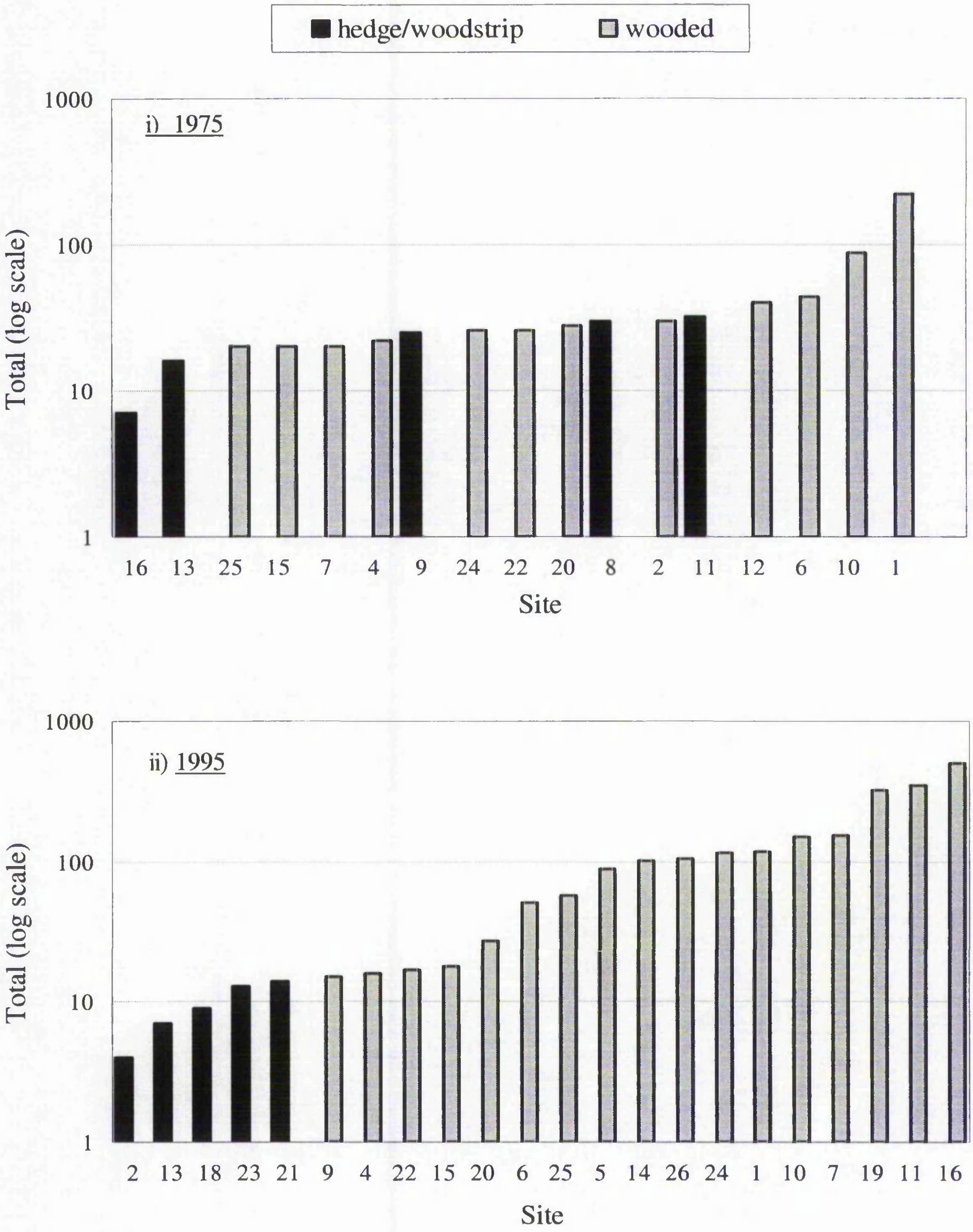
i) Total numbers

Three times as many *P. madidus* were trapped in 1995 compared with 1975 (Table B.1.i). The high 1995 numbers are partly explained by the extremely high activity at three sites (11, 16 and 19) where over half the total number of *P. madidus* was found. Site 19 was a new plantation in 1975, when *P. madidus* was apparently absent. Sites 11 and 16 were hedgerow sites in 1975, relocated to woods in 1995.

In fact, as shown in Fig 3.3, the highest activity of *P. madidus* was in woods and copses for both years. The contrast between woodland and "hedged" sites is particularly evident in 1995, when the activity of *P. madidus* was higher by a factor of 10 or more in over half the wooded sites compared with the hedgerow or narrow woodstrip habitats. Furthermore, two of the 1995 woods producing low numbers (Sites 2 and 12) were drainage areas and may have been too damp for *P. madidus* for at least part of the year.

Consequently, sites that trapped few *P. madidus* in 1995 were, where possible, relocated to woods and copses in 1996 when four times the number of *P. madidus* was trapped compared with 1975. Apart from the vandalised site (Site 3), only one relocation to a more wooded area (Site 21) failed to produce usable data. There was no accessible wood/copse at Site 8 (Ridgeway on the North Downs). Monitoring of a bushier hedge remnant at a different position along the Ridgeway still failed to trap the beetle. Notably, Sites 8 and 21 are both at high altitude relative to adjacent sites to the north and south (see Table A.2).

Fig 3.3 Comparison of number of *P.madidus* trapped in hedged and wooded sites along Transect 1 in i) 1975 and ii) 1995.



ii) 1996 sub-samples

Data from the 1996 sites where trapping was repeated over time or space have been pooled in Table B.1.i to give an overall frequency. Table 3.1 gives the results of a chi-squared test on the proportions of red and black-legged morphs from each sub-sample. This analysis shows no difference between proportions at the 5% level, although numbers were low at Site 22, giving an expected value of less than 5. At Site 1, two grids less than 20m apart were sampled over two different but overlapping time periods. Despite a large difference in the red-legged frequencies (46% compared with 75%), the significance level was only 0.07, questioning the validity of obtaining frequencies from numbers as low as 12 (Site 1A) – also see Fig C.1 and Section 2.6.3.

Table 3.1 Sampling results for 1996 Transect 1 sites where trapping was repeated over time or space, showing results of chi-squared analysis.

B = black-legged morph; R = red-legged morph; T = total P. madidus; P = significance level (probability).

SITE NO	LOCATION	SAMPLING DATE	SUB-SAMPLE	B	R	T	%R	value for χ^2 distribution	P
1	West End	25/7-7/8/96	A	3	9	*12*	**75.0**		
	(Southampton)	4/8-18/8/96	B	28	24	*52*	**46.2**		
			TOTAL	*31*	*33*	*64*	***51.6***	3.248	0.07
6	Greenham Common	4/8-18/896	A	43	58	*101*	**57.4**		
	(Berkshire)	4/8-18/8/96	B	12	17	*29*	**58.6**		
			TOTAL	*55*	*75*	*130*	***57.7***	0.013	0.91
22	Tonge	20/7-1/8/96		3	3	*6*			
	(Leicestershire)	1/8-16/8/96		14	4	*18*	**22.2**		
			TOTAL	*17*	*7*	*24*	***29.2***	1.681	0.19*
23	Elvaston	2/8-16/8/96		95	56	*151*	**37.1**		
	(Derbyshire)	16/8-29/8/96		74	51	*125*	**40.8**		
			TOTAL	*169*	*107*	*276*	***38.8***	0.397	0.53
24	Shipley	20/7-1/8/96		6	4	*10*			
	(Derbyshire)	1/8-16/8/96		21	10	*31*	**32.3**		
		17/8-29/8/96		65	38	*103*	**36.9**		
			TOTAL	*92*	*52*	*144*	***36.1***	0.223	0.64
26	Doe Lea	31/7-15/8/96		93	31	*124*	**25.0**		
	(Nottinghamshire)	15/8-29/8/96		155	66	*221*	**29.9**		
			TOTAL	*248*	*97*	*345*	***28.1***	0.930	0.33

* expected values under 5

iii) Comparison of 1975, 1995 and 1996 red-legged morph frequencies for Transect 1

Fig 3.4 shows that the red-legged morph frequency distribution was relatively stable between 1995 and 1996. From Table 3.2.i, of the 20 sites with sufficient data for both years, only Site 15 produced a significant difference in morph proportions. However, the total number of *P. madidus* for both years was low at this site and the expected values for the red-legged morph were less than 5. Except for Sites 6 and 7 in Berkshire, which produced a significant difference in morph proportions at the 5% level when the data were pooled (see last row of Table 3.2.i), the direction of morph frequency change was not consistent between adjacent sites.

Table 3.2 Temporal variability: results of chi-squared test on proportions of black and red-legged morphs at sites along Transect 1 comparing i) 1995 and 1996; ii) 1975 and 1995/6. (Shaded entries – at least one expected value is less than 5).

Asterisked P values indicate sites with significantly different morph proportions where: * = $P<0.05>0.01$; ** = $P<0.01>0.001$

i) 1995 and 1996

site	value for χ^2 distribution	P
1	2.998	0.083
2	1.058	0.304
4	1.889	0.169
5	0.099	0.753
6	3.120	0.077
7	1.738	0.187
9	0.025	0.874
10	0.059	0.808
11	1.371	0.242
13	1.475	0.225
14	0.864	0.353
15	10.035	0.002 **
16	0.387	0.534
19	1.825	0.177
20	0.001	0.975
22	0.000	1.000
23	0.000	1.000
24	0.329	0.566
25	0.167	0.683
26	3.548	0.060
6+7	*5.618*	*0.016* *

ii) 1975 and 1995/6

site	value for χ^2 distribution	P
1	0.288	0.592
2	7.366	0.007 **
4	0.207	0.649
6	0.432	0.511
7	1.876	0.171
9	2.058	0.151
10	10.111	0.001 **
11	5.039	0.025 *
12	0.640	0.424
13	3.463	0.063
15	3.009	0.083
20	0.006	0.938
22	0.310	0.578
24	2.440	0.118
25	0.538	0.463
6+7	*5.843*	*0.016* *
9+10	*4.312*	*0.038* *
22+24+25	*4.351*	*0.037* *

Fig 3.4 Comparison of 1995 and 1996 red-legged morph frequencies along Transect 1. Sites referred to in text are labelled by site number.

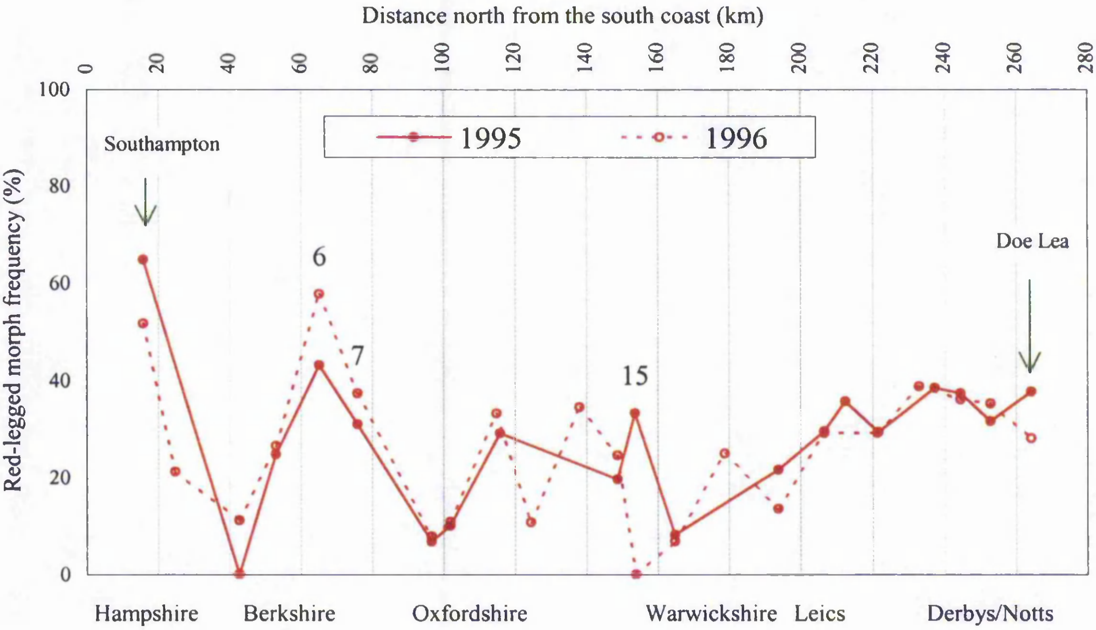
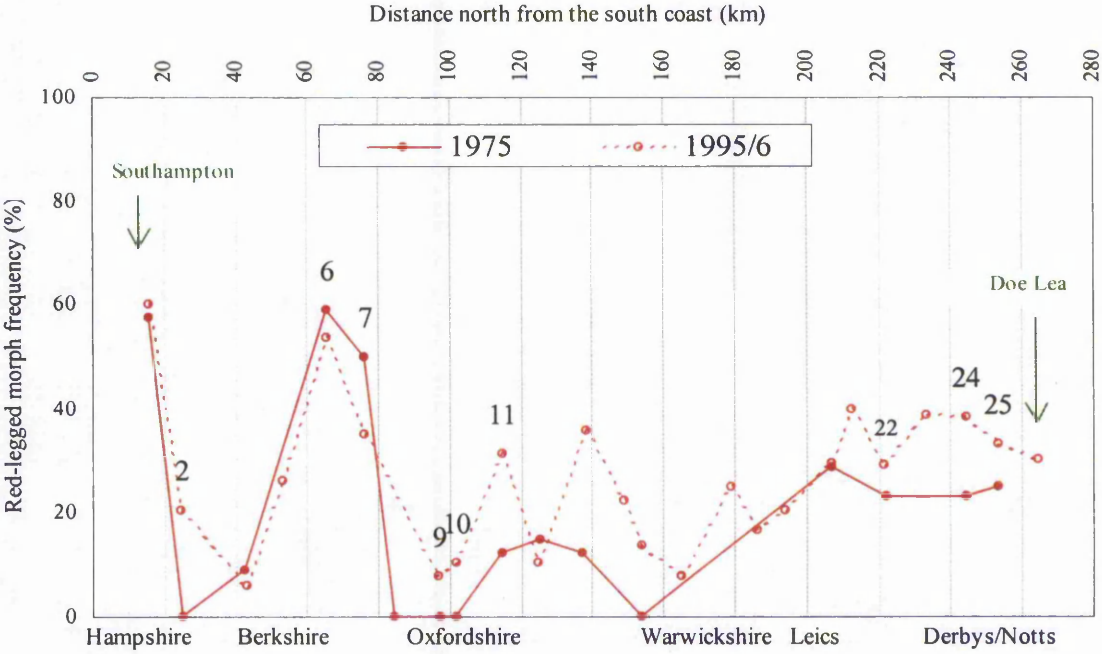


Fig 3.5 Comparison of 1975 and 1995/6 red-legged morph frequencies along Transect 1. Sites referred to in text are labelled by site number.



Given the morph frequency stability between 1995 and 1996, results for these years have been combined for Fig 3.5. Comparing 1975 with the mid-1990s, there has been an increase in the red-legged morph frequency at 10 of the 15 sites with sufficient data for comparison. The difference in morph proportions is significant for Site 2 (north of Southampton), for sites lying north and south of Oxford (Sites 9, 10 and 11) and for the Trent valley sites in the East Midlands (Sites 22, 24 and 25) when the data from these sites are pooled (see Table 3.2.ii). Of the sites which produced these differences, only Sites 2 and 11 had been moved to more wooded areas in 1995/6. This trend was reversed for Sites 6 and 7 in Berkshire, mainly due to the higher black-legged proportion in 1995 (see Fig 3.4 and Table B.1).

However, these changes over time are small compared with the spatial variability along the transect. Despite the 20-year gap between sampling dates, the same regions are subject to the same steep clines over relatively short distances of 10 to 20km. A higher red-legged morph frequency is associated with the maritime region of Southampton, the Trent river basin in Derbyshire and Nottinghamshire, and the wooded area around Newbury in Berkshire. These regions are also relatively urban. By contrast, a high black-legged morph frequency is associated with the intensive agricultural area of north Oxfordshire and Warwickshire, the open land of the North Downs in Berkshire and the higher ground inland from Southampton.

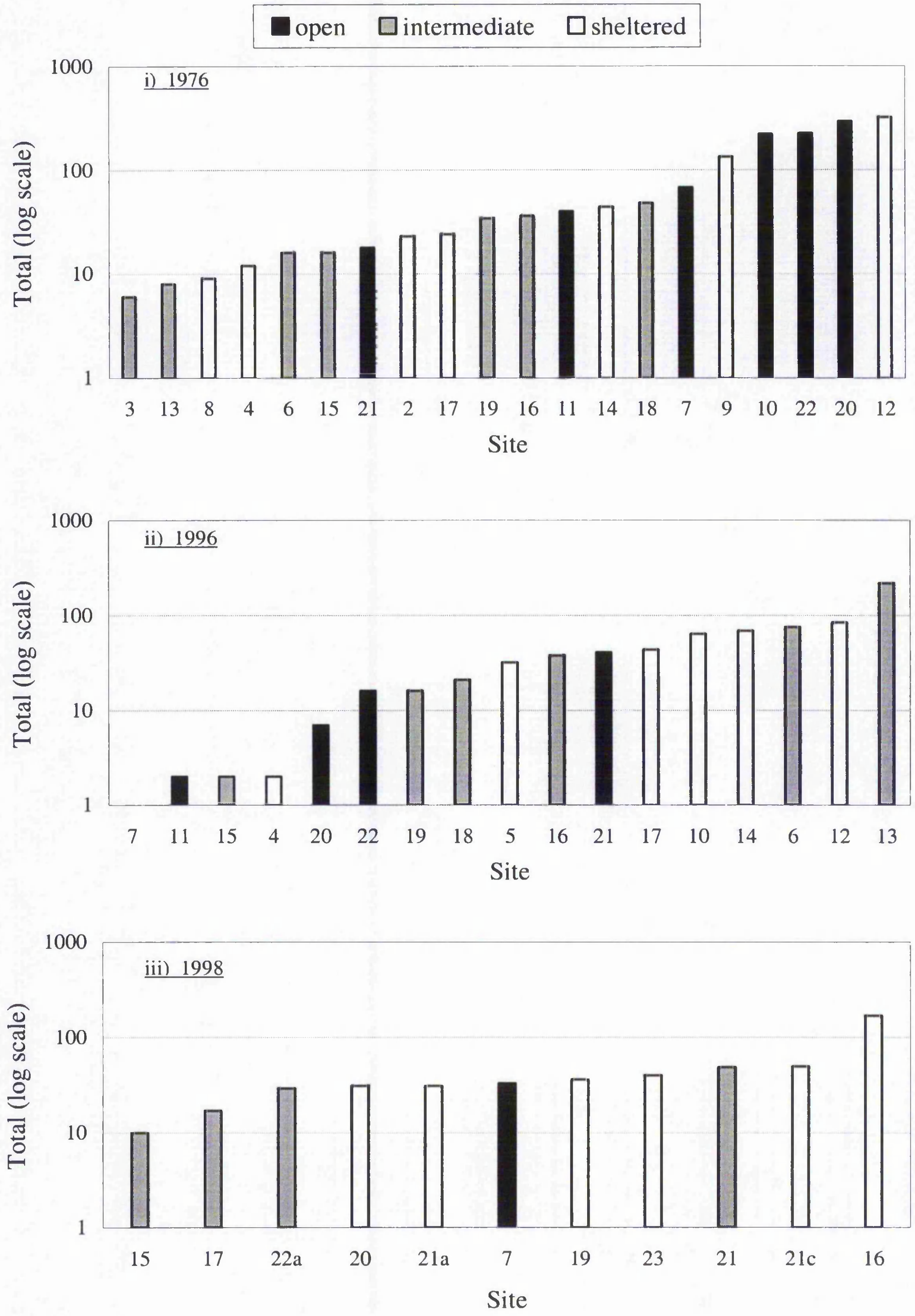
3.4.2 Transect 2 (West Dorset to East Sussex)

i) Total numbers

In contrast to Transect 1, there was a decline in the numbers of *P. madidus* trapped in 1996 compared with 1976 (Table B.2.i). An average of 33 *P. madidus* per site were trapped in 1996 compared with 69 per site in 1976. Although not strictly comparable because new sites were used, numbers were also lower in 1998, with 46 *P. madidus* trapped per site.

Classifying the sites as “open” (hedges, road verges, ditches and waste ground), “sheltered” (woods and copses) and “intermediate” (wood edges and other habitats adjacent to a wood), it is clear from Fig 3.6.i that *P. madidus* tended to be more active in open sites in 1976. In 1996 and 1998, there is a reversal, with a higher *P. madidus* activity in sheltered wooded sites compared with the more open sites (Fig 3.6.ii and iii).

Fig 3.6 Comparison of number of *P.madidus* trapped in open to more sheltered sites along Transect 2 i) 1976 ii) 1996 and iii) 1998.



ii) Comparison of 1976, 1996 and 1998 red-legged morph frequencies

As shown in Fig 3.7, the morph frequencies along Transect 2 were fairly stable between 1996 and 1998. Although there was a trend towards an increase in the black-legged morph proportions in 1998, Table 3.3.i shows that this is significant for only one site (Site 16). However, the number of comparable sites with good data sets for both years is limited.

Comparing 1996 with 1976, there was an overall increase in the red-legged morph frequency, particularly at the more eastern sites in West and East Sussex (Sites 14 to 18 – see Fig 3.7). This trend was significant at two sites (Sites 14 and 22b) and for Sites 16, 17 and 18 when the data for these sites are pooled (Table 3.3.ii). The trend is less evident when 1998 and 1976 are compared because of a swing back to black. Although Site 20 shows an increase in the red-legged morph proportion, which is significant at the 10% level (Table 3.3.iii), the red-legged frequency at Site 16 has reverted to that for 1976. At Sites 6 and 17, the 1998 frequencies are intermediate to those obtained in 1976 and 1996. In fact, as shown in Fig 3.7, the morph proportions for 1976 and 1998 are more remarkable for their similarities than for their differences.

Table 3.3 Temporal variability: results of chi-squared test on proportions of black and red-legged morphs at sites along Transect 2 comparing i) 1996 and 1998; ii) 1976 and 1996 and iii) 1976 and 1998. (Shaded entries – at least one expected value is less than 5).

Asterisked P values indicate sites with significantly different morph proportions where: * = P<0.05>0.01; ** = P<0.01>0.001

i) 1996 and 1998

site	value for χ^2 distribution	P
6	1.087	0.297
16	4.662	0.031*
17	0.263	0.608
19	0.674	0.412
21b	0.110	0.740

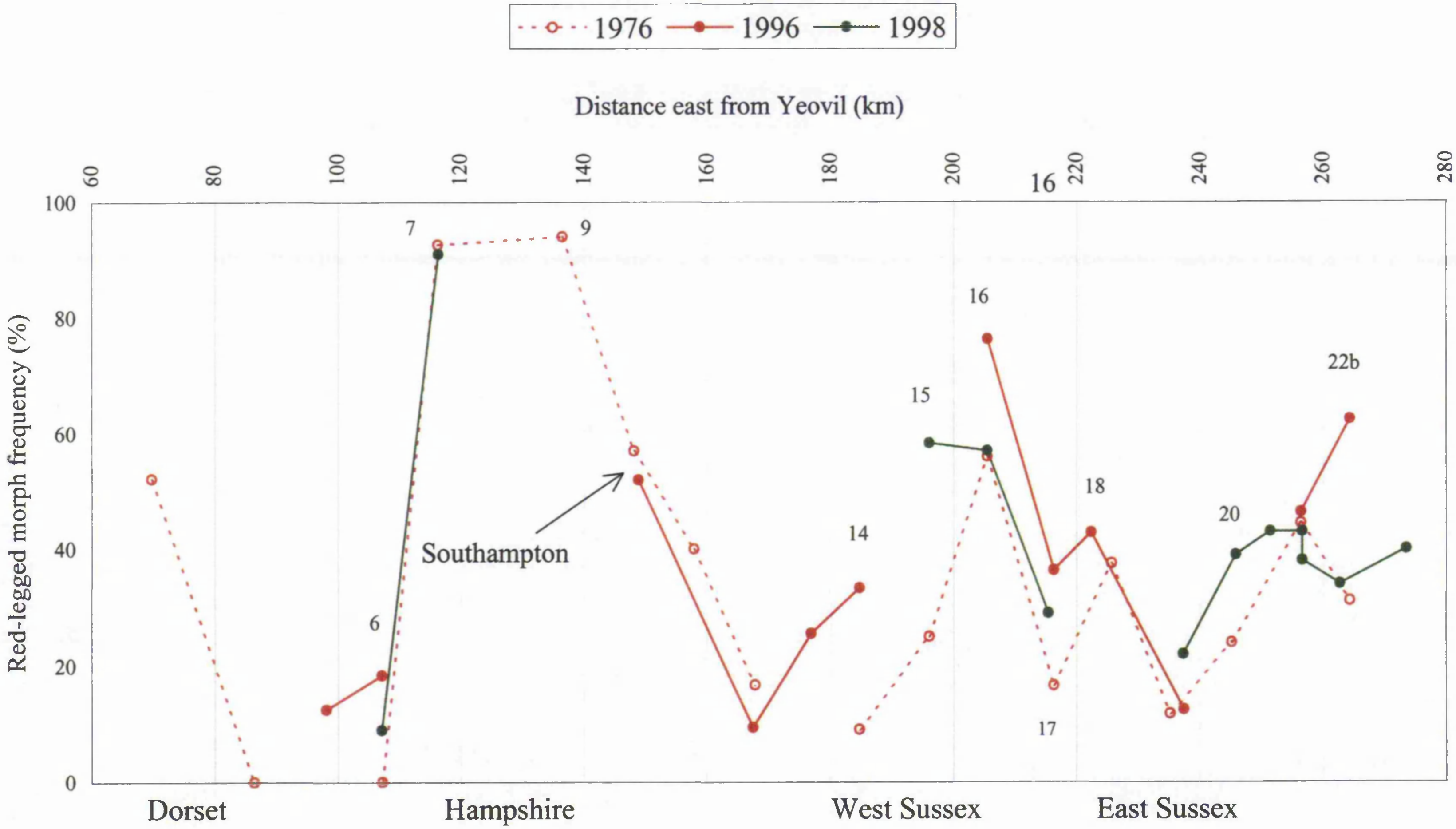
ii) 1976 and 1996

site	value for χ^2 distribution	P
6	3.476	0.062
10	0.702	0.402
12	2.82	0.093
14	8.683	0.003**
16	3.562	0.059
17	2.902	0.088
18	0.176	0.675
19	0.006	0.938
21b	0.018	0.893
22b	6.637	0.010*
16+17+18	*3.897*	*0.048**

iii) 1976 and 1998

site	value for χ^2 distribution	P
6	1.535	0.215
7	0.092	0.762
16	0.041	0.840
17	0.943	0.332
19	1.346	0.246
20	2.959	0.085
21b	0.014	0.906
15+16+17	*4.501*	*0.0340**

Fig 3.7 Comparison of 1976, 1996 and 1998 red-legged morph frequencies along Transect 2. Sites referred to in text are labelled by number. (A gap between data points indicates no data available for the intermediate sites).



In general, these results are comparable to those for Transect 1 with an overall increase in red-legged morph frequency occurring for some regions along the transects. Subsequent monitoring in 1996 (Transect 1) and 1998 (Transect 2) gives some evidence of a reversal to the frequencies of the mid-1970s.

As for Transect 1, the changes over time along Transect 2 are small compared with the spatial variation particularly at the western end of the transect in Dorset and Hampshire. There is less variation in morph frequencies at the eastern sites (West and East Sussex). When sites intermediate to Sites 20 to 22 were established in East Sussex in 1998 reducing the spatial resolution to about 5km, the change in morph frequency over space is more gradual (see Fig 3.7).

Transect 2 is topographically more complex than Transect 1 and this seems to be reflected in the greater fluctuations in morph frequencies from less than 10% to more than 90% over a distance of only 10km. However, consistent with the findings for Transect 1, high red-legged morph frequencies are associated with more wooded areas and river basins, such as the New Forest (Sites 7 and 9) and the River Arun in West Sussex (Site 16).

3.4.3 Male/female proportions along Transects 1 and 2 in 1995/6/8 (Figs 3.8 and 3.9)

Since August is a period of peak activity for *P. madidus* males (Luff, 1973) the sex ratio has been analysed in terms of male frequencies for each morph. As is clear from Tables B.1.ii and B.2.ii, overall male activity varied between sites and between years along both Transects.

For Transect 1, there is no evidence of a difference between the male activity of the morphs (see dotted lines in Fig 3.8). The correlation between the black- and red-legged male frequencies along the transect is highly significant (1995: $r = 0.796$, d.f. = 11, $P<0.01>0.001$; 1996: $r = 0.793$, d.f. = 17, $P<0.001$). Chi-squared analysis identified only one site where the difference in proportions was significant – Site 5 at Litchfield for 1996 ($\Sigma\chi^2 = 3.893$, $P<0.05>0.02$) - see Fig 3.8.ii.

Fig 3.8 Male frequencies among black-legged and red-legged morphs at increasing red-legged morph frequencies using Transect 1 data for i) 1995 and ii) 1996. (Sites are ordered by increasing red-legged morph frequency).

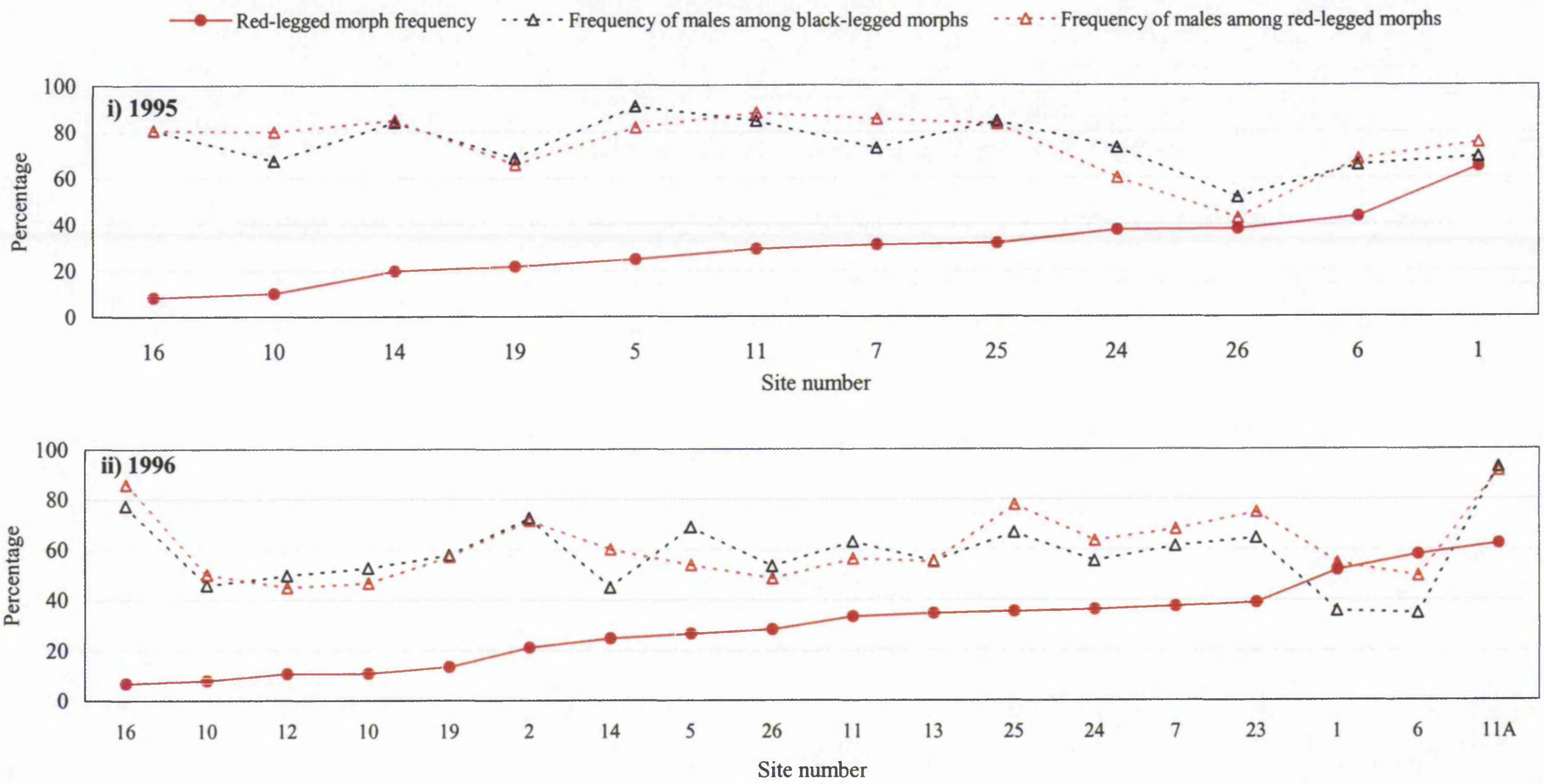
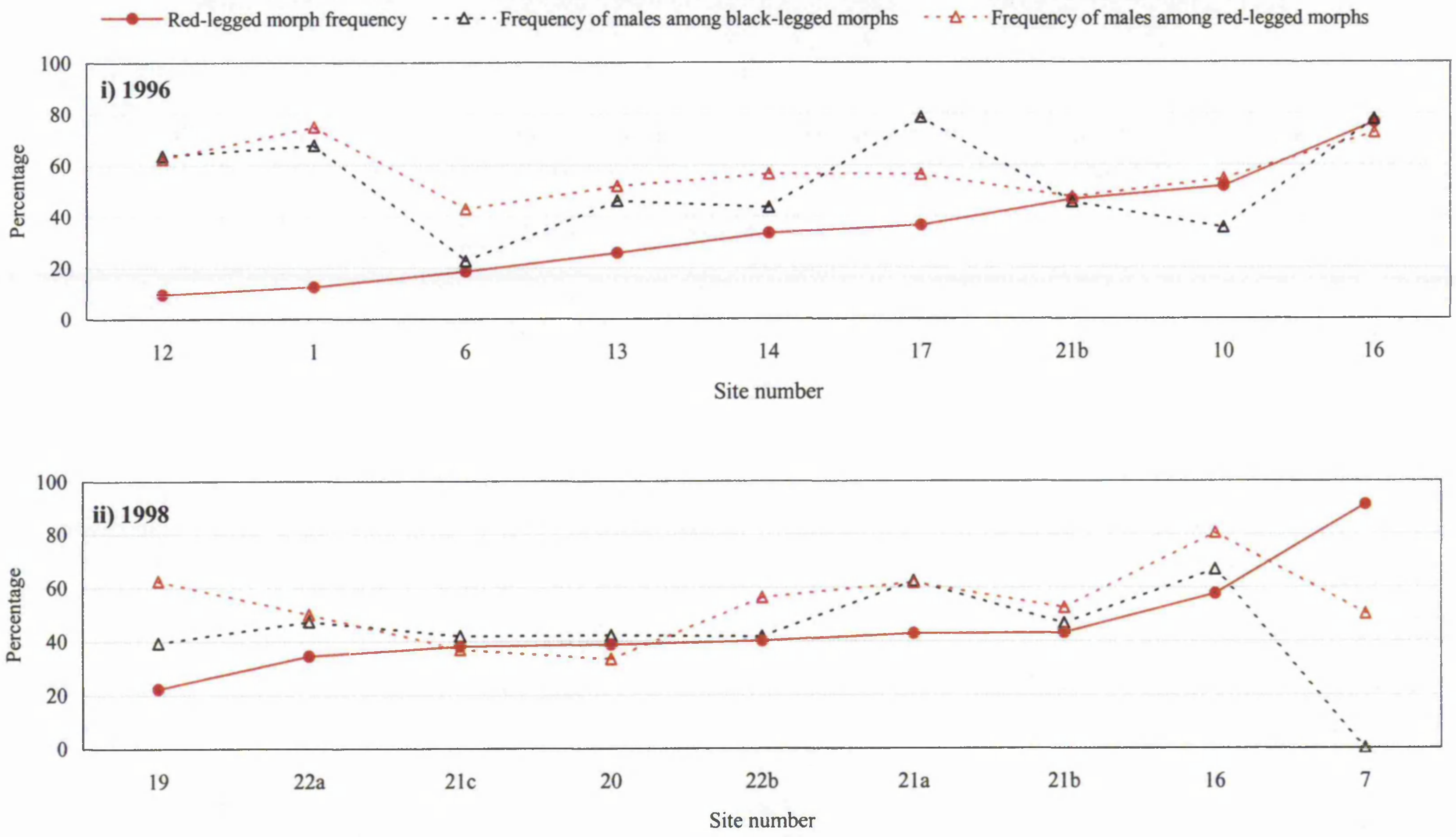


Fig 3.9 Male frequencies among black-legged and red-legged morphs at increasing red-legged morph frequencies using Transect 2 data for i) 1996 and(ii) 1998. (Sites are ordered by increasing red-legged frequency).



For Transect 2, there was a similar highly significant correlation between the black-legged and red-legged male frequencies in 1996 ($r = 0.765$, d.f. = 8, $P = 0.01$) but only a weak positive correlation in 1998 ($r = 0.436$, d.f. = 8, $P>0.1$). However, in 1998, numbers of *P. madidus* were less than 40 at 4 of the 9 sites, which may be giving spurious results for male frequencies in some instances.

In contrast to Transect 1, the overall male/female proportion for the two morphs was significantly different in 1998 ($\Sigma\chi^2 = 4.432$, $P<0.05>0.02$) and different at the 10% level in 1996 ($\Sigma\chi^2 = 3.519$, $P<0.1>0.05$) – see Table B.2.ii, final row headed 'Total'. This suggests a slight temporal difference in the peak male activity of the morphs in some years. Nevertheless, the difference in male/female proportions of the two morphs was not significant for any site in 1996 and was significant at one site only in 1998 - Site 16 at Wiggonholt Common ($\Sigma\chi^2 = 4.116$, $P < 0.05>0.02$) - see Fig 3.9.ii.

For both Transects, all years, there is no significant correlation between red-legged morph frequency (continuous line in Figs 3.8 and 3.9) and the male frequency of either morph.. Therefore, the variation in morph frequency along the Transects does not appear to be influenced by a higher activity of males (or females) of one morph relative to the other.

3.4.4 Close House, Northumberland

Table 3.4 summarises the annual data for Close House sampled by M. L. Luff and his team at the University of Newcastle upon Tyne, showing numbers of *Pterostichus madidus* by morph, red-legged frequencies and the results of statistical analyses.

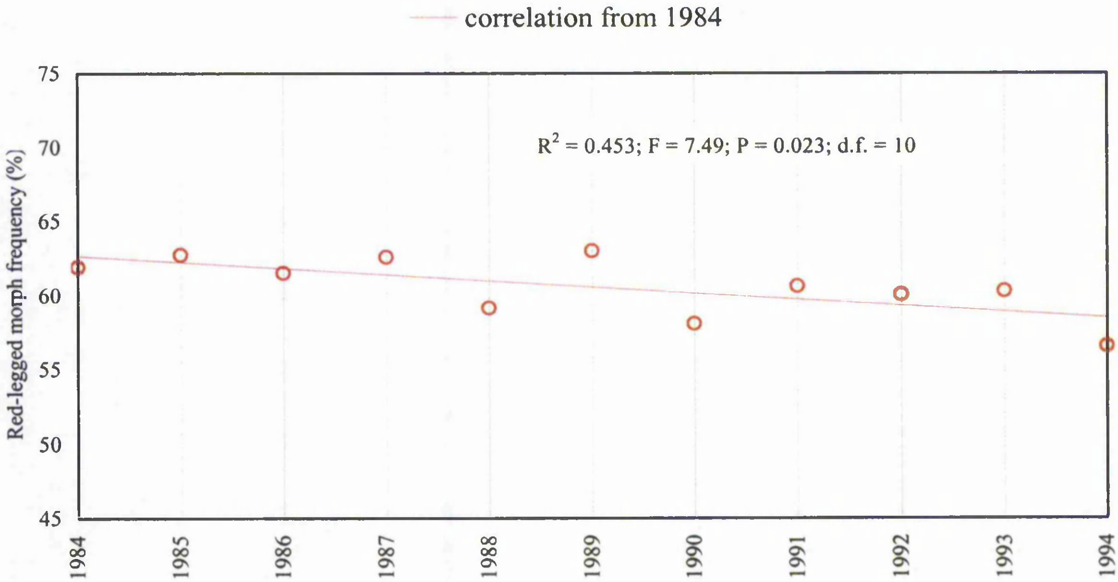
Although the red-legged morph frequencies fluctuated within a small range from 57 to 63% around a mean of 60% over the 14 years of sampling, chi-squared analysis shows a highly significant difference between the morph proportions ($\Sigma\chi^2 = 32.6$; d.f.= 13; $P = 0.002$). Even more interesting, there is a significant decrease in the red-legged frequency since 1984 ($P = 0.023$ – see Fig 3.10).

Table 3.4 Red-legged morph frequencies for each year of sampling at Close House, showing numbers of *P. madidus* trapped per site and a chi-squared analysis ($\Sigma\chi^2$) on morph proportions from 1981 to 1994. (*P. madidus* data from M.L.Luff, University of Newcastle upon Tyne).

B = black-legged morph; R = red-legged morph. S.D. = standard deviation; d.f. = degrees of freedom; P = significance level.

Year	Total B	Total R	Total *P. madidus*	%R
1981	427	674	1101	61.2
1982	228	330	558	59.1
1983	325	447	772	57.9
1984	247	401	648	61.9
1985	431	724	1155	62.7
1986	542	865	1407	61.5
1987	297	496	793	62.5
1988	451	652	1103	59.1
1989	855	1454	2309	63.0
1990	715	989	1704	58.0
1991	444	683	1127	60.6
1992	207	311	518	60.0
1993	471	714	1185	60.3
1994	944	1227	2171	56.5
all years	*6584*	*9967*	*16551*	*60.2*
			S.D	1.975
			$\Sigma\chi^2$	32.599
			d.f.	13
P			P	0.002

Fig 3.10: Annual morph frequencies from 1984 to 1994 at Close House, Northumberland, showing negative correlation with time.



However, it could be argued that these small fluctuations in morph frequencies are due to random error. The mean frequency for the 14 years of sampling is 60.3% with a standard deviation of 1.98 (Table 3.4). From the regression equation of Fig 2.5 (Section 2.5.3, p.50), the standard deviation is expected to fall below 1.98 when the sample number (*n*) exceeds 500 and to fall below 1.0 when *n* exceeds 900. On average, 1182 *P. madidus* were trapped per year at Close House, the lowest numbers occurring in 1982 (558) and 1992 (518), which had unexceptional red-legged morph frequencies of 59% and 60% respectively. In fact it is the two "good" years (1989 and 1994) - when over 2000 *P. madidus* were trapped - which made the greatest contribution to the $\Sigma\chi^2$ value, having the highest and lowest red-legged morph frequencies respectively. The standard deviation is predicted to become negligible when sample numbers are as high as this. It seems unlikely, therefore, that the small fluctuations in morph frequency over time are due to sampling error only.

As shown by the total numbers for 1989 and 1994 in Table 3.4, a "good" year for *P. madidus* does not consistently favour one morph over another and, not surprisingly, there was no correlation between total numbers and morph frequencies (r = -0.108).

3.4.5 ECN sites

Table B.6 in Appendix B shows the totals for *P. madidus* by leg colour and gives data for males and females where available. The red-legged morph frequencies and male frequencies for both morphs have been calculated for each year along each transect of the ECN sites. Overall frequencies for the sites from pooled transect and annual data are also shown.

Tables 3.5 and 3.6 are presented together at the end of Section 3.4.5 to allow for cross-referencing. Table 3.5 (p. 91) summarises the morph frequencies and gives the results of the chi-squared analysis on morph proportions for each transect and year of sampling. A chi-squared analysis has also been performed on pooled transect and annual data to ascertain whether there are overall differences (or similarities) over time or space. Table 3.6 (p. 92) presents the results of the chi-squared analysis on male/female proportions by morph. Total *P. madidus* and red-legged morph frequencies are also shown for comparison.

i) *P. madidus* activity

In general, transects with low-growing ground cover failed to trap *P. madidus* in high numbers. At Glensaugh, Sourhope, Porton Down and North Wyke, only one transect produced sufficient *P. madidus* data for analysis (see Table B.6). These were the mineral grassland transects at Glensaugh and Sourhope, the long grass transect at Porton Down and the transect adjacent to a scrub area at North Wyke. At Rothampsted and Wytham, there was higher activity at the woodland transects compared with the grassland transects. These results are consistent with findings in Section 3.4.1 that *P. madidus* has a preference for sites with good ground and/or canopy cover.

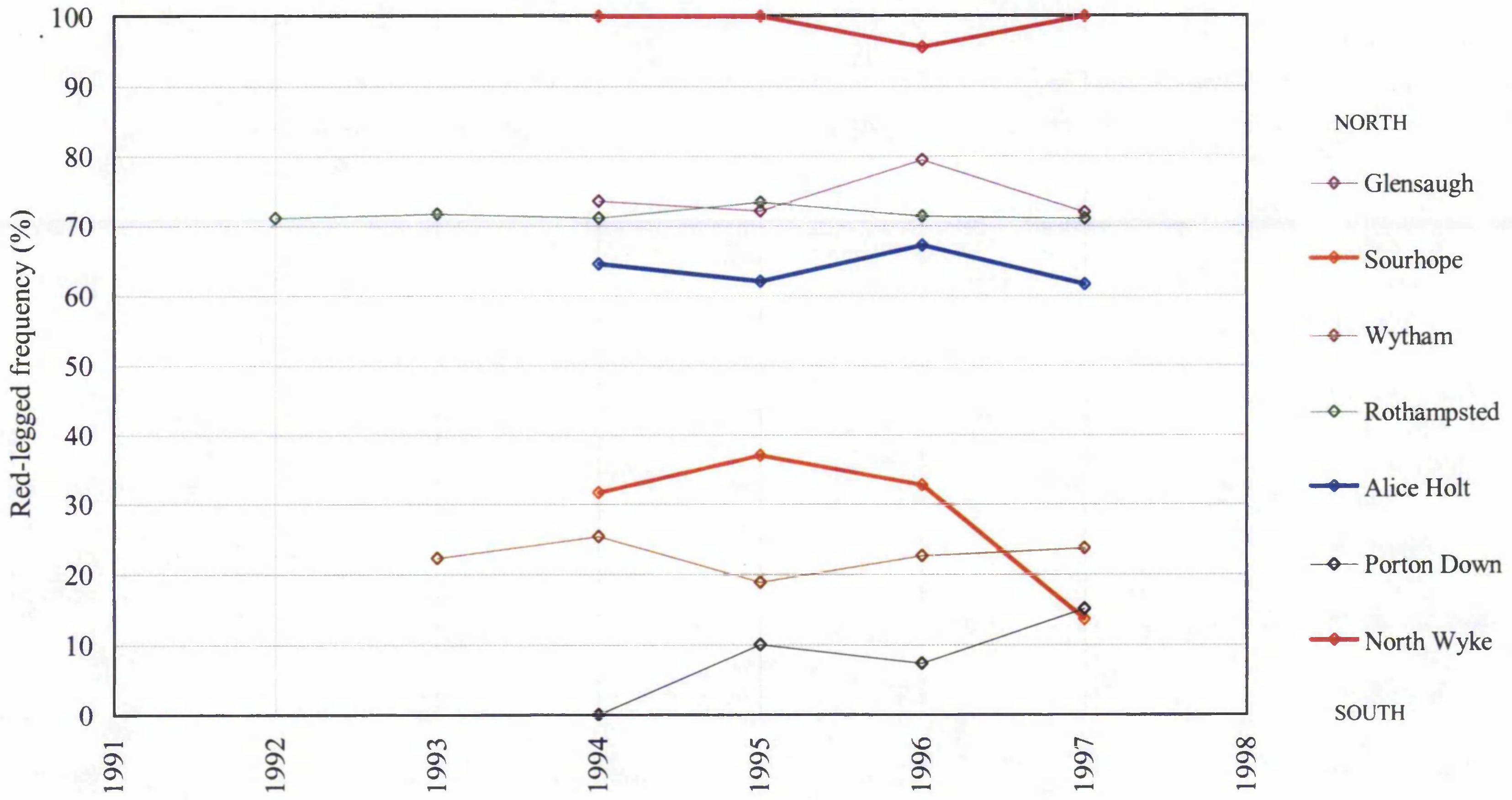
Favourable years for *P. madidus* activity also appear to vary according to habitat. Table B.6 shows that for wooded and hedged transects, 1997 produced the highest *P. madidus* activity. With the exception of Wytham, the highest activity in grassland transects occurred in 1996. At the Wytham grassland transect (Transect 2), the highest *P. madidus* activity was in 1997, although numbers were low for all years.

ii) Red-legged morph frequencies

Fig 3.11 shows the red-legged morph frequencies for each ECN site using pooled data for the 3 transects. Although the annual changes in morph proportions were found to be significant at only 3 sites – Alice Holt, North Wyke and Sourhope, the latter site showing a large fall in red-legged frequencies (see Table 3.5), there is clearly no consistent pattern between the 7 ECN sites. For each year, frequencies rose at some sites and fell at others.

Similarly, there is no evidence of a geographical trend. The highest and lowest red-legged morph frequencies (North Wyke and Porton Down respectively) are also the most southern sites. By contrast, red-legged morph frequencies at Rothampsted in Hertfordshire, southern England, are not significantly different to those at the most northern site, Glensaugh in the Grampians, Scotland. The only two sites where the pooled red-legged frequencies rose and fell in the same years (Glensaugh and Alice Holt in Surrey) are at opposite ends of the country. They are also represented by quite different habitats (grassland and woodland respectively).

Fig 3.11 Temporal change in red-legged morph frequencies at the ECN sites (see final column of Table B.6). Bold lines indicate sites with significant differences in morph proportions over time. In legend, sites are arranged from north to south.



Alice Holt, Wytham and Rothampsted, produced sufficient data at each transect for separate analysis. Fig 3.12 shows the annual morph frequencies for each transect at these ECN sites.

Alice Holt: The 3 woodland transects appear to be fairly homogenous. Although T3 trapped *P. madidus* in higher numbers and T2 is consistently the least favoured site (see Table B.6), the morph proportions of the 3 transects are not significantly different and the direction of frequency change over time is consistent between the transects.

Wytham: Frequencies were broadly similar and stable at the two woodland habitats (T1 and T3). However, the grassland transect (T2) not only produced the lowest *P. madidus* activity (Table B.6), its red-legged frequency also fluctuated significantly over time ($P = 0.04$, see Table 3.5). Given that the habitat of this transect is probably less favourable for *P. madidus*, it is interesting that the disadvantaged morph in this region (the red leg) is significantly at a higher frequency than the two woodland transects ($P = 0.04$, Table 3.5).

Rothampsted: The Rothampsted transects represent 3 different habitat types: hedgerow, woodland and grassland, the woodland transect (T2) obtaining the highest red-legged frequencies until 1997. From 1992 to 1995, there are significant differences in morph proportions between the transects, initially due to low red-legged frequencies at the hedgerow transect (T1) – see Fig 3.12. There are also significant differences in morph proportions between years at the hedgerow transect ($P < 0.01$), and the woodland transect ($P = 0.01$), but not the grassland transect ($P = 0.49$, see Table 3.5). However, when the data for the 3 Rothampsted transects are pooled, morph proportions are stable over time (see green line in Fig 3.11) and the small variation between years is not significant ($P = 0.40$, see row headed "all" in Table 3.5). Morph frequency differences between the transects may therefore be a consequence of a spatial redistribution of the two morphs over the period of monitoring.

It appears that the hedgerow (T1) was being colonised from 1993, initially by the black-legged morph. Fig 3.13 shows a steady rise in *P. madidus* numbers at the hedged transect from 1992 to 1995, which coincides with a rise in the red-legged morph frequency at this transect (Fig 3.12). The variation in *P. madidus* activity at the wooded and grassed sites follow each other closely until 1995, when the woodland and hedgerow red-legged frequencies become similar (Fig 3.13). By 1997, *P. madidus* numbers have decreased at the grassland transect, but have increased at the wooded and hedged transects, the hedgerow now achieving the highest red-legged frequency (Fig 3.12).

Fig 3.12 Change in red-legged morph frequencies over time by transect at Rothampsted (Roth), Wytham (Wyt) and Alice Holt (Ali). In bold: transects with significant differences in morph proportions over time. (For clarity, Alice Holt is on separate axis). W = wood; H = hedge; G = grass.

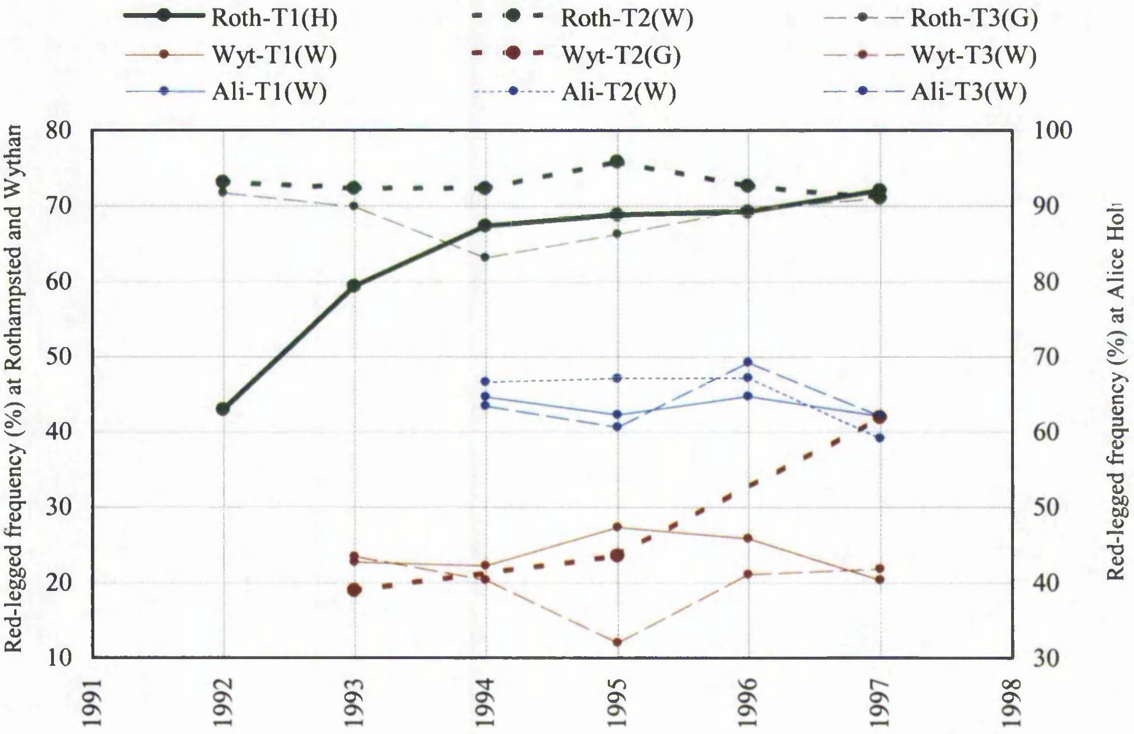
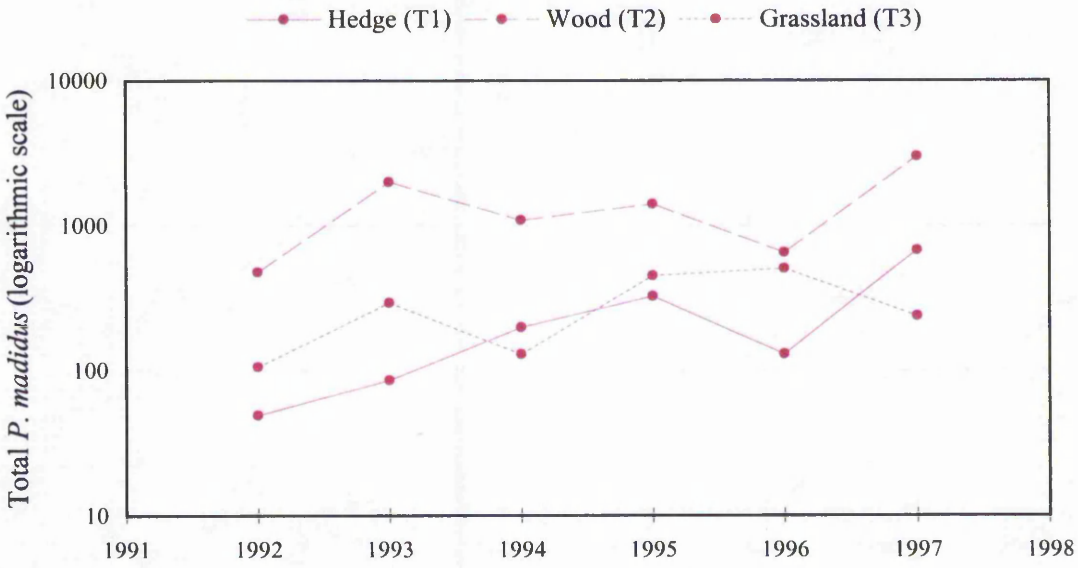


Fig 3.13 Number of *P. madidus* trapped per year along each transect at Rothampsted.



Finally, analysis by transect showed an interesting trend at two marginal sites where *P. madidus* activity was low for most years of monitoring, Sourhope (Transect 1) and North Wyke (Transect 2). At the Sourhope transect, the total number of *P. madidus* trapped per year declined by a factor of 4 between 1994 and 1996 (see Table B.6). By 1997, this decline coincided with a significant decrease in the red-legged morph frequency from 32% to 13.5% (P = 0.05, see Table 3.5). This suggests that, at marginal sites, the disadvantaged morph is driven out more rapidly under unfavourable conditions. Under favourable conditions, the converse may be true, such as Transect 3 at North Wyke where the disadvantaged morph (the black leg) was apparently absent even when total numbers of *P. madidus* trapped annually were as high as 38 (see Table B.6). In 1996, when the total increased to 83, the frequency of the disadvantaged morph increased disproportionately from 0 to 5% (P = 0.02, see Table 3.5).

iii) A comparison of male/female proportions by morph at the ECN sites (Table 3.6)

Male/female data were collected at 4 ECN sites, Porton Down, Wytham, Alice Holt and Rothampsted. From the pooled annual data (final row for each site in Table 3.6) the male frequency of both morphs along most transects is between 55 and 65%. Given that there was little or no sampling during early spring and late autumn when female activity is normally higher than the male's, it seems likely that the males and females of *P. madidus* are in 50:50 proportion.

From Table 3.6, there is clearly a variation in male activity between sites, transects and years. This may be due, in part, by the length of time over the season that the beetle was monitored. This varied between sites and years. It is possible, however, to examine whether there is a difference in the male/female proportions of the two morphs and, if so, whether morph frequencies are affected. The sites are examined in order of increasing red-legged frequency.

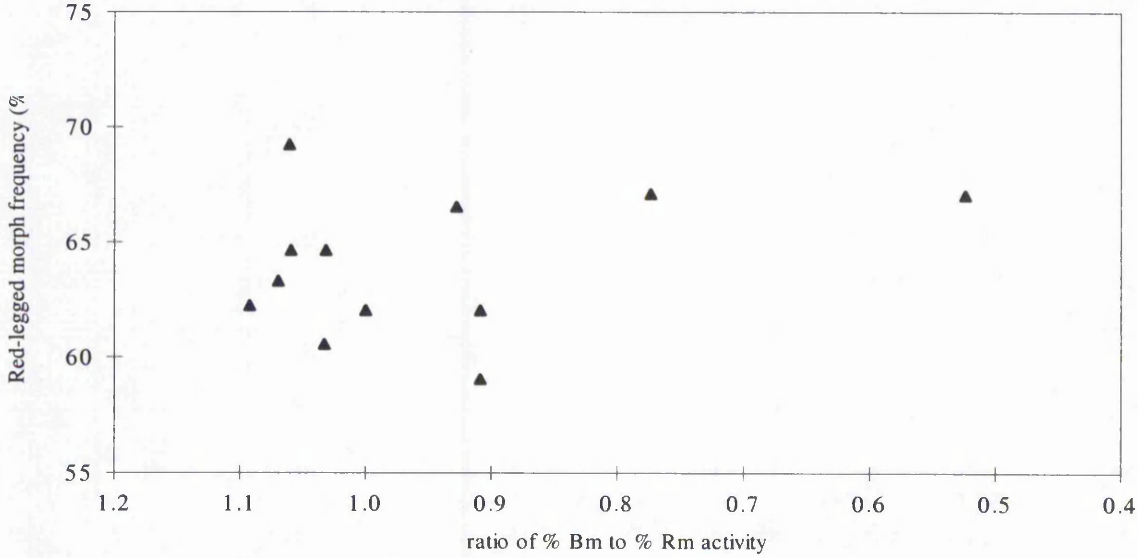
Porton Down: Over the 4 years of monitoring, male/female proportions by morph were not significantly different, although numbers were low.

Wytham: Over 5 years, a significant difference in the male/female proportions by morph occurred on one occasion only – Transect 3 in 1997. Even so, the red-legged morph frequency for that year (22%) was not significantly different to the morph proportions normally obtained along this transect.

Alice Holt: During the 4 years of monitoring, there was a consistently higher red-legged male activity compared with the black-legged male activity at the least favoured site (T2), although the overall difference in proportions is not quite significant (P = 0.08 – see final row for Alice Holt in Table 3.6). At T1 and T3, the converse is true until 1997, with the black-legged male consistently at higher frequency. Again, the difference in proportions is not significant. In 1997, *P. madidus* activity was high compared with previous years (see column headed *GT* in Table 3.6) and red-legged male activity increased disproportionately. At T1, the males of the red and black-legged morphs are now in the same proportion (P = 1.00). At T3, the red-legged male is significantly in higher proportion (P = 0.05).

Despite differences in the relative activity of the males of the two morphs, morph frequencies are not affected. As shown in Fig 3.14, there is no association between red-legged morph frequencies and the ratio of black-legged males to red-legged males ($r^2 = 0.09$).

Fig 3.14 Relationship between red-legged morph frequency and relative activity of the males of each morph at Alice Holt, using data for all transects and years.

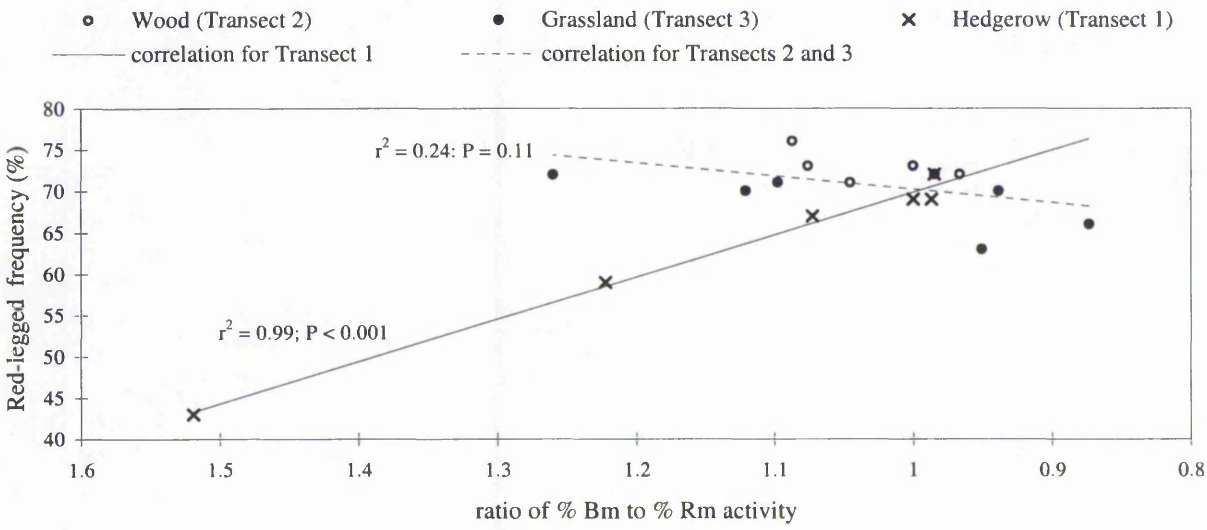


Rothampsted: This site has the longest time series (6 years). There is no consistent pattern for T2 and T3. Where significant differences occur, the red-legged male is in lower proportion relative to the black-legged male at T2 (the more favoured site), and in higher proportion at T3 (Table 3.6). At the hedgerow transect (T1), there is a clear directional trend with the black-legged male in higher proportion in 1992 (P = 0.05). Over time, this difference between the morphs decreases and, by 1996, it is the similarity in male/female proportions by morph which is significant (P = 0.971).

As noted in Section 3.4.5.ii, it is likely that the hedgerow at Rothampsted was being colonised by *P. madidus* from 1992 (Fig 3.13). There is also a high correlation between the red-legged morph frequency and the ratio of the black-legged male activity to the red-legged male activity ($r^2 = 0.99$; d.f. = 5; $P < 0.001$). This relationship is shown in Fig 3.15. As the red-legged male activity increased relative to that of the black-legged male, the red-legged frequency also increased. It appears, therefore, that the red-legged males were slower to colonise the hedgerow than the black-legged males.

Fig 3.15 also shows the correlation using pooled data for Transects 2 and 3. Although not significant ($r^2 = 0.24$; d.f. = 11; $P = 0.11$) a negative association is apparent. This may suggest that, in stable habitats in this region, the relatively high red-legged frequency is maintained by a higher female activity.

Fig 3.15 Relationship between red-legged morph frequency and relative activity of the males of each morph at Rothampsted.



In summary, the differences in male/female proportions between the two morphs are rarely significant. A slightly higher activity by the male of the favoured phenotype at less favourable but stable transects has occurred at Rothampsted and Alice Holt. With the exception of Transect 1 at Rothampsted, differences in morph male/female activity were not associated with red-legged frequencies. At Rothampsted, it appears that – despite being the less favoured morph in this region – the black-legged male colonised the hedgerow transect more rapidly than did the red-legged male. It is not known whether this is because conditions in adjacent habitats were less favourable to the black-legged male - which dispersed more readily as a consequence - or whether initial conditions in the hedgerow were less favourable for the red-legged male.

Table 3.5 **Red-legged morph frequencies for each year of sampling along each transect of the ECN sites, showing results of chi-squared analysis on morph proportions (1) along each transect over time (final 3 columns), (2) between transects for each year (final 3 rows of each site) and (3) using pooled data for all transects and years. Shaded boxes for P show significant results.**

W = woodland; H = hedge; G = grassland.

[1] one or more expected value less than 5; [2] data for years with 10% red-legged frequencies have been pooled.

* = data set too low (<10) to obtain frequencies;

"deleted" frequencies (e.g. ~~64.3~~) = small data set (<15). These have been excluded from the analysis.

ECN site	Transect	1992	1993	1994	1995	1996	1997	all	ΣX^2	d.f.	P
Alice Holt	T1 (W)			64.6	62.2	64.6	62.0	*63.1*	1.097	3	**0.78**
(Surrey)	T2 (W)			66.5	67.1	67.1	59.1	*63.2*	5.011	3	**0.17**
	T3 (W)			63.3	60.6	69.2	62.1	*63.2*	6.675	3	**0.08**
	all			*64.5*	*62.0*	*67.2*	*61.6*	***63.1***	*8.257*	*3*	***0.04***
	ΣX^2			0.806	1.217	1.382	1.145	*0.008*			
	d.f.			2	2	2	2	*2*			
	P			**0.67**	**0.54**	**0.50**	**0.56**	***1.00***			
Rothampsted	T1 (H)	42.9	59.3	67.3	68.8	69.2	71.9	68.6	22.029	5	**0.00**
(Herts)	T2 (W)	73.1	72.3	72.2	75.8	72.6	70.8	72.4	15.949	5	**0.01**
	T3 (G)	71.7	69.9	63.1	66.2	69.5	70.8	68.5	4.412	5	**0.49**
	all	*71.1*	*71.7*	*71.1*	*73.3*	*71.3*	*71.0*	***71.6***	*5.153*	*5*	***0.40***
	ΣX^2	20.257	7.411	6.396	20.609	1.780	0.298	*17.746*			
	d.f.	2	2	2	2	2	2	*2*			
	P	**0.00**	**0.02**	**0.04**	**0.00**	**0.41**	**0.86**	***0.00***			
Wytham	T1 (W)		22.7	22.2	27.3	25.8	20.3	22.1	0.963	4	**0.92**
(Oxfordshire)	T2 (G)		18.9	~~64.3~~	23.5	~~23.1~~	41.9	30.6	6.666	2	**0.04**
	T3 (W)		23.4	20.3	12.0	21.0	21.8	20.6	2.882	4	**0.58**
	all		*22.3*	*25.3*	*18.9*	*22.6*	*23.7*	***22.9***	*1.669*	*4*	***0.80***
	ΣX^2		0.454	0.072	3.038	0.277	9.179	*6.537*			
	d.f.		2	1	2	1	2	*2*			
	P		**0.80**	**0.79**	**0.22**	**0.60**	**0.01**	***0.04***			
Porton Down	T1 (G)			*	*	*	*	*			
(Wiltshire)	T2 (G)			*	9.7	9.2	21.4	*10.9*	3.524^1	2	**0.17**
	T3 (G)			*	11.8	3.8	8.7	*5.8*	1.617^1	2	**0.45**
	all			~~*0.0*~~	*10.0*	*7.3*	*15.1*	***8.8***	*2.945*	*2*	***0.23***
	ΣX^2				0.051^1	1.539^1	1.548^1	*2.105*			
	d.f.				1	1	1	*1*			
	P				**0.82**	**0.21**	**0.21**	***0.15***			
Glensaugh	T1 (G)			77.3	69.9	80.9	73.9	*75.8*	6.979	3	**0.07**
(Grampion	T2 (G)			62.1	*	62.5	*	*61.9*	0.001	1	**0.97**
Region)	T3 (G)			*	82.6	*	*	*73.1*	*		
	all			*73.5*	*72.0*	*79.5*	*71.9*	***74.6***	*4.825*	*3*	***0.19***
	ΣX^2			2.589	1.596	3.152^1	*	*5.943*			
	d.f.			1	1	1		*2*			
	P			**0.11**	**0.21**	**0.08**		***0.05***			
Sourhope	T1 (G)			32.0	36.8	39.0	13.5	32.7	8.012	3	**0.05**
(Borders	T2 (G)			*	*	*	*	*	*		
Region)	T3 (G)			*	*	*	*	*	*		
	all			*31.6*	*37.0*	*32.7*	*13.6*	***31.6***	*8.445*	*3*	***0.04***
	ΣX^2			*	*	*	*	*			
North Wyke	T1 (G)		*	*	*	*	*	*	*		
(Devon)	T2 (G)		*	*	*	*	*	*	*		
	T3 (G)		*	100.0	100.0	95.2	100.0	*97.9*	5.219^{1,2}	1	**0.02**
	all		*	*100.0*	*100.0*	*95.6*	*100.0*	***98.0***	*5.511*^{1,2}	*1*	***0.02***
	ΣX^2		*	*	*	*	*	*			

<u>Table 3.6</u> **Red-legged morph frequencies and total *P. madidus* compared with black and red-legged male frequencies for each morph at the four ECN sites. Table also shows 1) results of a chi-squared analysis of the male/female proportions by morph for each year along each transect and 2) results using pooled data for all transects and years for each site. Shaded boxes indicate significant results.**

GT = grand total; B = black-legged morph; R = red-legged morph; m = male; * = insufficient data (n<10); ^{1} = one or more expected value less than 5.

ECN site		Transect 1						Transect 2						Transect 3						All transects					
		%R	*GT*	%Bm	%Rm	ΣX^2	*P*	%R	*GT*	%Bm	%Rm	ΣX^2	*P*	%R	*GT*	%Bm	%Rm	ΣX^2	*P*	%R	*GT*	%Bm	%Rm	ΣX^2	*P*
Alice Holt	1994	65	387	58	54	0.382	0.54	67	269	39	42	0.225	0.64	63	518	56	52	0.646	0.42	64	1174	53	50	0.567	0.45
(Surrey)	1995	62	270	64	58	0.771	0.38	67	85	32	61	6.438	0.01	61	340	58	56	0.213	0.64	62	695	58	58	0.004	0.95
	1996	65	263	66	64	0.111	0.74	67	76	44	57	1.113	0.29	69	328	66	63	0.432	0.51	67	667	63	62	0.029	0.86
	1997	62	764	68	68	0.000	1.00	59	364	65	72	1.752	0.19	62	1057	60	66	3.873	0.05	62	2185	64	68	3.574	0.06
	all	63	1684	65	63	0.749	0.39	63	794	52	58	2.986	0.08	63	2243	59	61	0.379	0.54	63	4721	60	61	0.397	0.53
Rothampsted	1992	43	49	79	52	3.743	0.05	73	650	71	66	1.782	0.18	72	106	63	50	1.538	0.21	71	805	71	63	4.646	0.03
(Herts)	1993	59	86	77	63	1.998	0.16	72	2782	64	65	0.073	0.79	70	292	65	58	1.229	0.27	72	3160	65	64	0.129	0.72
	1994	67	199	74	69	0.418	0.52	72	1513	58	60	0.748	0.39	63	130	58	61	0.088	0.77	71	1842	60	61	0.320	0.57
	1995	69	324	75	76	0.011	0.92	76	1865	75	69	5.961	0.01	66	450	62	71	4.000	0.05	73	2639	72	70	1.073	0.30
	1996	69	130	58	58	0.001	0.97	73	894	75	75	0.001	0.97	70	502	46	49	0.226	0.63	71	1526	63	65	0.370	0.54
	1997	72	675	65	66	0.065	0.80	71	4224	69	66	4.715	0.03	71	236	45	41	0.355	0.55	71	5135	67	64	3.969	0.05
	all	69	1463	69	67	0.681	0.41	72	11928	68	66	3.765	0.05	69	1716	56	56	0.008	0.93	72	15107	67	65	2.999	0.08
Wytham	1993	23	141	55	63	0.560	0.45	19	53	51	70	1.162	0.28	23	111	44	42	0.012	0.91	22	305	50	56	0.680	0.41
(Oxfordshire)	1994	22	81	57	50	0.289	0.59	64	14	*	*			20	59	72	75	0.034^1	0.85	25	154	63	59	0.163	0.69
	1995	27	22	81	50	2.148^1	0.14	24	34	65	88	1.441^1	0.23	12	50	66	67	0.001^1	0.97	19	106	69	70	0.015	0.90
	1996	26	31	70	63	0.136^1	0.71	23	13	*	*			21	62	41	54	0.710	0.40	23	106	50	54	0.129	0.72
	1997	20	187	77	74	0.132	0.72	42	43	80	61	1.856	0.17	22	78	77	41	8.032	0.00	24	308	77	63	5.638	0.02
	all	22	462	66	64	0.251	0.62	31	157	61	65	0.230	0.63	21	360	58	51	1.188	0.28	23	979	63	60	0.532	0.47
Porton Down	1994	*	1	*	*			*	5	*	*			*	11	*	*			0	17	*	*		
(Wiltshire)	1995	*	2	*	*			10	31	29	33	*		12	17	*	*			10	50	38	60	0.926^1	0.34
	1996	*	4	*	*			9	120	30	45	1.064^1	0.30	4	53	59	50	*		7	177	39	46	0.256	0.61
	1997	*	2	*	*			21	28	23	17	1.103^1	0.29	9	23	71	50	*		15	53	49	25	1.564^1	0.21
	all	*	9	*	*			11	184	30	35	0.221	0.64	6	104	58	67	0.169^1	0.68	9	297	41	42	0.018	0.89

3.4.6 Comparison of red-legged morph frequencies and temperature data for Central England.

Given the between-year variability in temperature data and "noise" due to sampling error on morph frequency data (see Section 2.5.3), reasonable comparisons can only be made using *P. madidus* data that encompass a relatively long time period, i.e. Transects 1 and 2 morph frequency data, collected in the mid-1970s and mid-1990s (Sections 3.4.1 and 3.4.2), and the Close House data, which cover 14 years from 1981 to 1994 (Section 3.4.4).

i) Mean annual and seasonal temperature data for Central England since 1945

Fig 3.16, which shows the annual and seasonal mean temperatures for Central England since 1945, indicates an overall rise in annual and seasonal mean temperatures since the late 1980s. However, warm years occurred quite regularly from the late 1940s and throughout the 1950s, whereas the period from 1962 to the mid-1970s was generally cooler than previous years.

To give a better idea of the temperature changes for Central England since the mid-1970s, Fig 3.17 shows the temperature differences (anomalies) from 1975 using the 30-year average of 1945-1974 annual and seasonal mean temperatures as a baseline. This period was chosen because it precedes the earliest comprehensive records of *P. madidus* morph frequency.

For 9 of the 14 years from 1975 to 1988, annual and seasonal temperatures were lower than the 1945-74 average. The exceptions were 1975, 1976, 1982, 1983 and 1984. In general, the late 1970s and mid-1980s were characterised by cooler springs and summers, and include one exceptionally cold winter (1979). Since 1989, there has been a high incidence of warmer years mainly due to milder winters and warmer than average springs and summers. Nevertheless, lower than average seasonal temperatures have occurred in different years i.e. cool winters (1991 and 1996); a cool spring and summer (1993); a cool autumn (1997).

ii) Transects 1 and 2

Morph frequency data for Transect 1 (Southampton to north Nottinghamshire) were obtained in 1975, 1995 and 1996. For Transect 2 (West Dorset to East Sussex south coast transect), data collection took place in 1976, 1996 and 1998.

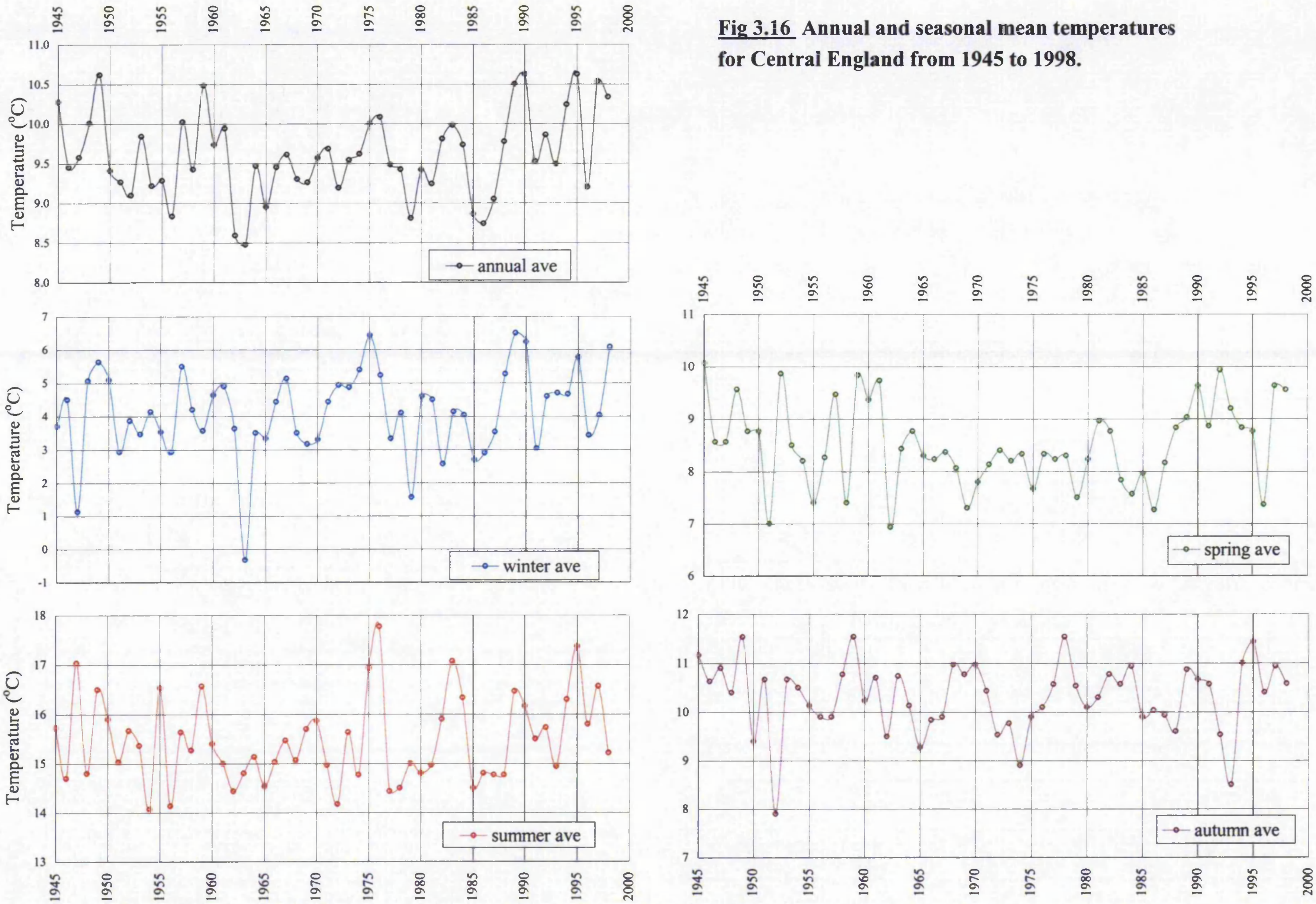
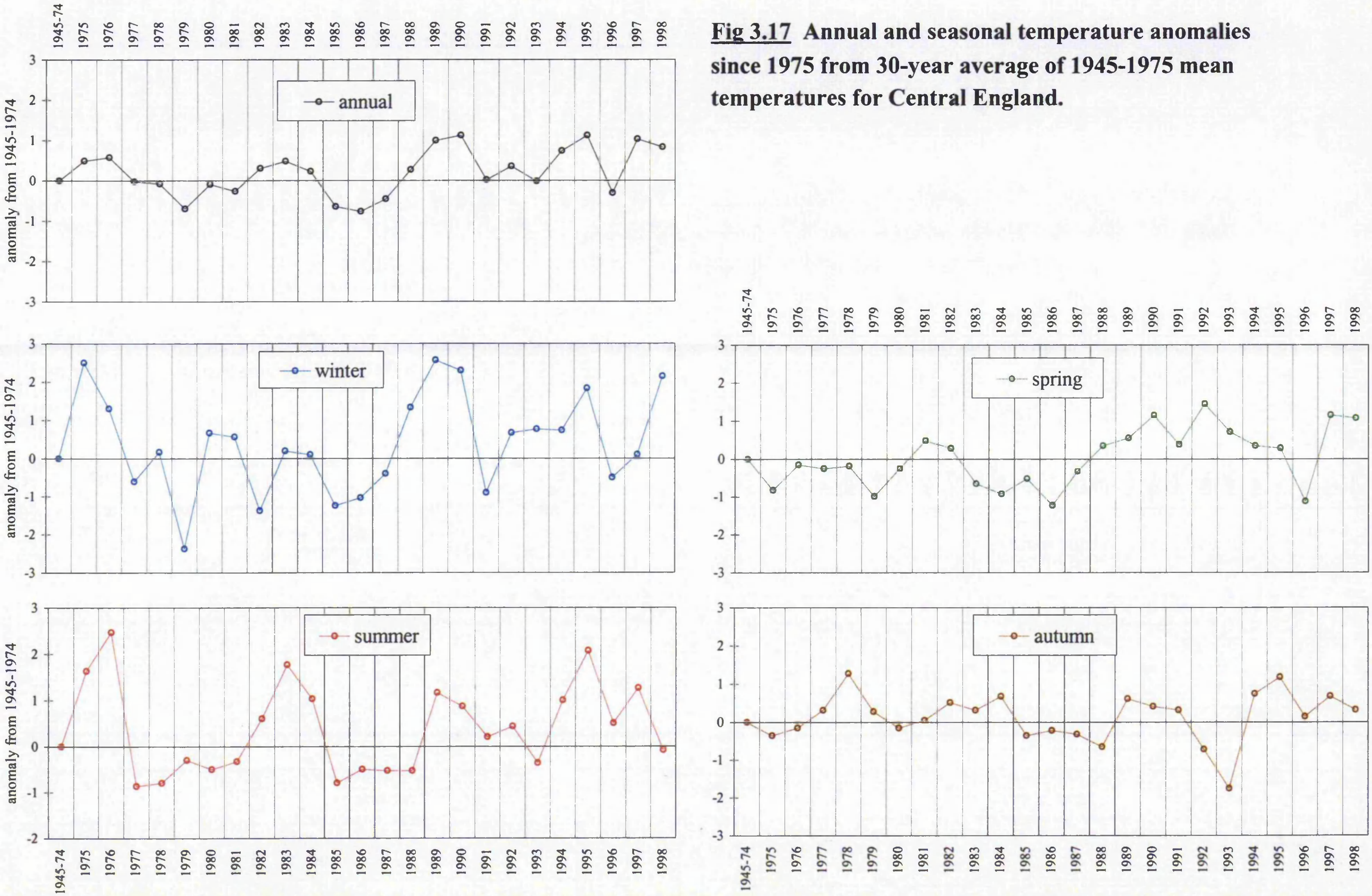


Fig 3.16 **Annual and seasonal mean temperatures for Central England from 1945 to 1998.**

Fig 3.17 **Annual and seasonal temperature anomalies since 1975 from 30-year average of 1945-1975 mean temperatures for Central England.**



As shown in Figs 3.16 and 3.17, 1975 was characterised by high summer and winter mean temperatures producing a higher than average annual mean compared with the 1945-74 baseline. Spring and autumn, however, were slightly cooler than average. The annual temperature for 1976 was even higher, with an exceptionally hot summer, a fairly mild winter, but only average temperatures for spring and autumn. 1995 showed a similar pattern to 1975, but also experienced higher than average autumn temperatures. The annual and seasonal mean temperatures for 1996 were lower than those for 1975 and 1995, although the summer mean is higher than the 30-year 1945-74 average. 1998 was one of the warmest years since 1989, primarily due to warmer than average winter and spring temperatures.

Comparing morph frequency data for the mid-1970s and mid-1990s, a trend towards a higher red-legged morph frequency was found in the 1990s at some sites along both transects (see Sections 3.4.1 and 3.4.2). What is not known is the variation in morph frequency over the 20-year period before re-establishment of the sites in the mid-1990s. Assuming leg colour is selected for, the intervening cooler years may have favoured the black-legged morph, with the red-legged morph frequency increasing since 1989. Given that both cooler and warmer years have occurred between the mid-1970s and mid-1990s, the relatively small difference in red-legged morph frequencies between these two periods is not surprising, if temperature is the main mechanism affecting morph distribution.

It is difficult to make a similar case for the induction of the leg colour phenotype by temperature. Although 1975 and 1976 were both warmer than average, as were 1995 and 1998, 1996 was not an exceptional year (Fig 3.17). The greater morph frequency difference that occurred between the mid-1970s and the mid-1990s than between 1995 and 1996 (Transect 1) and 1996 and 1998 (Transect 2) is more indicative of an evolutionary process than phenotypic induction.

iii) Close House (Northumberland)

The Close House morph frequency data is the only available long time series and covers 14 years from 1981 to 1994 (Fig 3.10, p. 82). Table 3.7.i shows the results of a regression analysis of red-legged morph frequencies against the 1981-94 seasonal and annual mean temperatures for Central England. An analysis was also performed using temperatures for the previous year (i.e.1980 temperatures for 1981 frequencies etc.).

As shown in Table 3.7, there is a significant and negative correlation between the red-legged morph frequency and the annual mean temperature (P = 0.05). A relatively strong association with the mean summer temperature (P = 0.10) is also apparent. Interestingly, using autumn temperatures for the previous year improved the significance level from 0.26 to 0.16. Autumn, of course, occurs after the main activity of *P. madidus.* A similar analysis for the other seasons and for annual data showed no association (P > 0.5 in all cases).

Table 3.7 Results of regression analysis on red-legged morph frequencies for Close House (Northumberland) against mean annual and seasonal temperature data for Central England for the period 1981 to 1994 i) using all years in the analysis and ii) excluding 1989 from the analysis. (Significant results are given in italics).

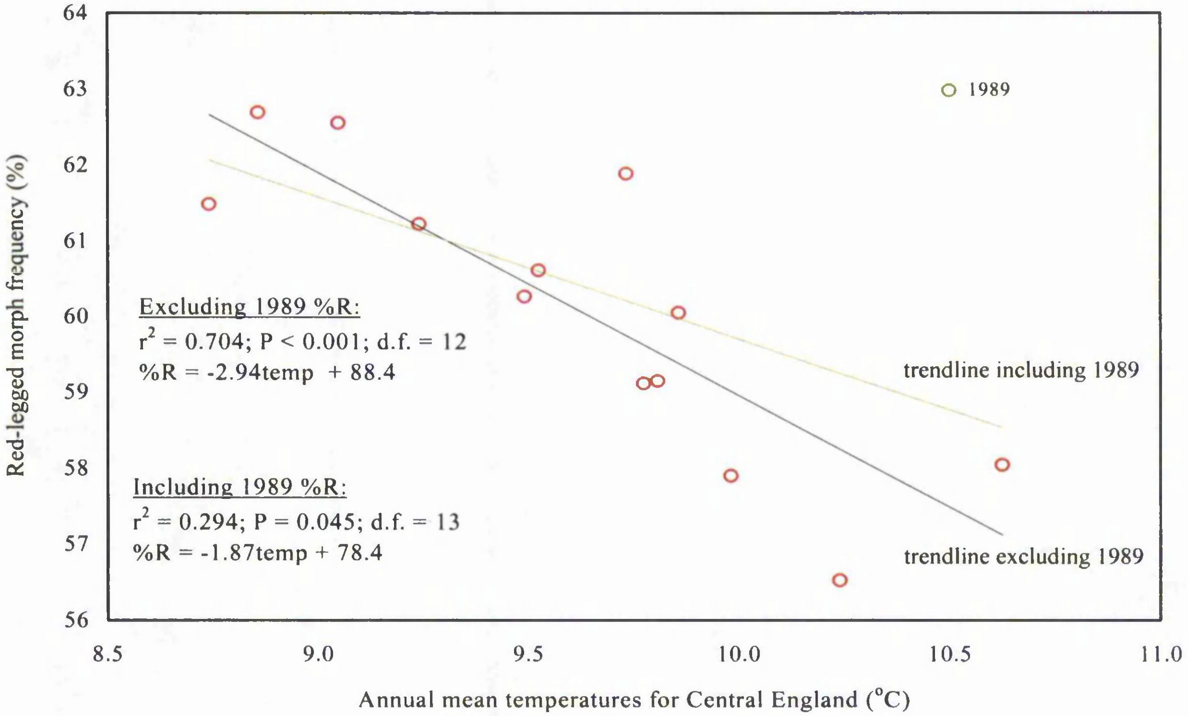
	i) all years (d.f. = 13)				ii) excluding 1989 (d.f. = 12)			
Temperature data	r^2 (%)	f-ratio	P	Association	r^2 (%)	f-ratio	P	Association
annual	*29*	*4.99*	*0.045*	+	*70*	*26.18*	*<0.001*	+
autumn	10	1.37	0.264		17	2.29	0.159	
winter	4	0.53	0.480		27	4.18	0.066	
spring	3	0.35	0.564		9	1.13	0.310	
summer	21	3.10	0.104		*43*	*8.35*	*0.015*	+
autumn*	16	2.25	0.160		*34*	*5.76*	*0.035*	+

* using temperatures for the previous year (i.e. 1980 autumn temperatures for 1981 frequencies etc)

From Fig 3.18, which shows the correlation between red-legged frequencies and the annual mean temperature data for Central England from 1981 to 1994 (black line in figure), a large residual on the 1989 data is apparent. Table 3.7.ii shows the results when 1989 is excluded from the regression analysis. For annual data, the coefficient of determination now becomes very highly significant (P < 0.001 - green line in Fig 3.18). A similar, albeit reduced significance was found when red-legged morph frequencies were regressed against summer mean temperatures (P = 0.02) and the previous year's autumn temperatures (P = 0.04).

The large residual on the 1989 data cannot easily be explained. 1989 is characterised by warmer than average temperatures for all the seasons (Fig 3.16), but this is also the case for 1990, 1994 and 1995, which did not produce anomalous results. It is possible that temperatures for Central England - presumed to represent regions as far north as Preston (latitude 53° 76`) - may not be typical for all years or seasons at Close House in Northumberland (latitude 55°). The mean maximum and minimum temperatures, which are not known, are also likely to be influential in different ways for different seasons or years.

Fig 3.18 Negative correlation between red-legged morph frequency at Close House from 1981 to 1994 and annual mean temperatures for Central England. Green trendline includes 1989 frequency (green circle); black trendline excludes results for1989.



Nevertheless, a good correlation has been found, strongly suggesting that temperature affects the temporal variation in morph frequencies at Close House, with the frequency of the black-legged morph increasing with increasing mean annual temperature. Furthermore, the closer association between frequencies and summer data compared with other seasonal data suggests that leg colour is inherited. Phenotypic induction of leg colour is expected to occur during the later stages of *P. madidus* development, but there was no association with spring temperatures.

3.4.7 Relative fitness of *Pterostichus madidus* morphs

The fluctuations in the 1981 to 1994 morph frequencies for Close House are not only significant, but also show a directional trend from 1984, the red-legged morph frequency decreasing significantly over time (Section 3.4.4 and Fig 3.10). The frequencies also correlate quite well with temperature data for Central England, with the black-legged morph favoured by higher annual mean temperatures (Section 3.4.6.ii and Fig 3.18).

Assuming leg colour is inherited and the fitness of the dominant homozygote and heterozygote is the same, the relative fitness (w) of the less favoured phenotype can be calculated for each year using Equation 3.1 (Section 3.3.5, p. 67). The fitness of the most favoured genotype is set at 1. One generation per year is assumed.

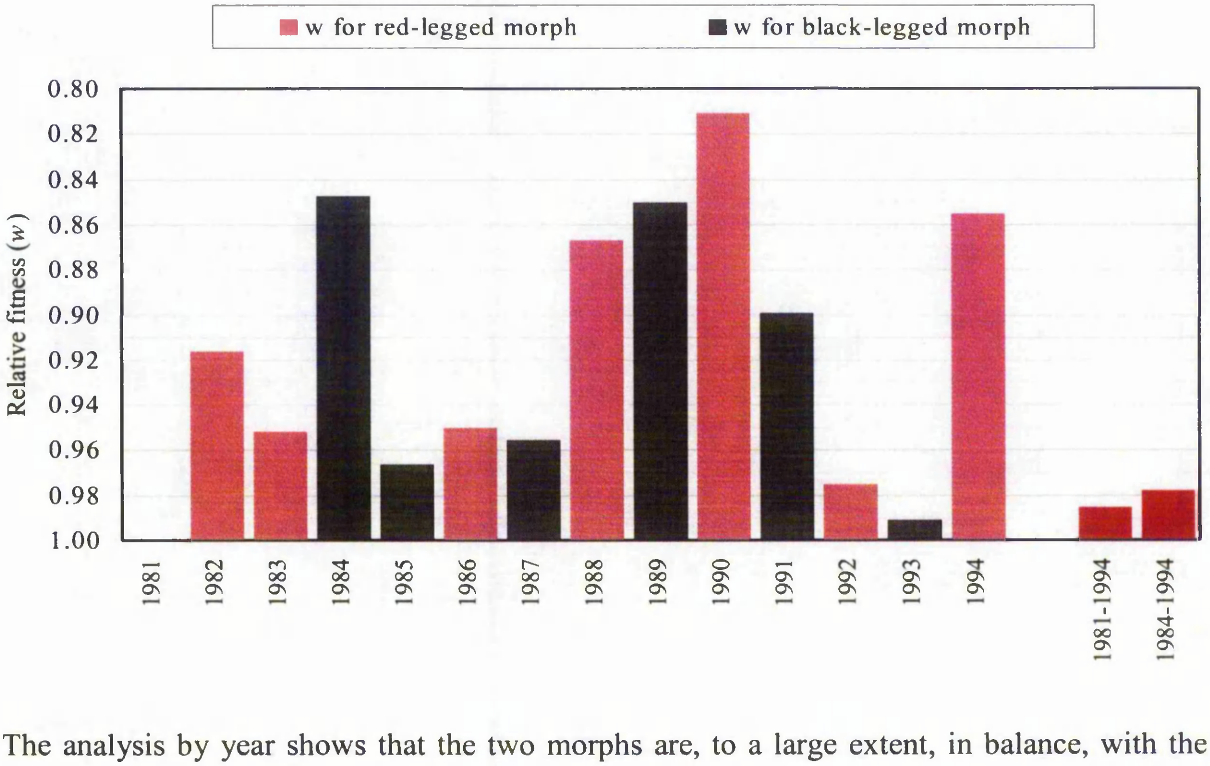
Fig 3.19 shows the results of these calculations as follows:

(1) by year using $\text{year}_{(n)}$ and $\text{year}_{(n+1)}$ as the initial and final frequencies, each year representing a generation;

(2) over the 14-year period, using 1981 and 1994 as the initial and final frequencies;

(3) over the 11-year period from 1984 when the directional trend is apparent (see red line in Fig 3.10), using 1984 and 1994 as the initial and final frequencies.

Fig 3.19 Relative fitness (w) of less favoured leg colour morph at Close House by year, assuming w for dominant homozygote and heterozygote is the same. In bold red: w over 14-year and 11-year period. (NB w for favoured morph is set at 1).



The analysis by year shows that the two morphs are, to a large extent, in balance, with the selective disadvantage tending to alternate between the morphs with each generation. The lowest value for w is 0.81 in 1990 representing a 17% disadvantage to the less favoured morph for that generation (the red legs). However, both morphs obtained relative fitness values of less than 0.90 in three separate years (1984, 1989 and 1991 for the black-legged morph; 1988, 1990, and 1994 for the red-legged morph).

Not surprisingly, therefore, the overall relative fitness of the disadvantaged red-legged morph from 1981 to 1994 (13 generations) is - at 0.985 - close enough to unity to suggest that selection against the red leg is undetectable over this period. For the period 1984 to 1994 (10 generations), when regression analysis found a significant directional trend, *w* has decreased to 0.978, giving a 2.2% selection against the red-legged morph.

Assuming this trend were to continue at the same rate, it is possible to calculate how many generations it would take before the red-legged morph became rare (< 5%) by running the model described in Section 3.3.5.ii, pp. 67-8 ('genetics1.xls' on CD-ROM). The initial red-legged morph frequency was set at 56.5% (the 1994 frequency) and the relative fitness was set at 0.978 (the average for 1984 to 1994). The results, shown in Fig 3.20, assume one of two conditions:

(1) red is dominant; black is recessive (bold lines)

(2) red is recessive; black is dominant

The fitness of the heterozygote was assumed to be the same as that for the dominant homozygote.

Fig 3.20 Expected change in morph frequencies over 300 generations (1) when red is dominant (bold lines) and (2) when black is dominant, from an initial red-legged frequency and relative fitness of 56.5% and 0.978 respectively.

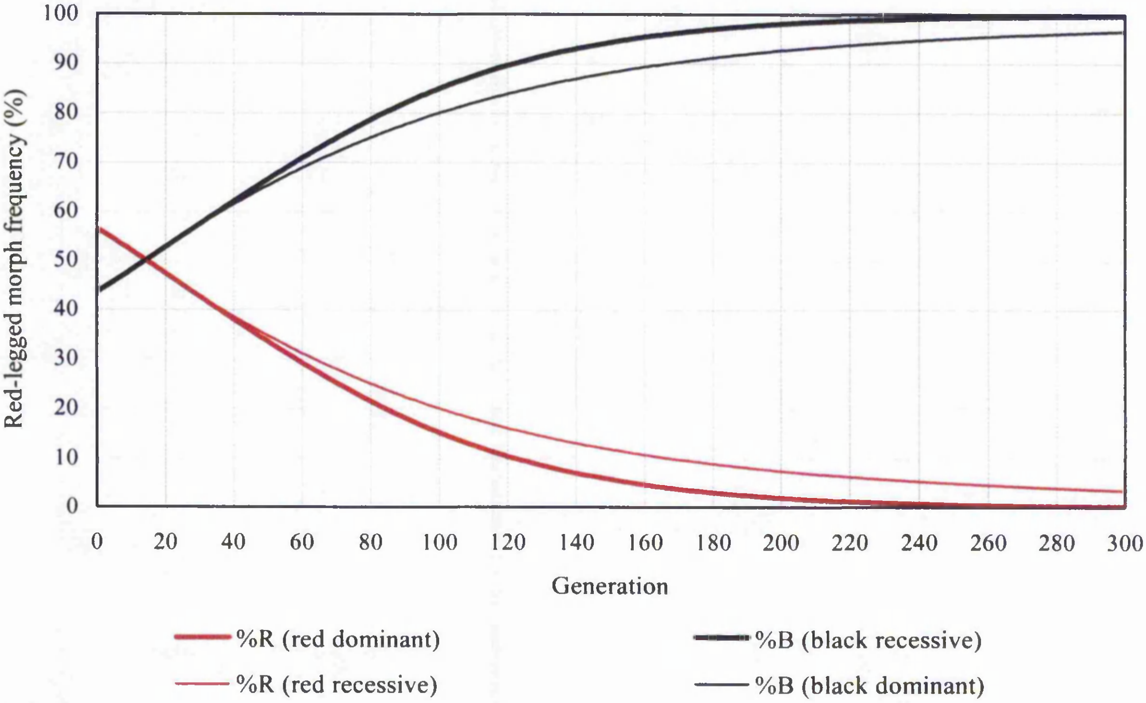


Fig 3.20 shows that, if red is dominant, it would take 160 generations before the red-legged morph frequency fell to below 5%. If red is recessive, 250 generations would be required before the red-legged morph became rare. The slower rate of decline for a disadvantageous recessive allele is because it is not expressed in the heterozygote, hence is retained in the population for a longer period compared with a similarly disadvantaged dominant allele.

Fig 3.20 also shows that the fastest rate of change occurs at intermediate frequencies, such as those for Close House. Even so, it would take 22 generations for the red-legged morph frequency to fall from 56.5% to 46.5%. Over 5 generations, the frequency falls by only 1% (to 54.3% if red is dominant and 54.4% if red is recessive). This would not be detectable, of course.

Obviously, this is a highly theoretical and speculative projection. For example, if climate warming continues, the relative fitness of the disadvantaged morph would be expected to decrease further, so increase the rate of morph frequency change. Nevertheless, it is clear from the calculations of relative fitness that it is unrealistic to expect to detect directional changes in morph frequencies from stable populations over relatively short time periods (e.g. less than 10 years), particularly when the frequencies are associated with highly variable climatic factors, which can favour one morph one year and the other the next.

3.5 Discussion

From his analysis of morph frequency data collected from the early 1970s to the mid-1980s, Terrell-Nield (1990a) proposed that the morph frequency distribution of *Pterostichus madidus* is associated with climatic factors. He assumed that the spatial variation in morph frequencies over-rode any temporal variation. Since this period, there has been general agreement that the climate in Britain is getting warmer. According to Terrell-Nield's hypothesis, there should be a concurrent change in the morph frequency distribution. However, Doberski & Gazzy (2000) have doubted the association of *P. madidus* morph frequency distributions with climate from their analysis of 2 sites sampled over 6 years in the 1990s. They found that the frequency change over time fluctuated within a narrow range about a mean and there was no directional trend. On the other hand, there were highly significant differences in the morph proportions of closely positioned sites which, it was assumed, would experience a similar microclimate.

The investigations by Terrell-Nield and Doberski & Gazzy (2000) suggest the following hypotheses:

(1) Leg colour in *P. madidus* has no direct or indirect selective advantage. The morph frequency obtained at a site simply reflects that of the initial colonisers. Providing populations remain isolated, these frequencies will be stable over time, any small fluctuations being a consequence of sampling error or genetic drift.

(2) As proposed by Terrell-Nield (1990a), the two leg colour morphs are adapted to different climatic conditions. The mechanism could be by Darwinian selection *or* phenotypic induction.

(3) As suggested by Doberski & Gazzy (2000), selection may be due to abiotic factors other than climate, such as soil type.

The analysis in this chapter has attempted to distinguish between these hypotheses by reference to three types of morph frequency data:

- data separated by 20 years (Transects 1 and 2);
- a longer time series of data collected over 14 years (Close House, Northumberland);
- data collected over 3 to 6 years from sites in England and Scotland.

Transects 1 and 2 extend over 250km in a north-south and east-west direction respectively, with a spatial resolution between 10 to 20km. Comparing data for the mid-1970s and the mid- to late 1990s, there has been an overall increase in the red-legged frequencies for most regions along both transects. There was, however, a significant increase in the black-legged morph in Berkshire (Transect 1). The fluctuations in morph frequency between the two periods are not known, but the stability between 1995 and 1996 (Transect 1) and relative stability between 1996 and 1998 (Transect 2) suggest that changes over time are gradual.

These results support Terrell-Nield's hypothesis of 1990 that the red-legged morph is favoured by higher minimum temperatures. Compared with the 1945-74 30-year average for mean temperatures, both cooler and warmer years have occurred between 1975 and 1998, climate warming only becoming detectable since 1989. Assuming the mean minimum temperature follows a similar pattern, a small overall increase in the red-legged morph frequency is expected. Terrell-Nield also found a negative association between red-legged morph frequencies and a wider temperature range, which might explain the rise in black-legged frequencies at the more exposed Berkshire sites.

However, the relative stability in morph frequencies for the two periods of sampling also supports the hypothesis that there is no selection between the morphs. Most sites were trapped on only one occasion. For 1975/6, *P.madidus* numbers were usually under 80 and could be as low as 12 (the target number when only one sample is taken). The frequencies are therefore likely to be subject to quite a high sampling error. Although sub-samples taken along Transect 1 in 1996 showed no significant difference in the morph proportions, their frequencies could be adrift by as much as 30%.

Arguably, the greater difference between sampling periods separated by 20 years compared with periods separated by only 1 or 2 years is due to random genetic drift whereby, as the number of generations increases, the populations begin to diverge from their initial allelic frequencies (Kimura, 1955). However, where there are only two alleles, the probability of an increase of one allele over another is 50%. It is therefore interesting that, for both transects, the frequency changes have tended towards increasing red-legged frequencies.

Compared with the temporal stability, the spatial variability in morph proportions along the transects is remarkable. Along Transect 1 (Nottingham to Southampton) and Transect 2 (Dorset to East Sussex), steep clines over short distances were maintained for each year of trapping, over-riding any temporal fluctuation between the periods of monitoring.

There is circumstantial evidence that climatic factors may be maintaining this spatial variability. This is suggested in two ways. Firstly, there was a far greater fluctuation in morph frequencies along the topographically complex Transect 2, particularly in the New Forest and on the South Downs where there are considerable altitudinal and aspect differences between the sites. It is reasonable to assume that there will be similarly steep climatic gradients. By contrast, the spatial variability between frequencies is not significant in the flatter, more topographically homogenous region of the Trent valley along Transect 1 (Sites 23 to 26).

Secondly, along both transects, higher red-legged frequencies were found at urban, maritime and river valley sites as well as the more wooded areas of the country. Higher black-legged frequencies occurred at more exposed sites, such as intensive agricultural areas where tree cover is sparse, the North Downs ridge and the higher ground inland from Southampton.

Maritime and urban regions, if not exposed to high wind flow, are characterised by a smaller range in diurnal and seasonal temperatures due to the warming effect of sea surfaces in coastal regions and the "heat island" effect of increased cloud cover in urban areas (Smith, 1976; Chandler, 1976). Sheltered habitats, such as woods, are also known to experience a smaller diurnal and seasonal temperature range. These climatic conditions are thought to favour the red-legged morph (Terrell-Nield, 1990a). If there is a constant micro-climatic gradient between two sites exerting a different selective pressure on one morph relative to the other, a difference in morph proportions between the sites becomes inevitable, particularly when the populations are isolated from one another. This could also explain why Doberski & Gazzy (2000) obtained a significant difference in morph proportions from two sites, characterised by different canopy and ground cover, in one forest.

The 14-year time series from 1981 to 1994 for Close House, Northumberland gives further evidence of a link between morph proportions and temperature factors. Although the red-legged morph frequency at Close House fluctuated within 3.5% of a mean of 60% for all the years of trapping, the difference between morph proportions by year was significant. A decline in the red-legged morph frequency from 1984 was also detectable. Given the high numbers of *P. madidus* trapped for each year, these frequency variations cannot be dismissed by reference to sampling error or genetic drift. Furthermore, there was a significant correlation between morph frequencies from 1981 to 1994 and the annual mean temperature for Central England for the same period, the black-legged morph favoured by higher mean temperatures. Only one year (1989) produced a high residual. Once excluded from the analysis, a highly significant coefficient of determination was obtained. Since both temperature and morph frequency data are subject to between-year variability, it is difficult to argue that the correlation is coincidental.

Terrell-Nield (1990) did not find any association between the black-legged morph frequency and mean temperature. The result for Close House could be due to the location of this site and the nature of the temperature data used in the analysis. South-facing and positioned in a walled garden within a low-lying, wooded region of the Tyne valley, the Close House site would experience an equitable microclimate (possibly reflected by the relatively high red-legged frequency). Although it cannot be presumed that the mean temperatures of Central England are representative of Northumberland, they could well be a better approximation of the microclimate of Close House than of more exposed regions at lower latitudes.

The warmer summers of recent years may have produced relatively high temperatures, which could be unfavourable to the red-legged morph or favourable to the black-legged morph in terms of mating behaviour or egg production. This is also suggested by the stronger association between morph frequency and summer compared with the other seasons. Unfortunately, maximum and minimum temperatures for Central England are not available. Knowledge of these data may also help identify why 1989 was atypical.

All in all, the results for Close House support the hypothesis that the leg colour morphs are adapted to different temperature conditions. The association between morph frequency and the summer mean - but not the spring mean – further suggests that phenotypic induction of leg colour is unlikely, summer being associated with reproduction rather than development.

If simple Darwinian selection can be assumed, on average, there has been a 2.2% selection against the red-legged morph at Close House from 1984 to 1994. According to model predictions, if this rate were to continue, after 20 years the red-legged frequency would have decreased by only 10% from an initial frequency of 56%. However, there are also large annual variations in the relative fitness of the morphs with the selective disadvantage tending to alternate between morphs, indicating a potential for recovery when conditions are more favourable. Consequently, directional trends over short periods are masked.

It is not surprising, therefore, that Doberski & Gazzy (2000) found no trend from 6 years of data collection. Similarly, the 3 to 6 years of monitoring of the ECN produced little evidence of consistent trends in morph frequency change over time, either within or between the different regions. However, at the Rothampsted hedgerow (T1) there was a significant increase in the red-legged frequency over the 6-year period of monitoring. When the data for all three transect habitats were pooled, the red-legged frequency was constant for each year of sampling. These results suggest that the spatial distribution of the morphs is an important factor. It appears that the black-legged male colonised the hedgerow more rapidly than the red-legged male.

Hedges are commonly used as corridors enabling movement of carabid beetles from one habitat to another (Petit, Rushton & Sanderson, 1997). It is possible that the black-legged morph has more rapid powers of dispersal. On the other hand, with a frequency of only 30%, this morph is less favoured at Rothampsted and its dispersal from an adjacent habitat may be due to unfavourable conditions at this site.

Overall, the spatial variability in morph frequencies is consistent with the hypothesis that climate - in particular temperature - is affecting the morph frequency distribution. The investigation into temporal changes in morph frequencies has also provided some fairly convincing evidence of an association with climatic factors as well as a theoretical framework for understanding why a directional change in morph frequency may not be perceptible from a short time series. If selection can be assumed, the difference in the fitness of the two morphs is small. Some morph frequency variation can also be accounted for by differences in the spatial activity pattern of the two morphs, i.e. in unfavourable years, one morph may disperse in greater proportion to an adjacent habitat where the microclimatic conditions are more favourable. This behavioural adaptation would tend to maintain stability in the morph proportions of an area, but not necessarily within one habitat of that area, and could explain the apparently inconsistent annual variations in morph frequencies between sites.

It is not known whether the morphs differ in their fertility, fecundity and/or survival under different temperature conditions. Male/female proportions were rarely different between the morphs. With the exception of the special case at Rothampsted, in instances where a significant difference in the sex ratio occurred, morph frequencies were not affected. However, it appears that, in some years, the peak activity of the red- and black-legged males (or females) is not synchronised. It is not known from these data whether this phenomenon can be linked to climate.

Other questions are also left unanswered. In particular, if temperature is the main mechanism influencing morph frequencies, it is curious that there are greater differences in morph proportions between sites less than 10km apart than between sites in the extreme north and south of the country. It is possible that, as for *Adalia bipunctata*, the variations in morph frequency are on a regional rather than national scale. Nor is it certain whether the morph frequency/temperature relationship at Close House in Northumberland is consistent with that found by Terrell-Nield (1990a). In an attempt to answer these questions, the spatial variability at national and regional levels in relation to climatic and other environmental factors is investigated further in Chapter 5.

CHAPTER 4: Investigation of environmental factors influencing the spatial variation in morph frequency

4.1 Aims

i) To investigate whether climatic or other environmental factors are associated with the spatial variation in the leg colour of *Pterostichus madidus* in different regions of England and Wales, using regression analysis.

ii) To investigate whether environmental factors can also explain small-scale variations in red-legged frequencies by examining morph proportions obtained from sites with a spatial resolution of 5 km or less in the East Midlands.

iii) To identify and rank the strongest predictors of the variations in morph frequency.

4.2 Introduction

Although the temporal variation in the morph frequencies of *Pterostichus madidus* appears to be relatively stable, a 14-year time series of annual red-legged frequencies at Close House in Northumberland was found to correlate negatively with annual mean temperatures for Central England over the same period (Section 3.4.6). Assuming selection is the mechanism influencing the frequencies, the difference in the relative fitness of the two morphs is neither large nor constant over time (Section 3.4.7). This, perhaps, explains the apparent stability between the mid-1970s and mid-1990s morph proportions along Transects 1 and 2 (Sections 3.4.1 and 3.4.2). By contrast, the leg colour morph proportions of sites within 10km of each other can show considerable variation (Sections 3.4.1 and 3.4.2). Could this be due to a climatic gradient between sites that is consistent over time?

Terrell-Nield (1990a) found that the spatial red-legged morph frequency in the UK correlated positively with the annual minimum temperature and rainfall, and negatively with the annual temperature range. Unlike the Close House temporal data, there was no association with the annual mean temperature. Terrell-Nield also found latitude to be an important factor, the red-legged morph correlating positively with increasing latitude. He suggests this is due to the more maritime nature of the British climate with more northern latitudes, i.e. although the mean temperature tends to decrease with increasing latitude, rainfall increases and the temperature range decreases, so favouring the red-legged morph.

Climatic factors are therefore strongly indicated as influential predictors of morph frequency but the associations are complex and not necessarily consistent between investigations. It is also difficult to explain why there can be a greater morph frequency difference between sites less than 20 km apart along Transects 1 and 2 (Sections 3.4.1 and 3.4.2) compared with sites separated by more than 500 km – e.g. Rothampsted in southern England and Glensaugh in Scotland (see Fig 3.11, Section 3.4.5). One would expect that the two latter regions would experience far greater climatic differences.

However, the microclimate of a habitat on the ground differs from the large-scale climate due to the influences on meteorological factors of a site's altitude, aspect, vegetation cover, soil type, and proximity to water-ways and urban dwellings. White & Smith (1982) estimate annual temperatures in Britain fall by 0.43°C for each 100m increase in altitude. The aspect and slope of a site also have important effects on temperature. For example, in Germany, a south-facing site at 20° inclination received twice as much radiation in January compared with a horizontal surface (Geiger, 1966). As described by both Geiger (1966) and Monteith (1975), vegetation cover affects incoming (solar) and outgoing (long-wave) radiation, eddy diffusion and soil water content. The temperature extremes beneath a canopy narrow with increasing height and density of vegetation due to reduced solar radiation reaching the ground. The reduced wind movement and an increase in evapotranspiration within a stand also raise the relative humidity. As a result, the microclimate within vegetated surfaces is milder and effectively more maritime.

Subsurface soil temperatures vary with the texture and moisture content of the soil. Thermal conductivity is directly related to soil moisture content and inversely related to soil texture (Geiger, 1966). Smith (1976) notes that the proximity of large water bodies can produce a thermal lag on a seasonal scale, due to their greater heat storage capacity. The "heat island" effect of heavily urbanised areas, which has resulted in lower insolation due to pollution but raised temperatures in winter due to the artificial supply of heat has been described by many workers (e.g. Geiger, 1966; Chandler, 1962, 1976).

It was not known which, if any of these influences on microclimate affect the morph frequency distribution of *P. madidus*. For example, Doberski & Gazzy (2000) were unable to explain consistent differences in morph frequencies at two sites in Thetford Forest by consideration of soil pH, ground and canopy cover and suggested there may be other factors influencing the frequencies, which are independent of climate.

This investigation attempts to identify the processes influencing both national and more localised variations in the leg colour proportions of *Pterostichus madidus*, using climatic and other environmental data and a more complex statistical analysis than the ones employed by Terrell-Nield (1990a) and Doberski & Gazzy (2000).

4.3 Method

The method for investigating the larger-scale spatial variations in morph frequencies (Aim i) is described in Section 4.3.1. The investigation of the small-scale variations in frequencies (Aim ii) is described in Section 4.3.2.

4.3.1 Statistical analysis of country-wide morph frequency variation: Independent variables

Simple and multiple regression analyses using Minitab version 12 were performed to ascertain whether the spatial variation in morph frequency correlated with geographical *and/or* soil factors *and/or* monthly *or* seasonal *or* annual climatic factors.

Multiple regression assumes a linear relationship between independent variables. Outliers – extreme values, which have little in common with the majority - can also have a strong influence on the regression equation. To avoid spurious results, the procedure for selection of variables, optimising and checking the validity of the multiple regression equations recommended by Iles (1993) was followed. This is summarised in Box C.1, Appendix C.

i) Independent variables for each site used in the analysis

Geographical parameters. These were:

northings; eastings; distance from the sea; altitude *or* adjusted altitude.

Northings and eastings were obtained from the OS map. The Euclidean distance of the site from the nearest sea or large harbour were estimated from the OS map as follows:

$$\text{Distance} = \sqrt{(\text{eastings of site} - \text{eastings of sea})^2 + (\text{northings of site} - \text{northings of sea})^2} \quad \text{eq 4.1}$$

Altitude (rounded to within 10m) was obtained from the OS map. The adjusted altitude, which takes into account the difference between the altitude of a site and that of the weather station, was calculated as follows:

$$\text{Altitude (adj)} = (\text{site altitude} - \text{weather station altitude}) + \text{constant} \qquad \text{eq. 4.2}$$

The constant is the greatest difference between the site and weather station altitudes so avoids negative values. This adjustment has the effect of increasing site altitude when the altitude of the weather station is low, and *vice versa.*

Climatic parameters. These were:

- mean maximum temperature and mean minimum temperature
  or mean average temperature (average of mean maximum and mean minimum)
  or mean range in temperature (difference between mean maximum and mean minimum);
- mean daily sunshine hours;
- total rainfall.

Annual, monthly and seasonal averages were calculated from the daily data. The method for calculating seasonal averages is given in Section 3.3.3. The meteorological data was obtained from BADC (British Atmospheric Data Centre), which supplies data from weather stations that comply with British Meteorological Office requirements (Meteorological Office, 1982).

Soil parameters. These were:

pH; % moisture retention; % organic content; texture.

Soil samples were collected from the East Anglian sites only. The topsoil to 10cm depth was taken from a number of points at the sampling site. The vegetation was removed and the soil mixed. The samples were stored in airtight polythene bags at 10°C until laboratory analysis.

The soil tests followed the procedures given in Carlile (1991):

pH: Air-dried soil was passed through a 2mm mesh sieve to remove small stones. 50ml of distilled water was added to 20ml of soil and the suspension shaken at frequent intervals for 15 minutes. The pH was then measured electrometrically using a Corning pH meter.

Moisture retention: Air dried soil was passed through a 2 mm sieve and dried at 80°C for at least 24 hours. The % moisture content was calculated as follows:

$$\frac{\text{initial weight of soil} - \text{weight of dried soil}}{\text{initial weight of soil}} \times 100 \qquad \text{eq.4.3}$$

Organic matter: This was determined by loss on ignition. Air-dried and sieved soil, which had been oven dried at 80°C for 24 hours, was ignited at 430°C using a muffle furnace. The % organic matter was calculated as follows:

$$\frac{\text{initial weight of oven dried soil} - \text{weight of soil after ignition}}{\text{initial weight of oven dried soil}} \times 100 \qquad \text{eq.4.4}$$

Soil texture: Sieved and air-dried soil samples were evaluated by hand texturing following the keys given in Carlile (1991) and Chalmers & Parker (1986). As shown in Table 4.1, the textures were given an arbitrary categorical rank, according to the relative proportions of sand, silt and clay, with the lowest ranks awarded to the coarser textured soils. Organic soils have been awarded intermediate ranks because they have the effect of reducing the overall particle size of sandy soils but increasing that for clay soils.

Table 4.1: Categorical scores given to texture of soil samples.

Mineral soils	**Rank**	**Organic soils**	**Rank**
Sand	1	sandy peat	2
loamy sand	2	organic loamy sand	2.5
sandy loam	3	organic sandy loam	3.5
sandy silt loam	4	organic sandy silt loam	4.5
silt loam	5	organic silt loam	5.5
Silt	6	organic silt	6.5
Loam	7	organic loam	7
sandy clay loam	8	organic sandy clay loam	7.5
silty clay loam	9	organic silty clay loam	8.5
clay loam	10	organic clay loam	9.5
sandy clay	11	organic sandy clay	10.5
silty clay	12	organic silty clay	11.5
Clay	13	organic clay	12.5

ii) Dependent variables used in the analysis

The dependent variables were the red-legged morph % frequency data representing different regions of the UK and two time periods (the mid-1970s and mid- to late 1990s). Sampling of sites followed the pitfall trapping method described in Section 2.4.1 (p.42).

As summarised in Table 4.2, an analysis was performed on each time period and region separately to ascertain whether the geographical and climatic predictors were consistent over time and space. For East Anglia only, the analysis also included soil factors.

There were insufficient weather stations operating in the mid- to late 1990s for a separate analysis of 1995/6 Transect 1 data. The reduction in the number of weather stations during the 1990s also meant that 1998 and - for some regions - 1997 climatic data could not be used. It was therefore assumed that spatial variation in climatic factors is fairly constant between closely adjacent years.

Table 4.2: **Summary of the multiple regression analysis of morph frequency data on environmental data.** (obs = observations).

Section	Year of monitoring	Region	Number of obs.	Period of climate data used in the analysis
4.4.1	1975	TRANSECT 1 (Nottingham to Southampton)	12	12 months preceding August 1975
4.4.1	1976	TRANSECT 2 (Dorset to East Sussex)	16	12 months preceding August 1976
4.4.2	1996; 1998	TRANSECT 2 (Dorset to East Sussex)	30	1995 (autumn only); 1996; 1997 (winter only)
4.4.3	1996-1998	TRANSECT 3 (Bethesda to Skegness)	24	1995 (autumn only); 1996
4.4.4	1998	EAST ANGLIA	12	1995 (autumn only); 1996; 1997

Tables A.1 to A.5 in Appendix A gives the location, grid reference, altitude and habitat type of each site in the regions sampled.

Tables 4.4 to 4.8 list the sites and weather stations used for each stage of the analysis, giving grid references, altitudes and, where appropriate, distance from the sea for both site and weather station. The red-legged morph frequencies for each observation are also given. The maps in Figs 4.1 and 4.2 show the position of the sites and weather stations for each region. To allow cross-referencing between Sections 4.3 and 4.4, these tables and figures have been placed at the end of Section 4.3 (pp. 120-125)

1975/6 data for Transect 1 and Transect 2 (Fig 4.1)

See Table A.2 in Appendix A for details of the 1975 sites along Transect 1 (Nottinghamshire to Southampton) and Table A.3.i for details of the 1976 sites along Transect 2 (West Dorset to East Sussex). These sites were sampled by C.Terrell-Nield of Nottingham Trent University.

Only sites within 27km of a weather station in a north-south direction (Transect 1) or east-west direction (Transect 2) were included in the analysis (see Tables 4.4 and 4.5). Fig 4.1 shows the location of sites and weather stations in relation to each other.

Along Transect 1, sometimes only one weather station was operating within the vicinity of more than one site. In these instances, the data from adjacent sites were pooled, providing they had broadly similar morph frequencies (i.e. observations 2, 6, 9 and 12 for Transect 1, see Table 4.4). A red-legged morph frequency was then calculated from the combined results and the geographical parameters of the sites were averaged.

In the case of observation 7 of Transect 1 (Sites 11 and 12), two weather stations were situated at equal distance from the sites in a north-east and south-west direction. A simple climatic gradient northwards and westwards was assumed and data from the two stations were averaged. Similarly, at two Transect 2 sites (Sites 6 and 10, observations 3 and 6 respectively, see Table 4.5), geographical and climatic data from two nearby weather stations were averaged.

The monthly, seasonal and annual weather data used in the analysis were taken from August 1974 to July 1975 for Transect 1 and August 1975 to July 1976 for Transect 2, i.e. the 12 months preceding the month of monitoring.

1995 to 1998 data for Transect 2, Transect 3 and East Anglia (Fig 4.2)

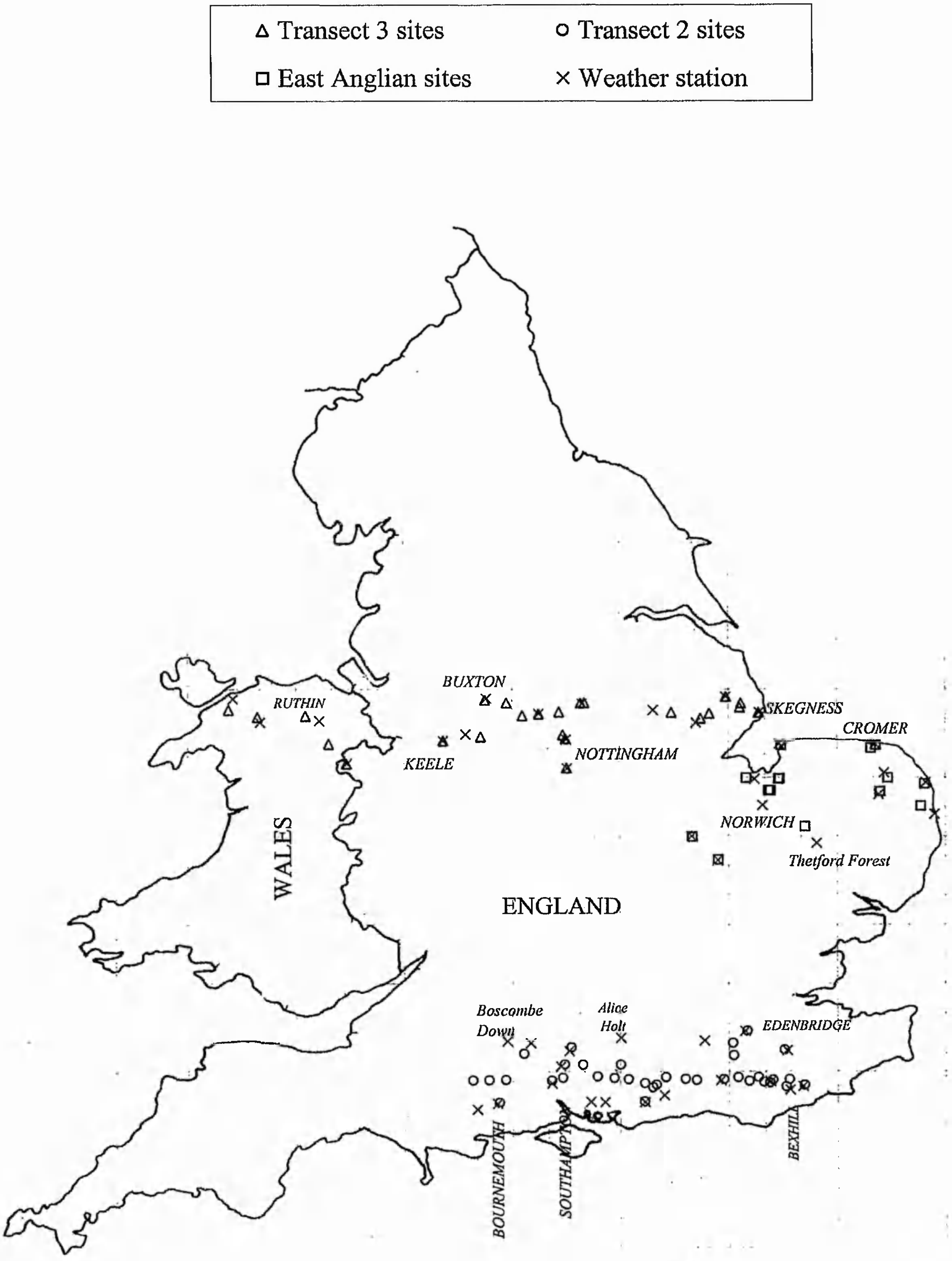
TRANSECT 2 (West Dorset to East Sussex): The 1976 transect was sampled again in 1996 and - in part – in 1998 (Table A.3.i). In 1998, new sites were established up to 35 km north and 15 km south of the transect line (see Table A.3.ii). The new sites either formed part of an altitudinal gradient or were situated within 5 km of an operating weather station.

Fig 4.1 Transects 1 and 2: Grid location of 1975/6 sampling sites and associated weather stations used in the regression analysis (see Tables 4.4 and 4.5).

Grids 20km x 20km

Fig 4.2 Transects 2, 3 and East Anglia: Grid location of 1996-1998 sampling sites and associated weather stations used in the regression analysis (see Tables 4.6-4.8).

Grids 20km x 20km



TRANSECT 3 (Bethesda to Skegness) - see Table A.4. A more northern east-west transect was established in 1996 from Bethesda (Gwynedd) to Skegness (Lincs), with 15 sites at approximately 20 km intervals. Because the higher altitude sites in Cheshire and Staffordshire and sites in the intensive agricultural areas of Lincolnshire failed to trap *P. madidus* in sufficient numbers for analysis, a broader belt of sampling sites was established in 1997 and 1998 (Table A.5). These were within 50 km south and 10 km north of the original transect line (i.e. there was an overall southward shift). Except for sites chosen because they formed part of an altitudinal gradient, the new sites were situated within 5 km of an operating weather station.

EAST ANGLIA was sampled in 1998 (Table A.5). This was because there were few records of red-legged morph frequencies in this eastern part of the country (Luff, 1998) and none had been included in the Terrell-Nield (1990a) analysis. A number of sites in Cambridgeshire, Norfolk and Suffolk, which lay within 5 km of an operating weather station, were chosen. Intermediate sites were also established to ascertain whether a morph frequency gradient occurred between weather stations. Soil samples were collected from each site.

P. madidus data collected over two or more years from sites along Transect 2 and 3 were pooled on the assumption that the temporal variation in morph proportions is small (see Section 3.4.1.iii, p. 72).

The sites and associated weather stations for the three regions are listed in Tables 4.6 to 4.8. Fig 4.2 shows their location in relation to each other. All sites were within 20km Euclidean distance of a weather station. If only one weather station was operating within a reasonable distance of two or more sites, data were pooled providing the morph proportions were not significantly different. The geographical parameters for the combined sites were calculated as follows:

$$\text{Parameter} = \frac{(\text{parameter} * \text{no. of red morphs for Site a}) + (\text{parameter} * \text{no. of red morphs for Site b})}{\text{total no. of red morphs for Sites a and b}} \qquad \text{(eq 4.4)}$$

If there were two meteorological stations within the vicinity of a site and within 5 km of each other, the data and other environmental factors of each station were weighted and used in the analysis as follows:

$$\text{Weighted value} = \frac{A}{(a+b)*b} + \frac{B}{(a+b)*a} \qquad \text{(eq 4.5)}$$

where A is data for met station 1
B is data for met station 2
a is distance of met station 1 from site
b is distance of met station 2 from site

iii) Statistical procedure

The overall procedure for finding associations between morph frequencies and the environmental variables was as follows (see also Box C1 in Appendix C):

- Simple regressions were performed to find the strongest single predictors among the geographical, climatic and soil factors.
- Multiple regressions were performed to find interactions between geographical *and/or* climatic *and/or* soil factors.

4.3.2 Statistical analysis of morph frequency variation in the East Midlands

i) Collection of morph frequency data in the East Midlands (dependent variables)

45 sites within a 25 x 25 km grid were established in the East Midlands Trent valley region, (Nottinghamshire, north Leicestershire and south-east Derbyshire). This region covers the intensively farmed Leicestershire wolds, which lie south of the River Trent, the River Trent plain, the city of Nottingham, which lies south and north of the river, and the ex-coal mining area north of Nottingham. Table A.7 in Appendix A lists the sites and gives their grid reference and habitat type.

The pitfall trapping method described in Section 2.4.1 was followed. Traps were laid at the beginning of August 1998. Samples were collected 2 weeks later (mid-August). A second and final collection was made at the end of August.

ii) Environmental parameters (independent variables)

Given the presence of only 3 meteorological stations in the region, it was not possible to use climatic data in the analysis. Therefore parameters which might be linked to microclimate (see Section 4.2) were obtained from geographical, topographical and soil measurements.

- The geographical parameters for each site were: position north, position east.
- The topographical parameters were: altitude, distance to the River Trent, aspect, % woodland within 1.5 km radius of the site, % urban area within 1.5 km radius of the site, %water body within 1.5km radius of the site.
- The soil parameters were: pH, % moisture retention, % organic content, texture.

Positions north and east were obtained from the site's eastings and northings on the OS map and were estimated to the nearest 0.1 km.

Altitude was estimated from the contours of the OS map to the nearest 10m.

Distance to the River Trent: This measurement attempted to identify sites in the river plain and was estimated to the nearest 0.5 km from the OS map.

Aspect was estimated from the contours of the OS map and ranked as shown in Table 4.3. The highest rank was given to a north-facing site, which was assumed to be the most exposed to cold wind; the smallest rank was given to a south-facing site, where temperatures are assumed to be warmer and milder.

Table 4.3: Categorical ranks given to the aspect of a site

Aspect	North	North East	North West	East	FLAT	South East	West	South West	South
Rank	9	8	7	6	5	4	3	2	1

% Woodland: Woodland was identified as green shading on the OS map. It is assumed that extensive woodland would produce a milder climate locally, i.e. lower maximum and higher minimum temperatures. The percentage woodland within 1.5km radius of a site was estimated from the OS map, using a circular transparent template (3cm diameter). The template was divided into quadrants to help quantify the proportion of woodland. The centre point of the template was placed on the grid point of the site. The percentage of green shading was estimated by eye, using the quadrants on the transparency as a guide.

% Urban: Urban areas were identified as brown shading on the OS map. It is assumed that extensive urbanisation close to the site would influence the local climate in a similar way to woodland. In addition, the heating of buildings may increase the overall mean temperature. The percentage urban area within 1.5 km radius of the site was estimated by the same method that was used for woodland.

% Water: It is assumed that extensive water bodies (i.e. lakes, reservoirs, rivers) would have a cooling effect on local climate. Identified by blue shading on the OS map, the percentage water body within 1.5km radius of the site was estimated by the same method used for woodland and urban areas.

Soil parameters: Soil samples were collected from the East Midlands sites and the pH, % organic content, % moisture retention and texture of the soil were measured in the laboratory. The collection, storage and analysis of the soil followed the method given in Section 4.3.1.

iii) Statistical procedure

Following the same procedure described in Section 4.3.1.iii and Box C1 in Appendix C, simple and multiple regressions were performed to find associations between morph frequency and geographical *and/or* topographical *and/or* soil parameters.

Table 4.4 **TRANSECT 1: 1975 sites and associated weather stations used in the regression analysis, showing eastings, northings, altitude and red-legged morph frequencies.**

Obs no	Site no	Trapping site	%R	East-ings	North-ings	Altitude (m)	Weather station	East-ings	North-ings	Altitude (m)	Distance from site (km)
1	1	West End	58	448.0	115.7	30	Southampton, Mayflower Park	441.6	111.2	3	7.8
2	2	Twyford/	4	449.1	124.9	37	Martyr Worthy	451.7	133.8	84	9.3
	4	Tidbury Ring		450.1	142.7	100					9.0
		mean		*449.6*	*133.8*	*68.5*	*mean*				*9.1*
3	6	Greenham	59	447.9	165.2	90	Shinfield	473.0	167.3	61	25.2
4	7	World's End	50	448.5	175.9	160	Reading	473.9	171.9	66	25.7
5	8	Ridgeway	0	448.3	184.4	130	Letcombe Regis	438.0	186.3	107	10.5
6	9	Frilford/	0	447.8	197.0	55	Abingdon	446.8	199.0	69	2.2
	10	Sandleigh		446.1	201.9	95					3.0
		mean		*447.0*	*199.5*	*75.0*	*mean*				*2.6*
7	11	Begbroke/	14	447.5	214.8	76	Brize Norton/	429.2	206.7	81	22.6*
	12	Steeple Aston		446.3	225.1	130	Grendon Underwood	468.2	221.6	67	22.0*
		mean		*446.9*	*220.0*	*103.0*	*mean*	*448.7*	*214.2*	*74.0*	*22.3*
8	13	Adderbury	13	447.2	236.9	110	Shipston-on-Sour	421.3	240.7	111	26.2
9	15	Priors Hardwick/	0	446.3	253.6	152	Moreton Morrell	430.6	255.3	85	15.8
	16	Stockton		444.5	264.3	90					16.6
		mean		*445.4*	*259.0*	*121.0*	*mean*				*16.2*
10	20	Barlestone	29	444.5	306.6	140	Newton Linford	453.0	309.5	119	9.0
11	22	Tonge	23	442.0	321.6	100	Sutton Bonington	450.7	325.9	48	9.7
12	24	Shipley	24	443.5	344.5	100	Watnall	450.3	345.6	117	6.9
	25	Somercotes		444.2	352.9	80					9.5
		mean		*443.9*	*348.7*	*90.0*	*mean*				*8.2*

* average distance for both sites

Table 4.5 **TRANSECT 2: 1976 sites and associated weather stations used in the regression analysis, showing eastings, northings, altitude, distance from sea and red-legged morph frequencies.**

Obs no	Site no	Trapping site	%R	East-ings	North-ings	Altitude (m)	Distance from sea (km)	Weather station	East-ings	North-ings	Altitude (m)	Distance from site (km)
1	2	Stourton Caundle	52	367.9	115.7	110	35	Yeovilton	355.1	123.7	18	15.1
2	4	Sutton Waldron	0	386.4	115.3	70	35	Poole	400.6	93.7	5	25.8
3	6	Cranbourne	0	407.1	114.2	95	24	Bournemouth, Hurn	411.7	97.8	10	17.0
								Bournemouth, Kings Park	412.4	92.7	40	22.1
								mean	*412.1*	*95.3*	*25*	*19.6*
4	7	Fordingbridge	93	416.3	114.3	45	23	Boscombe Down	417.2	140.3	49	26.0
								Christchurch	415.4	93.8	3	20.5
								mean	*412.1*	*117.1*	*26*	*23.3*
5	9	Nursling	94	435.8	116.3	7	21	Everton	430.2	94.7	16	22.3
6	10	West End	57	448.8	115.8	30	9	Southampton, Mayflower Park	441.8	111.4	3	8.3
								Southampton, Weather Centre	442.0	111.5	19	8.0
								mean	*441.9*	*111.5*	*11*	*8.1*
7	11	Shirral Heath	40	457.7	114.8	51	16	Martyr Worthy	451.7	133.8	84	19.9
8	12	Clanfield	17	467.4	116.6	110	16	Hayling Island	471.6	98.8	4	18.3
9	14	Colworth Down	9	484.7	114.7	92	18	Rogate	480.9	123.7	64	9.8
10	15	Duncton Down	25	496.0	116.2	76	17	Bognor Regis	493.4	98.9	7	17.5
11	16	Wiggonholt Common	56	505.5	116.1	20	15	Rustington	506.9	122.7	8	6.7
12	17	Guesses Farm	17	516.3	114.6	30	12	Worthing	516.0	103.5	2	11.1
13	18	Shave Wood	38	522.4	114.1	25	11	Gatwick	526.5	140.7	59	26.9
14	19	Brocks wood	12	537.3	114.7	54	11	Plumpton	535.7	113.6	76	1.9
15	20	Isfield	24	545.2	115.9	11	16	East Hoathly	550.9	114.4	40	5.9
16	22b	Bodle St. Green	31	564.3	114.2	30	10	Bexhill	573.7	107.2	4	11.7

Table 4.6 **1996-8 sites along or close to TRANSECT 2 and associated weather stations used in the regression analysis, showing eastings, northings, altitude, distance from sea and red-legged morph frequencies.** (Weighting for weather stations is calculated from equation 4.4).

Obs no	Site no	Trapping site	%R	East-ings	North-ings	Altitude (m)	Distance from sea (km)	Weather station	East-ings	North-ings	Altitude (m)	Distance from site (km)
1	5	Cashmoor	9	398.1	113.9	80	38	Poole	400.5	93.8	5	20.2
2	6	Cranbourne	16	407.1	114.2	95	24	Poole	400.5	93.8	5	21.4
								Bournemouth (Hurn)	411.7	97.8	10	17.0
								Poole/Bournemouth weighting	*406.7*	*96.0*	*7.8*	
3	26	Hurn (nr. Bournemouth)	78	412.8	98.7	15	7.4	Bournemouth (Hurn)	411.7	97.8	10	1.4
4	7	Fordingbridge	94	416.3	114.3	45	23	Bournemouth (Hurn)	411.7	97.8	10	17.1
								Boscombe Down	417.2	140.3	126	26.0
								Hurn/Boscombe weighting	*413.9*	*114.7*	*56.1*	
5	27	East Grimstead	80	419.3	128.2	80	34	Boscombe Down	417.2	140.3	126	12.3
6	28	The Common (Stockbridge)	58	426.5	132.2	82	39	Middle Wallop	430.1	139	90	7.7
7	30	Southampton Common	21	441.9	113.7	30	14	Southampton (WC)	442.0	111.5	19	2.2
8	10	West End (nr Southampton)	52	448.0	115.8	30	9	Southampton (WC)	442.0	111.5	19	7.4
								Otterbourne	446.8	123.3	34	7.6
								South'ton/Otterbourne weighting	*444.4*	*117.3*	*26.4*	
9	2(T1)	Twyford	21	449.1	124.8	40	18	Otterbourne	446.8	123.3	34	2.7
10	31	Michelover Wood	21	452.9	136.4	100	33	Martyr Worthy	451.7	133.8	90	2.9
11	32	Soberton Heath	52	459.3	124.7	60	15	Otterbourne	446.8	123.3	34	12.6
								Martyr Worthy	451.7	133.8	90	11.9
								Otterbourne/Martyr W. weighting	*449.3*	*128.7*	*62.8*	
12	12	Clanfield	9	467.4	116.6	110	16	Southsea	463.7	99.1	2	17.9
								Hayling Island	471.6	98.8	4	18.3
								Southsea/Hayling Is. weighting	*467.6*	*99.0*	*3.0*	
13	13	Compton	26	476.8	115.6	98	17	Hayling Island	471.6	98.8	4	17.6
14	33	Rogate	37	480.4	124.7	100	29	Alice Holt	480.5	142.7	115	18.0
15	34	Bognor Regis	7	493.7	99.5	2	0.6	Bognor Regis	493.8	98.8	7	0.7
16	14	Colworth Down	33	484.7	114.7	92	18	Bognor Regis	493.8	98.8	7	13.7
	35	Selhurst Park	30	493.8	111.9	160	13	Bognor Regis				12.7
	37	Fairmile Bottom	28	498.3	109.1	40	9.1	Bognor Regis				19.4
		weighting	*32*	*493.5*	*111.6*	*118.4*	*12.8*	*Bognor Regis*	*493.8*	*98.8*	*7*	

Table 4.6 (continued)

Obs no	Site no	Trapping site	%R	East-ings	North-ings	Altitude (m)	Distance from sea (km)	Weather station	East-ings	North-ings	Altitude (m)	Distance from site (km)
17	38	Houghton Forest	16	500.2	110.9	100	10	Bognor Regis	493.8	98.8	7	13.7
18	16	Wiggonholt Common	61	505.5	116.1	20	15	Rustington	504.5	103.4	3	12.7
19	17	Guesses Farm	34	516.3	114.9	40	12	Plumpton	535.7	113.6	76	19.4
								Rustington	504.5	103.4	7	16.5
								Plumpton/Rustington weighting	*518.8*	*108.1*	*38.7*	
20	18	Shave Wood/Duffield	43	522.4	114.1	25	11	Plumpton	535.7	113.6	76	13.3
21	19	Chiltington	19	537.3	114.5	50	12	Plumpton	535.7	113.6	76	1.8
22	42	Nr Edenbridge	66	550.3	147.5	45	50	Edenbridge	549.2	147.2	45	1.1
23	40	Hammer Wood	50	542.5	139.3	110	39	Edenbridge	549.2	147.2	45	10.4
								Gatwick	526.5	140.7	59	16.1
								Edenbridge/Gatwick weighting	*540.3*	*144.7*	*50.5*	
24	41	Wych Cross	59	542.8	130.9	190	31	Edenbridge	549.2	147.2	45	17.5
								Plumpton	535.7	113.6	76	18.7
								Edenbridge/Plumpton weighting	*542.7*	*131.0*	*60.0*	
25	20	Isfield/Nr Isfield	42	545.2	115.9	10	16	Plumpton	535.7	113.6	76	9.8
26	21a	East Hoathly	43	551.4	113.4	30	16	Plumpton	535.7	113.6	76	15.7
								Herstmonceux, West End	563.0	112.7	52	11.6
								Plumpton/Herst weighting	*551.4*	*113.1*	*62.2*	
27	21b	Hale Green	44	556.4	115.9	70	15	Herstmonceux, West End	563.0	112.7	52	7.3
	21c	Hellingly	38	559.4	112.7	40	11	Herstmonceux, West End	563.0	112.7	52	3.6
	22a	Herstmonceux	34	562.7	112.2	26	9	Herstmonceux, West End	563.0	112.7	52	0.6
	22b	Bodle St Green	63	564.3	114.2	30	10	Herstmonceux, West End	563.0	112.7	52	2.0
		weighting	*43*	*558.9*	*114.3*	*51.5*	*12.5*	*Herstmonceux, West End*	*563.0*	*112.7*	*52.0*	
28	43	Bedgebury Cross	36	570.8	134.7	56	27	Goudhurst	572.3	133.9	85	1.7
29	44	Nr Bexhill	36	571.6	109.4	40	2.9	Bexhill	573.7	107.2	4	3.0
30	23	Nr Battle	40	573.5	114.5	50	7.5	Bexhill	573.7	107.2	4	7.3
								Hastings	580.9	109.4	45	9.0
								Herstmonceux, West End	563.0	112.7	52	10.7
								Bexhill/Hastings/Herst weighting	*573.9*	*109.1*	*27.6*	
31	45	Alexandra Park (Hastings)	18	581.8	110.5	50	1.7	Hastings	580.9	109.4	45	1.4

Table 4.7 **TRANSECT 3: 1996-8 sites and associated weather stations used in the regression analysis, showing eastings, northings, altitude, distance from sea and red-legged morph frequencies.** (Weighting for weather stations is calculated from equation 4.4).

Obs no	Site no	Trapping site	%R	East-ings	North-ings	Altitude (m)	Distance from sea (km)	Weather station	East-ings	North-ings	Altitude (m)	Distance from site (km)
1	1	Bethesda	86	262.7	365.8	20	7	Aber	265.6	373.1	15	7.9
2	2	Llanwryst	59	278.9	360.9	30	18	Betws-y-coed	280.3	357.1	20	4.0
3	3	Pant past y nog	52	305.5	361.6	76	22	Ruthin	313.3	358.3	82	8.5
4	4	Llangollen	62	318.4	342.7	180	35	Ruthin	313.3	358.3	82	16.4
				318.4	342.7			Oswestry	328.5	329.3	139	16.8
								Ruthin/Oswestry weighting	*320.8*	*344.0*	*110.2*	
5	6	Oswestry	58	328.2	329.4	144	49	Oswestry	328.5	329.3	139	0.3
6	10	Keele University	71	381.8	344.9	180	70	Keele University	381.9	344.6	179	0.3
7	11	Froghall	63	402.7	347.8	150	88	Cellarhead	394.4	349.2	228	8.4
8	13	Buxton	57	405.1	372.5	300	85	Buxton	405.8	373.4	307	1.1
9	14	Taddington Vale	38	417.0	370.5	170	97	Buxton	405.8	373.4	307	11.6
								Ashover	434.9	362.9	178	19.4
								Buxton/Ashover weighting	*416.7*	*369.5*	*258.8*	
10	15	Birchover	20	425.8	361.8	150	107	Ashover	434.9	362.9	178	9.2
	16	Ashover	25	434.9	362.9	178	116		434.9	362.9	178	0.0
		mean	*23*	*430.4*	*362.4*	*164.0*	*111.5*					
11	17	Doe Lea	28	446.3	364.3	160	111	Ashover	434.9	362.9	178	11.5
								Warsop	459.1	369.9	46	14.0
								Ashover/Warsop weighting	*445.8*	*366.1*	*118.5*	
12	18	Watnall, lake	32	448.3	348.7	90	95	Nottingham (Watnall)	450.3	345.6	117	3.7
13	19	Watnall, copse	64	449.8	346.3	120	93	Nottingham (Watnall)	450.3	345.6	117	0.9
15	20	Sutton Bonington	29	450.4	326.5	43	98	Sutton Bonington	450.7	325.9	48	0.7
16	21	Warsop, Medon Vale	52	458.3	370.3	60	99	Warsop	459.1	369.9	46	0.9
17	22	Warsop, Budby	41	460.4	370.5	45	97	Warsop	459.1	369.9	46	1.4
18	25	Dunston Wood	53	508.9	363.9	70	48	Waddington	498.8	365.3	68	10.2
19	26	Mareham le Fen	69	525.0	359.9	10	32	Coningsby	522.4	356.8	6	4.0
20	27	Revesby Abbey	48	529.7	363.3	50	28	Coningsby	522.4	356.8	6	9.8
								Driby	538.7	374.5	41	14.4
								Coningsby/Driby weighting	*529.0*	*364.0*	*20.2*	
21	28	Driby	22	538.8	374.7	35	17	Driby	538.7	374.5	41	0.2
22	30	Candlesby	58	546.4	367.4	20	11	Driby	538.7	374.5	41	10.5
								Skegness	557.0	363.5	6	11.3
								Driby/Skegness weighting	*547.5*	*369.2*	*24.2*	
23	31	Welton Low Wood	36	547.1	370.2	13	10	Driby	538.7	374.5	41	9.4
								Skegness	557.0	363.5	6	12.0
								Driby/Skegness weighting	*546.8*	*369.7*	*25.6*	
24	32	Skegness	55	556.7	363.7	4	1	Skegness	557.0	363.5	6	0.4

Table 4.8 **EAST ANGLIA: 1998 sites and associated weather stations used in the regression analysis, showing eastings, northings, altitude, distance from sea and red-legged morph frequencies.** (Frequencies in brackets: total P. madidus < 20)

Weighting for frequencies calculated from proportion of *P. madidus* per site; weighting for weather stations is calculated from equation 4.4.

Obs no	Site no	Trapping site	%R	East-ings	North-ings	Altitude (m)	Distance from sea (km)	Weather station	East-ings	North-ings	Altitude (m)	Distance from site (km)
1	1	Terrington St Clement	19	550.0	319.7	3	7	Terrington St Clement	554.5	318.7	15	4.6
2	2	Watlington village	(90)	562.2	311.3	7	16	Terrington St Clement	554.5	318.7	15	10.7
	3	Watlington (lake)	86	563.3	311.2	9	16	Denver	559.0	300.9	3	11.2
		Weighted	*87*	*563.1*	*311.2*	*8.5*	*16.0*	*Terrington St-C/Denver weighting*	*556.7*	*310.0*	*9.1*	
3	4	Leziate (wood)	*94*	567.6	319.0	30	11	Terrington St Clement	554.5	318.7	15	13.1
	5	Leziate (copse)	95	568.2	319.3	20	11	Hunstanton	567.9	342.3	3	23.0
		Weighted	*95*	*568.0*	*319.2*	*22.9*	*11.0*	*Terrington St-C/Hunst weighting*	*559.4*	*327.3*	*10.6*	
	7	Old Hunstanton	79	569.1	341.9	10	1	Hunstanton	567.9	342.3	3	1.3
5	9	Cromer	(80)	621.6	341.8	30	0.7	Cromer	621.2	342.4	37	0.7
	10	Felbrigg Hall	73	618.9	339.8	60	3.4		621.2	342.4	37	3.5
		Weighted	*74*	*619.5*	*340.2*	*53.3*	*2.8*					
6	13	Coltishall	59	628.3	319.8	10	15	Coltishall	626.2	322.9	17	3.7
7	15	Norwich A	*76*	624.1	310.3	45	25	Norwich	623.3	308.2	35	2.3
	16	Norwich B	66	623.8	310.4	50	25					
		Weighted	*68*	*623.9*	*310.4*	*48.8*	*25.0*					
8	18	Ormesby St Margaret	55	648.7	315.7	8	3.1	Hemsby	649.3	316.2	5	0.8
9	19	Fritton (wood)	73	646.7	300.5	15	5.5	Lowestoft	654.3	294.6	25	9.6
10	24	Thetford Forest	92	582.3	286.6	51	58	Honington (from Aug 1997)	588.8	275.0	30	13.3
11	25	Boxworth	(25)	534.5	264.1	47	65	Boxworth	534.4	263.3	53	0.8
12	26	Monks Wood	(18)	520.3	279.6	43	56	Monks Wood	520.1	279.6	41	0.2

4. 4 Results

4.4.1 Analysis of 1975/6 data for Transects 1 and 2

i) Morph frequency variation along the transects

1975 and 1976 red-legged frequencies for Transects 1 and 2 respectively have been described in Section 3.4.1 (see also Tables B.1.i and B.2.i in Appendix B, Figs 3.5 and 3.7 in Section 3.4.1 – pp 73 and 77). In general, a higher red-legged frequency was associated with maritime and urban sites and a higher black-legged frequency with more exposed sites, i.e. higher altitude and/or sparsely wooded, intensively farmed regions.

ii) Regression analysis

The correlations producing the highest F-ratios for the number of variables included in the regressions are shown in Table 4.9 and represented graphically in Fig 4.3 (Transect 1) and Fig 4.4 (Transect 2).

Transect 1: The mean minimum temperature was the most influential variable in all the regressions, correlating positively with the red-legged morph frequency. As a single predictor, the annual minimum temperature explained about 50% of the variability along the transect but failed to predict the steep clines in morph frequency (see Fig 4.3.i). Using monthly mean minimum temperature data, the highest correlations were obtained from January through to June, but there was little improvement on the annual data (e.g. Prediction 2 in Table 4.9.i).

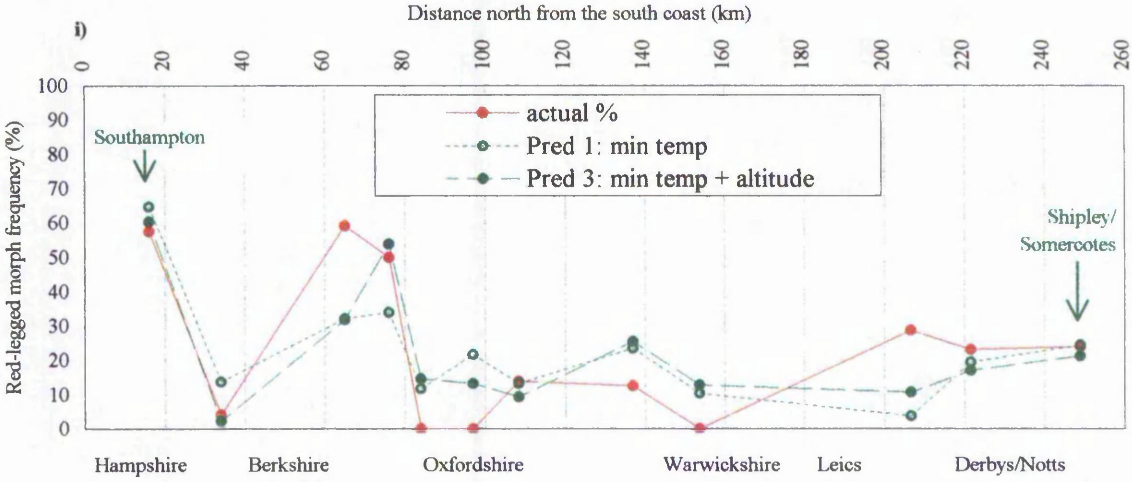
Use of two interacting variables, annual mean minimum temperature and site altitude, explained about two-thirds of the variability (Prediction 3, Fig 4.3.i). There was no further improvement using annual climatic data. However, highly significant correlations were obtained from multiple regressions on monthly or seasonal data. Two monthly climatic factors for March (mean minimum temperature and mean total rainfall) gave reasonable predictions of the clines in Hampshire and Berkshire (Prediction 4, Fig 4.3.ii).

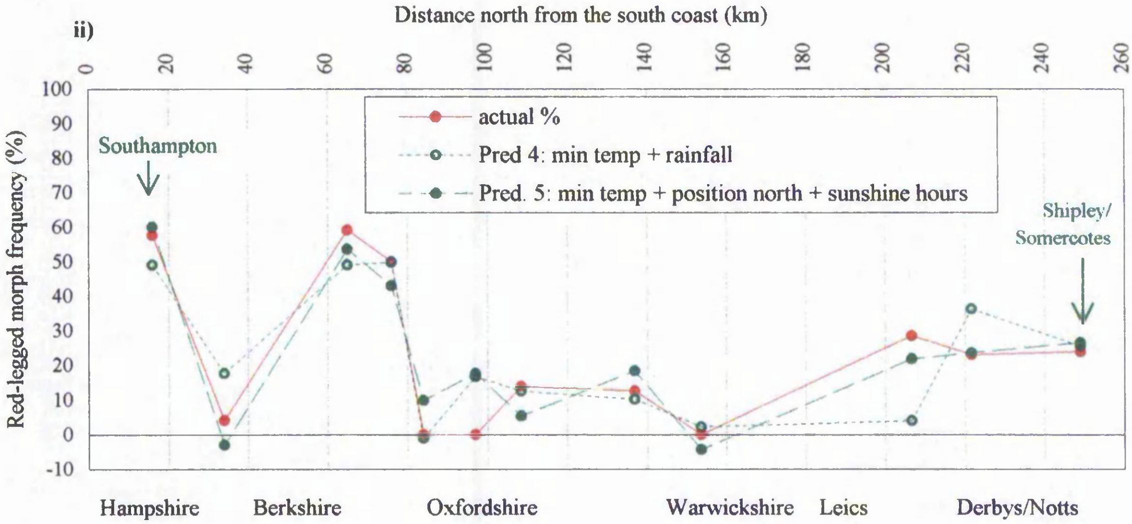
Table 4.9 **The most influential predictors of red-legged morph frequency for 1975/6 along i) Transect 1 and ii) Transect 2. (Partial predictors for the multiple regressions are listed in order of decreasing significance; in italics, P > 0.05).**

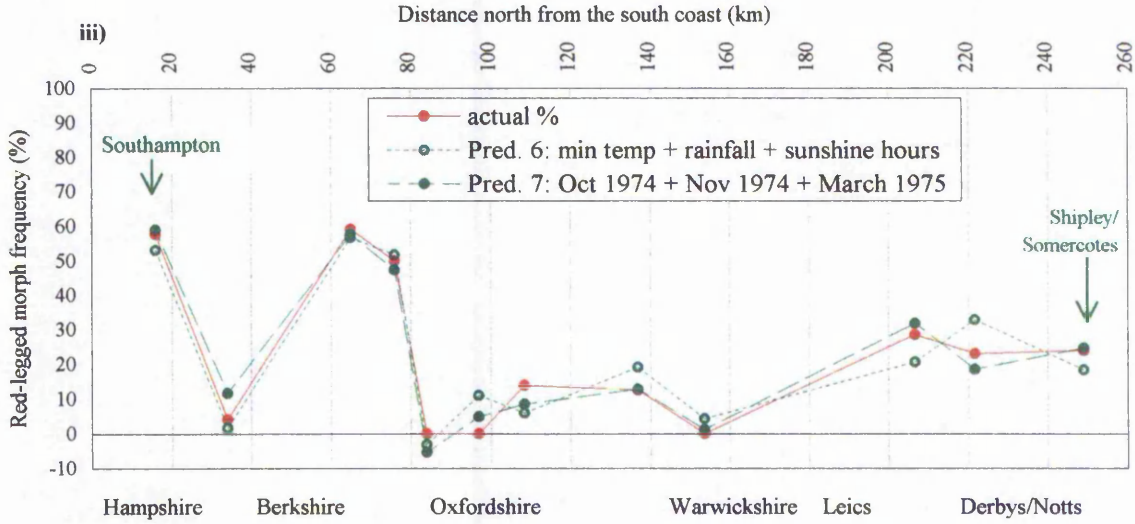
d.f. = degrees of freedom; min temp = mean minimum temperature; max temp = mean maximum temperature; rainfall = total rainfall; sunshine hours = total daily sunshine hours.

Prediction (see Figs 4.3, 4.4)	Time period	Variables	P (partial predictor)	Association (+/-)	F-ratio	P (regression equation)	r^2 (r^2-adj) %
i) TRANSECT 1: 1975 MORPH FREQUENCY DATA (d.f. = 12)							
1	Annual, (Aug 74 - Jul 75)	min temp	0.008	+	10.75	0.008	52 (47)
2	May, 1975	min temp	0.006	+	12.02	0.007	55 (50)
3	Annual, (Aug 74 - Jul 75)	min temp	0.003	+	8.40	0.009	65 (57)
		altitude	*0.097*	+			
4	March, 1975	min temp	0.003	+	12.45	0.003	73 (68)
		rainfall	0.031	+			
5	May, 1975	min temp	<0.001	+	17.24	0.001	87 (82)
		position north	0.003	+			
		sunshine hours	0.015	-			
6	Spring, 1975	min temp	<0.001	+	26.93	<0.001	91 (88)
		rainfall	0.006	-			
		sunshine hours	0.029	-			
7	Oct, 1974	Oct rainfall	<0.001	+	76.66	<0.001	97 (95)
	Nov, 1974	Nov sunshine hours	<0.001	-			
	March, 1975	March min temp	<0.001	+			
ii) TRANSECT 2: 1976 MORPH FREQUENCY DATA (d.f. = 15)							
1	Geographical	altitude	0.008	-	5.41	0.020	45 (37)
		position east	0.031	-			
2	Annual, (Aug 75 - Jul 76)	altitude	0.002	-	5.09	0.017	67 (54)
		sunshine hours	0.026	-			
		temp range	0.043	-			
		position east	*0.056*	-			
3	Annual, (Aug 75 - Jul 76)	altitude (adj)	0.001	-	5.59	0.013	69 (57)
		position east	0.017	-			
		rainfall	0.044	-			
		min temp	*0.057*	+			
4	April, 1976	altitude (adj)	<0.001	-	21.23	<0.001	89 (84)
		sunshine hours	<0.001	-			
		rainfall	<0.001	-			
		distance to sea	0.029	-			

Fig 4.3 TRANSECT 1(south to north): Predictions of red-legged morph frequency from regressions on 1975 frequencies using environmental data as predictors (see Table 4.9). Pred = prediction.







Inclusion of three variables (position north, mean minimum temperature and mean daily sunshine hours for May) modelled the clines in the north Midlands successfully, but not those in Oxfordshire (Prediction 5, Fig 4.3.ii). These were predicted using spring or interacting monthly data for three climatic factors: minimum temperature, total rainfall and daily sunshine hours (Predictions 6 and 7, Fig 4.3.iii). As shown in Table 4.9.i, excellent correlations were obtained ($r^2 > 90\%$; $P < 0.001$).

Transect 2: Significant correlations were not obtained when only one variable was used. However, nearly half the variability in morph frequency was explained by two interacting geographical factors - site altitude and the site's position eastwards along the transect (Prediction 1- see Table 4.9.ii). As shown in Fig 4.4.i, these modelled the steep cline from Southampton into East Sussex reasonably well. Inclusion of annual climatic data for sunshine and the temperature range (Prediction 2) or rainfall and the minimum temperature (Prediction 3) modelled Dorset *or* East Sussex, respectively (Fig 4.4.ii).

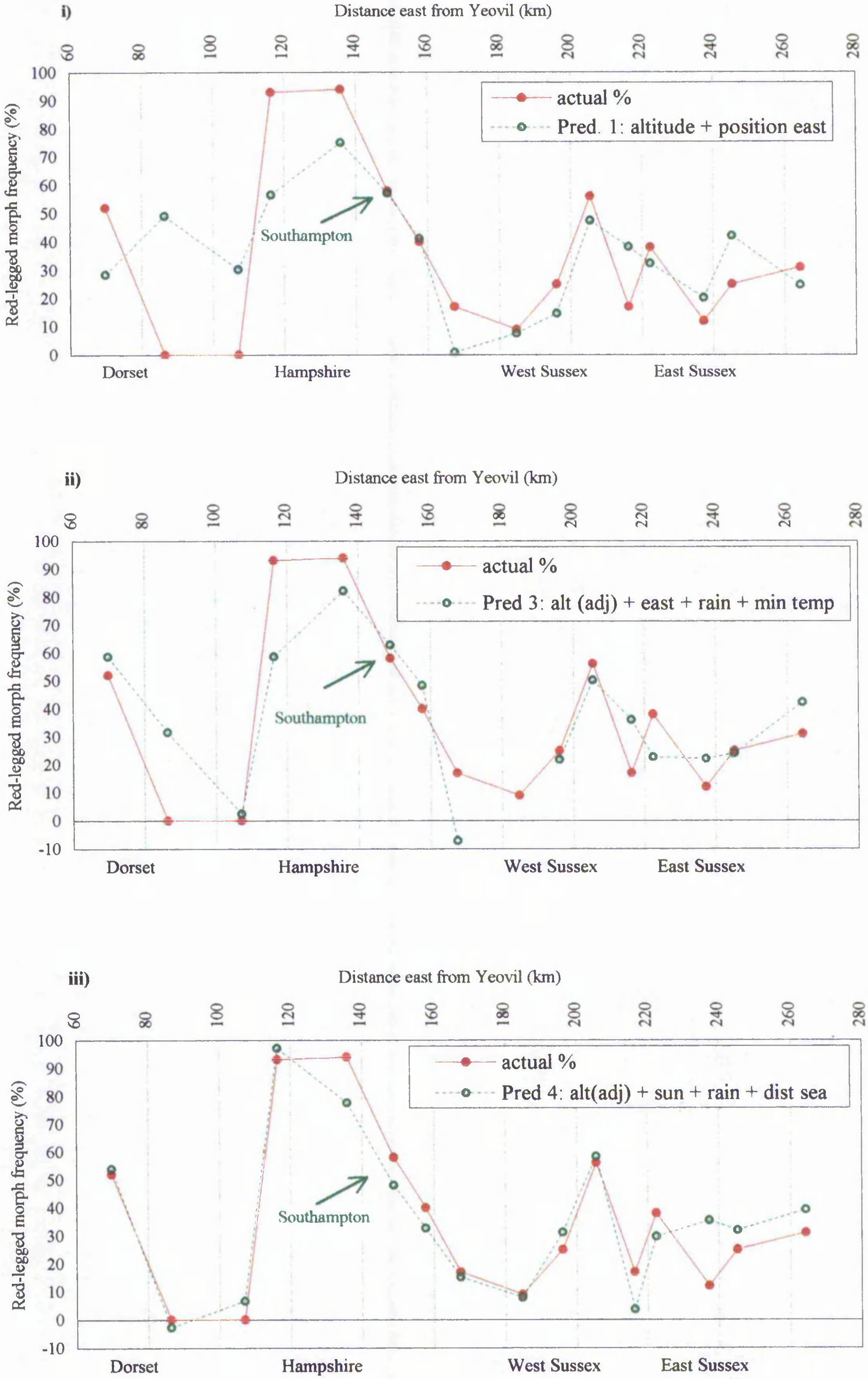
With the exception of April, use of monthly and seasonal climatic data did not improve these correlations. For April, a highly significant result ($r^2 = 89\%$) was obtained using sunshine and rainfall data (Prediction 4). Apart from one site near Plumpton in East Sussex (Site 19 at Brock's Wood), the complex variability in red-legged morph frequency along the transect was predicted remarkably well (see Fig 4.4.iii).

iii) Identification of environmental predictors of morph frequency along Transects 1 and 2

The spatial variability in the 1975/6 red-legged morph frequency along Transects 1 and 2 has been explained well by environmental factors. The mean minimum temperature was found to be the most influential single predictor along the north-south transect and became an important partial predictor for the south coast when site altitude was corrected for the weather station. The correlation was consistently positive, i.e. the red-legged frequency decreased with decreasing minimum temperatures.

The mean maximum temperature was an influential partial predictor along the south coast transect, associating negatively with red-legged morph frequency. The temperature range showed a negative association with Transect 1 red-legged frequency data, and could be an influential partial predictor for Transect 2, again showing negative association. The mean temperature (the average of the mean maximum and mean minimum) was rarely influential for either Transect.

Fig 4.4 TRANSECT 2 (west to east): Predictions of red-legged morph frequency from regressions on 1976 frequencies using environmental data as predictors (see Table 4.9). Pred = prediction; alt = altitude; east = position east; rain = rainfall; sun = sunshine hours.



The interaction of other climatic and geographical factors in the regressions can be explained in terms of their microclimatic effect on temperature. As a partial predictor, mean daily sunshine hours correlated negatively with the red-legged morph frequency. A large fluctuation in diurnal temperature can occur when sunny days are followed by clear nights, particularly during the rapid temperature changes of spring and autumn. These conditions are predicted to be less favourable for the red-legged morph.

Total rainfall could interact positively or negatively. Because the heat exchange of wet soils is slower than drier soils, rainfall retards the cooling of soil in autumn and the warming of soil in spring, and this could have different effects on the two morphs over the seasons. Notably, October rainfall showed a positive interaction for Transect 1.

There was a negative association between altitude and red-legged frequencies along Transect 2 but a weak positive association for Transect 1. However, the gradual rise of land with distance northwards along Transect 1 did not necessarily produce exposed conditions, many sites being located in river valleys, where temperatures are expected to be milder on average.

For Transect 2, the position east and distance from the sea both correlated negatively with the red-legged frequency. These variables may be indicators of continental-maritime climatic effects, a more continental climate occurring eastwards and inland from the south coast. The positive correlation of the red-legged morph with distance northwards along Transect 1 is less easy to explain. Although the British climate becomes increasingly maritime with latitude, this effect is less likely to be significant in the English Midlands.

Finally, the regressions identified periods during the year when climatic factors are most likely to have an influence. Excellent correlations were obtained for Transect 1 during early and late spring when the monthly mean minimum temperature can be low, but air temperatures are rising rapidly. A similarly high correlation was found for Transect 2 using meteorological data for April. Along this transect, a high maximum temperature and increased daily sunshine hours appear to be limiting factors for the red-legged morph.

The months of March to May represent critical developmental stages in the beetle, i.e. the late stages of Instar 3, pupation and emergence, as well as survival and emergence of the over-wintered adult female (Table 1.1, p. 6) and may be the period when selection between the morphs exerts its main pressure.

4.4.2 Analysis of 1996 to 1998 data for Transect 2 (Dorset to East Sussex)

Table B.2 in Appendix B includes the number of *P. madidus* morphs sampled at each site from 1996 to 1998 along the broad east/west band extending from Dorset to East Sussex. Table B.2.i shows results for the original transect. Table B.2.iii shows results for the new sites trapped in 1998. From chi-squared analysis, there were no significant differences in the 1996/7/8 morph proportions at sites sampled for two or more years. The annual data were therefore pooled for the analysis, giving the morph frequencies in Table 4.6 and Fig 4.5.

i) Morph frequency variation

Fig 4.5 shows a high variability in morph proportions between the western sites. Red-legged frequencies were low (9 and 16%) at the two Cranbourne Chase sites, and high (78 to 94%) at sites close to the River Arun and the New Forest (Fordingbridge, East Grimstead and Hurn - see Table 4.6). The red-legged frequency of sites located in and north of Southampton ranged from 21 to 52%. Sites on or close to the South Downs west of the River Arun generally have lower red-legged proportions than sites east of the river. These more eastern sites are located in the broad valley north of the Downs.

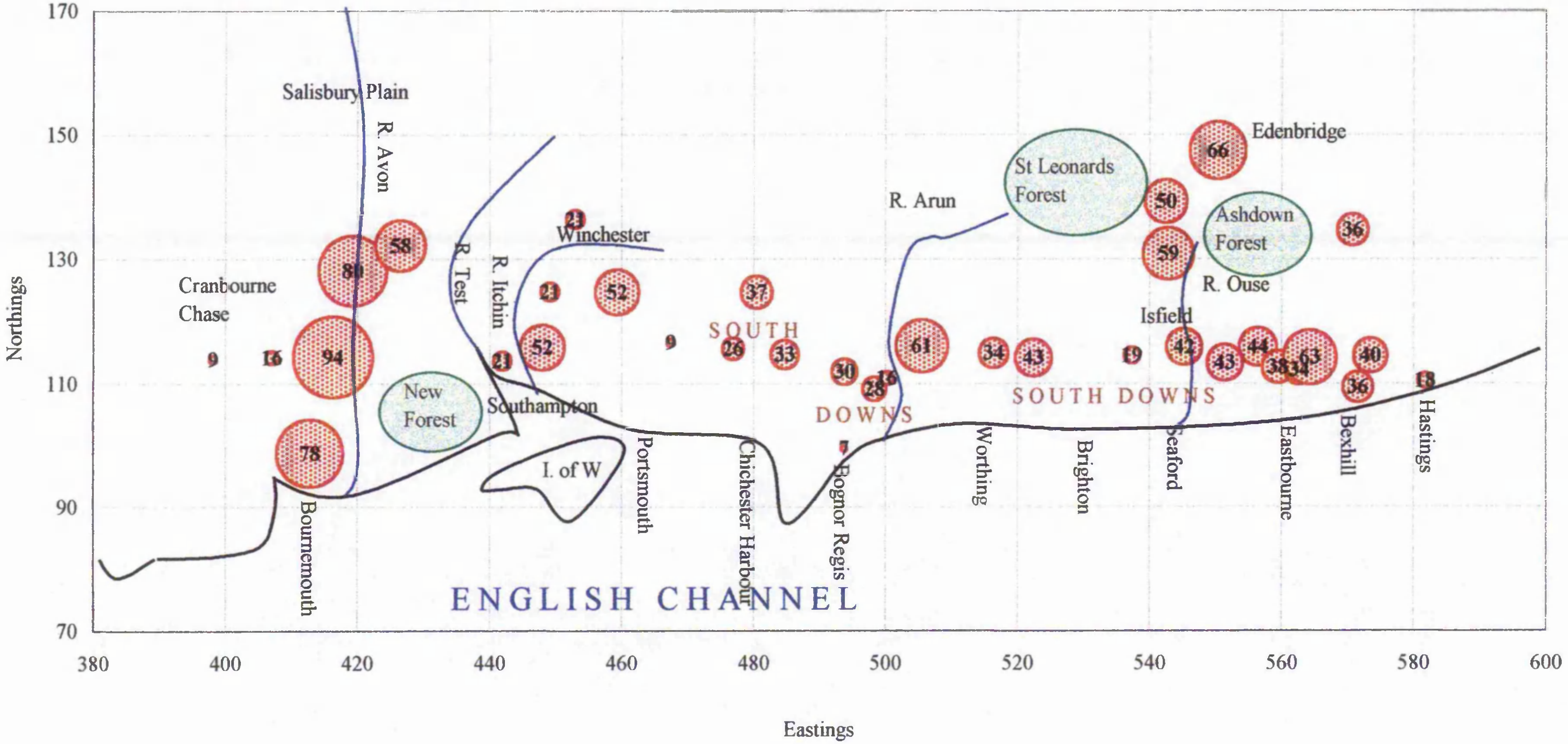
The red-legged frequencies of new sites in the South Downs region north of Seaford and Eastbourne (e.g. East Hoathly, Hellingly and Herstmonceux) are in the same range as those of the old sites (e.g. Isfield and Hale Green) indicating a more gradual change in morph proportions along this part of the transect (see also Tables B.2.i and B.2.iii). Although the red-legged frequency of Bodle St. Green is relatively high (63%), the number of *P. madidus* trapped was low (Table B.2.i) and the morph proportion is not significantly different.

The red-legged frequency for new sites in the forested area of Kent (Edenbridge, Hammer Wood and Wych Cross) ranges from 50 to 66%.

The relationship between morph proportions and urban areas or distance from the sea is clearly not simple. The lowest and highest frequencies are found in or near coastal towns e.g. 78% for Bournemouth and 7% for Bognor Regis (Fig 4.5). However, consistent with findings for 1976, the exposed sites on the Downs and Cranbourne Chase have lower red-legged frequencies, whereas extensively wooded areas in Hampshire and Kent have higher red-legged frequencies.

Fig 4.5 Red-legged morph frequencies along and close to Transect 2, showing coastline and major topographical features. Numbers in bubbles give red-legged frequencies. See Table B.2 in Appendix B for site details.

Grids = 20 x 20 km.



The establishment of new sites close to weather stations has raised problems. The site closest to the Hurn weather station at Bournemouth has a high red-legged frequency (78%). However, this station was used in the 1976 analysis to model the low red-legged frequency at Cashmoor. Similarly, a second site at Southampton (Southampton Common), which was established within 2.5km of the weather station, has a significantly lower red-legged morph proportion than that obtained from the original site at West End (23% compared with 52%).

ii) Regression analysis

The correlations producing the highest F-ratios are shown in Table 4.10.

As shown in Table 4.10.i, the annual mean maximum and minimum temperatures and the annual mean (the average of the mean maximum and minimum) were influential climatic factors, showing a negative association with red-legged morph frequencies. Use of the annual mean temperature with position east explained 38% of the variability (Prediction 1), rising to 46% when altitude was also included (Prediction 2). Both these geographical factors are negatively associated. The inclusion of four variables – position east, altitude, the annual mean maximum and minimum temperatures explained 56% of the variability (Prediction 3).

Fig 4.6.i shows how well Predictions 1 and 3 modelled morph frequencies at each site. Wiggonholt, Guesses Farm and the three Ashdown Forest sites did not have full weather data for 1996 so are excluded from the analysis.

Prediction 1 (annual mean temperature and position east) models the variability eastwards along the transect quite well but underestimates the highest red-legged frequencies (e.g. Hurn, Fordingbridge and Stockbridge) and overestimates the lowest frequencies (e.g. Twyford and Michelover Wood north of Southampton, Clanfield and Bognor Regis). The three new sites at the most eastern end of the transect are also poorly predicted.

Prediction 3, which includes the maximum and minimum temperatures as well as position east and altitude, improves the modelling of higher and lower frequencies for all sites except Bognor Regis. This was identified in the regression as having a large residual. Exclusion of Bognor Regis from the regression improved the coefficient of determination to 73% and the F-ratio to 31.47 (d.f. = 24).

Table 4.10 The most influential predictors of red-legged morph frequency for 1996-1998 along Transect 2. (Partial predictors for the multiple regressions are listed in order of decreasing significance. In italics, P > 0.05).

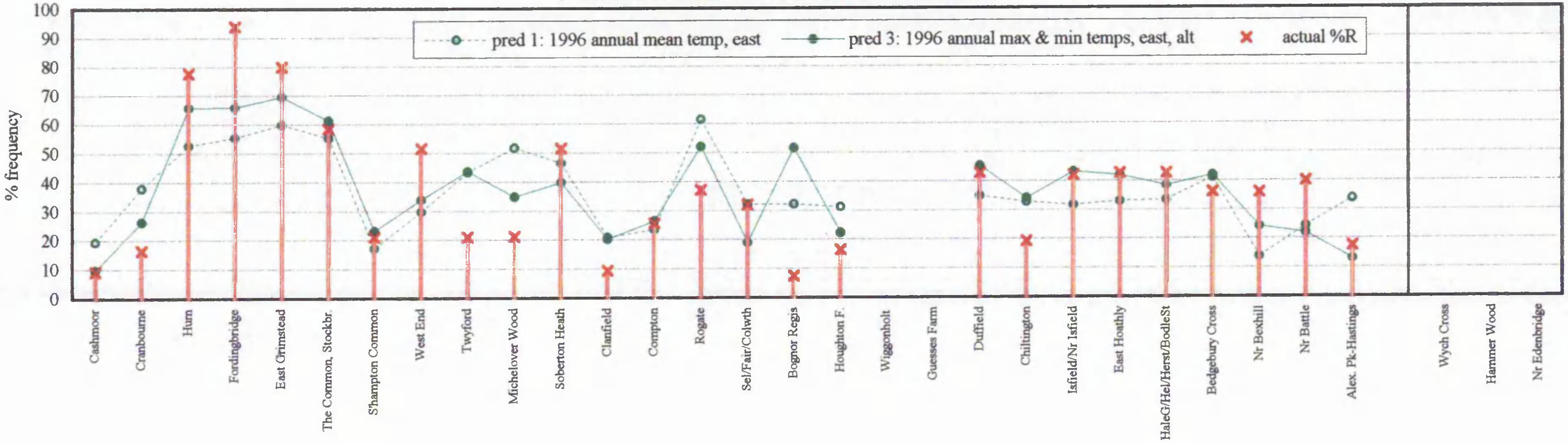
min temp = mean minimum temperature; max temp = mean maximum temperature;
mean temp = average of max and min temps; rainfall = total rainfall.

Prediction (Fig 4.6)	Time period	d.f.	Variables	P (partial predictor)	Association (+/-)	F-ratio	P (regression equation)	r^2 (r^2-adj) %
i) Annual climatic data:								
1	1996	25	mean temp	0.003	-	6.31	0.007	35 (30)
			position east	0.045	-			
2	1996	25	mean temp	0.002	-	6.19	0.003	46 (38)
			position east	0.012	-			
			altitude	*0.053*	-			
3	1996	25	position east	0.001	-	6.76	0.001	56 (48)
			max temp	0.002	-			
			min temp	0.003	-			
			altitude	0.013	-			
ii) Monthly climatic data:								
4	Sept, 1995	29	mean temp	< 0.001	-	18.09	< 0.001	38 (31)
5	May, 1996	28	rain	<0.001	-	16.74	< 0.001	38 (36)
6	May, 1996	28	rain	0.006	-	12.05	<0.001	48 (44)
			mean temp	0.035	-			
7	Sept, 1996	28	min temp	0.001	-	6.83	0.002	45 (38)
			max temp	0.003	-			
			position east	0.021	-			
8	April, 1997	28	mean temp	< 0.001	-	8.93	0.001	41 (36)
			position east	0.013	-			
9	May, 1996	28	May rain	0.002	-	14.80	< 0.001	53 (50)
	Dec, 1996		Dec min	0.008	-			

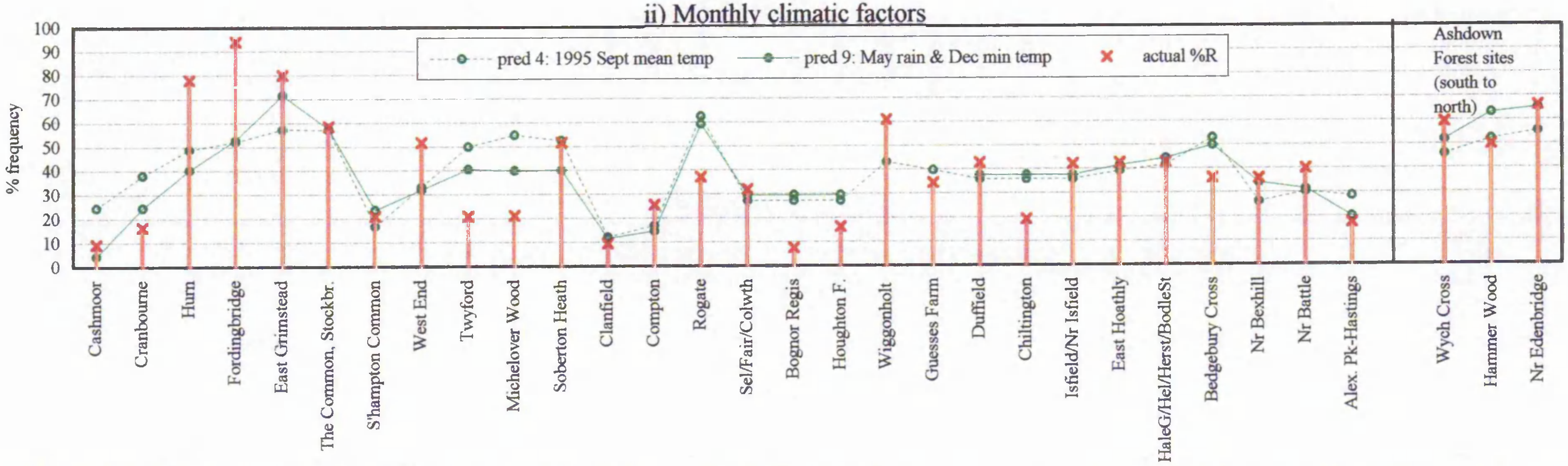
Table 4.10.ii shows that the same temperature factors were influential when monthly climatic variables were entered into the regression analysis. In addition, rainfall for May 1996 became important, showing a negative association with red-legged frequencies (Predictions 5 and 6). Position east was a significant partial predictor in Predictions 7 and 8, interacting negatively with temperature factors. Spring (April 1997 and May 1996), early autumn (September 1995, 1996) and early winter (December) were identified as the most influential times of the year. As single factors, the mean temperature for September 1995 (Prediction 4) and rainfall for May 19961 (Prediction 5) explained 38% of the variation in the region and produced the highest F-ratios. Predictions 6 and 9, which explained half the variability, also have high F-ratios.

Fig 4.6 TRANSECT 2 (west to east): Predictions of red-legged morph frequency from regressions on 1996-98 frequencies using environmental data as predictors. (Pred = prediction as numbered in Table 4.10).

i) Annual climatic and geographical factors



ii) Monthly climatic factors



The modelling of morph frequency at each site by Predictions 4 and 9 is shown in Fig 4.6.ii. Prediction 4 (1995 September mean temperature), which has climatic data for all the sites, fails to model the high frequencies of Hurn, Fordingbridge and Wiggonholt. Low frequencies are also poorly predicted. However, the Ashdown Forest sites are predicted well and there is an improvement for the three most eastern sites (Bexhill to Hastings). Prediction 9 (1996 May rainfall and December mean minimum temperature) generally improved the modelling of low red-legged frequencies, e.g. Cashmoor, Cranbourne, Twycross and Michelover Wood, though still over-estimates the latter two sites by 20%.

iii) Identification of environmental predictors of morph frequency along Transect 2

1996-8 red-legged frequencies in this region correlate with lower maximum, minimum and average temperatures and lower rainfall, suggesting that the red-legged morph is better adapted to cooler and drier conditions. Rainfall and the mean temperature were particularly influential for May and September respectively. These months produced high F-ratios and are, of course, critical periods in the life history of *P. madidus*, with pupation and emergence of over-wintering females expected in May, and oviposition in September. The mean maximum temperature always interacted with the mean minimum temperature. Because both these variables are negatively associated, the temperature range never became significant.

With the exception of rainfall, the association between red-legged frequencies and climatic factors is inconsistent with earlier findings for this region. Using data for 1975/6, the temperature range was found to be the most influential predictor of red-legged morph frequencies (see Section 4.4.1.ii). When interacting with the adjusted altitude, the direction of association with the mean minimum temperature was positive.

Of the geographical factors, position east and altitude were found to be important when interacting with temperature factors. Consistent with findings for the 1976 Transect 2 data, both these variables are negatively associated, i.e. the red-legged morph frequency tends to decrease eastwards and with increasing altitude. Distance inland was not a significant partial predictor. This, again, contrasts with the analysis of the 1976 data.

The negative correlation with the mean minimum temperature was not expected. Many more sites were sampled in the 1996-1998 period, including some further north, south and east of the original transect, and it could be argued that these new sites are influencing the association of red-legged frequencies with temperature factors in the opposite direction. In fact, when the new sites are excluded from the regression analysis, the negative association between red-legged frequencies and the mean minimum temperature continues to be significant. October 1996 had the highest F-ratio ($R^2 = 54\%$; F = 11.9; P = 0.006; d.f. = 11).

The weather stations used in the regressions recorded cooler temperatures for 1996 than for the 12-month 1975/6 period (see Table 4.11). However, the most striking differences between the two periods lie in the higher maximum and average temperatures for 1975/6, not the higher minimum temperature (compare results of t-tests in Table 4.11). Moreover, the minimum temperature was not a strong predictor in the analysis of the 1975/6 data and became a significant partial predictor only when the adjusted altitude was included in the regression (see Table 4.9, Section 4.4.1, p. 127).

Table 4.11: **Annual temperature data for Transect 2 obtained by weather stations used in the analysis of the two time periods (August 1975 to July 1976; January to December 1996), showing 1 standard error (s.e.) on the means and the results of t-tests for the two periods.**

Temperature (^{0} C)	mean maximum	s.e.	mean minimum	s.e.	average of max and min temperature	s.e.	mean temperature range	s.e.
Aug 1975 to July 1976	14.7	0.12	6.6	0.24	10.7	0.10	8.1	0.31
Jan to Dec, 1996	13.6	0.08	6.0	0.16	9.8	0.08	7.6	0.18
Distribution of t	7.778		2.297		6.631		1.117	
Probability (P)	< 0.0001		0.028		< 0.0001		n.s.	

Of the 30 Transect 2 sites, 18 are predicted quite well by at least one of the equations, particularly the Cranbourne Chase, South Downs and Ashdown Forest sites (see Fig 4.6). However, with the exception of Cashmoor and Cranbourne, the highest and lowest red-legged frequencies are poorly predicted. These more extreme frequencies were obtained from Hurn and Fordingbridge close to the River Avon and the New Forest, the three sites north of Southampton (West End, Twyford and Michelover Wood), Bognor Regis on the south coast, Wiggonholt close to the River Arun, and Chiltington on the South Downs (see Figs 4.5 and 4.6). Fordingbridge and Bognor Regis had the highest and lowest red-legged morph frequencies of 93 and 7% respectively.

Fordingbridge was identified as having a large residual in most of the equations. It is possible that the weather stations, which are 18 and 26 km away (Table 4.5), do not represent the climatic conditions of this site. On the other hand, the Hurn site, which had a red-legged frequency of 78%, is only 1.4km distance from the Bournemouth (Hurn) weather station (Table 4.5). In fact, this station gave a good prediction of the low red-legged frequency at Cranbourne in both this and the 1976 analysis (see Tables 4.9 and 4.10, Figs 4.5 and 4.6). Interestingly, the Hurn site is heavily wooded, whereas the Bournemouth weather station (an airport) and Cranbourne are situated in more open areas.

The weather stations for Twyford and Michelover Wood, north of Southampton, are also situated less than 3 km away (Table 4.5). The best prediction for Michelover Wood included the mean maximum temperature (Prediction 3, Fig 4.6.i). West End, also north of Southampton, was modelled well by the Southampton weather station in the 1976 analysis (Fig 4.4) but is underestimated by the two weather stations used in this analysis (Southampton and Otterbourne, both about 7km away – see Table 4.5). It is now the lower frequency of Southampton Common, the new 1998 site situated only 2.2km from Southampton weather station, which is predicted well (Table 4.5 and Fig 4.6).

Wiggonholt, 13 km away from the nearest weather station at Rustington, is included in only one equation - Prediction 4 (1995 September mean temperature – see Fig 4.6.i). This weather station had stopped operating by 1996. It is not known whether the inclusion of other variables would have improved the modelling of this site. However, Chiltington and Bognor Regis, both with low red-legged frequencies, were within 2 km of their respective weather stations, yet were poorly predicted.

Finally, the intermediate red-legged frequency of 37% at Rogate was overestimated by all the equations (Fig 4.6). This might be because the nearest weather station, Alice Holt, is situated in an extensively wooded area 18 km away. The Rogate weather station was not publishing data from 1995 but was used to predict the Colworth Down frequencies in the 1976 analysis (Table 4.4). This site had a low red-legged morph frequency of 9% in 1976 and was modelled quite well (Fig 4.4). The ECN frequency for Alice Holt is in fact in the 60% range that was predicted for Rogate in this analysis (see Table 3.5, p. 91).

There is no clear explanation for the poor predictions of red-legged frequencies of some sites that were within 3 km of a weather station. With the possible exception of Hurn, there is no reason to suppose that these stations are unrepresentative of the sites. On the contrary, the failure to identify the extremes in morph frequencies suggests that there is insufficient variability in the climatic data used in this analysis, and other factors are important. These could be sunshine hours - an important partial predictor in the 1976 analysis - or relative humidity. Non-climatic variables, such as soil factors, may also be having an influence.

Nevertheless, the red-legged frequencies of more than half the sites have been modelled well, indicating that the red-legged morph - in this region at least – is in higher proportion in lowland western areas, and where climatic conditions are cooler and drier. There is no association with variables considered to be indicative of maritime conditions – distance to sea and temperature range.

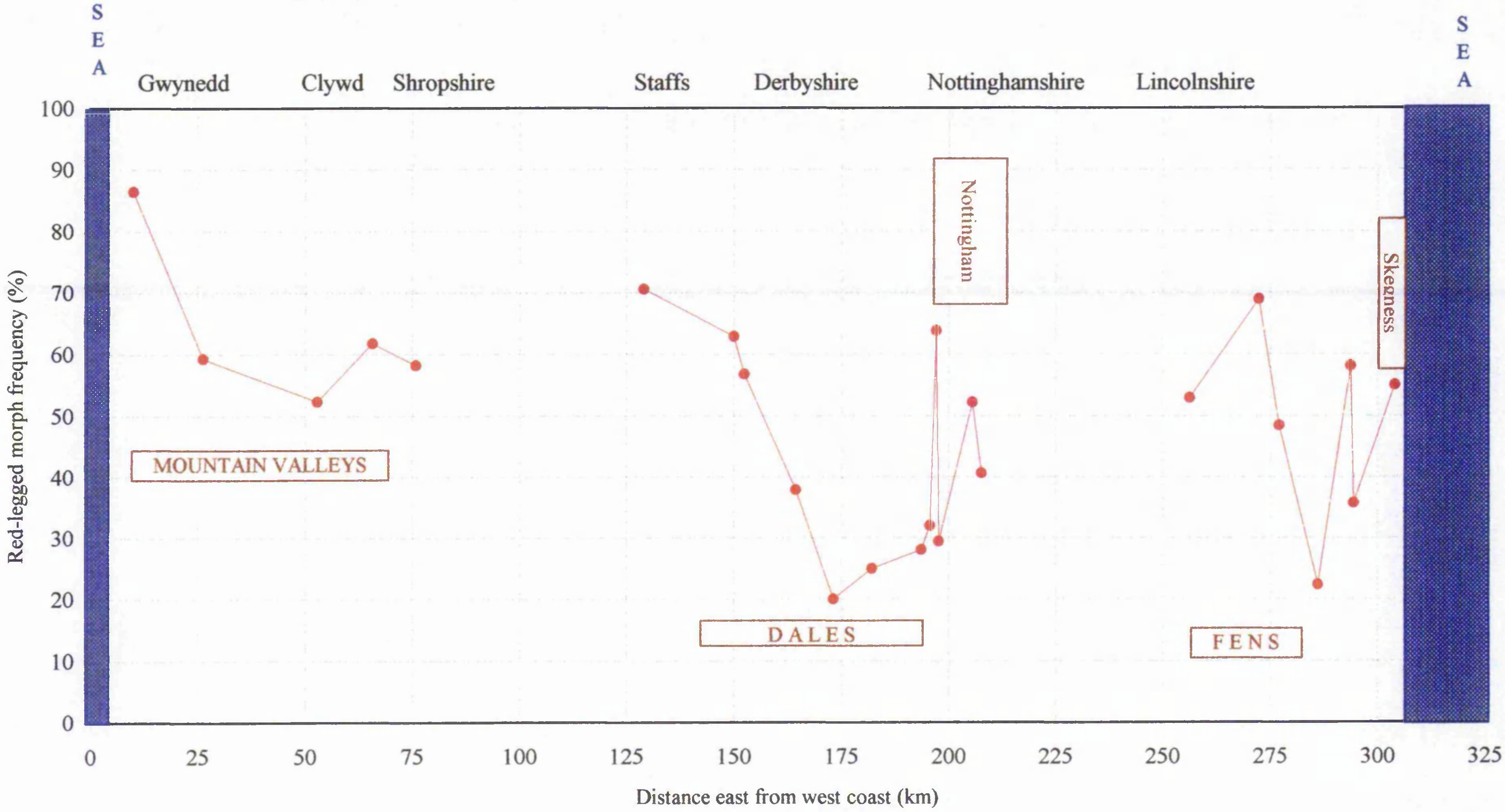
4.4.3 Analysis of 1996 to 1998 data for Transect 3

Table B.3 in Appendix B gives the number of *P. madidus* morphs sampled at each site from 1996 to 1998 along the broad east/west band extending from north Wales to Skegness. From chi-squared analysis, there were no significant differences in the 1996/7/8 morph proportions at sites sampled for two or more years. The annual data were therefore pooled for the analysis, giving the morph frequencies in Table 4.6.

i) Morph frequency variation

As shown in Fig 4.7, a high red-legged morph frequency (86%) was obtained at the most westerly site, 7km from the coast. Farther inland, moderately high frequencies of between 55% and 70% were maintained in Clwyd, Shropshire and Staffordshire. These are valley sites (the Welsh sites and Froghall in Staffordshire) or sites situated on the surburban edge of large towns (Sites 6 and 10 at Oswestry and Keele – see Table 4.6).

Fig 4.7 Red-legged morph frequency along Transect 3 (Bethesda to Skegness) using pooled 1996/7/8 data.



Although there was a relatively high red-legged frequency at one of the highest altitude sites (Buxton in the Derbyshire Dales – see Table 4.6), this site was in an urban woodland area within the town conurbation. Eastwards from Buxton, there is a sharp fall in the red-legged frequency, with a low of 20% at Birchover – a sparsely wooded site at 150m altitude in the Derbyshire Dales, and situated south-east of the Derwent valley (altitude = 95m). In the rural, agricultural regions north and south of the city of Nottingham (Doe Lea and Sutton Bonington), the red-legged morph frequencies were also relatively low.

Within the city, there was a highly significant difference in the morph proportions of two sites less than 3km apart - Sites 18 and 19 at Watnall ($\Sigma\chi^2 = 64.2$; $P<0.0001$; d.f. = 1). These had red-legged morph frequencies of 64% and 32% respectively. Site 18 was situated within a roadside copse in an urban area, whereas Site 19 was inside a large wood within 20 metres of Moorgreen Reservoir (area = 0.33km^2). In addition to the attenuation of temperature extremes due to canopy cover, there would be a thermal lag at the water body/ground surface boundary because of the slower response to changes in radiation by the body of water (Geiger, 1966; Smith, 1976).

North-east of Nottingham, in Warsop, two sites only 2 km apart (Sites 21 and 22) also showed significantly different morph proportions ($\Sigma\chi^2 = 4.353$; $P = 0.037$; d.f. = 1). The Medon Vale site, which had a red-legged morph frequency of 52%, was situated in a small area of hawthorn scrub between a housing estate and the colliery head. The Budby site, with a frequency 41%, was placed in a wood on the rural outskirts of Warsop.

Insufficient data were collected from the sparsely wooded, intensively farmed region of Lincolnshire, suggesting that the *P. madidus* population is low in this type of habitiat. Towards the east coast, morph frequencies show high variability from 69% in the Fens to 22% inland from Skegness. The Skegness site, which is only 4km from the sea, produced a relatively high frequency (55%) for this region. However, as shown in Fig 4.7, red-legged frequencies in the east are, on average, lower than those in the west.

In summary, maritime and urban areas are once again associated with higher red-legged frequencies whereas high black-legged frequencies are found in open and agricultural regions. However, steep clines occur on a local scale. The frequency differences may be a function of the specific topography affecting local climate, but climatic data are not available to distinguish the sites. Data from Sites 18, 19, 21 and 22 were therefore used individually in the analysis (see Table 4.6).

ii) Regression analysis

The correlations producing the highest F-ratios up to a maximum of five predictors are shown in Table 4.12. Mean daily sunshine hours were recorded at 5 weather stations only, so were not entered into the regressions.

Of the geographical factors, the strongest predictor was position eastwards, which correlated negatively with red-legged morph frequency (Prediction 1- see Table 4.12). Inclusion of distance to sea - also correlating negatively - improved the coefficient of determination to 34% (Prediction 2). As shown in Fig 4.8.i, the mid-point frequency for the regions was predicted reasonably well, but not the clines within the regions.

The mean minimum temperature is the strongest single climatic predictor when monthly data for late autumn, winter and spring are used. The highest coefficient of determination (33%) was obtained for January 1996 (Prediction 3). Although under-estimating the highest and lowest red-legged frequencies, this prediction improves the modelling of the direction of morph frequency change within the regions, especially at the eastern sites (see Fig 4.8.i). The temperature range becomes a stronger predictor from June to September 1996, showing a consistent, negative correlation with red-legged morph frequencies (e.g. Prediction 4). Rainfall, which is negatively associated, is significant only for December 1996 (Prediction 5).

Three predictors, using both climatic and geographical variables, explain about 50% of the variability (e.g. Predictions 6 to 10, Table 4.12). The temperature range, which interacts negatively with position east and distance to sea, is a significant partial predictor when data for December and February are used (compare Prediction 6 with Predictions 7 and 8 in Table 4.12). For January, minimum temperature is a positively associated partial predictor, interacting with site altitude and distance to sea (Prediction 9). Rainfall and temperature range are important for June, interacting negatively with position east (Prediction 10).

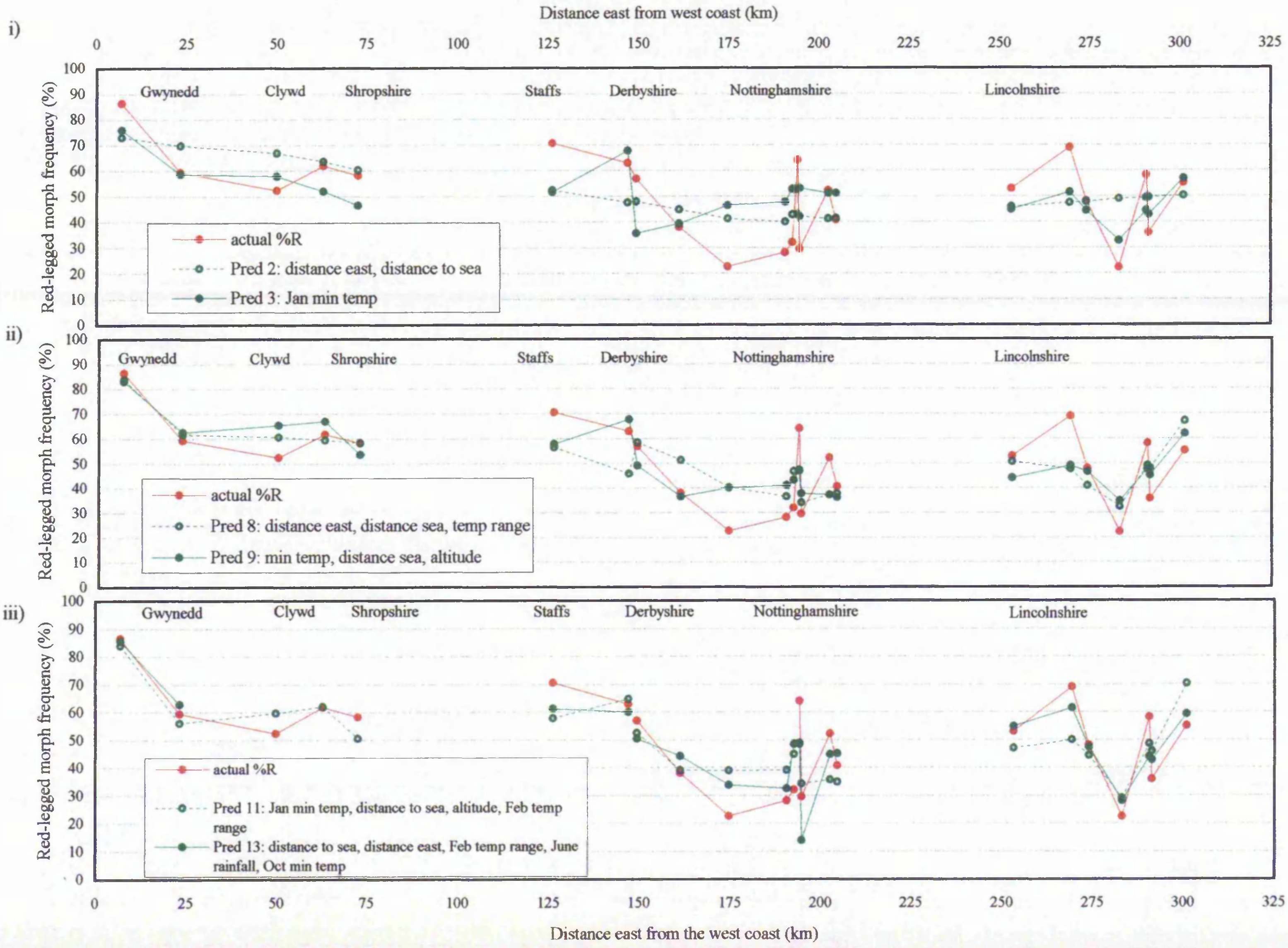
As shown in Fig 4.8.ii, Predictions 8 and 9 - the equations with three variables producing the highest F-ratios - predict higher red-legged frequencies more accurately and improve the modelling of frequency variation, including the direction of frequency change at the highly variable east Midlands sites in Nottinghamshire.

Table 4.12 **The most influential predictors of red-legged morph frequency for 1996/7/8 along Transect 3. (Partial predictors for the multiple regressions are listed in order of decreasing significance; in italics: $P > 0.05$).**

d.f. = degrees of freedom; min temp = mean minimum temperature; max temp = mean maximum temperature; rainfall = total rainfall.

Prediction (see Fig 4.8)	d.f.	Time period	Variables	P (partial predictor)	Association (+/-)	F-ratio	P (regression equation)	r^2 (r^2-adj) %
Geographical predictors								
1	23		position east	0.048	-	4.42	0.048	17 (13)
2	23		position east	0.027	-	5.10	0.016	34 (27)
			distance to sea	0.038	-			
Climatic predictors								
3	23	Jan, 1996	min temp	0.004	+	10.20	0.004	33 (30)
4	21	June, 1996	temp range	0.013	-	7.58	0.013	29 (25)
5	23	Dec, 1996	rainfall	0.006	-	9.13	0.007	30 (27)
Climatic and geographical predictors								
6	20	Annual, 1996	distance to sea	0.023	-	4.64	0.016	47 (37)
			position east	0.048	-			
			temp range	*0.106*	-			
7	23	Dec, 1996	position east	0.001	-	6.91	0.002	52 (45)
			distance to sea	0.002	-			
			temp range	0.014	-			
8	23	Feb, 1996	position east	0.001	-	7.14	0.002	53 (46)
			distance to sea	0.003	+			
			temp range	0.012	-			
9	23	Jan, 1996	min temp	0.001	+	8.70	0.001	58 (52)
			distance to sea	0.004	-			
			site altitude	0.017	+			
10	21	June, 1996	temp range	0.004	-	6.11	0.005	52 (43)
			position east	0.014	-			
			rainfall	0.030	-			
11	23	Jan, 1996	Jan min temp	0.001	+	7.41	0.001	62 (54)
			distance to sea	0.002	-			
			site altitude	0.024	+			
		Feb, 1996	Feb temp range	*0.167*	-			
12	20	spring 1996	spring min temp	0.001	+	6.76	0.003	64 (55)
			distance to sea	0.002	-			
		autumn 1995	autumn max temp	0.004	-			
		spring 1996	spring max temp	0.029	+			
13	20		distance to sea	< 0.001	-	8.23	0.001	75 (66)
			position east	0.001	-			
		Feb, 1996	Feb temp range	0.001	-			
		June, 1996	June rainfall	0.004	-			
		Oct 1995	Oct min temp	0.012	-			

Fig 4.8 TRANSECT 3 (west to east): Predictions of red-legged morph frequency from regressions on 1996 to 1998 frequencies using environmental data as predictors. (See Table 4.12 for predictions). Pred = prediction.



Higher coefficients of determination with four or five predictors are obtained only when interacting monthly or seasonal data are entered into the regressions (Predictions 11 to 13). The interaction of January minimum temperature and February temperature range with site altitude and distance to sea (Prediction 11), explains two-thirds of the variability and further improves the prediction of red-legged frequencies at sites which are modelled reasonably well by Predictions 8 and 9 (compare dotted line in Fig 4.8.iii with Fig 4.8.ii). However, the variability between adjacent sites continues to be poorly predicted and the partial predictor for February is weak ($P > 0.05$).

Prediction 12 produces a similar coefficient of determination, using spring and autumn seasonal data (see Table 4.12). In this equation, the maximum temperature interacts negatively for autumn and positively for spring. However, as shown in Table 4.13, the mean maximum temperature for autumn correlates significantly with the other partial predictors. This could be producing an artificially high coefficient of determination for this equation.

Table 4.13 Correlation between partial predictors of Prediction 12 (Table 4.12) with each other and with red-legged morph frequencies (%R). Significant correlations are shown in italics; the significance level (P) is denoted by asterisk.

* $P < 0.05 > 0.01$; ** $P < 0.01 > 0.001$; ***$P < 0.001$)

	%R	distance to sea	96 spring min temp	96 spring max temp	95 autumn max temp
%R	1				
distance to sea	-0.385	1			
96 spring min temp	*0.550***	-0.213	1		
96 spring max temp	0.130	-0.214	0.309	1	
95 autumn max temp	0.140	*-0.672****	*0.441**	*0.742****	1

Five predictors explain 75% of the morph frequency variation and also produce the highest F-ratios of the multiple regressions (Prediction 13). There is no significant correlation between the partial predictors. Fig 4.8.iii shows an improvement on Prediction 11 for the highly variable sites in the East Midlands and some improvement at the eastern sites. (Weather data for all these variables were not recorded at three of the western sites).

Interestingly, in Prediction 13, the association with the October minimum temperature is negative. The 1995 October minimum temperature for the weather stations was 1°C warmer, on average, than the 1996 October minimum (8.5°C compared with 7.5°C), the latter showing no significant correlation, either as a single or a partial predictor, with the red-legged frequency. Does this suggest that the red-legged morph is favoured by cooler October temperatures in warmer years? On the other hand, as a single predictor, October 1995 is positively associated with red-legged frequency ($R^2 = 24\%$; $F = 6.07$; $P = 0.023$), and it could be argued that its direction of association with the other partial predictors is an artefact of the multiple regression equation and has no biological meaning.

iii) Identification of environmental predictors of morph frequency along Transect 3

Of the climatic variables, the mean minimum temperature was found to be the most important predictor, particularly for the winter months, showing a positive correlation with red-legged frequencies. The mean temperature range became important in February and the summer months, especially June, and was negatively associated with red-legged frequencies. Rainfall was occasionally influential as a single or partial predictor, also showing negative correlation.

These climatic factors were also identified as influential for Transects 1 and 2, using 1975/6 morph frequencies and weather data. However, there is a shift in the time of year when they are most likely to have an influence. For Transects 1 and 2 (1975/6), this was predominantly spring; for Transect 3, the winter months and early summer (June) are more influential. Given that Transect 3 crosses the most northern site of Transect 1 (Doe Lea), this is not surprising. Temperatures along Transect 3 will be lower, on average, the winter minimum for the coldest months perhaps falling below a critical value, which distinguishes the two morphs. Development is also likely to be slower, with emergence possibly delayed until early summer.

Confirming findings for Transects 1 and 2 (1975/6), the red-legged morph also appears to be susceptible to a wider temperature range, particularly when overall temperatures are low (e.g. February, Prediction 8) and relatively high (June, Prediction 10). For June, rainfall was also an important partial predictor. Interestingly, in 1996, there was a sharp rise in temperature from a mean of 8.7^{0}C in May to 13.7°C in June. Due to the maximum temperature rising faster than the minimum, there was a wider temperature range in June (10.4°C) compared with May (8.4°C). However, the relationship is complex. Although rainfall, which was negatively associated with the red-legged frequency, would tend to reduce the temperature range, it would also retard soil warming.

The strength and direction of the association of geographical factors with red-legged morph frequencies, i.e. position east, distance to sea and site altitude, are also consistent with results for Transect 1 (site altitude) and Transect 2 (position east and distance to sea).

The positive interaction of site altitude is expected, the higher altitude valley sites, on the whole, being more sheltered than sites situated on the flat, lowlands of Lincolnshire, where black-legged frequencies are higher. The negative association with distance inland once again associates the red-legged morph with more maritime conditions. Exposure to the cooler east wind would be progressively more important moving from west to east along the transect.

However, as for Transect 2 (Section 4.4.2), the regressions of 1996 climate data on the Transect 3 morph frequencies did not produce the extraordinarily high F-ratios and coefficients of determination of the Transect 1 and 2 regressions using 1975/6 data. Although the reduced temporal resolution should not have a large effect on the significance level of the equations (Section 3.4.1), the use of 1996 climate data, which was closer to the years of sampling, produced more significant coefficients of determination than did data from 1995.

As for Transect 2 sampled in the 1990s, another source of error will be the steep clines in morph frequencies between neighbouring sites. This is shown by sites in the East Midlands - Sites 18 and 19 at Watnall (modelled by Watnall weather station) and Sites 21 and 22 at Warsop (modelled by Warsop weather station). Inevitably, there was a large residual on one or both of these pairs of sites in the analysis (see Fig 4.8). Omission of Site 19 from the analysis made no difference to the significance level. Omission of Site 18, with the higher frequency of 64%, increased the coefficient of determination in the regressions by about 7%. The copse (Site 18) was predicted to have a morph frequency within the 40 to 45% range – the average of the two values obtained for the Watnall sites. The red-legged morph frequency at Site 21 at Budby in Warsop was similarly under-estimated by all the regression equations (Fig 4.8). Yet, both Sites 18 and 21 were closer to their respective weather station than Sites 19 and 22, which were more accurately predicted (see Table 4.12).

In summary, the results for Transect 3 using data from the late 1990s, are consistent with findings for Transects 1 and 2 using data from the 1970s, but less consistent with those for Transect 1 sampled in the 1990s. Along the more northern, cooler east-west transect, the red-legged morph is associated with a higher minimum temperature, a narrower temperature range and the milder climate of western regions and coastal areas of the U.K.

4.4.4 Analysis of 1998 morph frequency data for East Anglia

Table B.4.i in Appendix B shows the number of *Pterostichus madidus* trapped at each site in East Anglia. Comparable with Transect 3, *P. madidus* activity is patchy, the beetle apparently concentrated in areas with fairly extensive deciduous woodland cover such as Thetford Forest (see also Luff, 1998).

There were no significant differences in morph proportions at sites positioned 1 to 3 km apart. These were Sites 2 and 3 at Watlington, Sites 4 and 5 at Leziate, Sites 9 and 10 at Cromer and Sites 15 and 16 at Norwich. Because of this stability, the morph proportions at these pairs of sites were combined and the sites' geographical parameters weighted using equation 4.4.

A red-legged morph frequency has been calculated for Site 25 (Boxworth) where only 8 *P. madidus* were trapped. This was because a weather station is situated nearby. However, the frequency (25%) is consistent with the frequency of 18% at Monks Wood (Site 26), also in south Cambridgeshire.

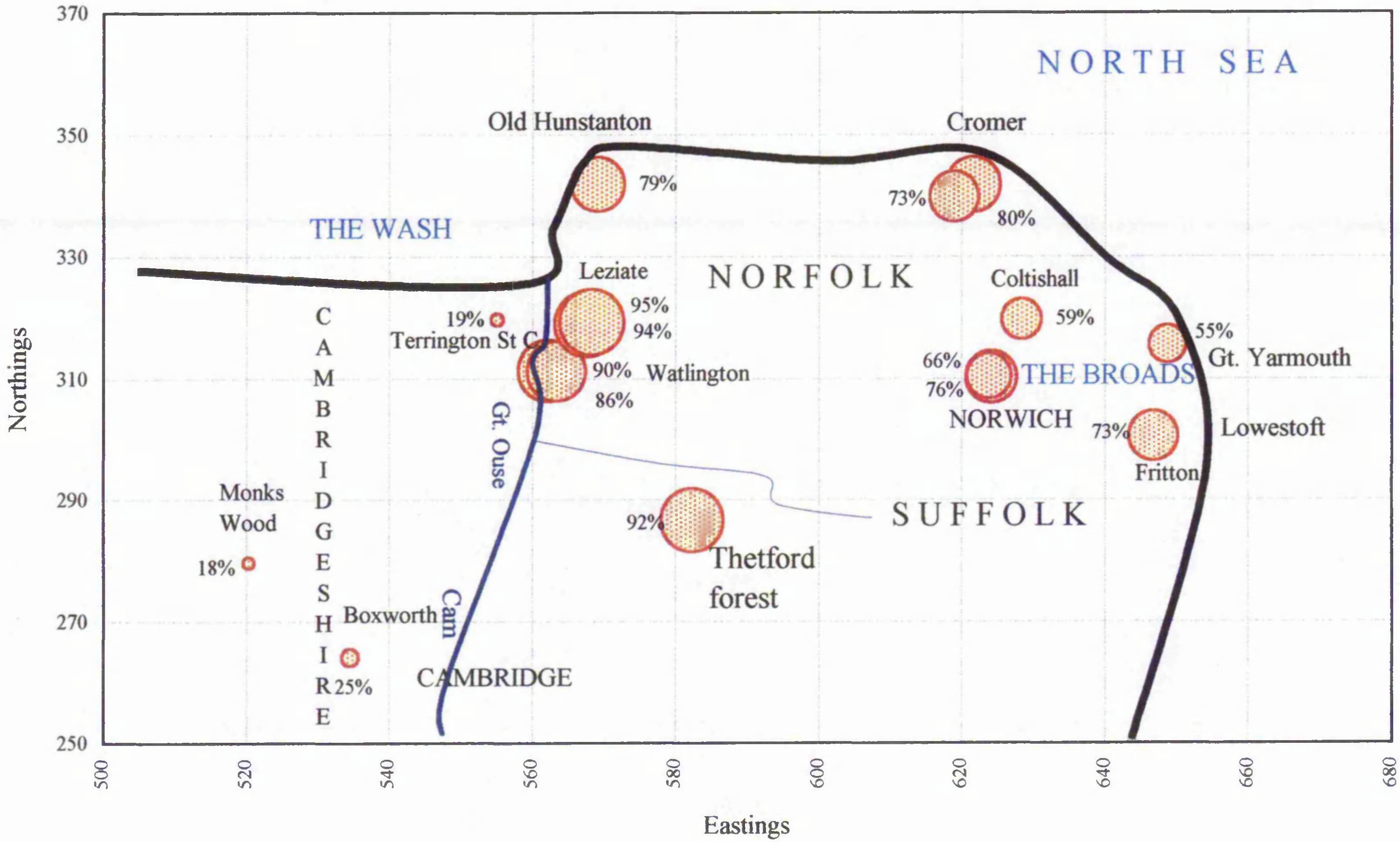
Table 4.7 shows the sites and associated weather stations used in the analysis, giving 12 observations in total. Soil factors for individual sites, which (with the exception of Site 25 at Boxworth) trapped at least 15 *P. madidus*, are given in Table B.4.ii in Appendix B (15 observations in total).

i) <u>Morph frequency variation</u>

Fig 4.9 is a schematic representation of morph proportions by area in East Anglia, the size of the bubble correlating with the size of the red-legged frequency (see Table 4.7).

Most noticeable is the high proportion of the red-legged morph throughout Norfolk and north Suffolk east of the rivers Ouse and Cam, the frequency ranging from about 60 to 95%. By contrast, the black-legged morph is in high proportion (> 75%) west of these rivers, both in the north (close to the Wash) and in the south, in Cambridgeshire. Although these results might suggest that the rivers are acting as gene flow barriers, they are also consistent with findings for Transects 1 and 3, i.e. the high black-legged areas are also the most intensively farmed. However, the remarkably steep cline from 19% at Terrington St Clement (Site 1) to 90% just 15km away at Watlington (Sites 2 and 3), was not expected.

Fig 4.9 Red-legged morph frequencies in East Anglia, 1998, showing coastline and the Rivers Ouse and Cam. Bubbles are sized with red-legged frequency. See Table B.4 in Appendix B for site details.
Grids = 20 x 20km.



ii) Regression analysis.

Regression analysis was performed on soil, geographical and climatic parameters separately to identify the strongest predictors. These were then entered into the multiple regression analysis to identify significant interactions between the parameters. The correlations producing the highest F-ratios up to a maximum of two predictors for 12 degrees of freedom and three predictors for 14 degrees of freedom are shown in Table 4.14. Fig 4.10 shows the red-legged frequencies generated by predictions with the highest R^2 for single and interacting predictors. In this figure, the sites have been grouped into three broad "transects":

- a south to north transect in north-west Norfolk (Sites 1 to 7);
- a south-east to north-west transect running from the Broadlands to the north coast in north-west Norfolk (sites 9 to 18);
- an east to west transect extending from the agricultural area west of the rivers Ouse and Cam in Cambridgeshire, through Thetford Forest in north west Suffolk to sites which were positioned close to the east coast in north Suffolk (Sites 19 to 26).

As shown in Table 4.14, among the soil factors, the strongest predictor is texture (Prediction 1). This explains 50% of the variability in red-legged frequencies. There were weaker associations with % moisture retention and pH ($P = 0.05$ and 0.06 respectively – Predictions 2 and 3). The associations are negative, i.e. a higher red-legged morph frequency is associated with sandy, acidic soils with a low moisture retention. Geographical factors only became significant when included with soil factors. The interaction of position north and site altitude with soil texture improves the coefficient of determination to 61% (Prediction 4). However, the F-ratio is much reduced from 13.5 to 5.7. Distance inland and position north were significant partial predictors when included with % moisture retention. This regression explains 55% of the variation (Prediction 5).

Fig 4.10.i shows that both Prediction 1 (texture only) and Prediction 4 (% moisture retention, position north and site altitude) give good predictions of Sites 19 to 26 in Cambridgeshire and north Suffolk. In north-east Norfolk, the high red-legged frequencies are slightly underestimated whereas the low frequency for Site 1 at Terrington-St-Clement is over-estimated, so the steepness of the cline between Sites 1 and 2/3 is not modelled well. However, the direction of frequency change is modelled. With the exception of the two sites close to the north coast (Sites 9 and 10), the frequencies in north Norfolk and the Broadlands are poorly predicted.

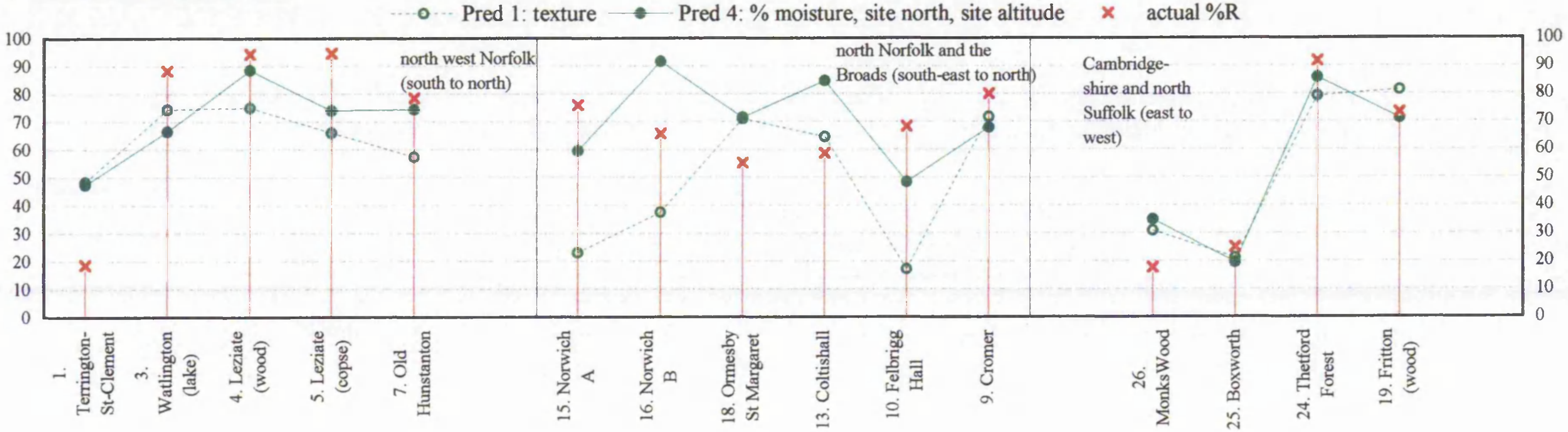
Table 4.14 The most influential predictors of 1998 red-legged morph frequencies in East Anglia. (Partial predictors for the multiple regressions are listed in order of decreasing significance; in italics, $P < 0.05$).

d.f. = degrees of freedom; min temp = mean minimum temperature; max temp = mean maximum temperature; rainfall = total rainfall.

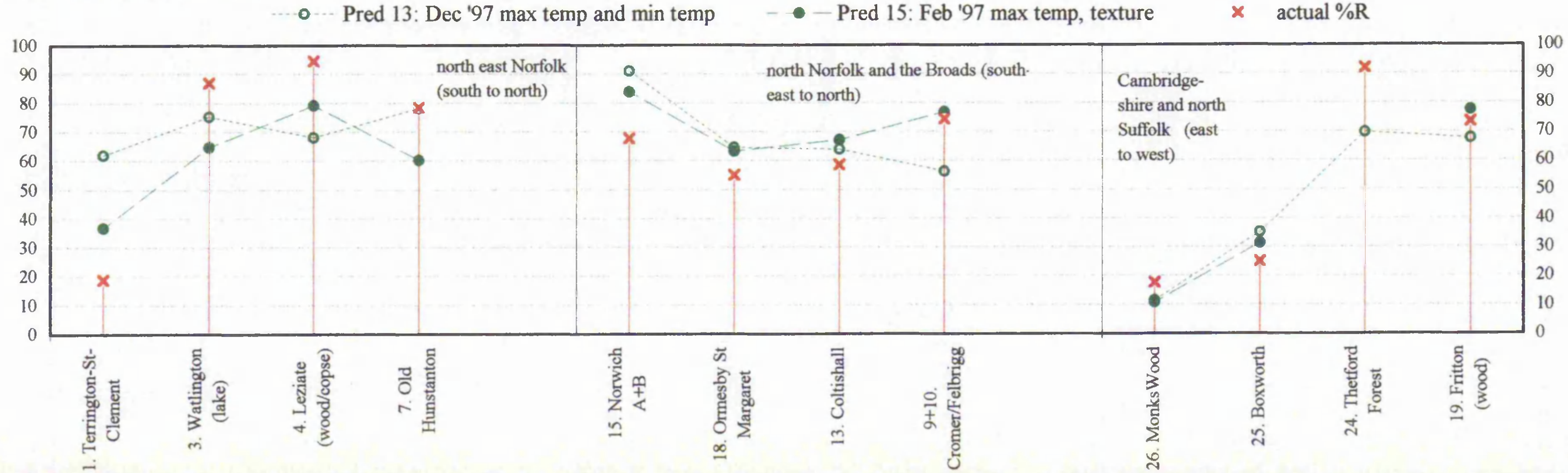
Prediction (see Fig 4.10)	Time period	d.f.	Variables	P (partial predictor)	Association (+/-)	F-ratio	P (regression equation)	r^2 (r^2-adj) %
i) Soil factors								
1		14	texture	0.003	-	13.48	0.003	51 (47)
2		14	% moisture retention	0.050	-	4.69	0.050	27 (21)
3		14	pH	*0.057*	-	4.37	*0.057*	25 (19)
ii) Interaction of soil and geographical factors								
4		14	% moisture retention	0.006	-	5.70	0.013	61 (50)
			position north	0.019	+			
			site altitude	0.044	+			
5		14	% moisture retention	0.013	-	4.45	0.028	55 (42)
			site altitude	0.040	+			
			distance inland	0.047	-			
iii) Climatic factors								
6	Feb, 1997	10	max temp	0.043	-	5.53	0.043	38 (31)
7	March, 1997	10	max temp	0.036	-	6.03	0.036	40 (33)
8	April, 1997	10	temp range	0.033	-	6.32	0.033	41 (35)
9	Dec, 1997	11	temp range	0.027	-	6.71	0.027	40 (34)
10	June, 1997	10	min temp	0.023	+	7.47	0.023	45 (39)
11	Sept, 1997	11	rainfall	0.033	-	6.12	0.033	38 (32)
12	Autumn, 1995	10	rainfall	0.025	-	7.21	0.025	45 (38)
13	Dec, 1997	11	min temp	0.008	+	5.75	0.025	56 (46)
			max temp	0.039	-			
iv) Interaction of soil and climatic factors								
14	Autumn, 1995	10	max temp	0.007	-	8.09	0.010	64 (56)
			pH	0.028	-			
15	Feb, 1997	10	texture	0.014	-	10.32	0.006	72 (65)
			max temp	0.032	-			

Fig 4.10 EAST ANGLIA: Predictions of red-legged morph frequency from regressions on 1998 frequencies using environmental data as predictors. (Site number is given by each site. Pred = prediction as numbered in Table 4.14).

i) **soil and geographical factors**



ii) **climate and soil factors**



Monthly temperature and rainfall data each produced good correlations for different times of the year, explaining about 40% of the variability in red-legged frequencies. The mean maximum temperature was the strongest predictor for February and March 1997 (Predictions 6 and 7) whereas the temperature range became the strongest predictor later in spring (Prediction 8) as well as early winter (Prediction 9). The mean minimum temperature was the strongest predictor in June 1997 (Prediction 10). Rainfall became the strongest predictor for September 1997 and – interestingly - autumn 1995 (Predictions 11 and 12). Use of both minimum and maximum temperatures for December improved the coefficient of determination to 56% (Prediction 13). Otherwise, there was little improvement when more than one climate variable was included in the analysis.

The association between red-legged frequencies and climatic factors are consistent for each equation - negative for rainfall, mean maximum temperature and temperature range and positive for mean minimum temperature.

Geographical parameters were not significant partial predictors when included with climatic data in the regression analysis. However, soil factors could be significant partial predictors; pH interacted with the maximum temperature for Autumn 1995 (Prediction 14), explaining almost two-thirds of the variability in red-legged frequencies, and texture interacted with the maximum temperature for February 1997 (Prediction 15), improving the coefficient of determination still further to 72%.

As shown in Fig 4.10.ii, the use of climatic factors improved the prediction of frequencies in north Norfolk and the Broads. However, morph data for Sites 15 and 16 at Norwich and the two sites close to the east coast (Sites 9 and 10) were combined for regressions that included climate data, giving only four observations for this "transect". Nevertheless, the red-legged frequencies and direction of frequency change are modelled quite accurately. Temperature factors also predicted the Cambridgeshire/north Suffolk transect quite well, though underestimated Thetford Forest (Site 24). The sites in north-east Norfolk were actually predicted more accurately by soil texture alone (Prediction 1 in Fig 4.10.i). It is clear that the mean maximum and minimum temperatures fail to explain the low red-legged frequency at Site 1 (Terrington-St-Clement). Equations which included the temperature range and rainfall (Predictions 8, 9, 10 and 11) did not improve the prediction for this site.

iii) Identification of environmental predictors of morph frequency in East Anglia

The wooded areas of East Anglia have coarsely textured soils with a poorer thermal conductivity, expected to produce a large temperature range. This is not consistent with high red-legged proportions. However, as with Transects 1 and 2, the red-legged morph tends to be at a higher frequency in these wooded areas. It is also positively associated with sandy, acidic soils (the black-legged morph was in higher proportion in the more clayey agricultural soils). Doberski & Gazzy (2000) also obtained a higher red-legged frequency at the more acidic Thetford Forest site. They point out that a low pH would slow down the decomposition rate of leaf litter. A deep litter layer, in turn, provides insulation against a large temperature range.

As for Transect 1, Transect 2 (1976) and Transect 3, a higher red-legged frequency is associated with a higher minimum temperature, a lower maximum temperature and a smaller temperature range, i.e. milder temperature conditions. Again, however, there is a seasonal shift, with a lower maximum temperature apparently favouring the red-legged morph in cooler months, and a higher minimum temperature favouring this morph in early summer. In contrast to findings for Transect 1 in 1975 but comparable with findings for Transect 2 (1996-8 data), the red-legged morph seems to be favoured by drier conditions in autumn, especially September. This preference for drier conditions is also suggested by the negative association between red-legged frequencies and % moisture retention of soil. However, dry conditions at this time of year are not expected to be advantageous to the red-legged morph, since they would produce a wider temperature range as well as accelerate soil cooling.

The direction of association with geographical parameters - significant only as partial predictors with soil factors - is consistent with previous findings. The association is negative with distance inland, implying a preference for more maritime conditions. Given the proximity of the north coast in East Anglia, the positive association with distance north also suggests an adaptation to more maritime conditions. The positive association with altitude is not unexpected, because woodland areas tend to be on higher ground in East Anglia (e.g Thetford Forest, the heath in Norwich), presumably not cleared for agriculture due to poor soil fertility and the potential for wind erosion. Since woods provide both canopy and ground cover for the beetle, their higher altitude is not necessarily an indicator of exposure.

In summary, the results for East Anglia are consistent with findings for Transects 1 and 2 in 1975/6 and Transect 3 in the mid-1990s with the red-legged morph in higher proportion in wooded areas and favoured by higher minimum and lower maximum temperatures. The positive correlation between the red-legged morph and soil factors typical of the sandy soils of the East Anglian woods sampled in this analysis may, therefore, be coincidental.

4.4.5 Comparison of annual temperatures for Transects 1, 2, 3 and East Anglia

The regression analysis identified an inconsistency between Transect 2 monitored from 1996 to 1998 and the other regions (Transects 1, 3 and East Anglia) as well as Transect 2 when monitored in 1976. The direction of association between the red-legged morph and the mean minimum temperature is negative for this region in the late 1990s as opposed to positive.

Table 4.15 compares the 1996 and 1997 annual temperature data averaged from the weather stations of regions analysed for these years. As explained in Sections 4.4.3 and 4.4.4 regression analyses were not performed using 1996 weather data for East Anglia and 1997 weather data for Transect 3. The direction of association with red-legged morph frequency and periods of strongest associations are also shown in Table 4.15. Table 4.16 gives the results of t-tests comparing the 1996 annual weather data for Transects 2 and 3 and 1997 data for Transects 2 and East Anglia.

As expected, the 1996 annual temperatures for Transect 2 – the south coast region – are higher than those for the more northern Transect 3 (Table 4.15), with the maximum and minimum temperatures warmer by 0.9 and 0.8°C respectively. However, as shown by the results of t-tests on these variables (Table 4.16), it is the maximum temperature that produces the most significant value for t. This is also the case when the 1997 weather data for Transect 2 and East Anglia are compared. Again, Transect 2 is warmer on average (Table 4.15), but a significant difference was found only for the maximum temperature (see Table 4.16).

In fact, the 1997 annual minimum temperatures for East Anglia and Transect 2 are almost the same (7.1°C and 7.0°C respectively), suggesting that relative rather than absolute temperature values are important. This is further suggested by the lower minimum temperature of 6.0°C for Transect 2 in 1996 (negatively associated with red-legged frequencies – see Table 4.10) compared with 6.6°C for 1975/6, which was positively correlated with red-legged frequencies, although the significance level is less than 0.05 (Table 4.9.ii).

Table 4.15 Annual temperature data for 1996 and 1997 averaged for weather stations used in regression analysis of Transect 2 (T2), East Anglia (EA) and Transect 3 (T3), showing direction of association with red-legged frequencies (%R) and period of strongest association.

max = mean maximum temperature; min = mean minimum temperature; range = difference between maximum and minimum temperatures.

YEAR	Region	max temp	min temp	average of max and min	temp range	Direction of association with %R and period of association			
						max temp	min temp	mean temp	temp range
1996	T2	13.6	6.0	9.8	7.6	*negative*	*negative*	*negative*	
						annual Sept	annual Sept Dec	annual May	
1996	T3	12.6	5.2	8.9	7.4	*negative*	*positive*		*negative*
						spring	spring Jan June Dec		annual Dec Feb June
1997	T2	15.2	7.0	11.1	8.2		*negative*	*negative*	
							Feb	April	
1997	EA	14.5	7.1	10.8	7.4	*negative*	*positive*		*negative*
						Feb March Dec	June Dec		April Dec

Table 4.16 Results of t-tests comparing 1996 annual temperature data for Transect 2 (T2) and Transect 3 (T3) and 1997 annual temperature data for Transect 2 and East Anglia (EA).

max = mean maximum temperature; min = mean minimum temperature;
range = difference between maximum and minimum temperatures.

t dist = distribution of t; P = significance level (probability); n.s. = not significant.

YEAR	Regions	t-test statistics	max temp	min temp	average of max and min	temp range
1996	T2 v T3	t dist P	5.031 0.0001	2.363 0.024	4.899 0.0002	1.319 n.s.
1997	T2 v EA	t dist P	3.754 0.0007	0.235 n.s.	1.716 n.s.	1.732 n.s.

The most consistent variable for the regions analysed is the *maximum temperature*, which has a negative influence on red-legged morph proportions regardless of year or region. This may be the dominant factor determining morph frequencies. It appears that the minimum temperature is positively associated with red-legged morph frequencies when the maximum temperature is relatively low (e.g. Transect 3 in relation to Transect 2 in 1996 and East Anglia in relation to Transect 2 in 1997), but negatively associated when the maximum temperature is relatively high (i.e. Transect 2 for 1996 and 1997).

As shown in Table 4.15, these temperature factors are more influential during early autumn, winter and spring, periods of the year when *P. madidus* is expected to be in the developmental and non-reproductive adult stages rather than the reproductive stage of its life cycle. Sharp changes in temperature are also more likely to occur during autumn and spring, for example April, June and September in 1996 and February, May, June, September and October in 1997 (see Fig 4.11).

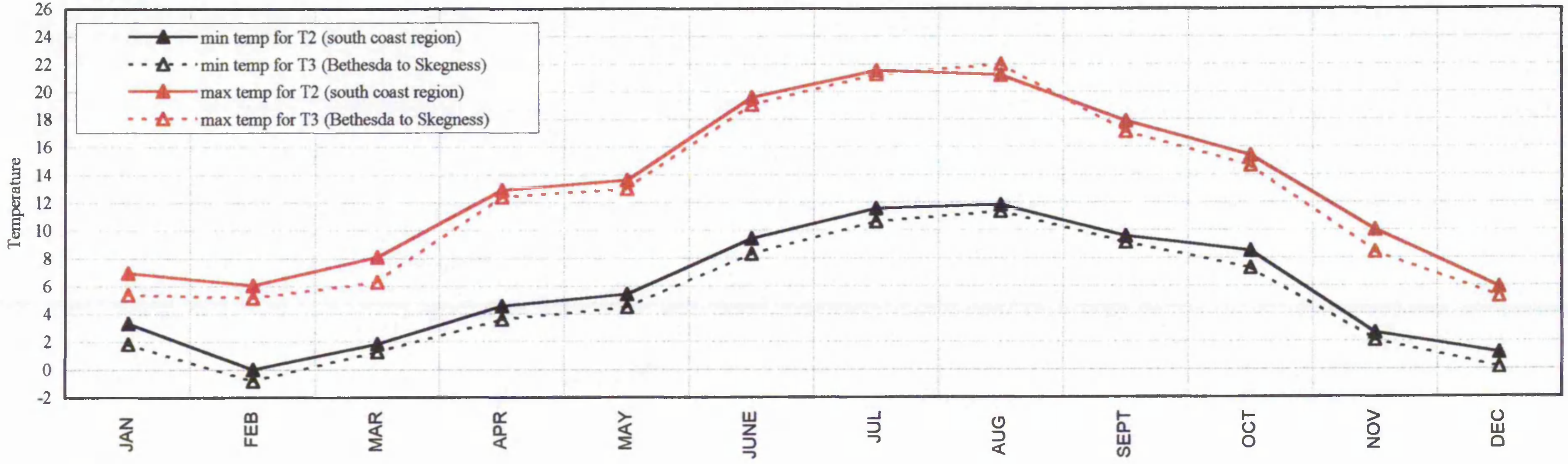
Table 4.15 also shows that the period of strongest association varied between the regions and years. For example, in 1996, September is important for the warmer south coast region, Transect 2, with the red-legged morph favoured by lower maximum and minimum temperatures. By contrast, winter (December, January and February) was important for the more northern, colder region, Transect 3, with the red-legged morph favoured by a higher minimum temperature and narrower temperature range.

Unusual temperature conditions can also produce significant correlations. February was exceptionally warm in 1997, and there had been a sharp rise in temperature from January (compare 1996 and 1997 in Fig 4.11). It is therefore interesting that cooler temperatures were positively correlated with the red-legged morph in February 1997. For Transect 2, the association was with a lower minimum temperature; for East Anglia, the association was with a lower maximum temperature (Table 4.15).

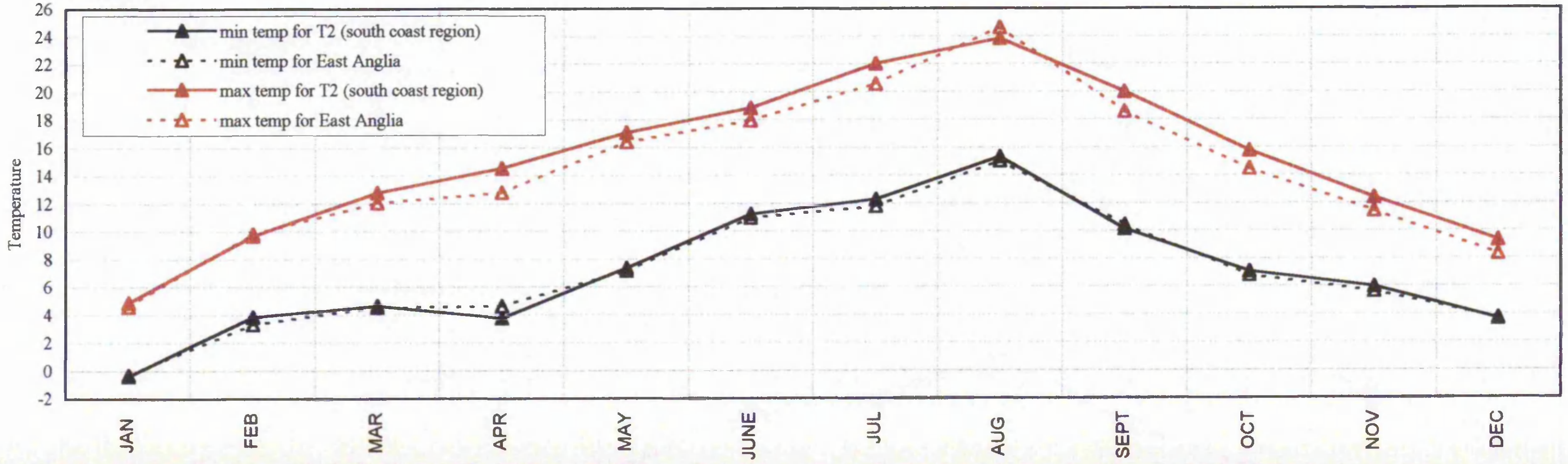
In summary, the anomaly between the regions for minimum temperature might be explained by the different temperature conditions of these regions, with a complex relationship between maximum and minimum temperatures indicated. What does seem to be consistent, however, is the association between the red-legged morph and cooler, milder climatic conditions during the developmental stages of the beetle's life cycle. There is also some indication that the red-legged morph is disadvantaged by rapid changes in temperature.

Fig 4.11 Comparison of averaged monthly maximum and minimum temperatures for i) Transect 2 and Transect 3 weather stations in 1996 and ii) Transect 2 and East Anglia weather stations in 1997.

i) 1996



ii) 1997



4.4.6 Analysis of 1998 morph frequency data for sites in the East Midlands

Table B.5 in Appendix B shows the number of black and red-legged morphs of *Pterostichus madidus* trapped during August 1998 at each of the 41 sites in the East Midlands. The geographical, topographical and soil parameters for each site are also given. Over 80 *P. madidus* were obtained from two-thirds of the sites (28 sites in total). Numbers were less than 40 at six sites: Keyworth (16), Epperstone (18), Borrowash (27), Attenborough (31), Selston Plantation (32) and Lowdham (36). The Borrowash and Attenborough sites were gardens. The Keyworth and Lowdham sites were hedgerows alongside ditches in open areas. Epperstone and Selston were trapped on one occasion only. Despite the low numbers at Keyworth and Epperstone, a morph frequency has been found for these sites. The 1998 red-legged frequency of 12.5% at Keyworth is known to be representative of this area (Terrell-Nield, pers. comm.).

i) Red-legged morph frequencies in the East Midlands

Table 4.17 gives the red-legged frequencies for each site in ascending order and the sites' major topographical and geographical parameters. Red-legged frequencies for the region ranged from 6.3% (Widmerpool) to 67.4% (the copse at Watnall), giving an overall average of 32.1%. However, as shown in Fig 4.12, the frequency for one-third of the sites fell within a narrow band from 31 to 40%. There is also skewing towards the lower frequencies, with nearly a quarter of the sites ranging between 21 and 30%. Because of this skewing, the actual red-legged morph frequency for the whole region is 30.5%.

Fig 4.12 Histogram showing range in red-legged frequencies at the East Midlands sites (41 sites in total).

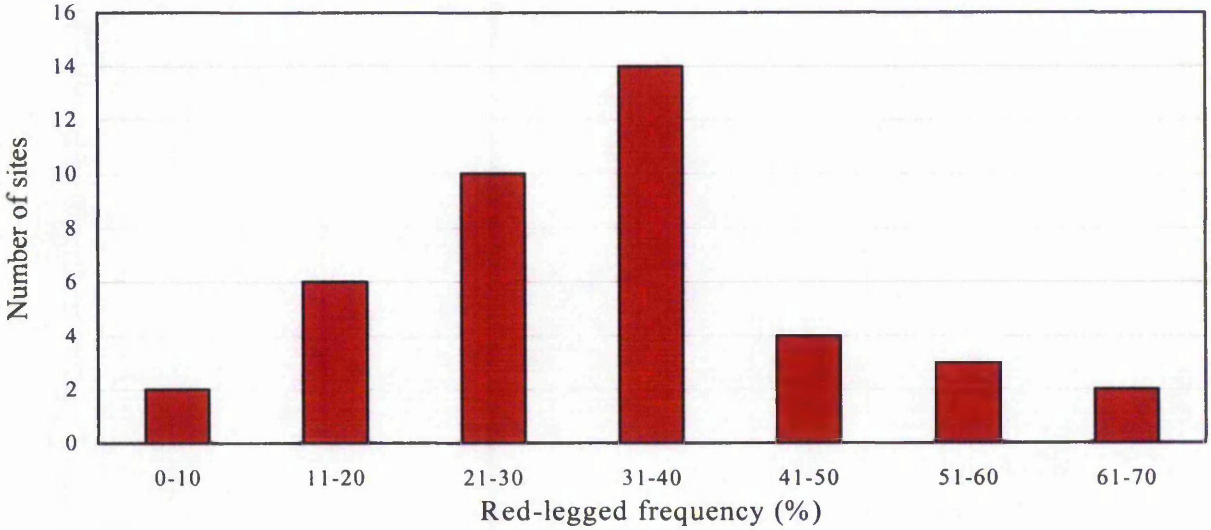


Table 4.17: Red-legged morph frequencies and major geographical and topographical parameters for each site in the East Midlands. (Sites are arranged from lowest to highest frequencies).

SITE NAME	%R	Type of area	Direction from River Trent	% wood	% urban	GRID REF		Alt (m)
						east	north	
Widmerpool	6	Wolds-rural	south	7	10	62.8	28.3	70
Borders wood	10	Wolds-rural	south	25	7	65.7	33.2	90
Borrowash	11	river plain (Derwent)	north	2	34	42.6	34.3	45
Keyworth	12	Wolds-suburban	south	1	31	62.3	32.3	55
Stragglethorpe	17	Wolds-rural	south	2	7	65.4	36.6	40
Ruddington Hall	18	suburban	south	2	45	57.8	34.2	40
Gotham	20	rural	south	4	11	53.8	31.3	45
Nottm Trent Univ (Clifton)	20	suburban	south	8	46	54.6	35.1	45
Glapton Wd (Clifton)	21	suburban	south	4	55	54.8	33.8	55
Roshoe Wd	22	Wolds-rural	south	4	15	64.8	29.4	85
Lockington	22	river plain (Trent)	south	2	15	46.4	27.7	45
Clifton Wood	23	suburban	south	8	30	53.8	34.6	65
Codnor	25	semi-rural	north	2	19	43.0	48.7	115
Sutton Bonington	27	rural	south	2	16	50.5	26.5	45
Epperstone	28	semi-rural (forestry)	north	17	18	63.3	50.2	65
Somercotes	29	suburban	north	4	47	43.4	53.2	113
Clifton Grove	30	suburban	south	6	43	54.2	34.8	35
Canning Circus (cemetery)	30	city	north	0	92	56.5	40.3	70
Burton Joyce	31	river plain (Trent)	north	4	20	63.7	43.2	30
Colwick racecourse	31	river plain (Trent)	north	10	25	60.4	39.3	60
Awsworth	33	suburban	north	1	28	48.8	44.7	70
Kirk Hallam	33	suburban	north	3	27	44.2	39.7	90
Selston Pl. (Bagthorpe)	34	suburban	north	12	23	47.6	51.6	100
Bunny Wood	35	rural	south	11	10	58.7	28.4	60
Bestwood Lodge	35	suburban	north	15	47	57.2	46.5	95
Attenborough	35	river plain (Trent)	north	4	41	51.8	34.9	25
Watnall (wood), by lake	37	semi-rural (lakeside)	north	19	7	48.3	48.7	80
Nr Hucknall	37	suburban	north	9	28	52.5	45.7	70
Nr Lowdham	39	semi-rural	north	1	18	65.0	47.9	30
King's Mill	40	river plain (Trent)	south	8	4	41.9	27.5	40
Elvaston Castle	40	river plain (Derwent)	north	10	5	40.9	32.8	40
Colwick Wd	40	city	north	11	51	59.7	39.8	85
Stapleford (Hemlock Stone)	41	suburban	north	7	32	50.0	38.5	60
Holme Pierrepoint	42	river plain (Trent)	south	3	12	61.5	38.7	20
Shipley	45	semi-rural (park)	north	14	14	43.6	44.2	110
Woodthorpe	50	city	north	0	97	58.1	44.8	80
Strelley	53	suburban	north	4	38	51.3	41.9	100
Sawley	53	river plain (Trent)	north	1	39	48.3	31.6	30
Nottm Univ	55	city	north	1	74	54.4	38.6	50
Harrison Plantation	66	city	north	3	71	53.1	40.3	45
Watnall (copse)	67	suburban	north	4	21	49.8	46.3	115

Fig 4.13 is a spatial representation of the frequencies in the East Midlands, the diameter of the bubble representing red-legged frequency. Rivers in the region are shown as blue lines, the River Trent running from the south-west to the north-east. Urban areas are shaded brown; suburban areas are shaded yellow. Woodland in this region was not extensive enough to be shown on the scale of this figure.

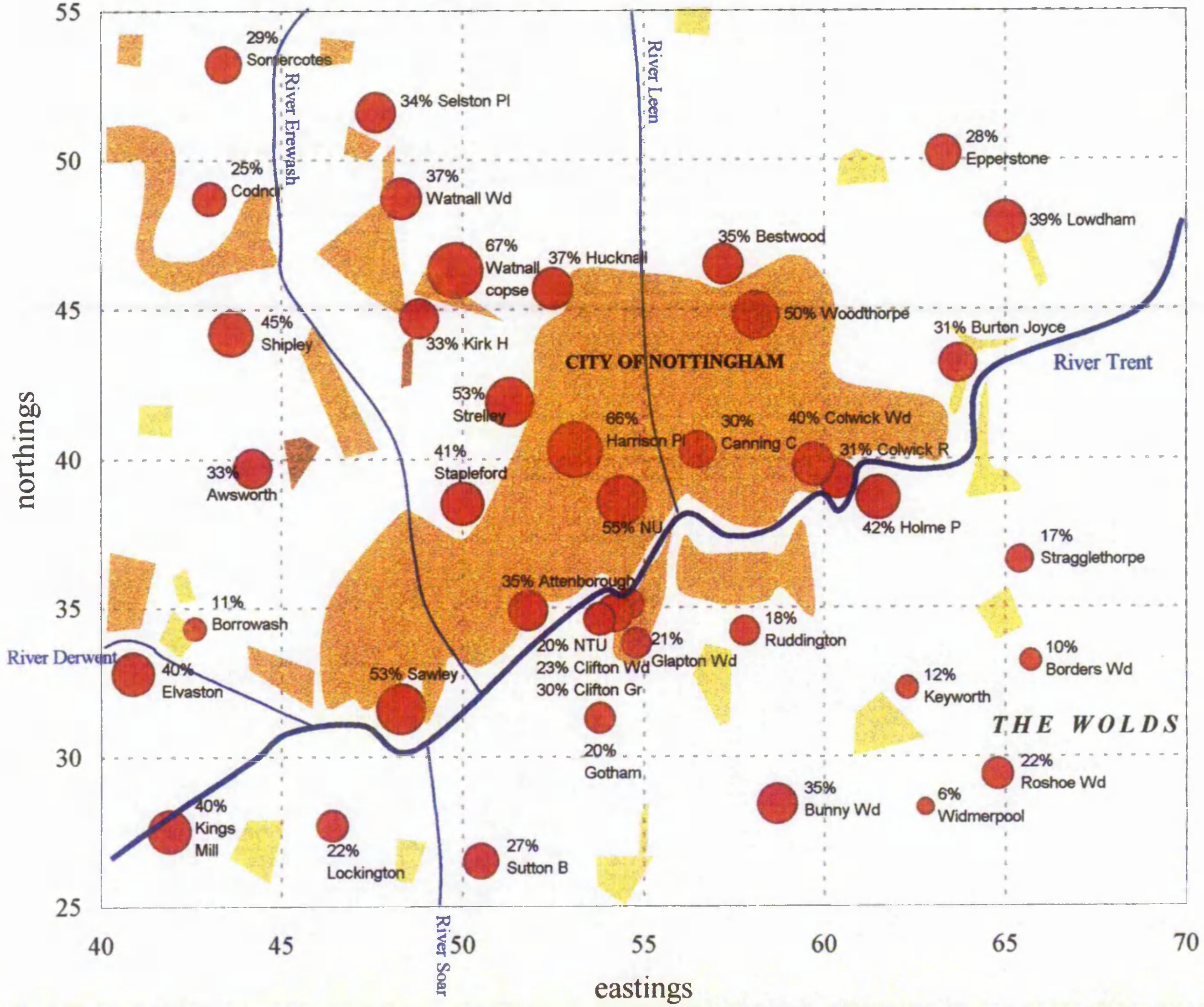


Fig 4.13 Red-legged morph frequencies at the East Midlands sites (see Table 4.17) where diameter of bubble represents size of frequency.
Dark shading = urban areas; light shading = surburban areas.

From Table 4.17 and Fig 4.13, there is clearly a lower than average red-legged morph frequency in the south east of the region (the Wolds) and a higher than average red-legged frequency in the city of Nottingham (the largest area of brown shading). North west of the city, where the urban areas become fragmented, the red-legged frequency remains relatively high. Areas between the urban patches are represented by features such as an open cast mine, sewage works and a high density of major roads including the M1 motorway. By contrast, the Wolds in the south-east is an intensively farmed area, with few buildings and few woods. The topography is fairly flat, and north-facing, i.e. the land rises gradually from the River Trent. The yellow shaded patches in Fig 4.13 represent small towns and villages within agricultural areas where, again, the red-legged morph frequency tends to be lower than average e.g. Borrowash, Lockington, Sutton Bonington and Epperstone (see also Table 4.17).

Although there is a gradient in red-legged frequencies across the Trent valley, it is not steep and there is no evidence from Fig 4.13 of a sudden discontinuity, which may be caused by the river acting as a barrier to gene mixing between areas. Morph frequencies are therefore more likely to be due to environmental conditions (selective factors) as opposed to gene flow (non-selective factors). Section 4.4.5.ii examines this hypothesis further.

ii) Regression analysis

Of the geographical, topographical and soil factors entered into the analysis, four are not significant predictors: site distance to the River Trent or nearest river, % water body within 1.5km radius of the site, % soil moisture retention and % soil organic content. Northings is an occasional significant predictor but was excluded from the analysis because identified by Minitab as exhibiting curvature, i.e. having a non-linear relationship with the other variables.

Table 4.18.i gives the correlations producing the highest F-ratios using all the East Midlands sites. Watnall copse, the site with the highest red-legged frequency, is identified in each equation as having a large residual (see final column of Table 4.18). A second analysis was then performed which excluded this site. These results are shown in Table 4.18.ii.

From Table 4.18.i, only soil texture and % urban area within 1.5km radius of the site are significant single predictors ($P = 0.017$ and 0.019 respectively). The correlation with red-legged frequencies is negative for soil texture and positive for urban topography.

Although the % woodland area within 1.5km radius of the site is never a significant single or partial predictor, it was found that combining "%wood" with "%urban" to produce the variable "%urban+wood" consistently improved the F-ratio and significance level (compare Predictions 1 and 3). Since both wooded and urbanised areas are likely to produce more equitable temperature conditions, a combination of these variables in the analysis was thought to be justified.

The new variable, %urban+wood, is always the most significant partial predictor in the regressions. Inclusion of pH *or* texture *or* eastings with %urban+wood improves the correlation of determination to around 25% (see Predictions 4, 5 and 6). As for Transects 1, 2, 3 and East Anglia, the interaction with distance east is negative. The interaction with pH and texture is also negative, which is consistent with the East Anglian results, i.e. the red-legged morph is associated with coarse-textured, acidic soils.

Inclusion of the variable, aspect, in three predictor equations (Predictions 7 and 8) increases the significance levels further and explains almost one-third of the variability in the data. Aspect is negatively associated, i.e. the red-legged morph appears to be favoured by more southerly and westerly facing sites. However, this variable is not in itself significant as a partial predictor ($P > 0.05$).

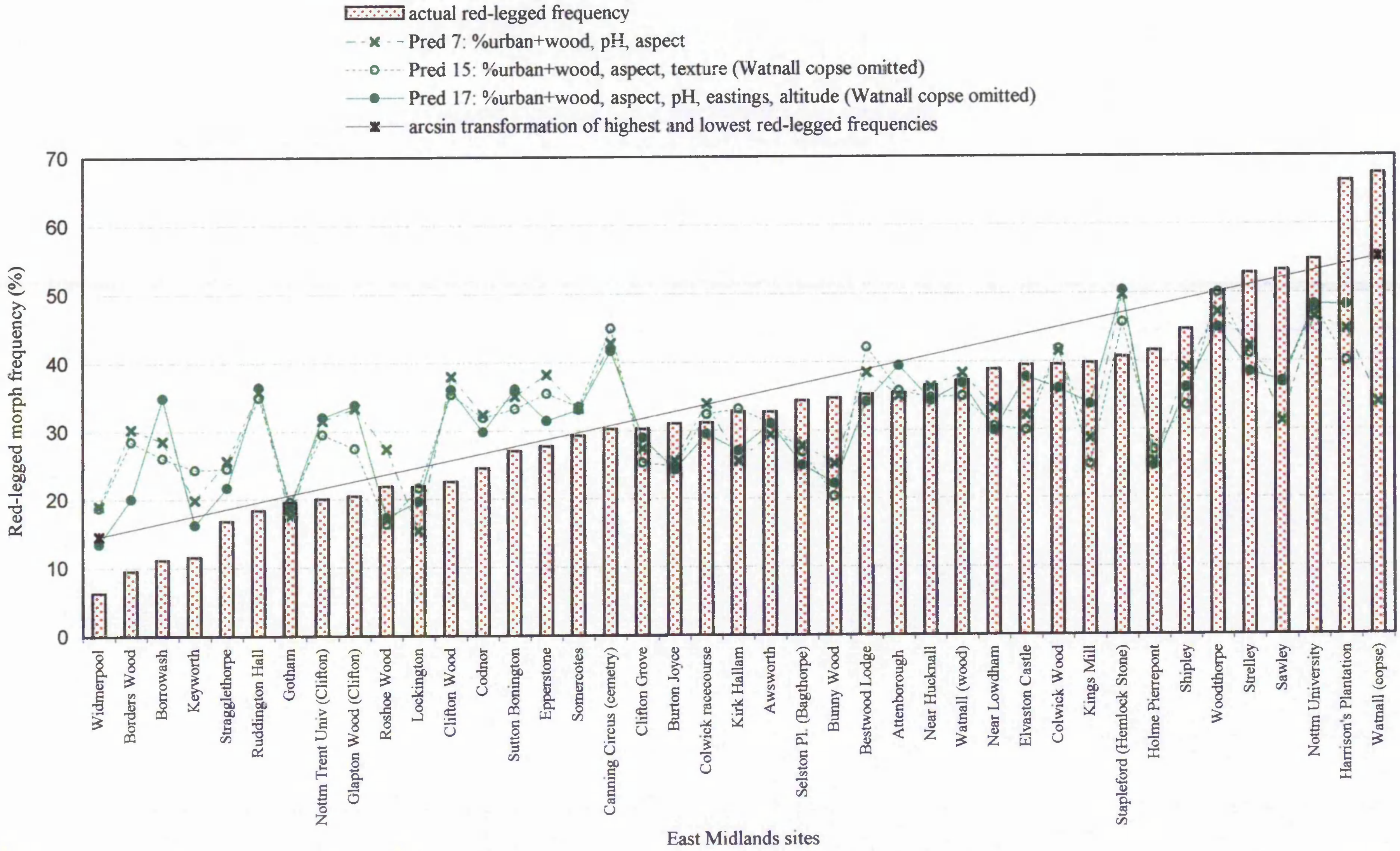
Fig 4.15 shows how Prediction 7 (%urban+wood, pH and aspect) models the East Midlands sites. The sites are ordered in increasing red-legged morph frequencies. It is clear from this figure that sites with low and high red-legged frequencies tend to be over- and under-predicted respectively.

As shown in Table 4.18.ii, exclusion of Watnall copse from the analysis improves the F-ratio and significance level of the regressions further (compare Predictions 9 to 17 with Predictions 1 to 8). The same variables are significant partial predictors, with %urban+wood the most influential predictor. As a single predictor, it explains almost one-quarter of the variability in red-legged frequencies (see Prediction 9), which is an improvement on the use of %urban alone (compare Predictions 9 and 10).

Table 4.18 The most influential predictors of red-legged morph frequency for the East Midlands. (Partial predictors for the multiple regressions are listed in order of decreasing significance; in italics, $P > 0.05$).

Pred-iction (Fig 4.14)	**Variables**	**P**	**Assoc-ation** (+/-)	**P (partial pred-ictor)**	**F-ratio**	**R^2 (R^2adj)**	**Observations with large residuals (R) or with strong influence (X)**
i) <u>Using all sites</u> (no. of observations = 41)							
One predictor:							
1	%urban+wood	0.009	+	0.009	7.53	16 (14)	R = Watnall Copse X = Woodthorpe, Canning Circus, Nottm Univ, Harrison's Pl
2	texture	0.017	-	0.017	6.18	14 (12)	R = Harrison's Pl, Watnall Copse
3	%urban	0.019	+	0.019	5.98	13 (11)	R = Watnall Copse X = Woodthorpe, Canning Circus
Two predictors:							
4	%urban+wood pH	0.006 0.041	+ -	0.004	6.33	25 (21)	R = Border's Wd, Watnall Copse
5	%urban+wood texture	0.027 *0.052*	+ -	0.005	6.07	24 (20)	R = Watnall Copse X = Harrison's Pl
6	%urban+wood eastings	0.006 *0.052*	+ -	0.005	6.07	24 (20)	R = Watnall Copse, Borrowash
Three predictors:							
7	%urban+wood pH aspect	0.002 0.035 *0.057*	+ - -	0.002	5.82	32 (27)	R = Watnall Copse
8	%urban+wood texture aspect	0.010 0.050 *0.067*	+ - -	0.003	5.51	31 (25)	R = Harrison's Pl, Watnall Copse
ii) <u>Excluding Watnall Copse</u> (no. of observations = 40)							
One predictor:							
9	%urban+wood	0.002	+	0.002	10.96	22 (20)	X = Woodthorpe, Canning Circus, Harrison's Pl
10	%urban	0.007	+	0.007	8.21	18 (16)	R = Harrison's Pl X = Woodthorpe, Canning Circus
11	texture	0.016	-	0.016	6.37	14 (12)	R = Borders Wd
Two predictors:							
12	%urban+wood aspect	0.001 0.042	+ -	0.001	8.20	31 (27)	
13	%urban+wood texture	0.007 *0.055*	+ -	0.001	7.87	30 (26)	R = Borders Wd X = Harrison's Pl
14	%urban+wood eastings	0.001 *0.058*	+ -	0.001	7.81	30 (26)	R = Borrowash
Three predictors:							
15	%urban+wood aspect texture	0.001 0.039 *0.051*	+ - -	0.001	7.29	38 (33)	R = Harrison's Pl
16	%urban+wood aspect pH	<0.001 0.035 *0.062*	+ - -	0.001	7.10	37 (32)	R = Sawley, Harrison's Pl
Five predictors:							
17	%urban+wood aspect pH eastings altitude	<0.001 0.027 0.050 *0.059* *0.107*	+ - - - -	0.001	5.71	46 (38)	R = Borrowash

Fig 4.14 Predicted red-legged morph frequencies for the East Midlands sites from regressions on actual frequencies using environmental data as predictors (See Table 4.17 for predictions). Pred = prediction.



Inclusion of two variables improved the coefficient of determination to around 30% (Predictions 12, 13 and 14), with aspect now becoming a significant partial predictor (Prediction 12). With three variables, the coefficient of determination increased to around 38% (Predictions 15 and 16). Inclusion of five variables explained almost half the variability in the data (Prediction 17). Altitude now becomes a negatively associated partial predictor, although its interaction with other partial predictors is weak ($P>0.05$).

Predictions 15 and 17 are modelled in Fig 4.14. Comparing Prediction 15 with Prediction 7, there is a small improvement at sites with red-legged frequencies at and below 20% (Border's Wood, Borrowash, NTU and Glapton). Otherwise, an improvement at some sites is balanced by a deterioration at others. The high frequency sites continue to be poorly modelled and Harrison's Plantation, which has the highest red-legged frequency, is identified as having a large residual (see Table 4.18).

Prediction 17 improves the modelling of four of the six sites with red-legged frequencies below 20% (Widmerpool, Border's Wood, Kegworth and Stragglethorpe). The Borrowash site, however, has an unusually large residual (see Table 4.18) and is over-estimated by 23%. At intermediate and higher frequencies, there is little evidence of a consistent improvement in the modelling. Nevertheless, the predicted frequencies of 28 (three-quarters) of the 41 sites fall within 10% of the actual frequency. The predictions deviate by more than 15% at five sites only - Borrowash, with an actual red-legged frequency of 11%, Ruddington Hall (18%), Holme Pierrepoint (42%), Sawley (53%) and Harrison's Plantation (66%).

In summary, regression analysis has succeeded in explaining nearly half the variability in morph frequencies of the East Midlands sites, when Watnall copse is excluded from the analysis. However, red-legged frequencies below 20% and above 50% are over- and under-estimated respectively. The poor modelling of extreme frequencies may be because the distribution of data sets in percentage form are usually not normal (Bishop, 1966). Arcsin transformation of the proportions at the ends of the frequency scale has the effect of raising the lower frequencies and reducing the higher frequencies (see Fig 4.14). Although this would give an actual frequency closer to the predicted frequencies, the consistent under- and over-estimations of frequencies from regression analysis would still occur. It is possible, therefore, that the relationship between morph proportions and environmental factors becomes non-linear at the edges of the frequency range in the East Midlands producing an inverse sigmoidal curve.

iii) Identification of environmental predictors of morph frequency in the East Midlands

Multiple regression analysis has identified a positive association between the red-legged morph and the more urban sites. The association is even stronger when these sites are also wooded. A more equitable aspect is also positively associated. The soil factors, pH and texture, could be important partial predictors, with the red-legged morph associated with acidic and coarse-textured soils. These soil types tend to be located in the city of Nottingham where the underlying rock is sandstone. There is also a negative association with distance east and altitude, although the latter variable appears only in the most complex regression (Prediction 17). Proximity to water courses (rivers, lakes and streams) was not found to be a significant factor. Nor was the organic content or % moisture retention of the soil.

These results suggest that the red-legged morph is favoured by regions within the East Midlands that are less exposed to the elements. This is consistent with findings for Transects 1, 2, 3 and the East Anglian sites (see Sections 4.4.1 to 4.4.5). However, there are two problems. Firstly, predictions are consistently poor for some individual sites, in particular Watnall copse. Secondly, the relationship between red-legged frequencies and the environmental factors identified by the analysis does not appear to be linear.

Poorly predicted sites might be explained by the type of land usage and disturbance that has occurred historically. For example, the copse site at Watnall was close to an extensive wood 120 years ago (O.S map, 1888) but was mostly cleared in the 1980s to make way for a new road (B600). Now, it is an isolated copse situated between the B600 and the original road. Both roads are adjacent to open fields and a small housing estate. During land clearance for development, the red-legged morph may have failed to adapt to the more open conditions, and remained in or retreated to small copses. This would raise the red-legged proportion of these habitats to a level that is not actually representative of this region of the East Midlands. Interestingly, the predicted red-legged frequencies for the site at Watnall wood are quite good (see Fig 4.14). This wood forms part of a private estate and has not experienced similar disturbance.

The poor predictions for the Borrowash site could also be due land disturbance. This site is located in a garden on a 1980s housing estate, which was built on land that had been entirely cleared for development (Kerr, pers. comm.). Possibly, only the black-legged morph survived such disturbance and - even though the gardens are now well established with shrubs, bushes and young trees – this morph remains in a higher than expected proportion today.

Obviously, it is not possible to explain away all the unusual site predictions in this way, but a factor that attempts to quantify the type and level of disturbance experienced by a site during its history could explain some of the variability in morph frequencies not accounted for by this analysis.

The second problem – the suggestion of inverse sigmoidal relationship between morph frequencies and environmental factors – suggests that there is a "cut-off" point where conditions become so unfavourable for one morph, selection against it is rapid. At intermediate frequencies, the difference in the fitness of the two morphs is relatively small.

Finally, the best prediction (Prediction 17) not only excluded Watnall copse, but also included five variables, two of which were not significant partial predictors at the 5% level. Two factors - %urban and %wood - have also been combined to produce one predictor. None of these predictors showed multicollinearity or high covariance. Although a multivariate analysis, such as Principal Components Analysis removes the problem of non-linearity, it was felt that this type of test is too open to subjective interpretation and would not improve on the identification of significant environmental factors affecting morph frequencies that was achieved by multiple regression analysis.

4.5 Discussion

Multiple regression analysis has been used as a diagnostic tool to identify the environmental factors that might be influencing the morph frequency variation in *Pterostichus madidus.* In general, the analysis has given consistent results regardless of the spatial resolution or time period of the region monitored. The red-legged morph tends to be in higher proportion in urban and wooded areas, while the black-legged morph appears to be better adapted to more exposed, intensively farmed regions. Regression of morph frequencies against climatic factors has identified temperature as the most influential factor, with a higher minimum temperature in cooler regions or periods and a lower maximum temperature in warmer regions or periods favouring the red-legged morph. The selection pressure also appears to be seasonal - often spring and winter, occasionally autumn, rarely summer - suggesting that selection occurs during the beetle's larval or pupal stage. There may also be selection pressure on the over-wintering adult female. Selection does not apparently take place during the reproductive phase, which occurs during July and August.

Regression analysis does not explain more than 50% of the variability in morph frequencies within a region when the data sets are large. There could be several reasons for this.

Firstly, most of the sampling occurred on one occasion only. An unrepresentative frequency for a site can be expected to occur occasionally, giving a large residual on the prediction. Even if the frequency obtained is representative, the standard deviation on the data will be ± 3 to 7 % of the mean frequency depending on the number of animals in the sample, which was sometimes low (see Fig 2.4, Section 2.5.3, p.50). Predictions within ± 10% of the actual frequency can, therefore, be regarded as a reasonable estimation.

Secondly, there are factors which can affect the micro-climate of a habitat that should, perhaps, have been included in the regressions. For example, dense ground and canopy cover could produce cooler conditions in the summer but protect a site against low temperatures in winter, due to the dampening effect of vegetation cover on temperature oscillations (Geiger, 1966). When Doberski & Gazzy (2000) recorded tree species, and the understorey/shrub and ground layers for experimental plots within two sites in Thetford Forest, they found a consistent *P. madidus* morph frequency for the plots within each site, regardless of vegetation type. The ground and air temperatures of these plots were not monitored. In this study however, the micro-climatic conditions of some sites may not be well represented by the published meteorological data of the nearest weather station. In order to standardise data between regions, the weather stations are sited in relatively open conditions (Meteorological Office, 1982).

Finally, the history of a site – whether or not it had been subjected to recent disturbance - might be important. This seemed to be the case for the Watnall copse site in the East Midlands (Section 4.4.5.iii).

Another problem with the regression analysis is that it assumes a straight-line relationship between the independent and dependent variables. In fact, an inverse sigmoidal relationship was indicated, as shown by the under- and over-estimations of higher and lower than average frequencies. This relationship is particularly noticeable for the East Midlands sites and sites close to the south coast (Transect 3), monitored in the late 1990s. At the more extreme frequencies, the favoured genotype in the population would be predominantly homozygous (whether dominant or recessive) – see Fig C.2 in Appendix C. This figure also shows that the change in frequencies of genotypes is not in a straight-line relationship at the extreme edges of the frequency range.

The high proportion of homozygotes at high and low frequencies might explain the sigmoidal effect, whereby conditions for one morph become disproportionately unfavourable. The rare morph is also more subject to catastrophic effects (e.g. Krebs, 2001). This, rather than insufficient variabilility in the environmental data sets used, is the more likely explanation for the poor predictions of extreme frequencies.

Although regression analysis has been useful for identifying factors that influence morph frequencies, it is not a powerful tool for making accurate predictions for sites or regions outside this study. There are clearly ecotypes of *Pterostichus madidus*, which are adapted to a particular temperature range within a region. For example, assuming temperature is the main factor affecting frequencies, one would not expect a higher red-legged morph frequency of 60% in Glensaugh, Scotland (Table 3.5, p.91) than the average red-legged frequency for sites in the East Midlands of England (31%). However, the results for the East Midlands show a considerable variation around the mean that is explained, in part, by the extent to which the site is open to the elements. In other words, adaptation of the morphs to temperature factors appears to be relative.

The direction of correlation between the red-legged morph and the mean minimum temperature gives further evidence of the relative nature of adaptation over time and space. The correlation was positive for all regions except Transect 2 monitored in 1995/6. Not only is this transect the warmest region monitored, but also the period of monitoring occurred in a warmer than average decade - the 1990s (see Figs 3.16 and 3.17 in Section 3.4.6, pp.94-5). Under these conditions, a higher minimum temperature appears to be no longer critical for the red-legged morph's survival.

The relative rather than absolute nature of the data might also explain why predictions of red-legged frequencies are more accurate for western sites compared with the eastern sites along the cooler more northern east-west transect, Transect 3. According to Gregory (1976), this region of Britain crosses at least four climatic zones: uplands, lowlands, urban and coastal regions. A straight-line relationship between these zones cannot be assumed.

For these reasons, the regression analysis was confined to regions or transects. An analysis of countrywide results simply would not give a high coefficient of determination and may miss environmental factors, which are more influential in one region than in another.

Nevertheless, regression analysis predicts that open, exposed areas within a region will have a higher than average black-legged morph frequency and less exposed areas will have a higher than average red-legged frequency, whatever the average is for that particular region. Exposed areas are defined here as intensively farmed with few woods, or only fragmented woodland. They may be at a higher altitude relative to the whole region, and/or be north or east facing. These areas are expected to experience higher maximum and lower minimum temperatures. Unexposed areas are defined as more wooded or urban, often situated at a lower altitude relative to the region; they are more likely to be west or south facing. These areas are expected to experience warmer temperatures and a narrower temperature range.

It was also found that the red-legged morph is associated with more western sites within a region. This is consistent with the climatic gradient of England and Wales, where a more continental climate is associated with easterly regions (Gregory, 1976). Although soil factors can be significant, with acid, coarse-textured soils favouring the red-legged morph, this association may be coincidental. Woods are commonly found growing on these soil types.

SECTION C

Laboratory work

CHAPTER 5: Investigation of the genetics of inheritance of leg colour in *Pterostichus madidus*

5.1 Aims

By conducting breeding experiments at various controlled temperatures:

i) To investigate the genetics of inheritance of leg colour in *Pterostichus madidus*.

ii) To examine the alternative hypothesis that leg colour may be induced by environmental conditions.

iii) To investigate the sex ratio of *P madidus.*

5.2 Introduction

It is not known whether leg colour in *P. madidus* is an example of environmentally induced or genetic polymorphism. If the former, morph frequency for any one year is simply a reflection of conditions earlier in that year. If the latter, it is not known whether the genes coding for melanin are dominant or recessive in this species. This knowledge is important for predictions about the rate of directional change. For example, dominant genes, if favoured, increase in frequency more rapidly than would recessive genes under equally favourable conditions, because they are expressed phenotypically in the heterozygote.

The standard method for identifying dominance and recessiveness is by mating adults of known phenotypes from wild populations. If there are only two phenotypes, simple Mendelian genetics predicts three genotypes will be present in the population: *AA* (dominant homozygote), *Aa* (heterozygote) and *aa* (recessive homozygote), where *A* denotes the dominant allele and *a* the recessive allele. Gene *A* is expressed phenotypically in the heterozygote. The inheritance pattern for two parental phenotypes is shown in Table C.1 of Appendix C. This assumes no mutation or other modification of the dominance relationships.

Assuming Mendelian genetics is operating, the proportion of homozygous to heterozygous dominants in a wild population is not known because both genotypes are phenotypically the same. However, the ratio can be estimated by reference to the Hardy-Weinberg Law, which predicts the allelic and genotypic frequencies of a large population in equilibrium (i.e. randomly mating and free from mutation, migration and natural selection).

The derivation of the Hardy-Weinberg Law is given in many texts (e.g. Russell, 1996). Using the symbols p for the dominant allele *A*, and q for the recessive allele, this law states that genotypic frequencies remain in a large, stable population in the proportions p^2 (frequency of *AA*), $2pq$ (frequency of *Aa*) and q^2 (frequency of *aa*). The sum of both the allelic frequencies and genotypic frequencies should be equal to 1 (i.e. $p + q = 1$, and $p^2 + 2pq + q^2 = 1$). Since $p = 1 - q$, the relationship between allelic frequencies and genotypic frequencies - hence the ratio of dominant homozygotes (p^2 or *AA*) to heterozygotes (pq or *Aa*) - can be calculated, provided the frequency of the recessive phenotype (q^2 or *aa*) is known. This relationship is shown in Fig C.2 of Appendix C.

The investigation of the genetics was part of a larger programme, the overall aim of which was to find the optimal environmental conditions for each developmental stage of *P. madidus* (see Chapter 6). This included:

1. An investigation under temperature and light conditions which were kept constant until late Instar 3, using parents of known leg colour (the constant temperature treatment).

2. An investigation under temperature and light conditions which more closely followed conditions in the field (the variable temperature treatment). This investigation also produced a number of emerged beetles from black-legged females who had mated with a male of unknown leg colour. Although not strictly part of the breeding programme, the results for leg colour of these emerged beetles are presented here.

5.3 Method

The constant and variable temperature treatments are termed C and V respectively throughout this chapter.

5.3.1 Laboratory conditions of adult females prior to breeding

Fertilised eggs have been found up to 4 months after the female's last contact with a male (Pudney, unpublished data), giving evidence that the female is able to store sperm. Thus, to be certain of the paternity of the larval offspring, females in their second year in the laboratory were used for breeding (in effect, over-wintered females).

Red and black-legged female adults of *Pterostichus madidus* were collected from the wooded and grassed areas of The Nottingham Trent University Clifton site (Grid Ref: SU456 351), which has a stable red-legged frequency of 23% (Pudney, unpublished data). Collection for the constant temperature treatments was between September and November 1994. For the variable temperature treatments, collection took place during July and August in 1995, 1996 and 1997.

After collection, the females were housed individually in petri dishes lined with agar to maintain a moist environment. The agar base was half-covered with a semi-circle of filter paper, which soaked up excess moisture from condensation. An upturned plastic spoon base was provided as cover for the beetle. The females were maintained in an incubator at a constant 12°C with a photoperiod of 16 hours light and 8 hours dark (16L 8D) until October. They were then transferred to a 10°C controlled temperature room at 12L 12D until used for breeding. Throughout their time in the laboratory, they were fed one live *Tenebrio* larva or ten live *Tribolium* larvae at approximately 10-day intervals.

5.3.2 Breeding

The types of breeding pairs are shown in Table 5.1. The symbols given are used throughout Chapters 5 and 6; the leg colour of the male is given first. Due to time constraints, the BR and RB breeding programme was not repeated for the variable temperature treatments.

For the BB, RR, BR and RB pairings, females who had not produced viable eggs during their 7 to 10 month period in the laboratory were selected for breeding. In order to simulate early summer conditions, they were moved to a 15°C incubator with a photoperiod of 16L 8D.

Table 5.1 **Types of breeding pairs of *Pterostichus madidus* at constant and variable temperature treatments.**

PARENTS	SYMBOL
i) Constant temperature treatment (1995)	
black-legged male x black-legged female	BB
black-legged male x red-legged female	BR
red-legged male x red-legged female	RB
red-legged male x black-legged female	RR
ii) Variable temperature treatment (1996/7/8)	
black-legged male x black-legged female	BB
red-legged male x red-legged female	RR
wild male of unknown leg colour phenotype x black-legged female	WB
wild male of unknown leg colour phenotype x red-legged female	WR

For the BB, BR, RR and RB pairs, red and black-legged males were collected from the same site as the females and given the laboratory conditions described in Section 5.3.1 for 14 days. One male was then housed with a female for 11 days. Copulation was observed in most cases. Only one male was given to each female. After breeding, any eggs laid were removed, placed in separate agar dishes and maintained at 7.5°, 10°, 15° or 20°C to within ± 5°C of the final temperature experienced by the larvae.

In addition to these breeding conditions, three laboratory-bred female beetles from the variable temperature treatment of 1996 (1 red and 2 black) were successfully mated with males of the same leg colour from the wild in 1997, giving one RR and two BB pairings. Attempts to breed emerged beetles from the constant temperature treatment and emerged males from either treatment failed.

Finally, the hypothesis of environmental induction of leg colour (aim ii) was tested by incubating the larvae of 1995/6/7 black- and red-legged females who had laid eggs shortly after collection. The leg colour phenotype of the male (termed W) is unknown. These pairings are termed WB or WR.

5.3.3 Larval and pupal rearing conditions

Sibling batches of hatched larvae were divided between temperature treatments within the C and V treatments (see Table D.1 in Appendix D).

There were four constant temperature treatments: C1 (7.5°C), C2 (10°C), C3 (15°C), and C4 (20°C). These temperatures were maintained until late Instar 3. Table D.1.i shows the day number (larval age) for changes in temperature and light conditions. At this stage, there were three and four sub-treatments given to the C2 and C3 larvae respectively (the 10° and 15°C treatments).

There were four variable temperature treatments: V1 (5°C start), V2 (7.5°C start), V3 (10°C start) and V4 (12.5°C start). As shown in Table D.1.ii, the temperature and light conditions were altered at regular intervals during larval development.

Treatments which failed to produce any emergence of the adult are also shown in the table.

Further details about the larval and pupal rearing conditions are given in Sections 6.3.2 and 6.3.3.

On emergence, the beetle was weighed and its sex and leg colour noted. It was then housed and fed as described for the other adults (see Section 5.3.1) .

5.3.4 Statistical analysis

Chi-squared analysis and t-tests were performed using Excel 95.

5.4 Results

Tables D.2 and D.3 in Appendix D give full details of the emerged adults for the constant and variable temperature treatments respectively. These are summarised in Table 5.2. In total, there were 64 emerged adults. None emerged from the BR and WR breeding pairs. In the latter case, this was due to an incubator breakdown in 1997, which led to heavy losses of larvae, including all the WR progeny.

Table 5.2 **Parentage and leg colour of emerged beetles and number of sibling batches per treatment.** (B = black; R = red; W = wild male of unknown leg colour phenotype; m = male; f = female).

	LEG COLOUR OF PARENTS:				
LEG COLOUR OF OFFSPRING:	Bm x Bf (BB)	Rm x Rf (RR)	Rm x Bf (RB)	Wm x Bf (WB)	TOTAL
Black	21	4	2	26	53
Red	0	7	4	0	11
TOTAL	21	11	6	26	64
No of sibling batches	*5*	*5*	*2*	*6*	

5.4.1 Emerged adults from RR and BB pairs

Two of the four RR pairs produced black-legged offspring (see Table 5.2). Assuming genetic polymorphism, this gives evidence that the genes coding for the red-legged morph are dominant. Since mutation is rare, such a result could only arise if both the red-legged parents of the black-legged offspring are heterozygous (*Aa* x *Aa*) and black is recessive (*aa*) - see Table C.1. The progeny of BB pairs should, therefore, be exclusively black-legged. As shown in Table 5.2, this is the case.

5.4.2 Emerged adults from mixed breeding pairs

No adults emerged from the BR breeding pairs and only six emerged from the RB pairs, four red-legged and two black-legged morphs (see Table 5.2). Of these, one sibling batch of three were red-legged. The two black-legs and fourth red-leg are from one or more RB pairing; their sibling status is therefore unknown.

These results are consistent with expectations if leg colour is inherited, but do not give any further information on the dominance relationships.

From mixed breeding pairs, the expected frequency of offspring with red legs depends on the ratio of homozygous to heterozygous dominants in the sample (see Table C.1, Appendix C). If red is dominant, the red-legged morph frequency of the progeny of RB pairs would be more than 50% provided a homozygous dominant was in the sample.

The converse would be true, of course, if the gene coding for black legs was dominant. Although the red to black leg colour ratio of the RB offspring is consistent with the observation that red is dominant, numbers are obviously too low for statistical analysis.

5.4.3 Emerged adults from WB pairs

All 26 offspring of the WB pair type were black-legged (see Table 5.2). This result concurs with the hypothesis that leg colour is inherited but, again, does not give information on which colour is dominant. This can be shown with reference to the Hardy-Weinberg principle, providing the assumptions of random mating and a large population in equilibrium are met.

The beetles were collected from a site where the red-legged morph frequency is stable at 23%. If red is dominant, black is the recessive *aa* genotype, with a frequency of 77%. The Hardy-Weinberg principle predicts that the frequencies of the red-legged *Aa* and *AA* genotypes would be 21.5% and 1.5% respectively (see Fig C.2, Appendix C). These genotypic proportions are shown in box 1 of Table 5.3.i.

The wild male of the WB pairs could be any of three genotypes, *AA*, *Aa*, and *aa*, giving three possible pairings. Box 2 of Table 5.3.i gives the predicted frequencies of the two leg colour morphs from these pairings. Note, if red is dominant, *aa* x *aa* can only produce black-legged morphs; *Aa* x *Aa* pairs produce only red-legs and *Aa* x *aa* produce red and black-legs in a 1:1 ratio (see Table C.1). Overall, the predicted red-legged morph frequency is 0.12 (12%).

Table 5.3 Predicted frequency of red-legged offspring of wild males of unknown leg colour phenotype x black-legged female in the laboratory calculated from Hardy-Weinberg predictions of genotype frequencies in a population where the red-legged morph is at a frequency of 0.23.

i) If red is dominant:

phenotype	black	red	red	*Total*
genootype	*aa*	*Aa*	*AA*	
proportion in field	0.770	0.215	0.015	*1.0*
proportion in lab	1.0	0.0	0.0	*1.0*

possible pairs †	*aa x aa*	aa x Aa	aa x AA	*Total*
offspring:				
black-legged morphs	0.770*	0.1075	0.000	**0.8775**
red-legged morphs	0.000	0.1075	0.015	**0.1225**
Total	0.770	0.215	0.015	***1.00***

* i.e. 0.77 x 1 = 0.77. All offspring would be *aa*, therefore black-legged in this example.

ii) If black is dominant

phenotype	red	black	black	*Total*
genootype	*aa*	*Aa*	*AA*	
proportion in field	0.230	0.499	0.271	*1.0*
proportion in lab	0.000	0.649	0.351	*1.0*

possible pairs †	*AA* x *AA*	*AA* x *Aa*	*AA* x *aa*	*Aa* x *AA*	*Aa* x *Aa*	*Aa* x *aa*	*Total*
offspring:							
black-legged morphs	0.095	0.175	0.081	0.176	0.243	0.075	**0.84**
red-legged morphs	0.000	0.000	0.000	0.000	0.081	0.075	**0.16**
Total	*0.095*	*0.175*	*0.081*	*0.176*	*0.324*	*0.149*	***1.00***

† First member of pair is in laboratory proportion; second member is in field proportion.

However, if black is dominant, a similar red-legged morph frequency is predicted. In this case, the black-legged female could be a dominant homozygote (*AA*) or a heterozygote (*Aa*), and could have mated with any of the three genotypes, giving six possible pair types.

The Hardy-Weinberg principle predicts that, in a population where the recessive phenotype is at a frequency of 23%, almost 50% of all beetles would be heterozygous dominants and 27% homozygous dominants, totalling 77% for the dominant phenotype (see Fig C.2). In the laboratory, only black-legged females were used, so the proportion of heterozygotes to homozygotes would be 0.65:0.35 [heterozygote fraction = (1.0/0.77) x 0.5 = 0.65; homozygote fraction = (1.0/0.77) x 0.27 = 0.35]. The genotypic proportions for the field males and laboratory females are shown in box 1 of Table 5.3.ii. Box 2 gives the predicted morph frequencies of the offspring for each possible pair, calculated from the product of the laboratory and field genotypes. The sum of the red-legged frequencies is 0.16 (16%).

So, from 26 emerged beetles, the expected number of red-legged offspring is around 3 if red is dominant, and 4 if black is dominant.

5.4.4 Induction of leg colour

Table D.4 in Appendix D gives a breakdown of the emerged adults by leg colour at each temperature and light treatment. These results do not support an alternative hypothesis that leg colour is induced by the temperature experienced during development.

As shown by rows 1, 2, 7 and 8 of Table D.4, BB parents produced only black-legged offspring regardless of the temperature at the initial and final stages of development. More significantly, red-legged offspring were produced only if one or both parents had red legs.

Both leg colour morphs emerged in four of the treatments, even though these varied considerably in their initial and final temperatures (see rows 1, 2, 6 and 7). The emergence of the three black-legged and three red-legged sibling offspring of an RR pair (row 6, see also Table D.3) is also inconsistent with the environmental induction hypothesis, since these larvae had experienced identical temperature and light conditions during development. By contrast, the leg colour of WB and BB siblings raised under different temperatures is identical (i.e. see Tables D.2 and D.3).

5.4.5 Sex ratio

As shown in Table 5.3, there were 27 male and 37 female emerged beetles in total, giving a male to female ratio of 0.72. The male to female proportion is not, however, significantly different to a 50:50 proportion ($\chi^2 = 1.563$; P = 0.2; d.f. = 2). Both morphs had a lower male to female ratio (0.83 and 0.70 for the red and black-legged morphs respectively). There was no difference between these male/female proportions ($\chi^2 = 0.058$; P = 0.81; d.f. = 2).

Table 5.4 **Sex of the emerged beetles by leg colour.**

	No. of males	No. of females
red-legged emerged beetles	5	6
black-legged emerged beetles	22	31
TOTAL	27	37

There is, however, a significant difference in the sex ratio between the constant and variable temperature treatments, which have a male to female ratio of 9:4 and 18:33 respectively ($\chi^2 = 4.892$; P = 0.03; d.f. = 1) – see final two columns of Table D.4. Under some environmental conditions, there may be a differential survival between males and females in their larval stages.

5.5 Discussion

The results of the breeding experiments give a strong indication that leg colour in *Pterostichus madidus* is an example of genetic polymorphism. There is no evidence that leg colour is induced by environmental conditions. This is not surprising given the unpredictable temperate climate of Britain. For example, an unusually cold winter does not predict a cold spring or summer.

The black-legged progeny of red-legged x red-legged pairs gives evidence that the genes coding for the red colour (or lack of melanin in the legs) are dominant. This is contrary to the usual finding that the melanic forms of polymorphic insect species are dominant (e.g. Kettlewell, 1961; Lus, 1932). However, Ford (1953) has shown that melanism can be controlled by recessives.

Assuming red is dominant, the red-legged progeny of the RB pairs would be heterozygous. The leg colour of these beetles was easily classified as red, suggesting that the inheritance pattern shows complete dominance. However, several workers (e.g. Terrell-Nield, pers. comm. and Doberski[1] pers. comm.) have speculated that a third morph is present in the population on the basis of observations that some morphs of *P. madidus* have brownish legs. The author has also found morphs with red femora but black tibiae. Although it is possible that these are examples of a third partially dominant morph comparable with the *insularia* form of *Biston betularia*, it can be postulated that partially red-legged forms are heterozygotes showing incomplete dominance. In monomorphic populations on the Isle of Mull, where only the red-legged form is present and must, therefore, be almost exclusively homozygous, the red colour of the legs appears brighter (Terrell-Nield, pers. comm.). There may, therefore, be some melanin production in the legs of the heterozygote but this is in too low a concentration to classify the colour as black by visual inspection.

The discovery that the red-legged phenotype may be dominant has implications when modelling the rate of directional change in morph frequency. Both the dominant and recessive phenotypes can be favoured depending on climatic conditions (see Chapters 3, 4 and 5). Although there is greater efficiency for selection of favourable dominant genes, because they are expressed phenotypically in the heterozygote, recessive genes - even when disadvantaged - are likely to remain in the population providing there is no linkage with deleterious dominant traits, i.e. there is no fixation. In fact, as shown from the countrywide monitoring (Chapters 3, 4 and 5), the black-legged phenotype is rarely absent.

Unlike melanism in *Biston betularia* and *Adalia bipunctata*, the direct adaptive value of leg colour in *P. madidus* is less obvious. However, if there is a pleiotropic gene influencing both leg colour and a physiological trait related to temperature, it cannot be presumed that the latter trait has the same dominance relationships. In other words, the heterozygote may be predominantly red-legged but could exhibit the physiological trait associated with the recessive black-legged phenotype.

The male to female ratio of emerged beetles for both morphs and for *Pterostichus madidus* as a species was not found to be significantly different from or similar to a 1:1 ratio, and there may be environmental conditions which favour or induce one sex over the other. This will be examined further in Chapter 6.

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CHAPTER 6 Growth, development and survival of *Pterostichus madidus* larvae under various temperature conditions.

6.1 Aims

i) To develop a successful method for rearing *Pterostichus madidus* larvae and pupae in the laboratory by identifying the optimal environmental conditions for pupation and emergence of the adult.

ii) To investigate the mortality, growth and developmental rates of the immature stages of *P. madidus* at various temperatures, which were kept constant until late Instar 3.

iii) To develop a model to predict the developmental rate of *P. madidus* larvae and pupae under different temperature conditions in the field.

iii) To test the model prediction by raising the immature stages of *P. madidus* under laboratory conditions that follow the external conditions of the seasons.

An overall aim was to identify any temperature-related difference between the *P. madidus* morphs in the immature stages by examining the mortality, growth and developmental rates of the offspring of parents of known leg colour.

6.2 Introduction

Carabid species have been broadly divided into spring and autumn breeders (e.g. Thiele, 1977). The two groups have fundamentally different life history tactics, the spring breeders having summer larvae and the autumn breeders having winter larvae. Often, the spring breeder needs to experience a photoperiodic change from short day (SD) to long day (LD) before reaching maturity. The converse is true for the autumn breeder. Paarman (1990) suggests that in the temperate zone, spring breeders evolved in areas with a continental climate whereas the autumn breeders are more abundant in areas with a maritime (Atlantic) climate. *Pterostichus madidus* is a Type 2 autumn breeder as described by Thiele (1977), i.e. a species with winter larvae, reproducing over summer and without an adult dormancy. However, as is the case for many carabid species (Den Boer, 1990), a proportion of the adult females hibernate over winter to resume reproductive activity the following summer. As the older beetles usually reproduce earlier in the summer than the callow individuals, there is an extended period of reproduction throughout the summer, with larval hatching from early autumn (primarily from older mothers) to early winter.

In order to synchronise the whole life cycle with the season, insects typically have periods of suppressed growth associated with diapause during one or more of their developmental stages (Tauber *et al*, 1986). In spring breeders, there is often a period of aestivation in the summer, which may be influenced by photoperiod and temperature. For autumn breeders, such as *P. madidus*, there is no summer adult diapause, but there may be a quiescent phase in the over-wintering larvae, which could be induced and/or terminated by photoperiod and/or temperatures. However, as described by Tauber *et al* (1986), there can be intraspecific variations related to the climatic conditions of the species, e.g. *Nebria brevicollis* does not show aestivation in Scandinavia, which is presumed to be an adaptation to the shorter summer in this region of Europe (Evans, 1965).

Finally, temperature affects the metabolic rate in poikilotherms and this has implications for each of the developmental stages of carabid beetles. In spring breeders with summer larvae, development is accelerated by rising temperatures (Thiele, 1977). However, as shown for *Pterostichus angustatus* and *P. oblongopunctatus*, the temperature requirements of the individual stages differ considerably, and represent adaptations to the temperature conditions in the field (Paarman, 1966, in Thiele, 1977). However, in *P. oblongopunctatus*, the third stage is most highly dependent on temperature, whereas in *P. angustatus*, the first stage requires higher temperatures. For the overwintering larvae of autumn breeders, a period of cold is often obligatory in order to overcome their larval dormancy (termed thermic hibernation parapause by Thiele). This has been shown for *P. melanarius* (Thiele & Krehan, 1969) and *P. niger* (Witzke, 1976, reported in Thiele).

Previous work has not developed a satisfactory method for raising the larvae of *Pterostichus madidus* to pupation and emergence (Luff, 1973; Terrell-Nield, pers. comm.). Luff raised *P. madidus* larvae in unlit incubators at constant temperatures (25°, 20°, 15°, 10°, and 5°C). The larvae were housed in petri dishes lined with moistened blotting paper and fed freshly killed, crushed worms. Only 4% of the larvae completed all the stages to emergence, mostly at 15°C. No emergence occurred at the extreme temperatures of 5° and 25°C.

Terrell-Nield raised 155 *P. madidus* larvae in petri dishes lined with agar and a semi-circle of filter paper. They were fed live animals - the larvae of the *Tribolium* beetle at first and second instar, and *Tenebrio* larvae (mealworm) at third instar. In an attempt to follow the external conditions of the seasons, the photoperiod of the incubator was gradually reduced from 17 to 7 hours of light per day, before being raised again. Following the same cycle, the temperature was lowered from 15° to 6.5°C, then increased. Only two of Terrell-Nield's larvae pupated. These did not survive to emergence.

Both Luff and Terrell-Nield found that the third instar larvae remained apparently fully-grown for a long time before dying or pupating. Luff estimated that if the rates of development in the laboratory were applied to the field, then pupation would not occur until September. However, adult emergence is known to occur around May (Greenslade, 1968; Luff, 1973). Luff attempted to speed up the development of *P. madidus* by chilling Instar 3 larvae, but the results were inconclusive. He suggested that *P. madidus* has a partial diapause in the third instar and that initiation of pupation is controlled by photoperiod.

Preliminary work to this investigation followed Terrell-Nield's method for raising *P. madidus* larvae, using temperatures of 15°C (Instar 1), 12°C (Instar 2) and 10°C (Instar 3). At third instar, it was found that *P. madidus* gained very little weight when fed on *Tenebrio* and *Tribolium* larvae. Nor did their weight increase when their diet was switched to an assortment of Diptera larvae obtained from soil samples, even though these organisms form part of the diet of *P. madidus* larvae (Luff, 1974). However, the larvae gained weight rapidly when given fresh worms (see Fig 6.3, Section 6.4). Choice chamber experiments showed that the larvae preferred live worms to crushed worms and, at all instar stages, chose moist soil substrates, which they burrowed into, in preference to agar or sand substrates (Gard, 1996). It was not clear, therefore, whether the failure of previous work was due to poor nutrition and unrealistic substrate conditions, rather than the temperature or light conditions provided.

The first stage of the investigation followed the method described by Luff (1973) but used a narrower temperature range and more natural rearing conditions. The mortality, growth and developmental rates of the immature stages of *P. madidus* reared under constant temperature conditions were examined in order to make predictions about the success of this species under different climatic conditions. These predictions were tested in the second stage of the investigation by raising the larvae under temperature and light conditions that followed seasonal conditions in the field. This method is similar to Terrell-Nield's though, again, more natural rearing conditions were provided. Also, initial temperatures were varied among treatments to represent the extensive period of egg hatching from late summer to late autumn.

Finally, it was hoped that knowledge of the leg colour of the parents would help identify any genetic correlation between the leg colour morph and larval development under different temperature conditions. However, many larvae failed to complete development to emergence. In the immature stages, the morphs are indistinguishable by eye and, assuming red is dominant and black is recessive (Section 5.4.1), the only certainty is that the offspring of black-legged parents are destined to be black-legged. The reasoning is as follows:

From Table C.1 (Appendix C), the offspring of two red-legged parents could be any of the three genotypes – dominant or recessive homozygotes or heterozygotes. The offspring of two black-legged parents would carry only the recessive genes (so black-legged). The offspring of mixed breeding pairs could be recessive homozygotes or heterozygous for red legs. Since the beetles were collected from a population where the black-legged, recessive phenotype was at a frequency of 77% (Section 5.3.1), the Hardy-Weinberg law predicts that the frequencies of the dominant homozygote and heterozygote will be 1.5% and 21.5% respectively (Fig C.2). So there is only a 1 in 14 chance that the red-legged beetles used in this investigation are homozygous, provided the assumptions of the Hardy-Weinberg law are met. Hence, there is a high probability that almost 25% of the offspring of red-legged parents and almost 50% of the offspring of mixed pairs will be black-legged.

6.3 METHOD

6.3.1 Investigation 1: Constant temperature treatments

i) Breeding and egg-laying

There were four types of breeding pairs used in the constant temperature treatments:

- black-legged male x black-legged female (BB);
- red-legged male x red-legged female (RR);
- red-legged male x black-legged female (RB);
- black-legged male x red-legged female (BR).

The symbols are used throughout this chapter; the male leg colour is given first. The laboratory conditions of the adults prior to and during breeding are described in Sections 5.3.1 and 5.3.2. After breeding, any eggs laid were removed, placed in separate agar dishes and maintained to within 5°C of the final temperature experienced by the larvae.

ii) Larval rearing conditions

Since the larvae are cannibalistic, they were removed from their siblings within a few days of hatching and transferred individually to 100ml sample jars half filled with a mixture of compost soil and moist vermiculite to limit moisture loss (100g dry vermiculite + 300g water + 800g compost soil). Third instar larvae were transferred to 150ml sample jars (see Plate 6.1) and a stone was placed on top of the soil to provide cover.

Plate 6.1 **Rearing conditions at third instar: 150ml sample jar containing soil/vermiculite mixture.**

As described in Section 5.3.3, four constant temperature treatments were given until late Instar 3: 7.5°C (C1), 10°C (C2), 15°C (C3) and 20°C (C4) – see Table D.1 in Appendix D. The larvae were housed in incubators at 7.5° and 20°C, and in constant temperature rooms at 10° and 15°C. The light conditions were 12 hours light and 12 hours dark for the 10°, 15° and 20°C treatments, and 8 hours light 16 hours dark for the 7.5°C treatment. Depending on numbers, sibling batches were divided between 2, 3 or 4 temperature treatments. To avoid bias from less viable batches, each treatment contained a minimum of three batches. Table 6.1 summarises these conditions and gives the initial number of larvae and sibling batch per breeding pair.

Table 6.1 Investigation 1: Temperature and light treatment given to the larval offspring of breeding pairs of *Pterostichus madidus*, and initial number of larvae and sibling batches per treatment.

Breeding pair	Number of larvae per treatment (in brackets, number of sibling batches)			
BB	21 (4)	25 (4)	27 (4)	23 (5)
RR	16 (4)	21 (5)	25 (5)	22 (6)
RB	20 (5)	21 (5)	22 (5)	23 (6)
BR	15 (3)	18 (4)	30 (4)	21 (4)
Treatment code	C1	C2	C3	C4
Temperature (°C)	7.5	10	15	20
Photoperiod	8L 16D	12L 12D	12L 12D	12L 12D

The larvae were fed excess live red worms obtained from Matchman Supplies (West Bridgford, Nottingham). The first and second instar larvae were inspected every seven days and the final instar was inspected fortnightly. At each inspection, the soil mixture was changed and worms added. The larva was weighed using a four-figure balance (Sartorius analytic) and the developmental stage noted. At instar change, if cuticle hardening had occurred before inspection, the mid-point between the dates of inspection was designated as the day of ecdysis and weight at instar change was the average of weights obtained before and after ecdysis.

Due to the high mortality rate of the faster developing 20°C larvae during late Instar 3, temperature and light conditions were altered for the remaining treatments to investigate whether a change in photoperiod and/or temperature initiated pupation. The new conditions and numbers of larvae remaining per treatment are shown in Table 6.2.

Because more eggs hatched than could be reared using the method described above, the later hatchlings were placed in aquarium tanks (23cm x 20cm x 20cm) in batches of 30 and maintained at 10°C, 12L 12D. The tanks were a third filled with the compost/vermiculite mixture and 30 worms per week were added. None of the larvae reared in tanks survived after 200 days due mainly to cannibalism. However, 7 BB and 9 RB larvae were recovered from their respective tanks at around 60 days, housed individually in jars, and maintained at 10°C in constant light. This treatment is C2.ii in Table 6.2, also referred to as "tank" larvae.

Table 6.2: Investigation 1: Initial and final treatments given to larvae, showing day number, degree-day and initial number of larvae per breeding group when final treatment started. (L = light; D = dark).

Initial treatment		Final treatment			Day no from hatching	Degree day from hatching	Number of larvae per group at treatment change				
Temp (°C)	Light	Treat-ment	Temp (°C)	Light		(Cd)	BB	RR	RB	BR	Total
7.5	8L 16D	C1	10	12L 12D	256	1920	6	3	5	3	***17***
10	12L 12D	C2.i	10	12L 12D	163	1630	8	7	3	5	*23*
		C2.ii (tank larvae)	10	24L	63	1630	8	5	4	4	*21*
		C2.iii	15	12L 12D	163	1630	8	6	4	4	*22*
		C2.iv	15	16L 8D	163	630	10	-	10	-	*20*
						total	*34*	*18*	*21*	*13*	***86***
15	12L 12D	C3.i	15	12L 12D	142	2130	7	6	4	8	*25*
		C3.ii	10	12L 12D	142	2130	6	6	5	6	*23*
		C3.iii	15	16L 8D	142	2130	8	7	5	7	*27*
						total	*21*	*19*	*14*	*21*	***75***
20	12L 12D	C4	no treatment change								

iii) Pupal conditions and emergence

Any pupae found were transferred to the agar petri dishes lined with filter paper. On emergence, the beetles were weighed, and their condition, sex and leg colour noted.

6.3.2 Investigation 2: Variable temperature treatments

i) Breeding and egg-laying

There were two types of breeding pairs used in the variable temperature treatments:

- black-legged male x black-legged female (BB);
- red-legged male x red-legged female (RR);

In addition, larvae of females who had laid eggs shortly after collection were incubated, primarily to test the hypothesis of environmental induction of leg colour (see Aim ii, Section 5.1). These pairings are termed:

- wild male of unknown leg colour phenotype x black-legged female (WB);
- wild male of unknown leg colour phenotype x red-legged female (WR);

The laboratory conditions of the adults have been described in Sections 5.3.1 and 5.3.2. Eggs laid were removed, placed in separate agar dishes and maintained to within $5^{o}C$ of the initial temperature experienced by the larvae.

ii) Larval rearing conditions

The larvae were reared individually in sample jars as described in Section 6.3.1, with inspection and feeding once every 4 weeks. Four variable temperature treatments were given: V1 ($5^{o}C$ start), V2 ($7.5^{o}C$ start), V3 ($10^{o}C$ start) and V4 ($12.5^{o}C$ start). The initial temperature conditions were intended to represent soil temperatures that may occur in the field, depending on the month of hatching where $12.5^{o}C$ approximates September, $10^{o}C$ approximates October, $7.5^{o}C$ approximates November and $5^{o}C$ approximates December. Table 6.3 summarises the temperature and light conditions throughout each treatment.

Table 6.4 gives the initial number of larvae and name of each sibling batch for each type of breeding pair - see Table D.2 in Appendix D for full explanation of naming system. Some females produced more than one batch of eggs. The first batch is termed b1, the second – b2, etc. Where possible, larval batches were divided between treatments. Part way through the investigation, the incubator that was maintaining larvae at $5^{o}C$ broke down. The larval batches affected were from the 7.5, 10 and $12.5^{o}C$ start temperatures and are shaded in Table 6.4. Most the larvae were found dead; the few survivors failed to thrive and died within 3 weeks.

Table 6.3: Investigation 2: Variable temperature and light treatments given to the larval offspring of breeding pairs of *Pterostichus madidus*, showing number of days spent at each temperature and photoperiod. (L = light; D = dark; ✓ indicates conditions for each treatment during immature stages).

Month represented	Sept	Oct	Nov	Dec/Jan/ Feb	March	April	May	June/ July
Temperature (°C)	12.5	10	7.5	5	7.5	10	12.5	12.5 to 15
Photoperiod	14L 10D	12L 12D	10L 14D	8L 16D	10L 14D	12L 12D	14L 10D	16L 8D
No. of days	30	30	30	90	30	30	30	until emergence
Treatment:								
V1				✓	✓	✓	✓	✓
V2			✓	✓	✓	✓	✓	✓
V3		✓	✓	✓	✓	✓	✓	✓
V4	✓	✓	✓	✓	✓	✓	✓	✓

Table 6.4: Investigation 2: Initial number of larvae from each sibling batch per variable temperature treatment. See Table D.2 for explanation of naming system.
Shading denotes larval batches affected by incubator breakdown at some stage during their development.

Treatment code	V1		V2		V3		V4	
Initial temperature (°C)	5		7.5		10		12.5	
Breeding pair	name of pair	no. of larvae	name of pair	no. of larvae	name of pair	no. of larvae	name of pair	no. of larvae
BB	-	-	-	-	BB(14.2)-b1	15	BB18-b1	10
					BB(14.2)-b2	3	BB18-b2	1
					BB(14.2)-b3	4		
					BB(14.4)-b1	18		
					BB(14.4)-b2	5		
					BB18-b1	10		
RR	RR(9.6)-b2	11	RR9-b1	9	RR6-b1	12	RR9-b2	10
					RR6-b2	10		
					RR9-b1	6		
					RR9-b2	5		
					RR18	9		
WB	WB12	10	WB2-b1	14	WB1	2	WB1	3
	WB13	8	WB3	2	WB2-b1	10	WB2-b2	8
	WB25	3	WB12	8	WB3	9	WB3	7
			WB14-b1	9	WB5	4	WB5	4
			WB14-b2	5	WB12	9	WB12	8
					WB14-b1	10	WB14-b1	5
					WB14-b2	8	WB14-b2	3
					WB140	4	WB120	1
							WB140	8
WR	-	-	WR14	4	WR17	4	WR14	6
					WR101	7	WR101	8
					WR103	4	WR103	3

iii) Pupal conditions and emergence

It was observed from Investigation 1 that, before pupation, the larva made a hollow beneath the stone provided on top of the jar or alongside the base of the jar (Plate 6.2.i). If left undisturbed, the larva pupated and emerged in the hollow (Plates 6.2.ii and 6.2.iii). In this investigation, the pupa was not disturbed and completed development *in situ* in the sample jar. On emergence, the beetle was weighed, and its condition, sex and leg colour noted.

6.3.3 Calculations and statistical analysis

i) Pre-reproductive mortality

To examine the sequential effects of the mortalities at each stage, a pre-reproductive mortality for each treatment was calculated by k-factor analysis as follows:

$$k = \log (N_{j-1}) - \log(N_j) \qquad \text{(eq 6.1)}$$

where k is the pre-reproductive mortality

N_j is the number of larvae at the development stage j

N_{j-1} is the number of larvae at the previous development stage

10^{-3} was substituted when N_j was zero, on the assumption that 1 in 1000 larvae would survive even extreme conditions.

ii) Thermal time

As the metabolic rate in poikilotherms is controlled by external temperatures, it is often more appropriate to measure the developmental rate in terms of thermal time (degree days) where:

$$\text{degree-days (Cd)} = \text{temperature } (^{o}\text{C}) \times \text{number of days (d)} \qquad \text{(eq 6.2)}$$

iii) Statistical analysis

Where appropriate, regression analysis and t-tests were performed using Excel 97. Chi-squared analysis was performed on Minitab version 13.

6.4 Results

Sections 6.4.1 to 6.4.3 examine the results for the constant temperature treatments. Section 6.4.1 considers all larvae at each developmental stage. Section 6.4.2 considers differences between the larval groups by leg colour of the parents. The development of *P. madidus* under optimum temperature conditions in the laboratory is compared with seasonal temperatures in the field in Section 6.4.3. Section 6.4.4 examines the results for the variable temperature treatments. The condition and developmental history of emerged adults from the constant and variable temperature treatments are considered in Sections 6.4.5 and 6.4.6 respectively.

Regular monitoring of larval weights gave evidence of a lengthy, pre-pupal quiescence during third instar, when the larvae stopped feeding or slowed down their feeding rate. Results for Instar 3 have therefore been separated into two phases: the growth phase (Instar 3.i) and the quiescent phase (Instar 3.ii).

6.4.1 Constant temperature treatments: survivorship, growth and developmental rates of *P. madidus* from Instar 1 to emergence.

For easier reference, tables and graphs showing the survivorship, development and growth rates for all the immature stages of *P. madidus* at each constant temperature treatment have been placed at the end of Section 6.4.3 (page 212) and are organised as follows:

- The results for the survivorship and pre-reproductive mortality of the larvae by breeding group are presented in Table 6.5 and summarised for all larvae in Fig 6.1.
- The average time for each breeding group to complete each developmental stage under different temperature conditions is given in Table 6.6 and summarised for all larvae in Fig 6.2. For comparison, Luff's 1973 results, from which thermal developmental times have been calculated, are also presented.
- Mean weights of the larvae at each instar change are given in Table 6.7. Growth rates measured in mg per degree-day (see Section 6.3.3) are shown in Fig 6.3. The slopes in Fig 6.3 have been calculated and are given in Table 6.8. A linear relationship for weight gain between instars has been assumed. However, comparable with *Abax ater* (Chaarbane *et al*, 1994), the rate of weight increase of *P. madidus* larvae was actually sigmoidal in each instar due to a non-feeding phase shortly before and after instar change.
- Figs 6.4 to 6.7 compare the means for growth and development over thermal time of the four breeding groups, showing each temperature separately.
- Tables and graphs specific to a developmental stage are placed with the text.

i) Instar 1

On average, *Pterostichus madidus* larvae weigh 3.5 mg on hatching (Table 6.7). Because earlier hatchlings within a batch may have fed on the agar substrate or siblings before weighing, analysis by temperature treatment is not appropriate,

10° and 15°C treatments. The 10° and 15°C Instar 1 larvae had the lowest mortality, with around 85% surviving to Instar 2 (Table 6.5.i). They also took fewer degree-days to develop to second instar and achieved the highest weights at instar change (see means for all larvae in Tables 6.6.i and 6.7). For both temperatures, there was a fivefold increase in larval weight from hatching to instar change and a growth rate of about 0.04mg/Cd (Table 6.7). The close similarities in the results at these temperatures are clear from Figs 6.1, 6.2.i and 6.3.

20°C treatment. Although the larvae reared at 20°C completed first instar in the fewest number of days, significantly more degree-days were required compared with the 10° and 15°C treatments (20° v 10°C: $t = 2.267$; $P<0.05>0.02$; 20° v 15°C: $t = 3.289$, $P<0.02>0.01$) – see Table 6.6.i and ii. There was also a slightly (albeit not significant) lower mean weight at instar change compared with larvae from the 10° and 15°C treatments (Table 6.7). As a consequence, the growth rate is reduced to 0.03mg/Cd (Table 6.8). These results, combined with a slight decrease in survival to around 70% (Table 6.5.ii), suggest that this temperature is becoming sub-optimal (see also Figs 6.1, 6.2.i and 6.3).

7.5°C treatment. At 7.5°C, only 40% of the first instar larvae survived to Instar 2 (Table 6.5.ii). Almost double the number of degree-days was required to complete development compared with the 10°, 15° and 20°C treatments (Table 6.6.ii). In each case, the critical value for *t* was exceeded giving a value of $P<0.001$. With only a fourfold increase in weight from hatching to instar change and less than half the growth rate of the 10° and 15°C treatments (Table 6.8), the mean weight of these larvae at instar change is significantly lower compared with the other temperatures (see Table 6.7, for each test, $P<0.01>0.001$).

Overall, 10°C and 15°C are identified as being within the optimum temperature range for the Instar 1 larvae used in this investigation, with some metabolic stress experienced at 20°C and considerable stress at 7.5°C. As shown in Fig 6.2.i, Luff's 1973 results for the number of degree-days to complete development at 15° and 20°C are very comparable. However, his 10° and 5°C larvae developed faster than those raised at 15° and 20°C, giving a strong indication that Luff's first instar larvae are better adapted to lower temperatures.

ii) Instar 2

10°C treatment. The lowest mortality, shortest thermal developmental time and most efficient metabolism occurred at 10°C (Tables 6.5.ii and 6.6.ii; Figs 6.1, 6.2.ii and 6.3). The mean weight of the larvae increased by a factor of 3.2, and the growth rate of 0.09mg/Cd is more than double the growth rate for the first instar at this temperature (Table 6.8).

15°C treatment. Survival at 15°C was also good (see k-value in Table 6.5.ii and Fig 6.1). The mean weight at instar change is similar to the mean weight for the 10°C larvae (Table 6.7), having increased by the same factor (Table 6.8). However, as shown in Fig 6.3, the metabolism of the 15°C larvae was slightly less efficient due to a slower thermal developmental time (a mean of 572 Cd compared with 393 Cd at 10°C; $t = 6.660$, $P<0.001$ – see Table 6.6), resulting in a reduction in the growth rate to 0.07mg/Cd (Table 6.8).

20°C treatment. The longest thermal time (921 Cd – see Table 6.6) and highest mortality occurred at 20°C, with almost half the sample dying at this temperature (Table 6.5, Fig 6.1). As shown in Table 6.7, the mean weight by ecdysis is significantly lower than weights for larvae reared at 10° and 15°C (20° v 10°C: $t = 2.671$, $P<0.01>0.001$; 20° v 15°C: $t = 4.412$, $P<0.001$) and is now comparable to the lower weight of the 7.5°C larvae. There has been no increase in the growth rate from Instar 1 (see Fig 6.3) so, despite the longer thermal period spent at this instar, the factor for weight increase is reduced to 2.8 (Table 6.8).

7.5°C treatment. The k-value for the 7.5°C larvae, although higher than the values for larvae maintained at 10° and 15°C, has fallen relative to Instar 1 (Table 6.5.iii). The overall survival of these larvae is therefore now not much lower than that for the 20°C larvae (Fig 6.1). The thermal time to complete development is comparable between the 7.5°C and 15°C larvae (Table 6.6), and the growth rate (0.06mg/Cd) has almost trebled compared with first instar (Table 6.8 – see also Fig 6.3). The mean weight at instar change continued to be significantly low compared with the 10°C and 15°C larvae (for both tests, $P<0.001$), but it has increased by a similar factor (3.3) – see Tables 6.7 and 6.8. If the maximum weight gain is in a fixed proportion between developmental periods, then the relatively low mean weight for the 7.5°C larvae at instar change is simply a consequence of the low initial weight.

Overall, the second instar larvae are adapted to a lower temperature range compared with Instar 1, 10°C being the optimum temperature within a 7.5° to 15°C range. At 20°C, the larvae are experiencing considerable metabolic stress comparable to the stress shown by first instar larvae reared at 7.5°C. Luff (1973) also noted that Instar 2 larvae appear to be adapted to lower temperatures, but concluded from the shorter number of days to complete development that 15°C was the optimum compared with 20°C at Instar 1 (see results for Luff in Table 6.6.i). In fact, when measured in degree-days, Luff's larvae continued to develop faster at 5°C, with the thermal time to complete development increasing exponentially with rising temperature. This is shown in Fig 6.3.ii.

iii) Instar 3.i (growth stage)

There was a high survival rate of around 90% at 7.5°, 10° and 15°C reduced to 66% at 20°C (Table 6.5.ii). At all temperatures, the mean weight of the larvae increased by a similar factor (2.3 to 2.4 – see Table 6.8), suggesting that the low final weights of 105mg for larvae maintained at 7.5° and 20°C are a result of the relatively poor growth at earlier instars (Instar 1 for the 7.5°C larvae; Instar 2 for the 20°C larvae). The main difference between the treatments is, in fact, the number of degree-days to complete the growing phase.

7.5° and 10°C treatments. The 7.5°C larvae spent the least number of degree-days at this phase (426Cd – see Table 6.6.ii), and had one of the fastest growth rates (0.14mg/Cd, Table 6.8). The 10°C larvae attained a similarly fast growth rate of 0.14mg/Cd, but a significantly longer thermal time of 565Cd was required ($t = 4.267$, $P<0.001$- see Table 6.6.ii). That this has not affected the fitness of the 10°C larvae suggests plasticity in the thermal time to quiescence. Assuming a limitation on the growth rate at each temperature (presumed to be around 0.14mg/Cd for the two lower temperatures), a higher initial mean weight would require more degree-days to attain a weight increase by the same factor.

15°C treatment. The growth rate of the 15°C larvae has slowed to 0.09mg/Cd (Table 6.8). However, their mean weight at the end of Instar 3.i is as high as that for the 10°C larvae (Table 6.7) due to the longer thermal time spent on this phase (823Cd compared with 565Cd for the 10°C larvae; $t = 7.293$; $P<0.001$) - see Table 6.6.ii and Fig 6.3. This suggests that the 15°C larvae are experiencing some metabolic stress, but thermal time to complete the growth phase of Instar 3 has been lengthened to compensate for the reduction in metabolic efficiency.

20°C treatment. At 20°C, the thermal time for completion of Instar 3.i was as short as that for the 10°C larvae (Table 6.6.ii), but the growth rate is reduced to 0.10mg/Cd, comparable with the growth rate for the 15°C larvae (Table 6.8 and Fig 6.3). Although the weight gain factor of 2.3 is similar to the other temperature treatments, the higher mortality, combined with the slower growth rate, suggests that 20°C is sub-optimal for Instar 3.i larvae.

There is, therefore, a reduction in metabolic efficiency for Instar 3.i larvae at higher temperatures. This appears to be compensated for by plasticity in the number of degree days spent in this phase, allowing the larvae to increase their weight by a factor of around 2.4 even at sub-optimal temperatures. This contrasts with the earlier instars, when a decrease in growth and developmental rates results in a reduction in the mean larval weight at the end of the developmental stage.

iii) Instar 3.ii (quiescence) to pupation and emergence

Quiescence is defined here as the later period during Instar 3 when there is no further weight increase (see Fig 6.3). During quiescence, the larva made a hollow beneath the stone provided or alongside the base of the jar (Plate 6.2.i), and maintained its weight by occasional feeding. Feeding also occurred shortly before pupation, when there was a slight gain in weight. If left undisturbed, the larva pupated and emerged in the hollow (Plates 6.2.ii and 6.2.iii).

As shown in Fig 6.3 and summarised in Table 6.9, the onset of quiescence occurred at around 1550 Cd at 7.5°C, 1300 Cd at 10°C, 1710 Cd at 15°C and 1880 at 20°C, the equivalent of 207, 130, 114 and 94 days for the four temperatures respectively. The 10°C group's entry into the quiescent phase is significantly earlier than all the other treatments ($P<0.05$). However, there is clearly flexibility over the timing of quiescence, the earliest entry (10°C group) and one of the later entries (15°C group) having the lowest accumulative k-values (Fig 6.1.ii) and the highest mean weights on onset (Fig 6.3).

Table 6.9 Mean accumulative degree-days for onset of quiescence under constant temperatures, showing 1 standard error (s.e.) on the mean.

Temperature until late Instar 3 (°C)	7.5	10	15	20
mean degree days for larvae that survived to pupation	1556	1299	1716	1880
s.e.	*81.5*	*38.6*	*61.3*	*75.9*

Plate 6.2.i Quiescent period of *Pterostichus madidus* larva during Instar 3

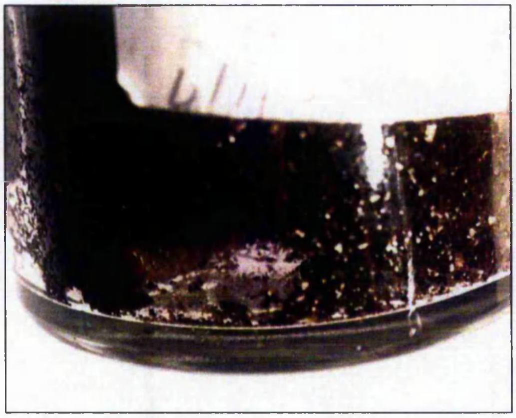
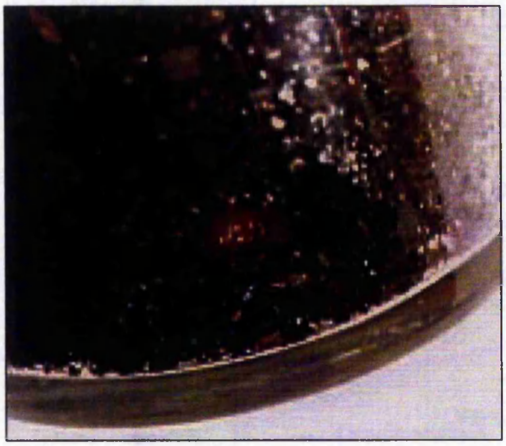


Plate 6.2.ii *Pterostichus madidus* pupa

Plate 6.2.iii Emergence of adult *Pterostichus madidus.*



Only 32 out of a total of 199 *P. madidus* larvae entering quiescence pupated (Table 6.5.i), the chance of pupation decreasing with increasing temperature treatment (Fig 6.1.ii). None pupated from the 20°C treatment. A poorer adaptation to higher initial temperatures could also be indicated by Fig 6.2.iii, which shows that the number of degree-days spent at Instar 3.i and 3.ii before pupation increased with increasing temperature, with highly significant differences between the temperature treatments (in all cases, $P<0.001$). By contrast, there is little difference between Luff's 10°, 15° and 20°C 1973 treatments, which produced similar results to the 10°C treatment of this investigation. However, this may be due to the methodology; there had been no increase in the photoperiod for Luff's larvae.

In this investigation, various temperature and photoperiod treatments were given during Instar 3 to the 7.5°, 10° and 15°C larvae (see Table 6.2 in Section 6.3.1). With the exception of the "tank" larvae (C2.ii), the changes were made *after* the onset of quiescence. The sub-treatments were not consistent between treatments because of limitations on the use of incubators as well as the need to have some successful. It is therefore not possible to draw conclusions about the pupal developmental times for each constant temperature treatment given in Table 6.6. As shown in Table 6.2, all the pupae were maintained at 10 or 15°C regardless of their initial constant temperature treatment. The decision to alter the temperature and light conditions of the treatments came after the losses of the 20°C larvae.

The results for pupation and emergence under each temperature and light condition are shown in Table 6.10. The mean accumulative degree-days at death for the non-pupating larvae is also given. Comparing the mean thermal time of death for the non-pupating larvae (column 11 of Table 6.10) with the means for successful pupations (column 7), it is clear that most larvae survived at least to the period when pupation would be expected to occur. Failure to pupate, therefore, is the main factor contributing to the high mortality.

C4 treatment (20°C). None of the 20 larvae from this treatment pupated. The mean thermal time of death at 2740 Cd was within the normal range for the constant temperature treatments – see column 11. The high mortality is therefore due to a failure to pupate, rather than any other stress factor at this temperature.

C3 treatment (15°C start). Only 2 of the initial 65 larvae pupated. These were from the C3.i and C3.ii sub-treatments. In neither case had the photoperiod been increased. The one pupa which emerged had actually experienced a fall in temperature to 10°C (C3.ii) but, compared with the emerged adults from C1 and C2.ii, almost double the number of degree days were required to complete development.

Table 6.10 **Constant temperature treatments: Results of sub-treatments given to Instar 3.ii larvae, showing initial numbers, survival and accumulative degree-days for each stage and the mean thermal time of death for non-pupating larvae.**

Sub-treatments are listed in ascending order of successful pupations (column 5). Codes for sub-treatments are the same as those in Table 6.2 (Section 6.3.1).
s.e. = standard error.

1	2		3	4		5	6	7		8	9	10		11	
Sub-treatment code	Initial treatment		Initial nos. at Instar 3.ii	Treatment at Instar 3.ii		No. pupated	% survival from Instar 3.ii	Mean no. of degree days at pupation		No. emerged	% survival from Instar 3.ii	Mean no. of degree days at emergence		Mean no. of degree days at death for non-pupating larvae	
	Temp (°C)	Light		Temp (°C)	Light			(Cd)	s.e.			(Cd)	s.e.	(Cd)	s.e.
C4	20	12L 12D	20	20	12L 12D	0	0%							2740	103.4
C3.iii	15	12L 12D	27	15	16L 8D	0	0%							3303	76.7
C3.ii	15	12L 12D	23	15	12L 12D	1	4%	3720	-	0	0%			3326	106.2
C3.i	15	12L 12D	25	10	12L 12D	1	4%	3985	-	1	4%	4400	-	3709	118.0
C2.i	10	12L 12D	23	10	12L 12D	2	9%	3270	113.0	1	4%	3510	-	2815	139.3
C2.iii	10	12L 12D	21	15	12L 12D	3	14%	2797	192.5	0	0%			2888	86.5
C2.iv	10	12L 12D	22	15	16L 8D	5	23%	2697	117.6	1	5%	2568	-	2723	85.7
C2.ii	10	12L 12D	20	10	24L 0D	8	40%	2460	94.8	5	25%	2746	141.4	2625	70.3
C1	7.5	8L 16D	17	10	12L 12D	11	65%	2291	50.5	6	35%	2594	31.1	2625	59.5

C2.i, C2.iii and C2.iv (10°C start). A poor survival rate to pupation (9%) and relatively long thermal time to pupation and emergence (respectively 3270Cd and 3510Cd) occurred for C2.i, the 10°C larvae, which were maintained at this temperature with no increase in the photoperiod. There was only one emergence. When the temperature was raised to 15°C (C2.iii), there was a slightly higher pupation rate (14%) and the mean number of degree-days to pupation fell to 2797Cd. None of the pupae completed development. Survival to pupation improved further to 23% for the 10°C larvae when both temperature and photoperiod were increased (C2.iv). Although only one of the five pupae successfully emerged, the thermal time to emergence was in the same range as that for C1 and C2.ii (2697 Cd).

C1 (7.5°C start) and C2.ii (10°C start). These sub-treatments were relatively successful, with around half the larvae pupating and two-thirds of the pupae emerging. The mean number of degree-days to pupation and emergence fell further compared with the other sub-treatments. The C1 larvae showed a slightly higher survival rate as well as a shorter mean thermal time to pupation and emergence compared with the C2.ii larvae, but these differences were not significant. In both sub-treatments, the larvae experienced an increase in day-length. For C1, there was an increase in both photoperiod and temperature. It is not known whether pupation would have occurred had the temperature been maintained at 7.5°C, but it is likely that the temperature has to be raised above a minimum threshold for successful pupation. Similarly, Luff's 5°C maintained larvae failed to pupate (see Table 6.6). Interestingly, the new photoperiod for CT1 was the same as the initial photoperiod for C2.ii (12L 12D), suggesting that the absolute number of hours of light per day is less important than a recognition that days are lengthening.

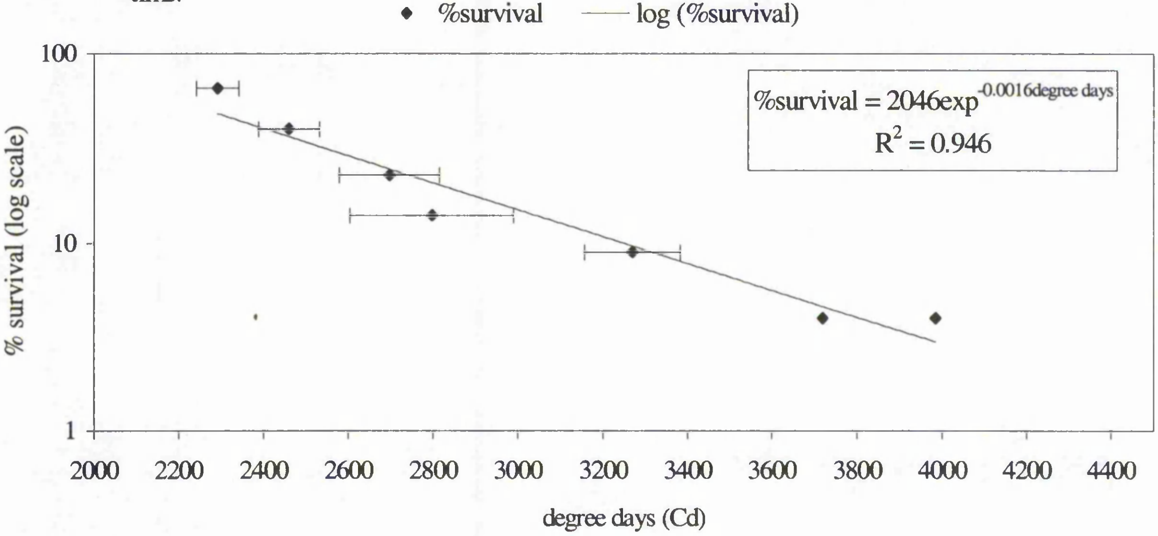
Sub-treatment C2.ii consisted of the 10°C "tank" larvae, which were transferred from tanks to individual jars and placed in constant light from Day 63 (see Table 6.2, Section 6.3.1). An increase in day-length before completion of Instar 3.i did not prevent the onset of quiescence. The apparent success of the C2.ii larvae compared with the other treatments may be due to the sibling predation conditions experienced by these larvae during first and second instar; they were the only survivors from the sibling batches placed in the tanks and were presumably the fittest individuals. The success of the C1 (formerly 7.5°C) larvae, when compared with their siblings maintained under different temperature/light conditions, can be attributed to differences in abiotic factors only.

It would be expected that the pupal developmental rate would increase with increasing temperature within the normal temperature range experienced by this beetle in the field (Thiele, 1977; Zaslavski, 1988). In fact, emergence from pupation was more likely to occur at the cooler temperature of 10°C (see column 10 of Table 6.10). There was only one emergence when the temperature was maintained or raised to 15°C. This might be a problem of the rearing method rather than an indication of the optimum temperature range for the pupae of this investigation. Luff (1973) obtained a higher survival rate to both pupation and emergence for larvae maintained at 15°C. However, the pupation rate of Luff's larvae, which had been kept in darkness, was low (12%, of which half emerged) and the additive mean thermal time to emergence of 3270 Cd was longer compared with the most successful treatments in this investigation (2643 Cd - see final column of Table 6.6.ii). The close similarity between the thermal developmental times of Luff's 15° and 10°C treatments and that for C2.i in this investigation (held at 10°C with no increase in day-length) suggest that development is delayed if there is no increase in photoperiod.

iv) Optimum conditions for growth and development of immature stages of *P. madidus*

It is reasonable to assume that growth rate, measured as weight gain per degree-day, is at its maximum at optimum temperatures (see Fig 6.3 and Table 6.8). It is also reasonable to assume that the optimum temperature range for each developmental stage is that which requires the shortest number of degree-days to complete each stage. For example, from Fig 6.8, there is a highly significant and negative correlation between the log percentage of third instar larvae surviving to pupation and the number of degree days to reach this stage ($r^2 = 0.95$; d.f. = 6; $P < 0.001$).

Fig 6.8 Relationship between thermal time to develop to pupation and percentage of Instar 3 larvae surviving to this stage, showing 1 standard error on the means for thermal time.



By making these two assumptions, it is possible to deduce the optimum conditions for the successful development of *P. madidus* in its immature stages, and the length of time, measured in degree-days, required to complete these stages.

Instar 1. From Figs 6.2, 6.3 and Table 6.8, it is clear that the first instar larvae used in this investigation are fairly well adapted to a wide temperature range, from 10°C to at least 20°C, with a maximum growth rate and minimum number of degree-days to complete this stage centred around 15°C. Under these conditions, the number of degree-days required to complete Instar 1 is 300-350 Cd (see mean values in Table 6.6.ii).

Instar 2. The optimum temperature range for Instar 2 is lower and narrower, centred at 10°C, when the number of degree-days to complete development is about 400 Cd (Fig 6.2 and Table 6.6.ii).

Instar 3.i. The growth rate of Instar 3.i larvae was at its highest at 7.5°C (Fig 6.3), with a drop in metabolic efficiency at temperatures above 10°C, suggesting an optimum temperature range at or below 7.5°C for this developmental stage. Quiescence occurs when the larval weight has increased by a factor of 2.4 (Table 6.8). At the most optimum temperature, this takes around 400 Cd (Table 6.6).

Instar 3.ii. The larvae that had been previously maintained at 7.5°C spent the shortest thermal time in this phase (around 700 Cd – see means for all these larvae in Table 6.6.ii). A qualitative photoperiodic reaction of the long-day type, as described by Zaslavski (1988), appears to aid termination of quiescence. A threshold minimum temperature is probably also required. If these environmental cues are not given, pupation is delayed or does not occur at all, the larvae eventually dying (Table 6.10).

Pupation and emergence. Under the most optimum conditions provided by this investigation, around 1100 degree-days were required to complete development at third instar for pupation to occur (Fig 6.2). This was for the larvae maintained at 7.5°C until Instar 3.ii when the photoperiod was increased and the temperature raised to 10°C. It is not known whether pupation can occur at temperatures as low as 7.5°C or whether 15°C is too high a temperature for the larvae of this investigation. Pupation proceeds to emergence successfully at 10°C, with a thermal time between 300 to 350 degree-days, for larvae previously maintained at 7.5° and 10°C (Table 6.6.ii).

Using these estimations, the expected number of degree-days to complete development from larval hatching to emergence under optimum temperature conditions is about 2200Cd. However, as indicated by Fig 6.2, thermal developmental time at Instar 3 may have been shorter had temperatures below 7.5°C been given during the growing phase.

Luff's results of 1973 show a different relationship. Fig 6.2 shows that his first and second instar larvae were better adapted to lower temperatures. His Instar 3 larvae were more likely to complete development at a higher temperature (15°C), and there was one successful emergence at 20°C.

6.4.2 Constant temperature treatments: analysis by larval group

This section attempts to identify any differences in the temperature requirements of the larvae of the different breeding pairs in terms of survival, growth and development. The eventual leg colour phenotype of larvae that failed to complete development is not known. However, on the basis of results presented in Section 5.4.1, it is assumed that the BB offspring carry only genes for black legs in the adult; a high proportion of the RR offspring was destined to be homozygous or heterozygous red-legged adults; the larvae from mixed pairs are presumed to be a mixture of heterozygous red-legged and homozygous black-legged morphs.

i) Instar 1

Mortality. Table 6.5.ii and iii shows a higher survival rate to second instar by the same leg colour breeding pairs (BB and RR) compared with the mixed breeding pairs (RB and BR) at all temperatures. BB had consistently fewer mortalities than RR, and RB fared the least well. However, chi-squared analysis did not find a significant difference in the proportion surviving to second instar between these groups either within the temperature treatments, or by totalling the larvae from all the treatments. The higher k-values for RB at 10°, 15° and 20°C were mainly due to the poor viability of one batch of siblings, which had been divided between these temperatures. Even so, the survivors within the RB group attained the lowest weights at instar change at all temperatures (Table 6.7), though only one result was found to be significant – that between RB and BB from the 10°C treatments ($t = 2.65$; $P<0.02>0.01$).

10°C treatment. Apart from the low weight for the RB group, the differences between the 10°C larvae are not significant (see Fig 6.4).

15° treatment. At 15°C, the BB larvae took significantly fewer thermal days to complete Instar 1 compared with the RR and RB offspring (BB v RR: $t = 2.941$, $P<0.01>0.001$; BB v RB: $t = 4.411$, $P<0.001$, see Table 6.6.ii), but the mean larval weights at instar change were not significantly different between the groups (Table 6.7 and Fig 6.5).

20°C treatments. At 20°C, the RR group was heavier, on average, than the other groups at instar change (Fig 6.6). This is significant only when compared with BB ($t = 2.003$, $P<0.05>0.02$, see Table 6.7). There are no differences between the groups in the number of degree-days to complete development (Table 6.6.ii).

7.5°C treatment. The RR offspring spent significantly fewer days to complete development (BB v RR: $t = 5.70$, $P<0.01>0.001$; RB v RR: $t = 2.83$, $P<0.02>0.001$; BR v RR: $t = 2.76$, $P<0.02>0.001$, see Table 6.6). By reaching a similar weight in a shorter period of time (Fig 6.3), this group also showed a better metabolic efficiency (defined as weight gain per degree-day - see Table 6.8). However, mortality for the RR larvae was as high as that for the slower developing BB offspring (Table 6.5.iii). There was also one low weight, slow developer from this group, which contributed to the unusually high standard errors on the means (see Fig 6.3). It is possible, therefore, that some RR individuals were no better adapted to this temperature than larvae from the other groups, but did not survive to instar change. Of the RR survivors, the fast developers were individuals from 3 (out of 4) separate sibling batches.

In summary, at 10°, 15° and 20°C there is little difference between the four larval groups in terms of growth and development, although there is a slightly better survival rate for the BB offspring, which are assumed to be exclusively black-legged. A more consistent result was obtained from the RR group maintained at 7.5°C which, on average, appeared to be better adapted to this lower temperature.

ii) Instar 2

Comparing larval groups, the BB larvae no longer show better survival (see k-values in Table 6.5.iii), and the surviving larvae of the RB group are now at similar weights to the other groups at all temperatures (Table 6.7).

10°C treatment. There were no differences between larval groups in terms of thermal time to complete development and mean weight at instar change (Tables 6.6.ii and 6.7; see also Fig 6.5).

7.5°C treatment. The fastest developing group is now RB (Table 6.6.ii), although this is significant only when compared with BB (t = 2.167, P<0.05>0.02). The RB and RR groups both achieved the highest growth rates (Fig 6.4), gaining weight at a rate of 0.064mg/Cd. Consequently, the RR group maintained its advantage in terms of the accumulative number of degree-days for instar change and mean weight at instar change. This is now significantly higher compared with the BB and BR groups (RR v BB: t = 2.166, P<0.05>0.02; RR v BR: t = 3.084, P<0.01>0.001 – see Table 6.7). The BB group, which spent longer at Instar 2 and achieved one of the lowest mean weights at ecdysis (Fig 6.4), has the least efficient metabolism (0.047mg/Cd).

15° treatment. At 15°C, RB spent longer thermal time at second instar than the other groups (Table 6.6.ii). This was significant when compared with the BB and BR groups (BB v RB: t = 4.344, P<0.001; RB v BR: t = 3.163, P<0.01>0.002). However, the mean weight of the RB group at instar change has not been adversely affected (Fig 6.6). Conversely, the BR group gained the most weight by instar change, significant in two instances (BR v BB: t = 4.014, P<0.001; BR v RR: t = 2.219, P<0.05>0.002 – see Table 6.7), but there was no advantage for this group in terms of thermal time to complete development (Fig 6.6).

20°C treatment. At 20°C, the BR group spent fewer days at second instar than the other groups (Table 6.6.ii). This was significant only when compared with RR (t = 2.171, P<0.05>0.02). Although the RR group attained the highest mean weight at instar change (Table 6.7), the high standard errors on the means for weight resulted in no significant difference between the larval groups at this temperature (Fig 6.7).

Overall, there were no consistent differences between the second instar larval groups in terms of survival, growth and development at the three higher temperatures. The RR and RB groups both maintained a good metabolic efficiency at the lower temperature of 7.5°C, although the weight advantage and earlier thermal time of ecdysis of the RR larvae are largely a result of the faster development of this group during first instar. By contrast, the second instar BB larvae seemed to be the least well adapted to this lower temperature.

iii) Instar 3.i (growth phase)

7.5^o and 10^oC treatments. At these lower temperatures, there were no significant differences between the groups in terms of mortality, growth and development (Tables 6.5 to 6.7). At 7.5°C, the RR group continued to maintain its earlier advantage (Fig 6.4), but there is clearly no difference in growth rates between the groups. Fig 6.5 shows a slightly slower growth rate for the 10°C BB and RB groups, resulting in lower weights at the end of the growth phase, but standard errors are high and differences are not significant.

15^o treatment. At 15°C, the BB group spent a higher number of degree-days on Instar 3.i (Table 6.6.ii). This was significant when compared with RR and RB (BB v RR, $t = 2.947$, $P<0.01>0,001$; BB v RB, $t = 2.629$, $P<0.02>0.01$). Hence, the shorter period spent on Instar 2 by the BB group has been balanced by a longer period spent at Instar 3.i (see Fig 6.6).

20^oC treatments. At 20°C, the RB group showed poor survival (Table 6.5.iii), but numbers are too low to draw conclusions. The high standard errors on the means for both thermal time and mean weights of the other larval groups indicate a large variation in response to high temperatures among the survivors (Fig 6.7).

Overall, therefore, there are no consistent trends differentiating the larval groups in the Instar 3.i developmental stage, although numbers are low.

Numbers are too low for analysis by breeding group of the final developmental stages (Instar 3.ii and pupation), though it is notable that the BR larval batches had poor viability at this stage. Only two pupated, neither of which emerged.

In summary, results for each development stage at the three higher temperatures are not consistent enough to identify any particular pattern differentiating the larval breeding groups. Other potential sources of variation include chance differences in the viability of the sibling batches and/or the eventual sex of the larvae. At the lowest temperature (7.5°C), the RR larvae showed greater metabolic efficiency in the first instar. Their siblings, maintained at higher temperatures, did not develop any faster than the other larval groups. Survivors to second instar in the RB group also showed good growth rates during Instar 2. By contrast, the BB larvae, which were destined to be black-legged, showed the poorest adaptation to 7.5°C during Instars 1 and 2. The emerged adults were red-legged from the RR and RB 7.5°C treatments and black-legged from the BB 7.5°C treatment (see Section 6.4.6).

Could the red-legged phenotype be better adapted to lower temperatures during first and second instar? Without knowing the phenotype of the RR and RB larvae that failed to emerge, this is speculation only.

6.4.3 Comparison of the development of *P. madidus* under optimum temperature conditions in the laboratory with seasonal temperatures in the field

Table 6.11 summarises the thermal time for the development of *P. madidus* larvae from investigation 1, based on the findings described in Section 6.4.1. Under these conditions, 2100-2200 degree-days would be required to complete development. By contrast, Luff's larvae were better adapted to lower temperatures at early instar (5 to 10°C) and higher temperatures at the later developmental stages (15 to 20°C).

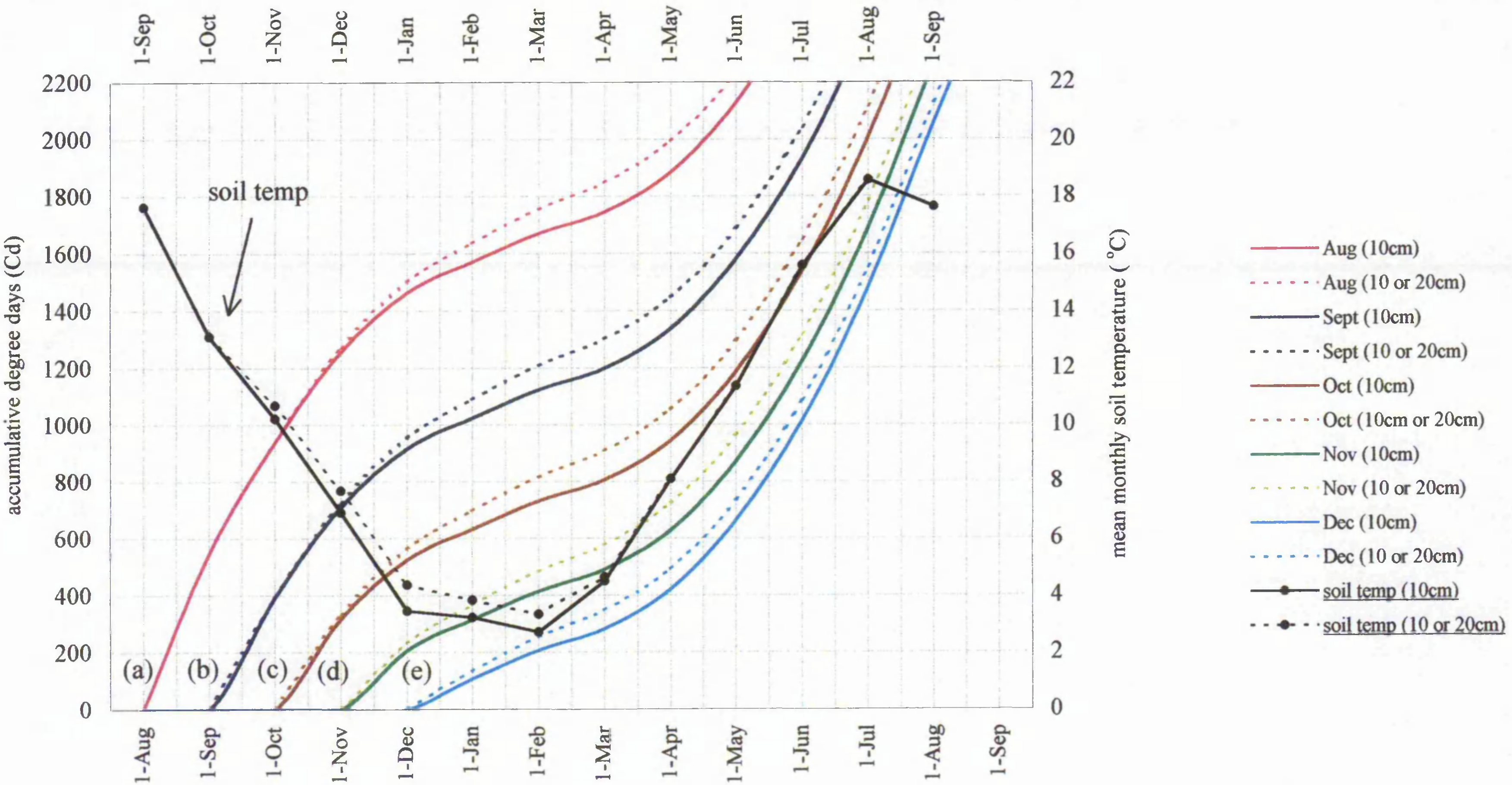
Table 6.11 Estimation of the thermal time required for the development of each of the immature stages of *P. madidus* under optimum light and temperature conditions of Investigation 1.

Developmental stage	Degree days (Cd)	Temperature	Photoperiod
Instar 1 to 2	300-350	10 – 20	
Instar 2 to 3.i	400	10	
Instar 3.i to 3.ii	400	7.5 (or less)	
Instar 3.ii to pupation	700	7.5 – 10	Increase in day length
Pupation to emergence	300	10	

The inconsistencies between the two investigations represent no more than a seasonal shift. This is shown in Fig 6.9, which predicts the month of emergence from larval hatchings using an average of 1994/5/6 mean monthly soil temperatures recorded at Sutton Bonington weather station in the East Midlands (data obtained from Nottingham University). Table C.3 in Appendix C gives the spreadsheet template of this model, using Excel 97.

The monthly soil temperatures, which have been converted to degree-days, have been accumulated for each month of hatching. The curves in Fig 6.9 show the accumulated number of degree-days up to 2200 Cd, which is the predicted time of emergence. Hence, curve (a) represents larvae that hatched at the beginning of August and are predicted to emerge as adults in May; curve (b) represents hatching at the beginning of September and emergence towards the end of June, etc.

Fig 6.9 Predicted month of eclosion from larval hatchings from August to December, using mean monthly soil temperatures for 1994/5/6 at i)10cm depth throughout year and ii)10cm depth May to September; 20cm depth October to April (dotted lines). (See Table C.3 in Appendix C).



Weather data from Sutton Bonington (north Leicestershire)

Curves for a constant 10cm soil depth are shown (plain lines). The larvae probably regulate their temperature conditions by moving towards the soil surface on warmer days, and burrowing further down the soil profile when surface temperatures are low. The dotted lines in Fig 6.9 show the predicted month of emergence using the warmer soil temperatures at 20cm depth from October to April.

From Fig 6.9, larvae hatching late autumn and early winter at the beginning of November and December (curves (d) and (e)) would experience low temperatures at Instars 1 and 2 (7°C down to 3.5°C at 10cm depth and 7.5° down to 4.5°C at 20 cm depth), but high temperatures from late Instar 3 to pupation (16° to 18°C). These seem to be the conditions that Luff's larvae were adapted to.

Late summer and early autumn hatchlings (curves (a) and (b)) would experience higher temperatures at Instars 1 and 2 (18°C down to 12°C at 10cm depth or down to 12.5°C at 20cm depth). Temperatures during late Instar 3 and pupation would be lower than those experienced by the later hatchlings (8° to 12°C). The larvae of this investigation seem better adapted to these conditions.

Also evident is the need for a lengthy quiescent period for larvae hatching during August. As shown in Table 6.9, these larvae are predicted to attain full growth at 1300-1700 Cd, when it is still early winter (see curve (a) of Fig 6.9).

There are some discrepancies with the model. Except for the August hatchlings, emergence is not predicted until late June to early August. Varying between soil depths to optimise soil temperatures leads to an earlier predicted emergence time of about two weeks for the August hatchlings but only one week for autumn and early winter hatchlings. Although Instar 3 larvae have been found in the field during mid-summer (Luff, 1973), this is a rare occurrence. It is possible that the late autumn and early winter hatchlings have a shorter quiescent period, if any at all, emerging as adults in fewer degree-days. Quiescence may be facultative - a period of reduced metabolism occurring only in larvae that have achieved full growth early in the season, and terminated by environmental cues representing spring. Some evidence for this is the shorter thermal time spent in quiescence by the larvae maintained at 7.5°C (Table 6.6.ii).

Investigation 2, in which *P. madidus* larvae are raised under temperature and light conditions that more closely follow that external temperature conditions of the seasons, examines these hypotheses further.

Table 6.5 Constant temperature treatments: Initial number, percentage survival and k-value for each developmental stage of larvae grouped by leg colour of parents.

1 = instar 1; 2 = instar 2; 3.i = growth stage of instar 3; 3.ii = quiescent stage of instar 3; P = pupation; E = emergence; n.a.= data not available.
See Section 6.3.1 for explanation of symbols for larval groups.

i) Number of larvae (grouped by leg colour of parents) at start of each developmental stage.

Developmental stage:

7.5°C*	1	2	3.i	3.ii	P	E
BB	21	10	7	6	4	2
RR	16	7	4	3	1	1
RB	20	7	5	5	5	3
BR	15	5	3	3	1	0
all larvae	**72**	**29**	**19**	**17**	**11**	**6**

10°C*	1	2	3.i	3.ii	P	E
BB	25	25	24	24	4	1
RR	21	20	20	18	3	1
RB	21	13	12	11	2	0
BR	18	15	14	13	1	0
all larvae	**85**	**73**	**70**	**66**	**10**	**2**

15°C*	1	2	3.i	3.ii	P	E
BB	27	27	22	21	0	0
RR	25	22	20	19	1	1
RB	22	17	16	14	1	0
BR	30	24	23	21	0	0
all larvae	**104**	**90**	**81**	**75**	**2**	**1**

20°C	1	2	3.i	3.ii	P	E
BB	23	21	10	6	0	
RR	22	16	9	7	0	
RB	23	10	6	2	0	
BR	21	14	7	6	0	
all larvae	**89**	**61**	**32**	**21**	**0**	

tank larvae:

10°C	1	2	3.i	3.ii	P	E
BB	-	10	10	10	5	2
RB	-	10	10	10	4	3
all larvae	-	**20**	**20**	**20**	**9**	**5**

ii) Percentage survival to eclosion

Developmental stage:

7.5°C*	1	2	3.i	3.ii	P	E
BB	100	48	33	29	19	10
RR	100	44	25	19	6	6
RB	100	35	25	25	25	15
BR	100	33	20	20	7	0
all larvae	**100**	**40**	**26**	**24**	**15**	**8**

10°C*	1	2	3.i	3.ii	P	E
BB	100	100	96	96	16	4
RR	100	95	95	86	14	5
RB	100	62	57	52	10	0
BR	100	83	78	72	6	0
all larvae	**100**	**86**	**82**	**78**	**12**	**2**

15°C*	1	2	3.i	3.ii	P	E
BB	100	100	81	78	0	
RR	100	88	80	76	4	4
RB	100	77	73	64	5	0
BR	100	80	77	70	0	
all larvae	**100**	**87**	**78**	**72**	**2**	**1**

20°C	1	2	3.i	3.ii	P	E
BB	100	91	43	26	0	
RR	100	73	41	32	0	
RB	100	43	26	9	0	
BR	100	67	33	29	0	
all larvae	**100**	**69**	**36**	**24**	**0**	

tank larvae:

10°C	1	2	3.i	3.ii	P	E
BB	n.a.	n.a.	100	100	50	20
RB	n.a.	n.a.	100	100	40	30
all larvae			**100**	**100**	**45**	**25**

iii) k-values for each developmental stage (values in italics: initial numbers < 5).

Developmental stage:

7.5°C*	1	2	3.i	3.ii	P	E
BB	0.322	0.155	0.067	0.176	0.301	1.021
RR	0.359	0.243	0.125	*0.477*	*0.000*	1.204
RB	0.456	0.146	0.000	0.000	0.222	0.824
BR	0.477	0.222	0.000	*0.477*	*3.000*	4.176
all larvae	**0.395**	**0.184**	**0.048**	**0.189**	**0.263**	**1.079**

10°C*	1	2	3.i	3.ii	P	E
BB	0.000	0.018	0.000	0.778	*0.602*	1.398
RR	0.021	0.000	0.046	0.778	*0.477*	1.322
RB	0.208	0.035	0.038	0.740	*3.301*	4.322
BR	0.079	0.030	0.032	1.114	*3.000*	4.255
all larvae	**0.066**	**0.018**	**0.026**	**0.820**	**0.699**	**1.628**

15°C*	1	2	3.i	3.ii	P	E
BB	0.000	0.089	0.020	4.322		4.431
RR	0.056	0.041	0.022	1.279	*0.000*	1.398
RB	0.112	0.026	0.058	1.146	*3.000*	4.342
BR	0.097	0.018	0.040	4.322		4.477
all larvae	**0.063**	**0.046**	**0.033**	**1.574**	***0.301***	**2.017**

20°C	1	2	3.i	3.ii	P	E
BB	0.040	0.322	0.222	3.778		4.362
RR	0.138	0.250	0.109	3.845		4.342
RB	0.362	0.222	0.477	*3.301*		4.362
BR	0.176	0.301	0.067	3.778		4.322
all larvae	**0.164**	**0.280**	**0.183**	**4.322**		**4.949**

tank larvae:

10°C	1	2	3.i	3.ii	P	E
BB	n.a.	n.a.	0.000	0.301	0.398	n.a.
RB	n.a.	n.a.	0.000	0.398	0.125	n.a.
all larvae			**0.000**	**0.347**	**0.255**	**n.a.**

* Temperature and light conditions were changed during instar 3.ii stage until eclosion (see Table 6.2 - Section 6.3.1)

Table 6.6 Constant temperature treatments: Mean number of days and degree days for larvae grouped by parental leg colour to complete each developmental stage, showing 1 standard error for thermal time. (Within-treatment values, which are significantly different, are identified by the same italicised letter).
s.e. = standard error; n.d. = no data. BB and RB "tank" larvae are included in 10°C data for Instar 3.ii. and pupa stages.

Temperature until late Instar III	Leg colour of parents	**(i) Days**					**(ii) Degree days**												
(°C)		Instar 1	Instar 2	Instar 3	Pupa	**Total	Instar 1	s.e.	Instar 2	s.e.	Instar 3.ii	s.e.	*Instar 3.ii	s.e.	*Instar 3	s.e.	*Pupa	s.e.	**Total
5	Luff (1973)	60.4	79.8	n.d.	n.d.	-	302	9.0	399	12.0					n.d.		n.d.		-
7.5	BB	82.8	80.0	132.7	32.5	328.0	621 *a*	29.3	600 *a*	29.4	461	32.6	659	77.1	1114	75.0	325	14.0	2660
	RR	55.3	71.3	129.5	37.5	293.6	414 *abc*	42.0	535	87.8	458	43.0	555	-	1058	-	375	-	2382
	RB	75.3	66.6	145.1	36.0	323.0	564 *b*	29.3	499 *a*	36.0	381	61.7	787	71.8	1168	57.9	360	-	2591
	BR	91.6	71.3	153.0	n.d.	-	687 *c*	89.3	535	48.0	403	38.8	n.d.		n.d.		n.d.		-
mean for all larvae		**77.3**	**73.3**	**142.5**	**35.1**	**328.2**	**580**	**29.7**	**550**	**26.4**	**426**	**25.4**	**713**	**53.6**	**1163**	**40.2**	**351**	**16.2**	**2643**
10	BB	31.7	40.2	192.6	32.2	296.7	317	9.0	402	22.0	602	38.6	1175	144.0	1831	152.1	320	33.0	2870
	RR	35.0	38.5	167.2	36.5	277.2	350	16.0	385	19.0	561	36.8	1426	211.6	1972	119.1	365	-	3072
	RB	36.5	39.5	171.0	30.8	277.8	365	25.0	395	15.0	493	44.9	1128	146.0	1905	7.8	290	51.9	2955
	BR	34.9	39.4	281.0	n.d.	-	349	43.0	394	22.0	561	32.8	2430	-	2810	-	n.d.		-
mean for all larvae		**34.1**	**39.3**	**189.6**	**38.3**	**301.3**	**341**	**7.6**	**393**	**10.6**	**565**	**20.4**	**1266**	**111.3**	**2160**	**108.5**	**314**	**28.0**	**3208**
	Luff (1973)	30.9	47.2	222.0	29.0	342.4	309	5.0	472	10.0					2220	118.0	290		3291
15	BB	18.8	37.0	n.d.	n.d.	-	282 *a*	10.5	555	54.0	946 *ab*	42.6	n.d.		n.d.		n.d.		-
	RR	23.2	41.0	254.0	41.5	359.7	348	19.5	615	51.0	730 *a*	59.9	1990	-	2875	-	415		4253
	RB	25.6	32.3	202.0	n.d.	-	384 *ab*	21.0	485	54.0	756 *b*	58.6	2385	-	3060	-	n.d.		-
	BR	20.5	40.7	n.d.	n.d.	-	308 *b*	12.0	611	34.5	832	55.2	n.d.		n.d.		n.d.		-
mean for all larvae		**21.4**	**38.1**	**228.8**	**41.5**	**329.8**	**321**	**7.7**	**572**	**24.7**	**823**	**28.9**	**2188**	**197.5**	**2968**	**65.4**	**415**	-	**4275**
	Luff (1973)	21.3	40.3	139.7	16.7	225.6	320	7.5	605	15.0					2096	91.5	251	48.0	3270
20	BB	18.5	47.4	n.d.		-	370	44.0	948	134.0	523	107.5	n.d.		n.d.		n.d.		-
	RR	19.3	46.1	n.d.		-	386	42.0	922	72.0	650	116.3	n.d.		n.d.		n.d.		-
	RB	20.3	46.2 *a*	n.d.		-	406	50.0	924 *a*	58.0	475	24.7	n.d.		n.d.		n.d.		-
	BR	21.1	37.1 *a*	n.d.		-	422	44.0	740 *a*	61.8	543	71.1	n.d.		n.d.		n.d.		-
mean for all larvae		**19.6**	**46.0**	**n.d.**		-	**392**	**9.7**	**921**	**11.0**	**567**	**55.2**	**n.d.**		**n.d.**		**n.d.**		-
	Luff (1973)	19.7	54.6	100.0	13.0	190.6	394	14.0	1092	52.0					2000		260		3812

* Multiplication factor is 10 or 15 from Day Number of treatment change (see Table 6.2 in Section 6.3.1 for details).

** Total shown is the sum of developmental stages within each row.

Table 6.7 **Constant temperature treatments: Mean weight of larvae (+/- 1 standard error) at start of each instar.**

(Within treatment values, which are significantly different, are identified by same italicised letter).

BB and RB "tank" larve are included in 10°C data from Instar 3.ii

Temperature treatment (°C) until Instar 3.ii *	Leg colour of parents			Weight at instar change (mg)									
		Instar 1	*s.e.*	Instar 2	*s.e.*	Instar 3.i	*s.e.*	Instar 3.ii	*s.e.*	Pupation	*s.e.*	Eclosion	*s.e.*
7.5	BB	3.5	*0.06*	13.0	*0.6*	41.3 *a*	*2.5*	107.0	*4.0*	108.4	*2.1*	94.6	*4.4*
	RR	3.6	*0.06*	14.0	*1.1*	48.1 *ab*	*1.9*	111.5	*9.8*	106.9	-	101.5	-
	RB	3.5	*0.10*	12.9	*0.8*	45.1	*3.4*	99.6	*4.2*	100.4	*6.7*	94.9	*7.9*
	BR	3.7	*0.15*	13.0	*0.8*	41.0 *b*	*1.3*	102.6	*0.8*	102.2	-	n.d.	
mean for all larvae		**3.5**	***0.05***	**13.2**	***0.4***	**43.9**	***1.5***	**104.8**	***2.8***	**104.1**	***2.3***	**95.9**	***4.3***
10	BB	3.6	*0.06*	18.1 *a*	*0.5*	53.7	*1.4*	134.3	*2.2*	121.3	*4.0*	119.2	*4.3*
	RR	3.4	*0.09*	17.0	*0.7*	55.0	*1.7*	141.0	*2.2*	122.0	*3.3*	123.4	-
	RB	3.6	*0.10*	15.6 *a*	*0.8*	53.5	*3.0*	132.9	*4.1*	122.8	*5.2*	115.6	*9.1*
	BR	3.5	*0.08*	17.0	*1.1*	54.7	*2.4*	138.4	*3.3*	120.6	-	n.d.	-
mean for all larvae		**3.5**	***0.04***	**17.1**	***0.3***	**54.2**	***1.0***	**132.6**	***1.8***	**121.8**	***2.6***	**118.1**	***4.8***
15	BB	3.6	*0.06*	17.6	*0.5*	53.1 *a*	*1.7*	127.3	*2.7*	n.d.		n.d.	
	RR	3.4	*0.07*	17.5	*0.5*	57.0 *b*	*1.8*	133.0	*3.5*	137.2	-	130.8	-
	RB	3.3	*0.11*	17.0	*0.5*	57.8 *c*	*1.7*	129.1	*5.0*	150.6	-	n.d.	
	BR	3.2	*0.12*	17.7	*0.5*	62.2 *abc*	*1.5*	138.6	*3.6*	n.d.		n.d.	
mean for all larvae		**3.4**	***0.05***	**17.5**	***0.3***	**57.6**	***0.9***	**132.2**	***1.9***	**143.9**	***4.7***	**130.8**	-
20	BB	3.4	*0.07*	15.8 *a*	*0.6*	50.6	*3.0*	98.4 *a*	*4.5*	n.d.		-	
	RR	3.6	*0.10*	17.5 *a*	*0.6*	54.5 *a*	*3.3*	99.1 *b*	*3.8*	n.d.		-	
	RB	3.5	*0.11*	15.3	*1.2*	42.3 a	*5.8*	128.2 *abc*	*4.0*	n.d.		-	
	BR	3.5	*0.09*	16.5	*0.9*	49.0	*2.7*	94.6 *c*	*7.2*	n.d.		-	
mean for all larvae		**3.5**	***0.05***	**16.6**	***0.7***	**46.6**	***3.4***	**104.9**	***6.2***	**n.d.**		-	

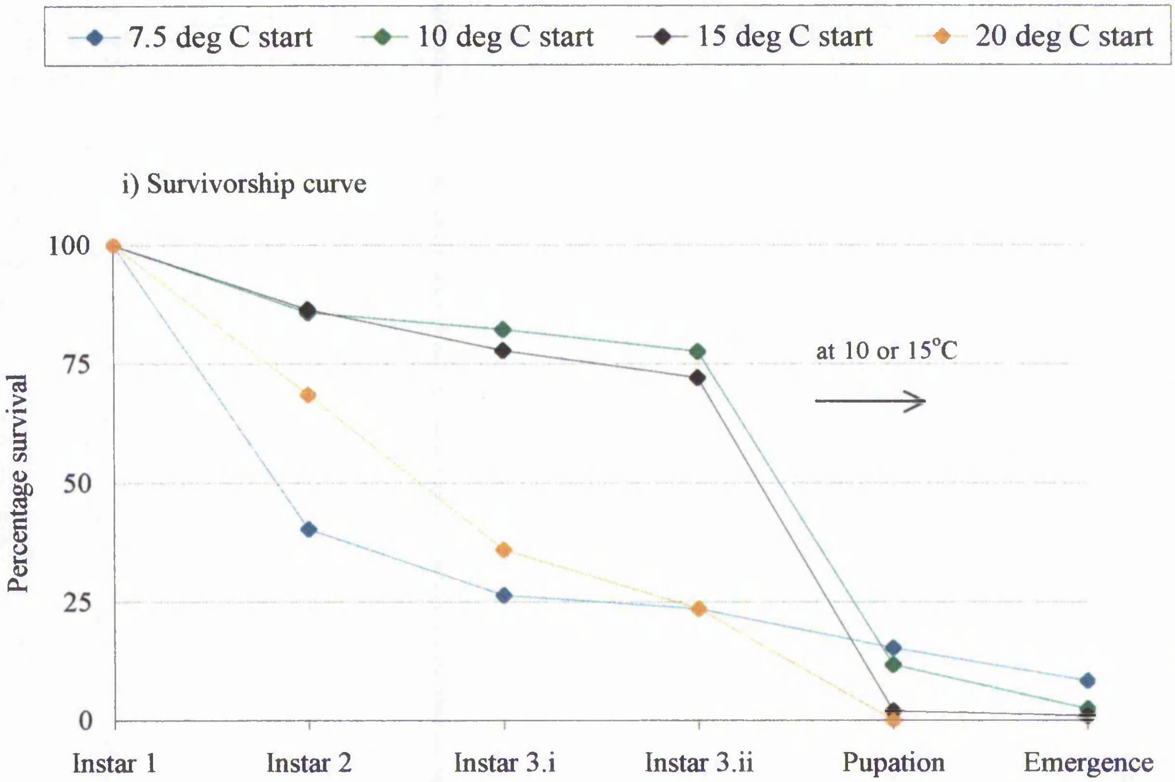
* See Table 6.2 in Section 6.3.1 for details of treatment change during Instar 3.ii

Table 6.8 Constant temperature treatments: growth rate and weight gain between developmental stages of *P. madidus* larvae.

Developmental stage	Temperature treatment (^{o}C)	Growth rate between developmental stages (mg/Cd)	Weight gain factor between developmental stages (final wt/initial wt)
Instar 1 - 2	7.5	0.017	3.77
	10	0.040	4.89
	15	0.044	5.15
	20	0.033	4.74
Instar 2 - 3.i	7.5	0.056	3.33
	10	0.094	3.17
	15	0.070	3.29
	20	0.033	2.81
Instar 3.i to 3.ii	7.5	0.143	2.39
	10	0.139	2.45
	15	0.090	2.30
	20	0.103	2.25

Fig 6.1 i) Survivorship curve and ii) accumulative k-values for immature stages of *Pterostichus madidus* under constant temperature conditions until Instar 3.ii.

i) Survivorship curve



ii) Accumulative k-values for each developmental stage

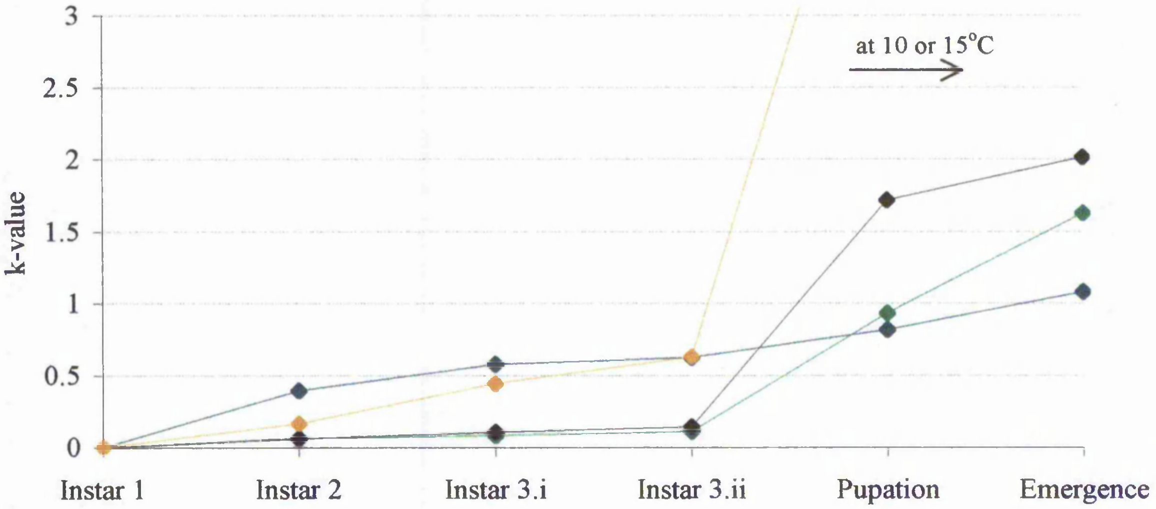


Fig 6.2 Thermal time to complete development from i) Instar 1 to 2, ii) Instar 2 to 3 and iii) Instar 3 to pupation, comparing Luff's 1973 results with those of this investigation. (NB: 7.5°C Instar 3 larvae raised to 10 or 15°C in this investigation).

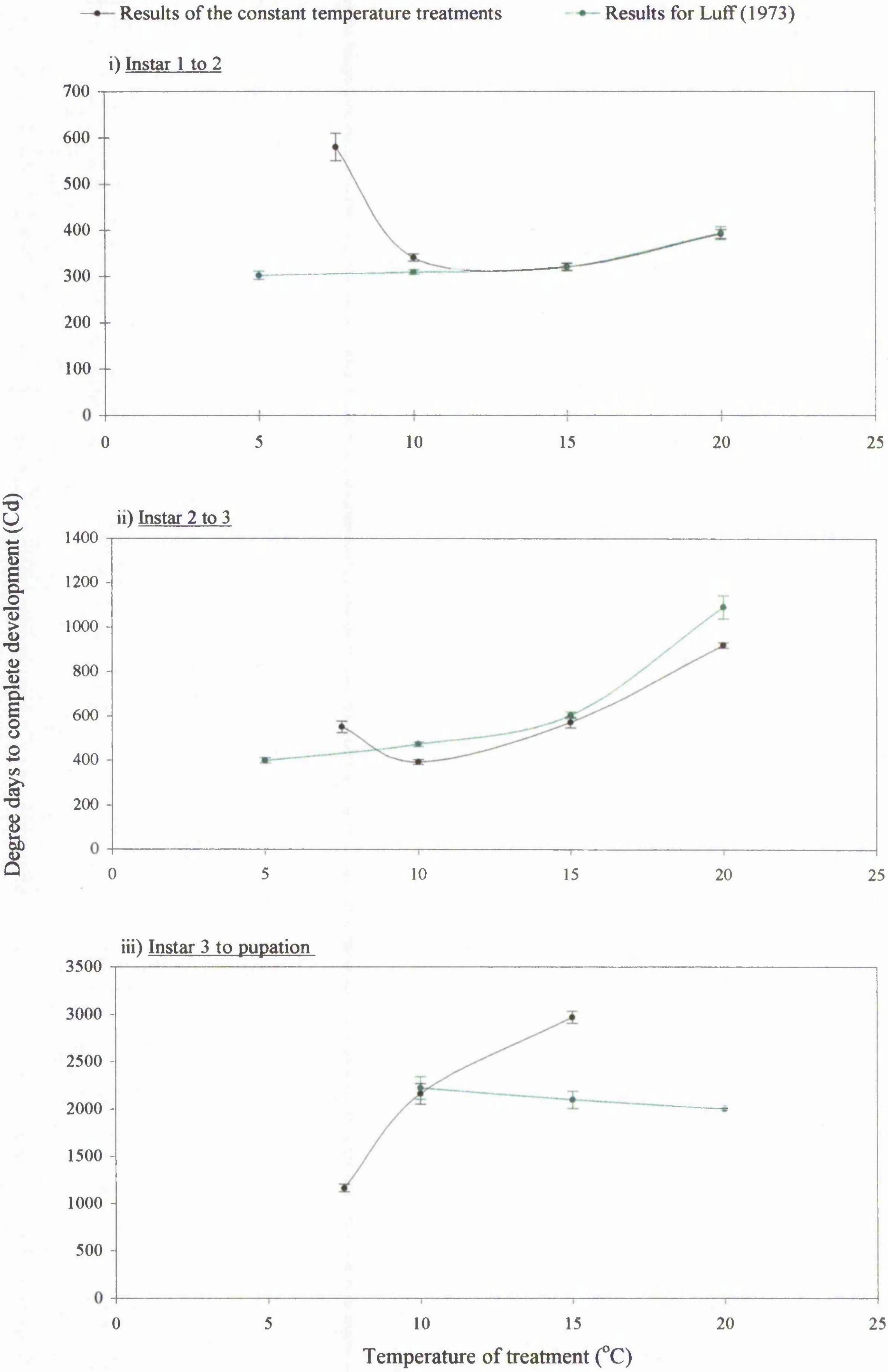


Fig 6.3 Growth and development of *P.madidus* larvae under constant temperature conditions until late Instar 3, measured in thermal time. (P = pupation; E = emergence).

Horizontal error bars show 1 standard error on the means for thermal time.
Vertical error bars show 1 standard error on the means for weight.

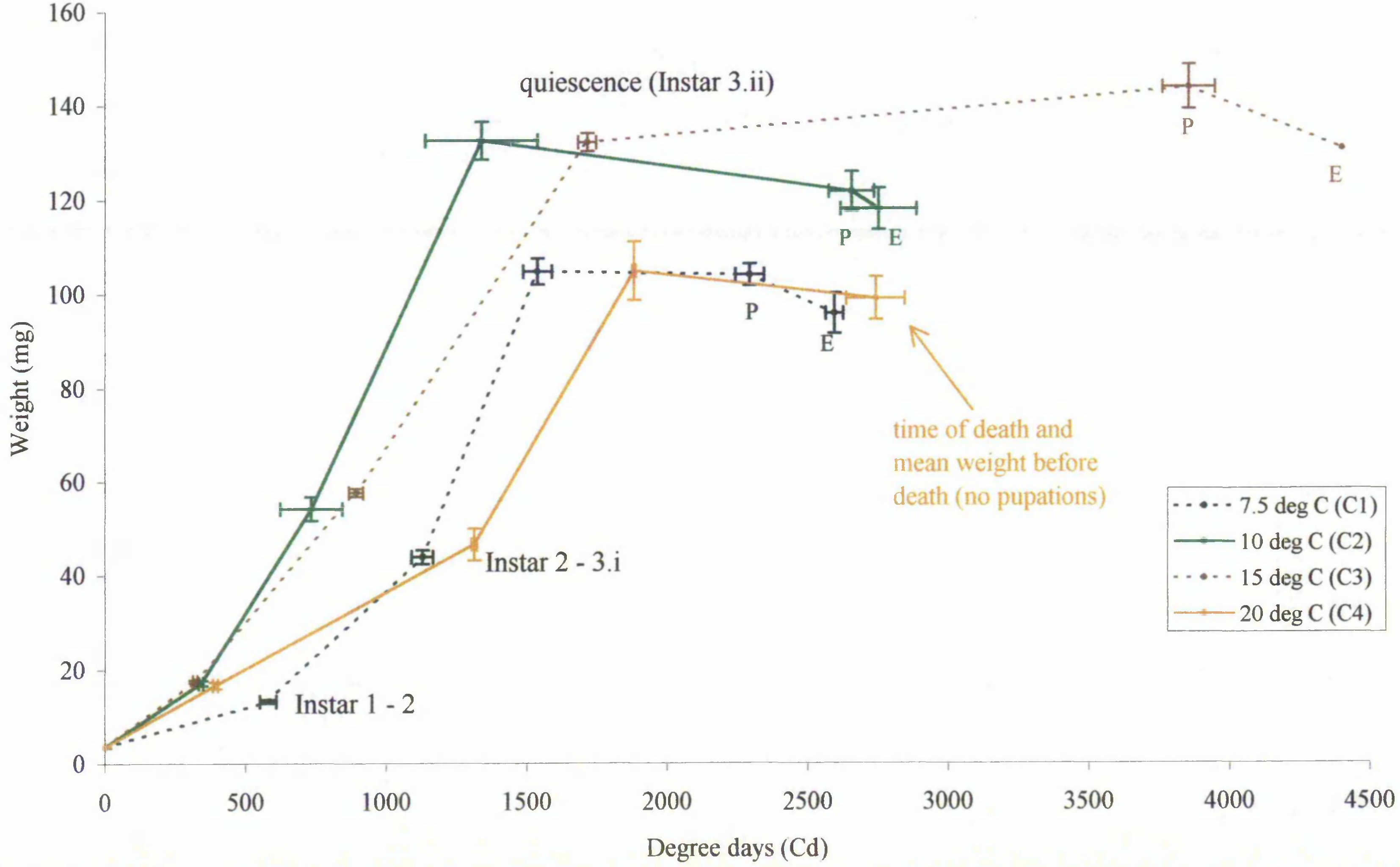


Fig 6.4 *7.5° C treatment (C1):* Growth and development of *P. madidus* larvae, grouped by leg colour of parents, measured in thermal time. Error bars show 1 standard error on the means for thermal time (horizontal bars) and weight (vertical bars).

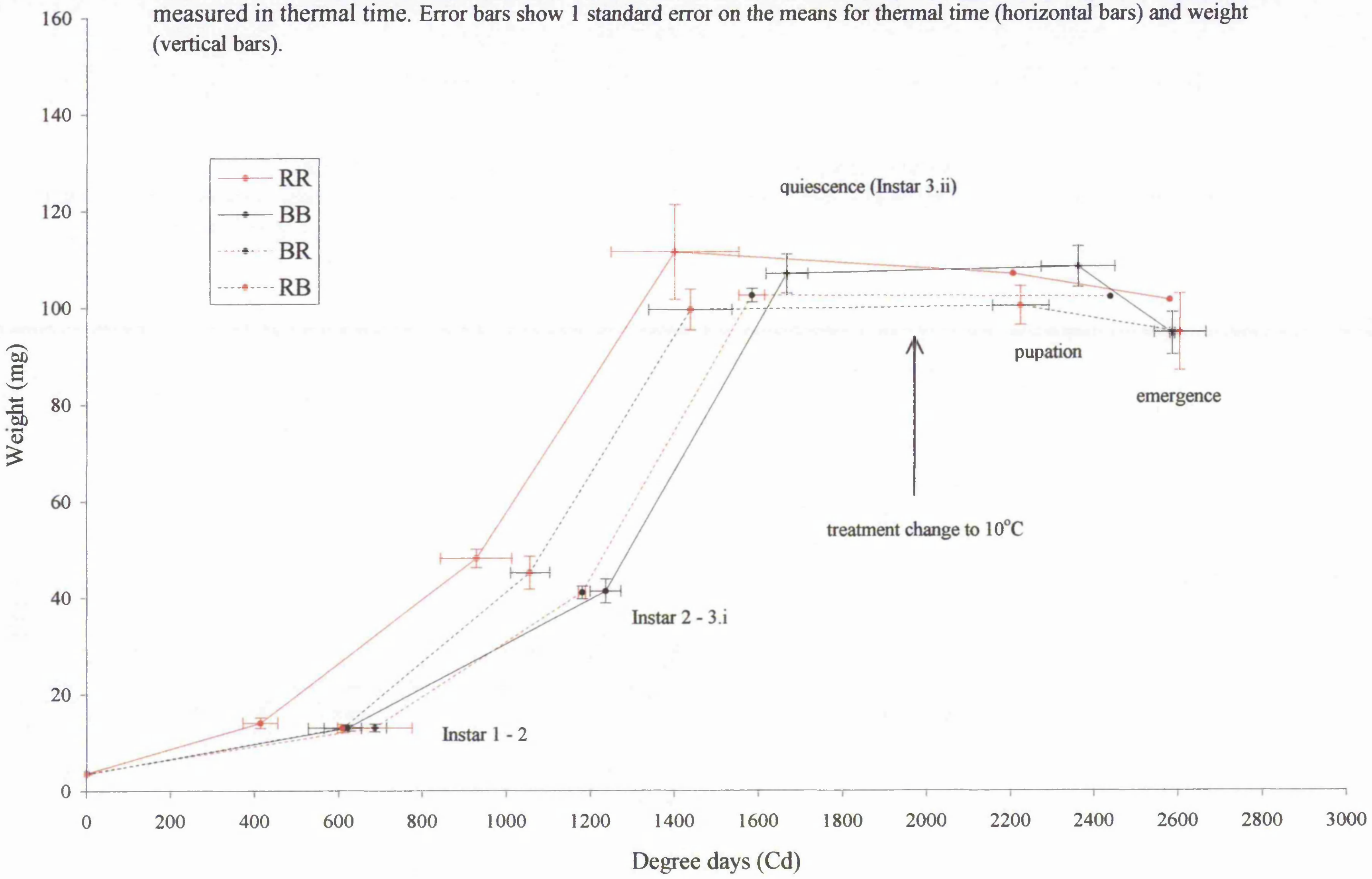


Fig 6.5 *10° C treatment (C2):* Growth and development of *P. madidus* larvae, grouped by leg colour of parents, measured in thermal time. Error bars show 1 standard error on the means for thermal time (horizontal bars) and weight (vertical bars).

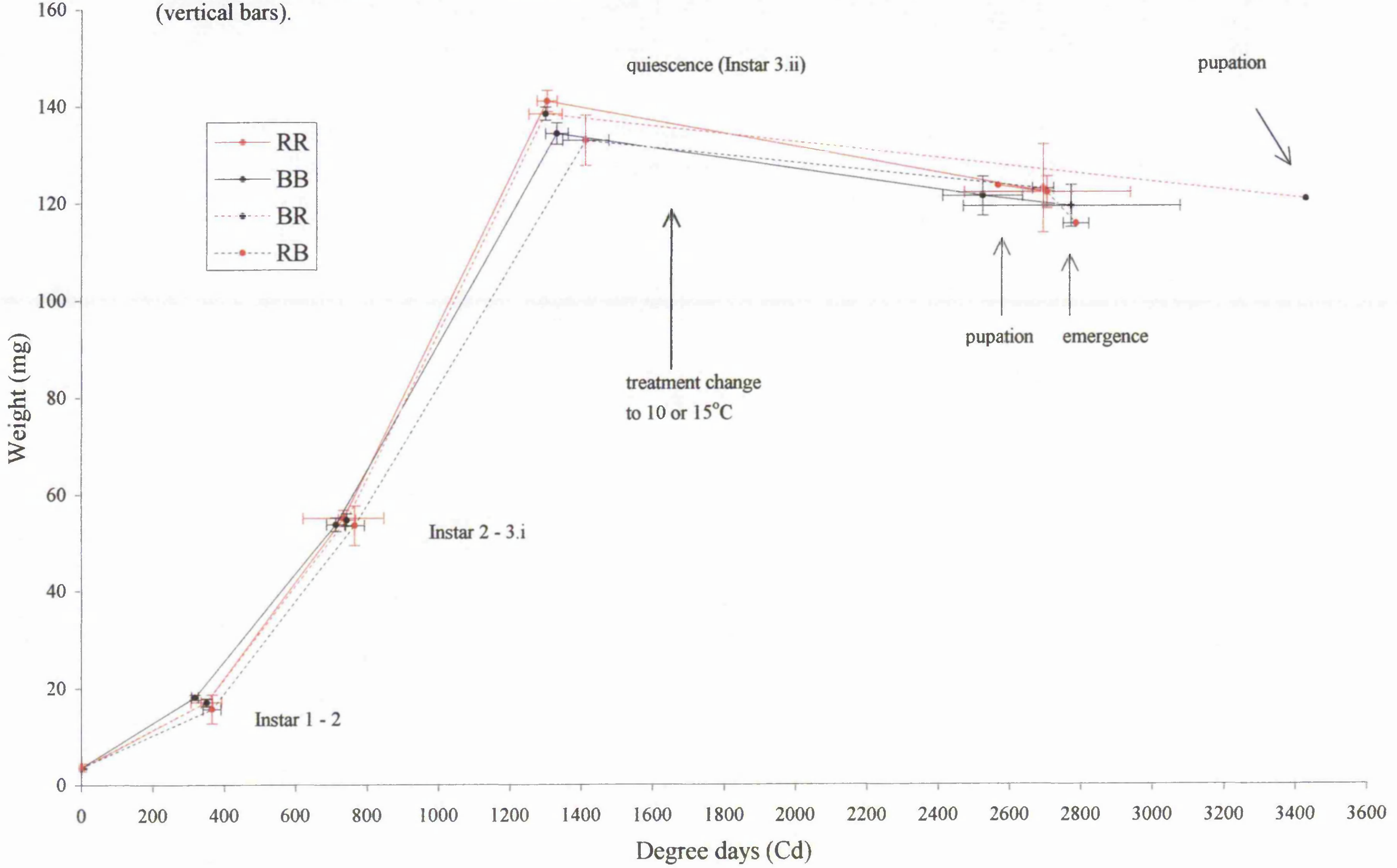


Fig 6.6 *15^o C treatment (C3)* : Growth and development of *P.madidus* larvae, grouped by leg colour of parents, measured in thermal time. Error bars show standard error on the means for thermal time (horizontal bars) and weight (vertical bars).

(Mean thermal time at death and mean weight before death is shown for BB and BR groups for which there were no pupations).

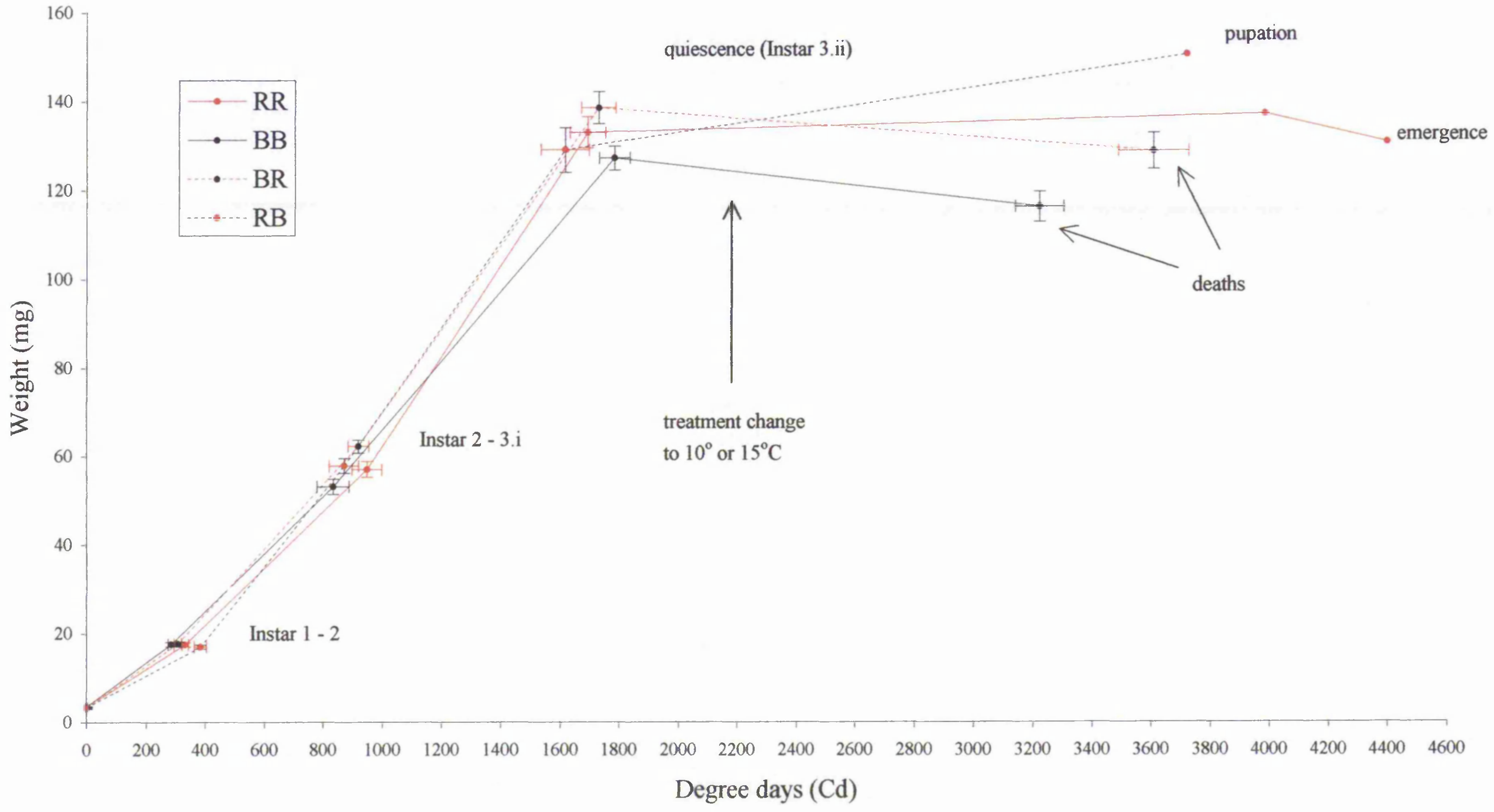
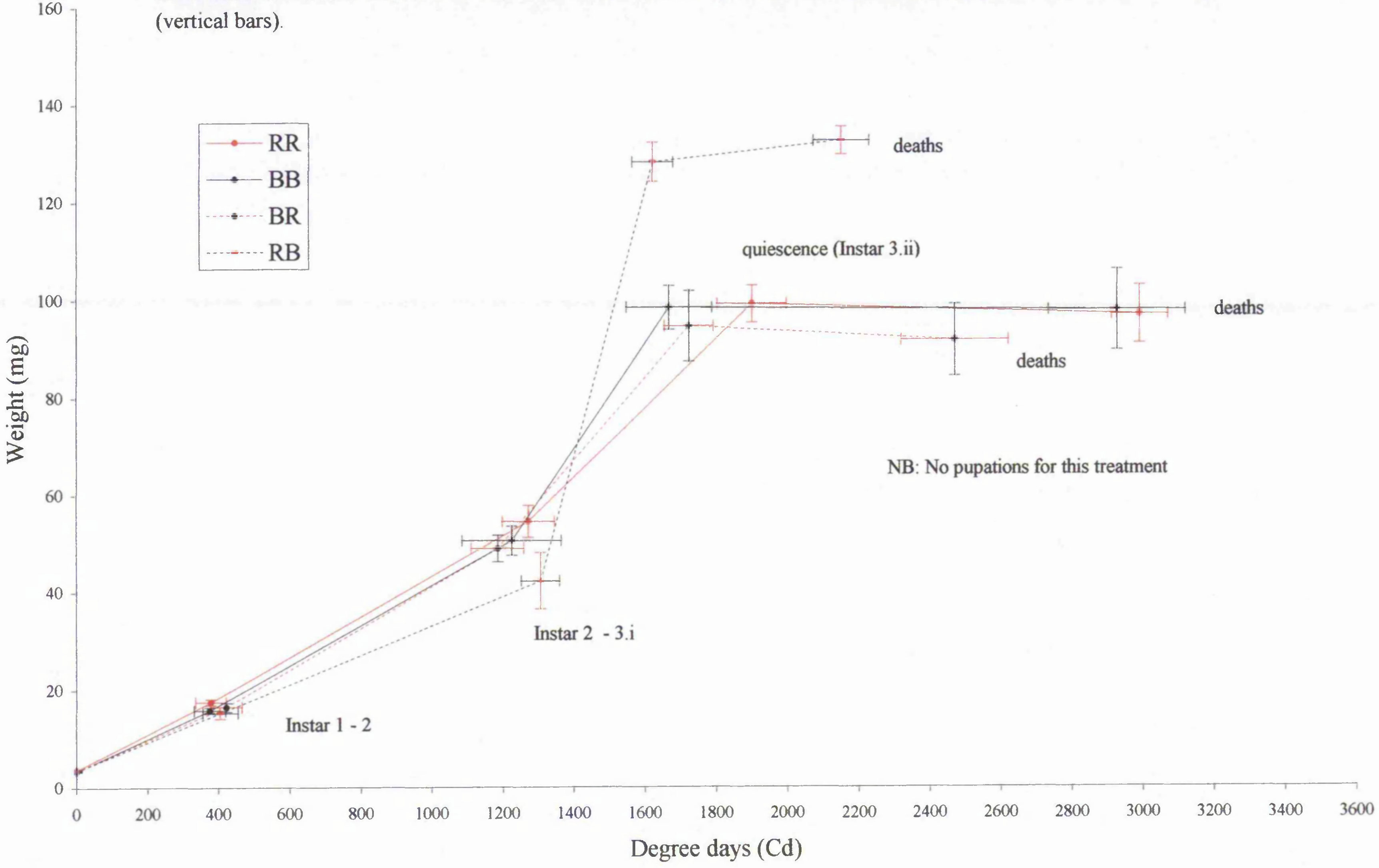


Fig 6.7 *20° C treatment (C4)* : Growth and development of *P. madidus* larvae, grouped by leg colour of parents, measured in thermal time. Error bars show standard errors on the means for thermal time (horizontal bars) and weight (vertical bars).



6.4.4 Results of the variable temperature treatments (Investigation 2)

Table 6.12 (p. 231) gives the full results for the survivorship of each larval group, classified by leg colour of parents and batch name (see Table D.2 in Appendix D for explanation). Larval batches, which failed to thrive due to incubator breakdown, have been excluded. The number, sex and leg colour of emerged adults for each larval group are also given. `

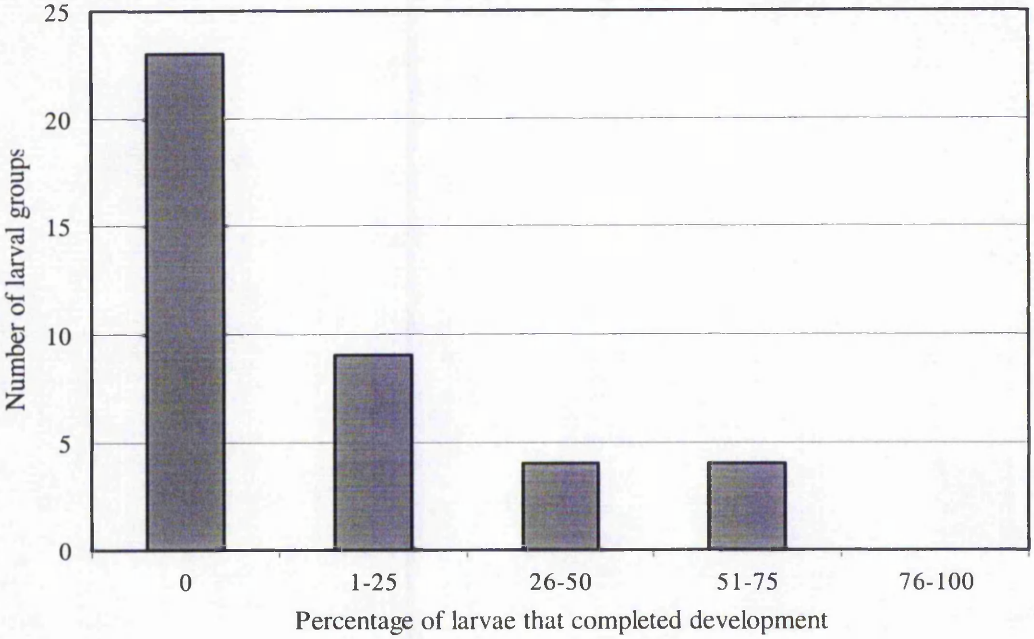
Table 6.13 (p. 232) gives the mean accumulative number of days and degree-days for all the larval groups to reach each stage development. Within each temperature treatment, the difference in the developmental rates of the larval groups classified by the leg colour of the parents (i.e. BB, RR, WB, WR groups) was not significant. Means and standard errors for each treatment have therefore been obtained for all the larvae, regardless of parentage.

i) <u>Survivorship of *P. madidus* larval groups, classified by leg colour of parents</u>

Table 6.12 and the histogram in Fig 6.10 show a high variability between the larval groups (40 in total). None of the larvae completed development from 23 of these groups, whereas more than half the larvae of 4 larval groups successfully emerged. These were: RR9-b1 from the 7.5°C initial treatment, BB(14.2)-b1 and WB14-b1 from the 10°C initial treatment, BB18 from the 12.5°C initial treatment, suggesting that developmental success is independent of the seasonal temperatures given or the leg colour of the parents. Nevertheless, larvae from 3 of the 4 larval groups from the 5°C initial treatment completed development as did larvae from 4 out of 5 larval batches from the 7.5°C initial treatment. By contrast, only 6 out of 20 and 4 out of 11 larval groups from the 10° and 12.5°C initial treatments achieved any emergence of the adult.

There could be two reasons for this. Firstly, with the exception of RR(9.6)-b2 from the 5°C start treatment, which achieved one adult emergence, later batches of larvae from the same female tended to be less successful than the first batch, e.g. BB(14.2)-b2, BB(14.2)-b3, BB(14.4)-b2, all from the 10°C treatment and WB2-b2 from the 12.5°C treatment). The 10°C treatment had five larval groups from the second or third batch of eggs; adults emerged from only one of these groups (RR9-b2). Secondly, a number of larval groups from female x wild male(s) matings may not have been viable, e.g. WB3, WB5, WR101 and WR103 from the 10° and/or 12.5°C variable temperature treatments. WB3 also failed under the 7.5°C variable temperature conditions. It is possible that the eggs of these parental groups were second or third batches.

Fig 6.10 Histogram showing percentage range of larvae that completed development under variable temperature conditions against the number of larval groups within this range.



There was one interesting difference in the survival rate to emergence of larvae from the RR9-b1 group, which had been divided between the temperature treatments. This group did well under the 7.5°C initial temperature conditions, with 6 out of 9 adults emerging. None of the RR9-b1 larvae completed development under the 10°C and 12.5°C initial temperature conditions (see Table 6.12). On the whole, however, the within-treatment variability for survivorship is too great and numbers of larval groups too low to make comparisons within or between each temperature treatment.

ii) Survivival rate for each developmental stage of *P. madidus* under each temperature condition

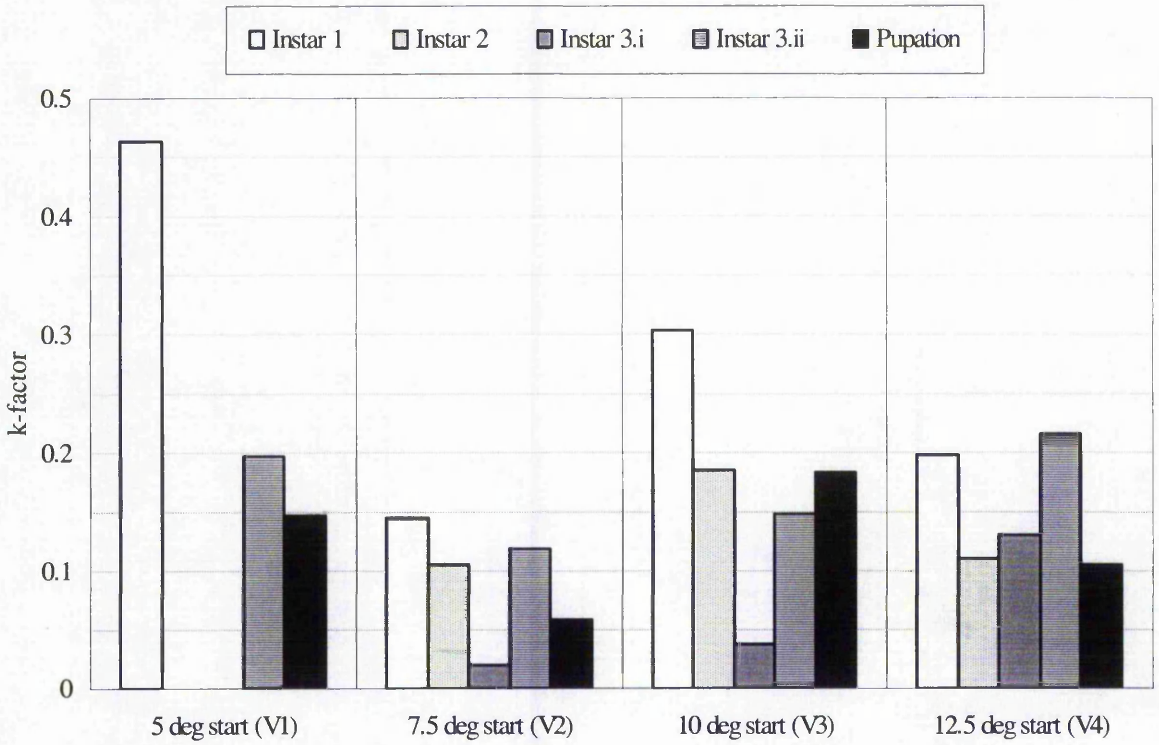
Table 6.14.i gives the percentage survival rate for each developmental stage of *P. madidus* under the four temperature conditions and Fig 6.11 shows the k-factor for each stage.

Treatment V1 (5°C start): When the initial temperature is as low as 5°C, Instar 1 is the most critical stage (Fig 6.10). Almost two-thirds of the larvae died (k = 0.46). Larvae that survived these initial conditions did well and there were no further losses until Instar 3.ii, when a third of the larvae failed to pupate (k = 0.2) and pupation, when the k-factor was almost 0.15, equivalent to 20% losses. Therefore, despite the high k-factor for Instar 1, the proportion of V1 larvae completing development is similar to the V3 and V4 proportions (see final column in Table 6.14.i).

Table 6.14 Percentage survival of larvae from all larval groups to each developmental stage for i) Variable and ii) Constant temperature treatments.

Initial temperatures:	Developmental stage:					
	1 (initial)	2	3.i	3.ii	P	E
	i) Variable temperature treatments					
5°C (V1)	100	34	34	34	22	16
7.5°C (V2)	100	72	56	54	41	36
10°C (V3)	100	50	32	30	21	14
12.5°C (V4)	100	63	49	37	22	17
	ii) Constant temperature treatments					
7.5°C (C1)	100	40	26	24	15	8
10°C (C2)	100	89	86	71	18	7
15°C (C3)	100	87	78	72	2	1
20°C (C4)	100	69	36	24	0	0

Fig 6.11 k-factor for completion of each immature stage of *Pterostichus madidus* under variable temperature conditions.



Treatment V2 (7.5°C start): Instar 1 is again the most critical stage although, with a k-factor of around 0.15, losses were small compared with V1. By Day 30, the temperature had been reduced to 5°C and ecdysis did not occur until Day 62 (s.e. ± 3.2) – see Table 6.13. A further 20% of larvae failed to reach Instar 3.i. During Instar 2, the temperature remained at 5°C until about Day 120, when it was raised to 7.5°C, then raised again to 10°C around Day 150. As shown in Table 6.13, ecdysis occurred on Day 179 (s.e. ± 4.1). The k-factor was low for Instar 3.i - in fact only 1 of the 22 remaining larvae died. Completing Instar 3.ii was the next most dangerous stage during this treatment (k = 0.12), although three-quarters of the larvae successfully pupated. The k-factor for completion of pupation was also low (k = 0.6); only 2 out of the 16 pupae failed to complete development. Despite the earlier losses, this was the most successful treatment, with one third of the larvae completing development (see Table 6.14.i).

Treatment V3 (10°C start): The high k-factor of 0.3 for Instar 1 - equivalent to 50% mortality rate - may be due to larval batches with low viability. The k-factor for Instar 2 is also unusually high, possibly for the same reason. There had also been a temperature reduction to 5°C. By the end of this developmental stage, all the larvae from 6 groups – BB(14.2)-b2, BB(14.2)-b3, BB(14.4)-b2, WB140, WR101 and WR103 - had died. The k-factors for the later developmental stages, Instar 3.i, Instar 3.ii and pupation, show some similarity to those for the V1 treatment. There was a low mortality during Instar 3.i, with a loss of only 2 out of 32 larvae. Around two-thirds of the larvae pupated and two-thirds of the pupae completed development to emergence.

Treatment V4 (12.5°C start): The k-factor of 0.2 for Instar 1 could also be artificially high due to the poor viability of some larval groups (e.g. WB3, WR101 – see Table 6.12). There was also a relatively high mortality of larvae during Instar 3.i (k = 0.13, equivalent to a loss of 25% of the larvae). The temperature was still at 7.5°C when most of the larvae entered this developmental stage (Day 83 (s.e. ± 4.6) - see Table 6.3), but was reduced to 5°C by Day 90. As shown by Fig 6.11, completion of Instar 3.ii was the most critical stage for this treatment. Nevertheless, 60% of the larvae pupated (k = 0.2). Of these, 77% emerged, giving a relatively low k-factor of 0.1 for this developmental stage.

Overall, Instar 1 was the most critical stage for all four variable temperature treatments (see Fig 6.11), though considerable losses occurred only during the V1 treatment, suggesting that the larvae of this investigation are poorly adapted to temperatures as low as 5°C in their first instar. Not surprisingly, the next most critical developmental stage was from Instar 3.i to pupation (V1, V2 and V4) or from pupation to emergence (V3). Both these processes require considerable reorganisation of the body, and would be susceptible to genetic as well as environmental factors.

iii) Comparison between Investigation 1 and Investigation 2 of the survival rate of *P. madidus* larvae to adult emergence.

One aim of Investigation 2 was to find out whether there would be an improvement in the survival rate to adult emergence when larvae are raised under more "natural" temperature and light conditions. From Table 6.14, it is clear that survival to completion of development is considerably improved under the variable temperature conditions. The emergence rate is around 15% for three treatments (V1, V3 and V4) and 36% for the 7.5°C treatment (V2). The highest survival rate for the constant temperature treatments is 8%. Overall, the success rate for the variable temperature treatments was 18% compared with 4% for the constant temperature treatments (or 5% if the 20°C treatment is excluded). This improvement is mainly due to the higher percentage of larvae completing Instar 3.ii and pupation .

However, Table 6.14 also shows that there was a higher survival rate until Instar 3.ii under the 10° and 15°C constant temperatures conditions compared with the 10° and 12.5°C variable temperature conditions. This may be due to the method in Investigation 1 – the sample jars were inspected more frequently, so the larvae would have been given more regular feeding (see Sections 6.3.1 and 6.3.2). There could be some metabolic stress in the variable temperature treatments when the start temperature was reduced during Instars 1 and 2. Or – as suggested earlier - it may simply be that a number of larval groups used in Investigation 2 had poor viability.

iii) Developmental rate of *P. madidus* from Instar 1 to emergence of the adult under variable temperature conditions.

The number of days and degree-days for all larvae to complete each stage of development are shown in Table 6.15. The means for the total number of days and degree-days to complete development, shown in the final column, are those for adults that completed development (see also Table 6.13).

Table 6.15 Variable temperature treatments: i) Mean number of days and ii) mean number of degree days (Cd) for larvae from all larval groups to complete each developmental stage with standard error (± 1 s.e.) given in brackets. iii) Temperature changes at each developmental stage within the four variable temperature treatments.

i) Number of days to complete each developmental stage

Initial temperature:		Instar 1	Instar 2	Instar 3.i	Instar 3.ii	Pupation	Total no. of days
5°C (V1)	mean *(s.e.)*	104 *(7.8)*	44 *(4.6)*	40 *(6.1)*	28 *(8.7)*	24 *(1.4)*	246 *(12.3)*
7.5°C (V2)	mean *(s.e.)*	63 *(3.2)*	115 *(5.2)*	49 *(3.7)*	43 *(4.4)*	23 *(1.4)*	289 *(1.2)*
10°C (V3)	mean *(s.e.)*	49 *(1.6)*	95 *(5.2)*	55 *(4.4)*	65 *(6.4)*	27 *(1.2)*	307 *(6.3)*
12.5°C (V4)	mean *(s.e.)*	37 *(1.3)*	47 *(4.5)*	82 *(4.5)*	106 *(7.3)*	20 *(1.7)*	315 *(4.5)*

ii) Number of degree days (Cd) to complete each developmental stage

Initial temperature:		Instar 1	Instar 2	Instar 3.i	Instar 3.ii	Pupation	Total no. of degree days
5°C (V1)	mean *(s.e.)*	550 *(56.6)*	385 *(55.7)*	436 *(75.0)*	366 *(124.8)*	256 *(13.1)*	2032 *(63.8)*
7.5°C (V2)	mean *(s.e.)*	455 *(19.6)*	676 *(40.4)*	466 *(37.7)*	520 *(50.1)*	329 *(47.8)*	2372 *(15.4)*
10°C (V3)	mean *(s.e.)*	469 *(11.5)*	610 *(31.2)*	418 *(24.2)*	745 *(54.6)*	379 *(21.6)*	2688 *(87.5)*
12.5°C (V4)	mean *(s.e.)*	452 *(11.0)*	373 *(28.2)*	499 *(25.5)*	1038 *(64.6)*	251 *(21.4)*	2779 *(59.7)*

iii) Temperature changes at each developmental stage

Initial temperature:		Instar 1	Instar 2	Instar 3.i	Instar 3.ii	Pupation	
5°C (V1)		5 → 7.5	7.5 → 10	10 → 12.5	12.5	12.5	
7.5°C (V2)		7.5 → 5	5 → 7.5	7.5 → 10	10 → 12.5	12.5	
10°C (V3)		10 → 7.5	7.5 → 5	7.5 → 10	10 → 12.5	12.5	
12.5°C (V4)		12.5 → 10	10 → 7.5	7.5 → 5	5 → 10 → 12.5	12.5	

Instar 1: The Instar 1 larvae were initially maintained at the start temperature of each treatment. Not surprisingly, the number of days to ecdysis decreased as the start temperatures increased (Table 6.15.i). However, the thermal time to complete development under the four temperature conditions, which ranged from around 450 to 550 degree-days, was not significantly different (Table 6.15.ii). As shown by the high standard error, there was considerable variability between the larvae maintained at 5°C (V1 treatment) which, combined with the high k-factor (Fig 6.11), may confirm that the newly hatched larvae used in this investigation experienced metabolic stress at this temperature.

As well as having a poorer survival rate than the larvae maintained at 10° and 15°C in the constant temperature treatments, the V3 (10°C start) and V4 (12.5°C start) larvae also took longer to complete this developmental stage (around 460 Cd compared with 320 Cd for the 10° and 15°C constant temperature larvae – see Tables 6.6.ii and 6.15). This may be because many of the variable temperature larvae experienced a fall in temperature during this developmental stage (see Table 6.15.iii).

Instar 2: Measured in thermal time, the V1 and V4 larvae took almost half the time to complete this stage compared with the V2 and V3 larvae (Table 6.15.ii). This difference was highly significant ($P < 0.0001$). As shown in Table 6.15.iii, during this developmental stage the temperature had been raised from 7.5° to 10°C for the V1 larvae and from 5° to 7.5°C for the V2 larvae. It was reduced from 7.5° to 5°C for the V3 larvae and from 10° to 7.5°C for the V4 larvae. The common factor appears to be the experience of 5°C by the V2 and V3 larvae. Although not strongly indicated by the k-factors for V2 and V3 (see Fig 6.11), it is possible that this temperature causes some metabolic stress for Instar 2 larvae, so lengthening the thermal time to complete development. The shorter thermal time of the V1 and V4 larvae to complete this developmental stage (around 380 Cd) is comparable with that for the larvae maintained at 10°C in the constant temperature treatments (393Cd)

Instar 3.i: For the growing phase of the third instar, the difference in the developmental rates of the treatments in terms of thermal time is only significant when the V3 larvae – the fastest developers - are compared with the V4 larvae - the slowest developers ($t = 2.304$, $P = 0.02$). Interestingly, the V4 larvae also suffered the highest mortality compared with the other groups during this stage (Fig 6.11). In general, however, the larvae seem to be well adapted to the various temperatures experienced during this stage of their development. In terms of thermal time, the developmental rate of the variable temperature larvae is similar to that for the C1 larvae in Investigation 1 (426Cd – see Table 6.6.ii). These larvae had been maintained at 7.5°C and were the fastest developers during Instar 3.i.

Instar 3.ii: With the exception of V1 and V2, the thermal time spent during the quiescence phase is significantly different between treatments ($P<0.01$). As shown by the high standard error (Table 6.15.ii), there was, again, a large variation between the V1 larvae. Table 6.15.ii shows that the number of degree-days spent in quiescence increased with increasing start temperature and ranged from 366 (s.e. = 124.8) Cd for the V1 larvae to 1038 (s.e .= 64.6) Cd for the V4 larvae. This was as predicted from Investigation 1 (see Section 6.4.3) and allows pupation to occur at approximately the same time of year regardless of month of hatching. During this developmental stage, the larvae of all four treatments were maintained at 12.5°C or experienced an increase in temperature to 12.5°C (Table 6.15.iii), as well as a lengthening of the photoperiod to 16 hours light and 8 hours dark (Table 6.3). It is possible, therefore, that the timing of pupation is dependent not only on environmental cues representing spring, but also on environmental conditions that occurred during the earlier developmental stages, e.g. Instar 1.

Pupation: Around 3 to 4 weeks were spent in the pupal phase (Table 6.15.i), the thermal time ranging between 250 to 380 Cd (Table 6.15.ii). Since the pupae were not disturbed, the exact day of emergence is not known for some larvae. The time spent on this developmental stage is probably an over-estimation, but is similar to the range found during Investigation 1 (around 300 to 350 Cd – see Table 6.6.ii).

Total number of days to complete development: (see final column of Table 6.15.i and ii) Emergence occurred on Day 246 (s.e. = 12.3) or 8½ months after hatching for the V1 treatment, Day 289 (s.e. = 1.2) or 9½ months after hatching for the V2 treatment, Day 307 (s.e. = 6.3) or 10 months after hatching for the V3 treatment and Day 315 (s.e. = 4.5) or 10½ months after hatching for the V4 treatment. Assuming the treatments represent September (V4), October (V3), November (V2) and December (V1) hatchings, then the larvae would emerge as adults within 30 days of each other towards the end of July and in early August. This is an improvement on the model shown in Fig 6.9, which predicts that larvae hatched in December would not emerge until September (curve (e)), and supports the hypothesis proposed in Section 6.4.3 of a flexible quiescence phase in order to co-ordinate adult emergence.

In summary, it appears that the larvae of this investigation are not well adapted to 5°C in their first and (possibly) second instar. However, larvae that survive these conditions do well, a large proportion successfully completing development. The Instar 3 larvae are well adapted to temperatures as low as 5°C. This is to be expected, given the seasonal temperatures normally experienced by *Pterostichus madidus* during its developmental stages in the region where sampling took place (East Midlands). This investigation has also given clear evidence of a flexible quiescence period, which enables larvae that were not hatched until late autumn and early winter to complete their development within 4 weeks of larvae hatched up to 4 months earlier (late summer and early autumn). Although *P. madidus* in its immature stages is well adapted to the wide range of temperature conditions that are typical of the temperate climate of Britain, it is also clear that, for successful development, this beetle has to be raised under conditions which follow the external seasonal conditions of temperature and light. These environmental cues influence the length of the quiescent period during Instar 3, hence the timing of pupation.

Table 6.12 **VARIABLE TEMPERATURE TREATMENTS: i) Initial number and ii) percentage survival of larvae for each developmental stage; iii) leg colour and sex of successful eclosions.** Larvae have been grouped by leg colour of parents and by batch number (b1, b2 etc). See Section 6.3.1 and Table D.3 for explanation of symbols and names for larval groups.

1 = instar 1; 2 = instar 2; 3.i = growth stage of instar 3; 3.ii = quiescent stage of instar 3; P = pupation; E = emergence.
B = black; R = red; W = wild (leg colour unknown); m = male; f = female.

i) Number of larvae (grouped by leg colour of parents) at start of each developmental stage.

ii) Percentage survival to emergence of adult.

iii) Number, leg colour and sex of emerged adults.

Developmental stage:

5°C start	1	2	3.i	3.ii	P	E
WB12	10	2	2	2	1	1
WB13	8	6	6	6	4	3
WB25	3	2	2	2	1	0
RR(9.6)-b2	11	1	1	1	1	1
all larvae	**32**	**11**	**11**	**11**	**7**	**5**

Developmental stage:

5°C start	1	2	3.i	3.ii	P	E
WB12	100	20	20	20	10	10
WB13	100	75	75	75	50	38
WB25	100	67	67	67	33	0
RR(9.6)-b2	100	9	9	9	9	9
all larvae	**100**	**34**	**34**	**34**	**22**	**16**

Emerged adults:

5°C start	Bm	Bf	Rm	Rf
WB12	1	0	0	0
WB13	1	2	0	0
WB25	0	0	0	0
RR(9.6)-b2	0	0	1	0
total	2	2	1	0

7.5°C start	1	2	3.i	3.ii	P	E
WB2-b1	14	12	8	8	6	4
WB3	2	0	0	0	0	0
WB14-b1	9	5	3	3	3	3
WB14-b2	5	3	3	2	1	1
RR9-b1	9	8	8	8	6	6
all larvae	**39**	**28**	**22**	**21**	**16**	**14**

7.5°C start	1	2	3.i	3.ii	P	E
WB2-b1	100	86	57	57	43	29
WB3	100	0	0	0	0	0
WB14-b1	100	56	33	33	33	33
WB14-b2	100	60	60	40	20	20
RR9-b1	100	89	89	89	67	67
all larvae	**100**	**72**	**56**	**54**	**41**	**36**

7.5°C start	Bm	Bf	Rm	Rf
WB2-b1	0	4	0	0
WB3	0	0	0	0
WB14-b1	1	2	0	0
WB14-b2	1	0	0	0
RR9-b1	2	1	1	2
total	4	7	1	2

10°Cstart	1	2	3.i	3.ii	P	E
BB(14.2)-b1	15	11	9	8	8	8
BB(14.2)-b2	3	1	0	0	0	0
BB(14.2)-b3	4	1	0	0	0	0
BB(14.4)-b1	18	7	6	6	5	1
BB(14.4)-b2	5	0	0	0	0	0
BB18-b1	10	6	3	3	3	1
WB1	2	2	0	0	0	0
WB2-b1	10	10	8	8	6	4
WB3	9	3	1	1	0	0
WB5	4	2	1	1	0	0
WB14-b1	10	7	6	6	6	6
WB140	4	1	0	0	0	0
RR6-b1	12	8	3	3	2	0
RR6-b2	10	1	1	1	0	0
RR9-b1	6	6	6	5	0	0
RR9-b2	5	5	2	2	1	1
RR18	9	1	1	1	1	0
WR17	4	2	2	0	0	0
WR101	7	0	0	0	0	0
WR103	4	1	0	0	0	0
all larvae	**151**	**75**	**49**	**45**	**32**	**21**

10°Cstart	1	2	3.i	3.ii	P	E
BB(14.2)-b1	100	73	60	53	53	53
BB(14.2)-b2	100	33	0	0	0	0
BB(14.2)-b3	100	25	0	0	0	0
BB(14.4)-b1	100	39	33	33	28	6
BB(14.4)-b2	100	0	0	0	0	0
BB18-b1	100	60	30	30	30	10
WB1	100	100	0	0	0	0
WB2-b1	100	100	80	80	60	40
WB3	100	33	11	11	0	0
WB5	100	50	25	25	0	0
WB14-b1	100	70	60	60	60	60
WB140	100	25	0	0	0	0
RR6-b1	100	67	25	25	17	0
RR6-b2	100	10	10	10	0	0
RR9-b1	100	100	100	83	0	0
RR9-b2	100	100	40	40	20	20
RR18	100	11	11	11	11	0
WR17	100	50	50	0	0	0
WR101	100	0	0	0	0	0
WR103	100	25	0	0	0	0
all larvae	**100**	**50**	**32**	**30**	**21**	**14**

10°Cstart	Bm	Bf	Rm	Rf
BB(14.2)-b1	2	6	0	0
BB(14.2)-b2	0	0	0	0
BB(14.2)-b3	0	0	0	0
BB(14.4)-b1	0	1	0	0
BB(14.4)-b2	0	0	0	0
BB18-b1	1	0	0	0
WB1	0	0	0	0
WB2-b1	2	2	0	0
WB3	0	0	0	0
WB5	0	0	0	0
WB14-b1	2	4	0	0
WB140	0	0	0	0
RR6-b1	0	0	0	0
RR6-b2	0	0	0	0
RR9-b1	0	0	0	0
RR9-b2	0	0	0	1
RR18	0	0	0	0
WR17	0	0	0	0
WR101	0	0	0	0
WR103	0	0	0	0
total	7	13	0	1

12.5°Cstart	1	2	3.i	3.ii	P	E
BB18-b1	10	9	9	9	7	7
BB18-b2	1	1	1	0	0	0
WB1	3	2	2	1	1	0
WB2-b2	8	4	4	4	0	0
WB3	7	1	1	1	1	1
WB5	4	3	1	1	1	1
WB120	1	1	0	0	0	0
WB140	8	7	3	3	2	2
RR9-b2	10	8	8	2	0	0
WR101	8	2	1	1	1	0
WR103	3	2	1	1	1	0
all larvae	**63**	**40**	**31**	**23**	**14**	**11**

12.5°Cstart	1	2	3.i	3.ii	P	E
BB18-b1	100	90	90	90	70	70
BB18-b2	100	100	100	0	0	0
WB1	100	67	67	33	33	0
WB2-b2	100	50	50	50	0	0
WB3	100	14	14	14	14	14
WB5	100	75	25	25	25	25
WB120	100	100	0	0	0	0
WB140	100	88	38	38	25	25
RR9-b2	100	80	80	20	0	0
WR101	100	20	10	10	10	0
WR103	100	67	33	33	33	0
all larvae	**100**	**63**	**49**	**37**	**22**	**17**

12.5°Cstart	Bm	Bf	Rm	Rf
BB18-b1	1	6	0	0
BB18-b2	0	0	0	0
WB1	0	0	0	0
WB2-b2	0	0	0	0
WB3	1	0	0	0
WB5	1	0	0	0
WB120	0	0	0	0
WB140	2	0	0	0
RR9-b2	0	0	0	0
WR101	0	0	0	0
WR103	0	0	0	0
total	5	6	0	0

Table 6.13 VARIABLE TEMPERATURE TREATMENTS: Mean accumulative number of days and degree days for each larval group to reach each developmental stage. Larvae have been grouped by the leg colour of the parents and by batch number (b1, b2 etc). See Section 6.3.1 and Table D.3 for explanation of symbols and names for larval groups.
1 = instar 1; 2 = instar 2; 3.i = growth stage of instar 3; 3.ii = quiescent stage of instar 3; P = pupation; E = emergence.

treat-ment		(i) Days					(ii) Degree days				
	larval group	Instar 2	Instar 3.i	Instar 3.ii	Pupation	Emergence	Instar 2	Instar 3.i	Instar 3.ii	Pupation	Emergence
5°C start (V1)	WB12	117	156	196	n.d.	226	624	988	1455	n.d.	1905
	WB13	85	139	176	206	235	408	806	1304	1688	2008
	WB25	121	151	179	197		707	1088	1333	1552	
	RR(9.6)-b2	154	185	259	n.d.	298	943	1293	1671	n.d.	2228
	mean	**104**	**148**	**188**	**204**	**246**	**550**	**935**	**1370**	**1661**	**2032**
	s.e.	7.8	4.6	8.5	5.2	12.3	56.6	54.1	72.7	74.6	63.8
	n	11	11	11	6	5	11	11	11	6	5
	larval group	Instar 2	Instar 3.i	Instar 3.ii	Pupation	Emergence	Instar 2	Instar 3.i	Instar 3.ii	Pupation	Emergence
7.5oC start (V2)	WB2-b1	58	184	241	268	289	437	1190	1758	2089	2373
	WB12	55	INCUBATOR BREAKDOWN				412	INCUBATOR BREAKDOWN			
	WB14-b1	84	184	235	263	287	584	1187	1689	2035	2348
	WB14-b2	76	170	226	263	292	541	1058	1573	2035	2398
	RR9-b1	65	173	210	269	291	466	1083	1416	2118	2388
	WR14	42	INCUBATOR BREAKDOWN				315	INCUBATOR BREAKDOWN			
	mean	**62**	**179**	**227**	**267**	**289**	**455**	**1139**	**1604**	**2069**	**2373**
	s.e.	3.2	4.1	4.1	0.9	1.2	19.6	36.0	46.8	10.0	15.4
	n	37	22	22	15	14	37	22	22	15	14
	larval group	Instar 2	Instar 3.i	Instar 3.ii	Pupation	Emergence	Instar 2	Instar 3.i	Instar 3.ii	Pupation	Emergence
10oC start (V3)	BB(14.2)-b1	60	189	239	308	334	586	1391	1876	2822	3178
	BB(14.2)-b2	58					490				
	BB(14.2)-b3	101					748				
	BB(14.4)-b1	59	178	208	261	275	491	1309	1641	2351	2540
	BB18	54	168	202	245	271	501	1102	1371	1883	2185
	WB1	35					350				
	WB2-b1	45	96	203	306	324	446	879	1392	2351	2711
	WB3	45	138	197			433	958	1403		
	WB5	35	203	239			350	1428	1813		
	WB12	44	113	INCUBATOR BREAKDOWN			431	831	INCUBATOR BREAKDOWN		
	WB14-b1	50	170	209	245	275	501	1133	1473	1874	2244
	WB14-b2	47	101	INCUBATOR BREAKDOWN			470	828	INCUBATOR BREAKDOWN		
	WB140	54					480				
	RR6-b1	42	184	234	275		408	1232	1841	2390	
	RR6-b2	34	185	199			340	1535	1710		
	RR9-b1	41	101	165			410	914	1277		
	RR9-b2	61	109	182	278	303	586	850	1205	2154	2473
	RR18	48	169	212	288		450	1150	1551	2501	
	WR17	38	163				365	1083			
	WR103	35					350				
	mean	**49**	**145**	**205**	**277**	**307**	**469**	**1087**	**1535**	**2321**	**2688**
	s.e.	1.6	5.7	4.8	4.9	6.3	11.5	34.2	39.2	57.7	87.5
	n	93	57	45	33	21	93	57	45	33	21
	larval group	Instar 2	Instar 3.i	Instar 3.ii	Pupation	Emergence	Instar 2	Instar 3.i	Instar 3.ii	Pupation	Emergence
12.5oC start (V4)	BB18-b1	36	96	189	302	320	450	938	1450	2640	2868
	BB18-b2	24	196				300	1400			
	WB1	36	112	221			450	1018	1625		
	WB2-b2	33	48	135			413	563	1131		
	WB3	28	52	110	275	293	350	585	1035	2265	2503
	WB5	58	112	251	298	320	593	1018	1925	2506	2813
	WB12	33	49	115	INCUB. BREAKDOWN		413	568	1058	INCUB.BREAKDOWN	
	WB14-b1	45	79	190	INCUB. BREAKDOWN		515	815	1498	INCUB.BREAKDOWN	
	WB14-b2	33	70	139	INCUB. BREAKDOWN		413	708	1068	INCUB.BREAKDOWN	
	WB120	43					538				
	WB140	33	96	180	278	303	409	859	1318	2278	2590
	RR9-b2	41	80	175			513	768	1373		
	WR14	37	82	INCUBATOR BREAKDOWN			463	854	INCUBATOR BREAKDOWN		
	WR101	36	112	221	310		450	928	1565	2233	
	WR103	36	168	221	318		450	1210	1565	2293	
	mean	**37**	**83**	**163**	**298**	**315**	**452**	**817**	**1301**	**2487**	**2779**
	s.e.	1.3	4.6	7.2	4.2	4.5	11.0	29.5	40.7	57.8	59.7
	n	60	51	36	13	11	60	51	36	13	11

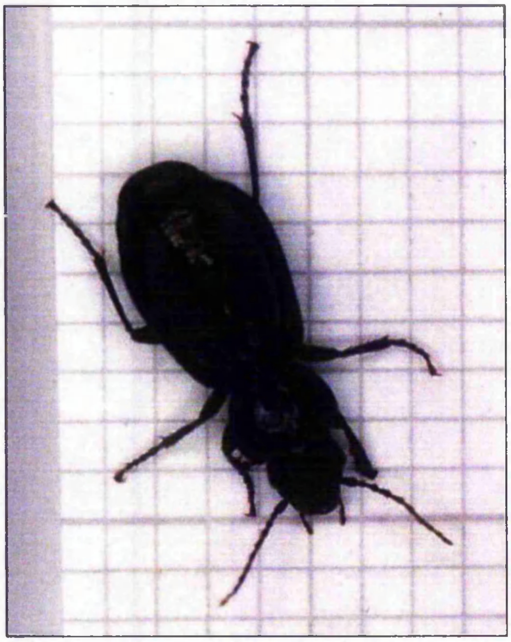
6.4.5 Constant temperature treatments: condition, growth and developmental rates of emerged beetles

The parentage, batch number, leg colour, sex, weight, thermal time to complete development and condition of beetles which emerged under constant temperature conditions are given in Table D.5 in Appendix D. As shown in Table D.2, the three RB tenerals from C1 are siblings. RR.1 from C1 and RR.1 from C2.iv are also from the same larval batch. It is not known whether the BB or RB tenerals from C2.ii (the 10°C "tank" treatment) are siblings.

i) Condition of emerged beetles

From Table D.5, only 2 of the 14 tenerals were perfectly formed. One beetle, which partially emerged, was so malformed the sex and leg colour could not be determined. Another emerged with no elytra and lived for only 14 days. Two beetles had large dents in one of their elytra, possibly due to an internal deformation. The elytra failed to fuse fully in the remaining 8 beetles (Plate 6.3). The elytral malformations may be due to disturbance of the larvae and pupae during the late developmental stages when they were weighed. Only 4 beetles survived for 8 months or longer, 3 males and 1 female. Attempts to mate the C1 RB siblings with each other failed, as did all other attempts at breeding the beetles.

Plate 6.3 **Incompletely developed elytra on a laboratory-reared *P. madidus***



iii) Comparison of the growth and development of the red and black-legged emerged beetles in their immature stages

Eleven of the 14 emerged beetles were reared under the same conditions within their respective sub-treatments, 6 from C1 (larvae initially maintained at 7.5°C) and 5 from C2.ii (the "tank" larvae maintained at 10°C). The data sets are obviously too low to attempt a statistical analysis on the sources of variation - temperature treatment during development, parentage, sibling batch, sex and leg colour of emerged beetle. Therefore, for comparison, the development and growth rate of each individual beetle are presented in Figs 6.12 (C1 and C2.ii) and 6.13 (C1 only).

C1 sub-treatment. The emerged beetles from C1 show a remarkable similarity in their timing to third instar during development (Fig 6.12). This was independent of parentage, sex or leg colour. The one RR emergence from this treatment (a red-leg) required fewer thermal days to complete Instar 1 and was heavier at instar change (see Fig 6.13). Although the three red-legged RB.1 siblings spent as long on Instar 1 as did the black-legged morphs, two of the siblings took fewer degree-days to complete Instar 2 compared with the BB and RR larvae (see Fig 6.12). There is no other indication of a difference in the growth and developmental rates between the red and black-legged morphs. The emerged adults from the RB parental group would, of course, be heterozygous if the genes coding for red are dominant. It is not known whether the RR emergence is heterozygous or homozygous for leg colour.

C2.ii sub-treatment. Fig 6.12 shows no difference in the total developmental time required by the red and black-legged morphs from C2.ii. The RB offspring, whether red or black-legged, required more degree days to develop to maturity than the BB offspring ($t = 3.84$; d.f. = 3; $P < 0.05$). Within the parental groups, there was, again, a remarkable similarity in the number of degree-days to complete development to Instar 3.

Although the emerged beetles from the C2.ii treatment were, on average, significantly heavier than those from the C1 treatment ($t = 2.262$; d.f. = 9, $P = 0.02$), the majority, if not all of the beetles reared under constant temperature conditions, showed poor viability in terms of survival and reproduction. There is no firm evidence of a difference in growth and developmental rates between red and black-legged *Pterostichus madidus*, although the emerged beetles from the 7.5^{0}C treatment (C1) do not contradict a hypothesis that the red-legged morph may be better adapted to this temperature in its early larval stages. However, there are clearly greater differences between sibling batches than between the two leg colour morphs.

Fig 6.12 Development of emerged beetles from i) C1 sub-treatment (7.5°C start) and ii) C2.ii sub-treatment (10°C "tank" larvae).

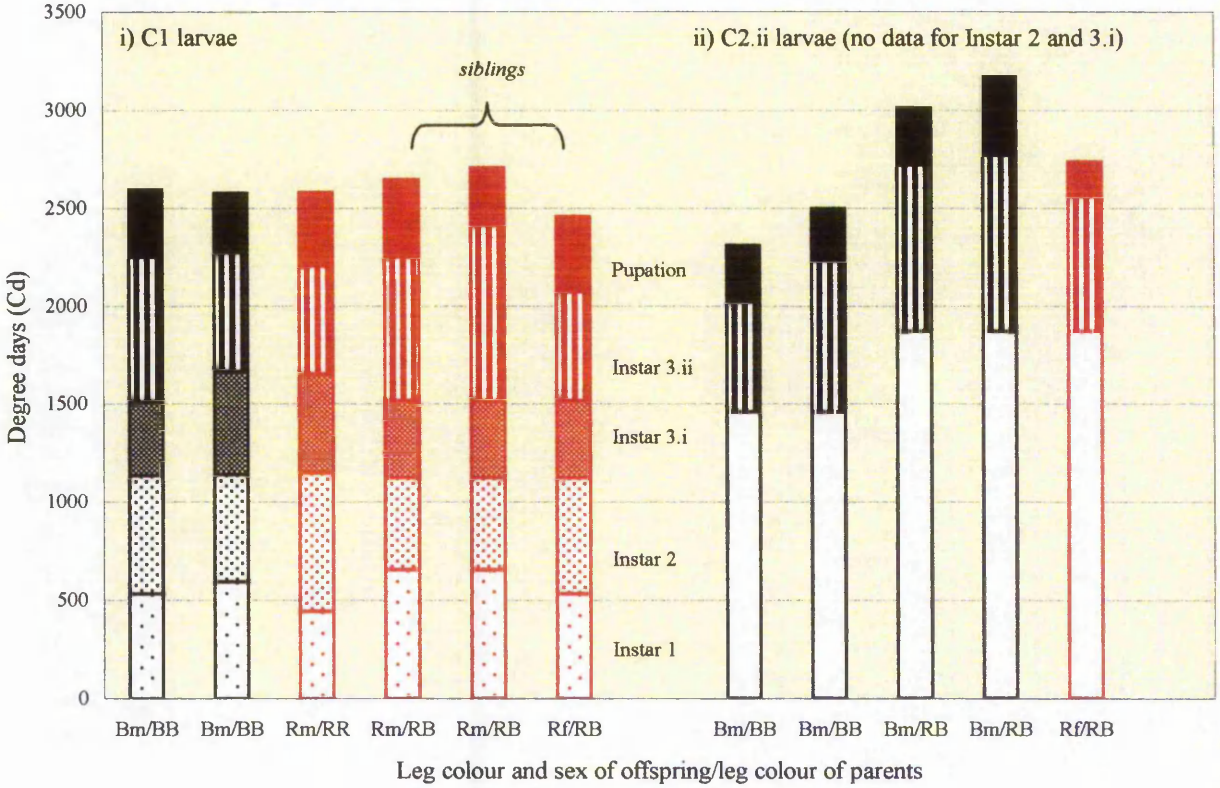
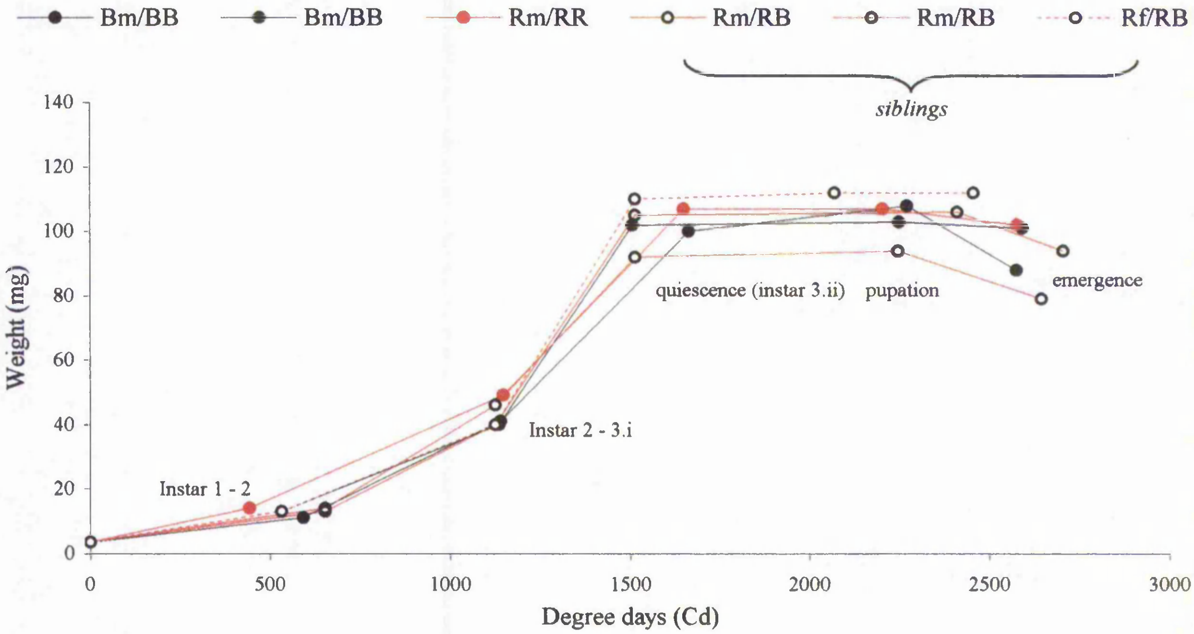


Fig 6.13 Growth rate of red and black-legged offspring from sub-treatment C1 (7.5°C constant temperature until Instar 3.i).



6.4.6 Variable temperature treatments: condition, growth and developmental of the larvae that completed development

Table D.6 gives the parentage, batch number, leg colour, thermal time to complete development, weight and condition of the beetles which emerged under constant temperature conditions. The day number of death after emergence is given where known. Several beetles were still alive at the end of the investigation and were released into the field. Emerged adults from the BB(14.2) and BB(14.4) groups were released immediately after feeding.

i) <u>Condition of emerged beetles</u>

From Table D.6, 35 of the 51 emerged beetles (69%) were perfectly formed. This is a considerable improvement on the constant temperature treatments and confirms that disturbance of the pupae and Instar 3 in its later stages has an adverse effect on development. However, some larval groups were more susceptible to failures in development than others, e.g. the WB13 group from V1, the WB2 group from V2 and V3 and the BB(14.2) and BB(14.4) groups from V3 (see Table D.6), so there could also be a genetic component.

The malformations that occurred were similar to those from the constant temperature treatments. A failure of the elytra to fuse fully, other elytral malformations or complete absence of an elytra were the main problems. Although seriously damaged beetles died within 30 days in the laboratory (Table D.6), beetles whose only apparent malformation was a poorly fused elytra could live for up to 6 months in the laboratory, e.g. WB2.6, WB2.7 and WB2.11.

ii) <u>Reproductive fitness of the emerged beetles</u>

To assess the reproductive fitness of the laboratory reared beetles, the females of groups WB2 (from V2 and V3), WB14 (from V2 and V3) and RR9-b1 (V3) were paired with various males of the same leg colour. Females paired with males are asterisked in Table D.6. Females who laid eggs are indicated by a tick in the final column of Table D.6.

Table 6.16 lists the females who were paired with males and/or who laid eggs. The listed males were housed individually with the females at various times during the investigation and include laboratory-reared males from the WB parental groups and wild males. Table 6.16 also shows the age of the female when eggs were laid, the number of eggs and the number of larvae hatched. The group name of the larval offspring and the number of larvae that completed development are given in the final two columns. Only a proportion of the hatched larvae was used in the various treatments.

Table 6.16 Names of laboratory-reared females paired with males and number and viability of eggs laid. Number of offspring that succeeded in completing development is also given. (See Tables D.3 for explanation of names for larvae).

Day numbers are counted from the emergence day of the female (= Day 1).
Eggs that the female wrapped in the agar substrate are termed "wrapped".

Name of female (Treatment is given in brackets)	Names of males (B = black R = red)	Day no. of female when eggs were laid	No. of eggs	No. of larvae hatched	Day no. of death of female	Name of larval group (Treatment is given in brackets)	No. of offspring that completed development
WB2.2 (V2)	WB14.25 WB12.8	no eggs	-	-	345	-	
WB2.6 (V2)	no males	185	6 – unwrapped	0	194	-	
WB2.8 (V3)	WB12.8 WB14.7 wild B males	no eggs	-	-	488	-	
WB2.11 (V3)	WB2.20	151	10 – unwrapped	0	181	-	
WB2.23 (V2)	WB12.8 wild B males	357	8 – unwrapped	0	370	-	
WB14.2 (V3)	WB12.8 WB14.51 wild B males	347	23 – wrapped	23	>620	BB(14.2) (V3)	8
		362	4 – wrapped	3			
		384	8 – wrapped	5			
		402	6 – wrapped	5			
WB14.4 (V3)	WB12.8 WB14.6 WB14.7 WB14.51 WB14.25 wild B males	397	27 – wrapped	25	493	BB(14.4) (V3)	1
		446	10 – wrapped	5			
WB14.5 (V3)	WB14.6 WB14.7 WB14.51	no eggs	-	-	149	-	
WB14.8 (V3)	WB14.6 WB14.7 WB14.51	no eggs	-	-	285	-	
WB14.21 (V2)	WB14.6 WB14.51 wild B males	no eggs	-	-	231	-	
WB14.22 (V2)	WB14.6 WB14.51	no eggs	-	-	221	-	
RR9.4 (V2)	RR9.1 RR9.3	419	2 – unwrapped	0	463	-	
RR9.6 (V2)	wild R males	355	22 – wrapped	15	503	RR(9.6)-b1	0
		395	27 - wrapped	24		RR(9.6)-b2	1
		425	4 – wrapped	not kept		(V1, V3)	

As shown in Table 6.16, 3 of the 12 females paired with males laid viable eggs. These eggs had been wrapped in agar by the female. Unwrapped eggs never hatched and are assumed to be unfertilised. For example, one female, WB2.6, which had an incompletely fused elytra (see Table D.6), was not paired with a male, yet laid 6 unwrapped eggs.

The fertilised eggs had been laid by WB14.2 and WB14.4, both black-legged and RR9.6, a red-legged morph. These females laid 4, 2 and 3 batches of eggs respectively. The first batch of eggs from the two WB14 beetles appears to be the more viable, being higher in number and having a higher eclosion rate (see Table 6.16). In the case of RR9.6, there were two healthy batches of eggs of 22 (batch 1) and 27 (batch 2). The second batch actually had a higher rate of eclosion of 89% compared with 68% from the first batch.

Perfectly formed adults eventually emerged from the first batch of eggs laid by WB14.2 and the second batch of eggs laid by RR9.6 – see Table D.6 (note, not all hatched larvae were raised in the laboratory). Four of the eight WB14.2 offspring, termed BB(14.2) in Tables D.3 and D.6, had elytral malformations, as did the one WB14.4 offspring (BB(14.4).7 in Tables D.3 and D.6). It is not known whether this is due to poor fitness or laboratory conditions.

It is not known whether any of the laboratory-reared males had reproductive fitness. Except for RR9.6, which was only paired with wild males, laboratory-reared males were paired with the females around 70 days after emergence of the female. At around Day 120, wild males were also introduced to the females. Given that the females are able to store sperm (see Section 5.3.1), any of the males listed in column 2 of Table 6.16 could have fertilised the eggs of WB14.2 and WB14.4.

What is interesting is the timing of egg development. Two females, WB2.6 and 2.11, laid unfertilised eggs 150 to 180 days or 5 to 6 months after their emergence. They died within 30 days of this event. If this timing were typical of field beetles then, assuming emergence in May/June, larval hatching would occur in October/November. The remaining 5 egg-laying females laid their fertilised or unfertilised eggs 12 to 14 months after emergence (between 347 and 419 days) – see Table 6.16. Larval hatching would therefore occur from August to September the year following the emergence of the female. Notably, four of the five non-egg-layers had died by this age.

These results confirm that a proportion of *P. madidus* females can adopt a biennial cycle, emerging one year but not breeding until the next (Luff, 1975; Butterfield, 1996). The biennial reproductive cycle may be induced by environmental conditions such as lower temperatures, as suggested by Butterfield (1996). However, WB2.6 and WB2.11, who adopted an annual egg-laying cycle, were maintained under exactly the same laboratory conditions as the "biennial" females. The annual and biennial reproductive cycles may, therefore, be genetically determined.

iii) Comparison of the growth and development of the emerged beetles in their immature stages under each temperature treatment

Table D.6 gives the thermal time to complete development and weights of the emerged beetles for the four variable temperature treatments. Only weights of emerged beetles that had not eaten before their first weighing are recorded in the table. As for the constant temperature treatments, the data sets are too low to attempt a statistical analysis on the sources of variation due to parentage, sibling batch and leg colour of the emerged beetle within each temperature treatment. The weights and thermal developmental times of the males and females within the treatments were not significantly different, although data sets were low.

Table 6.17 summarises the means for the number of degree-days to complete development and the weight of the emerged beetle, using data for all the beetles. It is clear from this table that the number of degree-days to complete development increased with increasing start temperature. The difference between Treatments V1 and V2 is highly significant ($t = 7.018$; $P < 0.0001$) as is the difference between Treatments V2 and V3 ($t = 2.846$; $P = 0.004$). Although consistent with the trend, the difference between V3 and V4 is not significant.

The longer thermal time to complete development with increasing start temperature is mainly due to the greater number of degree-days spent on quiescence (see Table 6.15 in Section 6.4.4). The significantly longer thermal time spent by V2 and V3 larvae on Instar 2 (Table 6.15) increased the difference between V1 and V2 larvae for total thermal time but reduced the difference between the V3 and V4 larvae.

Table 6.17 Means ± 1 standard error for thermal time to complete development and weight of emerged beetle under each variable temperature treatment.
(n = number in data set; s.e. = standard error).

Treatment	n	Degree-days (Cd)		Weight (mg) before feeding	
		mean	± 1 s.e.	mean	± 1 s.e.
V1 (5°C start)	5	2032	63.8	95	3.4
V2 (7.5°C start)	14	2373	15.4	123	3.0
V3 (10°C start)	21	2688	87.5	111	3.8
V4 (12.5°C start)	11	2779	59.7	136	6.2

The mean weights of the beetles on emergence are also significantly different between treatments. The V1 beetles are significantly lighter than the V2 and V4 beetles (V1 v V2: $t = 5.098$, $P<0.0001$; V1 v V4: $t = 4.165$, $P = 0.001$) as are the V3 beetles (V3 v V2: $t = 2.271$, $P = 0.03$; V3 v V4: $t = 3.465$, $P = 0.0009$). The difference in mean weights between the V1 and V3 beetles is almost significant ($t = 2.034$, $P = 0.054$). The mean weights of the V2 and V4 beetles are not significantly different ($P = 0.08$).

The lower mean weight of the newly emerged V1 beetles is probably due to the metabolic stress incurred during Instar 1 when there was a high mortality (Table 6.12 and Fig 6.11). The lower mean weight of the V3 beetles was not expected, but concurs with the relatively high mortality of larvae during this treatment (Table 6.12). Notably, both the V1 and V3 treatments have a high incidence of malformed beetles with almost half the V1 and V3 beetles malformed compared with only 20% of the V2 and V4 beetles (Table D.6).

iv) Comparison of the growth and development of the red and black-legged emerged beetles in their immature stages

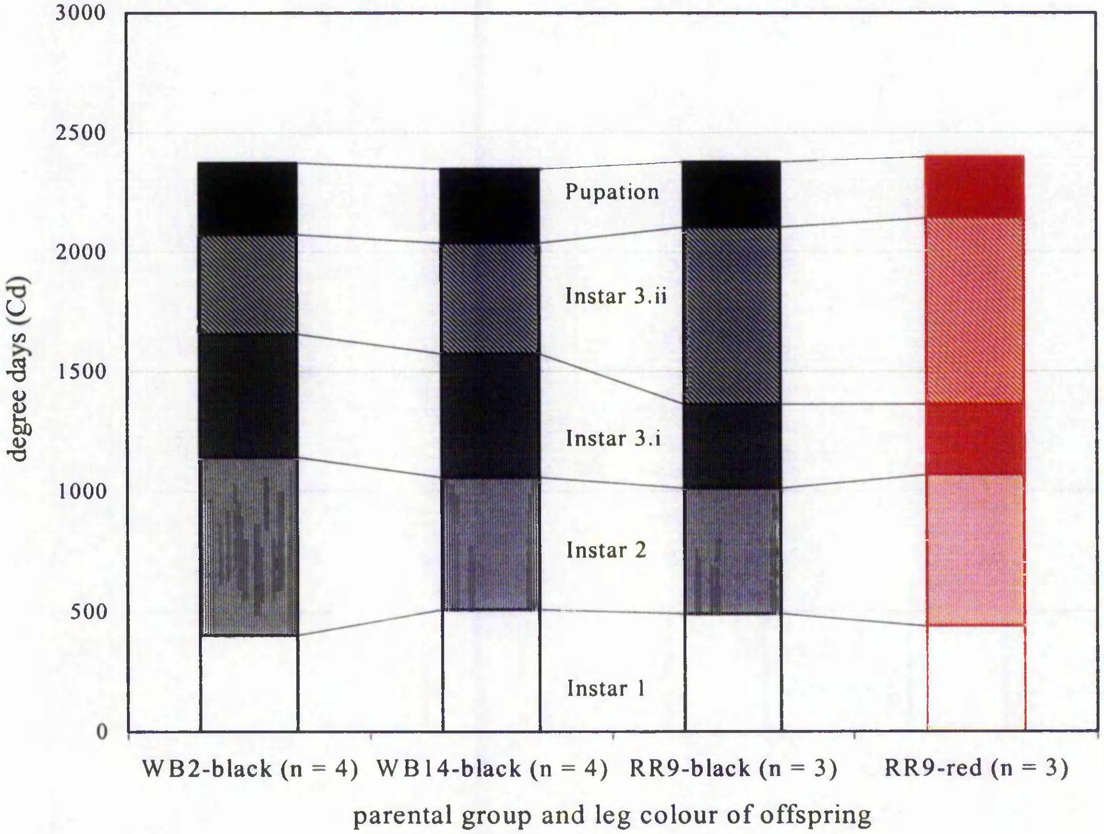
None of the emerged beetles from the 12.5°C treatment were red-legged and there were too few red-legged morphs from the other treatments to allow a statistical comparison of the growth and development of the black and red-legged emerged beetles. Nevertheless, there are some interesting observations.

V1 (5°C start) treatment: The one red-legged emergence, RR(9.6).28, took longer to complete development than the black-legged morphs from the WB12 and WB13 larval groups – 2228 Cd compared with 1905 Cd for WB12.8 and a mean of 2008 Cd for the WB13 group (Table 6.13) due to a longer time spent on Instar 1 - 943 Cd compared with 624 and 408 Cd for WB12.8 and the WB13 group respectively. On emergence, RR(9.6).28 was also one of the lightest beetles in this treatment (85mg – see Table D.6). However, it is obviously not possible to extrapolate from such a small data set.

V3 (10°C start) treatment: There was nothing exceptional about the developmental rate of the one red-legged emergence (see Tables 6.13 and D.6). Like some of the black-legged morphs from this treatment, it was light in weight (89mg), the elytra was not fused and it did not live long in the laboratory (53 days).

V2 (7.5°C start) treatment: From the results of the constant temperature treatments, there was some indication that, in terms of thermal developmental time and growth rate, red-legged morphs are better adapted to 7.5°C during instar 1 (see Section 6.4.2). This temperature was given to the larvae in the V2 treatment for the first 30 days only, after which the temperature was lowered to 5°C. Fig 6.14 shows the developmental time in degree-days of each immature stage of the emerged beetles from the various sibling groups maintained under the V2 temperature conditions. These are: the black-legged morphs from WB2 (n = 4) and WB14 (n = 4), the black-legged morphs from RR9 (n = 3) and the red-legged morphs from the same RR9 group (n = 3) – see Tables 6.13 and D.6.

Fig 6.14 Thermal time to complete development of each immature stage of the emerged beetles from sibling batches maintained under the V2 (7.5°C start) temperature conditions. (See Table 6.13 for names of larval groups).



Instars 1 and 2: The mean thermal time spent on Instars 1 and 2 by the four larval groups was not significant at the 5% level.

Instar 3.i: Most notable is the short developmental time spent on the growth stage of Instar 3 for the RR9 larvae regardless of leg colour phenotype in the adult. The difference between the larval groups is now significant (f-ratio = 3.708; P = 0.04; d.f. = 3).

Instar 3.ii: The longer time spent on the quiescent phase of Instar 3 by the two RR9 sibling batches (around 750Cd) compared with the WB2 and WB14 groups (around 440 Cd) is highly significant (f-ratio = 89.4; $P < 0.0001$; d.f. = 3). Again, the differences are between the larval groups not leg colour phenotypes.

Pupation: The earliest thermal time for pupation was 2035 Cd (WB14 group) and the latest was 2140 Cd (RR9-red). The difference between the means for the four groups is significant (f-ratio = 4.816; P = 0.03; d.f. = 3) but the actual source of variation is between the WB and the RR9 groups (WB2+WB14 v RR9-black+RR9-red: t = 3.050; P = 0.01; d.f. = 11).

Emergence: The four groups took a similar time to complete development to emergence, from 2348Cd (WB14 group) to 2399Cd (RR9-red group). The difference between the means is not significant (f-ratio = 2.437; P = 0.16).

Overall, the black-legged WB2 and red-legged RR9 showed similar development for Instars 2 and 3, whereas the RR9-black and RR9-red siblings showed a similar development from Instar 3 to emergence. These findings agree with those for the constant temperature treatments, when there was also a greater difference between sibling batches than between red and black-legged morphs (Figs 6.12 and 6.13).

One final observation is that mean weight of the three newly emerged red-legged RR9 beetles is significantly greater than the mean weight of the three black-legged beetles (138 mg ± 1 s.e. 1.70 compared with 118 mg ± 1 s.e. 2.05: t = 6.124, P = 0.002, d.f. = 4). This weight difference was maintained after feeding and throughout the beetles' time in the laboratory. Again, conclusions cannot be drawn from such small data sets.

6.5 Discussion

The results of the constant temperature treatments (Investigation 1), showed that larval rearing of *Pterostichus madidus* in the laboratory needs to take into account the seasonal conditions in the field, in particular temperature and (for the pre-pupal stage) photoperiod. From the results of both the constant and variable temperature treatments, it is clear that each immature stage is adapted to different temperature conditions.

Instar 1, which commonly develops during a period of rapid temperature change (late summer and autumn), tolerates a wide temperature range from 7.5 to 15°C. For the *P. madidus* larvae used in this investigation, a temperature as low as 5°C, which is typical of the soil temperature of early winter, causes high mortality. Instar 2 is better adapted to the lower but less wide-ranging temperature conditions of mid-autumn to early winter (5 to 10°C).

During its growth stage (phase 1), Instar 3 is well adapted to the soil temperatures of late December to early March, (4° to 5°C on average), but can tolerate temperatures up to 15°C typical of late spring/early summer. There is, however, a reduction in metabolic efficiency at higher temperatures. A lengthy period at cooler temperatures also appears to be a pre-requisite for normal development, as shown by the higher pupation and emergence rate and lower incidence of malformations in the emerged adult under variable temperature conditions.

The length of the quiescent phase of Instar 3 appears to be determined by the temperature experienced by the larva in its first instar. Larvae maintained at higher temperatures during Instar 1 (representing late summer and autumn eclosions) had a longer quiescent period than larvae maintained at low temperatures during Instar 1. This adaptation would allow adult emergence to coincide over a relatively short period regardless of the month of egg-laying.

The constant temperature treatments also found that termination of quiescence rarely occurs unless there is an increase in day-length. This response is probably qualitative and would allow adaptation to a wide latitudinal range. Temperature factors may also be involved, but could be a quantitative response i.e. a temperature threshold has to be reached before pupation occurs. In this investigation, successful pupations occurred at 10°C, though more commonly at 12.5°C. In the East Midlands, the soil is within this temperature range during May.

When Luff (1973) extrapolated the developmental time of his laboratory reared larvae to the field, he estimated that emergence would not occur before September. The results of the variable temperature treatments show that the number of thermal days to complete development is around 2100 Cd for larvae maintained at low temperatures during their first instar (representing December hatchlings) or 2500 Cd for larvae maintained at higher temperatures during Instar 1 (representing September/October hatchlings). Using realistic soil temperatures and depending on the month of hatching, 8 to 10 months would be required to reach full maturity, enabling emergence in June. This more closely represents what is presumed to occur in the field.

Luff's experimental larvae seemed better adapted to lower temperatures at Instars 1 and 2, compared with the larvae used in this investigation. There are two possible explanations.

First, Luff's beetles were from a higher latitude (Northumberland). His larvae may be showing an ecophysiological adaptation to the conditions of northern England, where winter temperatures can be assumed to be cooler than temperatures in the East Midlands. As a consequence, the larvae are likely to hatch later in autumn. This could also explain the higher red-legged proportion among the Close House population (see Section 3.4.4), assuming this morph is better adapted to a lower temperature range in the early instars.

However, Luff's beetles were obtained from a sheltered site (a walled garden) within a sheltered region (the Tyne valley). It is unlikely that the temperature difference between the Trent and Tyne valleys is so great as to delay hatching by up to two to three months in Northumberland.

An alternative explanation could be that larval batches have different adaptations depending on the time of year they are expected to hatch. In this investigation, a large proportion of the females used for breeding had been over-wintered in the laboratory. The exceptions were the females from the WB parental groups used in the variable temperature treatments. It is not known whether these females were in their first or second year of reproduction. The females in Luff's work were mated immediately after collection from the field and a large proportion would have been in their first year of reproduction.

It is believed that over-wintered females reproduce earlier in the year, probably from July onwards (Luff, 1973; Butterfield, 1996), when the ovaries of the newly emerged females are still maturing. The larvae of older mothers would therefore experience higher temperatures during first and second instar, whereas the offspring of beetles in their first year of reproduction would not hatch before mid-autumn. It is possible that the larvae of females in their second year of reproduction are pre-adapted to the seasonal conditions of late summer onwards, whereas those of newly emerged females are pre-adapted to autumn conditions.

This investigation also gave some evidence of a genetically determined biennial cycle in a proportion of the females. A number of laboratory-reared females from the variable temperature treatments did not lay eggs until one year after emergence, despite being housed with males two months after emergence.

Butterfield (1996) found that *P. madidus* adopts a biennial cycle at higher altitudes and argues that the success of this species is limited at these altitudes because of wear on the mandibles during autumn activity. If, due to cooler temperatures, the hatching of larvae from older mothers is delayed until autumn, it is likely that the fitness of *P. madidus* will be further reduced because the larvae are no longer synchronised with the season they are adapted to. For example, there appears to be a limitation on weight gain for each instar. This has been found to increase in a fixed proportion to the initial weight of each instar. Consequently, poor growth early on in development due to sub-optimal environmental conditions is not fully compensated for during later developmental stages even when conditions become optimal.

Apart from the significantly shorter time for the RR Instar 1 larvae to develop at 7.5°C in the C1 treatment, there was no consistent difference in the growth and developmental rates of the larvae of the four breeding pairs used in the constant temperature treatments. Data sets were too low in the variable temperature treatments to attempt any comparison.

There was no evidence of a difference in the growth and developmental rates between the red and black-legged emerged adults, although the data sets are small. The one RR larva to emerge under constant temperature conditions (C1, 7.5°C) was red-legged and may be homozygous for leg colour. This beetle, typical of its parental group, showed greater metabolic efficiency at first instar compared with the RB red-legged and BB black-legged morphs from the same sub-treatment. The red-legged RB emergence would, of course, be heterozygous. If a linked pleiotropic gene is dominant, there may be a heterozygous/ homozygous difference in development, with the heterozygote behaving more like the black-legged morph.

A comparison of the red and black-legged siblings raised under the V2 variable temperature conditions gave no evidence of a difference between the two morphs in terms of thermal time to complete each developmental stage, but there was a significant difference in the mean weights on emergence, the red-legged morphs being heavier on average. The one red-legged morph that emerged from V1 (5°C start) was clearly poorly adapted to the conditions of this treatment. It is not known whether the red-legged emerged beetles from the variable temperature treatments are homozygous or heterozygous.

In summary, a method for rearing *P.madidus* larvae under laboratory conditions has been developed. This needs to follow temperature and light conditions in the field. Under optimum conditions, a 33% emergence rate can be obtained. It is clear that the most critical period for development is from late Instar 3 to emergence. This occurs from late winter to early summer in the field, which is also the period that produced the strongest coefficient of determination in the multiple regressions (Sections 4.4.1 to 4.4.4). Unfortunately, the number of emerged beetles was too low to assess whether there are any differences between the morphs at the pupation/emergence stage of development.

SECTION D

Discussion

Conclusions

Further Work

CHAPTER 7 Discussion, Conclusions and Further Work

7.1 Discussion

7.1.1 Background and aims of study

Previous to this study, Terrell-Nield (1990a) had found a negative association between the red-legged morph of *Pterostichus madidus* and a wider annual temperature range in England and Wales, as well as a lower annual minimum temperature. In continental Europe, where the dominant leg colour form is red, the geographical range of *P. madidus* does not extend beyond 55° latitude (Lindroth, 1986). In Britain, *P. madidus* has succeeded in extending its range to 59° latitude in north Scotland and to more open country (Eyre *et al*, 1986; Luff *et al*, 1989), where the black-legged form can be in high proportion. The black-legged morph could therefore be an adaptation to the cooler climate of Britain. If this is the case, then it may be possible to use this species of beetle as an indicator of climate change.

Previous attempts to use the morph distribution of insect species (*Biston betularia* and *Adalia bipunctata*) as bio-indicators of environmental factors have run into difficulties. A limitation of the *B. betularia* study is the complexity of the biotic and abiotic factors that may be influencing the selective advantage of one morph over another. Even the selective mechanism is now being called into question (Section 1.5; also Hooper, 2002). The *A. bipunctata* study extended throughout Europe, but findings for one region were not necessarily consistent with those for another. Again, the selective mechanism remains uncertain (Section 1.6).

This study has attempted to circumvent these types of problems by limiting the investigation to regions within the U.K. It assumes that leg colour confers no specific advantage in terms of predator/prey relationships. However, there were a number of uncertainties. The genetics of leg colour in *P. madidus* was not known. Even assuming leg colour, or a gene linked to leg colour, influences selection between the morphs, the mechanism of selection was unknown. The only other published study of *P. madidus* morph frequency distribution has doubted the association of leg colour morph proportions with climate (Doberski & Gazzy, 2000).

In an attempt to resolve these uncertainties, this study has extended Terrell-Nield's work by (1) investigating morph frequency change in the U.K. over time and ascertaining whether changes in morph proportions reflect climatic data (Chapter 3) and (2) identifying associations between environmental factors and morph frequencies and investigating whether these are consistent within and between regions (Chapter 4). It also aimed to understand the genetics of leg colour polymorphism through breeding experiments (Chapter 5). Finally, by rearing the larvae of *P. madidus* under different temperature conditions, it aimed to improve our understanding of the whole ecology of this species of beetle and investigate whether there may be differences between the morphs in their immature stages (Chapter 6). The overall aim of the study was to find out whether *P. madidus* could act as a reliable bio-indicator of climatic factors.

7.1.2 Findings

There has been general agreement that the climate in Britain has been getting warmer since the first years of recorded morph frequencies (the mid-1970s). According to Terrell-Nield's hypothesis, there should be a concurrent change in the morph frequency distribution.

A comparison of the morph proportions for 1975 with 1995/6 data for Transect 1 (Southampton to Nottinghamshire) identified a small increase in the red-legged frequencies for some regions. For Transect 2 (Dorset to East Sussex) this trend is apparent only when 1976 morph frequencies are compared with those for 1996. By 1998, there had been a swing back to the black. The 3 to 6 years of monitoring of the ECN sites gave no evidence of consistent trends in morph frequency change either within or between the different regions. Similarly, Doberski & Gazzy (2000) found an overall stability in leg colour proportions over a 6-year period at two closely positioned sites within one forest.

By contrast, the longer 14-year time series from 1981 to 1994 inclusive for Close House, Northumberland showed a detectable decline in the red-legged morph frequency from 1984 and a positive correlation between the black-legged morph and the annual mean temperature for Central England for the same period. Even so, the year-by-year fluctuation in morph proportions around a mean, although significant ($P = 0.002$), is small (± 3.5%).

The changes in morph frequencies over time for *P. madidus* are clearly not as dramatic as those for the peppered moth and the two spot ladybird (e.g. Clarke *et al*, 1985; Brakefield & Lees, 1987). Doberski & Gazzy (2000) expected to detect a similar change in *P. madidus* morph proportions over 6 years of monitoring. However, the climate in Britain has not been subject to rapid directional change as was the case for smoke and sulphur dioxide pollution after the Clean Air Acts of 1956 and 1968 (Brakefield, 1990b). Mean monthly and annual temperatures for Central England varied considerably between 1975 and 1998, climate warming only becoming detectable since 1989 (Section 3.4.6).

Assuming simple Darwinian selection, it was calculated that there was a 2.2% selection against the red-legged morph at Close House for the period 1984 to 1994 (10 generations). Modelling this rate over 20 years, it was predicted that the red-legged frequency would have declined by only 10% from an initial frequency of 56% (Section 3.4.7). Clearly, 3 to 6 years of monitoring is not long enough to detect directional trends, especially when these are masked by annual variations in the relative fitness of the two morphs.

The direction of frequency change over time also appears to be inconsistent between the transects and Close House. However, monitoring of the transects and Close House took place over different time periods (1975/6 and 1995-8 for the transects; 1981-1995 for Close House). With the transect data, there is no knowledge of intermediate morph frequencies between the two periods of monitoring although a comparison of 1996 and 1998 Transect 2 data gives evidence of small fluctuations in morph proportions between years, as was the case for Close House. Furthermore, the data sets for some sites along Transects 1 and 2 were small, particularly for 1975/6, and therefore subject to high standard deviations. The results of the chi-squared analysis of morph proportions at some of these sites may thus be spurious.

The rise in black-legged frequencies at Close House and the more exposed sites along the transects might be explained by the negative association of red-legged morph frequencies with a wider temperature range (Terrell-Nield, 1990a). However, without knowledge of climate data for these regions, this interpretation is subjective, a criticism that was made of the interpretation of morph data correlations in the *B. betularia* and *A. bipunctata* investigations (Sections 1.5 and 1.6).

To compound matters further, the spatial variability in morph proportions over short distances is far greater than the temporal variability over long periods of time (Sections 3.4 and 4.4; also Doberski & Gazzy, 2000). On the other hand, there are similar morph proportions at sites located in different agroclimatic zones, where temperature differences would be considerable (Smith, 1986). This suggests that morph frequency variation is on a regional rather than national scale. There may be ecotypes of *P. madidus*, which are adapted to the particular climatic conditions of a region. Within a region, frequencies can range widely around a mean as shown by the East Midlands data (Section 4.4.6). The localised variations in morph proportions may be due to a constant micro-climatic gradient between closely positioned sites, where populations of *P. madidus* cannot mix because they are flightless. This would exert different selective pressures on the morphs. The discontinuous frequencies for *P. madidus* contrast with the smooth gradients found for *Biston betularia*, which has high mobility (Bishop, 1972).

Regardless of spatial scale or time period, the results of *P. madidus* sampling in 5 regions (Transects 1, 2, 3, East Anglia and the East Midlands) showed a consistent association between morph proportions and topographical features. Higher red-legged frequencies were found at the more urban and wooded sites. Higher black-legged frequencies occurred at sites located on higher ground and in intensive agricultural areas with sparse tree cover.

For Transects 1, 2, 3 and East Anglia, multiple regression analysis was employed as a diagnostic tool, using geographical, topographical, soil (East Anglia only) and annual or monthly climatic factors in order to identify the environmental factors that are associated with the red-legged morph.

Temperature was found to be the most influential climatic factor, with a positive correlation between the red-legged morph and a higher minimum temperature in cooler regions or periods and a lower maximum temperature in warmer regions or periods. The minimum temperature was negatively associated with the red-legged morph when the maximum and mean temperatures were relatively high (i.e. Transect 2 using 1996 and 1997 weather data). There was also an indication that the red-legged morph is disadvantaged by rapid changes in temperature. For example, the rapid temperature rise in February 1997 produced a positive correlation between cooler minimum and maximum temperatures and red-legged frequencies.

When monthly and seasonal climatic data were entered into the regressions, the months of spring, winter and (occasionally) autumn produced the highest F-ratios. Contrary to findings for the more northern site at Close House, an association with the climatic data for summer months was rare.

Of the geographical and topographical variables, the red-legged morph correlated positively with position north and negatively with position east and distance from sea. The association between altitude and red-legged frequencies was weak and could be positive (e.g. Transect 1) or negative (e.g. Transect 2). However, many of the inland higher altitude sites were located in river valleys, which are relatively sheltered. An altitude measurement that is relative to the highest point within the vicinity of the site might have been a more accurate predictor of exposed conditions.

Finally, for East Anglia, there was a positive association between the red-legged morph and acidic, coarse-textured soils. Doberski & Gazzy (2000) also found that the red-legged morph was in higher proportions on more acidic soils.

With 3 or 4 partial predictors, very high coefficients of determination for the 1975/6 Transects were produced ($R^2 > 85\%$). For the regions sampled in the mid-1990s, 50 to 60% of the morph frequency variability was explained. Compared with the 1975/6 analysis, there was a reduced temporal resolution in the weather data used in the 1995-8 analysis (see Table 4.2). When climate data closer to the years of sampling were used in the Transect 3 analysis, the coefficients of determination improved.

With the exception of East Anglia, however, there was a larger data set for the 1995-8 period compared with 1975/6 in terms of both number of sites and number of animals sampled at each site. Morph proportions obtained for the 1995-8 sites should therefore be more representative of the true proportions compared with the 1975/6 sites. Even so, for many sites, sampling occurred on one occasion only and the number of animals in the sample was less than 80. This would give a high standard deviation on the data (Section 2.4.2). Predictions within ± 10% of the actual frequency can, therefore, be regarded as reasonable.

It is also possible that the meteorological data did not represent the micro-climatic conditions of some of the sites, either because the weather stations were some distance from the site or because they were situated on open ground such as an airfield. This problem would affect both the 1975/6 and 1995-8 analyses.

Climate data could not take into account localised variations in morph frequency. For example, there were significant differences in the morph proportions of closely positioned sites along Transect 3 (Bethesda to Skegness). Inevitably, there was a large residual on one or both sites in the analysis because only one weather station could be used for each pair. It is this morph frequency variation over short distances that led Doberski & Gazzy (2000) to question the association of morph proportions with climatic factors.

To investigate this localised spatial variability, 41 sites with a spatial resolution of approximately 5km were sampled in the East Midlands (Section 4.4.6). Multiple regression analysis was again employed. The independent parameters were environmental factors relating to the sites' elevation, aspect and soil type, as well as surrounding topographical features such as waterways, woodland and urban dwellings.

The most significant association was between the red-legged morph and urban sites. The coefficient of determination increased further when these sites are also wooded. Other influential partial predictors associated with the red-legged morph were: a more equitable aspect, acidic and coarse textured soils, position west and a lower altitude. The association between the red-legged morph and acidic and coarse-textured soils may be coincidental, since these soil types tend to be located in the city of Nottingham where the underlying rock is sandstone. Similarly in East Anglia, higher red-legged frequencies were obtained from woods which, in this region, commonly grow on acidic, coarse-textured soils.

Only a third of the variation in morph frequencies could be explained using this analysis. There were two main problems. Firstly, the highest and lowest red-legged frequencies were under- and over-estimated respectively. Arcsin transformation of the percentage frequencies did not improve the modelling of these extreme frequencies. The topographically complex Transect 2 monitored in the 1990s similarly showed an inverse sigmoidal relationship between actual and predicted morph frequencies. It is possible that, at high and low frequencies, conditions for the rare morph become disproportionately unfavourable.

Secondly, there were unusually large residuals on some individual sites. This may be due to the type of land usage and disturbance that has occurred historically at a site, such as land clearance for development. A failure of the red-legged morph to adapt to more open conditions could raise its frequency in the surrounding areas, and lower its frequency in the once disturbed area. In a similar way, the ECN Rothampsted data gave evidence that the black-legged male colonised the hedgerow transect more rapidly than the red-legged male.

Other factors such as ground and canopy cover, which can affect the microclimate of a habitat, should perhaps have been included in the regressions. This is also suggested by the ECN data, which showed a higher black-legged morph frequency in grassland habitats compared to the woodland habitats in the same region. It could also explain why Doberski & Gazzy (2000) obtained a significant difference in morph proportions from two separate sites in Thetford Forest which were characterised by different canopy and ground cover.

However, it should not be assumed that microclimatic factors are influential throughout the year. The Colwick Wood sampling in 1995 identified an increase in the red-legged frequency with distance into the wood only when temperatures were rising rapidly (May and the early part of August). When Doberski & Gazzy (2000) recorded tree species and the understorey/shrub and ground layers for experimental plots *within* the two sites in Thetford Forest, they found a consistent *P. madidus* morph frequency, regardless of vegetation type. If the populations of morphs can freely mix, differences in the spatial distribution may occur only occasionally over the season and would be difficult to detect.

Despite its limitations, multiple regression analysis has been a powerful tool for identifying factors that influence the frequencies of the leg colour morphs of *P. madidus*. The findings for the East Midlands are consistent with those for Transects 1, 2 3 and the East Anglian sites and confirm the relationship between the red-legged morph and a more equitable climate, as found by Terrell-Nield (1990a) and with stronger levels of correlation.

The environmental factors strongly associated with the leg colour morphs are summarised in Table 7.1. Topography, geography and climate are, of course, linked. Maritime regions, providing they are not exposed to high wind flow, have a smaller diurnal and seasonal temperature range due to the warming effect of the sea surface. These temperature conditions are also known to occur in wooded areas. Urban areas also have a smaller range in diurnal and seasonal temperatures due to increased cloud cover and shelter from wind exposure by tall buildings. They are also likely to have a higher minimum temperature in winter compared to outlying areas due to the warming effect of heated buildings. The red-legged morph's association with more western sites within a region is consistent with the climatic gradient of England and Wales, where a less equitable climate is associated with more eastern regions (Gregory, 1976). Similarly Terrell-Nield (1990a) found a negative correlation between the red-legged frequency and Conrad's Index of Continentality.

Table 7.1: Summary of environmental factors associated with leg colour morphs of *Pterostichus madidus*.

Red-legged morph	Black-legged morph
CLIMATIC FACTORS	
▪ smaller annual and seasonal temperature range	▪ larger annual and seasonal temperature range
▪ higher minimum temperature in cooler years;	▪ lower minimum temperature in cooler years;
▪ lower minimum temperature in warmer years.	▪ higher minimum temperature in warmer years;
▪ lower maximum temperatures	▪ higher maximum temperature
▪ less tolerant of rapid changes in temperature	▪ more tolerant of rapid changes in temperature
TOPOGRAPHICAL/GEOGRAPHICAL FACTORS	
▪ wooded areas	▪ few woods or fragmented woodland
▪ urban areas	▪ intensively farmed areas
▪ maritime sites	▪ inland sites
▪ lower altitude relative to the region	▪ higher altitude relative to the region
▪ west or south facing sites	▪ north or east facing sites
▪ more western sites in a region	▪ more eastern sites in a region

Although multiple regression analysis has identified the many factors that influence morph frequencies, it is not possible to extrapolate from the equations to predict the kinds of temperatures that would affect the balance between the two leg colour morphs in any one region or year. Apart from the problem of non-linearity, temperature factors are relative not absolute values. This is also shown by the complex relationship between maximum and minimum temperatures and the localised variation in morph proportions by region. Although a multivariate analysis would remove the problems of non-linearity, it was not felt that this type of analysis would improve on the identification of significant environmental factors affecting morph frequencies.

Although the mechanism of selection influencing morph frequencies is not known, multiple regression analysis identified winter, spring and, for some regions and years, autumn as the most likely period of the year when selection between the leg colour morphs of *Pterostichus madidus* occurs. This suggests that selection pressure occurs during one or more of the developmental stages of the beetle and/or during over-wintering of the adult female. A link to temperature is also strongly indicated. For the more northern site at Close House, there was an association with the summer mean temperature for Central England, which suggests that the reproductive stage of the beetle can also be important.

This spread of associations over the year indicates that phenotypic induction of leg colour, termed "seasonal polyphenism" by Shapiro (1976), is unlikely. There was similarly no evidence of phenotypic induction from the breeding experiments. Regardless of temperature treatment, black-legged x black-legged parents produced only black-legged morphs. The progeny of red-legged x red-legged parents could be red or black-legged, even though they had been maintained under the same temperature conditions during their immature stages.

These results also give evidence that the genes coding for the red colour (or lack of melanin in the legs) are dominant. Although the leg colour of the emerged beetles was easily classified as red, there could be some melanin production in the legs of heterozygotes. This might explain the partially red-legged forms of *P. madidus* or forms with brownish legs that are occasionally found in the field (Terrell-Nield, pers. comm.; Doberski, pers. comm.).

Knowledge of the dominance/recessive relationship for leg colour has implications for modelling the rate of morph frequency change. Selection for or against the red-legged morph will be more efficient because dominant genes are expressed phenotypically in the heterozygote. Recessive genes - even when disadvantaged - are likely to remain in the population because they are not phenotypically expressed in the heterozygote. This lends support to the hypothesis that the black-legged form is an adaptation to the climatic conditions of the U.K (see Terrell-Nield, 1990a). The recessive gene for black would be present in the predominantly red-legged *P. madidus* populations on the European continent.

By rearing the larvae of *P. madidus* under conditions that take into account the seasonal conditions in the field, this study achieved a higher rate of survival to adult emergence than did Luff (1973) and Terrell-Nield (unpublished data). The critical stages during development were pupation and emergence, when most losses occurred. Under optimum conditions, the overall survival rate was around 25% (around 50% for the larvae and 50% for the pupae). This is similar to a survival rate of 24-28% for *Pterostichus melanarius* (Thiele & Krehan, 1969) but considerably lower than the rates reported by Heimbach (1994) for *Poecilus (Pterostichus) cupreus* (78% for larvae and 92% for pupae). Asteraki & Balsdon (1995) also obtained a relatively high survival rate of 69% for the pupae of *Nebria brevicollis* under optimum temperature and substrate conditions.

P. madidus is not, therefore, an easy beetle to raise under artificial conditions. It was found that its development is finely tuned to the seasonal temperature and light conditions of its native area, in this case, the East Midlands. Instar 1 tolerates a wide temperature range from 15° to 7.5°C, which represents the soil temperature from late summer to autumn. Instar 2 is better adapted to the lower temperature conditions of mid-autumn to early winter (10° to 5°C). During its growth stage, Instar 3 is well adapted to the soil temperatures of late December to early March, (4° to 5°C on average) but could tolerate temperatures up to 15°C. A lengthy period at cooler temperatures appears to be a pre-requisite for successful development. Similarly, *P. melanarius* achieved a higher emergence rate after experiencing cold temperatures representing winter during Instar 3 (Thiele & Krehan, 1969). The onset of quiescence during Instar 3 occurred when the larvae had reached their maximum weight. Termination of quiescence seemed to require an increase in day-length. This response is probably qualitative and would enable adaptation to a wide latitudinal range. The response to temperature may be quantitative. In this study, pupations occurred from 10° to 12.5°C, which is the typical soil temperature range for May in the East Midlands.

Larvae maintained at higher temperatures during Instar 1 (representing late summer and autumn hatchlings) had a longer quiescent period during Instar 3 than larvae maintained at low temperatures during Instar 1 (representing late autumn/early winter hatchlings). A model of the thermal rate to complete development using soil temperatures for the East Midlands showed that, depending on the month of hatching, 8 to 10 months is required to reach full maturity, adult emergence coinciding over a relatively short period in June. This represents what is presumed to occur in the field.

Clearly, populations of *P. madidus* are adapted (1) to their region of origin and (2) to the time of year they are expected to hatch. It is therefore interesting that Luff's experimental larvae were better adapted to lower temperatures at Instars 1 and 2, compared with the larvae used in this study. The obvious explanation is that the temperatures of northern England are cooler. However, the site at Close House has an equitable microclimate (Luff, pers. comm). A second explanation assumes that a large proportion of Luff's experimental female adults would have been in their first year of reproduction. Most females in this study had been over-wintered in the laboratory. In the field, over-wintered females are believed to reproduce earlier in the year from July onwards (Luff, 1973; Butterfield, 1996). The larvae of females in their second year of reproduction could be pre-adapted to the temperature conditions of late summer, whereas those of newly emerged females are pre-adapted to autumn conditions.

This study also produced some evidence that the biennial cycle in a proportion of the females could be genetically determined. This adaptation would be advantageous in cooler years and regions. A mixed population of annual and biennial females could also explain the large between-year variation in *P. madidus* numbers. For example, there could be a link between falling numbers and early onset of autumn the previous year in a predominantly "annual" population.

One final observation is that, for each instar, there appears to be a limitation on weight gain, which increased in a fixed proportion to the initial weight of each instar. Poor growth early on in development due to sub-optimal temperatures is therefore not fully compensated for during later developmental stages even when conditions become optimal. Again, this has implications for *P. madidus* numbers on a year-by-year basis.

Unfortunately, the data sets from the laboratory work were too small to identify any difference between the two morphs in terms of their development, growth or survival rates. Although the RR C1 (7.5°C) larvae spent a significantly shorter thermal time in Instar 1, it is not known whether they were destined to be red or black-legged. Only one larva emerged (a red leg). The red and black-legged siblings raised under the V2 variable temperature conditions showed no difference in the thermal time to complete each developmental stage, but there was a significant difference in the mean weight of the morphs on emergence, the red-legged morph being heavier. However, the data sets were too small to allow extrapolation. In fact, the similarities and differences within and between the sibling groups appear to be independent of leg colour. Clearly some sibling groups were well adapted to certain temperature conditions and had a high survival rate to emergence (>50%). Other groups suffered 100% mortality under the same conditions.

These results support the hypothesis that there are many ecotypes among the *P. madidus* population, each one adapted to different temperature conditions. This strategy of "spreading the risk" is described by den Boer (1993) for *Pterostichus oblongopunctatus* and ensures species' survival under unpredictable climatic conditions. It is not known from this study, whether the leg colour phenotypes are linked to specific ecotypes by pleiotropic genes. The localised variations in morph proportions suggest that they might be. A pleiotropic gene influencing both leg colour and a physiological trait related to temperature might not have the same dominance relationship, i.e. the red-legged heterozygote could exhibit the physiological trait associated with the recessive black-legged phenotype.

7.1.3 Could *Pterostichus madidus* be used as a bio-indicator of climate change?

Multiple regression analysis has found a consistent association between the red-legged morph of *Pterostichus madidus* and more equitable climatic conditions. These are usually characterised by higher minimum and lower maximum temperatures. However, in warmer years and regions, the correlation is with a lower minimum temperature, suggesting that this morph is favoured by cooler conditions providing the minimum temperature is not too low. The red-legged morph also appears to be disadvantaged by rapid changes in temperature.

The association with these climatic conditions could explain why *P.madidus* is a forest species in Europe, where it is predominantly red-legged (Lindroth, 1992). In Britain, *P. madidus* has been associated with the open country carabid communities (e.g. Luff, 1989; Butterfield, 1983). There is some evidence from this study of a difference in the activity pattern of the two morphs, with the black-legged morph dispersing more rapidly to more open habitats.

The use of *P. madidus* as a bio-indicator of climatic factors presents three problems. The first concerns the spatial scale of morph frequency variation. Because the relative frequency of each morph is on a regional rather than national scale, morph proportions for one region cannot be extrapolated to another. If, however, a number of sites in an area were sampled and the mean frequency obtained, then a higher than average red-legged frequency should identify a site as "equitable" with mild winter and spring temperatures. This knowledge could be useful in agriculture for selecting field crops.

The second problem concerns the use of the leg colour morphs to identify climate change. This study has shown the difference in the fitness of the two morphs is small. Given the year-by year variability in temperatures for the U.K. and the many genotypes in this species which, independently of leg colour, could enable the beetle to adapt to changing conditions in ways that cannot be predicted, a change in morph proportions may not be perceptible even after 20 years. Within this time scale, we are likely to have knowledge of climate change from rather more dramatic incidents (e.g. increased flooding). Monitoring insect bio-diversity over time would probably give more information.

Finally, the direction of frequency change will not be consistent between regions. Assuming temperatures are rising in the U.K., there should be a concurrent rise in red-legged frequency in cooler regions. In warmer regions, where the maximum temperature is already relatively high, the black-legged morph is more likely to increase in proportion.

In summary, the relative proportions of the leg colour morphs of *Pterostichus madidus* could be a good indicator of microclimatic conditions on a small spatial scale, which should be of assistance when making decisions about land use. As a bio-monitor of climate change over time, a change in the leg colour proportions could indicate whether climatic factors at a local level are becoming more maritime or continental. However, the response time is slow and the occurrence of other physiological and behavioural adaptations could over-ride leg colour selection.

7.2 Conclusions and Further Work

1. Multiple regression analysis has found an association between the frequencies of the leg colour morphs of *Pterostichus madidus* and environmental factores – in particular temperature - for a number of regions of England and Wales. The red-legged morph appears to be better adapted to a more equitable climate whereas the black-legged morph is adapted to more extreme temperatures. Further work needs to confirm whether these finding are consistent for regions that were not covered by this study, e.g. Scotland, south Wales, western and northern regions of England.

2. Due to year-by-year variability in climate and morph proportions, it was found that a long time series of 10 to 20 years is needed to identify (1) directional changes in morph proportions and (2) any correlation with climatic factors. Presently, *P. madidus* leg colour proportions and climate data are collected at the ECN sites (Sykes & Lane, 1996). This could form part of a long term study of the relationship between annual and seasonal temperature and morph proportions over time. Archaeological samples of *P. madidus* could also give information about the occurrence and distribution of the morphs in pre-history, providing leg colour is preserved.

3. This study has found some support for the hypothesis that the black-legged morph has better dispersal powers to more open country. Continuous monitoring of the spatial distribution of the two morphs over several years in a heterogeneous habitat where the morph populations can mix (e.g. interfaces of long and short grass; wood and field) could further test this hypothesis.

4. Breeding experiments have shown that the genes coding for red legs are dominant. A method for rearing *P.madidus* larvae under laboratory conditions has also been developed, which follows temperature and light conditions in the field. The most critical period for development is from late Instar 3 to emergence, which occurs from late winter to early summer in the field. This period also produced the strongest coefficient of determination in the multiple regressions. The number of emerged beetles was too low to assess whether there are any differences between the morphs during development. Knowledge of the mechanism of selection could be advanced by investigating the survival of *P. madidus* larvae to pupation and emergence under more extreme temperature conditions. To be more certain of the leg colour genotype of the larvae, and also compare adaptive strategies to different regional climates, the parents could be collected from exclusively red-legged and exclusively black-legged populations.

5. It is not known whether selection occurs in the adult. A number of laboratory studies could be performed to assess whether there are behavioural and/or physiological differences between the morphs, by investigating:
   - the activity and/or mating behaviour of the adult under different temperature conditions, using continuous monitoring techniques;
   - the metabolic rate of the two morphs under extreme temperature conditions, using respirometry;
   - the mortality rate of the over-wintering female at (1) low temperatures (<4°C) and (2) diurnally fluctuating temperatures.

Further work in this area could bring us closer to understanding the mechanism of selection influencing the morph frequencies of *Pterostichus madidus* and increase our knowledge of the evolutionary processes that enable species survival under changing climatic conditions.

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APPENDIX A

Tables showing *Pterostichus madidus* sampling sites

(for use with Chapters 2, 3, and 4)

Table A.1 **Description of trapping sites in Roughill and Colwick Wood.**

Site no.	Topography and aspect	Altitude (m)	Distance from wood edge (m)	Tree and shrub cover	Ground cover
ROUGHILL WOOD					
1	steep slope, SW facing	60	8	beech, ash, sycamore	dog's mercury, ivy, leaf litter
3	gentle slope, SW facing	65	28	oak, sycamore, elder	bluebells, celandine
COLWICK WOOD					
4	gentle slope, SW facing	75	53	birch, mature sycamore, sycamore saplings	mostly bare ground, sparse ground ivy
5	gentle slope, SW facing	70	40	widely spaced oak and sycamore	thick bramble, moss
7	level, close to edge of steep scarp, SW facing	65	18	sparse hawthorn scrub	grass, cleavers, brambles
8	level ground	65	13	dense hawthorn thicket	bare ground, thin layer leaf litter
10	gentle slope, NE facing 5m from firebreak (10m width)	60	20 (2m from firebreak)	silver birch plantation	grasses, nettles, sparse bramble
11	level ground, close to highest point of wood (SW aspect)	90	10	hawthorn scrub	brambles, nettles

Table A.2 Sampling sites along TRANSECT 1: Southampton to north Nottinghamshire.

(Shaded cells = no change in location between years; S = site; all woods are deciduous)

Site no.	Location	County	Years monitored	Grid Ref	Altitude (m)	Habitat description
1	West End, Southampton	Hampshire	1976	SU-480157	30	wood/waste ground
			1995	SU-487158	30	wood
			1996			
2	Twyford	Hampshire	1975	SU-491249	37	wood edge, by ditch
			1995			
			1996	SU-491248	40	copse
3	Wonston	Hampshire	1975	SU-460370	80	wood
			1995	SU-460370	80	double hedge
			1996	SU-460370	80	wood
4	Tidbury Ring	Hampshire	1975	SU-502427	100	copse
			1995	SU-505428	100	wood
			1996			
5	Litchfield	Berkshire	1975	SU-458530	120	wood
			1995			
			1996			
6	Greenham Common	Berkshire	1975	SU-479652	120	wood
			1995	SU-483652	120	wood
			1996 (S1)			
			1996 (S2)	SU-502655	110	wood
7	World's End	Berkshire	1975	SU-485759	160	wood edge
			1995			
			1996			
8	Illsley, Ridgeway	Berkshire/ Oxfordshire border	1975	SU-483844	130	hedge
			1995	SU-483844		hedge remnants
			1996	SU-418842	211	bushes
9	Frilford	Oxfordshire	1975	SU-478970	55	wood
	Marcham	Oxfordshire	1995	SU-448966	60	wood
			1996			
10	Sandleigh	Oxfordshire	1975	SP-461019	95	wood
			1995			
			1996			
11a	OXFORD		1996	SP-519062	58	small copse
11	Begbroke	Oxfordshire	1975	SP-475148	76	hedge, waste ground
			1995	SP-455158	85	wood
			1996			
12	Steeple Aston	Oxfordshire	1975	SP-463251	130	wood edge
			1995	SP-463245	130	wood edge, by ditch
			1996	SP-478247	100	large copse
13	Adderbury	Oxfordshire	1975	SP-472369	110	hedge
			1995	SP-471372	110	hedge
	Bodicote	Oxfordshire	1996	SP-449380	122	copse

Table A.2 (continued)

Site no.	Location	County	Years monitored	Grid Ref	Altitude (m)	Habitat description
14	Hanwell	Oxfordshire	1975	SP-443442	100	copse
	Mollington	Oxfordshire	1995	SP-437487	160	wood
			1996			
15	Priors Hardwick	Warwickshire/ Northants border	1975	SP-463536	152	wood
			1995			
			1996	SP-463538	152	wood
16	Stockton	Warwickshire	1975	SP-445648	90	hedge
			1995	SP-437648	90	wood
			1996			
17	Church Lawford	Warwickshire	1975	SP-445761	82	wood
			1995	SP-455758	90	woodstrip
	Brinklow	Warwickshire	1996	SP-444788	80	wood
18	Wibtoft	Leicestershire	1975	SP-463863	135	wood
			1995	SP-463863	135	woodstrip
			1996	SP-460855	125	wood
19	Hinckley	Leicestershire	1975	SP-461938	107	wood
			1995			
			1996			
20	Barlestone	Leicestershire	1975	SP-445066	140	wood
			1995			
			1996			
21	Coalville	Leicestershire	1975	SK-453124	170	double hedgerow
			1995	SK-458119	140	double hedgerow
	Copt Oak	Leicestershire	1996	SK-486132	210	wood
22	Tonge, Cloud Wood	Leicestershire	1975	SK-420216	100	wood
			1995			
			1996			
23	Ockbrook	Derbyshire	1975	SK-417372	100	double hedgerow
			1995			
	Elvaston Castle	Derbyshire	1996	SK-412329	37	wood
24	Shipley	Derbyshire	1975	SK-435445	100	wood
			1995			
			1996			
25	Somercotes	Nottinghamshire	1975	SK-442529	80	copse
			1995			
			1996			
26	Doe Lea	Nottinghamshire	1995(S1)	SK-463653	160	wood
			1996(S1)			
			1996(S2)	SK464643	160	wood

Table A.3.i Sampling sites along TRANSECT 2: Somerset to East Sussex.

(Shaded cells = no change in location between years; unless stated otherwise, all woods are deciduous).

Site no.	Location	County	Years monitored	Grid Ref	Alt-itude (m)	Distanc to sea (km)	Habitat description
1	Bradford Abbas	Somerset	1976	ST-579154	80	30	sandstone cliff bottom
			1996				
2	Stourton Caundle	West Dorse	1976	ST-697157	110	35	wood
			1996				
3	Sturminster Newton	North Dorse	1976	ST-796133	72	33	wood, by ditch
			1996				
4	Sutton Waldron	North Dorse	1976	ST-864153	70	35	wood (beech)
			1996				
5	Cashmoor	Dorset	1976	ST-970133	76	38	wood
			1996	ST-981139	80	38	wood
			1998				
6	Cranbourne	Dorset	1976	SU-072142	95	24	wood edge
			1996	SU-071142	95	24	wood edge
			1998				
7	Fordingbridge	Hampshire	1976	SU-162142	45	23	hedge bottom
			1996	SU-163143	45	23	hedge bottom
			1998				
8	Cadnam, New Forest	Hampshire	1976	SU-260144	97	22	holly copse
			1996	SU-260146	97	22	holly copse
9	Nursling	Hampshire	1976	SU-364163	7	21	wood
			1996	SU-358163	7	21	wood
			1998				
10	West End, Southampton	Hampshire	1976	SU-480157	30	9	wood/waste ground
			1995	SU-487158	30	9	wood
			1996				
11	Shirral Heath, Curdridge	Hampshire	1976	SU-578148	51	16	road verge
			1996	SU-577150	51	16	road verge
12	Clanfield	Hampshire	1976	SU-677166	110	16	wood
			1996	SU-674166	110	16	wood
13	Compton	West Susse	1976	SU-768155	98	17	wood edge
			1996	SU-768156	98	17	wood edge
14	Colworth Down	West Susse	1976	SU-847147	92	18	wood (beech)
			1996				
15	Duncton Down	West Susse	1976	SU-960162	76	17	wood edge
			1996				
			1998	SU-954162	100	17	wood
16	Wiggonholt Common	West Susse	1976	TQ-055161	20	15	wood, bank by road
			1996				
			1998	TQ-055160	20	15	wood
17	Guesses Farm	West Susse	1976	TQ-163149	30	12	coniferous and oak wood
			1996				
			1998	TQ-154146	40	12	wood edge
18	Shave Wood	West Susse	1976	TQ-257143	25	11	wood edge
	Duffield (Oreham Commo	West Susse	1996	TQ-224141	25	11	wood edge

Table A.3.i (continued)

Site no.	Location	County	Years monitored	Grid Ref	Alt-itude (m)	Distance to sea (km)	Habitat description
19	Brocks Wood	East Sussex	1976	TQ-351144	54	11	wood edge
	East Chiltington	East Sussex	1996	TQ-373143	50	12	wood edge
			1998	TQ-372147	50	12	small wood
20	Isfield	East Sussex	1976	TQ-451159	11	16	bushes
			1996	TQ-452159	11	16	bushes
	Nr Isfield	East Sussex	1998	TQ-458163	10	16	wood
21a	*East Hoathly*	*East Sussex*	*1998*	*TQ-514134*	*30*	*16*	*deciduous edge of coniferous forest*
21b	Hale Green	East Sussex	**1976** **1996**	**TQ-564159**	**70**	**15**	**trees by road verge**
			1998	TQ-566159	70	15	wood edge
21c	*Hellingly*	*East Sussex*	*1998*	*TQ-594127*	*40*	*11*	*woodland in park*
22a	*Herstmonceux*	*East Sussex*	*1998*	*TQ-627122*	*26*	*9*	*wood edge*
22b	Bodle Street Green	East Sussex	**1976** **1996**	**TQ-643142**	**30**	**10**	**ditch**
23	Battle	East Sussex	1998	TQ-735145	50	7.5	wood

Table A.3.ii Additional sites sampled in 1998 in the vicintiy of TRANSECT 2.

Site no.	Location	County	Years monitored	Grid Ref	Alt-itude (m)	Distance to sea (km)	Habitat description
24	West Alvington, Kingsbridge	Devon	1998	SX-724441	60	8.1 (S) 6.4 (E)	oak woodland
25	Taunton, Cheddon Road	Somerset	1998	ST-228268	28	37 (S) 27 (N)	garden (lawn, compost heap area)
26	Hurn (Nr Bournemouth)	Dorset	1998	SZ-128987	15	7.4	beech woodland
27	East Grimstead (Salisbury)	Hampshire	1998	SU-193282	80	34	damp beech woodland by forestry plantation
28	The Common (Stockbridge)	Hampshire	1998	SU-265322	82	39	damp beech woodland by forestry plantation
29	Leckford (Stockbridge)	Hampshire	1998	SU-387358	130	38	hedgerow strip by pasture
30	Southampton Common	City of Southampton	1998	SU-419137	30	14	woodland heath in urban area
31	Michelover Wood	Hampshire	1998	SU-529364	100	33	large deciduous copse at edge of conifereous forest
32	Soberton Heath	Hampshire	1998	SU-593135	60	15	deciduous edge of conifereous forest
33	Rogate	West Sussex	1998	SU-804247	100	29	mixed deciduous wood
34	Bognor Regis	West Sussex	1998	SZ-937995	2	0.6	ash/sycamore copse in park
35	Selhurst Park	West Sussex	1998	SU-938119	160	13	beech wood
36	Nr Slindon (on S. Downs)	West Sussex	1998	SU-966075	30	8.0	mixed decidous copse
37	Fairmile Bottom (on S. Downs)	West Sussex	1998	SU-983091	40	9.1	hawthorn/birch copse
38	Houghton Forest (on S. Downs)	West Sussex	1998	TQ-002109	100	10	mixed decidous copse in forestry plantation
39	Felbridge	Kent/East Sussex border	1998	TQ-351401	65	37	birch copse
40	Hammer Wood, nr East Grinstead	East Sussex	1998	TQ-425393	110	39	birch/ash wood
41	Wych Cross (Ashdown Forest)	East Sussex	1998	TQ-428309	190	31	deciduous copse by forestry plantation
42	Nr Edenbridge	Kent	1998	TQ-503475	45	50	birch sapling wood strip
43	Nr Goudhurst, Bedgebury Cross	Kent	1998	TQ-708347	56	27	mixed deciduous woodland
44	Nr Bexhill	East Sussex	1998	TQ-716094	40	2.9	mixed deciduous woodland
45	Alexandra Park, Hastings	East Sussex	1998	TQ-818105	50	1.7	holly/laurel copse in park

Table A.4 **Location and habitat description of sites monitored for three or more years by University of Newcastle upon Tyne and the Environmental Change Network (ECN).**

Location	Region	Years monitored	Sub-samples (T=Transect)	Grid Ref	Habitat description
i) Newcastle University site.					
Close House	Northumberland	1981-1995		4-120 5-650	walled garden
ii) ECN sites in Scotland and southern England.					
Glensaugh	Grampion Region	1994-1997	T1	3-670 7-790	mineral grassland
			T2		dry peat
			T3		wet peat
Sourhope	Borders Region	1994-1997	T1	3-860 6-220	dry peat
			T2		wet peat
			T3		mineral grassland
Wytham	Oxfordshire	1993-1997	T1	4-470 2-080	coppiced broadleaf wood
			T2		grassland (between track and hedge)
			T3		beech plantation
Rothampsted	Hertfordshire	1993-1997	T1	5-130 2-130	hedge (next to arable field)
			T2		old broadleaf woodland (Geescroft wilderness)
			T3		Park Grass grassland experiments, next to footpath
Alice Holt	Surrey	1994-1997	T1	4-800 1-430	mixed broadleaf woodland
			T2		mixed broadleaf woodland
			T3		mixed broadleaf woodland
Porton Down	Wiltshire	1994-1997	T1	4-200 1-360	short chalk grassland with scrub
			T2		open chalk grassland
			T3		rank tall chalk grassland with juniper
North Wyke	Devon	1993-1997	T1	2-650 0-990	next to grazed permanent pasture, near woodland strip
			T2		next to grazed permanent pasture
			T3		next to grazed permanent pasture and near scrub

Table A.5: Sampling sites along TRANSECT 3: Bethesda to Skegness. (In italics: additional sites to original 1996 transect line).

Site no.	Location	County	Years monitored	Grid Ref	Alt-itude (m)	Distance to sea (km)	Habitat description
1	Bethesda	Gwynedd	1996 1997	SH-627658	20	7	mixed deciduous wood
2	Llanwryst	Gwynedd	1996 1997	SH-789608	30	18	deciduous edge of forestry plantation
3	Pant past y nog	Clwyd	1996	SJ-055616	76	22	mixed deciduous/ coniferous wood
4	*Llangollen*	*Clwyd*	*1997*	*SJ-184427*	*180*	*35*	*beech decidous edge to forestry plantation*
5	Marsham	Clywd	1996	SJ-200610	200	17	deciduous wood
6	*Oswestry*	*Shropshire*	*1996* *1997*	*SJ-285293*	*144*	*49*	*edge of copse*
7	Eccleston	Cheshire	1996	SJ-407624	20	26	beech/sycamore/ larch woodland
8	Sandiway	Cheshire	1996	SJ-613696	70	42	oak/larch woodland
9	Brereton Heath	Cheshire	1996	SJ-795649	70	61	birch/oak woodland
10	*Keele University*	*Staffordshire*	*1997*	*SJ-817449*	*180*	*70*	*copse in university grounds*
11	*Froghall*	*Staffordshire*	*1997*	*SK-027478*	*228*	*88*	*decidous wooded valley*
12	Hollingsclough	Staffordshire	1996	SK-042666		85	birch/ash copse, wet
13	*Buxton*	*Derbyshire*	*1997*	*SK-051725*	*300*	*85*	*mixed deciduous country park*
14	*Taddington Vale*	*Derbyshire*	*1997*	*SK-170705*	*170*	*97*	*mixed deciduous woodland*
15	Birchover	Derbyshire	1996 1997	SK-258618	150	107	birch/hawthorn wood
16	*Ashover*	*Derbyshire*	*1997*	*SK-349629*	*178*	*116 (W)* *122 (E)*	*deciduous trees by cliff*
18	Doe Lea	Nottinghamshire	1996	SK-463643	160	127 (W) 111 (E)	mixed deciduous woodland
17	*Watnall, by lake*	*Nottinghamshire*	*1998*	*SK-483487*	*90*	*95*	*mixed deciduous woodland*
18	*Watnall, copse*	*Nottinghamshire*	*1996* *1998*	*SK-498463*	*120*	*93*	*large copse along roadside*
20	*Sutton Bonington*	*Leicestershire*	*1996* *1998*	*SK-504265*	*43*	*98*	*copse on grassland*

Table A.5 (continued)

Site no.	Location	County	Years monitored	Grid Ref	Alt-itude (m)	Distance to sea (km)	Habitat description
21	*Warsop, Medon Vale*	*Nottinghamshire*	*1998*	*SK-583703*	*60*	*99*	*hawthorn copse*
22	*Warsop, Budby*	*Nottinghamshire*	*1998*	*SK-604705*	*45*	*97*	*oak/birch/sycamore wood*
23	Rufford, Centre Park	Nottinghamshire	1996	SK-646657	60	93	mixed deciduous/ coniferous plantation
24	Norton Disney	Lincolnshire	1996 1998	SK-883607	16	59	young decidous plantation
25	Dunston Wood	Lincolnshire	1996 1998	TA-089639	70	48	sycamore/ash woodland
26	Mareham le Fen	Lincolnshire	1996 1998	TA-250599	10	32	large set-aside copse
27	*Revesby Abbey*	*Lincolnshire*	*1998*	*TA-297633*	*50*	*28*	*mature deciduous wood*
28	*Driby*	*Lincolnshire*	*1998*	*TA-388747*	*35*	*17*	*edge of deciduous wood*
29	Willoughby Wood	Lincolnshire	1996	TA-459708	30	10	edge of deciduous woodland
30	*Candlesby*	*Lincolnshire*	*1998*	*TA-464674*	*20*	*11*	*hedgerow by ditch*
31	*Welton Low Wood*	*Lincolnshire*	*1998*	*TA-471702*	*13*	*10*	*mixed deciduous woodland*
32	*Skegness*	*Lincolnshire*	*1998*	*TA-567637*	*4*	*1*	*birch/hawthorn scrub wasteland*

Table A.6: Sites sampled in EAST ANGLIA in 1998.

Site no.	Location	County	Grid Ref	Alt-itude (m)	Distance to sea (km)	Habitat description
1	Terrington St Clement	North West Norfolk	TF-550197	3	7	wide strip of deciduous woodland by road
2	Watlington village	North West Norfolk	TF-622113	7	16	deciduous wooded parkland, by church
3	Watlington, by lake	North West Norfolk	TF-633112	9	16	deciduous wood, close to lake
4	Leziate, wood	North West Norfolk	TF-676190	30	11	deciduous woodland, brow of hill
5	Leziate, copse	North West Norfolk	TF-682193	20	11	deciduous, by sewage works
6	Sandringham	North West Norfolk	TF-685283	50	5.5	oak/beech area of forested parkland
7	Old Hunstanton	North West Norfolk	TF-691419	10	1	deciduous copse by stream
8	East Runton	North East Norfolk	TG-202427	35	0.1	edge of scrub woodland by road
9	Cromer	North East Norfolk	TG-216418	30	0.7	sycamore/ash wood by golf course
10	Felbrigg Hall	North East Norfolk	TG-189398	60	3.4	beech plantation
11	Gunton Park	North East Norfolk	TG-240357	50	5.7	sycamore/oak edge of coniferous plantation
12	Swanton Abbott	North East Norfolk	TG-267252	12	12	small beech/oak wood
13	Coltishall	Norfolk, Broadlands	TG-283198	10	15	deciduous copse in wooded area
14	Nr Spixworth	Norfolk, Broadlands	TG-253151	19	21	small oak woodland
15	Norwich A (Mousehold Heath)	Norfolk, Broadlands	TG-241103	45	25	oak/birch area of heathland wood
16	Norwich B (Mousehold Heath)	Norfolk, Broadlands	TG-238104	50	25	mature oak/ash area of heathland wood
17	Filby	East Norfolk	TG-461137	5	6	by oak tree in damp shrub area by stream
18	Omesby St Margaret	East Norfolk	TG-487157	8	3.1	copse adjacent to deciduous wood
19	Fritton (wood)	South East Norfolk	TG-467005	15	6.8	beech/oak area of forestry plantation
20	Fritton (copse)	South East Norfolk	TM-483994	15	5.5	deciduous copse by field and edge of wood
21	Lowestoft	North East Suffolk	TM-542968	10	0.4	sycamore copse in pleasure park
22	Bridgham Heath (towards Thetford)	North West Suffolk	TL-935871	40	58	birch and pine trees by parking place
23	Barnham	North West Suffolk	TL-887792	20	66	sycamore/oak wood
24	Thetford Forest	North West Suffolk	TL-823866	51	58	deciduous edge of forestry plantation
25	Boxworth	South Cambridgeshire	TL-345641	47	65	edge of wood close to duck pond and green
26	Monks Wood	South Cambridgeshire	TL-203796	43	56	1. shrubs on road verge 2. dense woodland scrub (ditch between 2 areas)

Table A.7: **Sites sampled in the East Midlands in 1998.**

Site name	County, type of area, direction from River Trent	Grid Ref	Altitude (m)	Habitat description
Attenborough	Notts, river plain (Trent), north	SK-518349	25	herbaceous/shrubbed area of lawned garden
Awsworth	Notts, suburban, north	SK-488447	70	small deciduous wood/scrub (elder, birch)
Bestwood Lodge	Notts, suburban, north	SK-572465	95	deciduous wood (birch/sycamore/elder)
Borders Wood (beech)	Notts, Wolds-rural, south	SK-657332	90	beech plantation in deciduous wood
Borders Wood (larch)	Notts, Wolds-rural, south	SK-657332	90	larch area of deciduous wood
Borrowash	Derbys, river plain (Derwent), north	SK-426343	45	herbaceous/shrubbed area of lawned garden
Bunny Wood	Notts, rural, south	SK-587284	60	hawthorn area of deciduous wood
Burton Joyce	Notts, river plain (Trent), north	SK-637432	30	new plantation in deciduous wood
Canning Circus Cemetry, Nottm	Notts, city, north	SK-565403	70	under copper beech and shrubs, ground ivy patch
Clifton Grove	Notts, suburban, south	SK-542348	35	deciduous wood (beech/oak/sycamore)
Clifton Wood	Notts, suburban, south	SK-538346	65	larch and beech wood
Codnor	Notts, semi-rural, north	SK-430487	115	hawthorn and elder hedgerow/copse
Colwick racecourse	Notts, river plain (Trent), north	SK-604393	60	birch area of deciduous wood
Colwick Wood	Notts, city, north	SK-597398	85	elder/hawthorn area of deciduous woodland
Elvaston Castle	Derbys, river plain (Derwent), north	SK-409328	40	deciduous plantation (sycamore/rhododendron)
Epperstone	Notts, semi-rural, north	SK-633502	65	sycamore copse (Forestry Commission)
Glapton Wood, Clifton	Notts, suburban, south	SK-548338	55	deciduous wood (oak, beech)
Gotham	Notts, rural, south	SK-538313	45	small deciduous wood (beech, sycamore)
Harrison Plantation, Wollaton	Notts, city, north	SK-531403	45	deciduous wood (sycamore, hawthorn, cherry)
Hemlock Stone, Stapleford	Notts, suburban, north	SK-500385	60	deciduous wood (sycamore, holly, rhododendron)
Holme Pierrepoint	Notts, river plain (Trent), south	SK-615387	20	wood strip (deciduous) on grassed area by lake
Keyworth	Notts, Wolds-suburban, south	SK-623323	55	hawthorn/elder hedgerow/copse by railway
Kings Mill	Derbys, river plain (Trent), north	SK-419275	40	pine and sycamore/ash areas in woodland
Kirk Hallam	Notts, suburban, north	SK-442397	90	deciduous wood (oak, hawthorn, elder)
Lockington	Notts, river plain (Trent), south	SK-464277	45	small deciduous wood (sycamore/ash/hawthorn)
Near Hucknall	Notts, suburban, north	SK-525457	70	oak/beech/birch area of woodland

Table A.7 (continued)

Site name	County	Grid Ref (to 1 km)	Alt-itude (m)	Habitat description
Near Lowdham	Notts, semi-rural, north	SK-650479	30	hedgerow/copse by ditch alongside road
Nottingham Trent Univ, grounds	Notts, suburban, south	SK-546351	45	young ash plantation adjacent to grassland
Nottingham Univ, grounds	Notts, city, north	SK-544386	50	large deciduous copse (beech, holly, laurel)
Roshoe Wood	Notts, Wolds-rural, south	SK-648294	85	deciduous woodland (beech, sycamore, hawthorn)
Ruddington Hall	Notts, suburban, south	SK-578342	40	deciduous wood (hawthorn, beech)
Sawley	Derbys, river plain (Trent), north	SK-483316	30	birch and willow hedgerow/copse by railway
Selston Plantation, Bagthorpe	Notts, suburban, north	SK-476516	100	small deciduous wood
Shipley	Derbys, semi-rural, north	SK-436442	110	deciduous wood (oak)
Somercotes	Notts, suburban, north	SK-434532	113	hawthorn/ash copse
Stragglethorpe	Notts, Wolds-rural, south	SK-654366	40	large woodstrip by field (sycamore, beech, pine)
Strelley	Notts, suburban, north	SK-513419	100	yew, ash, beech, sycamore woodland
Sutton Bonington	Leics, rural, south	SK-505265	45	grassy copse (birch)
Watnall, copse	Notts, suburban, north	SK-498463	115	elder/hawthorn/sycamore copse
Watnall, wood	Notts, semi-rural, north	SK-483487	80	mixed woodland (pine, oak, beech sycamore) by lake
Widmerpoole	Notts, Wolds-rural, south	SK-628283	70	small deciduous wood (yew, beech) by church
Woodthorpe	Notts, city, north	SK-581448	80	shrubby, herbaceous area of garden

APPENDIX B

Tables giving results of countrywide and regional sampling of *Pterostichus madidus* morphs

(for use with Chapters 3 and 4)

Table B.1.i Results of 1975, 1995 and 1996 sampling of Transect 1: Southampton to north Nottinghamshire.

B = black-legged morph; R = red-legged morph; T = total *P. madidus* ; x = site not monitored.
W = wood; We = wood edge; Ws = woodstrip; C = copse; wG = waste ground; H = hedge; dH = double hedge; D = ditch

SITE	LOCATION		GRID REF	CLASS			1975				1995				1996				1995/6 summed			
NO			(for 1995/6)	1975	1995	1996	B	R	T	%R	B	R	T	%R	B	R	T	%R	B	R	T	%R
1	West End	Hants	4-480 1-158	W/wG	W	W	94	127	*221*	57	42	77	*119*	65	31	33	*64*	52	73	110	*183*	60
2	Twyford	Hants	4-491 1-248	W	We/D	C	30	0	*30*	0	4	0	*4*		105	28	*133*	21	109	28	*137*	20
3	Wonston	Hants	4-460 1-370	W	dH	W	0	0	*0*		2	0	*2*		no data (site vandalised)				2	0	*2*	
4	Tidbury Ring	Hants	4-450 1-428	C	W	W	20	2	*22*	9	16	0	*16*	0	16	2	*18*	11	32	2	*34*	6
5	Litchfield	Berks	4-458 1-153	W	W	W	0	0	*0*		67	22	*89*	25	150	54	*204*	26	217	76	*293*	26
6	Greenham Common	Berks	4-483 1-652	W	W	W	18	26	*44*	59	29	22	*51*	43	55	75	*130*	58	84	97	*181*	54
7	World's End	Berks	4-485 1-759	We	We	We	10	10	*20*	50	107	48	*155*	31	168	100	*268*	37	275	148	*423*	35
8	Illsley, Ridgeway	Oxon	4-486 1-844	H	H	H	30	0	*30*	0	0	0	*0*		0	0	*0*		0	0	*0*	
9	Frilford/Marcham	Oxon	4-489 1-966	W	W	W	25	0	*25*	0	14	1	*15*	7	118	10	*128*	8	132	11	*143*	8
10	Sandleigh	Oxon	4-461 2-019	W	W	W	88	0	*88*	0	135	15	*150*	10	249	30	*279*	11	384	45	*429*	10
11A	*Oxford*		4-519 2-062	x	x	C	x	x	x		x	x	x		28	46	*74*	62	28	46	*74*	62
11	Begbroke	Oxon	4-455 2-158	H/wG	W	W	28	4	*32*	13	246	101	*347*	29	229	114	*343*	33	475	215	*690*	31
12	Steeple Aston	Oxon	4-478 2-245	We	We/D	C	34	6	*40*	15	2	0	*2*		167	20	*187*	11	169	20	*189*	11
13	Adderbury/Bodicotes	Oxon	4-449 2-380	H	H	C	14	2	*16*	13	3	4	*7*		76	40	*116*	34	79	44	*123*	36
14	Hanwell/Mollington	Oxon	4-437 2-487	C	W	W	0	0	*0*		82	20	*102*	20	107	35	*142*	25	189	55	*244*	23
15	Priors Hardwick	Warwicks	4-463 2-538	W	W	W	20	0	*20*	0	12	6	*18*	33	26	0	*26*	0	38	6	*44*	14
16	Stockton	Warwicks	4-437 2-648	H	W	W	7	0	*7*		463	41	*504*	8	193	14	*207*	7	656	55	*711*	8
17	Church Lawford/Brinklow	Warwicks	4-447 2-788	W	Ws	W	0	0	*0*		0	0	*0*		15	5	*20*	25	15	5	*20*	25
18	Wibtoft	Leics	4-463 2-863	Ws	Ws	Ws	0	0	*0*		7	2	*9*		3	0	*3*		10	2	*12*	17
19	Hinckley	Leics	4-461 2-938	C	C	C	0	0	*0*		254	70	*324*	22	45	7	*52*	13	299	77	*376*	20
20	Barlestone	Leics	4-445 3-066	W	W	W	20	8	*28*	29	19	8	*27*	30	17	7	*24*	29	36	15	*51*	29
21	Coalville/Copt Oak	Leics	4-458 3-119	dH	dH	W	0	0	*0*		9	5	*14*	36	0	1	*1*		9	6	*15*	40
22	Tonge	Leics	4-420 3-216	W	W	W	20	6	*26*	23	12	5	*17*	29	17	7	*24*	29	29	12	*41*	29
23	Ockbrook/Elvaston	Derbys	4-412 3-329	dH	dH	W	0	0	*0*		8	5	*13*	38	169	107	*276*	39	177	112	*289*	39
24	Shipley	Derbys	4-435 3-445	W	W	W	20	6	*26*	23	67	40	*107*	37	92	52	*144*	36	159	92	*251*	37
25	Somercotes	Notts	4-442 3-529	C	C	C	15	5	*20*	25	39	18	*57*	32	33	18	*51*	35	72	36	*108*	33
26	Doe Lea	Notts	4-463 2-653	x	W	W	x	x	x		66	40	*106*	38	248	97	*345*	28	314	137	*451*	30

TOTAL (excluding Sites 11a & 26)	1975 B	1975 R	1975 T	1975 %R	1995 B	1995 R	1995 T	1995 %R	1996 B	1996 R	1996 T	1996 %R	1995/6 B	1995/6 R	1995/6 T	1995/6 %R
	493	202	695	29	1639	510	*2149*	24	2081	759	*2840*	27	3720	1269	*4989*	25

Table B.1.ii **Numbers of black-legged and red-legged males and females in 1995 and 1996 at sites along Transect 1: Southampton to north Nottinghamshire.**

B = black-legged morph; R = red-legged morph; m = male; f = female; T = total; GT = grand total; *P. madidus* ; x = site not monitored.

Calculation of frequency for males: %Bm = (no. of Bm/total no. of black-legged morphs) x 100; %Rm = (no. of Rm/total no. of red-legged morphs) x 100

SITE NO	LOCATION		GRID REF (for 1995/6)	1995										1996									
				Bm	Bf	BT	Rm	Rf	RT	GT	%Bm	%Rm	%R	Bm	Bf	BT	Rm	Rf	RT	GT	%Bm	%Rm	%R
1	West End	Hants	4-480 1-158	29	13	42	58	19	77	119	69	75	65	11	20	31	18	15	33	64	35	55	51
2	Twyford	Hants	4-491 1-248	4	0	4	0	0	0	4				76	29	105	20	8	28	133	72	71	21
3	Wonston	Hants	4-460 1-370	2	0	2	0	0	0	2				x	x	x	x	x	x	x	x	x	x
4	Tidbury Ring	Hants	4-450 1-428	3	13	16	0	0	0	16			0	9	7	16	0	2	2	18			11
5	Litchfield	Berks	4-458 1-153	61	6	67	18	4	22	89	91	82	25	103	47	150	29	25	54	204	69	54	26
6	Greenham Common	Berks	4-483 1-652	19	10	29	15	7	22	51	66	68	43	19	36	55	37	38	75	130	35	49	58
7	World's End	Berks	4-485 1-759	78	29	107	41	7	48	155	73	85	31	103	65	168	68	32	100	268	61	68	37
8	Illsley, Ridgeway	Oxon	4-486 1-844	0	0	0	0	0	0	0				0	0	0	0	0	0	0			
9	Frilford/Marcham	Oxon	4-489 1-966	5	9	14	1	0	1	15			7	54	64	118	5	5	10	128	46	50	8
10	Sandleigh	Oxon	4-461 2-019	91	44	135	12	3	15	150	67	80	10	131	118	249	14	16	30	279	53	47	11
11A	*Oxford*		4-519 2-062	x	x	x	x	x	x	x	x	x	x	26	2	28	42	4	*46*	74	93	91	62
11	Begbroke	Oxon	4-455 2-158	208	38	246	89	12	101	347	85	88	29	144	85	229	64	50	114	343	63	56	33
12	Steeple Aston	Oxon	4-478 2-245	2	0	2	0	0	0					83	84	167	9	11	20	187	50	45	11
13	Adderbury/Bodicotes	Oxon	4-449 2-380	3	0	3	2	2	4	7				42	34	76	22	18	40	116	55	55	34
14	Hanwell/Mollington	Oxon	4-437 2-487	69	13	82	17	3	20	102	84	85	20	48	59	107	21	14	35	142	45	60	25
15	Priors Hardwick	Warwicks	4-463 2-538	9	3	12	4	2	6	18			33	14	12	26	0	0	0	26			0
16	Stockton	Warwicks	4-437 2-648	373	90	463	33	8	41	504	81	80	8	149	44	193	12	2	14	207	77	86	7
17	Church Lawford/Brinklow	Warwicks	4-447 2-788	0	0	0	0	0	0	0				9	6	15	2	3	5	20			25
18	Wibtoft	Leics	4-463 2-863	7	0	7	2	0	2	9				2	1	3	0	0	0	3			
19	Hinckley	Leics	4-461 2-938	174	80	254	46	24	70	324	69	66	22	26	19	45	4	3	7	52	58	57	13
20	Barlestone	Leics	4-445 3-066	14	5	19	7	1	8	27			30	13	4	17	6	1	7	24			29
21	Coalville/Copt Oak	Leics	4-458 3-119	7	2	9	4	1	5	14			36	0	0	0	0	1	1	1			
22	Tonge	Leics	4-420 3-216	12	0	12	4	1	5	17			29	12	5	17	2	5	7	24			29
23	Elvaston/Ockbrook	Derbys	4-412 3-329	6	2	8	5	0	5	13			38	109	60	169	80	27	107	276	64	75	39
24	Shipley	Derbys	4-435 3-445	49	18	67	24	16	40	107	73	60	37	51	41	92	33	19	52	144	55	63	36
25	Somercotes	Notts	4-442 3-529	33	6	39	15	3	18	57	85	83	32	22	11	33	14	4	18	51	67	78	35
26	Doe Lea	Notts	4-463 2-653	34	32	66	17	23	40	106	52	43	38	132	116	248	47	50	97	345	53	48	28
	TOTAL (excl. S11a, S26)			1258	381	1639	397	113	510	2149	77	78	24	1230	851	2081	460	299	759	2840	59	61	27

Table B.2.i **Results of 1976, 1996 and 1998 sampling of Transect 2: Somerset to East Sussex.** (Grid references shown are for 1996 or 1998).

B = blacklegged morph; R = redlegged morph; T= total *P. madidus;* x = site destroyed.
W = wood; We = wood edge; C = copse; H = hedge; wG = waste ground; D = ditch; 'Br = bank by road; Bw = bank by wood; rV = road verge; cb = cliff bottom;

SITE NO	LOCATION		GRID REF	CLASS			1976				1996				1998			
				1976	1996	1998	B	R	T	%R	B	R	T	%R	B	R	T	%R
1	Bradford Abbas	Somerset	3-579 1-154	cb	cb		0	0	*0*		0	0	*0*					
2	Stourton Caundle	West Dorset	3-697 1-157	W	W		11	12	*23*	52	0	0	*0*					
3	Sturminster Newton	North Dorset	3-796 1-133	W/D	W/D		0	6	*6*		0	0	*0*					
4	Sutton Waldron/Claysmore	North Dorset	3-864 1-153	W	W		12	0	*12*	0	0	2	*2*					
5	Cashmoor	Dorset	3-981 1-139	W	W	W	0	0	*0*		28	4	*32*	13	11	0	*11*	
6	Cranbourne	Dorset	4-071 1-142	We	We	We	16	0	*16*	0	62	14	*76*	18	20	2	*22*	9
7	Fordingbridge	Hampshire	4-163 1-143	H	H	H	5	63	*68*	93	0	1	*1*		3	30	*33*	91
8	Cadnam, New Forest	Hampshire	4-260 1-146	C	C		0	9	*9*		0	0	*0*					
9	Nursling	Hampshire	4-364 1-163	W	W	W	8	126	*134*	94	x	x	*x*	x	1	2	*3*	
10	West End	Hampshire	4-480 1-158	wG	C		94	127	*221*	57	31	33	*64*	52				
11	Shirrell Heath	Hampshire	4-578 1-148	rV	rV		24	16	*40*	40	2	0	*2*					
12	Clanfield	Hampshire	4-674 1-166	W	W		268	54	*322*	17	77	8	*85*	9				
13	Compton	West Sussex	4-768 1-156	We	We		6	2	*8*		163	56	*219*	26				
14	Colworth Down	West Sussex	4-847 1-147	W	W		40	4	*44*	9	46	23	*69*	33				
15	Duncton Down	West Sussex	4-960 1-162	We	We	W	12	4	*16*	25	2	0	*2*		5	5	*10*	
16	Wiggonholt Common	West Sussex	5-055 1-161	W/Br	W/Br	W	16	20	*36*	56	9	29	*38*	76	72	97	*169*	57
17	Guesses Farm	West Sussex	5-163 1-149	W	W	We	20	4	*24*	17	28	16	*44*	36	12	5	*17*	29
18	Shave Wood/Duffield	West Sussex	5-224 1-141	We	We		30	18	*48*	38	12	9	*21*	43				
19	Brocks Wood/Chiltington	East Sussex	5-373 1-143	We	We	W	30	4	*34*	12	14	2	*16*	13	28	8	*36*	22
20	Isfield/Nr Isfield	East Sussex	5-452 1-159	bushes	bushes	W	222	72	*294*	24	3	4	7		19	12	*31*	39
21a	*East Hoathly*	East Sussex	5-514 1-134			*W*									109	81	*190*	43
21b	Hale Green	East Sussex	5-564 1-159	trees rV	trees rV	We	10	8	*18*	44	22	19	*41*	46	28	21	*49*	43
21c	*Hellingly*	East Sussex	5-594 1-127			*W*									31	19	*50*	38
22a	*Herstmonceux*	East Sussex	5-627 1-122			*We*									19	10	*29*	34
22b	Bodle Street Green	East Sussex	5-643 1-142	D	D		155	70	*225*	31	6	10	*16*	63				
23	*Battle*	East Sussex	5-735 1-145			*W*									24	16	*40*	40
						TOTAL	979	619	*1598*	39	505	230	*735*	31	358	292	*650*	45

Table B.2.ii Numbers of black-legged and red-legged males and females in 1996 and 1998 along Transect 2 (Dorset to East Sussex), and male frequency (%) for each morph. (Male frequencies found if total *P. madidus* >29. New sites for 1998 are shown in italics).

B = blacklegged morph; R = redlegged morph; m = male; f = female; BT = total B; RT = total R; GT= grand total *P. madidus*; x = site destroyed.

SITE NO	LOCATION		GRID REF	1996										1998									
				Bm	Bf	*BT*	Rm	Rf	*RT*	GT	%Bm	%Rm	%R	Bm	Bf	*BT*	Rm	Rf	*RT*	*GT*	%Bm	%Rm	%R
5	Cashmoor	Dorset	3-981 1-139	19	9	*28*	3	1	*4*	32	68	75	13	6	5	*11*	0	0	*0*	*11*			
6	Cranbourne	Dorset	4-071 1-142	14	48	*62*	6	8	*14*	76	23	43	18	8	12	*20*	1	1	*2*	*22*			9
7	Fordingbridge	Hampshire	4-163 1-143	0	0	*0*	0	1	*1*	1				0	3	*3*	15	15	*30*	*33*	0	50	91
9	Nursling	Hampshire	4-364 1-163	x	x	*x*	x	x	*x*	x	x	x	x	1	0	*1*	0	2	*2*	*3*			
10	West End	Hampshire	4-480 1-158	11	20	*31*	18	15	*33*	64	35	55	52										
11	Shirrell Heath	Hampshire	4-578 1-148	1	1	*2*	0	0	*0*	2													
12	Clanfield	Hampshire	4-674 1-166	49	28	*77*	5	3	*8*	85	64	63	9										
13	Compton	West Sussex	4-768 1-156	75	88	*163*	29	27	*56*	219	46	52	26										
14	Colworth Down	West Sussex	4-847 1-147	20	26	*46*	13	10	*23*	69	43	57	33										
15	Duncton Down	West Sussex	4-960 1-162	1	1	*2*	0	0	*0*	2				5	0	*5*	5	0	*5*	*10*			
16	Wiggonholt Common	West Sussex	5-055 1-161	7	2	*9*	21	8	*29*	38	78	72	76	48	24	*72*	78	19	*97*	*169*	67	80	57
17	Guesses Farm	West Sussex	5-163 1-149	22	6	*28*	9	7	*16*	44	79	56	36	9	3	*12*	3	2	*5*	*17*			29
18	Shave Wood/Duffield	West Sussex	5-224 1-141	6	6	*12*	3	6	*9*	21			43										
19	Brocks Wood/Chiltington	East Sussex	5-373 1-143	8	6	*14*	2	0	*2*	16			13	11	17	*28*	5	3	*8*	*36*	39	63	22
20	Isfield/Nr Isfield	East Sussex	5-452 1-159	2	1	*3*	4	0	*4*	7				8	11	*19*	4	8	*12*	*31*	42	33	39
21a	*East Hoathly*	East Sussex	5-514 1-134											68	41	*109*	50	31	*81*	*190*	62	62	43
21b	Hale Green	East Sussex	5-564 1-159	10	12	*22*	9	10	*19*	41	45	47	46	13	15	*28*	11	10	*21*	*49*	46	52	43
21c	*Hellingly*	East Sussex	5-594 1-127											13	18	*31*	7	12	*19*	*50*	42	37	38
22a	*Herstmonceux*	East Sussex	5-627 1-122											9	10	*19*	5	5	*10*	*29*	47	50	34
22b	Bodle Street Green	East Sussex	5-643 1-142	5	1	*6*	8	2	*10*	16			63										
23	*Battle*	East Sussex	5-735 1-145											10	14	*24*	9	7	*16*	*40*	42	56	40
			TOTAL	250	255	*505*	130	98	*228*	733	50	57	31	209	173	*382*	193	115	*308*	690	55	63	45

Table B.2.iii Results of 1998 sampling of new sites in the vicinity of Transect 2.

B = blacklegged morph; R = redlegged morph; T = total *P. madidus*
W = wood; Ws = wood strip; C = copse; H = hedge; P = park; glc = garden lawn/compost

SITE NO	LOCATION		GRID REF		CLASS	*P. madidus* sampled			
						B	**R**	**T**	**%R**
25	Taunton, Cheddon Road	Somerset	3-228	1-268	glc	2	2	*4*	
26	Hurn (Nr Bournemouth)	Dorset	4-128	0-987	W	4	14	*18*	*77.8*
27	East Grimstead (Salisbury)	Hampshire	4-193	1-282	W	6	24	*30*	80.0
28	The Common (Stockbridge)	Hampshire	4-265	1-322	W	49	69	*118*	58.5
29	Leckford (Stockbridge)	Hampshire	4-387	1-358	H	3	0	*3*	
30	Southampton Common	Hampshire	4-419	1-137	W	30	8	*38*	21.1
31	Michelover Wood	Hampshire	4-529	1-364	W	86	23	*109*	21.1
32	Soberton Heath	Hampshire	4-593	1-247	W	72	67	*139*	48.2
33	Rogate	West Sussex	4-804	1-247	W	49	29	*78*	37.2
34	Bognor Regis	West Sussex	4-937	0-995	CP	149	12	*161*	7.5
35	Selhurst Park	West Sussex	4-938	1-119	W	174	73	*247*	29.6
36	Nr Slindon (on S. Downs)	West Sussex	4-966	1-075	C	5	1	*6*	
37	Fairmile Bottom (on S. Downs)	West Sussex	4-983	1-091	C	78	30	*108*	27.8
38	Houghton Forest (on S. Downs)	West Sussex	5-002	1-109	C	97	19	*116*	16.4
39	Felbridge	East Sussex	5-351	1-401	C	1	0	*1*	
40	Hammer Wood, nr East Grinstead	East Sussex	5-425	1-393	W	13	13	*26*	50.0
41	Wych Cross (Ashdown Forest)	East Sussex	5-428	1-309	C	26	38	*64*	59.4
42	Nr Edenbridge	Kent	5-503	1-475	Ws	44	86	*130*	66.2
43	Nr Goudhurst, Bedgebury Cross	Kent	5-708	1-347	W	53	30	*83*	36.1
44	Nr Bexhill	East Sussex	5-716	1-094	W	55	31	*86*	36.0
45	Alexandra Park, Hastings	East Sussex	5-818	1-105	WP	33	7	*40*	17.5

Table B.3 **Results of 1996, 1997 and 1998 sampling of Transect 3: Anglesey to Skegness.** (In italics: additional sites to original 1996 transect line).

B = blacklegged morph; R = redlegged morph; T = total *P. madidus*.
W = wood; We = wood edge; C = copse; Ce = edge of copse; T = trees; B = bushes; wG = waste ground

SITE NO	LOCATION		GRID REF	CLASS	1996				1997				1998				1996/7/8			
					B	R	T	%R	B	R	T	%R	B	R	T	%R	B	R	T	%R
1	Bethesda	Gwynedd	2-627 3-658	W	6	23	29	79.3	0	15	15	100					6	38	44	86.4
2	Llanwryst	Gwynedd	2-789 3-608	W	9	14	23	60.9	2	2	4						11	16	27	59.3
3	Pant past y nog	Clwyd	3-055 3-616	W	21	23	44	52.3									21	23	44	52.3
4	*Llangollen*	*Clwyd*	*3-184 3-427*	*W*					26	42	68	61.8					26	42	68	61.8
5	Marsham	Clwyd	3-200 3-610	W	1	1	2										1	1	2	
6	*Oswestry*	*Shropshire*	*3-285 3-293*	*Ce*	63	83	146	56.8	24	38	62	61.3					87	121	208	58.2
7	Eccleston	Cheshire	3-407 3-624	W	1	3	4										1	3	4	
8	Sandiway	Cheshire	3-613 3696	W	2	3	5										2	3	5	
9	Brereton Heath	Cheshire	3-795 3-649	W	0	0	0										0	0	0	
10	*Keele University*	*Staffs*	*3-817 3-449*	*C*					5	12	17	70.6					5	12	17	70.6
11	*Froghall*	*Staffs*	*4-027 3-478*	*W*					13	22	35	62.9					13	22	35	62.9
12	Hollingsclough	Staffs	4-042 3-666	W	0	0	0										0	0	0	
13	*Buxton*	*Derbys*	*4-051 3-725*	*W*					16	21	37	56.8					16	21	37	56.8
14	*Taddington Vale*	*Derbys*	*4-170 3-705*	*W*					18	11	29	37.9					18	11	29	37.9
15	Birchover	Derbys	4-258 3-618	W	68	16	84	19.0	8	3	11						76	19	95	20.0
16	*Ashover*	*Derbys*	*4-349 3-629*	*T*					15	5	20	25.0					15	5	20	25.0
17	Doe Lea	Notts	4-463 3-643	W	248	97	345	28.1									248	97	345	28.1
18	*Watnall, lake*	*Notts*	*4-483 3-487*	*W*									157	74	231	32.0	157	74	231	32.0
19	*Watnall,copse*	*Notts*	*4-498 3-463*	*C*	99	153	252	60.7	11	18	29	62.1	73	151	224	67.4	183	322	505	63.8
20	*Sutton Bonington*	*Leics*	*4-504 3-265*	*C*	40	21	61	34.4					102	38	140	27.1	142	59	201	29.4
21	*Warsop, Medon Vale*	*Notts*	*4-583 3-703*	*C*									64	70	134	52.2	64	70	134	52.2
22	*Warsop,Budby*	*Notts*	*4-604 3-705*	*W*									117	80	197	40.6	117	80	197	40.6
23	Rufford, Centre Park	Notts	4-646 3-657	W	0	3	3										0	3	3	
24	Norton Disney	Lincs	4-883 3-607	W	1	1	2						1	1	2		2	2	4	
25	Dunston wood	Lincs	5-089 3-639	W	6	8	14	57.1					18	19	37	51.4	24	27	51	52.9
26	Mareham le Fen	Lincs	5-250 3-59.9	C	7	21	28	75.0					25	50	75	66.7	32	71	103	68.9
27	*Revesby Abbey*	*Lincs*	*5-297 3-633*	*W*									49	46	95	48.4	49	46	95	48.4
28	*Driby*	*Lincs*	*5-388 3-747*	*We*									129	37	166	22.3	129	37	166	22.3
29	Willoughby Wood	Lincs	5-459 3-708	We	5	4	9										5	4	9	
30	*Candlesby*	*Lincs*	*5-464 3-674*	*H*									26	36	62	58.1	26	36	62	58.1
31	*Welton Low Wood*	*Lincs*	*5-471 3-702*	*W*									36	20	56	35.7	36	20	56	35.7
32	*Skegness*	*Lincs*	*5-567 3-637*	*wG,B*									36	44	80	55.0	36	44	80	55.0

Table B.4.i **Results of 1998 sampling of East Anglia.** (Bracketed frequency values: Total *P. madidus* < 12).

B = blacklegged morph; R = redlegged morph; T = total *P.madidus*.
W = wood; Ws = wood strip; C = copse; Ce = edge of copse; H = hedge. Unless stated otherwise, all woods are deciduous.

SITE	LOCATION		GRID REF		*P. madidus* sampled			
NO				CLASS	BT	RT	T	%R
1	Terrington St Clement	NW Norfolk	5-550 3-197	Ws	26	6	*32*	**18.8**
2	Watlington village	NW Norfolk	5-622 3-113	W	1	9	*10*	***(90.0)***
3	Watlington (lake)	NW Norfolk	5-633 3-112	W	6	38	*44*	**86.4**
4	Leziate (wood)	NW Norfolk	5-676 3-190	W	2	33	*35*	**94.3**
5	Leziate (copse)	NW Norfolk	5-682 3-193	C	6	107	*113*	**94.7**
6	Sandringham	NW Norfolk	5-685 3-283	W conif	0	0	*0*	
7	Old Hunstanton	NW Norfolk	5-691 3-419	C	52	190	*242*	**78.5**
8	East Runton	NE Norfolk	6-202 3-427	W	1	0	*1*	
9	Cromer	NE Norfolk	6-216 3-418	W	3	12	*15*	**80.0**
10	Felbrigg Hall	NE Norfolk	6-189 3-398	W	18	49	*67*	**73.1**
11	Gunton Park	NE Norfolk	6-240 3-357	W decid/conif	1	2	*3*	
12	Swanton Abbott	NE Norfolk	6-267 3-252	W	4	6	*10*	***(60.0)***
13	Coltishall	Norfolk, Broadlands	6-283 3-198	C	41	58	*99*	**58.6**
14	Nr Spixworth	Norfolk, Broadlands	6-253 3-151	C	0	0	*0*	
15	Norwich A (Mousehold Heath)	Norfolk, Broadlands	6-241 3-103	W	6	19	*25*	**76.0**
16	Norwich B (Mousehold Heath)	Norfolk, Broadlands	6-238 3-104	W	36	69	*105*	**65.7**
17	Filby	E Norfolk	6-461 3-137	C	0	0	*0*	
18	Ormesby St Margaret	E Norfolk	6-487 3-157	C	35	43	*78*	**55.1**
19	Fritton (wood)	SE Norfolk	6-467 3-005	W	12	33	*45*	**73.3**
20	Fritton (copse)	SE Norfolk	6-483 2-994	C	0	4	*4*	
21	Lowestoft	NE Suffolk	6-542 2-968	C	0	0	*0*	
22	Brigham Heath	NW Suffolk	5-935 2-871	C decid/conif	0	0	*0*	
23	Barnham	NW Suffolk	5-887 2-792	W	0	0	*0*	
24	Thetford Forest	NW Suffolk	5-823 2-866	W	9	102	*111*	**91.9**
25	Boxworth	S Cambridgeshire	5-345 2-641	H	6	2	*8*	***(25.0)***
26	Monks Wood	S Cambridgeshire	5-203 2-796	Ws	14	3	*17*	**17.6**

Table B.4.ii **Results of analysis of soil samples from 1998 sites in East Anglia, showing grid references and red-legged morph frequencies for comparison.**

(Italicised frequencies: total *P. madidus* < 15).

Site no	Location	%R	Grid ref	% moisture retention	% organic content	pH	Texture class and rank (See Table 4.1)	
1	Terrington-St-Clement	18.8	5-550 3-197	7.37	17.77	6.9	silty clay loam	9
3	Watlington (lake)	88.4	5-633 3-112	2.83	12.65	4.2	sandy peat	2
4	Leziate (wood)	94.3	5-676 3-190	2.72	12.84	4.7	sandy peat	2
5	Leziate (copse)	94.7	5-682 3-193	4.29	22.28	3.8	sandy peat	2
7	Old Hunstanton	78.5	5-691 3-419	5.80	14.21	6.6	clay loam	10
9	Cromer	80.0	6-216 3-418	11.80	9.39	6.4	loamy sand	2
10	Felbrigg Hall	68.4	6-189 3-398	9.26	39.20	3.3	sandy peat	2
13	Coltishall	58.6	6-283 3-198	3.42	14.20	3.9	sandy loam	3
15	Norwich A	76.0	6-241 3-103	4.55	23.52	3.7	sandy peat	2
16	Norwich B	65.7	6-238 3-104	12.81	75.44	3.6	sandy peat	2
18	Ormesby St Margaret	55.1	6-487 3-157	3.31	9.26	4.7	sandy loam	3
19	Fritton (wood)	73.3	6-467 3-005	1.60	8.66	3.4	organic sandy loam	3.5
24	Thetford Forest	91.9	5-823 2-866	2.00	8.55	3.8	organic sandy loam	3.5
25	Boxworth	*25.0*	5-345 2-641	12.12	24.01	7.3	silty clay loam	9
26	MonksWood	17.6	5-203 2-796	10.44	22.04	5.0	clay loam	10

Table B.5 **Sites sampled in the East Midlands, giving number of *P. madidus*, red-legged morph frequencies, geographical, topographical and soil parameters for each site.** (See Section 4.3.1 for explanation of parameters; %W+U = %wood and %urban).

Site name	Habitat	total red	total black	grand total	% red	GRID REF east	GRID REF north	alt	aspect	aspect code	% wood	% urban	% water	km to river	% moisture	% organic	pH	texture class	texture rank
Attenborough	herbaceous/shrubbed area of lawned garden	11	20	31	35.5	51.8	34.9	25	FLAT	5	4	41	23	1	3.79	12.95	5.3	sandy silt loam	4
Awsworth	small deciduous wood/scrub (elder, birch)	20	41	61	32.8	48.8	44.7	70	W	3	1	28	0	2.2	8.78	27.45	7.1	organic sandy clay loam	7.5
Bestwood Lodge	deciduous wood (birch/sycamore/elder)	132	242	374	35.3	57.2	46.5	95	SE	4	15	47	0	2.5	6.81	10.73	6.3	sandy silt loam	4
Borders Wood	beech and larch plantations in deciduous wood	128	1216	1344	9.5	65.7	33.2	90	NW	7	25	7	0	7	6.29	28.72	4.2	organic sandy loam	3.5
Borrowash	herbaceous/shrubbed area of lawned garden	3	24	27	11.1	42.6	34.3	45	FLAT	5	2	34	5	0.75	4.86	15.57	6.0	sandy clay loam	8
Bunny Wood	hawthorn area of deciduous wood	16	30	46	34.8	58.7	28.4	60	FLAT	5	11	10	0	8	4.54	17.81	6.1	clay loam	10
Burton Joyce	new plantation in deciduous wood	46	102	148	31.1	63.7	43.2	30	SE	4	4	20	3	1	6.23	20.77	6.9	silty clay loam	9
Canning Circus Cemetery	under copper beech and shrubs, ground ivy patch	459	1055	1514	30.3	56.5	40.3	70	NE	8	0	92	1	2	2.86	9.96	5.1	loamy sand	2
Clifton Grove	deciduous wood (beech/oak/sycamore)	192	441	633	30.3	54.2	34.8	35	N	9	6	43	6	0.1	2.95	11.70	4.8	silt loam	5
Clifton Wood	larch and beech wood	24	82	106	22.6	53.8	34.6	65	FLAT	5	8	30	12	0.3	2.71	8.73	3.8	sandy loam	3
Codnor	hawthorn and elder hedgerow/copse	15	46	61	24.6	43.0	48.7	115	W	3	2	19	1	2.5	6.14	26.02	4.8	sandy silt loam	4
Colwick racecourse	birch area of deciduous wood	117	258	375	31.2	60.4	39.3	60	FLAT	5	10	25	21	0.5	8.67	21.54	4.3	organic sandy loam	3.5
Colwick Wood	elder/hawthorn area of deciduous woodland	19	29	48	39.6	59.7	39.8	85	FLAT	5	11	51	6	1	7.17	24.99	4.6	organic loamy sand	2.5
Elvaston Castle	deciduous plantation (sycamore/rhododendron)	107	169	276	38.8	40.9	32.8	40	FLAT	5	10	5	7	1	7.48	27.79	3.6	organic loamy sand	2.5
Epperstone	sycamore copse (Forestry Commission)	5	13	18	27.8	63.3	50.2	65	SE	4	17	18	0	1	6.37	29.85	3.5	organic loamy sand	2.5
Glapton Wood, Clifton	deciduous wood (oak, beech)	115	445	560	20.5	54.8	33.8	55	E	6	4	55	0	1.5	7.03	16.51	6.0	clay loam	10
Gotham	small deciduous wood (beech, sycamore)	19	78	97	19.6	53.8	31.3	45	E	6	4	11	0	2.5	3.89	12.62	7.3	sandy clay loam	8
Harrison Plantation	deciduous wood (sycamore, hawthorn, cherry)	61	31	92	66.3	53.1	40.3	45	E	6	3	71	1	4.75	5.93	24.14	5.7	sandy clay loam	8
Hemlock Stone, Stapleford	deciduous wood (sycamore, holly, rhododendron)	44	64	108	40.7	50.0	38.5	60	W	3	7	32	26	1.75	12.22	31.40	3.1	sandy peat	2
Holme Pierrepoint	wood strip (deciduous) on grassed area by lake	284	398	682	41.6	61.5	38.7	20	FLAT	5	3	12	0	0.5	6.96	16.93	5.4	sandy silt loam	4
Keyworth	hawthorn/elder hedgerow/copse by railway	2	14	16	12.5	62.3	32.3	55	NW	7	1	31	8	6.75	4.62	16.21	6.7	silt loam	5
Kings Mill	pine and sycamore/ash areas in woodland	117	177	294	39.8	41.9	27.5	40	NW	7	8	4	3	0.25	3.59	16.54	3.5	organic sandy loam	3.5
Kirk Hallam	deciduous wood (oak, hawthorn, elder)	19	39	58	32.8	44.2	39.7	90	SE	4	3	27	0	3.5	3.77	11.75	7.3	sandy silt loam	4
Lockington	small deciduous wood (sycamore/ash/hawthorn)	9	32	41	22.0	46.4	27.7	45	NE	8	2	15	3	2.5	7.46	14.85	6.8	sandy silt loam	4
Near Hucknall	oak/beech/birch area of woodland	171	297	468	36.5	52.5	45.7	70	E	6	9	28	0	3.5	9.09	47.81	3.7	sandy peat	2
Near Lowdham	hedgerow/copse by ditch alongside road	14	22	36	38.9	65.0	47.9	30	SE	4	1	18	1	4.5	2.40	9.36	4.6	silt loam	5
Nottm Trent Univ. grounds	young ash plantation adjacent to grassland	60	239	299	20.1	54.6	35.1	45	N	9	8	46	0	0.35	3.14	5.81	4.5	sandy silt loam	4
Nottm Univ. grounds	large deciduous copse (beech, holly, laurel)	51	42	93	54.8	54.4	38.6	50	E	6	1	74	0	2.75	3.28	8.81	4.8	loamy sand	2
Roshoe Wood	deciduous woodland (beech, sycamore, hawthorn)	18	64	82	22.0	64.8	29.4	85	NW	7	4	15	7	10.25	7.03	12.10	3.7	silty clay loam	9
Ruddington Hall	deciduous wood (hawthorn, beech)	102	452	554	18.4	57.8	34.2	40	SW	2	2	43	0	3	7.36	18.22	6.4	organic sandy clay loam	7.5
Sawley	birch and willow hedgerow/copse by railway	131	115	246	53.3	48.3	31.6	30	FLAT	5	1	39	7	0.75	3.83	26.03	6.1	organic loamy sand	2.5
Selston Pl., Bagthorpe	small deciduous wood	11	21	32	34.4	47.6	51.6	100	NW	7	12	23	0	3	3.32	13.44	4.7	sandy silt loam	4
Shipley	deciduous wood (oak)	80	99	179	44.7	43.6	44.2	110	W	3	14	14	2	3.5	9.72	16.70	3.3	sandy silt loam	4
Somercotes	hawthorn/ash copse	39	94	133	29.3	43.4	53.2	113	SE	4	4	47	2	1	9.21	23.69	6.7	organic clay loam	7.5
Stragglethorpe	large woodstrip by field (sycamore, beech, pine)	16	79	95	16.8	65.4	36.6	40	W	3	2	7	2	3.25	9.09	20.63	6.3	sandy clay loam	8
Strelley	yew, ash, beech, sycamore woodland	92	82	174	52.9	51.3	41.9	100	SE	4	4	38	2	3.5	14.22	31.23	4.1	organic loamy sand	2.5
Sutton Bonington	grassy copse (birch)	38	102	140	27.1	50.5	26.5	45	W	3	2	16	9	1.5	2.16	8.23	4.0	sandy loam	3
Watnall, copse	elder/hawthorn/sycamore copse	151	73	224	67.4	49.8	46.3	115	W	3	4	21	0	3.5	3.40	11.49	3.9	sandy silt loam	4
Watnall, wood (by lake)	mixed woodland (pine, oak, beech sycamore)	200	336	536	37.3	48.3	48.7	80	SW	2	19	7	4	3.25	6.66	18.23	3.8	organic sandy loam	3.5
Widmerpool	small deciduous wood (yew, beech) by church	6	89	95	6.3	62.8	28.3	70	NW	7	7	10	1	10.5	10.59	15.86	6.8	organic silty clay loam	8.5
Woodthorpe	shrubby, herbaceous area of garden	55	46	101	54.5	58.1	44.8	80	FLAT	5	0	97	0	3	2.14	8.30	5.8	sandy loam	3
	Total	3199	7298	10497	30.5														

Table B.6 Red-legged morph frequencies for each year at the ECN sites, showing number of *P. madidus* trapped at each transect and male/female data where available. Male frequencies given are for each morph. (See Table B.1 for abbreviations)

Red-legged frequencies not calculated if *P.madidus* total is less than 13. Male frequencies not calculated if total is less than 30.

Alice Holt 4-800 1-430	Transect 1 (wood)										Transect 2 (wood)										Transect 3 (wood)										All transects									
Year	Bm	Bf	BT	Rm	Rf	RT	GT	Bm %	Rm %	R %	Bm	Bf	BT	Rm	Rf	RT	GT	Bm %	Rm %	R %	Bm	Bf	BT	Rm	Rf	RT	GT	Bm %	Rm %	R %	Bm	Bf	BT	Rm	Rf	RT	GT	Bm %	Rm %	R %
1994	79	58	137	136	114	250	387	58	54	65	35	55	90	75	104	179	269	39	42	67	106	84	190	171	157	328	518	56	52	63	220	197	417	382	375	757	1174	53	50	64
1995	65	37	102	98	70	168	270	64	58	62	9	19	28	35	22	57	85	32	61	67	78	56	134	116	90	206	340	58	56	61	152	112	264	249	182	431	695	58	58	62
1996	61	32	93	108	62	170	263	66	64	65	11	14	25	29	22	51	76	44	57	67	67	34	101	142	85	227	328	66	63	69	139	80	219	279	169	448	667	63	62	67
1997	197	93	290	322	152	474	764	68	68	62	97	52	149	154	61	215	364	65	72	59	240	161	401	432	224	656	1057	60	66	62	534	306	840	908	437	1345	2185	64	68	62
Total	402	220	622	664	398	1062	1684	65	63	63	152	140	292	293	209	502	794	52	58	63	491	335	826	861	556	1417	2243	59	61	63	1045	695	1740	1818	1163	2981	4721	60	61	63

Rothampsted 5-130 2-130	Transect 1(hedge)										Transect 2 (wood)										Transect 3 (grass)										All transects									
Year	Bm	Bf	BT	Rm	Rf	RT	GT	Bm %	Rm %	R %	Bm	Bf	BT	Rm	Rf	RT	GT	Bm %	Rm %	R %	Bm	Bf	BT	Rm	Rf	RT	GT	Bm %	Rm %	R %	Bm	Bf	BT	Rm	Rf	RT	GT	Bm %	Rm %	R %
1992	22	6	28	11	10	21	49	79	52	43	125	50	175	313	162	475	650	71	66	73	19	11	30	38	38	76	106	63	50	72	166	67	233	362	210	572	805	71	63	71
1993	27	8	35	32	19	51	86	77	63	59	497	275	772	1305	705	2010	2782	64	65	72	57	31	88	118	86	204	292	65	58	70	581	314	895	1455	810	2265	3160	65	64	72
1994	48	17	65	93	41	134	199	74	69	67	243	177	420	659	434	1093	1513	58	60	72	28	20	48	50	32	82	130	58	61	63	319	214	533	802	507	1309	1842	60	61	71
1995	76	25	101	169	54	223	324	75	76	69	340	112	452	978	435	1413	1865	75	69	76	94	58	152	212	86	298	450	62	71	66	510	195	705	1359	575	1934	2639	72	70	73
1996	23	17	40	52	38	90	130	58	58	69	183	62	245	484	165	649	894	75	75	73	71	82	153	170	179	349	502	46	49	70	277	161	438	706	382	1088	1526	63	65	71
1997	123	67	190	319	166	485	675	65	66	72	851	381	1232	1963	1029	2992	4224	69	66	71	31	38	69	68	99	167	236	45	41	71	1005	486	1491	2350	1294	3644	5135	67	64	71
Total	319	140	459	676	328	1004	1463	69	67	69	2239	1057	3296	5702	2930	8632	11928	68	66	72	300	240	540	656	520	1176	1716	56	56	69	2858	1437	4295	7034	3778	10812	15107	67	65	72

Wytham 4-470 2-080	Transect 1(wood)										Transect 2 (grass)										Transect 3 (wood plantation)										All transects									
Year	Bm	Bf	BT	Rm	Rf	RT	GT	Bm %	Rm %	R %	Bm	Bf	BT	Rm	Rf	RT	GT	Bm %	Rm %	R %	Bm	Bf	BT	Rm	Rf	RT	GT	Bm %	Rm %	R %	Bm	Bf	BT	Rm	Rf	RT	GT	Bm %	Rm %	R %
1993	60	49	109	20	12	32	141	55	63	23	22	21	43	7	3	10	53	51	70	19	37	48	85	11	15	26	111	44	42	23	119	118	237	38	30	68	305	50	56	22
1994	36	27	63	9	9	18	81	57	50	22	2	3	5	5	4	9	14			64	34	13	47	9	3	12	59	72	75	20	72	43	115	23	16	39	154	63	59	25
1995	13	3	16	3	3	6	22	81	50	27	17	9	26	7	1	8	34	65	88	24	29	15	44	4	2	6	50	66	67	12	59	27	86	14	6	20	106	69	70	19
1996	16	7	23	5	3	8	31	70	63	26	5	5	10	1	2	3	13			23	20	29	49	7	6	13	62	41	54	21	41	41	82	13	11	24	106	50	54	23
1997	114	35	149	28	10	38	187	77	74	20	20	5	25	11	7	18	43	80	61	42	47	14	61	7	10	17	78	77	41	22	181	54	235	46	27	73	308	77	63	24
Total	239	121	360	65	37	102	462	66	64	22	66	43	109	31	17	48	157	61	65	31	167	119	286	38	36	74	360	58	51	21	472	283	755	134	90	224	979	63	60	23

Porton Down 4-200 1-370	Transect 1 (short grass)										Transect 2 (chalk grassland)										Transect 3 (long grass)										All transects									
Year	Bm	Bf	BT	Rm	Rf	RT	GT	Bm %	Rm %	R %	Bm	Bf	BT	Rm	Rf	RT	GT	Bm %	Rm %	R %	Bm	Bf	BT	Rm	Rf	RT	GT	Bm %	Rm %	R %	Bm	Bf	BT	Rm	Rf	RT	GT	Bm %	Rm %	R %
1994	0	1	1	0	0	0	1				3	2	5	0	0	0	5				5	6	11	0	0	0	11				8	9	17	0	0	0	17			0
1995	2	0	2	0	0	0	2				8	20	28	1	2	3	31	29	33	10	7	8	15	2	0	2	17			12	17	28	45	3	2	5	50	38	60	10
1996	1	3	4	0	0	0	4				33	76	109	5	6	11	120	30	45	9	30	21	51	1	1	2	53	59	50	4	64	100	164	6	7	13	177	39	46	7
1997	2	0	2	0	0	0	2				5	17	22	1	5	6	28	23	17	21	15	6	21	1	1	2	23			9	22	23	45	2	6	8	53	49	25	15
Total	5	4	9	0	0	0	9				49	115	164	7	13	20	184	30	35	11	57	41	98	4	2	6	104	58	67	6	111	160	271	11	15	26	297	41	42	9

Table B.6 (continued)

Glensaugh 3-670 7-790	Transect 1 (mineral grassland)										Transect 2 (dry peat)										Transect 3 (wet peat)										All transects									
Year	Bm	Bf	BT	Rm	Rf	RT	GT	Bm %	Rm %	R %	Bm	Bf	BT	Rm	Rf	RT	GT	Bm %	Rm %	R %	Bm	Bf	BT	Rm	Rf	RT	GT	Bm %	Rm %	R %	Bm	Bf	BT	Rm	Rf	RT	GT	Bm %	Rm %	R %
1994	n.a.	n.a.	20	n.a.	n.a.	68	88			77	n.a.	n.a.	11	n.a.	n.a.	18	29			62	n.a.	n.a.	0	n.a.	n.a.	0	0						31			86	117			74
1995	n.a.	n.a.	47	n.a.	n.a.	109	156			70	n.a.	n.a.	1	n.a.	n.a.	6	7				n.a.	n.a.	4	n.a.	n.a.	19	23			83			52			134	186			72
1996	n.a.	n.a.	46	n.a.	n.a.	195	241			81	n.a.	n.a.	6	n.a.	n.a.	10	16			63	n.a.	n.a.	1	n.a.	n.a.	0	1						53			205	258			79
1997	n.a.	n.a.	58	n.a.	n.a.	164	222			74	n.a.	n.a.	6	n.a.	n.a.	5	11				n.a.	n.a.	2	n.a.	n.a.	0	2						66			169	235			72
Total			171			536	707			76			24			39	63			62			7			19	26			73			202			594	796			75

Sourhope 3-860 6-220	Transect 1 (mineral grassland)										Transect 2 (dry peat)										Transect 3 (wet peat)										All transects									
Year	Bm	Bf	BT	Rm	Rf	RT	GT	Bm %	Rm %	R %	Bm	Bf	BT	Rm	Rf	RT	GT	Bm %	Rm %	R %	Bm	Bf	BT	Rm	Rf	RT	GT	Bm %	Rm %	R %	Bm	Bf	BT	Rm	Rf	RT	GT	Bm %	Rm %	R %
1994	n.a.	n.a.	123	n.a.	n.a.	58	181			32	n.a.	n.a.	3	n.a.	n.a.	1	4				n.a.	n.a.	2	n.a.	n.a.	0	2						128			59	187			32
1995	n.a.	n.a.	84	n.a.	n.a.	49	133			37	n.a.	n.a.	1	n.a.	n.a.	1	2				n.a.	n.a.	0	n.a.	n.a.	0	0						85			50	135			37
1996	n.a.	n.a.	25	n.a.	n.a.	16	41			39	n.a.	n.a.	0	n.a.	n.a.	0	0				n.a.	n.a.	8	n.a.	n.a.	0	8						33			16	49			33
1997	n.a.	n.a.	32	n.a.	n.a.	5	37			14	n.a.	n.a.	2	n.a.	n.a.	1	3				n.a.	n.a.	4	n.a.	n.a.	0	4						38			6	44			14
Total			264			128	392			33			6			3	9						14			0	14			0			284			131	415			33

North Wyke 2-650 0-990	Transect 1 (bet pasture/wood)										Transect 2 (grazed pasture)										Transect 3 (bet pasture/scrub)										All transects									
Year	Bm	Bf	BT	Rm	Rf	RT	GT	Bm %	Rm %	R %	Bm	Bf	BT	Rm	Rf	RT	GT	Bm %	Rm %	R %	Bm	Bf	BT	Rm	Rf	RT	GT	Bm %	Rm %	R %	Bm	Bf	BT	Rm	Rf	RT	GT	Bm %	Rm %	R %
1993	n.a.	n.a.	0	n.a.	n.a.	0	0				n.a.	n.a.	0	n.a.	n.a.	0	0				n.a.	n.a.	0	n.a.	n.a.	1	1						0			1	1			
1994	n.a.	n.a.	0	n.a.	n.a.	0	0				n.a.	n.a.	0	n.a.	n.a.	0	0				n.a.	n.a.	0	n.a.	n.a.	31	31			100			0			31	31			100
1995	n.a.	n.a.	0	n.a.	n.a.	0	0				n.a.	n.a.	0	n.a.	n.a.	0	0				n.a.	n.a.	0	n.a.	n.a.	37	37			100			0			37	37			100
1996	n.a.	n.a.	0	n.a.	n.a.	8	8				n.a.	n.a.	0	n.a.	n.a.	0	0				n.a.	n.a.	4	n.a.	n.a.	79	83			95			4			87	91			96
1997			0			4	4						0			2	2						0			38	38			100			0			44	44			100
Total			0			12	12			100			0			2	2						4			186	190			98			4			200	204			98

APPENDIX C

Statistical methods, inheritance patterns and models

(for use with Chapters 2, 3, 4, 5 and 6)

Table C.1 Inheritance pattern assuming Mendelian principles.

A = dominant phenotype	**a** = recessive phenotype
A = dominant allele	*a* = recessive allele

	Parental phenotypes	Parental genotypes	Offspring phenotypes	Offspring genotypes
1	**A** x **A**	*AA* x *AA*	all **A**	all *AA*
2	**A** x **A**	*AA* x *Aa*	all **A**	*AA* , *AA* , *Aa* , *Aa*
3	**A** x **A**	*Aa* x *Aa*	75% **A**, 25% **a**	*AA* , *Aa* , *Aa* , *aa*
4	**a** x **a**	*aa* x *aa*	all **a**	all *aa*
5	**A** x **a**	*AA* x *aa*	all **A**	all *Aa*
6	**A** x **a**	*Aa* x *aa*	50% **A**. 50% **a**	*Aa* , *Aa* , *aa*, *aa*

Table C.2 Method from Russell (1996) adapted for spreadsheet use to calculate change in allelic, genotypic and phenotypic frequency after natural selection.

Notation used is that given by Russell (1996) - see Section 3.3.5 for explanation. C = column; R = row. Unless indicated otherwise, calculations are made *within* each row.
See file 'genetic1' on disc to run model.

Column	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
		frequency of alleles:		freq of genotypes:			freq of phenotypes:			w_{11}	w_{12}	w_{22}	frequency after selection:				relative genotype frequency after selection:			
Row	generation	p	q	A_1A_1	A_1A_2	A_2A_2	dom	rec	total				A_1A_1	A_1A_2	A_2A_2	W	A_1A_1	A_1A_2	A_2A_2	total
1	0	p	q	$p2$	$2pq$	$q2$	p^2+pq	enter freq	C7+C8	enter values (0 to 1)			$w_{11}*p^2$	$w_{12}*pq$	$w_{22}*q^2$	$w_{11}+w_{12}+w_{22}$	$(w_{11}*p^2)$/W	$(w_{12}*pq)$/W	$(w_{22}*q^2)$/W	C17+C18+C19
2	1	R1: C17+1/2(C18)	R1: C19+1/2(C18)	$p2$	$2pq$	$q2$	p^2+pq	q^2	C7+C8	=R1, C10	=R1, C11	=R1, C12	$w_{11}*p^2$	$w_{12}*pq$	$w_{22}*q^2$	$w_{11}+w_{12}+w_{22}$	$(w_{11}*p^2)$/W	$(w_{12}*pq)$/W	$(w_{22}*q^2)$/W	C17+C18+C19
3	2	R2: C17+1/2(C18)	R2: C19+1/2(C18)	etc						etc (or change values)			etc							
4	3	etc																		
5	etc																			

Table C.3 **Model for predicting month of emergence from month of larval hatching, using monthly soil temperatures** (see Fig 6.9).

	B	C	D	E	F	G	H	I
4		average	(Accumulative degree day data for curves in Fig 6.9)					deg days
5		soil temp	Period of larval hatching:					per month
6	month	(°C)	beg Aug	beg Sept	beg Oct	beg Nov	beg Dec	(Cd)
7			0					
8	Aug	enter value	=I5	0				=C5*31
9	Sept	enter value	=D5+I6	=I6	0			=C6*30
10	Oct	enter value	=D6+I7	=E6+I7	=I7	0		=C7*31
11	Nov	enter value	=D7+I8	=E7+I8	=F7+I8	=I8	0	=C8*30
12	Dec	enter value	=D8+I9	=E8+I9	=F8+I9	=G8+I9	=I9	=C9*31
13	Jan	enter value	=D9+I10	=E9+I10	=F9+I10	=G9+I10	=H9+I10	=C10*31
14	Feb	enter value	=D10+I11	=E10+I11	=F10+I11	=G10+I11	=H10+I11	=C11*28
15	Mar	enter value	=D11+I12	=E11+I12	=F11+I12	=G11+I12	=H11+I12	=C12*31
16	Apr	enter value	=D12+I13	=E12+I13	=F12+I13	=G12+I13	=H12+I13	=C13*30
17	May	enter value	=D13+I14	=E13+I14	=F13+I14	=G13+I14	=H13+I14	=C14*31
18	Jun	enter value	=D14+I15	=E14+I15	=F14+I15	=G14+I15	=H14+I15	=C15*30
19	Jul	enter value		=E15+I16	=F15+I16	=G15+I16	=H15+I16	=C16*31
20	Aug	enter value			=F16+I17	=G16+I17	=H16+I17	=C17*31

Box C.1: Procedure for selection of variables, fitting and checking of multiple regression equations using Minitab 12.

Adapted from Iles (1993).

1. The dependent variable (y) was plotted against each predictor variable (x) in turn. Linear plots indicated important predictors. Curvature indicated the need for transformations.

2. Multi-collinearity was investigated by obtaining the correlation matrix of the predictor variables. If the correlation between two or more variables was significant at the 5% level, one or more of the variables were excluded from the analysis.

3. Optimal subsets of variables were identified using Best Subsets Regression. Selected regressions were those with the highest R^2 and a Mallows C_p close to $q + 1$, where q is the number of predictor variables. Number of predictor variables used depended on the number of dependent variables. The maximum ratio was 1:4.

4. Stepwise regression (forward selection and backward elimination) was used to confirm that there are no other optimal subset(s).

5. Full regression analysis for optimal subset(s) was calculated. The significance of the relationship between variables was checked using the F-ratio, the R^2 and adjusted R^2 (which takes the number of degrees of freedom into account), the significance level (P) and standard deviation(s) of the equation. The significance level (P) and the standard deviation on each to the partial coefficients were also checked.

6. Outliers and observations having a large influence on the calculation of partial regression coefficients were identified by Minitab 12. In the case of outliers, if the standardised residual was greater than 2, exclusion of the observation giving the high residual was considered.

7. The standardised residuals were checked for non-constant variance using plots of standardised residuals against predictor variables or plots of the dependent variable against the predictor variables where appropriate. If non-constant variance was indicated, transformation of the predictor variable(s) was considered.

8. The possibility of reducing the number of predictor variables was investigated.

9. An equation was regarded as optimum when the following conditions were met:
   - no correlation between the predictor variables;
   - no outliers or observations with a large influence;
   - a constant variance;
   - high F-ratio;
   - R^2 and the adjusted R^2 were close in value;
   - $P < 0.05$ for the regression equation, the constant and all the partial predictors.

Fig C.1 Relationship between morph frequency and number of individuals required per sample to achieve a 10% half width on the 95% confidence limits, using Equation 2.3 (Equation 2.11 in Southwood, 1978).

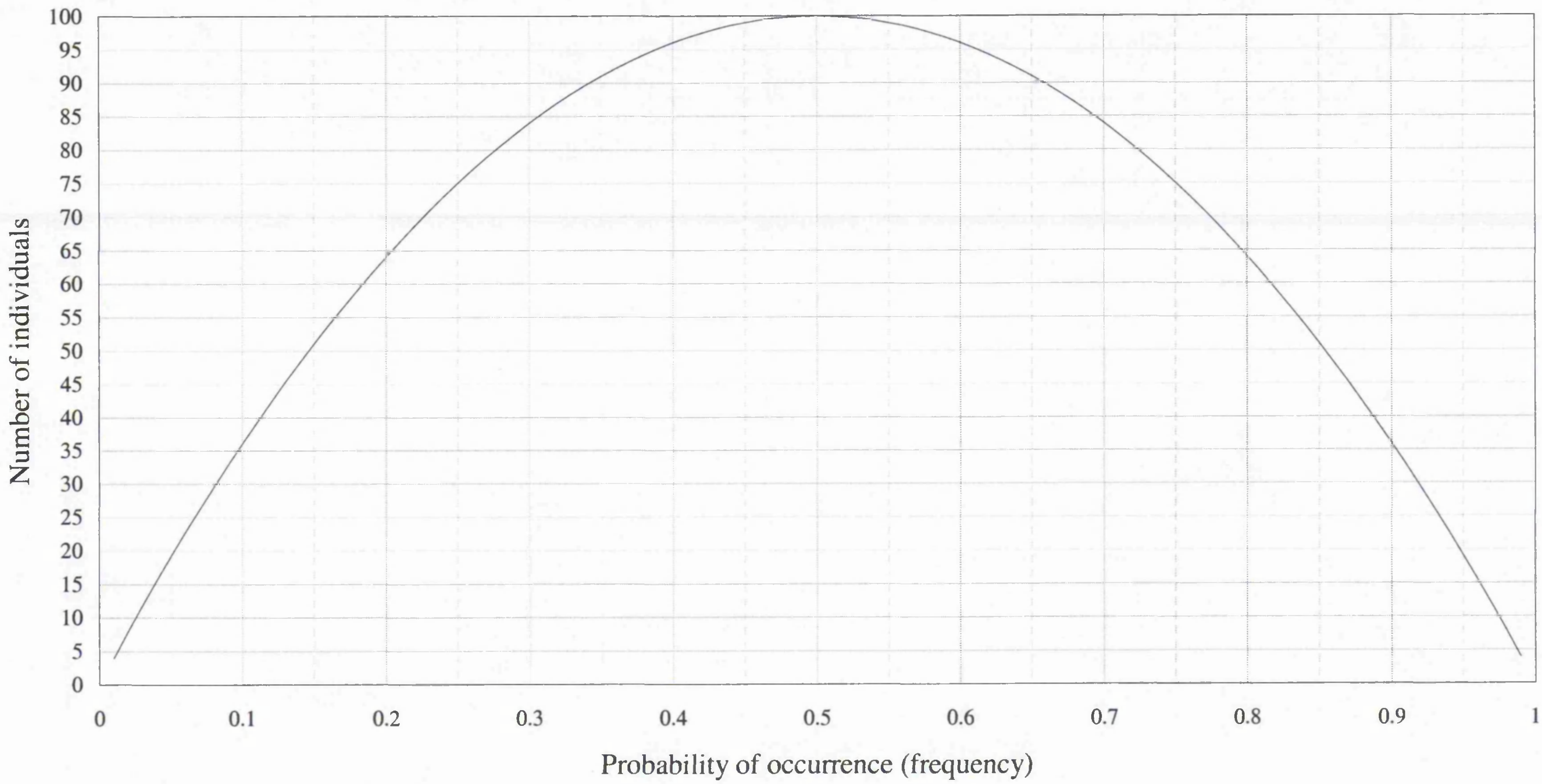
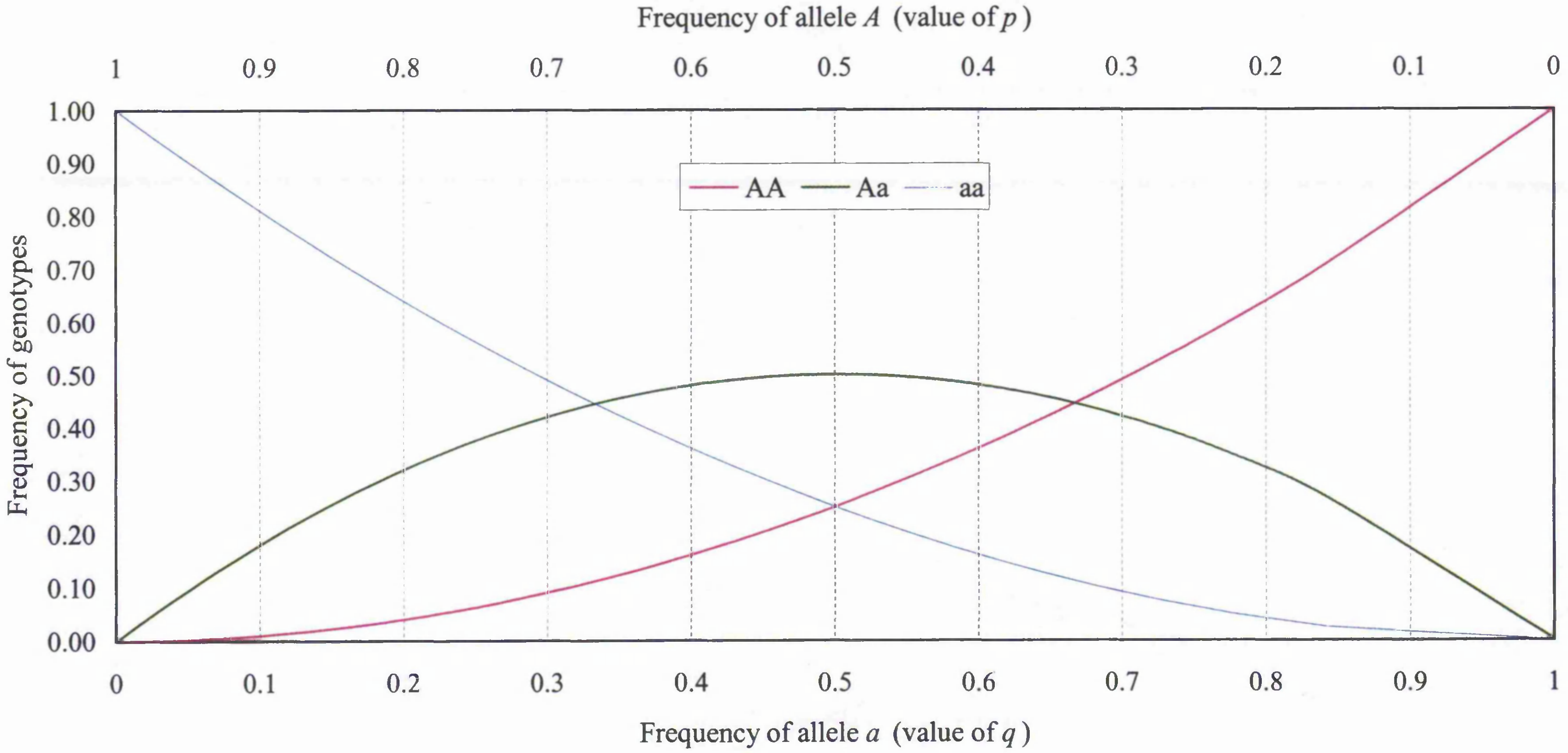


Fig C.2 Frequencies of genotypes AA (p^2), Aa ($2pq$) and aa (q^2) to the frequencies of alleles *A* (top axis) and *a* (bottom axis) in populations that meet the assumptions of the Hardy-Weinberg law.



APPENDIX D

Inheritance patterns and gene frequency models

(for use with Chapters 3, 5 and 6)

Table D.1 **Temperature and photoperiodic treatments given to larvae of breeding pairs until emergence or death, showing number of days spent at each temperature and photoperiod within each treatment.** (L = Light; D = Dark)

i) Constant temperature treatments until Instar 3

C1: 7.5°C			C2: 10°C			C3: 15°C			C4: 20°C		
temp (°C)	photoperiod	no of days	emp (°C	photoperiod	no of days	temp (°C)	photoperiod	no of days	temp (°C)	photoperiod	no of days
7.5	8L 16D	256	*subtreatment C2.i*			*subtreatment C3.i*			**20**	12L 12D	until death
10	12L 12D	to emergence	**10**	12L 12D	until death	**15**	12L 12D	until death			
			subtreatment C2.ii			*subtreatment C3.ii*					
			10	12L 12D	63	**15**	12L 12D	142			
			10	24L	to emergence	10	12L 12D	to emergence			
			subtreatment C2.iii			*subtreatment C3.iii*					
			10	12L 12D	163	**15**	12L 12D	142			
			15	12L 12D	until death	15	16L 8D	to emergence			
			subtreatment C2.iv								
			10	12L 12D	163						
			15	16L 8D	to emergence						

ii) Variable temperature treatments

V1: 5°C start			V2: 7.5°C start			V3: 10°C start			V4: 12.5°C start		
temp (°C)	photoperiod	no of days	emp (°C	photoperiod	no of days	temp (°C)	photoperiod	no of days	temp (°C)	photoperiod	no of days
5	24D	90	**7.5**	24D	30-60	**10**	24D	30	**12.5**	24D	30
7.5	24D	30	3-5	24D	90	7.5	24D	30	10	24D	30
10	12L 12D	30	7.5	24D	30	3-5	24D	90	7.5	24D	30
12.5	16L 8D	to emergence	10	12L 12D	30	7.5	24D	30	3-5	24D	90
			12.5	16L 8D	to emergence	10	12L 12D	30	7.5	24D	30
						12.5	16L 8D	to emergence	10	12L 12D	30
									12.5	16L 8D	to emergence

Table D.2 **CONSTANT TEMPERATURE TREATMENTS: Parentage, leg colour and sex of emerged adults.**

B = black; R = red; m = male; f = female; L = light; D = dark.

Same colour shading denotes sibling batches within and between treatments.

Treatment	temperature (°C)		photoperiod at late Instar III	leg colour of parents	leg colour of offspring	sex of offspring
	initial	final				
CT1	7.5	10.0	12L 12D	RR	R	m
				BB	B	m
				BB	B	m
				RB	R	f
				RB	R	m
				RB	R	m
CT2.ii	10.0	10.0	24L	BB	B	m
				BB	B	m
				RB	R	f
				RB	B	m
				RB	B	m
CT2.iv	10.0	15.0	16L 8D	RR	R	f
CT3.ii	15.0	10.0	16L 8D	RR	B	m

} sibling status unknown

Table D.3 **VARIABLE TEMPERATURE TREATMENTS: Parentage, leg colour and sex of emerged adults.**

Larvae are named as follows:

Leg colour of male parent is given first followed by leg colour of female parent.

Before decimal point is number given to the mother; after the decimal point is number given to larva.

Same colour shading denotes sibling batches within and between treatments.

As indicated by the arrows:

RR(9.6).28 is the offspring of RR9.6;

the BB(14.2) sibling batch is the progeny of BB14.2;

BB(14.4).7 is the offspring of BB14.4.

treatment code	temperature (°C)		photoperiod at late Instar III	name of larva *	leg colour of		sex
	initial	final			parents	offspring	
VT1	5.0	12.5	16L 8D	WB12.8	WB	B	m
				WB13.1	WB	B	m
				WB13.3	WB	B	f
				WB13.7	WB	B	f
VT2	7.5	12.5	16L 8D	WB2.2	WB	B	f
				WB2.6	WB	B	f
				WB2.13	WB	B	f
				WB2.23	WB	B	f
				WB14.21	WB	B	f
				WB14.22	WB	B	f
				WB14.25	WB	B	m
				WB14.51	WB	B	m
				RR9.1	RR	B	m
				RR9.3	RR	B	m
				RR9.4	RR	B	f
				RR9.6	RR	R	f
				RR9.7	RR	R	f
				RR9.8	RR	R	m
VT3	10.0	12.5	16L 8D	WB2.7	WB	B	f
				WB2.8	WB	B	f
				WB2.11	WB	B	f
				WB2.20	WB	B	f
				WB14.2	WB	B	f
				WB14.4	WB	B	f
				WB14.5	WB	B	f
				WB14.6	WB	B	m
				WB14.7	WB	B	m
				WB14.8	WB	B	f
				RR9.28	RR	R	f
				BB18.19	BB	B	m
VT4	12.5	12.5	16L 8D	WB14.7	WB	B	m
				WB14.8	WB	B	m
				WB5.3	WB	B	f
				WB3.13	WB	B	f
				BB18.9	BB	B	f
				BB18.10	BB	B	f
				BB18.12	BB	B	f
				BB18.14	BB	B	f
				BB18.15	BB	B	m
				BB18.17	BB	B	f
				BB18.18	BB	B	f

name of larva *	leg colour of		sex
	parents	offspring	
RR(9.6).28	RR	R	m
BB(14.2).2	BB	B	f
BB(14.2).3	BB	B	f
BB(14.2).4	BB	B	f
BB(14.2).5	BB	B	f
BB(14.2).8	BB	B	m
BB(14.2).12	BB	B	f
BB(14.2).14	BB	B	f
BB(14.2).15	BB	B	m
BB(14.4).7	BB	B	f

Table D.4 Initial and final temperature treatments given to the larvae that completed all developmental stages, showing leg colour of emerged adults and numbers of males and females. (See Section 5.3 for abbreviations; n.d. = no data).

		OFFSPRING				PARENTS						
Row no.	Treatment code	temperature treatment (°C)	initial	final	leg colour	BB	RR	RB	WB	*TOTAL*	*no. of males*	*no. of females*
1	C1	constant	7.5	10	B	2	0	0		2	2	0
					R	0	1	3		4	3	1
					total	2	*1*	*3*	*n.d.*	*6*	*5*	*1*
2	C2.ii	constant	10	10	B	2		2		4	4	0
					R	0		1		1	0	1
					total	2	*n.d.*	*3*	*n.d.*	*5*	*4*	*1*
3	C2.iv	constant	10	15	B		0			0	0	0
					R		1			1	0	1
					total	*n.d.*	*1*	*n.d.*	*n.d.*	*1*	*0*	*1*
4	C3.ii	constant	15	10	B		1			1	0	1
					R		0			0	0	0
					total	*n.d.*	*1*	*n.d.*	*n.d.*	*1*	*0*	*1*
					TOTAL	*4*	*3*	*6*	*n.d.*	*13*	*9*	*4*
5	V1	variable	5	12.5	B		0		4	4	2	2
					R		1		0	1	0	0
					total	*n.d.*	*1*	*n.d.*	*4*	*5*	*2*	*2*
6	V2	variable	7.5	12.5	B	8	3		8	19	6	13
					R	0	3		0	3	1	2
					total	*8*	*6*	*n.d.*	*8*	*22*	*7*	*15*
7	V3	variable	10	12.5	B	3	0		10	13	5	7
					R	0	1		0	1	0	1
					total	*3*	*1*	*n.d.*	*10*	*14*	*5*	*8*
8	V4	variable	12.5	12.5	B	7			4	11	3	8
					R	0			0	0	0	0
					total	*7*	*n.d.*	*n.d.*	*4*	*11*	*3*	*8*
					TOTAL	*18*	*8*	*n.d.*	*26*	*52*	*17*	*33*

Table D.5 **CONSTANT TEMPERATURE TREATMENTS: Parentage, leg colour, sex, weight and condition of emerged adults, showing thermal time to complete development and day number of death after emergence.** s.e. = 1 standard error.

Treat-ment	Leg colour of parents and larval group number	Leg colour of offspring	Sex	No. of degree-days (Cd) to complete development	Weight (mg) of emerged adult before feeding	Condition of emerged adult	Day number of death
C1 7.5°C start	BB.1	black	m	2595	97	dent in elytra	106
	BB.2	black	m	2578	88	elytra not fused	6
	RR.1	red	m	2580	102	perfect	329
	RB.1	red	m	2648	79	elytra not fused	123
	RB.1	red	m	2708	94	elytra not fused	37
	RB.1	red	f	2458	112	elytra not fused	303
			mean	**2594.5**	**95.9**		
			s.e.	*31.1*	*4.3*		
C2.ii * 10°C "tank" larvae	BB	black	m	2310	124	elytra not fused	249
	BB	black	m	2500	115	dent in elytra	11
	RB	black	m	3010	94	elytra not fused	80
	RB	black	m	3170	121	no elytra	14
	RB	red	f	2740	131	perfect	115
			mean	**2746.0**	**117.1**		
			s.e.	*141.4*	*5.6*		
C2.i 10°C start	BB.3	n.a.	n.a.	**3510**	n.a.	partial emergence (dead)	0
C2.iv 10°C start	RR.1	red	f	2568	123	elytra not fused	9
C3.ii 15°C start	RR.2	black	m	**4400**	**131**	elytra not fused	257

* sibling status unknown

Table D.6 **VARIABLE TEMPERATURE TREATMENTS: Parentage, leg colour, sex, weight and condition of emerged adults, showing thermal time to complete development and day number of death after emergence.** See Table D.3 for explanation of names for larvae. Asterisked females were paired with males during their time in the laboratory.
s.e. = 1 standard error; ✓ = eggs laid by female; n.d. = no data.

Treat-ment	Leg colour of parents and larval group number	Leg colour of offspring	Sex	No. of degree-days (Cd) to complete development	Weight (mg) of emerged adult before feeding	Condition of emerged adult	Day number of death
V1 5°C start	WB12.8	black	m	1905	87	perfect	287
	WB13.1	black	f	2150	104	perfect	>150
	WB13.3	black	f	1850	97	elytra damaged	9
	WB13.7	black	f	2025	101	elytra damaged	8
	RR(9.6).28	red	m	2228	85	perfect	released
			mean	**2032**	**94.8**		
			s.e.	*63.8*	*3.4*		
V2 7.5°C start	WB2.2 *	black	f	2358	108	malformed elytra	345
	WB2.6	black	f	2395	114	elytra not fused	194 ✓
	WB2.13	black	f	2345	n.d.	no elytra	3
	WB2.23 *	black	f	2395	115	perfect	370 ✓
	WB14.21-b1 *	black	f	2448	125	perfect	231
	WB14.22-b1 *	black	f	2260	120	perfect	221
	WB14.25-b1	black	m	2285	137	perfect	528
	WB14.51-b2	black	m	2398	113	perfect	354
	RR9.1-b1	black	m	2353	115	perfect	520
	RR9.3-b1	black	m	2416	117	perfect	192
	RR9.4-b1 *	black	f	2366	122	perfect	463 ✓
	RR9.6-b1 *	red	f	2316	143	perfect	503 ✓
	RR9.7-b1	red	f	2478	136	perfect	236
	RR9.8-b1	red	m	2403	135	perfect	205
			mean	**2373**	**123.1**		
			s.e.	*15.4*	*3.0*		
V3 10°C start	WB2.7	black	m	2880	106	elytra not fused	144
	WB2.8 *	black	f	2670	127	perfect	488
	WB2.11 *	black	f	2930	94	elytra not fused	181 ✓
	WB2.20	black	m	2583	105	dent in elytra	317
	WB14.2 *	black	f	2213	122	perfect	>620 ✓
	WB14.4 *	black	f	2013	144	perfect	493 ✓
	WB14.5 *	black	f	2288	143	perfect	149
	WB14.6	black	m	2275	124	perfect	410
	WB14.7	black	m	2388	99	protusion from abdomen	140
	WB14.8 *	black	f	2288	97	perfect	285
	BB(14.2).2	black	f	3330	106	perfect	released
	BB(14.2).3	black	f	2830	130	perfect	released
	BB(14.2).4	black	f	3330	99	perfect	released
	BB(14.2).5	black	f	2830	87	deformed head and elytra	released
	BB(14.2).8	black	m	2830	106	deformed head and elytra	released
	BB(14.2).12	black	f	3127	n.d.	no elytra	released
	BB(14.2).14	black	f	3127	n.d.	elytra not fused	released
	BB(14.20.15	black	m	3330	106	perfect	released
	BB(14.4).7	black	f	2540	102	malformed	released
	BB18.19	black	m	2185	121	perfect	>244
	RR9.28	red	f	2473	89	elytra not fused	53
			mean	**2688**	**110.9**		
			s.e.	*87.5*	*3.8*		
V4 12.5°C start	WB3	black	f	2503	181	perfect	133
	WB5	black	f	2813	99	perfect	>270
	WB140.7	black	m	2640	141	perfect	>225
	WB140.8	black	m	2540	129	perfect	>235
	BB18.9	black	f	2930	141	perfect	>162
	BB18.10	black	f	3030	127	perfect	120
	BB18.12	black	f	2745	n.d.	deformed head, no elytra	30
	BB18.14	black	f	2643	130	perfect	>200
	BB18.15	black	m	2768	131	perfect	>210
	BB18.17	black	f	2768	128	perfect	>205
	BB18.18	black	f	3193	150	perfect	>155
			mean	**2779**	**135.7**		
			s.e.	*59.7*	*6.2*		