

The thermal macrophysiology of core and marginal populations of the aphid *Myzus persicae* in Europe

by

Lucy Alford

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Abstract

Insects are ectotherms and have limited ability to regulate body temperature above or below ambient and are consequently greatly affected by temperature. The aphid *Myzus persicae* has an extensive distribution throughout Europe from Scandinavia to Southern Spain, representing three distinct climatic regions: sub-Arctic, temperate and Mediterranean. The aphid also has genetically distinct clones within its holocyclic (sexual) and anholocyclic (asexual) life cycles. This raises the possibility that aphids are regionally-adapted to distinct climatic zones along the latitudinal cline of its European distribution. Genetically distinct clones of *M. persicae* were collected from Sweden, UK and Spain. Indices of temperature tolerance (upper and lower lethal temperature₅₀, coma temperatures and mobility thresholds) were determined for each aphid clone at different rearing temperatures.

Acclimation at 10°C for one generation increased cold tolerance by depressing lower lethal, chill movement and chill coma temperatures when compared to 20°C and 25°C and further enabled mobility to be maintained to lower temperatures. Acclimation at 25°C for one generation increased heat tolerance by raising upper lethal, heat movement and heat coma temperatures when compared to 10°C and 20°C. Acclimation at 10°C also acted to raise upper lethal temperatures, indicating that the physiological processes conferring heat tolerance are induced at both high and low temperatures. Data did not support intergenerational acclimation to higher or lower temperatures. Lower thermal limits were more plastic than upper limits, enabling tolerance ranges to be increased following acclimation at 10°C, but reduced on acclimation at 25°C. Rates of change varied between clones, suggesting that certain clones could be more affected by climate change.

A relationship between thermal tolerance range and latitude was not supported by data on thermal traits investigated with the exception of heat coma temperature. This suggests that clonal mixing across Europe is extensive and prevents local adaptation, although long term populations could persist in the Mediterranean allowing increased heat tolerance. Clonal type, as identified by microsatellite analysis, did show a relationship with thermal tolerance, suggesting that clonal types could respond independently to climate change, affecting relative proportions of clones within populations.

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1 Introduction

Temperature is one of the most important abiotic factors affecting life on earth. Temperature extremes are a major threat to life, affecting both the distribution and abundance of species. This is especially true for insects which are ectothermic and have a limited ability to regulate body temperatures above or below ambient. Ectotherms are consequently greatly influenced by environmental conditions (Walther *et al.*, 2002), with winter temperatures often determining the limit to species' distributions (Bale, 1993b).

Temperature can affect insects in numerous ways, influencing reproduction, longevity, and development (Parish & Bale, 1993; Hutchinson & Bale, 1994), the latter of which may result in morphological abnormalities (Bale *et al.*, 1989). At low temperature extremes the probability of survival is dependent upon the interaction between temperature and duration of exposure and the cold hardiness of the individual (Bale, 1987). Bale (1987) defines cold hardiness as the attributes of an individual that prevent deleterious effects when exposed to low temperatures. However, the survival of the insect is of little ecological importance if the exposure results in the individual's failure to reproduce and contribute to the next generation (Bale, 1987).

Insects are regarded as the most successful taxa, with species distributed from tropical to polar climates. At present, approximately 10^6 insect species have been described, representing over half of all known species, although the total number of insect species on the Earth is estimated to lie in the region of 10^7 to 10^8 (Pimm *et al.*, 1995; Thomas, 2005). A major factor contributing to insect success is the development of a range of mechanisms to overcome the problems associated with temperature extremes.

1.1 Insect survival strategies at low temperatures

1.1.1 Classification of cold hardiness strategies

Insects survive at low temperatures either by an ability to tolerate the internal freezing of their body tissues and fluids or alternatively, to avoid such freezing by supercooling (Bale, 2002). Salt (1961) produced a classification scheme that categorized insects depending on their ability to survive the formation of extracellular ice, categorizing insects as either freeze tolerant or freeze avoiding (Salt, 1961). To determine the species' classification, the insect is cooled at a constant rate, typically $1^{\circ}\text{C min}^{-1}$ (Baust, 1986), to the temperature of crystallization or 'supercooling point' (SCP). The SCP is detected by the release of heat that occurs when water crystallizes to ice. The insects are then warmed and survival assessed, providing an indication of the cold hardiness of the insect and whether the insect is freeze tolerant or intolerant.

Freeze tolerant insects are those which can survive extracellular, and possibly intracellular, ice formation and in doing so are able to survive temperatures below the supercooling point. The majority of freeze tolerant insects occur in areas where extreme winter conditions are experienced, for example, the Arctic and sub-Arctic regions of America, Scandinavia, Russia and China (Bale, 1996). More recent work has suggested that freeze tolerance, in addition to being a strategy for surviving extreme Arctic conditions, is advantageous to surviving the milder conditions of the southern hemisphere where unpredictable cold spells can occur throughout the year (Sinclair *et al.*, 2003; Sinclair & Chown, 2005). Sinclair *et al.*, (2003) propose that freeze tolerance evolved in parallel in the two hemispheres, allowing survival during seasonal periods of extreme cold winters in the northern hemisphere and in the southern hemisphere, survival during unpredictable, intermittent cold spells. Freeze tolerance is achieved via the production of three main chemical compounds: ice nucleating agents (INAs), antifreeze proteins (AFPs) and polyhydroxy alcohols (polyols) and sugars, which are generally accumulated in the autumn and winter months.

Freeze intolerant or freeze avoiding insects as they are sometimes described, in contrast to freeze tolerant insects, are unable to survive extracellular ice formation and must actively avoid freezing via supercooling. The process of supercooling maintains the body tissues and fluids at a temperature below which freezing would occur and is achieved using polyols and AFPs. It is thought that most temperate insect species fall into this category (Bale, 1991).

The key difference, therefore, between freeze tolerant and freeze avoiding insects is the role of INAs. Within freeze tolerant insects INAs are synthesized and activated to allow for controlled extracellular freezing, whereas the converse is true in freeze avoiding insects where INAs are removed or masked to prevent freezing (Zachariassen, 1985). Unlike INAs, both freeze tolerant and freeze avoiding insects contain AFPs, polyols and sugars, although the role of these chemicals differs between the two strategies (Figure 1). In freeze tolerant species polyols, such as glucose, increase the insect's supercooling capacity and aid in the cryoprotection of tissue before INAs have become fully active and AFPs play a role in the prevention of recrystallization when body temperatures begin to rise at the end of winter (Bale, 1996). Polyols, in freeze avoiding insects, also enable insects to supercool to temperatures below which freezing would normally occur. The AFPs then act to stabilize the supercooled state (Zachariassen, 1985; Bale 1996).

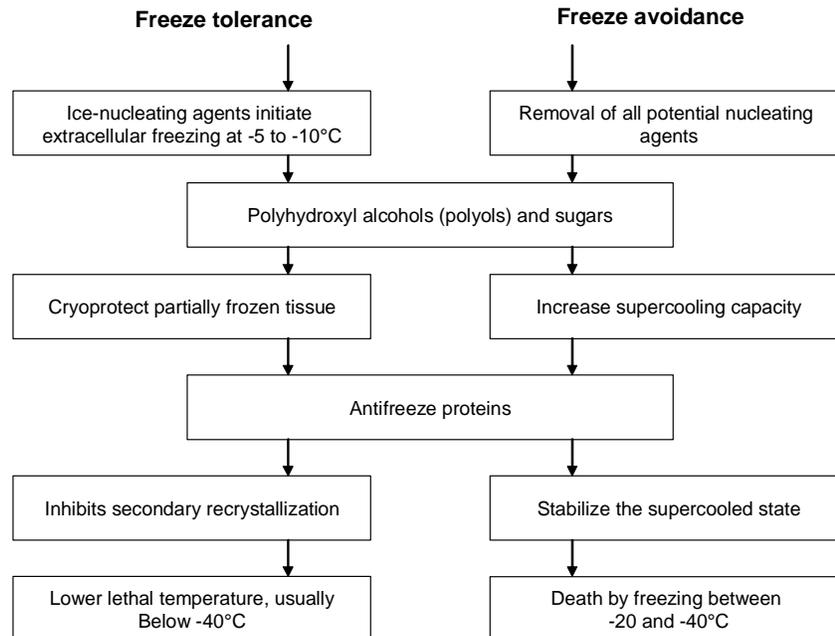


Figure 1. The biochemical mechanisms adopted by freeze tolerant and freeze avoiding insects (Bale 1996, 2002).

The classification scheme assumes that the only threat to insects at low temperature is freezing, although many insects in reality die at temperatures higher than their supercooling point, with death resulting from chill-related factors as opposed to actual freezing. The classification therefore incorrectly assumes that supercooling points are the lower limit of insect survival, when in fact they represent the lower limit to the insect's ability to supercool (Bale, 1996). The grain aphid, *Sitobion avenae*, is one such insect that experiences extensive pre-freeze mortality. This led Knight (1987) to the conclusion that supercooling points can be ecologically misleading because, in nature, *S. avenae* would die before freezing occurred. Similar pre-freeze mortality was evident in the peach-potato aphid *Myzus persicae* (Bale *et al.*, 1988). The cause of such pre-freeze mortality is unknown, although possible reasons include protein denaturation, membrane disruption, alterations to lipid fluidity or the decoupling of metabolic processes (Ring, 1980; Knight *et al.*, 1986). The classification of

insects as freeze intolerant was thus considered inappropriate due to failure of such insects to survive to temperatures as low as the supercooling point, leading to the production of a new classification scheme by Bale (1996).

The new classification scheme has retained the categories of freeze tolerance and freeze avoidance, although the criterion for freeze avoidance has been modified. Modification of the scheme has enabled incorporation of the knowledge that the effects of low temperature, i.e. chilling, and freezing are not synonymous, with death occurring as a consequence of short or prolonged exposure to low temperatures in the absence of freezing in many insects (Bale, 1991). Freeze tolerant insects are the most cold hardy species and withstand extracellular freezing initiated by INAs and include the larvae of the hoverfly *Syrphus ribesii*, which experiences 70% survival at -35°C (Hart & Bale, 1997, 1998). Under this new classification, insects are classified as freeze avoiding if low levels of mortality occur in the absence of freezing. An example of a freeze avoiding insect is the moth *Epirrita autumnata*, the eggs of which have a mean supercooling point of -35.5°C and do not die until freezing occurs (Virtanen *et al.*, 1998). For such freeze avoiding species, SCPs provide a reliable indicator of cold hardiness.

In addition to freeze tolerant and freeze avoiding, three new categories have been proposed: chill tolerant, chill susceptible and opportunistic survival. Chill tolerant insects are those that possess relatively low sub-zero supercooling points, around -20 to -30°C, although experience some mortality at temperatures above the supercooling point, for example, the mite *Alaskozetes antarcticus* and the beech weevil *Rhynchaenus fagi*. Overwintering adults of *R. fagi* possess the ability to supercool to low sub-zero temperatures, with a reported mean SCP of -25°C in mid-winter (Bale, 1991). However, 74% of the overwintering population were killed following 50 days at -15°C; a temperature 10°C above the mean SCP for the species (Bale, 1991). The SCP therefore becomes increasingly unreliable as an indicator of cold hardiness. Chill susceptible insects, including many aphid species such as *M. persicae* and *S. avenae*, also have the ability to supercool to low sub-zero temperatures, although experience relatively high levels of mortality at temperatures well above the supercooling point in very brief exposures of a few minutes or hours. For chill susceptible insects, mortality is unrelated to their SCP and as a consequence SCPs do not provide reliable

indicators regarding the cold tolerance of such species. The final category, opportunistic survival, includes all insects unable to survive below the threshold temperature required for normal metabolic activity, such as the housefly *Musca domestica*. In a study population of *M. domestica* pupae, 90% were killed within 4 days when held at 0° (Coulson & Bale, 1990). Such species actively seek out sheltered overwintering sites to avoid unfavourable conditions. Once again, the cold tolerance of the species is unrelated to their SCP.

More recently, Sinclair (1999) has suggested that the category of freeze tolerance, as with freeze intolerance, forms a separate continuum and can be subdivided according to insect SCPs and lower lethal temperatures (LLT). The resultant subcategories include partially freeze tolerant insects which can survive a small amount of body water freezing but will die when their body temperature reaches equilibrium with the environment, moderately freeze tolerant, whereby the insects die less than 10°C below their SCP, strongly freeze tolerant, whereby insects have LLTs 20°C or more below their SCP and finally, freeze tolerant insects that possess very low SCPs and can survive below these temperatures (Sinclair, 1999).

1.1.2 Survival mechanisms at low temperatures

Mechanisms to survive at unfavourable low temperatures can include both chemical (briefly mentioned previously) and behavioural responses. These mechanisms act either to increase the cold hardiness of the individual or to prevent exposure to the unfavourable conditions.

1.1.2.1 Chemical mechanisms

Chemical mechanisms act to increase the cold hardiness of the insect and include the use of INAs, polyols and sugars, and AFPs. INAs are proteins produced in the autumn by freeze tolerant insects in preparation for winter and were first described in the beetle *Eleodes blanchardi* by Zachariassen and Hammel (1976) and later purified in the hornet *Vespula maculata* (Duman & Patterson, 1978). Containing a high proportion of hydrophilic amino acids, mainly glutamate and glutamine, INAs are believed to function by their ability to

hydrogen bond with water molecules, thus creating organised embryo ice crystals (Duman, 2001). INAs act to initiate protective ice formation in extracellular areas at high sub-zero temperatures. In doing so, water is drawn out of cells via an osmotic gradient, concentrating cell contents and thus preventing potentially damaging intracellular freezing (Figure 2). In contrast to freeze tolerant insects, insects classified as freeze avoiding expel or mask INAs in autumn as opposed to producing them (Zachariassen, 1985).

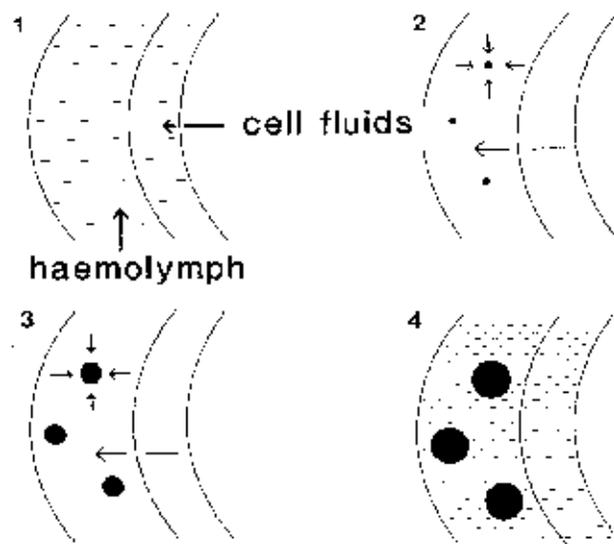


Figure 2. The activity of ice nucleating agents allowing for protective extracellular ice formation at sub-zero temperatures (Bale 1996). 1. Haemolymph and cell fluids are in osmotic equilibrium. 2. INAs initiate freezing in the haemolymph at sub-zero temperatures. 3. Growth in extracellular ice results in water being drawn from the cell into the haemolymph via an osmotic gradient. 4. Protective extracellular freezing within the haemolymph. Cellular fluids become concentrated preventing dangerous intracellular freezing and thus protecting the cell.

Polyols and sugars are found in both freeze tolerant and freeze avoiding species, with concentrations typically increasing in autumn months. Examples of polyols and sugars include glycerol, sorbitol, threitol, erythritol, fructose, sucrose and trehalose (Bale, 2002),

although glycerol is reported to be the most common and effective polyol due to its low molecular weight, allowing movement readily through cell membranes, its high solubility and low toxicity (Salt, 1957). In freeze tolerant insects the polyols act to protect partially frozen tissue and in freeze avoiding insects have an antifreeze function (Duman, 1982). Tissue protection in freeze tolerant species is brought about by the polyols preventing the potential causes of freeze damage, for example, mechanical damage, electrolyte imbalance and recrystallization (Bale, 2002). This is achieved via increasing the binding capacity of water, which in turn decreases the rate of ice formation, stabilizing protein structure, buffering electrolytes and decreasing transmembrane water flux (Baust, 1982; Bale, 2002). Glycerol further acts as a solvent, keeping potentially harmful salts in solution as they become more concentrated during ice formation (Miller & Smith, 1975).

Also found in both freeze tolerant and freeze avoiding insects are AFPs which lower the freezing point of fluids in relation to the melting point, creating a thermal hysteresis (Zachariassen & Husby, 1982). AFPs are adsorbed onto the surface of embryonic ice crystals, depressing the nucleation temperatures and thus preventing increased ice crystal growth (Duman & DeVries, 1972). Bale (2002) suggests that the AFPs offer protection to freeze tolerant insects in autumn months prior to INA production.

The process of vitrification, rather than freezing, can further aid insect survival at sub zero temperatures (Duman *et al.*, 1991) and provides an alternative strategy for managing internal water at sub zero temperatures (Hawes & Bale, 2007). Vitrification is the process by which body water is supercooled and forms a glass-like, amorphous structure without the occurrence of crystallization. In doing so, the haemolymph is prevented from becoming too concentrated and thus protects against enzyme denaturation and maintains cell hydration. Vitrification has been reported in larvae of the gall fly *Eurosta solidaginis* (Wasylyk *et al.*, 1988) and the Alaskan beetle *Cucujus clavipes* (Bennett *et al.*, 2005).

The restructuring of cell membranes aids insect cold acclimation and assists in the protection of membranes from perturbation. In ectotherms, cold acclimation commonly acts to increase membrane fluidity via modification of membrane lipids by increasing the proportion of unsaturated fatty acids (Los & Murata, 2004). This is achieved by increasing the proportion

of *cis* unsaturated and long chain fatty acids and increasing the phosphatidylethanolamine content relative to phosphatidylcholine (Los & Murata, 2004; Lee *et al.*, 2006). Changes to the levels of unsaturated fatty acids maintain cell membranes in a liquid phase, preventing transition to a gel phase, which is considered a major cause of cold injury in insects in the absence of freezing (Drobnis *et al.*, 1993; Clark & Worland, 2008). Other reported changes to membrane structure include increased proportions of glycerophosphoethanolamines in relation to glycerophosphocholines, decreased proportions of plasmalogens in relation to diacyl-glycerophospholipids, and changes to the cholesterol content, all believed to function in the protection of the cell membrane (Hazel, 1989; 1995).

Additionally, insects can enter diapause, a period of suspended growth and development, to endure unfavourable conditions. Facultative and obligatory diapause exists with facultative diapause being induced by changes in the environment and obligatory diapause being genetically programmed, occurring at specific times of the year, independent of environmental conditions (Bale & Hayward, 2010). In temperate and Polar Regions, day length provides the main cue for facultative diapause, with short day lengths providing a reliable cue for the onset of winter and is termed photoperiodism (Denlinger, 2002; Bale & Hayward, 2010). Declining temperatures, in combination with day length cues, also contribute to inducing diapause, although temperature alone does not induce diapause (Bale & Hayward, 2010).

Diapause enables growth and development to be coordinated with favourable environmental conditions and, in many insects, acts to increase cold tolerance during these unfavourable periods (Denlinger, 2002). Increased cold tolerance is attributed to the synthesis of cryoprotectants such as trehalose, galactose and glucose (Khani *et al.*, 2007; Zeng *et al.*, 2008), upregulation of heat proteins (Denlinger, 2002; Rinehart *et al.*, 2000; 2007) and changes to the lipid cell membrane (Michaud & Denlinger, 2006; Tomčala *et al.*, 2006). The larvae of the codling moth *Cydia pomonella*, for example, experience a threefold increase in trehalose when in full diapause, acting to increase supercooling capacity, survival at low temperature and chilling tolerance (Khani *et al.*, 2007a). Additionally, changes to the lipid cell membrane occur, with diapausing larvae of the moth having increased proportions of

unsaturated fatty acids, acting to increase membrane fluidity at low temperatures (Khani *et al.*, 2007b).

The chemical mechanisms discussed above are seasonal adaptations allowing survival through predictable cold spells of winter months. In addition to seasonal adaptations, short term protection is possible in many insects via rapid cold hardening (RCH). RCH was first described in the flesh fly *Sarcophaga crassipalpis* by Lee *et al.* (1987) who defined the process as a rapid protective mechanism against cold injury in response to changing environmental temperatures on an hourly to daily basis (Lee *et al.*, 1987). RCH has since been reported in many insect species including the fruit fly *Drosophila melanogaster* (Czajka & Lee, 1990), the housefly *M. domestica* (Coulson & Bale, 1991), the monarch butterfly *Danaus plexippus* (Larsen & Lee, 1994), western flower thrips *Frankliniella occidentalis* (McDonald *et al.*, 1997), the migratory locust *Locusta migratoria* (Wang & Kang, 2003), the grain aphid *S. avenae* (Powell & Bale, 2004) and a Karoo beetle *Afrinus* sp (Sinclair & Chown, 2006), the majority of which are chill susceptible (Wang *et al.*, 2003). RCH is further reported outside of the Class Insecta, although rather limited to the Arthropoda, in the Class Arachnida, for example, in the mites *Euseius finlandicus* (Broufas & Koveos, 2001), *Alaskozetes antarcticus* and *Halozetes belgicae* (Worland & Convey, 2001).

It is suggested that RCH allows chill susceptible insects to survive unpredictable and unfavourable changes in temperature and is especially important when seasonal adaptation is incomplete during spring and autumn months (Coulson & Bale, 1990). Such short term adaptation is considered to be of great importance to aphid species with short generation times (Coulson & Bale, 1990) where not all aphids experience winter conditions and enables a rapid response to sudden changes in temperature (Powell & Bale, 2008). Proposed mechanisms involved in RCH include changes to the composition of phospholipid bilayers, increases in haemolymph osmolalities and the production of polyols (Wang *et al.*, 2003).

1.1.2.2 Behavioural mechanisms

Behavioural responses can act to increase the cold hardiness of the insect or to prevent exposure to unfavourable conditions and include the expulsion of gut contents and migration.

The cessation of feeding and expulsion of gut contents increases cold hardiness via removal of potential ice nucleators in freeze intolerant species. Insects that adopt this method include most of those that rely on supercooling to aid winter survival such as the beech weevil *R. fagi* (Bale, 1980). In *R. fagi*, the cessation of feeding lowers the supercooling point by approximately 3°C (Bale, 1980). However, a previous study on the grain aphid, *S. avenae*, has revealed that starvation of the insect did not act to lower the supercooling point (Knight, 1987). Aphids feed on phloem sap which is known to be deficient in nucleators (Sømme & Zachariassen, 1981) and high in cryoprotectant carbohydrates (Danks, 1978). The composition of phloem sap thus enables aphids to continue feeding throughout unfavourable low temperatures without compromising the supercooling ability of the individual.

The selection of overwintering sites increases survival by preventing exposure to unfavourable conditions. Migration in search of overwintering sites can occur over varying scales, with the monarch butterfly *D. plexippus* displaying extensive annual migrations over thousands of kilometres from breeding sites in North America to overwintering sites in Mexico (Larsen & Lee, 1994). Conversely, migrations over very small scales are also evident with insects often migrating down into the soil or up host plants to higher air temperatures to avoid ground frosts. *M. persicae* is one such insect that migrates up plants, although this migration also has nutritional advantages as leaves lower down the plant senesce (Harrington & Taylor, 1990). However, it should be noted that aphids such as *M. persicae* may also engage in large scale migrations, although, over which, they have very little control and are commonly transported large distances on low level jet streams (Berry & Taylor, 1968; Zhu *et al.*, 2006), giving them the name ‘aerial plankton’ (Drake & Farrow, 1989).

1.2 Insect survival strategies at high temperatures

The survival of insects at high temperatures has received comparatively less research interest than at low temperatures. As a consequence, the available literature and knowledge base is somewhat poor, although research interest on the subject matter is increasing with the focus being primarily on drosophilid species.

Following exposure to a heat stress, death can occur as a result of protein denaturation, affecting enzyme and substrate binding, and alterations to the fluidity of phospholipid bilayers (Dahlgaard *et al.*, 1998; Neven, 2000). In addition, structural damage to DNA, RNA and carbohydrates may arise and critical pH and ion concentrations can become distorted (Neven, 2000).

Heat shock proteins, synthesized following exposure to a heat stress, play an important role in survival at unfavourably high temperatures. In addition to extreme high temperatures, heat shock proteins are induced by a variety of stresses which include low temperature extremes, depletion of cellular energy, and extreme concentrations of ions, osmolytes, gases and toxic substances (Feder & Hofmann, 1999). It should be noted that not all heat shock proteins are induced by stress (Feder & Hofmann, 1999).

Of the heat shock proteins known, proteins belonging to the Hsp70 family are best characterized. Hsp70 proteins are believed to act as molecular chaperones, aiding in the transportation of denatured or non-functional proteins to lysosomes for degradation, to prevent aggregations of denatured proteins by binding with hydrophobic domains (Feder & Hoffmann, 1999; Bahrndorff *et al.*, 2009b) or to assist the re-folding of proteins on removal of the heat stress (Sørensen *et al.*, 2003; see Neven 2000 for review). In the flesh fly *S. crassipalpis* exposure to a heat stress led to increased thermal tolerance at a previously lethal exposure regime. The newly acquired heat tolerance was found to decline with time, although persisted long after the heat shock proteins had degraded, suggesting that additional mechanisms could be involved in the acquisition of heat tolerance (Yocum & Delinger, 1992).

A trade off exists between the benefits conferred by the upregulation of Hsps enabling survival at unfavourably high temperatures and the associated costs. These costs include detrimental affects on rates of growth, development and reproduction, and provide an explanation as to why cells remove Hsp70 in the absence of stress (Feder *et al.*, 1992; Sørensen *et al.*, 2003). Such costs associated with the stress response are suggested to be a consequence of normal cell functions shutting down during the response, toxicity of Hsps70

in high concentrations, or due to requiring large amounts of energy (Feder & Hoffmann, 1999).

1.3 Indices of insect thermal tolerance

A number of indices are commonly adopted in the laboratory to measure insect thermal tolerance. Such indices enable comparisons between species or populations of the same species and allow the effects of acclimation and experimental treatments on thermal tolerance to be determined. The focal indices discussed below include the supercooling point, lethal temperatures and times, critical temperatures and the discriminating temperature which is used in rapid cold hardening studies.

1.3.1 Supercooling point

The supercooling point or crystallization temperature is the lowest temperature before spontaneous freezing of body water occurs. It is determined in the laboratory by cooling the insect at a constant rate until freezing occurs. With careful monitoring of the insect's body temperature, the supercooling point can be readily identified as the exotherm caused by the latent heat of crystallization. Determination of insect supercooling points has previously been used in the classification of insect cold hardiness into the categories of freeze tolerance and freeze avoiding depending on whether the insect can survive following exposure at the supercooling point.

1.3.2 Lethal temperatures and times

The lethal temperature (LT) is the temperature at which a specific percentage of an insect sample population is killed. The LT_{50} , for example, is the temperature at which an experimental population experiences 50% mortality. Insects are cooled or heated at a set rate to a predetermined temperature, held at the temperature for a desired length of time, and then

returned to the start temperature, usually at the same rate. Insect survival is assessed following a recovery period. The LT_{50} is estimated by exposing samples to a number of decreasing or increasing temperatures depending on whether cold or heat tolerance is being assessed. From the acquired data set, a graph of insect survival against exposure temperature can be produced which follows a typically sigmoidal dosage-mortality curve. Probit analysis is commonly used to linearise the sigmoidal curve and enables an accurate estimate of the LT_{50} value. Following a similar principle, lethal times are determined by exposing an insect sample to a pre-determined stressful temperature (e.g. 0°C , -5°C , -10°C) for varying lengths of times before re-warming. Insect survival is then assessed and Probit analysis performed.

1.3.3 Critical temperatures

The ability to move at increasingly higher or lower temperatures also provides an indication of the thermal tolerance of an insect. Critical temperatures are estimated by cooling or heating a sample population at a set rate and recording the temperature at which movement of an individual insect ceases or coma is induced, depending on the critical temperature being studied. The temperature at which insects lose the ability to walk is known as the CT_{\min} or CT_{\max} , referring to the movement threshold at low and high temperature respectively. Additional cooling or heating will eventually result in the insect entering a state of coma, referred to respectively as the chill or heat coma, and is defined as the last twitch of an appendage. In the literature critical temperatures can also refer to the temperature at which an individual insect can no longer right itself after being artificially knocked over. Due to the numerous characterizations of critical temperature values, it is vital that, when using such indices, the adopted definition and experimental procedure be stated clearly to avoid ambiguity. The measurement of critical temperatures perhaps provides more ecologically relevant information on thermal tolerance since survival is of little importance if the individual is unable to move to find food, a mate or escape predation.

1.3.4. Discriminating temperatures and rapid cold hardening

When assessing an insect's ability to rapidly cold harden, the discriminating temperature is an essential measurement that enables the RCH response to be identified and quantified. The discriminating temperature is the temperature which results in 80-90% mortality in a population sample when transferred directly from the rearing temperature to a particular sub-zero temperature. The discriminating temperature is therefore a reference point to determine changes in mortality following treatment to induce RCH.

1.4 Aphids and the study species *Myzus persicae*

1.4.1 Aphid lifecycles

The majority of aphid species so far studied are considered chill susceptible species due to experiencing high mortality at temperatures above the supercooling point. Aphids can be classified as monoecious or heteroecious, depending on the occurrence of host-alternation during the lifecycle. Monoecious species, such as *S. avenae*, remain on the same herbaceous host species throughout the year, although this life strategy is only possible if the herbaceous host is present throughout the year. Heteroecious species, such as *M. persicae*, alternate between a summer herbaceous host and a winter woody host plant. Aphids that possess the host-alternating lifecycle have a potential nutritional advantage over species with a monoecious lifecycle, although they are required to return to the primary woody host to lay eggs (Shaposhnikov, 1987). Conversely, it could be argued that the monoecious lifecycle is advantageous because the mortality associated with a migrating stage is avoided. It should, however, be noted that some species of monoecious aphids such as *S. avenae* do migrate.

Many aphid species, although not all, can overwinter as one of two lifecycle types that differ in cold tolerance. The first overwintering strategy used by most aphid species is as a holocyclic (sexual) egg, produced by the mating of an ovipara and a male. The second strategy involves overwintering as an anholocyclic or asexual aphid which arises due to a

stable genetic mutation affecting the photoperiodic switch leading to a failure to produce sexual forms in autumn (Moran, 1992; Dixon, 1998). The anholocyclic aphids do not seek out hibernation sites to overwinter, but instead remain active on the underside of leaves as they do for the remainder of the year (Harrington & Taylor, 1990). The holocyclic egg is more cold tolerant than the overwintering anholocyclic aphid (Strathdee *et al.*, 1995), with aphid eggs supercooling to sub-zero temperatures as low as -35°C (Hutchinson & Bale, 1994). Overwintering as an egg therefore increases the chance of surviving through the winter under unfavourable conditions. Although less cold hardy, the anholocyclic aphid can continue to feed and reproduce throughout the winter, albeit at a lower rate than in summer. The two lifecycles represent a trade off between increased probability of winter survival (the holocyclic lifecycle) and increased population size through continuous reproduction and thus greater spring dispersal (the anholocyclic lifecycle).

The proportion of holocyclic and anholocyclic clones in a population is governed by the severity of winter conditions. Consequently, locations experiencing milder winters would be predicted to have increased proportions of anholocyclic clones compared to locations experiencing severe winters (Broadbent & Heathcote, 1955). At more northerly latitudes where low temperature extremes would be experienced more frequently, the proportion of holocyclic clones is proposed to increase (Walters & Dewar, 1986). Understanding the complex interactions between winter severity and overwintering strategy can assist in the prediction of spring population abundance and aid the production of reliable forecasting systems for aphid outbreaks (Walters & Dewar, 1986; Harrington *et al.*, 1990; Cocu *et al.*, 2005).

1.4.2 The study species *Myzus persicae*

Myzus persicae (Sulzer) (Hemiptera: Aphididae) is a heteroecious aphid host alternating between the primary peach *Prunus persica* host in winter and various herbaceous hosts, belonging to approximately 40 different families, which include brassicas, potatoes and sugar beet, in summer (Blackman & Eastop, 2000). Due to the primary peach host originating from Asia, it is believed that *M. persicae* also originates from the region

(Blackman & Eastop, 2000). The aphid can overwinter as both holocyclic eggs and anholocyclic aphids (Figure 3).

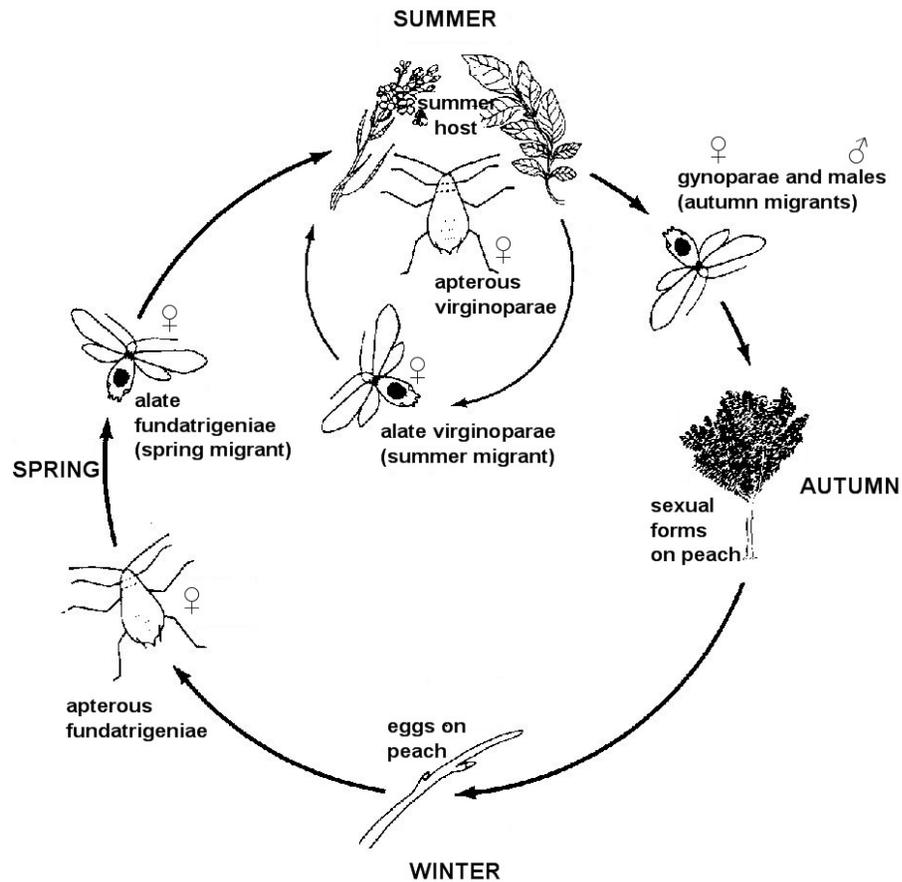


Figure 3. The lifecycle of the peach-potato aphid *Myzus persicae*.

In contrast to the majority of insect species, functional roles, such as sexual reproduction, migration etc., are not allocated to specific stages in the lifecycle such as in Lepidoptera where dispersal is largely restricted to the adult life stage. Due to the short generation times, an aphid, for example in autumn, will not survive through to the following spring and as a

consequence specific roles are instead partitioned between generations. Throughout the year a sequence of morphs are produced. Males and gynoparae migrate in winter to the primary peach host. Once on the winter host, the gynoparae produce oviparae via parthenogenesis. The oviparae are sexual, egg laying female forms which mate with the males to produce overwintering eggs. When conditions become favourable in spring, the eggs hatch into apterous (wingless) fundatrices. The fundatrices reproduce parthenogenically for several generations throughout spring, producing apterous fundatrigeniae. Eventually, alate (winged) fundatrigeniae are produced which migrate to the summer, herbaceous host. Throughout the summer, aphids reproduce via parthenogenesis, producing both alate and apterous forms, with the apterous virginoparae producing both males and gynoparae in the autumn in response to decreasing photoperiod in preparation for migration to the winter host. A separate annual cycle co-occurs in which apterous virginoparae fail to produce sexual forms and continue reproducing parthenogenically throughout the year, overwintering as anholocyclic individuals with intermittent alate generations. In some aphid species, including *M. persicae*, a third and fourth lifecycle exists known as the androcyclic and intermediate lifecycle. With the androcyclic lifecycle, anholocyclic clones produce males in addition to virginoparae. Males are also produced in addition to virginoparae in the intermediate lifecycle, although alates have the ability to produce both apterous offspring and sexual female morphs under winter conditions; this is an intermediate of the anholocyclic lifecycle in which only apterous offspring are produced and the holocyclic lifecycle in which sexual female morphs are produced. The third and fourth lifecycle types will not be discussed hereafter.

Some aphid species, including *M. persicae*, are distributed widely across the globe. In Europe, *M. persicae* can be found from northern Scandinavia to southern Spain. With knowledge of the lifecycle and physiology of *M. persicae*, predictions can be made on interpopulational variation along the latitudinal cline. For example, regional adaptations in thermal tolerance and changes to the ratio of anholocyclic to holocyclic clones could be expected. Such knowledge would further allow the level of gene flow between populations to be deduced. Significant variation in thermal tolerance and lifecycle predominance between populations would suggest a lack of gene flow, and would provide the basis for future speciation.

1.5 Geographic variation in thermal tolerance

Geographic variation in thermal tolerance has recently been the focus of research in a number of species from the Phylum Arthropoda, including drosophilid species (Davidson, 1990; Goto & Kimura, 1998; Gibert & Huey, 2001; Gibert *et al.*, 2001ab; Bublily *et al.*, 2002; Ayrinhac *et al.*, 2004), the common woodlouse *Porcellio laevis* (Castañeda *et al.*, 2004, 2005), the pea leafminer *Liriomyza huidobrensis* (Chen & Kang, 2004), the springtail *Orchesella cincta* (Bahrndorff *et al.*, 2006, 2009a) and European diving beetles (Calosi *et al.*, 2010).

Studies concerning variation in the thermal tolerance of drosophilid species have primarily focused on the differences in lower thermal limits between tropical and temperate populations, both within and between species. In *D. melanogaster*, temperate populations were more resistant to cold and desiccation stress than tropical populations (Davidson, 1990). Similarly, in a comparison of 26 temperate drosophilid species and 48 tropical species, temperate species displayed shorter recovery times following a cold induced coma (Gibert *et al.*, 2001b). A limited number of comparative studies have been conducted comparing populations along latitudinal and altitudinal gradients. In *Drosophila subobscura*, the temperature inducing chill coma declined with increasing latitude, indicating that populations from high latitudes were increasingly cold tolerant (Gibert & Huey, 2001). In *D. melanogaster*, both mortality and recovery time following a cold shock decreased at temperate latitudes and mortality decreased and knockdown time increased following a heat shock at tropical latitudes (Hoffmann *et al.*, 2002; Ayrinhac *et al.*, 2004). Also within the Order Diptera, cold tolerance of the pea leafminer *L. huidobrensis*, this time measured by lower lethal temperature, was again found to increase with latitude (Chen & Kang, 2004).

Literature regarding geographical variation in thermal tolerance for species outside the Order Diptera is further limited, although research is less biased towards lower thermal limits. Castañeda *et al.* (2004) studied the crustacean *P. laevis* along a 10° latitudinal gradient through Chile. *P. laevis* from low latitudes were found to display lower cold tolerance and higher optimum temperatures and were consequently more adapted towards high temperatures. *P. laevis* from high latitudes had greater cold tolerance and lower optimum

temperatures. Likewise, recovery time from chill coma revealed an inverse relationship with latitude (Castañeda *et al.* 2005), suggesting that populations along a latitudinal gradient display local adaptations in thermal physiology.

More recently, Bahrndorff *et al.* (2006, 2009a) studied populations of the springtail *O. cincta* collected along a 2000 km latitudinal gradient throughout Europe from Denmark to Southern Italy. The studies revealed that individuals from high latitudes exhibited the greatest cold shock resistance, but lowest resistance to heat shock. The reverse was true of *O. cincta* from low latitudes, once again suggesting adaptation to local climate. Similar relationships are, in addition to along latitudinal gradients, also evident in altitudinal gradients. Cold tolerance increased with increasing altitude, as indicated by declining values of CT_{min} , in species of scarab dung beetle along an altitudinal gradient in South Africa; dung beetles from high altitude populations could remain active to lower temperatures and avoid entering a state of cold induced torpor for longer (Gaston & Chown, 1999). CT_{max} was revealed to vary less with altitude than CT_{min} , resulting in increased thermal tolerance at high altitude (Gaston & Chown, 1999).

In the majority of insect species studied, upper thermal limits show much less variation with regard to genetic variation and phenotypic plasticity than lower lethal limits, indicating that, in insects, thermal limits are ‘decoupled’ (Addo-Bediako *et al.*, 2000). Put simply, the relationship between upper and lower thermal limits appears not to be a fixed one, with one limit not necessarily moving in accordance with the other. Given that upper lethal limits are generally less variable than lower lethal limits, insects experiencing lower temperatures (i.e. at high latitude or altitude) are expected to display greater differentials between upper and lower limits due to lower lethal limits being lowered to a greater extent in relation to the upper lethal limit. This decoupling of thermal limits has been attributed to the increased climatic variability associated with increasing latitude, resulting in a need for greater thermal tolerance (Addo-Bediako *et al.*, 2000).

1.6 Applications of insect thermal biology

The limited ability of insects to maintain body temperature above or below the environmental temperature results in insects being greatly influenced by the environment (Walther *et al.*, 2002). An understanding of insect thermal biology can thus provide valuable information for predictive models for the forecasting of pest outbreaks, the establishment of invasive species, shifts in range patterns, and the potential implications of global climate change.

Over the past century the Earth's climate has warmed by approximately 0.6°C (Easterling *et al.*, 2000; Walther *et al.*, 2002). However, this trend in rising temperature has not been a steady process and since the mid 1970s parts of the world have experienced a rapid increase to a rate of warming of 0.2°C per decade (Karl *et al.*, 2000). Using mid range climate warming scenarios, it is predicted that 15-37% of species and taxa could become extinct by 2050 (Thomas *et al.*, 2004). In addition to warming, climate change can encompass changes to climatic variability, atmospheric composition, land cover and land use and can occur over varying temporal and spatial scales (Schermer *et al.*, 2000). The impacts of global change are likely to have profound effects on distribution patterns and the pest and invasive status of insect species. It is therefore understandable that knowledge of the variability in insect thermal tolerance and acclimation ability would provide valuable information for such areas.

1.6.1 Forecasting pest outbreaks

The development of a forecasting system for predicting pest outbreaks is of particular importance for aphid species. Aphids feed on the phloem sap of host plants using modified mouthparts known as stylets which penetrate the plant phloem, allowing passive feeding on phloem sap due to the positive pressure within the phloem. By feeding on host plants, aphids can result in the stunting of plant growth, the lowering of crop yields and aid the transmission of viral diseases (Block *et al.*, 1992).

Aphids are vectors in the transmission of approximately 275 plant virus species from 19 genera, equating to more than 50% of all insect transmitted plant viruses, many of which are commercially important (Nault, 1997). The short lifecycles of aphids, in addition to high rates of population increase and dispersal, contribute to the success of aphids as one of the main groups of plant disease vectors (Feres & Moreno, 2009). Aphid-transmitted viruses can be classified as either stylet-borne or circulative. Stylet-borne viruses, commonly referred to as non-persistent viruses, are short-lived within the aphid, with the vector often remaining infectious for less than 24 hours and include sugarcane mosaic virus and maize dwarf mosaic strain (Slykhuis, 1976). Circulative viruses, referred to as persistent viruses, are those which are ingested into the aphid gut and pass to the saliva via the haemolymph, allowing infection of the plant during subsequent aphid feeding. Once infectious, an aphid can remain so for a long time and such viruses are retained through moulting to later instars and adults. Circulative viruses include barley yellow dwarf virus, wheat yellow dwarf virus, maize leaf fleck virus and beet mild yellowing virus (Russell, 1962; Slykhuis, 1976).

Perhaps the most common and widespread of crop viral diseases is barley yellow dwarf (BYD), caused by barley yellow dwarf viruses (BYDV) belonging to the Luteoviridae family (Edwards, 2001) and transmitted by over 20 species of aphid (Irwin, 1990). Symptoms of infected plants include yellowing of the leaves and stunted growth. In addition to yield loss, BYDV can further be economically damaging through reduction in crop quality. Since the 1970s the disease has become more widespread throughout Britain as a consequence of increased abundance of a major vector, *S. avenae*. However, although widespread, large scale yield losses in Britain are infrequent (Knight *et al.*, 1996). For the subject species *M. persicae*, it is the transmission of the potato viruses that are the most damaging. The potato leaf roll virus (PLRV), also belonging to the Luteoviridae family, is a persistent, circulative virus and has contributed to declining potato productivity over the last century (Radcliffe & Ragsdale, 2002; Chatzivassiliou *et al.*, 2008). Perhaps more damaging is the potato virus Y (PVY) belonging to the Potyviridae family. This non-persistent, stylet-borne virus is transmitted by mainly winged aphids (Saucke & Döring, 2004) and it is reported to cause yield losses of up to 80% (Takacs, 2000).

In addition to reducing crop yields and transmitting viral diseases, many pest insects cause extensive damage via defoliation resulting in a direct loss of biomass, but further indirect loss by leaving the host plant susceptible to fungal infection. The majority of insect defoliators belong to the Order Lepidoptera, with examples including the Autumnal moth *E. autumnata*, a major defoliator of mountain birch in Scandinavia (Virtanen *et al.*, 1998) and the gypsy moth *Lymantria dispar*, the main defoliator of broadleaved trees in Eastern North America (Kegg, 1971; Kenis *et al.*, 2009). Other examples of major insect defoliators outside the Lepidoptera include the sawflies *Neodiprion sertifer* (Eklundh *et al.*, 2009), *Perga affinis* (Jordan *et al.*, 2002), *Diprion pini* and *Pristiphora abietina* (Dajoz, 2000) to name a few, gall midges of the family Cecidomyiidae (Dajoz, 2000) and a variety of weevils, leaf beetles, and chafers of the Coleoptera (Dajoz, 2000).

The study species *M. persicae* gains its pest status due to the efficacy with which it spreads viral diseases in crop plants. It alone is a vector in the transmission of approximately 100 plant diseases and is consequently a pest of major economic importance (Kennedy *et al.*, 1962). The development of effective forecasting systems would decrease the need for prophylactic insecticide spraying, which can result in the death of non-target organisms, contribute to the pollution of freshwater and can lead to increased insecticide resistance amongst target organisms (Block *et al.*, 1992).

The lifecycle type prevalent in an aphid population will further have implications for the severity of potential pest outbreaks. As previously discussed, the overwintering anholocyclic clones of pest aphids such as *M. persicae* and *S. avenae* are less cold hardy than overwintering holocyclic eggs (Strathdee *et al.*, 1995) and consequently, their proportions are dependent upon winter (Walters & Dewar, 1986). On arrival of spring conditions, anholocyclic clones respond more quickly to higher temperatures, resulting in increased numbers, an earlier production of the migratory alates and consequent earlier spring migration to the summer host. For *M. persicae*, almost 80% of the variation in the timing of first capture in suction traps, a sampling method by which air is continually sampled at specific heights above ground, can be explained by mean winter temperatures (Harrington *et al.*, 1990). Holocyclic clones, originating from the overwintering egg, require several generations before production of migratory alates can commence. In contrast, production of

migratory alates from anholocyclic clones can begin as soon as conditions become favourable. A trade-off therefore exists between the two clonal types regarding winter survival and spring migration.

During mild winters, greater survival of anholocyclic clones leads to earlier migration of potentially infectious aphids to the summer host (Figure 4). Transmission of viruses, for example BYDV, is most likely to occur via the progeny of anholocyclic clones as opposed to aphids originating from virus-free holocyclic eggs. BYDV causes the greatest damage when infection of the barley plant occurs at a young age due to mature plants being less susceptible to viral infections (McKirdy and Jones, 1996; McKirdy *et al.*, 2002). This is particularly problematic when milder winters lead to a greater prevalence of anholocyclic aphids which are, not only more prone to carrying the virus, but also more likely to infect the younger barley plants through earlier spring migrations. Likewise, large numbers of winged aphids are required to successfully colonise sugar beet and as a consequence, mild winter conditions will have implications for *M. persicae* populations and the spread of beet yellow viruses (BYV) (Williams *et al.*, 2000).

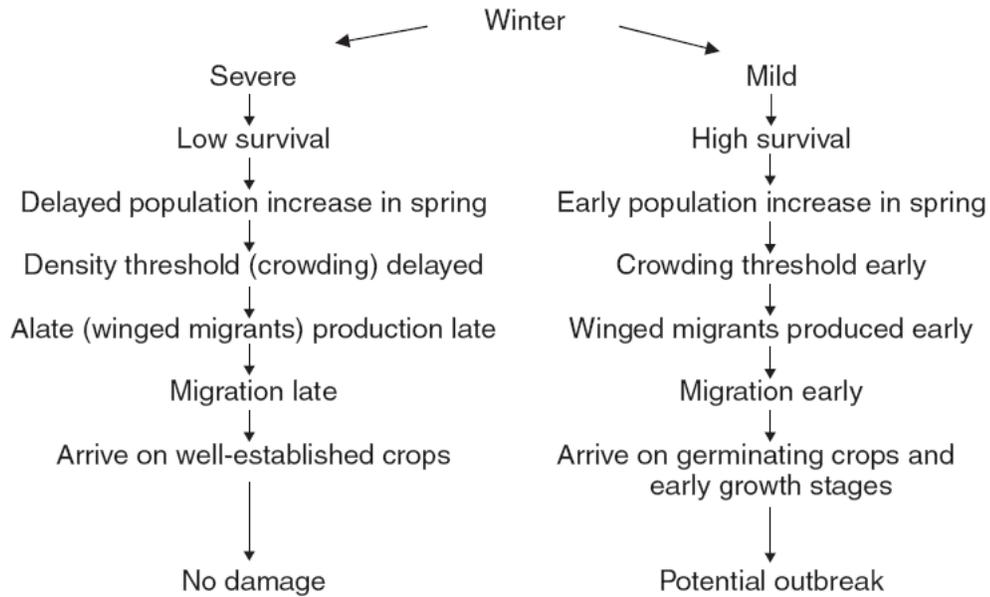


Figure 4. The effects of mild and severe winters on spring and summer populations and related damage potential of aphids overwintering as anholocyclic clones (Bale & Hayward, 2010).

Changes in temperature will further impact the number of generations per year, known as voltinism, with voltinism being positively correlated with temperature (Jonsson *et al.*, 2009; Sand & Brittain, 2009). Additional generations would lead to greater population growth and accelerate potential pest outbreaks (Altermatt, 2009). It is therefore understandable that knowledge of aphid thermal biology is vital to the understanding of how aphids may react to potential climate change with implications for the prediction of pest outbreaks.

1.6.2 Species distribution

In the face of global change, species are expected to track changing climate, either through adapting to new conditions or by shifting distribution (Walther *et al.*, 2002), since failure to

do so could result in population extinction. Bio-indicator species, such as Lepidoptera, provide information on alterations to species distribution. In a study of 35 non-migratory butterfly species, 63% were found to have shifted their range northwards by between 35-240km over the previous century, which is considered a direct impact of regional warming (Parmesan *et al.*, 1999). Additionally, the southern green stinkbug *Nezara viridula* has extended its range 70km further north in Japan since the 1960s, displacing the closely related *Nezara antennata* (Musolin, 2007). Similar range shifts have been reported for bird species, for example, with the northern margins of British breeding birds having extended northwards by an average of 18.9km over a 20 year period (Thomas & Lennon, 1999).

Climate tracking is not always achievable and species re-distribution can be prevented when geographical formations, habitat loss and fragmentation impede movement or when movement is limited by additional factors, for example, light limitation preventing the re-distribution of reef building corals (Hoegh-Guldberg, 1999). Failure to reach climatically favourable habitats is apparent in the forests of Monteverde, Costa Rica, where 20 of 50 species of toads and frogs have become locally extinct and one species, the golden toad *Bufo periglenes*, has become globally extinct, as a consequence of cloud bank migration to higher altitudes (Pounds *et al.*, 1999). In addition to species distribution, climate change is expected to impact the phenology and physiology of organisms, community interactions, and ecosystem dynamics (Walther *et al.*, 2002). Information on variation in thermal tolerance of *M. persicae* along a latitudinal gradient and the potential differences in acclimation ability will provide an indication of how the distribution of the species could change in a changing global climate, with implications for pest management.

1.6.3 Alien species

Species are moved beyond their natural ranges, both deliberately and accidentally, by human interference and this too is true of aphids. For example, the spotted alfalfa aphid *Therioaphis trifolii* was introduced into the USA in the 1950s, the Russian wheat aphid *Diuraphis noxia* into South Africa in the 1970s (Blackman & Eastop, 2000), and the rose-grain aphid *Metopolophium dirhodum* into New Zealand as recently as the 1980s (Nicol *et al.*, 1997).

Fortunately, most invasions fail with successful establishment of alien species being prevented when new habitats are not climatically matched to the native range. The ‘tens rule’ provides a general rule of thumb to the success of invasions, with invasions progressing through a series of stages: import to the country, escape into the wild, establishment of a population and finally development into a pest species. The rule theorizes that only 10% of invasive species successfully progress to the next stage (Williamson & Brown, 1986; Williamson, 1996, 2006). Nonetheless, it is estimated that naturalized alien species constitute approximately 2-33% of the flora of continental areas and 80% of islands (Vitousek *et al.*, 1997).

Alien species can have detrimental ecological impacts on native species via direct predation, competition, spreading diseases, hybridization and altering the habitat. Economic impacts can further be imposed. Introduction of the zebra mussel *Dreissena polymorpha* into North America resulted in costs of up to \$2 billion annually for the removal of the species from fouled water pipes and inlets (Lodge *et al.*, 1993; MacIsaac, 1996). Insects, in particular, can cause great economic losses due to many species being pests of important agricultural crops. The introduction of the Mexican rice borer *Eoreuma loftini* into Louisiana, Southern America, is predicted to cost the rice industry \$45 million in lost revenue once all of Louisiana is infested (Reay-Jones *et al.*, 2008). Global change and shifting temperatures could alter current patterns of climatic matching, potentially facilitating the successful establishment of alien species. This highlights once again the importance of understanding the thermal biology of such a prolific and major agricultural pest as *M. persicae*, should global climate change result in expansion outside of its current range.

1.7 Study hypotheses and experimental considerations

Previous research on aphid thermal biology has focused on the variation of cold hardiness between different life stages and the effects of rapid cold hardening. To date, no study has focused on variation in thermal biology within a single aphid species along a latitudinal gradient. Such knowledge is important in the forecasting of how the distribution of pest species such as *M. persicae* will be impacted by global climate change and will further

provide information regarding the level of gene flow between populations and the potential for future speciation.

It can be hypothesised that populations of *M. persicae* will display regional adaptations. At the northern end of the range, it would be predicted that populations would have greater cold tolerance (indicated by lower lethal and coma temperatures) and lower developmental thresholds. At high latitude, populations would further be expected to display a greater ability to acclimate at lower temperatures, although a lesser ability to acclimate to higher temperatures, than populations at the range centre and southerly range margin. The reverse would be predicted for populations from lower latitudes. It would therefore be expected that a gradient of increasing cold tolerance but decreasing heat tolerance would become apparent as *M. persicae* populations progress from Mediterranean, to temperate and then sub-polar climates. It could also be predicted that a shift from anholocycly to holocycly would occur from southerly latitudes to more northerly latitudes due to extreme low temperatures selecting against the anholocyclic lifecycle in sub-polar regions and favourable temperatures preventing natural climatic control of anholocyclic clones in the Mediterranean.

On careful consideration of the predictions stated above, the reality is perhaps less straightforward. Mediterranean countries, such as Spain, would be expected to have an abundance of anholocyclic clones due to a lack of winter stress. However, Spain also has a plentiful supply of the primary host, the peach tree, with Spain being the fourth largest producer of peaches in the world after China, Italy and the USA (Llacer *et al.*, 2009). Availability of the primary host will favour existence of the holocyclic lifecycle, and thus, anholocyclic and holocyclic lifecycles could both be common in Spain. As a result, extensive migrations are not required and regionally adapted populations displaying greater heat tolerance could be expected. At the northerly range margin of *M. persicae* in Scandinavia, holocycly would be favoured due to extreme winter temperatures which would select against the anholocyclic lifecycle. However, peach trees are required to support the holocyclic lifecycle, and, these are uncommon in Scandinavia. If holocyclic aphids cannot persist due to a lack of the primary host and likewise anholocyclic aphids due to extreme sub-zero winter temperatures, it is likely that *M. persicae* populations cannot reside all year round in Scandinavia, unless in protected areas such as glasshouses, and an annual influx of aphids

must therefore occur in order to build up the populations that are found outdoors in summer. If annual immigrations into Scandinavia occur from other parts of Europe, aphids collected in Scandinavia would not be expected to display greater levels of cold tolerance or reduced heat tolerance.

The current study is designed to determine inter-clonal variation in the thermal biology of *M. persicae* in relation to geographical variation. However, aphid clones display variation in biological characteristics that, although are not the target of the investigation, have the potential to impact on thermal tolerance. As a consequence, aphid characteristics including lifecycle type, clonal type, insecticide resistance status, colour morphology and the presence of symbionts, need to be considered prior to the investigation to ensure that results observed are a consequence of geographical variation associated with the sites of collection.

1.7.1 Aphid lifecycle

M. persicae exists as two distinct lifecycle types, anholocycly and holocycly, with a further two intermediate lifecycle types, androcycly and intermediate. It is well recognized that anholocyclic clones are less cold tolerant than overwintering holocyclic eggs, at least in their respective overwintering stages (Hutchinson & Bale, 1994). The lifecycle of all clones used in the current study must be determined because failure to determine the lifecycle of study clones will prevent any interpopulational variation in temperature tolerance being attributed to geographical distribution, since disparity in lifecycle type could also account, at least in part, for observed differences.

1.7.2 Aphid clonal type

The ability of *M. persicae* to reproduce clonally, both within the anholocyclic and holocyclic lifecycles, results in the production of genetically distinct aphid clones within a population (Fenton *et al.*, 1998; Fenton *et al.*, 2005; Kasprowicz *et al.*, 2008). All aphid clones collected for study will be subjected to microsatellite analysis to determine variation at the chosen loci

and to assign a 'type'. Aphids with identical microsatellite patterns at the loci examined are classified as the same type and, although this does not indicate that the clones are genetically identical, it does determine that clones originated from the same stem mother through asexual propagation (Kasprowicz *et al.*, 2008). Such information will further allow investigation into whether aphids of the same type are more alike in relation to thermal tolerance than clones of different types.

1.7.3 Insecticide resistance

Aphids are a major pest worldwide owing to the ease in which they transmit plant viral diseases and due to resultant structural damage caused to the plant when feeding. The control of aphid pests using insecticides has led to an increase in insecticide resistant aphid clones.

Recent studies on aphid insecticide resistance suggest a trade off between resistance and thermal tolerance. In *M. persicae*, winter survival is inversely related to insecticide resistance, leading to the conclusion that selection for insecticide resistance is counteracted by the selection pressures of British winters (Foster *et al.*, 1996). In addition, the mobility of *M. persicae* at low temperatures is inversely related to levels of insecticide resistance (Foster *et al.*, 1997). All study clones will therefore be resistance typed prior to use in experiments.

1.7.4 Colour morphology

Many aphid species exist in distinct and stable colour morphs. Examples include the pea aphid *Acyrtosiphon pisum* which has green and red forms (Dixon, 1998), the walnut aphid *Chromaphis juglandicola* with yellow and white forms (Hougardy & Mills, 2008) and the study species *M. persicae* which also has green and red forms (Blackman, 1987). The colour of the study clones will be noted in case colour morphology influences thermal tolerance.

1.7.5 Bacterial symbionts

All phloem feeding Homoptera possess symbiotic microorganisms (Douglas, 1998). In aphids, the primary symbiont is the bacterium *Buchnera aphidicola*. Transmitted maternally via a process known as transovarial transmission, the bacteria are housed in specialised cells known as bacteriocytes or mycetocytes and provide the aphids with nutrients and essential amino acids lacking from the phloem sap diet (Douglas, 1998). This symbiotic relationship is essential to aphid development, without which growth and reproductive rates are low (Moran & Dunbar, 2006). *Buchnera* are sensitive to high temperatures, with relatively short exposures of a few hours at high temperatures (37°C) being sufficient enough to kill *Buchnera* and have detrimental affects on the aphids (Ohtaka & Ishikawa, 1991).

In addition to the *Buchnera* primary symbiont, aphids are host to a variety of secondary symbionts. Such secondary symbionts have been found to confer resistance against parasitic wasps (Oliver *et al.*, 2005) and fungal infections (Scarborough *et al.*, 2005), expand the number of host plants available for utilization (Tsuchida *et al.*, 2004), and even impact life history traits such as dispersal and mating habits which would reduce gene flow and ultimately aid speciation (Leonardo & Mondor, 2006). Additionally, specific symbionts have been demonstrated to increase heat tolerance (Chen *et al.*, 2000; Montllor *et al.*, 2002; Russell & Moran, 2006).

1.8 Aims, objectives and hypotheses tested

The main aim of the study is to investigate aspects of the thermal biology of the aphid *Myzus persicae* to ascertain how tolerance to extreme temperatures varies between clones collected from different locations along the aphid's latitudinal distribution in Europe.

Specific objectives include:

- To establish in culture nine clones of *M. persicae*, three collected from each of three regions of the aphid's European distribution: Southern Spain, Britain and Scandinavia (Sweden).
- To estimate the lower and upper lethal temperatures (LT₅₀ min and LT₅₀ max) of these clones.
- To determine the movement thresholds and chill and heat coma temperature of the clones.
- To determine the mobility of the clones at varying temperatures (0°C to 25°C).
- To study the effects of acclimation at 10°C and 25°C for one and three generations on the above thresholds of all clones.

In meeting these objectives, four main hypotheses will be tested:

- Clones collected from Sweden will be more cold tolerant than those collected from Britain or Spain.
- Clones collected from Southern Spain will be more heat tolerant than clones collected from Britain or Sweden.
- No latitudinal trend in thermal tolerance will be evident. Severe winter temperatures and a lack of the primary host plant in Scandinavia will prevent permanent *M. persicae* populations existing and therefore populations will rely on annual migrations from other parts of Europe to persist. Extensive annual seasonal migrations results in an annual redistribution of aphid clones that will override any regional adaptation.
- Aphid clones of the same type, as determined by microsatellite analysis, will be more similar with respect to thermal tolerance than clones of differing types.

2 General materials and methods

2.1 Aphid culture and maintenance

Individual *Myzus persicae* clones were collected from three locations within Europe to represent a latitudinal gradient spanning approximately 21°. Nine clones were collected in total, three from each geographically distinct region. The Spanish clones (Span 1, 2 and 3) were collected from Almeria, Spain (approx. 36°), from sweet pepper *Capsicum annuum* in 2006, and the remaining two from the Murcia region (approx 37°), 40km apart, also on sweet pepper in 2008. The British clones (UK 1, 2 and 3) were collected from Suffolk, England (approx. 52°), on sugar beet *Beta vulgaris* in 2007, from Angus, Scotland (approx. 57°), on Swede *Brassica napus* var. napobrassica in 2000 and from wild mustard *Sinapis arvensis* in 2001. The Scandinavian clones (Swed 1, 2 and 3) were collected from Skåne County, Sweden (approx. 55°), on *Brassica oleracea* var. calabrese and *Brassica oleracea* var. Victoria Pigeon in 2008, and the third clone in 2009 on *Brassica oleracea*, although variety unknown. Meteorological data for locations nearest to aphid collection sites are provided in Table 1, providing an indication of the variation in local climate between collection sites. All clones were transported back to the School of Biosciences, Birmingham University, for culturing.

Table 1. Meteorological data for areas of aphid collection along a latitudinal gradient in Europe (Data: Weatherbase)

Location	Latitude	Average temperature January (°C)	Average temperature July (°C)	Average number of days per year below 0°C
Scandinavia				
Malmo, Sweden	55°	-2	14	99
Britain				
Perth, Scotland	56°	3	15	59
Ipswich, England	52°	3	16	54
Mediterranean				
Almeria, Spain	36°	12	25	1

Single monoclonal lineages were established for each of the nine experimental clones and were maintained in the laboratory at a culture temperature of $20 \pm 0.5^\circ\text{C}$, photoperiod LD 16:8, representing an intermediate temperature for the three locations. Aphid culturing was carried out in 'Blackman boxes' (Blackman, 1988) for which aphids were enclosed within a ventilated box containing a single leaf of food material. Aphid numbers within Blackman boxes were kept to five per leaf so as to prevent overcrowding. The food material used throughout the study was Chinese cabbage (*Brassica rapa* var. Wingbok) grown by the School of Biosciences (Horticultural Services) glasshouses from seed in net covered cages. Fresh plants were supplied once a week to the laboratory. Glasshouse plants were inspected weekly to prevent contamination with wild aphids. Prior to use in the Blackman boxes, selected leaves were cut, washed and further inspected under a microscope for the presence of wild aphids to prevent contamination of the culture populations. Aphids were transferred with a fine paintbrush to reduce potential handling damage to Blackman boxes containing newly cut *B. rapa* leaves every 5-7 days to ensure a fresh food supply.

2.2 General experimental procedures

2.2.1 Acclimation to different temperatures

To study the effects of acclimation on aphid thermal tolerance, first instar nymphs (less than 24h old) were removed from the culture temperature of 20°C and transferred to either 10°C (low temperature) or 25°C (high temperature). Aphids were left to develop at the selected temperature for one and three generations, thus allowing distinction between acclimation within a single generation and intergenerational acclimation. The aphids used in all experiments were the offspring of individuals reared under these acclimation treatments and were collected between days 3 and 6 of the adults' reproductive cycle, since aphids born earlier in the reproductive life of the adults are reported to differ physiologically (Clough *et al.*, 1990; Powell & Bale, 2008) (See section 3.2 for more information and preliminary work). First instar nymphs were thus available for experimentation from five different acclimation treatments: constant 20°C , 10°C for one generation, 10°C for three generations, 25°C for one generation and 25°C for three generations. Due to the slower development of

aphids reared at 10°C, first instar nymphs were left to develop for 48h prior to use in experiments as opposed to 24h in the other treatment regimes.

2.2.2 Exposure to temperature extremes

First instar nymphs were placed within plastic 0.5ml Eppendorf tubes at densities of ten nymphs per tube which, in turn, were placed within a glass boiling tube. Pieces of sponge were used to stopper the boiling tubes to limit air circulation and ensure a more stable internal temperature within the tubes. The boiling tubes were held within a test tube holder and lowered into an alcohol bath (Haake Phoenix 11 P2; Thermo Electron Corp., Germany). The alcohol bath was programmed to cool or heat aphids to a set temperature at a set rate. The alcohol bath could further be programmed to hold the aphids at a selected maximum or minimum temperature for a required time period. A thermocouple was placed within an empty Eppendorf tube, set up in the same way as the tubes containing the aphids, enabling the temperature the aphids were experiencing to be monitored.

Following exposure, nymphs were transferred to a recovery tray. Recovery trays were constructed from *B. rapa* leaf discs (approximately 30mm in diameter) placed on damp tissue paper. Specially constructed Perspex cages were placed over the leaf discs to prevent aphid escape. Mortality was assessed 72h after exposure.

2.2.3 Recording activity thresholds

The effect of temperature on activity thresholds was determined using a method described by Hazell *et al.* (2008). An aluminium block arena (Figure 5) was created which attached to an alcohol bath, enabling pumping of heated or cooled alcohol fluid throughout channels drilled into the block and thus allowing fine control over the temperature experienced within the arena. The walls of the arena were coated with Fluon (Blades Biological, UK) to prevent aphids climbing up the arena sides. A thermocouple (Tecpel, Taiwan) was placed within the arena to accurately monitor the exposure temperature experienced. A camera (Infinity 1-1;

Lumenera Scientific, Canada) was positioned directly over the arena which connected to video recording software (Studio Capture DT; Studio86designs, UK) on a desktop computer. First instar nymphs were placed within the arena (depth 7.5mm, diameter 25mm) covered with a thin sheet of Perspex, and the desired temperature ramp programmed into the alcohol bath. For all experiments, the initial temperature of the arena was set to the rearing temperature of the aphids. Once the temperature ramp was in progress, the camera could be activated to record aphid movement within the arena. The video was logged to the computer with both time and temperature displayed on the screen. The video could be played back at a later date to investigate aphid activity in relation to temperature and duration of exposure.

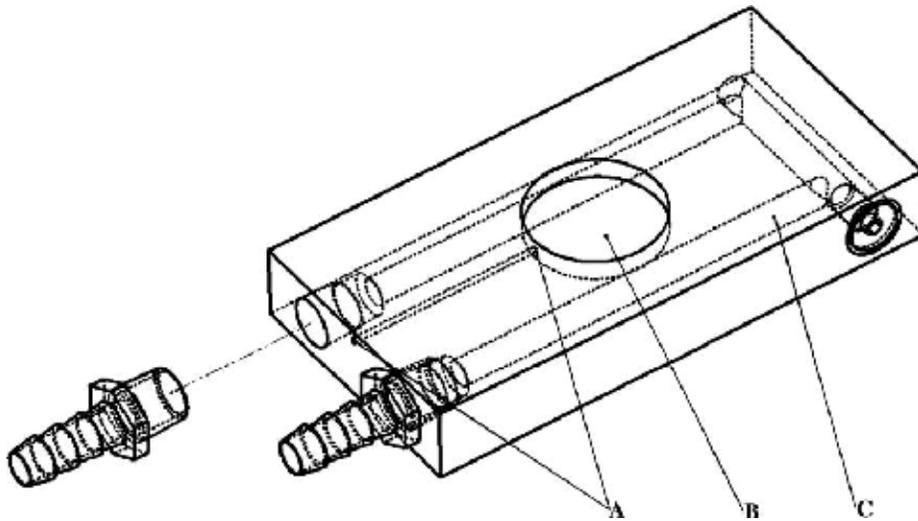


Figure 5. Aluminium block design used to measure aphid activity thresholds. A. channel for the thermocouple to measure arena temperature; B. Aphid arena; C. Channel for pumping alcohol fluid to enable fine temperature control over the arena (Hazell *et al.*, 2008).

3 Preliminary experiments: characterisation of clones and birth sequence effects on thermal tolerance

3.1 Determination of aphid characteristics

3.1.1 Genetic variability

The genetic variability of aphid clones was determined using microsatellite DNA analysis performed at the Scottish Crop Research Institute in Dundee, Scotland. DNA was extracted and amplified from ethanol preserved aphids. Three microsatellite loci, chosen for resolution, were selected (M49, M63 and M86) and amplified using fluorochrome primers labelled at the 5' end of the reverse primer and PCR ready to go beads, and then analysed on an automated sequencer using the method detailed in Kasprowicz *et al.* (2008)

3.1.2 Lifecycle type

The lifecycle type of all *M. persicae* clones used in experiments was determined using a method previously described by Vorburger *et al.*, (2003). Reproducing adults (known as generation G0) were placed under conditions of short day length (LD 12:12h) and lower temperature (14.5°C) and allowed to reproduce for 48h within freshly prepared Blackman boxes. Such conditions are known to induce the production of sexual morphs in *M. persicae* if the clone is able to do so i.e. if the clone is holocyclic. Adults were removed after 48h and nymphs (G1 generation) allowed to develop into wingless parthenogenetic females under these conditions. On commencing reproduction, 4 adults of the G1 generation were retained for each clone and allowed to reproduce for a total of 20 days. All nymphs produced (G2) were retained and allowed to develop until adult ecdysis to determine the morph type. Aphid morphs were classified as alate (winged) or apterae (wingless) or male. Of the alate forms produced, all, excluding 4 individuals, were preserved in 70% ethanol following aphid morph scoring in case further identification was required. The excluded 4 alates were

transferred in to separate Blackman boxes and allowed to reproduce for sufficient time to produce approximately 5 nymphs each. The resultant nymphs (G3) were allowed to develop until adult ecdysis and the aphid morphs were again classified as apterous parthenogenetic females or sexual females. Figure 6 displays the different lifecycle types that could have been identified for *M. persicae* and details the different morphs that would be expected at each generation.

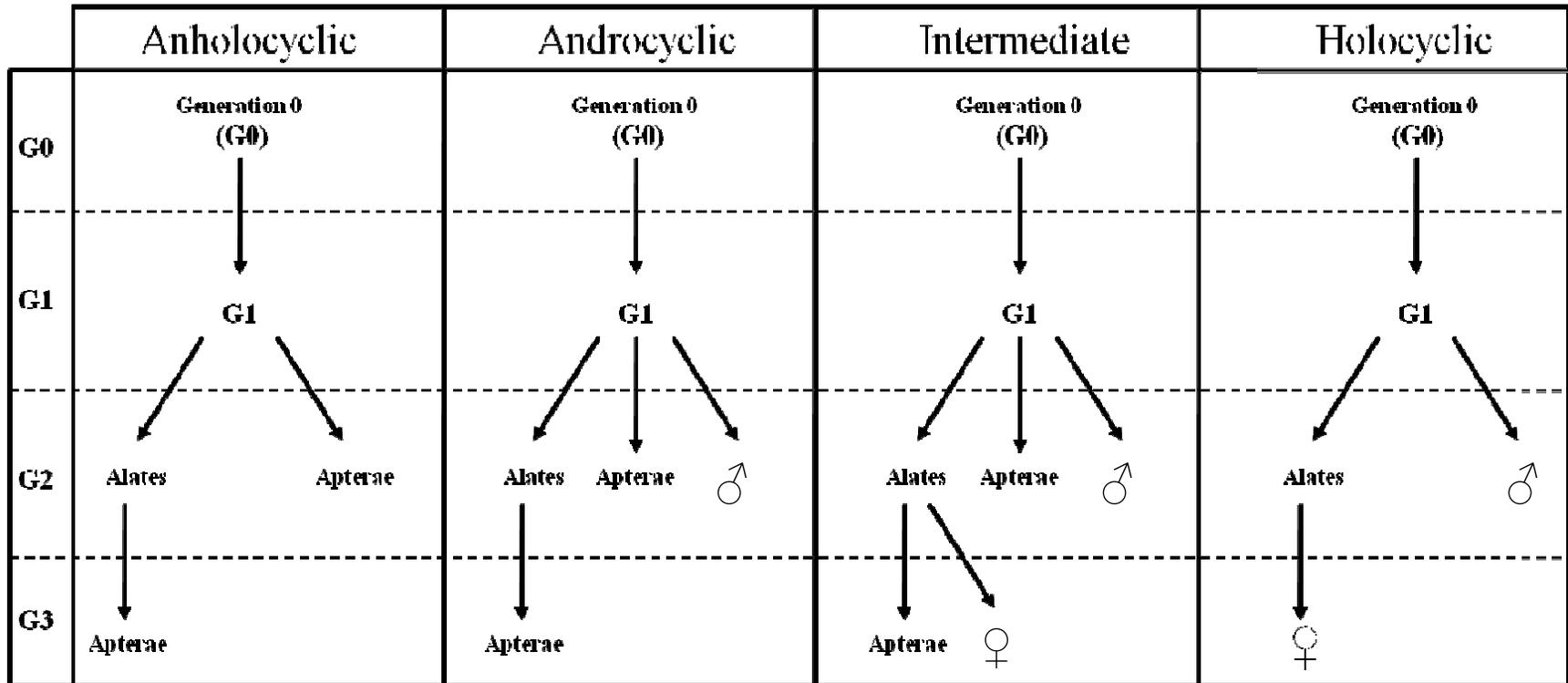


Figure 6. The succession of aphid morph production across generations G0 to G3 for *Myzus persicae* when held under conditions of reduced daylight (LD 12:12h) and lower temperature (14.5°C) to determine lifecycle type. Lifecycle type could be classified as one of four types: holocyclic (sexual), anholocyclic (asexual) or as a transitional lifecycle type of androcyclic or intermediate. Alates of G2 in the holocyclic lifecycle will be exclusively gynoparae. Alates of G2 in the intermediate lifecycle may include gynoparae.

3.1.3 Resistance type

All clones used in experiments were characterised for insecticide resistance mechanisms by Rothamsted Research in Hertfordshire, England, using a method described in van Toor *et al.* (2008). The level of carboxylesterase, conferring resistance to organophosphates and carbamates, was measured using an esterase assay by absorbance at 450nm. Aphid clones were then categorised as either susceptible (S), with medium resistance (R1), high resistance (R2) or with extreme levels of resistance (R3). The presence of modified acetylcholinesterase (MACE) was determined using a kinetic assay and scored as either Mace or Non-Mace. Resistance to pyrethroids was determined via identification of Kdr and s-kdr mutations using an allelic discrimination PCR assay on L1014F and M918T in the voltage gated sodium channel protein (for more detailed information on all resistance typing experiments see van Toor *et al.*, 2008).

Information regarding genetic variability, lifecycle type and resistance type obtained from the preliminary experiments discussed above are summarised in Table 2. Also presented is information detailing location and dates of aphid collection, food plants from which the aphids were collected and aphid colour morphs. In addition, Table 3 provides the detailed results of microsatellite analysis from which clonal types were assigned.

Table 2. Characteristics of aphid clones used in experiments including aphid collection information, genetic variability, lifecycle type, and insecticide resistance where available. Genetic type is indicated by a universal letter code, with clones of the same letter proving identical at the loci examined. Clones classified as unique proved individual from microsatellite analysis and have not, as of yet, been assigned a genetic type. Insecticide resistance was determined by four resistance mechanisms. Carboxylesterase levels are indicated by classifying clones as either susceptible (s), of medium resistance (R1), high resistance (R2) or extreme resistance (R3). The presence of modified acetylcholinesterase (MACE) is indicated by clones being classified as either mace or non mace. Knockdown resistance (Kdr) and super Kdr (s-Kdr) is indicated by clones being classified as either super susceptible (SS) or super resistance (SR).

<i>M. persicae</i> clone	Location of collection	Date of collection	Food plant on which collected	Genetic Type	Life cycle type	Colour Morph	Insecticide resistance status:			
							carboxylesterase	MACE	Kdr	s-Kdr
Sub-Arctic										
Swed 1	Skåne County	2008	<i>Brassica oleracea</i>	O	anholocyclic	Green	R1	Mace	SS	SS
Swed 2	Skåne County	2008	<i>Brassica oleracea</i>	C	anholocyclic	Green	R1	Non-Mace	SR	SS
Swed 3	Skåne County	2009	<i>Brassica oleracea</i>	O	anholocyclic	Green	R1	Mace	SS	SS
Temperate										
UK 1	Suffolk	2007	<i>Brassica napus</i>	C	anholocyclic	Green	R1	Non-Mace	SR	SS
UK 2	Angus	2000	<i>Beta vulgaris</i>	C	anholocyclic	Green	R1	Non-Mace	SR	SS
UK 3	Perthshire	2001	<i>Sinapis arvensis</i>	J	anholocyclic	Green	R1	Non-Mace	SS	SS
Mediterranean										
Span 1	Almeria	2006	<i>Capsicum annuum</i>	unique	anholocyclic	Green	R3	Mace		
Span 2	Murica	2008	<i>Capsicum annuum</i>	unique	anholocyclic	Red				
Span 3	Murica	2008	<i>Capsicum annuum</i>	unique	anholocyclic	Red				

Table 3. Results of microsatellite analysis using loci M49, M63 and M86 on the nine experimental clones of *Myzus persicae*. From microsatellite analysis, clones were assigned a universal letter code, with clones of the same letter proving identical at the examined loci. Unique clones are clones which have not, as of yet, been assigned a genetic type.

<i>M. persicae</i> Clone	M49		M63		M86		Type
Sub-Arctic							
Swed 1	176	211	164	174	99	101	O
Swed 2	153	166	166	170	136	141	C
Swed 3	176	211	164	174	99	101	O
Temperate							
UK 1	153	165	166	170	136	140	C
UK 2	153	166	166	170	136	140	C
UK 3	153	F	158	172	115	138	J
Mediterranean							
Span 1	120	170	174	176	103	126	unique
Span 2	114	115	166	182	114	124	unique
Span 3	113	115	168	182	99	115	unique

3.2 Variation in thermal tolerance across the birth sequence

It is documented that aphid nymphs produced at the beginning of an adult's reproductive life can differ physiologically from later born progeny (Clough *et al.*, 1990; Powell & Bale, 2008). First born nymphs of *M. persicae* have been shown to display LT_{50} values up to 7°C lower than later born nymphs (-15.9°C on day 1 compared to -8.3°C by day 4), suggesting first born nymphs to be more cold tolerant (Clough *et al.*, 1990).

An experiment was subsequently conducted to determine if the thermal tolerances of first instar nymphs of the collected clones differed depending on position in the birth sequence and consequently would verify which days of nymph production could be combined for subsequent experiments. Only the first clone collected to represent each of the three climatic regions along the latitudinal gradient in Europe was included in the birth sequence experiment.

First instar nymphs born within a 24h period were collected from the constant 20°C culture. The nymphs were allowed to develop into newly moulted adults at densities of one aphid per leaf. Harvesting the aphids in such a way synchronised the aphids to within 24h. On development to adulthood, Blackman boxes were examined daily to determine when reproduction commenced. Holding the aphids at densities of one per Blackman box enabled accurate determination of the day in which individual aphids commenced reproduction. At the onset of reproduction, the resultant nymphs were collected daily for each successive day of the reproductive cycle and survival at selected temperature extremes determined.

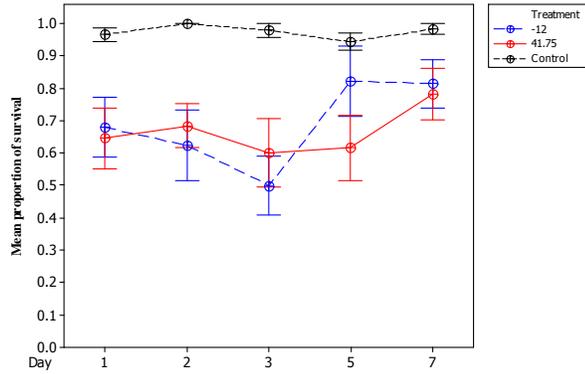
Based on previous studies of *M. persicae* at Birmingham University, two temperatures were selected that would result in considerable mortality at high and low temperatures: 41.75°C and -12°C respectively. First instar nymphs were subjected to the exposure temperature using an alcohol bath whilst held within Eppendorf tubes at densities of 10 nymphs per tube, a method previously described in the general materials and methods. Six replicates of 10 aphids were used for each treatment. Nymphs were cooled or warmed to the predetermined temperature at a rate of 0.5°C min⁻¹ and then held at the selected temperature. At -12°C nymphs were held for 30 min before being returned to the culture temperature of 20°C at 0.5°C min⁻¹. At 41.75°C the nymphs were held for only 10 sec before returning to 20°C. The decision to hold nymphs for 10 sec as opposed to 30 min at the high temperature exposures was based on previous work in the Arthropod Ecophysiology Laboratory at the University of Birmingham. The studies suggested that a brief exposure of 10 sec to high temperature extremes was all that was required to achieve mortality values comparable to a longer exposure duration of 30 min at low temperature (Hazell, unpublished data), based on the time required for all aphids in the sample to experience the desired exposure temperature (Piyaphongkul, unpublished data). A handling control was conducted each day for which 6 replicates of 10 aphids within Eppendorf tubes were set up. The control tubes were left at the culture temperature for the duration of the exposure experiments. Following exposure, nymphs were placed on recovery trays and mortality assessed after 72h.

Exposure to the high and low temperature plus a handling control was repeated using the newly born nymphs produced on days 1,2,3,5 and 7 of the reproductive cycle. From this experiment, fitness variation of newly born nymphs across the birth sequence could be

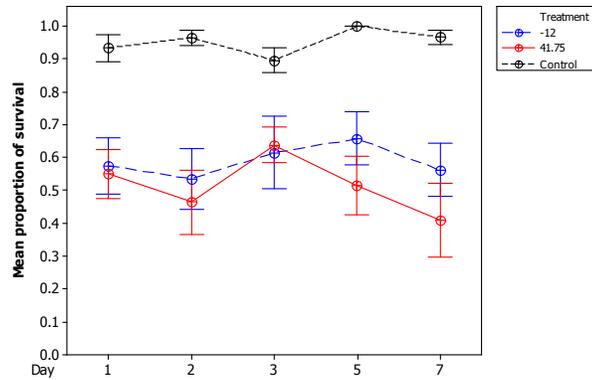
determined. Results would indicate the combinability of nymphs from different days of the cycle for subsequent experiments. A general linear model ANOVA was performed using MINITAB v.14 to determine significant differences between fitness across the birth sequence.

Figure 7 shows the variation in survival of I. Span 1, II. UK 1, and III. Swed 1 first instar nymphs from a 20°C culture collected on successive days of the reproductive cycle when exposed to either 41.75°C or -12°C. An ANOVA revealed no significant difference between the thermal tolerances of nymphs of the Span 1, UK 1 and Swed 1 clones when collected across the birth sequence up to day 7.

I.



II.



III.

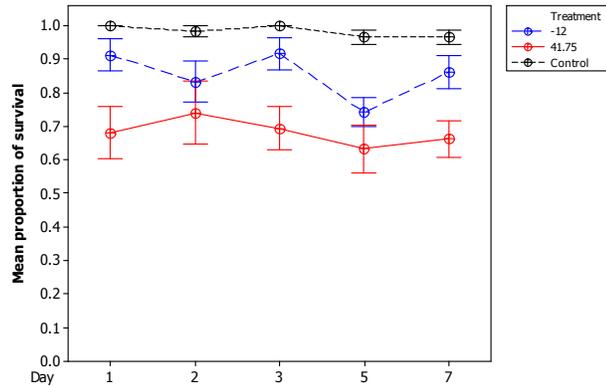


Figure 7. Mean proportion of survival for individual *Myzus persicae* clones I. Span 1, II. UK 1 and III. Swed 1 when nymphs were collected on successive days of the mothers' reproductive cycle and exposed to a high temperature (41.75°C) and a low temperature (-12°C). Error bars represent 1 standard error of the mean.

The thermal tolerances of nymphs born of adults reared at 20°C did not significantly differ across the birth sequence up to day 7 for clones Span 1, UK 1 and Swed 1, indicating that nymphs from different reproductive adults and across different days of the reproductive cycle could be combined for subsequent experiments. This supports recent studies on *S. avenae* which revealed no significant difference between progeny born on differing days of the birth sequence for adults maintained at 20°C (Powell & Bale, 2008). However, differences between nymphal cold hardiness in *S. avenae* became apparent when acclimated at 10°C, with first born nymphs proving to be significantly more cold tolerant than later born nymphs (Powell & Bale, 2008). Powell & Bale (2008) suggest that, under winter conditions, a reproducing aphid is unlikely to live long enough to fulfil its reproductive potential and it is therefore advantageous to invest greater levels of resources at the beginning of the reproductive cycle and produce the more cold tolerant progeny first, thus allowing the first born nymphs to survive at low temperatures that could have killed the parents or later born siblings (Powell & Bale, 2008). It was consequently decided that nymphs born on days 3-6 of the birth sequence would be used for all experiments to avoid the use of first born nymphs and those born later in the reproductive cycle.

4 Effect of latitude and acclimation on the lethal temperatures of the peach-potato aphid *Myzus persicae*

4.1 Summary

When *Myzus persicae* was acclimated at 10°C for one generation, the lower lethal temperature was significantly depressed when compared to aphids reared at 20°C (from a mean of -12.3°C to -14.3°C). Additional acclimation over three generations did not further increase cold tolerance, with intergenerational acclimation significantly depressing LLT₅₀ in only three out of the nine clones (Span 1 from -14.8°C to -16.4°C, Span 2 from -12.9°C to -14.8°C and Swed 1 from -14.1°C to -16.7°C). Cold tolerance was lost following one generation at 25°C when compared to 20°C rearing, leading to a significant increase in LLT₅₀ (from a mean of -12.3°C to -11.2°C). Additional acclimation over three generations did not further decrease cold tolerance with the only significant increase in LLT₅₀ being observed for Swed 2 (from -11.3°C to -10.9°C).

The ULT₅₀ of *M. persicae* proved less plastic than LLT₅₀, although could be significantly increased in all nine clones after one generation at 25°C, resulting in an increase in heat tolerance (from a mean of 41.8°C to 42.1°C). Low temperature acclimation also significantly increased heat tolerance, raising the ULT₅₀ of five out of the nine clones following one generation at 10°C and in seven of the nine clones after three generations at 10°C.

Due to greater plasticity in ULT₅₀, thermal tolerance ranges were expanded following low temperature acclimation and contracted following high temperature acclimation. This resulted in aphids reared at 10°C surviving over a temperature range approximately 2°C to 6°C greater than aphids reared at 25°C. There was no clear relationship between lethal temperature thresholds and latitude.

4.2 Introduction

Temperature is the most important abiotic factor affecting aphid physiology. Due to a limited ability to regulate body temperature above or below ambient, severity of winter conditions greatly influences aphid survival, with implications for resultant spring populations and potential pest outbreaks (Walters & Dewar, 1986; Harrington *et al.*, 1990; Cocu *et al.*, 2005). Furthering our understanding of aphid thermal biology and elucidating the interactions between their cold tolerance and winter severity with the implications for pest control has become a primary focus of recent research on aphids.

It is known that aphids including *Sitobion avenae*, *Myzus persicae* and *Aphis fabae* freeze at low sub-zero temperatures, with supercooling points of around -25°C (Knight *et al.*, 1986; Bale, 1987; Bale *et al.*, 1988). With the exception of enabling classification of insect cold tolerance (Salt, 1961; reclassification by Bale, 1993a, 1996; Sinclair, 1999), supercooling points are somewhat ecologically irrelevant when studying aphid thermal biology due to aphids experiencing high levels of pre-freeze mortality, and are therefore used more as a theoretical limit (Knight, 1987). Lethal temperatures such as the LLT_{50} (the low temperature which results in 50% mortality of an experimental population) have consequently received much attention in recent years due to providing, not only more ecologically relevant information, but enabling a reference point to study variation and plasticity of aphid thermal tolerance (Powell & Bale, 2008; Hazell *et al.*, 2010a).

Aphid biology contrasts to the majority of insects due to a succession of functionally distinct morphs being produced throughout the year. As a consequence of short generation times, an aphid at the onset of winter is unlikely to survive to the following spring. This led Powell & Bale (2004, 2008) to conclude that seasonal cold hardening is less important in aphids and that short term acclimation plays a greater role in thermal tolerance. Data concerning a single clone of *S. avenae* supported this statement, with the LLT_{50} declining from -8.5°C when reared at 20°C to -13.6°C after acclimation to 10°C for one generation and further again to -16.4°C after acclimation to 10°C for three generations (Powell & Bale, 2008). Due to the phenomenon known as ‘telescoping of generations’ whereby an aphid begins development within its grandmother (Kindlmann & Dixon, 1989), an aphid has the potential to begin the

acclimation process prior to birth and consequently gain thermal tolerance with each successive generation.

Lower thermal limits play an important role in determining aphid distribution and abundance. However, with average temperature increasing, many species are exhibiting range expansions as low temperature limits are elevated (Cannon, 1998; Parmesan, 1999; Thomas and Lennon, 1999; Battisti *et al.*, 2005; Musolin, 2007). It is suggested that increasing temperatures, in conjunction with a rise in heat wave incidence, could lead to an increase in the importance of upper thermal limits in determining species distribution and abundance (Hazell *et al.*, 2010b). In addition to increasing the knowledge baseline regarding upper thermal limits, understanding how thermal biology varies with latitude is also integral to predicting how insect populations will be affected by climate change and has provided the focus for a number of recent studies (e.g. Addo-Bediako *et al.*, 2000; Gibert & Huey, 2001; Chen & Kang, 2004; Bahrndorff *et al.*, 2006, 2009a). For such an agriculturally important pest species as *M. persicae* with an extensive worldwide distribution, it is surprising that research into variation in thermal biology along its latitudinal distribution is lacking.

This study aims to provide a detailed investigation into the lower and upper lethal temperatures of *M. persicae*. As highlighted in Hazell *et al.* (2010b), few studies to date have considered the effect of extreme high temperatures on insect populations. In this chapter, experiments designed to investigate the variation in the ULT₅₀ and LLT₅₀ of nine clones collected along a latitudinal gradient in Europe are detailed. The levels of intra and intergenerational plasticity following low (10°C) and high (25°C) temperature acclimation are determined, in addition to geographic variation in aphid thermal tolerance.

4.3 Materials and methods

The nine experimental clones of *M. persicae* were kept as a stock culture at 20°C. First instar nymphs (less than 24h old) were taken from the stock culture and acclimated at either 10°C or 25°C for one or three generations prior to use of their offspring in experiments, as detailed

in the general materials and methods chapter. All nymphs used in experiments were first instars.

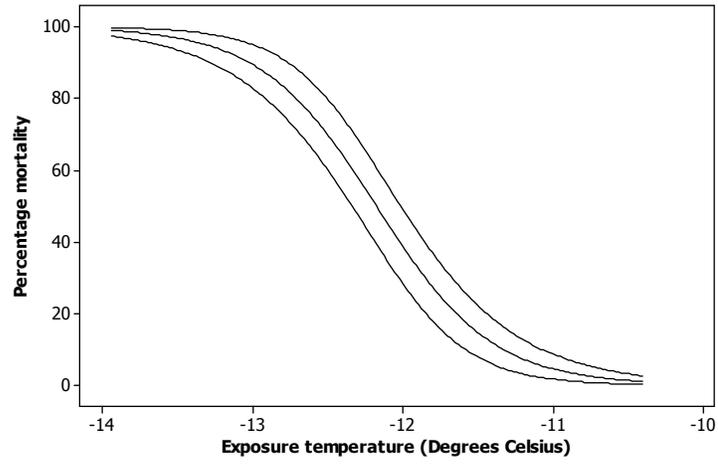
4.3.1 Determining the lethal temperature (LLT₅₀ and ULT₅₀)

To determine the lower and upper LT₅₀, first instar nymphs were exposed to a range of low temperatures (-9, -10, -11, -12, -13, -14, -15, -16, -17 and -18°C) and a range of high temperatures (41.25, 41.50, 41.75, 42.00, 42.25, 42.50, 42.75 and 43.00°C). The temperatures selected to determine the LLT₅₀ increased at intervals of 1°C as opposed to 0.25°C when determining the ULT₅₀ due to the range over which 0 to 100% mortality occurs at high temperature being relatively compressed.

For each temperature treatment 60 first instar nymphs were collected and placed within plastic 0.5ml Eppendorf tubes to produce 6 replicates of 10 aphids. The nymphs were placed within an alcohol bath programmed to the desired temperature as described in the general materials and methods. Nymphs within the alcohol bath were cooled or heated from their culture temperature to the pre-determined exposure temperatures at a rate of 0.5°C min⁻¹. On reaching the desired temperature, nymphs were held at the low exposure temperatures (-9 to -18°C) for 30 min and at the high exposure temperatures (41.25 to 43.00°C) for 10 sec. These exposure temperatures were selected on the basis of preliminary experiments that indicated the times required for all aphids in the sample to experience the desired exposure temperatures i.e. there is a time lag between the temperature of the circulating fluid in the alcohol bath and the loss or gain in temperature of the aphids. Following the exposure duration, nymphs were returned to their culture temperature at a rate of 0.5°C min⁻¹. Nymphs were subsequently transferred to recovery trays and allowed to recover at their culture temperature (10°C, 20°C or 25°C). Survival was assessed 72h after exposure. The procedure was repeated for each exposure temperature. LLT₅₀ and ULT₅₀ values were obtained for all *M. persicae* clones held at each of the five acclimation treatments (i.e. constant 20°C and 10°C and 25°C for one and three generations). A handling control was set up for each treatment/day of experiments as detailed above with the exception of the aphids being kept at acclimation temperature for the duration of the experiment.

On obtaining values for the proportion of aphid survival at each exposure temperature, a graph of survival against exposure temperature was produced, displaying a typical sigmoidal dosage-mortality curve. From the resultant data, the temperature resulting in 50% mortality (the LT_{50}) was determined for the high and low exposure temperatures using Probit analysis in MINITAB 15. Probit transformations act to linearise sigmoidal curves, allowing more accurate estimation of the LT_{50} temperature. Figure 8 displays example graphs illustrating the probit transformations from the sigmoidal graph of raw data (8I) to the linearised Probit graph (8II). Handling controls resulted in approximately 99% survival across all treatments. The natural response rate was therefore assumed to be close to zero and not included in the model (Hazell *et al.*, 2010a). Significant differences in mortality were identified by non-overlapping 95% fiducial limits (Hart *et al.*, 2002; Hughes *et al.*, 2009).

I.



II.

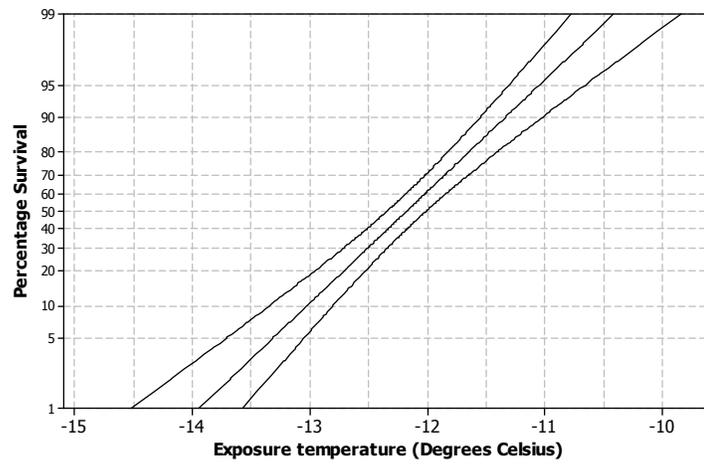


Figure 8. Example graphs illustrating Probit transformations from the raw mortality against exposure temperature data ($\pm 95\%$ Fiducial CI) shown in I. to the linearised data ($\pm 95\%$ Fiducial CI) shown in II.

4.4 Results

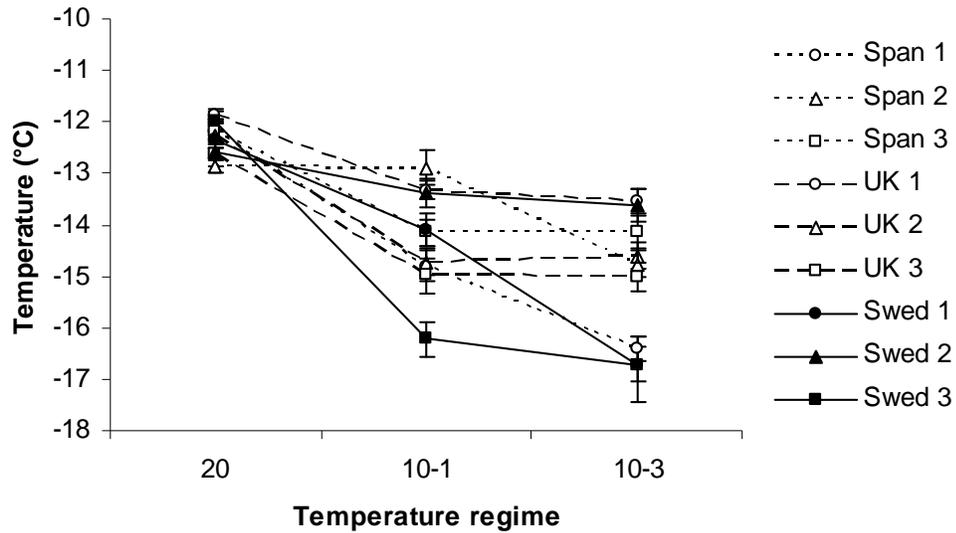
4.4.1 Clonal differences

Significant interclonal differences in LLT_{50} were apparent between the nine experimental clones (indicated by non-overlapping 95% fiducial limits) (Figure 9). However, although clonal differences existed, these were not consistently correlated with latitude or country of origin. At 20°C, Span 2 proved to be the most cold tolerant with a LLT_{50} of $-12.9 \pm 0.2^\circ\text{C}$. This LLT_{50} proved significantly lower than all other clones with the exception of UK 3. UK 1 was the least cold tolerant with a LLT_{50} of $-11.9 \pm 0.1^\circ\text{C}$, significantly higher than all other clones excluding Swed 3. At 20°C, the range across all LLT_{50} values was 1.0°C. Following acclimation to 10°C for one generation, interclonal variation became more pronounced, with the LLT_{50} variation varying by 3.3°C. Swed 3 was the most cold tolerant clone with a LLT_{50} of $-16.2 \pm 0.3^\circ\text{C}$, significantly lower than all other clones. Clones Span 2, UK 1 and Swed 2 proved to be the least cold tolerant, with LLT_{50} values of $-12.9 \pm 0.3^\circ\text{C}$, $-13.3 \pm 0.2^\circ\text{C}$ and $-13.4 \pm 0.3^\circ\text{C}$ respectively, significantly higher than all other clones. After further acclimation to 10°C for three generations Swed 3 remained one of the most cold tolerant of clones, with the addition of Swed 1 and Span 1. Respective LLT_{50} values for these clones were $-16.7 \pm 0.3^\circ\text{C}$, $-16.7 \pm 0.6^\circ\text{C}$ and $-16.4 \pm 0.2^\circ\text{C}$, significantly lower than all the remaining clones. UK 1 remained one of the least cold tolerant with a LLT_{50} of $-13.5 \pm 0.2^\circ\text{C}$, significantly higher than all other clones with the exception of Swed 2.

Following acclimation to 25°C for one generation, variation in LLT_{50} was reduced to 0.9°C. Swed 1 displayed the lowest LLT_{50} of $-11.6 \pm 0.2^\circ\text{C}$, although this was not significantly lower than Swed 2, Swed 3 and UK 2. Span 3, with a LLT_{50} of $-10.7 \pm 0.2^\circ\text{C}$, was the least cold tolerant with a LLT_{50} significantly lower than all other clones with the exception of UK 1. Acclimation for three generations at 25°C resulted in a further reduction in the variation in LLT_{50} to 0.5°C. Swed 1 displayed the lowest LLT_{50} of $-11.3 \pm 0.1^\circ\text{C}$ and Span 1 the highest of $-10.8 \pm 0.1^\circ\text{C}$. Although these two clones differed significantly in relative cold tolerance, there was considerable overlap in clonal thermal tolerance following three generations at 25°C and consequently few significant differences between clones.

No consistent relationship between LLT_{50} and latitude was evident. However, UK 1 was one of the least cold tolerant of clones across all treatments. Following low temperature acclimation to 10°C clones Span 1 and Swed 3 were consistently among some of the most cold tolerant of clones. Following high temperature acclimation to 25°C Span 3 was consistently one of the least cold tolerant of clones and Swed 1 one of the most cold tolerant.

I.



II.

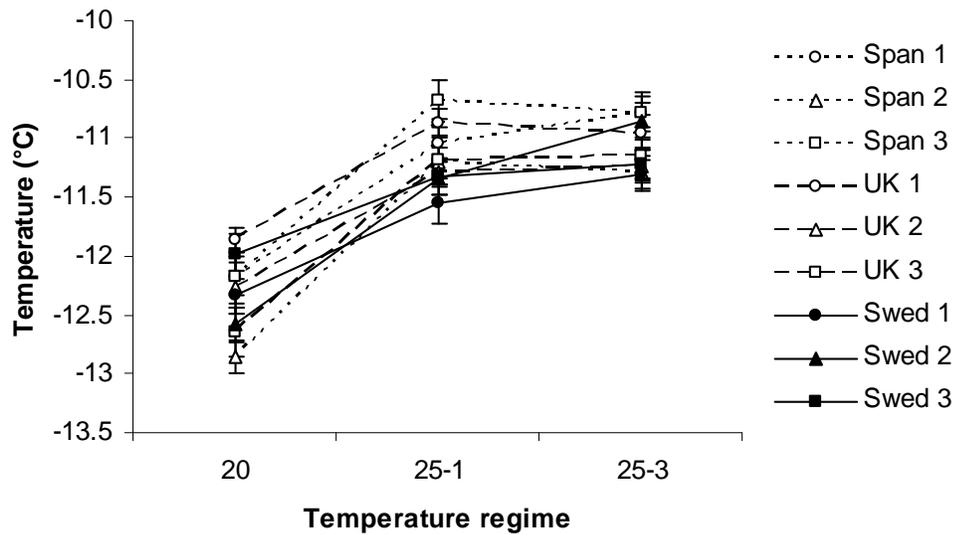
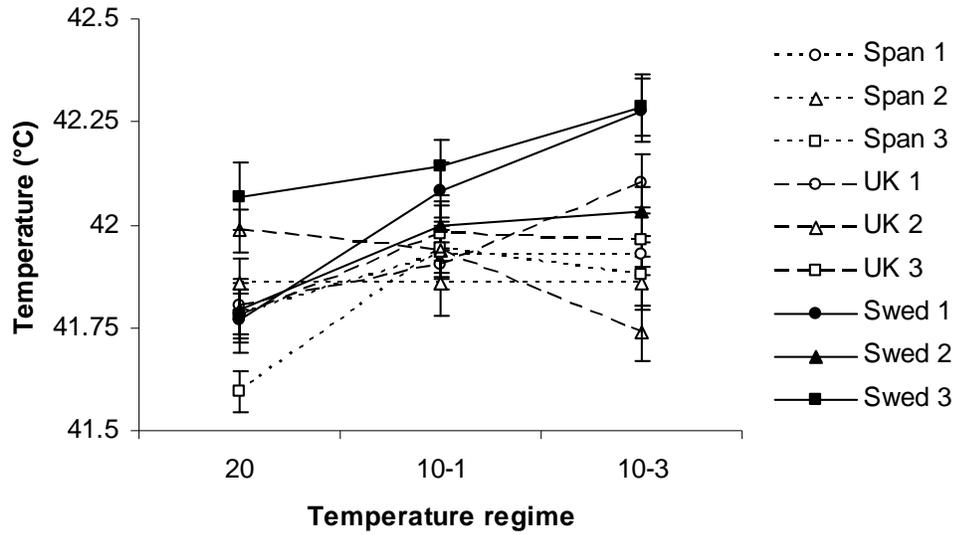


Figure 9. Effects of acclimation on lower lethal temperature following acclimation from 20°C to I. 10°C for one and three generations and II. 25°C for one and three generations. Data points represent LLT₅₀ (±95% Fiducial CI) for *Myzus persicae* clones collected from Spain, UK and Sweden (codes for clones are shown in the figure). Temperature regime is displayed as the acclimation temperature with the number of generations at that temperature indicated after the dash symbol.

Interclonal variation in ULT_{50} was much compressed compared to LLT_{50} . Nevertheless, significant interclonal differences were apparent (as indicated by non-overlapping 95% fiducial limits) (Figure 10). At 20°C, Span 3 was the least heat tolerant with an ULT_{50} of $41.6 \pm 0.1^\circ\text{C}$, significantly lower than all other clones. Swed 3 and UK 2 were the most heat tolerant with ULT_{50} values of $42.1 \pm 0.1^\circ\text{C}$ and $42.0 \pm 0.1^\circ\text{C}$ respectively. Following acclimation to 10°C for one generation, variation in ULT_{50} reduced to 0.2°C and as a consequence few significant differences were detected. An ULT_{50} of $41.9 \pm 0.1^\circ\text{C}$ was observed for Span 1, Span 2, Span 3, UK 1 and UK 2. The most heat tolerant clone was Swed 3 with an ULT_{50} of $42.1 \pm 0.1^\circ\text{C}$ which proved significantly higher than all other clones with the exception of Swed 1. Further acclimation to 10°C for three generations resulted in an increase in interclonal variation to 0.6°C. Swed 1 and Swed 3 were the most heat tolerant, both displaying an ULT_{50} of $42.3 \pm 0.1^\circ\text{C}$, significantly higher than all other clones. UK 2 was the least heat tolerant with an ULT_{50} of $41.7 \pm 0.1^\circ\text{C}$ which was significantly lower than all other clones with the exception of Span 2 and Span 3 (both displaying an ULT_{50} of $41.9 \pm 0.1^\circ\text{C}$).

Following acclimation to 25°C for one generation, Swed 1 was the most heat tolerant of clones with an ULT_{50} of $42.4 \pm 0.1^\circ\text{C}$, significantly higher than all other clones with the exception of UK 2 (ULT_{50} of $42.3 \pm 0.1^\circ\text{C}$). Span 3 was the least heat tolerant clone with an ULT_{50} of $41.8 \pm 0.1^\circ\text{C}$, significantly lower than all other clones with the exception of Span 1 (ULT_{50} of $41.9 \pm 0.1^\circ\text{C}$). Additional acclimation to 25°C for three generations lead to a reduction in interclonal variation from 0.6°C to 0.4°C, resulting in a high level of overlap between clonal ULT_{50} values. Swed 1 remained the most heat tolerant clone with an ULT_{50} of $42.2 \pm 0.1^\circ\text{C}$, although this was only significantly higher than clones UK 3, Span 1, Swed 2 and Span 3 (ULT_{50} values of $42.0 \pm 0.1^\circ\text{C}$, $41.9 \pm 0.1^\circ\text{C}$, $41.9 \pm 0.1^\circ\text{C}$ and $41.8 \pm 0.1^\circ\text{C}$ respectively). Span 3 remained the least heat tolerant clone with an ULT_{50} of $41.8 \pm 0.1^\circ\text{C}$ which proved significantly lower than all other clones with the exception of Swed 2 and Span 1.

I.



II.

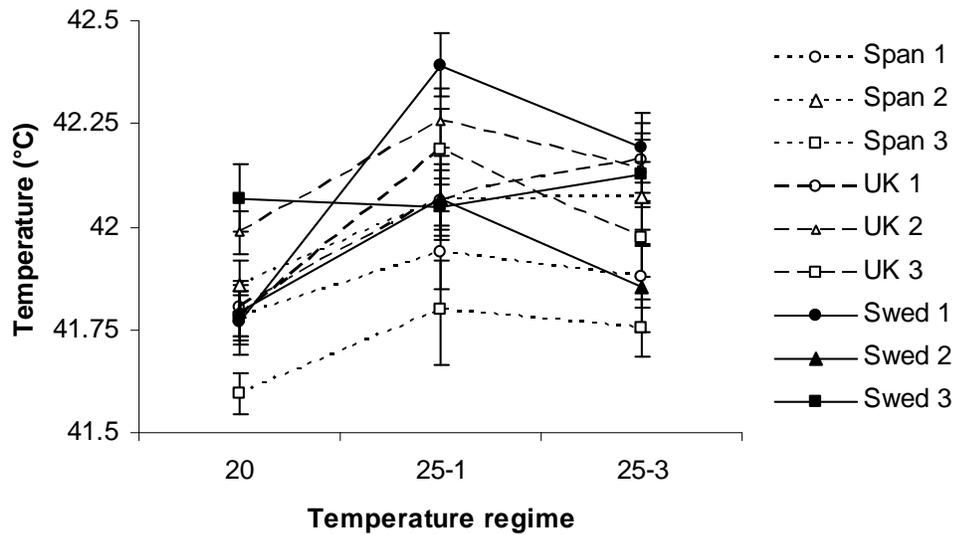


Figure 10. Effects of acclimation on upper lethal temperature following acclimation from 20°C to I. 10°C for one and three generations and II. 25°C for one and three generations. Data points represent ULT₅₀ (±95% Fiducial CI) for *Myzus persicae* clones collected from Spain, UK and Sweden (codes for clones are shown in the figure). Temperature regime is displayed as the acclimation temperature with the number of generations at that temperature indicated after the dash symbol.

As with LLT_{50} , a consistent relationship between ULT_{50} and latitude was not apparent. Following high temperature acclimation to 25°C, clones Span 3 and Span 1 were consistently the least heat tolerant of clones and Swed 1 and UK 2 the most heat tolerant of clones. Following low temperature acclimation to 10°C, only Swed 3 displayed a constant trend with acclimation treatment, proving to be consistently the most heat tolerant.

4.4.2 Acclimation

The LLT_{50} of *M. persicae* clones was significantly depressed following acclimation to 10°C for one generation leading to increased cold tolerance. However, cold tolerance was lost on acclimation to 25°C causing a significant rise in LLT_{50} (as indicated by non-overlapping 95% fiducial limits) (Figure 9).

All clones, with the exception of Span 2 where no change was observed, were significantly more cold hardy following acclimation to 10°C for one generation when compared to the culture temperature of 20°C. The largest effect of acclimation was observed for Swed 3, with LLT_{50} decreasing by 4.2° from $-12.0 \pm 0.2^{\circ}\text{C}$ at 20°C to $-16.2 \pm 0.3^{\circ}\text{C}$ at 10°C. For the remaining clones where LLT_{50} significantly decreased, reductions ranged from 0.8°C (for Swed 2) to 2.6°C (for Span 1). Further acclimation to 10°C for three generations failed to further significantly increase cold tolerance in all but three clones. The LLT_{50} of Span 2, although the only clone not to display a significant reduction in LLT_{50} following one generation at 10°C, did show a significant reduction when acclimated for three generations; LLT_{50} decreased by 1.9°C from $-12.9 \pm 0.3^{\circ}\text{C}$ to $-14.8 \pm 0.3^{\circ}\text{C}$. Swed 1 and Span 1 displayed a further decrease in LLT_{50} taking their respective LLT_{50} values down to $-16.7 \pm 0.3^{\circ}\text{C}$ and $-16.4 \pm 0.2^{\circ}\text{C}$, corresponding to a reduction of 2.6°C and 1.6°C respectively.

Acclimation to 25°C resulted in a significant loss in cold tolerance for all clones. Increases in LLT_{50} , when compared to respective values obtained at 20°C, ranged from 0.7°C for Swed 3, which resulted in a LLT_{50} of $-11.3 \pm 0.2^{\circ}\text{C}$, to -1.7°C for Span 2, which resulted in an LLT_{50} of $-11.2 \pm 0.1^{\circ}\text{C}$. Further acclimation to 25°C for three generations failed to reduce cold tolerance significantly further in all clones excluding Swed 2. Swed 2 experienced a

significant increase in LLT_{50} of 0.4°C , increasing LLT_{50} from $-11.3 \pm 0.1^{\circ}\text{C}$ following one generation at 25°C to $-10.9 \pm 0.2^{\circ}\text{C}$ after generations at 25°C .

The effects of acclimation on ULT_{50} were much compressed compared to LLT_{50} , with variation between treatments being less than 1°C . Despite this reduced between-treatment variation, the ULT_{50} of *M. persicae* could still be significantly increased following high temperature acclimation to 25°C for one generation, leading to an increase in clonal heat tolerance (as indicated by non-overlapping 95% fiducial limits). Heat tolerance also significantly increased in 5 of the 9 clones following acclimation to 10°C for one generation, and in 7 of the 9 clones by three generations at 10°C (Figure 10).

Acclimation to 25°C for one generation resulted in a significant increase in heat tolerance for all clones with the exception of Swed 3 where no change was recorded. Significant increases in ULT_{50} ranged from 0.1°C in Span 1, increasing ULT_{50} from $41.8 \pm 0.1^{\circ}\text{C}$ to $41.9 \pm 0.1^{\circ}\text{C}$, to 0.6°C for Swed 1, increasing ULT_{50} from $41.8 \pm 0.1^{\circ}\text{C}$ to $42.4 \pm 0.1^{\circ}\text{C}$. Further acclimation for three generations at 25°C resulted in a further significant increase in heat tolerance for UK 1, with ULT_{50} increasing from $42.1 \pm 0.1^{\circ}\text{C}$ to $42.2 \pm 0.1^{\circ}\text{C}$. However, for clones UK 3, Swed 1 and Swed 2, a significant decrease in ULT_{50} was observed of 0.2°C , reducing ULT_{50} to $42.0 \pm 0.1^{\circ}\text{C}$, $42.2 \pm 0.1^{\circ}\text{C}$ and $41.9 \pm 0.1^{\circ}\text{C}$ respectively following acclimation over three generations at 25°C .

Low temperature acclimation to 10°C for one generation, rather counter intuitively, acted to increase heat tolerance in the majority of clones and significantly so in five clones: Span 1, Span 3, UK 1, Swed 1 and Swed 2. Increases in ULT_{50} ranged from 0.1°C for Span 1, raising ULT_{50} from $41.8 \pm 0.1^{\circ}\text{C}$ to $41.9 \pm 0.1^{\circ}\text{C}$, to 0.3°C for Swed 1, increasing ULT_{50} from $41.8 \pm 0.1^{\circ}\text{C}$ to $42.1 \pm 0.1^{\circ}\text{C}$. For the majority of clones, further acclimation for three generations at 10°C did not significantly alter ULT_{50} . However, for clones UK 1, Swed 1 and Swed 3, additional acclimation resulted in a further significant increase in ULT_{50} . The ULT_{50} of UK 1 increased from $41.9 \pm 0.1^{\circ}\text{C}$ to $42.1 \pm 0.1^{\circ}\text{C}$, the same level of heat tolerance gained after three generations at 25°C . The ULT_{50} of clones Swed 1 and Swed 3 increased to $42.3 \pm 0.1^{\circ}\text{C}$, conferring greater levels of heat tolerance than when acclimated at 25°C .

4.4.3 Differentials between LLT_{50} and ULT_{50}

Due to varying effects of long term acclimation on lethal temperatures, the relationships between upper and lower thresholds were investigated using three generation acclimation data. Owing to the phenomenon known as ‘telescoping of generations’ whereby aphids begin development within their grandparents, acclimation to three generations removes any maternal effects and will consequently allow detection of potential relationship between rearing temperature and thermal tolerance range.

The thermal tolerance ranges of aphids were calculated by subtracting LLT_{50} from ULT_{50} . The differentials were plotted against rearing temperature and are displayed in Figure 11. Variation in ULT_{50} is much lower compared with the LLT_{50} and, as a consequence, thermal tolerance ranges can be expanded following acclimation to a low temperature (10°C) compared to a high temperature (25°C). For clones Span 2, Span 3, UK 1, UK 2, UK 3 and Swed 2, thermal tolerance range decreased at a rate of approximately 0.15°C to 0.25°C per 1°C increase in rearing temperature. Aphids acclimated to 10°C were therefore able to survive over temperature ranges approximately 2.25°C to 3.75°C greater than when acclimated at 25°C . Clones Span 1, Swed 1 and Swed 3 displayed a decrease in the tolerance range of approximately 0.4°C per 1°C increase in rearing temperature. Therefore, the temperature range over which aphid clones Span 1, Swed 1 and Swed 3 could survive was approximately 6°C greater when acclimated to 10°C compared to 25°C . The greater rates observed for Span 1, Swed 1 and Swed 3 are a consequence of the clones being the most cold tolerant with LLT_{50} values below -16°C .

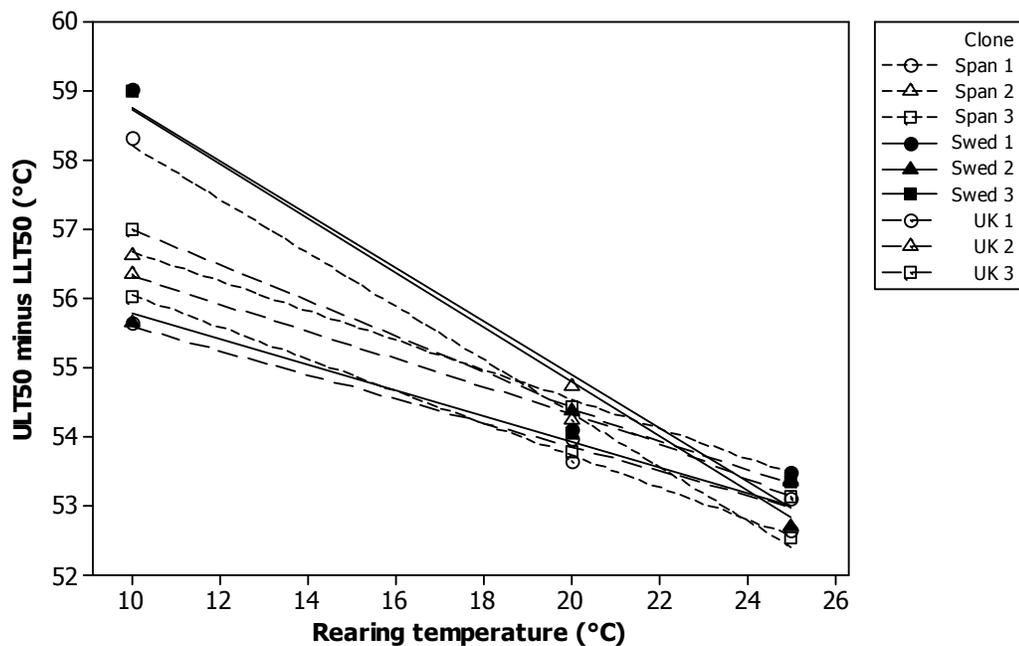


Figure 11. ULT_{50} minus LLT_{50} plotted against rearing temperature for *Myzus persicae* clones collected from Spain, UK and Sweden (codes for clones are shown in the figure). Lines were fitted using the regression fitting procedure in Minitab 15.

4.5 Discussion

In this chapter, the results of experiments designed to study the variation and plasticity of lethal temperatures of *Myzus persicae* clones from various latitudes in Europe are presented. The aims of the experiments were to: 1. Determine the level of plasticity in aphid lethal temperature thresholds and to elucidate how the traits are affected by rearing temperature. 2. Establish if lethal temperature thresholds are related to latitude. 3. Investigate relationships between the upper and lower lethal temperatures and variation in the thermal tolerance ranges with rearing temperature.

4.5.1 Plasticity of lethal temperatures

In the event of climate change, the level of plasticity in the lethal temperature thresholds of *M. persicae* will have profound implications for how the species will be affected. Failure to adapt will ultimately result in changes to mortality, impacting species abundance, distribution and pest status. The current study confirms that lethal temperature thresholds of *M. persicae* are not fixed traits and can be altered following acclimation at low (10°C) or high temperatures (25°C). Cold tolerance could be increased following acclimation at low temperature, with one generation at 10°C being sufficient to significantly decrease LLT₅₀ (reductions in LLT₅₀ ranged from 0.8°C to 4.2°C), indicating a level of phenotypic plasticity within a single generation.

Aphids are atypical in the insect world due to having telescoping of generations. This means that a viviparous, parthenogenetically reproducing female aphid has within her developing daughters, which in turn have their daughters developing inside them. As a consequence, aphids begin development within their grandmothers (Kindlmann & Dixon, 1989). For that reason, an aphid can, in theory, begin acclimation prior to birth and progressively increase levels of acclimation with each subsequent generation (Hazell *et al.*, 2010a). This was supported by data collected on *S. avenae* whereby cold tolerance increased with each successive generation at 10°C (LLT₅₀ declined from -8.5°C when reared at 20°C to -13.6°C after one generation at 10°C, to -15.0°C after two generations, and to -16.4°C after three generations) (Powell & Bale, 2008). Due to the short generation time of aphids, this short term acclimation, allowing a very rapid response to a change in temperature, was proposed to be of much greater importance to aphid species than long term acclimation processes. Powell and Bale (2008) state that the ability to respond rapidly to sudden changes in temperature (i.e. single generation acclimation) and the capacity to increase cold tolerance throughout successive generations to track the declining environmental temperatures experienced from summer to winter (i.e. intergenerational acclimation) are vital to the winter survival of anholocyclic (asexual) aphids. The current study confirms that *M. persicae* is capable of rapid acclimation within a single generation. However, data do not support consistent intergenerational acclimation. Further acclimation to three generations at 10°C acted to significantly reduce LLT₅₀ further in only three of the nine experimental clones, emphasising

that aphid cold tolerance differs between clones, and also explaining how previous studies on single clones (Clough *et al.*, 1990; Powell & Bale, 2008) observed changes in cold hardiness over successive generations when aphids were maintained in an acclimation regime.

M. persicae could rapidly respond to declining temperatures with increasing cold tolerance. However, cold tolerance was lost following acclimation at high temperatures (25°C). One generation at 25°C caused a reduction in LLT₅₀ ranging between 0.7°C to 1.7°C when compared to results obtained at 20°C. A rapid loss of acquired cold tolerance would be expected given that resource investment in maintaining unnecessary cold tolerance on cessation of winter temperatures is of little benefit. Detrimental effects have been associated with mechanisms of heat tolerance, with induced heat shock proteins causing a reduction in cell growth rate (Feder *et al.*, 1992). It is plausible that investment of resources in unnecessary low temperature protection could lead to ecological costs in the form of reduced development, longevity and fecundity. Natural selection is unlikely to favour unnecessary thermal tolerance and as a consequence, on cessation of winter temperatures, any cold tolerance acquired should be lost, as is evident in the current study. Additional acclimation over three generations did not lead to any further significant loss in cold tolerance in all but one of the nine experimental clones, once again suggesting a lack of intergenerational acclimation in *M. persicae*.

In addition to the acquisition of cold tolerance, data suggest that *M. persicae* can gain heat tolerance in a similar way following acclimation at high temperatures. Although less responsive than LLT₅₀, ULT₅₀ could still be significantly raised following one generation at 25°C, leading to increased clonal heat tolerance (increases in ULT₅₀ ranged between 0.1°C and 0.6°C). Interestingly, heat tolerance was also increased following acclimation at 10°C, significantly increasing ULT₅₀ in five of the nine experimental clones after one generation and in seven out of the nine clones by three generations when compared to data collected at 20°C (increases in ULT₅₀ ranged between 0.1°C and 0.5°C). This observation would suggest that the physiological process involved in conferring heat tolerance is also induced at low temperatures. Exposure to a heat stress results in the up-regulation of heat shock proteins (Feder *et al.*, 1992; Sørensen *et al.*, 2003; Bahrndorff *et al.*, 2009b). These heat shock proteins, the most common of which is Hsp70, aid survival at unfavourably high

temperatures by functioning as molecular chaperones, assisting in the re-folding of proteins once the heat stress is removed and preventing protein aggregation (Neven, 2000; Sørensen *et al.*, 2003). Acclimation at 25°C could prove stressful enough to *M. persicae* to induce up-regulation of heat shock proteins, resulting in greater subsequent survival at high temperatures and causing the observed rise in ULT₅₀. Heat shock proteins are also induced by low temperature extremes (Feder & Hofmann, 1999) and are considered vital for winter survival in the diapausing flesh fly *Sacophaga crassipalpis* (Delinger, 2007), offering a possible explanation for the observed rise in ULT₅₀ following acclimation at 10°C.

4.5.2 Geographic variation in thermal tolerances

In order to make predictions about how insect populations will be affected by climate change, an understanding of how thermal biology varies with latitude is required. Such information will further provide insight into the level of gene flow and clonal mixing between aphid populations.

If clonal mixing is limited across Europe, local adaptation in aphid populations would be expected. As hypothesised, in a Mediterranean climate where long-term populations could persist, or at least, be possible, increased heat tolerance and reduced cold tolerance would be predicted. Aphids of Scandinavian origin could be predicted to display increased cold tolerance and reduced heat tolerance and temperate clones to show intermediate levels of thermal tolerance. However, due to harsh winters controlling the numbers of anholocyclic clones and a lack of primary host plants preventing persistence of holocyclic clones in Scandinavia, it is possible that Scandinavian populations do not persist continuously and that aphids found outdoors in summer are the result of annual immigration from other parts of Europe. It is this method by which outdoor *M. persicae* populations persist in the northern Great Plains of America, with spring migrations from the south re-establishing populations annually (Zhu *et al.*, 2006). As a consequence, Scandinavian clones would not be expected to display greater cold tolerance than their Mediterranean and temperate counterparts as the aphids found at this most northerly location would be of annually mixed origin.

Data collected in the current study do not suggest a relationship with latitude. UK 1 was one of the least cold tolerant clones, consistently displaying some of the highest LLT₅₀ temperatures across all acclimation treatments. Swed 3 proved to be a relatively thermal tolerant clone following low temperature acclimation displaying both high ULT₅₀ values and low LLT₅₀ values, indicating a high level of both cold and heat tolerance. Likewise, Swed 1 displayed high levels of heat tolerance following low temperature acclimation and high levels of both cold and heat tolerance following high temperature acclimation. Conversely, Span 3 exhibited limited thermal tolerance following high temperature acclimation, displaying low levels of heat tolerance and cold tolerance. With regard to lethal temperatures, it must be concluded that hypothesis 1 and 2 stated on page 32 of the Introduction are not supported i.e. hypothesis 3 is supported stating that there is no direct relationship between thermal tolerance and the latitudinal site of collection. Greater heat tolerance was not evident in the Spanish clones and, against predictions, the Swedish clones proved to be some of the most heat and cold tolerant. Due to the absence of any clear relationship with latitude, it is concluded that clonal mixing throughout Europe is extensive so as to prevent local adaptations in thermal tolerance with aphids experiencing widespread dispersals on low-level jet streams (Zhu *et al.*, 2006).

Microsatellite analyses to determine clonal 'type' indicated that both Swed 1 and Swed 3 were Type O clones, suggesting that the two clones originate from the same stem mother through asexual propagation (Kasprowicz *et al.*, 2008). The LLT₅₀ values of Swed 1 and Swed 3 did not significantly differ following acclimation to 25°C both over one and three generations and to 10°C over three generations. Similarly, the ULT₅₀ values of Swed 1 and Swed 3 did not significantly differ following acclimation to 10°C both over one and three generations and to 25°C for three generations. Due to the comparatively high levels of thermal tolerance displayed by both clones and the lack of significant differences across the majority of acclimation treatments, thermal tolerance may be better described by clonal type rather than latitude.

The other predominant clonal type of experimental clones was Type C and included the clones Swed 2, UK 1 and UK 2. Swed 2 and UK 1 did not significantly differ in their relative cold hardiness following low temperature acclimation or in their relative heat

tolerance following acclimation to all temperature treatments with the exception of 25°C after three generations. UK 2 only proved non-significantly different to Swed 2 with regards to LLT₅₀ values. Microsatellite analysis determines if clones are identical at the loci tested and, as a consequence, does not conclude that the clones are genetically identical, but instead, that they share the same stem mother. A lack of conformity between all Type C clones could therefore be a consequence of some variation in genetic similarity between clones.

The remaining four clones were genetically distinct, being different from one another and to the Type O and Type C clones, with UK 3 being a Type J and Span 1, 2 and 3 being unique i.e. different microsatellite patterns that have not, as of yet, been assigned a type. These four clones therefore cannot provide information on the idea that clones of the same type are more similar in their levels of thermal tolerance. However, they can provide an insight into the level of dissimilarity between clones of different types. No consistent trend between clones of differing types was observed with the exception of UK 2 (a Type C) and UK 3 (a Type J) with the LLT₅₀ values of both clones not significantly differing across all acclimation treatments, suggesting that a more complex interaction between clonal type and latitude could be at play. To determine if thermal tolerance is related to clonal type and to test for potential interactions between clonal type and latitude, a more comprehensive study involving a greater number of clonal types and replicates of each type would be required and could form the focus of future research. Accordingly, if clonal types are revealed to vary in thermal tolerance, clonal types could respond independently to climate change, changing the relative ratios of types within populations of *M. persicae*.

4.5.3 Relationship between LLT₅₀ and ULT₅₀

The lower thermal limits of many insects show greater variability and are more responsive to acclimation than upper thermal limits (Addo-Bediako *et al.*, 2000). This is certainly true of *M. persicae* and suggests that the thermal limits are ‘decoupled’ as opposed to fixed. Since variation in ULT₅₀ was less marked than variation in LLT₅₀, thermal tolerance ranges could be expanded following low temperature acclimation. For clones Span 1, Swed 1 and Swed 3

the temperature range over which aphids could survive increased by 0.4°C for every 1°C decrease in rearing temperature. The remaining clones experienced a reduced rate of 0.15 - 0.25°C increase per 1°C decrease in rearing temperature. Current estimates of climate change predict that global mean surface air temperatures could increase by between 1°C and 3.5°C by 2100 in comparison to 1990 temperatures resulting in greater abundance of pest insects (Cannon, 1998). However, a reduction in the thermal tolerance range of *M. persicae* could result in greater susceptibility to unpredictable bouts of extreme weather such as frosts and heat waves.

The evolution of plasticity is predicted to be more common in environments that are predictably variable (Deere *et al.*, 2006). Where environmental cues are unreliable, the evolution of plasticity will not be favoured. The ability to acclimate should therefore be more common in temperate species where variations in temperature occur on a predictable, seasonal basis. Should this theory apply to aphid clones, temperate clones should display the greatest levels of plasticity and Mediterranean clones the least. However, of the nine experimental clones in the current study, it is Span 1, Swed 1 and Swed 3 that prove to be the most plastic, displaying greater acclimation responses at low temperatures and consequently greater tolerance ranges. Current data do not suggest a relationship between plasticity and environmental predictability, once again supporting the idea that clonal intermixing is extensive throughout Europe.

In summary, data demonstrate that lethal temperature thresholds are plastic in *M. persicae* and can be altered through short term acclimation. ULT_{50} could be increased through both high temperature and low temperature acclimation, suggesting that the physiological processes involved in heat resistance are also induced by low temperature acclimation. Due to LLT_{50} being more plastic than ULT_{50} , thermal tolerance ranges are compressed with increasing rearing temperature, which could have implications for the thermal tolerance of the species if current trends in global warming continue. Data did not provide evidence for a relationship between temperature lethality and latitude, suggesting that clonal mixing occurs over large scales throughout Europe preventing local adaptation. A possible relationship with clonal type was suggested, although this requires vigorous testing to confirm and could provide the focus for future work.

5 Comparison of the thermal activity thresholds of clones of the peach-potato aphid *Myzus persicae* collected along a latitudinal gradient in Europe

5.1 Summary

Warming of Earth's temperature, in conjunction with a decline in the frequency of winter frosts, could put increased importance on sub-lethal activity thresholds over lethal temperatures in predicting the distribution and abundance of aphids such as *M. persicae*. For an aphid to survive at temperatures within the range of potentially lethal temperatures, it is crucial that their ability to walk and to avoid temperature-induced torpor is not compromised. Results from the current study demonstrate that low temperature activity thresholds could be decreased following low temperature (10°C) acclimation as indicated by depressed movement threshold (e.g. from 8.8°C to 2.5°C for UK 1 and from 6.5°C to 0.7°C for Span 3 after one generation at 10°C) and chill coma temperatures (e.g. from 4.8°C to 2.0°C for UK 2 and from 1.7°C to -0.8°C for Swed 2 after one generation at 10°C). High temperature activity thresholds could be increased following high temperature (25°C) acclimation, as indicated by raised heat movement threshold temperatures (e.g. from 40.1°C to 41.1°C for Swed 1 and from 40.2°C to 40.9°C for Span 2 and Span 3 after one generation at 25°C) and heat coma temperatures (e.g. from 41.4°C to 42.3°C for Swed 1 and from 41.9°C to 42.6°C for Span 2 after one generation at 25°C). However, data did not provide any evidence of intergenerational acclimation in *M. persicae*.

A relationship between activity thresholds and latitude was not evident with the exception of data on heat coma where Scandinavian clones Swed 2 and 3 consistently had some of the lowest heat coma temperatures and Mediterranean clones Span 1, 2 and 3 some of the highest (e.g. median heat coma values at 20°C were 42.1, 41.9 and 42.5°C respectively for Span 1, Span 2 and Span 3, and 41.4, 41.3 and 41.3°C for Swed 1, Swed 2 and Swed 3 respectively). Either clonal mixing occurs over large scales across Europe preventing local adaptation, or local adaptation is limited to the Mediterranean where conditions enable long

term persistence of clonal populations and thus adaptation to higher temperatures. Temperate clones UK 1 and 2 consistently showed some of the highest low temperature activity thresholds, perhaps suggesting that clonal type more strongly affects aphid thermal tolerance rather than location of collection.

High temperature activity thresholds were less plastic than low temperature activity thresholds. Consequently, thermal activity ranges could be expanded following low temperature acclimation. For the temperature range over which coma was avoided, this expanded at a rate of approximately 0.2°C per 1°C decrease in rearing temperature. Consequently aphids reared at 10°C avoided coma over a range 3°C greater than when reared at 25°C. For the temperature range over which movement was retained, the range at 10°C was 4.5 to 6°C greater than at 25°C.

5.2 Introduction

It is widely recognised that temperature greatly affects insect survival and subsequently insect populations, with survival being dependent upon the temperature and duration of exposure (Bale, 1987). With much research focusing on the detection of lethal temperature values (e.g. Knight *et al.*, 1986; Bale *et al.*, 1988; Powell & Bale, 2008) it must not be assumed that death is the only threat to insects at temperature extremes and the only index relevant to understanding aphid thermal biology. This is especially true in Mediterranean and temperate regions where temperatures are rarely extreme enough to result in direct mortality.

When insects are exposed to extreme temperatures, for example increasingly sub-zero temperatures, insects pass through a continuum of measurable behavioural traits before death occurs as a result of physiological damage. Firstly, activity levels decline as indicated by walking speed, eventually leading to a complete loss of walking ability rendering the insect immobile. Finally, a state of cold induced coma will occur (chill coma) as indicated by the last twitch of an appendage. Chill coma is usually a reversible condition from which insects will make a full recovery if warmed, although if temperatures continue to decrease, death will occur. A similar continuum, linked to neurophysiological failures, occurs following high

temperature exposure with insects first experiencing a loss of control over activities causing locomotor arrhythmicity. Uncontrollable spasms then begin, as defined by CT_{max} , and finally a heat induced coma (heat coma) occurs due to complete loss of neuromuscular activity (Hazell *et al.*, 2010b). Recent work has shown that the upper lethal temperatures and temperatures of heat coma do not differ significantly in aphids (Hazell *et al.*, 2010b), suggesting that, unlike chill coma, heat coma is not a reversible process and will result in insect death.

It is the measurement of these non-lethal thermal thresholds that is perhaps more important when trying to understand aphid thermal biology since they provide more ecologically relevant information (Macdonald *et al.*, 2004). Survival at temperature extremes is of little importance if the surviving insect is rendered inactive since it would be unable to move to find food, find a mate and thus contribute to the next generation, or to escape from predation (Mellanby, 1939; Bale, 1987). The measurement of thermal thresholds is further proposed to be more relevant than lethal temperatures in the determination of insect distribution and abundance in the event of climate change (Hazell *et al.*, 2010a) as the frequency of winter frosts decline and mean winter temperatures increase (Easterling, 2002), which will have implications for the pest and invasive status of many insects.

The majority of research into the onset of temperature induced comas has focused on the 'righting response'. This involves observation of the temperatures at which subjects lose the ability to right themselves after being artificially knocked over. The common practice is for test subjects to be heated or cooled, usually within an alcohol or water bath, and to be removed at regular intervals to test for the righting response (e.g. Gibert & Huey, 2001; Klok & Chown, 2001; Castañeda *et al.*, 2005). However, this method is not only labour intensive and accountable to error, but creates problems via the repeated disturbances of test insects which can impact body temperature, physiological status and thus the trait being measured (Hazell *et al.*, 2008). The present study adopts a method described in detail in Hazell *et al.* (2008) which, not only allows for mass testing, but reduces disturbance to the test insects and further allows for continual monitoring of insects to enable the study of hard to define behavioural traits.

To date, no study has investigated the variation in thermal thresholds of behavioural traits along a latitudinal gradient within a single aphid species. This study details a series of experiments that investigate the variation in thermal thresholds between clones collected from distinct climatic regions and the levels of intra and intergenerational plasticity following both low (10°C) and high (25°C) temperature acclimation. The thresholds investigated are detailed in Table 4.

Table 4. Thermal behaviours observed in clones of the aphid *Myzus persicae*.

Behaviour	Definition
Chill movement threshold / CTmin	Low temperature at which an insect stops gross movement (walking) and becomes immobile
Chill coma	Low temperature at which the last twitch of an appendage (antenna, leg) is observed
Chill coma recovery	Temperature at which the first twitch of an appendage (antenna, leg) is observed following chill coma
Movement (walking) recovery	Temperature at which an insect begins gross movement (walking) following chill coma
Heat movement threshold / CTmax	High temperature at which an insect stops gross movement (walking) and becomes immobile
Heat coma	High temperature at which the last twitch of an appendage (antenna, leg) is observed

5.3 Materials and methods

The nine experimental clones of *M. persicae* were kept as a stock culture at 20°C. First instar nymphs (less than 24h old) were taken from the culture and acclimated at either 10°C or 25°C for one or three generations prior to use of their offspring in experiments, as detailed in the general materials and methods chapter. All nymphs used in experiments were first instars.

5.3.1 Determination of chill movement threshold (CT_{min}) and chill coma temperature

The temperature of chill movement threshold and chill coma was determined by subjecting aphids, positioned in an aluminium arena, to a decreasing temperature regime whilst recording aphid activity using a camera which logged videos to a desktop computer. 40 first instar nymphs were placed within the aluminium arena pre-set to the culture temperature from which the nymphs were obtained. An alcohol bath was programmed to decrease the temperature of the aluminium block from the culture temperature to 10°C at a rate of 0.5°Cmin⁻¹. On reaching 10°C the aluminium block was further cooled to -6°C at a rate of 0.1°Cmin⁻¹. This minimum temperature was selected as it was low enough to guarantee that all aphids had long since entered chill coma. When using aphids from the 10°C acclimation regime, the initial cooling to 10°C was not applicable and cooling began at a rate of 0.1°Cmin⁻¹ to -6°C. During the cooling phase a camera positioned over the arena recorded aphid movement whilst logging the video, along with changing time and arena temperature, to a desktop computer. Videos were recorded at a rate of one frame per 5 sec.

On completion of the experiment, the video recording was stopped, saved to computer and the nymphs removed from the arena. The stored videos were saved in reverse format prior to playback (Using StudioPlayer, Studio86designs, UK). Videos could be played back on a desktop computer at a later date and the chill movement threshold and the chill coma temperature of individuals deduced. Videos were viewed in reverse to allow for more precise identification of the temperature at which walking ceased and chill coma commenced. Once movement was identified, the video could be paused and the arena temperature at that

specific moment in time read from the screen. Thermal thresholds were determined for 30 aphids for each clone by treatment combination.

5.3.2 Determination of heat movement threshold (CT_{max}) and heat coma temperature

The temperature of heat movement threshold and heat coma was determined by subjecting aphids positioned in the aluminium arena to an increasing temperature regime whilst, once again, recording aphid activity. 40 first instar nymphs were placed within the aluminium arena pre-set to the aphid culture temperature. An alcohol bath was programmed to increase the temperature of the aluminium block from the culture temperature to 35°C at a rate of 0.5°Cmin⁻¹. On reaching 35°C the aluminium block was further heated to 45°C at a rate of 0.1°Cmin⁻¹. The camera was positioned over the arena to record the movement of nymphs during the heating phase, at a rate of one frame per 5 sec, with the video being logged to a desktop computer.

Following heating of the aluminium block, the video recording was stopped, saved to computer and the nymphs removed from the arena. The video was saved in reverse format using StudioPlayer and the video subsequently viewed to determine the temperature at which movement ceased (heat movement threshold) and heat coma was induced. Thermal thresholds were determined for 30 aphids for each clone by treatment combination.

5.3.3 Detection of chill coma recovery

Following the experiment to determine the chill movement threshold (CT_{min}) and chill coma temperature, a new sample of 40 aphids was placed within the arena. The alcohol bath was pre-programmed to decrease the temperature of the aluminium block from culture temperature to a pre-determined sub-zero temperature at a rate of 0.5°C min⁻¹. To allow for comparisons between clones a single sub-zero temperature was selected for each acclimation regime, across all clones. These temperatures were determined using information obtained from the chill coma experiments and preliminary chill coma recovery experiments, and were

selected so that all clones, originating from the same acclimation temperature, would enter a non-lethal chill coma following a 30 min exposure to the temperature. The selected temperatures were -6°C for 10°C cultures, -4°C for 20°C cultures and -3°C for 25°C cultures.

It was originally thought that individual exposure temperatures should be determined for each clone at each acclimation treatment to standardise the level of stress experienced by each aphid. However, it was decided to subject all clones from a specific acclimation temperature to the same sub-zero temperature. The experiment was conducted in this way, firstly, to allow for comparisons between clones and, secondly, because in nature, the temperature to which living organisms are exposed does not discriminate between an individual's (or a clone's) thermal tolerance. Subjecting all clones from a specific acclimation treatment to the same exposure temperature would therefore provide ecologically meaningful results with real life implications.

Following the 30 min holding period at the minimum exposure temperature, the aluminium block was returned to culture temperature at a rate of $0.1^{\circ}\text{Cmin}^{-1}$. During the warming phase, the camera was activated and the video of aphid movement logged to the desktop computer. On reaching culture temperature the video recording was stopped and the nymphs removed from the arena.

To determine chill coma recovery, the recorded video was not saved in a reverse format, but viewed in 'correct' chronology. From video playback the temperature at which nymphs exited chill coma (as determined by the first movement of antenna or a leg) and the temperature at which they resumed walking was determined. The temperature of chill coma recovery was determined for 30 aphids for each clone by treatment combination. Unlike chill coma, heat coma is not a reversible process, resulting in insect death (Hazell *et al.*, 2010b). As a consequent, investigation of heat coma recovery was not possible.

5.3.4 Statistical analysis

Distribution fitting analysis was performed using MINITAB 15 for each thermal threshold treatment group to determine which distribution best described the data. Data were first converted from a value of temperature to duration of exposure to make data suitable for the distribution fitting analysis. Parametric distribution analysis was subsequently performed using the appropriate distribution, in all cases logistic, to allow for comparison of scale and location parameters. Individual comparisons were made using Bonferroni 95% confidence intervals. To investigate how aphid thermal activity ranges are affected by temperature, chill coma values were subtracted from heat coma, and chill movement threshold values subtracted from heat movement threshold and residuals were plotted against rearing temperature.

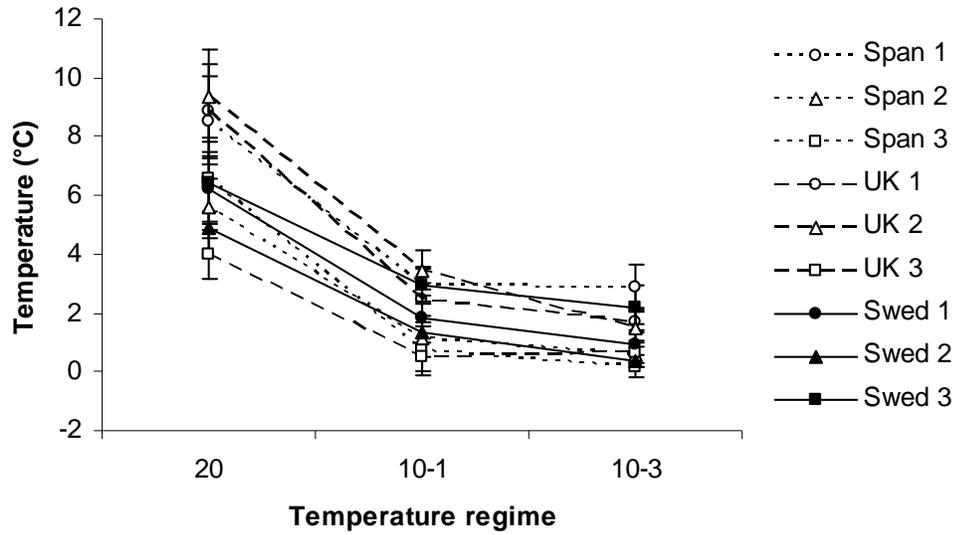
5.4 Results

5.4.1 Chill movement threshold (CT_{min}) and chill coma temperature

5.4.1.1 Clonal differences in low temperature activity thresholds

Strongly significant differences between the chill movement threshold of different clones were observed at 20°C ($\chi^2_8 = 74.50$, $p < 0.0001$) and following acclimation to 10°C (one generation: $\chi^2_8 = 100.65$, $p < 0.0001$; three generations: $\chi^2_8 = 81.19$, $p < 0.0001$) and 25°C (one generation: $\chi^2_8 = 108.20$, $p < 0.0001$; three generations: $\chi^2_8 = 63.47$, $p < 0.0001$) (Figure 12). Comparison of the Bonferroni corrected 95% confidence intervals indicated that at 20°C, UK 3 retained activity at the lowest temperatures (median 4.0°C), significantly lower than temperatures obtained for clones Swed 3, Span 1, UK 1 and UK 2. UK 2 had the highest values of chill movement threshold, with movement ceasing at a median temperature of 9.4°C, significantly higher than the chill movement thresholds for clones Span 2, Swed 2 and UK 3.

I.



II.

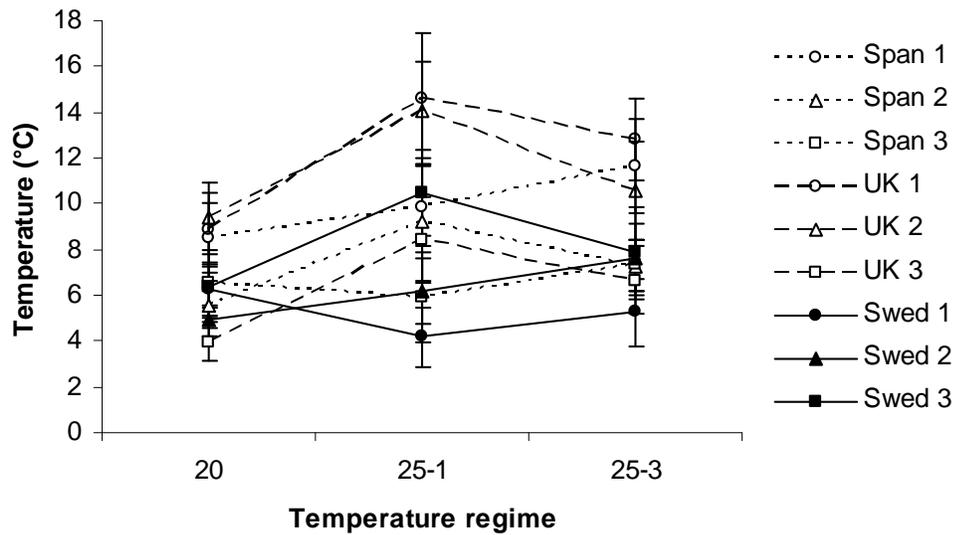


Figure 12. Effects of acclimation on chill movement threshold following acclimation from 20°C to I. 10°C for one and three generations and II. 25°C for one and three generations. Data points represent median chill movement ($\pm 95\%$ CI) for *Myzus persicae* clones collected from Spain, UK and Sweden (codes for clones are shown in figure). Temperature regime is displayed as the acclimation temperature with the number of generations at that temperature indicated after the dash symbol.

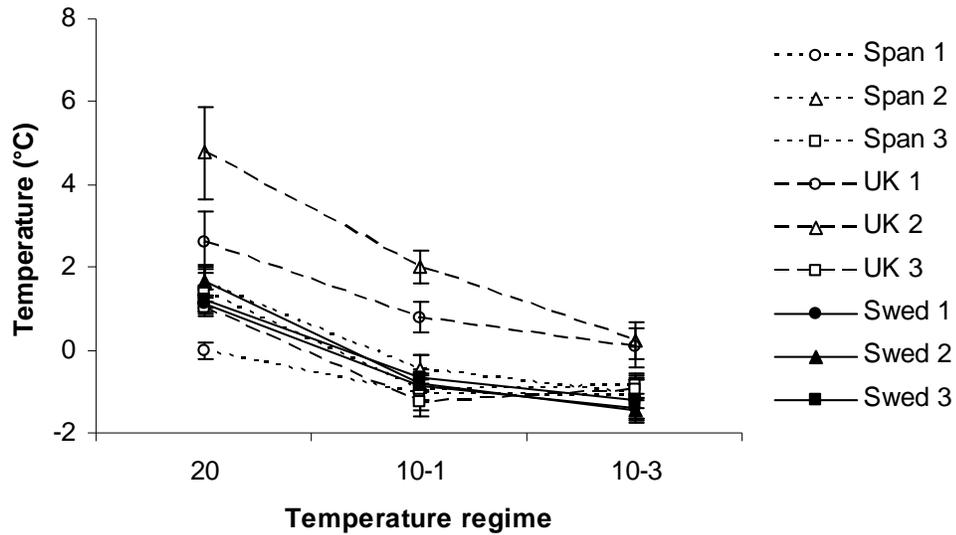
Following acclimation to 10°C for one generation, clone UK 3 once again had some of the lowest activity threshold temperatures and UK 2 some of the highest. Clones UK 3, Span 3 and Span 2 ceased movement at significantly lower temperatures (median values 0.5, 0.7 and 1.1°C respectively) than clones Swed 3, Span 1 and UK 2 (median values 2.9, 3.0 and 3.5°C respectively). Clones Swed 2, Swed 1 and UK 1 proved intermediate in their activity thresholds. Acclimation to 10°C for three generations resulted in no further significant decrease in activity thresholds. Span 3, Swed 2, Span 2 and UK 3 remained active to the lowest temperatures (median chill movement temperature 0.2, 0.4, 0.6 and 0.6°C respectively), significantly lower than UK 1, Swed 3 and Span 1 (median chill movement temperature 1.7, 2.2 and 2.8°C respectively). Therefore, clones Span 1, UK 1, UK 2 and Swed 3 proved to be the least able to maintain activity to low temperatures, having some of the highest chill movement threshold temperatures in the majority of culture and low temperature treatments. Clones Span 2, Span 3, UK 3 and Swed 2 were consistently the best able to remain active at the lowest temperatures.

After acclimation to 25°C for one generation, clones UK 1 and UK 2 ceased movement at higher temperatures than all other clones (medians 14.6 and 14.1°C respectively), although these proved only significantly higher than clones UK 2, Span 3, Swed 2 and Swed 1. Swed 1 and Swed 2 maintained the lowest activity threshold temperatures (medians 4.2 and 6.2°C respectively). Additional acclimation over three generations at 25°C resulted in Swed 1 once again ceasing movement at the lowest temperatures of all the clones (median 5.3°C) and UK 1 ceasing movement at the highest of temperatures (median 12.8°C) suggesting that the first sub-Arctic clone can remain active to 7.5°C below the first temperate clone.

Significant differences in the temperature of chill coma were observed between the different clones at the culture temperature of 20°C ($\chi^2_8 = 185.58$, $p < 0.0001$) and following acclimation to 10°C (one generation: $\chi^2_8 = 250.27$, $p < 0.0001$; three generations: $\chi^2_8 = 70.47$, $p < 0.0001$) and 25°C (one generation: $\chi^2_8 = 92.87$, $p < 0.0001$; three generations: $\chi^2_8 = 151.60$, $p < 0.0001$) (Figure 13). Comparison of the Bonferroni corrected 95% confidence intervals indicated that at 20°C, clone Span 1 coma values were significantly lower than all other clones (median 0.0°C) suggesting the clone to be the best able to avoid entering a cold induced coma at low

temperatures. UK 2 was the least able to avoid a cold induced coma (median 4.8°C), entering chill coma at significantly higher temperatures than all other clones excluding UK 1.

I.



II.

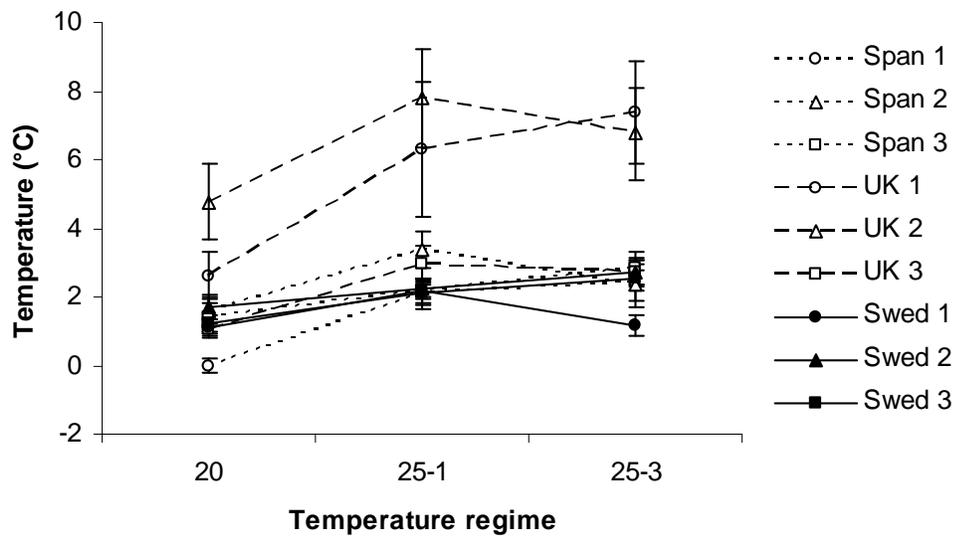


Figure 13. Effects of acclimation on chill coma threshold following acclimation from 20°C to I. 10°C for one and three generations and II. 25°C for one and three generations. Data points represent median chill coma ($\pm 95\%$ CI) for *Myzus persicae* clones collected from Spain, UK and Sweden (codes for clones are shown in figure). Temperature regime is displayed as the acclimation temperature with the number of generations at that temperature indicated after the dash symbol.

Following acclimation at 10°C for one generation clones UK 1 and UK 2 were the least tolerant to low, coma inducing temperatures (respective medians 0.8 and 2.0°C), entering chill coma at significantly higher temperatures than all other clones (medians ranging between -0.5°C for Span 2 to -1.3°C for UK 3). Additional acclimation at 10°C over three generations resulted in clones UK 1 and UK 2 once again having the highest chill coma temperatures (respective medians of 0.1 and 0.2°C), significantly higher than all other clones (medians ranging between -0.9°C for Span 3 and UK 3 to -1.5°C for Swed 2). After acclimation to 25°C for one generation, UK 2 was the least tolerant (median 7.8°C), entering chill coma at significantly higher temperatures than all other clones excluding UK 1. Following additional acclimation for three generations at 25°C, chill coma values for both UK 1 and UK 2 were significantly higher than all other clones (respective medians 7.4 and 6.8°C). Swed 1 had the lowest temperatures of chill coma (median 1.2°C) with chill coma values significantly lower than all other clones excluding Span 1.

5.4.1.2 Effect of acclimation on low temperature activity thresholds

A relationship of decreasing activity threshold with decreasing acclimation temperature was evident for chill coma (Figure 13) and, to a lesser extent, for chill movement threshold (CT_{min}) (Figure 12). Activity threshold was significantly depressed in all clones following acclimation to 10°C for one generation. The greatest effect was observed for UK 1, with the chill movement threshold decreasing from 8.8°C at 20°C to 2.5°C following one generation at 10°C enabling the clone to maintain the ability to walk to 6.3°C lower. The smallest effect of acclimation was observed for UK 3 and Swed 3. Activity thresholds were depressed by 3.5°C from 4.0 to 0.5°C for UK 3 and 6.4 to 2.9°C for Swed 3. Acclimation for three generations at 10°C failed to further depress the activity threshold in all clones with the exception of UK 2 and Swed 2 for which chill movement threshold was depressed by an additional 2.0°C and 0.9°C respectively. Data suggest that intergenerational acclimation has little effect on activity threshold.

The relationship between acclimation temperature and chill movement threshold is less clear following high temperature acclimation. No significant change in chill movement

temperature was observed in clones Span 1, Span 3, Swed 1 and Swed 2, whereas significant increases were observed for the remaining five clones, with increases ranging from 3.6°C for Span 2 (from 5.6°C at 20°C to 9.2°C at 25°C for one generation) to 5.8°C for UK 1 (from 8.8°C at 20°C to 14.6°C at 25°C for one generation). Acclimation at 25°C could therefore result in the aphid losing the ability to walk at up to almost 6°C above which it would maintain activity at 20°C. Further acclimation to three generations at 25°C did not result in any significant change to the chill movement threshold temperature when compared to values obtained at 25°C after one generation, again suggesting a lack of intergenerational acclimation.

Acclimation to 10°C for one generation resulted in a significant decrease in chill coma temperatures for all clones, with differences of between 1.1°C and 2.8°C when compared to populations maintained at 20°C. The greatest decrease in chill coma was observed for UK 2 which entered chill coma 2.8°C lower following acclimation at 10°C (median 4.8°C at 20°C and 2.0°C at 10°C for one generation). The smallest change of 1.1°C was for Span 1, lowering the chill coma temperature from 0.0°C at 20°C to -1.1°C at 10°C for one generation. Further acclimation to 10°C for three generations failed to further significantly depress the temperature of chill coma for all clones excluding UK 2, which decreased from 2.0 to 0.2°C, and Swed 2, which decreased from -0.8 to -1.5°C, suggesting that intergenerational acclimation has little effect on chill coma temperature.

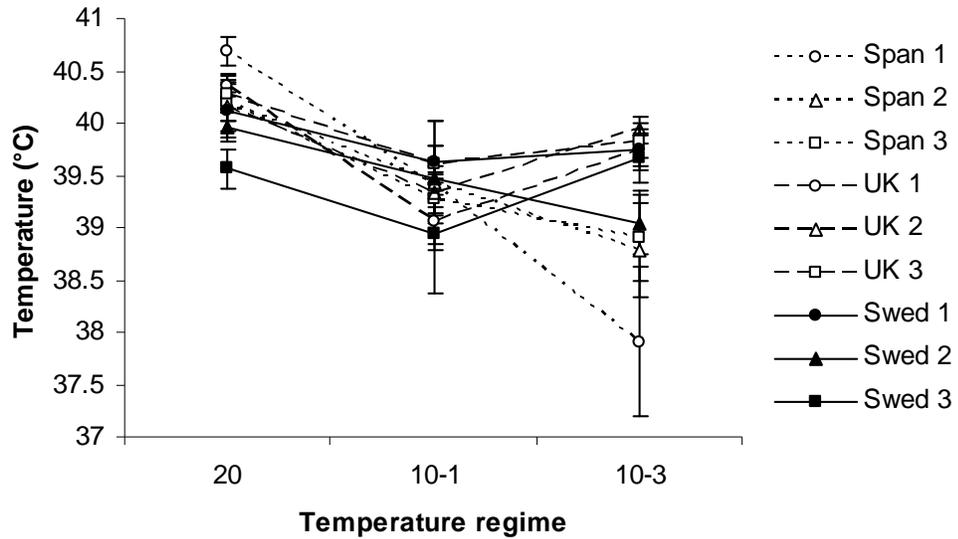
Acclimation to 25°C for one generation resulted in an increase in chill coma temperatures when compared to 20°C, although proved non-significant for Span 3 and Swed 2. The greatest significant increases were observed for UK 1 and UK 2 with the temperature of chill coma being raised by 3.7°C and 3.0°C (from 2.6 to 6.3°C and from 4.8 to 7.8°C for UK 1 and UK 2 respectively). Further acclimation over three generations at 25°C did not result in any further increase in the temperatures inducing chill coma, once again suggesting a lack of intergenerational acclimation.

5.4.2 Heat movement threshold (CT_{max}) and heat coma temperature

5.4.2.1 Clonal differences in high temperature activity thresholds

Strongly significant differences between the heat movement threshold (CT_{max}) of different clones were observed at 20°C ($\chi^2_8 = 110.10$, $p < 0.0001$) and following acclimation to 10°C (one generation: $\chi^2_8 = 22.68$, $p = 0.004$; three generations: $\chi^2_8 = 89.43$, $p < 0.0001$) and 25°C (one generation: $\chi^2_8 = 95.58$, $p < 0.0001$; three generations: $\chi^2_8 = 45.30$, $p < 0.0001$) (Figure 14). Comparison of the Bonferroni corrected 95% confidence intervals revealed that at 20°C Swed 1 ceased activity at temperatures significantly lower than all other clones (median 39.6°C). Span 1 was best able to retain the ability to walk at high temperatures, maintaining activity to 40.7°C, significantly higher than all other clones.

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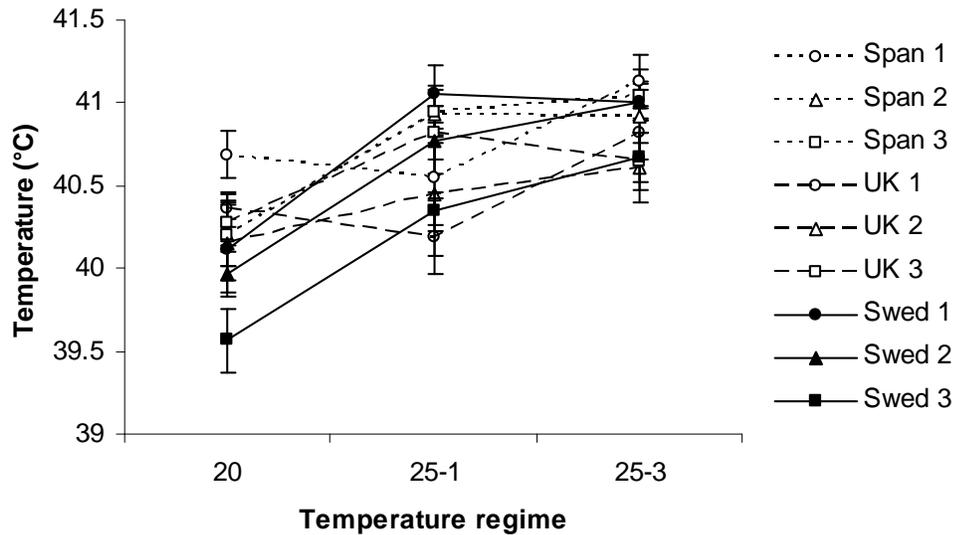


Figure 14. Effects of acclimation on heat movement threshold following acclimation from 20°C to I. 10°C for one and three generations and II. 25°C for one and three generations. Data points represent median heat movement ($\pm 95\%$ CI) for *Myzus persicae* clones collected from Spain, UK and Sweden (codes for clones are shown in figure). Temperature regime is displayed as the acclimation temperature with the number of generations at that temperature indicated after the dash symbol.

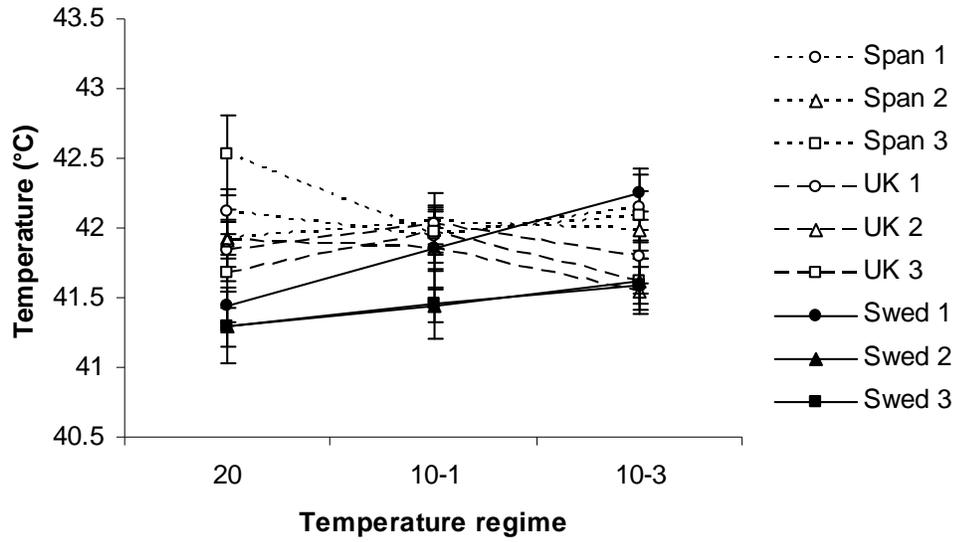
When acclimated at 10°C for one generation, temperatures of heat movement threshold did not significantly differ between clones, with the exception of Swed 1, which had the highest heat movement threshold temperature of all clones and UK 1 which had the lowest of all clones (median heat movement threshold of 39.6°C and 39.1°C respectively). Following additional acclimation at 10°C over three generations, the Mediterranean clones (medians of 37.9, 38.8 and 38.9°C respectively for Span 1, 2 and 3), ceased movement at temperatures significantly lower than UK 1, Swed 1, UK 3 and, the clone active to the highest temperatures, UK 2 (median values 39.7, 39.7, 39.8 and 39.9°C respectively).

High temperature acclimation at 25°C for one generation resulted in no significant difference between the heat movement threshold for all clones with the exception of UK 1 and Swed 3 (respective medians 40.2 and 40.3°C), which ceased movement at significantly lower temperatures than Swed 2, UK 3, Span 2, Span 3 and Swed 1 (respective medians of 40.8, 40.8, 40.9, 40.9 and 41.1°C). Further acclimation over three generations reduced the range between the clone exhibiting the highest heat movement threshold and the clone exhibiting the lowest from 0.9°C after one generation (Swed 1 median of 41.1°C, UK 1 median of 40.2°C) to 0.5°C after three generations (Span 1 median of 41.1°C, UK 2 median 40.6°C). Regardless of the reduction in the range of movement thresholds, UK 2 (median 40.6°C) still ceased movement at temperatures significantly lower than Swed 2, Swed 1, Span 3 and Span 1 (respective medians 41.0, 41.0, 41.0 and 41.1°C). Span 1 (median 41.1°C) ceased movement at temperatures significantly higher than UK 1, Swed 3, UK 3 and UK 2 (respective medians 40.8, 40.7, 40.6 and 40.6°C).

Significant differences in the temperature of heat coma were observed between the different clones at the culture temperature of 20°C ($\chi^2_8 = 131.14$, $p < 0.0001$) and following acclimation to 10°C (one generation: $\chi^2_8 = 69.17$, $p < 0.0001$; three generations: $\chi^2_8 = 75.62$, $p < 0.0001$) and 25°C (one generation: $\chi^2_8 = 113.77$, $p < 0.0001$; three generations: $\chi^2_8 = 129.69$, $p < 0.0001$) (Figure 15). Comparison of the Bonferroni corrected 95% confidence intervals indicated that at 20°C, Swed 2, Swed 3 and Swed 1 had the lowest median values of heat coma (medians 41.3, 41.3 and 41.4°C respectively), entering heat coma at temperatures significantly lower than the Mediterranean clones (medians for Span 1, Span 2 and Span 3 are 42.1, 41.9 and 42.5°C respectively). The temperate clones showed heat coma values

intermediate of the Mediterranean and Scandinavian clones and only significantly differed from Span 3, the clone with the highest heat coma temperature and Swed 2, the clone with the lowest heat coma temperature.

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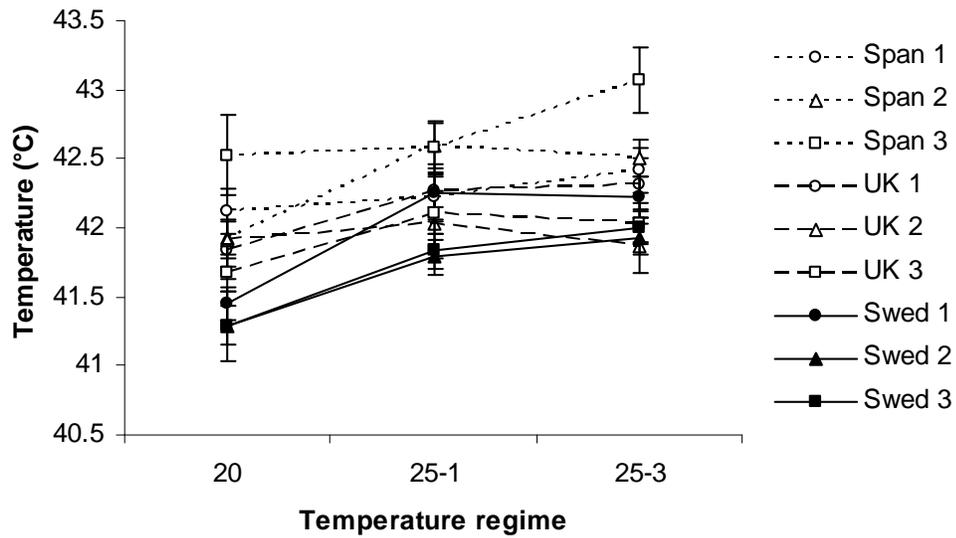


Figure 15. Effects of acclimation on heat coma threshold following acclimation from 20°C to I. 10°C for one and three generations and II. 25°C for one and three generations. Data points represent median heat coma ($\pm 95\%$ CI) for *Myzus persicae* clones collected from Spain, UK and Sweden (codes for clones are shown in figure). Temperature regime is displayed as the acclimation temperature with the number of generations at that temperature indicated after the dash symbol.

Following acclimation to 10°C for one generation, there was little evidence of the latitudinal pattern observed at 20°C. Temperatures of heat coma did not significantly differ between clones with the exception of Swed 2 and Swed 3. Swed 2 had the lowest activity threshold (median 41.4°C), entering heat coma at significantly lower temperatures than clones Swed 1, Span 1, Span 3, UK 3, UK 1 and Span 2 (respective medians 41.9, 41.9, 42.0, 42.0 and 42.1°C). Heat coma temperatures of Swed 3 were significantly lower than clones Span 1, UK 3, UK 1 and Span 2. Additional acclimation at 10°C over three generations resulted in Swed 1 having the highest high temperature activity thresholds, entering heat coma at 42.2°C, significantly higher than clones UK 2, Swed 3, Swed 2, UK 3 and UK 1 (respective medians 41.5, 41.6, 41.6, 41.6 and 41.8°C). The Mediterranean clones also showed high activity threshold temperatures (medians 42.0, 42.1 and 42.1°C respectively for Span 2, Span 3 and Span 1), although only proved significantly higher than clones UK 2, Swed 3, Swed 2 and UK 3.

Acclimation to 25°C for one generation resulted in Swed 2 and Swed 3 having the lowest threshold, with each clone having a median heat coma temperature of 41.8°C, proving significantly lower than clones Span 1, Swed 1, UK 1, Span 3 and Span 2. Span 2 and Span 3 had the highest threshold (median of 42.6°C for both) avoiding coma to temperatures 0.8°C higher than Swed 1 and 2. Heat coma temperatures for Span 2 and Span 3 were significantly higher than clones UK 3, UK 2, Swed 3 and Swed 2. After acclimation at 25°C over three generations, Swed 2 remained the clone with the lowest heat coma threshold (median 41.9°C), although only proved significantly lower than UK 1, Span 1, Span 2 and Span 3. Span 3 had the highest heat coma threshold, avoiding coma until 43.1°C, a significantly higher temperature than all clones with the exception of Span 2.

Across all acclimation treatments, clones Span 3 and Span 2 consistently had the highest high temperature activity thresholds, entering heat coma at higher temperatures and thus avoiding coma for longer. Swed 2, Swed 3 and UK 2 had consistently low activity thresholds at high temperatures, suggesting that latitudinal differences in heat tolerance do occur.

5.4.2.2 Effect of acclimation on high temperature activity thresholds

Variation in heat movement threshold and heat coma was much reduced in contrast to variation in the lower thresholds. In addition, the temperature of heat coma did not appear to show any clear relationship with rearing temperature (Figure 15), as was observed for the lower activity thresholds. The high temperature activity threshold (CT_{max}) appeared more plastic than heat coma, with acclimation treatment significantly altering the temperature at which aphids ceased movement (Figure 14). Clear effects of acclimation on heat movement threshold were evident, with acclimation at 10°C acting to decrease the heat movement threshold and acclimation at 25°C increasing the threshold when compared to aphid activity at 20°C in the majority of clones. Acclimation to 10°C significantly reduced the activity threshold for all clones, excluding Span 2 and Swed 3, resulting in aphid clones ceasing movement at lower temperatures. The largest reduction of 1.3°C was observed for clones Span 1 and UK 1, with median heat movement thresholds declining from 40.7 to 39.4°C and from 40.4 to 39.1°C respectively. Additional acclimation over three generations resulted in further significant losses in activity thresholds for Span 1 (reduction in median heat movement threshold from 39.4 to 37.9°C) and for Swed 2 (reduction in median heat movement threshold from 39.5 to 39.0°C). Conversely, UK 1 and UK 2 experienced an increase in heat movement threshold following acclimation over three generations at 10°C, with UK 1 increasing from 39.1 to 39.7°C and UK 2 from 39.3 to 39.9°C.

Movement was maintained to the highest temperatures following acclimation at 25°C. Acclimation for one generation increased heat movement threshold in all clones, with the exception of Span 1, UK 1 and UK 2, suggesting that high temperature acclimation increases high temperature activity thresholds. The greatest increases were observed for clones Swed 1 and Swed 2 with aphids remaining active for 0.8°C to 1.0°C higher when compared to aphids reared at 20°C (increase in median heat movement threshold from 40.0 to 40.8°C for Swed 2 and from 40.1 to 41.1°C for Swed 1). Additional acclimation for three generations resulted in further increases in activity threshold for only three clones: Span 1, UK 1 and Swed 3 (increase in median heat coma from 40.6 to 41.1°C, from 40.2 to 40.8°C and from 40.3 to 40.7°C respectively).

Heat coma temperature appeared less plastic than heat movement threshold. Acclimation to 10°C failed to significantly alter the temperature of heat coma in all clones with the exception of Span 3 and Swed 1. In Span 3, acclimation to the lower temperature resulted in a decrease in activity thresholds, with the temperature inducing heat coma being reduced from 42.5°C at 20°C to 42.0°C following one generation at 10°C. The reverse was true for Swed 1, with heat coma temperature actually increasing following low temperature acclimation (median heat coma 41.4°C at 20°C, increasing to 41.9°C after one generation at 10°C). Additional acclimation over three generations also failed to significantly alter heat coma temperature in all clones excluding Swed 1 and UK 3. Swed 1 showed a further increase in activity thresholds, raising the heat coma temperature by an additional 0.3°C to 42.2°C. UK 3 experienced a significant loss in heat coma tolerance, with median temperatures declining from 42.0°C following one generation to 41.6°C following three generations.

Acclimation to 25°C had a more directional effect on heat coma, significantly raising heat coma in clones Span 2, UK 1, UK 3, Swed 1, Swed 2 and Swed 3. The greatest increase of 0.9°C was observed for Swed 1, increasing median heat coma from 41.4°C at 20°C to 42.3°C after one generation at 25°C. Additional acclimation for three generations failed to further raise heat coma temperatures in all clones with the exception of Span 3, where heat coma was raised by 0.5°C from 42.6°C to 43.1°C.

5.4.3 Differentials between thermal activity thresholds

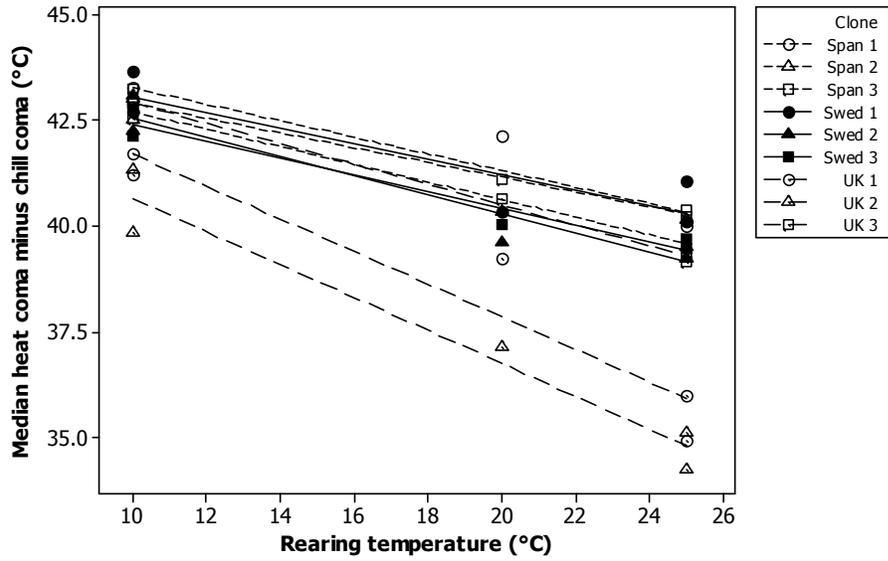
Six out of thirty six comparisons of coma temperatures showed an effect of intergenerational acclimation and nine out of thirty six comparisons of activity threshold. The data for first and third generation responses were combined to determine possible relationships between the activity thresholds and to establish any general effect of rearing temperature on aphid thermal tolerance range.

The activity threshold ranges of aphids were calculated by subtracting the chill coma from the heat coma temperature, and the chill movement threshold from the heat movement

threshold temperature. The differentials were plotted against rearing temperature and are shown in Figure 16. Variation in heat coma was less marked than chill coma and, as a consequence, activity ranges could be expanded following acclimation at lower temperatures (i.e. at 20°C when compared to 25°C, or at 10°C when compared to 20°C and 25°C). For all clones, with the exception of UK 1 and UK 2, activity range decreased at approximately 0.2°C per 1°C increase in rearing temperature. Aphids acclimated at 10°C were therefore able to avoid entering a temperature induced coma over a temperature range approximately 3.0°C greater than when acclimated at 25°C. UK 1 and UK 2 showed a greater rate of change in activity range of 0.4°C, indicating that the activity range decreased at approximately 0.4°C per 1°C increase in rearing temperature. Therefore, the temperature range over which UK 1 and UK 2 aphids avoided entering coma was 6°C less when acclimated to 25°C compared to 10°C.

With regard to movement thresholds, variation in the upper limit was once again less pronounced when compared to the lower limit, resulting in activity ranges increasing following acclimation to lower temperatures. Slopes of between -0.3 to -0.4 were observed for clones Span 1, Span 2, Span 3, Swed 2, Swed 3 and UK 3. Therefore, the temperature range over which aphids retained the ability to move decreased by between 0.3 and 0.4°C for every 1°C increase in rearing temperature. Aphids acclimated to 10°C were consequently able to move over a temperature range 4.5 to 6°C greater than aphids acclimated to 25°C. Clones UK 1 and UK 2 showed a greater negative correlation with a slope of -0.6 and -0.7 respectively. The temperature range over which movement was maintained therefore declined by between 9.0 to 10.5°C when acclimated to 25°C compared to at 10°C. The smallest slope of -0.15 was observed for Swed 1, indicating that the thermal range over which movement was maintained only declined by 0.15°C per 1°C increase in rearing temperature, a loss of only 2.25°C when reared at 25°C compared to 10°C. This is due to the relative ability of Swed 1 aphids to maintain cold tolerance following high temperature acclimation.

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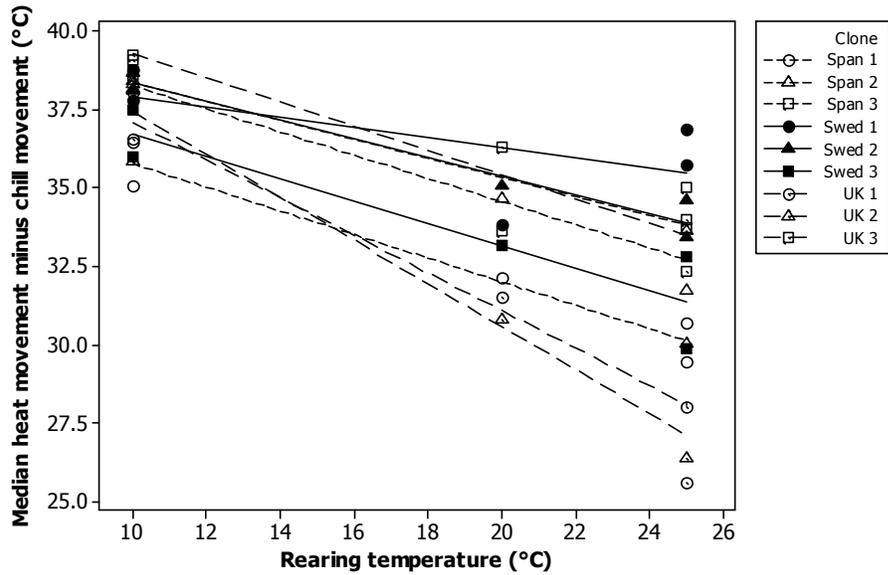


Figure 16 I. Median heat coma minus median chill coma plotted against rearing temperature and II. median heat movement threshold minus median chill movement threshold plotted against rearing temperature for *Myzus persicae* clones collected from Spain, UK and Sweden (codes for clones are shown in figure). Lines were fitted using the regression fitting procedure in Minitab 15.

5.4.4 Chill Coma Recovery

Significant differences in the time duration before aphids exited a state of chill coma, as indicated by the first twitch of a leg or antenna, were revealed between clones at the culture temperature of 20°C ($\chi^2_8 = 102.92$, $p < 0.0001$), following acclimation to 10°C (one generation $\chi^2_8 = 63.81$, $p < 0.0001$; three generations $\chi^2_8 = 91.95$, $p < 0.0001$) and 25°C (one generation $\chi^2_8 = 118.93$, $p < 0.0001$; three generations $\chi^2_8 = 28.31$, $p < 0.0001$). The time duration before aphids began spontaneous walking following chill coma also revealed significant differences between clones at the culture temperature of 20°C ($\chi^2_8 = 81.53$, $p < 0.0001$), following acclimation to 10°C (one generation $\chi^2_8 = 264.23$, $p < 0.0001$; three generations $\chi^2_8 = 228.72$, $p < 0.0001$) and 25°C (one generation $\chi^2_8 = 171.78$, $p < 0.0001$; three generations $\chi^2_8 = 151.56$, $p < 0.0001$).

At the culture temperature of 20°C, median time duration to exit chill coma ranged from 78.5 to 100.5 min (Figure 17). Following chill coma induction at -4°C, aphids therefore did not exit chill coma until temperatures between 3.9°C and 6.1°C were reached when warmed at a rate of 0.1°Cmin⁻¹. Clones Span 2, UK 2, UK 3, Swed 1 and Swed 3 took the longest duration to recover from chill coma (respective medians of 96.3, 98.4, 97.4, 97.5 and 100.5 min) and did not significantly differ in duration, although took significantly more time to recover than clones UK 1 (with the exception of Swed 1 and UK 2), Swed 2, UK 1, and Span 3. Swed 1 recovered from chill coma after the shortest duration (median 78.5 min which approximates to 3.9°C), a significantly shorter duration than the other clones with the exception of Span 1, Span 3, UK 1 and Swed 2 (respective medians of 83.4, 83.4, 82.4 and 78.5 min).

Spontaneous walking for aphids acclimated to 20°C occurred after a median time duration of between 124.4 and 155.0 min, approximating temperatures ranging from 8.4°C to 11.5°C (Figure 17). Clones Span 3 and Swed 2 were the first to regain movement (median time duration of 124.4 and 125.3 min respectively), significantly less than clones Swed 3, UK 3, UK 2 and Swed 1. Swed 1 took the greatest time to spontaneously walk following chill coma with a median time duration of 155.0 min, approximating to 11.5°C, which proved significantly longer than all clones with the exception of Span 1.



Figure 17. Time duration (min) following which aphids exited chill coma $\pm 95\%$ CI (as indicated by the top of the white bar) and spontaneously move $\pm 95\%$ CI (as indicated by the top of the grey bar) following exposure to -4°C for 30 min and then re-warmed to acclimation temperature at a rate of $0.1^{\circ}\text{C min}^{-1}$ for all clones acclimated to 20°C .

Aphids acclimated to 10°C were subjected to -6°C for 30 min to induce chill coma and re-warmed to 10°C at a rate of $0.1^{\circ}\text{C min}^{-1}$. Recovery time from chill coma following acclimation to 10°C for one generation ranged from a median of 68.8 to 88.5 min, approximating a temperature of 0.9°C to 2.9°C (Figure 18I). The first clones to exit from chill coma were Swed 2 and UK 3 (median duration of 68.8 and 69.7 min respectively), although this proved significantly different only from Span 2 (median duration of 88.5 min) and, for Swed 2, significantly different from Span 3 (median duration of 85.7). Span 2, taking 88.5 min to exit chill coma, took significantly longer than clones Swed 2, UK 3, Swed 1, UK 1 and Span 1.

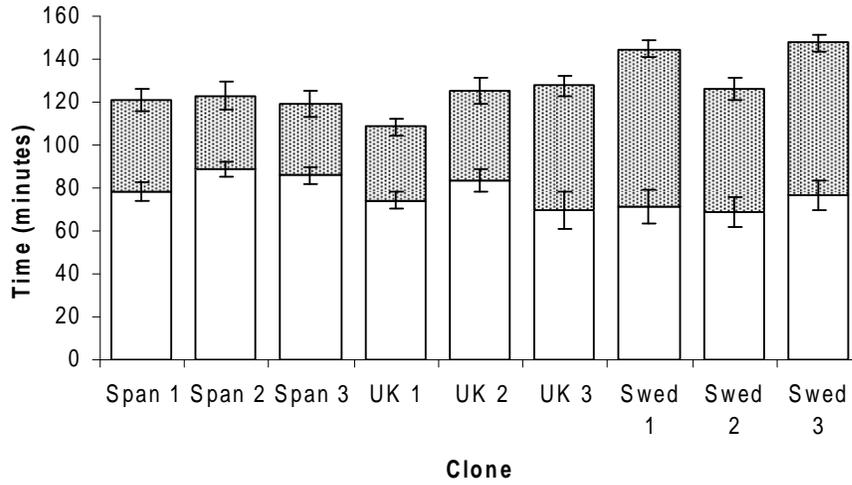
The time taken until aphids spontaneously walked following chill coma ranged from 108.5 to 147.5 min, approximating to 4.9°C to 8.8°C (Figure 18I). UK 1 took the shortest duration of 108.5 min to engage in spontaneous walking, significantly shorter than all clones with the exception of Span 3 and Span 1. Scandinavian clones Swed 1 and Swed 3 took significantly longer to spontaneously walk than all clones, with median durations of 144.6 and 147.5 min

respectively. Although Swed 1 and Swed 3 exited chill coma at relatively low temperatures, results would suggest that they require more time until they regain the ability to walk.

Following further acclimation over three generations at 10°C, the time required until aphids exited chill coma was significantly reduced for clones Span 2 (from 88.5 to 77.7 min), Span 3 (from 85.7 to 76.2 min) and Swed 3 (from 76.3 to 55.1 min). Conversely, the time required to exit chill coma significantly increased for Swed 1, increasing from 71.2 to 89.4 min. For all remaining clones, significant intergenerational effects were not observed. The median duration to exit chill coma ranged from 55.1 to 89.4 min, approximating temperatures of -0.5°C to 2.9°C (Figure 18II). Swed 1 required the longest duration of 89.4 min to exit chill coma, significantly longer than all clones with the exception of UK 2 (median 83.6 min). Swed 3 showed the shortest chill coma recovery duration of 55.1 min, significantly less than all clones excluding Swed 2 and UK 3 (respective medians 65.8 and 65.6 min).

No intergenerational effects were observed in the time taken until spontaneous walking following chill coma, with further acclimation to three generations failing to either increase or decrease the duration significantly. The median duration until spontaneous walking occurred ranged from 107.7 to 140.9 min, approximating to 4.8°C to 8.1°C (Figure 18II). The longest durations until spontaneous walking were observed in the three Scandinavian clones. Swed 1 and Swed 3 took significantly longer than all clones, with median durations of 140.9 and 138.2 min respectively until spontaneous walking. Although Swed 2 had a median duration of 129.9 min, it only proved significantly greater than UK 1. UK 1 required the shortest duration until spontaneous walking following chill coma, with a median of 107.7 min, significantly less time than all other clones with the exception of Span 3. Once again, although the Scandinavian clones exited chill coma after relatively short durations, they required longer time until normal walking could resume.

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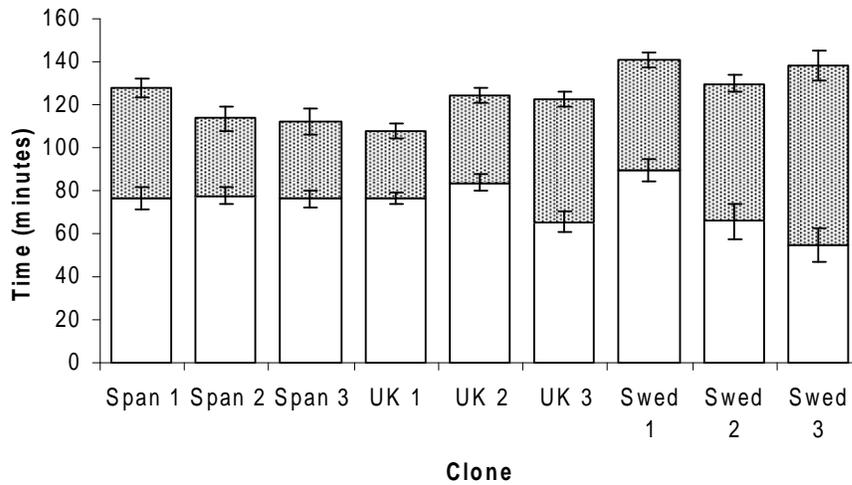


Figure 18. Time duration (min) following which aphids exit chill coma $\pm 95\%$ CI (as indicated by the top of the white bar) and spontaneously move $\pm 95\%$ CI (as indicated by the top of the grey bar) following exposure to -6°C for 30 min and then re-warmed to acclimation temperature at a rate of $0.1^{\circ}\text{C min}^{-1}$ for all clones when acclimated to I. 10°C for one generation and II. 10°C for three generations.

Aphids acclimated to 25°C were subjected to -3°C for 30 min to induce chill coma and re-warmed to 25°C at a rate of 0.1°Cmin⁻¹. Recovery time from chill coma following acclimation to 25°C for one generation ranged from a median of 65.2 to 99.7 min, approximating a temperature of 3.5°C to 7.0°C (Figure 19I). The shortest duration of 65.2 min until chill coma was exited was displayed by UK 1, which proved significantly shorter than Span 3, UK 2, Swed 1, Swed 2 and UK 3 (respective medians of 81.9, 83.8, 88.3, 94.4 and 99.7 min). UK 3 required the greatest amount of time to recover from chill coma (median 99.7 min), significantly more time than UK 1, Span 1, Span 2, Swed 3 and Span 3 (respective medians of 65.2, 70.6, 72.0, 79.8 and 81.9 min).

The duration until aphids spontaneously walked following chill coma ranged from 103.5 to 161.5 min, approximating 7.4°C to 13.2°C (Figure 19I). UK 1 took the shortest duration of 103.5 min to spontaneously walk, significantly less than all other clones. Although clones Span 1 and Swed 1 required the longest duration until spontaneous walking following chill coma (median value 161.5 and 159.5 min respectively), this proved only significantly longer than that required by UK 1, Span 2, Swed 2, UK 2 and, for Swed 1, Span 3.

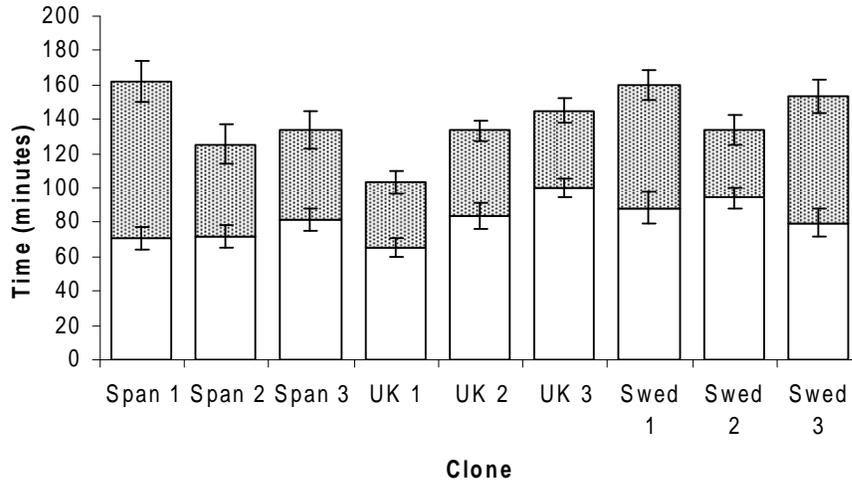
Following further acclimation to 25°C over three generations, no significant intergenerational effect on the duration required until the aphid exited chill coma or engaged in spontaneous walking was observed. The duration required for aphids to exit chill coma ranged from a median value of 74.8 to 92.8 min, approximating 4.5°C to 6.3°C (Figure 19II). No significant differences were observed between the durations with the exception of Swed 2 (median 92.8 min) taking significantly greater time to exit chill coma than UK 1 and Span 2 (medians 76.6 and 74.8 min respectively).

The duration until aphids, acclimated to 25°C for three generations, spontaneously walked following chill coma ranged from 114.9 to 166.2 min, approximating 8.5°C to 13.6°C (Figure 19II). As observed following acclimation for one generation, following acclimation to three generations, clone UK 1 once again required the shortest duration to spontaneously walk (median 114.9 min). This proved significantly less than all other clones with the exception of Span 2 and UK 2. The longest durations were required by Swed 1 and Swed 3 (medians 160.8 and 166.2 min respectively). Swed 3 required a significantly longer duration

to exit chill coma than all other clones excluding Swed 1 and UK 3. Swed 1 was shown to only require significantly greater time to spontaneous walk than UK 1, Span 2 and UK 2.

When comparing across all clones and treatments, clones UK 3 and Swed 2 consistently exited chill coma after shorter durations following low temperature acclimation (10°C) and clones UK 1 and Span 2 following high temperature acclimation (25°C). Clones Swed 1, Swed 2 and UK 2 consistently required longer durations to exit chill coma following acclimation to high temperatures. With regard to duration until spontaneous walking occurred following chill coma recovery, UK 1 was consistently one of the quickest clones to regain the ability to walk following acclimation to both high and low temperatures. Span 3 was consistently one of the quickest clones following low temperature acclimation and Span 2 one of the quickest following high temperature acclimation. After both high and low temperature acclimation, clones Swed 1 and Swed 3 consistently required the longest duration to spontaneously walk following chill coma.

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II.

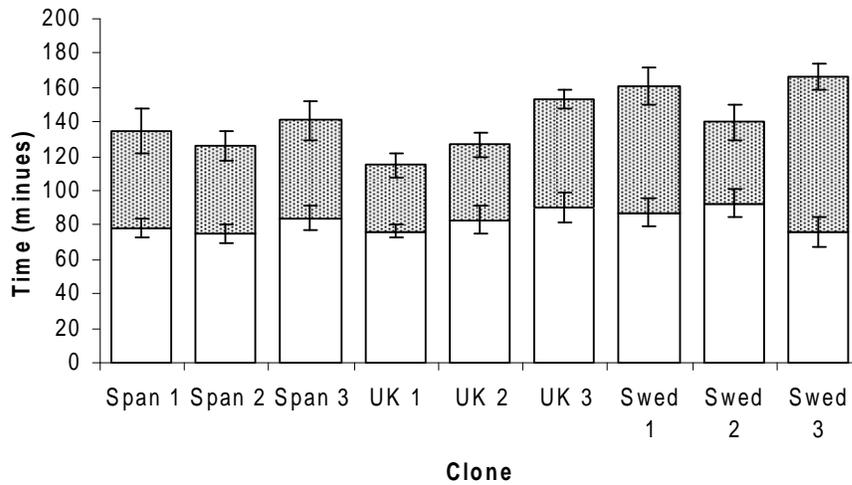


Figure 19. Time duration (min) following which aphids exit chill coma $\pm 95\%$ CI (as indicated by the top of the white bar) and spontaneously move $\pm 95\%$ CI (as indicated by the top of the grey bar) following exposure to -3°C for 30 min and then re-warmed to acclimation temperature at a rate of $0.1^{\circ}\text{C min}^{-1}$ for all clones when acclimated to I. 25°C for one generation and II. 25°C for three generations.

5.5 Discussion

In this chapter, the results of experiments designed to investigate the behavioural ecophysiology of *Myzus persicae* clones collected along a latitudinal gradient across Europe have been described. The experiments aimed to: 1. Determine levels of plasticity in activity thresholds and how these thresholds are affected by rearing temperature. 2. Establish if clones collected from latitudinally distinct regions differ in their ability to tolerate temperature extremes. 3. Investigate the relationships between the activity thresholds to ascertain how temperature tolerance range changes when aphids are acclimated to different temperatures and if relationships show interclonal variation.

5.5.1 Plasticity of activity threshold

The capacity of aphids to retain the ability to walk and avoid cold induced comas at low temperatures is crucial to winter survival if aphids are to escape predation and engage in fine-scale migrations from senescing leaves to fresh food sources (Bale, 1987; Harrington & Taylor, 1990). In *M. persicae*, the chill movement threshold was shown to be plastic within a single generation and could be depressed following acclimation at 10°C, thus allowing the aphid to remain active to lower temperatures. This ability of aphids to track changing environmental temperatures is, as previously discussed, of great ecological importance if the aphids are to remain active throughout cold spells, enabling the continuation of feeding, avoiding predation and finding suitable mates (applicable to sexual morphs of the holocyclic lifecycle). No consistent directional effect of high temperature acclimation on chill movement threshold was evident, although the majority of clones did experience a reduction in the ability to remain active to low temperatures. Heat movement threshold was shown to be plastic within a single generation with acclimation at 10°C decreasing heat movement threshold and acclimation at 25°C increasing heat movement threshold.

Chill coma temperatures were shown to be plastic within a single generation, with the temperatures inducing chill coma decreasing following acclimation at 10°C and increasing following acclimation at 25°C relative to the culture temperature of 20°C, as previously

demonstrated for chill coma temperatures in *Drosophila* species (Gibert & Huey, 2001), aphids of the genus *Myzus* (Hazell *et al.*, 2010a) and for lower lethal temperatures in *S. avenae* (Powell & Bale, 2008) and *M. persicae* (Chapter 4 of current thesis). In contrast to chill coma, variation in heat coma was much lower. Although the temperature of heat coma was not decreased through low temperature acclimation, high temperature acclimation within a single generation did raise heat coma temperature in the majority of clones and thus increasing activity thresholds.

In a previous study comparing heat movement and heat coma temperatures in aphids of the genus *Myzus*, *M. persicae* was found to exhibit the highest thresholds and thus greatest levels of heat tolerance, although had the lowest ability to acclimate at high temperatures (Hazell *et al.*, 2010b). From this, the authors concluded that *M. persicae* exist near to their upper thermal limit (Hazell *et al.*, 2010b), resulting in a trade off between physiological limit and plasticity (Stillman, 2003). As indicated in the current study, plasticity of upper limits is much compressed in comparison to lower limits, with acclimation often only acting to change upper limits by less than 1°C. With current trends of increasing global temperatures (Karl *et al.*, 2000), should the physiological limit and plasticity trade off apply, upper thermal limits could play an increasingly important role in limiting populations of *M. persicae*.

Aphids begin development within their grandmothers, a phenomenon known as ‘telescoping of generations’ (Kindlmann & Dixon, 1989). As a result, aphids have the potential to begin acclimating to environmental conditions prior to birth. Intergenerational acclimation is described in a study on *S. avenae* where the LLT₅₀ of a single clone decreased with each successive generation (LLT₅₀ declined from -13.6°C after one generation at 10°C, to -15.0°C after two generations and to -16.4°C after three generations) (Powell & Bale, 2008). However, data concerning lethal temperatures of nine *M. persicae* clones (presented in Chapter 4 of the current thesis) failed to provide consistent evidence for a role of intergenerational acclimation. Likewise, data presented in this chapter did not support intergenerational acclimation. Acclimation at 10°C over three generations did not significantly decrease chill coma, chill movement threshold or heat coma in the majority of clones. Intergenerational acclimation did impact heat movement threshold (4 out of 9

comparisons proved significant) although was not consistent in its effect. As with acclimation at 10°C, acclimation at 25°C for three generations did not significantly increase chill coma, chill movement or heat coma in the majority of clones. Intergenerational acclimation resulted in increasing heat movement in only three of the nine clones. The lack of evidence provided in the current study to suggest an effect of intergenerational acclimation is supported by previous work on the three aphid species *M. persicae*, *M. ornatus* and *M. polaris*, for which only one out of thirty long term acclimation tests suggested the possibility of intergenerational acclimation (Hazell *et al.*, 2010a). It can be concluded that the majority of aphids of the genus *Myzus* have little or no ability to continue the acclimation process over successive generations.

5.5.2 Geographic variation in activity thresholds

Geographic variation in insect thermal tolerance and activity thresholds has provided the focus for recent research in Coleoptera (Calosi *et al.*, 2010), Collembola (Bahrndorff *et al.*, 2006; 2009a) and Diptera (Goto & Kimura, 1998; Gibert & Huey, 2001; Gibert *et al.*, 2001b; Chen & Kang, 2004) with research suggesting that measures of thermal tolerance and activity levels are related to latitude. An understanding of the relationship between thermal tolerance and geography in the major pest species *M. persicae* provides, not only information on how species distribution and pest outbreaks could alter in a changing climate, but will also indicate levels of gene flow between geographically separate aphid populations and thus the potential for speciation.

As previously discussed, aphids inhabiting a region, such as the Mediterranean, where harsh winter conditions are a rarity would be unlikely to experience sub-zero temperatures and, as a consequence, tolerance to such temperatures would provide few advantages and could even prove costly in terms of resources. Investment in heat tolerance and maintaining activity at high temperatures would be more beneficial to a Mediterranean aphid. Conversely, aphids inhabiting regions such as Scandinavia and temperate Britain have a greater probability of experiencing sub-zero temperatures, and thus would be predicted to show greater levels of low temperature tolerance and a greater ability to maintain activity at low temperatures,

especially if there was little or no immigration to resident populations. Due to comparatively mild summer temperatures, investment in heat tolerance and high temperature activity would provide few advantages for Scandinavian clones. However, should outdoor Scandinavian populations rely on annual migrations from other parts of Europe for persistence, it would be expected that Scandinavian clones would not demonstrate greater levels of cold tolerance or low temperature activity levels than their temperate and Mediterranean counterparts. Likewise, should aphid redistribution be extensive throughout Europe, geographic adaptation in thermal tolerance and activity thresholds would not be expected at all.

In the current study, interclonal variation in the temperature at which movement ceased and chill coma was induced at low temperatures was apparent, although did not appear to show a consistent trend with latitude. Temperate clones UK 1 and UK 2 consistently had some of the highest low temperature activity thresholds, both ceasing movement and entering chill coma at higher temperatures than the remaining clones following all acclimation treatments. The clones with consistently low activity threshold temperatures included the Mediterranean clones Span 2 and 3 and the temperate clone UK 3, with these clones retaining the ability to walk to lower temperatures. Span 1 and Swed 1 proved the most tolerant with regard to chill coma, avoiding chill coma to lower temperatures than the remaining clones. The data therefore do not support the hypothesis that Scandinavian clones are more cold tolerant or better adapted to low temperatures than temperate or Mediterranean clones. This is most likely due to an inability of Scandinavian aphid populations to overwinter, unless in protected areas, owing to a combination of a lack of primary host plants to support the holocyclic lifecycle and harsh winter temperatures acting on anholocyclic clones. Local adaptation to harsh winters would not occur if aphids overwinter in protected areas or if immigrations from other parts of Europe are required to increase or renew post-winter populations.

No relationship between latitude and the temperature at which aphids ceased to move at high temperatures was apparent. However, a clear relationship was seen with the temperature of heat coma. Scandinavian clones Swed 2 and Swed 3 were consistently the least active and tolerant to high temperatures, entering heat coma at temperatures lower than the remaining clones. The three Mediterranean clones, Span 1, 2 and 3 had the highest activity thresholds at

high temperature, avoiding heat coma to higher temperatures at the majority of acclimation treatments. Results would suggest latitudinal variation in the temperature inducing heat coma, with Mediterranean clones having higher thresholds than their temperate and Scandinavian counterparts, and the Scandinavian clones having lower thresholds than their temperate and Mediterranean counterparts. This supports the hypothesis that Mediterranean populations could persist continuously in the more favourable Mediterranean climate without the need for extensive migrations, allowing opportunity to adapt to local conditions in the form of increased heat tolerance and activity levels.

In addition to influencing coma temperatures, the literature details studies demonstrating the effects of rearing temperature and latitude on recovery times from chill coma, with recovery times being positively related to rearing temperatures and negatively related to latitude, although with a focus on *Drosophila* species (David *et al.*, 1998; Gibert *et al.*, 2001b; Ayrinhac *et al.*, 2004; Macdonald *et al.*, 2004; Rako & Hoffmann, 2006). As previously stated, the ability to regain movement following cold shock is ecologically important if an insect is to survive and contribute to the next generation (Bale, 1987). If recovery from chill coma in *M. persicae* is related to latitude, it would be predicted that clones collected from Scandinavia, where harsh winters are a common occurrence, would show quicker recovery from chill coma than Mediterranean clones. This was not supported by the current study, with Scandinavian clones Swed 1 and Swed 3 consistently having some of the longest complete recovery times from chill coma and UK 1 some of the shortest complete recovery times, disproving the hypothesis stated in the Introduction that Scandinavian clones would be more cold tolerant than clones collected from other regions, most likely due to the annual influx of aphids from other parts of Europe.

All clones in the current study were categorized into different clonal types using microsatellite analysis, with clones of the same type being those genetically identical at the loci examined. Although clones of the same type can not be confirmed as being genetically identical, they are classified as having originated from an identical stem mother (Kasproicz *et al.*, 2008). This information enabled testing into the hypothesis stated on page 32 of the Introduction (that clones of the same type are more similar with regard to thermal tolerance than clones of different types) and therefore, whether aphid thermal tolerance traits could

better be explained in relation to clonal type than latitude. In the previous chapter, clones Swed 1 and Swed 3, both Type O clone, had comparatively high levels of thermal tolerance with regard to lethal temperatures when compared to other clones of different types. In the current study Swed 1 and Swed 3 were once again better adapted to low temperatures, entering chill coma at relatively low temperatures across all acclimation treatments. However, these two clones required some of the longest recovery times from chill coma. Likewise, clones UK 1 and UK 2, both Type C clones, displayed similar activity levels with regard to heat and chill movement and coma temperatures across all acclimation treatments, most notably with their inability to maintain activity to temperatures as low as all other clones. The third temperate clone, UK 3, a Type J clone, differed from the Type C temperate clones, for example, having significantly lower chill movement and chill coma temperatures after the majority of acclimation treatments. Data therefore suggest that clonal type could be related to thermal tolerance and activity thresholds. However, Swed 2, also a Type C clone, did not show similar patterns in activity thresholds as seen for UK 1 and UK 2. Since clones of the same type are not necessarily genetically identical, only at the loci examined, variation between clones of the same type could be a result of variation in the level of genetic similarity between clones.

The three Mediterranean clones were classified as unique indicating that the clones were different types, although had not previously been assigned a type letter code. If clones of different types do differ in their thermal tolerances and activity thresholds, it would be expected that the three Mediterranean clones would show different patterns in movement and coma threshold temperatures across acclimation treatments. Although different patterns were often observed, all three Mediterranean clones showed high levels of activity as indicated by high heat movement and coma temperatures. As discussed above, it is suggested that this is because Mediterranean clones can persist continuously in the favourable climate, allowing adaptation to the local climate, with such local adaptation in heat tolerance and activity thresholds possibly overriding any genetic effect of clonal type.

Due to the increased levels of high temperature activity observed for the Mediterranean clones of different types and due to Swed 2 proving dissimilar to the temperate clones of the same type, it is possible that a more complex interaction between clonal type and latitude is

at play. To better understand the relationship between thermal tolerance and clonal type, a more extensive study would be required involving a larger number of clonal types and replicates and could provide the focus for future work.

5.5.3 Relationships between activity threshold

It is reported in the literature that upper lethal temperatures of insects are less variable than lower lethal temperatures (Gaston & Chown, 1999; Addo-Bediako *et al.*, 2000; Chown 2001; Terblanche *et al.*, 2005). This was shown to be the case with lethal temperatures of *M. persicae* (presented in Chapter 4) and is also true with respect to coma and movement threshold temperatures. Both heat movement threshold and heat coma were less responsive to acclimation treatment than chill movement threshold and chill coma, with variation occurring over a much condensed temperature range. This is most probably due to the high association between heat coma and lethality resulting in a reduced capacity for acclimation. Hazell *et al.*, (2010b) found no significant difference between temperatures of heat coma and upper lethal limits (LT₅₀) when aphids were reared at 15°C and 20°C, suggesting that heat coma is not a reversible process like chill coma and results in irreparable physiological damage, leading to insect death.

Since variation in heat movement threshold and heat coma temperature was less marked than for chill movement threshold and chill coma temperature, activity ranges could be expanded following acclimation to low temperatures. For all aphid clones with the exception of UK 1 and UK 2 (which displayed a greater reduction of 0.4°C per 1°C increase in rearing temperature) the temperature range over which aphids could avoid a temperature-induced coma decreased at a rate of 0.2°C per 1°C increase in rearing temperature. As a consequence, aphids reared at 10°C showed an activity range 3°C greater than aphids reared at 25°C. The greater rate of decline in activity range for UK 1 and UK 2 was also observed with movement threshold temperatures. As discussed above, UK 1 and UK 2 were both Type C clones. This provides further evidence in the support of the hypothesis that clones of the same type are more similar with respect to thermal tolerance and activity thresholds than clones of different types, acknowledging that a larger sample size would be required to test

this hypothesis more fully. With Earth's climate warming at a rate of approximately 0.2°C per decade (Karl *et al.*, 2000), there could be a reduction in aphid activity ranges, which could prove more pronounced for common temperate clones such as type C if the current results are to be assumed.

In addition to tolerance ranges increasing with acclimation, it is reported that tolerance ranges increase with latitude (Addo-Bediako *et al.*, 2000) and altitude (in dung beetles: Gaston & Chown, 1999; in frogs Navas, 1996ab, 1997), although ultimately linked to temperature. One would expect Scandinavian *M. persicae* clones to display the largest activity temperature range, the British clones an intermediate range, and finally the Mediterranean clones to have the smallest range. However, current data do not indicate any clear latitudinal relationship with activity range, further supporting the hypothesis that clonal mixing occurs over such a large scale in Europe that local adaptation is prevented. The comparatively reduced activity ranges of clones UK 1 and UK 2 are most likely a consequence of their genetics linked to clonal type rather than latitude or an interaction of the two factors.

In summary, the data demonstrate that thermal activity thresholds show levels of intragenerational plasticity (i.e. within one generation), with chill coma and chill movement threshold temperatures decreasing following low temperature acclimation and heat coma and heat movement thresholds increasing following high temperature acclimation. Intergenerational plasticity was not supported by the data. Evidence for latitudinal effects on these thresholds was lacking with the exception of data concerning heat coma. Data therefore support the hypothesis that gene flow and clonal mixing between populations occurs over large scales across Europe preventing any distinct patterns in macrophysiology being observed. Due to the high levels of heat coma tolerance observed in Spanish clones, the hypothesis that local adaptation can occur in the Mediterranean because of an ability of clonal populations to persist and locally adapt is supported. Data also suggest that clonal type, as determined by microsatellite analysis, could impact activity thresholds, although more extensive studies are required to confirm this. Furthermore, results demonstrate that high temperature activity thresholds are less plastic than low temperature activity thresholds,

allowing the temperature range over which activity can be maintained to increase following low temperature acclimation.

6 Comparison of the relative walking speeds of clones of the peach-potato aphid *Myzus persicae*

6.1 Summary

This study investigated the variation in relative walking speeds of clones of *M. persicae* collected along the species' latitudinal distribution in Europe and the level of plasticity in walking speeds after acclimation to different temperatures (10°C, 20°C and 25°C). Walking speeds declined with decreasing temperature, with maximum performance at temperatures closest to acclimation temperature (fastest median walking speed of 5.8 cm min⁻¹ was recorded for UK 3 at 25°C after acclimating to 25°C for one generation). Following acclimation at both 20°C and 25°C aphids became immobile at temperatures as high as 7.5°C and 12.5°C. However, acclimation at 10°C enabled mobility to occur to temperatures as low as 0°C, which would have rendered aphids acclimated to 20°C and 25°C immobile. Clonal variation was revealed with clone UK 3 outperforming the majority of clones across all temperatures at which mobility was maintained following acclimation at 25°C for one generation (median walking speeds of 5.8, 3.2, 2.3, 1.0 and 0.7 cm min⁻¹ recorded at temperatures 25, 22.5, 20, 17.5 and 15°C respectively), at 10°C for one generation (median walking speeds of 4.6, 3.7, 2.0 and 1.0 cm min⁻¹ recorded at temperatures 10, 7.5, 5, and 2.5°C respectively) and at 10°C over three generations (median walking speeds of 4.1, 2.7, 1.7 and 1.3 cm min⁻¹ recorded at temperatures 10, 7.5, 5, and 2.5°C respectively). Following acclimation at 25°C for three generations, UK 3 performed poorly, displaying some of the slowest walking speeds (median walking speeds of 1.3, 0.7 and 0.6 cm min⁻¹ recorded at temperatures 25, 22.5 and 20°C). The Scandinavian clones, however, consistently outperformed their temperate and Mediterranean counterparts at the majority of temperatures following acclimation for three generations at 25°C (e.g. median walking speeds of 4.2, 4.5 and 3.5 cm min⁻¹ recorded at 25°C for Swed 1, Swed 2 and Swed 3 respectively). There was no relationship between mobility and latitude of origin.

6.2 Introduction

Earth's climate has warmed by approximately 0.6°C over the past century (Easterling *et al.*, 1997, 2000; Walther *et al.*, 2002), although this warming has not been a steady process and since the mid 1970s parts of the world have experienced a rapid increase to a rate of warming of 0.2°C per decade (Karl *et al.*, 2000). Due to aphids, and indeed all insects, being ectothermic, they possess a limited ability to regulate body temperature above or below ambient. As a consequence, climate warming is likely to have profound effects on distribution patterns and the pest and invasive status of insect species (e.g. Williams & Liebhold, 1995; Parmesan, 1996; Hill *et al.*, 1999; Parmesan *et al.*, 1999; Yamamura & Yokozawa, 2002; Battisti *et al.*, 2005; Vanhanen *et al.*, 2007; Tougou *et al.*, 2009).

The majority of research into insect thermal biology has focused on the lethal effects of low temperatures and the ability of insect species to exhibit seasonal and rapid cold hardening (Bale *et al.*, 1988; McDonald *et al.*, 1997; Kelty & Lee, 1999; Powell & Bale, 2004, 2008). However, as discussed in the previous chapter, non-lethal thermal thresholds must not be overlooked in favour of lethal thermal thresholds. Due to the limited ability to regulate body temperature, an insect's thermal environment will affect many aspects of their biology and behaviour. It is the non-lethal thermal tolerance traits such as temperatures of induced coma and movement thresholds that provide ecologically relevant information since survival is of little benefit if movement is compromised, resulting in mortality through inability to feed or escape predation or parasitism.

A new method described in Hazell *et al.* (2008) has enabled the continuous recording and measurement of non-lethal thermal tolerance traits in small insects. This method permits multiple specimens to be cooled or heated within an arena and behavioural traits to be recorded by video capture technology without disturbance to the specimens. The method has already proven successful in determining movement and coma thresholds, which can otherwise be hard to define (Hazell *et al.*, 2010ab). More recently, the method has been adopted to investigate relative walking speeds of predator and prey species to determine the potential of a predatory insect to act as a biological control agent (Hughes *et al.*, 2010).

This study will adopt this method to investigate relative walking speeds of *M. persicae* clones collected along a latitudinal gradient in Europe to determine if locomotor function can be altered via acclimation and if latitudinal adaptations exist. Due to the recent development of the method, no study exists whereby latitudinal variation in relative walking speeds of an insect species has been determined. Such information will be useful in increasing the baseline knowledge on aphid thermal biology with implications for pest control and the prediction of how and which clones or populations of *M. persicae* will be affected by climate change.

6.3 Materials and methods

The nine experimental clones of *M. persicae* were kept as a stock culture at 20°C. First instar nymphs (less than 24h old) were taken from the stock and acclimated at either 10°C or 25°C for one or three generations prior to use of their offspring in experiments, as detailed in the general materials and methods chapter. All nymphs used in experiments were first instars.

6.3.1 Walking speed

A sample of 5 aphids was placed within the arena set to the culture temperature. The arena temperature was lowered from culture temperature at a rate of 0.5°C min⁻¹. At 2.5°C intervals, cooling was temporarily halted and the arena held at the specific temperature for 5 min, before cooling continued to the next temperature 2.5°C lower. Cooling continued in this way until 0°C was reached. During the 5 min holding periods the camera (Infinity 1-1; Lumenera Scientific, Canada) recorded aphid movement within the arena at a rate of 1 frame per 5 sec and videos logged to a desktop computer. This was repeated with a new sample of aphids to produce a total of 10 aphids for each clone by treatment combination.

Videos of aphid movement were played back using StudioMeasure (Studio86designs, UK), frame by frame, with arena temperature and time displayed on the screen. Distance travelled was measured using the computer mouse to mark the location of the aphid on screen. The

video was then moved on one frame and the new location of the aphid marked. This was continued for the entire video. When marking aphid position the tip of the head was selected as the point of marking because this was an easily identifiable reference point for each specimen.

The resultant computer output was exported to Microsoft Excel in the form of a series of values of distance travelled (arbitrary units) per frame for each holding temperature. Distance travelled in arbitrary units was converted to millimetres where 1 arbitrary unit equated to 0.269 mm. Data from 4 min of film were used for each holding temperature for subsequent analyses.

6.3.1 Statistical analysis

Walking speed data were analysed using the Scheirer-Ray-Hare extension of the Kruskal Wallis test, previously detailed by Hughes *et al.* (2010). Post hoc comparisons were performed using a Mann-Whitney U test and adopting a Bonferroni procedure to adjust significance levels to the number of comparisons.

6.4 Results

Results of aphid mobility are presented in Figures 20 - 22. From the graphs, it is evident that both median walking speed and spread of the data, as indicated by the inter-quartile ranges, decrease with declining temperature for any one treatment. The fastest rates of walking were observed at 25°C for aphids acclimated to 25°C for one generation (e.g. fastest median walking speed of 5.8 cm min⁻¹ was recorded for UK 3). However, walking speeds for aphids acclimated at 20°C or 25°C, although initially relatively rapid, quickly decreased. On reaching temperatures of 10°C and below, most aphid clones acclimated at 20°C and 25°C displayed median walking speeds of 0.0 cm min⁻¹ with little variation around the medians, indicating that the majority of individuals were immobile. Aphids acclimated at 10°C,

however, maintained greater relative rates of walking and greater variation to temperatures as low as 0°C.

For nymphs reared at a constant 20°C, Sheirer-Ray-Hare tests revealed significant effects of temperature ($p < 0.001$) and clone ($p < 0.001$) on walking speed (Figure 20). Post-hoc Mann-Whitney U tests revealed that Span 1 displayed the fastest median walking speed of 5.2 cm min⁻¹ at 20°C, significantly faster than all other clones ($p \leq 0.0002$ for all comparisons). Span 2 displayed the slowest median walking speed of 1.0 cm min⁻¹, proving significantly different to all clones with the exception of UK 2 and UK 3 (medians of 1.6 and 2.0 cm min⁻¹ respectively). When exposed to 17.5°C, the interclonal variation in walking speed compressed and walking speed declined for all clones excluding Span 2 which maintained a walking speed of 1.0 cm min⁻¹. Span 1 remained the fastest clone with a reduced median walking speed of 2.6 cm min⁻¹, significantly differing from all other clones ($p \leq 0.0002$ for comparisons proving significant) with the exception of UK 3 (median 2.0 cm min⁻¹). At 15°C, UK 3 displayed the fastest walking speed (median 1.9 cm min⁻¹), but was only significantly different from clones Span 2, Span 3, Swed 1 and Swed 2 (median values 0.0, 0.6, 0.9 and 0.7 cm min⁻¹ respectively). Span 2 displayed a median of 0.0 cm min⁻¹ indicating that the majority of individuals could not walk at 15°C. At 12.5°C interclonal variation further reduced; Swed 3 and Span 2 had the faster rate of 1.3 cm min⁻¹, significantly differing from all other clones with the exception of Span 1 ($p \leq 0.0001$ for comparisons proving significant). Span 3, UK 1 and Swed 2 did not walk at 12.5°C. At 10°C, all clones were immobile with the exception of UK 3 with a median of 0.6 cm min⁻¹. When exposed to temperatures of 7.5°C, 5.0°C, 2.5°C and 0.0°C all clones were immobile.

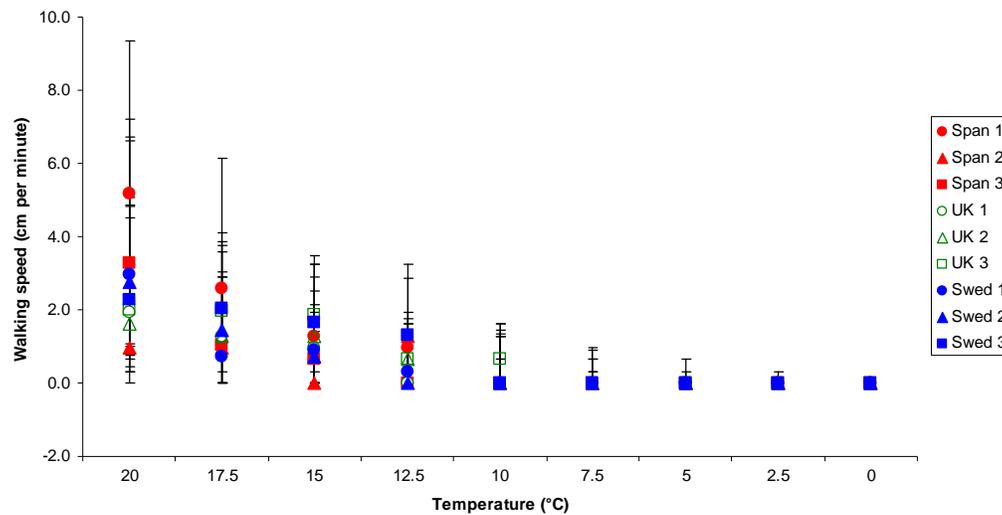


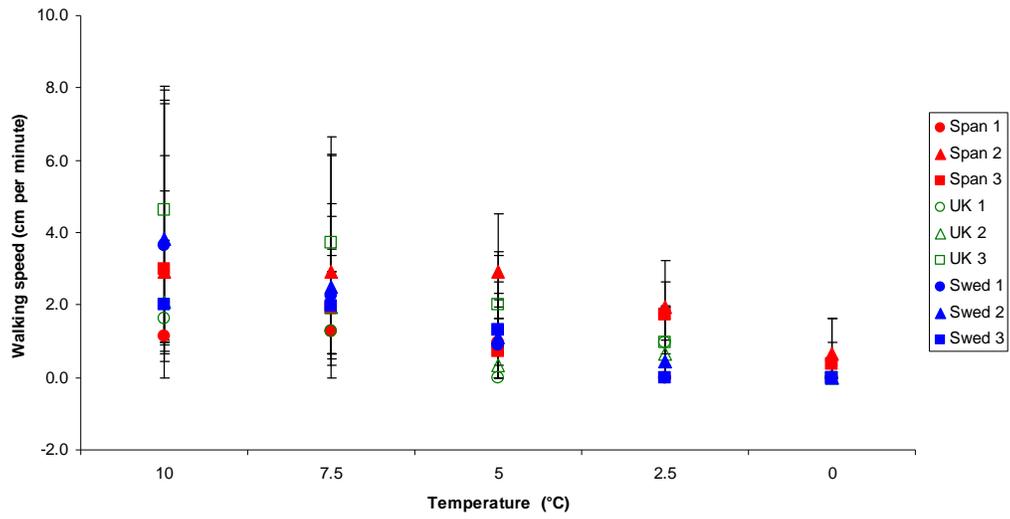
Figure 20. Walking speed (median \pm quartiles) of *M. persicae* nymphs at a range of constant temperatures when acclimated to 20°C. Spanish clones are represented by closed red symbols, British clones by open green symbols and Swedish clones by closed blue symbols.

For nymphs reared at 10°C for one generation, Sheirer-Ray-Hare tests revealed significant effects of temperature ($p < 0.001$) and clone ($p < 0.001$) on walking speed. The results, shown in Figure 21, suggest that UK 3 was consistently one of the fastest clones across all temperatures, although, at temperatures of 5°C and below, Span 2 increased in relative activity, consequently becoming the fastest clone. Post-hoc Mann Whitney U tests revealed that UK 3 displayed the fastest rate of walking at 10°C (median 4.6 cm min⁻¹) which proved significantly faster than all other clones with the exception of Swed 2 (median 3.8 cm min⁻¹) ($p \leq 0.0003$ for comparisons proving significant). The slowest rate was displayed by Span 1 (median 1.1 cm min⁻¹) which proved significantly different to the rates shown by all other clones ($p \leq 0.0011$ for all comparisons). As the temperature declined to 7.5°C, UK 3 remained the faster clone, displaying a median walking speed of 3.7 cm min⁻¹, significantly different to all clones with the exception of Span 2 and Swed 2 (respective rates of 2.9 and 2.5 cm min⁻¹) ($p \leq 0.0001$ for comparisons proving significant). Span 1 was once again the slowest clone, in addition to UK 1, both having median walking speeds of 1.3 cm min⁻¹. At 5°C, two temperate clones displayed the slowest walking speeds, with UK 1 remaining the slowest clone and displaying a median of 0.0 cm min⁻¹, indicating that the majority of individuals were immobile, and UK 2 having a median rate of 0.3 cm min⁻¹. These rates proved

significantly slower than all other clones with the exception of Swed 1 (median 0.9 cm min⁻¹) ($p \leq 0.0011$ for comparisons proving significant). The fastest rate of 2.9 cm min⁻¹ was displayed by Span 2, which proved significantly faster than all other clones excluding UK 3 (median 2.0 cm min⁻¹) ($p \leq 0.0001$ for comparisons proving significant). At 2.5°C, clonal walking speeds separated out in relation to region: Span 2 and Span 3 displayed the fastest walking speeds (1.9 and 1.7 cm min⁻¹ respectively), Swed 1, Swed 2 and Swed 3 the slowest walking speeds (0.0, 0.5 and 0.0 cm min⁻¹ respectively) and UK 1, UK 2 and UK 3 intermediate rates (1.0, 0.6 and 1.0 cm min⁻¹ respectively). Span 1, with a median of 0.00 cm min⁻¹, did not fit the pattern. However, due to the lack of evidence supporting a relationship between walking speed and region for aphids acclimated to 10°C for one generation at other temperatures, it must be concluded that the observed pattern at 2.5°C is coincidental. On reaching 0°C, all clones had become immobile with the exception of Span 3 (median 0.4 cm min⁻¹) and Span 1 (median 0.6 cm min⁻¹).

Further acclimation at 10°C over three generations also resulted in significant effects of temperature ($p < 0.001$) and clone ($p < 0.001$) on walking speed (Figure 21). Once again, UK 3 showed the fastest rate of walking across the majority of temperatures. Post-hoc Mann Whitney U tests revealed UK 3 to be the fastest clone at 10°C when acclimated to 10°C over three generations (median 4.1 cm min⁻¹), as previously observed when acclimated to 10°C for one generation (median 4.6 cm min⁻¹). This proved significantly greater than all other clones excluding Swed 1 and Swed 2 (median 3.6 and 3.5 cm min⁻¹ respectively) ($p \leq 0.0002$ for comparisons proving significant). The slowest walking speeds were observed for the Mediterranean clones, Span 1, Span 2 and Span 3 (median 1.9, 1.7 and 1.9 cm min⁻¹ respectively). At 7.5°C, few significant differences were revealed between median walking speeds which ranged from 1.8 cm min⁻¹ (observed for Span 2) to 2.7 cm min⁻¹ (observed for UK 3). On reaching 5°C, Span 2 displayed the fastest walking speed of 3.0 cm min⁻¹, significantly faster than all other clones ($p \leq 0.0002$ for all comparisons). Swed 3 and UK 2 proved the slowest clones, both with a median of 0.5 cm min⁻¹ respectively. By 2.5°C median walking speeds had further declined and variation had compressed, with medians ranging from 0.0 cm min⁻¹ (observed for Span 2, UK 1 and Swed 3) to 1.3 cm min⁻¹ (observed for Span 3 and UK 3). On reaching 0.0°C, all clones were immobile with the exception of Span 3 and UK 3 which displayed median rates of 0.3 cm min⁻¹.

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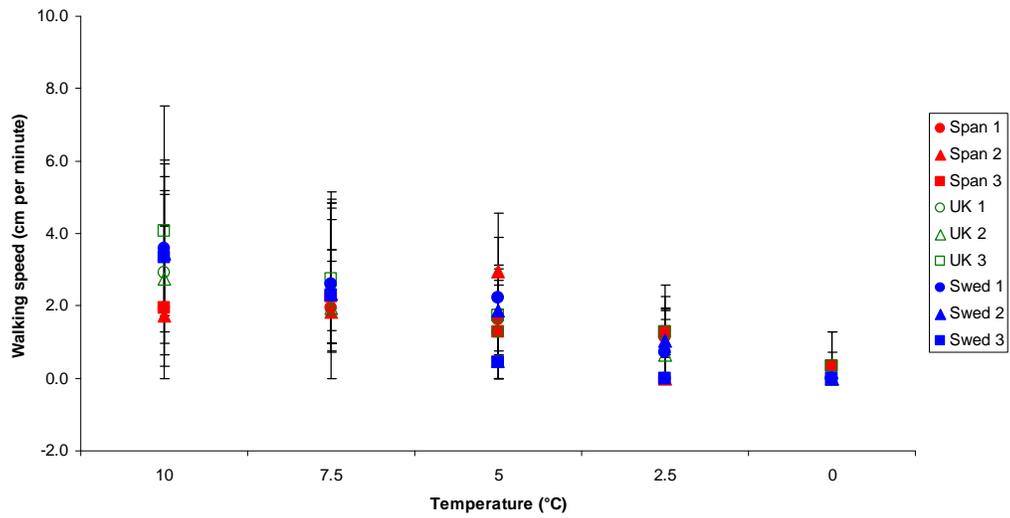


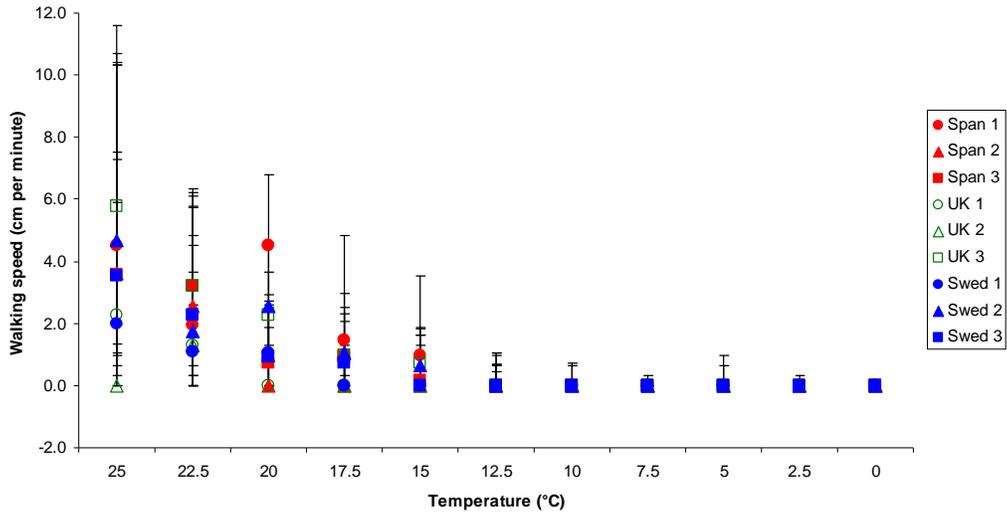
Figure 21. Walking speed (median \pm quartiles) of *M. persicae* nymphs at a range of constant temperatures when acclimated to 10°C for I. one generation and II. three generations. Spanish clones are represented by closed red symbols, British clones by open green symbols and Swedish clones by closed blue symbols.

For nymphs reared at 25°C for one generation, Sheirer-Ray-Hare tests revealed significant effects of temperature ($p < 0.001$) and clone ($p < 0.001$) on walking speed. The results, displayed in Figure 22, suggest that clones UK 3, Span 1 and Swed 2 were consistently the fastest clones at temperatures where mobility was maintained and Swed 1 showed some of the slowest rates. Post-hoc Mann Whitney U tests revealed UK 3 to be the fastest clone at 25°C, displaying a median walking speed of 5.8 cm min⁻¹, significantly faster than all other clones with the exception of Swed 2 and Span 1 (medians 4.7 and 4.5 cm min⁻¹ respectively) ($p \leq 0.0008$ for comparisons proving significant). UK 1 was the slowest clone, proving to be significantly slower than all other clones with a median of 0.0 cm min⁻¹ ($p \leq 0.0007$ for all comparisons). At 22.5°C, variation in median walking speed was reduced, in addition to inter-quartile ranges. UK 3 displayed the fastest median rate of 3.2 cm min⁻¹, but was not significantly different to the remaining clones. The slowest rate was observed for Swed 1 (median 1.1 cm min⁻¹), although, as with the highest rate observed for UK 3, was not significantly different to the other clones. On reaching 20°C, Span 1 was the fastest clone with a median of 4.5 cm min⁻¹, significantly faster than all other clones ($p \leq 0.0001$ for all comparisons). Swed 2 and UK 3 displayed relatively fast rates of 2.5 and 2.3 cm min⁻¹ respectively, proving significantly slower than Span 1, but significantly faster than all other clones. Clones Span 2 and UK 1 were immobile at 20°C. Span 1 was, once again, the fastest clone at 17.5°C, displaying a walking speed of 1.5 cm min⁻¹, significantly faster than all clones ($p \leq 0.0001$ for all comparisons). Clones Span 2, UK 1, UK 2 and Swed 2 did not walk at 17.5°C. At 15°C, five clones had become immobile: Span 2, UK 1, UK 2, Swed 1 and Swed 3. Span 1 displayed the fastest median walking speed of 1.0 cm min⁻¹, a rate significantly faster than all clones ($p \leq 0.0001$ for all comparisons). At temperatures of 12.5°C and below, all clones had become immobile.

Significant effects of temperature ($p < 0.001$) and clone ($p < 0.001$) on walking speed were also observed following acclimation at 25°C over three generations. The results, showed in Figure 22, reveal that UK 3 performed poorly when acclimated over three generations at 25°C and was no longer one of the fastest clones, with the temperate clones displaying some of the lowest median walking speeds across all temperatures (e.g. median walking speeds of 1.3, 1.2 and 1.3 cm min⁻¹ recorded for UK 1, UK 2 and UK 3 at 25°C). Scandinavian clones, in particular Swed 1, had some of the fastest walking speeds across all temperatures (e.g.

median walking speeds of 4.2, 4.5 and 3.5 cm min⁻¹ recorded for Swed 1, Swed 2 and Swed 3 at 25°C). Using post-hoc Mann Whitney U tests, it was revealed that Swed 2 was the fastest clone at 25°C with a median walking speed of 4.5 cm min⁻¹, although this did not significantly differ from the remaining Scandinavian clones, Swed 1 and Swed 3 (medians 4.2 and 3.5 cm min⁻¹ respectively) and Span 2 (median 2.5 cm min⁻¹). The temperate clones had the slowest walking speeds (medians 1.3, 1.2 and 1.3 cm min⁻¹ respectively for UK 1, UK 2 and UK 3), suggesting a relationship between clonal origin and mobility, with Mediterranean clones having intermediate walking speeds to the Scandinavian and temperate clones (medians 1.9, 2.5 and 1.4 cm min⁻¹ respectively for Span 1, Span 2 and Span 3). At 22.5°C, the Scandinavian clones remain the fastest of all clones, with median walking speeds of 2.6, 1.3 and 2.1 cm min⁻¹ for Swed 1, Swed 2 and Swed 3 respectively. Swed 1 proved significantly faster than all other clones with the exception of the remaining Scandinavian clones and Span 1 (median 1.3 cm min⁻¹) ($p \leq 0.0002$ for comparisons proving significant). Span 2 did not walk at 22.5°C and significantly differed from all other clones as a result ($p \leq 0.0001$ for all comparisons). At 20°C, Swed 3 was the fastest clone (median 2.0 cm min⁻¹), significantly faster than all other clones excluding Span 1, Swed 1 and Swed 2 (respective medians 1.3, 1.5 and 0.6 cm min⁻¹). Both UK 1 and Span 2 did not move at 20°C. On reaching 17.5°C, Span 2, UK 2 and UK 3 were immobile. Swed 1, with a median of 2.0 cm min⁻¹, proved the fastest clone, significantly faster than all clones ($p \leq 0.0001$ for all comparisons). At 15°C, Swed 1 once again proved significantly faster than all other clones (median 2.0 cm min⁻¹) ($p \leq 0.0001$ for all comparisons). Clones Span 3, UK 1, UK 2, UK 3 and Swed 2 all displayed median walking speeds of 0.0 cm min⁻¹. By 12.5°C, all clones with the exception of Swed 1 and Span 1 (medians 1.3 and 1.0 cm min⁻¹ respectively) were immobile. On reaching 10.0°C, once again Swed 1 and Span 1 were the only clones to still be active (respective medians 0.7 and 0.6 cm min⁻¹). At temperatures 7.5°C and below, all clones had become immobile.

I.



II.

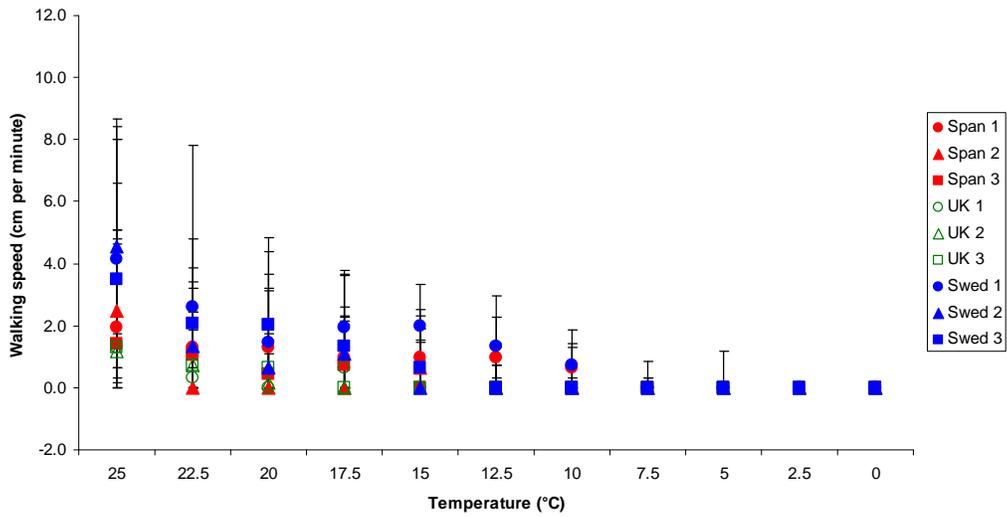


Figure 22. Walking speed (median \pm quartiles) of *M. persicae* nymphs at a range of constant temperatures when acclimated to 25°C for I. one generation and II. three generations. Spanish clones are represented by closed red symbols, British clones by open green symbols and Swedish clones by closed blue symbols.

6.5 Discussion

In this chapter, the results of experiments designed to investigate the variation and plasticity in the walking speeds of *M. persicae* clones from various latitudes in Europe are described. The experiment aimed to: 1. Ascertain how walking speed is affected by ambient temperature. 2. Determine the level of plasticity in aphid walking speed and how walking speed is affected by rearing temperature. 3. Establish if walking speed varies along the aphid's geographical distribution in Europe.

The mobility of *M. persicae*, as indicated by walking speed, declined with decreasing temperature and therefore aphid performance was maximal at temperatures closest to the acclimation temperature, with departure from optimal temperature conditions causing a decline in aphid performance, as previously reported for *D. melanogaster* (Crill *et al.*, 1996; Gilchrist *et al.*, 1997; Dillon & Frazier, 2006). Acclimation at the culture temperatures of 20°C and at 25°C resulted in mobility ceasing at temperatures as high as 12.5°C, with aphids stopping walking at 12.5°C following acclimation at 25°C for one generation and at 7.5°C following acclimation at 20°C or 25°C over three generations. Results suggest that further acclimation over three generations at 25°C enables aphids to retain mobility to temperatures 5.0°C lower when compared to aphids acclimated over just one generation or at 20°C. Acclimation at 10°C for both one and three generations resulted in mobility being maintained to temperatures as low as 0°C. If current trends of global warming continue, with implications for reduced winter severity and summer heat wave incidence (Easterling *et al.*, 1997; Cannon, 1998; Easterling *et al.*, 2000; Karl *et al.*, 2000), any unpredictable cold spell event could render aphids immobile at higher temperatures with the potential to impede migration and increase susceptibility to predation, with consequent implications for pest status.

Recent work by Hughes *et al.* (2010) investigated relative walking speeds of pest insects and their predators to determine the potential of the predator for use as a biological control agent. Traits considered desirable in a successful predator included a lower developmental threshold, increased voltinism and the capacity to retain mobility at temperatures below those at which the prey are rendered immobile (Hughes *et al.*, 2010). The current study

suggests that, with increasing temperatures, aphids could lose locomotor function at increasingly higher temperatures. Therefore, an understanding of how the mobility of aphid predator species will be impacted will provide useful information in understanding how the predator-prey balance could change with global climate change and would prove an insightful extension to this study.

No clear relationship between latitude and mobility was revealed. At 25°C, following acclimation for three generations at 25°C, Scandinavian clones displayed the fastest walking speeds, temperate clones the slowest and Mediterranean clones intermediate walking speeds. This trend was loosely continued until walking ceased at 7.5°C and below, although was most evident at 25°C. However, this is perhaps contrary to what would be expected if a relationship with latitude did exist. Data regarding walking speeds in *D. melanogaster* revealed that tropical flies (collected from the Congo) displayed faster walking speeds than temperate flies (collected from France) when reared at 25°C (Gibert *et al.*, 2001a). It would therefore be predicted that Scandinavian clones would be the least adapted towards high temperatures and to display the lowest walking speeds at 25°C following high temperature acclimation, the opposite of what was observed in the current study. Additionally, in the current study, Mediterranean clones had the fastest walking speeds at 2.5°C following acclimation at 10°C for one generation, Scandinavian the slowest and temperate clones intermediate walking speeds. In the study by Gibert *et al.* (2001a), temperate *D. melanogaster* outperformed the tropical counterparts when reared at a 'low' temperature of 18°C, indicating that flies display maximal performance in relation to the climate of collection origin. Likewise, holarctic tree frogs outperformed southern counterparts with regard to jumping performance at low temperatures (John-Alder *et al.*, 1988). Once again, the results here do not support this type of relationship. Scandinavian clones would be predicted to be well adapted to low temperatures and therefore to outperform temperate and Mediterranean clones when acclimated to low temperatures and then held at low ambient temperatures and not to display the slowest walking speeds as was observed. With the exception of the two examples listed above whereby walking speeds could be grouped in relation to latitude, no overall relationship with latitude was evident across all acclimation treatments and temperatures. It is concluded that such patterns were coincidental and that mobility is unrelated to latitude of collection origin.

Although no clear relationship between walking speed and latitude was found, clonal variations were evident. Following low temperature acclimation at 10°C for both one and three generations, UK 3 was consistently one of the fastest clones across all temperatures where mobility was maintained. UK 3 also displayed high rates of mobility following acclimation at 25°C for one generation, although, when acclimation was continued to three generations at 25°C, UK 3 became one of the slowest clones. Following acclimation at 25°C for one generation clones Span 1 and Swed 2 also displayed consistently faster walking speeds across all temperatures, in addition to UK 3. This is consistent with data concerning chill coma movement threshold, presented in the previous chapter, whereby UK 3 maintained activity at lower temperatures than remaining clones when acclimated to 20°C and 10°C, although did not when acclimated to 25°C. A different trend was observed following acclimation to three generations at 25°C whereby the three Scandinavian clones outperformed their temperate and Mediterranean counterparts across the majority of temperatures and the temperate clones performed poorly across all temperatures.

Due to the lack of any consistent relationship between latitude and mobility, it must be concluded that clonal mixing occurs over large scales in Europe as a result of jet stream dispersal (Zhu *et al.*, 2006), thus preventing local adaptations in mobility. As discussed in Chapter 4 concerning temperature lethality and Chapter 5 with regard to activity thresholds, clonal type, rather than latitude, could prove important when explaining observed patterns in the thermal biology of *M. persicae*. Type O clones Swed 1 and Swed 3 displayed high levels of thermal tolerance as indicated by lower and upper thermal tolerances. Type C clones UK 1 and UK 2 showed a greater rate of range reduction between high and low coma and movement thresholds with increasing temperature.

With the mobility data presented in this Chapter, at 20°C, the temperate Type C clones (UK 1 and UK 2) did not significantly differ at all temperatures at which mobility was maintained when acclimated to 20°C, 10°C for one generation, 25 °C for three generations and to 25°C for one generation with the exception of at 25°C. The remaining Type C clone, Swed 2, did not fit this pattern as tightly, only proving non-significantly different following acclimation at 20°C and non-significantly different to either one or both of the clones following acclimation to 10°C for three generations. Due to the strong association between the mobility

of UK 1 and UK 2, although a weaker association with Swed 2, it is plausible that UK 1 and UK 2 are more genetically alike than Swed 2, although all originate from a common stem mother. Likewise, the Type O clones, Swed 1 and Swed 2, showed a level of similarity with their respective walking speeds, proving not significantly different at the majority of holding temperatures following acclimation to 10°C and 25°C for both one and three generations.

In addition, UK 3 proved well adapted to retaining activity at low temperatures and to maintaining fast walking speeds relative to the other experimental clones. Microsatellite analysis revealed UK 3 to be a Type J clone. If thermal tolerance is related to clonal type, it is possible that Type J clones are well adapted to maintaining activity levels at low temperatures. However, only one Type J clone was included in the current study and more Type J clones would be needed to confirm this. If thermal biology is a factor related to clonal type as opposed to latitude, distinct clonal types would respond independently to climate change, thus affecting the relative proportions of *M. persicae* clonal types within populations.

In summary, data demonstrate that the mobility of *M. persicae*, as indicated by walking speed, declined with decreasing temperature and that maximum performance was reached at temperatures closest to acclimation temperature with walking speeds declining with departure from optimum temperatures. Acclimation to lower temperatures further enabled aphids to maintain mobility at lower temperatures which would have rendered the aphids acclimated to higher temperatures immobile. Such findings could have implications for pest status if increasing global temperatures raise aphid activity thresholds, affecting aphid ability to cope with unpredictable cold spells. Data did not provide consistent evidence for a relationship between walking speed and latitude, suggesting that large scale migrations occur between aphid populations in Europe preventing local adaptation. Although a relationship with latitude was not supported, clonal variation was observed, the most prominent of which being UK 3 consistently displaying some of the fastest walking speeds following the majority of acclimation treatments and Scandinavian clones outperforming their temperate and Mediterranean counterparts following acclimation at 25°C over three generations. A high level of similarity was observed between clones of the same type, although more extensive testing is required to confirm if locomotor activity is related to clonal type.

7 General discussion

With global climate change already well documented, which in turn will greatly affect the spatial and temporal distribution of aphid species, it is surprising that a detailed study investigating the variation in aphid thermal biology along a latitudinal gradient is lacking. The current study investigated the latitudinal variation in the thermal tolerance of nine clones of *Myzus persicae* collected from Sweden, Britain and Spain and the extent to which thermal tolerance levels can be altered through acclimation. In addition to an extensive, global distribution, *M. persicae* has genetically distinct clones in both the sexual and asexual lifecycles, leading to the possibility of four main outcomes when investigating large scale variation in thermal tolerance: 1) a relationship between thermal tolerance and latitude exists and regional adaptation occurs, 2) clonal mixing throughout Europe is extensive and no relationship between thermal tolerance and latitude is observed, 3) Scandinavian clones are no more cold tolerant than their temperate and Mediterranean counterparts due to being unable to reside year round in Scandinavia, but increased heat tolerance is evident in Mediterranean clones due to year round persistence enabling regional adaptation and 4) clones differ in thermal tolerance according to their genetic ‘type’ and, as a consequence, any pattern in thermal tolerance depends on the composition of the population at the time of collection. In addition, the study further provided investigation into aphid thermal tolerance at high temperatures and elucidated the relationships between upper and lower limits and across different indices of thermal tolerance, an area of study also lacking in the literature.

7.1 Plasticity of thermal tolerance traits

Due to the short generation times of aphids, their ability to respond rapidly to sudden changes in temperature over just one generation is considered to be of much greater importance than long term acclimation processes (Powell & Bale, 2008). The current study found that increased cold tolerance could be acquired following just one generation at a low temperature (10°C), acting to decrease the lower lethal, chill movement (CT_{min}) and chill coma temperatures. Low temperature acclimation also enabled aphids to maintain mobile at lower temperatures. However, the level of cold tolerance was reduced on acclimation to a

high temperature (25°C). Likewise, the level of heat tolerance could be increased after one generation of acclimation at a high temperature, acting to increase upper lethal, heat coma and heat movement temperatures, although high temperature tolerance proved much less plastic than lower thermal limits. Interestingly, low temperature acclimation also acted to raise heat tolerance in the form of elevated upper lethal temperatures, indicating that the physiological processes involved in conferring heat tolerance are also induced at low temperatures. This is most likely due to the up-regulation of heat shock proteins through exposure to both high and low stressful temperatures (Feder *et al.*, 1992; Feder & Hofmann, 1999; Sørensen *et al.*, 2003; Bährndorff *et al.*, 2009b). Although acclimation over one generation was evident in *M. persicae*, further acclimation over three generations was not, suggesting that the species is unable to increase levels of acclimation with each subsequent generation. With global temperatures rising, these results suggest that aphids could experience a reduced level of cold tolerance, making them more susceptible to unpredictable cold spells. Heat tolerance is likely to increase, but with a limited ability to acclimate, this cannot occur indefinitely and upper thermal limits could become increasingly important in governing aphid abundance and distribution, as suggested by Hazell *et al.* (2010b).

This study further revealed that the upper thermal limits of *M. persicae* are much less plastic and variable than the lower limits. A high association between heat coma temperature and lethality has been reported, indicating that heat coma is not a reversible process like chill coma and results in irreparable physiological damage and even insect death (Hazell *et al.*, 2010b). As a consequence of the greater plasticity of lower thermal limits, thermal tolerance ranges with regard to both lethality and behavioural traits can be increased following acclimation at low temperatures and reduced following acclimation at high temperatures. With current estimates of climate change forecasting increased surface and air temperatures (Cannon, 1998), a reduction in the thermal tolerance range of *M. persicae* could be expected, resulting in a greater susceptibility of aphids to unpredictable bouts of extreme weather such as frosts and heat waves. More interestingly, changes to the thermal tolerance range varied between clones, with the temperate clones UK 1 and UK 2, both Type C clones, displaying the greatest rate of range reduction for coma and movement temperatures with increasing temperature. This finding suggests that climate change is unlikely to have a uniform effect on all *M. persicae* clones, with common temperate clones such as Type C being more

strongly affected by increasing temperatures. It was hypothesised in the Introduction that clones of the same type would be more similar with respect to thermal tolerance than clones of differing types. The finding above (and the similarity between clones of Type O discussed in section 7.3) supports the hypothesis, although the inference is weak and a more extensive study involving a greater number of clonal types would be required to accept the hypothesis and could provide the basis for future work.

7.2 Relationship between indices

Information concerning the relationships between mortality and activity thresholds in pest aphid species is limited to a small number of studies (Powell & Bale, 2008; Hazell *et al.*, 2010ab), none of which have encompassed the number of indices of thermal tolerance used in the current study. A clear relationship between indices of thermal tolerance was reported for three aphid species of the genus *Myzus* where positive correlations were observed between lower lethal and chill coma temperatures (Hazell *et al.*, 2010a). In the study, *M. ornatus* was shown to be the least cold tolerant, having higher LLT₅₀ and chill coma temperatures than *M. persicae* and *M. polaris*. Although correlations could be made between thermal tolerance traits of different aphid species, it is unclear if such correlations are evident between clones of the same species. The current study, not only provided opportunity to investigate such relationships within a single species, but enabled correlations to be observed using a more extensive set of thermal tolerance traits than used in previous studies.

Evidence in favour of a relationship between the different indices within any individual clone of *M. persicae* was limited when comparisons were made using 20°C data to remove any effect of acclimation. Few correlations existed between lethal temperatures and activity thresholds and, consequently, the clone with the lowest LLT₅₀ temperature, for instance, did not necessarily have lower activity threshold temperatures. Where correlations were observed, the correlations were not consistent across all indices. For example, Span 1 showed low temperatures of chill movement threshold and chill coma, and a short chill coma recovery time, however, this was not coordinated with high levels of cold tolerance in the form of a low LLT₅₀. Likewise, UK 2 entered chill coma at a high temperature and required

a longer recovery period following chill coma. Although this did not correspond with a reduction in cold tolerance and a high LLT₅₀, UK 2 did have a high ULT₅₀ and level of heat tolerance.

Direct correlations between indices may be lacking in the literature, but differential effects of treatments on activity thresholds and mortality can provide information on possible linkages between traits. Studies on *Drosophila melanogaster* found that cold hardening could improve mortality, but had little effect on recovery from chill coma (Rako & Hoffmann, 2006). Similarly, long term acclimation had little effect on chill coma temperatures, although could reduce mortality (Powell & Bale, 2008; Hazell *et al.*, 2009, 2010a). Hazell *et al.* (2010a) suggest that this indicates that at least two distinct processes determine thermal tolerance and could offer explanation as to why correlations were evident between indices of activity thresholds in the current study, yet why so few correlations between lethal temperatures and activity thresholds existed. This further supports the idea that climate change is unlikely to affect *M. persicae* clones uniformly.

7.3 Latitudinal variation in thermal tolerance

Geographic variation in insect thermal tolerance has received great interest in recent years, with research on the Coleoptera (Calosi *et al.*, 2010), Collembola (Bahrndorff *et al.*, 2006; 2009) and Diptera (Davidson, 1990; Goto & Kimura, 1998; Gibert & Huey, 2001; Gibert *et al.*, 2001b; Bublly *et al.*, 2002; Ayrinhac *et al.*, 2004; Chen & Kang, 2004) suggesting that thermal tolerances are related to latitude. Results concerning geographic variation in *M. persicae* thermal tolerance would prove useful, not only in providing information on how and which populations are most likely to be affected by climate change, but also in suggesting the level of clonal mixing throughout Europe.

Overall, data support hypothesis 3 that no latitudinal trend in thermal tolerance is evident, with no observed relationship between the latitude of clone collection and either lethal temperature, movement thresholds, chill coma and walking speeds. This also disproves hypothesis 1 which states that Scandinavian clones would be more cold tolerant than their

temperate and Mediterranean counterparts and is best explained by the unfavourable conditions in Scandinavia resulting in the need for annual migrations from other parts of Europe to maintain populations, thus preventing local adaptation to the Scandinavian climate. It is by this process of annual migration that outdoor populations of *M. persicae* are able to persist in the northern Great Plains of America, with populations being annually re-established by spring migrations from the south (Zhu *et al.*, 2006). Large scale migrations by aphids are also supported by data on *S. avenae* from samples collected in England (Llewellyn *et al.*, 2004) and the grain aphid *Macrosiphum miscanti* in Eastern China (Guo *et al.*, 2005). Conversely, data concerning *M. miscanti* in Western China (Guo *et al.*, 2005) and *M. persicae* in Italy (Angela *et al.*, 2006) suggest a lack of clonal mixing and gene flow in sexual forms, leading to the production of isolated populations, which could favour speciation.

Although this study did not find a link between the thermal tolerance traits discussed above and latitude, it did provide some evidence supporting latitudinal variation with Mediterranean clones proving to be the most heat tolerant of clones in terms of their heat coma temperature. This finding supports hypothesis 2 and suggests that Mediterranean populations can persist in the favourable local climate without the need for extensive migrations, allowing adaptation in the form of increased heat tolerance.

With the exception of heat coma data, there was no evidence in favour of a relationship with latitude, indicating that clonal mixing across Europe must be extensive, at least across Northern Europe, to override or mask any local adaptation. Although a relationship with latitude was lacking, there was some indication of a relationship with clonal type, offering support for hypothesis 4. The current study included nine clones of six different clonal types: Type C (UK 1, UK 2 and Swed 2), Type O (Swed 1 and Swed 3), Type J (UK 3) and three unique clones (Span 1, Span 2 and Span 3). The Mediterranean clones were unique, indicating that they were different types, although none of them had been previously assigned a type letter code. If a relationship between thermal tolerance and clonal type did exist, clones of the same type would be expected to be more similar in thermal tolerance than clones of different types. As discussed above, UK 1 and UK 2, both Type C clones, displayed the greatest rate of reduction in thermal tolerance range with increasing

temperature, differing from all other clones. Additionally, a high level of thermal tolerance was evident in Swed 1 and Swed 3, both Type O clones, in the form of lethal temperatures. Also, UK 3, a Type J clone, showed high thermal tolerance in the form of low chill coma temperatures and high rates of mobility across most acclimation treatments which significantly differed from the Type C temperate clones at the majority of acclimation temperatures.

Although clones of the same type did display similarities in thermal tolerance, Swed 2, a Type C clone, was sometimes significantly different to the temperate Type C clones UK 1 and UK 2, notably with regard to the chill movement threshold and chill coma temperature. Since clones of the same type are not necessarily genetically identical, only at the loci examined, such variation in thermal tolerance of clones of the same type could be a result of variation in the level of genetic similarity between clones. In addition, as discussed above, the Mediterranean clones which proved to be of different types, possessed high levels of heat tolerance, providing evidence in favour of local adaptation in regions where populations can persist outside due to favourable conditions even though populations may be of different clonal types.

The type O clone, a particularly thermal tolerant clone in the current study, is a MACE carrying genotype new to Britain, first appearing in 2007. Numbers have since increased and Type O now accounts for almost 95% of the clonal composition found within the UK, with the clonal type fast displacing the previously common clones Type C, I and J (Fenton *et al.*, 2010). Part of the reason for the success of Type O is that it lacks the Kdr based resistance mechanisms thought to be deleterious to aphids, affecting their response to environmental cues (Fenton *et al.*, 2010). As a consequence, aphids possessing Kdr mechanisms are believed less fit and could explain the lack of thermal tolerance observed for Type C clones in the current study. In addition, the Type J clone (UK 3), which displayed high thermal tolerance with regard to mobility thresholds, also lacks the Kdr resistance mechanism, further supporting the idea that such mechanisms are detrimental to aphids carrying them. However, since clones of Type O are fast displacing those of Type J, it would appear that factors additional to the presence of Kdr mechanisms must also be contributing to the fitness of Type O clones.

As previously discussed, aphids possess the symbiotic bacterium *Buchnera aphidicola*, which provides the aphid with nutrients and essential amino acids which are deficient in their phloem sap diet (Douglas, 1998). Transmitted maternally via transovarial transmission, genetic lines of *Buchnera* are therefore likely to be closely associated with distinct aphid clonal types. It is reported that a mutation in *Buchnera* of the Pea aphid *A. pisum* consisting of a single nucleotide deletion dramatically alters thermal tolerance, conferring fitness advantages under cool temperature conditions, although proving detrimental following heat shock (Dunbar *et al.*, 2007). In addition, aphids are host to a variety of secondary symbionts known to confer fitness advantages (Tsuchida *et al.*, 2004; Oliver *et al.*, 2005; Scarborough *et al.*, 2005), in particular, altering thermal tolerance (Chen *et al.*, 2000; Montllor *et al.*, 2002; Russell & Moran, 2006; Burke *et al.*, 2010). If bacterial symbionts form a close association with specific aphid clonal types, variation in the symbionts specific to clonal types could offer some explanation towards the variation in aphid thermal biology observed in the current study.

The relationship between thermal tolerance and clonal type is therefore likely to be a complex one. Interactions could exist between clonal type and latitude and it is possible that patterns in clonal thermal tolerance could be masked when local adaptation is supported, as observed with the Mediterranean clones. More intricately, variation in the thermal biology of clonal types could be linked to variation in insecticide resistance mechanisms or the genetics of bacterial symbionts associated with each type. However, should thermal tolerance be related to clonal type, it could be expected that clonal types would respond independently to climate change, thus affecting the relative proportions of *M. persicae* clonal types within populations.

7.4 Future work

This study investigated variation in the thermal tolerance of *M. persicae* along a latitudinal gradient. Although little evidence in support of latitudinal variation and regional adaptation in thermal tolerance was observed, variation between distinct clonal types was apparent. Variation between clonal types could be partially explained by differences in insecticide

resistance, with Type O clones gaining a fitness advantages through lacking the Kdr mechanism and the associated costs of the mutation (Fenton *et al.*, 2010). However, this does not explain why Type O clones are successfully displacing Type J clones in Britain (Fenton *et al.*, 2010), a clone that proved very thermal tolerant in the current study and one which also lacks the Kdr mechanism. Since mutations in *Buchnera* are reported to alter the thermal biology of the pea aphid (Dunbar *et al.*, 2007), it is plausible that genetic variation in the *Buchnera* associated with different clonal types of *M. persicae* could further offer explanation into the observed variation in thermal biology. To elucidate whether thermal tolerance is best explained by clonal type, a more extensive study should be conducted by selecting clones of the same type along a latitudinal gradient for comparative laboratory experiments. Data concerning bacterial symbionts and resistance status of experimental clones would further prove useful in determining the underlying factors that result in clonal variation. Such information would prove useful in furthering our understanding of why certain clonal types have increased in fitness in recent years and also enable prediction into how climate change is likely to impact aphid distribution and clonal composition.

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