Intraspecific variation in traits in relation to environment and sex in an insect

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Declaration

I declare that this thesis is the result of my own work and my own research. Any help received in data collection or analysis is fully acknowledged. I certify that no part of this thesis has been submitted to any other university in application for any degree.

Signature:

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1st April 2022

Summary

Individuals within species frequently show differences in traits. Yet, until relatively recently, researchers treated all conspecifics as ecologically equivalent. I investigated how environmental variation, temperature and sex may influence intraspecific variation in behavioural, morphological and physiological traits, and their covariance, in the ground beetle *Carabus hortensis* - a species that is currently undergoing range expansion.

By examining intraspecific variation in morphological traits across the *C. hortensis* expansion front, I showed that male, but not female, body size increased with proximity to the range edge. This may suggest that males evolved larger bodies and longer legs to increase mate searching efficiency where female density is low. Secondly, I found intraspecific variation in *C. hortensis* thermal biology, with males being active over a wider range of temperatures than females, and large females being more thermally sensitive than smaller females. Additionally, I showed that male and female movement in the wild is differentially influenced by temperature, and that laboratory measures of animal personality differences can be predictive of intraspecific variation in movement patterns in the wild.

I then tested the relationships between metabolic rate, exploratory behaviour and morphology, finding that the strength, direction, and temperature dependency of relationships differed between the sexes. Finally, I demonstrated that that relationships between metabolic rate and body mass are uninfluenced by temperature, and that individuals with high average metabolic rates and exploratory behaviour are more thermally sensitive.

My findings provide new insights into the roles of sex and thermal sensitivity in shaping intraspecific variation in traits, and their implications for individual fitness and population dynamics under continued climate change. Overall, my results suggest that increasing temperatures may select for smaller individuals and those with lower average metabolic rates and exploratory behaviour.

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Publications

The following publications have arisen from this thesis*:

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*Please note that minor changes have been made to the published versions of these manuscripts to improve the continuity of this thesis.

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Chapter 1: General introduction

1.1 Intraspecific variation in traits

Individuals within species differ from one another. That much is clear – you only have to glance around a room full of people to notice that they come in many different shapes and sizes, or talk to different individuals to realise that they have different personalities. The same applies to non-human animal species: they show intraspecific, or between-individual, variation in traits.

Individuals within species may differ from one another in many different ways: they may differ morphologically, in terms of their shape, size, or colouration; physiologically, consistently differing from one another in terms of their metabolic rate or hormonal profiles; and in their life history traits, such as age and size at maturity, growth pattern, and longevity. Individuals may additionally display behavioural differences from one another that are consistent across time and/or contexts. Such consistent behavioural differences are known as 'animal personality differences' (Dall *et al*., 2004; Eysenck & Eysenck, 1985), and are usually measured across five major axes: exploration-avoidance, shyness-boldness, sociability, aggressiveness and activity (Réale *et al*., 2007). Finally, individuals may differ from one another in their level of phenotypic plasticity – their range of behavioural and physiological responses to environmental conditions (Pigliucci, 2005). Evidence suggests that different intraspecific traits may covary with one another, forming pace-of-life syndromes (POLS) (reviewed in: Réale *et al*., 2010) that may arise due to correlational selection, via gene pleiotrophy, or due to external selection pressures (reviewed in: Immonen *et al*., 2018).

Examples of intraspecific trait variation can be observed in oystercatchers (*Haematopus ostralegus*), which employ one of two available foraging tactics to break open mussels (*Mytilus edulis*) (Sutherland & Ens, 1987), and in peppered moths (*Biston betularia*), which exist in two distinct colour morphs; a white morph speckled with black dots, and a melanized black form (Edleston, 1864). These examples are, of course, of traits that fall into distinct categories. Other traits, such as personality (*e.g.* Réale *et al*., 2007), vary along a spectrum within species.

1.2 Why study intraspecific variation in traits and their covariance?

In this thesis, I aim to increase knowledge and understanding of the factors that affect intraspecific variation in traits and their covariance. Doing so is important because intraspecific variation in the allocation of energy towards energetically expensive traits (*e.g.* active personality types, large body sizes and high metabolic rates) may cause a trade-off in allocation of energy towards survival and reproduction (Burton *et al*., 2011; Stearns, 1992). Intraspecific variation in traits and their covariance may result in intraspecific variation in individual fitness. For example, individuals with larger body sizes are more likely to win in competitive interactions (Shackleton *et al*., 2005) and less likely to become injured than smaller conspecifics, and may have increased longevity and higher chance of survival as a result. Individual differences in traits and their covariance may also influence how different individuals cope with environmental stressors, including environmental warming (*e.g.* Sih *et al*., 2012). Clearly, intraspecific variation in traits has important consequences for individual fitness.

In influencing individual fitness, the factors that contribute towards intraspecific variation in traits have ramifications for population dynamics (Burton *et al*., 2011). Intraspecific variation, and hence the factors that influence that variation, also play an important role in the structure and function of ecosystems: within species, individual differences in traits can influence ecologically important processes including interspecific competition (*e.g.* Duffy, 2010), predator-prey interactions (*e.g.* Post *et al*., 2008), and host-parasite dynamics (*e.g.* Duffy & Sivars-Becker, 2007). In fact, intraspecific trait variation may be as important as species diversity in shaping ecosystems (Des Roches *et al*., 2018). Understanding the factors that affect intraspecific variation in traits and their covariance will broaden understanding of why ecosystems are shaped as they are and function as they do.

Intraspecific variation in traits is also important in evolutionary biology: because the majority of traits contain a heritable element (Lynch & Walsh, 1998), and influence individual fitness, intraspecific variation in traits provides the raw material upon which natural selection can act. Understanding the factors that affect intraspecific variation in traits and their covariance is therefore important as intraspecific trait variation determines the rate and direction of evolution, and allows adaptation to environmental change (Jump *et al*., 2009). Intraspecific trait variation and trait covariances, and hence the extrinsic and intrinsic factors affecting intraspecific trait variation, may therefore influence how species as a whole respond to environmental change.

Despite universal variation in traits between members of the same species, and the clear implications for intraspecific variation for individual fitness, species' ecology and evolution, the significance of individual differences in traits has been recognised only within the past three decades (*e.g.* Wilson, 1998). As such, the factors that affect

intraspecific variation in traits and their covariance are still not fully understood. Investigating the extrinsic and intrinsic factors affecting intraspecific variation in traits and their covariance will increase understanding of the potential implications of that variation for species' ecology, evolution, and responses to climate change. I therefore ask: what intrinsic and extrinsic factors affect, and how do they affect, intraspecific variation in and the covariance of traits?

1.3 Extrinsic factors influencing intraspecific variation in traits and their covariance

A range of extrinsic factors during both development and adulthood combine to cause differences in the ways that individuals look, behave, and function physiologically (Bolnick *et al.*, 2003). For instance, exposure of different individuals to different population densities (*e.g.* Denno *et al.*, 1985), parental predation risks (*e.g.* Giesing *et al.*, 2011), or oxygen availabilities (*e.g.* Frazier *et al.*, 2001) during development can result in different individuals having different trait values or expressions. Because successive generations often develop under different environmental conditions, it is common for traits that are influenced by environmental conditions to vary between individuals within populations.

Spatial variation may also lead to variation within populations. For instance, individuals within species undergoing range expansions/shifts may show differences in trait values or expressions depending upon their position relative to the range edge: invasive cane toads (*Rhinella marina*) at the range edge have higher body conditions (Brown *et al.*, 2013), move in longer, straighter paths (Alford *et al.*, 2009; Brown *et al.*,

2014; Lindstrom *et al*., 2013), and have longer legs (Phillips *et al*., 2006) than individuals farther back in the species' range. Likewise, changes in environmental conditions across geographic gradients have been shown to influence average trait values between populations (*e.g.* Berven & Gill, 1983; Conover & Present, 1990; Huey *et al*., 2000; Roff, 1980). For instance, house finch (*Haemorhous mexicanus*) bills increase in length and decrease in width with an increase in urbanisation (Giraudeau *et al*., 2014). Hence, both stable differences in environmental conditions between populations, and fluctuations of environmental conditions within populations, can contribute towards intraspecific variation in traits.

Like average trait values, the relationships between traits may change across environmental gradients. This suggests that environmental conditions may play an important role in influencing intraspecific variation in trait covariances (Hämäläinen *et al*., 2020), and may help to explain why strength and direction of trait covariances often differ between species and/or studies (Hämäläinen *et al*., 2020; Killen *et al*., 2013). Trait covariances are expected to increase in unfavourable habitats, because the survival advantage/disadvantage of some trait values may become evident only in harsh environments (Dammhahn *et al*., 2018). A reduction in resource acquisition and associated allocation of energy towards growth, reproduction and metabolic rates should also increase the strength of trait covariances (reviewed in: Hämäläinen *et al*., 2020). However, only very few studies have investigated trait covariances across different environmental conditions and contexts, and thus far evidence for environmental effects remains weak. Weak associations between environmental conditions and trait covariances may, however, be attributed to differences amongst studies in the types of species (*i.e.* invertebrates *versus* vertebrates, ectotherms *versus* endotherms) and

environmental conditions studied (Hämäläinen *et al*., 2020). There is therefore a call to study the influence of different environmental conditions and contexts on the intraspecific variation of trait covariances of many species from different taxa. In this thesis I investigate intraspecific variation in the influence of environmental conditions on the relationships between traits in an insect, adding substantially to this field.

1.3.1 Temperature

Temperature is an environmental factor that may contribute to between-individual variation in traits and their covariance, with long- and short-term effects (discussed below). Given that animals living in temperate regions experience large daily fluctuations in temperature, and that environmental temperatures are on the rise (IPCC, 2022), environmental temperature has ecological implications for individuals and species. The effect of environmental temperature on intraspecific traits and their covariances may be especially important for ectotherms, because their body temperature, which determines the rate of biological reactions, is strongly dependent upon that of the external environment.

1.3.1.1 Long term effects of temperature on intraspecific traits and their covariance

Because individuals can acclimatise to their environmental conditions during development (*e.g.* Muir *et al*., 2014), and because temperature during development may exert long-term effects on intraspecific traits (*e.g.* Brakefield, 1996; Prudic *et al*., 2011), temperature during development may contribute towards intraspecific trait variation. For instance, individuals that develop under and therefore acclimatise to consistently low environmental temperatures demonstrate higher metabolic rates than warm-developed

individuals when compared in an intermediate thermal environment. This is because: (1) under low developmental temperatures, individuals generally upregulate mitochondria and enzyme production to sustain a relatively high metabolic rate in the cold (Clarke, 1993); and (2) metabolic rate increases with temperature within species (Clarke & Fraser, 2004). In influencing individual metabolic rate, and by association, its trade-off with the allocation of energy towards maintenance, growth, behaviour and reproduction, environmental temperature can influence the growth and development of ectotherms (Gillooly *et al*., 2001), causing trait covariances. Indeed, individuals that develop at cold temperatures tend to have reduced growth rates and longer development times, and attain a larger body size than conspecifics that develop under warmer temperatures (Atkinson, 1994). Likewise, in affecting individual metabolic rate, environmental temperature may influence individual personality. Overall, environmental temperature exerts great influence over the average trait values of individuals and is an important factor contributing to intraspecific variation in traits and their covariance.

In addition to environmental temperature during development influencing average-level trait values, the degree to which environmental temperature varies during development may influence metabolic and behavioural thermal plasticity (Beaman *et al*., 2016): the extent to which individual metabolic rate and behaviour changes with temperature in the short term. For instance, individuals that develop under extremely variable thermal conditions may respond to a range of temperatures with low behavioural and metabolic plasticity (*i.e.* behaviour and metabolic rate remain consistent across temperatures), whilst individuals that are acclimated to a particular temperature should typically demonstrate large behavioural and metabolic plasticity when experiencing temperatures outside of their developmental range (Beaman *et al*., 2016;

Gabriel, 2006). Intraspecific variation in thermal plasticity can be studied using 'reaction norms' (Schlichting & Pigliucci, 1998) that investigate the interaction between genotype and environment. This is because individual reaction norms capture information on two different components of each individual phenotype: (1) the average expression of a phenotype under average environmental conditions (*i.e.* individual average animal personality, average metabolic rate etc); and (2) the change in the expression of a phenotype with a change in environmental conditions (*i.e.* individual plasticity) (Nussey *et al*., 2007). Importantly, individuals of similar personalities (*e.g.* Dingemanse *et al*., 2010) and metabolic rates (*e.g.* Réveillon *et al*., 2019) seem to respond to temperature in similar ways, indicating that average animal personality and metabolic rate may be related to thermal plasticity. For instance, common lizards (*Zootoca vivipara*) with higher maximal sprint speeds at average temperatures demonstrate increasingly higher maximal sprint speeds as temperatures increase when compared to individuals with lower maximal sprint speeds at average temperatures (Artacho *et al*., 2013). In summary, environmental temperature can have long-term effects in influencing intraspecific variation in average level traits, trait plasticity, and trait covariance.

1.3.1.2 Short term effects of temperature on intraspecific traits and their covariance

Temperature not only exerts long-term effects by influencing intraspecific variation in traits, it has short-term effects, too. In the short term, environmental temperature can cause individual traits such as metabolic rate and behaviour to fluctuate by influencing the speed at which biochemical and physiological processes occur. The influence of temperature upon animal personality has been studied extensively in the laboratory (*e.g.* Franken *et al*., 2018; Lann *et al*., 2011; Lyons *et al*., 2012; Maebe *et al*.,

2021; Marden, 1995; Nyamukondiwa & Terblanche, 2009; Sgrò *et al*., 2010; Sinclair *et al*., 2012). However, studying how temperature fluctuations influence animal personality in the natural environment is far less studied, though such measures should be far more ecologically relevant than those obtained in the laboratory. Investigating the effects of short-term temperature fluctuations on personality in the natural environment therefore forms an important component of this thesis.

The rate at which environmental temperature changes intraspecific traits depends upon individual thermal plasticity. However, if traits that covary differ in their rate of change with temperature (*i.e.* their thermal plasticity), then temperature may also influence the strength or even direction of trait covariances in the short term. The short-term effects of temperature on intraspecific trait covariances are therefore directly linked to the long-term effects of temperature on intraspecific traits (Beaman *et al*., 2016). Differences in the rate of change of covarying traits with temperature may have implications for individual fitness. For instance, if individuals experience an increase in metabolic rate but are unable to sustain resource acquisitioning behaviours to fuel their heightened metabolic rate, then they will starve. Despite the fact that temperature-induced changes in the covariance of intraspecific metabolic rate and behaviour may have implications for individual fitness, and hence the evolution of species' under continued climate warming, covariance in the thermal plasticity of metabolic rate and behaviour has yet to be investigated (Careau *et al*., 2020). This therefore represents a major gap in the study of intraspecific trait covariances. I am to fill this gap through the work presented in this thesis.

In addition to influencing trait covariances, environmental temperature fluctuations may also change our perception of the level of intraspecific trait variation in

a population in the short term. This is because, when individuals vary in their metabolic (*e.g.* Careau *et al*., 2014; Kar *et al*., 2021; Shik *et al*., 2019) and behavioural (*e.g.* Artacho *et al*., 2013; Baškiera & Gvoždík, 2019; Biro *et al*., 2010; Briffa *et al*., 2013; Cornwell *et al*., 2019; Dingemanse *et al*., 2010) thermal plasticity, fluctuations of environmental temperature may exacerbate differences in animal personality and metabolic rate between individuals. In some circumstances, individuals may appear to have a high metabolism, or a more bold, exploratory or aggressive personality under one temperature but a relatively low metabolic rate and relatively shy and inactive personality under another (*e.g.* Biro *et al*., 2010). Such effects can be observed in the lemon damselfish (*Pomacentrus moluccensis*), where temperature increases of just three degrees centigrade alter the rank-order of individual personality, as some individuals become more aggressive and bold as temperature increases whilst the behaviour of other individuals remains stable (Biro *et al*., 2010). Clearly, understanding how temperature affects intraspecific variation in traits and their covariance, and our perception of those traits and covariances, will be integral to properly understand the potential implications of intraspecific trait variation for species' ecology, evolution, and their responses to climate change.

1.4 Intrinsic factors influencing intraspecific variation in traits and their covariance

In addition to extrinsic factors, a range of intrinsic factors also contribute towards intraspecific trait variation and trait covariances. Individuals of different ages (reviewed in: Glazier, 2005), sexes (*e.g.* Rusterholz & Erhardt, 2000), body conditions (*e.g.* Cotton *et al*., 2004) and reproductive states (*e.g.* Videlier *et al*., 2019) frequently differ from one

another in the ways that they look, behave and function. For example, the metabolic rate of mated *Drosophila melanogaster* females is considerably higher than that of virgin females (Videlier *et al*., 2019), and in many species, differences in body composition between different age groups can lead to age-specific differences in metabolic rate (reviewed in: Glazier, 2005). Intraspecific variation in traits may also be attributable to genetics; most traits are at least intermediately heritable (Lynch & Walsh, 1998). Certainly, animal personality is moderately heritable across taxa (*e.g.* Ariyomo *et al*., 2013; Brodie, 1993; Dingemanse *et al*., 2002; Drent *et al*., 2003; Fairbanks *et al*., 2004; Niemela *et al*., 2015; Sinn *et al*., 2006), with average heritability across species being estimated at 52% (Dochtermann *et al*., 2015). Heritability of life history, physiological, and morphological traits across invertebrates, amphibians and birds are estimated to be 27%, 31% and 51%, respectively (Mousseau & Roff, 1987).

1.4.1 Body size

One of the most notable intrinsic factors influencing intraspecific variation in traits and their covariance is body size. Body size may vary amongst individuals due to genetics (*e.g.* Mousseau & Roff, 1987), sex (*e.g.* Sadowski *et al*., 1999), or environmental conditions during development (*e.g.* Atkinson, 1994).

Intraspecific variation in body size influences intraspecific variation in metabolic rate. Both amongst and within species, metabolic rate consistently scales with body mass (a measure combining both body size and body condition) such that metabolic rate = aM^b, where a is the coefficient, and b is the mass-scaling exponent (Kleiber, 1932). The exact rate to which metabolic rate scales with body mass is highly debated (Brown *et al*., 2004; Glazier, 2005, 2010; West *et al*., 1997), with some researchers reporting exponents

of ¾ (Brown *et al*., 2004; Kleiber, 1932; West *et al*., 1997), whist others report a scaling exponent of ⅔ (Dodds *et al*., 2001; Heusner, 1982). Yet still, others report that metabolic rate likely scales with body mass at different rates in different species (*e.g.* Bokma, 2004; Clarke & Johnston, 1999; reviewed in: Glazier, 2005). At the very least, two things are clear: both within and among species, metabolic rate increases with organ size such that larger individuals have higher metabolic rates, but, because organs typically scale with body size with negative allometry (*i.e.* organs are relatively larger in smaller rather than larger individuals), smaller individuals generally have higher metabolic rates relative to their body size (Kleiber, 1932). Body size is therefore a great source of variation in individual metabolic rate.

As well as influencing intraspecific variation in metabolic rate, intraspecific variation in body size may lead to animal personality differences. This may occur if: (1) individuals of different body sizes differ in their expected future fitness (Wolf *et al*., 2007); (2) individuals of different body sizes consistently differ in the cost-benefit ratios of competitive interactions; and (3) individuals with different body sizes consistently differ in their energy requirements (Schmidt-Nielsen, 1984). Individuals should therefore behave differently from one another in a way that is consistent over time and/or contexts. Because individuals with high expected future fitness have relatively more to lose than conspecifics with lower expected future fitness, they are generally expected to behave cautiously, whilst those with less to lose readily take more risks (Wolf *et al*., 2007). If individual metabolic rate and animal personality do not scale with body mass to the same rate, then body mass may also influence the covariance of metabolic rate and animal personality.

1.4.2 Sex

In addition to body size, sex is a major intrinsic factor influencing intraspecific variation in and the covariation of traits. This is because males and females show differences in reproductive investment - males typically invest in energetically cheap sperm whilst females typically produce large and expensive eggs (Bateman, 1948) – which causes sex-differences in the energetic requirements of reproduction. A widespread and widely documented product of sex differences in reproductive investment is intraspecific variation in the form of sexual dimorphisms (reviewed in: Shine, 1989).

1.4.2.1 Sex differences in the acquisition and allocation of energy

Species may display sexual dimorphisms in traits and their covariances because sex-differences in the energetic requirements of reproduction may cause the sexes to allocate energy differentially amongst metabolism, growth and behaviour. This may result in the sexes displaying, on average, different metabolic rates (*e.g.* Burggren *et al*., 2017), body sizes (*e.g.* Teder & Tammaru, 2005), and animal personalities (*e.g.* Videlier *et al*., 2019). For instance, because female insects frequently invest greater energy into growth than their male counterparts, female insects generally display a strong relationship between body size and fecundity (Forrest, 1987; Honěk, 1993). Sex differences in reproductive investment may additionally cause the sexes to occupy different ecological niches in order to meet their different energy requirements (Darwin, 1871). Indeed, male and female Adonis blue butterflies (*Polyommatus bellargus*) display different foraging patters (Rusterholz & Erhardt, 2000). Moreover, sexes that predate upon different species or sizes of prey may exhibit different personalities or

morphologies. For instance, female mosquitofish (*Gambusia affinis*) preferentially select for larger prey (Bence & Murdoch, 1986) and are both more active (Etheredge *et al*., 2018) and larger in size (Fryxell *et al*., 2015) than males. Sex overall presents a large source of intraspecific variation in traits.

1.4.2.2 Sexual selection

Sex differences in the energetic requirements of reproduction may also generate dimorphic traits via sexual selection. Individuals that invest more into gamete production and survival (typically females) should be more selective with whom they mate, whilst individuals that invest relatively less in reproduction (typically males), and who are the subject of mate choice, should compete with other members of the same sex to win access to mates (Andersson, 1994; Darwin, 1871; Trivers, 1972). The two mechanisms, inter-sexual and intra-sexual selection respectively, may generate sexual dimorphisms, thereby generating intraspecific variation in traits.

Sexual selection may additionally influence intraspecific variation in traits within sexes. Inter-sexual selection may influence intraspecific variation because, although male traits may accurately signal individual ability to provide parental care (*e.g.* Møller & Jennions, 2001), or the quality of individual genes to potential mates (Fisher, 1930), the cost of producing traits that accurately signal quality is often high (Zahavi & Zahavi, 1997), and not all males can afford to produce such costly signals. For example, although female Midas cichlids (*Cichlasoma citrinellum*) prefer aggressive males (Barlow, 1986), aggression varies widely amongst males (Holder *et al*., 1991). Similarly, the eye spans of male stalk-eyed flies (*Cyrtodiopsis dalmanni*) become more exaggerated with increasing body condition (Cotton *et al*., 2004). Intraspecific variation in both average level and

consistency of female choosiness may also generate intraspecific variation in male traits. Moreover, males may demonstrate intraspecific variation in traits that do not confer information on male quality if females differ in their preferences for different trait values or expressions (reviewed in: Schuett *et al*., 2010). As with traits that are subject to inter-sexual selection, traits that improve competitive advantage are often energetically expensive, meaning that intra-sexual selection may contribute towards intraspecific variation in male traits. For instance, intra-sexual selection may lead to the evolution of alternative reproductive tactics in males (reviewed in: Gross, 1996) which involve different personalities and morphologies (Sih & Bell, 2008). On the whole, sex is a major factor that influences intraspecific variation in traits and their covariance. Studying how sex and its interaction with environmental conditions impact trait covariances is therefore essential to broaden knowledge and understanding of how intraspecific traits affect species' ecology, evolve, and the capacity of species to survive environmental change.

1.4.2.3 Sex and intraspecific variation in trait covariances

In addition to causing intraspecific variation in traits, sex-differences in the energetic requirements of reproduction may cause sex-differences in the fitness benefits of trait-covariances. This may lead to sex-differences in trait covariances as a result. However, the role of sex in influencing intraspecific variation in trait covariances has thus far been neglected (Hämäläinen *et al*., 2018). Furthermore, whether or not sex interacts with environmental conditions to influence intraspecific traits and their covariances is unknown. Ignoring the potential influence of sex on trait covariances and how sex-specific trait covariances are influenced by environmental conditions could lead to erroneous

conclusions on the strength or even direction of trait covariances and their implications for the individual, the ecosystem, and the survival of species under continued environmental change. In this thesis I investigate the above stated gaps in the literature, increasing knowledge and understanding of how important sex may be in influencing intraspecific trait covariances, and the sensitivity of those covariances to environmental conditions.

1.5 Study outline

In this thesis I use sex-specific laboratory and field-based measures of intraspecific traits, experimentally manipulated temperatures, and environmental conditions in the natural habitat, to study sex differences in and the effect of environmental conditions on intraspecific variation in traits and their covariance, using *Carabus hortensis* as a model organism. I use the results of my findings to predict how intraspecific variation in traits may impact *C. hortensis* in a changing world. Specifically, I investigate intraspecific differences in metabolic rate, personality (exploratory behaviour in a novel environment), body mass and body size, and their covariance.

The first three chapters in my thesis focus on sex differences in and the effects of environmental conditions on intraspecific trait variation. Being based on an insect system, these first three chapters contribute much towards a field in both insects and ectotherms more generally are currently understudied.

In Chapter 2, I demonstrate for the first time in an insect that morphological traits associated with movement may increase across an expanding range and towards the range edge, in just one sex. This demonstrates that environmental conditions interact

with sex differences in reproductive biology to produce differential distributions of male and female morphology in space.

In Chapter 3, I use radio-telemetry methods to obtain individual thermal tolerance values (*i.e.* the lower, upper, optimum and range of temperatures at or over which individuals are active) from the wild, and assess sex differences in both intraspecific variation in these traits, and in the thermal dependency of repeatable movement patterns, in the wild. Though I found no sex differences in average level or the level of intraspecific variation in thermal tolerance values, males and females differed in the thermal dependency of movement patterns. To the best of my knowledge, this is the first time that insect thermal tolerance values have been obtained from the wild. The methods used here could be helpful for future studies looking to predict species' responses to environmental warming, because thermal tolerance values obtained from the wild should more accurately describe the capacity of individuals or species to deal with high temperatures than those obtained in the laboratory.

In Chapter 3, I found that individuals consistently differed from one another in the ways in which they moved in the natural habitat, over time. The finding that different movement patterns in the wild could be described as personality traits provided the basis for Chapter 4, in which I investigate the link between those field-derived personality traits and a personality trait commonly measured in the laboratory: exploratory behaviour in a novel environment. I know of only one other study that has investigated the link between exploratory behaviour in the laboratory and personality traits measured in the wild in an insect (Fisher *et al.*, 2015). I show that exploratory behaviour measured in the laboratory is predictive of sex-specific movement parameters that differ consistently among

individuals. I therefore demonstrated that exploration in a novel environment has different ecological relevance for the sexes.

The following three chapters contribute several novel findings to the field of intraspecific variation in traits and their covariances. The scope of Chapters 5-7 broadened from that of Chapters 2-4 to investigate sex differences in and the effects of environmental temperature on not only intraspecific variation in traits, but their covariances. Given that sex is rarely considered when investigating the relationships between intraspecific traits (Hämäläinen *et al*., 2018), especially so in insects (Royauté *et al*., 2015), and that studies investigating trait covariances across different environmental contexts remain rare (Hämäläinen *et al*., 2020), Chapters 5-7 contribute substantial information to this field of study.

In Chapters 5-7, I invested both sex differences in the relationships between and the sex-specific effects of temperature on the relationships between metabolic rate and other intraspecific traits. I included repeated measures of resting metabolic rate (RMR; the metabolic rate of an animal that it is not digesting, during a period of inactivity), which is probably the most commonly used measure of metabolic rate in intraspecific studies, but also repeated measures of active metabolic rate (AMR; the metabolic rate of an animal that it is not digesting, during a period of activity). Active metabolic rate could influence the energy available for growth and reproduction, and could be tightly linked to personality, because it describes the energetic cost of activity or behaviour. However, to the best of my knowledge, no other studies test the relationships between AMR and personality on a sex-specific basis. This thesis therefore makes a novel contribution to the study of factors influencing intraspecific trait covariance in that, for the first time, the sex-

specificity of: (1) the relationships between repeatable AMR and personality (Chapter 5); (2) the thermal dependency of metabolic rate scaling with body mass (Chapter 6); and (3) the covariance in metabolic and behavioural thermal plasticity (Chapter 7) are investigated.

This thesis demonstrates the importance of conducting all intraspecific trait and covariance analysis on a sex-specific basis, and of taking into consideration that different individuals respond to temperature in different ways. For instance, in Chapter 3, I observed that there was intraspecific variation in the temperatures below and beyond which individual movement slowed. In Chapter 5, I demonstrated that combining data from both sexes can lead to erroneous conclusions when investigating intraspecific trait covariances. In Chapter 7, I used reaction norms approaches to test for the first time the covariance of metabolic and behavioural thermal plasticity. Together these results demonstrate that ignoring the presence of between-individual variation in thermal sensitivity could lead to erroneous conclusions on the effects of temperature on species' trait covariances. This may have consequences for predictions to be made about individuals that experience large daily fluctuations in temperature, on the survivability of species under continued environmental warming. The results in this these may also help to explain why the strength and direction of trait covariances often differ between studies and species, and may be used to develop a framework for obtaining more accurate results concerning how intraspecific traits relate to one another.

1.6 Study species

Figure 1. Female *C. hortensis.*

C. hortensis is a flightless, predatory, and nocturnally active ground beetle (Freude *et al*., 1976) (Figure 1), that is active from mid-July until the end of October (Günther & Assmann, 2000; Larsson, 1939), reproductively active from August – September (Turin *et al*., 2003), and eurytopic within forests (Lindroth, 1985). *C. hortensis* is an ideal study species for investigating sex differences in and the effects of environmental conditions

on intraspecific variation in traits and their covariance because males and females differ morphologically - females are larger than males, who are distinguishable from females by the presence of their dilated pro-tarsi. Findings from other, closely related ground beetle species suggest that the behaviour of male and female ground beetles may be driven by different factors (Baumgartner, 2000; Drees & Huk, 2000), meaning that male and female *C. hortensis* may also demonstrate differences in personality and its relation to other intraspecific traits. Furthermore, *C. hortensis* are ectothermic and, being a temperate species (occurring naturally from Russia and Finland in the North to the Balkan Peninsula in the South, and across eastern, central and northern Europe (Turin *et al*., 2003)), should experience large daily fluctuations in temperature that may affect individual traits and their covariance. Moreover, evidence suggests that the species is still expanding its range westward: *C. hortensis* can now be found 85km south-west of and 150km north-west of its 1920's range edge in Ulm, southern Germany (Trautner, 1992), and was shown to expand its range westward by approximately 2.7km in the Lüneburger Heide nature reserve, northern Germany, from 1995-2017 (Völler *et al*., 2018). This system provides the opportunity to compare intraspecific traits across an environmental gradient, being the species' expansion front. Finally, *C. hortensis* can easily be kept in the laboratory, and hence are suitable for studies involving experimental manipulations.

Chapter 2: Sex differences in morphology across an expanding range edge in the flightless ground beetle, *Carabus hortensis*

2.1 Abstract

Species' ranges are dynamic, changing through range shifts, contractions and expansions. Individuals at the edge of a species' shifting range often possess morphological traits that increase movement capacity, that are not observed in individuals farther back within the species' range. Although morphological traits that increase in proportion towards the range edge may differ between the sexes, such sex differences are rarely studied. Here, we test the hypotheses that body size and condition increase with proximity to an expanding range edge in the flightless ground beetle, *Carabus hortensis*, and that these trait changes differ between the sexes. Male, but not female, body size increased with proximity to the range edge. Body size was positively correlated to male front and mid tibia length and to female hind tibia length, indicating that body size is indicative of movement capacity in both sexes. Body condition (relative to body size) decreased with increasing population density in males but not females. Population density was lowest at the range edge. Our results indicate that sex is an important factor influencing patterns in trait distribution across species' ranges, and future studies should investigate changes in morphological traits across expanding range margins separately for males and females. We discuss the implications for sex differences in resource allocation and reproductive rates for trait differentiation across species' shifting ranges.

2.2 Introduction

Species' ranges are dynamic, flexible, and capable of change through range shifts, contractions and expansions (Andrewartha & Birch, 1954; Sexton *et al*., 2009). Range shifts can occur as the result of stochastic processes, whereby random individuals at the edge of the species' range (hereafter: range edge) slowly expand the range over time through random movements (Skellam, 1951). Yet, range shifts may often be driven by a subset of individuals residing at the range edge, who are characterised by traits that increase capacity for forward movement, not possessed by individuals farther back within the species' range (Chuang & Peterson, 2016; Phillips *et al*., 2006; Shine *et al*., 2011). Such traits are often associated with morphology.

Understanding how traits associated with morphology differ across expanding or shifting ranges is important because such patterns in trait distribution may alter the pace of range changes (Bowler & Benton, 2005; Roff, 1984; Zera & Denno, 1997) and influence population dynamics (*e.g.* Gadgil & Bossert, 1970; Stearns, 1976). Intraspecific (Bolnick *et al*., 2003) and interspecific (Rudolf, 2007) interactions, including predator-prey interactions (Cohen *et al*., 1993), resource use (Polis, 1984), and individual capacity to overcome environmental change (Huey & Kingsolver, 1989), may also be affected by trait differentiation at range edges. In invasive species, traits associated with morphology that propel the species forward could amplify the negative effects that the invader has upon native flora and fauna (Phillips *et al*., 2006). Hence, traits that drive range shifts at range edges can have large-scale ecological impacts.

Differences in traits associated with morphology among individuals from the centre or 'core' of a species' range *versus* the range edge have been documented across

different taxa (*e.g.* Berthouly-Salazar *et al*., 2012; Bonte *et al*., 2012; Brandner *et al*., 2013; Hill *et al*., 1999; Phillips *et al*., 2006). For example, across homogenous environments, individual body condition may increase towards the range edge (*e.g.* Brown *et al*., 2013) where population densities and associated competition for resources are typically low. Individuals that have increased locomotor capacity (*i.e.* those with longer legs (Phillips *et al*., 2006), increased flight muscle mass and/or wing size (Heidinger *et al*., 2018; Hill *et al*., 1999) also increase in frequency with proximity to the range edge. Patterns in trait distribution across species' ranges may occur through: (a) trait dependent dispersal (*e.g.* Heidinger *et al*., 2018), whereby only individuals with traits that confer the highest dispersal capacity disperse to the range edge (Heidinger *et al*., 2018); (b) phenotypic plasticity to environmental variation (*e.g.* Tejedo Madueño *et al*., 2010), in which individuals plastically respond to environmental differences in the core *versus* the edge of the range; or (c) the process of spatial sorting (Phillips *et al*., 2008; Shine *et al*., 2011), whereby genes that improve movement propensity become sorted in space, such that individuals with a greater capacity for forward movement reach the range edge at a time where the only available mates are similarly adapted individuals. Assortative mating (Fisher, 1918) then occurs at the range edge.

We may observe a stronger gradient in the distribution of traits associated with movement in one sex over the other, if species' range shifts are primarily driven by one sex (Berthouly-Salazar *et al*., 2012). Such sex-biases in movement may arise because: (a) sexual traits selected for in males and females are often divergent due to fundamental differences in male and female reproductive investment (Bateman, 1948; Darwin, 1871; Maynard Smith, 1978) where males generally maximise reproductive fitness through increasing mating opportunities, whilst female reproductive success depends on egg and

offspring production (Trivers, 1972); and (b) some sexually dimorphic traits, such as behaviour or body size, affect movement capacity, and may therefore enhance the propensity for one sex to disperse (Bowler & Benton, 2005). Strong sexual disparities in trait distribution across species' ranges may be especially prominent in scramble-competition type mating systems, in which males evolve adaptations that improve locomotion to increase mate searching efficiency (Husak & Fox, 2008). Although sex differences in morphological traits at range edges have been observed in a few studies, results differ and studies are largely restricted to vertebrates (*e.g.* Bodden & Puschendorf, 2019; Campbell & Echternacht, 2003; Gunnarsson *et al*., 2012; Miller *et al*., 2017; Simberloff *et al*., 2000, but see Laparie *et al*., 2013 for a study on an insect species). This may be explained by differences in reproductive and mating systems across the different taxa. Consequently, further investigation of patterns in trait distribution across species' ranges in taxa with different reproductive and mating systems is required if we are to fully understand the mechanisms underlying morphological differentiation across species' shifting ranges.

Here we study sex-specific changes in body size and body condition along an expanding range margin of the flightless ground beetle, *Carabus hortensis* L., at its western distribution edge in northern Germany. Previous monitoring of this population (Völler *et al*., 2018) allows us to pinpoint the precise range edge of the species in previous years, meaning that traits of individuals from the centre or 'core' of the range can be systematically compared to traits of individuals from the range edge. We predict that individuals at the range edge should be larger in body size than those from the 'core', if body size is directly related to leg length and associated movement capacity. We test this prediction, assessing the correlation between leg length and body size.

Male *C. hortensis,* like other *Carabus* species (*e.g.* Drees & Huk, 2000; Weber & Heimbach, 2001), are generally more active than females (Szyszko *et al*., 2004). Because males of other *Carabus* species are known to actively search for females with whom to mate (Turin *et al*., 2003), male *C. hortensis* activity may be an adaptation to increasing mate searching capacity. Male *C. hortensis* are therefore likely to be the more dispersive sex owing to mate searching behaviour (Turin *et al*., 2003). Thus, we predict that the change in body size across the *C. hortensis* range will be stronger in males than females, with body size increasing towards the range edge.

We further predict that population density will decline with proximity to the expansion front. Thus, we predict that, as long as conditions at the range edge are suitable, and population density is lower at the range edge than at the core, individuals will have better body condition at the range edge than at the core of the species' range, owing to reduced intraspecific competition for resources (Brown, 1984).

2.3 Methods

2.3.1 Study Species, Trapping and Maintenance

Carabus hortensis Linnaeus, 1758 (Coleoptera, Carabidae) ground beetles were studied from August to September 2018 in the Lüneburger Heide, Lower Saxony, Germany, where the species has expanded its range westward from ancient forests into adjacent forested areas at a constant pace over the last 25 years (Völler *et al*., 2018).

To sample individuals from the range edge and from regions farther back in the species' range (*i.e.* across the expansion front) rows of live pitfall traps (hereafter 'trap rows') were installed parallel to the most westerly edge of the species' range, starting

from the leading edge of the expansion and spanning across 3km to the point at which the species was first observed in this area in 1995 (Völler *et al*., 2018). A map of the study site with some trap rows included in our study can be found in Völler *et al*. (2018). Habitat across the sample area consisted of coherent forests of coniferous, broad leaved and mixed stands, and there were no clear systematic habitat differences across the expansion front. We installed 17 trap rows, which reflected the positions of the *C. hortensis'* westerly range edge for the years 1995, 1999, 2001, 2003, 2005, and 2007-2018. Because *C. hortensis* has dispersed westward by approximately 130m each year (Völler *et al*., 2018), an additional trap row was placed 130m beyond 'trap row 2018' where we expected beetles to arrive the following year, in 2019: 'trap row 2019'. This trap row mainly served to assess whether *C. hortensis* had expanded its range further than expected and to ensure that, if it had, we would catch those individuals. Thus, we installed 18 trap rows in total. Each trap row contained 12 live pitfall traps that were separated by 10m to span 120m. To ensure that beetles were caught at the range edge where population densities were expected to be low, 12 additional pitfall traps were positioned at each of the five most westerly trap rows, such that trap rows 2015-2019 contained 24 traps, and all other rows contained 12 traps. We found beetles at 'trap row 2018', which was the expected range edge when our study took place in 2018, but not 'trap row 2019'. This suggested that *C. hortensis* were still expanding their range westward by at least 130m per year, but not as far as 260m per year.

Live pitfall traps (10cm diameter, 500ml plastic cup inside) were dug into the ground so that they were level with the surface soil. A drainage tube around the cup served as a structural support and water drained through holes in the bottom of each

trap. A metal mesh cover prevented small vertebrates, leaves and sticks from falling into the traps. All traps were baited with a piece of cellulose soaked in red wine, and were emptied and re-baited once every 7-8 days (Schuett *et al*., 2018).

The total number of individuals caught at each trap row from August to September 2018 was used as a proxy for population density (Baars, 1979a). However, because sampling efforts at each trap row differed depending on whether the trap row contained 12 or 24 pitfall traps, the population density at each trap row was divided by the total number of pitfall traps present in that row and this was divided by the total number of days over which each trap row was sampled. This provided the number of beetles caught per trap and trapping day for each trap row, which was used as a proxy for population density. The female to male sex ratio at each trap row was also quantified, by dividing the total number of females caught at each trap row by the total number of males caught at that trap row. When more than 30 individuals (15% of the cases) were caught at a particular trap row in one week, we did not record their sex. Consequently, our measure of sex ratio is only an estimate in these cases.

Individuals were either taken to the laboratory for further studies or released to the site of capture. Released individuals were marked using permanent marker pens (Edding 781, Edding International GmbH, Ahrensburg, Germany), to avoid re-testing upon re-capture. Each week, where possible (based on the number of individuals in a trap row), the body size and mass were measured for four females and four males selected randomly from each trap row. In total 161 female and 92 male *C. hortensis* were weighed to the nearest milligram (CA-103 Phoenix Instrument, Phoenix Instrument GmBH, Garbsen, Germany). Individual pronotum width was then measured as a proxy for body

size. Pronotum width has previously been used as a proxy for movement ability in other studies of flightless carabid beetles (*e.g.* Laparie *et al*., 2013), because it describes the space available for locomotor muscles (Berwaerts *et al*., 2002). Dorsal photos of each individual were taken over a laminated page of mm grid paper using a camera phone (Wileyfox Swift 2X, Wileyfox, London, UK), and the widest section of the pronotum was later measured to the nearest 0.1mm, using ImageJ (Schneider *et al*., 2012). To assess our prediction that pronotum width was indicative of movement capacity, we later measured the leg lengths of retained specimens. The tibia and femur of the front, mid and hind leg from the left-hand side of each beetle was carefully removed and mounted upon a piece of card using insect glue. Photos of each leg were taken using a digital camera (Canon EOS 7D; Canon, Tokyo, Japan) mounted on a stereoscopic microscope (Nikon SMZ-U; Nikon Corp., Tokyo, Japan), and the length of each front, mid and hind tibia and femur were measured to the nearest 0.1mm using ImageJ. Two photographs taken of each leg showed that the measurements were significantly repeatable. Each leg length was then calculated as the mean of the two measurements.

Individual body condition scores (relative mass to body size, in g) were calculated separately for males and females. Several different methods to obtain measures for body condition exist, including taking direct measurements of energy stores (*e.g.* Weatherhead & Brown, 1996), calculating body condition from the residuals from reduced major axis regressions of body mass *versus* body size (Green, 2001), and calculating body condition as the residuals from ordinary least-squares (OLS) regression of body mass *vs* body size (*e.g.* Cordero *et al*., 1999; Dobson *et al*., 1999). Here, we employ the latter, more commonly used method, using residual scores from a linear model (LM) of body mass

against pronotum width (males: $y = 0.027*x + 0.295g$, $R^2 = 0.096$, $F_{1,90} = 9.673$, $p = 0.003$, $N = 92$; females: $y = 0.030*x + 0.377g$, $R^2 = 0.087$, $F_{1,159} = 15.360$, $p < 0.001$, $N = 161$) to calculate body condition. We note, however, that, as with other methods, calculating body condition by this means is not without its caveats. For example, several assumptions must be made to permit calculation of body condition from OLS mass/ body size residuals (outlined in: Green, 2001). Moreover, some variation in body condition calculated via OLS mass/ body size residuals may be attributed to intraspecific variation in lean dry body mass (*e.g.* Schulte-Hostedde *et al*., 2005), meaning that OLS mass/ body size residuals may somewhat inaccurately describe lipid stores and therefore body condition (Moya-Laraño *et al*., 2008).

2.3.2 Statistical Analysis

All statistical analyses were carried out using R version 3.3.2 (R Core Team, 2019). We performed Spearman's rank correlations (Spearman, 1904) to assess the relationships between body condition, body size and body mass, and between body size and leg lengths. Spearman's rank correlations were used because not all data followed a normal distribution. Body condition and body size were not highly correlated ($R_s < 0.3$, Table A.1). Body condition and body mass were highly positively correlated ($R_s > 0.9$), and body mass and body size were significantly positively correlated in both male and female *C. hortensis* (Table A.1). To avoid multicollinearity, only data concerning individual body size and body condition were analysed further. Our measures of pronotum width were significantly positively correlated with leg length (Table A.1). To corroborate our hypothesis that male *C. hortensis* may be the more dispersive sex, owing to mate

searching behaviour (Turin *et al*., 2003), we performed additional Spearman's rank correlations to assess the relationship between the female to male sex ratio at each trap row and position along the expansion front.

We predicted that *C. hortensis* population density should decline with increasing proximity to the range edge. The effect of position along the expansion front upon population density was determined using a LM, with population density as the response variable, and the position along the expansion front as the explanatory variable. Position along the expansion front was a discrete variable, in which the positions of the *C. hortensis* westerly range edge for the years 1995, 1999, 2001, 2003, 2005, and 2007-2018 were the values ('trap row 2019' was excluded from analyses as no beetles were caught there), 2018 was the range edge, and the greatest distance was between 2018 and 1995 (Völler *et al*., 2018). For the purpose of analysis, the position along the expansion front was treated as a continuous variable.

To determine whether individuals from different positions along the expansion front differed in their body size and body condition, we performed linear mixed models (LMMs) with body size and body condition as the response variables, and the position along the expansion front as the main explanatory variable. The week (week 1 to week 6) in which individuals were collected was included as a random term. In 34% of the cases, beetles were collected from traps from which at least one other beetle was collected in the same week. To account for any potential interdependence of beetles collected from the same trap on the same week, the trap from which individuals were collected nested within the week of collection (week 1 to week 6) was included as a second random term. Again, the position along the expansion front was treated as a continuous variable during

analyses. Population density was included in the models as a covariate (Table 1.1, Table 1.2). To test whether the relationships between body size/body condition and position along the expansion front differed between the sexes, we added the explanatory variable of sex as well as its interaction with position along the expansion front.

The sex-specificity of the effect of position along the expansion front on individual body size and condition was determined by using two additional LMMs per response variable using only female or male data. The structure of the models was the same as above excluding 'sex' and its interaction with the position along the expansion front as explanatory variables.

Because a significant negative relationship was found between population density and position along the expansion front, population density might mask the effects of position along the expansion front. Thus, body size and body condition LMMs, for male and female combined data, female data alone and male data alone, were re-run without population density as an explanatory variable. Removal of population density from the maximal models for body size (Table 1.1) or body condition (Table 1.2) did not qualitatively change our results (Table A.2; Table A.3).

2.4 Results

As predicted, population density decreased with proximity to the *C. hortensis* expansion front in the Lüneburger Heide (LM; $R^2 = 0.406$, $F_{1,15} = 10.250$, $p = 0.006$; Figure 1.1). The female to male sex ratio was negatively correlated with position along the expansion front ($R_S = -0.492$, $p = 0.044$, $N = 17$), meaning that proportionally fewer females were found at the range edge.

Female *C. hortensis* were larger than males (Table 1.1). Female pronotum width was 8.0 ± 0.1mm (mean ± SE) (range: 5.8 – 9.8mm), whilst male pronotum width was 7.8 ± 0.1mm (range: 5.8 – 9.2mm). Males and females did not significantly differ in their body condition (Table 1.2).

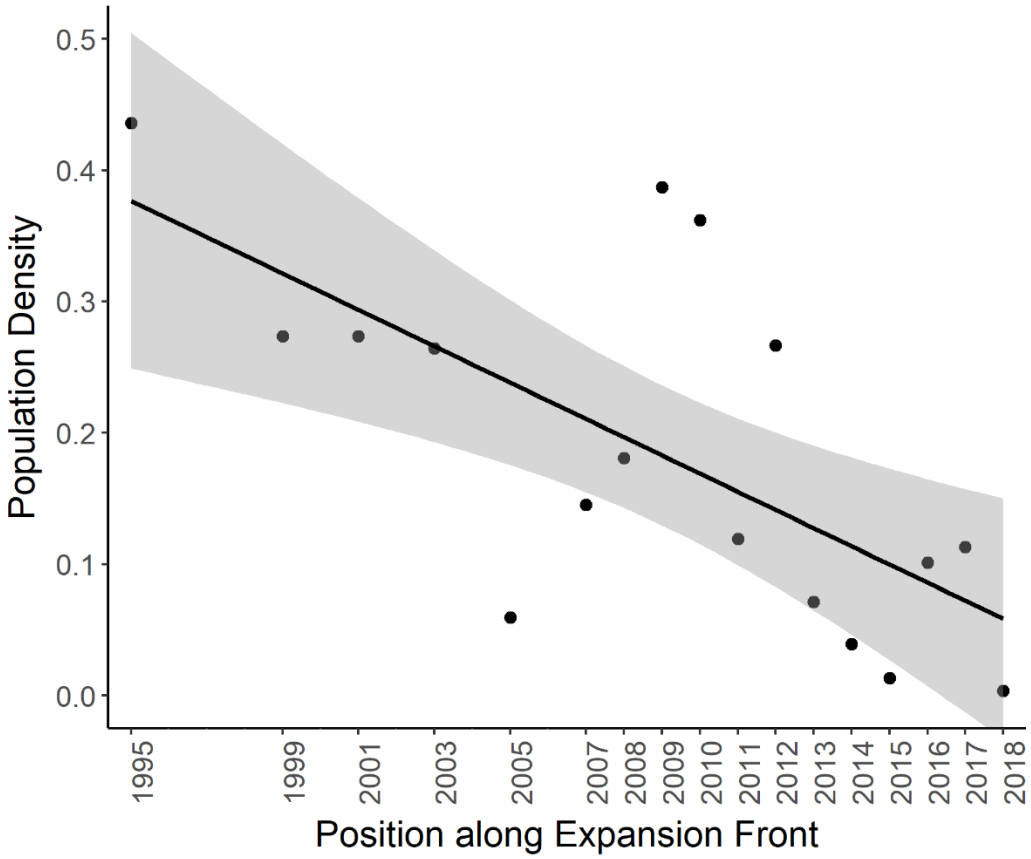

Figure 1.1. The relationship between *C. hortensis* population density and position along the expansion front (N = 17). Years denote the previous locations of the westerly range edge of *C. hortensis* in that year, such that 2018 is the range edge in 2018. Population density is the mean number of beetles per trap and trapping day across a trap row. Predicted line is fitted using outputs from LM estimates. 95% confidence interval is shown in grey.

Table 1.1. Summary of test statistics from LMMs with the pronotum width as a proxy for body size as a response in males and females (M+F), females alone (F) and males alone (M). Sex, position along the expansion front (Position) and population density (the number of beetles caught per trap and trapping day for each trap row), were used as fixed terms. Coefficients (Coeff.) in square brackets belong to non-significant terms just before dropping those terms from the model. Bold p-values denote significant terms. Variance (Var.) of the random terms 'Week' and 'Week/trap' (the trap from which individuals were collected nested within the week of collection) and residuals are presented.

Response Variable	Sex	Random Term	Var.	Fixed Term	Coeff.	χ^2	DF	p-value
Pronotum Width	M+F	Week	0.020	Intercept	-27.18			
(*N* = 253)		Week/trap	0.177	Sex (males): Position	[0.02]	2.92	1	0.088
		Residual	0.331	Sex (males)	-0.23	6.30	1	**0.012**
				Population Density	[-0.33]	0.49	1	0.485
				Position	0.02	5.06	1	**0.024**
Pronotum Width	F	Week	0.003	Intercept	8.04			
(*N* = 161)		Week/trap	0.203	Position	[0.01]	0.40	1	0.529
		Residual	0.352	Population Density	[<-0.11]	0.05	1	0.829
Pronotum Width	M	Week	0.048	Intercept	-66.62			
(*N* = 92)		Week/trap	0.134	Position	0.04	9.88	1	**0.002**
		Residual	0.264	Population Density	[-0.65]	0.91	1	0.340

Table 1.2. Summary of test statistics from LMMs with body condition as a response in males and females (M+F), females alone (F) and males alone (M). Sex, position along the expansion front (Position), and population density (the number of beetles caught per trap and trapping day for each trap row), are used as fixed terms. Coefficients (Coeff.) in square brackets belong to non-significant terms just before dropping those terms from the model. Bold p-values denote significant terms. Variance (Var.) of the random terms 'Week' and 'Week/trap' (the trap from which individuals were collected nested within the week of collection) and residuals are presented. Bold p-values denote significant terms.

Response Variable	Sex	Random Term	Var.	Fixed Term	Coeff.	χ^2	DF	p-value
Body Condition	M+F	Week	0.002	Intercept	0.02			
(*N* = 253)		Week/trap	0.002	Sex (males): Position	[<-0.01]	0.01	1	0.918
		Residual	0.005	Population Density	-0.14	10.02	1	**0.002**
				Sex (males)	[<0.01]	0.01	1	0.922
				Position	[<-0.01]	0.71	1	0.399
Body Condition	F	Week	0.002	Intercept	-0.01			
(*N* = 161)		Week/trap	0.002	Population Density	[<-0.12]	3.54	1	0.060
		Residual	0.007	Position	[<-0.01]	0.24	1	0.623
Body Condition	M	Week	<0.001	Intercept	0.03			
(*N* = 92)		Week/trap	0.002	Population Density	-0.17	9.95	1	**0.002**
		Residual	0.002	Position	[<-0.01]	1.02	1	0.313

The body size of all beetles (Table 1.1) and male beetles (Table 1.1, Figure 1.2) increased towards the range edge. However, female body size did not significantly change with position along the expansion front (Table 1.1, Figure 1.2). There was a marginally significant trend for an interactive effect of sex upon the relationship between body size and position along the expansion front. Body size was independent of population density in both sexes (Table 1.1). Male front tibia length, male mid tibia length and female hind tibia length were positively correlated to body size (Table A.1).

There was no significant relationship between body condition and position along the expansion front for either males or females (Table 1.2). However, the body condition of all beetles (Table 1.2), and male beetles alone (Table 1.2, Figure 1.3) increased with decreasing population density. Female body condition, however, was independent of population density (Table 1.2, Figure 1.3).

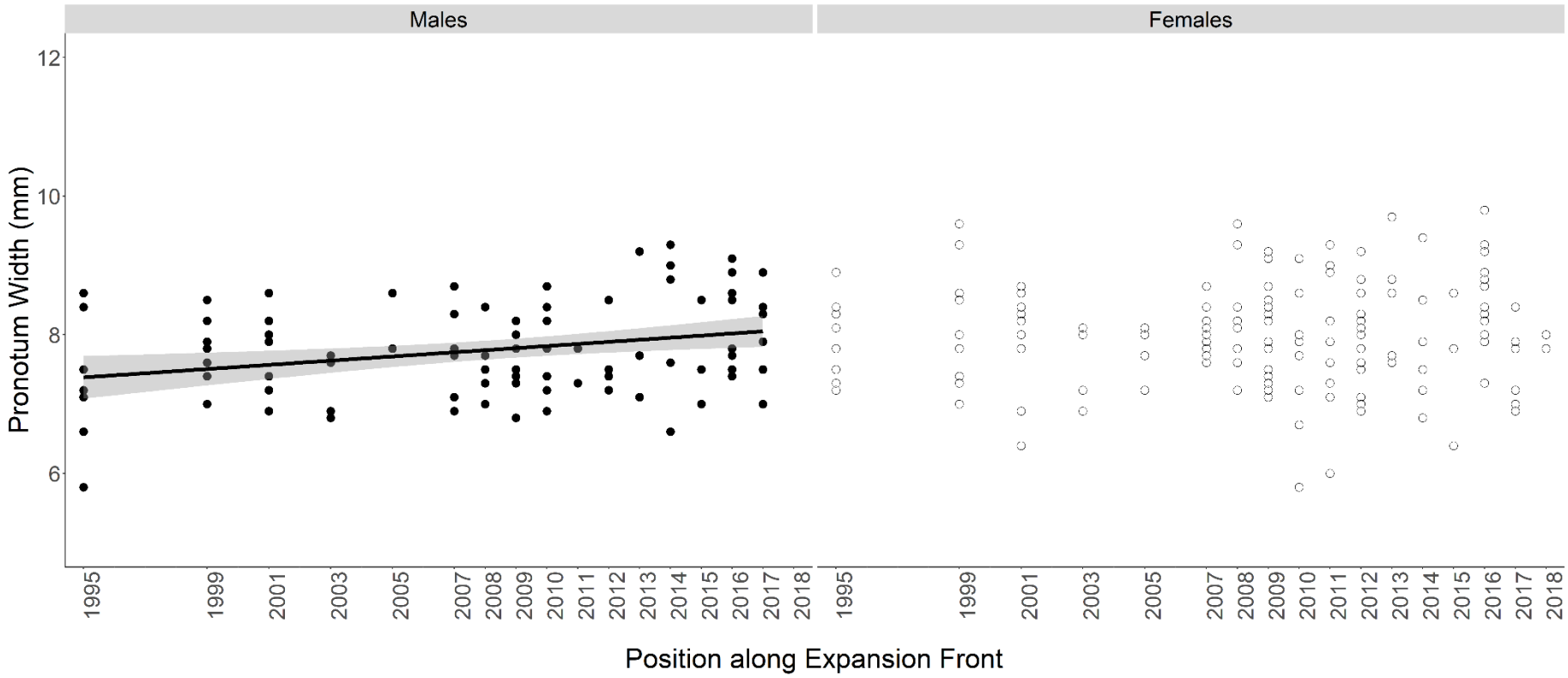

Figure 1.2. The relationship between individual *C. hortensis* body size and position along the expansion front in males (N = 92) and females (N = 161). Years denote the previous locations of the westerly range edge of *C. hortensis* in that year, such that 2018 is the range edge at the time of study, in 2018. Predicted line is fitted using outputs from LMM estimates. 95% confidence interval is shown in grey.

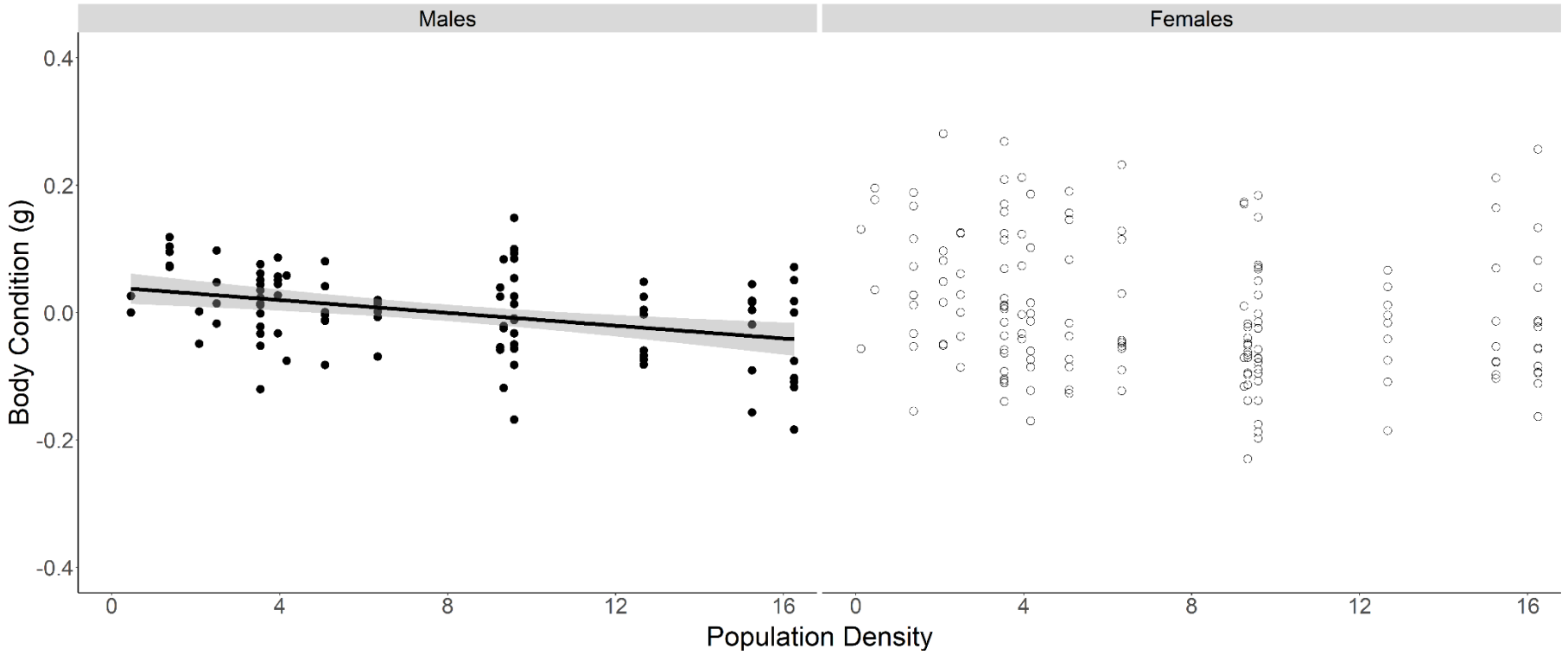

Figure 1.3. The relationship between individual *C. hortensis* body condition and population density in males (N = 92) and females (N = 161). Population density is the mean number of beetles per trap and trapping day across a trap row. Predicted line is fitted using outputs from LMM estimates. 95% confidence interval is shown in grey.

2.5 Discussion

We investigated variation of traits associated with morphology across an expansion front on a sex-specific basis in the ground beetle *Carabus hortensis*. As we hypothesised, body size increased with proximity to the range edge in males but not in females. Although body condition did not increase with proximity to the range edge in either sex as we had predicted, male body condition alone improved with decreasing population density, which was lowest at the edge of the *C. hortensis* range. This may indicate that male body condition was generally higher in areas with low intraspecific competition. Sex differences in the relationships between body size and position along the expansion front, and between body condition and population density, may be rooted in sex differences in activity. Together, our findings provide evidence of sex-specific relationships between morphology and position along an expansion front.

Consistent with our predictions, male but not female *C. hortensis* were larger at the range edge than towards the core of the species' range. Such sex-specific changes in body size distribution across the *C. hortensis* range could occur if divergent selection pressures act upon males and females to produce differences in traits associated with morphology between the sexes (Bateman, 1948; Darwin, 1871; Trivers, 1972) that alter movement capacity. Moreover, differences in male and female activity could underpin the differences between the sexes in the distribution of body sizes across the *C. hortensis* range. Male *C. hortensis* are the more active sex (Szyszko *et al*., 2004); if higher activity levels in males support a male-led range expansion, male body size may change more strongly than female body size across the *C. hortensis* range. That the female to male sex ratio declined with proximity to the range edge supports that range expansion by *C. hortensis* in the Lüneburger Heide may be mainly male led. Increases of male body size

towards the *C. hortensis* range edge may be further reinforced through sexual selection (*e.g.* Hengeveld & Haeck, 1982); where population densities and mate availability is lower at the range edge, males may be sexually selected for larger body size, which may improve movement capacity and related mate searching ability (*e.g.* Arnold *et al*., 2017; Zollikofer, 1994). Again, this hypothesis is supported by our findings that the female to male sex ratio decreased towards the range edge. To understand the mechanisms underlying sex-specific traits differentiation across species' shifting ranges, more studies investigating species with different mating systems are needed.

In line with previous studies of intraspecific competition effects on carabid beetles (Lenski, 1984), male *C. hortensis* from trap rows with lower population densities had a better body condition. Female body condition, however, was unrelated to population density. Population density influences body condition both by altering competition for resources (Iba *et al*., 1995), and by influencing individual activity level (Le Galliard *et al*., 2015; Tuda & Shima, 2002) and associated energy expenditure. Consequently, sex differences in the relationships between population density and body condition may arise if there are: (a) sex differences in activity level; and/or (b) sex differences in the motivations for activity, because population density will influence activity (and associated energy expenditure) differentially between the sexes. For instance, because male *C. hortensis* activity is likely associated with reproductive success, males may be similarly motivated to be active irrespective of whether they are in areas of high or low population density. This may create an imbalance between energy consumption and expenditure under high population densities, because males living under high population densities will experience high intraspecific competition, leading to lower resource availability and associated energy intake than males living under low population densities that have

similar energy expenditure levels. In contrast, female *C. hortensis* may adjust their activity levels to match the population density and related resource availability. Our results suggest that sex can be an important factor in determining how population density will relate to body condition, where males and females differ in activity level. Further investigations into the effects of population density on body condition in systems where males and females differ substantially in their behaviours and life histories, could help to reinforce our findings.

Very few studies have investigated patterns in sex-specific traits associated with morphology across invertebrates' shifting ranges and, thus far, results are mixed. Some studies report that differential morphological traits between the sexes increased with proximity to the range edge (Hughes *et al*., 2003), whilst others state that the same morphological traits increased towards the range edge in both sexes, but with a stronger effect in one sex over the other (Laparie *et al*., 2013). We believe that our study is the first to report the increase in size of morphological traits associated with movement towards a species' range edge in just one sex, in an insect. Some vertebrate studies of morphological changes across species' range expansions are in line with our own, reporting that only male morphological traits increase with proximity to the range edge of an expanding or shifting range (Bodden & Puschendorf, 2019; T. S. Campbell & Echternacht, 2003; Gunnarsson *et al*., 2012). Conversely, for other vertebrates, traits that increased with proximity to the range edge did so in both sexes, but the effect was stronger in males than females (*e.g.* Padilla *et al*., 2019; Simberloff *et al*., 2000). In general, it appears that traits such as body size, wing length and muscle mass (*i.e.* traits that improve movement propensity) are most likely to increase with proximity to the range edge in males. Still, there are too few studies to draw conclusions upon the role of

sex in the distribution of morphological traits across species' ranges, especially in insects. Further work evaluating sex-specific patterns in trait distribution across species' shifting ranges in a range of species with different mating systems will help to further this field.

Ours is the first study of an insect species to report that morphological traits associated with movement may change across a species' range in just one sex. We demonstrated that body size increased across the expansion front in male but not female *C. hortensis* beetles. Males at the range edge of the expansion front were larger than conspecifics farther back in the species' range. Furthermore, male body condition declined with increasing population density. In contrast, we found no significant relationship between female body size and position along the expansion front, and no significant relationship between female body condition and population density. We argue that the observed differences between male and female *C. hortensis* may be linked to differences in the reproductive biology of the sexes and sex-differences in activity level, leading to differential distributions of male and female body size in space (Bateman, 1948; Trivers, 1972). Our results move the field forwards, demonstrating that sex and sex differences in behaviour play an important role in determining the distribution of morphological traits across species' shifting ranges.

2.6 Ethics

The study was carried out under permits from the Heidekreis and Harburg nature conservation *authorities and* the Lower Saxon State Department for Waterway, Coastal and Nature Conservation authorities (number: H72.2220212019) which allowed entry

into the Lüneburger Heide nature reserve and collection of beetles of the genus *Carabus* therein, respectively.

Chapter 3: Intraspecific variation in insect thermal tolerances and temperature dependency of movement in the natural environment

3.1 Abstract

Temperate zones experience large fluctuations in both daily and seasonal temperatures that influence the movement patterns of ectotherms, including insects. However, individuals within species may differ in the temperature-dependency of their movement patterns, potentially influencing reproduction or survival. Such intraspecific variation is usually assessed within laboratory environments, so there is little information from natural environments. We used radio-telemetry to track the movements of individual male and female *Carabus hortensis* ground beetles in their natural habitat. This allowed us to characterise intraspecific variation in field-derived thermal tolerance values, consistent individual differences in path straightness and distances covered, the effects of temperature upon these movement patterns, as well as links between thermal tolerance values and movement patterns. *C. hortensis* showed intraspecific variation in all thermal tolerance values (*i.e.* thermal minima, maxima, optima and operating ranges), and showed the largest variation in thermal maxima. Individual beetles consistently differed in their path straightness and their distance travelled, and these movement parameters were temperature dependent in a sex-specific way. Beetles with broader operating ranges travelled shorter distances over the tracking period than conspecifics with narrower operating ranges. Our findings show that there is intraspecific variation in thermal tolerances and temperature-dependent movement patterns in *C. hortensis* and

emphasise the role of sex in such variation. We discuss potential consequences of our findings for the survival of *C. hortensis* under future climate heating.

3.2 Introduction

Movement is an essential aspect of life (Wilkinson, 2016). It affects multiple scales of organisation from individuals (*e.g.* foraging, habitat choice and reproduction) (reviewed in: Shaw, 2020) to species distributions (Soberon & Peterson, 2005; reviewed in: Bruneel *et al*., 2018) and ecosystems (*e.g.* Lundberg & Moberg, 2003). Animal movements are influenced by environmental factors, including temperature. This is especially true for ectotherms, whose body temperature is strongly dependent upon that of the external environment. Terrestrial ectotherms can buffer themselves against fluctuations in environmental temperature using behavioural strategies, such as basking (*e.g.* Forsman *et al*., 2002; Kevan & Shorthouse, 1970; Kührt *et al*., 2005) or aggregation (*e.g.* Klok & Chown, 1999). However, when environmental temperatures become either too cold or too hot to support the biochemical reactions underpinning key physiological processes, such as skeletal muscle contraction, terrestrial ectotherms cease to move (Bennett, 1985; Rome, 1990).

Ectotherms may show inter-individual differences in their thermal tolerances (*e.g.* Franken *et al*., 2018; Nyamukondiwa & Terblanche, 2009; Sgrò *et al*., 2010; Sinclair *et al*., 2012), including the upper (*e.g.* Franken *et al*., 2018; Nyamukondiwa & Terblanche, 2009) and lower (*e.g.* Maebe *et al*., 2021; Nyamukondiwa & Terblanche, 2009) temperatures beyond which movement capacity declines. They may also differ in the range of temperatures over which they operate and those at which they reach peak movement performance; their operating range and optimal performance temperature (*e.g.* Marden, 1995), respectively. Thus, some individuals may be better adapted to survive changing global temperatures, if they tolerate broader temperature ranges than conspecifics.

Insects are the largest group of terrestrial organisms and therefore the largest group of terrestrial ectotherms. The few available studies that investigate intraspecific variation in insect thermal tolerance values have been carried out in the laboratory (*e.g.* Franken *et al*., 2018; Lann *et al*., 2011; Lyons *et al*., 2012; Maebe *et al*., 2021; Marden, 1995; Nyamukondiwa & Terblanche, 2009; Sgrò *et al*., 2010; Sinclair *et al*., 2012). However, field-derived measures of intraspecific variation in both thermal tolerance values and in the thermal dependency of movement patterns should be far more ecologically relevant than laboratory-based measures, especially if field-based measures accurately describe the thermal dependency of behaviours that influence individual fitness. With recent advancements in radio-tracking technology (*i.e.* a reduction in the size of radio-tags), intraspecific variation in both ecologically relevant thermal tolerance values and the temperature dependency of insect movement patterns can now be measured *in-situ*.

By affecting the movement of terrestrial ectotherms, temperature also directly impacts their foraging (*e.g.* Jayatilaka *et al*., 2011; Vogt *et al*., 2003; Willmer & Stone, 2004), predator avoidance (*e.g.* Christian & Tracy, 1981), communication (*e.g.* Doherty & Callos, 1991; Dolbear, 1897; Edmunds, 1963), and locomotion (*e.g.* Baars, 1979; Lailvaux & Irschick, 2007; Taylor, 1963) with important ramifications for individual fitness (Huey & Kingsolver, 1989). Consequently, understanding how temperature influences ectotherm movement is especially important for species that experience large fluctuations in both daily and seasonal temperatures, such as those living in temperate zones.

That individuals differ in their thermal tolerances, and thermal tolerances influence the temperatures at which movement capacities increase, peak, and decrease, also means that individuals may differ in the thermal dependency of movement. This may

then impact when, and how efficiently, individuals compete for access to mates (*e.g.* Willmer, 1991), and could affect individual capacities to forage, search for potential mates, and avoid predation. Studies examining both the extent of and factors influencing intraspecific variation in thermal tolerance values and in the temperature dependency of insect movement parameters are therefore required to understand how species are likely to respond to climate change.

Here we investigate individual differences in field-derived thermal tolerance values and in the temperature-dependency of movement in an insect, using radio-telemetry to track movement patterns of *Carabus hortensis* Linnaeus, 1758 ground beetles in their natural habitat. Intraspecific differences in the temperature-dependency of field-derived movement patterns have previously been captured in other ground beetles using radioisotopes (*e.g.* Baars, 1979), whilst radio-telemetry (*e.g.* Negro *et al*., 2017; Riecken & Raths, 1996; Růžičková & Veselý, 2018), harmonic radar (*e.g.* Wallin & Ekbom, 1988; Weber & Heimbach, 2001), capture-mark-recapture (*e.g.* Rijnsdorp, 1980; Weber & Heimbach, 2001) or direct *in situ* observation (*e.g.* Drees *et al*., 2008) have been employed to investigate inter-individual differences in movement patterns in ground beetles. Here we ask: (a) what is the extent of intraspecific variation in field-derived thermal tolerance values? (b) do individuals consistently differ in their movement? (c) how does temperature influence individual *C. hortensis* movement? and (d) how do thermal tolerance values relate to movement in the wild?

Given that insects are capable of acclimatising to local conditions (*e.g.* Overgaard *et al*., 2008; Seebacher *et al*., 2015), we predict that population thermal optima for movement will be close to the average temperature conditions experienced in the field.

We also expect to find intraspecific variation in field-derived thermal tolerance values, and in the temperature dependency of movement. Finally, because evidence suggests that male and female *C. hortensis* may have different ecological needs (*e.g.* the relationship between metabolic rate and exploratory behaviour is temperature dependent in females, but not males (Yarwood et al., 2021b), and that male *C. hortensis* (Szyszko *et al*., 2004), like males of other carabid beetles (*e.g.* Drees & Huk, 2000; Gerlach *et al*., 2009; Lagisz *et al*., 2010) are more active than females, we expect to find differences in the temperature dependency of movement between the sexes.

3.3 Methods

3.3.1 Trapping and Maintenance

Movements of individual *Carabus hortensis* (Coleoptera, Carabidae) ground beetles were studied in their natural woodland habitat in the Lüneburger Heide, Lower Saxony, Germany (N53°10'53.32'', E9°53'08.06''), at the western range edge of the species' distribution (Völler *et al*., 2018). Individuals were studied between 10th August and 14th October 2019, during the active and reproductive phase of the species (Turin *et al*., 2003). Each individual's movement was followed for ca. two weeks (see below).

Individuals were captured from 5th August – 30th September 2019, using six rows of live pitfall traps (hereafter 'trap rows') that ran parallel to the species' westerly range edge. Trap rows were positioned in two study sites separated by ~800m; this distance was rarely covered by closely related ground beetles over the space of one month (Negro *et al*., 2017), and we considered that, because *C. hortensis* are flightless, they would be

unlikely to cover this distance over the course of our study. Within study sites, trap rows were separated by ~130m, aside from the first and second trap row in the second study site, which were separated by ~260m, due to logistical constraints (*i.e.* low habitat quality) associated with placing the trap rows 130m apart. Trap rows each contained 10-15 pitfall traps separated by 10m. Pitfall trap structure, baiting and trap monitoring schedules followed those outlined in a previous study by Yarwood *et al*. (2021a) (Chapter 2).

In total, 52 *Carabus hortensis* beetles (28 females and 24 males) were collected and returned to the field station where they were housed separately in the dark, in 10(L) x 7.5(W) x 4.5(H) cm transparent containers containing peat. The soil was regularly sprayed with water to ensure a moist environment and was changed every few weeks. Individuals were fed *Tenebrio molitor* pupae *ad libitum.* Temperatures that beetles were housed under ranged from 17.6 – 32.3°C with a mean temperature of 23.34°C ± 1.76 SD.

Individuals were weighed to the nearest milligram (CA-103 Phoenix Instrument, Phoenix Instrument GmbH, Garbsen, Germany) twice, 2-5 days apart. The average of these two values was taken as individual body mass. *C. hortensis* individuals were kept at the field station for 13.8 days ± 7.5 (mean ± SD; range 1-44 days) prior to release for radio tracking. The number of days that individuals were kept at the field station prior to release into the field did not significantly impact the total distance travelled by females (linear regression; $F_{1,26} = 1.939$, $p = 0.176$, $N = 28$) or males (linear regression; $F_{1,20} = 1.835$, $p = 0.191$, $N = 22$) in the wild.

3.3.2 Tagging

Figure 2.1. *Carabus hortensis* with an attached radio tag.

To track beetle movements in the field, individuals were outfitted with a Micro-Pip radio-tag transmitter (Biotrack Ltd., Wareham, United Kingdom), each with a specific radio frequency (Figure 2.1). A precision drill (PROXXON GmbH, Föhren, Germany) was used to gently scratch the surface of the elytra. The elytra were cleaned with ethanol, before tags were fitted to the roughened elytra with a thin layer of car sealant (CAR SYSTEM UNIFLEX-PU 'Klebe und Dichtmasse', Voss Chemie GmbH, Uetersen, Germany). Individuals were restrained and placed in a refrigerator overnight, to prevent the tag from becoming dislodged, and to ensure that the car sealant would dry. Radio-tags combined with the car sealant weighed 0.275 ± 0.88g (mean ± SD) (Table B.1). Radio tags weighed on average 47% and 53% of female and male body mass, respectively, similar to the percentage body mass of radio tags used on other ground beetle species (*e.g.* Negro *et al*., 2007, 2017; Riecken & Raths, 1996). Individuals were removed from the refrigerator and the restraint ~8 hours prior to release into the field. Food was removed from beetles

24 hours prior to release for tracking to standardise the effect of hunger level on the activity of the beetles.

3.3.3 Pilot Study

To assess when *C. hortensis* were most active, we monitored the number of beetles in 15 live pitfall traps every 2-4 hours from the 7th-9th August 2019. Pitfall traps were distanced 10m from each other and located between the two study sites. We found that *C. hortensis* were trapped most frequently between sunset and the subsequent eight hours (Figure 2.2) confirming that *C. hortensis* is night active (Turin *et al*., 2003). This informed the beetle tracking schedule in our study.

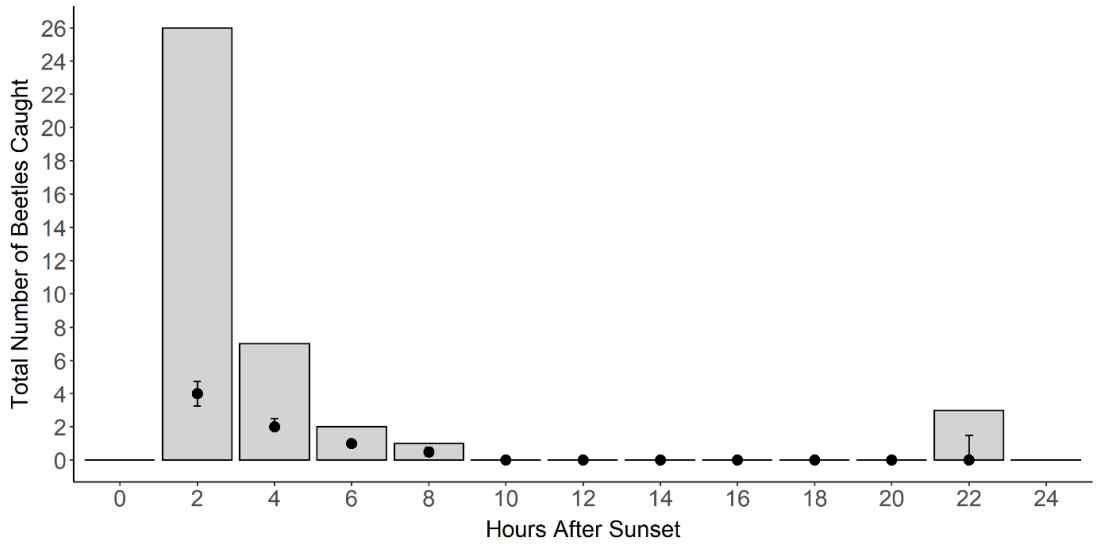

Figure 2.2. *Carabus hortensis* were most frequently trapped in pitfall traps 2-8 hours after sunset. Bars show the total number of *C. hortensis* beetles caught in pitfall traps across three days and nights in relation to the time in minutes past sunset. Median values and interquartile ranges are also presented.

3.3.4 Movement Data

Radio telemetry was conducted from 10th August – 14th October 2019. Up to nine individuals were released into their natural woodland habitat each week for a period of eight weeks (Figure B.1, see Table B.1 for further detail) during the *C. hortensis* active season (Figure B.2), and were collected after approximately two weeks of being tracked in the field. Two male *C. hortensis* were predated upon during their first day of release into the field, and were therefore removed from all analysis.

Tagged beetles were released into their site of capture to ensure that our measurements of movement in the field were indicative of an animal in its preferred environment. Individuals were held inside a section of round 10(D) x 15(H) cm PVC drainage tube, placed on the forest ground approximately 5m North-East from the pitfall trap where the individual had been previously caught, for 1 hour, to re-acclimatise to the field environment. Tubes were removed 1 hour before sunset and the GPS location of each individuals' release point was recorded.

C. hortensis were radio-tracked once every 2 hours for 8 hours from sunset, five nights per week. To control for the change in day length, radio-tracking started from 20:00 onwards from the 10th August – 8th September 2019 and was conducted from 18:00 onwards from the 9th September - 14th October 2019. Each individual was located using a Sika radio-tracking receiver attached to a Yagi antenna (Lotek, Wareham, United Kingdom), to an accuracy of <20cm^2. Numbered, flagged bamboo sticks were placed where a beetle was located. Due to limitations in the accuracy of radio telemetry, we placed an additional numbered, flagged bamboo stick only when beetles had moved at least 20cm away from their previous location. Metre rulers and measuring tape and a

compass were used the following day, during daylight, to quantify the distance (m) and direction (°TN) travelled between successive locations. Headlamps with red light were used when tracking beetles at night to guide our path whilst ensuring that beetles would not be disturbed by our presence: studies show that red light does not apparently disturb the behaviour of carabid beetles (Drees *et al*., 2008; Hasselmann, 1962).

We recorded the locations of beetles 2180 times, including both instances in which beetles did not move (35% of cases), and repeated visits to the same locations by the same beetle. We found that beetles moved only 0.5m or less from their previous location in 78% of all location events, and in 45% of those cases beetles did not move from their previous location at all.

Once per week, individuals were located every 4 hours from the end of the 8-hour observation period and until the beginning of the next 8-hour observation period. We halved the distance travelled by individuals across the 4-hour period, giving the distance travelled every 2 hours. We then combined these data with data collected once every 2 hours for 8 hours, providing information on the movements of each individual over 24 hours. The median distance travelled by all beetles in 2-hour intervals relative to sunset was then calculated and plotted to determine when *C. hortensis* are most active (Figure 2.3A). These data were used to corroborate the results of our pilot study alone and was not utilised in further analysis.

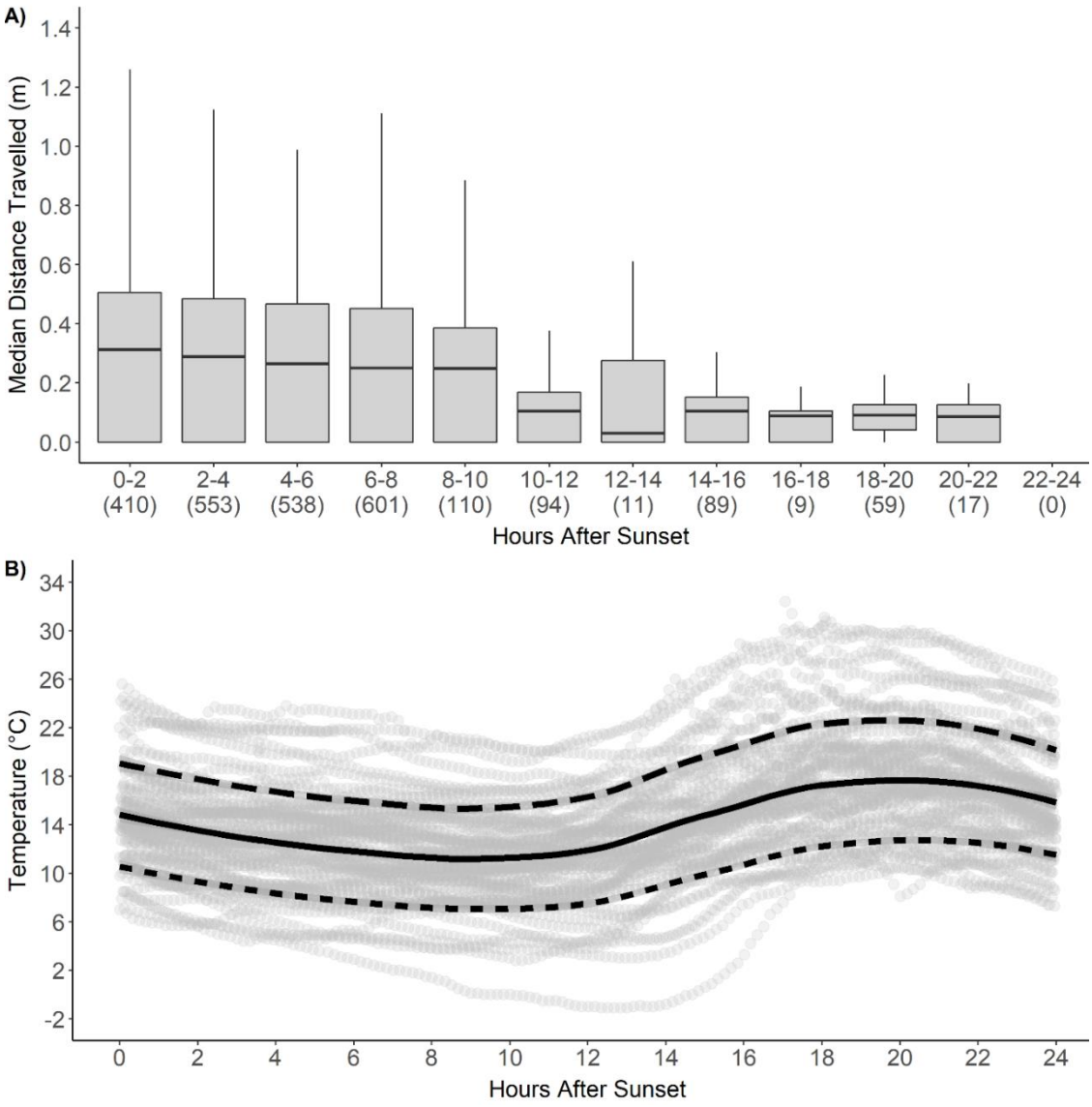

Figure 2.3. *Carabus hortensis* beetles travelled farthest on average between 0 hours and 10 hours after sunset, when temperatures in the field were lowest. A) Median and interquartile range distances travelled by *C. hortensis* beetles within a 2-hour window in relation to sunset. The number of data points per individual ranged from 41-88 with a mean of 61. The number of data points per observation hour are indicated in brackets. B) The temperature in relation to sunset over the whole study period. Temperature data collected once every 10 minutes from the beginning to the end of the study (10th August – 14th October) (open grey circles), along with the mean temperature (solid black line), mean + 1SD (long black dashes) and mean -1SD (short black dashes) are given.

3.3.4 Temperature data

We recorded the temperature every 10 minutes during the study using data loggers (VOLTCRAFT DL-210TH, Hirschau, Germany), placed ca. 10 cm above the forest floor at the centre of each study site. Temperatures between sunset and 8 hours post-sunset from the 10th August – 14th October 2019 ranged from -0.5 – 29.6°C in one study site and from 0.0 – 28.5°C in the other study site. There was no significant difference in the average temperatures recorded during 8 hour tracking periods each day, between study sites (Student's t-test, $t_{124} = 0.056$, $p = 0.955$). The average temperature recorded across study sites during this time was 13.47°C ± 4.90 SD. Temperatures recorded at the exact times at which beetles were located ranged from 4.4 – 29.1°C across study sites. The mean temperature recorded at the exact times at which beetles were located across study sites was 12.62°C ± 4.11 SD. Because movement parameters were calculated over several hours (see: 'Calculating movement parameters'), we took the average temperature (hereafter: temperature) between the time at which the beetle was first and last located on that particular day and used this as the temperature value in our analyses.

3.3.5 Data Analyses

3.3.5.1 Calculating thermal tolerance values

Four different values were calculated to investigate intraspecific variation in *C. hortensis* thermal tolerances: (1) The field-derived thermal minimum for movement (hereafter: thermal minimum); (2) the field-derived thermal maximum for movement (hereafter: thermal maximum); (3) the optimal temperature for movement (thermal optimum); and (4) the field-derived operating range (hereafter: operating range).

All thermal tolerance values (°C) were estimated by fitting sigmoid curves of the cumulative distance travelled by an individual over the entire radio-tracking period, against increasing temperature (Figure 2.4B, C). The R^2 of plotted sigmoid curves ranged from 0.77 – 1.00 with a mean of 0.96 ± 0.05 SD. Cumulative distance was calculated as the sum total of raw distances travelled between each successive location. The temperature at which the slope of the sigmoid curve was maximal was defined as the individual thermal optimum. The thermal minimum and maximum were the temperatures at which 10% and 90% of the total cumulative distance travelled by an individual had been reached, respectively. The operating range was calculated as the thermal maximum minus the minimum. In some instances (9 females, 7 males, Table B.1), beetles moved at low and high temperatures but did not move at intermediate temperatures, meaning that it was difficult to obtain accurate thermal maximum, thermal minimum, and operating range values. The thermal maximum, thermal minimum, and operating ranges of these individuals were not used in our analysis.

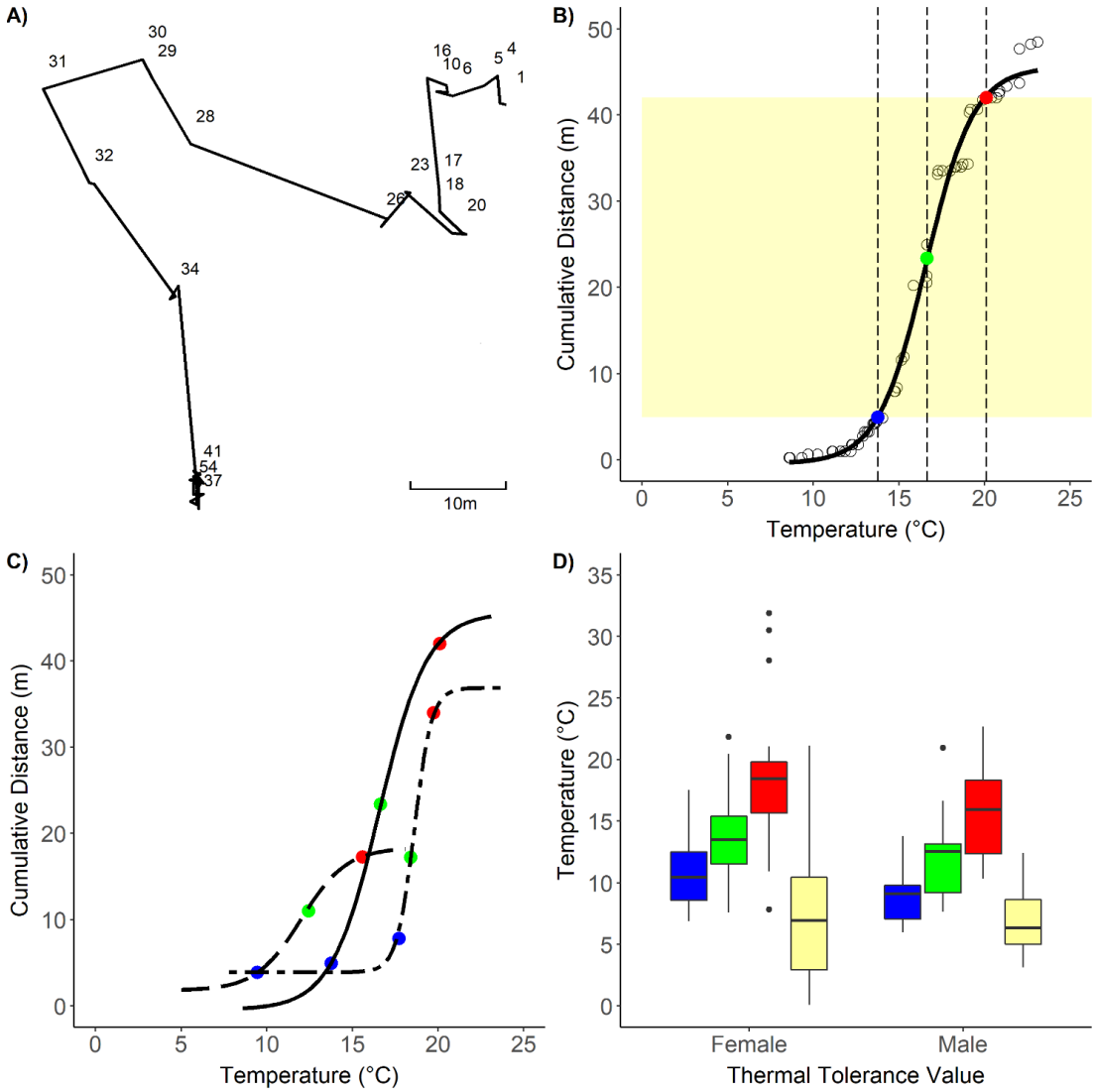

Figure 2.4. Individual *C. hortensis* ground beetles differed in their thermal tolerance values. A) the movements of a single individual throughout tracking, relative to its start position. Numbers denote the order of individual movements. B) Sigmoid curve fitted to the cumulative distance travelled against temperature in a single individual (R^2 = 0.963). Thermal tolerance values for that individual are indicated (thermal minimum (blue), thermal optimum (green), thermal maximum (red)). The operating range is shaded in yellow. Unfilled circles denote individual data points of distance travelled at different temperatures. C) Sigmoid curves of the cumulative distance travelled by three different individuals against temperature. Thermal tolerance values are highlighted for each individual (thermal minimum (blue), thermal optimum (green), thermal maximum (red)). D) Distributions of thermal minima (blue: female N = 19, male N = 15), thermal optima (green: female N = 28, male N = 22), thermal maxima (red: female N = 19, male

N = 15), and operating ranges (yellow: female N = 19, male N = 15). Solid lines in graphs A-C represent data gathered from the same individual.

3.3.5.2 Calculating movement parameters

We calculated four different variables to quantify individual *C. hortensis* movement in the field: (1) distance travelled over 2 hours (DT2h); (2) distance travelled over 8 hours (DT8h); (3) the total distance travelled over 8 hours per day for two weeks (TDT); and (4) path straightness.

We calculated DT2h as the distance in metres between each successive location over approximately 2 hours, while DT8h was calculated as the sum-total distances (in metres) between each successive location for approximately 8 hours post-sunset. Because some beetle locations were recorded earlier or later than every 2 hours, DT2h and DT8h were adjusted to the distance covered in 2 hours and 8 hours, respectively. To do so, we divided the sum total of raw distances travelled over 2 or 8 hours by the total of time in minutes over which those raw distances were travelled and multiplied this value by 120 or 480 respectively. Path straightness was calculated by dividing the Euclidean distance (in metres) between the first location of the individual, recorded at sunset, and the last location of the individual, recorded 8 hours post-sunset, for each day by the raw, unadjusted DT8h (Frizzi, 2018). Raw unadjusted values of DT8h were used in the calculation of path straightness instead of adjusted DT8h, because we could not be certain of the distance between the first location of the individual and the last location when DT8h was adjusted. We were, therefore, unable to calculate the Euclidean distance from the first and last location of the individual using adjusted DT8h.

Beetle locations could not be found in 4% (89 out of 2180) of all location attempts, such that the time between successive beetle locations was approximately 4 hours instead of 2 hours. In such instances, we linearly interpolated the data to estimate the distance travelled every 2 hours. Interpolating the data ensured that for each individual, measures of path straightness and DT8h were calculated from five movement sections per night.

We calculated TDT (in metres) as the sum total of raw DT8h measures per individual over the entire tracking period. Some individuals were located on more days than others (range = 7-16 days), with the majority of individuals being located approximately once every 2 hours for 8 hours a day and located on 10 - 14 days (Table B.1). We therefore adjusted TDT for each beetle to the distance travelled for 8 hours a day for 14 tracking days.

The number of days that individuals were kept at the field station prior to release into the field did not significantly impact female (linear regression; $F_{1,26} = 1.939$, $p = 0.176$, $N = 28$) or male (linear regression; $F_{1,20} = 1.835$, $p = 0.191$, $N = 22$) TDT.

3.3.6 Statistical Analyses

All analyses were carried out in R version 3.3.2 (R Core Team, 2019). We performed one linear mixed effects model (LMM) on data from combined male and female data (hereafter: all beetles), using the lme4 package (Bates *et al*., 2015), with TDT as the response variable. We additionally performed two generalised linear mixed effect models (GLMMs), one using a Poisson error structure and the number of times that an individual was located as the response variable, and the other using a binomial error

structure, with the percentage of instances in which the individual was located but had not moved from its previous location as the response variable. In each of the models, sex was included as a fixed term, and the week (week 1 – week 8) in which beetles were released for radio-tracking was included as a random term to control for seasonal temperature changes throughout the study period.

To determine whether the extent of individual variation was significantly different among different thermal tolerance values, we performed Levene's tests of variance using the car package (Fox & Weisberg, 2019) on separate female and male data. We additionally used Levene's tests of variance to ascertain whether the extent of individual variation in each thermal tolerance value significantly differed between the sexes.

To examine sex differences in average thermal tolerance values, we performed four LMMs on data from all beetles. Thermal maxima, thermal minima, thermal optima, and operating range were the response variables, and sex was the main fixed term in each model. Body mass was included as a fixed term because individuals with different body sizes have different surface area to volume ratios, and hence lose body heat at different rates (reviewed in: Angilletta, 2009), meaning that individuals of different body sizes may have different thermal tolerances. Week was included as a random term. In the models with thermal optima and operating range as the response variables, thermal minima and thermal maxima were also included as fixed terms. In the model with thermal minima as the response variable, thermal maxima was included as a fixed term, and *vice versa*. The sex-specificity of the effect of body mass and of thermal tolerance values on one another was determined by performing models as described above, on separate male and female datasets, with sex removed as an explanatory variable. Model

assumptions were checked visually and, in order to meet model assumptions, operating range data was log-transformed within each model.

To determine whether individuals consistently differed in their movements over time, we used LMMs and the rptR package (Stoffel *et al*., 2017) to estimate repeatability of path straightness, DT2h and DT8h, using temperature as a covariate. Repeatability of each movement parameter was estimated for all beetles as well as for females and males separately. ID was included as a random term. Because individuals may not have been motivated to move due to factors such as satiety, predation threat, and weather, only instances in which we were able to detect beetle movement were included in the repeatability analyses. Repeatability estimates for DT2h, DT8h and path straightness were calculated from all days in which an individual's movements were tracked, when we were able to detect individual movement. We therefore calculated repeatability estimates of DT2h, DT8h and path straightness from 7-14 measures taken 1-8 days apart from another. Because individual *C. hortensis* activity changed throughout the night (Figure 2.2, Figure 2.3A), we measured only the repeatability of DT2h recorded 2 hours after approximate sunset. Confidence intervals of 95% were used to infer the significance of the repeatability. If the confidence interval included zero, the trait was considered not repeatable.

To measure the effects of temperature on path straightness, we conducted LMMs on data from all beetles. Individual beetle ID, and the week in which beetles were released for radio-tracking were included as random intercepts. Temperature, temperature^2 and sex were included as fixed terms. Models with DT2h and DT8h as response variables were carried out as described for path straightness. However, because

there were many instances in which individuals did not move between location events, our datasets for DT2h and DT8h were over-dispersed with zeros. To correct for this, we fit hurdle models to the DT2h and DT8h generalised linear mixed models (GLMMs), using the glmmTMB package (Brooks *et al*., 2017). Because hurdle models cannot be fit to non-integer data, DT2h and DT8h were multiplied by 100 and then rounded, giving integers of DT2h and DT8h in cm. The same models were also fit for the separate male and female datasets, using temperature and temperature^2 as the only fixed terms.

To determine the relationships between thermal tolerance values and movements in the field, we conducted LMMs on separate male and female data, with each of operating range, thermal optima, thermal minima and thermal maxima as the response variables and TDT as the fixed term. As with other LMMs and GLMMs, the week in which beetles were released for radio-tracking were included as a random term allowing for random intercepts. We did not fit LMMs with thermal tolerance values as the response variables and TDT as the fixed term on combined male and female data, because sex differences in average thermal tolerance values were explored in the models above.

Stepwise model simplification was performed on all LMMs and GLMMs; fixed terms were removed from the model in stages and compared to the previous model using likelihood ratio tests (Crawley, 2007). At each stage, the most non-significant fixed term, whose removal did not significantly reduce the power of the model, was removed. Effects sizes (R^2 values) were calculated for all linear relationships.

3.4 Results

On average and across all individuals, individual beetles were located 43.6 ± 7.68 times (mean ± SD), with no differences between the sexes (GLMM; $\chi^2 = 0.207$, DF = 1, p = 0.649). Males and females also did not differ in TDT (LMM; $\chi^2 = 0.366$, DF = 1, p = 0.545), or in the percentage of location recordings in which they did not move (GMM; $\chi^2 = 1.514$, DF = 1, p = 0.219). We used our measurements to calculate both thermal tolerance values and movement parameters (Table 2.1), as well as the temperature dependency of those movement parameters.

Table 2.1. Summary statistics for thermal tolerance values and movement parameters. Summary statistics are given for the field-derived thermal minima for movement (thermal minima (°C)), the field-derived thermal maxima for movement (thermal maxima (°C)), the thermal optima for movement (thermal optima (°C)), the field-derived operating range (operating range (°C)), path straightness, distance travelled over 2 hours (DT2h (m)) distance travelled over 8 hours (DT8h (m)) and the total distance travelled over 8 hours per day for two weeks (TDT (m)), for male and female combined data (M+F), female data (F) and male data (M). Variance (Var.), number of individuals (N_{ID}) and number of observations (N_{OBS}) are given. An asterisk denotes one instance in which DT2h is larger than DT8h due to the methods with which DT2h and DT8h were calculated (please see 'Methods: Calculating movement parameters' for further information).

Response Variable	Sex	Mean ± SD	Range	Var.	N_{ID} (N_{OBS})
Thermal Minima	M + F	9.84 ± 2.70	5.97 – 17.52	7.10	34 (34)
	F	10.73 ± 2.86	6.89 – 17.52	7.92	19 (19)
	M	8.71 ± 2.05	5.97 – 13.77	4.22	15 (15)
Thermal Maxima	M + F	17.17 ± 4.82	7.82 – 31.88	27.61	34 (34)
	F	18.21 ± 5.35	7.82 – 31.88	34.62	19 (19)
	M	15.85 ± 3.83	10.32 – 22.65	14.64	15 (15)

Thermal Optima	M + F	12.97 ± 3.52	7.60 – 21.85	12.36	50 (50)
	F	13.78 ± 3.68	7.60 – 21.85	13.55	28 (28)
	M	11.93 ± 3.07	7.65 – 20.95	9.41	22 (22)
Operating Range	M + F	7.33 ± 4.60	0.08 – 21.12	21.14	34 (34)
	F	7.47 ± 5.66	0.08 – 21.12	32.02	19 (19)
	M	7.14 ± 2.93	3.14 – 12.41	8.60	15 (15)
Path Straightness	M + F	0.535 ± 0.33	0.00 – 1.00	0.11	50 (547)
	F	0.516 ± 0.32	0.00 – 1.00	0.10	28 (322)
	M	0.562 ± 0.33	0.00 – 1.00	0.11	22 (225)
DT2h	M + F	0.51 ± 2.97	0.00 – 128.76*	8.80	50 (2180)
	F	0.50 ± 3.71	0.00 – 128.76*	13.78	28 (1288)
	M	0.52 ± 1.27	0.00 – 17.44	1.62	22 (892)
DT8h	M + F	2.18 ± 6.06	0.00 – 115.96*	36.76	50 (547)
	F	2.04 ± 6.91	0.00 – 115.96*	47.69	28 (322)
	M	2.39 ± 4.60	0.00 – 41.05	21.20	22 (225)
TDT	M + F	30.49 ± 29.64	8.89 – 153.34	879.40	50 (50)
	F	28.57 ± 27.95	10.09 – 131.07	749.21	28 (28)
	M	33.23 ± 31.78	8.89 – 153.34	1073.16	22 (22)

3.4.1 Variability of field-derived thermal tolerance values and their relationships

Individual beetles differed in their thermal minima, maxima, optima and operating ranges (Table 2.1, Figure 2.4C, 2.4D). However, thermal maxima were significantly more variable than other thermal tolerance values (Levene's Test: $F_{3,150}$ = 2.921, p = 0.036). The average thermal optimum of the population (12.97°C ± 3.52 SD), which, compared to the thermal maximum, varied relatively little intraspecifically (Table 2.1), reflected the average night-time temperature experienced in the field (13.47°C ± 4.90 SD). There was no significant difference between males and females in the variance

of thermal minima (Levene's Test: $F_{1,33}$ = 1.350, p = 0.254), thermal maxima (Levene's Test: $F_{1,33}$ = 2.999, p = 0.588), thermal optima (Levene's Test: $F_{1,48}$ = 0.345, p = 0.560), or operating ranges (Levene's Test: $F_{1,32}$ = 3.732, p = 0.062).

Whilst males had significantly broader mean operating ranges than females (Table B.2, Figure 2.4D), males and females did not differ in their mean thermal minimum (Table B.4, Figure 2.4D), mean thermal maximum (Table B.5, Figure 2.4D) or mean thermal optimum (Table B.3, Figure 2.4D).

Body mass was not significantly related to thermal minima (Table B.4), thermal optima (Table B.3) or operating range (Table B.2) in either sex. Female (Table B.5, Figure 2.5A), but not male (Table B.5, Figure 2.5B) thermal maxima were, however, negatively related to body mass; heavier females had a lower thermal maximum than lighter females.

Individuals with broader operating ranges had both higher thermal maxima (Table B.2) and lower thermal minima across all beetles (Table B.2), and for the sexes separately (Table B.2, Figure 2.6A, 2.6B, 2.6C, 2.6D). Thermal optima were positively related to both thermal minima and thermal maxima in all beetles, females and males (Table B.3). Although there was no significant relationship between thermal minima and thermal maxima in all beetles or females, thermal minima were positively related to thermal maxima in males (Table B.4).

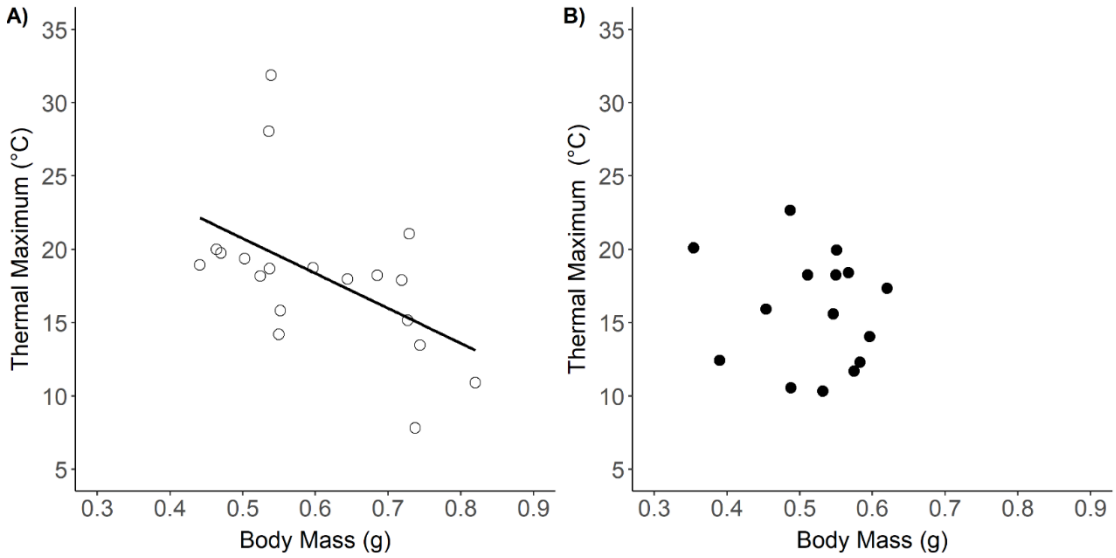

Figure 2.5. Thermal maxima decline with increasing body mass in females. Sex-specific relationships between thermal maxima and body mass in (A) females (N = 19, unfilled circles) and (B) males (N = 15, filled circles). Predicted line is fitted using outputs from LMM estimates.

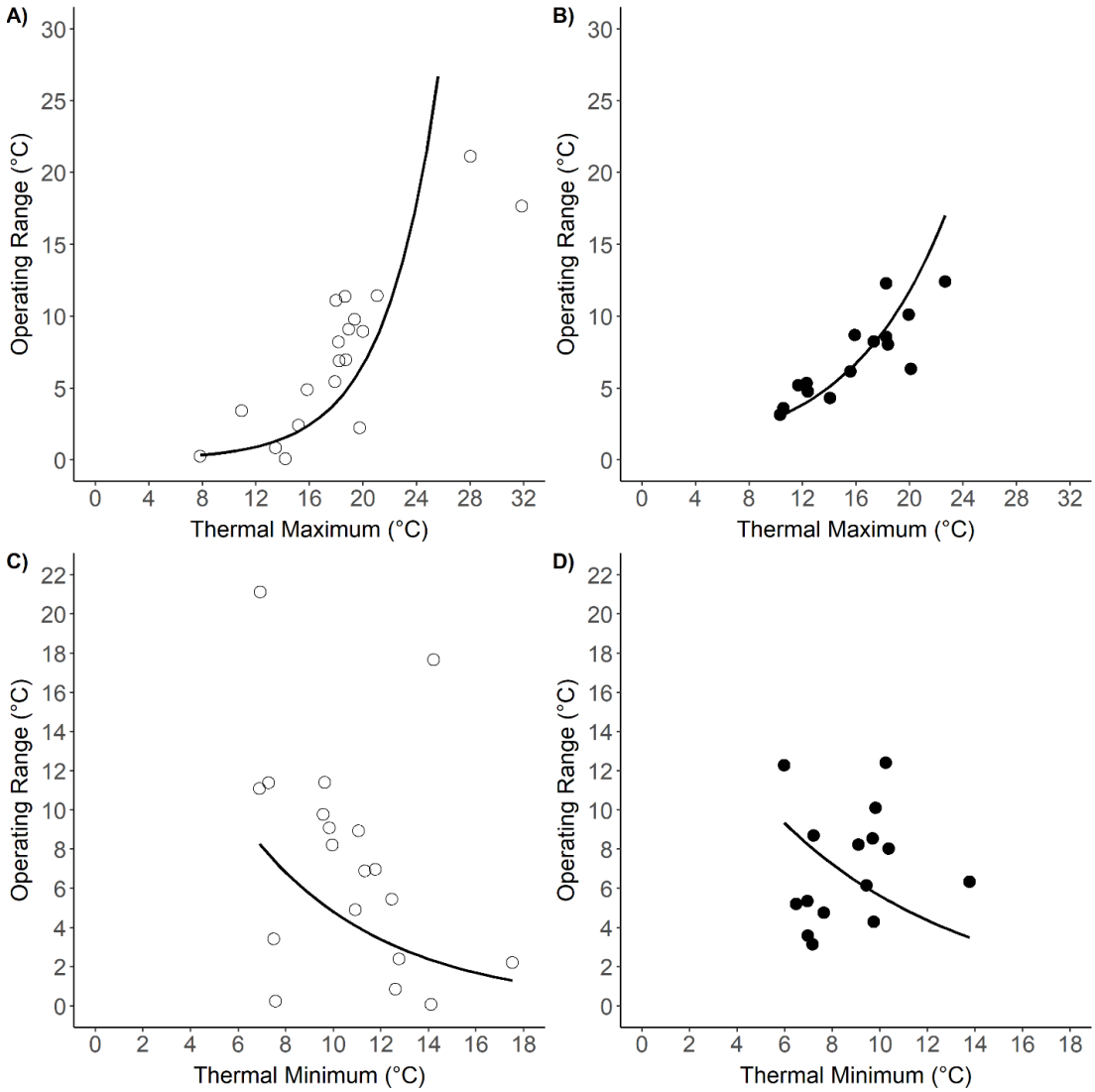

Figure 2.6. Operating ranges increase with an increase in thermal maxima and a decrease in thermal minima in both sexes. The operating range in relation to A) Female TDT (N_{ID} = 19), B) Male TDT (N_{ID} = 15), C) Female thermal maxima (N_{ID} = 19), D) Male thermal maxima (N_{ID} = 15), E) Female thermal minima (N_{ID} = 19) and F) Male thermal minima (N_{ID} = 15). Females are represented by unfilled circles whilst males are represented by filled circles. Lines represent the predicted relationships from model outputs, back-transformed from a model with a log-transformation of the response variable (operating range).

3.4.2 Individual consistency in movement parameters

Path straightness and distances covered (DT2h and DT8h) were repeatable over time across all beetles (Table 2.2). The distances covered were also repeatable within each sex.

Table 2.2. Repeatability estimates (± 95% confidence intervals) from linear mixed effects models. Repeatability estimates are given for path straightness, distance travelled over 2-hour intervals (DT2h) and distance travelled over a whole day (DT8h) adjusted with temperature, for male and female data combined (M + F) female-only data (F), and male-only data (M). Repeatability tests were conducted on only data when individuals moved. Bold values denote significance. N_{ID}, number of individuals; N_{Obs}, number of observations.

Response Variable	Dataset	Repeatability	95% CI	N_{ID} (N_{Obs})
Path Straightness	M + F	**0.061**	**0.004 – 0.121**	50 (513)
	F	0.064	0.000 – 0.148	28 (302)
	M	0.058	0.000 – 0.151	22 (211)
DT2h	M + F	**0.160**	**0.063 – 0.258**	50 (376)
	F	**0.120**	**0.015 – 0.247**	28 (227)
	M	**0.212**	**0.088 – 0.386**	22 (149)
DT8h	M + F	**0.205**	**0.121 – 0.295**	50 (514)
	F	**0.161**	**0.057 – 0.270**	28 (303)
	M	**0.270**	**0.107 – 0.424**	22 (211)

3.4.3 Relationships between movement parameters and temperature

As predicted, we found temperature dependency of movement patterns, and these were sex-specific. The path straightness of all beetles and females (Table B.6, Figure 2.7A) followed an inverse temperature optimum curve, in which path straightness was

greatest at lower and higher temperatures and lowest at mid-range temperatures. Male path straightness (Table B.6, Figure 2.7B) was unrelated to temperature.

For all beetles, DT2h (Table B.7) and DT8h (Table B.8) increased with temperature but in a sex-specific way. Female DT2h followed a temperature optimum curve (Table B.7, Figure 2.7C), while male DT2h increased linearly with temperature (Table B.7, Figure 2.7D). Temperature increases accounted for 16% of variation in male DT2h when they moved and 16% of variation in male DT2h both when males did and did not move. The DT8h increased with temperature across all beetles (Table B.8) and in males (Table B.8, Figure 2.7F) but not in females (Table B.8, Figure 2.7E). Temperature increases accounted for 27% of variation in male DT8h when males moved and 31% of variation in male DT8h both when males did and did not move.

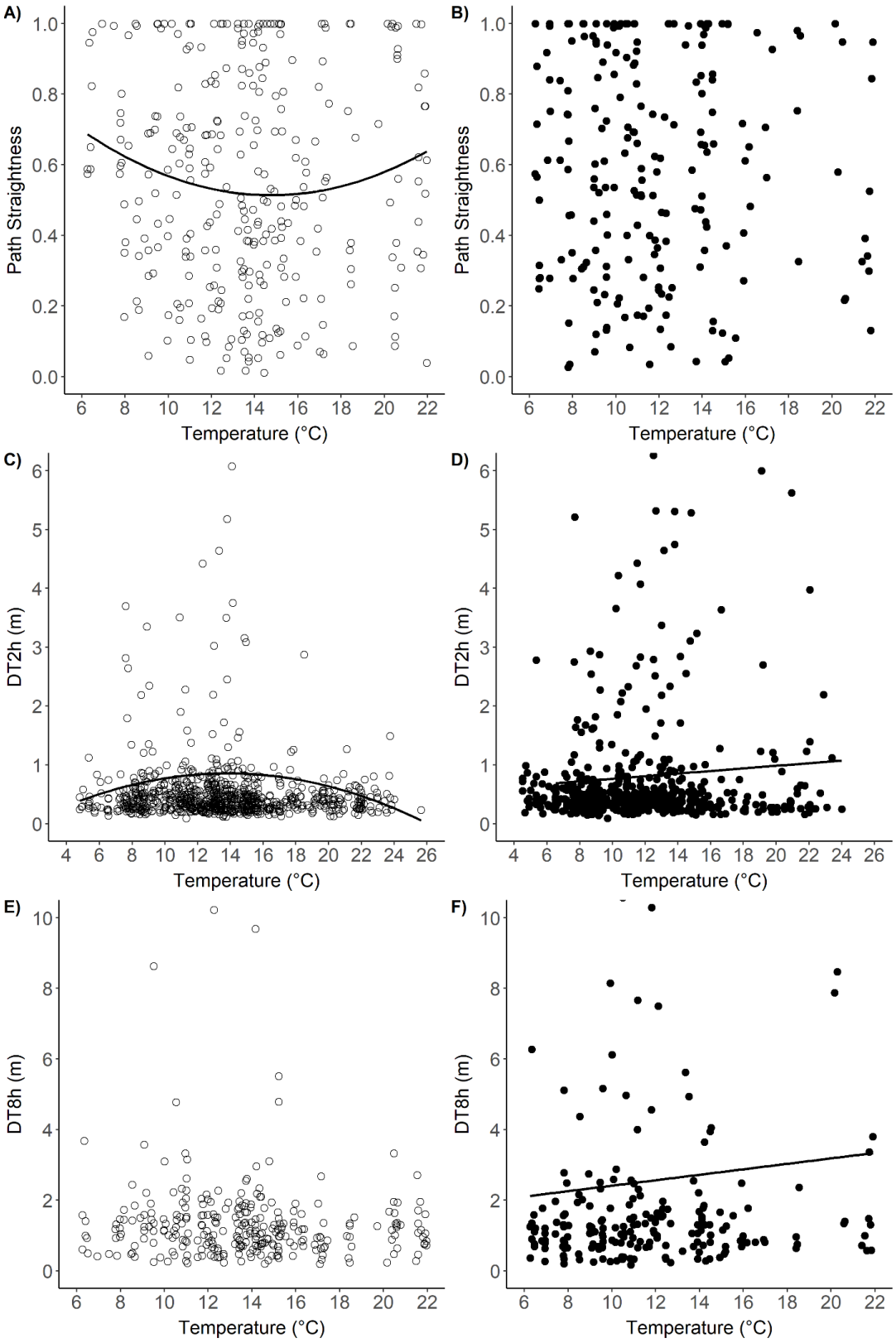

Figure 2.7. Different movement parameters are related to temperature in males and females. Path straightness in relation to the temperature (°C) in A) Females (N_{ID} = 28, N_{Obs} = 302) and B) Males (N_{ID} = 22, N_{Obs} = 211). The distance travelled over 2 hours

(DT2h) in relation to the temperature (°C) in C) Females (N_{ID} = 28, N_{Obs} = 858) and D) Males (N_{ID} = 22, N_{Obs} = 555). The distance travelled over 8 hours (DT8h) in relation to the temperature (°C) in E) Females (N_{ID} = 28, N_{Obs} = 303) and F) Males (N_{ID} = 22, N_{Obs} = 211). Females are represented by unfilled circles whilst males are represented by filled circles. Instances where beetles did not move are removed from the figures to improve clarity of the figure. To improve clarity of the figures, eleven data points are removed from Figure 7C, and nine data points are removed from Figure 7D, thirteen data points are removed from Figure 7E, and twelve data points are removed from Figure 7F. Predicted lines are fitted using outputs from LMM estimates from the models excluding instances when individuals did not move.

3.4.4 Relationships between thermal tolerance values and total distance travelled

Across all beetles (Table B.9) and in females (Table B.9, Figure 2.8A), but not in males (Table B.9, Figure 2.8B), TDT was negatively related to operating range. Females with broader operating ranges travelled shorter distances over the tracking period than did female conspecifics with narrower operating ranges. However, TDT was not significantly related to the thermal optima across all beetles, or in females and males alone (Table B.10). Whilst TDT was positively related to the thermal minima (Table B.11) and negatively related to the thermal maxima (Table B.12) of all beetles, TDT was not related to female or male thermal minima (Table B.11) or maxima (Table B.12).

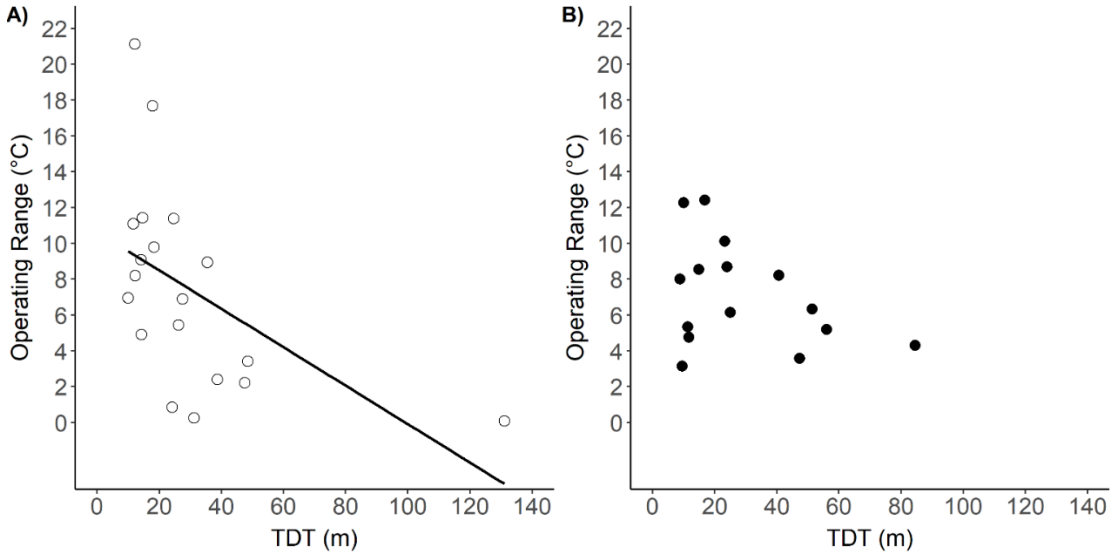

Figure 2.8. Females with wider operating ranges cover shorter distances in the field. The operating range in relation to TDT in A) Females (N_{ID} = 19), and B) Males (N_{ID} = 15). Females are represented by unfilled circles whilst males are represented by filled circles. Predicted line is fitted using outputs from LMM estimates.

3.5 Discussion

Here we provide field-derived thermal tolerance values and measures of intraspecific variation in the thermal dependency of movement, by monitoring *Carabus hortensis* ground beetles in their natural woodland habitat during their activity phase. We found that intraspecific variation in thermal tolerance values was independent of sex, but that males and females differed in some mean thermal tolerance values and in the temperature dependency of different movement parameters, which were consistent over time. The total distance travelled by females, but not males, was negatively related to operating range. Significant relationships between the total distance travelled by beetles and other thermal tolerance values were significant only across all beetles. Contrary to previous findings from *C. hortensis* (Szyszko *et al*., 2004), and from closely

related ground beetle species (*e.g.* Drees & Huk, 2000; Gerlach *et al*., 2009; Lagisz *et al*., 2010), we found that males were not more active (*i.e.* they did not travel significantly farther distances overall) than females.

Characterising (1) intraspecific variation in insect thermal tolerances, (2) whether individuals consistently differ in their movement patterns, and (3) the relationships between thermal tolerance values and movement patterns, is integral to understand how temperature influences individual fitness and population dynamics, and to predict how species may respond to changing global temperatures. Having been obtained from a wild population in their realised thermal niche, our measures should accurately inform how individuals respond to temperature and the extent of intraspecific variation in these responses. Although inter-individual variation in the effects of temperature on insect movement has been studied previously (*e.g.* Baars, 1979b), our study is, to the best of our knowledge, the first to investigate intraspecific variation in field-derived thermal tolerance values, in an insect.

3.5.1 The ecological significance of our thermal tolerance values

We obtained field-derived measures of *C. hortensis* thermal minima, maxima, optima and operating ranges by monitoring individual movement at a range of temperatures in the field. Thermal tolerance values are usually calculated in the laboratory, and with a different set of experimental methods to those used in our study. We quantified the thermal minimum and maximum as the temperatures at which 10% and 90% of the total cumulative distance travelled by an individual had been reached, respectively. In contrast, laboratory measures of thermal tolerances are usually obtained

by gradually heating or cooling the animal from a given temperature, until individuals lose muscular control and movements cease (*e.g.* Piyaphongkul *et al*., 2014; Terblanche *et al*., 2007). Our field-derived measures should be far more ecologically relevant than those obtained from the laboratory for several reasons. Firstly, because both the temperature from which the individual is heated/cooled and the rate of heating/cooling can influence thermal tolerance values (*e.g.* Terblanche *et al*., 2007), thermal tolerance values estimated in the natural environment with natural rates of heating/cooling are likely to be more accurate than those estimated in the laboratory. Second, insects are capable of acclimatising to local conditions (*e.g.* Overgaard *et al*., 2008; Piyaphongkul *et al*., 2014) meaning that laboratory rearing temperatures may influence thermal tolerance values. Third, laboratory conditions often control for important factors that would otherwise impact individual movement, such as food availability and humidity, leading to a disparity between these laboratory-calculated individual thermal tolerance values and those of animals within a natural environment. Overall, our measures of thermal tolerance values may be better used to assess the vulnerability of *C. hortensis* to climate change.

3.5.2 How do our thermal tolerance values compare to those obtained in the laboratory?

Our field-derived thermal tolerance values are comparable to laboratory-based studies of insect thermal tolerance values in that we found: (1) large intraspecific variation in thermal maxima but relatively low intraspecific variation in thermal minima (*e.g.* Oyen & Dillon, 2018); (2) that the average thermal optima reflected the average environmental temperature (*e.g.* Overgaard *et al*., 2008; Seebacher *et al*., 2015); and (3)

that the sexes did not differ in their average thermal minima, maxima and optima (*e.g.* Lann *et al*., 2011; Nyamukondiwa & Terblanche, 2009; Porter *et al*., 2019).

3.5.3 To what extent can you rely on our measures?

Despite the many advantages of obtaining thermal tolerance values and information on the thermal dependency of movement parameters directly from the field, doing so has some limitations. Some may question the validity of our movement parameter estimates, given that: (1) we located beetles only once every 2 hours; (2) beetles could reasonably travel over far distances within a 2-hour period; and (3) more intensive location rates would improve accuracy in movement parameter estimates by accounting for small scale movements. However, we found that, in the majority of cases, beetles moved only 0.5m or less from their previous location. Moreover, our sample rate was far more frequent than those previously used to monitor ground beetle movement via radio-telemetry (*e.g.* Negro *et al*., 2007, 2017; Riecken & Raths, 1996; but see: Růžičková & Veselý, 2018). Thus, we conclude that our sample rate of once every 2 hours is sufficient for estimating *C. hortensis* movement parameters.

The addition of the radio-tags may have affected the behaviour of *C. hortensis* and hence compromised the ecological significance of our measures. However, in our study, radio-tags weighed 47% and 53% of female and male body weight respectively, which is comparable with the percentage body weight of radio-tags used to track closely related and similarly sized *Carabus* species (*e.g.* Negro *et al*., 2017; Riecken & Raths, 1996). Moreover, we observed that tagged individuals ran up tree trunks on multiple occasions, covered distances of up to approximately 130m over 2 hours, and dug deep into the substrate. We also observed predation of only two *C. hortensis*, which were

excluded from all analysis. We therefore conclude that the added weight of the radio-tags is unlikely to substantially impair *C. hortensis* movement, and that our measures of the influence of temperature on their movement can be considered reliable.

3.5.4 Sex differences in the temperature dependency of movement parameters

As predicted, we found that the temperature dependency of movement parameters differed between male and female *C. hortensis.* Female path straightness followed the inverse of a temperature optimum curve, meaning that at mid-range temperatures, females walked in more complex and tortuous paths. Conversely, male path straightness was not significantly related to temperature. We should be cautious in interpreting these findings, because path straightness was not repeatable in females and males, and was only marginally repeatable across all beetles. Low repeatability in path straightness may be explained by a change in behaviour with changes in satiety and hunger, and/ or changes in behaviour based upon recent foraging success. Other carabid beetles have been shown to travel with straight, fast movements, and then switch to slow and tortuous movements for short periods of time upon encountering food resources (Mitchell, 1963). Cautiously, we suggest that, based on the hypothesis that ground beetle foraging success increases with the performance of short movements that constantly change in direction (*i.e.* a 'random walk') (Baars, 1979b), which are likely analogous to our measure of low path straightness, female *C. hortensis* may enjoy greater foraging success at mid-range temperatures than at low or high range temperatures. Moreover, because ground beetles are thought to switch from a random walk to long spurts of movement in a single direction when environmental conditions such as temperature or

humidity are sub-optimal within otherwise high quality habitats (Weber & Heimbach, 2001), our results may indicate that female *C. hortensis* actively switch from random walk movement patterns to directed movement to escape regions where temperatures are sub-optimal. Our results therefore suggest that environmental temperature may have direct consequences for foraging success in female *C. hortensis*.

That females both travelled farther within 2 hours and walked in more tortuous paths when temperatures were intermediate, suggests that females cover more ground through random walking patterns than via directed movement. This indicates that females are driven to move by the need to consume large amounts of food to fuel egg production. In other carabid species, sex-differences have been found in both the satiety-dependency activity, with the activity or speed of females, but not males, increasing with hunger (Mauremooto *et al*., 1995; Wallin & Ekbom, 1994), and in the proportion of satiated individuals, with comparatively more females having fuller stomachs than males (Sunderland, 1975).

In contrast to females, males travelled farther distances over both 2 and 8 hours at high temperatures. This is in line with findings that carabid beetles are caught in pitfall traps in higher numbers at higher temperatures (Baars, 1979a). Such sex differences in the temperature dependency of movement patterns could arise if movement patterns are ecologically different between the sexes, or if males and females have different motivations for their movement. For instance, movement in male *C. hortensis* may be more driven by the need to locate mates, as has been hypothesised for other *Carabus* species (Drees & Huk, 2000), rather than the need to consume large amounts of food.

3.5.5 Potential implications of our findings for *C. hortensis* under climate change

In our study, temperature optima were independent of both sex and body mass, indicating that males and females, and individuals of different body sizes, may be similarly capable of adapting to changing environmental temperatures through the thermal optima acclimation. This is supported by the fact that the average *C. hortensis* thermal optima reflected the average night-time temperature measured during the species' active season. Combined with the finding that temperature increases accounted for a relatively small proportion of the variation in distances travelled by males, and that substantial variation in *C. hortensis* thermal maxima provides the raw material for natural selection to act, our results suggest that *C. hortensis* may have the capacity to overcome temperature increases in their current environment in the near future.

Acclimation of thermal optima to changing environmental temperatures and an increase in the heat tolerance of the species may, however, be insufficient in securing the survival of *C. hortensis* under continued climate change. Other, winged, ground beetle species in the Lower Saxony region have shifted their ranges poleward in response to regional warming of just 1°C within the last 50-100 years (Drees *et al*., 2011), and summer temperatures in the Lüneburger Heide are expected to increase by a further 1-1.5°C in the next 29 years and by 2.5-3°C from 2071-2100 (European Environment Agency, 2012). *C. hortensis* may similarly have to adapt to regional temperature changes through range shifts, however, unlike the species mentioned above, *C. hortensis* are unable to fly.

The capacity for *C. hortensis* to engage in range shifts to overcome environmental warming may be limited. This is because both the distances travelled by females, at least over 2 hours, and the movement of larger bodied females, who should have longer legs and should therefore be capable of covering greater distances than smaller bodied

conspecifics (Yarwood *et al.*, 2021a; Chapter 2), became constrained at higher temperatures. Females that travelled the farthest distances overall also had the smallest operating ranges and lowest tolerance to high temperatures, indicating that *C. hortensis* range shifts may be further limited by the movement capacity of females able to withstand higher temperatures. Differences in the effects of temperature on the performance of male and female *C. hortensis* may have consequences for the ability of the species to adapt to climate change through range expansion. Any adaptation towards changing environmental temperatures through the thermal optima acclimation or an increase in tolerance to heat may therefore be crucial for the survival of *C. hortensis*, especially given that the species' dispersal capacity is limited by its inability to fly.

In addition to suggesting that the capacity for *C. hortensis* to undergo range expansion may be limited, our results suggest that climate change may cause a shift in female *C. hortensis* body size. Our results indicate that smaller females are less sensitive to heat (*i.e.* have higher thermal maxima), and should therefore be selected for in warmer environments, whilst larger individuals may be less able to withstand rising temperatures. Indeed, climate change has caused a reduction in body size of other invertebrate species (*e.g.* Brans *et al.*, 2017). A potential reduction in the body size of female *C. hortensis* may have further implications for the population dynamics of the species, because, in insects, large body sizes are linked to fecundity (Forrest, 1987; Honěk, 1993): we may therefore expect population densities of *C. hortensis* to fall as environmental temperature continue to rise.

Finally, that we found larger intraspecific variation in thermal maxima than in other thermal tolerance values suggests that intraspecific variation in *C. hortensis* movement patterns should rise with increasing temperatures, as has been found in other

ground beetle species (*e.g.* Baars, 1979b). This may have implications at the individual level, if temperature increases cause a change in the rank order of individual vulnerability in risky situations.

We studied intraspecific variation in both insect thermal tolerances and the effects of temperature on insect movement, using field derived data. We observed larger intraspecific variation in thermal maxima than in thermal minima or thermal optima for movement, and found that temperature influenced movement patterns in different ways in males and females, which may be caused by sex-differences in the motivations for activity. Although both an average population thermal optimum close to that of average nightly temperatures and intraspecific variation in thermal maxima could equip *C. hortensis* beetles with an adaptive capacity to overcome rising temperatures, sex differences in the effects of temperature on movement may constrain the ability of the species to respond to climate warming through range expansions/shifts. More studies investigating the effects of temperature on intraspecific movement patters and thermal tolerance values using field derived data are required if we are to predict and understand species' responses to climate change, as well as more general consequences for individual fitness and population dynamics that results from individual differences in the thermal dependency of movement. Here we provide an example of how to approach such studies.

Chapter 4: Does exploratory behaviour in the laboratory predict movement patterns in the wild in an insect?

4.1 Abstract

Laboratory-based studies are frequently used to study animal personality differences (*i.e.* consistent behavioural differences among individuals) under a single temperature. Nevertheless, whether such laboratory-based measures readily predict measures of personality differences in the wild, such as animal movement, is rarely investigated - especially in insects. The effects of different laboratory conditions on such links have received even less attention. We repeatedly tested exploratory behaviour in the laboratory, at a series of different temperatures, and used radio telemetry to track the movements of 48 radio-tagged *Carabus hortensis* ground beetles in their natural habitats over 14 days each, to assess correlations between the behaviour in the laboratory and repeatable movement parameters (*i.e.* personality traits) in the wild. One male personality trait measured in the wild correlated negatively with personality differences in exploratory behaviour in the laboratory. Our results demonstrate that exploratory behaviour measured in the laboratory can be used to predict personality traits in insects in the wild, but indicate that conditions in the laboratory can influence such relationships.

4.2 Introduction

Individuals within-species frequently display differences in behaviour that are consistent across time and/or contexts (Eysenck & Eysenck, 1985). Such consistent differences in behaviour are termed 'animal personality differences' (Gosling, 2001). Animal personality differences have a range of important ecological and evolutionary consequences both at the individual and population level (reviewed in: Dall *et al*., 2004; Schuett *et al*., 2010; Wolf & Weissing, 2012).

Animal personality traits are often measured in the laboratory (Carter *et al*., 2013; Niemelä & Dingemanse, 2014), because measuring animal personality in the natural environment can be labour intensive and time consuming. There are, however, potential pitfalls of measuring animal personality traits in the laboratory. For example, there may be an assumption that behaviours expressed in the laboratory are analogous to those expressed in the natural environment. Laboratory tests may involve stimuli that would not otherwise be encountered by individuals in the natural environment (Niemelä & Dingemanse, 2014). Because behaviour is often context specific (Dingemanse *et al*., 2010), studies of animal personality in captive environments conducted under carefully controlled conditions may not represent animal personality traits in the natural environment. Different laboratory conditions (*e.g.* temperature) may affect individuals' expression of animal personality (reviewed in: Carter *et al*., 2013), potentially altering the relationships between the personalities in captivity *versus* those in natural environments. Finally, individual differences in acclimation to laboratory conditions may lead to false interpretation of animal personalities (Niemelä & Dingemanse, 2014). In general, the use of laboratory studies to collect information on animal personalities may lead to erroneous conclusions about their ecological and evolutionary significance (Niemelä & Dingemanse,

2014). However, laboratory estimates of animal personalities can be informative if they are predictive of animal personalities in natural environments (*e.g.* Aplin *et al*., 2013; Dingemanse *et al*., 2003; Fisher *et al*., 2015; Fraser *et al*., 2001; Herborn *et al*., 2010; Hollander *et al*., 2008; McCowan *et al*., 2015; Quinn *et al*., 2011; Schirmer *et al*., 2019; Schuett *et al*., 2012; van Overveld & Matthysen, 2010; Wilson & McLaughlin, 2007; Yuen *et al*., 2016). Links between animal personality measured in the laboratory and in natural environments should, therefore, be assessed if we are to understand the ecological relevance of animal personality measures obtained in the laboratory.

Exploratory behaviour is a personality trait that has important consequences for individual survival and fitness in many species (Smith & Blumstein, 2008), affecting, for instance, resource acquisition (*e.g.* van Overveld & Matthysen, 2010), and predation risk (*e.g.* Dingemanse *et al*., 2004). Exploratory behaviour is frequently measured in a novel environment in the laboratory (*e.g.* Dingemanse *et al*., 2002, 2003; Verbeek *et al*., 1994), and has been linked to individual differences in space use in natural environments (*e.g.* Spiegel *et al*., 2015), for instance in sleepy lizards (*Tiliqua rugosa*) (Spiegel *et al*., 2015). In other species, exploratory behaviour measured in the laboratory predicts activity (*e.g.* Fisher *et al*., 2015), risk taking propensity (*e.g.* Hollander *et al*., 2008), sociability (*e.g.* Aplin *et al*., 2013; McCowan *et al*., 2015), foraging patterns or behaviour (*e.g.* Herborn *et al*., 2010; Schirmer *et al*., 2019; van Overveld & Matthysen, 2010; Wilson & McLaughlin, 2007) or capacity to disperse (*e.g.* Dingemanse *et al*., 2003; Fraser *et al*., 2001; Quinn *et al*., 2011) in the natural environment.

The ecological significance of laboratory-based measures of exploratory behaviour may differ depending upon a species' habitat use and its ecology. Importantly, poor

understanding of the ecological significance of species' space-use may lead to erroneous interpretations of exploratory behaviour measured in the laboratory. For example, individuals of species that predominantly forage in sheltered areas, or that rely upon social interactions to gain information about their surroundings, may move little in a novel environment, but do not necessarily suffer a reduction in resource acquisition when compared to more exploratory individuals (Dall & Griffith, 2014). Furthermore, because sex-differences in reproductive strategies and investment often lead to sex-differences in behaviour (Bateman, 1948), the ecological significance of exploratory behaviour measured in the laboratory may additionally differ between the sexes (Dingemanse *et al*., 2003; van Overveld *et al*., 2014; Wat *et al*., 2020). Understanding the ecological significance of species' behaviour, and that of males and females individually, is therefore of importance when determining the relevance of exploratory behaviour measured in the laboratory.

The few studies that have investigated the link between exploratory behaviour in the laboratory and personality traits measured in natural environments have been based predominantly on birds (*e.g.* Aplin *et al*., 2013; Dingemanse *et al*., 2003; Herborn *et al*., 2010; Hollander *et al*., 2008; McCowan *et al*., 2015; Quinn *et al*., 2011; Schuett *et al*., 2012; van Overveld & Matthysen, 2010), with little focus on insects (but see: Fisher *et al*., 2015). Yet, the relationships between exploratory behaviour in the laboratory and personality traits measured in natural environments may differ between taxonomic groups or species, such as birds *versus* insects, owing to differences in both foraging and habitat use ecology, and in the endothermy of birds *versus* the ectothermy of insects. Because the body temperature of insects reflects that of their external environment, environmental temperature directly impacts insect behaviour (Huey & Kingsolver, 1989).

Moreover, insects demonstrate intraspecific differences thermal tolerance values (*e.g.* Franken *et al*., 2018; Nyamukondiwa & Terblanche, 2009; Sgrò *et al*., 2010; Sinclair *et al*., 2012), meaning that different individuals move in different ways at different temperatures. Therefore, temperature-controlled laboratory estimates of exploratory behaviour may not necessarily predict insect behaviour in the natural environment, where temperatures fluctuate. In other ectotherms, an increase in temperature has been shown to elevate aggressiveness, boldness, and the activity of some, but not all, individuals, thereby affecting personality differences (*e.g.* Biro *et al*., 2010). Such problems may be less likely to arise when studying the relationships between exploratory behaviour in the laboratory and personality traits measured in natural environments in birds or other endotherms, who are able to regulate their own internal body temperature via physiological processes. Hence, exploring the link between insect personality measured in the natural environment, and insect personality that has been measured in the laboratory under different temperature treatments, will be important going forward.

Here we use radio-telemetry to track the short-term and long-term movements of male and female *Carabus hortensis* Linnaeus, 1758 ground beetles in the natural environment, and repeatedly test their exploratory behaviour in the laboratory at ambient temperature and at a series of different temperatures, to assess: (1) whether personality differences in exploratory behaviour measured *ex situ* and in movement behaviours measured in the natural environment are present; (2) whether exploratory behaviour measured in the laboratory is indicative of *C. hortensis* movement behaviour after recently being re-released into its habitat (which may capture exploratory behaviour in natural environments), and/or movement behaviour over the longer term; and (3) whether the temperature at which exploratory behaviour is measured may influence the

relationship between exploratory behaviour measured in the laboratory and movement behaviours measured in natural environment.

We predict that all behavioural traits measured will be repeatable across time, conforming to distinct animal personalities. We expect that exploratory behaviour will be negatively related to repeatable movement parameters, because individuals that more thoroughly explore their immediate environment may travel less far than individuals that move quickly through the environment. Finally, because male and female *C. hortensis* (Szyszko et al., 2004; but see Chapter 3), and males and females of other carabid beetles (*e.g.* Drees & Huk, 2000; Gerlach *et al*., 2009; Lagisz *et al*., 2010) differ in activity level, with males being more active than females, and activity increases with hunger in female but not male carabids (*e.g.* Mauremooto *et al*., 1995), we consider the potential that male and female exploratory behaviour in the laboratory will be indicative of different movement behaviours in natural environments resulting in sex-specific relationships between those traits.

4.3 Methods

4.3.1 Trapping, Maintenance and General Procedure

Carabus hortensis (Coleoptera, Carabidae) ground beetles were collected between the 5th August - 30th September 2019, from the western range edge of the species' distribution (Völler *et al*., 2018) in the Lüneburger Heide, Lower Saxony, Germany (N53°10'53.32'', E9°53'08.06''). The beetles were tracked by means of radio telemetry in

their natural woodland habitat between the 10th August - 14th October 2019, during the *C. hortensis* active and reproductive season (Turin *et al*., 2003).

In order to collect individuals, live pitfall traps were arranged into six rows of traps (hereafter: trap rows) which ran parallel to the westerly range of the species. Trap rows were separated by 130m, apart from the third and fourth trap rows, which were separated from one another by approximately 800m for reasons beyond the scope of this study, and the fourth and fifth trap rows, which were placed approximately 260m apart due to low habitat quality. Each trap row contained 10-15 pitfall traps that were placed 10m apart. Pitfall trap structure, and the schedules for baiting and emptying pitfall traps are outlined in detail in Yarwood *et al*., (2021a) (Chapter 2). In total, 48 beetles (26 females, 22 males) were collected from the pitfall traps for this study. Collected individuals were returned to the field station, where they were housed separately at ambient temperature in 10(L) x 7.5(W) x 4.5(H) cm containers. Containers were filled with peat that was regularly sprayed with water and was changed every few weeks. Beetles were fed *Tenebrio molitor* pupa *ad libitum.*

Beetles were then tested for their exploratory behaviour at ambient laboratory temperature (see: 'Exploratory behaviour tests and temperature treatments: Exploratory behaviour prior to radio-tracking') before being released into the natural environment for radio-tracking. Following radio-tracking, individuals were tested again for their exploratory behaviour (see: 'Exploratory behaviour tests and temperature treatments: Exploratory behaviour following radio-tracking') at a series of ecologically relevant temperatures in the laboratory.

4.3.2 Tagging procedure and movement data

To track individual beetle movements in the natural environment using radio telemetry, a Micro-Pip radio-tag transmitter (Biotrack Ltd., Wareham, United Kingdom) emitting a specific radio frequency was adhered to the elytra of each individual. The surface of the elytra was gently scratched using a precision drill (PROXXON GmbH, Föhren, Germany), and was cleaned with ethanol. A thin layer of car sealant (CAR SYSTEM UNIFLEX-PU 'Klebe und Dichtmasse', Voss Chemie GmbH, Uetersen, Germany) was then used to apply the tag to the roughened elytra. To prevent the tag from becoming dislodged, individuals were restrained throughout the tag-fitting process, and were placed in their restraints in a refrigerator until approximately 8 hours before release into the natural environment. The average weight of the radio-tags plus the weight of the car sealant was 0.275 ± 0.88g (mean ± SD). To standardise the effect of hunger level on the activity of the beetles, access to food was restricted for 24 hours prior to release. Individuals were kept at the field station on average for 13.8 days ± 7.5 SD (range: 1-44 days) prior to release.

Each week for eight weeks from the 10th August – 14th October 2019, up to nine individuals were released approximately 5m North-East from the pitfall trap from which they were collected (See Table B.1 for detailed information on the dates and times of individual beetle release and recapture), during the *C. hortensis* active season and daily activity period (See Figure 2.2, Figure 2.3A, and Figure B.2). A Sika radio-tracking receiver attached to a Yagi antenna (Lotek, Wareham, United Kingdom) was used to pinpoint the location of each individual once every 2 hours for 8 hours from sunset, 5 days per week, for approximately two weeks. Numbered and flagged bamboo sticks were placed at each

position at which beetles were located, when beetles had moved at least 20cm from their previous location. During daylight, a compass was used to measure the direction (°TN) of travel between successive locations, whilst the distance between successive locations was measured in metres. Throughout radio-tracking, temperature was recorded once every 10 minutes using data loggers (VOLTCRAFT DL-210TH, Conrad Electronic SE, Hirschau, Germany) that were placed ca. 20cm above the ground at the second and fifth trap rows. We used headlamps with red light to guide our path when tracking beetles at night to ensure that the beetles were not disturbed by our presence: carabid beetle behaviour is not apparently disturbed under red light (Drees *et al.*, 2008; Hasselmann, 1962). Beetles were recaptured following approximately two weeks of being in the natural environment, and their radio-tags were removed.

4.3.3 Exploratory behaviour tests and temperature treatments

To assess the effects of temperature upon the relationship between laboratory measures of exploratory behaviour and behaviour measured in natural environments, *C. hortensis* exploratory behaviour was assessed in two distinct phases; (1) prior to radio-tracking, twice at ambient 'laboratory' temperature (°C); and (2) following radio-tracking, twice at each of four ecologically relevant temperatures. To assess exploratory behaviour in each instance, individuals were placed at the centre of an open white 37.5(L) x 26.0(W) cm plastic box with a 28 x square grid, and the number of squares visited, including repeated visits to the same square, were recorded during observation for 90 seconds (Harris *et al.*, 2020; Schuett *et al.*, 2018; Yarwood et al., 2021b).

4.3.3.1 Exploratory behaviour prior to radio-tracking

Prior to radio tracking, exploratory behaviour was measured twice at ambient laboratory temperature, once 6.43 ± 2.12 days (Mean ± SD) prior to radio tracking, the second time 2-5 days later. The repeated tests allowed to test whether individual differences in exploratory behaviour are consistent over time. Ambient laboratory temperature ranged from 18.90 – 27.30°C (mean ± SD: 22.78 ± 1.74°C) and was recorded once every 10 minutes using data loggers (VOLTCRAFT DL-210TH, Conrad Electronic SE, Hirschau, Germany). All exploratory behaviour tests at ambient laboratory temperature were conducted after sunset from 18:00 – 02:00.

4.3.3.2 Exploratory behaviour following radio-tracking

Throughout September and October 2019, following radio-tracking, 36 of the 48 *C. hortensis* individuals (22 female, 14 males) were taken to the University of Hamburg to assess exploratory behaviour repeatedly under a range of four different controlled temperatures. Beetles were maintained under a 10h : 15h, 12°C : 6°C light: dark regime reflecting average autumn temperatures and daylight hours at the Schneverdingen weather station (Weather Underground, n.d.) in the Lüneburger Heide region for 2019. Maintaining beetles at average autumn temperatures and daylight hours ensured that the beetles remained in the same phase of activity under which they were tracked in the natural environment. Because *C. hortensis* are night-active, and we wanted to conduct exploratory behaviour when *C. hortensis* were most active, we reversed the day-night cycle of the beetles. We slowly shifted daylight hours by 62 minutes once per week from

the 14th October 2019 until 23rd December 2019, ensuring that there was limited disruption to individual physiology.

C. hortensis exploratory behaviour was measured throughout January - February 2020 in a controlled climate chamber (Weiss WK2T Climate Chamber WeisScientific Ltd., Timrat, Israel) at 3°C, 10°C, 17°C and 23°C. These controlled temperatures were chosen as they reflect the minimum (4.5°C), intermediate (10°C, 17°C), and maximum (22°C) temperatures that we recorded at the study site between September – October 2019 during the 8 hours following sunset (*i.e.* the main daily active period of the beetles; Figure 2.3A, Figure B.2). We only considered temperatures recorded at night and during the *C. hortensis* active season to ensure that individuals were only exposed to temperatures within their normal temperature range during active periods. Individuals were tested for their exploratory behaviour in the climate chamber at a different temperature once every 4-5 days until all beetles had been tested at each of the four temperatures twice. Individuals experienced temperature treatments in one of four different orders: (1) 3°C, 10°C, 17°C, 23°C; (2) 10°C, 17°C, 23°C, 3°C; (3) 17°C, 23°C, 3°C, 10°C; or (4) 23°C, 3°C, 10°C, 17°C. Repeated measures at each of 3°C, 10°C, 17°C and 23°C were taken 18-19 days after the first measure to assess whether individual differences in exploratory behaviour are consistent over time. Exploratory behaviour tests were conducted from 08:00 - 18:00, under the reversed day-night cycle.

4.3.4 Data Analyses

4.3.4.1 Calculation of movement parameters

Individuals may behave differently in the first few hours of release into the natural environment following a short period of captivity to how they may behave in the natural environment over the longer term. We therefore calculated three different variables to quantify individual *C. hortensis* movement in the natural environment during the first day of release: (1) distance travelled over the first 2 hours of release into the natural environment (day 1 DT2h); (2) distance travelled over the first 8 hours of release into the natural environment (day 1 DT8h); and (3) path straightness over the first 8-hours of release into the natural environment (day 1 path straightness). Individuals were released into the natural environment at approximate sunset. We calculated measures for both day 1 DT2h and day 1 DT8h because individuals may have behaved differently in the first two hours of re-release into the natural environment following a period in the laboratory but may have re-acclimatised to their environment after 8 hours.

For each day that the individual was tracked in the natural environment (excluding the first day in the natural environment, for which values of day 1 DT2h, day 1 DT8h, and day 1 path straightness had already been calculated), we additionally calculated measures of: (1) distance travelled over 2 hours post-sunset (DT2h+); (2) distance travelled over 8 hours post-sunset (DT8h+); and (3) path straightness+ over 8 hours post-sunset. Measures of DT2h+, DT8h+ and path straightness+ were used to estimate the repeatability of those behaviours over time and for the purposes of calculating variables to quantify individual *C. hortensis* movement in the natural environment over the longer term. Measures of DT2h+, DT8h+ and path straightness+ were not used to assess the

relationships between exploratory behaviour and each of day 1 DT2h, day 1 DT8h and day 1 path straightness.

We calculated all DT2h and DT8h as the distances in metres between each successive location over approximately 2 hours and 8 hours post-sunset each day, respectively. Because we recorded successive locations only approximately once every 2 hours, DT2h and DT8h were adjusted to the distance covered in exactly 2 hours and 8 hours, respectively. We calculated path straightness for each day as the Euclidean distance (in metres) between the first point at which the individual was located (*i.e.* at sunset), and the last point at which the individual was located (*i.e.* 8 hours post-sunset), divided by the raw, unadjusted DT8h (*e.g.* Frizzi, 2018).

In addition to DT2h, DT8h and path straightness, we calculated two different variables to quantify individual *C. hortensis* movement over the longer term: (1) total distance travelled over two weeks (TDT); and (2) average path straightness over two weeks (average path straightness). Average path straightness was calculated as the average of path straightness values calculated for each day that the beetle was tracked and was calculated from all available path straightness data (*i.e.* from both day 1 path straightness and path straightness+). The TDT for each individual was calculated as the sum total of raw DT8h measures for each day that a beetle was tracked and was calculated from all available DT8h data (*i.e.* from both day 1 DT8h and DT8h+). To control for the fact that some individuals were tracked for longer than others (range = 7-16 days), we adjusted TDT to the distance travelled for 8 hours a day for 14 tracking days. We chose to correct TDT to the distance travelled over two weeks because the majority of

individuals (N = 42 out of N = 48) were in the natural environment for two weeks before re-collection.

In 4% (86 out of 2064) of location attempts, beetles could not be located, resulting in the time between successive locations doubling from approximately 2 hours to approximately 4 hours. To estimate missing data points, we linearly interpolated the data rather than halving the distance travelled by the individual: doing so obtained more realistic estimates of how individuals may have moved over time. Estimating missing data points ensured that all measures of DT8h and path straightness were calculated from five individual movements and were therefore comparable.

4.3.5 Statistical Analyses

All analyses were carried out in R version 3.3.2 (R Core Team, 2019). Spearman's rank correlations (Spearman, 1904) were performed to assess whether there was any collinearity amongst day 1 DT2h, day 1 DT8h and day 1 path straightness, average path straightness and TDT (Table C.1). Average values of DT2h and DT8h over two weeks were also quantified, however, because these were highly correlated (R>0.90) with both one another and TDT (Table C.1), these variables were not analysed to avoid multicollinearity.

4.3.5.1 Repeatability and rank consistency of exploratory behaviour and movement parameters

We used the rptR package (Stoffel *et al*., 2017) with linear mixed effects models (LMMs) to estimate the repeatability of exploratory behaviour at each of the temperature treatments (at ambient laboratory temperature after 2-5 days and at each of 3°C, 10°C, 17°C and 23°C after 18-19 days) as well as exploratory behaviour that was averaged

across all temperatures (hereafter: average exploratory behaviour), with two measures per individual and temperature treatment. We additionally estimated the repeatability of: (1) the movement parameters in the natural environment; and (2) exploratory behaviour across all temperature treatments, over time, including temperature as a covariate. Repeatability of DT2h+, DT8h+ and path straightness+ was calculated from 6-14 measures per individual taken 1-4 days apart from another. Measures of day 1 DT2h, day 1 DT8h and day 1 path straightness were excluded from repeatability analysis to control for the fact that individuals may not have acclimated to and therefore behaved differently in their environment during this time. Because individuals may not have been motivated to move at a given temperature due to various factors, only instances where individuals moved were included in the repeatability analyses for DT2h+, DT8h+ and path straightness+. Beetle ID was included as a random term in all repeatability analyses. Confidence intervals of 95% were used to infer the repeatability significance: if the confidence interval included zero, the trait was considered not repeatable. Spearman's rank correlations were also used to assess the rank consistency of exploratory behaviour over all temperatures and at each individual temperature treatment. Repeatability estimates and Spearman's rank correlation coefficients were calculated for combined male and female data (hereafter: all beetles), as well as separately for each sex. Because we performed Spearman's rank correlations on the same datasets but at multiple temperatures, we corrected for multiple testing by performing false discovery rate adjustments on all significant p-values (Benjamini & Hochberg, 1995). Using the false discovery rate method, the threshold p-value for significance of Spearman's rank correlations was set to 0.008. Results stated refer to those following the false discovery rate correction, however unadjusted p-values are provided for comparison.

4.3.5.2 Relationships between exploratory behaviour and movement parameters

We investigated the relationships between each of day 1 DT2h, day 1 DT8h and day 1 path straightness , as well as average path straightness and TDT (response variables) and exploratory behaviour (fixed term) by conducting LMMs on data from all beetles as well as on separate male and female data, using the lme4 package (Bates *et al*., 2015). The relationships between each of DT2h+, and DT8h+ and exploratory behaviour were not explored due to high correlations between both average day 1 DT2h and day 1 DT8h and TDT (Table C.1). Six sets of LMMs were conducted per response variable and data set (*i.e.* all beetles, females and males) to test the effects of temperature treatment on the relationship between behaviour measured in the natural environment and exploratory behaviour, in which one of the first measurements taken of: (1) exploratory behaviour measured prior to radio-tracking, interacting with ambient laboratory temperature; or exploratory behaviour following radio-tracking, measured at: (2) 3°C; (3) 10°C; (4) 17°C; (5) 23°C; or (6) average exploratory behaviour, was included as the main fixed term. The average temperature in the natural environment over which the response variable was calculated (2 hours for DT2h; 8 hours for path straightness and DT8h), was included as a fixed term in all LMMs. Sex was included in as an additional fixed term in models exploring data from all beetles. The week (week 1 – week 8) in which beetles were released for radio-tracking was included as a random term in all LMMs, controlling for seasonal temperature changes throughout the study period. Stepwise model simplification was performed on all LMMs; fixed terms were removed from the model in stages and compared to the previous model using likelihood ratio tests (Crawley, 2007). At each stage, the most non-significant fixed term, whose removal reduced the power of the

model the least, was removed. One female was removed from day 1 DT2h analysis as the average temperature over which day 1 DT2h for that individual was measured was missing. A single outlier was removed from day 1 DT8h LMMs for all beetles and for females alone. Because we performed LMMs on the same datasets but at multiple temperatures, we corrected for multiple testing by performing false discovery rate adjustments on all significant p-values (Benjamini & Hochberg, 1995). Using the false discovery rate method, the threshold p-value for significance was set to 0.008. Results stated refer to those following the false discovery rate correction, however unadjusted p-values are provided for comparison.

4.4 Ethics

This study was carried out under permits from the Heidekreis (permit number: 2019-0168) and Harburg (permit number: 2019-0218-Kr) nature conservation *authorities and* the Lower Saxon State Department for Waterway, Coastal and Nature Conservation authorities (permit number: H72.22202/2019-Drees).

4.5 Results

The range and mean ± SD of male and female movement parameters, as well as exploratory behaviour at each of 3°C, 10°C, 17°C, 23°C and ambient laboratory temperature can be found in Table 3.1. The temperatures in the natural environment over which day 1 DT2h was measured ranged from 12.00 – 27.25°C (mean ± SD: 16.35 ± 4.11°C), whilst the temperatures in the natural environment over which both day 1 DT8h and day 1 path straightness were measured ranged from 9.60 – 20.62°C (mean ± SD:

13.44 ± 3.28°C). The temperatures in the natural environment over which both TDT and average path straightness were recorded ranged from 8.60 – 19.50°C (mean ± SD: 13.54 ± 3.13°C).

Table 3.1. The range and mean ± SD of movement parameters measured in the natural environment and exploratory behaviour (number of square visits in a novel environment) at each of 3°C, 10°C, 17°C, 23°C, lab temperature (Lab_{Temp}; °C), and averaged across temperature treatments (°C). Values given for exploratory behaviour are across both first and repeated measures. N_{ID}, number of individuals; N_{Obs}, number of observations.

Behaviour	Sex	Range	Mean ± SD	N_{ID} (N_{Obs})
Day 1 DT2h (m)	F	0.00 – 1.37	0.39 ± 0.39	26 (26)
	M	0.00 – 0.86	0.32 ± 0.31	22 (22)
Day 1 DT8h (m)	F	0.00 – 96.39	6.58 ± 18.32	26 (26)
	M	0.00 – 24.78	3.52 ± 5.47	22 (22)
Day 1 Path Straightness	F	0.00 – 1.00	0.47 ± 0.40	26 (26)
	M	0.00 – 1.00	0.47 ± 0.30	22 (22)
DT2h+ (m)	F	0.09 – 16.96	0.62 ± 1.23	26 (759)
	M	0.09 – 17.44	0.82 ± 1.50	22 (540)
DT8h+ (m)	F	0.20 – 14.99	1.58 ± 2.03	26 (252)
	M	0.17 – 41.05	2.38 ± 4.57	22 (192)
Path Straightness+	F	0.00 – 1.00	0.55 ± 0.30	26 (252)
	M	0.03 – 1.00	0.60 ± 0.31	22 (192)
TDT (m)	F	10.09 – 131.07	28.81 ± 28.27	26 (26)
	M	8.89 – 153.34	33.28 ± 32.76	22 (22)
Average Path Straightness	F	0.31 – 0.73	0.52 ± 0.11	26 (26)
	M	0.33 – 0.84	0.56 ± 0.11	22 (22)
Exploratory Behaviour (3°C)	F	1.00 – 31.00	12.30 ± 6.55	22 (22)
	M	5.00 – 24.00	12.75 ± 4.49	14 (14)
Exploratory Behaviour (10°C)	F	1.00 – 52.00	24.23 ± 11.04	22 (22)

	M	1.00 – 69.00	30.43 ± 15.12	14 (14)
Exploratory Behaviour (17°C)	F	1.00 – 72.00	41.48 ± 17.95	22 (22)
	M	28.00 – 97.00	55.25 ± 20.57	14 (14)
Exploratory Behaviour (23°C)	F	11.00 – 100.00	48.64 ± 18.44	22 (22)
	M	1.00 – 113.00	59.93 ± 28.47	14 (14)
Exploratory Behaviour (Lab_{Temp})	F	1.00 – 134.00	44.94 ± 35.85	26 (26)
	M	2.00 – 114.00	35.36 ± 30.23	22 (22)
Average Exploratory Behaviour	F	1.00 – 55.33	32.67 ± 12.60	26 (26)
	M	5.00 – 50.17	33.08 ± 11.46	22 (22)

4.5.1 Repeatability and rank consistency of exploratory behaviour and movement parameters

All results reported are those after applying the false discovery rate correction (Benjamini & Hochberg, 1995). *C. hortensis* exploratory behaviour was not repeatable but was rank consistent for all beetles and females alone, but not males alone, at ambient laboratory temperature (after 2-5 days). Average *C. hortensis* exploratory behaviour was repeatable but not rank consistent for all beetle and females alone. The average exploratory behaviour of males was not rank consistent or repeatable. However, *C. hortensis* exploratory behaviour was neither repeatable nor rank consistent for all beetles, females alone and males alone at 3°C, 10°C, 17°C or 23°C (after 18-19 days). When assessed over all combined temperature treatments, *C. hortensis* exploratory behaviour was rank consistent, but not repeatable, in all beetles, and in females alone. Male exploratory behaviour was not rank consistent or repeatable when assessed over all combined temperature treatments (Table 3.2). DT2h+ and DT8h+ (after 1-4 days) were repeatable over time in all beetles, and for females and males separately, while path straightness+ (after 1-4 days) was repeatable only for data from all beetles (Table 3.2).

Table 3.2. Repeatability estimates (± 95% confidence intervals) from linear mixed effects models for exploratory behaviour (number of square visits in a novel environment) at each of 3°C, 10°C, 17°C, 23°C, lab temperature (Lab_{Temp}; °C), and averaged across temperature treatments (Average; °C), as well as exploratory behaviour across all temperature treatments (All_{Temps}; °C), path straightness+, DT8h+ and DT2h+, with temperature as a covariate. Repeatability tests for path straightness+, DT8h+ and DT2h+ were conducted only when individuals moved. Spearman's rank correlation coefficients (R_s) and associated p-values are included. Bold values denote significance. Results are given for all beetles (M + F), females (F) and males (M). N_{ID}, number of individuals; N_{Obs}, number of observations. Asterisks on p-values denote which correlations remain significant following False Discovery Rate testing (Benjamini & Hochberg, 1995)

Response Variable	Sex	Repeatability	95% CI	R_s	p-value	N_{ID} (N_{Obs})
Exploratory Behaviour (3°C)	M + F	0.193	0.000 - 0.473	-0.134	0.434	36 (72)
	F	0.234	0.000 - 0.603	-0.322	0.143	22 (44)
	M	0.103	0.000 - 0.567	0.236	0.416	14 (28)
Exploratory Behaviour (10°C)	M + F	<0.001	0.000 - 0.317	-0.136	0.429	36 (72)
	F	<0.001	0.000 - 0.388	-0.317	0.151	22 (44)
	M	0.025	0.000 - 0.514	-0.020	0.946	14 (28)
Exploratory Behaviour (17°C)	M + F	0.115	0.000 - 0.420	0.128	0.458	36 (72)
	F	<0.001	0.000 - 0.378	0.235	0.292	22 (44)
	M	0.296	0.000 - 0.661	-0.026	0.929	14 (28)
Exploratory Behaviour (23°C)	M + F	0.290	0.000 - 0.563	-0.224	0.244	36 (72)
	F	0.223	0.000 - 0.565	-0.192	0.431	22 (44)
	M	0.291	0.000 - 0.691	-0.195	0.590	14 (28)
Exploratory Behaviour (Lab_{Temp})	M + F	0.124	0.000 - 0.396	**0.617**	**<0.001***	48 (91)
	F	0.118	0.000 - 0.492	**0.622**	**0.001***	26 (49)
	M	0.121	0.000 - 0.536	**0.560**	**0.010**	22 (42)

Exploratory Behaviour (Average)	M + F	**0.629**	**0.430 – 0.774**	**0.328**	**0.026**	48 (94)
	F	**0.721**	**0.484 – 0.864**	**0.369**	**0.064**	26 (51)
	M	0.331	0.000 – 0.663	0.364	0.115	22 (43)
Exploratory Behaviour (All_{Temps})	M + F	0.034	0.000 - 0.102	**0.527**	**<0.001***	48 (473)
	F	0.026	0.000 – 0.117	**0.525**	**<0.001***	26 (276)
	M	0.026	0.000 – 0.147	**0.530**	**<0.001***	22 (197)
DT2h+	M + F	**0.216**	**0.136 – 0.302**	NA	NA	48 (1299)
	F	**0.226**	**0.124 – 0.337**	NA	NA	26 (759)
	M	**0.193**	**0.082 – 0.308**	NA	NA	22 (540)
DT8h+	M + F	**0.233**	**0.131 – 0.341**	NA	NA	48 (444)
	F	**0.178**	**0.059 – 0.298**	NA	NA	26 (252)
	M	**0.291**	**0.122 – 0.448**	NA	NA	22 (192)
Path Straightness+	M + F	**0.075**	**0.012 - 0.145**	NA	NA	48 (444)
	F	0.078	0.000 – 0.176	NA	NA	26 (252)
	M	0.066	0.000 – 0.181	NA	NA	22 (192)

4.5.2 Relationships between movement parameters and average exploratory behaviour

After applying the false discovery rate correction (Benjamini & Hochberg, 1995), day 1 DT2h was not significantly related to average exploratory behaviour, in all beetles or in either sex (Table 3.3). Whilst we found no significant correlation between female day 1 DT8h and average exploratory behaviour (Table 3.4, Figure 3.1A), the day 1 DT8h of all beetles (Table 3.4) and of males alone (Table 3.4, Figure 3.1B) were significantly, negatively correlated to average exploratory behaviour. Day 1 path straightness (Table 3.5), TDT (Table 3.6) and average path straightness (Table 3.7) were not significantly correlated with average exploratory behaviour in either sex or in all beetles.

Table 3.3. Summary of test statistics from LMMs with the distance travelled in the first two hours of release into the natural environment (day 1 DT2h) by all beetles (M + F), females alone (F) or males (M) as the response. Exploratory behaviour measured at ambient laboratory temperature (Lab_{Temp}) or averaged over all temperature treatments (Average), and the average temperature of the natural environment ($Field_{Temp}$) for the time over which day 1 DT2h was measured were included as fixed terms. Coefficients (Coeff.) in square brackets belong to non-significant terms just before dropping those terms from the model. N_{ID}, number of individuals used in analysis. Variance (Var.) of the random term 'Week' and Residuals are presented. Bold p-values denote significant terms.

Dataset	Random Term	Var.	Fixed Term	Coeff.	χ^2	DF	p-value
M + F (Lab_{Temp}) (N_{ID} = 47)	Week	0.02	Intercept	0.38			
	Residual	0.11	Exploratory behaviour: Lab_{Temp}	[<0.01]	0.18	1	0.672
			$Field_{Temp}$	[0.01]	0.47	1	0.494
			Exploratory behaviour	[<-0.01]	0.02	1	0.881
			Lab_{Temp}	[0.02]	0.01	1	0.911
			Sex (males)	[-0.11]	1.18	1	0.277
F (Lab_{Temp}) (N_{ID} = 25)	Week	0.02	Intercept	0.44			
	Residual	0.14	Exploratory behaviour: Lab_{Temp}	[<0.01]	1.57	1	0.210

			$Field_{Temp}$	[-0.01]	0.12	1	0.731
			Exploratory behaviour	[<-0.01]	0.27	1	0.600
			Lab_{Temp}	[-0.02]	0.53	1	0.817
M (Lab_{Temp})	Week	0.01	Intercept	0.33			
(N_{ID} = 22)	Residual	0.09	Exploratory behaviour: Lab_{Temp}	[<-0.01]	0.15	1	0.702
			$Field_{Temp}$	[0.03]	2.66	1	0.103
			Exploratory behaviour	[<0.01]	0.08	1	0.784
			Lab_{Temp}	[0.05]	1.46	1	0.228
M + F (Average)	Week	0.02	Intercept	0.38			
(N_{ID} = 47)	Residual	0.11	Exploratory behaviour	[<-0.01]	0.70	1	0.402
			$Field_{Temp}$	[0.01]	0.60	1	0.440
			Sex (males)	[-0.11]	1.18	1	0.277
F (Average)	Week	0.02	Intercept	0.44			
(N_{ID} = 25)	Residual	0.14	Exploratory behaviour	[-0.01]	0.96	1	0.328
			$Field_{Temp}$	[-0.01]	0.06	1	0.810
M (Average)	Week	0.01	Intercept	0.33			
(N_{ID} =22)	Residual	0.09	Exploratory behaviour	[<-0.01]	0.01	1	0.937
			$Field_{Temp}$	[0.03]	2.66	1	0.103

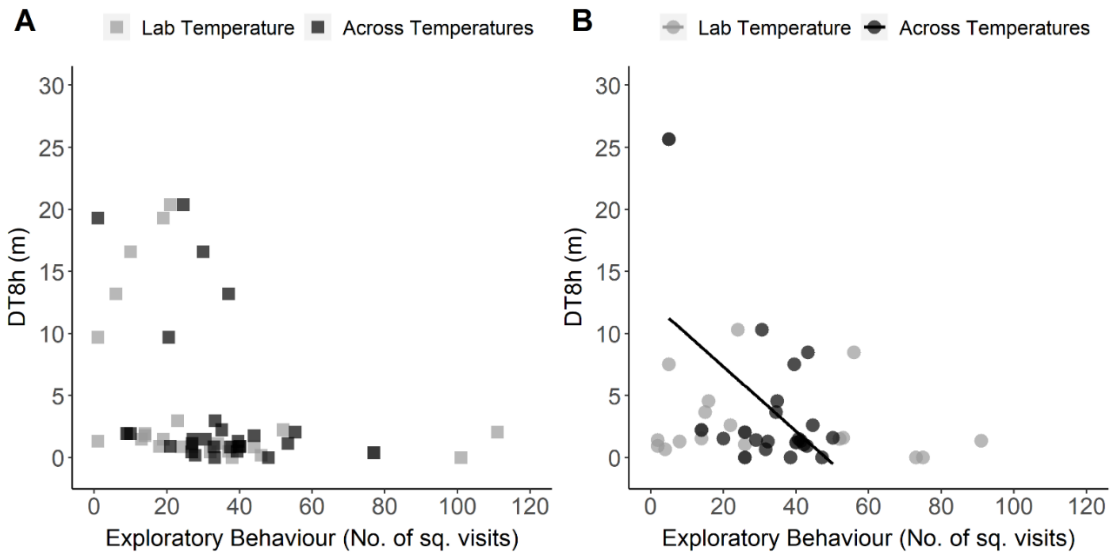

Figure 3.1. Distance travelled over 8 hours in the first day of release into the natural environment is negatively related to exploratory behaviour in male but not female *C. hortensis*. A) females and B) males, at ambient laboratory temperature (light grey; female N = 26, male N = 22), and averaged across all temperature treatments (dark grey; female N = 26, male N = 22). Female data points are represented by squares whilst male data points are represented by circles. One point is removed from Figure 1A to improve clarity of the figure. Predicted lines are fitted using outputs from LMM estimates.

4.5.3 Relationships between movement parameters and exploratory behaviour at individual temperatures

After applying the false discovery rate correction (Benjamini & Hochberg, 1995), day 1 DT2h was not significantly correlated with exploratory behaviour at ambient laboratory temperature (Table 3.3), 3°C, 10°C, 17°C or 23°C (Table C.2), in all beetles or in either sex. Likewise, day 1 DT8h (Table 3.4, Table C.3) and day 1 path straightness (Table 3.5, Table C.4) were not significantly correlated with exploratory behaviour at any

individual temperature in all beetles or either sex. The TDT of all beetles, females and males was not significantly correlated with exploratory behaviour at ambient laboratory temperature (Table 3.6), 3°C, 10°C, 17°C or 23°C (Table C.5). Finally, we found no significant correlation between the average path straightness of all beetles, females or males and exploratory behaviour measured at ambient laboratory temperature (Table 3.7), 3°C, 10°C, 17°C or 23°C (Table C.6).

Table 3.4. Summary of test statistics from LMMs with the distance travelled in the first day hours of release into the natural environment (day 1 DT8h) by all beetles (M + F), females (F) or males (M) as the response. Exploratory behaviour measured at either ambient laboratory temperature (Lab_{Temp}) or averaged over all temperature treatments (Average), and the temperature of the natural environment ($Field_{Temp}$) for the time over which day 1 DT8h was measured were included as fixed terms. Coefficients (Coeff.) in square brackets belong to non-significant terms just before dropping those terms from the model. N_{ID}, number of individuals used in analysis. Variance (Var.) of the random term 'Week' and Residuals are presented. Bold p-values denote significant terms. Asterisks on p-values denote which correlations remain significant following False Discovery Rate testing (Benjamini & Hochberg, 1995).

Dataset	Random Term	Var.	Fixed Term	Coeff.	χ^2	DF	p-value
M + F (Lab_{Temp})	Week	<0.01	Intercept	5.88			
(N_{ID} = 47)	Residual	32.78	Exploratory behaviour: Lab_{Temp}	[0.01]	0.89	1	0.346
			$Field_{Temp}$	[0.43]	2.87	1	0.090
			Exploratory behaviour	-0.06	4.76	1	**0.029**
			Lab_{Temp}	[-0.07]	0.02	1	0.891
			Sex (males)	[-0.53]	0.11	1	0.743
F (Lab_{Temp})	Week	<0.01	Intercept	-6.92			
(N_{ID} = 25)	Residual	35.23	Exploratory behaviour: Lab_{Temp}	[0.02]	0.99	1	0.319

			$Field_{Temp}$	0.80	4.24	1	**0.039**
			Exploratory behaviour	[-0.06]	2.71	1	0.099
			Lab_{Temp}	[-0.07]	0.01	1	0.911
M (Lab_{Temp})	Week	<0.01	Intercept	3.61			
(N_{ID} = 22)	Residual	31.91	Exploratory behaviour: Lab_{Temp}	[0.02]	0.27	1	0.601
			$Field_{Temp}$	[0.14]	0.15	1	0.700
			Exploratory behaviour	[-0.05]	1.47	1	0.226
			Lab_{Temp}	[-0.13]	0.03	1	0.868
M + F (Average)	Week	<0.01	Intercept	11.32			
(N_{ID} = 47)	Residual	28.50	Exploratory behaviour	-0.23	11.34	1	**<0.001***
			$Field_{Temp}$	[0.38]	2.71	1	0.100
			Sex (males)	[0.08]	<0.01	1	0.958
F (Average)	Week	<0.01	Intercept	-0.58			
(N_{ID} = 25)	Residual	30.99	Exploratory behaviour	-0.18	4.32	1	**0.038**
			$Field_{Temp}$	0.76	4.58	1	**0.032**
M (Average)	Week	<0.01	Intercept	13.00			
(N_{ID} =22)	Residual	22.41	Exploratory behaviour	-0.28	8.85	1	**0.003***
			$Field_{Temp}$	[0.03]	0.01	1	0.922

Table 3.5. Summary of test statistics from LMMs with the path straightness in the first day hours of release into the natural environment of all beetles (M + F), females (F) or males (M) as the response. Exploratory behaviour measured at either ambient laboratory temperature (Lab_{Temp}), or averaged over all temperature treatments (Average), and the average temperature of the natural environment ($Field_{Temp}$) for the time over which day 1 path straightness was measured were included as fixed terms. Coefficients (Coeff.) in square brackets belong to non-significant terms just before dropping those terms from the model. N_{ID}, number of individuals used in analysis. Variance (Var.) of the random term 'Week' and Residuals are presented. Bold p-values denote significant terms. Asterisks on p-values denote which correlations remain significant following False Discovery Rate testing (Benjamini & Hochberg, 1995).

Dataset	Random Term	Var.	Fixed Term	Coeff.	χ^2	DF	p-value
M + F (Lab_{Temp})	Week	<0.01	Intercept	0.51			
(N_{ID} = 48)	Residual	0.12	Exploratory behaviour: Lab_{Temp}	[<0.01]	0.98	1	0.323
			$Field_{Temp}$	[0.02]	1.43	1	0.232
			Exploratory behaviour	[<-0.01]	3.51	1	0.061
			Lab_{Temp}	[-0.03]	0.82	1	0.365
			Sex (males)	[-0.10]	1.02	1	0.312
F (Lab_{Temp})	Week	0.01	Intercept	0.55			
(N_{ID} = 26)	Residual	0.15	Exploratory behaviour: Lab_{Temp}	[<0.01]	0.23	1	0.632
			$Field_{Temp}$	[0.04]	3.02	1	0.082
			Exploratory behaviour	[<-0.01]	0.02	1	0.881

			Lab_{Temp}	[-0.05]	1.46	1	0.226
M (Lab_{Temp})	Week	<0.01	Intercept	0.63			
(N_{ID} = 22)	Residual	0.07	Exploratory behaviour: Lab_{Temp}	[<0.01]	0.59	1	0.441
			$Field_{Temp}$	[<0.01]	<0.01	1	0.963
			Exploratory behaviour	-0.01	5.71	1	**0.017**
			Lab_{Temp}	[-0.03]	0.65	1	0.420
M + F (Average)	Week	<0.01	Intercept	0.51			
(N_{ID} = 48)	Residual	0.12	Exploratory behaviour	[-0.01]	2.37	1	0.124
			$Field_{Temp}$	[0.02]	1.12	1	0.289
			Sex (males)	[-0.07]	0.52	1	0.472
F (Average)	Week	0.01	Intercept	0.55			
(N_{ID} = 26)	Residual	0.15	Exploratory behaviour	[<-0.01]	0.19	1	0.661
			$Field_{Temp}$	[0.04]	3.02	1	0.082
M (Average)	Week	<0.01	Intercept	0.84			
(N_{ID} =26)	Residual	0.09	Exploratory behaviour	-0.01	4.54	1	**0.033**
			$Field_{Temp}$	[-0.01]	0.41	1	0.522

Table 3.6. Summary of test statistics from LMMs the total distance travelled over the field season (TDT) by all beetles (M + F), females (F) or males (M) as the response. Exploratory behaviour measured at either ambient laboratory temperature (Lab_{Temp}) or averaged over all temperature treatments (Average), and the average temperature of the natural environment ($Field_{Temp}$) for the time over which TDT was measured were included as fixed terms. Coefficients (Coeff.) in square brackets belong to non-significant terms just before dropping those terms from the model. N_{ID}, number of individuals used in analysis. Variance (Var.) of the random term 'Week' and Residuals are presented. Bold p-values denote significant terms. Asterisks on p-values denote which correlations remain significant following False Discovery Rate testing (Benjamini & Hochberg, 1995).

Dataset	Random Term	Var.	Fixed Term	Coeff.	χ^2	DF	p-value
M + F (Lab_{Temp})	Week	0.01	Intercept	3.40			
(N_{ID} = 26)	Residual	0.43	Exploratory behaviour: Lab_{Temp}	[<0.01]	0.56	1	0.455
			$Field_{Temp}$	[0.02]	0.26	1	0.608
			Exploratory behaviour	-0.01	5.36	1	**0.021**
			Lab_{Temp}	[0.04]	0.49	1	0.483
			Sex (males)	[0.07]	0.16	1	0.687
F (Lab_{Temp})	Week	<0.01	Intercept	3.10			
(N_{ID} = 26)	Residual	0.43	Exploratory behaviour: Lab_{Temp}	[<0.01]	0.39	1	0.535
			$Field_{Temp}$	[0.02]	0.11	1	0.740
			Exploratory behaviour	[-0.01]	2.43	1	0.119
			Lab_{Temp}	[-0.01]	0.01	1	0.925

M (Lab_{Temp})	Week	<0.01	Intercept	3.20			
(N_{ID} = 22)	Residual	0.56	Exploratory behaviour: Lab_{Temp}	[0.01]	0.76	1	0.384
			$Field_{Temp}$	[0.02]	0.11	1	0.744
			Exploratory behaviour	[-0.10]	3.00	1	0.083
			Lab_{Temp}	[0.10]	1.03	1	0.310
M + F (Average)	Week	<0.01	Intercept	3.73			
(N_{ID} = 48)	Residual	0.45	Exploratory behaviour	-0.02	4.71	1	**0.030**
			$Field_{Temp}$	[<0.01]	0.02	1	0.901
			Sex (males)	[0.11]	0.34	1	0.561
F (Average)	Week	<0.01	Intercept	3.10			
(N_{ID} = 26)	Residual	0.43	Exploratory behaviour	[-0.01]	0.89	1	0.345
			$Field_{Temp}$	[-0.01]	0.04	1	0.838
M (Average)	Week	20.10	Intercept	78.14			
(N_{ID} =26)	Residual	858.00	Exploratory behaviour	-1.37	5.56	1	**0.018**
			$Field_{Temp}$	[-0.51]	0.04	1	0.843

Table 3.7. Summary of test statistics from LMMs with average path straightness over the field season of all beetles (M + F), females (F) or males (M) as the response. Exploratory behaviour measured at ambient laboratory temperature (Lab_{Temp}) or averaged over all temperature treatments (All_{Temps}), and the average temperature of the natural environment ($Field_{Temp}$) for the time over which average path straightness was measured were included as fixed terms. Coefficients (Coeff.) in square brackets belong to non-significant terms just before dropping those terms from the model. N_{ID}, number of individuals used in analysis. Variance (Var.) of the random term 'Week' and Residuals are presented. Bold p-values denote significant terms. Asterisks on p-values denote which correlations remain significant following False Discovery Rate testing (Benjamini & Hochberg, 1995).

Dataset	Random Term	Var.	Fixed Term	Coeff.	χ^2	DF	p-value
M + F (Lab_{Temp})	Week	<0.01	Intercept	1.12			
(N_{ID} = 48)	Residual	0.01	Exploratory behaviour: Lab_{Temp}	[<0.01]	0.58	1	0.447
			$Field_{Temp}$	[<-0.01]	0.01	1	0.928
			Exploratory behaviour	[<-0.01]	0.67	1	0.415
			Lab_{Temp}	-0.03	8.96	1	**0.003***
			Sex (males)	[0.02]	0.40	1	0.526
F (Lab_{Temp})	Week	<0.01	Intercept	1.18			
(N_{ID} = 26)	Residual	0.01	Exploratory behaviour: Lab_{Temp}	[<-0.01]	0.36	1	0.549
			$Field_{Temp}$	[-0.01]	1.09	1	0.297
			Exploratory behaviour	[<0.01]	1.54	1	0.214
			Lab_{Temp}	-0.03	6.59	1	**0.010**

M (Lab_{Temp})	Week	<0.01	Intercept	0.62			
(N_{ID} = 22)	Residual	0.01	Exploratory behaviour: Lab_{Temp}	[<0.01]	0.20	1	0.656
			$Field_{Temp}$	[<0.01]	0.22	1	0.635
			Exploratory behaviour	<-0.01	5.95	1	**0.015**
			Lab_{Temp}	[-0.01]	1.18	1	0.277
M + F (All_{Temps})	Week	<0.01	Intercept	0.54			
(N_{ID} = 48)	Residual	0.01	Exploratory behaviour	[<0.01]	0.11	1	0.739
			$Field_{Temp}$	[<-0.01]	0.80	1	0.372
			Sex (males)	[0.04]	1.69	1	0.193
F (All_{Temps})	Week	<0.01	Intercept	0.52			
(N_{ID} = 26)	Residual	0.01	Exploratory behaviour	[<0.01]	3.42	1	0.064
			$Field_{Temp}$	[-0.01]	1.34	1	0.244
M (All_{Temps})	Week	<0.01	Intercept	0.57			
(N_{ID} =26)	Residual	0.01	Exploratory behaviour	[<-0.01]	1.54	1	0.214
			$Field_{Temp}$	[<-0.01]	<0.01	1	0.998

4.6 Discussion

Laboratory-based studies are frequently used to study animal personality, but whether the outcome of such studies readily predict measures of animal movement in natural environments has been investigated in only a few of cases (*e.g.* Aplin *et al*., 2013; Dingemanse *et al*., 2003; Fisher *et al*., 2015; Fraser *et al*., 2001; Herborn *et al*., 2010; Hollander *et al*., 2008; McCowan *et al*., 2015; Quinn *et al*., 2011; Schirmer *et al*., 2019; Schuett *et al*., 2012; van Overveld & Matthysen, 2010; Wilson & McLaughlin, 2007; Yuen *et al*., 2016). Our study is one of the first to investigate the relationships between laboratory and natural environment-derived measures of some animal personality traits, in an insect. We repeatedly assayed exploratory behaviour in a novel environment at a range of temperatures and tracked individual *C. hortensis* movement parameters within the natural environment for up to two weeks. Movement parameters measured in natural environment were repeatable over time and therefore form personality traits. We found that the exploratory behaviour of all beetles and of males alone was significantly related to one of our movement parameters. However, we failed to find any general trends or many connections between movement parameters and exploratory behaviour measured at each of 3°C, 10°C, 17°C and 23°C. This may be due to: (1) the lack of repeatability of exploratory behaviour at those temperatures; (2) the fact that individuals were tested for their exploratory behaviour at each of 3°C, 10°C, 17°C and 23°C several months after the measurement of personality traits in natural environments; and (3) the data presented in this study were not gathered to test the hypothesis that personality measured in the laboratory may predict personality in the natural environment. Given the lack of results for exploratory behaviour measured at 3°C, 10°C, 17°C and 23°C, we focus our discussion on the relationships between movement

parameters and exploratory behaviour both at ambient laboratory temperature and averaged across all temperatures.

In line with other studies of insect (Fisher *et al.*, 2015), bird (*e.g.* Aplin *et al.*, 2013; Dingemanse *et al.*, 2003; Herborn *et al.*, 2010; Hollander *et al.*, 2008; McCowan *et al.*, 2015; Quinn *et al.*, 2011; Schuett *et al.*, 2012; van Overveld & Matthysen, 2010), mammal (*e.g.* Schirmer *et al.*, 2019; Yuen *et al.*, 2016), and fish (*e.g.* Fraser *et al.*, 2001) personality differences, we found that exploratory behaviour measured in the laboratory can be used to predict an insect personality trait in natural environment: average exploratory behaviour was negatively related to the distance travelled by all beetles and by males during the first 8 hours of release into the natural environment. Because: (1) we also found this relationship in all beetles; (2) the average exploratory behaviour of all beetles was found to be repeatable or rank consistent; and (3) the exploratory behaviour of males was rank consistent when measured across all temperatures, we consider the lack of repeatability and rank consistency in average male exploratory behaviour to be the by-product of low sample size. In contrast with males and all beetles, we found no significant relationships between female exploratory behaviour and personality traits in measured in the natural environment. That male exploratory behaviour was related to distance travelled in the natural environment over the shorter, but not longer, term, indicates that, upon being released into the natural environment, males, moved and behaved differently to their movements over the following two weeks. Our results therefore suggest that exploration in a novel environment describes the short-term response of males to a new or different environment. Ground beetles have been shown to switch from a 'random walk', characterised by small-scale movements paired with continually changing or 'random' directions to 'directed movement' characterised by long stints of

movement in a single direction, upon entering habitats with unfavourable vegetation structure (*e.g.* Baars, 1979b; Rijnsdorp, 1980), or low population densities (*e.g.* Charrier *et al*., 1997). However, the chance of encountering resources or potential mates is thought to increase with the 'random walk' movement pattern (Baars, 1979b; Charrier *et al*., 1997; Rijnsdorp, 1980). Given that our measures of short movement distances might be analogous to a random walk and opposite to directed movement, our results suggest that: (1) some males may respond to being placed in a new or different environment with directed movement; and (2) exploration in a novel environment may be used to predict male *C. hortensis* foraging or mate searching behaviours or strategies, at least upon entering a new environment. Exploratory behaviour has previously been shown to relate to foraging behaviour in vertebrate species (*e.g.* Herborn *et al*., 2010; Schirmer *et al*., 2019; Tanner & Jackson, 2012; van Overveld & Matthysen, 2010; Wilson & McLaughlin, 2007).

That exploratory behaviour was predictive of male movement over the shorter-term means that exploratory behaviour may also be used to infer information on the predation risk of individual *C. hortensis* males when entering new environments. For instance, we may predict that males that are highly exploratory (*i.e.* those that travel short distances in the natural environment over the short term) should have low predation risk and limited acquisition of high-quality resources. This is because, in general, individuals that walk in more tortuous paths and cover less distance in natural environments exploit and deplete resources in their immediate environment. These individuals therefore have lowered predation risk (Smith & Blumstein, 2008), and are thought to have limited chance of finding superior resource patches to those that they already exploit (Sih *et al*., 2015). Conversely, we may predict that males that demonstrate

low exploration should have high predation risk and increased chances of encountering high quality resources, because individuals that walk in straighter paths and cover more ground tend to forage in short periods at multiple, spread-out locations (Biro & Stamps, 2010; Réale *et al*., 2010; Smith & Blumstein, 2008). Alternatively, exploratory behaviour may inform individual differences in the perception of risk within the same environment (Wilson & McLaughlin, 2007). These predictions are, however, valid only over the shorter term, when males enter into a new habitat.

Had we not measured the relationships between exploratory behaviour and personality traits measured in natural environment on a sex-specific basis, as well as across multiple apparently analogous personalities in the natural environment, we, like other studies (*e.g.* McCowan *et al*., 2015), may have failed to find a link between exploratory behaviour and personality in the natural environment. Certainly, we found no relationship between female exploratory behaviour and movement patterns, and would not have observed any relationship between exploratory behaviour and male movement had measures not been conducted over the shorter term. Exploration in a novel environment may predict more personality traits in *C. hortensis* not measured here. Alternatively, we may have failed to find relationships that would have otherwise been present, because the data analysed here was not collected for the purposes of assessing links between personality in the laboratory and personality in the natural environment. Our results clearly show that the ecological significance of exploration in a novel environment may differ even within species, between the sexes. Our results exemplify the importance of relating laboratory-based measures of personality to multiple measures of personality in the natural environment, to do so on a sex-specific basis, to truly capture the ecological significance of behaviours measured in the laboratory.

Chapter 5: Sex-specific covariance between metabolic rate, behaviour and morphology in the ground beetle Carabus hortensis

5.1 Abstract

Individuals within the same species often differ in their metabolic rates, which may covary with behavioural traits (such as exploration), that are consistent across time and/ or contexts, and morphological traits. Yet, despite the frequent occurrence of sexual dimorphisms in morphology and behaviour, few studies have assessed whether and how sexes differ in metabolic trait covariances. We investigated sex-specific relationships among resting or active metabolic rate (RMR and AMR, respectively) with exploratory behaviour, measured independently of metabolic rate in a novel environment, body size and body mass, in *Carabus hortensis* ground beetles. RMR, AMR and exploratory behaviour were repeatable among individuals across time, except for male RMR which was unrepeatable. Female RMR neither correlated with exploratory behaviour nor body size/body mass. In contrast, AMR was correlated with both body size and exploratory behaviour. Males with larger body sizes had higher AMRs, whereas females with larger body sizes had lower AMRs. Both male and female AMR were significantly related to exploratory behaviour, though the relationships between AMR and exploration were body mass-dependent in males and temperature-dependent in females. Differences between sexes exist in the covariances between metabolic rate, body size and exploratory behaviour. This suggests that selection acts differently on males and females to produce these trait covariances with potentially important consequences for individual fitness.

5.2 Introduction

Individuals within a species often display consistent differences in metabolic rate (Biro & Stamps, 2010; Burton *et al*., 2011; Nespolo & Franco, 2007). Metabolic rate is an important element of life-history that exists in a trade-off with growth, reproduction and survival (Burton *et al*., 2011; Stearns, 1992). Understanding the processes that produce individual differences in metabolism are therefore important because intraspecific differences in metabolism may influence individual fitness. Intraspecific variation in metabolic rate may also have significant impacts at the population level by influencing individual reproductive rates and survival (Burton *et al*., 2011).

Intraspecific variation in metabolic rate may be associated with traits such as body mass (*e.g.* Killen *et al*., 2010; reviewed in: Glazier, 2005), or linked to distinct 'personalities' (Careau *et al*., 2008). In this context, 'personality' refers to consistent individual differences in a behavioural trait across time (and/or context) (*e.g.* Bell, 2007; Dall *et al*., 2004; Réale *et al*., 2007; Schuett *et al*., 2010; Stamps & Groothuis, 2010; reviewed in: Sanchez-Tojar *et al*., 2021).

Three different hypotheses attempt to explain the relationship between RMR and personality differences: (1) the 'performance hypothesis' (positive relationship between RMR and personality: high RMRs drive behaviours that feed-back high energy input); (2) the 'allocation hypothesis' (negative relationship between RMR and personality: energy is a finite resource that is split between the two) (Careau *et al*., 2008), and; (3) the 'independent hypothesis of energy management' (no relationship) (Careau & Garland, 2012). Each of these three hypotheses are supported by evidence from the literature. For instance, some studies support the performance hypothesis (*e.g.* Careau *et al*., 2011; Videlier *et al*., 2019), whilst others observed a negative correlation between personality

and RMR, thereby supporting the allocation hypothesis (*e.g.* Biro *et al*., 2020; Bouwhuis *et al*., 2014). Still, other studies support the independent hypothesis of energy management in that they found no significant relationship between personality and RMR (*e.g.* Agnani *et al*., 2020; Bouwhuis *et al*., 2014; Videlier *et al*., 2019). Consequently, the relationships between intraspecific RMR, body mass/body size and animal personality traits remain largely unclear. Even more unclear are the potential associations between active metabolic rate (AMR), body mass/body size and animal personality traits. Nevertheless, intraspecific differences in AMR may be more tightly linked to independently measured personality differences than RMR, because the energetic cost of movement should influence whether an individual engages in more or less energy expending behaviours. Studying the links between AMR and personality traits is important because intraspecific differences in energy expenditure during movement may affect the energy available for growth, somatic maintenance, and reproduction.

Despite the increasing interest surrounding the relationships between metabolism, personality, and morphology, the majority of studies investigating metabolic trait covariances neglect one important factor: sex (Hämäläinen *et al*., 2018). This is particularly surprising given sex differences are often considered in studies investigating solely RMR (*e.g.* Hill *et al*., 2020), personality traits (*e.g.* Schuett & Dall, 2009), or morphology (*e.g.* Yarwood *et al*., 2021a; Chapter 2). Differences in reproductive strategies and investment between the sexes arise as consequences of anisogamy (Bateman, 1948; Maynard Smith, 1978), which may lead to differences in traits associated with reproduction (Bateman, 1948), and/or differences in the fitness benefits of investing in metabolic rate, personality traits and morphological traits (Hämäläinen *et al*., 2018). The latter case may lead to sex-specific trait-covariances; the strength (and potentially

direction) of correlations between traits differing between the sexes because the trait-covariance is more beneficial to one sex than the other (Hämäläinen *et al*., 2018). Furthermore, the majority of studies investigating relationships between metabolic rate, and both personality traits and morphological traits have focused on endothermic vertebrates, largely ignoring insects (but see: Royauté *et al*., 2015). This is despite clear differences in the physiology and morphology of insects in comparison to endothermic vertebrates (Schmidt-Nielsen, 2007), that might affect metabolic trait covariances (*e.g.* Mathot *et al*., 2019).

Here we investigate the relationships between metabolic rate (RMR and AMR), exploratory behaviour in a novel environment, body size and body mass in both males and females of the predatory, nocturnal ground beetle *Carabus hortensis* Linnaeus, 1758. *C. hortensis* are flightless (Turin *et al*., 2003), obviating the need to measure flight metabolic rate to obtain active metabolic rate measures. Moreover, in other closely-related flightless ground beetle species, individual exploratory behaviour has been found to: (a) be repeatable across individuals over time, meaning that individuals display personality differences in exploration; and (b) relate to another behavioural trait – risk taking (Schuett *et al*., 2018). The body size of male, but not female *C. hortensis* has been shown to increase towards range edges, with which the male to female sex ratios also increased. Body size may therefore be more important to male than female reproductive success (Yarwood *et al*., 2021a; Chapter 2). Furthermore, males of closely-related carabid beetles show higher locomotory activity than females (*e.g.* Drees & Huk, 2000; Gerlach *et al*., 2009; Lagisz *et al*., 2010), which likely serves to increase the rate at which individuals encounter potential mating partners (Drees & Huk, 2000). If *C. hortensis* show similar sex differences in the ecological significance of movement (*e.g.* Chapter 4), then

males may expend more energy and have greater fitness benefits associated with exploration than females. These sex-differences in behaviour and the selection-pressures upon morphological traits may cause sex-differences in *C. hortensis* average trait values and in the direction and slope of trait covariances.

We measured metabolic rate and exploratory behaviour independently of one another to reduce the possibility that correlations between them are caused by immediate influences of one on the other. Such correlations could occur regardless of whether individuals consistently differ in behaviour and metabolism and hence could produce erroneous conclusions. We measured the repeatability of RMR, AMR, and exploratory behaviour in a novel environment over time across individuals, assessing the presence of intraspecific differences in metabolism and personality. We first analysed the relationships between metabolic rates, exploratory behaviour, body mass and body size with both male and female combined data to assess whether the sexes differ in their average trait values. We then measured the relationships between traits using separate male and female data to determine sex-specific metabolic trait covariances.

We hypothesise that: (1) *C. hortensis* individuals show consistent personality differences in exploratory behaviour and consistent differences in metabolic rates; (2) *C. hortensis* metabolic rate scales with body size/body mass; (3) RMR and AMR are positively ('performance hypothesis') or negatively ('allocation hypothesis') correlated with exploratory behaviour (Careau *et al.*, 2008); (4) if the relationship between metabolic rate and exploratory behaviour is positive, then average RMR and AMR may be higher in males than females; (5) if the relationship between metabolic rate and exploratory behaviour is negative, then females may have higher average RMR and AMR than males; and (6) the

relationships between metabolic rate, exploratory behaviour, body size, and body mass are stronger in males than in females.

5.3 Materials & Methods

5.3.1 Study Species, Trapping and Maintenance

Carabus hortensis (Coleoptera, Carabidae) Linnaeus, 1758 ground beetles were collected from the Lüneburger Heide, Lower Saxony, Germany (N53°10'53.32'', E9°53'08.06'') (Yarwood *et al*., 2021a; Chapter 2). In total, 62 females and 26 males were caught between August-September 2018 during the reproductive season of the beetles (Günther & Assmann, 2000), using live pitfall traps (Schuett *et al*., 2018; Yarwood *et al*., 2021a; Chapter 2). Traps were baited with cellulose soaked in red wine and were emptied/ re-baited every 7-8 days (*e.g.* Ernst & Buddle, 2013; Marcus *et al*., 2015; Schuett *et al*., 2018). Collected individuals were housed separately in 10(L) x 7.5(W) x 4.5(H) cm containers containing peat, and regularly sprayed with water to ensure a moist environment. Beetles were fed mealworm (*Tenebrio molitor*) pupae *ad libitum.* The light and temperature at which individuals were stored was reduced in increments over time, once per week, to mimic daylight and temperature changes in the natural environment, thereby promoting natural behaviours and metabolic rates. The experiment lasted from October 2018 to February 2019 during which time the conditions in which the beetles were kept changed from a 12 hour 13.8:6.6°C light-dark regime to an 8.5:15.5 hour light-dark regime at 5.8°C. Prior to making behavioural and metabolic measurements, beetles were starved for two days to ensure that they were in a post-absorptive state.

5.3.2 Behavioural Tests

All behavioural tests were conducted immediately before all metabolic measures. To measure individual exploratory behaviour, individuals were placed at the centre of an open white 37.5 (length) x 26 (width) cm plastic box with a 28 x square grid on the base (Schuett *et al*., 2018). The number of squares visited, including repeated visits to the same square, were counted during observation for 90 seconds to assess individual exploratory behaviour. Temperature was recorded once every 10 minutes throughout behavioural trials using data loggers (Voltcraft DL-210TH, Conrad Electronic SE, Hirschau, Germany) and ranged from 11.5-24.1°C (17.3±2.3°C mean±SD). Two measurements of exploratory behaviour were taken 13-15 days apart to assess whether differences among individuals were consistent over time.

5.3.3 Measuring Metabolic Rate

A L1-7000 dual channel CO_2 infra-red gas analyser (LI-COR, Lincoln, NE, USA), operating in differential mode at 2Hz with two identical chambers was used to measure individual *C. hortensis* metabolic rates (Perl & Niven, 2018). One chamber was empty acting as a reference chamber whilst the other chamber contained the beetle, allowing a differential measurement of CO_2. Chambers were 115 (length) x 30 (width) mm, with a 50ml capacity, allowing ample space for beetle movement. Air was pumped into the chambers using a SS4 Subsampler (Sable Systems International, Las Vegas, Nevada, USA) through soda lime and Drierite (W.A. Hammond Drierite, Xenia, USA) scrubbing columns, to remove CO_2 and H_2O, respectively, before it was split between two mass flow controllers (GFC17; Aalborg, New York, USA) that maintained airflow into two chambers

at 100ml min^{-1}. Temperature was recorded once every 10 minutes using Voltcraft DL-210TH data loggers (Conrad Electronic SE, Hirschau, Germany), and ranged from 14.4-23.3°C (mean = 18.1 ± 2.0°C SD). Temperatures at which metabolic measurements and behavioural measurements were taken differed because metabolic and behavioural measurements were conducted in different rooms. Individuals were allowed to move freely throughout metabolic measurements. We filmed the metabolic rate chamber with a high-speed camera (JVC GC-PX100, JVC Ltd, Yokohama, Japan) operating at 72 frames per second to classify periods when beetles were stationary and when they were moving.

RMR measures were conducted over 30 minutes between 08:00 and 16:00, immediately after assessing individual exploratory behaviour. An LED work light (Sealey WL483D 230V, Sealey Tools, Bury St Edmunds, UK) was used to replicate daylight. AMR was measured over 12 hours and took place during the night (20:00-08:00), during the *C. hortensis* active period, after assessing individual exploratory behaviour. AMR trials took place over 12 hours rather than 30 minutes due to difficulties with conducting multiple 30-minute metabolic measurements throughout the night. A red lamp was used for illumination: ground beetles are apparently undisturbed by the wave lengths of red light (*e.g.* Drees *et al*., 2008; Hasselmann, 1962). All 88 beetles were tested twice during the day (with 13-15 days between repeated trials) to assess repeatability of RMR over time; 43 of the 88 beetles (25 females and 18 males) were also tested once for their AMR and behaviour overnight, so that these 43 individuals were tested for their metabolic rate three times (with RMR measured twice and AMR measured once). The night-time AMR of the remaining 45 individuals was not measured.

5.3.4 Metabolic Rate Analysis

Videos of the beetles within chambers were analysed offline using JWatcher software (Blumstein *et al*., 2000). Measurements of RMR were made only when beetles were stationary during daytime metabolic measurements. Conversely, AMR estimates were obtained from periods when beetles were active during the night-time metabolic measurements. Estimates of RMR and AMR in CO_2µm min^{-1} production were calculated using Origin(Pro) 2016 from time periods when individuals were at rest or active, respectively. Resting metabolic rate was measured when beetles were at rest for 3 minutes or longer and was estimated from the last minute of inactivity. Resting metabolic rate was averaged across all periods of inactivity within a single 30-minute trial. Active metabolic rate was averaged across all periods of activity within a single 12-hour trial. Concentrations of CO_2µm min^{-1} for separate periods of activity and rest were converted to provide the total volume produced per hour.

C. hortensis beetles performed three different types of ventilation; continuous, discontinuous, and pulsatile (Gudowska *et al*., 2017b). Ventilation patterns produced by beetles were visually classified in Origin(Pro) 2016 (OriginLab Corporation, Northampton, MA, USA) software. Traces were classified as continuous respiration where we visually observed that CO_2 output was continuous, and troughs did not reach 0µl min^{-1}. We classified respiration patterns as discontinuous when we visually observed multiple cycles within a 30-minute time period of CO_2 µl min^{-1} decreasing sharply to and plateauing at 0µl min^{-1} for 100 seconds or longer, before sharply increasing. We classified respiration patterns as pulsatile when we visually observed rise and falls in CO_2 µl min^{-1} similar to discontinuous respiration, but in which: (a) the length of time over which CO_2 µl min^{-1} plateaued at 0 µl min^{-1} was almost equal to the time where CO_2 µl min^{-1} was above 0µl

min^{-1}; and (b) where there were obvious, individual peaks of CO_2 output. Although some studies have shown that ventilation pattern has no significant effect on metabolic rate scaling (Gudowska *et al*., 2017a), others have shown that metabolic rate can scale differently with body mass when CO_2 production values from continuous, discontinuous and pulsatile ventilation patterns are analysed together *versus* separately (*e.g.* Perl & Niven, 2018). We therefore measured the trait covariances of CO_2 production values from continuous ventilation patterns separately from those of CO_2 production values from discontinuous and pulsatile ventilation patterns. Due to the small number of instances in which beetles performed discontinuous or pulsatile respiration (15 RMR traces, 7 AMR traces), these breathing patterns were excluded from analysis.

Sample sizes available for different analyses differed. Twenty-one females and nine males were excluded from RMR analyses because they either: (a) remained active throughout both RMR trials (14 females, 9 males); (b) performed discontinuous or pulsatile respiration throughout both RMR trials (2 females); (c) remained active throughout one RMR trial and performed discontinuous or pulsatile respiration throughout the other RMR trial (3 females); or (d) remained active throughout one RMR trials and died before a second could be taken (2 females). RMR analyses were, therefore, conducted on 58 individuals (41 females, 17 males).

Nine females and two males were excluded from AMR analyses because they either: (a) remained inactive throughout the AMR trial (2 females); (b) performed discontinuous or pulsatile respiration (5 females, 2 males); or (c) died shortly afterward (2 females). AMR analyses were therefore conducted on 32 individuals (16 females, 16 males).

5.3.5 Measurements of Body Mass and Pronotum Width

We measured both body mass and pronotum width as a proxy for body size (Yarwood *et al*., 2021a) to investigate the relationships between body size/body mass and metabolic rate. Dorsal photos were taken of each individual over a laminated page of mm grid paper using a Wileyfox Swift 2x camera phone (Wileyfox, London, UK). ImageJ (Schneider *et al*., 2012) was used to measure the widest section of the pronotum to the nearest 0.1mm. To account for changes in body mass during metabolic measurements, beetles were weighed (Precisa 125A, Precisa Limited, Livingston, UK) to the nearest milligram, immediately before and afterward. These two weight measurements were then averaged to provide a measure for average body mass for the duration of the metabolic measurement.

5.3.6 Statistical Analysis

All statistical analyses were carried out in R version 3.3.2 (R Core Team, 2019).

5.3.6.1 Consistency of exploratory behaviour and metabolic rates over time

Linear mixed effects models (LMMs) were used with the rptR package (Stoffel *et al*., 2017) to estimate repeatability of RMR, AMR and exploratory behaviour for combined male and female data as well as separately for each sex. For AMR, repeatability estimates were obtained from samples 4-8 hours apart: the first from 0-2 hours from the start of metabolic testing and the second from 6-10 hours. To account for differences in the temperature at which metabolic rate and behavioural trials were conducted between repeated tests, temperature was included as a covariate in all cases, thus adjusting repeatability. Beetle identity ('ID') was included as a random term. Confidence intervals

of 95% were used to infer the significance of the repeatability of exploratory behaviour and metabolic rates; if the confidence interval included zero, the trait was considered not repeatable.

Male RMR was not repeatable over time, however, the sample size was considerably smaller than that of female RMR (Table 4.1). To assess whether a small sample size affected male RMR repeatability, we performed 1000 permutations of repeatability on subsets of female data, where the subset size equalled the total male sample size (*i.e.* 17 individuals). From these tests, we determined that female RMR was repeatable in only 43% of cases in which the sample size was 17, suggesting that low sample size may explain why male RMR was unrepeatable.

5.3.6.2 Collinearity of traits

Body size and body mass are frequently correlated. To check for collinearity of body size (pronotum width) and body mass, we performed Spearman's rank correlations on female data alone and male data alone. We reasoned that collinearity of traits was present if the R_s value was equal to or higher than 0.7.

5.3.6.3 Relationships between metabolic rate and exploratory behaviour/body mass/body size

To assess whether relationships between metabolic rate and body size/mass, and metabolic rate and exploratory behaviour exist across combined male and female data, we performed a linear mixed effects model (LMM), using RMR as the response variable. The LMM was performed on collated male and female data, and sex was included as a fixed term. The temperature at which measurements of metabolic rate were made

(hereafter: metabolic temperatures) was included as a fixed term because metabolic rates are influenced by temperature (reviewed in: Schmidt-Nielsen, 2007). Pronotum width (as a proxy for body size) and body mass were also included as fixed terms. Temperatures impact also ectotherm behaviour (reviewed in: Abram *et al*., 2017) and may influence links between metabolism and behaviour (Hämäläinen *et al*., 2020). Exploratory behaviour interacting with the temperature at which exploratory behaviour was observed (hereafter: behavioural temperature) was, therefore, included as a fixed term. Personality traits have been shown to relate to morphological traits (*e.g.* Kern *et al*., 2016). We therefore included exploratory behaviour as a fixed term interacting with body mass in our model. To account for changes in the temperature and light-dark conditions experienced by individuals over time, the week (week 1-10) in which beetles' metabolism was measured, and their identity ('ID') were included as random terms. Removal of one outlier from the dataset did not qualitatively change the results (not presented).

We performed a generalised linear mixed model (GLMM) using AMR as the response variable. We used a gamma error structure with a log link in our GLMM to account for increased AMR variability with increasing exploratory behaviour, such that the AMR data were log-transformed. Fixed and random terms for the GLMM with AMR as response were as described for the LMM, however, as beetles were tested for their AMR only once, beetle ID was not included as a random term.

The sex-specificity of the effect of exploratory behaviour, body size and body mass on both RMR and AMR was determined by performing models as described above, on separate male and female datasets, with sex removed as an explanatory variable.

Because male RMR was not repeatable, the effects of exploratory behaviour, body size and body mass on male RMR was not assessed.

5.3.6.4 Model simplification

Stepwise model simplification was performed on LMMs and GLMMs; fixed terms were removed from these models in stages and compared to the previous model using likelihood ratio tests (Crawley, 2007). At each stage, the least significant fixed term, with the smallest effect on the model's power was removed. All models were carried out using the lme4 package (Bates *et al*., 2015). Effects sizes of minimum adequate models were calculated using the MuMIn package (Barton, 2009).

5.4 Ethics

The collection of beetles utilised in this study was carried out with a permit granted by the Lower Saxon State Department for Waterway, Coastal and Nature Conservation authorities (number: H72.2220212019).

5.5 Results

Individual *C. hortensis* showed consistent differences in exploratory behaviour across 13-15 days, and in AMR across 4-8 hours, for combined male and female data (hereafter: all beetles; Table 4.1) and each sex separately. RMR was repeatable over 13-15 days across all beetles and in females but not in males (Table 4.1). Body size (pronotum width) was significantly, positively correlated with body mass in males (Spearman rank correlation; $R_S = 0.433$, $p = 0.005$, $N = 22$), but not in females ($R_S = 0.119$, $p = 0.323$, $N =$

46). Average body size, body mass and their ranges are reported separately for males and females in Table D.1.

Table 4.1. Repeatability estimates (±95% confidence intervals) from linear mixed effects models for active metabolic rate (AMR), resting metabolic rate (RMR) and exploratory behaviour. Repeatability tests were carried out on male and female combined data (M + F), female data alone (F) and male data alone (M), and were adjusted with ambient temperature (°C). The mean temperature (Mean Temp) ± one standard deviation (1SD) at which behavioural and metabolic tests were measured is given. Bold values denote significance. N_{ID}, number of individuals; N_{Obs}, number of observations.

Response Variable	Dataset	Mean Temp ± 1SD	Repeatability	95% CI	N_{ID} (N_{Obs})
AMR	M + F	21.5 ± 0.8	**0.644**	**0.332 – 0.856**	32 (50)
	F	21.5 ± 0.9	**0.696**	**0.324 – 0.902**	16 (29)
	M	21.5 ± 0.7	**0.698**	**0.017 – 0.960**	16 (21)
RMR	M + F	17.5 ± 1.7	**0.419**	**0.043 – 0.709**	58 (80)
	F	17.6 ± 1.9	**0.524**	**0.055 – 0.830**	41 (55)
	M	17.4 ± 1.2	0.111	0.000 – 0.784	17 (25)
Exploration	M + F	17.3 ± 2.3	**0.367**	**0.169 – 0.544**	88 (171)
	F	17.4 ± 2.4	**0.247**	**0.009 – 0.478**	62 (119)
	M	17.1 ± 2.1	**0.484**	**0.123 – 0.738**	26 (52)

More exploratory individuals had lower RMR for all beetles (Table D.2), but there was no significant relationship between female RMR and exploratory behaviour (Table D.2). AMR was significantly related to exploratory behaviour for all beetles (Table 4.2), and for females alone (Table 4.2, Figure 4.1A). In both cases, the relationship between AMR and exploratory behaviour depended upon behavioural temperature. Male AMR was also significantly related to exploratory behaviour, however, this relationship depended upon body mass (Table 4.2, Figure 4.1B).

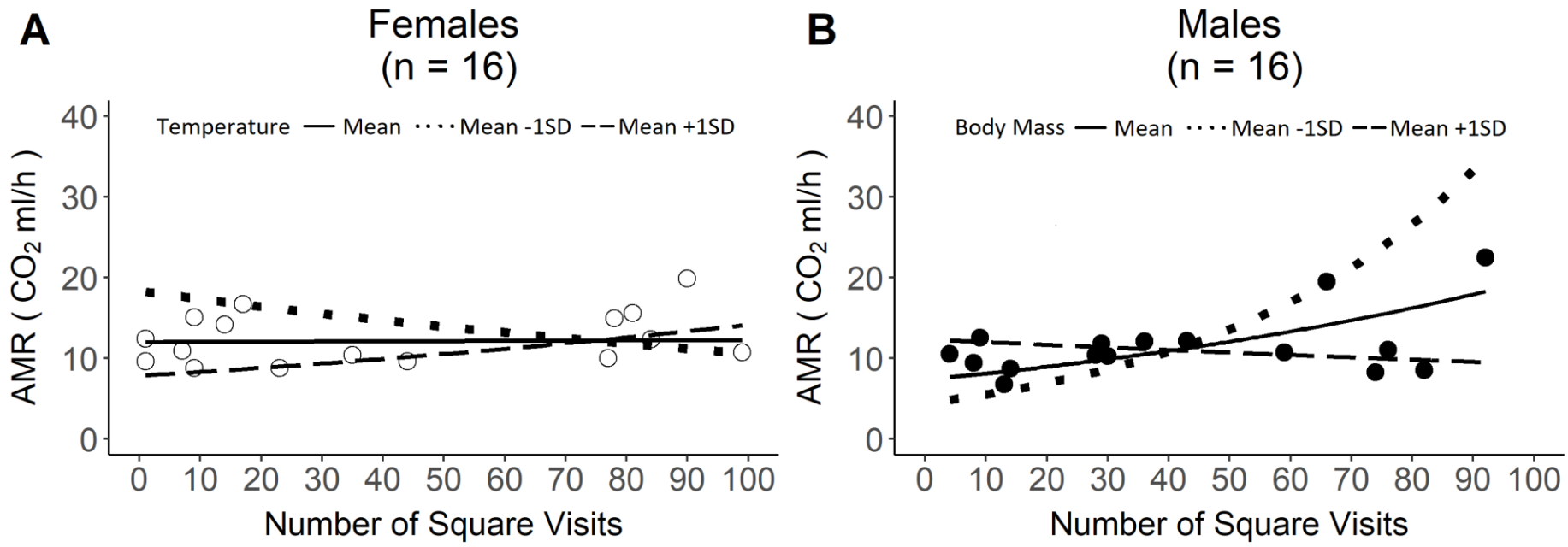

Figure 4.1: Sex-specific relationships between active metabolic rate (AMR) and exploratory behaviour. (A) The relationship between AMR and exploratory behaviour at the mean temperature, mean +1 SD temperature, and mean −1 SD temperature in females (n = 16). (B) The relationship between AMR and exploratory behaviour at the mean body mass, mean +1 SD body mass, and mean −1 SD body mass, in males (n = 16). Lines represent the predicted relationships from model outputs, back-transformed from a model with a log-link function.

AMR was unrelated to body size or mass for all beetles (Table 4.2). However, AMR did scale with both body mass and size in both males and females separately but did so differently between the sexes. Females with larger body sizes had significantly lower AMR (Table 4.2, Figure 4.2A), whereas males with larger body sizes had significantly higher AMR (Table 4.2, Figure 4.2B). Both male and female AMR increased with body mass: heavier females had significantly higher AMR (Table 4.2, Figure 4.2C), as did heavier males with average exploratory behaviour (Table 4.2, Figure 4.2D). However, per gram increase in body mass, the AMR of males that performed average exploratory behaviour (Table 4.2, Figure 4.2D) increased more than female AMR (Table 4.2, Figure 4.2C).

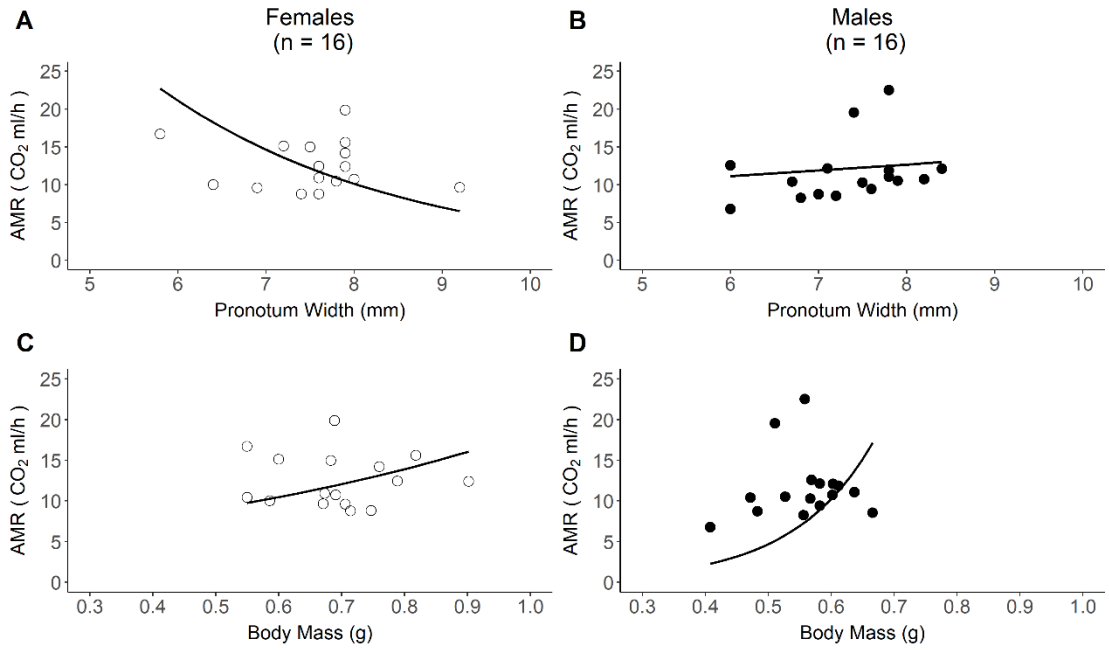

Figure 4.2: Sex-specific relationships between active metabolic rate (AMR) and both body size and body mass. (A) The relationship between AMR and body size (measured as pronotum width) in females (n = 16). (B) The relationship between AMR and body size in males (n = 16). (C) The relationship between AMR and body mass in females (n = 16). (D) The relationship between AMR and body mass at average exploratory

behaviour in males (n = 16). Lines represent the predicted relationships from model outputs, back-transformed from a model with a log-link function.

Table 4.2. GLMMs for active metabolic rate (AMR) (CO2 ml/h) for males and female combined data (M + F), female data alone (F) and male data alone (M). Data were log-transformed during analysis with the use of a log-link function. Coefficients (Coeff.) shown are not backtransformed. Behavioural temperature, B_{Temp}; exploratory behaviour (number of square visits in a novel environment); metabolic temperature, M_{Temp}; number of individuals, N; variance of random terms, Var. Coefficients (Coeff.) in square brackets belong to non-significant terms just before dropping those terms from the model. Bold p values denote significant terms.

Dataset	Random Term	Var.	Fixed Term	Coeff.	χ^2	DF	p-value
M + F	Week	0.008	Intercept	3.05			
N = 32	Residual	0.054	B_{Temp}: Exploratory Behaviour	<0.01	6.57	1	**0.010**
			Body Mass: Exploratory behaviour	[-0.01]	1.40	1	0.237
			Body Mass	[-0.28]	0.24	1	0.623
			Exploratory Behaviour	-0.03			
			Pronotum Width	[0.04]	0.22	1	0.641
			M_{Temp}	[0.08]	1.50	1	0.221
			B_{Temp}	-0.03			
			Sex	[-0.12]	1.73	1	0.189
F	Week	0.072	Intercept	2.67			
N = 16	Residual	0.018	B_{Temp}: Exploratory Behaviour	<0.01	11.77	1	**<0.001**

			Body Mass: Exploratory Behaviour	[-0.07]	2.01	1	0.156
			Body Mass	1.42	4.74	1	**0.030**
			Exploratory Behaviour	-0.04			
			Pronotum Width	-0.37	10.71	1	**0.001**
			M_{Temp}	0.21	6.52	1	**0.011**
			B_{Temp}	-0.15			
M	Week	0.164	Intercept	4.77			
N = 16	Residual	0.005	B_{Temp}: Exploratory Behaviour	[<0.01]	1.40	1	0.237
			Body Mass: Exploratory Behaviour	-0.19	41.93	1	**<0.001**
			Body Mass	7.84			
			Exploratory Behaviour	0.12			
			Pronotum Width	0.06	9.06	1	**0.003**
			M_{Temp}	-0.24	18.81	1	**<0.001**
			B_{Temp}	-0.12	15.10	1	**<0.001**

5.6 Discussion

The relationships between metabolic rate and personality traits (*e.g.* Biro *et al*., 2020; Cornwell *et al*., 2020), and between metabolic rate and morphology (*e.g.* Baktoft *et al*., 2016; Bergstrom *et al*., 2019), have been studied in different taxa, but rarely in insects (but see: Krams *et al*., 2017; Royauté *et al*., 2015), or on a sex-specific basis (Hämäläinen *et al*., 2018), despite: (a) differences in physiology between insects and more commonly studied vertebrates (Schmidt-Nielsen, 2007); and (b) differences between males and females that might influence trait covariances (Hämäläinen *et al*., 2018). Consequently, our study is among the first to investigate sex-specific metabolic trait covariances with both a personality trait and morphology, in insects. *C. hortensis* AMR was related to body size/mass, though these relationships differed between sexes in terms of directionality for body size. Moreover, the relationship between AMR and exploratory behaviour depended upon behavioural temperature in females, but on body mass in males. Against our prediction, male RMR was not repeatable and hence its relationship with exploration or morphology not assessed. Though repeatable, female RMR was unrelated to exploratory behaviour or body size/mass. Conversely, exploratory behaviour and AMR were repeatable in both sexes. Given the lack of female RMR trait covariances, and the lack of male RMR repeatability, we focus our discussion on AMR trait covariances.

The majority of studies of the relationships between metabolic rate, personality traits, body mass and size combine data from males and females (*e.g.* Timonin *et al*., 2011) or analyse metabolic trait covariances in one sex alone (*e.g.* Royauté *et al*., 2015; Wells & Taigen, 1989; White *et al*., 2016). By comparing metabolic trait covariances in all beetles with that of males and females alone, we show that these relationships differed

and that combining data for both sexes can lead to erroneous conclusions. This may help to explain why several studies across different taxa fail to find relationships between metabolic rate and personality traits/body mass (*e.g.* McDevitt & Speakman, 1996; Timonin *et al.*, 2011; Wells & Taigen, 1989). For example, the AMR-exploration relationship was temperature-dependent for all beetles but was body mass-dependent for males alone. Furthermore, AMR was unrelated to body mass when analysing all beetles but was significantly related to body mass when the sexes were considered separately. Such differences between sexes may arise from differences in reproductive strategies and investment as a consequence of anisogamy (Bateman, 1948; Hämäläinen *et al*., 2018; Maynard Smith, 1978).

In line with our predictions, differences in the relationship between AMR and exploratory behaviour occurred between sexes. The male AMR-exploration relationship was influenced by body mass, suggesting males of different weights have different proportions of metabolically active tissues. Conversely, the female AMR-exploration relationship was temperature-dependent. Such differences in the AMR-exploration relationship may arise from sex differences in the ecological significance of movement (*e.g.* Chapter 4). Males of other *Carabus* species are thought to search for females with whom to mate (*e.g.* Drees & Huk, 2000), meaning that exploration or activity may influence the reproductive success of males more than that of females. Male *C. hortensis* exploratory behaviour may therefore remain relatively stable across the context of temperature in comparison to female exploratory behaviour.

Although some studies have previously shown sex-specific relationships between AMR and behaviours (*e.g.* Methling *et al*., 2020; Moschilla *et al*., 2019; Niitepõld *et al*., 2011), these behaviours were not tested repeatedly. Thus, to our knowledge, ours is the

first to investigate the relationship between AMR and repeatable behaviour or personality traits on a sex-specific basis. As predicted, our results demonstrate that sex can be an important factor in the relationships between (active) metabolic rate and personality traits. We hypothesise that the extent to which sexes diverge in their metabolic rate-personality trait relationships depends on the strength of difference between male and female reproductive success or survival associated with the personality trait; the greater the difference in the association between a personality trait and fitness between the sexes, the greater potentially the divergence in the metabolic rate-personality trait relationships between males and females.

The relationships between *C. hortensis* AMR and both body size and mass differed between males and females. Males that had larger body sizes had greater AMR, whilst larger bodied females had lower AMR. Both male and female AMR increased with body mass, such that heavier individuals had higher AMR, yet the relationship was stronger in males than in females. Our findings are in line with both: (1) our prediction that relationships between metabolic rate and body mass should be stronger in males in than in females; and (2) findings of the only comparable insect-based study, in which the relationship between AMR and body mass was stronger in male eucalyptus-boring beetles (*Phoracantha semipunctata*) than in females (Rogowitz & Chappell, 2000). In contrast, other studies on vertebrates have found no significant difference in the AMR scaling relationships between males and females (*e.g.* Gifford *et al*., 2013; Peterson *et al*., 1998).

Sex differences in the relationships between AMR and body mass/size may be explained by sex differences in the proportions and benefits of metabolically active tissues. Evidence across taxa (*e.g.* Streicher *et al*., 2012; reviewed in: Glazier, 2005)

indicates that mass dependence of metabolic rates changes with body composition. Males of other *Carabus* species seem to actively search for females with whom to mate (*e.g.* Drees & Huk, 2000), which is likely an adaptation to increase mate searching capacity. If male *C. hortensis* are more active than females as we hypothesise (but see Chapter 2), then males may invest heavily in musculature (*i.e.* metabolically costly tissue) to sustain increased bouts of movement and to increase chances of locating a potential mate. In contrast, female *C. hortensis* are more likely to store energy as lipids (*i.e.* metabolically less-costly tissues) to fuel egg production (Turin *et al*., 2003). Female *C. hortensis* remain relatively inactive until hungry (Szyszko *et al*., 2004), which may be an adaptation to retaining energy resources that should be allocated towards egg production. Heavier females, but not males, may store proportionally more lipids than lighter individuals, thus potentially explaining sex differences in AMR scaling. Our arguments would benefit from further investigation of sex-differences in body composition, as direct measures of body composition were not obtained in this study. Sex differences in AMR scaling could have also been explained by intraspecific variation in the proportions of eggs carried by heavier *versus* lighter females, however, it is highly unlikely that females in our study were carrying any eggs as our measures of body mass were recorded outside of the reproductive season. The negative relationship observed between female AMR and body size may be caused by a trade-off between the two: large bodies incur high metabolic costs, but may not be beneficial to female *C. hortensis* fecundity because in gravid carabids, the abdomen often becomes distended to accommodate large numbers of eggs (Goulet, 1976). Fecundity itself is thought to increase with metabolic rate in animals in general (Réale *et al*., 2010).

We sought to explore the sex-specific relationships between metabolic rate (RMR and AMR), body size, body mass and exploratory behaviour in *Carabus hortensis* ground beetles. We found that males and females had different AMR trait associations: males with larger body sizes had higher AMR, while the opposite was true of females. Moreover, while the relationship between male AMR and exploratory behaviour was body mass-dependent, the relationship between female AMR and exploratory behaviour was temperature-dependent. Our results are suggestive of sexually antagonistic selection, meaning that individuals may be unable to reach their optimum trait expression and trait correlations and may suffer reduced fitness as a result. This may be especially true in cases where the direction of trait covariances differ between the sexes (Hämäläinen *et al*., 2018). Our results emphasise that sex plays an important role in intraspecific AMR trait covariances, and may help to explain why studies across many taxa fail to find relationships between metabolic rate and personality traits or body mass/size (McDevitt & Speakman, 1996; Wells & Taigen, 1989). Future studies of the relationships between metabolic rate, personality traits and body mass/size should therefore be careful to analyse data from males and females separately.

Chapter 6: The thermal dependency of metabolic scaling in an insect

6.1 Abstract

The allometric scaling of metabolic rate has been intensively studied both within and across species. Yet, there are considerable differences among studies in the scaling exponents obtained. Species' metabolic allometric scaling exponents may be influenced by multiple factors, including intraindividual variation in body mass, inconsistencies in the phenotypic flexibility of metabolic rate under different temperatures, and the thermal dependency of metabolic rate scaling. Whether or not each of these factors influence allometric scaling of metabolic rate has yet to be investigated in insects. We used reaction norm approaches in *Carabus hortensis* ground beetles to assess the thermal dependency in the allometric scaling exponents of resting metabolic rate and active metabolic rate, and the extent to which they are influenced by intraindividual variation in body mass. We found that phenotypic flexibility of resting and active metabolic rates to temperature was repeatable over time, and that scaling of resting and active metabolic rate was independent of temperature and not influenced by intraindividual variation in body mass. Our results suggest that consistent phenotypic flexibility of metabolic responses to temperature fosters the temperature-independence of metabolic rate scaling with body mass.

6.2 Introduction

Metabolic rate (MR) is an important element of animal life-history, controlling the conversion of resources from the environment (McNiell Alexander, 1999) into energy for growth, reproduction, and behaviour (Schmidt-Nielsen, 1984). In so doing, MR may influence life-history evolution (Kozłowski *et al*., 2004) and the densities of populations (White & Seymour, 2004). Metabolic rate is, however, affected by a range of factors, one of the most influential being body mass. Given the impact that MR can have on species ecology and evolution, understanding how body mass influences MR is of great importance.

Metabolic rate scales with body mass (M) such that $MR = aM^b$, where '*a*' is the coefficient, and '*b*' is the allometric scaling exponent (Kleiber, 1932). The relationship between MR and body mass has been studied intensively since the seminal work of Kleiber, (1932). Yet, the precise value of *b* remains the subject of debate. Although both Kleiber, (1932) and the metabolic theory of ecology (Brown *et al*., 2004; West *et al*., 1997) posit that the standard MR of organisms should scale both inter- and intraspecifically to the power of 0.75, there is considerable and growing evidence that interspecific (Clarke *et al*., 2010; Isaac & Carbone, 2010; reviewed in: Glazier, 2005) and intraspecific (Bokma, 2004; Clarke & Johnston, 1999; reviewed in: Glazier, 2005) MR allometric-scaling exponents vary. Maximum aerobic MR is also expected to scale with body mass, and, although it is understudied in comparison to the allometric scaling of standard MR, evidence suggests that the power to which maximum aerobic MR scales with body mass varies among species too (*e.g.* Glazier, 2005, 2009; Norin & Gamperl, 2018; Weibel *et al*.,

2004; Weibel & Hoppeler, 2005). The causes or drivers of variation in MR allometric-scaling exponents among species, are, however, not fully understood.

Differences in the allometric scaling exponents among species may be driven by intraindividual variation in body mass, which produces intraindividual variation in the allometric scaling of MR. The additive effects of intraindividual variation may affect allometric scaling relationships at the species level, thereby contributing to heterogeneity in allometric scaling exponents among species (Kar *et al.*, 2021) (Figure 5.1). Intraindividual variation in body mass therefore cannot be ignored when estimating species' or population allometric scaling exponents.

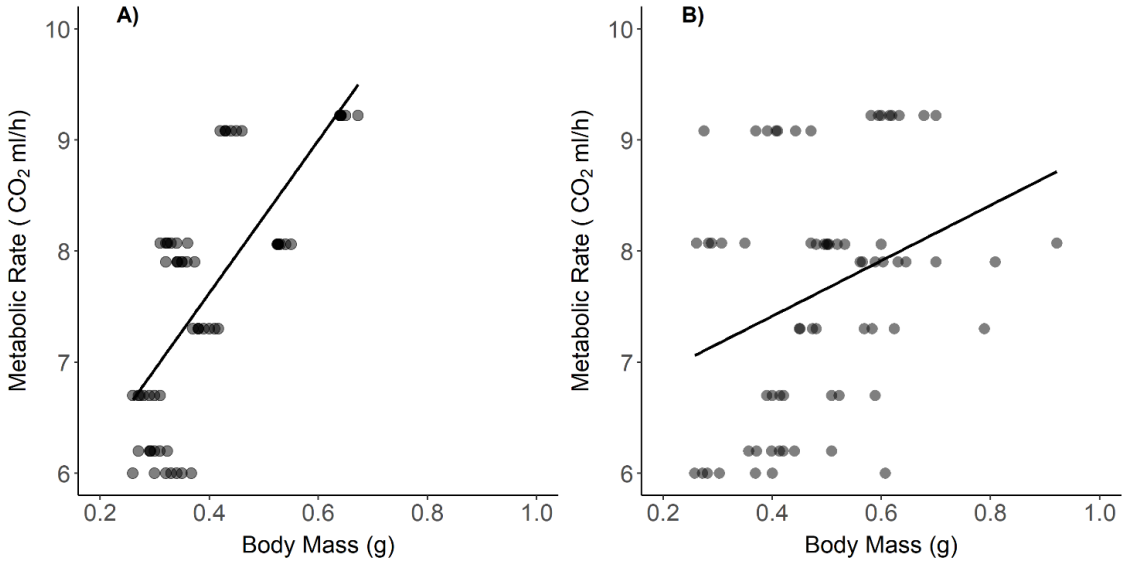

Figure 5.1. An example scenario in which large intraindividual variation in body mass influences the allometric scaling of metabolic rate with body mass at the population level. The relationships between metabolic rate and A) body mass with low intraindividual variation and B) body mass with large interindividual variation.

In addition to intraindividual variation in body mass, differences in the allometric scaling exponents among species may be explained if allometric scaling exponents are

temperature dependent. The metabolic theory of ecology postulates that the allometric scaling of MR is independent of temperature (Brown *et al*., 2004; West *et al*., 1997). However, evidence shows that intraspecific allometric scaling exponents can and do in fact fluctuate under different temperatures (*e.g.* Killen *et al*., 2010; Lindmark *et al*., 2018; Ohlberger *et al*., 2012; reviewed in: Glazier, 2010), at least in some species. This has important implications for understanding how temperature influences individual fitness, population dynamics, and how populations and species may respond to changing global temperatures.

Differences in allometric scaling exponents among species may also be caused if intraspecific differences in the reversible plasticity of, or phenotypic flexibility in, metabolic rate (*i.e.* individual differences in the range of potential metabolic responses to temperature) lack consistency (Clarke, 2004). For simplicity, we follow the nomenclature set out by others investigating the thermal dependency of metabolic rate scaling *e.g.* Kar *et al*. (2021) and henceforth refer to phenotypic flexibility of an individual's metabolic rate to temperature as 'metabolic thermal plasticity'. Lack of consistency in metabolic thermal plasticity may increase the level of variation in MR across temperatures, which may alter the thermal dependency of allometric scaling exponents at the population or species-level. Alternatively, consistent individual differences in metabolic thermal plasticity may 'cancel out' temperature dependency of MR scaling at the population or species-level, if the direction of the relationship between MR and temperature differs between individuals. Incorporating intraindividual variation in body mass, the consistency of intraspecific variation metabolic thermal plasticity, and the thermal dependency of species' allometric scaling exponents, into analysis will be required to obtain accurate species-specific or population-specific allometric scaling

exponents. However, we know of only one instance in which all such factors were incorporated into the calculation of allometric scaling exponents: research by Kar *et al*. (2021).

Here we follow the approach taken by Kar *et al*. (2021) to investigate the thermal dependency of MR scaling at the population-level, extending analysis to resting metabolic rate (RMR) and active metabolic rate (AMR; our estimate of maximal aerobic metabolic rate) in an invertebrate system. We repeatedly measured RMR and AMR in male and female *Carabus hortensis* ground beetles across a range of temperatures to assess whether metabolic thermal plasticity differed consistently among individuals, and used reaction norm approaches to assess whether population-level allometric scaling exponents are thermally dependent, and whether intraindividual variation in body mass influences the thermal dependency and values of allometric scaling exponents at the population-level.

6.3 Methods

6.3.1 Study Species, Trapping and Maintenance

Carabus hortensis (Coleoptera, Carabidae) Linnaeus, 1758 ground beetles were collected in the Lüneburger Heide, Lower Saxony, Germany, at the most westerly edge of the species' range (Völler *et al*., 2018), from August – October 2019. The active season of the species runs from July to October, and individuals are night-active (Turin *et al*., 2003). In total, 100 beetles (52 females, 48 males) were collected using live pitfall traps (Schuett

et al., 2018; Yarwood *et al*., 2021a; Chapter 2), which were emptied and re-baited every 7-8 days.

Collected beetles were housed individually in 10(L) x 7.5(W) x 4.5(H) cm containers filled with peat. Containers were regularly sprayed with water to ensure a moist environment and beetles were maintained on an *ad libitum* diet of *Tenebrio molitor* pupa. Individuals were kept in the laboratory throughout September and October 2019, where MR and body mass were measured. To ensure that beetles remained in their most active phase for the remainder of the study (Günther & Assmann, 2000), individuals were maintained at average autumn temperatures obtained from the Schneverdingen weather station (Weather Underground, n.d.) (at 12°C during the day and at 6°C during the night), and daylight hours (under a 10h : 15h light : dark regime) for the Lüneburger Heide region. So that metabolic trials could be conducted when *C. hortensis* are most active (*i.e.* at night), the day-night cycles of the study subjects were reversed. Each week from the 14th October 2019 until 23rd December 2019, daylight hours were gradually shifted by 62 minutes, so that individuals experienced fully reversed day-night cycles for 1.5 weeks prior to the onset of metabolic trials. This gradual shift in timing of daylight hours was implemented to limit disruption to individual physiology.

Resting metabolic rate can only be accurately recorded during the post-absorptive state, where no energy expenditure is allocated to digestion (Wang *et al*., 2001). Before the onset of metabolic trials, therefore, beetles were starved for 24-48 hours: this was in line with methodology utilised in other RMR studies (Gudowska *et al*., 2017; Yarwood *et al*., 2021b; Chapter 5).

6.3.2 Temperature Treatments

In order to assess the effects of temperature on MR scaling, individual MR was recorded in a climate chamber (Weiss WK2T Climate Chamber WeisScientific Ltd., Timrat, Israel) at a series of four different temperatures (3°C, 10°C, 17°C and 23°C) within the natural temperature range that the beetles experience, twice per temperature and individual. Temperature treatments were informed by minimum (4.5°C), maximum (22.0°C) and intermediate (10°C, 17°C) temperatures recorded during the 8 hours following sunset (*i.e.* during the daily active period of the beetles) (Figure 2.3A, Figure B.2) from September - October 2019, at the trapping site of study animals. Individuals were held under each temperature treatment for at least 30 minutes prior to MR tests.

6.3.3 Metabolism tests, metabolic rate analysis and body mass measures

The RMR and AMR of individual *C. hortensis* beetles were measured for 15 minutes using a flow-through respirometer at each of the four temperatures (3°C, 10°C, 17°C and 23°C). Individuals were tested under one temperature treatment, and after 4-5 days were tested under another temperature treatment, until they had been tested under all temperature treatments. Individuals experienced temperature treatments in one of four different orders: (1) 3°C, 10°C, 17°C, 23°C; (2) 10°C, 17°C, 23°C, 3°C; (3) 17°C, 23°C, 3°C, 10°C; or (4) 23°C, 3°C, 10°C, 17°C. To assess repeatability of results over the four temperatures, the temperature treatment cycle was repeated such that individual MR was measured at each temperature twice, 18-19 days apart. Trials were conducted between 08:00 – 17:00 and under red light so that trials could be carried out with minimal disturbance to beetle activity (Drees *et al*., 2008; Hasselmann, 1962).

Methods used to capture MR measurements were identical to those described in Yarwood *et al*. (2021b) (Chapter 5). In short, air was pumped through sodalime and Drierite (W.A. Hammond Drierite, Xenia, USA) scrubbing columns before being split between two identical 7ml chambers, one of which contained one *C. hortensis* individual whilst the other remained empty. A L1-7000 dual channel CO_2 infra-red gas analyser (LI-COR, Lincoln, NE, USA), sampled the air in the two respective chambers at a sample rate of 2 Hz. LI-COR software was then used to record differences in the CO_2 between chambers, thereby calculating the volume of CO_2 excreted from the beetle. A camera was fixed above the test chamber to record *C. hortensis* activity during metabolic trials. Following methods outlined in Yarwood *et al*. (2021b) (Chapter 5), MR traces were then visually classified into continuous, discontinuous and pulsatile respiration types, and video recordings were analysed to separate metabolic traces into periods of activity and rest, allowing accurate calculation of RMR and AMR from periods in which individuals were respectively at rest or active. Both RMR and AMR were estimated only from continuous respiration patterns, and only from instances where individuals remained stationary or active, respectively, for 3 minutes or longer. Both RMR and AMR were estimated from the 3rd minute of inactivity or activity onwards for the duration of the period at rest/activity. Where individuals had multiple bouts of inactivity or activity that lasted 3 minutes or longer within the same metabolic trial, we calculated RMR or AMR from the average volume of CO_2 excreted from the beetle across those bouts of inactivity or activity.

The body mass of *C. hortensis* beetles was measured (Precisa 125A, Precisa Limited, Livingston, UK) to the nearest milligram immediately before metabolic trials so that we obtained eight measurements of body mass per individual. We quantified

individual average body mass as the mean of these eight body mass measures. Eight measures of intraindividual variation in body mass were obtained by subtracting each measurement of body mass from the average body mass value.

6.3.4 Statistical analyses

All statistical analyses were carried out in R version 3.3.2 (R Core Team, 2019) and were conducted on male and female data separately. We calculated temperature-specific RMR and AMR allometric scaling exponents at the population-level as the slopes of linear models with log-transformed average RMR or AMR at the given temperature as the response variable, and log-transformed average body mass at the given temperature as the explanatory variable. We chose to calculate allometric scaling exponents from logged data for comparison with other studies. Allometric scaling exponents were compared with the value of 0.75 (*i.e.* the power to which RMR was proposed to scale with body mass by Kleiber (1932) and Brown *et al*. (2004)), using the smatr package in R (Warton *et al*., 2012). Confidence intervals of 95% were used to infer whether scaling exponents were significantly different from 0.75; we considered that the scaling exponent was not significantly different from 0.75 where the confidence interval included 0.75.

Repeatability of RMR over time was estimated using a multivariate response model, using the brms package (Bürkner, 2017), and following steps in Kar *et al*. (2021). The multivariate response model was composed of four different linear mixed effects models (LMMs), with RMR measured at 3°C, 10°C, 17°C and 23°C as the response variables, body mass measured at the corresponding temperature as covariate, and beetle ID as a random term. Repeatability of AMR was estimated following steps outlined

above. Repeatability of RMR and AMR thermal plasticity (*i.e.* repeatability of the slope of RMR/AMR *versus* temperature) over time was estimated by fitting generalised linear mixed effects models (GLMMs) using the MCMCglmm package (Hadfield, 2010), with either RMR or AMR as the response variable and with temperature as a covariate. Beetle ID and the test series for that particular beetle ID (*i.e.* whether the RMR/AMR measurement was a repeated measure) were included as random terms allowing for random intercepts. Temperature was included as a random term allowing for random slopes twice (*e.g.* Kar *et al*., 2021). Repeatability of metabolic thermal plasticity was then estimated using equations provided in the supplemental information of Kar *et al*. (2021). Confidence intervals of 95% were used to infer the significance of all repeatability estimates; we considered traits to be non-repeatable where the confidence interval included zero.

Reaction norms were used to assess the impact of temperature on RMR and AMR scaling whilst allowing for intraindividual variation in body mass, following steps in Kar *et al*. (2021). For each response variable (*i.e.* RMR and AMR) and sex, we fitted and compared two general linear mixed models (GLMMs; hereafter: pairs of GLMMs). Each pair of GLMMs contained a 'mixed effects model' and an 'interaction model', which had different fixed terms but contained the same random terms: intraindividual variation in body mass, allowing for random slopes, and ID, allowing for random intercepts. The mixed effects model contained average body mass, intraindividual variation in body mass and temperature as fixed effects whilst the interaction model contained two interactions: one between average body mass and temperature and another between intraindividual variation in body mass and temperature.

To determine whether intraindividual variance in body mass impacted on the thermal dependency of MR scaling, we fitted two further pairs of GLMMs for each sex and response variable: a 'random slopes and intercepts mass model' and a 'random intercepts mass model'. Here, pairs of GLMMs both contained the fixed effects structure of the 'interaction model' described above - but had different random terms. The random slopes and intercepts mass model included intraindividual variation in body mass as a random term allowing for random slopes and ID as a random term allowing for random intercepts, whilst the random intercepts mass model included ID as a random term allowing for random intercepts alone. To assess whether intraindividual variation in body mass impacted the population-level allometric scaling exponent its-self, we fitted a further pair of GLMMs for each sex and response variable, each with the fixed effects structure of the 'mixed effects model'. One GLMM contained the random terms structure of the random slopes and intercepts mass model whilst the other contained the random terms structure of the random intercepts mass model.

Watanabe-Akaike information criterion (WAIC) and leave-one-out cross validation (LOO) values were used to compare all pairs of GLMMs for best fit: the model with the smallest WAIC and LOO values, regardless of being positive or negative, was deemed as the model with the best fit. Because the question of how much WAIC (Whalen & Hoppitt, 2016) and LOO values should differ in order to select one model over the other remains open, we based our model selection criteria on those conservatively used for model selection using DIC values (MRC Biostatistics Unit, n.d.; Spiegelhalter *et al*., 2002). Taking a conservative approach, we considered that WAIC and LOO values should differ by at least ten in order to select the interaction model/random slopes and intercepts model over the mixed effects model/random intercepts model.

6.4 Ethics

This study was carried out under permits from the Heidekreis (permit number: 2019-0168) and Harburg (permit number: 2019-0218-Kr) nature conservation *authorities and* the Lower Saxon State Department for Waterway, Coastal and Nature Conservation authorities (permit number: H72.22202/2019-Drees).

6.5 Results

Body mass ranged from 0.31 – 0.93g (mean ± SD: 0.63 ± 0.11g) in females and from 0.27 – 0.81g (mean ± SD: 0.56 ± 0.10g) in males. Intraindividual variation in body mass ranged from -0.28 – 0.25g (mean ± SD: <-0.01 ± 0.09g) in females and from -0.23 – 0.19g (mean ± SD: <-0.01 ± 0.08g) in males. The range and mean ± SD for male and female RMR and AMR at each of 3°C, 10°C, 17°C and 23°C can be found in Table 5.1. The allometric-scaling exponents for female RMR, male RMR, female AMR, and male AMR can be found in Table 5.2. Allometric scaling exponents did not significantly differ from 0.75 for female or male RMR or AMR (Table 5.2).

Table 5.1. Summary statistics for female (F) and male (M) resting metabolic rate (RMR; CO_2 ml/h) and active metabolic rate (AMR; CO_2 ml/h) at each temperature (Temp; °C).

Sex	Temperature	RMR		AMR	
		Mean ± SD	Range	Mean ± SD	Range
F	3	3.96 ± 1.53	0.67 – 7.75	6.62 ± 1.42	3.78 – 9.16
	10	6.48 ± 2.31	2.24 – 12.84	11.18 ± 2.76	5.45 – 19.28
	17	8.14 ± 2.68	3.80 – 15.75	16.45 ± 4.29	5.41 – 25.14
	23	11.64 ± 3.98	4.92 – 25.46	22.08 ± 7.08	9.94 – 38.10
M	3	3.36 ± 1.71	0.49 – 8.31	6.17 ± 1.55	3.67 – 10.33
	10	6.71 ± 2.59	2.12 – 11.47	12.25 ± 4.81	2.92 – 26.96
	17	7.79 ± 3.28	2.84 – 15.77	15.45 ± 6.93	4.09 – 39.01
	23	11.36 ± 4.39	0.50 – 19.11	21.89 ± 6.99	11.06 – 42.13

Table 5.2. Temperature-specific population-level allometric scaling exponents ± 95% confidence intervals (95% CI) for female (F) and male (M) resting metabolic rate (RMR) and active metabolic rate (AMR) in comparison to an allometric scaling exponent of 0.75. Allometric scaling exponents are given. Bold CI denote that the allometric scaling exponent obtained from logged data is significantly different from 0.75. N_{ID}, number of individuals.

Metabolic Rate	Sex	Temperature	Scaling Exponent	95% CI	N_{ID}
RMR	F	3	1.189	-0.732 - 3.111	29
		10	0.945	-0.604 - 2.495	26
		17	0.428	-0.974 - 1.830	38
		23	0.031	-0.924 - 0.985	34
RMR	M	3	0.941	-0.332 - 2.214	20
		10	0.836	-1.061 - 2.733	26
		17	0.275	-1.138 - 1.687	33
		23	1.185	-14.586 - 18.665	26
AMR	F	3	0.759	0.304 - 1.213	33
		10	0.130	-0.564 - 0.825	41
		17	0.175	-1.064 - 1.414	27
		23	0.820	-0.111 - 1.752	25
AMR	M	3	0.309	-0.201 - 0.818	42
		10	0.030	-1.280 - 1.340	38
		17	0.511	-0.686 - 1.708	27
		23	0.434	-0.477 - 1.345	33

RMR and AMR were repeatable at each temperature in both sexes (Table 5.3). Thermal plasticity of RMR and AMR was consistent over time in both males and females (Table 5.4). Comparison of WAIC and LOO values revealed that there was no significant difference between interaction and mixed effects models. Temperature therefore had no

significant influence on the scaling of RMR (Table 5.5, Figure 5.2A, Figure 5.2B) or AMR (Table 5.5, Figure 5.2C, Figure 5.2D) with body mass at the population-level in either males or females. Comparison of WAIC and LOO values revealed that accounting for intraindividual variation in body mass did not influence the allometric scaling of RMR (Table 5.6, Figure 5.3) or AMR (Table 5.6, Figure 5.3), or the thermal dependency of MR scaling at the population-level (Table 5.7, Figure 5.3).

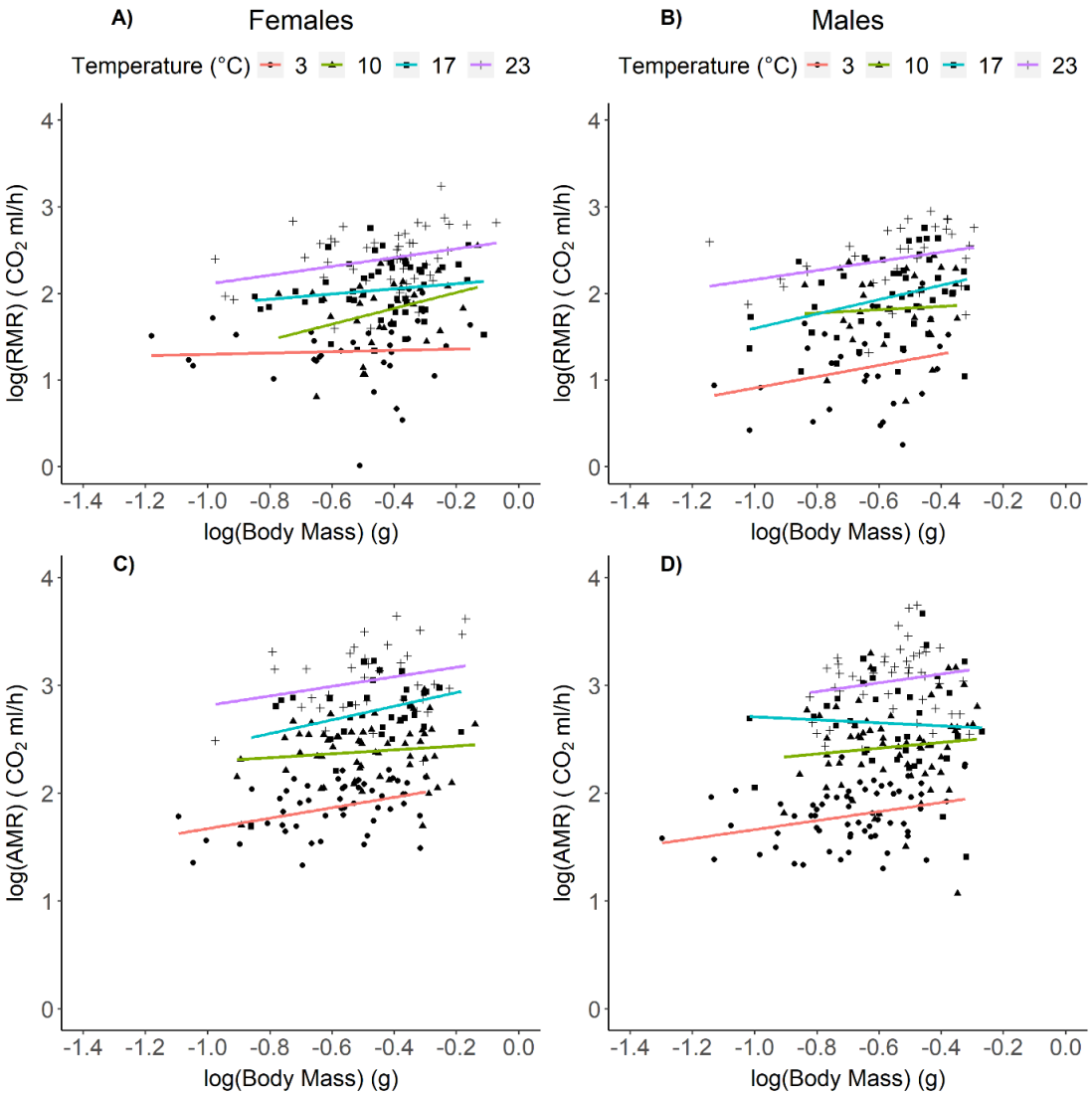

Figure 5.2. Temperature does not influence the allometric scaling of MR at the population-level. The relationship between in A) female RMR B) male RMR C) female

AMR and D) male AMR and body mass at a series of different temperatures. Circles, triangles, squares and crosses represent data points at 3°C, 10°C, 17°C and 23°C, respectively. Pink, green, blue and purple lines represent relationships between RMR/AMR and body mass at each of 3°C, 10°C, 17°C and 23°C, respectively. Two datapoints are provided per individual and temperature for each of the RMR-body mass and AMR-body mass relationships presented. Predicted lines are fitted using outputs from LMM estimates.

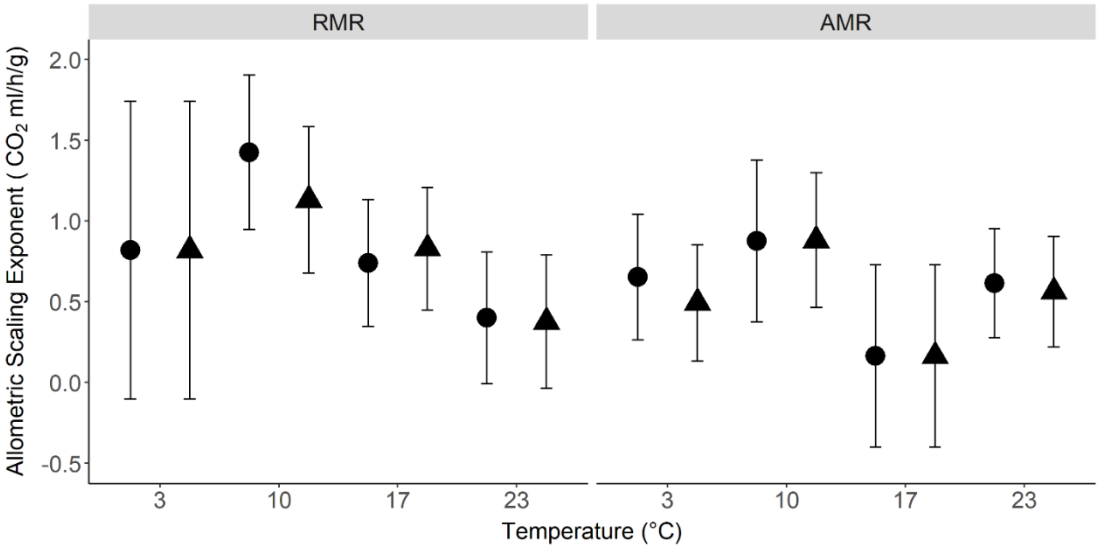

Figure 5.3. Intraindividual variation in body mass does not influence *C. hortensis* RMR and AMR allometric scaling exponents. Triangles denote population-level RMR and AMR allometric scaling exponents that were calculated from LMMs including both intraindividual variation in body mass as a random term allowing for random intercepts, and individual as a random term allowing for random intercepts. Circles denote population-level RMR and AMR allometric scaling exponents that were calculated from LMMs including individual as a random term allowing for random intercepts alone. Error bars represent standard error.

Table 5.3. Repeatability estimates (R^2) (± 95% credible intervals (95% CI)) from multivariate response models female (F) and male (M) resting metabolic rate (RMR) and active metabolic rate (AMR) across four measurement temperatures. Bold values denote significance. N_{ID}, number of individuals; N_{Obs}, number of observations.

MR	Sex	Temperature	R^2	95% CI	N_{ID}	N_{Obs}
RMR	F	3	**0.295**	**0.025 - 0.684**	29	34
		10	**0.405**	**0.012 - 0.706**	26	31
		17	**0.427**	**0.078 - 0.626**	39	49
		23	**0.324**	**0.063 - 0.574**	34	47
RMR	M	3	**0.400**	**0.067 - 0.705**	20	29
		10	**0.413**	**0.034 - 0.756**	25	27
		17	**0.341**	**0.098 - 0.573**	33	47
		23	**0.500**	**0.155 - 0.689**	26	33
AMR	F	3	**0.361**	**0.052 - 0.575**	33	50
		10	**0.318**	**0.032 - 0.528**	43	58
		17	**0.306**	**0.071 - 0.557**	27	32
		23	**0.195**	**0.008 - 0.452**	25	36
AMR	M	3	**0.288**	**0.072 - 0.536**	41	63
		10	**0.687**	**0.436 - 0.830**	38	61
		17	**0.262**	**0.052 - 0.469**	27	37
		23	**0.276**	**0.026 - 0.504**	33	46

Table 5.4. Repeatability estimates (± 95% confidence intervals) for individual female (F) and male (M) resting metabolic rate (RMR)-temperature and active metabolic rate (AMR)-temperature slopes over time. Bold values denote significance. N_{ID}, number of individuals; N_{Obs}, number of observations.

MR	Sex	R^2	95% CI	N_{ID}	N_{Obs}
RMR	F	**0.674**	**0.115 - 0.999**	**47**	**161**
	M	**0.697**	**0.114 - 1.000**	**45**	**136**
AMR	F	**0.156**	**<0.001 - 0.516**	**49**	**224**
	M	**0.199**	**<0.001 - 0.604**	**45**	**177**

Table 5.5. Comparison of models including either an interaction between average body mass and temperature and between intraindividual variation in body mass and temperature (Interaction Model) or with temperature, average body mass, and intraindividual variation in body mass as fixed terms without an interaction (Mixed Effects Model). Expected log pointwise predictive density (ELPD) Watanabe-Akaike information criterion (WAIC; Watanabe, 2010) and leave-one-out cross validation (LOO) values and standard error (SE) are presented. Differences between the WAIC and LOO values (ELPD.diff: Random Intercepts Model – Random Slopes and Intercepts Model) are given.

		Interaction Model				*Mixed Effects Model*			
		WAIC		LOO		WAIC		LOO	
Response Variable	Sex	ELPD	SE	ELPD	SE	ELPD	SE	ELPD	SE
RMR	F	-33.6	6.6	-31.7	6.3	-35.3	6.2	-33.7	6.0
	M	-31.1	6.2	-29.0	5.9	-22.5	6.3	-21.3	6.0
AMR	F	-14.1	8.3	-13.5	8.1	-11.0	8.4	-10.4	8.3
	M	-59.2	9.5	-57.3	9.0	-62.5	11.8	-61.5	11.7

		Mixed Effects Model – Interaction Model			
		WAIC		LOO	
Response Variable	Sex	ELPD.diff	SE	ELPD.diff	SE
RMR	F	1.7	-0.4	2	-0.3
	M	-8.6	0.1	-7.7	0.1
AMR	F	-3.1	0.1	-3.1	0.2
	M	3.3	2.3	4.2	2.7

Table 5.6. Comparison of models including temperature, average body mass and intraindividual variation in body mass as fixed terms and either intraindividual variation in body mass as a random slope and individual as a random intercept (Random Slopes and Intercepts Mass Model), or individual as a random intercept alone (Random Intercepts Mass Model). Expected log pointwise predictive density (ELPD) Watanabe-Akaike information criterion (WAIC; Watanabe, 2010) and leave-one-out cross validation (LOO) values and standard error (SE) are presented. Differences between the WAIC and LOO values (ELPD.diff: Random Intercepts Model – Random Slopes and Intercepts Model) are given.

		Random Slopes and Intercepts Mass Model				*Random Intercepts Mass Model*			
		WAIC		LOO		WAIC		LOO	
Response Variable	Sex	ELPD	SE	ELPD	SE	ELPD	SE	ELPD	SE
RMR	F	-36.0	7.3	-34.9	7.1	-30.7	6.7	-29.2	6.3
	M	-26.8	7.2	-24.0	6.2	-20.0	4.8	-18.9	4.6
AMR	F	-10.1	7.3	-9.4	7.2	-10.5	8.0	-9.6	7.8
	M	-54.9	9.3	-53.9	9.0	-61.6	12.1	-59.6	11.3

		Random Intercepts Mass Model - Random Slopes and Intercepts Mass Model			
		WAIC		LOO	
Response Variable	Sex	ELPD.diff	SE	ELPD.diff	SE
RMR	F	-5.3	-0.6	-5.7	-0.8
	M	-6.8	-2.4	-5.1	-1.6
AMR	F	0.4	0.7	0.2	0.6
	M	6.7	2.8	5.7	2.3

Table 5.7. Comparison of models including interactions between average body mass and temperature and between intraindividual body mass and temperature, and either intraindividual variation in body mass as a random slope and individual as a random intercept (Random Slopes and Intercepts Mass Model), or individual as a random intercept alone (Random Intercepts Mass Model). Expected log pointwise predictive density (ELPD) Watanabe-Akaike information criterion (WAIC; Watanabe, 2010) and leave-one-out cross validation (LOO) values and standard error (SE) are presented. Differences between the WAIC and LOO values (ELPD.diff: Random Intercepts Model – Random Slopes and Intercepts Model) are given.

		Random Slopes and Intercepts Mass Model				*Random Intercepts Mass Model*			
		WAIC		LOO		WAIC		LOO	
Response Variable	Sex	ELPD	SE	ELPD	SE	ELPD	SE	ELPD	SE
RMR	F	-33.9	6.2	-33.1	6.1	-34.7	6.6	-33.5	6.4
	M	-34.9	6.4	-33.6	6.2	-28.2	5.6	27.0	5.4
AMR	F	-11.6	7.4	10.5	7.1	12.1	8.4	-11.0	8.1
	M	-62.7	10.4	-60.5	9.7	-59.9	9.3	-57.8	8.5

Random Intercepts Mass Model - Random Slopes and Intercepts Mass Model					
		WAIC		LOO	
Response Variable	Sex	ELPD.diff	SE	ELPD.diff	SE
RMR	F	0.8	0.4	0.4	0.3
	M	-6.7	-0.8	-6.6	-0.8
AMR	F	0.5	1	0.5	1
	M	-2.8	-1.1	-2.7	-1.2

6.6 Discussion

The value of species' MR allometric scaling exponent *b* may be attributed to several factors, most notably that allometric scaling exponents may be temperature dependent. We investigated the repeatability of individual metabolic thermal plasticity over time, and assessed the potential influence of intraindividual variation in body mass on the thermal dependency of MR scaling at the population-level, in both male and female *C. hortensis* ground beetles. Allometric scaling of male and female RMR and AMR was independent of temperature at the population level – a finding that was uninfluenced by intraindividual variation in body mass. To the best of our knowledge, this is the first time that both intraindividual variation in body mass and the consistency of individual metabolic thermal plasticity have been considered as potential influential factors contributing towards the thermal dependency of MR scaling, in an insect.

The metabolic theory of ecology postulates that RMR should scale with body mass intraspecifically to the power of 0.75. However, in many species (Bokma, 2004; Clarke & Johnston, 1999; reviewed in: Glazier, 2005), including carabid species that are closely related to *C. hortensis* (*Abax ovalis; b* = 0.58*, Pterostichus burmeisteri; b* = 0.91*, Carabus linnei; b* = 0.60*, Carabus nemoralis; b* = 1.31*,* and *Carabus violaceus; b* = 1.0) (Gudowska *et al*. 2017) RMR allometric scaling exponents significantly differ from 0.75. Moreover, although AMR often scales with body mass to a power greater than 0.75, the exact value of *b* varies from species to species (*e.g.* Glazier, 2005, 2009; Norin & Gamperl, 2018; Weibel *et al*., 2004; Weibel & Hoppeler, 2005). Our findings suggest that the power to which *C. hortensis* RMR and AMR scales with body mass at each of 3°C, 10°C, 17°C and 23°C does not significantly differ from 0.75. However, because the allometric scaling exponents estimated in our study produced very large confidence intervals, whether or

not *C. hortensis* RMR and AMR truly scale with body mass to the power of 0.75 remains uncertain. Increasing sample size and the number of measurements of RMR and body mass recorded per individual may improve clarity on *C. hortensis* RMR allometric scaling exponents.

In line with findings from other studies on ectotherms (*e.g.* Barneche *et al*., 2017; Gifford *et al*., 2013; Grigoriou & Richardson, 2009; Kar *et al*., 2021; Li *et al*., 2020; Melzner *et al*., 2007; Ohlberger *et al*., 2012; Paranjape, 1967), and in line with one of the main assumptions of the metabolic theory of ecology (Brown *et al*., 2004; West *et al*., 1997), we found that the population-level allometric scaling of RMR and AMR was independent of temperature in males or females. However, other studies of ectotherms still have found that allometric scaling exponents decline (*e.g.* Barneche *et al*., 2017; G. Li *et al*., 2018; Ohlberger *et al*., 2007, 2012; Xiaojun & Ruyung, 1990), or even increase (*e.g.* Barneche *et al*., 2017) with increasing temperatures. Differences in the thermal dependence of MR scaling between studies may be attributable to inconsistencies in metabolic thermal plasticity (Kar *et al*., 2021). In our study we found that interindividual differences in thermal plasticity of *C. hortensis* RMR and AMR were repeatable over time, but other studies fail to test the consistency of metabolic thermal plasticity over time (*e.g.* Ohlberger *et al*., 2012). Additionally, differences in the thermal dependence of MR scaling among species may be caused by differences in the methods used to assess the effect of temperature on allometric scaling exponents. For example, in some studies, the thermal dependency of MR scaling is determined by comparing the allometric scaling exponents of populations of individuals which have been kept at different temperatures (*e.g.* Li *et al*., 2018; Li *et al*., 2020; Ohlberger *et al*., 2007; Xiaojun & Ruyung, 1990). In other studies such as our own, however, the thermal dependency of MR scaling is

determined by subjecting all individuals to a series of increasing temperatures (*e.g.* Gifford *et al*., 2013; Grigoriou & Richardson, 2009; Kar *et al*., 2021; Melzner *et al*., 2007; Paranjape, 1967). The range of temperatures over which the thermal dependency of MR scaling is investigated also varies considerably from study to study and may therefore influence results. Moreover, in some studies, study subjects are obtained from the wild (*e.g.* Gifford *et al*., 2013; Kar *et al*., 2021; Ohlberger *et al*., 2012; Xiaojun & Ruyung, 1990), whilst in others they are reared in the laboratory (*e.g.* Grigoriou & Richardson, 2009; Melzner *et al*., 2007; Ohlberger *et al*., 2007).

Intriguingly, differences in allometric scaling exponents among species may be attributable to intraindividual variation in body mass, and its effect on individual allometric scaling exponents and their thermal dependency (Kar *et al*., 2021). However, removal of intraindividual variation in body mass from our models did not significantly alter MR scaling, nor our conclusions that MR scaling in *C. hortensis* is not influenced by temperature. This indicates that, at least in our study, intraindividual variation in body mass had little influence on the allometric scaling of MR. Our findings are in line with those of Kar *et al*. (2021), the only other study that we know of in which intraindividual variation in body mass was accounted for when investigating the thermal dependency of MR scaling, who found that temperature did not significantly influence the allometric scaling of metabolic rate. However, we build upon the study by Kar *et al*. (2021), to investigate the effects of intraindividual variation in body mass on the thermal dependency of both RMR and AMR scaling, in an invertebrate system, and on a sex-specific basis. Clearly, further studies investigating influence of intraindividual variation in body mass on MR scaling and its thermal dependency are required to help determine potential sources of variation in allometric scaling exponents among species.

Differences in allometric scaling exponents among species may be attributable to differences in the thermal dependency of MR scaling. We found that MR scaling was not thermally dependent in *C. hortensis* ground beetles, suggesting that differently sized individuals are not likely to be adversely affected by rising temperatures in the future. Moreover, we found that intraindividual variation in body mass had no effect on allometric scaling exponents nor their thermal dependency. Further studies investigating the potential factors influencing the thermal dependency of MR scaling are required to help uncover the source of variation in allometric scaling exponents among species.

Chapter 7: Covariation of metabolic and behavioural thermal reaction norms in an insect

7.1 Abstract

Environmental temperature influences the metabolic rate and behaviour of ectothermic insects, with consequences for energy allocation and fitness. However, individuals may respond to temperature in different ways, owing to individual differences in the reversible plasticity or phenotypic flexibility of potential behavioural and metabolic responses to different temperatures, defined here as behavioural or metabolic thermal plasticity. Despite potential associations among metabolic rate, behaviour, and their thermal plasticity, intraspecific differences have yet to be studied. Here, using reaction norm approaches we studied intraspecific variation in the thermal plasticity of exploratory behaviour, resting metabolic rate (RMR), and active metabolic rate (AMR) in male and female *Carabus hortensis* ground beetles. We also investigated covariance of thermal plasticity in exploratory behaviour and RMR, and in exploratory behaviour and AMR. Individuals differed consistently in their exploratory behaviour, RMR and AMR at different temperatures, and in the thermal plasticity of those traits. Individuals that had high average exploration or metabolic rates across temperatures exhibited the greatest thermal plasticity. Females with high thermal plasticity in exploratory behaviour exhibited high thermal plasticity in RMR and AMR, but the thermal plasticity of traits was not correlated in males. Our results show that within species individuals differ in their metabolic and behavioural thermal plasticity, that plasticity is related to personality and average metabolic rate, and suggest that intraspecific differences in thermal plasticity can alter the strength of metabolic rate – behaviour relationships. Our results may help to

explain why evidence for metabolic rate - personality relationships to date have been mixed, and may have important consequences for species in a warming world.

7.2 Introduction

Temperature is an important environmental factor that influences many aspects of animal life history and fitness. This is especially true for ectotherms, whose body temperature is strongly dependent upon that of the external environment. Consequently, environmental temperature influences the speed at which biochemical and physiological processes occur in ectotherms, thereby affecting both movement or behaviour (Mellanby & Gardiner, 1939) and energy metabolism. Because metabolic rate increases with temperature within species (Clarke & Fraser, 2004), and because an increase in energy consumption through increased metabolic rate or behavioural frequency/ speed restricts energy allocation towards other important processes, small increases in environmental temperature can have large impacts on ectotherm growth (*e.g.* Miller *et al*., 2009; Pörtner *et al*., 2001) and reproduction (*e.g.* Lemoine & Burkepile, 2012; Pörtner *et al*., 2001), with wider implications for population growth. As global temperatures are expected to rise by 2-3 °C by the year 2100 (Sherwood *et al*., 2020), understanding how temperature affects ectotherm behaviour and metabolism is essential to predict how species are likely to respond to future climate warming.

Individuals within species may not all respond to temperature changes in similar ways. This is because individuals frequently differ in their reversible plasticity or phenotypic flexibility of potential behavioural (*e.g.* Artacho *et al*., 2013; Baškiera & Gvoždík, 2019; Biro *et al*., 2010; Briffa *et al*., 2013; Cornwell *et al*., 2019; Dingemanse *et al*., 2010) and metabolic responses (*e.g.* Careau *et al*., 2014; Kar *et al*., 2021; Shik *et al*., 2019) to different temperatures. This is in addition to the fact that: (1) species often display consistent individual differences in their average behavioural level, meaning that they show personality differences (reviewed in: Dall *et al*., 2004; Eysenck & Eysenck,

1985); (2) species often display consistent individual differences in average level metabolic rate (reviewed in: Biro & Stamps, 2010); and (3) intraspecific differences in behaviour and metabolism may be tightly linked (Biro & Stamps, 2010; Careau *et al*., 2008; Réale *et al*., 2010). Thus, the relationship between metabolic rate and behaviour may also change with temperature in different ways for different individuals (Killen *et al*., 2013). Henceforth we follow the nomenclature set out by others investigating individual differences in the thermal dependency of animal personality *e.g.* Dingemanse *et al*., (2010) and metabolism *e.g.* (Kar *et al*., 2022) and refer to reversible plasticity or phenotypic flexibility of an individual's behaviour or metabolic rate to temperature as 'behavioural or metabolic thermal plasticity'.

Inter-individual variability in behavioural or metabolic thermal plasticity, or inter-individual variability in 'thermal reaction norms' (Schlichting & Pigliucci, 1998), likely have important ecological consequences for both population stability and population persistence (Dingemanse & Wolf, 2013), as populations with large levels of intraspecific variation should be better equipped to overcome climate change than those with lower intraspecific variation (Forsman & Wennersten, 2016). Moreover, populations that exhibit intraspecific differences in the influence of temperature on metabolic rate – behaviour relationships may also have an adaptive advantage to climate change, because temperature-induced changes in the relationship between metabolic rate and behaviour may augment intraspecific differences in metabolic rate and behaviour, thereby increasing raw material upon which natural selection can act (Killen *et al*., 2013). However, intraspecific variation in thermal reaction norms may also impose constraints on evolution; because the two reaction norm components (*i.e.* the average level behaviour/ metabolic rate, and the behavioural/ metabolic plasticity) are often correlated

(*e.g.* Artacho *et al*., 2013; Biro *et al*., 2010; Briffa *et al*., 2013; Cornwell *et al*., 2019; Dingemanse *et al*., 2010; Réveillon *et al*., 2019; Shik *et al*., 2019), it is unlikely that optimal phenotypes of both reaction norm components will be ever be expressed within the population (Dingemanse & Wolf, 2013). Intraspecific variation in thermal reaction norms also has consequences for individual fitness. For instance, actively changing metabolic rate and behaviour, and indeed maintaining the ability to change metabolic rate and behaviour, may create trade-offs with traits that influence individual fitness (Norin & Metcalfe, 2019). The consequences of behavioural or metabolic plasticity for individual fitness may also be amplified or diminished if individuals experience differences in the influence of temperature on metabolic rate – behaviour relationships. For example, in some individuals, temperature changes may cause an increase in the metabolic cost of performing essential behaviours, while others remain relatively unaffected.

Despite likely ecological, evolutionary and individual level consequences, whether or not intraspecific variation in metabolic and behavioural thermal plasticity covary, and hence whether or not temperature influences metabolic rate – behaviour relationships in different ways, has yet to be investigated. Our study aims to fill this gap by utilising reaction norm approaches in *Carabus hortensis* Linnaeus, 1758 ground beetles to assess: (a) how temperature influences the consistency of exploratory behaviour, resting metabolic rate (RMR; the metabolic rate of the animal when inactive and during the post-absorptive phase), and active metabolic rate (AMR; the metabolic rate of the animal when active and during the post-absorptive phase) over time; (b) the extent to which different individuals respond to temperature in different ways (*i.e.* intraspecific variation in plasticity) in terms of their exploratory behaviour, RMR and AMR; (c) the relationships between personality and its thermal plasticity and between metabolic rate and its

thermal plasticity; and (d) the rank order of individual thermal plasticity in exploratory behaviour *versus* the rank order of individual thermal plasticity in RMR and AMR. Here we choose exploratory behaviour as the animal personality trait of interest as it has demonstrable consequences for individual survival and fitness (Dingemanse *et al*., 2004; Smith & Blumstein, 2008; van Overveld & Matthysen, 2010). Because links between metabolic rate and animal personality may differ between the sexes (Hämäläinen *et al*., 2018), we investigate metabolic and behavioural thermal plasticity, and the thermal plasticity of metabolic rate – behaviour relationships, on a sex-specific basis.

Given that individuals respond differentially to temperature in terms of their behaviour (*e.g.* Artacho *et al*., 2013; Baškiera & Gvoždík, 2019; Biro *et al*., 2010; Briffa *et al*., 2013; Cornwell *et al*., 2019) and metabolic rate (*e.g.* Careau *et al*., 2014; Kar *et al*., 2021; Shik *et al*., 2019), we expect significant intraspecific variation in the responses of *C. hortensis* exploratory behaviour, RMR and AMR to temperature. Because evidence demonstrates that plasticity in metabolic rate is correlated with average metabolic rate (*e.g.* Careau *et al*., 2014; Shik *et al*., 2019), and behavioural plasticity is correlated with personality type (*e.g.* Artacho *et al*., 2013; Biro & Stamps, 2010; Briffa *et al*., 2013; Cornwell *et al*., 2019), we predict that individual average metabolic rate and animal personality will be correlated with thermal plasticity. Finally, we hypothesise that temperature should influence the relationship between metabolic rate and behaviour in different ways in different individuals. We therefore expect to find no correlation in the rank-order of thermal plasticity in one trait and the rank-order of thermal plasticity in another.

7.3 Methods

7.3.1 Study Species, Trapping and Maintenance

Carabus hortensis (Coleoptera, Carabidae) Linnaeus, 1758 ground beetles were collected Lüneburger Heide, Lower Saxony, Germany, at the most westerly edge of the species' range (Völler *et al*., 2018; Yarwood *et al*., 2021a), from August – October 2019. The active season of the species runs from July to October, and individuals are night-active (Turin *et al*., 2003). 100 beetles (52 females, 48 males) were collected in total using live pitfall traps (Schuett *et al*., 2018; Yarwood *et al*., 2021a), which were emptied and re-baited every 7-8 days.

Collected beetles were housed individually in a field station, in 10(L) x 7.5(W) x 4.5(H) cm containers filled with peat. Containers were regularly sprayed with water to ensure a moist environment and beetles were maintained on an *ad libitum* diet of *Tenebrio molitor* pupa. Individuals were returned to the laboratory throughout September and October 2019, where behavioural and metabolic rate trials would later take place (See: 'Behaviour Tests' and 'Metabolism Tests'). To ensure that beetles remained in their most active phase for the remainder of the study (Günther & Assmann, 2000), individuals were maintained at average autumn temperatures obtained from the Schneverdingen weather station (at 12°C during the day and at 6°C during the night), and daylight hours (under a 10h: 15h light: dark regime) for the Lüneburger Heide region. So that behavioural and metabolic trials could be conducted when *C. hortensis* are most active, the day-night cycles of the study subjects were reversed. Each week from the 14th October 2019 until 23rd December 2019, daylight hours were gradually shifted by 62 minutes, so that individuals experienced fully reversed day-night cycles, with periods in

darkness lasting from 04:00 – 18:00, for 1.5 weeks prior to the onset of metabolic and behavioural trials. This gradual shift in timing of daylight hours was implemented to limit disruption to individual physiology.

RMR can only be accurately recorded during the post-absorptive state, where no energy expenditure is allocated to digestion (Wang *et al*., 2001). Before the onset of metabolic trials, therefore, beetles were starved for 24-48 hours: this was in line with methodology utilised in other carabid metabolic rate studies (*e.g.* Gudowska *et al*., 2017; Yarwood *et al*., 2021b; Chapter 5).

7.3.2 Temperature Treatments

Individual metabolic rate and exploration were recorded at a series of four different temperatures (3°C, 10°C, 17°C and 23°C) in a climate chamber (Weiss WK2T Climate Chamber WeisScientific Ltd., Timrat, Israel), twice per temperature and individual. Temperature treatments were informed by temperatures that *C. hortensis* experience in their natural habitat. These were obtained from minimum (4.5°C), maximum (22.0°C) and intermediate (10°C, 17°C) temperatures recorded every 2 hours after sunset for 8 hours during for September and October 2019 (*i.e.* during the active period of the beetles), at the trapping site of study animals. Individuals were held under each temperature treatment for at least 30 minutes prior to behavioural and metabolism tests. Individuals were tested under one temperature treatment, and after 4-5 days were tested under the next temperature until they had been tested under all four temperature treatments. Individuals experienced temperature treatments in one of four different orders: (1) 3°C, 10°C, 17°C, 23°C; (2) 10°C, 17°C, 23°C, 3°C; (3) 17°C, 23°C, 3°C, 10°C; or

(4) 23°C, 3°C, 10°C, 17°C. To assess repeatability of results over the four temperatures, this cycle was repeated such that individual metabolic rate and behaviour was measured at each temperature twice, 18-19 days apart.

7.3.3 Behavioural Tests

Exploration for each beetle was assessed using previously established novel environment tests for ground beetles (outlined in Schuett *et al*., (2018) and Yarwood, *et al*., (2021b)), immediately before metabolic trials. Individuals were placed in the centre of an open white 37.5(L) x 26(W) cm plastic box split into a 28x square grid, and the total number of squares visited in the gird within a 90 second period, including repeated visits to the same square, was counted during observation.

Behavioural trials were conducted on all individuals at each of 3°C, 10°C, 17°C and 23°C, and were repeated at each temperature 18-19 days later to ascertain the presence of consistent personality differences over time. Trials were conducted between 08:00 – 17:00. During trials, red light was used to illuminate the climate chamber so that trials could be carried out with minimal disturbance to beetle activity: studies show that red light does not apparently disturb carabid beetle behaviour (Drees *et al*., 2008; Hasselmann, 1962).

7.3.4 Metabolism Tests, Respiration Classification and Metabolic rate Analysis

Immediately following behavioural trials, individual *C. hortensis* beetles were weighed (CA-103 Phoenix Instrument, Phoenix Instrument GmbH, Garbsen, Germany) to the nearest milligram and then underwent metabolic trials. The RMR and AMR of

individual *C. hortensis* beetles were measured for 15 minutes using a flow-through respirometer. As with behavioural trials, metabolic trials were conducted between 08:00 – 17:00 and took place at each of the four temperatures (3°C, 10°C, 17°C and 23°C) under red light and were repeated at each temperature 18-19 days later.

Methods used to capture metabolic rate measurements were identical to those described in Yarwood *et al*., (2021b) (Chapter 5). In short, air was pumped through Sodalime and Drierite (W.A. Hammond Drierite, Xenia, USA) scrubbing columns before being split between two identical 7ml chambers, one of which contained one *C. hortensis* individual whilst the other remained empty. A L1-7000 dual channel CO_2 infra-red gas analyser (LI-COR, Lincoln, NE, USA), sampled the air in the two respective chambers at a sample rate of 2 Hz. Li-COR software was then used to record differences in the volume of CO_2 between chambers, thereby calculating the volume of CO_2 excreted from the beetle. A camera was fixed above the test chamber to record *C. hortensis* activity during metabolic trials. Following methods outlined in Yarwood *et al*., (2021b) (Chapter 5), metabolic rate traces were then visually classified into different respiration types (continuous, discontinuous and pulsatile), and video recordings were analysed to separate metabolic traces into periods of activity and rest, allowing accurate calculation of RMR and AMR from periods in which individuals were respectively at rest or active. RMR and AMR were estimated only from instances where individuals remained stationary or active, respectively, for 3 minutes or longer, and were estimated from the 3rd minute of inactivity or activity onwards for the duration of the period at rest/activity. If individuals were inactive/active for 3 minutes or longer on multiple occasions within same metabolic trial, RMR/ AMR were calculated from the average volume of CO_2 excreted.

7.3.5 Statistical analyses

All statistical analyses were carried out in R version 3.3.2 (R Core Team, 2019) and were conducted on male and female data separately. Repeatability of exploratory behaviour, RMR and AMR at each temperature over time was estimated using three multivariate response models, using the brms package (Bürkner, 2017; Kar *et al.*, 2021). Each multivariate response model was composed of four different linear mixed effects models (LMMs), with one of either exploratory behaviour, RMR or AMR measured at 3°C, 10°C, 17°C and 23°C as the response variables, body mass measured at the corresponding temperature as covariate, and beetle ID as a random term. Confidence intervals of 95% were used to infer the significance of the repeatability; we considered the trait to be repeatable where the confidence interval did not include zero.

Repeatability of the thermal plasticity of exploratory behaviour, RMR, AMR were estimated by fitting generalised linear mixed effects models (GLMMs) using the MCMCglmm package (Hadfield, 2010). They either contained exploratory behaviour, RMR, or AMR as the response variable and temperature as a covariate. Both beetle ID and beetle ID interacting with test series (*i.e.* whether the metabolic rate/behavioural measurement was a repeated measure) were included as random terms allowing for random intercepts, while temperature was included as a random term allowing for random slopes twice *e.g.* Kar *et al.*, (2021). Repeatability was then estimated using equations provided in the supplemental information of Kar *et al.*, (2021).

To test whether RMR, AMR and exploratory behaviour changed with temperature, we fit separate male and female linear mixed effects models (LMMs) with

each of RMR, AMR and exploratory behaviour as the response variables. Temperature was included as the fixed effect, and beetle ID was included as a random term allowing for random intercepts.

Reaction norms were used to determine the extent of individual variation in the plasticity of *C. hortensis* RMR, AMR and exploratory responses to temperature. Analysis was carried out following steps in (Roche *et al.*, 2016), using the MCMCglmm package (Hadfield, 2010). For each sex, and response variable (*i.e.* RMR, AMR and exploration), we fit two GLMMs (hereafter: pairs of GLMMs): a 'random slopes and intercepts' model and a 'random intercepts model'. Random terms differed between pairs of GLMMs: the 'random slopes and intercepts' model included temperature as a random term allowing for random slopes (*i.e.* allowed for intraspecific differences in RMR/AMR/exploration thermal reaction norms) and beetle ID as a random term allowing for random intercepts, whilst the 'random intercepts model' included beetle ID as a random term allowing for random intercepts alone (*i.e.* did not allow for intraspecific differences in RMR/AMR/exploration thermal reaction norms). Temperature was included as a fixed term in all models. Because GLMMs were performed on separate male data and female data alone for each response variable, we performed a total comparison of six pairs of GLMMs.

Deviance information criteria (DIC) values were then used to compare pairs of GLMMs for best fit: the model with the smallest DIC value was deemed as the model with the best fit (we considered that DIC's should differ by at least ten in order to select the random slopes and intercepts model (MRC Biostatistics Unit, n.d.)). Response variables and continuous variables were mean centred prior to analysis, as outlined in Roche *et al*. (2016). In instances where the random slopes and intercepts model had the best fit, we

calculated the correlation coefficient (R) and confidence interval for R between the intercept and the slope. The correlation was considered as non-significant where the confidence interval included zero. This tested whether individuals with high average RMR/ AMR/ exploration show a higher increase in RMR/ AMR/ exploration with exposure to higher temperatures than conspecifics with lower average trait values.

To investigate whether the relationship between metabolic rate and behaviour is likely to change with temperature in different ways in different individuals, we then used Spearman's rank correlations (Spearman, 1904) to test whether the rank-order of individual thermal plasticity of exploratory behaviour was correlated with the rank-order of individual thermal plasticity of RMR and AMR.

To assess the robustness of our findings across analytical methods, we repeated all reaction norm analysis following steps in Kar *et al*. (2021), using Watanabe-Akaike information criterion (WAIC; Watanabe, 2010) and leave-one-out cross validation (LOO) values to compare the pairs of GLMMs for best fit. The model with the smallest WAIC and LOO values, regardless of being positive or negative, was deemed as the model with the best fit. Following the reasoning in Chapter 6, we considered that WAIC and/or LOO values should differ by at least ten in order to select the random slopes and intercepts model over the random intercepts model. Results were qualitatively different for male and female exploratory behaviour when following steps in Kar *et al*. (2021) – using the Kar *et al*. (2021) methodology, we found no significant intraspecific variation in male or female exploration thermal reaction norms (Table E.1).

7.4 Ethics

This study was carried out under permits from the Heidekreis (permit number: 2019-0168) and Harburg (permit number: 2019-0218-Kr) nature conservation *authorities and* the Lower Saxon State Department for Waterway, Coastal and Nature Conservation authorities (permit number: H72.22202/2019-Drees).

7.5 Results

The range and mean ± SD of exploratory behaviour, RMR and AMR for males and females at each individual measurement temperature can be found in Table 6.1. Exploratory behaviour, RMR and AMR were repeatable at each temperature in both sexes (Table 6.2). The thermal plasticity of male and female exploratory behaviour, RMR, and AMR were also repeatable over time (Table 6.3).

Table 6.1. Summary statistics for female (F) and male (M) exploratory behaviour (number of square visits in a novel environment), resting metabolic rate (RMR; CO_2 ml/h) and active metabolic rate (AMR; CO_2 ml/h) at each of four measurement temperatures (°C). N_{ID}, number of individuals; N_{Obs}, number of observations.

Response Variable	Sex	Temperature	Mean ± SD	Range	N_{ID}	N_{Obs}
Exploratory	F	3	12.89 ± 7.03	1 – 31	50	100
Behaviour		10	27.07 ± 12.26	1 - 58	50	100
		17	44.48 ± 20.45	1 – 111	50	100
		23	51.9 ± 21.71	11 - 136	50	100
Exploratory	M	3	12.83 ± 6.06	1 – 29	48	96
Behaviour		10	28.88 ± 13.81	1 – 69	48	96
		17	46.43 ± 20.48	1 – 97	48	96
		23	63.11 ± 29.32	1 - 148	48	96

RMR	F	3	3.95 ± 1.53	0.67 – 7.75	29	34
		10	6.48 ± 2.31	2.24 – 12.84	26	31
		17	8.14 ± 2.68	3.80 – 15.75	39	49
		23	11.64 ± 3.98	4.92 – 25.46	34	47
RMR	M	3	3.36 ± 1.71	0.49 – 8.31	20	29
		10	6.71 ± 2.59	2.12 – 11.47	25	27
		17	7.79 ± 3.28	2.84 – 15.77	33	47
		23	11.36 ± 4.39	0.50 – 19.11	26	33
AMR	F	3	6.62 ± 1.42	3.78 – 9.16	33	50
		10	11.18 ± 2.76	5.45 – 19.28	43	58
		17	16.45 ± 4.29	4.41 – 25.14	27	32
		23	22.08 ± 7.08	9.94 – 38.10	25	36
AMR	M	3	6.17 ± 1.55	3.67 – 10.33	41	63
		10	12.25 ± 4.81	2.92 – 26.96	38	61
		17	15.45 ± 6.93	4.09 – 39.01	27	37
		23	21.89 ± 6.99	11.06 – 42.13	33	46

Table 6.2. Repeatability estimates (R^2) (± 95% credible intervals (95% CI)) from multivariate response models. Repeatability estimates are given for female (F) and male (M) exploratory behaviour (number of square visits in a novel environment), resting metabolic rate (RMR; CO_2 ml/h) and active metabolic rate (AMR; CO_2 ml/h) at each of four measurement temperatures (°C). Bold values denote significance. N_{ID}, number of individuals; N_{Obs}, number of observations.

Response Variable	Sex	Temperature	R^2	95% CI	N_{ID}	N_{Obs}
Exploratory Behaviour	F	3	**0.396**	**0.296 - 0.495**	50	100
		10	**0.217**	**0.064 - 0.356**	50	100
		17	**0.324**	**0.086 - 0.633**	50	100
		23	**0.308**	**0.042 - 0.659**	50	100
Exploratory Behaviour	M	3	**0.284**	**0.102 - 0.425**	48	96
		10	**0.310**	**0.041 - 0.465**	48	96

		17	**0.233**	**0.012 - 0.507**	48	96
		23	**0.288**	**0.051 - 0.612**	48	96
RMR	F	3	**0.361**	**0.039 - 0.599**	29	34
		10	**0.284**	**0.046 - 0.506**	26	31
		17	**0.463**	**0.144 - 0.632**	39	49
		23	**0.355**	**0.069 - 0.520**	34	47
RMR	M	3	**0.322**	**0.038 - 0.524**	20	29
		10	**0.319**	**0.089 - 0.578**	25	27
		17	**0.365**	**0.139 - 0.516**	33	47
		23	**0.463**	**0.125 - 0.627**	26	33
AMR	F	3	**0.527**	**0.252 - 0.676**	33	50
		10	**0.365**	**0.110 - 0.559**	43	58
		17	**0.414**	**0.091 - 0.657**	27	32
		23	**0.249**	**0.078 - 0.523**	25	36
AMR	M	3	**0.213**	**0.013 - 0.408**	41	63
		10	**0.588**	**0.440 - 0.707**	38	61
		17	**0.174**	**0.011 - 0.381**	27	37
		23	**0.241**	**0.026 - 0.490**	33	46

Table 6.3. Repeatability estimates (± 95% confidence intervals) for the thermal plasticity of individual female (F) and male (M) exploratory behaviour (number of square visits in a novel environment), resting metabolic rate (RMR; CO_2 ml/h) and active metabolic rate (AMR; CO_2 ml/h) over time. Bold values denote significance. N_{ID}, number of individuals; N_{Obs}, number of observations.

Response Variable	Sex	R^2	95% CI	N_{ID}	N_{Obs}
Exploratory Behaviour	F	**0.087**	**<0.001 – 0.305**	50	400
	M	**0.044**	**<0.001 – 0.161**	48	384
RMR	F	**0.672**	**0.149 – 0.999**	47	161
	M	**0.776**	**0.247 – 0.999**	45	136
AMR	F	**0.184**	**<0.001 – 0.574**	48	176
	M	**0.211**	**<0.001 – 0.640**	47	207

Female (LMM; Intercept = 2.546, slope = 0.381, χ^2 = 119.08, p = <0.001, DF = 1; Figure 6.1A, Figure 6.2C) and male (LMM; Intercept = 2.415, slope = 0.361, χ^2 = 80.095, p = <0.001, DF = 1; Figure 6.1A, Figure 6.2D) RMR, female (LMM; Intercept = 3.871, slope = 0.770, χ^2 = 188.56, p = <0.001, DF = 1; Figure 6.1A, Figure 6.2E) and male (LMM; Intercept = 4.000, slope = 0.759, χ^2 = 165.26, p = <0.001, DF = 1; Figure 6.1A, Figure 6.2F) AMR, and female (LMM; Intercept = 7.395, slope = 2.014, χ^2 = 246.54, p = <0.001, DF = 1; Figure 6.1B, Figure 6.2A) and male (LMM; Intercept = 4.557, slope = 2.510, χ^2 = 258.88, p = <0.001, DF = 1; Figure 6.1B, Figure 6.2B) exploratory behaviour increased with increasing temperature. However, the exploratory behaviour (Table 6.4, Figure 6.2A, B, Figure 6.3A, B), RMR (Table 6.4, Figure 6.2C, D, Figure 6.3C, D), and AMR (Table 6.4, Figure 6.2E, F, Figure 6.3E, F) of both males and females increased with temperature at different rates in different individuals. Individuals with higher average exploration (Table 6.4, Figure 6.4A, B), with higher average RMR (Table 6.4, Figure 6.4C, D), and that had higher

average AMR (Table 6.4, Figure 6.4E, F), had higher thermal plasticity in those traits than conspecifics with lower average exploration, RMR or AMR values.

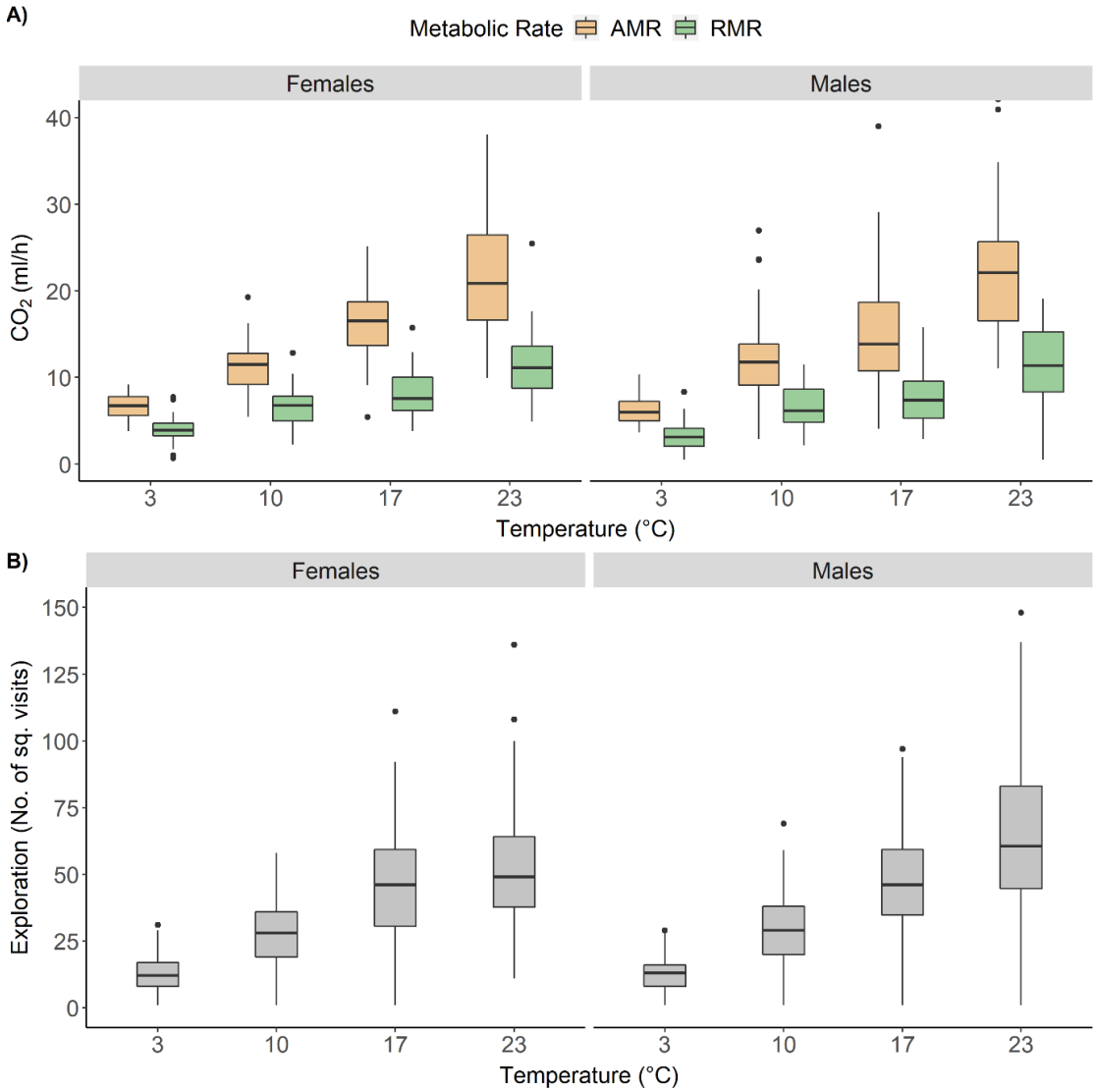

Figure 6.1. Male and female RMR, AMR and exploratory behaviour increase with temperature. Distributions of A) female RMR (N = 47) and AMR (N = 50) and male RMR (N = 40) and AMR (N = 48), B) female (N = 50) and male (N = 48) exploratory behaviour, at different temperature treatments.

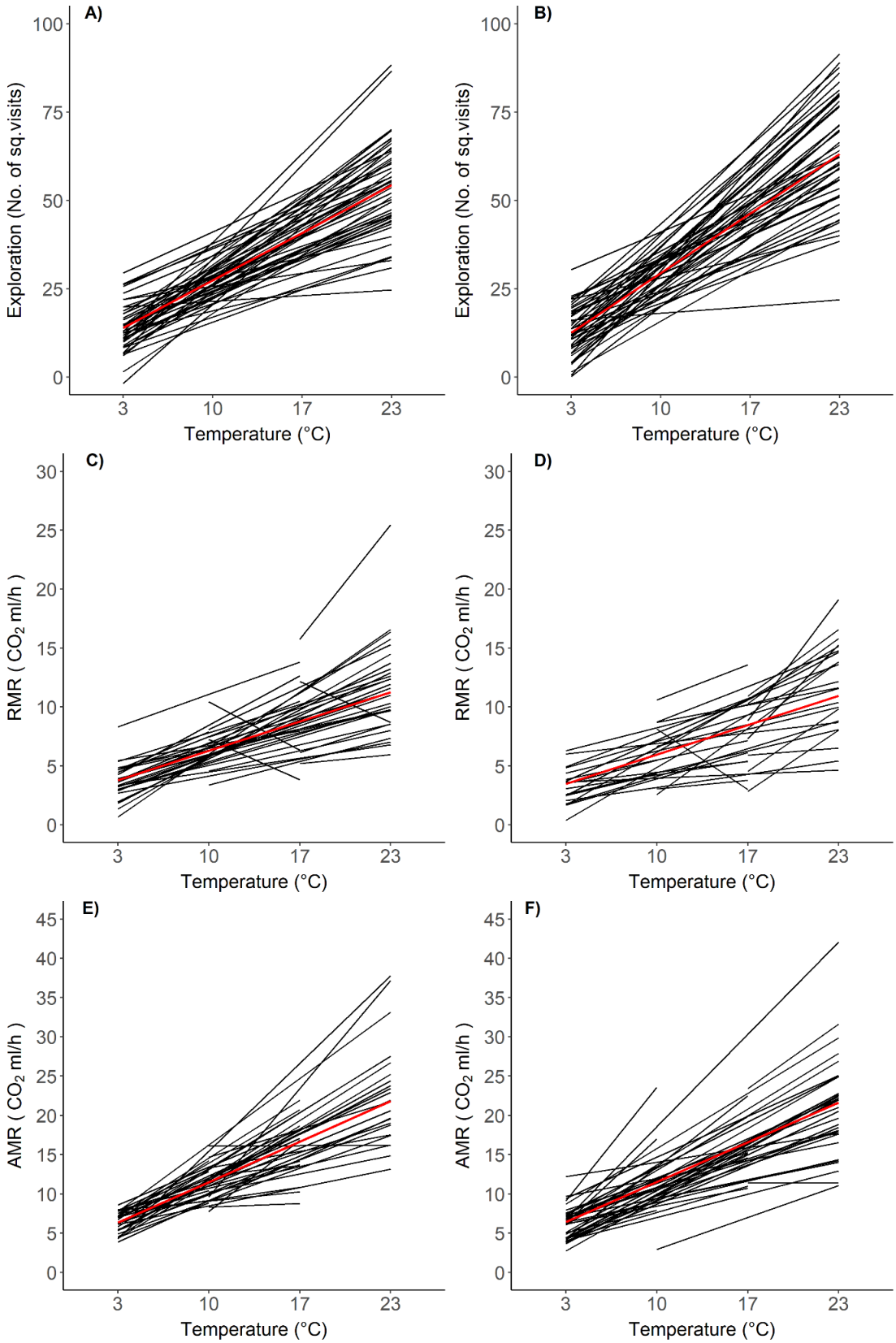

Figure 6.2. Individuals differ in their responses of exploration and metabolism to temperature (black lines), while exploration and metabolism increase with increasing temperature overall (red lines). A) female exploration (N=50), B) male exploration

(N=48), C) female RMR (N=47), D) male RMR (N=40), E) female AMR (N=50) and F) male AMR (N=48) in response to temperature. Each black line represents a single individual. Lines are plotted using linear model estimates for each individual. Red lines represent the population average response to temperature.

The rank-order of thermal plasticity in female (Spearman's rank correlation; Rs = 0.295, p = 0.045), but not male (Spearman's rank correlation; Rs = -0.132, p = 0.387) RMR was positively correlated with the rank-order of thermal plasticity in exploratory behaviour. Likewise, the rank-order of thermal plasticity in female (Spearman's rank correlation; Rs = 0.395, p = 0.005), but not male (Spearman's rank correlation; Rs = 0.131, p = 0.380) AMR was positively correlated with the rank-order of thermal plasticity in exploratory behaviour.

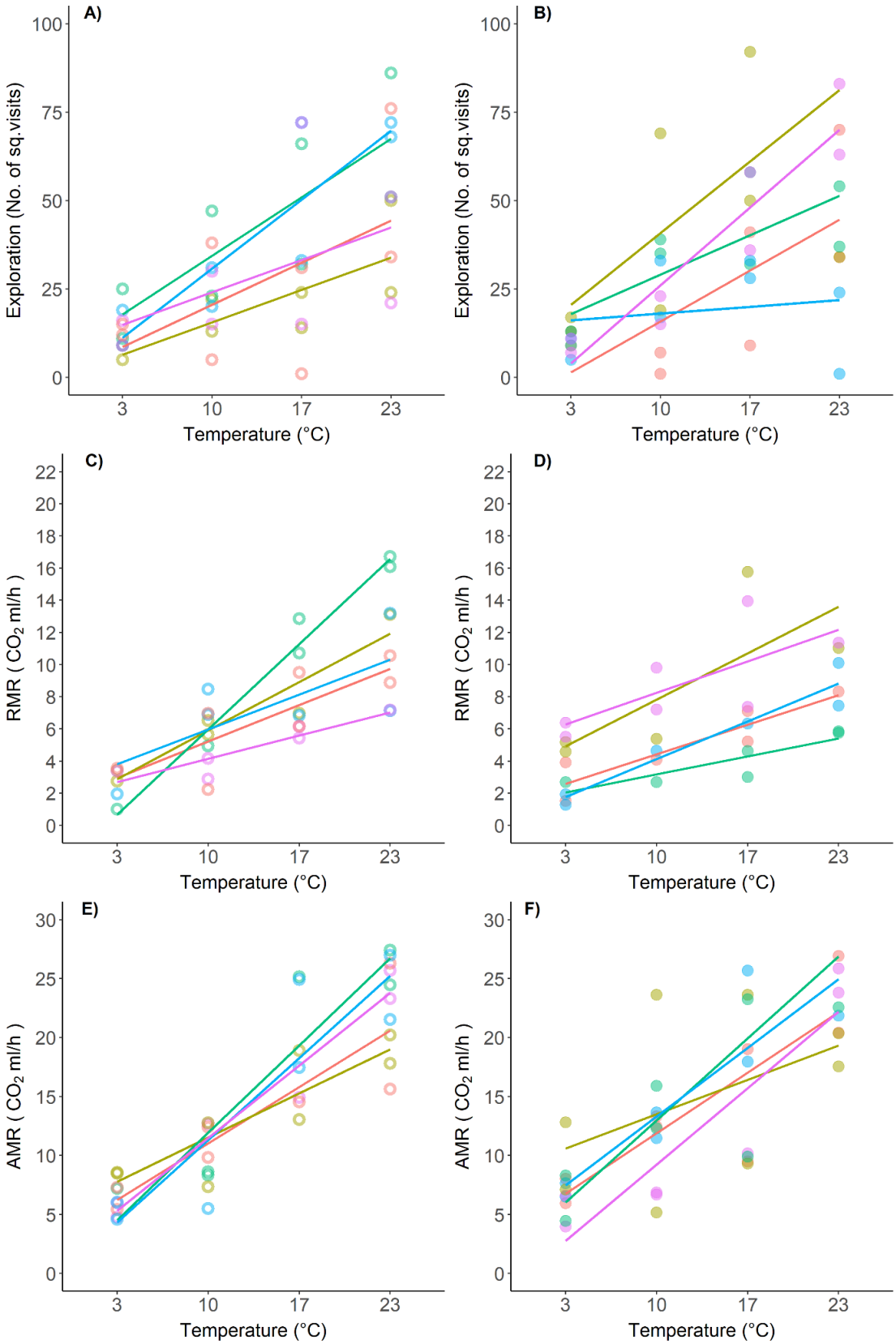

Figure 6.3. Individuals differ in their responses of exploration and metabolism to temperature. A) exploration of five randomly chosen females, B) exploration of five

different males, C) RMR of five different females, D) RMR of five different males, E) AMR of five different females and F) AMR of five different males, in response to temperature. Each line represents a single individual. Females are represented by open circles whilst males are represented by filled circles. Lines are plotted using linear model estimates for each individual.

Table 6.4. Differences in deviance information criterion (DIC) values between generalised linear mixed models (GLMMs) with temperature included as a random slope and individual included as a random intercept (Random Slopes and Intercepts Model), and GLMMs with individual included as a random intercept with no random slopes (Random Intercepts Model). DIC values are given for separate female (F) and male (M) GLMMs with exploratory behaviour (number of square visits in a novel environment), resting metabolic rate (RMR; CO_2 ml/h) and active metabolic rate (AMR; CO_2 ml/h) as the response variables and temperature as the fixed term. Differences between DIC values between the Random Intercepts Model and Random Slopes and Intercepts Model (DIC.diff: Random Intercepts Model – Random Slopes and Intercepts Model) are given. Correlation coefficients (R^2) and confidence intervals (CI) are given denoting the correlation between intercept and slope (individual and temperature). Bold DIC.diff values denote a significant difference between the Random Intercepts Model and Random Slopes and Intercepts Model whilst bold R and CI values significant correlations between intercept and slope.

Response Variable	Sex	Random Slopes and Intercepts DIC	Random Intercepts DIC	DIC.diff	R^2	95% CI
Exploratory Behaviour	F	879.112	894.338	**15.226**	**0.947**	**0.400 – 0.996**
	M	823.217	836.337	**13.120**	**0.934**	**0.335 – 0.996**
RMR	F	295.946	323.102	**27.156**	**0.927**	**0.589 – 0.997**
	M	266.402	288.746	**22.344**	**0.777**	**0.425 – 0.988**
AMR	F	264.732	316.145	**51.413**	**0.980**	**0.843 – 0.997**
	M	396.109	428.134	**32.025**	**0.965**	**0.662 – 0.998**

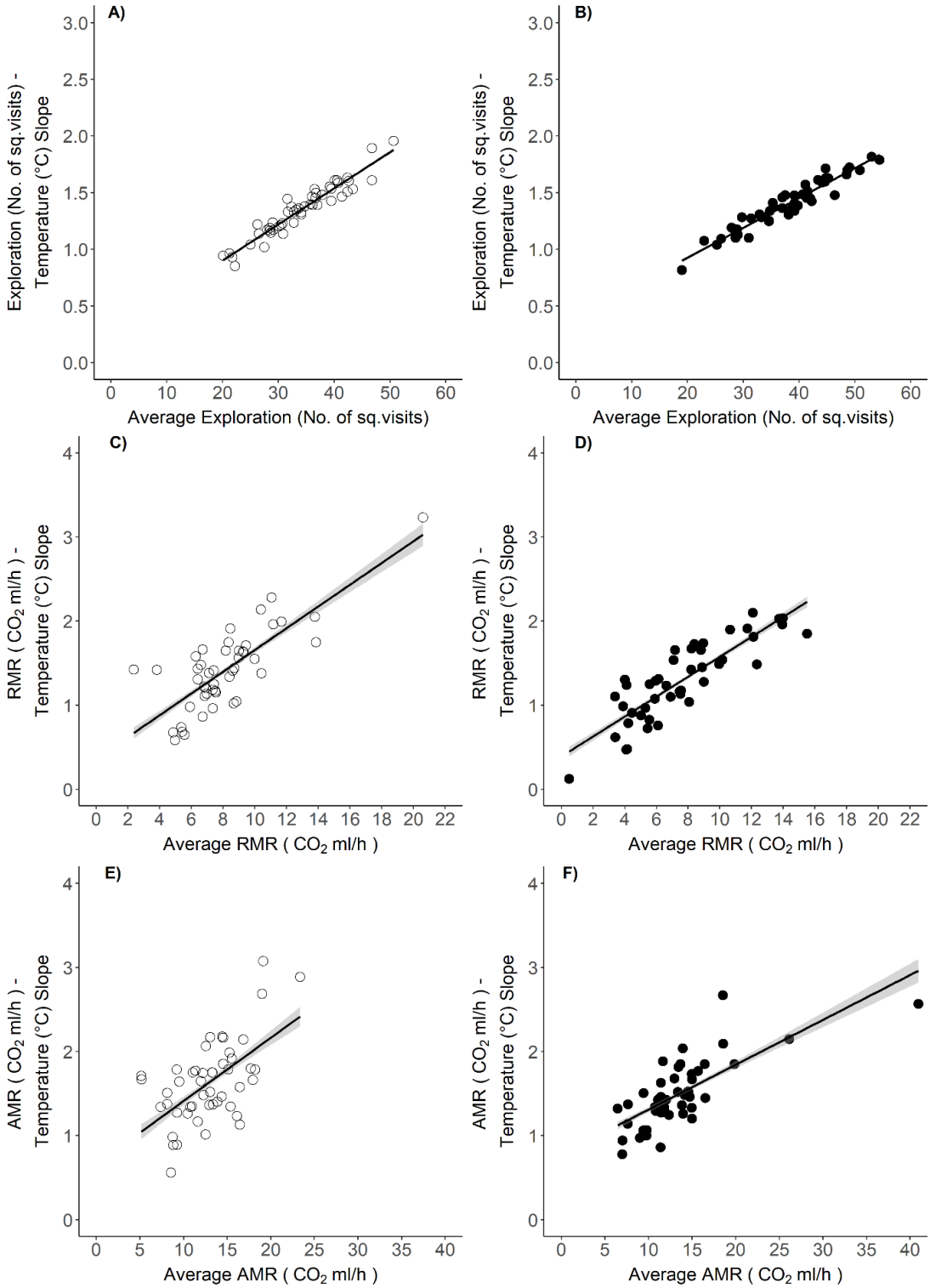
A)
B)
C)
D)
E)
F)
Exploration (No. of sq.visits) - Temperature (°C) Slope
Average Exploration (No. of sq.visits)
RMR (CO_2 ml/h) - Temperature (°C) Slope
Average RMR (CO_2 ml/h)
AMR (CO_2 ml/h) - Temperature (°C) Slope
Average AMR (CO_2 ml/h)

Figure 6.4. Individuals with higher average exploration, RMR and AMR experience sharper increases in those traits as temperature increases. Relationships between the average trait value of A) female exploration (N = 50), B) male exploration (N = 48), C) female RMR (N = 47), D) male RMR (N = 40), E) female AMR (N = 50), and F) male AMR (N = 48), and the slope of their thermal reaction norms. Predicted lines are fitted using outputs from LM estimates. 95% confidence intervals are shown in grey.

7.6 Discussion

We investigated intraspecific variation in male and female *C. hortensis* exploration, RMR, and AMR thermal reaction norms. Male and female exploration, RMR and AMR were repeatable both at different temperatures and in their thermal plasticity across temperatures. In both sexes, exploration, RMR, and AMR changed with temperature in different ways for different individuals. Individuals with the highest average exploration, RMR and AMR expressed the sharpest increase in those traits as temperature increased, indicating that both personality and metabolic rate can be related to plasticity. Individual thermal plasticity in exploratory behaviour was weakly, positively correlated with thermal plasticity in RMR and AMR in females, but not males.

In line with findings across studies of insects (Nespolo, 2003), reptiles (Careau *et al*., 2014) and fish (Seppänen *et al*., 2010), *C. hortensis* beetles consistently differ from one another in their behavioural and metabolic thermal plasticity over time. Moreover, *C. hortensis* males and females with higher average exploratory behaviour, RMR or AMR experience sharper changes in those traits as temperature increases than conspecifics with lower average trait values. These findings reflect advancements in both the fields of animal personality and animal metabolism that demonstrate that intraspecific variation in behavioural or metabolic plasticity is correlated with animal personality (*e.g.* Artacho

et al., 2013; Biro *et al*., 2010; Briffa *et al*., 2013; Cornwell *et al*., 2019) and average level metabolic rate (*e.g.* Careau *et al*., 2014; Shik *et al*., 2019) respectively, and suggest that plasticity forms part of animal personality and average metabolic rate its-self. We advance the field further, showing for the first time that intraspecific variation in the thermal plasticity of exploratory behaviour is weakly or not correlated with that of metabolic rate. This means that within individuals, metabolic rate and exploratory behaviour change with temperature at different rates. This, in combination with the fact that we found significant intraspecific variation in metabolic and behavioural thermal plasticity, suggests that temperature may change the relationships between both RMR and exploratory behaviour and AMR and exploratory behaviour at different rates in different *C. hortensis* individuals. Our findings therefore support the notion that evidence for relationships between metabolic rate and personality have been mixed thus-far due to intraspecific differences in plasticity of responses to environmental contexts (Killen *et al*., 2013).

The temperatures over which we measured metabolic rate and exploratory behaviour were ecologically relevant to *C. hortensis.* Our findings therefore indicate that individual survival and fitness relative to other individuals in the population may fluctuate with daily temperature changes in the wild. For instance, at the hottest part of the day, the risk of predation of an individual with high thermal plasticity – here, individuals with high average metabolic rates and exploratory behaviour - may be larger than that of individuals with low thermal plasticity, low average metabolic rates, and low average exploratory behaviour, because high temperatures should exacerbate between-individual differences in exploratory behaviour and hence time spent out in the open. Likewise, because an individual's competitive ability is related to its metabolic rate (Biro

& Stamps, 2010), the competitive advantage that an individual with high thermal plasticity has over an individual with low thermal plasticity will change with daily fluctuations in temperature. Where individual reaction norms cross (*i.e.* when individuals with higher average level trait values have lower thermal plasticity than individuals with relatively lower average level trait values) as seen in some instances in our study, daily temperature fluctuations will alter which individual is at the greatest risk of predation or has the greatest competitive advantage (Biro *et al*., 2010). Thus, our findings highlight the importance of studying intraspecific variation in metabolic rate and behaviour over a range of ecologically relevant temperatures. If we had measured metabolic rate and exploratory behaviour at just one temperature, our estimates of animal personality and average level metabolic rate of one individual in comparison to another would have been impacted by the test temperature. Our interpretation of intraspecific differences in animal personality and average level metabolic rate would also be incorrect where thermal reaction norms cross, and the rank order of individual exploratory behaviour and metabolic rate changes with temperature. Overall, we show that the study of intraspecific variation in animal personality and average metabolic rate requires a shift in methodology to include measurements over multiple ecologically relevant temperatures, or more generally, environmental gradients (Hämäläinen *et al*., 2020).

In addition to fluctuations in daily temperatures impacting the fitness and predation risk of individuals relative to one another, our findings have important consequences for the evolution and survival of the species under continued climate change. Summer temperatures in the Lüneburger Heide are expected to increase by 1-1.5°C in the next 29 years and by 2.5-3°C from 2071-2100 (European Environment Agency, 2012), and other related, flighted ground beetle species in the region have

engaged in poleward range shifts in response to recent warming (Drees *et al*., 2011). However, because *C. hortensis* are unable to fly (Turin *et al*., 2003), it is unlikely that the species will adapt to climate change by the same means. Yet, because intraspecific differences in thermal plasticity were consistent over time, *C. hortensis* may respond and adapt to climate change through natural selection of both metabolic and behavioural thermal plasticity. Our results suggest that individuals with high average trait values may be selected against under continued environmental warming, because, at higher temperatures, those individuals should experience large increases in energy expenditure, and suffer a reduction in the allocation of resources towards reproduction as a result. Certainly, temperature-induced shifts in energy allocation have been observed to reduce fecundity in other ectothermic species (Pörtner *et al*., 2001). Moreover, our findings suggest that, at higher temperatures, some individuals may be unable to acquire enough resources through exploratory behaviour to meet the energy requirements of heightened metabolic rate, leading to starvation. Clearly, intraspecific variation in the thermal plasticity may have important consequences for *C. hortensis* under continued climate change. In our study, increases of 7°C exacerbated intraspecific differences in, and changed the rank order of, individual animal personality/metabolic rate. We may therefore observe the effects climate change on *C. hortensis* populations within a relatively short space of time, after regional increases in just a few degrees centigrade.

How has this study changed how we should measure animal personality, metabolic rate and the relationship between the two? First, because higher temperatures exacerbate the differences in behavioural and metabolic traits among individuals, and in the relationships among behaviour and metabolic rate, we suggest that behaviour and metabolic rate tests should either be conducted at a standardised temperature, or be

conducted at a range of temperatures, to make results comparable between studies. Second, we propose a change in methods used in repeatability analysis. Repeatability analysis is often performed on behavioural and metabolic data to confirm the presence of distinct personality and metabolic types, and, when tests are conducted across a range of temperatures, repeatability tests are temperature corrected under the assumption that all individuals respond to temperature in similar ways. However, because different individuals respond to temperatures in different ways, individual temperature corrections that reflect each individual's rate of change in behaviour or metabolic rate with temperature should be used to more accurately temperature correct data and obtain more reliable repeatability estimates. Third, we advocate that all analysis should be conducted separately on male and female data. Doing so, in conjunction with the above-described changes, should lead to a better understanding of animal personality, metabolic rate, and the relationship between the two.

Our study is the first to investigate links between intraspecific variation in the thermal plasticity of personality and metabolic rate, and to measure the sex-specificity reaction norms. We suggest that differences in the thermal plasticity of metabolic rate and personality within individuals may alter the strength of metabolic rate – behaviour relationships at different temperatures and suggest that these factors may help to explain why evidence for metabolic rate – behaviour relationships remain mixed (Killen *et al*., 2013). We move the field of personality and metabolism forward, demonstrating the importance of studying intraspecific relationships that usually only consider intercept-level differences between individuals under a reaction norms perspective.

Chapter 8: General discussion

In this thesis, I aimed to: (1) investigate sex differences in and the effect of environmental conditions on intraspecific variation in traits and their covariance in *Carabus hortensis* ground beetles; and (2) understand what the ecological consequences of extrinsic and intrinsic factors influencing intraspecific variation in traits and their covariance could be for *C. hortensis,* in a changing world.

I have shown that male and female *Carabus hortensis* differ in their body sizes (Chapter 2), in the range of temperatures over which they are active (Chapter 3), in the ecological significance of personality (Chapter 4), and in the relationships between intraspecific traits (Chapter 5). I have demonstrated that individual differences in traits arise due to different environmental pressures (Chapter 2), that temperature can change the relationships between metabolic rate and personality (Chapter 5), and that *C. hortensis* individuals with different personalities and metabolic rates respond to temperature in different ways (Chapter 3, 7), which exacerbates the level of intraspecific variation perceived in the population (Chapter 7). Together these findings support the hypothesis that intraspecific variation in traits can be attributed to sex, temperature and other environmental factors, highlight the importance of studying traits, their relationships with environmental variables such as temperature at the intraspecific level, and provide insight into the potential consequences of climate warming for *C. hortensis* at both the individual and population levels. Below, I use the findings of my thesis to suggest measures that should be taken when investigating intraspecific traits and their covariance going forward, identify areas for further research, and describe predictions for how *C. hortensis* may fair under increasing environmental change.

8.1 Discussions of results and future directions

8.1.1 The importance of intraspecific variation in thermal plasticity

Over several thesis chapters I investigated intraspecific traits, their inter-relationships at the population-level, and their thermal dependency, allowing for individual differences in average-level traits. In Chapter 7, however, I investigated the thermal dependency of intraspecific traits at the population as well as the individual levels, using a reaction norms approach. I found that different individuals demonstrated different levels of plasticity in their responses of metabolic rate and personality to temperature. Importantly, I found that such intraspecific differences in thermal plasticity altered both the perception of individual traits relative to other individuals within the population, sometimes altering the rank-order of individual metabolic rate and personality. However, I found only weak (females) or no (males) evidence to suggest that metabolic and behavioural thermal plasticity were similarly ranked within individuals, suggesting that the relationships between metabolic rate and personality may change in different individuals in different ways across increasing temperatures.

That metabolic rate changed with temperature in different ways in different individuals (Chapter 7) means that individuals may also differ in thermal dependency of metabolic rate scaling with body mass. Intraspecific variation in the thermal dependency of metabolic rate scaling should have additive effects in calculating both species' allometric scaling exponents and their thermal dependency. For instance, if many individuals within a species experience a reduction in the allometric scaling exponent with increasing temperatures whilst others experience the opposite, then species-specific

allometric scaling exponents may appear uninfluenced by temperature. Intraspecific variation in the thermal dependency of metabolic scaling may therefore help to explain why: (1) I (Chapter 6), and many others studying intraspecific metabolic scaling in ectotherms (*e.g.* Barneche *et al*., 2017; Gifford *et al*., 2013; Grigoriou & Richardson, 2009; Kar *et al*., 2021; Li *et al*., 2020; Melzner *et al*., 2007; Ohlberger *et al*., 2012; Paranjape, 1967) find no thermal dependency of metabolic rate scaling; and (2) that intraspecific scaling exponents vary from study to study. However, I know of no study that investigates the influence of interindividual variation in the thermal dependency of metabolic scaling and its potential influence on both the thermal dependency of species' metabolic scaling and on species' allometric scaling exponents. This therefore represents a major gap in the study of allometric scaling that must be addressed going forward.

The above findings and hypotheses stress the importance of considering individual differences in plasticity to different environmental contexts in studies investigating intraspecific traits and their covariance going forward. If individual traits and their covariances are not assessed over a range of environmental contexts, then researchers may wrongly interpret individual traits, population-level relationships, and form incorrect conclusions on both the fitness consequences of intraspecific trait variation, and evolutionary and ecological consequences of climate change for species. Although population-level relationships can be considered useful, I suggest that results obtained from studies that do not incorporate reaction norms analyses be considered with caution. I suggest that all future studies investigating intraspecific traits and their covariances at the population-level incorporate reaction norms analysis to determine to what extent the population-level relationships can be trusted, and to draw higher quality predictions and/or hypotheses from study findings.

8.1.2 The importance of sex differences in intraspecific traits and their covariance

Throughout this thesis I demonstrated that males and females often differ in the average level traits that they exhibit, in the strength and direction of relationships between intraspecific traits, and in the influence of temperature on their movement in the wild. In Chapter 3, I found that male and female *C. hortensis* do not differ in the distances that they cover, nor in the frequency of their movements. However, sex differences in and the relationships between traits may arise due to sex-differences in investment of differential tissues, or in the ecological significance of movement. For instance, in Chapter 4, I found that exploratory behaviour in the laboratory described a personality trait measured in the natural environment in males, but found no such relationship in females. Such differences in the ecological significance of movement may also help to explain why relationships between exploratory behaviour and metabolic rate differed between males and females (Chapter 5). Regardless of the cause of sex-differences in traits, their covariances and their thermal sensitivity, my findings exemplify the importance of differentiating between males and females when studying relationships between intraspecific traits or when predicting species' responses to climate change. However, studies that do not differentiate between males and females are still the norm.

8.1.3 Repeatability

In Chapters 4 and 5, I found that female, but not male, RMR and exploratory behaviour were repeatable over time. By definition, animals show personality differences when they demonstrate individual differences in behaviour that are repeatable over time

and/or contexts (Dall *et al.*, 2004; Eysenck & Eysenck, 1985). Many researching animal personality and other intraspecific traits, including myself, therefore exclude data from analysis when it is not significantly repeatable. However, when repeated measures are taken across variable temperatures, as was the case in Chapters 4 and 5, lack of repeatability may in fact indicate high thermal plasticity across study subjects. Lack of male but presence of female exploratory behaviour and RMR repeatability in Chapters 4 and 5 may therefore suggest that male *C. hortensis* experience greater changes in their behaviour and metabolic rate across thermal contexts than females. Given that plasticity is often highly correlated with animal personality and metabolic rate (*e.g.* Chapter 7), it may be wrong to exclude such data from further analysis and doing so may skew results. Repeatedly measuring intraspecific traits across variable or uncontrolled temperatures and other environmental contexts should therefore be avoided, so that data are not excluded from analyses unnecessarily. Instead, repeated measures should be taken at a series of carefully controlled temperatures or environmental conditions.

8.2 Consequences of temperature influences for *C. hortensis* under continued environmental warming

In this thesis, I provided explanations as to how the sex-dependence of and influence of temperature on intraspecific traits and their covariance may impact the fitness of individual *C. hortensis* beetles, as well as the survival of the species as a whole, under continued environmental warming. Here I bring together my findings to summarise the consequences of temperature's influence on intraspecific traits and their covariance for *C. hortensis* under climate change.

In Chapter 3, I discussed how greater sensitivity of larger *C. hortensis* females, who should have larger bodies and legs and should therefore be capable of travelling over farther distances, to higher temperatures could limit the ability for the species to adapt to climate change through range expansions and shifts. However, the capacity for male *C. hortensis* to adapt to climate change through range expansions/ shifts may too become limited at higher environmental temperatures, since traits that are often positively related to body size (*i.e.* high metabolic rate and exploratory behaviour) (*e.g.* Sinn *et al*., 2006) are likely to be selected against under warmer climates owing to the high energetic cost of their high thermal plasticity (Chapter 7). We may further expect an overall reduction in the body size of *C. hortensis* beetles under climate change because, as unpredictable weather events increase (Ummenhofer & Meehl, 2017), the inflexible and often energetically expensive nature of trait covariances may negatively impact fitness. This may cause selection away from fixed trait strategies and their associated covariances, towards trait flexibility (Hämäläinen *et al*., 2020), to which small body size is often related (*e.g.* Baldwin *et al*., 2022). Because, in insects, large body sizes are linked to fecundity (Forrest, 1987; Honěk, 1993) this may have further negative consequences for *C. hortensis* population densities.

Despite the above-described constraints on the ability of *C. hortensis* to adapt to climate change through range expansions, they may be able to do so via other means. In Chapter 3, I found large intraspecific variation in *C. hortensis* upper thermal tolerance values, and that the average thermal optima of the population reflected the average environmental temperature experienced by active beetles. In Chapter 7, I also found large intraspecific variation in the thermal plasticity of exploratory behaviour and metabolic rate. Given that intraspecific variation provides the raw material for natural

selection to act, these findings suggest that *C. hortensis* may be able to adapt to climate change through a shift in thermal tolerance values and thermal plasticity. Indeed, populations that demonstrate large inter-individual variation are generally more successful in overcoming environmental change than those with low levels of variation (Forsman & Wennersten, 2016).

8.3 Predicting responses of other species to climate change: a case-by-case basis

To what extent can the predictions outlined in this thesis be applied to species more generally? Integrating the biology of the study species, including sex differences in ecology, will be essential when predicting the potential responses of species' to climate change, and some predictions presented in this thesis may be applicable only to *C. hortensis* and closely related species. For instance, many predictions presented in this thesis are based upon the 'random walk' versus 'directed movement' of carabid beetles, and would not be applicable to species that: (1) do not move in similar ways; or (2) do move in similar ways, but differ from *C. hortensis* in the ecological significance of that movement. Nevertheless, some predictions made in this thesis may be generalisable across species. For instance, my hypothesis that that we should observe declines in *C. hortensis* population densities under increasing environmental temperatures (Chapter 3) may be extended to other species, because the factors that contributed towards this prediction (*i.e.* that larger females were more sensitive to heat, and larger individuals are often more fecund) are generally true of many species. Other predictions made based on sex-differences in this thesis may also be applicable to other species, because divergent selection pressures (*i.e.* females typically investing more into reproduction than males)

often act upon males and females to produce differences in traits (Bateman, 1948; Darwin, 1871; Trivers, 1972).

8.4 Conclusions

I have demonstrated that sex differences, temperature and other environmental conditions do influence intraspecific variation in traits and their covariance in *C. hortensis.* Clearly, sex differences and individual differences in thermal plasticity have important implications for how we perceive intraspecific traits and their covariances. I have therefore outlined a framework for future studies investigating intraspecific variation to incorporate information on thermal plasticity on a sex-specific basis, which will hopefully increase understanding of the true relationships between traits. I hope that the results and interpretations presented in this thesis will motivate further studies investigating the roles of intrinsic and extrinsic factors in influencing intraspecific variation in traits and their covariance, to better understand the evolutionary and ecological consequences of intraspecific variation both for individual fitness and under climate change.

References

Abram, P. K., Boivin, G., Moiroux, J., & Brodeur, J. (2017). Behavioural effects of temperature on ectothermic animals: Unifying thermal physiology and behavioural plasticity. *Biological Reviews*, *92*(4), 1859–1876. https://doi.org/10.1111/brv.12312

Agnani, P., Thomson, J., Schradin, C., & Careau, V. (2020). The fast and the curious II: Performance, personality, and metabolism in Karoo bush rats. *Behavioral Ecology and Sociobiology*, *74*(10), 123. https://doi.org/10.1007/s00265-020-02908-y

Alford, R. A., Brown, G. P., Schwarzkopf, L., Phillips, B. L., & Shine, R. (2009). Comparisons through time and space suggest rapid evolution of dispersal behaviour in an invasive species. *Wildlife Research*, *36*(1), 23. https://doi.org/10.1071/WR08021

Andersson, M. (1994). *Sexual selection*. Princeton University Press.

Andrewartha, H. G., & Birch, L. C. (1954). *The distribution and abundance of animals*. University of Chicago Press.

Angilletta, M. J. (2009). *Thermal adaptation: A theoretical and empirical synthesis.* Oxford University Press.

Aplin, L. M., Farine, D. R., Morand-Ferron, J., Cole, E. F., Cockburn, A., & Sheldon, B. C. (2013). Individual personalities predict social behaviour in wild networks of great tits (*Parus major*). *Ecology Letters*, *16*(11), 1365–1372. https://doi.org/10.1111/ele.12181

Ariyomo, T. O., Carter, M., & Watt, P. J. (2013). Heritability of boldness and aggressiveness in the zebrafish. *Behavior Genetics*, *43*(2), 161–167. https://doi.org/10.1007/s10519-013-9585-y

Arnold, P. A., Cassey, P., & White, C. R. (2017). Functional traits in red flour beetles: The dispersal phenotype is associated with leg length but not body size nor metabolic rate. *Functional Ecology*, *31*(3), 653–661. https://doi.org/10.1111/1365-2435.12772

Artacho, P., Jouanneau, I., & Le Galliard, J.-F. (2013). Interindividual variation in thermal sensitivity of maximal sprint speed, thermal behavior, and resting metabolic rate in a lizard. *Physiological and Biochemical Zoology: Ecological and Evolutionary Approaches*, *86*(4), 458–469. JSTOR. https://doi.org/10.1086/671376

Atkinson, D. (1994). Temperature and organism size—A biological law for ectotherms? In M. Begon & A. H. Fitter (Eds.), *Advances in Ecological Research* (Vol. 25, pp. 1–58). Academic Press. https://doi.org/10.1016/S0065-2504(08)60212-3

Baars, M. A. (1979a). Catches in pitfall traps in relation to mean densities of carabid beetles. *Oecologia*, *41*(1), 25–46. https://doi.org/10.1007/BF00344835

Baars, M. A. (1979b). Patterns of movement of radioactive carabid beetles. *Oecologia*, *44*(1), 125–140. https://doi.org/10.1007/BF00346411

Baktoft, H., Jacobsen, L., Skov, C., Koed, A., Jepsen, N., Berg, S., Boel, M., Aarestrup, K., & Svendsen, J. C. (2016). Phenotypic variation in metabolism and morphology correlating with animal swimming activity in the wild: Relevance for the OCLTT (oxygen- and capacity-limitation of thermal tolerance), allocation and performance models. *Conservation Physiology*, *4*(1), cov055. https://doi.org/10.1093/conphys/cov055

Baldwin, J. W., Garcia-Porta, J., & Botero, C. A. (2022). Phenotypic responses to climate change are significantly dampened in big-brained birds. *Ecology Letters*, *n/a*(n/a). https://doi.org/10.1111/ele.13971

Barlow, G. W. (1986). Mate choice in the monogamous and polychromatic Midas cichlid, *Cichlasoma citrinellum*. *Journal of Fish Biology*, *29*(sa), 123–133. https://doi.org/10.1111/j.1095-8649.1986.tb05004.x

Barneche, D. R., White, C. R., & Marshall, D. J. (2017). Temperature effects on mass-scaling exponents in colonial animals: A manipulative test. *Ecology*, *98*(1), 103–111. https://doi.org/10.1002/ecy.1624

Barton, K. (2009). *Mu-MIn: Multi-model inference.* (R Package Version 0.12.2/r18) [Computer software]. http://R-Forge.R-project.org/projects/mumin/

Baškiera, S., & Gvoždík, L. (2019). Repeatability of thermal reaction norms for spontaneous locomotor activity in juvenile newts. *Journal of Thermal Biology*, *80*, 126–132. https://doi.org/10.1016/j.jtherbio.2019.01.010

Bateman, A. J. (1948). Intra-sexual selection in *Drosophila*. *Heredity*, *2*(3), 349–368. https://doi.org/10.1038/hdy.1948.21

Bates, D., Maechler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*(1), 1–48. https://doi.org/10.18637/jss.v067.i01

Baumgartner, R. (2000). Sexual attraction in *Carabus auronitens* F.: Males lured by females. In P. Brandmayr, G. L. Lövei, T. Zetto-Brandmayr, A. Casale, & A. Vigna Taglianti (Eds.), *Natural history and applied ecology of carabid beetles. Proceedings of IX European Carabidologists' Meeting.* (pp. 139–145). Pensoft.

Beaman, J. E., White, C. R., & Seebacher, F. (2016). Evolution of plasticity: Mechanistic link between development and reversible acclimation. *Trends in Ecology & Evolution*, *31*(3), 237–249. https://doi.org/10.1016/j.tree.2016.01.004

Bell, A. M. (2007). Future directions in behavioural syndromes research. *Proceedings of the Royal Society B: Biological Sciences*, *274*(1611), 755–761. https://doi.org/10.1098/rspb.2006.0199

Bence, J. R., & Murdoch, W. W. (1986). Prey size selection by the mosquitofish: Relation to optimal diet theory. *Ecology*, *67*(2), 324–336. https://doi.org/10.2307/1938576

Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: A practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society: Series B (Methodological)*, *57*(1), 289–300.

Bennett, A. F. (1985). Temperature and muscle. *Journal of Experimental Biology*, *115*(1), 333–344. https://doi.org/10.1242/jeb.115.1.333

Bergstrom, C. A., Alba, J., Pacheco, J., Fritz, T., & Tamone, S. L. (2019). Polymorphism and multiple correlated characters: Do flatfish asymmetry morphs also differ in swimming performance and metabolic rate? *Ecology and Evolution*, *9*(8), 4772–4782. https://doi.org/10.1002/ece3.5080

Berthouly-Salazar, C., van Rensburg, B. J., Le Roux, J. J., van Vuuren, B. J., & Hui, C. (2012). Spatial sorting drives morphological variation in the invasive bird, *Acridotheris tristis*. *PLoS ONE*, *7*(5), 1–9. https://doi.org/10.1371/journal.pone.0038145

Berven, K. A., & Gill, D. E. (1983). Interpreting geographic variation in life-history traits. *American Zoologist*, *23*(1), 85–97. https://doi.org/10.1093/icb/23.1.85

Berwaerts, K., Dyck, H. V., & Aerts, P. (2002). Does flight morphology relate to flight performance? An experimental test with the butterfly *Pararge aegeria*. *Functional Ecology*, *16*(4), 484–491. https://doi.org/10.1046/j.1365-2435.2002.00650.x

Biro, P. A., Beckmann, C., & Stamps, J. A. (2010). Small within-day increases in temperature affects boldness and alters personality in coral reef fish. *Proceedings of the Royal Society B: Biological Sciences*, *277*(1678), 71–77. https://doi.org/10.1098/rspb.2009.1346

Biro, P. A., & Stamps, J. A. (2010). Do consistent individual differences in metabolic rate promote consistent individual differences in behavior? *Trends in Ecology & Evolution*, *25*(11), 653–659. https://doi.org/10.1016/j.tree.2010.08.003

Biro, P. A., Thomas, F., Ujvari, B., Adriaenssens, B., & Beckmann, C. (2020). Spontaneous activity rates and resting metabolism: Support for the allocation model of energy management at the among-individual level. *Ethology*, *126*(1), 32–39. https://doi.org/10.1111/eth.12957

Blumstein, D., Evans, C., & Daniel, J. (2000). *JWatcher* (0.9) [Computer software]. Animal Behaviour Laboratory, Macquarie University.

Bodden, V., & Puschendorf, R. (2019). Morphological divergence and reduced ectoparasite prevalence in an introduced population of a Caribbean anole. *Journal of Zoology*, *308*(3), 188–196. https://doi.org/10.1111/jzo.12664

Bokma, F. (2004). Evidence against universal metabolic allometry. *Functional Ecology*, *18*(2), 184–187. https://doi.org/10.1111/j.0269-8463.2004.00817.x

Bolnick, D. I., Svanbäck, R., Fordyce, J. A., Yang, L. H., Davis, J. M., Hulsey, C. D., & Forister, M. L. (2003). The ecology of individuals: Incidence and implications of individual specialization. *The American Naturalist*, *161*(1), 1–28. https://doi.org/10.1086/343878

Bonte, D., Dyck, H. V., Bullock, J. M., Coulon, A., Delgado, M., Gibbs, M., Lehouck, V., Matthysen, E., Mustin, K., Saastamoinen, M., Schtickzelle, N., Stevens, V. M., Vandewoestijne, S., Baguette, M., Barton, K., Benton, T. G., Chaput-Bardy, A., Clobert, J., Dytham, C., … Travis, J. M. J. (2012). Costs of dispersal. *Biological Reviews*, *87*(2), 290–312. https://doi.org/10.1111/j.1469-185X.2011.00201.x

Bouwhuis, S., Quinn, J. L., Sheldon, B. C., & Verhulst, S. (2014). Personality and basal metabolic rate in a wild bird population. *Oikos*, *123*(1), 56–62. https://doi.org/10.1111/j.1600-0706.2013.00654.x

Bowler, D. E., & Benton, T. G. (2005). Causes and consequences of animal dispersal strategies: Relating individual behaviour to spatial dynamics. *Biological Reviews*, *80*(2), 205–225. https://doi.org/10.1017/S1464793104006645

Brakefield, P. M. (1996). Seasonal polyphenism in butterflies and natural selection. *Trends in Ecology & Evolution*, *11*(7), 275–277. https://doi.org/10.1016/0169-5347(96)30025-6

Brandner, J., Cerwenka, A. F., Schliewen, U. K., & Geist, J. (2013). Bigger is better: Characteristics of round gobies forming an invasion front in the Danube river. *PLoS ONE*, *8*(9), 1–15. https://doi.org/10.1371/journal.pone.0073036

Brans, K. I., Jansen, M., Vanoverbeke, J., Tüzün, N., Stoks, R., & De Meester, L. (2017). The heat is on: Genetic adaptation to urbanization mediated by thermal tolerance and body size. *Global Change Biology*, *23*(12), 5218–5227. https://doi.org/10.1111/gcb.13784

Briffa, M., Bridger, D., & Biro, P. A. (2013). How does temperature affect behaviour? Multilevel analysis of plasticity, personality and predictability in hermit crabs. *Animal Behaviour*, *86*(1), 47–54. https://doi.org/10.1016/j.anbehav.2013.04.009

Brodie, E. D. (1993). Homogeneity of the genetic variance-covariance matrix for antipredator traits in two natural populations of the garter snake *Thamnophis ordinoides*. *Evolution*, *47*(3), 844–854. https://doi.org/10.1111/j.1558-5646.1993.tb01238.x

Brooks, M. E., Kristensen, K., van Benthem, K. J., Magnusson, A., Berg, C. W., Nielsen, A., Skaug, H. J., Maechler, M., & Bolker, B. M. (2017). GlmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *The R Journal*, *9*(2), 378–400.

Brown, G. P., Kelehear, C., & Shine, R. (2013). The early toad gets the worm: Cane toads at an invasion front benefit from higher prey availability. *Journal of Animal Ecology*, *82*(4), 854–862. https://doi.org/10.1111/1365-2656.12048

Brown, G. P., Phillips, B. L., & Shine, R. (2014). The straight and narrow path: The evolution of straight-line dispersal at a cane toad invasion front. *Proceedings of the Royal Society B: Biological Sciences*, *281*(1795), 20141385. https://doi.org/10.1098/rspb.2014.1385

Brown, J. H. (1984). On the relationship between abundance and distribution of species. *The American Naturalist*, *124*(2), 255–279.

Brown, J. H., Gillooly, J. F., Allen, A. P., Savage, V. M., & West, G. B. (2004). Toward a metabolic theory of ecology. *Ecology*, *85*(7), 1771–1789. https://doi.org/10.1890/03-9000

Bruneel, S., Gobeyn, S., Verhelst, P., Reubens, J., Moens, T., & Goethals, P. (2018). Implications of movement for species distribution models—Rethinking environmental data tools. *The Science of the Total Environment*, *628–629*, 893–905. https://doi.org/10.1016/j.scitotenv.2018.02.026

Burggren, W., Souder, B. M., & Ho, D. H. (2017). Metabolic rate and hypoxia tolerance are affected by group interactions and sex in the fruit fly (*Drosophila melanogaster*): New data and a literature survey. *Biology Open*, *6*(4), 471–480. https://doi.org/10.1242/bio.023994

Bürkner, P.-C. (2017). brms: An R package for Bayesian multilevel models using stan. *Journal of Statistical Software*, *80*, 1–28. https://doi.org/10.18637/jss.v080.i01

Burton, T., Killen, S. S., Armstrong, J. D., & Metcalfe, N. B. (2011). What causes intraspecific variation in resting metabolic rate and what are its ecological consequences? *Proceedings of the Royal Society B: Biological Sciences*, *278*(1724), 3465–3473. https://doi.org/10.1098/rspb.2011.1778

Campbell, T. S., & Echternacht, A. C. (2003). Introduced species as moving targets: Changes in body sizes of introduced lizards following experimental introductions and historical invasions. *Biological Invasions*, *5*(3), 193–212.

Careau, V., & Garland, T. (2012). Performance, personality, and energetics: Correlation, causation, and mechanism. *Physiological and Biochemical Zoology*, *85*(6), 543–571. https://doi.org/10.1086/666970

Careau, V., Gifford, M. E., & Biro, P. A. (2014). Individual (co)variation in thermal reaction norms of standard and maximal metabolic rates in wild-caught slimy salamanders. *Functional Ecology*, *28*(5), 1175–1186. https://doi.org/10.1111/1365-2435.12259

Careau, V., Mariette, M. M., Crino, O., Buttemer, W. A., & Buchanan, K. L. (2020). Repeatability of behavior and physiology: No impact of reproductive investment. *General and Comparative Endocrinology*, *290*, 113403. https://doi.org/10.1016/j.ygcen.2020.113403

Careau, V., Thomas, D., Humphries, M. M., & Réale, D. (2008). Energy metabolism and animal personality. *Oikos*, *117*(5), 641–653. https://doi.org/10.1111/j.0030-1299.2008.16513.x

Careau, V., Thomas, D., Pelletier, F., Turki, L., Landry, F., Garant, D., & Réale, D. (2011). Genetic correlation between resting metabolic rate and exploratory behaviour in deer mice (*Peromyscus maniculatus*). *Journal of Evolutionary Biology*, *24*(10), 2153–2163. https://doi.org/10.1111/j.1420-9101.2011.02344.x

Carter, A. J., Feeney, W. E., Marshall, H. H., Cowlishaw, G., & Heinsohn, R. (2013). Animal personality: What are behavioural ecologists measuring? *Biological Reviews*, *88*(2), 465–475. https://doi.org/10.1111/brv.12007

Charrier, S., Petit, S., & Burel, F. (1997). Movements of *Abax parallelepipedus* (Coleoptera, Carabidae) in woody habitats of a hedgerow network landscape: A radio-tracing study. *Agriculture, Ecosystems & Environment*, *61*(2), 133–144. https://doi.org/10.1016/S0167-8809(96)01101-2

Christian, K. A., & Tracy, C. R. (1981). The effect of the thermal environment on the ability of hatchling Galapagos land iguanas to avoid predation during dispersal. *Oecologia*, *49*(2), 218–223. https://doi.org/10.1007/BF00349191

Chuang, A., & Peterson, C. R. (2016). Expanding population edges: Theories, traits, and trade-offs. *Global Change Biology*, *22*(2), 494–512. https://doi.org/10.1111/gcb.13107

Clarke, A. (1993). Seasonal acclimatization and latitudinal compensation in metabolism: Do they exist? *Functional Ecology*, *7*(2), 139–149. https://doi.org/10.2307/2389880

Clarke, A. (2004). Is there a universal temperature dependence of metabolism? *Functional Ecology*, *18*(2), 252–256. https://doi.org/10.1111/j.0269-8463.2004.00842.x

Clarke, A., & Fraser, K. P. P. (2004). Why does metabolism scale with temperature? *Functional Ecology*, *18*(2), 243–251. https://doi.org/10.1111/j.0269-8463.2004.00841.x

Clarke, A., & Johnston, N. M. (1999). Scaling of metabolic rate with body mass and temperature in teleost fish. *Journal of Animal Ecology*, *68*(5), 893–905. https://doi.org/10.1046/j.1365-2656.1999.00337.x

Clarke, A., Rothery, P., & Isaac, N. J. B. (2010). Scaling of basal metabolic rate with body mass and temperature in mammals. *Journal of Animal Ecology*, *79*(3), 610–619. https://doi.org/10.1111/j.1365-2656.2010.01672.x

Cohen, J. E., Pimm, S. L., Yodzis, P., & Saldana, J. (1993). Body sizes of animal predators and animal prey in food webs. *The Journal of Animal Ecology*, *62*(1), 67. https://doi.org/10.2307/5483

Conover, D. O., & Present, T. M. C. (1990). Counter gradient variation in growth rate: Compensation for length of the growing season among Atlantic silversides from different latitudes. *Oecologia*, *83*(3), 316–324. https://doi.org/10.1007/BF00317554

Cordero, P. J., Wetton, J. H., & Parkin, D. T. (1999). Extra-pair paternity and male badge size in the house sparrow. *Journal of Avian Biology*, *30*(1), 97–102. https://doi.org/10.2307/3677248

Cornwell, T. O., McCarthy, I. D., & Biro, P. A. (2020). Integration of physiology, behaviour and life history traits: Personality and pace of life in a marine gastropod. *Animal Behaviour*, *163*, 155–162.

Cornwell, T. O., McCarthy, I. D., Snyder, C. R. A., & Biro, P. A. (2019). The influence of environmental gradients on individual behaviour: Individual plasticity is consistent across risk and temperature gradients. *Journal of Animal Ecology*, *88*(4), 511–520. https://doi.org/10.1111/1365-2656.12935

Cotton, S., Fowler, K., & Pomiankowski, A. (2004). Condition dependence of sexual ornament size and variation in the stalk-eyed fly *Cyrtodiopsis dalmanni* (diptera: Diopsidae). *Evolution*, *58*(5), 1038–1046. https://doi.org/10.1111/j.0014-3820.2004.tb00437.x

Crawley, M. J. (2007). *The R Book*. John Wiley & Sons, Ltd.

Dall, S., & Griffith, S. (2014). An empiricist guide to animal personality variation in ecology and evolution. *Frontiers in Ecology and Evolution*, *2*, 3. https://doi.org/10.3389/fevo.2014.00003

Dall, S. R. X., Houston, A. I., & McNamara, J. M. (2004). The behavioural ecology of personality: Consistent individual differences from an adaptive perspective. *Ecology Letters*, *7*(8), 734–739. https://doi.org/10.1111/j.1461-0248.2004.00618.x

Dammhahn, M., Dingemanse, N. J., Niemelä, P. T., & Réale, D. (2018). Pace-of-life syndromes: A framework for the adaptive integration of behaviour, physiology and life history. *Behavioral Ecology and Sociobiology*, *72*(3), 62, s00265-018-2473-y. https://doi.org/10.1007/s00265-018-2473-y

Darwin, C. (1871). *The descent of man, and selection in relation to sex*. John Murray.

Denno, R. F., Douglas, L. W., & Jacobs, D. (1985). Crowding and host plant nutrition: Environmental determinants of wing-form in *Prokelisia marginata*. *Ecology*, *66*(5), 1588–1596. https://doi.org/10.2307/1938021

Des Roches, S., Post, D. M., Turley, N. E., Bailey, J. K., Hendry, A. P., Kinnison, M. T., Schweitzer, J. A., & Palkovacs, E. P. (2018). The ecological importance of intraspecific variation. *Nature Ecology & Evolution*, *2*(1), 57–64. https://doi.org/10.1038/s41559-017-0402-5

Dingemanse, N. J., Both, C., Drent, P. J., & Tinbergen, J. M. (2004). Fitness consequences of avian personalities in a fluctuating environment. *Proceedings of the Royal Society B: Biological Sciences*, *271*(1541), 847–852.

Dingemanse, N. J., Both, C., Drent, P. J., Van Oers, K., & Van Noordwijk, A. J. (2002). Repeatability and heritability of exploratory behaviour in great tits from the wild. *Animal Behaviour*, *64*, 929–938. https://doi.org/10.1006/anbe.2002.2006

Dingemanse, N. J., Both, C., van Noordwijk, A. J., Rutten, A. L., & Drent, P. J. (2003). Natal dispersal and personalities in great tits (*Parus major*). *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *270*(1516), 741–747. https://doi.org/10.1098/rspb.2002.2300

Dingemanse, N. J., Kazem, A. J. N., Réale, D., & Wright, J. (2010). Behavioural reaction norms: Animal personality meets individual plasticity. *Trends in Ecology & Evolution*, *25*(2), 81–89. https://doi.org/10.1016/j.tree.2009.07.013

Dingemanse, N. J., & Wolf, M. (2013). Between-individual differences in behavioural plasticity within populations: Causes and consequences. *Animal Behaviour*, *85*(5), 1031–1039. https://doi.org/10.1016/j.anbehav.2012.12.032

Dobson, F. S., Risch, T. S., & Murie, J. O. (1999). Increasing returns in the life history of Columbian ground squirrels. *Journal of Animal Ecology*, *68*(1), 73–86. https://doi.org/10.1046/j.1365-2656.1999.00268.x

Dochtermann, N. A., Schwab, T., & Sih, A. (2015). The contribution of additive genetic variation to personality variation: Heritability of personality. *Proceedings of the Royal Society B: Biological Sciences*, *282*(1798), 20142201. https://doi.org/10.1098/rspb.2014.2201

Dodds, P. S., Rothman, D. H., & Weitz, J. S. (2001). Re-examination of the "3/4-law" of Metabolism. *Journal of Theoretical Biology*, *209*(1), 9–27. https://doi.org/10.1006/jtbi.2000.2238

Doherty, J. A., & Callos, J. D. (1991). Acoustic communication in the trilling field cricket, *Gryllus rubens* (Orthoptera: Gryllidae). *Journal of Insect Behavior*, *4*(1), 67–82. https://doi.org/10.1007/BF01092552

Dolbear, A. E. (1897). The Cricket as a Thermometer. *The American Naturalist*, *31*(371), 970–971. https://doi.org/10.1086/276739

Drees, C., Brandmayr, P., Buse, J., Dieker, P., Gurlich, S., Habel, J., Harry, I., Hardtle, W., Matern, A., Meyer, H., Pizzoloto, R., Quante, M., Shafer, K., Schuldt, A., Taboada Palomares, A., & Assmann, T. (2011). Poleward range expansion without a southern contraction in the ground beetle *Agonum viridicupreum* (Coleoptera, Carabidae). *ZooKeys*, *100*, 333–352. https://doi.org/10.3897/zookeys.100.1535

Drees, C., & Huk, T. (2000). Sexual differences in locomotory activity of the ground beetle *Carabus granulatus* L. In P. Brandmayr, G. L. Lovei, T. Z. Brandmayr, A. Casale, & A. Vigna Taglianti (Eds.), *Natural history and applied ecology of carabid beetles.* (pp. 133–138). Pensoft Publishers.

Drees, C., Matern, A., & Assmann, T. (2008). Behavioural patterns of nocturnal carabid beetles determined by direct observations under red-light conditions. In L. Penev, T. Erwin, & T. Assmann (Eds.), *Back to the Roots and Back to the Future? Towards a New Synthesis between Taxonomic, Ecological and Biogeographical Approaches in Carabidology* (pp. 415–429). Pensoft Publishers.

Drent, P. J., Oers, K. van, & Noordwijk, A. J. van. (2003). Realized heritability of personalities in the great tit (Parus major). *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *270*(1510), 45–51. https://doi.org/10.1098/rspb.2002.2168

Duffy, M. A. (2010). Ecological consequences of intraspecific variation in lake Daphnia. *Freshwater Biology*, *55*(5), 995–1004.

Duffy, M. A., & Sivars-Becker, L. (2007). Rapid evolution and ecological host–parasite dynamics. *Ecology Letters*, *10*(1), 44–53. https://doi.org/10.1111/j.1461-0248.2006.00995.x

Edleston, R. S. (1864). First carbonaria melanic of moth *Biston betularia*. *Entomologist*, *2*, 150.

Edmunds, L. N., JR. (1963). The relation between temperature and flashing intervals in adult male fireflies, *Photinus pyralis*. *Annals of the Entomological Society of America*, *56*(5), 716–718. https://doi.org/10.1093/aesa/56.5.716

Ernst, C. M., & Buddle, C. M. (2013). Seasonal patterns in the structure of epigeic beetle (Coleoptera) assemblages in two subarctic habitats in Nunavut, Canada. *The Canadian Entomologist*, *145*(2), 171–183. https://doi.org/10.4039/tce.2012.111

Etheredge, R. I., Avenas, C., Armstrong, M. J., & Cummings, M. E. (2018). Sex-specific cognitive-behavioural profiles emerging from individual variation in numerosity discrimination in *Gambusia affinis*. *Animal Cognition*, *21*(1), 37–53. https://doi.org/10.1007/s10071-017-1134-2

European Environment Agency. (2012). *Climate change, impacts and vulnerability in Europe 2012* (EEA Report No. 1725–9177; pp. 1–304). European Environment Agency.

Eysenck, H. J., & Eysenck, M. W. (1985). *Personality and individual differences: A natural science approach.* Plenum Press.

Fairbanks, L. A., Newman, T. K., Bailey, J. N., Jorgensen, M. J., Breidenthal, S. E., Ophoff, R. A., Comuzzie, A. G., Martin, L. J., & Rogers, J. (2004). Genetic contributions to social impulsivity and aggressiveness in vervet monkeys. *Biological Psychiatry*, *55*(6), 642–647. https://doi.org/10.1016/j.biopsych.2003.12.005

Fisher, D. N., James, A., Rodríguez-Muñoz, R., & Tregenza, T. (2015). Behaviour in captivity predicts some aspects of natural behaviour, but not others, in a wild cricket population. *Proceedings of the Royal Society B: Biological Sciences*, *282*(1809), 20150708. https://doi.org/10.1098/rspb.2015.0708

Fisher, R. A. (1918). The correlation between relatives on the supposition of Mendelian inheritance. *Transactions of the Royal Society of Edinburgh*, *52*(2), 399–433.

Fisher, R. A. (1930). *The Genetical Theory of Natural Selection*. Oxford University Press.

Forrest, T. G. (1987). Insect size tactics and developmental strategies. *Oecologia*, *73*(2), 178–184. https://doi.org/10.1007/BF00377505

Forsman, A., Ringblom, K., Civantos, E., & Ahnesjo, J. (2002). Coevolution of color pattern and thermoregulatory behavior in polymorphic pygmy grasshoppers *Tetrix undulata*. *Evolution*, *56*(2), 349–360. https://doi.org/10.1111/j.0014-3820.2002.tb01345.x

Forsman, A., & Wennersten, L. (2016). Inter-individual variation promotes ecological success of populations and species: Evidence from experimental and comparative studies. *Ecography*, *39*(7), 630–648. https://doi.org/10.1111/ecog.01357

Fox, J., & Weisberg, S. (2019). *An R Companion to Applied Regression* (Third). Sage.

Franken, O., Huizinga, M., Ellers, J., & Berg, M. P. (2018). Heated communities: Large inter- and intraspecific variation in heat tolerance across trophic levels of a soil arthropod community. *Oecologia*, *186*(2), 311–322. https://doi.org/10.1007/s00442-017-4032-z

Fraser, D. F., Gilliam, J. F., Daley, M. J., Le, A. N. T., & Skalski, G. T. (2001). Explaining leptokurtic movement distributions: Intrapopulation variation in boldness and exploration. *The American Naturalist*, *158*(2), 124–135. https://doi.org/10.1086/321307

Frazier, M. R., Woods, H. A., & Harrison, J. F. (2001). Interactive effects of rearing temperature and oxygen on the development of *Drosophila melanogaster*. *Physiological and Biochemical Zoology: PBZ*, *74*(5), 641–650. https://doi.org/10.1086/322172

Freude, H., Harde, K. W., & Lohse, G. A. (1976). *Die Käfer Mitteleuropas, Band 2, Adephaga 1: Carabidae*. Goecke & Evers.

Frizzi, F. (2018). Complexity of searching movement in the European harvester ant *Messor wasmanni*: Effect of temperature and body size. *Insectes Sociaux*, *65*(2), 263–273. https://doi.org/10.1007/s00040-018-0609-8

Fryxell, D. C., Arnett, H. A., Apgar, T. M., Kinnison, M. T., & Palkovacs, E. P. (2015). Sex ratio variation shapes the ecological effects of a globally introduced freshwater fish. *Proceedings of the Royal Society B: Biological Sciences*, *282*(1817), 20151970. https://doi.org/10.1098/rspb.2015.1970

Gabriel, W. (2006). Selective advantage of irreversible and reversible phenotypic plasticity. *Archiv Hydrobiol*, 1–20.

Gadgil, M., & Bossert, W. H. (1970). Life historical consequences of natural selection. *The American Naturalist*, *104*(935), 1–24. https://doi.org/10.1086/282637

Gerlach, A., Voigtländer, K., & Heidger, C. M. (2009). Influences of the behaviour of epigeic arthropods (Diplopoda, Chilopoda, Carabidae) on the efficiency of pitfall trapping. *Soil Organisms*, *81*(3), 773–790.

Giesing, E. R., Suski, C. D., Warner, R. E., & Bell, A. M. (2011). Female sticklebacks transfer information via eggs: Effects of maternal experience with predators on offspring. *Proceedings of the Royal Society B: Biological Sciences*, *278*(1712), 1753–1759. https://doi.org/10.1098/rspb.2010.1819

Gifford, M. E., Clay, T. A., & Peterman, W. E. (2013). The effects of temperature and activity on intraspecific scaling of metabolic rates in a lungless salamander. *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology*, *319*(4), 230–236. https://doi.org/10.1002/jez.1787

Gillooly, J. F., Brown, J. H., West, G. B., Savage, V. M., & Charnov, E. L. (2001). Effects of size and temperature on metabolic rate. *Science*, *293*(5538), 2248–2251. https://doi.org/10.1126/science.1061967

Giraudeau, M., Nolan, P. M., Black, C. E., Earl, S. R., Hasegawa, M., & McGraw, K. J. (2014). Song characteristics track bill morphology along a gradient of urbanization in house finches (*Haemorhous mexicanus*). *Frontiers in Zoology*, *11*(1), 83. https://doi.org/10.1186/s12983-014-0083-8

Glazier, D. S. (2005). Beyond the '3/4-power law': Variation in the intra- and interspecific scaling of metabolic rate in animals. *Biological Reviews*, *80*(4), 611–662. https://doi.org/10.1017/S1464793105006834

Glazier, D. S. (2009). Activity affects intraspecific body-size scaling of metabolic rate in ectothermic animals. *Journal of Comparative Physiology. B, Biochemical, Systemic, and Environmental Physiology*, *179*(7), 821–828. https://doi.org/10.1007/s00360-009-0363-3

Glazier, D. S. (2010). A unifying explanation for diverse metabolic scaling in animals and plants. *Biological Reviews*, *85*(1), 111–138. https://doi.org/10.1111/j.1469-185X.2009.00095.x

Gosling, S. D. (2001). From mice to men: What can we learn about personality from animal research? *Psychological Bulletin*, *127*(1), 45–86. https://doi.org/10.1037/0033-2909.127.1.45

Goulet, H. (1976). A method for rearing ground beetles (*Coleoptera: Carabidae*). *The Coleopterists Bulletin*, *30*(1), 33–36. JSTOR.

Green, A. J. (2001). Mass/length residuals: Measures of body condition or generators of spurious results? *Ecology*, *82*(5), 1473–1483. https://doi.org/10.1890/0012-9658(2001)082[1473:MLRMOB]2.0.CO;2

Grigoriou, P., & Richardson, C. A. (2009). Effect of body mass, temperature and food deprivation on oxygen consumption rate of common cuttlefish *Sepia officinalis*. *Marine Biology*, *156*(12), 2473–2481. https://doi.org/10.1007/s00227-009-1272-4

Gross, M. R. (1996). Alternative reproductive strategies and tactics: Diversity within sexes. *Trends in Ecology & Evolution*, *11*(2), 92–98. https://doi.org/10.1016/0169-5347(96)81050-0

Gudowska, A., Schramm, B. W., Czarnoleski, M., Antoł, A., Bauchinger, U., & Kozłowski, J. (2017a). Mass scaling of metabolic rates in carabid beetles (*Carabidae*) – the importance of phylogeny, regression models and gas exchange patterns. *The Journal of Experimental Biology*, *220*(18), 3363–3371. https://doi.org/10.1242/jeb.159293

Gudowska, A., Schramm, B. W., Czarnoleski, M., Kozłowski, J., & Bauchinger, U. (2017b). Physical mechanism or evolutionary trade-off? Factors dictating the relationship between metabolic rate and ambient temperature in carabid beetles. *Journal of Thermal Biology*, *68*, 89–95. https://doi.org/10.1016/j.jtherbio.2016.11.009

Gunnarsson, T. G., Sutherland, W. J., Alves, J. A., Potts, P. M., & Gill, J. A. (2012). Rapid changes in phenotype distribution during range expansion in a migratory bird. *Proceedings of the Royal Society B: Biological Sciences*, *279*(1727), 411–416. https://doi.org/10.1098/rspb.2011.0939

Günther, J. M., & Assmann, T. (2000). Competition in the woodland? Phenology, body mass and body length of coexisting *Carabus* species – preliminary results (Coleoptera, Carabidae). In P. Brandmayr, G. L. Lövei, T. Zetto-Brandmayr, A. Casale, & A. Vigna Taglianti (Eds.), *Natural history and applied ecology of Carabid beetles* (pp. 185–195). Pensoft.

Hadfield, J. D. (2010). MCMC methods for multi-response generalized linear mixed models: The MCMCglmm R package. *Journal of Statistical Software*, *33*(1), 1–22. https://doi.org/10.18637/jss.v033.i02

Hämäläinen, A., Guenther, A., Patrick, S. C., & Schuett, W. (2020). *Environmental effects on the covariation among pace-of-life traits*. https://doi.org/10.1111/eth.13098

Hämäläinen, A., Immonen, E., Tarka, M., & Schuett, W. (2018). Evolution of sex-specific pace-of-life syndromes: Causes and consequences. *Behavioral Ecology and Sociobiology*, *72*(3), 50. https://doi.org/10.1007/s00265-018-2466-x

Harris, C., Liedtke, J., Drees, C., & Schuett, W. (2020). Exploratory behaviour is not related to associative learning ability in the carabid beetle *Nebria brevicollis*. *Behavioural Processes*, *180*, 104224. https://doi.org/10.1016/j.beproc.2020.104224

Hasselmann, E.-M. (1962). Über die relative spektrale Empfindlichkeit von Käfer- und Schmetterlingsaugen bei verschiedenen Helligkeiten. *Zoologische Jahrbücher*, *69*, 537–576.

Heidinger, I. M. M., Hein, S., Feldhaar, H., & Poethke, H.-J. (2018). Biased dispersal of *Metrioptera bicolor*, a wing dimorphic bush-cricket. *Insect Science*, *25*(2), 297–308. https://doi.org/10.1111/1744-7917.12412

Hengeveld, R., & Haeck, J. (1982). The distribution of abundance. I. Measurements. *Journal of Biogeography*, *9*(4), 303–316. https://doi.org/10.2307/2844717

Herborn, K. A., Macleod, R., Miles, W. T. S., Schofield, A. N. B., Alexander, L., & Arnold, K. E. (2010). Personality in captivity reflects personality in the wild. *Animal Behaviour*, *79*(4), 835–843. https://doi.org/10.1016/j.anbehav.2009.12.026

Heusner, A. A. (1982). Energy metabolism and body size I. Is the 0.75 mass exponent of Kleiber's equation a statistical artifact? *Respiration Physiology*, *48*(1), 1–12. https://doi.org/10.1016/0034-5687(82)90046-9

Hill, J. K., Thomas, C. D., & Blakeley, D. S. (1999). Evolution of flight morphology in a butterfly that has recently expanded its geographic range. *Oecologia*, *121*(2), 165–170. https://doi.org/10.1007/s004420050918

Hill, S. J., Silcocks, S. C., & Andrew, N. R. (2020). Impacts of temperature on metabolic rates of adult *Extatosoma tiaratum* reared on different host plant species. *Physiological Entomology*, *45*(1), 7–15. https://doi.org/10.1111/phen.12310

Holder, J. L., Barlow, G. W., & Francis, R. C. (1991). Differences in aggressiveness in the Midas cichlid fish (*Cichlasoma citrinellum*) in relation to sex, reproductive state and the individual. *Ethology*, *88*(4), 297–306. https://doi.org/10.1111/j.1439-0310.1991.tb00284.x

Hollander, F. A., Overveld, T. V., Tokka, I., & Matthysen, E. (2008). Personality and nest defence in the great tit (*Parus major*). *Ethology*, *114*(4), 405–412. https://doi.org/10.1111/j.1439-0310.2008.01488.x

Honěk, A. (1993). Intraspecific variation in body size and fecundity in insects: A general relationship. *Oikos*, *66*(3), 483–492. https://doi.org/10.2307/3544943

Huey, R. B., Gilchrist, G. W., Carlson, M. L., Berrigan, D., & Serra, § Luís. (2000). Rapid evolution of a geographic cline in size in an introduced fly. *Science*, *287*(5451), 308–309. https://doi.org/10.1126/science.287.5451.308

Huey, R. B., & Kingsolver, J. G. (1989). Evolution of thermal sensitivity of ectotherm performance. *Trends in Ecology & Evolution*, *4*(5), 131–135. https://doi.org/10.1016/0169-5347(89)90211-5

Hughes, C. L., Hill, J. K., & Dytham, C. (2003). Evolutionary trade-offs between reproduction and dispersal in populations at expanding range boundaries. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *270*(Suppl_2). https://doi.org/10.1098/rsbl.2003.0049

Husak, J. F., & Fox, S. F. (2008). Sexual selection on locomotor performance. *Evolutionary Ecology Research*, *10*(2), 213–228.

Iba, M., Nagao, T., & Urano, A. (1995). Effects of population density on growth, behavior and levels of biogenic amines in the cricket, *Gryllus bimaculatus*. *Zoological Science*, *12*(6), 695–702. https://doi.org/10.2108/zsj.12.695

Immonen, E., Hämäläinen, A., Schuett, W., & Tarka, M. (2018). Evolution of sex-specific pace-of-life syndromes: Genetic architecture and physiological mechanisms. *Behavioral Ecology and Sociobiology*, *72*(3), 60. https://doi.org/10.1007/s00265-018-2462-1

IPCC. (2022). *Climate Change 2022: Impacts, Adaptation and Vulnerability*.

Isaac, N. J. B., & Carbone, C. (2010). Why are metabolic scaling exponents so controversial? Quantifying variance and testing hypotheses. *Ecology Letters*, *13*(6), 728–735. https://doi.org/10.1111/j.1461-0248.2010.01461.x

Jayatilaka, P., Narendra, A., Reid, S. F., Cooper, P., & Zeil, J. (2011). Different effects of temperature on foraging activity schedules in sympatric *Myrmecia* ants. *Journal of Experimental Biology*, *214*(16), 2730–2738. https://doi.org/10.1242/jeb.053710

Jump, A. S., Marchant, R., & Peñuelas, J. (2009). Environmental change and the option value of genetic diversity. *Trends in Plant Science*, *14*(1), 51–58. https://doi.org/10.1016/j.tplants.2008.10.002

Kar, F., Nakagawa, S., Friesen, C. R., & Noble, D. W. A. (2021). Individual variation in thermal plasticity and its impact on mass-scaling. *Oikos*, *130*(7), 1131–1142. https://doi.org/10.1111/oik.08122

Kar, F., Nakagawa, S., & Noble, D. W. A. (2022). Impact of developmental temperatures on thermal plasticity and repeatability of metabolic rate. *Evolutionary Ecology*. https://doi.org/10.1007/s10682-022-10160-1

Kern, E. M. A., Robinson, D., Gass, E., Godwin, J., & Langerhans, R. B. (2016). Correlated evolution of personality, morphology and performance. *Animal Behaviour*, *117*, 79–86. https://doi.org/10.1016/j.anbehav.2016.04.007

Kevan, P. G., & Shorthouse, J. D. (1970). Behavioural thermoregulation by high Arctic butterflies. *Arctic*, *23*(4), 268–279.

Killen, S. S., Atkinson, D., & Glazier, D. S. (2010). The intraspecific scaling of metabolic rate with body mass in fishes depends on lifestyle and temperature. *Ecology Letters*, *13*(2), 184–193. https://doi.org/10.1111/j.1461-0248.2009.01415.x

Killen, S. S., Marras, S., Metcalfe, N. B., McKenzie, D. J., & Domenici, P. (2013). Environmental stressors alter relationships between physiology and behaviour. *Trends in Ecology & Evolution*, *28*(11), 651–658. https://doi.org/10.1016/j.tree.2013.05.005

Kleiber, M. (1932). Body size and metabolism. *Hilgardia*, *6*(11), 315–353.

Klok, C. J., & Chown, S. L. (1999). Assessing the benefits of aggregation: Thermal biology and water relations of anomalous Emperor Moth caterpillars. *Functional Ecology*, *13*(3), 417–427. https://doi.org/10.1046/j.1365-2435.1999.00324.x

Kozłowski, J., Czarnołęski, M., & Dańko, M. (2004). Can optimal resource allocation models explain why ectotherms grow larger in cold? *Integrative and Comparative Biology*, *44*(6), 480–493. https://doi.org/10.1093/icb/44.6.480

Krams, I. A., Niemelä, P. T., Trakimas, G., Krams, R., Burghardt, G. M., Krama, T., Kuusik, A., Mänd, M., Rantala, M. J., Mänd, R., Kekäläinen, J., Sirkka, I., Luoto, S., & Kortet, R. (2017). Metabolic rate associates with, but does not generate covariation between, behaviours in western stutter-trilling crickets, *Gryllus integer*. *Proceedings of the Royal Society B: Biological Sciences*, *284*(1851), 20162481. https://doi.org/10.1098/rspb.2016.2481

Kührt, U., Samietz, J., & Dorn, S. (2005). Thermoregulation behaviour in codling moth larvae. *Physiological Entomology*, *30*(1), 54–61. https://doi.org/10.1111/j.0307-6962.2005.00431.x

Lagisz, M., Wolff, K., Sanderson, R. A., & Laskowski, R. (2010). Genetic population structure of the ground beetle, *Pterostichus oblongopunctatus*, inhabiting a fragmented and polluted landscape: Evidence for sex-biased dispersal. *Journal of Insect Science*, *10*(105). https://doi.org/10.1673/031.010.10501

Lailvaux, S. P., & Irschick, D. J. (2007). Effects of temperature and sex on jump performance and biomechanics in the lizard *Anolis carolinensis*. *Functional Ecology*, *21*(3), 534–543. https://doi.org/10.1111/j.1365-2435.2007.01263.x

Lann, C. L., Roux, O., Serain, N., Alphen, J. J. M. V., Vernon, P., & Baaren, J. V. (2011). Thermal tolerance of sympatric hymenopteran parasitoid species: Does it match seasonal activity? *Physiological Entomology*, *36*(1), 21–28. https://doi.org/10.1111/j.1365-3032.2010.00758.x

Laparie, M., Renault, D., Lebouvier, M., & Delattre, T. (2013). Is dispersal promoted at the invasion front? Morphological analysis of a ground beetle invading the Kerguelen Islands, *Merizodus soledadinus* (Coleoptera, Carabidae). *Biological Invasions*, *15*(8), 1641–1648. https://doi.org/10.1007/s10530-012-0403-x

Larsson, S. G. (1939). Entwicklungstypen und Entwicklungszeiten der Dänischen Carabiden. *Entomologiske Meddelelser*, *20*, 277–560.

Le Galliard, J. F., Paquet, M., Cisel, M., & Montes-Poloni, L. (2015). Personality and the pace-of-life syndrome: Variation and selection on exploration, metabolism and locomotor performances. *Functional Ecology*, *27*(1), 136–144. https://doi.org/10.1111/1365-2435.12017@10.1111/(ISSN)1365-2435.LOCOMOTIONUNPLUGGED

Lemoine, N. P., & Burkepile, D. E. (2012). Temperature-induced mismatches between consumption and metabolism reduce consumer fitness. *Ecology*, *93*(11), 2483–2489. https://doi.org/10.1890/12-0375.1

Lenski, R. E. (1984). Food limitation and competition: A field experiment with two *Carabus* species. *Journal of Animal Ecology*, *53*(1), 203–216. https://doi.org/10.2307/4352

Li, G., Lv, X., Zhou, J., Shen, C., Xia, D., Xie, H., & Luo, Y. (2018). Are the surface areas of the gills and body involved with changing metabolic scaling with temperature? *Journal of Experimental Biology*, *221*(8). https://doi.org/10.1242/jeb.174474

Li, Q., Zhu, X., Xiong, W., Zhu, Y., Zhang, J., Djiba, P. K., Lv, X., & Luo, Y. (2020). Effects of temperature on metabolic scaling in black carp. *PeerJ*, *8*, e9242. https://doi.org/10.7717/peerj.9242

Lindmark, M., Huss, M., Ohlberger, J., & Gårdmark, A. (2018). Temperature-dependent body size effects determine population responses to climate warming. *Ecology Letters*, *21*(2), 181–189. https://doi.org/10.1111/ele.12880

Lindroth, C. H. (1985). The Carabidae (Coleoptera) of Fennoscandia and Denmark I. *Fauna Entomologica Scandinavica*, *15*(1), 1–226.

Lindstrom, T., Brown, G. P., Sisson, S. A., Phillips, B. L., & Shine, R. (2013). Rapid shifts in dispersal behavior on an expanding range edge. *Proceedings of the National Academy of Sciences*, *110*(33), 13452–13456. https://doi.org/10.1073/pnas.1303157110

Lundberg, J., & Moberg, F. (2003). Mobile link organisms and ecosystem functioning: Implications for ecosystem resilience and management. *Ecosystems*, *6*(1), 0087–0098. https://doi.org/10.1007/s10021-002-0150-4

Lynch, M., & Walsh, B. (1998). *Genetics and Analysis of Quantitative Traits* (Vol. 68). Sinauer Associates, Inc. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1235294/

Lyons, C. L., Coetzee, M., Terblanche, J. S., & Chown, S. L. (2012). Thermal limits of wild and laboratory strains of two African malaria vector species, *Anopheles arabiensis* and *Anopheles funestus*. *Malaria Journal*, *11*(1), 226. https://doi.org/10.1186/1475-2875-11-226

Maebe, K., De Baets, A., Vandamme, P., Vereecken, N. J., Michez, D., & Smagghe, G. (2021). Impact of intraspecific variation on measurements of thermal tolerance in bumble bees. *Journal of Thermal Biology*, *99*, 103002. https://doi.org/10.1016/j.jtherbio.2021.103002

Marcus, T., Boch, S., Durka, W., Fischer, M., Gossner, M. M., Müller, J., Schöning, I., Weisser, W. W., Drees, C., & Assmann, T. (2015). Living in heterogeneous woodlands – are habitat continuity or quality drivers of genetic variability in a flightless ground beetle? *PLoS ONE*. https://doi.org/10:e0144217

Marden, J. (1995). Large-scale changes in thermal sensitivity of flight performance during adult maturation in a dragonfly. *Journal of Experimental Biology*, *198*(10), 2095–2102. https://doi.org/10.1242/jeb.198.10.2095

Mathot, K. J., Dingemanse, N. J., & Nakagawa, S. (2019). The covariance between metabolic rate and behaviour varies across behaviours and thermal types: Meta-analytic insights. *Biological Reviews*, *94*(3), 1056–1074. https://doi.org/10.1111/brv.12491

Mauremooto, J. R., Wratten, S. D., Worner, S. P., & Fry, G. L. A. (1995). Permeability of hedgerows to predatory carabid beetles. *Agriculture, Ecosystems & Environment*, *52*(2), 141–148. https://doi.org/10.1016/0167-8809(94)00548-S

Maynard Smith, J. (1978). *The evolution of sex*. Cambridge University Press.

McCowan, L. S. C., Mainwaring, M. C., Prior, N. H., & Griffith, S. C. (2015). Personality in the wild zebra finch: Exploration, sociality, and reproduction. *Behavioural Ecology*, *26*(3), 735–746.

McDevitt, R. M., & Speakman, J. R. (1996). Summer acclimatization in the short-tailed field vole, *Microtus agrestis*. *Journal of Comparative Physiology B*, *166*(4), 286–293. https://doi.org/10.1007/BF00262873

McNiell Alexander, R. (1999). *Energy for animal life*. Oxford University Press.

Mellanby, K., & Gardiner, J. S. (1939). Low temperature and insect activity. *Proceedings of the Royal Society of London. Series B - Biological Sciences*, *127*(849), 473–487. https://doi.org/10.1098/rspb.1939.0035

Melzner, F., Bock, C., & Pörtner, H.-O. (2007). Allometry of thermal limitation in the cephalopod *Sepia officinalis*. *Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology*, *146*(2), 149–154. https://doi.org/10.1016/j.cbpa.2006.07.023

Methling, C., Blažek, R., Řežucha, R., & Reichard, M. (2020). Individual-level pace-of-life syndromes in annual killifish are mediated by intersexual and interspecific differences. *Evolutionary Ecology*, *34*(5), 745–761. https://doi.org/10.1007/s10682-020-10059-9

Miller, G. A., Clissold, F. J., Mayntz, D., & Simpson, S. J. (2009). Speed over efficiency: Locusts select body temperatures that favour growth rate over efficient nutrient utilization. *Proceedings of the Royal Society B: Biological Sciences*, *276*(1673), 3581–3589. https://doi.org/10.1098/rspb.2009.1030

Miller, K. A., Duran, A., Melville, J., Thompson, M. B., & Chapple, D. G. (2017). Sex-specific shifts in morphology and colour pattern polymorphism during range expansion of an invasive lizard. *Journal of Biogeography*, *44*(12), 2778–2788. https://doi.org/10.1111/jbi.13075

Mitchell, B. (1963). Ecology of two carabid beetles, *Bembidion lampros* (Herbst) and *Trechus quadristriatus* (Schrank). *Journal of Animal Ecology*, *32*(2), 289–299. https://doi.org/10.2307/2542

Møller, A. P., & Jennions, M. D. (2001). How important are direct fitness benefits of sexual selection? *Die Naturwissenschaften*, *88*(10), 401–415. https://doi.org/10.1007/s001140100255

Moschilla, J. A., Tomkins, J. L., & Simmons, L. W. (2019). Sex-specific pace-of-life syndromes. *Behavioral Ecology*, *30*(4), 1096–1105. https://doi.org/10.1093/beheco/arz055

Mousseau, T. A., & Roff, D. A. (1987). Natural selection and the heritability of fitness components. *Heredity*, *59*(2), 181–197. https://doi.org/10.1038/hdy.1987.113

Moya-Laraño, J., Macías-Ordóñez, R., Blanckenhorn, W. U., & Fernández-Montraveta, C. (2008). Analysing body condition: Mass, volume or density? *The Journal of Animal Ecology*, *77*(6), 1099–1108. https://doi.org/10.1111/j.1365-2656.2008.01433.x

MRC Biostatistics Unit, U. of C. (n.d.). *DIC: Deviance Information Criteria*. MRC Biostatistics Unit, University of Cambridge. https://www.mrc-bsu.cam.ac.uk/software/bugs/the-bugs-project-dic/

Muir, A. P., Biek, R., Thomas, R., & Mable, B. K. (2014). Local adaptation with high gene flow: Temperature parameters drive adaptation to altitude in the common frog (*Rana temporaria*). *Molecular Ecology*, *23*(3), 561–574. https://doi.org/10.1111/mec.12624

Negro, M., Caprio, E., Leo, K., Maritano, U., Roggero, A., Vacchiano, G., Palestrini, C., & Rolando, A. (2017). The effect of forest management on endangered insects assessed by radio-tracking: The case of the ground beetle *Carabus olympiae* in European beech *Fagus sylvatica* stands. *Forest Ecology and Management*, *406*, 125–137.

Negro, M., Casale, A., Migliore, L., Palestrini, C., & Rolando, A. (2007). The effect of local anthropogenic habitat heterogeneity on assemblages of carabids (Coleoptera, Caraboidea) endemic to the Alps. *Biodiversity & Conservation*, *16*, 3919–3932.

Nespolo, R. F. (2003). Intrapopulational variation in the standard metabolic rate of insects: Repeatability, thermal dependence and sensitivity (Q10) of oxygen consumption in a cricket. *Journal of Experimental Biology*, *206*(23), 4309–4315. https://doi.org/10.1242/jeb.00687

Nespolo, R. F., & Franco, M. (2007). Whole-animal metabolic rate is a repeatable trait: A meta-analysis. *Journal of Experimental Biology*, *210*(11), 2000–2005. https://doi.org/10.1242/jeb.02780

Niemelä, P. T., & Dingemanse, N. J. (2014). Artificial environments and the study of 'adaptive' personalities. *Trends in Ecology & Evolution*, *29*(5), 245–247. https://doi.org/10.1016/j.tree.2014.02.007

Niemela, P. T., Lattenkamp, E. Z., & Dingemanse, N. J. (2015). Personality-related survival and sampling bias in wild cricket nymphs. *Behavioral Ecology*, *26*(3), 936–946. https://doi.org/10.1093/beheco/arv036

Niitepõld, K., Mattila, A. L. K., Harrison, P. J., & Hanski, I. (2011). Flight metabolic rate has contrasting effects on dispersal in the two sexes of the Glanville fritillary butterfly. *Oecologia*, *165*(4), 847–854. https://doi.org/10.1007/s00442-010-1886-8

Norin, T., & Gamperl, A. K. (2018). Metabolic scaling of individuals vs. populations: Evidence for variation in scaling exponents at different hierarchical levels. *Functional Ecology*, *32*(2), 379–388. https://doi.org/10.1111/1365-2435.12996

Norin, T., & Metcalfe, N. B. (2019). Ecological and evolutionary consequences of metabolic rate plasticity in response to environmental change. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *374*(1768), 20180180. https://doi.org/10.1098/rstb.2018.0180

Nussey, D. H., Wilson, A. J., & Brommer, J. E. (2007). The evolutionary ecology of individual phenotypic plasticity in wild populations. *Journal of Evolutionary Biology*, *20*(3), 831–844. https://doi.org/10.1111/j.1420-9101.2007.01300.x

Nyamukondiwa, C., & Terblanche, J. S. (2009). Thermal tolerance in adult Mediterranean and natal fruit flies (*Ceratitis capitata* and *Ceratitis rosa*): Effects of age, gender and feeding status. *Journal of Thermal Biology*, *34*(8), 406–414. https://doi.org/10.1016/j.jtherbio.2009.09.002

Ohlberger, J., Mehner, T., Staaks, G., & Hölker, F. (2012). Intraspecific temperature dependence of the scaling of metabolic rate with body mass in fishes and its ecological implications. *Oikos*, *121*(2), 245–251.

Ohlberger, J., Staaks, G., & Hölker, F. (2007). Effects of temperature, swimming speed and body mass on standard and active metabolic rate in vendace (*Coregonus albula*). *Journal of Comparative Physiology B*, *177*(8), 905–916. https://doi.org/10.1007/s00360-007-0189-9

Overgaard, J., Tomčala, A., Sørensen, J. G., Holmstrup, M., Krogh, P. H., Šimek, P., & Koštál, V. (2008). Effects of acclimation temperature on thermal tolerance and membrane phospholipid composition in the fruit fly *Drosophila melanogaster*. *Journal of Insect Physiology*, *54*(3), 619–629. https://doi.org/10.1016/j.jinsphys.2007.12.011

Oyen, K. J., & Dillon, M. E. (2018). Critical thermal limits of bumble bees (*Bombus impatiens*) are marked by stereotypical behaviors and are unchanged by acclimation, age, or feeding status. *Journal of Experimental Biology*, jeb.165589. https://doi.org/10.1242/jeb.165589

Padilla, P., Courant, J., & Herrel, A. (2019). Allocation trade-offs impact organ size and muscle architecture in an invasive population of *Xenopus laevis* in Western France. *Journal of Anatomy*, *235*(6), 1057–1064. https://doi.org/10.1111/joa.13063

Paranjape, M. A. (1967). Molting and respiration of Euphausiids. *Journal of the Fisheries Research Board of Canada*, *24*(6), 1229–1240. https://doi.org/10.1139/f67-105

Perl, C. D., & Niven, J. E. (2018). Metabolic rate scaling, ventilation patterns and respiratory water loss in red wood ants: Activity drives ventilation changes, metabolic rate drives water loss. *The Journal of Experimental Biology*, *221*(18), jeb182501. https://doi.org/10.1242/jeb.182501

Peterson, C. C., Walton, B. M., & Bennett, A. F. (1998). Intrapopulation variation in ecological energetics of the garter snake *Thamnophis sirtalis*, with analysis of the precision of doubly labeled water measurements. *Physiological Zoology*, *71*(4), 333–349. https://doi.org/10.1086/515426

Phillips, B. L., Brown, G. P., Travis, J. M. J., Shine, R., & Price, T. D. (2008). Reid's paradox revisited: The evolution of dispersal kernels during range expansion. *The American Naturalist*, *172*(S1), S34–S48. https://doi.org/10.1086/588255

Phillips, B. L., Brown, G. P., Webb, J. K., & Shine, R. (2006). Invasion and the evolution of speed in toads. *Nature*, *439*(7078), 803–803. https://doi.org/10.1038/439803a

Pigliucci, M. (2005). Evolution of phenotypic plasticity: Where are we going now? *Trends in Ecology & Evolution*, *20*(9), 481–486. https://doi.org/10.1016/j.tree.2005.06.001

Piyaphongkul, J., Pritchard, J., & Bale, J. (2014). Effects of acclimation on the thermal tolerance of the brown planthopper *Nilaparvata lugens* (Stål). *Agricultural and Forest Entomology*, *16*(2), 174–183. https://doi.org/10.1111/afe.12047

Polis, G. A. (1984). Age structure component of niche width and intraspecific resource partitioning: Can age groups function as ecological species? *The American Naturalist*, *123*(4), 541–564. https://doi.org/10.1086/284221

Porter, J. D., Owen, C. A., Compton, S. G., & Coetzee, J. A. (2019). Testing the thermal limits of *Eccritotarsus catarinensis*: A case of thermal plasticity. *Biocontrol Science and Technology*, *29*(6), 565–577. https://doi.org/10.1080/09583157.2019.1572712

Pörtner, H. O., Berdal, B., Blust, R., Brix, O., Colosimo, A., De Wachter, B., Giuliani, A., Johansen, T., Fischer, T., Knust, R., Lannig, G., Naevdal, G., Nedenes, A., Nyhammer, G., Sartoris, F. J., Serendero, I., Sirabella, P., Thorkildsen, S., & Zakhartsev, M. (2001). Climate induced temperature effects on growth performance, fecundity and recruitment in marine fish: Developing a hypothesis for cause and effect relationships in Atlantic cod (*Gadus morhua*) and common eelpout (*Zoarces viviparus*). *Continental Shelf Research*, *21*(18), 1975–1997. https://doi.org/10.1016/S0278-4343(01)00038-3

Post, D. M., Palkovacs, E. P., Schielke, E. G., & Dodson, S. I. (2008). Intraspecific variation in a predator affects community structure and cascading trophic interactions. *Ecology*, *89*(7), 2019–2032.

Prudic, K. L., Jeon, C., Cao, H., & Monteiro, A. (2011). Developmental plasticity in sexual roles of butterfly species drives mutual sexual ornamentation. *Science*, *331*(6013), 73–75. https://doi.org/10.1126/science.1197114

Quinn, J. L., Cole, E. F., Patrick, S. C., & Sheldon, B. C. (2011). Scale and state dependence of the relationship between personality and dispersal in a great tit population. *Journal of Animal Ecology*, *80*(5), 918–928.

R Core Team. (2019). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. https://www.R-project.org/.

Réale, D., Garant, D., Humphries, M. M., Bergeron, P., Careau, V., & Montiglio, P.-O. (2010). Personality and the emergence of the pace-of-life syndrome concept at the population level. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*(1560), 4051–4063. https://doi.org/10.1098/rstb.2010.0208

Réale, D., Reader, S. M., Sol, D., McDougall, P. T., & Dingemanse, N. J. (2007). Integrating animal temperament within ecology and evolution. *Biological Reviews*, *82*(2), 291–318. https://doi.org/10.1111/j.1469-185X.2007.00010.x

Réveillon, T., Rota, T., Chauvet, É., Lecerf, A., & Sentis, A. (2019). Repeatable inter-individual variation in the thermal sensitivity of metabolic rate. *Oikos*, *128*(11), 1633–1640. https://doi.org/10.1111/oik.06392

Riecken, U., & Raths, U. (1996). Use of radio telemetry for studying dispersal and habitat use of *Carabus coriaceus* L. *Annales Zoologici Fennici*, *33*, 109–116.

Rijnsdorp, A. D. (1980). Pattern of movement in and dispersal from a Dutch forest of *Carabus problematicus Hbst.* (Coleoptera, Carabidae). *Oecologia*, *45*(2), 274–281. https://doi.org/10.1007/BF00346470

Roche, D. G., Careau, V., & Binning, S. A. (2016). Demystifying animal 'personality' (or not): Why individual variation matters to experimental biologists. *Journal of Experimental Biology*, *219*(24), 3832–3843. https://doi.org/10.1242/jeb.146712

Roff, D. (1980). Optimizing development time in a seasonal environment: The ups and downs of clinal variation. *Oecologia*, *45*(2), 202–208. https://doi.org/10.1007/BF00346461

Roff, D. A. (1984). The cost of being able to fly: A study of wing polymorphism in two species of crickets. *Oecologia*, *63*(1), 30–37.

Rogowitz, G. L., & Chappell, M. A. (2000). Energy metabolism of eucalyptus-boring beetles at rest and during locomotion: Gender makes a difference. *Journal of Experimental Biology*, *203*(7), 1131–1139.

Rome, L. C. (1990). Influence of temperature on muscle recruitment and muscle function in vivo. *The American Journal of Physiology*, *259*(2 Pt 2), R210-222. https://doi.org/10.1152/ajpregu.1990.259.2.R210

Royauté, R., Greenlee, K., Baldwin, M., & Dochtermann, N. A. (2015). Behaviour, metabolism and size: Phenotypic modularity or integration in *Acheta domesticus*? *Animal Behaviour*, *110*, 163–169. https://doi.org/10.1016/j.anbehav.2015.09.027

Rudolf, V. H. W. (2007). Consequences of stage-structured predators: Cannibalism, behavioral effects, and trophic cascades. *Ecology*, *88*(12), 2991–3003. https://doi.org/10.1890/07-0179.1

Rusterholz, H.-P., & Erhardt, A. N. dreas. (2000). Can nectar properties explain sex-specific flower preferences in the Adonis Blue butterfly *Lysandra bellargus*? *Ecological Entomology*, *25*(1), 81–90. https://doi.org/10.1046/j.1365-2311.2000.00233.x

Růžičková, J., & Veselý, M. (2018). Movement activity and habitat use of *Carabus ullrichii* (Coleoptera: Carabidae): The forest edge as a mating site? *Entomological Science*, *21*(1), 76–83. https://doi.org/10.1111/ens.12286

Sadowski, J. A., Moore, A. J., & Brodie, E. D. (1999). The evolution of empty nuptial gifts in a dance fly, *Empis snoddyi* (Diptera: Empididae): Bigger isn't always better. *Behavioral Ecology and Sociobiology*, *45*(3–4), 161–166. https://doi.org/10.1007/s002650050549

Sanchez-Tojar, A., Moiron, M., & Niemelä, P. T. (2021). *Ambiguous terminology in animal personality research: A self-report questionnaire and a systematic review* [Preprint]. https://doi.org/10.32942/osf.io/9srpy

Schirmer, A., Herde, A., Eccard, J. A., & Dammhahn, M. (2019). Individuals in space: Personality-dependent space use, movement and microhabitat use facilitate individual spatial niche specialization. *Oecologia*, *189*(3), 647–660. https://doi.org/10.1007/s00442-019-04365-5

Schlichting, C., & Pigliucci, M. (1998). *Phenotypic Evolution: A Reaction Norm Perspective*. Sinauer.

Schmidt-Nielsen, K. (1984). *Scaling: Why is animal size so important?* Cambridge University Press.

Schmidt-Nielsen, K. (2007). *Animal Physiology* (5th ed.). Cambridge University Press.

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, *9*(7), 671–675. https://doi.org/10.1038/nmeth.2089

Schuett, W., & Dall, S. R. X. (2009). Sex differences, social context and personality in zebra finches, *Taeniopygia guttata*. *Animal Behaviour*, *77*(5), 1041–1050. https://doi.org/10.1016/j.anbehav.2008.12.024

Schuett, W., Delfs, B., Haller, R., Kruber, S., Roolfs, D., Timm, D., Willmann, M., & Drees, C. (2018). Ground beetles in city forests: Does urbanization predict a personality trait? *PeerJ*, *6*, e4360. https://doi.org/10.7717/peerj.4360

Schuett, W., Laaksonen, J., & Laaksonen, T. (2012). Prospecting at conspecific nests and exploration in a novel environment are associated with reproductive success in the jackdaw. *Behavioral Ecology and Sociobiology*, *66*(9), 1341–1350. https://doi.org/10.1007/s00265-012-1389-1

Schuett, W., Tregenza, T., & Dall, S. R. X. (2010). Sexual selection and animal personality. *Biological Reviews*, *85*(2), 217–246. https://doi.org/10.1111/j.1469-185X.2009.00101.x

Schulte-Hostedde, A. I., Zinner, B., Millar, J. S., & Hickling, G. J. (2005). Restitution of mass–size residuals: Validating body condition indices. *Ecology*, *86*(1), 155–163. https://doi.org/10.1890/04-0232

Seebacher, F., White, C. R., & Franklin, C. E. (2015). Physiological plasticity increases resilience of ectothermic animals to climate change. *Nature Climate Change*, *5*(1), 61–66. https://doi.org/10.1038/nclimate2457

Seppänen, E., Piironen, J., & Huuskonen, H. (2010). Consistency of standard metabolic rate in relation to life history strategy of juvenile Atlantic salmon *Salmo salar*. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *156*(2), 278–284. https://doi.org/10.1016/j.cbpa.2010.02.014

Sexton, J. P., McIntyre, P. J., Angert, A. L., & Rice, K. J. (2009). Evolution and ecology of species range limits. *Annual Review of Ecology, Evolution, and Systematics*, *40*(1), 415–436. https://doi.org/10.1146/annurev.ecolsys.110308.120317

Sgrò, C. M., Overgaard, J., Kristensen, T. N., Mitchell, K. A., Cockerell, F. E., & Hoffmann, A. A. (2010). A comprehensive assessment of geographic variation in heat tolerance and hardening capacity in populations of *Drosophila melanogaster* from eastern Australia: Variation in heat tolerance and hardening capacity. *Journal of Evolutionary Biology*, *23*(11), 2484–2493. https://doi.org/10.1111/j.1420-9101.2010.02110.x

Shackleton, M. A., Jennions, M. D., & Hunt, J. (2005). Fighting success and attractiveness as predictors of male mating success in the black field cricket, *Teleogryllus commodus*: The effectiveness of no-choice tests. *Behavioral Ecology and Sociobiology*, *58*(1), 1–8. https://doi.org/10.1007/s00265-004-0907-1

Shaw, A. K. (2020). Causes and consequences of individual variation in animal movement. *Movement Ecology*, *8*(1), 12. https://doi.org/10.1186/s40462-020-0197-x

Sherwood, S. C., Webb, M. J., Annan, J. D., Armour, K. C., Forster, P. M., Hargreaves, J. C., Hegerl, G., Klein, S. A., Marvel, K. D., Rohling, E. J., Watanabe, M., Andrews, T., Braconnot, P., Bretherton, C. S., Foster, G. L., Hausfather, Z., Heydt, A. S. von der, Knutti, R., Mauritsen, T., … Zelinka, M. D. (2020). An assessment of Earth's climate sensitivity using multiple lines of evidence. *Reviews of Geophysics*, *58*(4), e2019RG000678. https://doi.org/10.1029/2019RG000678

Shik, J. Z., Arnan, X., Oms, C. S., Cerdá, X., & Boulay, R. (2019). Evidence for locally adaptive metabolic rates among ant populations along an elevational gradient. *Journal of Animal Ecology*, *88*(8), 1240–1249. https://doi.org/10.1111/1365-2656.13007

Shine, R. (1989). Ecological causes for the evolution of sexual dimorphism: A review of the evidence. *The Quarterly Review of Biology*, *64*(4), 419–461.

Shine, R., Brown, G. P., & Phillips, B. L. (2011). An evolutionary process that assembles phenotypes through space rather than through time. *Proceedings of the National Academy of Sciences*, *108*(14), 5708–5711. https://doi.org/10.1073/pnas.1018989108

Sih, A., & Bell, A. M. (2008). Insights for Behavioral Ecology from Behavioral Syndromes. *Advances in the Study of Behavior*, *38*, 227–281. https://doi.org/10.1016/S0065-3454(08)00005-3

Sih, A., Cote, J., Evans, M., Fogarty, S., & Pruitt, J. (2012). Ecological implications of behavioural syndromes. *Ecology Letters*, *15*(3), 278–289. https://doi.org/10.1111/j.1461-0248.2011.01731.x

Sih, A., Mathot, K. J., Moirón, M., Montiglio, P.-O., Wolf, M., & Dingemanse, N. J. (2015). Animal personality and state–behaviour feedbacks: A review and guide for empiricists. *Trends in Ecology & Evolution*, *30*(1), 50–60. https://doi.org/10.1016/j.tree.2014.11.004

Simberloff, D., Dayan, T., Jones, C., & Ogura, G. (2000). Character displacement and release in the small Indian mongoose *Herpestes javanicus*. *Ecology*, *81*(8), 14.

Sinclair, B. J., Williams, C. M., & Terblanche, J. S. (2012). Variation in thermal performance among insect populations. *Physiological and Biochemical Zoology*, *85*(6), 594–606. https://doi.org/10.1086/665388

Sinn, D. L., Apiolaza, L. A., & Moltschaniwskyj, N. A. (2006). Heritability and fitness-related consequences of squid personality traits. *Journal of Evolutionary Biology*, *19*(5), 1437–1447. https://doi.org/10.1111/j.1420-9101.2006.01136.x

Skellam, J. G. (1951). Random dispersal in theoretical populations. *Biometrika*, *38*(1–2), 196–218. https://doi.org/10.1093/biomet/38.1-2.196

Smith, B. R., & Blumstein, D. T. (2008). Fitness consequences of personality: A meta-analysis. *Behavioral Ecology*, *19*(2), 448–455. https://doi.org/10.1093/beheco/arm144

Soberon, J., & Peterson, A. T. (2005). Interpretation of models of fundamental ecological niches and species' distributional areas. *Biodiversity Informatics*, *2*. https://doi.org/10.17161/bi.v2i0.4

Spearman, C. (1904). The proof and measurement of association between two things. *The American Journal of Psychology*, *15*(1), 72–101.

Spiegel, O., Leu, S. T., Sih, A., Godfrey, S. S., & Bull, C. M. (2015). When the going gets tough: Behavioural type-dependent space use in the sleepy lizard changes as the season

dries. *Proceedings of the Royal Society B: Biological Sciences*, *282*(1819), 20151768. https://doi.org/10.1098/rspb.2015.1768

Spiegelhalter, D. J., Best, N. G., Carlin, B. P., & Van Der Linde, A. (2002). Bayesian measures of model complexity and fit. *Journal of the Royal Statistical Society: Series B (Statistical Methodology)*, *64*(4), 583–639. https://doi.org/10.1111/1467-9868.00353

Stamps, J., & Groothuis, T. G. G. (2010). The development of animal personality: Relevance, concepts and perspectives. *Biological Reviews*, *85*(2), 301–325. https://doi.org/10.1111/j.1469-185X.2009.00103.x

Stearns, S. C. (1976). Life-history tactics: A review of the ideas. *The Quarterly Review of Biology*, *51*(1), 3–47. https://doi.org/10.1086/409052

Stearns, S. C. (1992). *The evolution of life histories*. Oxford University Press.

Stoffel, M. A., Nakagawa, S., & Schielzeth, H. (2017). RptR: repeatability estimation and variance decomposition by generalized linear mixed-effects models. *Methods in Ecology and Evolution*, *8*, 1639–1644. https://doi.org/10.1111/2041-210X.12797

Streicher, J. W., Cox, C. L., & Birchard, G. F. (2012). Non-linear scaling of oxygen consumption and heart rate in a very large cockroach species (*Gromphadorhina portentosa*): Correlated changes with body size and temperature. *The Journal of Experimental Biology*, *215*(Pt 7), 1137–1143. https://doi.org/10.1242/jeb.061143

Sunderland, K. D. (1975). The diet of some predatory arthropods in cereal crops. *Journal of Applied Ecology*, *12*(2), 507–515. https://doi.org/10.2307/2402171

Sutherland, W. J., & Ens, B. J. (1987). The criteria determining the selection of mussels *Mytilus edulis* by Oystercatchers *Haematopus ostralegus*. *Behaviour*, *103*(1/3), 187–202.

Szyszko, J., Gryuntal, S., & Schwerk, A. (2004). Differences in locomotory activity between male and female *Carabus hortensis* (Coleoptera: Carabidae) in a pine forest and a beech forest in relation to feeding state. *Environmental Entomology*, *33*(5), 1442–1446. https://doi.org/10.1603/0046-225X-33.5.1442

Tanner, C. J., & Jackson, A. L. (2012). Social structure emerges via the interaction between local ecology and individual behaviour. *Journal of Animal Ecology*, *81*(1), 260–267. https://doi.org/10.1111/j.1365-2656.2011.01879.x

Taylor, L. R. (1963). Analysis of the effect of temperature on insects in flight. *Journal of Animal Ecology*, *32*(1), 99–117. https://doi.org/10.2307/2520

Teder, T., & Tammaru, T. (2005). Sexual size dimorphism within species increases with body size in insects. *Oikos*, *108*(2), 321–334. https://doi.org/10.1111/j.0030-1299.2005.13609.x

Tejedo Madueño, M., Marangoni, F., Pertoldi, C., Richter-Boix, A., Laurila, A., Orizaola Pereda, G., González Nicieza, A. C., Álvarez Fernández, D., & Gómez Mestre, I. (2010). Contrasting effects of environmental factors during larval stage on morphological plasticity in post-metamorphic frogs. *Climate Research*, *43*, 31–39. https://doi.org/10.3354/cr00878

Terblanche, J. S., Deere, J. A., Clusella-Trullas, S., Janion, C., & Chown, S. L. (2007). Critical thermal limits depend on methodological context. *Proceedings of the Royal Society B: Biological Sciences*, *274*(1628), 2935–2943. https://doi.org/10.1098/rspb.2007.0985

Timonin, M. E., Carrière, C. J., Dudych, A. D., Latimer, J. G. W., Unruh, S. T., & Willis, C. K. R. (2011). Individual differences in the behavioural responses of meadow voles to an

unfamiliar environment are not correlated with variation in resting metabolic rate: Activity and RMR in meadow voles. *Journal of Zoology*, *284*(3), 198–205. https://doi.org/10.1111/j.1469-7998.2011.00792.x

Trautner, J. (1992). *Rote liste und artenverzeichnis der laufkäfer Baden-Württembergs (Col., Carabidae s. Fat.). -Weikersheim: Margraf.*

Trivers, R. L. (1972). *Sexual selection and the descent of man* (B. Campbell, Ed.).

Tuda, M., & Shima, K. (2002). Relative importance of weather and density dependence on the dispersal and on-plant activity of the predator *Orius minutus*. *Population Ecology*, *44*(3), 251–257. https://doi.org/10.1007/s101440200028

Turin, H., Penev, L., Casale, A., Arndt, E., Assmann, T., Makarov, K. V., Mossakowski, D., Szél, G., & Weber, F. (2003). Species accounts. In H. Turin, L. Penev, & A. Casale (Eds.), *The genus* Carabus *in Europe—A synthesis.* (pp. 151–283). Pensoft Publishers & European Invertebrate Survey.

Ummenhofer, C. C., & Meehl, G. A. (2017). Extreme weather and climate events with ecological relevance: A review. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *372*(1723), 20160135. https://doi.org/10.1098/rstb.2016.0135

van Overveld, T., Careau, V., Adriaensen, F., & Matthysen, E. (2014). Seasonal- and sex-specific correlations between dispersal and exploratory behaviour in the great tit. *Oecologia*, *174*(1), 109–120. https://doi.org/10.1007/s00442-013-2762-0

van Overveld, T., & Matthysen, E. (2010). Personality predicts spatial responses to food manipulations in free-ranging great tits (*Parus major*). *Biology Letters*, *6*(2), 187–190. https://doi.org/10.1098/rsbl.2009.0764

Verbeek, M. E. M., Drent, P. J., & Wiepkema, P. R. (1994). Consistent individual differences in early exploratory behaviour of male great tits. *Animal Behaviour*, *48*(5), 1113–1121. https://doi.org/10.1006/anbe.1994.1344

Videlier, M., Rundle, H. D., & Careau, V. (2019). Sex-specific among-individual covariation in locomotor activity and resting metabolic rate in *Drosophila melanogaster*. *The American Naturalist*, *194*(6), E164–E176. https://doi.org/10.1086/705678

Vogt, J. T., Smith, W. A., Grantham, R. A., & Wright, R. E. (2003). Effects of temperature and season on foraging activity of red imported fire ants (Hymenoptera: Formicidae) in Oklahoma. *Environmental Entomology*, *32*(3), 447–451. https://doi.org/10.1603/0046-225X-32.3.447

Völler, E., Boutaud, E., & Assmann, T. (2018). The pace of range expansion: A long-term study on the flightless ground beetle *Carabus hortensis* (Coleoptera: Carabidae). *Journal of Insect Conservation*, *22*(1), 163–169. https://doi.org/10.1007/s10841-017-0043-7

Wallin, H., & Ekbom, B. (1994). Influence of hunger level and prey densities on movement patterns in three species of *Pterostichus* beetles (Coleoptera: Carabidae). *Environmental Entomology*, *23*(5), 1171–1181. https://doi.org/10.1093/ee/23.5.1171

Wallin, H., & Ekbom, B. S. (1988). Movements of carabid beetles (Coleoptera: Carabidae) inhabiting cereal fields: a field tracing study. *Oecologia*, *77*(1), 39–43. https://doi.org/10.1007/BF00380922

Wang, Z., Heshka, S., Zhang, K., Boozer, C. N., & Heymsfield, S. B. (2001). Resting energy expenditure: Systematic organization and critique of prediction methods. *Obesity Research*, *9*(5), 331–336. https://doi.org/10.1038/oby.2001.42

Warton, D. I., Duursma, R. A., Falster, D. S., & Taskinen, S. (2012). smatr 3: An R package for estimation and inference about allometric lines. *Methods in Ecology and Evolution*, *3*(2), 257–259. https://doi.org/10.1111/j.2041-210X.2011.00153.x

Wat, K. K. Y., Herath, A. P. H. M., Rus, A. I., Banks, P. B., & Mcarthur, C. (2020). Space use by animals on the urban fringe: Interactive effects of sex and personality. *Behavioral Ecology*, *31*(2), 330–339. https://doi.org/10.1093/beheco/arz194

Weather Underground. (n.d.). *Schneverdingen, Lower Saxony, Germany Weather History*. Weather Underground. https://www.wunderground.com/history/daily/de/soltau/ISCHNE23/date/

Weatherhead, P. J., & Brown, G. P. (1996). Measurement versus estimation of condition in snakes. *Canadian Journal of Zoology*, *74*(9), 1617–1621. https://doi.org/10.1139/z96-179

Weber, F., & Heimbach, U. (2001). Behavioural, reproductive and developmental seasonality in *Carabus auronitens* and *Carabus nemoralis* (Col., Carabidae). A demographic comparison between two co-existing spring breeding populations and tests for intra- and interspecific competition and for synchronizing weather events. *Mitteilungen Aus Der Biologischen Bundesanstalt Für Land- Und Forstwirtschaft*, *382*, 1–192.

Weibel, E. R., Bacigalupe, L. D., Schmitt, B., & Hoppeler, H. (2004). Allometric scaling of maximal metabolic rate in mammals: Muscle aerobic capacity as determinant factor. *Respiratory Physiology & Neurobiology*, *140*(2), 115–132. https://doi.org/10.1016/j.resp.2004.01.006

Weibel, E. R., & Hoppeler, H. (2005). Exercise-induced maximal metabolic rate scales with muscle aerobic capacity. *Journal of Experimental Biology*, *208*(9), 1635–1644. https://doi.org/10.1242/jeb.01548

Wells, K. D., & Taigen, T. L. (1989). Calling energetics of a neotropical treefrog, *Hyla microcephala*. *Behavioral Ecology and Sociobiology*, *25*(1), 13–22. https://doi.org/10.1007/BF00299706

West, G. B., Brown, J. H., & Enquist, B. J. (1997). A general model for the origin of allometric scaling laws in biology. *Science*, *276*(5309), 122–126. https://doi.org/10.1126/science.276.5309.122

Whalen, A., & Hoppitt, W. J. E. (2016). Bayesian model selection with network based diffusion analysis. *Frontiers in Psychology*, *7*. https://www.frontiersin.org/article/10.3389/fpsyg.2016.00409

White, C. R., & Seymour, R. S. (2004). Does basal metabolic rate contain a useful signal? Mammalian BMR allometry and correlations with a selection of physiological, ecological, and life-history variables. *Physiological and Biochemical Zoology: Ecological and Evolutionary Approaches*, *77*(6), 929–941. https://doi.org/10.1086/425186

White, S. J., Kells, T. J., & Wilson, A. J. (2016). Metabolism, personality and pace of life in the Trinidadian guppy, *Poecilia reticulata*. *Behaviour*, *153*(13–14), 1517–1543. https://doi.org/10.1163/1568539X-00003375

Wilkinson, M. (2016). *Restless creatures: The story of life in ten movements.* Basic Books.

Willmer, P. (1991). Thermal biology and mate acquisition in ectotherms. *Trends in Ecology & Evolution*, *6*(12), 396–399. https://doi.org/10.1016/0169-5347(91)90161-P

Willmer, P. G., & Stone, G. N. (2004). Behavioral, ecological, and physiological determinants of the activity patterns of bees. In *Advances in the Study of Behavior* (Vol. 34, pp. 347–466). Academic Press. https://doi.org/10.1016/S0065-3454(04)34009-X

Wilson, A. D. M., & McLaughlin, R. L. (2007). Behavioural syndromes in brook charr, *Salvelinus fontinalis*: Prey-search in the field corresponds with space use in novel laboratory situations. *74*(4), 689–698. https://doi.org/10.1016/j.anbehav.2007.01.009

Wilson, D. S. (1998). Adaptive individual differences within single populations. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *353*(1366), 199–205. https://doi.org/10.1098/rstb.1998.0202

Wolf, M., van Doorn, G. S., Leimar, O., & Weissing, F. J. (2007). Life-history trade-offs favour the evolution of animal personalities. *Nature*, *447*(7144), 581–584. https://doi.org/10.1038/nature05835

Wolf, M., & Weissing, F. J. (2012). Animal personalities: Consequences for ecology and evolution. *Trends in Ecology & Evolution*, *27*(8), 452–461. https://doi.org/10.1016/j.tree.2012.05.001

Xiaojun, X., & Ruyung, S. (1990). The bioenergetics of the southern catfish (*Silurus meridionalis* Chen). I. Resting metabolic rate as a function of body weight and temperature. *Physiological Zoology*, *63*(6), 1181–1195.

Yarwood, E., Drees, C., Niven, J. E., Gawel, M., & Schuett, W. (2021a). Sex differences in morphology across an expanding range edge in the flightless ground beetle, *Carabus hortensis*. *Ecology and Evolution*, *11*(15), 9949–9957. https://doi.org/10.1002/ece3.7593

Yarwood, E., Drees, C., Niven, J. E., & Schuett, W. (2021b). Sex-specific covariance between metabolic rate, behaviour and morphology in the ground beetle *Carabus hortensis*. *PeerJ*, *9*, e12455. https://doi.org/10.7717/peerj.12455

Yuen, C. H., Pillay, N., Heinrichs, M., Schoepf, I., & Schradin, C. (2016). Personality traits are consistent when measured in the field and in the laboratory in African striped mice (*Rhabdomys pumilio*). *Behavioral Ecology and Sociobiology*, *70*(8), 1235–1246. https://doi.org/10.1007/s00265-016-2131-1

Zahavi, A., & Zahavi, A. (1997). *The handicap principle: A missing piece of Darwin's puzzle.* Oxford University Press.

Zera, A. J., & Denno, R. F. (1997). Physiology and ecology of dispersal polymorphism in insects. *Annual Review of Entomology*, *42*(1), 207–230. https://doi.org/10.1146/annurev.ento.42.1.207

Zollikofer, C. (1994). Stepping patterns in ants—Influence of body morphology. *Journal of Experimental Biology*, *192*(1), 107–118.A

Appendix A: Chapter 2

Table A.1. Sex-specific Spearman's rank correlations between morphological traits measured in *Carabus hortensis*. Correlations using data from females alone (F) and data from males alone (M) are presented. Bold p-values denote significant correlations. Bold R_s values denote strong correlations (R > 0.7). Asterisks on p-values denote which correlations remain significant following False Discovery Rate testing (Benjamini & Hochberg, 1995).

Sex	1st Variable	2nd Variable	R_s	p-value	N
F	Body Condition (g)	Body Mass (g)	**0.936**	**<0.001***	161
	Pronotum Width (mm)	Body Mass (g)	0.275	**<0.001***	161
	Body Condition (g)	Pronotum Width (mm)	-0.035	0.661	161
	Pronotum Width (mm)	Front Femur	0.239	0.118	44
	Pronotum Width (mm)	Mid Femur	0.199	0.195	44
	Pronotum Width (mm)	Hind Femur	0.178	0.207	44
	Pronotum Width (mm)	Front Tibia	0.157	0.310	44
	Pronotum Width (mm)	Mid Tibia	0.165	0.285	44
	Pronotum Width (mm)	Hind Tibia	0.385	**0.010***	44
M	Body Condition (g)	Body Mass (g)	**0.941**	**<0.001***	92
	Pronotum Width (mm)	Body Mass (g)	0.267	**0.010***	92
	Body Condition (g)	Pronotum Width (mm)	-0.018	0.883	92
	Pronotum Width (mm)	Front Femur	-0.178	0.427	22
	Pronotum Width (mm)	Mid Femur	0.182	0.418	22
	Pronotum Width (mm)	Hind Femur	0.220	0.324	22
	Pronotum Width (mm)	Front Tibia	0.610	**0.003***	22
	Pronotum Width (mm)	Mid Tibia	0.547	**0.008***	22
	Pronotum Width (mm)	Hind Tibia	0.268	0.228	22

Table A.2. Summary of test statistics from LMMs with the pronotum width as a proxy for body size as a response in males and females (M + F), females alone (F) and males alone (M). Sex and position along the expansion front (Position) were used as fixed terms. Coefficients (Coeff.) in square brackets belong to non-significant terms just before dropping those terms from the model. Bold p-values denote significant terms. Variance (Var.) of the random terms 'Week' and 'Week/trap' (the trap from which individuals were collected nested within the week of collection) and residuals are presented.

Sex	Random Term	Var.	Fixed Term	Coeff.	χ^2	DF	p-value
M + F	Week	0.020	Intercept	-27.18			
(N=253)	Week/trap	0.177	Sex (males): Position	[0.02]	2.94	1	0.086
	Residual	0.308	Sex (males)	-0.23	6.30	1	**0.012**
			Position	0.02	5.06	1	**0.024**
F	Week	0.003	Intercept	8.04			
(N=161)	Week/trap	0.203	Position	[0.01]	0.40	1	0.529
	Residual	0.352					
M	Week	0.048	Intercept	-66.62			
(N=92)	Week/trap	0.134	Position	0.04	9.88	1	**0.002**
	Residual	0.264					

Table A.3. Summary of test statistics from LMMs with body condition as a response in males and females (M + F), females alone (F) and males alone (M). Sex and position along the expansion front (Position) are used as fixed terms. Coefficients (Coeff.) in square brackets belong to non-significant terms just before dropping those terms from the model. Bold p-values denote significant terms. Variance (Var.) of the random terms 'Week' and 'Week/trap' (the trap from which individuals were collected nested within the week of collection) and residuals are presented. Bold p-values denote significant terms.

Sex	Random Term	Var.	Fixed Term	Coeff.	χ^2	DF	p-value
M + F	Week	0.002	Intercept	-0.02			
(N=253)	Week/trap	0.002	Sex (males): Position	[<-0.01]	0.01	1	0.928
	Residual	0.005	Sex (males)	[<-0.01]	0.01	1	0.936
			Position	[<0.01]	1.77	1	0.183
F	Week	0.002	Intercept	-0.01			
(N=161)	Week/trap	0.002	Position	[<0.01]	0.59	1	0.443
	Residual	0.007					
M	Week	<0.001	Intercept	-0.02			
(N=92)	Week/trap	0.002	Position	[<0.01]	1.53	1	0.216
	Residual	0.002					

Appendix B: Chapter 3

Table B.1. Dates and times of beetle release and recapture for each beetle ID. The number of times the beetle was located, number of days tracked in the field, the thermal minima (T. Min), maxima (T. Max), and optima (T. Opt), the total distance travelled in the field over two weeks (TDT) (m), the average distance travelled over 2 hours (Avg DT2h) (m), the body mass and the body mass with the radio tag fitted to the elytra are provided for each individual. An asterisk (*) next to the beetle ID indicates that an operating range could not be calculated for this individual.

Beetle ID	Sex	Week	Release Date and Time		Recapture Date and Time		Times Located	Days Tracked	T. Min	T. Max	T. Opt	TDT (m)	Avg DT2h (m)	Body Mass (g)	Tagged Body Mass (g)
OP19.1.1	F	1	10/08/19	18:46	27/08/19	15:11	80	16	11.06	19.99	15.70	35.7	0.39	0.464	0.768
O23.1.1	F	1	10/08/19	18:52	26/08/19	15:24	80	15	12.45	17.89	15.70	27.5	0.47	0.719	1.009
O23.1.2	F	1	10/08/19	18:52	26/08/19	22:32	80	15	11.76	18.72	14.75	9.6	0.18	0.597	0.907
O16.2.1	M	2	19/08/19	18:53	02/09/19	17:06	70	14	13.77	20.10	16.65	51.4	0.59	0.354	0.634
O19.2.1*	F	2	19/08/19	19:00	02/09/19	17:03	65	14	NA	NA	13.60	14.9	0.26	0.611	0.836
O20.1.1*	F	2	19/08/19	19:03	02/09/19	17:00	70	14	NA	NA	20.45	12.7	0.23	0.745	NA
I2.1.1*	F	2	19/08/19	19:21	02/09/19	14:23	70	14	NA	NA	19.65	18.4	0.33	0.573	0.792
I5.1.1	F	2	19/08/19	19:22	02/09/19	14:29	65	14	14.21	31.88	19.65	17.6	0.32	0.539	0.846
I7.1.1	F	2	19/08/19	19:24	02/09/19	14:30	70	14	14.11	14.19	14.00	111.8	0.30	0.550	0.885
P16.2.1*	M	3	26/08/19	18:51	09/09/19	11:00	60	12	NA	NA	20.95	22.3	0.39	0.499	0.837
P18.2.1	F	3	26/08/19	18:58	09/09/19	11:04	60	12	9.84	18.92	13.05	14.2	0.24	0.441	0.791
P19.2.1	M	3	26/08/19	18:59	09/09/19	11:06	60	12	10.25	22.65	13.40	16.8	0.30	0.487	0.879
I3.1.1*	F	3	26/08/19	19:15	09/09/19	10:10	60	12	NA	NA	21.85	13.6	0.24	0.699	0.986
I1.3.1*	M	3	26/08/19	19:17	09/09/19	10:08	60	12	NA	NA	13.55	22.0	0.39	0.465	0.785

I8.3.1	F	3	26/08/19	19:22	09/09/19	10:14	55	12	17.52	19.74	18.10	51.1	0.25	0.470	0.811
P15.3.1	M	4	02/09/19	17:00	16/09/19	10:35	50	10	9.82	19.93	13.00	23.2	0.37	0.551	0.785
P20.3.1	F	4	02/09/19	17:02	16/09/19	10:40	50	10	9.96	18.16	13.40	12.3	0.21	0.524	0.781
P21.2.1	F	4	02/09/19	17:03	16/09/19	10:42	50	10	10.93	15.83	13.40	14.6	0.25	0.552	0.826
F3.2.1	F	4	02/09/19	17:06	16/09/19	10:17	50	10	9.58	19.36	13.30	18.7	0.33	0.503	0.786
P16.4.1*	M	5	09/09/19	17:07	23/09/19	10:43	50	10	NA	NA	12.60	153.3	1.41	0.496	NA
P18.3.2	M	5	09/09/19	17:08	23/09/19	10:45	50	10	7.22	15.92	7.80	24.0	0.44	0.454	0.49
P19.3.1*	M	5	09/09/19	17:08	23/09/19	10:47	50	10	NA	NA	12.70	12.6	0.23	0.557	0.609
G1.4.1	F	5	09/09/19	17:15	23/09/19	10:05	50	10	7.28	18.67	10.65	24.6	0.44	0.537	NA
G1.4.2	F	5	09/09/19	17:15	23/09/19	10:04	50	10	6.92	28.04	15.30	11.8	0.22	0.536	NA
G6.4.1	M	5	09/09/19	17:17	23/09/19	10:06	50	10	9.44	15.59	12.45	25.0	0.45	0.546	0.678
G9.4.1	M	5	09/09/19	17:20	23/09/19	10:16	50	10	9.11	17.33	12.95	40.6	0.58	0.620	0.642
G7.5.1	F	6	16/09/19	16:30	28/09/19	10:16	50	10	9.64	21.05	13.00	14.7	0.26	0.729	0.979
G7.5.2	M	6	16/09/19	16:30	30/09/19	10:13	50	10	10.38	18.39	13.15	8.9	0.16	0.567	0.852
G9.4.2	M	6	16/09/19	16:30	30/09/19	10:16	50	10	9.70	18.26	13.15	14.9	0.27	0.511	0.78
P13.5.1*	F	6	16/09/19	17:00	30/09/19	11:11	50	10	NA	NA	8.00	15.1	0.29	0.585	1.003
P14.5.1*	F	6	16/09/19	17:00	30/09/19	11:13	50	10	NA	NA	8.00	17.8	0.32	0.625	0.801
P15.5.1	F	6	16/09/19	17:00	30/09/19	11:02	50	10	12.61	13.46	12.80	24.2	0.43	0.744	0.979
G10.6.1	F	7	23/09/19	17:00	07/10/19	10:15	50	10	7.58	7.82	7.60	31.2	0.31	0.737	0.992
G10.6.2	M	7	23/09/19	17:00	07/10/19	10:10	50	10	5.97	18.25	11.05	10.0	0.18	0.550	0.833
G5.7.2	F	7	23/09/19	17:00	07/10/19	10:06	50	10	11.33	18.22	13.65	27.5	0.49	0.685	0.893
G6.6.1	F	7	23/09/19	17:00	07/10/19	10:13	50	10	12.76	15.16	13.65	38.8	0.43	0.727	1.07
P15.7.1*	F	7	23/09/19	17:00	07/10/19	10:40	50	10	NA	NA	13.65	102.0	1.21	0.815	1.159
P18.5.1*	M	7	23/09/19	17:00	07/10/19	10:50	50	10	NA	NA	11.55	28.4	0.49	0.464	0.728
P18.5.4*	F	7	23/09/19	17:00	07/10/19	10:45	50	10	NA	NA	11.55	20.5	0.37	0.782	1.04

P19.1.2*	F	7	23/09/19	17:05	07/10/19	10:47	50	10	NA	NA	11.50	13.7	0.26	0.532	0.771
G7.4.1	F	7	09/09/19	17:18	23/09/19	10:08	50	10	6.89	17.98	10.65	11.7	0.21	0.644	NA
G10.4.1	M	8	30/09/19	16:30	14/10/19	10:10	50	10	7.64	12.41	9.20	11.7	0.21	0.390	0.797
G10.8.1	M	8	30/09/19	16:30	14/10/19	10:10	50	10	7.18	10.32	9.05	9.6	0.17	0.532	0.797
G4.8.1	F	8	30/09/19	16:30	14/10/19	10:10	50	10	7.49	10.91	9.25	48.5	0.34	0.820	1.157
G7.7.1	M	8	30/09/19	16:30	14/10/19	10:25	50	10	9.75	14.04	9.20	84.4	0.67	0.596	0.904
G7.7.3*	M	8	30/09/19	16:30	09/10/19	14:06	35	7	NA	NA	11.15	20.2	0.42	0.469	0.904
P15.7.2	M	8	30/09/19	17:38	12/10/19	11:06	50	10	6.95	12.30	8.80	11.4	0.20	0.583	0.952
P17.7.1*	M	8	30/09/19	17:38	14/10/19	11:11	50	10	NA	NA	13.65	38.0	0.24	0.593	0.863
P18.7.2	M	8	30/09/19	17:38	14/10/19	11:20	50	10	6.97	10.56	8.80	47.3	0.25	0.488	0.742
Q24.9.1	M	8	01/10/19	13:03	13/10/19	18:21	40	8	6.48	11.68	7.65	56.1	1.01	0.575	0.882

Table B.2. Summary of test-statistics from LMMs with field-derived operating range as the response variable. Test statistics are given for male and female data combined (M + F) female-only data (F), and male-only data (M). Body mass, sex, the total distance travelled over 8 hours per day for two weeks (TDT) (m), the field-derived thermal minima for movement (thermal minima) (°C) and the field-derived thermal maxima for movement (thermal maxima) (°C) were included as fixed terms. Coefficients (Coeff.) in square brackets belong to non-significant terms just before dropping those terms from the model. Variance (Var.) of the random term 'Week' and residuals are presented. The number of individuals (N) used in analysis are given. Bold p-values denote significant terms.

Dataset	Random Term	Var.	Fixed Term	Coeff.	χ^2	DF	p-value
M + F	Week	0.163	Intercept	-0.61			
(N = 34)	Residuals	0.345	Body Mass	[<-0.10]	<0.01	1	0.982
			Thermal Minima	-0.17	8.67	1	**0.003**
			Thermal Maxima	0.21	35.83	1	**<0.001**
			Sex (males)	0.53	4.12	1	**0.042**
F	Week	0.595	Intercept	-1.21			
(N = 19)	Residuals	0.393	Body Mass	[1.52]	0.55	1	0.460
			Thermal Minima	-0.17	6.26	1	**0.012**
			Thermal Maxima	0.25	20.26	1	**<0.001**
M	Week	0.002	Intercept	0.77			
(N = 15)	Residuals	0.005	Body Mass	[0.09]	0.27	1	0.606
			Thermal Minima	-0.13	29.87	1	**<0.001**
			Thermal Maxima	0.14	40.45	1	**<0.001**

Table B.3. Summary of test-statistics from LMMs with the field-derived thermal optima for movement as the response variable. Test statistics are given for male and female data combined (M + F) female-only data (F), and male-only data (M). Body mass and sex were included as fixed terms. Coefficients (Coeff.) in square brackets belong to non-significant terms just before dropping those terms from the model. Variance (Var.) of the random term 'Week' and residuals are presented. The number of individuals (N) used in analysis are given. Bold p-values denote significant terms.

Dataset	Random Term	Var.	Fixed Term	Coeff.	χ^2	DF	p-value
M + F	Week	<0.001	Intercept	-0.01			
(N = 50)	Residuals	0.776	Body Mass	[-0.63]	0.15	1	0.698
			Sex (males)	[-0.37]	1.37	1	0.242
			Thermal Maxima	0.35	48.46	1	**<0.001**
			Thermal Minima	0.67	49.57	1	**<0.001**
F	Week	0.209	Intercept	0.52			
(N = 28)	Residuals	0.236	Body Mass	[-0.36]	0.07	1	0.793
			Thermal Maxima	0.34	45.70	1	**<0.001**
			Thermal Minima	0.63	44.28	1	**<0.001**
M	Week	<0.001	Intercept	-0.11			
(N = 22)	Residuals	1.304	Body Mass	[0.85]	0.05	1	0.825
			Thermal Maxima	0.31	6.39	1	**0.011**
			Thermal Minima	0.72	11.38	1	**0.001**

Table B.4. Summary of test statistics from LMMs with the field-derived thermal minima as the response variable. Test statistics are given for male and female data combined (M + F) female-only data (F), and male-only data (M). Body mass and sex were included as fixed terms. Coefficients (Coeff.) in square brackets belong to non-significant terms just before dropping those terms from the model. Variance (Var.) of the random term 'Week' and residuals are presented. The number of individuals (N) used in analysis are given. Bold p-values denote significant terms.

Dataset	Random Term	Var.	Fixed Term	Coeff.	χ^2	DF	p-value
M + F	Week	4.252	Intercept	10.33			
(N = 34)	Residuals	3.571	Body Mass	[1.27]	0.04	1	0.846
			Sex (males)	[-1.10]	2.26	1	0.133
			Thermal Maxima	[0.01]	0.03	1	0.865
F	Week	2.928	Intercept	10.74			
(N = 19)	Residuals	5.782	Body Mass	[-3.43]	0.45	1	0.501
			Thermal Maxima	[0.04]	0.07	1	0.795
M	Week	3.999	Intercept	0.85			
(N = 15)	Residuals	0.834	Body Mass	[0.52]	<0.01	1	0.984
			Thermal Maxima	0.47	5.95	1	**0.015**

Table B.5. Summary of test statistics from LMMs with the field-derived thermal maxima as the response variable. Test statistics are given for male and female data combined (M + F) female-only data (F), and male-only data (M). Body mass and sex were included as fixed terms. Coefficients (Coeff.) in square brackets belong to non-significant terms just before dropping those terms from the model. Variance (Var.) of the random term 'Week' and residuals are presented. The number of individuals (N) used in analysis are given. Bold p-values denote significant terms.

Dataset	Random Term	Var.	Fixed Term	Coeff.	χ^2	DF	p-value
M + F	Week	7.499	Intercept	17.74			
(N = 34)	Residuals	15.421	Body Mass	[-8.45]	1.43	1	0.232
			Sex (males)	[-2.88]	2.86	1	0.091
			Thermal Minima	[0.30]	1.05	1	0.306
F	Week	<0.001	Intercept	32.67			
(N = 19)	Residuals	22.37	Body Mass	-23.85	5.74	1	**0.017**
			Thermal Minima	[0.10]	0.07	1	0.797
M	Week	8.532	Intercept	11.64			
(N = 15)	Residuals	0.689	Body Mass	[1.72]	0.17	1	0.680
			Thermal Minima	0.69	6.37	1	**0.012**

Table B.6. Summary of test statistics from LMMs with path straightness as a response variable. Test statistics are given for male and female data combined (M + F) female-only data (F), and male-only data (M). Coefficients (Coeff.) in square brackets belong to non-significant terms just before dropping those terms from the model. Variance (Var.) of the random terms 'Week' and 'ID' and Residuals are presented. The number of individuals (N) used in analysis are given. Bold p-values denote significant terms. Temperature, average daily temperature.

Data set	Random Term	Var.	Fixed Term	Coeff.	χ^2	DF	p-value
M + F	Week	<0.001	Intercept	0.89			
(N = 50)	ID	0.002	Temperature^2	<0.01	5.89	1	**0.015**
	Residual	0.103	Temperature	-0.06			
			Sex (males)	[0.04]	1.42	1	0.234
F	Week	0.001	Intercept	0.89			
(N = 28)	ID	0.002	Temperature^2	<0.01	5.22	1	**0.022**
	Residual	0.100	Temperature	-0.06			
M	Week	0.002	Intercept	0.57			
(N = 22)	ID	<0.001	Temperature^2	[<0.01]	0.73	1	0.394
	Residual	0.107	Temperature	[<-0.01]	0.23	1	0.630

Table B.7. Summary of test statistics from GLMMs with the distance travelled in 2 hours (cm) as a response variable. Test statistics are given for male and female data combined (M + F) female-only data (F), and male-only data (M), both for models in which instances in which individuals didn't move were retained (Zero inflated model output) and for models in which instances in which individuals didn't move were removed (Conditional model output). Average daily temperature^2, average daily temperature, and sex were included as fixed terms. Data were analysed first with collated male and female data and then separately. Model estimates and test statistics reported for conditional models are from terms after dropping those terms from the model, however, because terms could not be dropped from zero-inflated models, model estimates and test statistics reported for zero-inflated models are from the maximal model. Model coefficients (Coeff.) from conditional models that are in square brackets belong to non-significant terms just before dropping those terms from the model. Variance (Var.) of the random terms 'Week', and 'ID' are presented. The number of individuals (N) used in analysis are given. Bold p-values denote significant terms.

Dataset	Random Term	Var.	Fixed Term	Coeff.	χ^2	DF	p-value
M + F	Week	0.010	**Zero inflated model output**				
(N = 50)	ID	0.305	Intercept	-0.98			
			Temperature^2	<-0.03	2.07	1	0.151
			Temperature	0.06	0.96	1	0.327
			Sex (males)	0.15	2.71	1	0.100
			Conditional model output				
			Intercept	3.08			
			Temperature^2	<-0.01	17.40	1	**0.001**
			Temperature	0.14			
			Sex (males)	[0.19]	1.23	1	0.268
F	Week	0.004	**Zero inflated model output**				
(N = 28)	ID	0.325	Intercept	-0.98			

			Temperature^2	<-0.01	1.68	1	0.195
			Temperature	0.07	0.89	1	0.347
			Conditional model output				
			Intercept	3.29			
			Temperature^2	<-0.01	8.81	1	**0.003**
			Temperature	0.12			
M	Week	0.034	**Zero inflated model output**				
(N = 22)	ID	0.313	Intercept	-0.58			
			Temperature^2	<-0.01	0.47	1	0.493
			Temperature	0.04	0.17	1	0.681
			Conditional model output				
			Intercept	3.46			
			Temperature^2	[<-0.01]	2.42	1	0.120
			Temperature	0.07	35.83	1	**<0.001**

Table B.8. Summary of test statistics from GLMMs with the distance travelled in 8 hours (cm) as a response variable. Test statistics are given for male and female data combined (M + F) female-only data (F), and male-only data (M), both for models in which instances in which individuals didn't move were retained (Zero inflated model output) and for models in which instances in which individuals didn't move were removed (Conditional model output). Average daily temperature^2, average daily temperature, and sex were included as fixed terms. Data were analysed first with collated male and female data and then separately. Model estimates and test statistics reported for conditional models are from terms after dropping those terms from the model, however, because terms could not be dropped from zero-inflated models, model estimates and test statistics reported for zero-inflated models are from the maximal model. Model coefficients (Coeff.) from conditional models that are in square brackets belong to non-significant terms just before dropping those terms from the model. Variance (Var.) of the random terms 'Week', and 'ID' are presented. The number of individuals (N) used in analysis are given. Bold p-values denote significant terms.

Dataset	Random Term	Var.	Fixed Term	Coeff.	χ^2	DF	p-value
M + F	Week	<0.001	**Zero inflated model output**				
(N = 50)	ID	0.379	Intercept	-6.69			
			Temperature^2	-0.03	3.45	1	0.063
			Temperature	0.75	2.93	1	0.087
			Sex (males)	-0.03	0.01	1	0.934
			Conditional model output				
			Intercept	4.87			
			Temperature^2	[<-0.01]	2.81	1	0.094
			Temperature	0.026	3.95	1	**0.047**
			Sex (males)	[0.17]	0.77	1	0.379
F	Week	<0.001	**Zero inflated model output**				

(N = 28)	ID	0.330	Intercept	-3.09			
			Temperature^2	-0.01	0.48	1	0.487
			Temperature	0.19	0.18	1	0.673
			Conditional model output				
			Intercept	5.14			
			Temperature^2	[<-0.01]	0.19	1	0.662
			Temperature	[-0.01]	0.12	1	0.726
M	Week	0.032	**Zero inflated model output**				
(N = 22)	ID	0.415	Intercept	-16.89			
			Temperature^2	[-0.11]	3.40	1	0.065
			Temperature	2.50	3.50	1	0.061
			Conditional model output				
			Intercept	4.25			
			Temperature^2	[<-0.01]	1.80	1	0.180
			Temperature	0.08	14.60	1	**<0.001**

Table B.9. Summary of test statistics from LMMs with the field-derived operating range as the response variable. Test statistics are given for male and female data combined (M + F) female-only data (F), and male-only data (M). Total distance travelled (TDT, m) was included as a fixed term. Coefficients (Coeff.) in square brackets belong to non-significant terms just before dropping those terms from the model. Variance (Var.) of the random term 'Week' and residuals are presented. The number of individuals (N) used in analysis are given. Bold p-values denote significant terms.

Dataset	Random Term	Var.	Fixed Term	Coeff.	χ^2	DF	p-value
M + F	Week	2.258	Intercept	10.15			
(N = 34)	Residuals	14.810	TDT	-0.09	9.12	1	**0.003**
F	Week	12.750	Intercept	12.00			
(N = 19)	Residuals	14.560	TDT	-0.14	7.32	1	**0.007**
M	Week	8.375	Intercept	8.71			
(N = 15)	Residuals	0.964	TDT	[<-0.01]	0.03	1	0.853

Table B.10. Summary of test statistics from LMMs with the field-derived thermal optima as the response variable. Test statistics are given for male and female data combined (M + F) female-only data (F), and male-only data (M). Total distance travelled (TDT, m) was included as a fixed term. Coefficients (Coeff.) in square brackets belong to non-significant terms just before dropping those terms from the model. Variance (Var.) of the random term 'Week' and residuals are presented. The number of individuals (N) used in analysis are given. Bold p-values denote significant terms.

Dataset	Random Term	Var.	Fixed Term	Coeff.	χ^2	DF	p-value
M + F	Week	7.160	Intercept	13.35			
(N = 50)	Residuals	5.829	TDT	[<-0.01]	0.11	1	0.736
F	Week	6.610	Intercept	13.73			
(N = 28)	Residuals	7.463	TDT	[-0.01]	0.34	1	0.561
M	Week	4.692	Intercept	12.71			
(N = 22)	Residuals	5.136	TDT	[0.01]	0.122	1	0.727

Table B.11. Summary of test statistics from LMMs with the field-derived thermal minima as the response variable. Test statistics are given for male and female data combined (M + F) female-only data (F), and male-only data (M). Total distance travelled (TDT, m) was included as a fixed term. Coefficients (Coeff.) in square brackets belong to non-significant terms just before dropping those terms from the model. Variance (Var.) of the random term 'Week' and residuals are presented. The number of individuals (N) used in analysis are given. Bold p-values denote significant terms.

Dataset	Random Term	Var.	Fixed Term	Coeff.	χ^2	DF	p-value
M + F	Week	3.746	Intercept	9.35			
(N = 34)	Residuals	3.174	TDT	0.03	5.10	1	**0.024**
F	Week	2.928	Intercept	10.74			
(N = 19)	Residuals	5.782	TDT	[0.04]	2.67	1	0.102
M	Week	4.718	Intercept	9.36			
(N = 15)	Residuals	1.272	TDT	[0.03]	3.11	1	0.078

Table B.12. Summary of test statistics from LMMs with the field-derived thermal maxima as the response variable. Test statistics are given for male and female data combined (M + F) female-only data (F), and male-only data (M). Total distance travelled (TDT, m) was included as a fixed term. Coefficients (Coeff.) in square brackets belong to non-significant terms just before dropping those terms from the model. Variance (Var.) of the random term 'Week' and residuals are presented. The number of individuals (N) used in analysis are given. Bold p-values denote significant terms.

Dataset	Random Term	Var.	Fixed Term	Coeff.	χ^2	DF	p-value
M + F	Week	10.290	Intercept	19.86			
(N = 34)	Residuals	12.530	TDT	-0.07	5.10	1	**0.024**
F	Week	<0.001	Intercept	18.21			
(N = 19)	Residuals	28.580	TDT	[-0.11]	3.65	1	0.056
M	Week	11.322	Intercept	18.10			
(N = 15)	Residuals	1.359	TDT	[0.02]	2.08	1	0.149

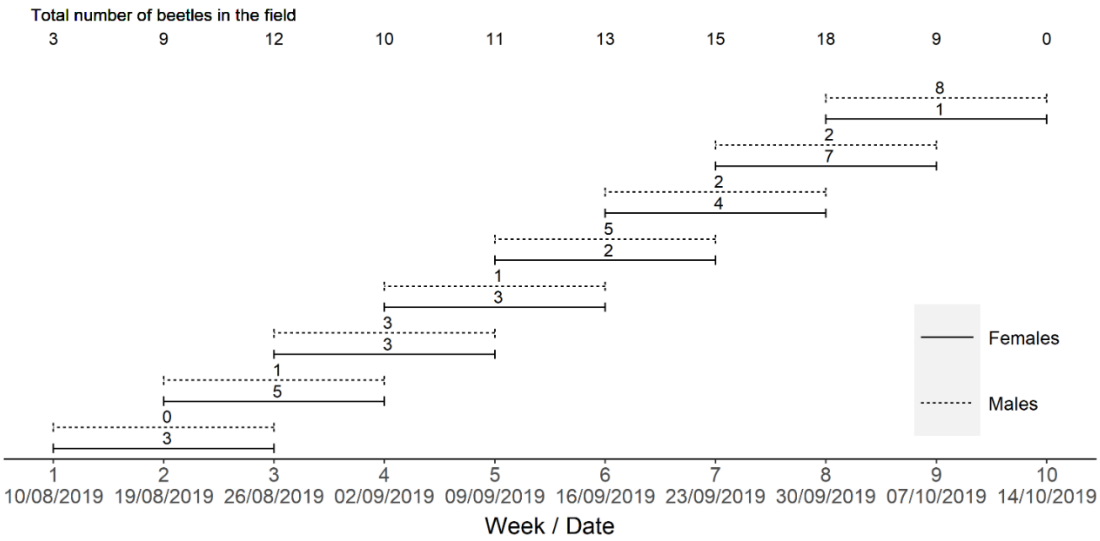

Figure B.1. Release and recapture schedule for *Carabus hortensis* individuals used in radio tracking. The study week and date on which male (dashed line) and female (solid line) *C. hortensis* were released into their natural environment, the duration over which those individuals were tracked via radio telemetry, and the study week and date on which those individuals were recaptured. Numbers above male and female lines denote the number of males or females released and tracked during the given time period. Numbers at the top of the figure denote the total number of beetles being tracked via radio telemetry in the field during the given study week.

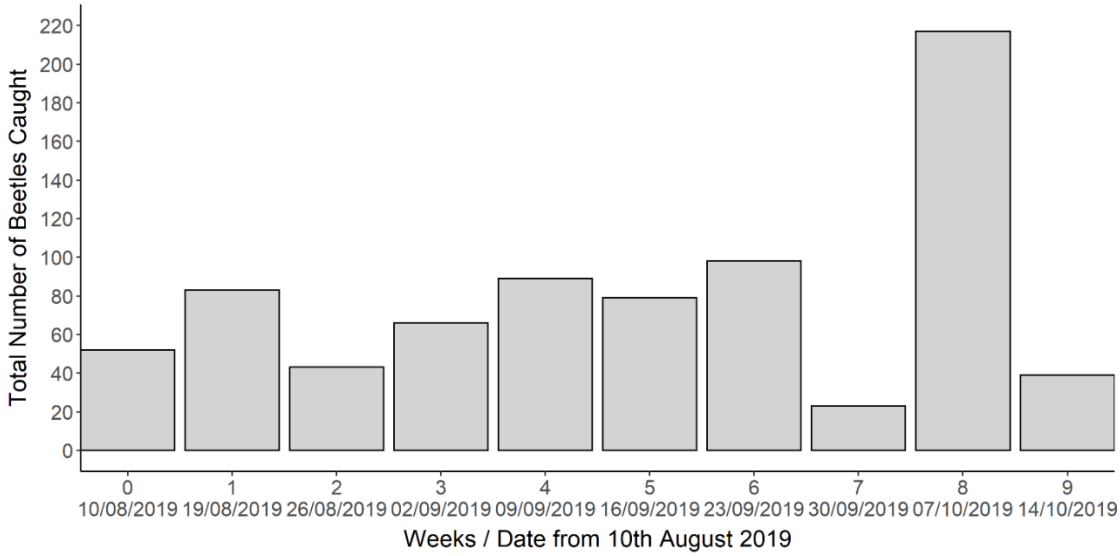

Figure S2. The total number of beetles caught each week for 9 weeks from 10th August 2019.

Appendix C: Chapter 4

Table C.1. Spearman's rank correlations between female (F) male (M) *C. hortensis* day 1 DT2h (distance travelled in the first 2 hours in the natural environment), day 1 DT8h (distance travelled in the first day in the natural environment), day 1 path straightness (path straightness of the first day in the natural environment), TDT (sum-total of DT8h for the entire tracking period), average path straightness over the entire tracking period, average DT2h (average DT2h over the entire tracking period), and average DT8h (average DT8h over the entire tracking period). Bold R_S values denote strong correlations (R > 0.7). Asterisks on p-values denote which correlations remain significant following False Discovery Rate testing (Benjamini & Hochberg, 1995)

Sex	Variable 1	Variable 2	R_S	p-value
F	Day 1 DT2h	Day 1 DT8h	0.282	0.163
	Day 1 DT2h	Day 1 Path Straightness	0.177	0.388
	Day 1 DT8h	Day 1 Path Straightness	0.234	0.272
	Average DT2h	Average DT8h	**0.984**	**<0.001***
	Average DT2h	Average Path Straightness	0.048	0.818
	Average DT8h	Average Path Straightness	-0.002	0.992
	Average DT2h	TDT	**0.998**	**<0.001***
	Average DT8h	TDT	**0.986**	**<0.001***
	Average Path Straightness	TDT	-0.002	0.992
M	Day 1 DT2h	Day 1 DT8h	0.037	0.871
	Day 1 DT2h	Day 1 Path Straightness	-0.468	0.028
	Day 1 DT8h	Day 1 Path Straightness	0.435	0.043
	Average DT2h	Average DT8h	**0.915**	**<0.001***
	Average DT2h	Average Path Straightness	0.077	0.732
	Average DT8h	Average Path Straightness	0.096	0.671
	Average DT2h	TDT	**1.000**	**<0.001***
	Average DT8h	TDT	**0.915**	**<0.001***
	Average Path Straightness	TDT	0.096	0.671

Table C.2. Summary of test statistics from LMMs with the distance travelled in the first two hours of release into the natural environment (day 1 DT2h) by all beetles (M + F), females (F) or males (M) as the response. Exploratory behaviour measured at one of 3°C, 10°C, 17°C, or 23°C, and the average temperature of the natural environment ($Field_{Temp}$) for the time over which day 1 DT2h was measured were included as fixed terms. Coefficients (Coeff.) in square brackets belong to non-significant terms just before dropping those terms from the model. N, number of individuals used in analysis. Variance (Var.) of the random term 'Week' and Residuals are presented. Bold p-values denote significant terms. Asterisks on p-values denote which correlations remain significant following False Discovery Rate testing (Benjamini & Hochberg, 1995)

Dataset	Random Term	Var.	Fixed Term	Coeff.	χ^2	DF	p-value
M + F	Week	0.01	Intercept	0.38			
(3°C)	Residual	0.12	Exploratory behaviour	[0.01]	1.64	1	0.200
(N = 36)			$Field_{Temp}$	[0.01]	0.57	1	0.450
			Sex (males)	[-0.02]	0.06	1	0.805
F	Week	0.19	Intercept	0.11			
(3°C)	Residual	0.06	Exploratory behaviour	0.03	4.97	1	**0.026**
(N = 22)			$Field_{Temp}$	[-0.2]	0.45	1	0.502
M	Week	<0.01	Intercept	-0.11			
(3°C)	Residual	0.07	Exploratory behaviour	[<0.01]	0.06	1	0.810
(N = 14)			$Field_{Temp}$	0.03	4.67	1	**0.031**
M + F	Week	0.01	Intercept	0.381			
(10°C)	Residual	0.12	Exploratory behaviour	[<-0.01]	0.51	1	0.476
(N = 36)			$Field_{Temp}$	[0.01]	0.82	1	0.364
			Sex (males)	[0.01]	0.12	1	0.730
F	Week	0.05	Intercept	0.42			
(10°C)	Residual	0.12	Exploratory behaviour	[<-0.01]	0.37	1	0.541

(N = 22)			$Field_{Temp}$	[-0.01]	0.07	1	0.798
M	Week	<0.01	Intercept	-0.11			
(10°C)	Residual	0.07	Exploratory behaviour	[<0.01]	0.03	1	0.860
(N =14)			$Field_{Temp}$	0.03	4.67	1	**0.031**
M + F	Week	0.01	Intercept	0.38			
(17°C)	Residual	0.12	Exploratory behaviour	[-0.01]	3.42	1	0.064
(N = 36)			$Field_{Temp}$	[<0.01]	0.04	1	0.847
			Sex (males)	[0.02]	0.07	1	0.796
F	Week	0.09	Intercept	1.17			
(17°C)	Residual	0.08	Exploratory behaviour	-0.01	5.32	1	**0.021**
(N = 22)			$Field_{Temp}$	[-0.02]	0.81	1	0.367
M	Week	<0.01	Intercept	-0.11			
(17°C)	Residual	0.07	Exploratory behaviour	[<0.01]	0.24	1	0.621
(N = 14)			$Field_{Temp}$	0.03	4.67	1	**0.031**
M + F	Week	0.01	Intercept	0.38			
(23°C)	Residual	0.12	Exploratory behaviour	[<0.01]	0.32	1	0.572
(N = 36)			$Field_{Temp}$	[0.01]	0.82	1	0.364
			Sex (males)	[-0.01]	<0.01	1	0.965
F	Week	0.05	Intercept	0.42			
(23°C)	Residual	0.12	Exploratory behaviour	[<0.01]	0.04	1	0.841
(N = 22)			$Field_{Temp}$	[-0.01]	0.14	1	0.708
M	Week	<0.01	Intercept	-0.11			
(23°C)	Residual	0.07	Exploratory behaviour	[<0.01]	0.44	1	0.509
(N =14)			$Field_{Temp}$	0.03	4.67	1	**0.031**

Table C.3. Summary of test statistics from LMMs with the distance travelled in the first day hours of release into the natural environment (day 1 DT8h) by all beetles (M + F), females (F) or males (M) as the response. Exploratory behaviour measured at one 3°C, 10°C, 17°C, or 23°C, and the average temperature of the natural environment ($Field_{Temp}$) for the time over which day 1 DT8h was measured were included as fixed terms. Coefficients (Coeff.) in square brackets belong to non-significant terms just before dropping those terms from the model. N, number of individuals used in analysis. Variance (Var.) of the random term 'Week' and Residuals are presented. Bold p-values denote significant terms.

Dataset	Random Term	Var.	Fixed Term	Coeff.	χ^2	DF	p-value
M + F	Week	0.04	Intercept	0.934			
(3°C)	Residual	0.58	Exploratory behaviour	[0.01]	0.05	1	0.816
(N = 36)			$Field_{Temp}$	[0.03]	0.63	1	0.428
			Sex (males)	[-0.10]	0.136	1	0.713
F	Week	0.11	Intercept	0.94			
(3°C)	Residual	0.72	Exploratory behaviour	[0.01]	0.04	1	0.843
(N = 22)			$Field_{Temp}$	[0.06]	0.93	1	0.334
M	Week	0.12	Intercept	0.82			
(3°C)	Residual	0.22	Exploratory behaviour	[0.03]	0.62	1	0.432
(N = 14)			$Field_{Temp}$	[<0.01]	<0.01	1	0.995
M + F	Week	0.04	Intercept	0.93			
(10°C)	Residual	0.58	Exploratory behaviour	[<0.01]	0.03	1	0.873
(N = 36)			$Field_{Temp}$	[0.03]	0.63	1	0.428
			Sex (males)	[-0.10]	0.14	1	0.713
F	Week	0.11	Intercept	0.094			
(10°C)	Residual	0.72	Exploratory behaviour	[-0.01]	0.21	1	0.644
(N = 22)			$Field_{Temp}$	[0.06]	0.93	1	0.334

M	Week	0.12	Intercept	0.82			
(10°C)	Residual	0.22	Exploratory behaviour	[0.10]	0.53	1	0.466
(N = 14)			$Field_{Temp}$	[-0.01]	0.03	1	0.855
M + F	Week	0.04	Intercept	0.93			
(17°C)	Residual	0.58	Exploratory behaviour	[<0.01]	<0.01	1	0.973
(N = 36)			$Field_{Temp}$	[0.03]	0.63	1	0.428
			Sex (males)	[-0.10]	0.14	1	0.713
F	Week	0.11	Intercept	0.94			
(17°C)	Residual	0.72	Exploratory behaviour	[-0.02]	3.03	1	0.082
(N = 22)			$Field_{Temp}$	[0.06]	0.81	1	0.368
M	Week	0.12	Intercept	0.82			
(17°C)	Residual	0.22	Exploratory behaviour	[0.01]	3.32	1	0.068
(N = 14)			$Field_{Temp}$	[0.03]	0.48	1	0.489
M + F	Week	0.04	Intercept	0.93			
(23°C)	Residual	0.58	Exploratory behaviour	[0.01]	0.87	1	0.352
(N = 36)			$Field_{Temp}$	[0.04]	0.80	1	0.370
			Sex (males)	[-0.30]	1.00	1	0.316
F	Week	0.11	Intercept	0.94			
(23°C)	Residual	0.72	Exploratory behaviour	[0.01]	0.78	1	0.379
(N = 22)			$Field_{Temp}$	[0.06]	0.93	1	0.334
M	Week	0.12	Intercept	0.82			
(23°C)	Residual	0.22	Exploratory behaviour	[0.01]	1.25	1	0.263
(N = 14)			$Field_{Temp}$	[<0.01]	<0.01	1	0.946

Table C.4. Summary of test statistics from LMMs with the path straightness in the first day hours of release into the natural environment of all beetles (M + F), females (F) or males (M) as the response. Exploratory behaviour measured at one 3°C, 10°C, 17°C, or 23°C, and the average temperature of the natural environment ($Field_{Temp}$) for the time over which day 1 path straightness was measured were included as fixed terms. Coefficients (Coeff.) in square brackets belong to non-significant terms just before dropping those terms from the model. N, number of individuals used in analysis. Variance (Var.) of the random term 'Week' and Residuals are presented. Bold p-values denote significant terms.

Dataset	Random Term	Var.	Fixed Term	Coeff.	χ^2	DF	p-value
M + F	Week	<0.01	Intercept	0.49			
(3°C)	Residual	0.13	Exploratory behaviour	[0.01]	0.30	1	0.581
(N = 36)			$Field_{Temp}$	[0.01]	0.51	1	0.478
			Sex (males)	[-0.16]	1.78	1	0.183
F	Week	0.02	Intercept	0.54			
(3°C)	Residual	0.15	Exploratory behaviour	[0.01]	0.31	1	0.580
(N = 22)			$Field_{Temp}$	[0.04]	2.50	1	0.114
M	Week	<0.01	Intercept	0.39			
(3°C)	Residual	0.07	Exploratory behaviour	[<0.01]	0.02	1	0.881
(N = 14)			$Field_{Temp}$	[-0.03]	2.06	1	0.151
M + F	Week	<0.01	Intercept	0.49			
(10°C)	Residual	0.13	Exploratory behaviour	[<0.01]	0.26	1	0.610
(N = 36)			$Field_{Temp}$	[0.01]	0.50	1	0.478
			Sex (males)	[-0.16]	1.78	1	0.183
F	Week	0.02	Intercept	0.54			
(10°C)	Residual	0.15	Exploratory behaviour	[<0.01]	0.06	1	0.805
(N = 22)			$Field_{Temp}$	[0.04]	2.50	1	0.114

M	Week	<0.01	Intercept	0.39			
(10°C)	Residual	0.07	Exploratory behaviour	[<-0.01]	0.17	1	0.682
(N = 14)			$Field_{Temp}$	[-0.03]	2.06	1	0.151
M + F	Week	<0.01	Intercept	0.49			
(17°C)	Residual	0.13	Exploratory behaviour	[<0.01]	0.17	1	0.681
(N = 36)			$Field_{Temp}$	[0.01]	0.50	1	0.478
			Sex (males)	[-0.16]	1.78	1	0.183
F	Week	0.02	Intercept	0.54			
(17°C)	Residual	0.15	Exploratory behaviour	[<-0.01]	0.36	1	0.549
(N = 22)			$Field_{Temp}$	[0.04]	2.50	1	0.114
M	Week	<0.01	Intercept	0.39			
(17°C)	Residual	0.07	Exploratory behaviour	[<0.01]	0.12	1	0.731
(N = 14)			$Field_{Temp}$	[-0.03]	2.06	1	0.151
M + F	Week	<0.01	Intercept	0.49			
(23°C)	Residual	0.13	Exploratory behaviour	[<0.01]	0.46	1	0.497
(N = 36)			$Field_{Temp}$	[0.01]	0.50	1	0.478
			Sex (males)	[-0.16]	1.78	1	0.183
F	Week	0.02	Intercept	0.54			
(23°C)	Residual	0.15	Exploratory behaviour	[<-0.01]	0.32	1	0.572
(N = 22)			$Field_{Temp}$	[0.04]	2.50	1	0.114
M	Week	<0.01	Intercept	0.39			
(23°C)	Residual	0.07	Exploratory behaviour	[<0.01]	3.03	1	0.082
(N = 14)			$Field_{Temp}$	[-0.02]	1.16	1	0.281

Table C.5. Summary of test statistics from LMMs with the total distance travelled over the field season (TDT) by all beetles (M + F), females (F) or males (M) as the response. Exploratory behaviour measured at one of 3°C, 10°C, 17°C, or 23°C, and the average temperature of the natural environment ($Field_{Temp}$) for the time over which TDT was measured were included as fixed terms. Coefficients (Coeff.) in square brackets belong to non-significant terms just before dropping those terms from the model. N, number of individuals used in analysis. Variance (Var.) of the random term 'Week' and Residuals are presented. Bold p-values denote significant terms. Asterisks on p-values denote which correlations remain significant following False Discovery Rate testing (Benjamini & Hochberg, 1995).

Dataset	Random Term	Var.	Fixed Term	Coeff.	χ^2	DF	p-value
M + F	Week	<0.01	Intercept	2.98			
(3°C)	Residual	0.31	Exploratory behaviour	[-0.01]	0.55	1	0.459
(N = 36)			$Field_{Temp}$	[-0.05]	2.75	1	0.097
			Sex (males)	[-0.14]	0.57	1	0.449
F	Week	<0.01	Intercept	4.12			
(3°C)	Residual	0.29	Exploratory behaviour	[-0.01]	0.29	1	0.590
(N = 22)			$Field_{Temp}$	-0.08	4.02	1	**0.045**
M	Week	<0.01	Intercept	21.89			
(3°C)	Residual	151.10	Exploratory behaviour	[-0.36]	0.28	1	0.599
(N = 14)			$Field_{Temp}$	[-0.94]	0.43	1	0.512
M + F	Week	<0.01	Intercept	2.98			
(10°C)	Residual	0.31	Exploratory behaviour	[0.01]	1.49	1	0.222
(N = 36)			$Field_{Temp}$	[-0.05]	2.75	1	0.097
			Sex (males)	[-0.17]	0.87	1	0.351
F	Week	<0.01	Intercept	4.12			
(10°C)	Residual	0.29	Exploratory behaviour	[0.01]	1.29	1	0.257

(N = 22)			$Field_{Temp}$	-0.08	4.02	1	**0.045**
M	Week	<0.01	Intercept	21.89			
(10°C)	Residual	151.10	Exploratory behaviour	[0.36]	1.04	1	0.307
(N = 14)			$Field_{Temp}$	[-0.98]	0.51	1	0.475
M + F	Week	<0.01	Intercept	2.98			
(17°C)	Residual	0.31	Exploratory behaviour	[-0.01]	1.01	1	0.316
(N = 36)			$Field_{Temp}$	[-0.05]	2.75	1	0.097
			Sex (males)	[-0.11]	0.32	1	0.571
F	Week	<0.01	Intercept	4.12			
(17°C)	Residual	0.29	Exploratory behaviour	[-0.01]	2.04	1	0.153
(N = 22)			$Field_{Temp}$	-0.08	4.02	1	**0.045**
M	Week	<0.01	Intercept	21.89			
(17°C)	Residual	151.10	Exploratory behaviour	[0.03]	0.05	1	0.828
(N = 14)			$Field_{Temp}$	[-0.94]	0.43	1	0.512
M + F	Week	<0.01	Intercept	2.98			
(23°C)	Residual	0.31	Exploratory behaviour	[<0.01]	0.95	1	0.330
(N = 36)			$Field_{Temp}$	[-0.05]	2.75	1	0.97
			Sex (males)	[-0.29]	1.86	1	0.173
F	Week	<0.01	Intercept	4.12			
(23°C)	Residual	0.29	Exploratory behaviour	[0.01]	0.50	1	0.478
(N = 22)			$Field_{Temp}$	-0.08	4.02	1	**0.045**
M	Week	<0.01	Intercept	21.89			
(23°C)	Residual	151.10	Exploratory behaviour	[0.17]	1.62	1	0.203
(N = 14)			$Field_{Temp}$	[-0.83]	0.37	1	0.542

Table C.6. Summary of test statistics from LMMs with the average path straightness over the field season of all beetles (M + F), females (F) or males (M) as the response. Exploratory behaviour measured at one 3°C, 10°C, 17°C, or 23°C, and the average temperature of the natural environment ($Field_{Temp}$) for the time over which average path straightness was measured were included as fixed terms. Coefficients (Coeff.) in square brackets belong to non-significant terms just before dropping those terms from the model. N, number of individuals used in analysis. Variance (Var.) of the random term 'Week' and Residuals are presented. Bold p-values denote significant terms. Asterisks on p-values denote which correlations remain significant following False Discovery Rate testing (Benjamini & Hochberg, 1995).

Dataset	Random Term	Var.	Fixed Term	Coeff.	χ^2	DF	p-value
M + F	Week	<0.01	Intercept	0.55			
(3°C)	Residual	0.01	Exploratory behaviour	[<0.01]	0.10	1	0.753
(N = 36)			$Field_{Temp}$	[-0.01]	0.74	1	0.391
			Sex (males)	[-0.01]	0.06	1	0.813
F	Week	<0.01	Intercept	0.55			
(3°C)	Residual	0.01	Exploratory behaviour	[<-0.01]	0.97	1	0.324
(N = 22)			$Field_{Temp}$	[<-0.01]	0.45	1	0.505
M	Week	<0.01	Intercept	0.37			
(3°C)	Residual	0.01	Exploratory behaviour	0.01	5.73	1	**0.017**
(N = 14)			$Field_{Temp}$	[-0.01]	1.00	1	0.318
M + F	Week	<0.01	Intercept	0.55			
(10°C)	Residual	0.01	Exploratory behaviour	[<0.01]	0.28	1	0.598
(N = 36)			$Field_{Temp}$	[-0.01]	0.74	1	0.391
			Sex (males)	[-0.01]	0.10	1	0.747
F	Week	<0.01	Intercept	0.55			
(10°C)	Residual	0.01	Exploratory behaviour	[<0.01]	1.25	1	0.264

(N = 22)			$Field_{Temp}$	[-0.01]	0.89	1	0.346
M	Week	0.01	Intercept	0.55			
(10°C)	Residual	0.01	Exploratory behaviour	[<-0.01]	0.76	1	0.383
(N = 14)			$Field_{Temp}$	[<-0.01]	0.05	1	0.829
M + F	Week	<0.01	Intercept	0.55			
(17°C)	Residual	0.01	Exploratory behaviour	[<0.01]	1.63	1	0.202
(N = 36)			$Field_{Temp}$	[<-0.01]	0.44	1	0.507
			Sex (males)	[-0.02]	0.27	1	0.606
F	Week	<0.01	Intercept	0.55			
(17°C)	Residual	0.01	Exploratory behaviour	[<-0.01]	0.05	1	0.822
(N = 22)			$Field_{Temp}$	[<-0.01]	0.35	1	0.557
M	Week	0.01	Intercept	0.55			
(17°C)	Residual	0.01	Exploratory behaviour	[<0.01]	2.33	1	0.127
(N = 14)			$Field_{Temp}$	[<0.01]	0.03	1	0.866
M + F	Week	<0.01	Intercept	0.55			
(23°C)	Residual	0.01	Exploratory behaviour	[<0.01]	0.42	1	0.519
(N = 36)			$Field_{Temp}$	[-0.01]	0.74	1	0.391
			Sex (males)	[-0.02]	0.37	1	0.541
F	Week	<0.01	Intercept	0.55			
(23°C)	Residual	0.01	Exploratory behaviour	[<0.01]	0.11	1	0.745
(N = 22)			$Field_{Temp}$	[<-0.01]	0.35	1	0.557
M	Week	0.01	Intercept	0.55			
(23°C)	Residual	0.01	Exploratory behaviour	[<0.01]	0.66	1	0.418
(N = 14)			$Field_{Temp}$	[<-0.01]	0.12	1	0.730

Appendix D: Chapter 5

Table D.1. The mean, standard deviation (SD), and range of female (F) (n = 46) and male (M) (n = 22) pronotum width (mm) and body mass (g). Data includes only those individuals used in analysis.

	Pronotum Width (mm)		Body Mass (g)	
Sex	Average ± SD	Range	Average ± SD	Range
F	7.5 ± 0.79	5.8 - 9.5	0.703 ± 0.10	0.481 - 0.910
M	7.3 ± 0.73	5.8 - 8.4	0.558 ± 0.06	0.408 - 0.666

Table D.2. LMMs for resting metabolic rate (RMR) (CO_2 ml/h) for male and female data combined (M + F) and female-only (F) data B_{Temp}, Behavioural temperature; exploratory behaviour (number of square visits in a novel environment); $M_{Temp,}$ metabolic temperature; n, number of individuals; Var, variance of random terms. Coefficients (Coeff.) in square brackets belong to non-significant terms just before dropping those terms from the model. Bold p-values denote significant terms.

Dataset	Random Term	Var.	Fixed Term	Coeff.	χ^2	DF	p-value
M + F	Week	1.397	Intercept	5.92			
(N = 58)	ID	4.801	B_{Temp}: Exploratory Behaviour	[<0.01]	0.40	1	0.529
	Residual	3.231	Body Mass: Exploratory Behaviour	[0.20]	1.66	1	0.197
			Body Mass	[0.56]	0.04	1	0.846
			Exploratory Behaviour	-0.04	7.87	1	**0.005**
			Pronotum Width	[-0.36]	0.62	1	0.430
			M_{Temp}	[0.30]	2.81	1	0.094
			B_{Temp}	[-0.10]	0.23	1	0.632
			Sex (M)	[-0.38]	0.26	1	0.611
F	Week	1.293	Intercept	5.19			
(N = 41)	ID	6.519	B_{Temp} : Exploratory Behaviour	[<0.01]	0.25	1	0.615
	Residual	3.162	Body Mass: Exploratory Behaviour	[0.08]	0.16	1	0.692
			Body Mass	[-1.04]	0.02	1	0.896
			Exploratory Behaviour	[-0.03]	2.30	1	0.129
			Pronotum Width	[-0.72]	1.51	1	0.220
			M_{Temp}	[0.30]	2.07	1	0.150
			B_{Temp}	[-0.01]	0.02	1	0.881

Appendix E: Chapter 7

Table E.1. Comparison of a model including temperature as a random slope and individual as a random intercept (Random Slopes and Intercepts Model) with a model with individual as a random intercept alone (Random Intercepts Model). Expected log pointwise predictive density (ELPD) Watanabe-Akaike information criterion (WAIC; Watanabe, 2010) and leave-one-out cross validation (LOO) values and standard error (SE) are presented. Differences between the WAIC and LOO values (ELPD.diff: Random Intercepts Model – Random Slopes and Intercepts Model) are given. Bold values denote a significant difference between the Interaction and Mixed Effects Model.

		Random Slopes and Intercepts Model				*Random Intercepts Model*			
		WAIC		LOO		WAIC		LOO	
Response Variable	Sex	ELPD	SE	ELPD	SE	ELPD	SE	ELPD	SE
Exploratory Behaviour	F	-445.8	22.1	-446.4	22.1	-447.0	21.6	-447.1	21.6
Exploratory Behaviour	M	-419.2	22.0	-420.1	22.1	-419.0	20.8	-419.3	20.8
RMR	F	-151.1	10.2	-154.5	10.7	-163.1	12.1	-163.6	11.9
RMR	M	-135.9	8.3	-139.5	8.8	-146.5	8.9	-147.7	9.1
AMR	F	-150.7	9.8	-153.4	10.0	-163.2	12.1	-164.3	12.3
AMR	M	-134.5	8.3	-136.9	8.5	-146.4	9.0	-147.4	9.0

		Random Intercepts Model - Random Slopes and Intercepts Model			
		WAIC		LOO	
Response Variable	Sex	ELPD.diff	SE	ELPD.diff	SE
Exploratory Behaviour	F	1.2	-0.5	0.6	-0.5
Exploratory Behaviour	M	-0.2	-1.2	-1.1	-1.3
RMR	F	**12**	**1.9**	8.6	1.2
RMR	M	**10.6**	**0.6**	7	0.3

AMR	F	12.5	2.3	9.8	2.3
AMR	M	11.9	0.7	9.5	0.5