CHAPTER 5

Genetic and plastic responses of insects to climate change

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5.1 Introduction

Ever since insects emerged around 480 million years ago, they have been a key component of terrestrial ecosystems and have become one of the most speciose groups of animals (Misof et al., 2014). This evolutionary success story speaks to their resilience and ability to evolve in response to environmental change. However, the speed at which environments have been changing in the last 150 years or so has imposed major challenges on insect populations. Indeed, there is mounting evidence that insect populations have been plummeting in the last few decades and that even once common species now face the threat of extinction (Hallmann et al. 2017; Wagner et al. 2021; Warren et al. 2021). Because changes in insect abundances have immediate ecosystem-level consequences, including ecosystem services (e.g., Losey and Vaughan 2006), these observations are reason for concern. Consequently, the mechanisms that allow insects to cope with changing environments are of great interest to entomologists, evolutionary biologists, and ecologists alike.

In general, insects have three options to cope with climatic changes. The first option, if possible, is to track changes in environmental conditions through *shifts in geographic distribution ranges*. These shifts are not due to active migration but primarily a consequence of some populations experiencing increasingly unsuited conditions facing extinction, while others disperse and establish in areas where environmental conditions were previously unsuited. Although climate change-mediated distribution shifts have long been recognized and are common (Parmesan and Yohe 2003), relocation is unlikely to be an option for most insects due to habitat fragmentation, as well as natural and anthropogenic barriers to dispersal. In cases where relocation is not possible, insects may rely on *phenotypic plasticity*. Insects have evolved mechanisms to deal with environmental variation within their lifetime and can adjust various aspects of their morphology, physiology, or behavior to deal with climatic challenges. If adaptive, such within-generation responses may buffer against rapid environmental change and allow populations to persist. Lastly, insects may also withstand rapid environmental changes through *adaptation*. That is, insects can evolve in response to novel conditions through genetic (i.e., heritable) changes. Although adaptation is often a very slow process, there is ample evidence that populations can

evolve on the scale of just a few decades (so-called 'ecological timescales'; Carroll et al. 2007; Hendry 2017).

While Chapters 6 and 11 of this book focus on changes in phenology and geographic distribution ranges, this chapter focuses on genetic (i.e., evolutionary) and plastic (i.e., environmental) responses of insects to climate change. Sections 5.2 and 5.3 provide a brief quantitative genetic overview on genetic variation, heritability and adaptation. In sections 5.4–5.7, we turn to plasticity and why plastic changes are so abundant. In the remainder of the chapter, we discuss how plasticity, once evolved, shapes further evolutionary change and how genetic accommodation may contribute to rapid evolutionary responses to climate change.

5.2 Adaptation: Genetic responses to selection

In its widest sense, adaptation refers to the process of organisms becoming better suited to their habitats through evolutionary processes (Dobzhansky 1968). This process is driven by the joint action of heredity (i.e., the passing on of characteristics from one generation to the next) and natural selection (i.e., the non-random survival and reproduction of individuals with regard to their phenotype). Together, these two processes allow organisms that are better suited to their environment not only to contribute more offspring to the next generation but to also pass on the genes (and possibly other factors) to their offspring that made them better at thriving in their habitat. Over time, this leads to populations becoming better able to perform in their environment. Adaptation is often regarded as a slow process but it can be very fast and is thus an important mechanism that can allow insects to adapt to novel environments on short timescales (Hendry 2017). This includes rapid evolution of resistance to insecticides (e.g., in mosquitos; Weetman et al. 2015), adaptation to urban habitats (reviewed in Diamond et al. 2022) and rapid adaptation in recently introduced invasive species (e.g., Gibert et al. 2016; Olazcuaga et al. 2022). Below, we start by providing a brief quantitative genetic perspective on adaptation, discuss how evolutionary potential can be quantified and then provide several examples of how insects may adapt to changing climates.

5.2.1 *Identifying and predicting adaptation: An evolutionary quantitative genetic perspective*

Evolutionary quantitative genetics provides a powerful framework to predict evolutionary change in most continuous polygenic traits (Walsh and Lynch 2018). Although quantitative genetics largely ignores the molecular developmental basis of trait variation, it is a very successful discipline that continues to lay the foundation of modern evolutionary and ecological genetics (Hill and Kirkpatrick 2010). One of the cornerstones of the field is the so-called *breeder's equation* and its derivatives. In essence, it predicts that the response to selection (i.e., change in mean trait value, $\Delta\mu$) is not only dependent on the magnitude and direction of selection (S) but also on the degree to which traits are inherited from parents to their offspring (h^2) (Falconer 1960).

$$\Delta\mu = h^2 S$$

Heritability (and in particular, as we will see below, genetic variation) is thus a major determinant of responses to selection and is likely to play a key role in shaping the magnitude

and speed of evolutionary responses to climate change. Below, we provide a brief primer on why genetic variation is an important estimate of evolvability and how the amount of heritable variation can be estimated.

5.2.2 *Quantifying heritable variation*

Heritability can be assessed by quantifying the extent to which phenotypic variation is explained by genetic relatedness. Shared variation among relatives can be estimated, for instance, by rearing groups of individuals that vary in their degree of relatedness (e.g., clones, inbred lines, full-sib or half-sib families) under standardized conditions in a laboratory or greenhouse. These approaches are often referred to as 'common garden experiments'. Using statistical approaches (e.g., standard analysis of variance (ANOVA) or mixed model procedures (Walsh and Lynch 2018)), the total phenotypic variance (V_P) observed among animals reared in common garden conditions can be decomposed into a genetic (i.e., heritable) component (V_G) and a residual (or error) term (V_ε) that subsumes non-genetic sources of variation as:

$$V_P = V_G + V_{\varepsilon.}$$

The genetic variance component V_G captures the amount of variation explained by mean differences in trait expression between genotypes (or other genetic groupings, depending on the experimental design). In this simplistic example, the non-genetic variance component V_ε consists of measurement error and environmental variation (see section 5.4) and various other factors. Importantly, the term '*genetic* variation' is used to indicate the amount of *heritable* variation. This entity does *not* indicate whether the development of a trait *per se* is related to genes or gene products in any way. For example: like many scarabs, *Onthophagus* dung beetles develop shovel-like foretibiae with four tooth-like structures that are used to dig through soil. When beetles are collected in the field, the number, shape and size of these tibial teeth varies greatly (Figure 5.1A,B, and C), indicating large amounts of phenotypic variation. However, when rearing these beetles in the laboratory, each individual develops exactly four pointed tibial teeth (Figure 5.1D and E). It turns out that the variation observed in the field is caused by wear of tibial teeth when digging through compact soil. Most of the variation among individuals we observe in nature is thus driven by wear and tear and has no heritable basis. Consequently, the genetic variation is nil (or at least very small). It is important to note that, at the same time, the number of tibial teeth is under tight genetic control. For instance, knocking down the embryonic patterning gene *mex3* leads to the disappearance of two out of the four tibial teeth (Linz et al. 2019). This suggests that while genes and gene products play a role in the development of tibial teeth, there is just no heritable (i.e., genetic) variation in these developmental processes that selection could act on.

The heritability that features prominently in the breeder's equation can be calculated by dividing the variation attributable to genetic factors by the total phenotypic variation. Heritability comes in two different flavors. *Broad-sense* heritability (H^2) is the ratio between total genetic variation and total phenotypic variation calculated as:

$$H^2 = V_G/V_P$$

This proportion indicates the degree to which phenotypic variation among individuals is due to shared heritable factors. However, the genetic component V_G is composed of several different types of genetic effects and it is useful to differentiate between them. The
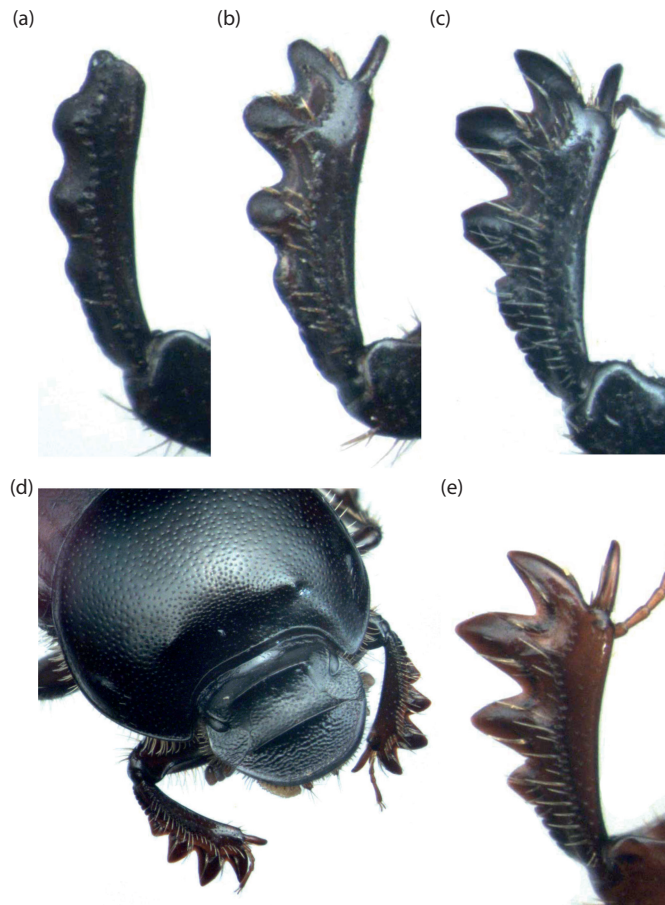
Figure 5.1 The forelegs (specifically the tibiae) of the bull-headed dung beetle *Onthophagus taurus* are equipped with four exaggerated tibial teeth that are used to construct underground breeding tunnels. In natural populations, the morphology of the forelegs differs strongly among individuals (a–c), indicating large amount of phenotypic variation. However, when females (d) from various families and populations are reared under laboratory conditions, they all develop a very similar morphology (e). This is because the variation in the field is caused by the wear of the tibia when digging through hard soil. The large phenotypic variation observed in the field thus does not necessitate to be heritable.

most important one is the variance attributable to additive genetic variance V_A; that is, the variance due to the additive effects of segregating alleles. In contrast to non-additive sources of variance (such as dominance and epistasis), additive effects take center stage in evolutionary quantitative genetics because they are primarily responsible for the resemblance among relatives and respond to selection the fastest (Hill et al. 2008; Walsh and Lynch 2018). Most of the quantitative literature thus focuses on the *narrow-sense* heritability (h^2) that is calculated by dividing the additive genetic variance by the total phenotypic variance.

$$h^2 = V_A/V_P$$

Calculating the additive genetic variance component requires more complex breeding designs, such as full-sib/half-sib designs or so-called animal models (Wilson et al. 2010) which require pedigree information. Because V_A is only one component of V_G, h^2 is always smaller than H^2. Note that most quantitative genetic studies in insects are performed in heavily controlled and often artificial (laboratory) environments. As genetic (and phenotypic) variance components are contingent on the environment they are measured in, it is often unclear how findings made in the laboratory translate into the wild. This continues to be a major caveat, especially because laboratory conditions rarely resemble the complex and fluctuating conditions in nature (Rodrigues and Beldade 2020).

5.2.3 *Genetic variation, heritability, and evolvability*

As evident from the breeder's equation, the amount of additive genetic variation relative to the total phenotypic variance (i.e., h^2) is a key determinant of the magnitude of the response to selection. However, while heritability estimates measure how much of the *variance* in a trait is heritable, they do not tell us how V_A compares to the *mean* phenotypic value. It is thus useful to also consider genetic variance relative to the mean trait values. A useful measure in this regard is *evolvability (I_A)* (Hansen and Pélabon 2021; Houle 1992). I_A is calculated by dividing the additive genetic variance by the square of the mean trait value (m):

$$I_A = V_A/m^2$$

The value of I_A is the expected percentage change in a trait under a unit strength of selection. I_A is thus a more intuitive measure of evolutionary potential than heritability because it focuses on changes in mean trait values rather than changes in variances. Even though evolvability and heritability are just different ways of standardizing genetic variances, they may lead to very different conclusions. For instance, while morphological traits tend to have higher heritability compared to many life-history traits, their evolvability tends to be smaller. These inconsistencies lead to the lack of a correlation between h^2 and I_A (Hansen and Pelabon 2021). Consequently, comparing V_A across species, environments and traits is not necessarily straightforward and the method of standardization (mean- or variance) should be chosen with care.

5.2.4 *Testing for adaptation*

Per definition, adaptation refers to the process of organisms becoming better able to perform in their habitats through evolutionary processes (Dobzhansky 1968). In order to infer adaptation, investigators at minimum need to demonstrate two things: First, it needs to be shown that populations or species differ due to evolutionary change (as opposed to, for example, environmental effects). This can, for instance, be achieved by estimating heritable differences between populations using common garden experiments described above. Secondly, it must be demonstrated that the observed heritable differences increase fitness in the respective environment. This point is often challenging but remains crucial to understand the adaptive value of heritable trait differences (Gould and Lewontin 1979). It is thus preferable to directly estimate fitness in different environmental conditions. One way of doing so is to subject populations to reciprocal transplant experiments (Johnson

et al. 2022). If populations perform better in their own environments relative to all other populations, this indicates strong support for local adaptation. For instance, using reciprocal transplants, Via (1991) showed that pea aphid strains collected either on alfalfa or red clover performed best when reared on the host plant on which they were collected. Similar findings are common across study systems, indicating that populations are often adapted to their local environment (Hereford 2009; Olazcuaga et al. 2022). In the next section (5.3), we briefly review some examples of how insects adapt to their environment (also see Chapter 12).

5.3 Examples of adaptation

5.3.1 *Physiological adaptations*

A key factor with regard to climate change is physiological resistance to temperature or desiccation stress. Populations and species often differ in their thermal limits (Blanckenhorn et al. 2021; Garbuz et al. 2003; Sgrò et al. 2010), suggesting that these traits can evolve. Indeed, thermal tolerance thresholds often have additive genetic variation and thus, in principle, have the capacity to respond to selection (Diamond 2017). For instance, *Drosophila* species occurring in cooler habitats evolved a lower critical thermal minimum indicating that physiological resistance to cool environments play a major role in local adaptation (Figure 5.2; MacLean et al. 2019). Similar responses are also found within species. For example, Hangartner and Hoffmann (2016) show that the fruit fly *Drosophila melanogaster* can evolve higher heat resistance in the laboratory (+0.5 °C) (also see e.g., Mesas et al. 2021), suggesting that populations—in principle—can evolve in response to heat stress. However, Hangartner and Hoffmann (2016) also report that the response to selection reached a plateau after eight to ten generations, probably due to the erosion of V_A. This indicates that while initial responses to selection may be rapid, it remains unclear whether responses will be sustained in the long term. In addition, heritability estimates often vary among species. Kellermann et al. (2009) showed that, in contrast to cosmopolitan generalists, tropical *Drosophila* species have very little genetic variation for
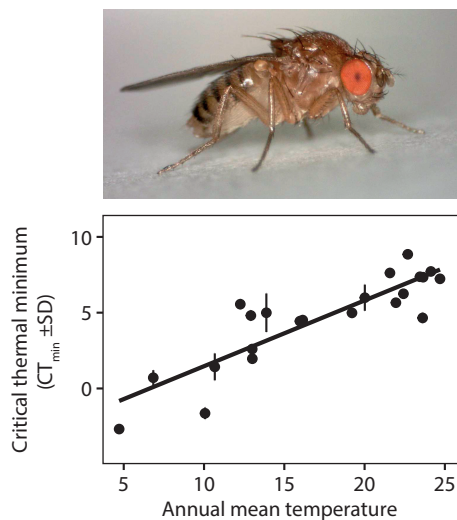
Figure 5.2 MacLean et al. (2019) demonstrate that *Drosophila* species occurring in different thermal habitats differ in their critical thermal minima indicating that physiological resistance to temperature plays a major role in local adaptation (picture of female *Drosophila melanogaster* by Hanna Davis CC-BY-SA-4.0).

desiccation and cold tolerance. Species adapted to narrow thermal niches may thus be severely constrained by low heritabilities which are expected to constrain the speed at which populations can evolve in response to environmental change.

5.3.2 *Morphological adaptations*

In addition to physiology, morphology also plays a major role in adaptation to climate change. This is especially true for traits that facilitate physiological or behavioral thermoregulation. For instance, desert ants of the genus *Cataglyphis* and *Ocymyrmex* evolved disproportionately long legs compared to other ant species. The increase in leg length not only reduces heat exposure by increasing the distance between the body and the hot ground but also by increasing convective cooling and foraging time (Sommer and Wehner 2012).

Another example of how morphology can facilitate thermoregulation is the evolution of relative wing size. Flight is a main contributor to thermoregulation in winged insects because it facilitates microhabitat choice. However, flight is also heavily limited at cool temperatures due to energetic constraints. One way to circumvent these biophysical constraints it to increase relative wing size, which allows insects to take off at cooler temperatures (Dudley 2002; Neve and Hall 2016). Such patterns have evolved convergently in two species of sepsid flies *Sepsis punctum* and *Sepsis fulgens* (Rohner et al. 2019), European populations of *Drosophila subobscura* (Gilchrist and Huey 2004) and the dung beetle *Onthophagus taurus* (Rohner and Moczek 2020). Similar patterns are also found across species of drosophilids (Rohner et al. 2018a). Evolutionary changes in relative wing size may thus contribute to adaptation to changing climates. Other aspects of wing morphology, such as coloration, have also been shown to relate to local adaptation to climatic differences (e.g., Ellers and Boggs 2004).

These examples highlight how populations and species can adapt to changing environments (also see Chapter 12 in this book). However, in addition to genetic responses, insects also have the capacity to adjust their phenotype using plastic within-generation mechanisms. In section 5.4, we discuss how plasticity arises and how it can be estimated.

5.4 Phenotypic plasticity

Phenotypic plasticity refers to the capacity of a single genotype to produce different phenotypes depending on the environment (West-Eberhard 2003). Plasticity is exceptionally common in insects (and organisms in general; see de Jong and van der Have 2009; Pfennig 2021; Sultan 2015). Examples include developmental adjustments to cuticular pigmentation to match substrate coloration in caterpillars (Figure 5.3; Noor et al. 2008), size-dependent development of many male secondary sexual structures (Rohner and Blanckenhorn 2018; Figure 5.3), or immune responses to the presence of pathogens (Rolff et al. 2009).

Plastic responses can be complex and non-linear. For instance, locusts that develop in a crowded environment develop into gregarious (i.e., swarming) adults while individuals experiencing low population densities develop into a solitary morph (Verlinden et al. 2009). In this case, plasticity to crowding results in a polyphenism—the presence of multiple discrete alternative morphs that are induced by environmental conditions. Similar polyphenisms are common in species with alternative reproductive tactics where large 'fighter' morphs develop elaborate secondary sexual traits while small 'sneaker'
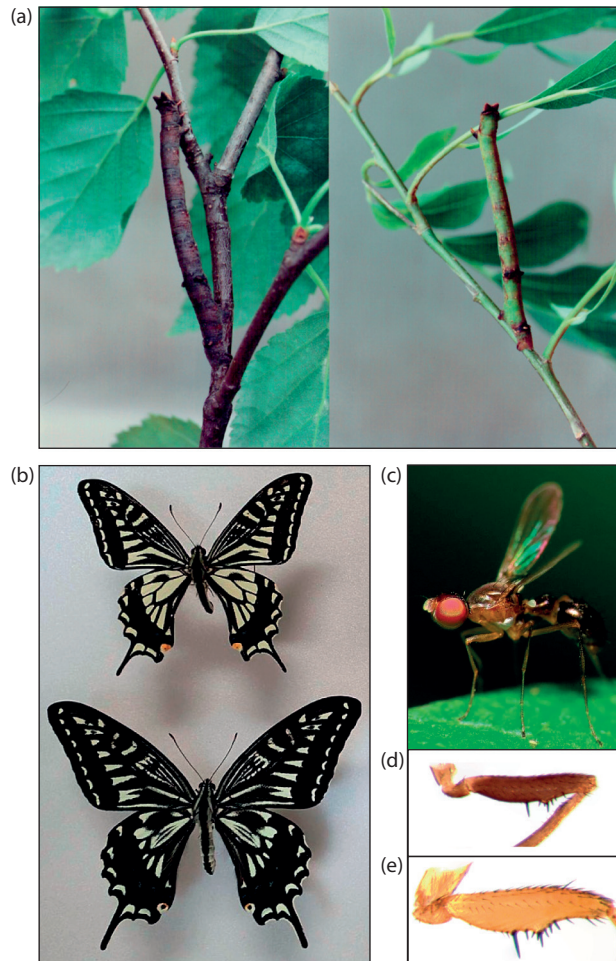
Figure 5.3 Phenotypic plasticity can have major effects on phenotypic variation. This includes a) plastic adjustments of cuticular coloration to substrate coloration as in *Biston betularia*; b) seasonal polyphenism in *Papilio xuthus* (spring form on top, summer form on the bottom); and c) differences in ornament morphology between small (d) and large (e) sepsid flies (Rohner 2022; Rohner and Blanckenhorn 2018) (image credit: a) *Biston betularia* by M. A. F. Noor, R. S. Parnell, and B.S. Grant 2008, CC-BY-2.5; b) *Papilio xuthus* by S. Komata and T. Sota 2017, CC-BY-4.0; c) *Sepsis pyrrhosoma* male by K. Schulz 2015, CC-BY-2.0; d) and e) by P. Rohner).

morphs invest in clandestine mating behaviors (e.g., in horned and horn-less dung beetles (Moczek and Emlen 2000)). Similarly, there are many instances of seasonal morphs in butterfly wing coloration patterns (Brakefield and Reitsma 1991; Komata and Sota 2017; Figure 5.3). The induction of hibernation behavior can also be regarded as a polyphenism if individuals switch between a diapause and a direct development path (Kivelä et al. 2017).

Whether plasticity takes a continuous or discrete form, it is often a major contributor to phenotypic variation and can confound genetic differences among populations or species. That is, if environmental conditions are not accounted for, differences in

population or species means across geographic regions (e.g., warmer versus cooler climates) or temporal samples (e.g., historical museum collections versus contemporary populations) are unsuited to infer *evolutionary* changes and adaptation. Consequently, this has major impacts on how we assess insects' responses to ongoing climate change in the field in that relatively few studies are able to disentangle plastic from genetic responses in nature (e.g., Blanckenhorn 2015; Bradshaw and Holzapfel 2001). Plasticity has thus been traditionally considered a confounding factor when studying genetics and evolution. However, the relative contribution of plasticity to phenotypic variation can be assessed in the laboratory. Below, we briefly outline a classic quantitative genetic approach.

5.4.1 *Quantifying phenotypic plasticity*

The contribution of phenotypic plasticity to the total phenotypic variation can be assessed by rearing closely related individuals (e.g., clones, inbred lines, or full-sib/half-sib families) in different environment. This allows to decompose the total phenotypic variation V_P into variance components due to genetic factors, the environment, and genotype-by-environment interactions (see Figure 5.4).

$$V_P = V_G + V_E + V_{G \times E} + V_\varepsilon$$
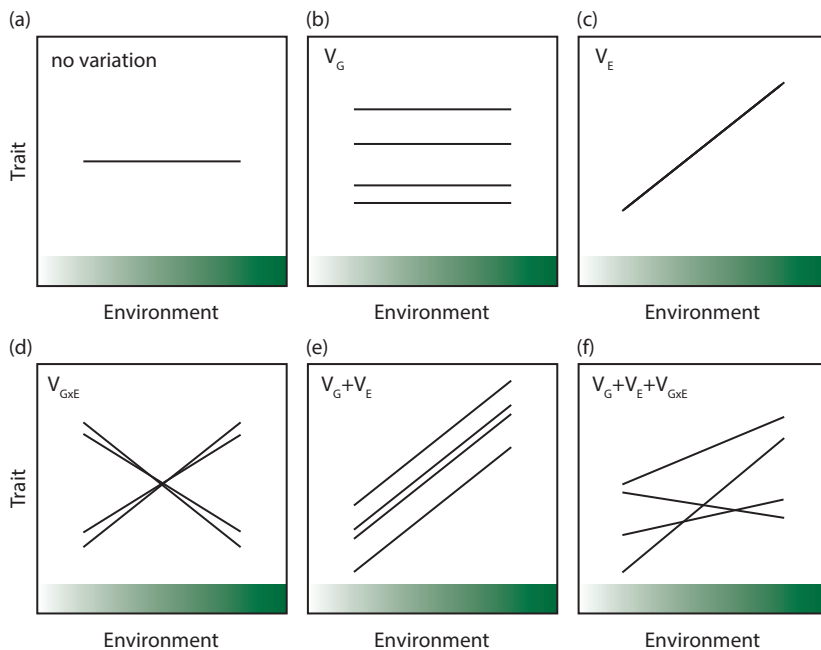
Figure 5.4 Examples of reaction norms under varying contributions of genetic differentiation, plasticity and G × E interactions. Each line represents a single genotype's reaction norm. a) No variation; b) only genetic differences among genotypes; c) plasticity in the absence of genetic variation; d) only genotype-by-environment interactions; e) genetic differences and a common plastic response; f) genetic differences, plasticity and G × E interactions.

V_E captures variation explained by the shared plastic response while $V_{G \times E}$ captures variation explained by heritable differences in plastic responses among related individuals. The latter is generally regarded as a component of plasticity and can account for a significant amount of the phenotypic variance. For instance, measuring temperature-specific thorax length in 196 *Drosophila melanogaster* lines, Lafuente et al. (2018) computed that the variance component associated to the genotype-by-temperature interaction component ($V_{G \times E} = 6.0 \times 10^{-4}$) was three times larger than V_G (2.0×10^{-4}, Figure 5.5). For abdomen length, which the authors measured as well, $V_{G \times E}$ (6.2×10^{-3}) was even six times larger than V_G (1.0×10^{-3}). This suggests substantial heritable variation for thermal plasticity in body size, a phenomenon found in many species (Rodrigues and Beldade 2020; DeWitt and Scheiner 2004).

Although often documented, the contribution of phenotypic plasticity (V_E and $V_{G \times E}$) to phenotypic variation is difficult to predict and depends strongly on the type of environmental variable and the range at which it is investigated. However, some environmental variables most likely to be affected by climate change, such as temperature, humidity, and food availability, often have strong and predictable effects on many life-history and morphological traits. Below we briefly highlight common forms of plasticity as a response to temperature.
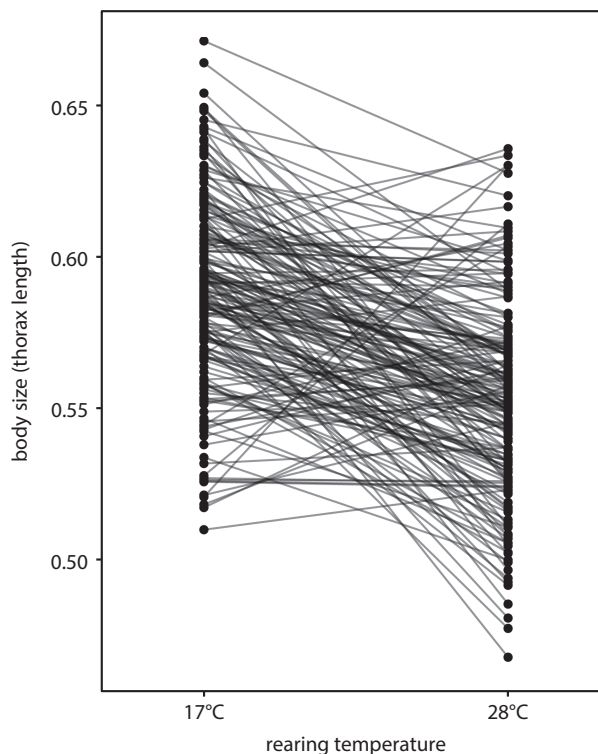
Figure 5.5 Differences in thermal plasticity in body size among 196 isogenic *Drosophila melanogaster* strains. Each line represents the thermal response of a genetic strain. The finding that lines differ in their response to rearing temperature indicates significant levels of genetic variation for plasticity (Data from Lafuente et al. 2018).

5.4.2 *Examples of common plastic responses: Thermal reaction norms*

The development and physiology of insects is strongly dependent on ambient temperature. This is especially true for egg-to-adult development time and growth rate (de Jong and van der Have 2009; Hochachka and Somero 2014). Plastic responses of these traits to temperature are so widespread that they can be expected *a priori*. As an example, Buckley et al. (2017) found that contemporary climate changes cause phenological advancements and an increase in the number of generations per year almost universally across the globe. Such plastic increases in developmental rates thus have major effects on entire ecosystems.

In addition to growing slower, insects (and other ectotherms) also tend to grow to larger sizes at cold temperatures. This response is so strong that the phenomenon has been dubbed the 'temperature size rule' (Atkinson 1994). The physiological mechanisms underpinning the temperature size rule diverge across insects. In the tobacco hornworm *Manduca sexta*, the temperature size rule is caused by temperature-dependent growth occurring late in development (Davidowitz and Nijhout 2004). In contrast, in *Drosophila*, it is caused by temperature-dependent plasticity in the size at which metamorphosis is initiated (Ghosh et al. 2013). The observation that different insects utilize different mechanisms to achieve the temperature size rule suggests that the temperature size rule may be adaptive. However, the precise mechanisms and their adaptive value remain disputed (Angilletta and Dunham 2003; Horne et al., 2015; Verberk et al. 2021).

Although most insects follow the temperature size rule, there are many exceptions (e.g., Walters and Hassall 2006). In addition, thermal plasticity in growth-related traits is usually nonlinear. Specifically, thermal performance curves take a typical asymmetric bell shape (Angilletta 2006; David and Clavel 1967). Estimating thermal plasticity using only two or just a few temperatures can be misleading and is insufficient to locate a species' thermal niche. Estimating full thermal performance curves is difficult and often challenging but provides important insights into the effect of environmental variation on development, morphology and fitness.

5.4.3 *Is plasticity adaptive?*

From an evolutionary genetic perspective, plasticity is expected to evolve if organisms occupy variable but predictable habitats, if selection favors alternative phenotypes in different environments, and if no phenotype is best suited across all levels of the environmental variable (DeWitt and Scheiner 2004; Ghalambor et al. 2007). Such adaptive plasticity enables an insect to take advantage of cues in its current environment to make predictions about future conditions, enabling adaptive adjustments to developmental trajectories. For instance, the flesh fly *Sarcophaga bullata* takes advantage of daylength as a cue to initiate diapause (i.e., hibernation) whenever there are less than around thirteen hours of light per day (Denlinger 1972). This is most likely to be an adaptive response that is found in many insects (Tauber et al. 1986). Similarly, the mayfly *Drunella coloradensis* takes advantage of chemical cues indicating the presence of fish predators to develop longer caudal filaments—the development of which reduces predation risk (Dahl and Peckarsky 2002). Any plastic response that relates to environment-specific survival (e.g., predator avoidance, immune responses, etc.), are most likely adaptive responses to selection. However, our increasing understanding of development and physiology demonstrates that developmental systems are generally sensitive to environmental conditions (Bateson and Gluckman 2011; Nijhout et al. 2021). Plasticity to variables that affect developmental

processes directly, such as temperature, can thus be regarded as the default, not the exception. For instance, in a classic experiment, Waddington (1953) exposed *Drosophila* pupae to heat shock and found that some adults developed wings with unusual morphology (i.e., missing cross-veins). This is certainly a form of plasticity, but probably of little adaptive value. Hence, while many plastic responses to climatic variables are probably under selection and therefore have the potential to be adaptive, it needs to be demonstrated that the phenotypic changes have fitness consequences. In section 5.8, we will consider how plasticity, once evolved, impacts future evolutionary change.

5.5 Evolution of (and through) plasticity

Plasticity decouples an organisms' phenotype from its genotype. Consequently, plasticity impacts evolutionary changes in a variety of ways (Crispo 2008; Ghalambor et al. 2007; West-Eberhard 2003). For instance, adaptive plastic responses to climate change are thought to facilitate subsequent adaptation by maintaining a population's fitness until novel, beneficial mutations emerge (e.g., Corl et al. 2018). Plasticity can thus 'buy time'—which is of the essence because adaptation is often slow. However, plasticity may also hamper adaptation, for instance if plastic responses buffer the phenotypic effects of deleterious mutations. By decoupling phenotypes visible to selection from an organism's genotype, plasticity can prevent selection from removing maladaptive alleles (e.g., Huey et al. 2003). Plasticity thus has complex effects on evolutionary trajectories. In addition, plasticity itself can evolve, potentially changing the phenotypic variation visible to selection and influencing direction and magnitude of adaptive responses. Below, we first outline how the evolution of plasticity can contribute to local adaptation. Next, we discuss how robustness shapes evolutionary capacitance, and lastly, we touch on plasticity's ability to precede and 'lead' future evolution.
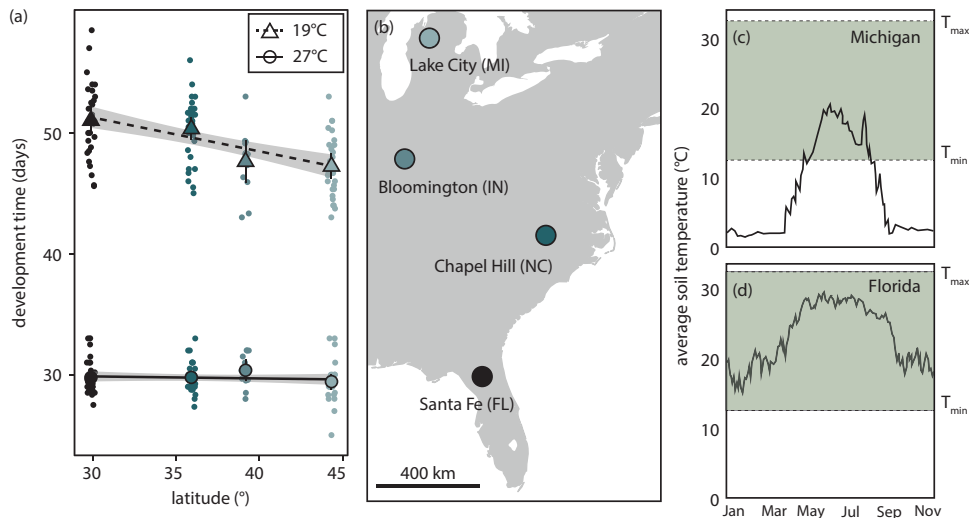
5.5.1 *Evolution of plasticity*

Plastic responses often differ between species, ecotypes, sexes and traits, demonstrating that plasticity has large potential to evolve (e.g., Foquet et al. 2021; Rohner et al. 2018b). While evolution of plasticity is often documented both across and within species, *how* plasticity evolves is still poorly understood, especially under natural conditions and on 'ecological timescales' (Fox et al. 2019). This is because to study its evolution, plasticity must first be quantified in the ancestral population and then be contrasted to patterns of plasticity after evolution has taken place. This can be done in the laboratory using artificial selection or laboratory evolution experiments (Mallard et al. 2020; Suzuki and Nijhout 2006; Waddington 1952), but it has been exceedingly difficult to investigate how plasticity evolves in nature.

The evolution of plasticity can be studied in the field using longitudinal approaches. For instance, Bradshaw and Holzapfel (2001) sampled pitcher plant mosquitos repeatedly over a time span of around thirty years. Rearing wild-caught populations under various controlled laboratory conditions, the authors were able to show that populations evolved a modified photoperiod threshold for diapause induction to match more southern day lengths, a finding consistent with the hypothesis that plasticity evolved rapidly and adaptively in response to climate change. Such longitudinal studies are very insightful and allow us to track adaptation to climate change in real time. However, these approaches are

logistically and experimentally challenging. An alternative is to study species that successfully and rapidly invaded new habitats that are climatically different from the ancestral range. Studying invasions offers an exceptional opportunity to study how organisms cope with and adapt to rapidly changing environments (Gilchrist et al. 2001; Kingsolver and Buckley 2017), and, provided that the ancestral source population (or a proxy thereof) is known and still accessible, even allows to investigate the role of ancestral plasticity therein (Moczek 2007).

One example is the invasion of the dung beetle *Onthophagus taurus* in the eastern United States. This species, which is native to the Mediterranean region, was accidentally introduced in Florida in the early 1970s. Upon its introduction, it rapidly expanded its range towards the North, and within forty years—which corresponds to about eighty to one hundred generations—reached the Canadian border (Figure 5.6; Rohner and Moczek 2020). A common garden experiment revealed that this rapid invasion coincided with a reduction in development time in the North when beetles were reared at 19 °C (Figure 5.6). This is likely to be adaptive because northern climates are too short for typical southern populations to complete their reproductive cycle. However, no differences were found when rearing populations at 27 °C which represents the average temperature during the breeding season at the southern range edge (Figure 5.6). This indicates that *O. taurus* adapted to short seasons in the North by an evolutionary reduction of thermal plasticity in development time (Rohner and Moczek 2020). Such countergradient variation (or genetic compensation; Grether 2005) allows northern populations to complete their reproductive cycle despite shorter seasons. Similar responses are expected to be common in general and contribute to climate change (Kelly 2019).

Figure 5.6 The invasion and rapid range expansion of *O. taurus* in the eastern United States involved an evolutionary reduction of thermal plasticity in development time, allowing northern populations to complete their reproductive cycle despite shorter seasons (Rohner and Moczek, 2020). Panels on the right show average soil temperature at a depth of 9 cm throughout the year 2020 in Michigan (c) and Florida (d). The shaded area indicates the temperature range between the minimal and maximal thermal limits of *O. taurus* (soil temperature data from the National Ecological Observatory Network).

5.5.2 *Robustness, cryptic genetic variation, and evolutionary potential*

Before we continue discussing how plasticity shapes evolution, it may be useful to discuss the complex relationship between plasticity and robustness. Robustness (i.e., the apparent insensitivity of phenotype expression to environmental variation) is often interpreted as the absence of plasticity. However, robustness and plasticity are in a very complex relationship because the mechanisms that make insects robust are often themselves plastic and the mechanisms that make a developmental system plastic are often surprisingly robust. Robustness and plasticity are thus not just flip sides of the same phenomenon but are in a reciprocal relationship (see: Bateson and Gluckman 2011; Schwab et al. 2019).

Robustness is important for evolution because it enables organisms to withstand environmental as well as genetic (e.g., mutational) perturbations. For instance, individuals within a population may carry various non-synonymous mutations but can still be indistinguishable on the phenotypic level. Such robustness is rooted in the way genes affect phenotypes through development. Genes don't affect phenotypes directly but act through developmental genetic networks that are riddled with redundant interactions and feedback loops such that one component can compensate for another (Bateson and Gluckman 2011; Nijhout 2002; Gursky et al. 2012). This allows developmental systems to buffer against deleterious variation and prevents mutational perturbations from affecting phenotypes. Because some of these mutations are not visible to selection, they can accumulate and form so-called *cryptic genetic variation*. This variation is referred to as *cryptic* because it usually has no effects on phenotypes and thus remains invisible.

Cryptic genetic variation can have major evolutionary implications. That is because the capacity to which developmental systems can buffer against perturbations is not limitless. If systems are disturbed too much (e.g., through the exposure to a novel environment), the cryptic variation that was previously buffered against can be released and hit phenotypes with full force. This can lead to an increase in heritable variation in the new environment. Most of this heritable variation will be deleterious or neutral but some decrypted effects are likely to bring an organism closer to the optimal trait value in the new environment. The release of previously cryptic variation can thus fuel adaptation to novel habitats or changing environments (Paaby and Rockman 2014).

One example where cryptic genetic variation shapes phenotypic variation in a novel climatic environment was documented in the yellow dung fly *Scathophaga stercoraria*. Females of this species typically develop three sperm storage organs (i.e., spermathecae), but sometimes they develop a fourth one. In nature, the frequency of females developing a fourth spermatheca (the so-called 4S phenotype) is nearly zero (Berger et al. 2011). However, when offspring of wild-caught 3S females are reared in the laboratory, the frequency of the 4S phenotype increases strongly. This increase is especially pronounced at warm temperatures (Figure 5.7) outside the species' preferred temperature range. Crucially, high temperatures not only increase the total phenotypic variance in 4S phenotype expression but also the relative amount of genetic variance. This leads to an increase of heritability with rearing temperature, suggesting that genetic variation that remains cryptic at low temperatures close to the species' optimum is released when encountering thermal stress. In the case of the yellow dung fly, this increase in heritability could fuel adaptation if favored by selection. Indeed, some studies suggest that females with four spermathecae have a fitness advantage via genetic benefits through female choice despite a fecundity cost (Ward 2007; Ward et al. 2008; but see Walters et al.
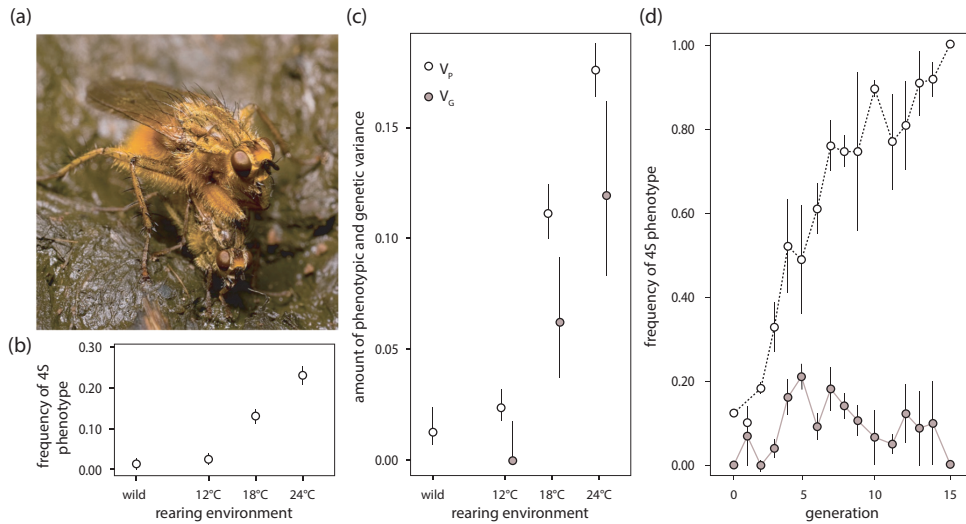
Figure 5.7 Female yellow dung flies (a) develop either three (3S) or four (4S) spermathecae. b) The frequency of the 4S phenotype is almost nil in nature but increases when females are reared in the laboratory, especially at warm temperatures. c) The increase in 4S phenotype expression with temperature not only increases the total phenotypic variance but also the relative amount of the genetic variance. This leads to an increase of heritability with rearing temperature, suggesting that genetic variation that remains cryptic at optimal low temperatures is released when developing flies encounter thermal stress. Interestingly, this released genetic variation can respond rapidly to artificial selection (d). (data from Berger et al. 2011 and War, 2000; picture of Scathophaga stercoraria by David Evans CC-BY-2.0).

2022). The context-dependent expression of spermatheca number in the yellow dung fly exemplifies how plasticity and robustness can shape genetic variation and adaptive potential. Next, we explore how plasticity may generally facilitate evolution through genetic accommodation.

5.5.3 *Plasticity-led evolution: Genetic accommodation and assimilation*

A large body of literature documents how plasticity emerges as a product of evolution. However, once evolved, plasticity may also precede and 'lead' subsequent evolutionary change. These ideas are relatively old (e.g., Baldwin 1896; Morgan 1896; Waddington 1942) but received reinvigorated interest and scrutiny since the early 2000s (Pfennig et al. 2010; West-Eberhard 2003). Most commonly, plasticity-led evolution is discussed in the context of genetic accommodation and genetic assimilation—two similar but distinct mechanisms. Let's first consider a hypothetical example of how genetic accommodation is thought to 'lead' evolution.

We begin with a hypothetical insect population that is adapted to a particular environment (Figure 5.8a). Because selection has removed unfit genotypes over time, most genotypes produce a phenotype close to the fitness optimum (in Figure 5.8a). Exposure to a novel environment—for example, a much hotter environment or unprecedented desiccation stress—will induce plastic responses. However, not all genotypes will respond equally to the environmental change. Some may be totally unaffected by environmen-
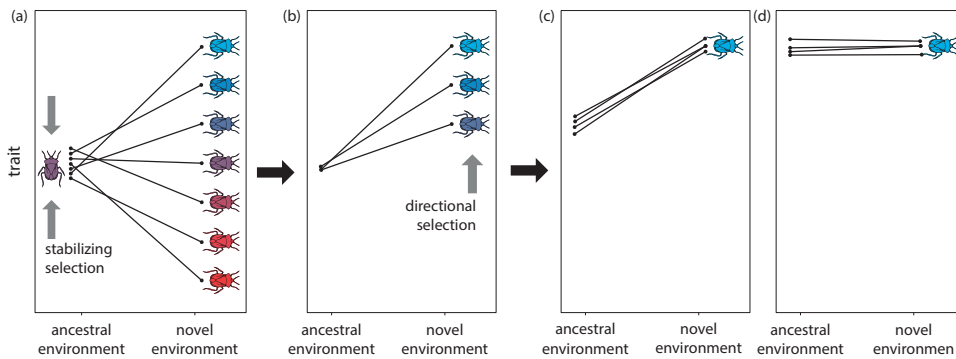
Figure 5.8 a) A hypothetical population adapted to its habitat is exposed to a novel environment. Exposure to this new environment induces plastic responses and releases cryptic genetic variation that is now visible to selection. b) Directional selection in the new environment favors an increase in the trait value and favors genotypes with an increased plastic response. c) The result is a population that adapted to a novel environment through genetic modification of an ancestral plastic response (i.e., genetic accommodation). d) If genetic accommodation leads to environment-insensitive trait expression, this process is referred to as genetic assimilation.

tal variation, while others may vary in the strength and direction of the change. The differences in the reaction norms among genotypes are due to previously cryptic genetic variation. The release of this cryptic variation manifests in increased phenotypic variation that is now visible to selection. In the example shown in Figure 5.8, selection will favor an increase in the trait value and favors genotypes with an increased plastic response. The result is a population that adapted to a novel environment through genetic modification of an ancestral plastic response. In other words, the trait has undergone *genetic accommodation*.

The process of genetic accommodation contrasts with classic neo-Darwinian frameworks in that new selectable variants initially arise through plasticity in a novel environment without the need for novel mutations (Moczek 2007). In this sense, plasticity precedes and has the potential to bias future genetic change (note, however, that plasticity-first evolution can be approached using standard quantitative genetics; e.g., Lande 2009). Genetic accommodation is also thought to be much faster than 'typical' neo-Darwinian modes of evolution which depend on new genetic variants entering the population one at a time through mutation and gene flow. That is because selection in a new environment can act on potentially large amounts of 'decrypted' variation that have accumulated over many generations in an entire population (as opposed to novel alleles entering the population at low frequency). To provide an empirical example of how fast responses to selection can be when fueled by cryptic genetic variation, we shall revisit variation in spermatheca number in the yellow dung fly. Ward (2000) selected on the 4S phenotype (which is almost completely cryptic in the field) and was able to fix the 4S phenotype within just fifteen generations (see Figure 5.8d). This highlights cryptic genetic variation's potential as an evolutionary capacitor.

Crucially, selection in the new environment can also change the phenotype produced when genotypes are again exposed to the ancestral environment. Because selection only 'sees' phenotypes in the new environment, mutations that increase trait values will be favored. Some of these will do so constitutively and consequently produce genotypes that in turn produce an overall higher level of trait expression irrespective of the environmental conditions. These responses can be so strong that traits become completely environment insensitive (Figure 5.8d). In that case, this process is referred to as *genetic assimilation*. Genetic assimilation is simply a form of accommodation where previously plastic traits evolve to become expressed constitutively across environments. In that sense, the concept of genetic accommodation can be seen as a generalization of genetic assimilation (Braendle and Flatt 2006).

Genetic accommodation has been demonstrated under laboratory conditions, but there are very few cases where it has been demonstrated in the field. This may suggest that genetic accommodation is not important. However, plasticity-led evolution via genetic accommodation is expected to be rapid and the products of plasticity-first and neo-Darwinian evolution cannot be distinguished once evolution has taken place. Based on these challenges, Levis and Pfennig (2016) provide four key criteria that need to be demonstrated in order to demonstrate plasticity-first evolution in nature. First, plasticity must have been present in the ancestral population (in the case of thermal plasticity this is very likely). Exposing an ancestral population to the new environment should thus induce the novel phenotype, although not necessarily to the same degree. Secondly, release of cryptic genetic variation must have occurred in the derived environment. Thirdly, it needs to be demonstrated that plasticity evolved, and, lastly, the evolved form of plasticity should increase fitness in the derived environment relative to ancestral plasticity. Based on these promising criteria, future work in natural populations will hopefully reveal how much plasticity-first evolution contributes to adaptation to climate change.

5.6 Conclusions

Although climate change is only one of many factors contributing to the rapid decline of insect populations across the globe, understanding how insects respond to changes in climate is crucial. Laboratory studies document that many key physiological, morphological or life-history traits harbor some degree of genetic variation. This indicates that insect populations have the potential to respond adaptively to rapid environmental changes. Many fitness-related traits also show plastic responses to climatic changes. Some (but certainly not all) of these plastic responses are adaptive and will be able to buffer against the potentially negative effects of climate change. Furthermore, many studies demonstrate genetic variation for plasticity, indicating that plasticity itself may be able to evolve in response to selection. Some studies even indicate that this is possible on ecological timescales. In addition, plasticity may facilitate adaptive evolution through genetic accommodation, a process potentially faster than classical neo-Darwinian evolution. Taken together, there are good reasons to believe that many insects will be able to adapt to novel climatic conditions—either via plasticity, genetic changes, or adaptation through the evolution of plasticity. However, whether these responses are going to be sufficiently strong and rapid enough remains unclear.

Key reflections

- There is accumulating evidence that insect populations can adapt to rapidly changing environments. However, our understanding of the evolutionary potential insect populations (e.g., the amount of genetic variation) is mostly limited to a few heavily studied groups of insects (e.g., drosophilids). Predicting adaptive responses more broadly will require more research in diverse insect groups.
- Environmental responses to temperature, humidity, and nutrient availability are so common that plastic responses to climate change can be expected *a priori*. This implies that changes in trait means observed in nature over time or space are unsuited to infer *evolutionary* diversification.
- Laboratory studies often estimate plastic and genetic responses under constant conditions and one variable at a time. However, natural environments are complex and often fluctuating. Plastic and evolutionary responses to climatic variability remain poorly understood, especially when several variables change simultaneously. The degree to which laboratory studies reflect the natural situation thus often, unfortunately, remains unclear.
- Plasticity can both hamper and facilitate adaptation to climate change. In addition, plasticity itself can evolve, potentially changing the phenotypic variation visible to selection and influencing direction and magnitude of adaptive responses. Finally, plasticity can precede and 'lead' future genetic changes. Although there are many examples that are consistent with a plasticity-first scenario, there is little unambiguous evidence for genetic accommodation in the field. Future research in diverse species will be necessary to reveal how often plasticity contributes to, hampers, or leads evolution.

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