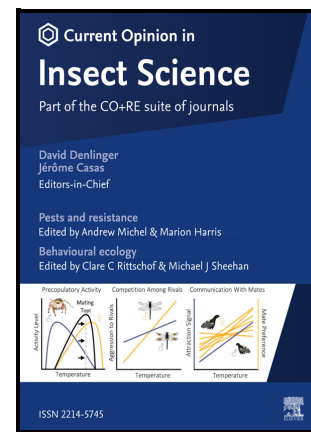


Untangling plastic responses to combined thermal and dietary stress in insects
Short Title: Plasticity to combined stress

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Title: Untangling plastic responses to combined thermal and dietary stress in insects

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Short Title: Plasticity to combined stress

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Abstract: Animals are exposed to changes in their environmental conditions daily. Such changes will become increasingly more erratic and unpredictable with ongoing climate change. Responses to changing environments are influenced by the genetic architecture of the traits under selection, and modified by a range of physiological, developmental, and behavioural changes resulting from phenotypic plasticity. Furthermore, the interactions between multiple environmental stressors to which organisms are exposed can generate unexpected phenotypic responses. Understanding how genetic and plastic variation contributes to the response to combined environmental stress will be key to predicting how animals will cope with climate change, and ultimately will define their ability to persist. Here, we review the approaches used to explore how animals respond to combined stressors, specifically nutrition and temperature, the physiological mechanisms that underlie such plastic responses, and how genetic variation alters this plasticity.

Introduction

Organisms face variable and often suboptimal environmental conditions, which is made worse with ongoing climate change (Chevin et al., 2010; Foden et al., 2013; Scheffers et al., 2016; Hoffmann and Bridle 2021*). Changes in temperature will have significant impacts on small-bodied ectotherms like insects, which cannot regulate their body temperature (Huey and Pascual 2009; Huey and Tewksbury 2009). Furthermore, because they represent the

most abundant class of animals on the planet (Erwin 1983), the impact of unpredictable thermal conditions on insects has the potential to change whole ecosystems by altering nutrient cycling, predator/prey interactions, and insect pollinator dynamics.

In addition to the direct impacts of temperature on animal biology, nutritional quality and abundance of resources for herbivorous and frugivorous animals will be affected because both temperature and CO₂ can alter the macronutrient content of plants (Du et al., 2020; Lenhart, 2017; DaMatta et al., 2010). Such changes will influence rates of ingestion, assimilation of nutrients, and growth rates in animals that depend on plants for their primary food source (Dijkstra et al., 2012). This has the potential to ramify up the food web by altering the body composition of prey items available to predators (Jensen et al., 2011; 2012). Understanding how animals will respond to changes in their environment represents a key challenge to evolutionary and conservation biologists and is a necessary pre-requisite for predicting at-risk ecosystems.

One mechanism by which organisms may survive shifts in their environment is to modify their development, physiology, or behaviour via phenotypic plasticity (Bradshaw 1965). The extent to which animals can mount a plastic response depends on their genetic makeup, with some genotypes exhibiting stronger plastic responses, characterised by steeper reaction norms, in response to certain environmental conditions than others (Falconer and Mackay 1996; Hoffmann and Bridle 2021*). Despite its importance, the way genotypes vary in their plastic response to combined stress is rarely characterised. We know even less about how genetic variation in plasticity and the proximate causes of differences in plasticity constrain or facilitate adaptation to environmental change.

Here, we discuss whether and how genetic variation in plasticity, particularly to combined thermal and nutritional stress, can be used to predict population responses to global change. We first outline how thermal and nutritional environments influence life-history traits on their own. Then, we consider the complexity of responses to combined environmental stressors. We discuss three approaches to understand effects of combined stress: a) physiological and developmental mechanisms, b) genetics and c) their interactions. Finally, we propose that predicting responses to combined thermal and nutritional stress

requires an integration of perspectives from evolutionary genetics and understanding of the underlying proximate mechanisms that regulate plasticity.

Phenotypic plasticity as a first response to changes in nutritional and thermal environments

Animal responses to environmental change, in the short to midterm, are underpinned by phenotypic plasticity (Chevin et al., 2010; Hoffmann and Sgro 2011; Sgro et al., 2016). Plasticity can enable animals to alter physiological, morphological, and behavioural phenotypes towards those that are best suited to the environment in which they live (Min et al., 2021*; Kuczyk et al., 2021*). However, plasticity need not always be adaptive. Plastic responses to environmental conditions can also result from dysregulation of developmental or metabolic processes (Pfennig 2021*). Thus, plasticity can benefit or harm animals, depending on the trait, condition, or genotype of the exposed individual.

Insects and other invertebrates exhibit a variety of plastic responses to changes in temperature. For example, many invertebrates initiate diapause to survive harsh, unfavourable environments, usually triggered by changes in temperature and photoperiod (Karp 2021*). Several species of butterflies develop different coloration in their wings in response to developmental temperature (Oostra et al., 2018; Bhardwaj et al., 2020). Furthermore, insects are known to regulate their body and organ sizes in response to the thermal conditions in which they are reared, with larger adults arising from lower developmental temperatures (Wonglersak et al., 2022*).

The nutritional environment also induces plastic shifts in insects, affecting a range of fitness-related traits like growth, survival, development time, body size, fecundity, and stress resistance amongst others (K.M. Kapheim, et al., 2020; Verspagen et al., 2020; Mirth et al., 2021*; Pfennig 2021*; Littler et al., 2021*; Pullock et al., 2023*; Ben-Yosef et al., 2023*). Importantly, while restricting the calories available for consumption reduces some traits like growth and fecundity, it increases other traits like lifespan (May et al., 2015; Hosking et al., 2019). Thus, the response to low calorie diets differs between traits. This highlights how traits might trade-off in response to changes in food availability.

Nutrition is more than just calories. The nutritional combinations of protein, carbohydrate, fat, and micronutrients required to optimise trait values also vary with species, development stage, and trait under study (Gray et al., 2018; Kim et al., 2020; Cheon et al., 2022*; Carey et al., 2022*; reviewed in Raubenheimer et al., 2022*). For example, when fruit flies (*Drosophila melanogaster*) were reared across a range of protein to carbohydrate (P:C) ratios, development rate was highest on intermediate protein, intermediate carbohydrate concentration diets, whereas body size peaked on intermediate protein, low carbohydrate diets (Gray et al., 2018). Taken together, food quality is equally as important as food availability for insect trait plasticity. Further, capturing species' susceptibility to changes in the nutritional environment requires measuring multiple fitness-related traits.

Plastic responses to combinations of dietary and thermal stress

Given that animals in the wild are rarely faced with a single stressor, researchers have recently asked whether combined stress changes plastic responses in ways that could not be predicted by additive effects of single stressors. Using Nutritional Geometry (Kutz et al. 2019; Kim et al., 2020; Chakraborty et al., 2020; Min et al., 2021*) to map trait responses across a broad quantitative range of nutrient contents in the diet, these studies identified that the responses of life history traits to diet change significantly with temperature. For some life history traits, like wing size in *D. melanogaster*, increasing the rearing temperature from 25°C to 28°C narrowed the range of diets that produce the largest wings (Kutz et al., 2019). The higher temperature also worsened the impacts of the poor diets, reducing wing size more than would be expected from the effects of either temperature or diet alone (Kutz et al., 2019). Temperature also alters the impact of diet on male and female lifespan, egg production rate, and development rate (Kim et al., 2020). Moreover, temperature and nutrition can independently interact with other abiotic and biotic stressors affecting life-history traits (Silva et al., 2024*; Halsch et al., 2023*). Because the effects of combined thermal and nutritional stress are often non-additive, this makes it difficult to predict whether and how changes in temperature will affect plastic responses to diet.

Since interactions between only two environmental stressors can alter plasticity of a range of life history traits in unpredictable ways, this raises the question of how many stressors

need to be studied in tandem to understand the impacts of climate change on species survival? In principle, the combined effects of stressors could eventually saturate, as there are a limited number of physiological processes that can be impacted by stressors (Kaunisto et al., 2016). Alternatively, stressors could accelerate the effects on life history traits as the number of synergistic interactions between stressors increases (Kaunisto et al., 2016). To distinguish between these hypotheses requires increasing the number of stressors and measuring the impact across a range of life history traits.

Experimentally validating the impact of increasing dimensions of stressors on life history traits rapidly becomes intractable. A more tractable approach would be to predict the impacts of combinations of stressors by understanding the proximate mechanisms they affect to regulate plastic responses. By identifying how stressors impact the proximate mechanisms that modify traits, we can begin to build a greater understanding of when and why some stressors might elicit the same effects, and when we might expect combinations of stressors to act synergistically.

Proximate mechanisms regulating plastic responses to environmental stress

Understanding the proximate mechanisms regulating animal responses to multiple stressors can potentially enable us to predict a priori the effects of unfamiliar combinations of environmental stressors, or to speculate on the generalisability of the effects of stressors across taxa (Todgham and Stillman 2013). For many traits, our understanding of proximate mechanisms regulating plastic response to even single stressors is not yet sufficient to apply this approach (Todgham and Stillman 2013). However, for a subset of traits, like insect body size, we have a detailed understanding of the physiological and genetic mechanisms regulating plasticity.

The proximate mechanisms underlying body size plasticity can be characterised at different levels, from the whole-animal processes, down to alterations in the molecular interactions that govern these processes. For example, variation in body size could result from differences in the behaviour of food intake (Mahishi and Huetteroth 2019, and references therein), the physiological mechanisms that dictate food assimilation and absorption, (Trier and Mattson 2003; Jensen and Hessen 2007) or the developmental processes regulating the

duration of the growth period and the rate of growth (Davidowitz and Nijhout 2004). The relative contribution of each of these processes to generate variation in body size plasticity is unknown.

To cope with changing nutritional needs, developing animals respond by modulating their food-related behaviours according to developmental stage and the nutritional environment (Raubenheimer and Simpson, 1999; Raubenheimer and Browne 2000; Carvalho and Mirth 2017). For example, *D. melanogaster* larvae modulate their feeding rates to regulate macronutrient consumption, increasing their food intake when faced with diets with reduced protein concentration; while younger larvae regulate their protein intake more tightly than older larvae (Carvalho and Mirth, 2017).

When animals face limited food availability due to biotic (predators or competitors) or abiotic factors (microclimatic conditions), or restricted foraging capacity (reviewed in Clissold et al., 2010), they must cope with suboptimal food resources. They compensate for nutrient imbalances through post-ingestive adjustments in the gastrointestinal tract or via metabolism and excretion (Trier and Mattson 2003; Jensen and Hessen 2007). For example, the locust *Locusta migratoria* downregulates digestive enzymes like amylase and chymotrypsin in response to excess carbohydrates or proteins respectively (Clissold et al., 2010). Various insects, including grasshoppers and fruit flies, convert excess carbohydrates to lipids (reviewed in Behmer 2009; Talal et al., 2021), thereby rebalancing nutrients post-ingestively when they cannot regulate food intake to meet nutritional needs (Raubenheimer & Simpson 1993; Simpson & Raubenheimer 1993).

Regardless of how much food an animal consumes or assimilates, final adult body size is determined by two major developmental processes, development time and growth rate, throughout the nymphal or larval stages (Hanna et al., 2023). Development time itself affects final body size by determining the length of time available for larval growth, while growth rate affects final body size by modulating the rate at which mass accumulates (Mirth and Riddiford, 2007; Shingleton et al, 2008).

Importantly, the relative contribution of these two developmental processes can vary with environmental factors like temperature and nutrition (reviewed in Shingleton 2013). In tobacco hornworm *Manduca sexta*, at lower temperatures development time is the major determinant of body size, whereas at higher temperatures growth rate contributes more to body size (Davidowitz & Nijhout 2004). This is because high temperatures increase growth rate but decrease development time generating a smaller body size (Davidowitz et al. 2004).

On the other hand, both quality and quantity of diet influence growth rate and development time across a wide range of animals (Nijhout and Grunert 2010; Holmes et al., 2020; Gutiérrez et al., 2020; Pocas et al., 2022*; Bordiean et al., 2022*). High quality diets generate a larger body size by accelerating growth rate, whereas low quality diets prolong development time and dramatically decrease growth rate generating smaller body sizes (Davidowitz et al., 2004).

Finally, temperature and nutrition can act synergistically to affect the developmental processes that generate final body size. In *Spodoptera exigua* caterpillars, growth rates are highest on diets that are intermediate in their protein to carbohydrate ratio. However, at elevated temperatures (30°C) growth rates increase disproportionately in caterpillars reared on intermediate protein to carbohydrate diets when compared to those reared at lower temperatures (below 26°C) (Lee and Roh, 2010; Lemoine and Shantz, 2016). This is likely because of the combined effects of high temperature and intermediate protein to carbohydrate ratio diets on growth rate. This highlights how we can use these proximate mechanisms to predict why the combination of the two environmental conditions might act synergistically to affect body size.

In insects, we have an extensive understanding of the molecular pathways that act to regulate growth rates and developmental timing. Growth rates in all animals studied to date is regulated by highly conserved pathways that control cell division and cellular growth (Cobham and Mirth 2020). Ultimately, stress-induced changes in growth rate will impact the pathways that regulate cell division and cell size (Ren et al., 2020).

Developmental timing in insects is a product of the secretion of pulses of the steroid moulting hormone ecdysone (Yamanaka et al 2013, Kannangara et al., 2021*). These pulses

induce key transitions, such as moults between nymphal/larval stages and onset of metamorphosis, thereby regulating the pace of development (Yamanaka et al 2013, Kannangara et al., 2021*). Changes in developmental timing ultimately results from altering the timing of ecdysone secretion (Kannangara et al., 2021*).

This means that for body size we can model the potential impacts of combined stressors by assessing how each stressor affects the development cell growth, cell division, and developmental timing. We can gain further insights by identifying the signalling pathways that regulate these responses and comparing whether these pathways differ in their response to individual stressors. Stressors that alter the same developmental processes are likely to saturate plastic responses when combined. Stressors that differ in how they regulate cell growth, cell division, and developmental timing are likely to generate synergistic, potentially unpredictable effects when combined. Similar approaches could be applied to other traits for which we understand the developmental or physiological underpinnings.

Using genetic variation in plasticity to understand responses to combined thermal and dietary stress

For traits wherein the proximate mechanisms regulating responses to environmental stressors are poorly understood, uncovering how genetic variation modifies trait responses to the environment offers an important alternative. Plasticity varies across genotypes, often described as genotype by environment interactions, GxE (Falconer and Mackay 1996, Hoffmann and Bridle 2022*). In this section we discuss two ways of looking at GxE interactions: 1) examining variation across genetically-diverse, locally-adapted populations, and 2) determining how genetic variation within a population alters plastic responses to combined thermal and nutritional stress.

Some of the best evidence for genetic variation in plasticity of life-history traits comes from studies of insect populations sampled along environmental gradients. Studies show that populations differ in their plastic responses to temperature for a range of life-history traits like development time, growth rate, and body weight (Papadogiorgou et al., 2023*; Sgro et al., 2016; De Jong et al., 2010; Fischer and Karl 2010; Shama et al., 2011; Kivelä et al., 2012).

Recent studies have also shown that populations have unique plastic responses to combinations of environmental stressors. For example, when two geographically distinct populations of butterflies (*Pieris napi*) were reared on host-plants at two different temperatures and water regimes, they showed population-specific differences in plasticity for development time, growth rate, and body size (Kuczyk et al., 2021*). Thus, the extent of plasticity differs in genetically diverse populations across multiple life-history traits.

These differences among populations have led to theoretical predictions of how plasticity should evolve in response to environmental gradients. Generally, theory predicts that populations adapted to more variable environments should exhibit higher plasticity (environmental variability hypothesis, Sørensen et al. 2016). Following this, we would predict the highest plasticity in temperate environments, which show higher variation in temperature and the availability of nutritional resources when compared to tropical environments (Robinson and Partridge 2001; Rosenblatt and Schmidt 2016).

Research aimed at testing this hypothesis has produced mixed results (Hoffmann and Bridle 2022*; Chakraborty et al., 2020). Comparing plastic responses to combined thermal and nutritional stress in tropical, sub-tropical, and temperate populations of *Drosophila melanogaster* showed that the populations showing highest plasticity was trait and condition specific (Chakraborty et al., 2020). When reared at 18°C, the sub-tropical population exhibited the highest plasticity for wing area in response to dietary conditions, with the temperate and tropical populations showing lower plasticity. Rearing animals at 25°C reduced the plastic response of wing area to diet, but only in the tropical population. Plasticity also differed across traits and macronutrient type: at 25°C, the temperate population had the highest plasticity for femur size in response to carbohydrate, but the lowest plasticity in response to protein (Chakraborty et al., 2020). This suggests that we currently do not have a framework to accurately predict how plasticity should evolve across populations (Hoffmann and Bridle 2022*).

If we aim to predict how plasticity might evolve in response to combined stress, an alternative approach would be to examine plastic responses within a population. The extent

to which plasticity can evolve among populations depends on the type and amount of genetic variation for plasticity present within a population (Dreyer et al., 2016; Lafuente et al., 2018; Frankino et al., 2019). Further, the distribution of individual plastic responses within a population dictates adaptation by providing a range of phenotypic adjustments that can be selected for under varying environmental conditions. A wider distribution of plastic responses increases genetic and phenotypic diversity, enhancing the population's ability to adapt to and survive environmental changes (Frankino et al., 2019).

Across a wide range of taxa, individuals within a population can vary in their plastic responses to temperature and nutrition for numerous traits, including body size, development time, growth rate, mating success, olfactory behaviour, longevity, and stress resistance (Camus et al., 2017; Ørsted et al., 2018; 2019; Lafuente et al., 2018; Thompson 2019; Frankino et al., 2019; Cunningham et al., 2020). For example, the grasshopper *Melanoplus sanguinipes* shows genetic variation in their plasticity to nutrition for growth rate and across several body-size traits like head size and leg length (Thompson 2019). Similarly, *D. melanogaster* shows genetic variation among individuals for body size plasticity in response to combinations of temperature and nutrition (Chakraborty et al., 2022*). Genetic variation in plasticity is important, as it can dictate how populations respond to sustained changes in their environment (Dreyer et al., 2016).

Examining genetic variation either across or within populations allows us to examine fundamentally different questions. Looking across populations tells us how much the adaptive history of the population shapes current plastic responses. Within population variation allows us to predict whether plasticity can evolve. Both approaches are highly valuable but have distinct implications.

The physiological basis of genetic variation in plasticity in response to developmental temperature and nutrition

Examining either the proximate mechanisms regulating plasticity or genetic variation in plasticity represent important approaches for understanding responses to combined thermal and nutritional stress. For some traits, it is possible to explore both. Importantly, genetic variation in body size plasticity in *Drosophila* and other insect species can be found

both within and across populations (Chakraborty et al., 2020, 2021*, 2023*). This provides the potential to capitalise on our extensive knowledge of the proximate mechanisms regulating body size to understand genetic variation in plasticity within and between populations.

To affect body size plasticity, adaptation to combinations of thermal and nutritional conditions must also generate variation in either developmental timing, growth rate, or both. When adapting *D. melanogaster* flies to combinations of high and low temperatures and poor and good quality foods, male flies show the strongest decrease in body size in response to high rearing temperatures when they were adapted to high temperatures and poor-quality food (Bochdanovits and de Jong, 2003). This is because growth rate, but not developmental time, is more strongly reduced in the high temperature, poor food adapted flies. This demonstrates how adaptation to combined stress can alter plastic responses.

Interestingly, genotypes can differ in the mechanisms they use to achieve either high or low plasticity even within a population (Chakraborty et al., 2023*). In *D. melanogaster*, genotypes that showed high plasticity to combinations of diet and temperature could achieve this response either by altering developmental time or growth rate (Chakraborty et al., 2023*). Thus, seemingly similar plastic responses can be achieved more than one way within a population. This has important implications for those employing genome wide association approaches to uncover loci responsible for genetic variation in plasticity; understanding proximate mechanisms for plasticity is crucial for separating whether there are multiple ways within a population to achieve the same phenotypic outcome for plasticity. Future work examining the molecular pathways through which genotypes achieve these differences in plastic response to stressors will allow us to begin predicting how populations might adapt to combined stress.

Concluding remarks

As already appreciated by many in the field, predicting how insects will respond to ongoing global change is complex. To accurately do so, we need to include more than one environmental variable. However, it remains unclear how many stressors should be considered and in what combination to avoid diminishing returns. Furthermore, while we

can manipulate temperature and diet independently in the lab to explore the effects of combined stress, in the wild temperature and diet will covary, as temperature will alter the quality and abundance of animal diets. This makes it more difficult to disentangle the direct effects of combined stress on animal traits and the indirect effects of temperature on diet quality and availability. We propose that understanding either the proximate mechanisms regulating plastic response to stressors, genetic variation in plastic responses, or both provides powerful approaches to enhance our predictions of how stressors interact.

Declaration of interests statement

None

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Used nutritional geometry to manipulate protein and carbohydrates content in adult diet, to visualise dietary effects on life-history traits like lifespan and reproduction. Both males and females increased their lifespan with increasing carbohydrate. Male offspring production rate and the total number of offspring produced also increased with carbohydrate percentage in the diet. Interestingly, female egg production rate was optimised in a relatively narrow area of intermediate P:C ratio, whereas total egg production was maximised over a much larger area in the nutritional space.

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This was one of the first studies to examine within-population genetic variation in plastic responses to combinations of temperature and diet. The authors looked at wing area plasticity to combinations of diet and temperature across isogenic lines from a wild caught population. They further investigated the proximate mechanisms that could explain the genetic variation in body size plasticity. Interestingly, there is a diversity of ways in which plasticity can be achieved, genetic variation in body size plasticity is regulated by mechanisms that are at least partially independent from those regulating plasticity in development time

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One of the few studies that investigate more than two interacting abiotic stressors. The authors study the additive and interactive effects of diet (native and non-native host plant), temperature (three thermal regime) and three concentrations of pesticide exposure on survival, development time and body mass in caterpillars. This study provides a pragmatic data point for understanding interactive threats in small-bodied non-migratory butterfly.

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Novel experiment wherein the authors grow the insect's host plant at different temperatures and water regimes, to test for the effect of food quality on the insects life history traits. This experiment tries to replicate ecological scenario wherein climate change will not only cause difference in temperature but also precipitation which in turn will affect

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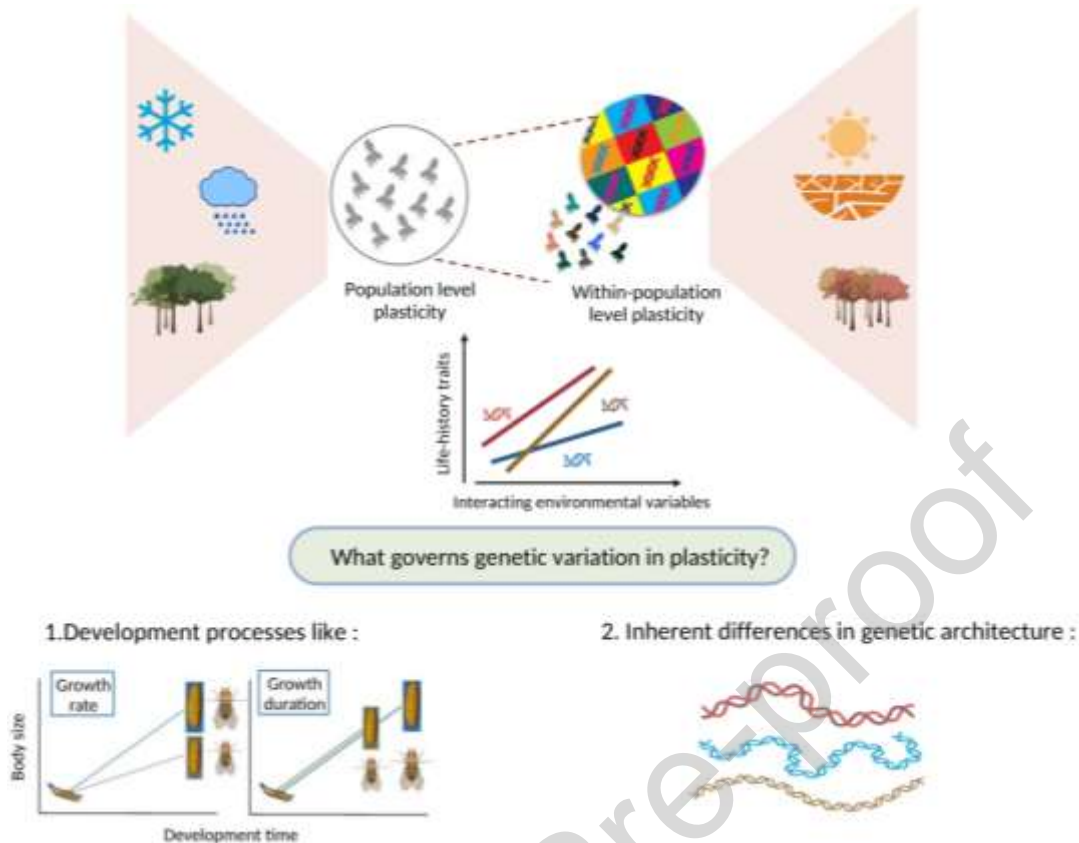


Figure 1: Insects face unpredictable changes in their environmental conditions with ongoing climate change. They exhibit phenotypic plasticity, in response to multiple interacting environmental variables like precipitation, nutritional quality and quantity, temperature etc. Locally-adapted, genetically-diverged populations differ in their plastic responses. Even within a population each distinct individual of a particular genotype can show a specific plastic response. This generates genotype by environment (GxE) interactions that can be observed with non-parallel intersecting reaction norms. Further, this plasticity is trait-specific, i.e., life-history traits respond with varying degrees of independence to combinations of environmental stressors, which again is dependent on the genetic makeup. Understanding what governs this genetic variation in plasticity requires us to take a multifaceted approach of looking into development process like growth rate and development time that can regulate body size plasticity, along with the genomic architecture of an individual.

Declaration of interests statement

None

Highlights

1. Insects exhibit plastic responses in life-history traits to both temperature and nutrition.
2. Combinations of nutrition and temperature shape plastic responses in unpredictable ways.
3. Genetic variation in plasticity in response to nutrition and temperature exists both across and within populations.
4. The plastic response of a trait to nutrition and temperature can be achieved by modulating multiple physiological processes.
5. Understanding the proximate mechanisms regulating plastic responses promises to enhance our predictions of how organisms will respond to interacting stressors.