



Adaptation to the abiotic environment in insects: the influence of variability on ecophysiology and evolutionary genomics

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Advances in tools to gather environmental, phenotypic, and molecular data have accelerated our ability to detect abiotic drivers of variation across the genome-to-phenome spectrum in model and non-model insects. However, differences in the spatial and temporal resolution of these data sets may create gaps in our understanding of linkages between environment, genotype, and phenotype that yield missed or misleading results about adaptive variation. In this review we highlight sources of variability that might impact studies of phenotypic and 'omic environmental adaptation, challenges to collecting data at relevant scales, and possible solutions that link intensive fine-scale reductionist studies of mechanisms to large-scale biogeographic patterns.

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Introduction

“It is simpler to examine most biological phenomena in uniform or invariable environments than under variable conditions” – Beardmore and Levine [1]

Methodological advances have greatly aided the quest to link genotype to phenotype in diverse organisms, providing new tools for addressing major questions in ecology and evolution [2,3]. High-throughput DNA sequencing has enabled rapid determination of individual genotypes at genome-wide resolution [4–6], facilitating comparisons

among individuals, populations, and species. Linking genotypes with relevant phenotypes is a powerful approach for inferring adaptation of insects to the abiotic environment [7]. However, the abiotic environment, insect phenotypes, and the 'omics that determine those phenotypes are often far more variable in time and space than commonly appreciated, complicating inferences of adaptation. A better understanding of variation at all of these levels will be crucial to deciphering how organisms adapt to the abiotic environment.

Insects experience some degree of spatial and temporal environmental variation whether they have broad or restricted distributions [8–10]. Such variation includes large-scale bioclimatic gradients for species with broad geographic ranges, such as temperature changes with latitude or elevation [11], small-scale local extremes for species that occupy multiple microhabitats during a day [12^{••}], or seasonal extremes for long-lived or multivoltine species [13]. Individuals or populations must thus express physiological traits emerging from underlying 'omic variation (e.g. genomic, transcriptomic, proteomic, metabolomic) that facilitate tolerance of variation over space and time [14].

However, an explicit appreciation for variation in abiotic environments, in phenotypes necessary to live in those environments, and in the genetic basis of those phenotypes is rare in the literature. Abiotic variables like temperature are rarely measured at spatial and temporal scales relevant to insects [8,10]. Phenotypes are generally measured for a small number of individuals from a few populations of relatively few species, and, for many species, it may not even be clear what phenotypes are most important to measure. The ability to characterize molecular variation from large numbers of individuals sampled across abiotic gradients has provided many examples of genes that facilitate abiotic adaptation [15^{••},16^{••},17[•],18^{••}]; however, analytical challenges (such as multiple, co-occurring population genetic processes) and a lack of high resolution environmental data mean we may often be missing much of the picture. Methodological issues can be overcome, but conceptual advances will also be necessary to better account for variation (at all these levels) in studies of insect adaptation to the abiotic environment. Understanding ecological and evolutionary responses to abiotic variation across relevant spatial and temporal scales will improve our capacity to predict how species will respond to local and global environmental change [19–21].

In this review we discuss how abiotic variability may influence variation in phenotypes and genotypes, and how integration of experimental physiology and genomic approaches can yield novel insights into environmental adaptation. We focus on temperature because it has been best studied from characterization of environments (including microclimates) to phenotypic and genotypic responses. Other abiotic variables, including precipitation [22], oxygen [23], wind speed, solar radiation, acidity, and salinity, or anthropogenic stressors like pesticides [24[•]] or urbanization [25], may similarly create fine-grained landscapes of stress to which insects must respond, and the principles discussed here should largely apply to studies of these other factors.

Variation in the abiotic environment at the insect scale

Variation in phenotypes across individuals, populations, and species reflects, to a large extent, tolerance to abiotic conditions, which vary geographically [26]. It is only recently becoming clear that variation per se may be more important than mean conditions in determining organisms responses to climate [19,27,28] and that organisms respond to variation at much finer temporal and spatial scales (seconds to hours and mm to meters) than those typically considered (monthly to annual and km to continents) [12^{••},29]. Despite this growing appreciation of how fine scale variation can determine traits critical for population persistence [8,9,21,27,30], characterizing spatial and temporal variation in abiotic variables and incorporating those key elements of variation in studies of adaptation remains a hurdle (but see Refs. [31,32,33^{••}]).

The capacity to gather and analyze data at fine temporal and spatial scales currently limits mapping of temperatures at insect scales [8,10,34]. Although countless studies have likely measured, for example, field temperatures at high temporal frequencies (1/s), that temporal variation is usually lost in statistical summaries in publications [10,35], leaving few data sets with temperatures measured at high temporal frequency and with broad geographic coverage (but see Refs. [34,36,37]). Fortunately, the recent development of low-cost thermal imaging will facilitate characterization of temperature variation over spatial scales relevant to insects [38,39].

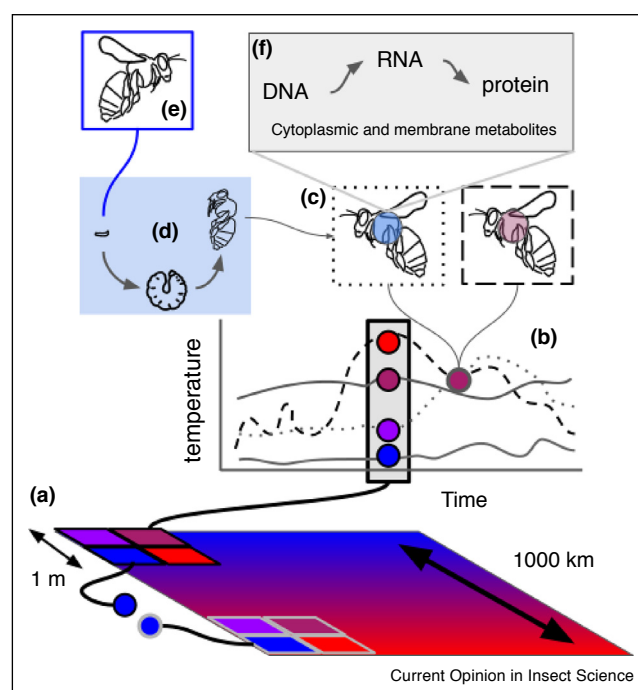
Insect body temperatures are determined by complex interactions between biophysical characteristics and behavior and a mosaic of ambient conditions that dictate heat (or moisture or respiratory gas) transfer between the environment and the organism. New approaches for predicting landscapes of insect body temperatures from mechanistic biophysical models [40,41] and organismal climatology [42], combined with methods for extracting important metrics of variation [31,43–45], will facilitate more insect-centric characterization and manipulation of abiotic variables. These approaches have rarely been applied in evolutionary ‘omics studies (but see e.g.

[46]) and require continued refinement to make them broadly available [47]. A key challenge will be identifying the habitats and spatial scales over which variation is predictable [43,48], as continuous measurement of all sites at high frequency and at relevant scales is currently impractical. Characterization of abiotic stressors at the appropriate spatial and temporal scales not only provides a more accurate picture of what insects are adapting to, but also directly informs measurements of physiological traits of insects living in those environments (Figure 1).

Insect physiological responses to environmental variation

Mounting evidence suggests that fine-scale variation in temperature (and likely other abiotic factors: precipitation

Figure 1



Spatial and temporal variation in abiotic stressors challenges characterization of environments, of insect phenotypes, and therefore of inference of abiotic adaptation. (a) Within a square meter, an insect could experience as much temperature variation as is found across 1000 km [39]. Therefore, insects from warm (red) climates (gray border squares) may rely on cooler (blue) microclimates to maintain similar body temperatures (gray outlined point) as insects from cool climates (black outlined point). (b) Even after sampling across habitats, current temperatures (gray box) may not reflect past temperatures or predict future conditions. Insects sampled from apparently similar thermal environments (magenta points) could have strikingly different thermal histories, resulting in differences in thermal traits. (c) The enhanced heat tolerance of the insect on the right could reflect previous exposure to hot temperatures (dashed line), and the enhanced cold tolerance of the insect on the left could reflect previous cold exposure as an adult (dotted line), or during development (d), or (e) thermal experience of its parents. (f) Ultimately, these physiological differences arise from cellular level differences in genotype, in transcribed RNA and proteins, and in interacting metabolites, all of which can be measured with ‘omics approaches.

[22], oxygen [23]) often drives insect physiological responses [9,12^{••},49,50], calling into question the standard approach of characterizing physiology via ‘thermal performance curves’ (TPCs) based on static temperature treatments [27,51–53]. A single TPC is likely insufficient to characterize responses of insect species or populations because insects rarely experience constant temperatures but rather have adapted to the temporal and spatial variations in body temperatures (including extremes) experienced in their lifetimes [54,55]. For example, in the case of heat tolerance, both the shapes of TPCs and the optimal temperatures for performance depend more strongly on temperature variation, than on mean temperature [53,56].

Over shorter timescales, TPCs of individual insects can vary due to recent temperatures experienced during a heat/cold shock [57], diurnal cycles [58], a heat or cold front [59], or temperatures experienced in previous life stages (developmental plasticity, e.g. [60]) or by the parent (transgenerational or epigenetic, e.g. [61]) (Figure 1). Physiological traits can also vary over short spatial scales. For example, ants and bees in urban environments tolerate higher temperatures than those in nearby rural areas [62,63] and canopy ants are more heat tolerant than those on the forest floor [64]. Even at the level of individual leaves, insects can differ in upper lethal temperature by up to 8°C, despite separation of only a few centimeters [12^{••}]. It will be critical to incorporate these thermal history effects into estimates of the physiological responses of insects to environmental temperatures [51,53,65] if we are to appropriately characterize phenotypes and their adaptive variation in response to abiotic stressors.

Broadly speaking, these differences reflect responses to thermal history via trait plasticity at different time scales. The extent to which physiological traits are plastic, whether trait plasticity varies consistently across geographic gradients, and whether plasticity itself evolves to facilitate persistence in the face of abiotic stressors are all critical questions that have engaged physiologists for decades [66–68]. Environmental variation itself can also drive both plasticity and its evolvability [69]. For example, in ants, both thermal tolerance *and* plasticity of thermal tolerance evolve rapidly in response to urban heat clines [70]. However, plasticity can vary strikingly between traits [71], insects may tradeoff constitutive (local adaptation) and induced tolerance (plasticity), and traits may evolve together [72] or be uncorrelated [73], all of which potentially muddle inferences about insect adaptation to temperature or other abiotic stressors [74].

Complicating the matter further, short duration extreme events may exert exceptionally strong selection pressure, resulting in phenotypes adapted to levels of abiotic stress not measured in a particular study [27,64,65].

Surviving exposure to extremes requires mounting a physiological response and can result in physiological damage, both of which likely alter responses to subsequent exposures to abiotic stressors in complicated ways [27,75–77]. And organisms may adapt to the predictability of extreme fluctuations rather than their amplitude. For example, flies exposed to unpredictable temperature fluctuations were less stress-resistant than those exposed to predictable temperature fluctuations of the same magnitude [78].

Individual insects are also likely responding to multiple, often interrelated abiotic challenges, and may vary in the measured phenotype in time and space for reasons that may be unrelated to a focal abiotic stressor [22]. For example, body size [74,79,80], age [81], nutritional state [82], pathogenic and beneficial microorganisms [83], reproductive state, biotic [84] and anthropogenic chemicals [85] can all alter tolerance traits. Cross-talk and cross-tolerance between traits [86] can alter insect responses to stressors but are rarely considered, particularly in the context of detecting adaptation. Awareness of these diverse effects when designing studies (see below) and implementation of standardized approaches to measuring relevant physiological traits [87,88] will help overcome potential confounding effects and generate robust evidence for physiological adaptation to abiotic stressors.

Separating signal from noise to detect adaptation with ‘-omics’ data

Population genomics of local adaptation

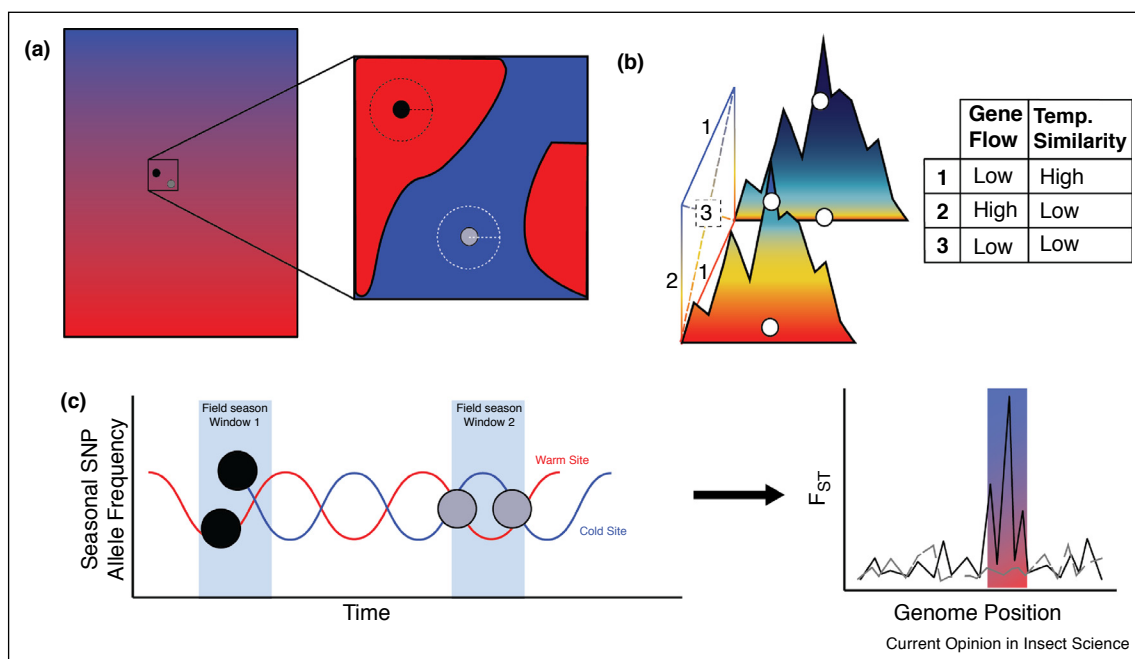
The search for genes influencing responses to environmental pressures like temperature has a long history from traditional genetics approaches in model insects like *Drosophila melanogaster* [89[•]]. More recently, ‘omics tools have led to major advancements in understanding environmental adaptation across levels of molecular organization, from the effects of DNA sequence changes on protein coding variation, to changes in expression of this variation across environments. In this section we focus on population genomics, which has leveraged these developments for discovery of environment-associated loci in natural populations of model and non-model organisms [90[•]], especially where traditional genetics experiments are challenging. Statistical population genomics methods to detect loci (usually single nucleotide polymorphisms, or SNPs) with strong allele frequency differentiation among populations in different environments have aided the search for genes underlying local adaptation [91–93]. In just over a decade, investigations of adaptive variation have become nearly as commonplace in molecular ecology as estimating neutral demographic processes like dispersal [94]. Molecular tools to genotype SNPs in species with limited genome resources have facilitated such studies by reducing the genome to bioinformatically tractable subsets [6,95,96], and as more insect genomes are sequenced [97], whole genome

resequencing will likely become the norm. Even with whole genome data, however, uncovering the genomic basis of locally adapted phenotypes remains challenging. Discussion of these challenges [7,91–93] has focused primarily on methodological and statistical concerns, with less consideration for appropriately sampling genotypes in time and space [98*,99].

Sampling individuals across spatial-environmental gradients is currently the most common approach to population genomic detection of adaptation. Insect examples include adaptation to high elevations in honey bees [18**], latitudinal gradients in *Drosophila* [100*], and gradients in temperature and precipitation in damselflies [101] and midges [17*]. Spatial variation represents the low hanging fruit for genome-environment association analyses, but still can present challenges. One issue is that abiotic gradients may correlate with spatial population structure, confounding isolation by distance and environmental adaptation [93,102]. Statistical approaches can model

population structure when detecting environmental adaptation (e.g. [103,104]), but distance and environment can be further decoupled with creative sampling. Comparative analyses of multiple species can detect concordance across the same environmental gradient [105,106], the same abiotic gradient can be sampled in widely separated transects [107], or paired designs can leverage landscape heterogeneity by sampling genotypes from distinct environments at multiple spatial scales [92,108] (Figure 2). Such approaches are most effective when selection drives parallel changes across replicated environments, however, and may miss population-specific mutations. A second issue is that detecting local adaptation across heterogeneous landscapes requires relevant environmental data. But, as discussed above, environmental data are often only available at coarse spatial or temporal resolutions [109]. Whether this ‘spatial gap’ [8] is a problem for adaptation studies depends on the study question and organismal traits (e.g. dispersal distance, longevity). For long-lived species, genetic variation may reflect

Figure 2



Some issues of spatial and temporal complexity when sampling environmental gradients for genomic adaptation scans. **(a)** The potential spatial gap [8] between available environmental data and local-scale realities when sampling across an abiotic gradient (see also Figure 1a). In this case two genotypes from an intermediate value environment actually segregate by habitat, with activity ranges (dashed lines) restricted to discrete microenvironments, potentially masking any signature of local adaptation using coarse spatial data. **(b)** Mountains illustrate how complex environmental landscapes can be used to design paired sampling studies. In this case, pairing elevation and latitude extremes allows genotypes to be sampled from different temperatures at multiple spatial scales, in part decoupling confounding effects of gene flow when testing for environmental adaptation. This hypothetical species also illustrates how using latitude as a proxy for temperature without considering elevation could produce misleading adaptation scan results. **(c)** Temporally fluctuating selection pressures can produce oscillating allele frequencies [110**] for multivoltine species that can produce misleading signatures of local environmental adaptation in genomic data that depends on when samples are taken during a single field collection trip. Black genotype samples might produce a strong signal of differentiation (F_{ST}) near the seasonal locus, while gray genotypes show no evidence of differentiation between environments. Both results are misleading, the former because a conclusion would be made about the strength of local adaptation when both populations are responding similarly to the same environmental fluctuations at different times, and the latter because while no local adaptation is detected, another interesting form of selection from the environmental variable of interest is overlooked.

long-term bioclimatic patterns captured by annual means and extremes at large spatial scales. However, many insects will experience microclimates at finer scales than are represented in such data sets (Figures 1 and 2), and multivoltine species may experience temporal fluctuations in selection within a year. For such species, it will be important to both fully characterize relevant environmental variables, and carefully design collections so that samples are comparable among populations (Figure 1).

Many examples of rapid genomic evolution in response to environmental change in insects [101,110^{••},111[•]] suggest that effects of fluctuating selection on population genetic data may be underappreciated [98[•]]. For species with short generation times relative to environmental changes, intra-season fluctuations in allele frequencies from standing genetic variation could confound statistical genotype-environment associations, depending on when sampling is conducted (Figure 2). Importantly, the timing of such fluctuations may vary across a species range; Bergland *et al.* [110^{••}] has provided some of the clearest evidence for seasonal selection on insect genomes using *D. melanogaster* populations from a latitudinal cline, with repeatable oscillations of allele frequencies observed within and between years at seasonal loci throughout the genome that could be recapitulated with spatial comparisons. In addition to selection favoring different genotypes in different environments, some genetic variants may be selected on by variation itself, including loci associated with seasonal rhythms or traits like diapause and circadian clocks [112,113]. Migratory species like monarch butterflies have additional complexities, as selection will shape responses to local climates [114], requiring consideration of the question ‘which environment is local?’ with respect to genomic variation. Finally, range expansions (e.g. of insect pests) highlight the importance of environmental adaptation associated with colonization of new environments [101,115]. Expansions produce spatially and temporally non-equilibrium scenarios that may complicate population genetics analyses; however, certain population genomics tools may be particularly effective at detecting adaptation in such situations [116]. So, spatio-temporally fluctuating selection can be strong in insect populations, but methods to detect its effects are in their infancy. Models incorporating time-series data or chronological sampling are potentially promising but require further development [117[•]].

Beyond genome sequence variation

Other ‘omic technologies provide additional opportunities for uncovering the basis of environmental adaptation. DNA sequence variation can clearly contribute to local adaptation across environments through structural protein coding changes or mutations in regulatory regions that alter gene expression. However, mechanistic insights into tolerance of abiotic variation may often be more clearly revealed in other parts of the ‘omic spectrum,

including transcriptomics (gene expression), methylomics (epigenetics), or metabolomics (small molecules). While DNA sequences are stable within an individual, other layers of the molecular spectrum are variable across space, environment, time, and tissue [118,119]. This inherent responsiveness makes these methods ideal for determination of mechanisms underlying organismal sensitivity to environmental pressures, further illuminating links between genotype and phenotype. Ample evidence suggests that gene expression regulation under environmental stress contributes to adaptive phenotypes. For example, differences in transcription in flies are substantial after cold shock (−10°C), but rare following prolonged cold hardening (0°C) [120]. Population (i.e. genotypic background) by environment effects also reveal that adaptive evolution may shape gene expression responses in individuals from regions with different conditions [121,122]. Gene expression is not only influenced by spatial variation [16^{••}], but also responds to temporal (seasonal) variation [123]. For some traits, adaptation may not be detectable at genomic or transcriptional levels, requiring assays of other levels of the ‘omic spectrum [120,124,125].

A multi-scale integrative approach

Many insects have large geographic distributions that span environmental gradients, so understanding adaptation across the species range is important. Recording abiotic data at high spatio-temporal resolution at each collecting locality may be impractical at these scales, where samples are often taken over multiple years or even from natural history collections [126]. Likewise, collecting physiology data, genome sequences, transcriptome sequences, and so on in all populations is infeasible, and traditional laboratory genetics experiments are difficult for many species. However, a multi-scale integrative approach may help address these limitations. One part of a project could include a biogeographic clinal adaptation study of many individuals and populations, incorporating population genomics to detect adaptation candidate genes and measurement of traits hypothesized *a priori* to be physiologically relevant (e.g. body size or wing size variation with latitude or altitude: [127]). Such observational studies could be combined with extensive spatial and temporal sampling of individuals and environmental conditions in focal populations, perhaps at the abiotic extremes of the study region. A more comprehensive suite of molecular assays, including RNAseq, epigenomics, and metabolomics in these focal populations could be directly tied to experimentally measured physiological variation, and cross-validated against results from large-scale analyses to predict how different sources of variation enable adaptation to abiotic conditions. Of course, fully addressing the question of adaptation ultimately requires fitness measurements for genotypes and phenotypes across environments; this will be a major challenge for most non-model organisms. Developing

approaches to overcome such challenges and integrate distinct data types collected at different spatial and temporal resolutions will be necessary if we hope to make accurate and actionable predictions (e.g. [128,129,130*]) about insect evolution in contemporary and future landscapes.

Conflict of interest statement

Nothing declared.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Beardmore JA, Levine L: **Fitness and environmental variation. 1. A study of some polymorphic populations of *Drosophila pseudoobscura*.** *Evolution* 1963, **17**:121-129.
 2. Matz MV: **Fantastic beasts and how to sequence them: ecological genomics for obscure model organisms.** *Trends Genet* 2018, **34**:121-132.
 3. Rudman SM, Barbour MA, Csilléry K, Gienapp P, Guillaume F, Hairston Nelson Glo Jr, Hendry AP, Lasky JR, Rafajlović M, Räsänen K *et al.*: **What genomic data can reveal about eco-evolutionary dynamics.** *Nat Ecol Evol* 2018, **2**:9.
 4. Goodwin S, McPherson JD, McCombie WR: **Coming of age: ten years of next-generation sequencing technologies.** *Nat Rev Genet* 2016, **17**:333-351.
 5. Sedlazeck FJ, Lee H, Darby CA, Schatz MC: **Piercing the dark matter: bioinformatics of long-range sequencing and mapping.** *Nat Rev Genet* 2018, **19**:329.
 6. Andrews KR, Good JM, Miller MR, Luikart G, Hohenlohe PA: **Harnessing the power of RADseq for ecological and evolutionary genomics.** *Nat Rev Genet* 2016, **17**:81-92.
 7. Tiffin P, Ross-Ibarra J: **Advances and limits of using population genetics to understand local adaptation.** *Trends Ecol Evol* 2014, **29**:673-680.
 8. Potter KA, Arthur Woods H, Pincebourde S: **Microclimatic challenges in global change biology.** *Global Change Biol* 2013, **19**:2932-2939.
 9. Woods HA, Dillon ME, Pincebourde S: **The roles of microclimatic diversity and of behavior in mediating the responses of ectotherms to climate change.** *J Therm Biol* 2015, **54**:86-97.
 10. Kearney MR, Matzelle A, Helmuth B: **Biomechanics meets the ecological niche: the importance of temporal data resolution.** *J Exp Biol* 2012, **215**:922-933.
 11. Jackson JM, Pimsler ML, Oyen KJ, Koch-Uhuad JB, Herndon JD, Strange JP, Dillon ME, Lozier JD: **Distance, elevation and environment as drivers of diversity and divergence in bumble bees across latitude and altitude.** *Mol Ecol* 2018, **27**:2926-2942.
 12. Pincebourde S, Casas J: **Narrow safety margin in the phyllosphere during thermal extremes.** *PNAS* 2019, **116**:5588-5596.
- Outstanding example of integration of microclimate, physiology, and biotic interactions into predictions of thermal safety margins. Upper lethal limits of 7 insect species living on apple leaves differed by as much as 8 °C and matched the microclimates each species inhabited, with leaf surface temperature in part determined by the plant's reaction to different types of herbivory.
13. Buckley LB, Huey RB: **Temperature extremes: geographic patterns, recent changes, and implications for organismal vulnerabilities.** *Global Change Biol* 2016, **22**:3829-3842.
 14. Somero GN: **The physiology of global change: linking patterns to mechanisms.** *Annu Rev Mar Sci* 2012, **4**:39-61.
 15. MacMillan HA, Knee JM, Dennis AB, Udaoka H, Marshall KE, Merritt TJS, Sinclair BJ: **Cold acclimation wholly reorganizes the *Drosophila melanogaster* transcriptome and metabolome.** *Sci Rep* 2016, **6**:28999.
- This study found that long term cold acclimation improved *D. melanogaster* cold tolerance and led to massive regulatory changes in the transcriptome and metabolome. The study points to ways in which temporal environmental variability (eg. short vs long term cold exposure) and variability in tissues can influence molecular changes.
16. Lancaster LT, Dudaniec RY, Chauhan P, Wellenreuther M, Svensson EI, Hansson B: **Gene expression under thermal stress varies across a geographical range expansion front.** *Mol Ecol* 2016, **25**:1141-1156.
- The study examines gene expression of thermal tolerance in damselfly (*Ischnura elegans*) populations in northern Europe. The study incorporates spatial and temporal variability by sampling responses to thermal stress across a geographic temperature gradient in a range expanding species.
17. Waldvogel A-M, Wieser A, Schell T, Patel S, Schmidt H, Hankeln T, Feldmeyer B, Pfenninger M: **The genomic footprint of climate adaptation in *Chironomus riparius*.** *Mol Ecol* 2018, **27**:1439-1456.
- Powerful approach combining comparisons among populations with demography and drift to assess genomic evolution across a cline for a midge.
18. Wallberg A, Schöning C, Webster MT, Hasselmann M: **Two extended haplotype blocks are associated with adaptation to high altitude habitats in East African honey bees.** *PLoS Genet* 2017, **13**:e1006792.
- A detailed whole-genome population genomic study of altitudinal adaptation in honey bees that accounts for spatial variability by replicating relevant abiotic gradients across elevations for multiple mountains and identifies genetic changes at high elevations that are rare or absent in most of the globe.
19. Vasseur DA, DeLong JP, Gilbert B, Greig HS, Harley CDG, McCann KS, Savage V, Tunney TD, O'Connor MI: **Increased temperature variation poses a greater risk to species than climate warming.** *Proc R Soc B* 2014, **281**:20132612.
 20. Estay SA, Lima M, Bozinovic F: **The role of temperature variability on insect performance and population dynamics in a warming world.** *Oikos* 2014, **123**:131-140.
 21. Suggitt AJ, Wilson RJ, Isaac NJB, Beale CM, Auffret AG, August T, Bennie JJ, Crick HQP, Duffield S, Fox R *et al.*: **Extinction risk from climate change is reduced by microclimatic buffering.** *Nat Clim Change* 2018, **8**:713.
 22. Barton MG, Terblanche JS, Sinclair BJ: **Incorporating temperature and precipitation extremes into process-based models of African Lepidoptera changes the predicted distribution under climate change.** *Ecol Modell* 2019, **394**:53-65.
 23. Harrison JF, Greenlee KJ, Verberk WCEP: **Functional hypoxia in insects: definition, assessment, and consequences for physiology, ecology, and evolution.** *Annu Rev Entomol* 2018, **63**:303-325.
 24. Crossley MS, Chen YH, Groves RL, Schoville SD: **Landscape genomics of Colorado potato beetle provides evidence of polygenic adaptation to insecticides.** *Mol Ecol* 2017, **26**:6284-6300.
- This paper uses a combination of landscape genomics and RNAseq to detect candidate genes for rapid adaptation in insecticide resistance in an invasive pest. Selection in this case is spatially and temporally variable, and the paper highlights how a combination of standing and novel genetic variation is likely used in adaptation to such environments.
25. Theodorou P, Radzevičiūtė R, Kahnt B, Soro A, Grosse I, Paxton RJ: **Genome-wide single nucleotide polymorphism scan suggests adaptation to urbanization in an important**

- pollinator, the red-tailed bumblebee (*Bombus lapidarius* L.).** *Proc R Soc B Biol Sci* 2018, **285**:20172806.
26. Andrewartha HG, Birch LC: *The Distribution and Abundance of Animals*. Chicago, IL: University of Chicago Press; 1954.
 27. Williams CM, Buckley LB, Sheldon KS, Vickers M, Pörtner H-O, Dowd WW, Gunderson AR, Marshall KE, Stillman JH: **Biological impacts of thermal extremes: mechanisms and costs of functional responses matter.** *Integr Comp Biol* 2016, **56**:73-84.
 28. Dowd WW, King FA, Denny MW: **Thermal variation, thermal extremes and the physiological performance of individuals.** *J Exp Biol* 2015, **218**:1956-1967.
 29. Sears MW, Angilletta MJ, Schuler MS, Borchert J, Dilliplane KF, Stegman M, Rusch TW, Mitchell WA: **Configuration of the thermal landscape determines thermoregulatory performance of ectotherms.** *PNAS* 2016, **113**:10595-10600.
 30. Barton MG, Clusella-Trullas S, Terblanche JS: **Spatial scale, topography and thermoregulatory behaviour interact when modelling species' thermal niches.** *Ecography* 2019, **42**:376-389.
 31. Dillon ME, Woods HA, Wang G, Fey SB, Vasseur DA, Telemeco RS, Marshall K, Pincebourde S: **Life in the frequency domain: the biological impacts of changes in climate variability at multiple time scales.** *Integr Comp Biol* 2016, **56**:14-30.
 32. Wang G, Dillon ME: **Recent geographic convergence in diurnal and annual temperature cycling flattens global thermal profiles.** *Nat Clim Change* 2014, **4**:988-992.
 33. Koussoroplis A-M, Pincebourde S, Wacker A: **Understanding and predicting physiological performance of organisms in fluctuating and multifactorial environments.** *Ecol Monogr* 2017, **87**:178-197.
- This paper develops a clear mathematical framework for predicting how variance in single environmental factors as well as covariance among environmental factors may affect physiological performance. The approach allows for quantitative separation of variance and covariance effects, generating testable experimental predictions on the effects of variability (abiotic and biotic) on organism physiology.
34. Isaak DJ, Wenger SJ, Young MK: **Big biology meets microclimatology: defining thermal niches of ectotherms at landscape scales for conservation planning.** *Ecol Appl* 2017, **27**:977-990.
 35. Nadeau CP, Urban MC, Bridle JR: **Coarse climate change projections for species living in a fine-scaled world.** *Global Change Biol* 2017, **23**:12-24.
 36. NOAA: <ftp://ftp.ncdc.noaa.gov/pub/data/noaa/isd-lite/2012/>. 2012.
 37. Dillon ME, Wang G, Huey RB: **Global metabolic impacts of recent climate warming.** *Nature* 2010, **467**:704-706.
 38. Faye E, Rebaudo F, Yáñez-Cajó D, Cauvy-Fraunié S, Dangles O: **A toolbox for studying thermal heterogeneity across spatial scales: from unmanned aerial vehicle imagery to landscape metrics.** *Methods Ecol Evol* 2016, **7**:437-446.
 39. Pincebourde S, Suppo C: **The vulnerability of tropical ectotherms to warming is modulated by the microclimatic heterogeneity.** *Integr Comp Biol* 2016, **56**:85-97.
 40. Kearney MR, Porter WP: **NicheMapR – an R package for biophysical modelling: the microclimate model.** *Ecography* 2017, **40**:664-674.
 41. Kearney MR, Shamakhya A, Tingley R, Karoly DJ, Hoffmann AA, Briggs PR, Porter WP: **Microclimate modelling at macro scales: a test of a general microclimate model integrated with gridded continental-scale soil and weather data.** *Methods Ecol Evol* 2014, **5**:273-286.
 42. Helmuth B, Broitman BR, Yamane L, Gilman SE, Mach K, Mislán KAS, Denny MW: **Organismal climatology: analyzing environmental variability at scales relevant to physiological stress.** *J Exp Biol* 2010, **213**:995-1003.
 43. Bramer I, Anderson BJ, Bennie J, Bladon AJ, De Frenne P, Hemming D, Hill RA, Kearney MR, Körner C, Korstjens AH *et al.*: **Chapter three - advances in monitoring and modelling climate at ecologically relevant scales.** In *Advances in Ecological Research*. Edited by Bohan DA, Dumbrell AJ, Woodward G, Jackson M. Academic Press; 2018:101-161.
 44. Carter A, Kearney M, Mitchell N, Hartley S, Porter W, Nelson N: **Modelling the soil microclimate: does the spatial or temporal resolution of input parameters matter?** *Front Biogeogr* 2015, **7**.
 45. Rubalcaba JG, Gouveia SF, Olalla-Tárraga MA: **Upscaling microclimatic conditions into body temperature distributions of ectotherms.** *Am Nat* 2019, **193**:677-687.
 46. Campbell-Staton SC, Bare A, Losos JB, Edwards SV, Cheviron ZA: **Physiological and regulatory underpinnings of geographic variation in reptilian cold tolerance across a latitudinal cline.** *Mol Ecol* 2018, **27**:2243-2255.
 47. Chown SL, Gaston KJ: **Macrophysiology - progress and prospects.** *Funct Ecol* 2016, **30**:330-344.
 48. Pincebourde S, Murdock CC, Vickers M, Sears MW: **Fine-scale microclimatic variation can shape the responses of organisms to global change in both natural and urban environments.** *Integr Comp Biol* 2016, **56**:45-61.
 49. Bonebrake TC, Boggs CL, Stamberger JA, Deutsch CA, Ehrlich PR: **From global change to a butterfly flapping: biophysics and behaviour affect tropical climate change impacts.** *Proc R Soc B* 2014, **281**:20141264.
 50. Colinet H, Sinclair BJ, Vernon P, Renault D: **Insects in fluctuating thermal environments.** *Annu Rev Entomol* 2015, **60**:123-140.
 51. Kingsolver JG, Woods HA: **Beyond thermal performance curves: modeling time-dependent effects of thermal stress on ectotherm growth rates.** *Am Nat* 2016, **187**:283-294.
 52. Sinclair BJ, Marshall KE, Sewell MA, Levesque DL, Willett CS, Slotsbo S, Dong Y, Harley CDG, Marshall DJ, Helmuth BS *et al.*: **Can we predict ectotherm responses to climate change using thermal performance curves and body temperatures?** *Ecol Lett* 2016, **19**:1372-1385.
 53. Kellermann V, Chown SL, Schou MF, Aitkenhead I, Janion-Scheepers C, Clemson A, Scott MT, Sgrò CM: **Comparing thermal performance curves across traits: how consistent are they?** *J Exp Biol* 2019, **222**:jeb193433.
 54. Levins R: *Evolution in Changing Environments: Some Theoretical Explorations*. Princeton University Press; 1968.
 55. Gilchrist GW: **Specialists and generalists in changing environments. I. Fitness landscapes of thermal sensitivity.** *Am Nat* 1995, **146**:252-270.
 56. Kingsolver JG, Ragland GJ, Diamond SE: **Evolution in a constant environment: thermal fluctuations and thermal sensitivity of laboratory and field populations of *Manduca sexta*.** *Evolution* 2009, **63**:537-541.
 57. Teets NM, Denlinger DL: **Physiological mechanisms of seasonal and rapid cold-hardening in insects.** *Physiol Entomol* 2013, **38**:105-116.
 58. Esch C, Jimenez JP, Peretz C, Uno H, O'Donnell S: **Thermal tolerances differ between diurnal and nocturnal foragers in the ant *Ectatomma ruidum*.** *Insect Soc* 2017, **64**:439-444.
 59. Dillon ME, Cahn LRY, Huey RB: **Life history consequences of temperature transients in *Drosophila melanogaster*.** *J Exp Biol* 2007, **210**:2897-2904.
 60. Slotsbo S, Schou MF, Kristensen TN, Loeschcke V, Sørensen JG: **Reversibility of developmental heat and cold plasticity is asymmetric and has long-lasting consequences for adult thermal tolerance.** *J Exp Biol* 2016, **219**:2726-2732.
 61. Cavieres G, Alruiz JM, Medina NR, Bogdanovich JM, Bozinovic F: **Transgenerational and within-generation plasticity shape thermal performance curves.** *Ecol Evol* 2019, **9**:2072-2082.
 62. Hamblin AL, Youngsteadt E, López-Urbe MM, Frank SD: **Physiological thermal limits predict differential responses of bees to urban heat-island effects.** *Biol Lett* 2017, **13**:20170125.
 63. Baudier KM, D'Amelio CL, Malhotra R, O'Connor MP, O'Donnell S: **Extreme insolation: climatic variation shapes the evolution of**

- thermal tolerance at multiple scales.** *Am Nat* 2018, **192**:347-359.
64. Kaspari M, Clay NA, Lucas J, Yanoviak SP, Kay A: **Thermal adaptation generates a diversity of thermal limits in a rainforest ant community.** *Global Change Biol* 2015, **21**:1092-1102.
 65. Colinet H, Rinehart JP, Yocum GD, Greenlee KJ: **Mechanisms underpinning the beneficial effects of fluctuating thermal regimes in insect cold tolerance.** *J Exp Biol* 2018, **221**: jeb164806.
 66. Morgan Kelly: **Adaptation to climate change through genetic accommodation and assimilation of plastic phenotypes.** *Philos Trans R Soc B Biol Sci* 2019, **374** 20180176.
 67. Schou MF, Mouridsen MB, Sørensen JG, Loeschcke V: **Linear reaction norms of thermal limits in *Drosophila*: predictable plasticity in cold but not in heat tolerance.** *Funct Ecol* 2017, **31**:934-945.
 68. Sgrò CM, Terblanche JS, Hoffmann AA: **What can plasticity contribute to insect responses to climate change?** *Annu Rev Entomol* 2016, **61**:433-451.
 69. Diamond SE: **Evolutionary potential of upper thermal tolerance: biogeographic patterns and expectations under climate change.** *Ann N Y Acad Sci* 2017, **1389**:5-19.
 70. Diamond SE, Chick L, Perez A, Strickler SA, Martin RA: **Rapid evolution of ant thermal tolerance across an urban-rural temperature cline.** *Biol J Linn Soc* 2017, **121**:248-257.
 71. Sørensen JG, Kristensen TN, Overgaard J: **Evolutionary and ecological patterns of thermal acclimation capacity in *Drosophila*: is it important for keeping up with climate change?** *Curr Opin Insect Sci* 2016, **17**:98-104.
 72. Tobler R, Hermisson J, Schlötterer C: **Parallel trait adaptation across opposing thermal environments in experimental *Drosophila melanogaster* populations.** *Evolution* 2015, **69**:1745-1759.
 73. Diamond SE, Chick LD: **The Janus of macrophysiology: stronger effects of evolutionary history, but weaker effects of climate on upper thermal limits are reversed for lower thermal limits in ants.** *Curr Zool* 2018, **64**:223-230.
 74. Esperk T, Kjærsgaard A, Walters RJ, Berger D, Blanckenhorn WU: **Plastic and evolutionary responses to heat stress in a temperate dung fly: negative correlation between basal and induced heat tolerance?** *J Evol Biol* 2016, **29**:900-915.
 75. Buckley LB, Huey RB: **How extreme temperatures impact organisms and the evolution of their thermal tolerance.** *Integr Comp Biol* 2016, **56**:98-109.
 76. Grant PR, Grant BR, Huey RB, Johnson MTJ, Knoll AH, Schmitt J: **Evolution caused by extreme events.** *Philos Trans R Soc B Biol Sci* 2017.
 77. Grant PR, Grant BR, Huey RB, Johnson MTJ, Knoll AH, Schmitt J: **Evolution caused by extreme events.** *Philos Trans R Soc B Biol Sci* 2017, **372**:20160146.
 78. Manenti T, Sørensen JG, Moghadam NN, Loeschcke V: **Predictability rather than amplitude of temperature fluctuations determines stress resistance in a natural population of *Drosophila simulans*.** *J Evol Biol* 2014, **27**:2113-2122.
 79. Klockmann M, Günter F, Fischer K: **Heat resistance throughout ontogeny: body size constrains thermal tolerance.** *Global Change Biol* 2017, **23**:686-696.
 80. Oyen KJ, Giri S, Dillon ME: **Altitudinal variation in bumble bee (*Bombus*) critical thermal limits.** *J Therm Biol* 2016, **59**:52-57.
 81. Bowler K, Terblanche JS: **Insect thermal tolerance: what is the role of ontogeny, ageing and senescence?** *Biol Rev Cambridge Philos Soc* 2008, **83**:339-355.
 82. Glass JR, Stahlschmidt ZR: **Should I stay or should I go? Complex environments influence the developmental plasticity of flight capacity and flight-related trade-offs.** *Biol J Linn Soc* 2019, **128**(1):59-69.
 83. Glass JR, Stahlschmidt ZR: **Should I stay or should I go? Complex environments influence the developmental plasticity of flight capacity and flight-related trade-offs.** *Biol J Linn Soc* 2019, **128**:59-69.
 84. Rane RV, Pearce SL, Li F, Coppin C, Schiffer M, Shirriffs J, Sgrò CM, Griffin PC, Zhang G, Lee SF *et al.*: **Genomic changes associated with adaptation to arid environments in cactophilic *Drosophila* species.** *BMC Genomics* 2019, **20**:52.
 85. Fournier-Level A, Good RT, Wilcox SA, Rane RV, Schiffer M, Chen W, Battlay P, Perry T, Batterham P, Hoffmann AA *et al.*: **The spread of resistance to imidacloprid is restricted by thermotolerance in natural populations of *Drosophila melanogaster*.** *Nat Ecol Evol* 2019, **3**:647.
 86. Sinclair BJ, Ferguson LV, Salehipour-shirazi G, MacMillan HA: **Cross-tolerance and cross-talk in the cold: relating low temperatures to desiccation and immune stress in insects.** *Integr Comp Biol* 2013, **53**:545-556.
 87. Moretti M, Dias ATC, de Bello F, Altermatt F, Chown SL, Azcárate FM, Bell JR, Fournier B, Hedde M, Hortal J *et al.*: **Handbook of protocols for standardized measurement of terrestrial invertebrate functional traits.** *Funct Ecol* 2017, **31**:558-567.
 88. Sinclair BJ, Coello Alvarado LE, Ferguson LV: **An invitation to measure insect cold tolerance: methods, approaches, and workflow.** *J Therm Biol* 2015, **53**:180-197.
 89. Hoffmann AA, Sørensen JG, Loeschcke V: **Adaptation of *Drosophila* to temperature extremes: bringing together quantitative and molecular approaches.** *J Therm Biol* 2003, **28**:175-216.
- A detailed review of quantitative genetics and selection experiments on thermal adaptation in *Drosophila*.
90. Ahrens CW, Rymer PD, Stow A, Bragg J, Dillon S, Umbers KDL, Dudaniec RY: **The search for loci under selection: trends, biases and progress.** *Mol Ecol* 2018, **27**:1342-1356.
- A recent review of patterns and trends in population genomic analyses, including summaries of data types, analyses, and reporting of local adaptation signatures. The authors have several important recommendations for conducting and reporting the results of population genomic scans for environmental adaptation in a standardized way.
91. Forester BR, Jones MR, Joost S, Landguth EL, Lasky JR: **Detecting spatial genetic signatures of local adaptation in heterogeneous landscapes.** *Mol Ecol* 2016, **25**:104-120.
 92. Lotterhos KE, Whitlock MC: **The relative power of genome scans to detect local adaptation depends on sampling design and statistical method.** *Mol Ecol* 2015, **24**:1031-1046.
 93. Rellstab C, Gugerli F, Eckert AJ, Hancock AM, Holderegger R: **A practical guide to environmental association analysis in landscape genomics.** *Mol Ecol* 2015, **24**:4348-4370.
 94. Schoville SD, Bonin A, François O, Lobreaux S, Melodelima C, Manel S: **Adaptive genetic variation on the landscape: methods and cases.** *Annu Rev Ecol Evol Syst* 2012, **43**:23-43.
 95. Jones MR, Good JM: **Targeted capture in evolutionary and ecological genomics.** *Mol Ecol* 2016, **25**:185-202.
 96. Pimsler ML, Jackson JM, Lozier JD: **Population genomics reveals a candidate gene involved in bumble bee pigmentation.** *Ecol Evol* 2017, **7**:3406-3413.
 97. González VL, Devine AM, Trizna M, Mulcahy DG, Barker KB, Coddington JA: **Open access genomic resources for terrestrial arthropods.** *Curr Opin Insect Sci* 2018, **25**:91-98.
 98. Messer PW, Ellner SP, Hairston NG: **Can population genetics adapt to rapid evolution?** *Trends Genet* 2016, **32**:408-418.
- A review discussing some of the many challenges of using traditional population genetic models for detecting the basis of rapid phenotypic evolution in populations under variable selection pressures, highlighting the need to consider ecology and to take advantage of inexpensive sequencing for large scale analysis of genetic variation across space and time.

99. Pélissié B, Crossley MS, Cohen ZP, Schoville SD: **Rapid evolution in insect pests: the importance of space and time in population genomics studies.** *Curr Opin Insect Sci* 2018, **26**:8-16.
 100. Reinhardt JA, Kolaczowski B, Jones CD, Begun DJ, Kern AD:
 - **Parallel geographic variation in *Drosophila melanogaster*.** *Genetics* 2014, **197**:361-373.
 This paper uses spatial replication of an abiotic gradient by sampling *D. melanogaster* along latitudinal clines on two continents. Numerous parallels are identified that suggests repeatable selection by similar environmental pressures on standing genetic variation.
 101. Dudaniec RY, Yong CJ, Lancaster LT, Svensson EI, Hansson B: **Signatures of local adaptation along environmental gradients in a range-expanding damselfly (*Ischnura elegans*).** *Mol Ecol* 2018, **27**:2576-2593.
 102. O'Brien C, Bradshaw WE, Holzapfel CM: **Testing for causality in covarying traits: genes and latitude in a molecular world.** *Mol Ecol* 2011, **20**:2471-2476.
 103. Günther T, Coop G: **Robust identification of local adaptation from allele frequencies.** *Genetics* 2013, **195**:205-220.
 104. Frichot E, Schoville SD, Bouchard G, François O: **Testing for associations between Loci and environmental gradients using latent factor mixed models.** *Mol Biol Evol* 2013, **30**:1687-1699.
 105. Gamboa M, Watanabe K: **Genome-wide signatures of local adaptation among seven stoneflies species along a nationwide latitudinal gradient in Japan.** *BMC Genomics* 2019, **20**:84.
 106. Yeaman S, Hodgins KA, Lotterhos KE, Suren H, Nadeau S, Degner JC, Nurkowski KA, Smets P, Wang T, Gray LK *et al.*: **Convergent local adaptation to climate in distantly related conifers.** *Science* 2016, **353**:1431-1433.
 107. Adrion JR, Hahn MW, Cooper BS: **Revisiting classic clines in *Drosophila melanogaster* in the age of genomics.** *Trends Genet* 2015, **31**:434-444.
 108. Keller I, Alexander JM, Holderegger R, Edwards PJ: **Widespread phenotypic and genetic divergence along altitudinal gradients in animals.** *J Evol Biol* 2013, **26**:2527-2543.
 109. Fick SE, Hijmans RJ: **Worldclim 2: new 1-km spatial resolution climate surfaces for global land areas.** *Int J Climatol* 2017.
 110. Bergland AO, Behrman EL, O'Brien KR, Schmidt PS, Petrov DA:
 - **Genomic evidence of rapid and stable adaptive oscillations over seasonal time scales in *Drosophila*.** *PLoS Genetics* 2014, **10**:e1004775.
 The authors use whole genome sequencing of pooled samples (pool-seq) to detect oscillating allele frequencies at "seasonal SNPs" distributed throughout the *D. melanogaster* genome that are seemingly driven by temporal climatic fluctuations. The authors sample a latitude gradient to show that seasonally varying SNPs within a population likewise vary across latitude, illustrating the complex interactive effects of spatial and temporal heterogeneity on genomic variation.
 111. Mallard F, Nolte V, Tobler R, Kapun M, Schlötterer C:
 - **A simple genetic basis of adaptation to a novel thermal environment results in complex metabolic rewiring in *Drosophila*.** *Genome Biol* 2018, **19**:119.
 Measured responses of fruit flies to 60 generations of laboratory natural selection in cold or hot conditions. Linked differences in whole organism respiration to changes in oxidative phosphorylation, TCA cycle and regulation of key glycolytic enzymes.
 112. Prüsscher P, Nylin S, Gotthard K, Wheat CW: **Genetic variation underlying local adaptation of diapause induction along a cline in a butterfly.** *Mol Ecol* 2018, **27**:3613-3626.
 113. Kaiser TS, Poehn B, Szkiba D, Preussner M, Sedlazeck FJ, Zrim A, Neumann T, Nguyen L-T, Betancourt AJ, Hummel T *et al.*: **The genomic basis of circadian and circalunar timing adaptations in a midge.** *Nature* 2016, **540**:69-73.
 114. Zhan S, Zhang W, Niitepöld K, Hsu J, Haeger JF, Zalucki MP, Altizer S, de Roode JC, Reppert SM, Kronforst MR: **The genetics of monarch butterfly migration and warning colouration.** *Nature* 2014, **514**:317-321.
 115. Friedline CJ, Faske TM, Lind BM, Hobson EM, Parry D, Dyer RJ, Johnson DM, Thompson LM, Grayson KL, Eckert AJ: **Evolutionary genomics of gypsy moth populations sampled along a latitudinal gradient.** *Mol Ecol* 2019, **28**:2206-2223.
 116. Frichot E, Schoville SD, de Villemereuil P, Gaggiotti OE, François O: **Detecting adaptive evolution based on association with ecological gradients: orientation matters!** *Heredity* 2015, **115**:22-28.
 117. Gompert Z: **Bayesian inference of selection in a heterogeneous environment from genetic time-series data.** *Mol Ecol* 2016, **25**:121-134.
- Develops a statistical approach to identify environmental adaptation in genomic data using time series samples from wild populations. The method has many assumptions, but is a first step in modeling rapid evolution in spatially and temporally heterogeneous environments in nature.
118. Angers B, Castonguay E, Massicotte R: **Environmentally induced phenotypes and DNA methylation: how to deal with unpredictable conditions until the next generation and after.** *Mol Ecol* 2010, **19**:1283-1295.
 119. Gayral P, Weinert L, Chiari Y, Tsagkogeorga G, Ballenghien M, Galtier N: **Next-generation sequencing of transcriptomes: a guide to RNA isolation in nonmodel animals.** *Mol Ecol Resour* 2011, **11**:650-661.
 120. Teets NM, Peyton JT, Ragland GJ, Colinet H, Renault D, Hahn DA, Denlinger DL: **Combined transcriptomic and metabolomic approach uncovers molecular mechanisms of cold tolerance in a temperate flesh fly.** *Physiol Genomics* 2012, **44**:764-777.
 121. Telonis-Scott M, Clemson AS, Johnson TK, Sgrò CM: **Spatial analysis of gene regulation reveals new insights into the molecular basis of upper thermal limits.** *Mol Ecol* 2014, **23**:6135-6151.
 122. Kelly MW, Pankey MS, DeBiasse MB, Plachetzki DC: **Adaptation to heat stress reduces phenotypic and transcriptional plasticity in a marine copepod.** *Funct Ecol* 2017, **31**:398-406.
 123. Toxopeus J, Des Marteaux LE, Sinclair BJ: **How crickets become freeze tolerant: the transcriptomic underpinnings of acclimation in *Gryllus veletis*.** *Comp Biochem Physiol Part D Genomic Proteomics* 2019, **29**:55-66.
 124. Brankatschk M, Gutmann T, Knittelfelder O, Palladini A, Prince E, Grzybek M, Brankatschk B, Shevchenko A, Coskun Ü, Eaton S: **A temperature-dependent switch in feeding preference improves *Drosophila* development and survival in the cold.** *Dev Cell* 2018, **46**:781-793.e4.
 125. Teets NM, Yi S-X, Lee RE, Denlinger DL: **Calcium signaling mediates cold sensing in insect tissues.** *PNAS* 2013, **110**:9154-9159.
 126. Cridland JM, Ramirez SR, Dean CA, Sciligo A, Tsutsui ND: **Genome sequencing of museum specimens reveals rapid changes in the genetic composition of honey bees in California.** *Genome Biol Evol* 2018, **10**:458-472.
 127. Gilchrist GW, Huey RB: **Plastic and genetic variation in wing loading as a function of temperature within and among parallel clines in *Drosophila subobscura*.** *Integr Comp Biol* 2004, **44**:461-470.
 128. Manel S, Andreello M, Henry K, Verdelet D, Darracq A, Guerin P-E, Desprez B, Devaux P: **Predicting genotype environmental range from genome-environment associations.** *Mol Ecol* 2018, **27**:2823-2833.
 129. Bay RA, Harrigan RJ, Underwood VL, Gibbs HL, Smith TB, Ruegg K: **Genomic signals of selection predict climate-driven population declines in a migratory bird.** *Science* 2018, **359**:83-86.
 130. Razgour O, Forester B, Taggart JB, Bekaert M, Juste J, Ibáñez C,
 - Puechmaile SJ, Novella-Fernandez R, Alberdi A, Manel S: **Considering adaptive genetic variation in climate change vulnerability assessment reduces species range loss projections.** *PNAS* 2019, **116**:10418-10423.
 Integrates climate-based distribution modeling with neutral and adaptive genetic variation to develop an approach for forecasting species range shifts based on evolutionary potential.