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## A genomic perspective on the generation and maintenance of genetic diversity in herbivorous insects

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### Abstract

Understanding the processes that generate and maintain genetic variation within populations is a central goal in evolutionary biology. Theory predicts that some of this variation is maintained as a consequence of adapting to variable habitats. Studies in herbivorous insects have played a key role in confirming this prediction. Here, we highlight theoretical and conceptual models for the maintenance of genetic diversity in herbivorous insects, empirical genomic studies testing these models, and pressing questions within the realm of evolutionary and functional genomic studies. To address key gaps, we propose an integrative approach combining population genomic scans for adaptation, genome-wide characterization of targets of selection through experimental manipulations, mapping the genetic architecture of traits influencing fitness, and functional studies. We also stress the importance of studying the maintenance of genetic variation across biological scales—from variation within populations to divergence among populations—to form a comprehensive view of adaptation in herbivorous insects.

### Keywords

Evolutionary Genomics; Genetic Variation; Experimental Evolution; Local Adaptation; Plant-Herbivore Interactions; Population Genomics

## 1. INTRODUCTION: INSECT DIVERSITY

Insects constitute the most species-rich guild of multicellular organisms on the planet. Though the factors driving their extraordinary evolutionary success remain the subject of debate, the evolution of herbivory has likely played a particularly important role (Mitter et al. 1988; Labandeira and Sepkowski 1993; Mayhew 2007; Wiens et al. 2015). Herbivorous insect clades exhibit faster rates of net diversification and higher species richness compared

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to clades of non-herbivorous insects, and roughly half of all extant insect species are herbivorous (Mitter et al. 1988; Wiens et al. 2015).

Why do herbivorous insect clades diversify so rapidly? Fraenkel (1959), and later Ehrlich and Raven (1964), suggested that species radiations of plants and insect herbivores resulted from reciprocal, antagonistic interactions between the two. Herbivorous insects are, by and large, parasites of plants—reliant on living plants as both habitat and food (Price 1980). Accordingly, selective pressures on insects are strongly shaped by their host plants. Fraenkel’s initial theory posited that bottom-up selective agents—specifically, plant defensive chemicals, which vary among plant species—largely mediate the outcome of plant-herbivore interactions (see sidebar: “bottom-up agents of selection”). Under this assumption, insect evolution is driven by pressure to overcome specific plant defensive chemicals, and lineages that do so diversify as they spread across new niches. Bernays and Graham (1988) later hypothesized that variation in top-down forces associated with different plant species might be stronger selective agents on herbivorous insects (see sidebar: “top-down agents of selection”). Although the development of more precise, mechanistic explanations for herbivorous insect diversification continues, most theories share a common theme: insect diversification is closely tied to evolutionary shifts between host plant species (Janz et al. 2011).

More recently, a growing body of evidence suggests that plant-herbivore interactions generate diversity at another level: genetic variation within insect species and populations. Theories to explain this process, just as those focused on insect species diversity, are heavily motivated by the idea that host plants exert strong and variable selective forces on herbivorous insects.

In this review, we describe how foundational models for adaptation in heterogeneous environments (e.g., Levene 1953), when applied to plant-herbivore interactions, predict that interactions with host plants can maintain genetic variation within herbivorous insects. We also highlight more recent conceptual, empirical, and theoretical advances in evolutionary genomics that clarify and support these predictions. These insights arise from studies both of genetic variation within insect populations, and genetic divergence between insect populations, driven by adaptation to host plants. Importantly, we emphasize that processes influencing genetic variation at both of these scales should be embraced together, so that a more complete picture can be gained of adaptation in herbivorous insects. Lastly, we discuss experimental approaches that can be integrated to help understand how much genetic variation in herbivorous insects is maintained through interactions with host plants.

**SIDEBAR: Bottom-up Agents of Selection.** The major bottom-up agents of selection on insect herbivores are host plant-associated traits, which can vary dramatically within and between species. Plants protect themselves against herbivores via structural characters (e.g., trichomes) and defensive chemicals. Production of these defenses happens constitutively and in response to attack, regulated through an intricate signaling network (Thaler et al. 2012; Schuman and Baldwin 2015). Plant defensive traits and herbivore counter-adaptations are typically polymorphic and hypothesized to be the result of co-evolutionary

dynamics (Flor 1956; Ehrlich and Raven 1964; Stahl et al. 1999; Karasov et al. 2014). Arms race dynamics at the genomic level have been observed in several plant-herbivore systems (You et al. 2013; Edger et al. 2015; Zhao et al. 2015).

Plants also differ nutritionally from one another and from other food sources such as microbes. In response to these differences, many herbivores show signs of adaptive evolution in digestive enzymes and transporters (International Aphid Genomics Consortium 2010), and have evolved more or less intimate relationships with microbial (endo-) symbionts (Pauchet and Heckel 2013), which could also assist with the subversion of plant defenses (Hansen and Moran 2014).

**SIDEBAR: Top-down agents of selection.** Predators, parasitoids and pathogens pose unique selective pressures by themselves and act as selective agents in combination with bottom-up, plant-derived characters. Attack by pathogens and parasitoids has clear long-term effects on the evolution of immune system genes (Sackton et al. 2007; International Aphid Genomics Consortium 2010). Combinations of top-down and bottom-up selection pressures have led to many conspicuous adaptations in herbivores that are specialized on a restricted number of host plants. Host plant-specialized herbivores have either gained the ability to sequester plant-produced chemicals to defend themselves against attack by the third trophic level—often advertised through aposematic coloration—or they employ detoxification mechanisms and evasive strategies such as camouflage or mimicry (Farkas et al. 2013; Petschenka and Agrawal 2016). Although the genomic consequences of these combinations of selection pressures are just starting to be explored (Joron et al. 2011; Heliconius Genome Consortium 2012; Kunte et al. 2014; Soria-Carrasco et al. 2014; Zhan et al. 2014), it seems probable that top-down forces, including generalist predators such as birds, are especially important (Bernays and Graham 1988; Singer et al. 2014).

## 2. THE LINK BETWEEN HABITAT VARIATION AND GENETIC VARIATION

Balancing selection, broadly defined, encompasses the suite of evolutionary processes that maintain genetic variation within species. Such processes include heterozygote advantage, negative frequency dependent selection, and spatially and temporally varying selection (Charlesworth 2006; Delph and Kelly 2014). Balancing selection contrasts against another, non-adaptive mechanism that maintains genetic variation within populations: the balance between the continuous input of new, predominately deleterious genetic variation by mutation, and the purging of that variation by genetic drift (Lande 1975).

Although many forms of balancing selection may be widespread in herbivorous insects, we focus specifically on **spatially varying selection** (SVS). SVS has clear relevance for plant-herbivore interactions: different host plant species, individuals, or tissues can be thought of as spatially distinct habitats.

Population genetic models predict that SVS can maintain genetic variation when the fitness effects of alleles at a locus trade off across habitats, such that no single allele is always superior in every habitat (Hedrick 2006). In this case, SVS causes divergence of allele

frequencies among habitats, which is counteracted by inter-habitat migration (Hedrick 2006). Even with random mixing of genotypes across habitats each generation (e.g., panmictic mating), variation can be maintained when the costs or benefits of each genotype across habitats are not too imbalanced (Levene 1953). By reducing gene flow among habitats, both habitat choice and limited migration increase the potential for SVS to maintain genetic variation (Christiansen 1974). However, an important restriction is that most individuals do not passively experience the full range of environments during a single life stage, for example due to rapid environmental fluctuations or frequent movement of individuals between habitats. If this assumption is violated, selection will favor the genotype that maximizes fitness across environments (Futuyma and Moreno 1988).

Initially, herbivorous insects helped inspire models for the maintenance of genetic polymorphism within panmictic populations where individuals are dispersed across multiple host plant species (Dobzhansky 1951; Levene 1953; Prout and Savolainen 1996). Later, they helped illuminate how populations can diverge into sympatric races occupying different host plant species, evolving increased reproductive isolation in the face of gene flow (Futuyma and Moreno 1988; Drès and Mallet 2002). They also provided foundational evidence for local adaptation in species interactions (Edmunds and Alstad 1978). The genomic consequences of these three processes in herbivorous insects are discussed in detail in the remainder of this review, with an emphasis on evolution driven by host plant variation.

### 3. DIVERGENCE OF HOST-ASSOCIATED POPULATIONS AND RACES

Host plants are generally viewed as the most relevant component of an herbivorous insect's habitat, so variation between different host species is posited as the major source of habitat variation for insect herbivores (Feeny 1976; Hunter et al. 2012). In a seminal review, Drès and Mallet (2002) delimited three key phases of divergence among subsets of a parasite population that occupy different host species. First, a population of parasites that uses multiple host species, but which disperses or mates freely across hosts such that any genetic differentiation among individuals on different host species is due to a single generation of selection, is termed a polymorphic population. Second, genetically differentiated, sympatric populations of parasites that use different hosts, but between which there is appreciable gene flow, are defined as host races. Third, increasing divergence between populations can ultimately lead to the formation of full species associated with different hosts and which share little to no gene flow. Although the evolutionary transition from polymorphic populations to separate species is a continuous process (Drès and Mallet 2002; Feder et al. 2014), treating different points on this continuum as stages is useful for conceptualizing how diversification proceeds.

Herbivorous insects offer key advantages for disentangling processes contributing to divergence among host-associated populations, in particular for elucidating the relative importance of host species as a selective agent driving divergence. Habitats are clearly delimited, especially at the level of host species; probable selective pressures are readily identifiable; and large-scale experiments can finely map the targets of selection across the genome when combined with parallel studies in the field (Gloss et al. 2013).

### 3.1 Natural History of Model Systems

New empirical, genomic insights highlighting the importance of adaptive evolution during divergence-with-gene-flow have emerged primarily from the study of three herbivorous insect clades: stick insects (Phasmidae: *Timema cristinae* and sibling species), tephritid fruit flies (Tephritidae: *Rhagoletis pomonella* and sibling species), and pea aphids (Aphididae: *Acyrtosiphon pisum*). Although these are not the only lineages to give insight into host race formation, we restrict our discussion to these three because patterns of divergence have been characterized in-depth at a genomic scale.

Pea aphids form a complex of at least 8 host races and 3 possible species associated with 20 plant species across Western Europe and North America, which diverged from a common ancestor 8–16 thousand years ago and experience variable rates of gene flow among races (Peccoud et al. 2009a; Peccoud et al. 2009b; Via et al. 2012). Tephritid fruit flies (Fig. 1A) comprise a complex spanning from host races exhibiting partial reproductive isolation to sibling species. Multiple host races are associated with different native hawthorn species and with introduced apple in North America (Powell et al. 2014). Stick insects (Fig. 1B) form host races on two sympatric rosid host species. Pairs of host-associated populations form repeatedly across the landscape, and populations cluster phylogenetically by geography rather than by host plant; this scenario is indicative of repeated parallel divergence (Nosil et al. 2012; Soria-Carrasco et al. 2014).

The strongest selective agents underlying host-associated divergence likely differ for each lineage. These selective agents include diapause timing in *Rhagoletis* (Fig. 1C; Egan et al. 2015), predation in *Timema* (Fig. 1D; Nosil et al. 2012), and host plant nutritional and defensive properties in *Acyrtosiphon* (Hawthorn and Via 2001).

### 3.2 Natural Selection and the Genomic Landscape of Divergence

Two major goals in genomic studies of host race formation are to characterize patterns of **genomic divergence** between host-associated populations or races, and to explain how these patterns arise (Via 2012; Feder et al. 2013).

Genome scans in multiple insect species (Michel et al. 2010; Via et al. 2012; Soria-Carrasco et al. 2014; Gompert et al. 2014; Egan et al. 2015) have revealed that the genomic landscape of divergence among host-associated populations is heterogeneous, with interspersed peaks and valleys (e.g., Fig. 1E,F). Why doesn't divergence proceed uniformly across the genome? Proponents of sympatric host race formation have long argued that reproductive isolation among host races is driven by adaptation to different host species (Bush 1969; Berlocher and Feder 2002). From a genomic perspective, adaptation to different hosts (or to different habitats more generally) is expected to reduce gene flow and increase divergence at loci underlying habitat-specific fitness tradeoffs (Bengtsson 1985). This effect should extend to nearby neutral loci but weaken as the distance between a neutral locus and selected locus increases, since greater distance from a divergently selected site allows more opportunities for a neutral allele to recombine into haplotypes containing alternatively selected alleles (Charlesworth et al. 1997; Via 2012). Similarly, **linkage disequilibrium** (LD) between nearby selected sites can enhance divergent selection at each locus (Via 2012). Accordingly,

heterogeneous divergence might reflect an uneven distribution of divergently selected sites across the genome, variation in the magnitude of habitat-specific fitness costs and benefits of alleles at each site, and LD between neutral loci and nearby selected sites (Via 2012).

However, a heterogeneous genomic landscape of divergence is not necessarily evidence that adaptation is driving genomic divergence among host races. Variation across the genome in other properties or processes – such as the rate of mutation and recombination, levels of standing genetic variation, and genetic drift – could produce a similar pattern, in some cases even without the action of divergent selection (Seehausen et al. 2014). Toward resolving this issue, genomic studies of herbivorous insects have produced some of the most convincing evidence that divergent selection contributes to the heterogeneous landscape of genomic divergence among sympatric races. Dense genome re-sequencing in *Timema* and *Rhagoletis*, and marker-based studies in *Acyrtosiphon*, showed that divergence was strikingly widespread across the genome, rather than clustered at just a few outlier regions with large effects on fitness (Michel et al. 2010; Soria-Carrasco et al. 2014; Egan et al. 2015). In all three genera, loci underlying host-specific fitness tradeoffs are enriched in the most differentiated genomic regions between populations or races on different host species (shown for *Rhagoletis* in Fig. 1E,G). Further, genetic divergence in natural populations of *Rhagoletis* – and to a lesser extent, *Timema* – was correlated with allele frequency change over a single generation of experimental selection (Soria-Carrasco et al. 2014; Egan et al. 2015). Overall, this pattern supports the hypothesis that polygenic adaptation is an important process driving heterogeneous genomic divergence.

Simulations of divergent selection acting on a pair of populations occupying two habitats further support a role for natural selection driving heterogeneous genomic divergence during host race formation (Flaxman et al. 2014; Feder et al. 2014). In the simulations, adaptive trait variation was assumed to arise from a low, continuous input of new mutations with small effects on fitness. Importantly, these simulations modeled chromosomes within individuals within populations, rather than individual loci: the method thus incorporated the various divergence-promoting effects of physical and statistical LD among co-selected alleles (Barton 1983; Via 2012; Yeaman 2013). Divergence among selected loci accumulated slowly and in few genomic regions at first, but then increased precipitously across the genome, consistent with genome-wide patterns of divergence observed between sympatric host races.

In the future, genomic simulations of selection and migration across divergent habitats (e.g., host species) could be parameterized with empirical maps of genome-wide variation in mutation rate, recombination, and habitat-specific selection coefficients (Gompert et al. 2014; Egan et al. 2015), and inferred models of demography and gene flow (Sousa and Hey 2013). Robust, carefully-parameterized simulations may help further establish predicted genome-wide patterns of divergence under probable demographic scenarios, clarify the relative importance of divergent selection and other processes in generating observed patterns of divergence between host races in nature, and more accurately pinpoint genomic regions showing exceptional divergence due to adaptation (Hsieh et al. 2016).

### 3.3 Reconciling Different Patterns Observed Across Study Systems

Although empirical studies and modeling point to natural selection as a major driver of genomic differentiation during divergence with gene flow, fundamental differences in patterns of divergence in *Acyrtosiphon*, *Rhagoletis*, and *Timema* remain to be explained. Many patterns discussed in this section may reflect how different selective agents shape genomic divergence differently within each of these three lineages.

The concordance between the genome-wide response to experimental selection and genome-wide patterns of divergence in nature was much stronger in *Rhagoletis* than *Timema*. Egan et al. (2015) hypothesize that higher LD in *Rhagoletis* – particularly due to extensive polymorphism for chromosomal inversions – may enable a strong response to divergent selection that extends across broad windows of linked adaptive and neutral sites. Though perhaps not a prerequisite, chromosomal inversions can facilitate ecological divergence by locally suppressing recombination and preserving LD among alleles conferring an advantage in the same habitat (Kirkpatrick and Barton 2006), particularly if loci captured in the inversions have already adaptively diverged in allopatry (Feder et al. 2011). In fact, inversions harboring genetic variation that influences diapause timing arose during local adaptation in allopatry in a southern *Rhagoletis* population and were infused into northern populations through secondary contact (Feder et al. 2003). Alternatively, life history (i.e. diapause timing), which is divergently selected between *Rhagoletis* host races, is likely a highly complex trait; as a result, many genomic regions may harbor loci affected by divergent selection, leading to strong genome-wide concordance between natural and experimental divergence (Egan et al. 2015).

Biological functions also differed among divergently selected loci in *Timema*, *Rhagoletis*, and *Acyrtosiphon*. Specifically, divergently selected loci were enriched for olfactory receptors and salivary proteins in *Acyrtosiphon* (Jaquiere et al. 2012), loci affecting diapause in *Rhagoletis* (Michel et al. 2010; Egan et al. 2015), and ion binding, possibly underlying divergently selected cryptic pigmentation phenotypes, in *Timema* (Gompert et al. 2014; Soria-Carrasco et al. 2014). This pattern suggests that phenotypic and genetic targets of divergent selection are highly dependent on the ecology of the interacting plant and herbivore species.

Divergent genomic regions may differ not just among species, but also among instances of parallel host-driven adaptation within a single species. Genome sequencing across four between-host pairs of natural *Timema* populations revealed that exceptionally divergent genomic regions were frequently unique, rather than shared, between pairs (Soria-Carrasco et al. 2014). This could result from different genetic routes to parallel phenotypic changes, different phenotypes being divergently selected among host pairs in each locality (although phenotypic convergence in parallel host adaptation is high in *Timema*; Nosil et al. 2012), or the genomic regions exhibiting highest host-associated divergence arising through stochastic processes (e.g., genetic drift) more often than adaptation.



### 3.4 The Role of Plant Defensive Chemicals: Recently Overlooked or Historically Overhyped?

Although plant chemicals are thought to be a major barrier to colonization of new food plants by insects, concrete evidence is so far lacking to support a major role for plant defensive chemistry driving genomic divergence within each of these three model systems. However, functional studies of divergent loci are needed to examine the role of plant defensive chemicals. Specifically, *Acyrtosiphon* salivary proteins and *Timema* ion binding proteins could both function in suppressing plant defense (Elzinga et al. 2014), possibly to avoid the production of defensive chemicals. *Acyrtosiphon* olfactory receptors may detect plant defensive chemicals to mediate host finding, given that host preference varies among *Acyrtosiphon* races (Hawthorne and Via 2001). Host preference also differs among *Rhagoletis* races, although the genetic basis of this variation has not been uncovered yet (Forbes and Feder 2006).

Alternatively, top-down selective agents such as predators and parasitoids, which regularly drive the evolution of plant defensive chemical sequestration and crypsis in herbivores, could play comparatively larger roles in driving divergence among insect populations (Bernays and Graham 1988). Predatory birds are proposed to maintain divergence in regions of the genome controlling pigmentation among populations of *Heliconius*, *Papilio* and *Danaus* butterflies (Heliconius Genome Consortium 2012; Kunte et al. 2014, Zhan et al. 2014), in addition to acting as a selective agent maintaining pigmentation divergence among host-associated *Timema* populations. Field studies have revealed different parasitoid communities associated with different host races of a single herbivorous insect species (Ohshima et al. 2015).

Overall, studies of insect herbivores that consume chemically well-defended tissues are also needed before drawing broad conclusions. Ideal study systems to test for host chemicals driving insect host-race divergence might include leaf-chewing herbivore species, which often face maximal exposure to host chemicals (Ali and Agrawal 2012).

## 4. MAINTENANCE OF GENETIC VARIATION WITHIN POPULATIONS

Herbivorous insects have yielded insight into the general problem of how populations can diverge in the face of gene flow – but the buildup of genomic divergence and reproductive isolation among individuals occupying sympatric host species (or other types of variable habitats) is far from ubiquitous (Drès and Mallet 2002). In this section, we explore expectations for how habitat variation might maintain variation *within* insect populations, as opposed to promoting divergence *between* populations as discussed previously.

### 4.1 The Conceptual Basis for Studies of SVS in Insects

The framework for adaptation to habitat variability emerged from population genetic theory. Well before DNA sequencing allowed direct observation of genetic variation, Dobzhansky (1951) posited that SVS was a major force responsible for the maintenance of genetic diversity within natural populations. He drew on observations of tremendous heterozygosity for chromosomal inversions in Amazonian *Drosophila* species. Dobzhansky and colleagues

speculated that this variation resulted from adaptation to the diversity of fruit in the Amazon (which can be viewed as spatially varying habitats) used as feeding substrates (Da Cunha et al. 1950). Single-locus population genetic models soon confirmed his intuition (Fig. 2): if habitats vary in space (or time), and no one allele is most fit in every habitat, selection can maintain multiple alleles at equilibrium frequencies in a panmictic population occupying those habitats (Levene 1953; Dempster 1955).

Tests for the maintenance of genetic variation *sensu* Levene's (1953) model in oligophagous and polyphagous herbivorous insects have been rare and inconclusive (Prout and Savolainen 1996). This is likely due in part to a focus, when applicable, on the maintenance of variation in structured populations with reduced gene flow. Models of the balance between migration and selection, in which inter-habitat divergence in allele frequency is not erased by random dispersal and mating each generation, are more appropriate for insects that exhibit biased mate or habitat choice or that experience ecological scenarios (e.g., coarse habitat patchiness) that reduce gene flow between habitats (Felsenstein 1976). As a result of the focus on migration-selection balance, however, the amount of genetic variation maintained by spatially and temporally varying selection in approximately panmictic insect populations remains largely unexplored.

#### 4.2 How Widespread Are Conditions Required for Maintenance of Genetic Variation by SVS?

We evaluate evidence for three major conditions required for SVS to be a common mechanism maintaining genetic variation within insect populations.

1. *Insects frequently inhabit spatially variable environments.* While the distinct habitats offered by different host species may be the most obvious source of plant-driven SVS in herbivorous insects, the effects of phenotypic variation *within* each host species can also be substantial (see sidebar: "More than just host species"). The process of adaptation driven by habitat variation warrants major consideration as a general property of plant-herbivore interactions, even in insects that narrowly specialize on a single host plant species.

**SIDEBAR:** More than just host species: the breadth of biotic habitat variation for herbivorous insects. Host plants shape the environment – and, accordingly, selective pressures – for herbivorous insects. Differences among host plant species have featured prominently in conceptual models and empirical studies of how habitat variation drives insect adaptation. However, variation may be substantial *within* individual host plant species as well. Such variation may arise through plant ontology (i.e., differences among tissues and organs), phenology, genotype, interactions with the biotic and abiotic environment (which alter plant growth and defense), and spatiotemporal variation in communities that function in extended plant defense against herbivory, such as parasitoids and predators (Schuman and Baldwin 2015).

2. *Insect populations in spatially variable environments do not always rapidly diverge into distinct populations specialized on each habitat.* If the formation of

divergent, partially reproductively isolated populations were an inevitable and rapid consequence of SVS, models of long-term maintenance of genetic variation by SVS in approximately panmictic populations would have limited ecological relevance. However, current theory and evidence from nature suggests population divergence is not ubiquitous. Genomic simulations show that if migration between habitats is sufficiently high, the formation of divergent habitat-associated populations may proceed extremely slowly (Feder et al. 2014), potentially precluding the formation of habitat-specialized races or species in many cases. Various ecological scenarios have also been proposed that favor the evolution of oligophagy or polyphagy over specialization on a single host species (Forister et al. 2012). This is consistent with the observation that 90% of herbivorous insect species are oligophagous; the remainder are monophagous or polyphagous (Bernays 1998). Phylogenetic analyses reveal that while specialist insects evolve from generalists, generalists also frequently evolve from specialists (Janz and Nylin 2008). And population genomic approaches have revealed a range of host-associated differentiation, sometimes including the absence of host-associated differentiation, in polyphagous insect species (Antwi et al. 2015). Overall, while host- or habitat-driven adaptation likely drives divergence among sympatric populations in some cases (Berlocher et al. 2002; Feder et al. 2013), a classic population genetic prediction remains relevant: when migration is frequent, the homogenizing effects of gene flow generally prevent divergence among populations (Yeaman and Otto 2011).

3. *Fitness trades off across habitats at individual loci.* Studies measuring performance of large numbers of insect genotypes on two or more host species often fail to observe host-associated tradeoffs (Scheirs et al. 2005; Forister et al. 2012). However, such studies often suffer from four limitations. First, tests for tradeoffs are typically conducted at a subset of an insect's life stages, which may not always be the stage at which tradeoffs occur (Scheirs et al. 2005; Forister et al. 2012). Second, performance-related traits (e.g., larval growth rate or weight) are often measured, but these may not be adequate proxies for fitness. Third, fitness or related traits are measured under more benign conditions than in nature (Agrawal et al. 2010). And fourth, genetic variation at loci controlling insect fitness in a host-*independent* manner may mask host-*dependent* fitness tradeoffs when tests for tradeoffs are conducted at the level of whole-organism genotypes, rather than for many loci across the genome individually (Houle 1991; Agrawal et al. 2010).

Alternatively, many studies convincingly demonstrating fitness tradeoffs across hosts have employed selection experiments and quantitative trait mapping (Hawthorne and Via 2001; Scheirs et al. 2005), which overcome one or more of these three limitations. Powerful genomic techniques – such as “evolve and resequence” studies (Schlötterer et al. 2015) and genome-wide association studies (GWAS) with individuals from genetically diverse natural populations – can provide genome-wide tests for tradeoffs by narrowly mapping loci underlying fitness variation and quantifying their effects across habitats. Loci exhibiting host-dependent tradeoffs in larval performance were rare in a recent study (Gompert et al.

2015), although this does not preclude tradeoffs at other life stages, and much larger sample sizes may be required to adequately test for tradeoffs if fitness is controlled by many loci each with small effect.

### 4.3 Empirical Support for Adaptive Within-Population Polymorphism Maintained by SVS

Recently, convincing evidence for the maintenance of within-population variation via SVS was demonstrated for a melanized/green color polymorphism in *T. cristinae*, which feeds on two host species (Comeault et al. 2015b; Fig. 1B). While crypsis in the green *Timema* morph involves a dorsal stripe phenotype that is favored on one host and disfavored on another (Fig. 1D), no such divergent selection acts on the melanized morph: on both host species, melanized individuals are cryptic on stems, disperse more effectively across soil, are resistant to a fungal pathogen, and have a mating advantage. Consequently, the proportion of individuals with the melanized phenotype, the genetic basis of which mapped to a single locus, showed a remarkable lack of inter-host differentiation among geographically paired populations on each host species. This suggests that divergent selection does not act on the melanistic phenotype, so melanized individuals could act as a “genetic bridge” to facilitate gene flow between host-associated populations and limit the extent of divergence between those populations. Agent-based modeling (i.e., modeling of populations of individual organisms) confirmed this prediction. The different modes of selection on cryptic pigmentation in *T. cristinae* are a striking example of the interplay between adaptive variation within populations and divergence among populations.

Interestingly, variation underlying the green/melanized polymorphism localizes to the same genomic region in another stick insect, *Timema podura*, but the melanized phenotype is divergently selected among host-associated populations rather than within them: the green morph is cryptic on leaves of both host species and green bark of one host, while the melanized morph is only cryptic on the brown bark of the other host (Comeault et al. 2015a). Thus, the same traits and underlying genetic loci may experience different modes of selection under different ecological scenarios. Combined, these contrasting modes of selection on pigmentation in different *Timema* species suggest an intriguing, broadly-applicable hypothesis: alternating epochs of divergent selection between and within populations may maintain the same genetic variants over long evolutionary timescales, which may help preserve genetic variation that facilitates the frequent host shifts or host breadth expansions seen in herbivorous insects (Janz and Nylin 2008).

## 5. LOCAL ADAPTATION

When geographic distance or barriers limit migration between habitats, the buildup of adaptive divergence among populations in different habitats can lead to **local adaptation** (Kawecki and Ebert 2004). From a population genetic perspective, loci can only remain adaptively differentiated among populations if they confer a fitness tradeoff across habitats; otherwise, gene flow will homogenize allele frequencies across populations (Kawecki and Ebert 2004; Savolainen et al. 2013).

The rich theoretical framework for local adaptation in host-parasite interactions is highly relevant to herbivorous insects (Kaltz and Shykoff 1998; Greischar and Koskella 2007). In

fact, to our knowledge, the first parasite shown to be locally adapted was an herbivore: intra- and inter-tree transfer experiments revealed that pineleaf scale insects were more fit on their natal host tree than other conspecific trees, presumably due to adaptation to intraspecific variation in defenses in their long-lived hosts (Edmunds and Alstad 1978). Local adaptation to individual trees, termed adaptive deme formation, may be relatively common (Mopper 1996). Though studies identifying genomic regions underlying local adaptation and the selective pressures driving their evolution are generally lacking in insect herbivores, population-level matches observed between wild parsnip chemical profiles and parsnip webworm detoxification enzyme repertoires suggest plant defensive chemicals, in particular, can drive herbivore local adaptation (Berenbaum and Zangerl 1998).

While local adaptation by parasites is often observed in host-parasite systems, it is far from a general rule (Kaltz and Shykoff 1998). In fact, theory suggests cases in which hosts, rather than parasites, may be expected to maintain an advantage in the co-evolutionary arms race (i.e., host resistance evolves faster than parasite exploitation traits; Gilman et al. 2012). Consistent with this expectation, a test of local adaptation considering both an herbivorous insect and its host plant found cases of both herbivore local adaptation and maladaptation (Garrido et al. 2012). Thus, a comprehensive view of geographic variation in interactions with host plants should encompass such a dynamic mosaic of outcomes (Thompson 2005).

Empirical genomic insights into local adaptation in species interactions are rare; they require knowledge of natural history, experimental evidence for fitness differences across environments, and availability of genomic resources and population genomic data. Direct links between local selective forces, adaptive phenotypic differentiation among populations, and adaptive genomic variation underlying phenotypic differentiation has not yet been fully elucidated in any single herbivorous insect species. However, genomic analyses highlighted below have made strides toward closing the genotype-phenotype-fitness loop (Barrett and Hoekstra 2011) in a few herbivorous insect species.

### 5.1 Example 1: Local Adaptation through the Evolution of Gene Expression

Cactophilic *Drosophila* species feed on microbiota on cactus rots. While they are not true herbivores, important aspects of their habitat – such as toxins, volatiles, and nutrients – are shaped by their host plants. A common garden experiment revealed that both viability and life history strategy differed among populations of *D. mojavensis*, which uses different cactus species in different geographic locations (Etges et al. 2010). Adaptation to local host plant species appeared to partially drive this pattern: inter-population differences in life history strategy depended on the host species on which flies were reared, in a direction indicative of local adaptation. Differences in gene expression paralleled phenotypic differences between populations: inter-population differences in expression of some genes involved in metabolism and larval development also depended on the host species on which flies were reared (Etges et al. 2015). A similar study found a diverse suite of genes – enriched for functions in detoxification, metabolism, and sensory perception – that exhibited host-dependent expression level differences between populations of *D. mettleri* specializing on different host cacti (Hoang et al. 2015). While these transcriptomic studies revealed insights into the likely physiological basis of local adaptation in cactophilic *Drosophila*,

population genomic scans coupled with genome-wide measurements of allele frequency change during exposure to local and non-local hosts could definitively identify the targets of selection and genomic architecture of local adaptation. Host-driven local adaptation may be a precursor to speciation in this system: natural history observations and demographic modeling suggest that geographically isolated populations of an ancestral cactophilic *Drosophila* diverged into a pair of sister species associated with different species of cacti, with some evidence that lineage-specific inversions reduced initial post-divergence gene flow (Lohse et al. 2015).

## 5.2 Example 2: Local Adaptation through Exceptional Divergence at Few Loci

On the African island of Mayotte, a population of *Drosophila yakuba* recently specialized on toxic *Morinda citrifolia* (Rubiaceae) fruit, which are absent in its ancestral range in mainland Africa (Yassin et al. 2016). Demographic modeling revealed that isolation from mainland populations began recently (~29,000 years ago), with continuing gene flow since then. After excluding genomic regions with low recombination rate, a genome scan revealed a few small genomic windows exhibiting exceptionally rapid genomic divergence unique to the *Morinda*-specialized population. Strikingly, these genomic regions overlap more often than expected by chance with loci that contribute to *Morinda* tolerance in *D. sechellia*, a distantly related *Drosophila* species also specialized on *Morinda*. Some of these same genomic regions were implicated in the physiological and evolutionary response of the drosophilid *Scaptomyza flava* to dietary mustard oils, particularly genes encoding Osiris proteins, consistent with a role in toxin resistance more generally (Whiteman et al. 2012). These results, combined with phenotypic convergence for *Morinda* tolerance in *D. sechellia* and Mayotte *D. yakuba*, suggests that adaptation to a novel host shaped the genomic landscape of divergence during local adaptation in *D. yakuba*.

## 5.3 Example 3: Local Adaptation through Many Loci with Small Fitness Effects

Gompert et al. (2015) dissected the **genetic architecture** of local adaptation in the Melissa blue butterfly (*Lycaeides melissa*). In addition to feeding on a small number of native legume species in western North America, *L. melissa* colonized naturalized alfalfa within the past 200 years. Larvae from two populations (one associated with a native host, and one with alfalfa) were reared on both host plant species, and a GWAS was employed to identify nucleotide polymorphisms affecting larval performance. Larval survival was highest when feeding on the natal (“home”) plant species for each population, consistent with local adaptation. Strikingly, loci associated with variation in larval performance in one environment were almost always neutral in the other environment, with only a few loci conferring performance tradeoffs across host plants. This is surprising, given that locally adaptive genetic variation is expected to persist in the face of gene flow only at loci conferring fitness tradeoffs across environments (Kawecki and Ebert 2004). However, this finding warrants a cautious interpretation, as experimental and statistical issues make tests for tradeoffs prone to false negative results (Colautti et al. 2012).

As studies of local adaptation in herbivorous insects extend to many populations within single study species, it will be particularly interesting to determine whether plant-herbivore local adaptation occurs primarily through escalating divergence among populations or

through spatiotemporal cycling of standing genetic variants shared by many populations. An example is a geographic mosaic of phenotypes oscillating under frequency-dependent selection, as modeled in many host-parasite interactions (Stahl et al. 1999).

## 6. HOW MUCH GENETIC VARIATION IS MAINTAINED BY SVS IN HERBIVOROUS INSECTS?

### 6.1 The Genetic Architecture of Adaptation Driven by SVS: Population and Quantitative Genetic Perspectives

A recent analysis applying both population genetic and quantitative genetic analyses to genomic data in *Drosophila melanogaster* suggested that balancing selection makes a large contribution to genetic variation affecting fitness (Charlesworth 2015). Accordingly, the possibility exists that SVS, as a form of balancing selection, might have large effects on explaining patterns of functional genomic variation within species. Although studies highlighted in this review strongly support a role for SVS in shaping genomic patterns of **functional genetic variation** within and between populations, its genome-wide impact relative to other forces (such as mutation-selection balance) remains unclear. Does SVS increase functional genetic variation within populations and species primarily by acting on a few loci with large effect on fitness? Or does SVS directly affect allele frequencies at many (hundreds or even many thousands) of polymorphic sites across the genome, each with very small effects on fitness individually but with very large effects collectively?

Population genetic theory generally predicts that alleles with large effects on fitness (relative to migration rate) are more likely to establish and persist within populations, while alleles with small effects on fitness are likely to be lost to genetic drift or swamped out by migration between habitats (Yeaman and Otto 2011; Yeaman and Whitlock 2011). This does not, however, necessarily mean that large effect alleles will account for the majority of genetic and phenotypic variation maintained by SVS. If new mutations or standing polymorphisms with small effects on fitness are abundant (a realistic model for complex traits: Flint and Mackay 2009; Rockman et al. 2012), different expectations arise. At least for cases of relatively low gene flow, simulations show that SVS causes adaptive population divergence for fitness-related traits primarily by slowing the rate at which many alleles with small effects on fitness are lost through genetic drift, rather than by preserving large effect variants for long periods of time (Kelly 2006; Delph and Kelly 2014; Yeaman 2015).

### 6.2 Methods for Detecting Variation Maintained by SVS

A range of polymorphism-based methods can be used to detect functional genetic variation maintained by SVS. Population genomic scans, which refer to statistical comparisons of genome sequences of many individuals from natural populations, can reveal two indicators of divergent selection: genomic windows of elevated divergence between habitat-associated populations (e.g., Yassin et al. 2016), and non-random geographic correlation of individual SNPs with environmental variables (Wang and Bradburd 2014; Rellstab et al. 2015). Quantitative trait locus (QTL) mapping involves the detection of statistical associations between genotype and phenotype, and typically now involves interrogating hundreds of thousands or millions of polymorphic sites across the genome. Methods to map QTL require

genetic variation in the mapping population, which can be either a genetically diverse sampling from a natural population (as in GWAS; Comeault et al. 2014; Gompert et al. 2015) or recombinant offspring of a cross between phenotypically divergent individuals (Mackay 2001). Experimental evolution, combined with genome sequencing of evolved populations (“evolve and resequence”), can identify genomic regions responding to specific selective agents (Schlötterer et al. 2015). Experiments can range in duration from single to many generations, and be conducted in the lab (Egan et al. 2015) or in nature (Soria-Carrasco et al. 2014). In vivo functional genomics is increasingly feasible in non-model systems thanks to rapid advances in genome-editing technology. Introducing different alleles into a common genetic background can isolate the phenotypic effects of each allele. Alternatively, properly designed crosses between outbred individuals and a lab strain harboring a loss of function mutation can isolate effects of different alleles at a locus (see Turner 2014); this less laborious approach takes advantage of the ease of introducing knockout mutations through CRISPR/Cas9.

### 6.3 Limitations to Methods for Detecting Variation Maintained by SVS

With the exception of *in vivo* functional genomics, approaches above are complicated by LD between multiple sites underlying variation in fitness (or a fitness-related trait), and by LD between these sites and neutral sites. Effects of LD can limit the resolution (i.e., narrowness of the genomic location) to which loci affecting fitness can be mapped, and they impede the identification of loci with relatively small effects on fitness (Table 1). Accordingly, using *in vivo* functional genomic techniques to validate candidate loci identified through other approaches will be necessary to understand how much functional genetic variation is maintained by SVS. For example, Chakraborty and Fry (2015) used genome-editing to demonstrate a habitat-dependent fitness tradeoff at two alleles of a detoxification enzyme in *D. melanogaster*, the tradeoff had been predicted based on the distribution of the two alleles across habitats in nature. Using experimental fitness measurements for each allele to parameterize Levene’s (1953) model, they showed the magnitude of this tradeoff was likely sufficient to maintain both alleles in natural populations.

However, approaches largely devoid of bias toward discovering large effect loci are needed to gain elusive insight into the importance of loci with very small habitat-dependent fitness effects in herbivorous insects. These may include, for example, the brute-force approach of using genome-editing to test for habitat-dependent fitness effects of different haplotypes at randomly chosen genomic intervals, which would reveal if loci with very small effects on fitness are widespread throughout the genome.

Both the promise and limitations for characterizing the genetic architecture of traits impacted by SVS are illustrated through two recent GWAS. Comeault et al. (2014) explored the genetic basis of 10 traits divergently selected across host plant species in *T. cristinae*, while Gompert et al. (2015) explored the basis of host-dependent larval performance phenotypes in two populations of *L. melissa* that are locally adapted to different host species. In both studies, 10–60% of variation at each trait was explained by tens (but likely not hundreds) of SNPs per trait. The explained variation was generally controlled by a few loci of large effect and many loci of small but detectable effect. However, the possibility



remains that unexplained phenotypic variation, which exceeded the variation explained by detected SNPs for many traits, may be underpinned by hundreds to many thousands of SNPs whose effects were too small to be individually detected using GWAS.

#### 6.4 Benefits of Studying SVS within Populations

A greater emphasis on empirical genomic studies of SVS within populations, as opposed to the current focus on host race formation and local adaptation, may offer benefits beyond filling a gap in our understanding of how genetic variation is maintained in herbivorous insects. In fact, insect populations associated with multiple hosts are likely ideal for identifying loci involved host-driven adaptation in plant-herbivore interactions for two reasons. First, theory generally predicts (Levene 1953) that alleles maintained by SVS in panmictic populations will be at relatively intermediate frequency. Second, LD should decay quickly around these loci (Charlesworth 2006). This contrasts with cases of host race formation, where reduced inter-habitat gene flow facilitates LD among co-selected alleles at different loci (Feder et al. 2014). Techniques to map loci influencing fitness, such as GWAS (assuming high marker density) and “evolve and resequence” approaches, have high power and fine-scale mapping resolution for common alleles in low LD with nearby loci.

To leverage these advantages, we recommend identifying genetic variation maintained by SVS in panmictic populations through the following approach: (1) Use mapping techniques, such as GWAS and “evolve and resequence”, to identify candidate loci underlying host-associated fitness tradeoffs. (2) Identify loci showing parallel host-associated divergence across many pairs of natural populations. (3) For loci shared between the first two approaches, use genome-editing, if possible, to validate fitness tradeoffs. (4) Using estimated selection coefficients for each locus (Gompert et al. 2014), and (optionally) information about the abundance of each host species, apply Levene’s (1953) model to determine if SVS is predicted to maintain variation at these loci. Although this approach is unlikely to detect loci with small effects on fitness, it could offer an initial first look at the extent of genome-wide variation maintained by SVS in insect populations associated with multiple host plants.

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#### DEFINITIONS

##### **Spatially varying selection (SVS)**

the scenario in which selective pressures differ among spatially distinct habitats

##### **Genomic divergence**

differences in allele frequencies or the number of fixed nucleotide differences between populations or species, interrogated across the genome. can be thought of as spatially distinct habitats, es.cts that associate with multiple hosts, a

**Linkage disequilibrium**

non-random association of alleles at different loci. Alleles in LD co-occur in a chromosome more often than expected by chance

**Local adaptation**

the scenario in which populations have higher fitness in their native habitat than do populations introduced from other locations

**Genetic architecture**

The genetic basis of variation for a phenotype, including the identity and number of loci influencing the trait, and the sizes of their effects on trait variation

**Functional genetic variation**

genetic variation that affects phenotypes or fitness; e.g., non-neutral variation

**LITERATURE CITED**

- Agrawal, AA., Conner, JK., Rasmann, S. Tradeoffs and negative correlations in evolutionary ecology. In: Bell, MA. Futuyma, DJ. Eanes, WF., Levinton, JS., editors. *Evolution since Darwin: The First 150 years*. Sunderland, MA: Sinauer Associates; 2010. p. 243–268.
- Ali JG, Agrawal AA. Specialist versus generalist insect herbivores and plant defense. *Trends Plant Sci*. 2012; 17:293–302. [PubMed: 22425020]
- Antwi JB, Sword GA, Medina RF. Host-associated differentiation in a highly polyphagous, sexually reproducing insect herbivore. *Ecol Evol*. 2015; 5:2533–2543. [PubMed: 26257868]
- Barrett RD, Hoekstra HE. Molecular spandrels: tests of adaptation at the genetic level. *Nat Rev Gen*. 2011; 12:767–780.
- Barton NH. Multilocus clines. *Evolution*. 1983; 37:454–471. [PubMed: 28563316]
- Bengtsson, BO. The flow of genes through a genetic barrier. In: Greenwood, PJ. Harvey, PH., Slatkin, M., editors. *Evolution: essays in honour of John Maynard Smith*. Cambridge, UK: Cambridge University Press; 1985.
- Berenbaum MR, Zangerl AR. Chemical phenotype matching between a plant and its insect herbivore. *PNAS*. 1998; 95:13743–13748. [PubMed: 9811871]
- Berlacher SH, Feder JL. Sympatric speciation in phytophagous insects: moving beyond the controversy. *Annu Rev Entom*. 2002; 47:773–815.
- Bernays E, Graham M. On the evolution of host specificity in phytophagous arthropods. *Ecology*. 1988; 69:886–892.
- Bloom JS, Ehrenreich IM, Loo WT, Lite TLV, Kruglyak L. Finding the sources of missing heritability in a yeast cross. *Nature*. 2013; 494:234–237. [PubMed: 23376951]
- Brachi B, Morris GP, Borevitz JO. Genome-wide association studies in plants: the missing heritability is in the field. *Genome Biol*. 2011; 12:1–8.
- Bush GL. Sympatric host race formation and speciation in frugivorous flies of the genus *Rhagoletis* (Diptera: Tephritidae). *Evolution*. 1969; 23:237–251. [PubMed: 28562891]
- Chakraborty M, Fry JD. Evidence that Environmental Heterogeneity Maintains a Detoxifying Enzyme Polymorphism in *Drosophila melanogaster*. *Current Biology*. 2015; 26:219–223. [PubMed: 26748852]
- Charlesworth B. Causes of natural variation in fitness: evidence from studies of *Drosophila*. *PNAS*. 2015; 112:1662–1669. [PubMed: 25572964]
- Charlesworth D. Balancing selection and its effects on sequences in nearby genome regions. *PLoS Genetics*. 2006; 2:e64. [PubMed: 16683038]
- Charlesworth B, Nordborg M, Charlesworth D. The effects of local selection, balanced polymorphism and background selection on equilibrium patterns of genetic diversity in subdivided populations. *Genetical Research*. 1997; 70:155–174. [PubMed: 9449192]

- Christiansen FB. Sufficient conditions for protected polymorphism in a subdivided population. *Am Nat.* 1974; 108:157–166.
- Colautti RI, Lee C, Mitchell-Olds T. Origin, fate, and architecture of ecologically relevant genetic variation. *Curr Opin Plant Biol.* 2012; 15:199–204. [PubMed: 22341792]
- Comeault AA, Soria-Carrasco V, Gompert Z, Farkas TE, Buerkle CA, Parchman TL, Nosil P. Genome-wide association mapping of phenotypic traits subject to a range of intensities of natural selection in *Timema cristinae*. *Am Nat.* 2014; 183:711–727. [PubMed: 24739202]
- Comeault A, Ferreira C, Dennis S, Soria-Carrasco V, Nosil P. Evolution of color phenotypes in two distantly related species of stick insect: different ecological regimes acting on similar genetic architectures. *bioRxiv.* 2015a 023481.
- Comeault AA, Flaxman SM, Riesch R, Curran E, Soria-Carrasco V, Gompert Z, Farkas TE, Muschick M, Parchman TL, Schwander T. Selection on a genetic polymorphism counteracts ecological speciation in a stick insect. *Current Biology.* 2015b; 25:1975–1981. [PubMed: 26119745]
- Da Cunha AB, Burla H, Dobzhansky T. Adaptive chromosomal polymorphism in *Drosophila willistoni*. *Evolution.* 1950:212–235.
- Delph LF, Kelly JK. On the importance of balancing selection in plants. *New Phytologist.* 2014; 201:45–56. [PubMed: 23952298]
- Dempster ER. Maintenance of genetic heterogeneity. *Cold Spring Harbor Symp Quant Biol.* 1955; 20:25–32. [PubMed: 13433552]
- Dobzhansky, T. *Genetics and the Origin of Species.* New York: Columbia University Press; 1951.
- Drès M, Mallet J. Host races in plant-feeding insects and their importance in sympatric speciation. *Phil Trans R Soc B.* 2002; 357:471–492. [PubMed: 12028786]
- Edmunds GF Jr, Alstad DN. Coevolution in insect herbivores and conifers. *Science.* 1978; 199:941–945. [PubMed: 17752349]
- Edger PP, Heide-Fischer HM, Bekaert M, Rota J, Glockner G, Platts AE, Heckel DG, Der JP, Wafula EK, Tang M, et al. The butterfly plant arms-race escalated by gene and genome duplications. *PNAS.* 2015; 112:8362–8366. [PubMed: 26100883]
- Egan SP, Ragland GJ, Assour L, Powell TH, Hood GR, Emrich S, Nosil P, Feder JL. Experimental evidence of genome-wide impact of ecological selection during early stages of speciation-with-gene-flow. *Ecol Lett.* 2015; 18:817–825. [PubMed: 26077935]
- Ehrlich PR, Raven PH. Butterflies and plants: a study in coevolution. *Evolution.* 1964; 18:586–608.
- Etges WJ, De Oliveira CC, Noor MA, Ritchie MG. Genetics of incipient speciation in *Drosophila mojavensis*. III Life-history divergence in allopatry and reproductive isolation. *Evolution.* 2010; 64:3549–3569. [PubMed: 20681983]
- Etges WJ, Trotter MV, Oliveira CC, Rajpurohit S, Gibbs AG, Tuljapurkar S. Deciphering life history transcriptomes in different environments. *Mol Ecol.* 2015; 24:151–179. [PubMed: 25442828]
- Farkas TE, Mononen T, Comeault AA, Hanski I, Nosil P. Evolution of camouflage drives rapid ecological change in an insect community. *Current Biology.* 2013; 23:1835–1843. [PubMed: 24055155]
- Feder JL, Berlocher SH, Roethele JB, Dambroski H, Smith JJ, Perry WL, Gavrilovic V, Filchak KE, Rull J, Aluja M. Allopatric genetic origins for sympatric host-plant shifts and race formation in *Rhagoletis*. *PNAS.* 2003; 100:10314–10319. [PubMed: 12928500]
- Feder JL, Gejji R, Powell TH, Nosil P. Adaptive chromosomal divergence driven by mixed geographic mode of evolution. *Evolution.* 2011; 65:2157–2170. [PubMed: 21790566]
- Feder JL, Flaxman SM, Egan SP, Comeault AA, Nosil P. Geographic mode of speciation and genomic divergence. *Annu Rev Ecol Evol Syst.* 2013; 44:73–97.
- Feder JL, Nosil P, Wacholder AC, Egan SP, Berlocher SH, Flaxman SM. Genome-wide congealing and rapid transitions across the speciation continuum during speciation with gene flow. *J Hered.* 2014; 105(Suppl 1):810–820. [PubMed: 25149256]
- Feeny, P. Plant apparency and chemical defense. In: Wallace, JW., Mansell, RL., editors. *Biochemical interaction between plants and insects.* Vol. 10. Springer; 1976. p. 1-40.
- Felsenstein J. Skepticism towards Santa Rosalia, or why are there so few kinds of animals? *Evolution.* 1981; 35:124–138. [PubMed: 28563447]

- Felsenstein J. The theoretical population genetics of variable selection and migration. *Annu Rev Genet.* 1976; 10:253–280. [PubMed: 797310]
- Flaxman SM, Wacholder AC, Feder JL, Nosil P. Theoretical models of the influence of genomic architecture on the dynamics of speciation. *Mol Ecol.* 2014; 23:4074–4088. [PubMed: 24724861]
- Flint J, Mackay TF. Genetic architecture of quantitative traits in mice, flies, and humans. *Genome Research.* 2009; 19:723–733. [PubMed: 19411597]
- Flor H. The complementary genic systems in flax and flax rust. *Adv Genet.* 1956; 8:29–54.
- Forbes AA, Feder JL. Divergent preferences of *Rhagoletis pomonella* host races for olfactory and visual fruit cues. *Entomol Exp Appl.* 2006; 119:121–127.
- Forister M, Dyer L, Singer M, Stireman J III, Lill J. Revisiting the evolution of ecological specialization, with emphasis on insect-plant interactions. *Ecology.* 2012; 93:981–991. [PubMed: 22764485]
- Fraenkel GS. The Raison d’Etre of Secondary Plant Substances: These odd chemicals arose as a means of protecting plants from insects and now guide insects to food. *Science.* 1959; 129:1466–1470. [PubMed: 13658975]
- Franssen SU, Nolte V, Tobler R, Schlötterer C. Patterns of linkage disequilibrium and long range hitchhiking in evolving experimental *Drosophila melanogaster* populations. *Mol Biol Evol.* 32:495–509.
- Futuyma DJ, Moreno G. The evolution of ecological specialization. *Annu Rev Ecol Syst.* 1988; 19:207–233.
- Garrido E, Andraca-Gómez G, Fornoni J. Local adaptation: simultaneously considering herbivores and their host plants. *New Phytol.* 2012; 193:445–453. [PubMed: 21988566]
- Gilman RT, Nuismer SL, Jhvueng D. Coevolution in multidimensional trait space favours escape from parasites and pathogens. *Nature.* 2012; 483:328–330. [PubMed: 22388815]
- Gloss AD, Nelson Dittrich AC, Goldman-Huertas B, Whiteman NK. Maintenance of genetic diversity through plant–herbivore interactions. *Curr Opin Plant Biol.* 2013; 16:443–450. [PubMed: 23834766]
- Gompert Z, Comeault AA, Farkas TE, Feder JL, Parchman TL, Buerkle CA, Nosil P. Experimental evidence for ecological selection on genome variation in the wild. *Ecol Lett.* 2014; 17:369–379. [PubMed: 24354456]
- Gompert Z, Jahner JP, Scholl CF, Wilson JS, Lucas LK, Soria-Carrasco V, Fordyce JA, Nice CC, Buerkle CA, Forister ML. The evolution of novel host use is unlikely to be constrained by trade-offs or a lack of genetic variation. *Mol Ecol.* 2015; 24:2777–2793. [PubMed: 25877787]
- Greischar MA, Koskella B. A synthesis of experimental work on parasite local adaptation. *Ecology Letters.* 2007; 10:418–434. [PubMed: 17498141]
- Groen SC, Whiteman NK. Using *Drosophila* to study the evolution of herbivory and diet specialization. *Curr Op Insect Sci.* 2016; 14:66–72.
- Hansen AK, Moran NA. The impact of microbial symbionts on host plant utilization by herbivorous insects. *Mol Ecol.* 2014; 23:1473–1496. [PubMed: 23952067]
- Hawthorne DJ, Via S. Genetic linkage of ecological specialization and reproductive isolation in pea aphids. *Nature.* 2001; 412:904–907. [PubMed: 11528477]
- Hedrick PW. Genetic polymorphism in heterogeneous environments: the age of genomics. *Annu Rev Ecol Evol Sys.* 2006; 37:67–93.
- Heliconius Genome Consortium. Butterfly genome reveals promiscuous exchange of mimicry adaptations among species. *Nature.* 2012; 487:94–98. [PubMed: 22722851]
- Hoang K, Matzkin LM, Bono JM. Transcriptional variation associated with cactus host plant adaptation in *Drosophila mettleri* populations. *Mol Ecol.* 2015; 24:5186–5199. [PubMed: 26384860]
- Houle D. Genetic covariance of fitness correlates: what genetic correlations are made of and why it matters. *Evolution.* 1991; 45:630–648. [PubMed: 28568816]
- Hunter, MD., Ohgushi, T., Price, PW. Effects of resource distribution on animal plant interactions. San Diego, CA: Academic Press Inc.; 2012.

- Hsieh P, Veeramah KR, Lachance J, Tishkoff SA, Wall JD, Hammer MF, Gutenkunst RN. Whole-genome sequence analyses of Western Central African Pygmy hunter-gatherers reveal a complex demographic history and identify candidate genes under positive natural selection. *Genome Research*. 2016; 26:279–290. [PubMed: 26888263]
- International Aphid Genomics Consortium. Genome sequence of the pea aphid *Acyrtosiphon pisum*. *PLoS Biol*. 2010; 8:e1000313. [PubMed: 20186266]
- Janz N, Nylin S. The oscillation hypothesis of host-plant range and speciation. In: Tilmon, KJ., editor. *Specialization, speciation, and radiation: The evolutionary biology of herbivorous insects*. Los Angeles: University of California Press; 2008. p. 203-215.
- Janz N. Ehrlich and Raven revisited: Mechanisms underlying codiversification of plants and enemies. *Annu Rev Ecol Evol Sys*. 2011; 42:71–89.
- Jaquiere J, Stoeckel S, Nouhaud P, Mieuzet L, Maheo F, Legeai F, Bernard N, Bonvoisin A, Vitalis R, Simon JC. Genome scans reveal candidate regions involved in the adaptation to host plant in the pea aphid complex. *Mol Ecol*. 2012; 21:5251–5264. [PubMed: 23017212]
- Joron M, Frezal L, Jones RT, Chamberlain NL, Lee SF, Haag CR, Whibley A, Becuwe M, Baxter SW, Ferguson L. Chromosomal rearrangements maintain a polymorphic supergene controlling butterfly mimicry. *Nature*. 2011; 477:203–206. [PubMed: 21841803]
- Kaltz O, Shykoff JA. Local adaptation in host–parasite systems. *Heredity*. 1998; 81:361–370.
- Karasov TL, Kniskern JM, Gao L, DeYoung BJ, Ding J, Dubiella U, Lastra RO, Nallu S, Roux F, Innes RW. The long-term maintenance of a resistance polymorphism through diffuse interactions. *Nature*. 2014; 512:436–440. [PubMed: 25043057]
- Kawecki TJ, Ebert D. Conceptual issues in local adaptation. *Ecol Lett*. 2004; 7:1225–1241.
- Kelly JK. Geographical variation in selection, from phenotypes to molecules. *Am Nat*. 2006; 167:481–495. [PubMed: 16670992]
- Kirkpatrick M, Barton N. Chromosomal inversions, local adaptation and speciation. *Genetics*. 2006; 173:419–434. [PubMed: 16204214]
- Kofler R, Schlötterer C. A practical guide for the design of evolve and resequencing studies. *Mol Biol Evol*. 2014; 31:474–483. [PubMed: 24214537]
- Kunte K, Zhang W, Tenger-Trolander A, Palmer D, Martin A, Reed R, Mullen S, Kronforst M. Doublesex is a mimicry supergene. *Nature*. 2014; 507:229–232. [PubMed: 24598547]
- Labandeira CC, Sepkoski JJ. Insect diversity in the fossil record. *Science*. 1993; 261:310–315. [PubMed: 11536548]
- Lande R. The maintenance of genetic variability by mutation in a polygenic character with linked loci. *Genet Res*. 1975; 26:221–235. [PubMed: 1225762]
- Levene H. Genetic equilibrium when more than one ecological niche is available. *Amer Nat*. 1953; 87:331–333.
- Levy SF, Blundell JR, Venkataram S, Petrov DA, Fisher DS, Sherlock G. Quantitative evolutionary dynamics using high-resolution lineage tracking. *Nature*. 2015; 519
- Li X, Fan D, Zhang W, Liu G, Zhang L, Zhao L, Fang X, Chen L, Dong Y, Chen Y, Ding Y. Outbred genome sequencing and CRISPR/Cas9 gene editing in butterflies. *Nature Communications*. 2015; 6:8212.
- Lohse K, Clarke M, Ritchie MG, Etges WJ. Genome-wide tests for introgression between cactophilic *Drosophila* implicate a role of inversions during speciation. *Evolution*. 2015; 69:1178–1190. [PubMed: 25824653]
- Louthan AM, Kay KM. Comparing the adaptive landscape across trait types: larger QTL effect size in traits under biotic selection. *BMC Evol Biol*. 2011; 11:60. [PubMed: 21385379]
- Mackay TFC. Quantitative trait loci in *Drosophila*. *Nat Rev Gen*. 2001; 2:11–20.
- Mayhew PJ. Why are there so many insect species? Perspectives from fossils and phylogenies. *Biol Rev Camb Philos Soc*. 2007; 82:425–454. [PubMed: 17624962]
- Michel AP, Sim S, Powell THQ, Taylor MS, Nosil P, Feder JL. Widespread genomic divergence during sympatric speciation. *PNAS*. 2010; 107:9724–9729. [PubMed: 20457907]
- Mitter C, Farrell B, Wiegmann B. The phylogenetic study of adaptive zones: Has phytophagy promoted insect diversification. *Am Nat*. 1988; 132:107–128.

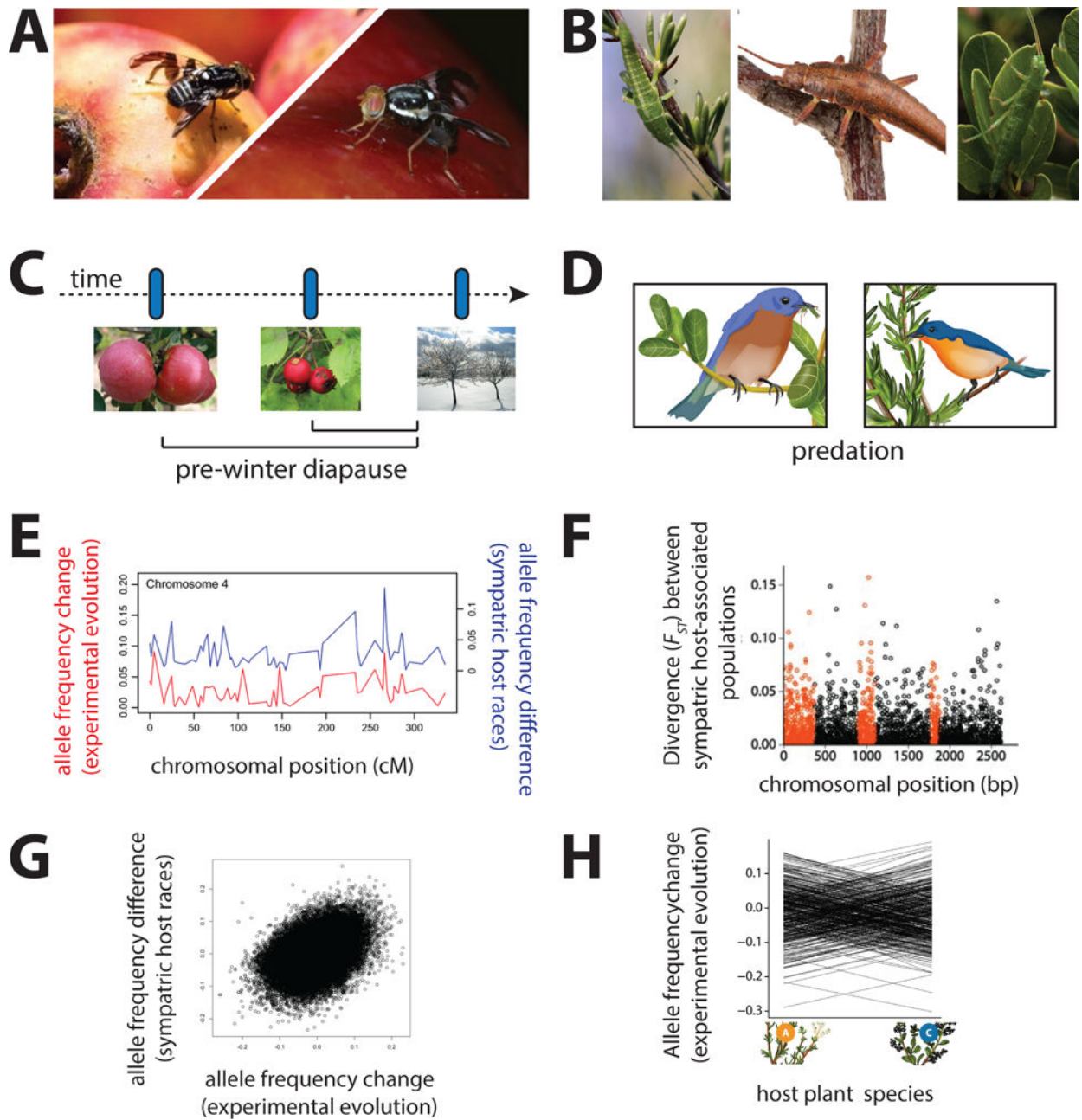
- Mopper S. Adaptive genetic structure in phytophagous insect populations. *Trends Ecol Evol.* 1996; 11:235–238. [PubMed: 21237824]
- Nosil P, Gompert Z, Farkas TE, Comeault AA, Feder JL, Buerkle CA, Parchman TL. Genomic consequences of multiple speciation processes in a stick insect. *Proc Biol Sci.* 2012; 279:5058–5065. [PubMed: 22696527]
- Ohshima I, Watanabe K, Kawamura T. Distinct parasitoid communities associated with host races of the leaf-mining moth *Acrocercops transecta* on distantly related host plants (Juglandaceae and Ericaceae). *J Nat Hist.* 2015; 49:815–828.
- Pauchet Y, Heckel DG. The genome of the mustard leaf beetle encodes two active xylanases originally acquired from bacteria through horizontal gene transfer. *Proc Biol Sci.* 2013; 280:20131021. [PubMed: 23698014]
- Peccoud J, Ollivier A, Plantegenest M, Simon JC. A continuum of genetic divergence from sympatric host races to species in the pea aphid complex. *PNAS.* 2009a; 106:7495–7500. [PubMed: 19380742]
- Peccoud J, Simon J, McLaughlin HJ, Moran NA. Post-Pleistocene radiation of the pea aphid complex revealed by rapidly evolving endosymbionts. *PNAS.* 2009b; 106:16315–16320. [PubMed: 19805299]
- Petschenka G, Agrawal AA. How herbivores coopt plant defenses: natural selection, specialization, and sequestration. *Curr Opin Ins Sci.* 2016; 14:17–24.
- Powell TH, Forbes AA, Hood GR, Feder JL. Ecological adaptation and reproductive isolation in sympatry: genetic and phenotypic evidence for native host races of *Rhagoletis pomonella*. *Mol Ecol.* 2014; 23:688–704. [PubMed: 24351094]
- Price, PW. *Evolutionary Biology of Parasites.* Princeton University Press; Princeton, New Jersey: 1980.
- Prout T, Savolainen O. Genotype-by-environment interaction is not sufficient to maintain variation: Levene and the leafhopper. *Am Nat.* 1996; 148:930–936.
- Ragland GJ, Egan SP, Feder JL, Berlocher SH, Hahn DA. Developmental trajectories of gene expression reveal regulatory candidates for diapause termination, a key life history transition in the apple maggot fly, *Rhagoletis pomonella*. *J Exp Biol.* 2011; 214:3948–3960. [PubMed: 22071185]
- 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. [PubMed: 26184487]
- Rockman M. The QTN program and the alleles that matter for evolution: all that's gold does not glitter. *Evolution.* 2012; 66:1–17. [PubMed: 22220860]
- Sackton TB, Lazzaro BP, Schlenke TA, Evans JD, Hultmark D, Clark AG. Dynamic evolution of the innate immune system in *Drosophila*. *Nat Genet.* 2007; 39:1461–1468. [PubMed: 17987029]
- Savolainen O, Lascoux M, Merilä J. Ecological genomics of local adaptation. *Nat Rev Genet.* 2013; 14:807–820. [PubMed: 24136507]
- Scheirs J, Jordaens K, De Bruyn L. Have genetic trade-offs in host use been overlooked in arthropods? *Evol Ecol.* 2005; 19:551–561.
- Schlötterer C, Kofler R, Versace E, Tobler R, Franssen SU. Combining experimental evolution with next-generation sequencing: a powerful tool to study adaptation from standing genetic variation. *Heredity.* 2015; 114:431–440. [PubMed: 25269380]
- Schuman MC, Baldwin IT. The Layers of Plant Responses to Insect Herbivores. *Annu Rev Entomol.* 2015 (available online Dec 11).
- Seehausen O, Butlin RK, Keller I, Wagner CE, Boughman JW, Hohenlohe PA, Peichel CL, Saetre G, Bank C, Brännström Å. Genomics and the origin of species. *Nat Rev Genet.* 2014; 15:176–192. [PubMed: 24535286]
- Singer MS, Lichter-Marck IH, Farkas TE, Aaron E, Whitney KD, Mooney KA. Herbivore diet breadth mediates the cascading effects of carnivores in food webs. *PNAS.* 2014; 111:9521–9526. [PubMed: 24979778]
- Soria-Carrasco V, Gompert Z, Comeault AA, Farkas TE, Parchman TL, Johnston JS, Buerkle CA, Feder JL, Bast J, Schwander T, et al. Stick insect genomes reveal natural selection's role in parallel speciation. *Science.* 2014; 344:738–742. [PubMed: 24833390]

- Sousa V, Hey J. Understanding the origin of species with genome-scale data: modeling gene flow. *Nat Rev Genet.* 2013; 14:404–414. [PubMed: 23657479]
- Stahl EA, Dwyer G, Mauricio R, Kreitman M, Bergelson J. Dynamics of disease resistance polymorphism at the *Rpm1* locus of *Arabidopsis*. *Nature.* 1999; 400:667–671. [PubMed: 10458161]
- Terhorst J, Schlötterer C, Song YS. Multi-locus analysis of genomic time series data from experimental evolution. *PLoS Genet.* 2015; 11:e1005069. [PubMed: 25849855]
- Thaler JS, Humphrey PT, Whiteman NK. Evolution of jasmonate and salicylate signal crosstalk. *Trends Pl Sci.* 2012; 17:260–270.
- Thompson, JN. *The geographic mosaic of coevolution.* Chicago: University of Chicago Press; 2005.
- Turner TL. Fine-mapping natural alleles: quantitative complementation to the rescue. *Mol Ecol.* 23:2337–2382.
- Via S. Divergence hitchhiking and the spread of genomic isolation during ecological speciation-with-gene-flow. *Philos Trans R Soc Lond B.* 2012; 367:451–460. [PubMed: 22201174]
- Via S, Conte G, Mason-Foley C, Mills K. Localizing FST outliers on a QTL map reveals evidence for large genomic regions of reduced gene exchange during speciation-with-gene-flow. *Mol Ecol.* 2012; 21:5546–5560. [PubMed: 23057835]
- Wang JJ, Bradburd GS. Isolation by environment. *Mol Ecol.* 2014; 23:5649–5662. [PubMed: 25256562]
- Whiteman NK, Gloss AD, Sackton TB, Groen SC, Humphrey PT, Lapoint RT, Sonderby IE, Halkier BA, Kocks C, Ausubel FM, Pierce NE. Genes involved in the evolution of herbivory by a leaf-mining, drosophilid fly. *Gen Biol Evol.* 2012; 4:788–804.
- Wiens JJ, Lapoint RT, Whiteman NK. Herbivory increases diversification across insect clades. *Nat Comm.* 2015; 6:8370.
- Yassin A, Debat V, Bastide H, Gidaszewski N, David JR, Pool JE. Recurrent specialization on a toxic fruit in an island *Drosophila* population. *PNAS.* 2016 In press.
- Yeaman S. Local Adaptation by Alleles of Small Effect\*. *Am Nat.* 2015; 186:S74–S89. [PubMed: 26656219]
- Yeaman S, Whitlock MC. The genetic architecture of adaptation under migration–selection balance. *Evolution.* 2011; 65:1897–1911. [PubMed: 21729046]
- Yeaman S, Otto SP. Establishment and maintenance of adaptive genetic divergence under migration, selection, and drift. *Evolution.* 2011; 65:2123–2129. [PubMed: 21729066]
- Yeaman S. Genomic rearrangements and the evolution of clusters of locally adaptive loci. *PNAS.* 2013; 110:1743–1751.
- You M, Yue Z, He W, Yang X, Yang G, Xie M, Zhan D, Baxter SW, Vasseur L, Gurr GM. A heterozygous moth genome provides insights into herbivory and detoxification. *Nat Genet.* 2013; 45:220–225. [PubMed: 23313953]
- 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. [PubMed: 25274300]
- Zhao C, Escalante LN, Chen H, Benatti TR, Qu J, Chellapilla S, Waterhouse RM, Wheeler D, Andersson MN, Bao R. A massive expansion of effector genes underlies gall-formation in the wheat pest *Mayetiola destructor*. *Current Biology.* 2015; 25:613–620. [PubMed: 25660540]

### SUMMARY POINTS

- Theory predicts that habitat variation, such as variation among host species, can maintain genetic variation within and between herbivorous insect populations.
- Genomic studies of host races and locally adapted populations, which involve low rates of gene flow among habitats, generally support this prediction.
- The amount of genetic variation maintained by habitat variation *within* herbivorous insect populations, a situation characterized by high rates of gene flow among habitats, is unknown. Limited empirical studies suggest this process occurs in natural insect populations, at least for a few genes, but their generality is unclear. Genomic studies in this area are urgently needed.
- An integrative approach combining experimental, population, functional, and theoretical genomics is ideal for detecting genetic variation maintained by habitat variation within insect populations. Insect populations associated with multiple habitats are naturally well-suited for finely mapping loci underlying host- or habitat-associated adaptation.





**Figure 1. Genomic evidence that adaptation drives divergence in *Rhagoletis pomonella* (left) and *Timema cristinae* (right)**

(A) *R. pomonella* oviposits in fruits of apple and hawthorn, where larvae develop. (B) *T. cristinae* feeds externally on *Adenostoma* sp. (left) and *Ceanothus* sp. (right) plants. On leaves, green morphs with (on *Adenostoma*) and without (*Ceanothus*) a dorsal stripe are most cryptic. A third morph (center) is cryptic on stems. (C,D) The length of pre-winter diapause and crypsis to avoid predatory birds are traits under strong divergent, host-associated selection in *R. pomonella* and *T. cristinae*, respectively. (E,G) Across the genome, allele frequency changes between populations experimentally exposed to long and short diapause match allele frequency differences among natural host races in *R. pomonella*.

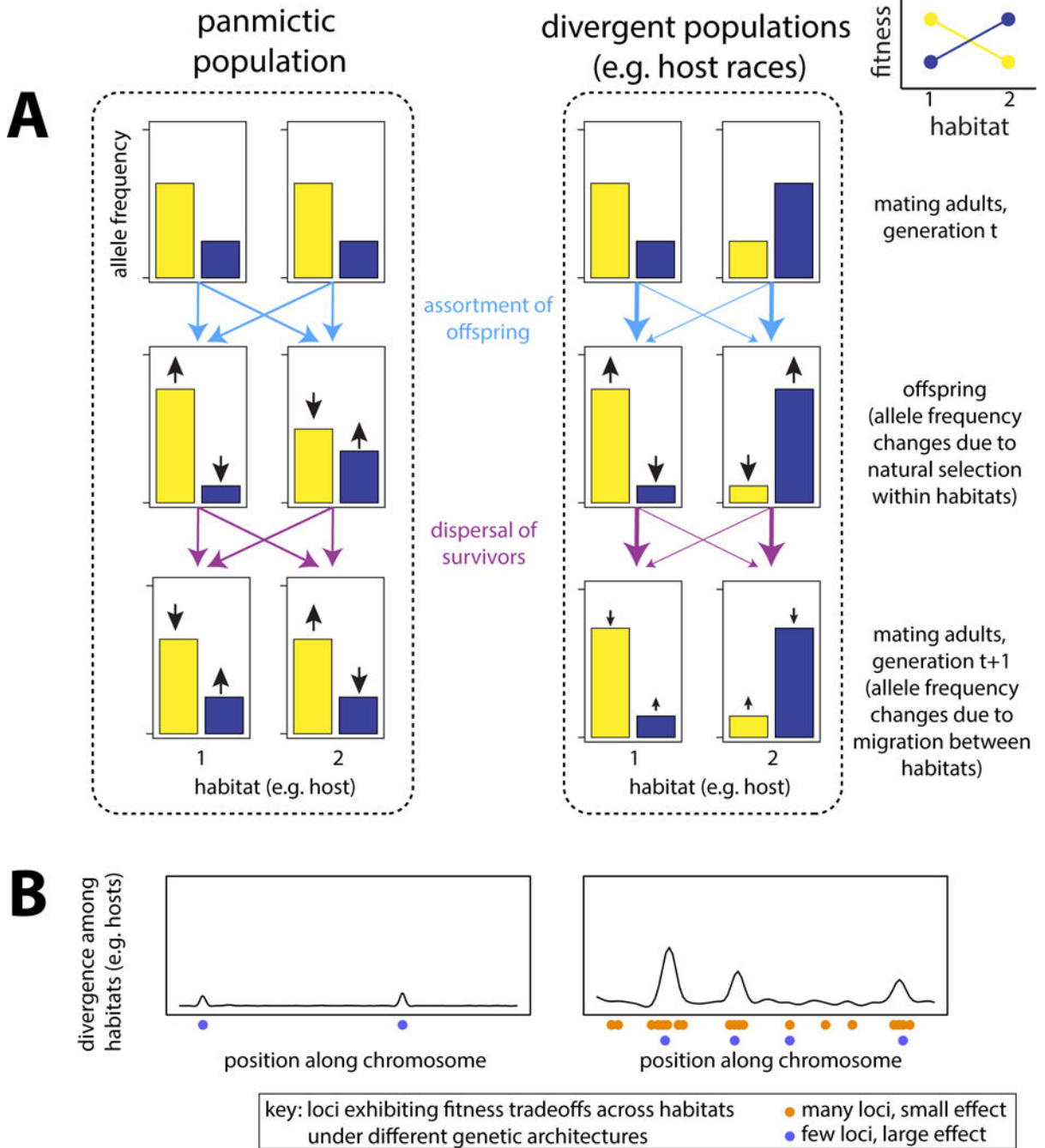
**(F)** Regions of high (orange) and low (black) divergence between sympatric host-associated *T. cristinae* populations are small and finely interspersed throughout the genome. **(H)** Loci exhibiting exceptional allele frequency change across host species in *T. cristinae* transplant experiments. Figures are reproduced from Soria-Carrasco et al. 2014 and Egan et al. 2015. Photographs in panels A and B, from left to right, were taken by: Hannes Schuler, Andrew Forbes, Aaron Comeault, Moritz Muschick, Aaron Comeault.

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**Figure 2. Trajectories of habitat-specific genetic differentiation under two modes of spatially varying selection**

A panmictic population is shown on the left, while divergent habitat-associated populations with low migration, such as host races or locally adapted populations, are shown on the right. (A) Changes in frequencies in each habitat for a locus exhibiting a fitness tradeoff across habitats. Left: allele frequencies will diverge due to natural selection favoring different alleles in habitat 1 and 2, but random dispersal of survivors each generation resets allele frequencies to an equilibrium level (determined by selection coefficients and the

proportion of survivors in each habitat). Right: low migration between habitats enables divergence to increase every generation. Note that although a single locus is shown, allele frequency trajectories will be greatly affected by selection at linked sites as genome-wide divergence builds up in the latter scenario. **(B)** Simple cartoon representation of the heterogeneous landscape of divergence across a chromosome segment. Exceptional levels of divergence are expected to be narrowly centered on targets of selection under panmixia (left), but can subsume broad regions (including targets of selection and physically linked neutral sites) in differentiated host races or locally adapted populations (right).

Table 1

Genomic methods to detect loci responding to spatially varying selection.

Method	Power to detect loci with small effects on fitness or weak response to selection <sup>a</sup>	Key benefits from large sample sizes or numbers of replicates	Key conceptual or empirical evaluations of power, mapping resolution
Population genomic scans	Usually <i>low</i> , but possibly <i>moderate</i> when (1) individuals are sampled from different habitats many times independently, and (2) the population is panmictic across habitats or habitat doesn't strongly correlate with population structure. <sup>b</sup>	Replicating inter-habitat allele frequency comparisons across many sites helps distinguish allele frequency differences due to selection from differences due to genetic drift or population structure	Rellstab et al. 2015
Genome wide association studies (GWAS)	<i>Low</i> , especially for rare alleles	Large mapping population reduces spurious LD, increases power to detect weak associations.	Brachi et al. 2011
Quantitative trait locus (QTL) mapping using recombinant lines	<i>Moderate</i>	Many recombinant inbred lines (or a highly outbred mapping population, if employing pooled sequencing) narrows mapping resolution and increases power to detect weak associations.	Bloom et al. 2013
Experimental evolution ("evolve and resequence")	<i>Low</i> , although possibly <i>moderate</i> with very high replication and/or evolving population sizes, little LD in founding population, and enormously high sequencing depth	Large, diverse founding populations limit LD (which causes responses to selection at many neutral sites) at onset of experiment. High replication distinguishes changes due to selection from those due to drift.	Franssen et al. 2014; Kofler and Schlötterer 2014; Terhorst et al. 2015; Levy et al. 2015
<i>In vitro</i> functional genomics (e.g., genome-editing)	<i>High</i> , but only interrogates a single locus chosen <i>a priori</i> rather than the whole genome		Turner 2014; Li et al. 2015

<sup>a</sup>We assume that sample sizes, sequencing depth, and replication are not highly limiting. We also note that even under this assumption, loci with *extremely* small effects on fitness will not be detected by any of these methods (Rockman et al. 2012).

<sup>b</sup>A caveat: if fitness is highly polygenic (such that mutations affecting fitness are frequent) or has high genetic redundancy, different alleles will respond to SVS in different populations (Kelly 2006; Yeaman 2015), greatly reducing power to detect small effect loci in structured (i.e., non-panmictic) populations