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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.

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 & Sepkoski 1993, Mayhew 2007, Wiens et al. 2015). Herbivorous insect clades exhibit faster rates of net diversification and higher species richness compared with clades of nonherbivorous 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 & 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 the 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 & Graham (1988) later hypothesized that variation in top-down forces associated with different plant species might be stronger selective agents on herbivorous insects (see the 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 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 like 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

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 and is regulated through an intricate signaling network (Thaler et al. 2012, Schuman & Baldwin 2016). Plant defensive traits and herbivore counteradaptations are typically polymorphic and hypothesized to be the result of coevolutionary dynamics (Flor 1956, Ehrlich & 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 symbionts (endosymbiosis) (Pauchet & Heckel 2013). These relationships could also assist with the subversion of plant defenses (Hansen & Moran 2014).

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 either have gained the ability to sequester plant-produced chemicals to defend themselves against attack by the third trophic level—often advertised through aposematic coloration—or have employed detoxification mechanisms and evasive strategies such as camouflage or mimicry (Farkas et al. 2013, Petschenka & 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 & Graham 1988, Singer et al. 2014).

support these predictions. These insights arise from studies of both 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.

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 & Kelly 2014). Balancing selection contrasts with another, nonadaptive 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 and purifying selection (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 interhabitat 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

Spatially varying selection (SVS): the scenario in which selective pressures differ among spatially distinct habitats

Local adaptation:

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

a single life stage, for example, owing to rapid environmental fluctuations or frequent movement of individuals between habitats. If this assumption is violated, selection favors the genotype that maximizes fitness across environments (Futuyma & Moreno 1988).

Initially, herbivorous insects helped inspire models for the maintenance of genetic polymorphism within panmictic populations in which individuals are dispersed across multiple host plant species (Dobzhansky 1951, Levene 1953, Prout & Savolainen 1996). Later, they helped illuminate how populations can diverge into sympatric races occupying different host plant species and evolve increased reproductive isolation in the face of gene flow (Futuyma & Moreno 1988, Drès & Mallet 2002). They also provided foundational evidence for local adaptation in species interactions (Edmunds & 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 & 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 that are associated with different hosts and share little to no gene flow. Although the evolutionary transition from polymorphic populations to separate species is a continuous process (Drès & Mallet 2002, Feder et al. 2014), treating different points on this continuum as stages is useful to conceptualize how diversification proceeds.

Herbivorous insects offer key advantages for disentangling processes that contribute to divergence among host-associated populations, in particular for elucidating the relative importance of host species as a selective agent that drives 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 (Timematidae: *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 experienced variable rates of gene flow among races (Peccoud et al.

2009a, 2009b; Via et al. 2012). Tephritid fruit flies (**Figure 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 (**Figure 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—a scenario 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* (**Figure 1c**; Egan et al. 2015), predation in *Timema* (**Figure 1d**; Nosil et al. 2012), and host plant nutritional and defensive properties in *Acyrtosiphon* (Hawthorne & Via 2001).

3.2. Natural Selection and the Genomic Landscape of Divergence

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

Genome scans in multiple insect species (Michel et al. 2010, Via et al. 2012, Gompert et al. 2014, Soria-Carrasco 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., **Figure 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 & 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 trade-offs (Bengtsson 1985). This effect should extend to nearby neutral loci but weaken as the distance between a neutral locus and selected locus increases, as 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 to different host species 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 resequencing 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, Via et al. 2012, Soria-Carrasco et al. 2014, Egan et al. 2015). In all three genera, loci underlying experimentally detected, host-specific fitness trade-offs are enriched in the most differentiated genomic regions between populations or races on different host species (shown for *Rhagoletis* in **Figure 1e,g**). Further, genome-wide patterns of genetic divergence in natural populations of *Rhagoletis*—and to a lesser extent *Timema*—were correlated with allele frequency change

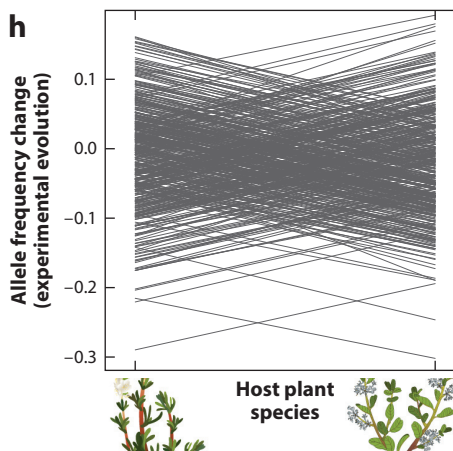
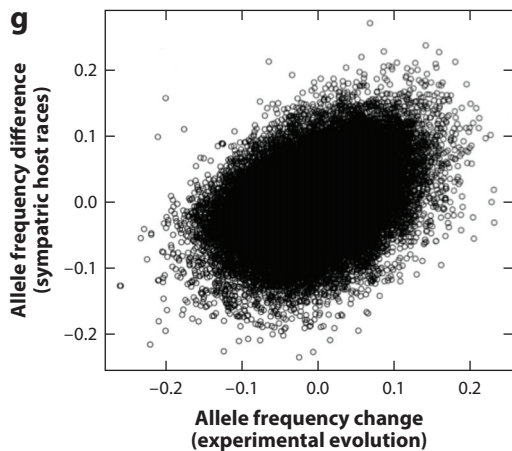
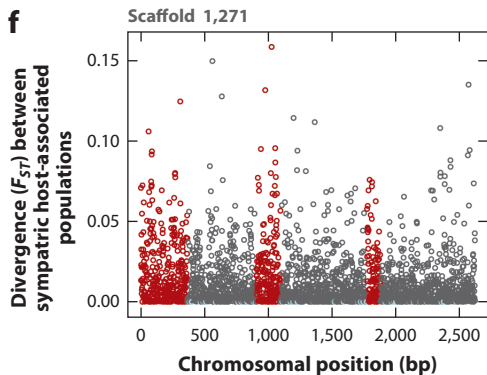
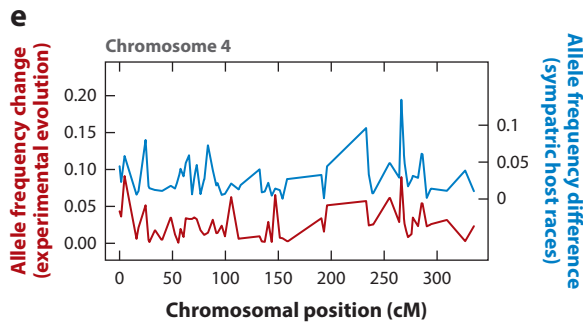
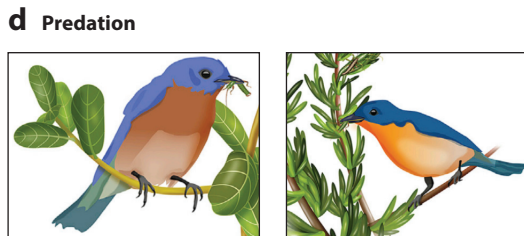
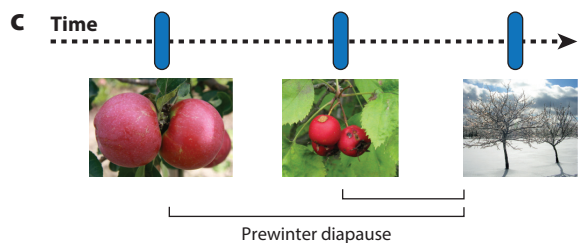
Genomic divergence:

differences in allele frequencies or the number of fixed nucleotide differences between populations or species, as interrogated across the genome

Linkage

disequilibrium (LD):

nonrandom association of alleles at different loci; alleles in LD co-occur in a chromosome more often than expected by chance



over a single generation of experimental selection (Soria-Carrasco et al. 2014, Egan et al. 2015; **Figure 1g**). 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 that occupy two habitats further support a role for natural selection to drive heterogeneous genomic divergence during host race formation (Feder et al. 2014, Flaxman 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 coselected 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 with inferred models of demography and gene flow (Sousa & 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 (e.g., 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*, *Rbagoletis*, 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 *Rbagoletis* than *Timema*. Egan et al. (2015) hypothesize that higher LD in *Rbagoletis*—particularly due to extensive polymorphism

Figure 1

Genomic evidence that adaptation drives divergence in *Rbagoletis pomonella* (left) and *Timema cristinae* (right). (a) *R. pomonella* oviposits in apple and hawthorn fruit, in which larvae develop. (b) *T. cristinae* feeds externally on *Adenostoma* sp. (left) and *Ceanothus* sp. (right). On leaves, green morphs with a dorsal stripe are most cryptic on *Adenostoma*, and morphs without a dorsal stripe are most cryptic on *Ceanothus*. A third morph (b, center) is cryptic on stems. (c,d) The length of prewinter 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 (red) and low (gray) divergence (F_{ST}) between sympatric host-associated *T. cristinae* populations are small and finely interspersed throughout the genome. (h) Loci exhibiting exceptional allele frequency change in *T. cristinae* populations experimentally transplanted onto two host plant species, *Adenostoma* (left) and *Ceanothus* (right). Photographs in panels a and b (left to right), were taken by Hannes Schuler, Andrew Forbes, Aaron Comeault, Moritz Muschick, and Aaron Comeault. Panel b (center) © Moritz Muschick, 2012. Panel d modified and reprinted from Comeault et al. (2015b) with permission from Elsevier. Panels e and g reprinted from Egan et al. (2015) with permission from John Wiley and Sons. Panels f and h reproduced from Soria-Carrasco et al. (2014) with permission from the American Association for the Advancement of Science.

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 that confer an advantage in the same habitat (Kirkpatrick & 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 (Ragland et al. 2011); 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* (Jaquiery et al. 2012), loci affecting diapause were enriched in *Rhagoletis* (Michel et al. 2010, Egan et al. 2015), and loci affecting ion binding, possibly underlying divergently selected cryptic pigmentation phenotypes, were enriched 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 only 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 finding could result from different genetic routes to parallel phenotypic changes, divergent selection of different phenotypes among host pairs in each locality (although phenotypic convergence in parallel host adaptation is high in *Timema*; Nosil et al. 2012), or a strong contribution of stochastic processes (e.g., genetic drift) to the emergence of genomic regions exhibiting high host-associated divergence.

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 supporting a major role for plant defensive chemistry in driving genomic divergence is so far lacking within each of these three model systems. However, functional studies of divergent loci are needed to examine the role of plant defensive chemicals. Specifically, both *Acyrtosiphon* salivary and *Timema* ion-binding proteins could function in suppressing plant defense (Elzinga & Jander 2013), 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 & Via 2001). Host preference also differs among *Rhagoletis* races, although the genetic basis of this variation has not yet been uncovered (Forbes & 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 & Graham 1988). Predatory birds have been 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 selective agents that maintain 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 host-race divergence in insects 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 different host species (or other types of variable habitats) is far from ubiquitous (Drès & 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 (Da Cunha et al. 1950) 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. Single-locus population genetic models soon confirmed his intuition (**Figure 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 & 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 interhabitat 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 the 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.

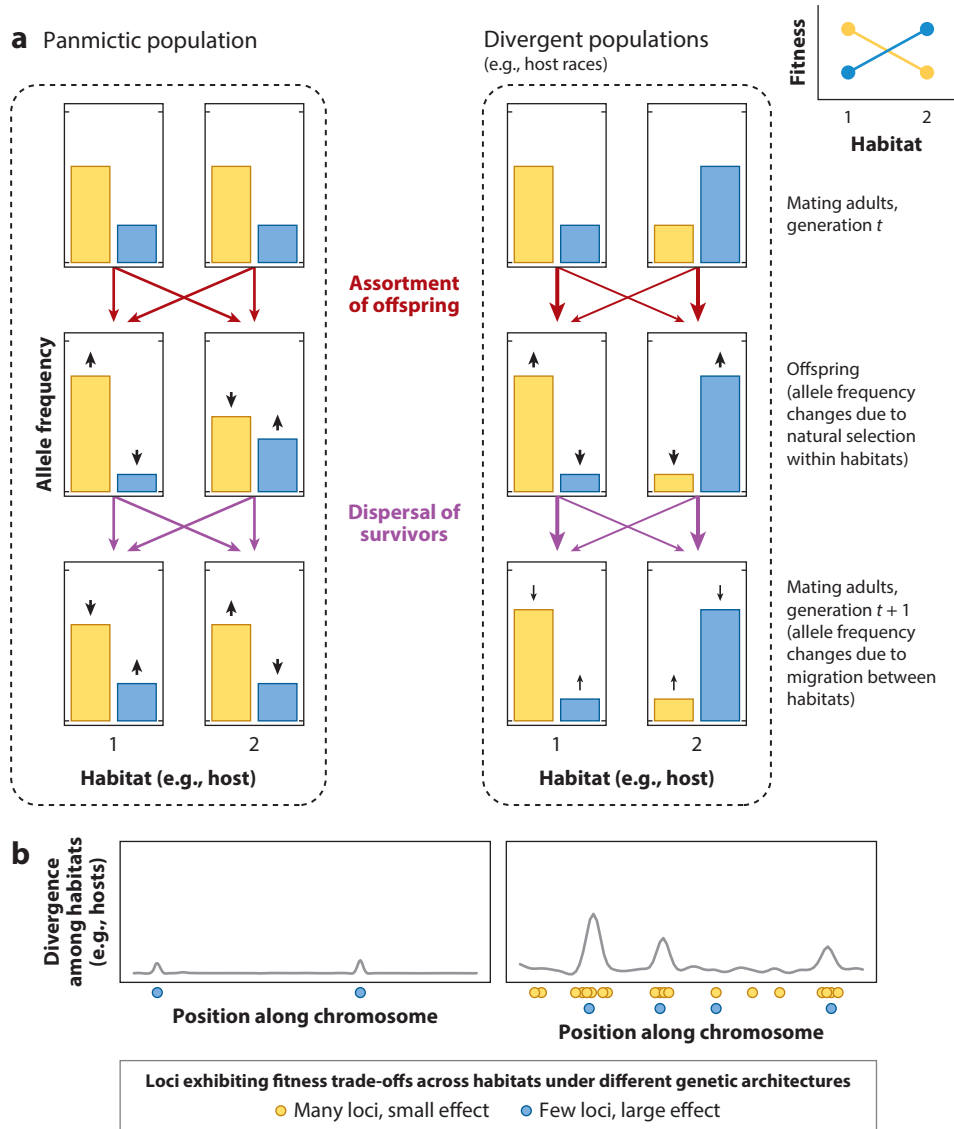


Figure 2

Trajectories of habitat-specific genetic differentiation under two modes of spatially varying selection. A panmictic population is shown on the left, whereas 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 trade-off across habitats. (Left) Allele frequencies diverge due to natural selection favoring different alleles in habitats 1 and 2, but random dispersal of survivors in 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 are greatly affected by selection at linked sites as genome-wide divergence builds up in the latter scenario. (b) 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 (left) panmixia but can subsume broad regions (including targets of selection and physically linked neutral sites) in (right) differentiated host races or locally adapted populations.

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 & Baldwin 2016).

Condition 1: Insects frequently inhabit spatially variable environments. Although 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 the sidebar, More Than Just Host Species). The process of adaptation driven by habitat variation thus warrants major consideration as a general property of plant–herbivore interactions, even in insects that narrowly specialize on a single host plant species.

Condition 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 was 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 suggest that 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 hypothesis that specialization may not always be the optimal feeding strategy is consistent with the observation that 90% of herbivorous insect species are oligophagous; the remainder are monophagous or polyphagous (Bernays & Graham 1988). Phylogenetic analyses reveal that although specialist insects evolve from generalists, generalists also frequently evolve from specialists (Janz & 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, although adaptation likely drives divergence among sympatric host- or habitat-associated populations in some cases (Berlocher & Feder 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 & Otto 2011).

Condition 3: Alleles at individual loci exhibit fitness trade-offs across habitats. SVS is only expected to maintain genetic variation within a population (e.g., Levene 1953) at loci conferring a fitness trade-off across habitats. Studies measuring performance of large numbers of insect genotypes on two or more host species often fail to observe host-associated trade-offs (Scheirs et al. 2005, Forister et al. 2012). However, such studies often suffer from four limitations. First, tests for trade-offs are typically conducted at a subset of an insect's life stages, which may not

always be the stage at which trade-offs 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 always 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 trade-offs when tests for trade-offs 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 trade-offs across hosts have employed selection experiments and quantitative trait mapping (Hawthorne & Via 2001, Scheirs et al. 2005), which overcome one or more of these four 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 trade-offs by narrowly mapping loci underlying fitness variation and quantifying their effects across habitats. Loci exhibiting host-dependent trade-offs in larval performance were rare in a recent GWAS study (Gompert et al. 2015). However, this finding does not preclude trade-offs at other life stages, and much larger sample sizes may be required to adequately test for trade-offs 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 green/melanized polymorphism in *T. cristinae*, which feeds on two host species (Comeault et al. 2015b; **Figure 1b**). Although crypsis in the green *Timema* morph involves a dorsal stripe phenotype favored on one host and disfavored on another (**Figure 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 interhost differentiation among geographically paired populations on each host species. This finding 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 (Comeault et al. 2015b). 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 on the green bark of one host, whereas the melanized morph is cryptic only 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 for 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 & 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 & Ebert 2004). From a population genetic perspective, loci can remain adaptively differentiated among populations only if they confer a fitness trade-off across habitats; otherwise, gene flow homogenizes allele frequencies across populations (Kawecki & Ebert 2004, Savolainen et al. 2013).

The rich theoretical framework for local adaptation in host–parasite interactions is highly relevant to herbivorous insects (Kaltz & Shykoff 1998, Greischar & Koskella 2007). In fact, to our knowledge, the first parasite shown to be locally adapted was an herbivore: Intra- and intertree 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 & Alstad 1978). Local adaptation to individual trees, termed adaptive deme formation, may be relatively common (Mopper 1996). Though studies of insect herbivores that identify genomic regions underlying local adaptation and the selective pressures driving their evolution are generally lacking, 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 & Zangerl 1998).

Although local adaptation by parasites is often observed in host–parasite systems, it is far from a general rule (Kaltz & Shykoff 1998). In fact, theory suggests cases in which hosts, rather than parasites, may be expected to maintain an advantage in the coevolutionary 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 a 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 & 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. Although 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 *Drosophila 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, as interpopulation differences in life history strategy depended on the host species on which flies were reared (e.g., a genotype \times environment interaction), in a direction indicative of local adaptation. Differences in gene expression paralleled phenotypic differences between populations: Interpopulation 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

Genetic architecture:

genetic basis of phenotype variation, including identity and number of loci influencing the trait and effects on trait variation

in detoxification, metabolism, and sensory perception—that exhibited host-dependent expression level differences between populations of *Drosophila mettleri* specializing on different host cacti (Hoang et al. 2015). Although 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 postdivergence 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 rates, 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 *Drosophila 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; this finding is consistent with a general role for these loci in toxin resistance (Whiteman et al. 2012). These results, combined with phenotypic convergence for *Morinda* tolerance in *D. sechellia* and Mayotte *D. yakuba* also observed by Yassin et al. (2016), suggest 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 (*Lycia 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 study was employed to identify nucleotide polymorphisms affecting larval performance on each host. 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 trade-offs across host plants. This observation is surprising, given that locally adaptive genetic variation is expected to persist in the face of gene flow only at loci conferring fitness trade-offs across environments (Kawecki & Ebert 2004). However, this finding warrants a cautious interpretation, as experimental and statistical issues make tests for trade-offs prone to false negative results (Colautti et al. 2012).

As studies of local adaptation in herbivorous insects are extended to many species and populations, 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. For example, a geographic mosaic of phenotypes oscillating under frequency-dependent selection has been 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 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 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, SVS's 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, whereas alleles with small effects on fitness are likely to be lost to genetic drift or swamped out by migration between habitats (Yeaman & Otto 2011, Yeaman & Whitlock 2011). This does not, however, necessarily mean that large-effect alleles 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; see Flint & Mackay 2009, Rockman 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 & 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 (Table 1). 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 nonrandom geographic correlation of individual alleles with environmental variables (Wang & 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; see Comeault et al. 2014, Gompert et al. 2015) or the 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

Functional genetic variation: genetic variation that affects phenotypes or fitness (e.g., non-neutral variation)

Table 1 Genomic methods to detect loci responding to spatially varying selection (SVS)

Method	Power to detect loci with small effects on fitness or weak response to selection ^a	Key benefits from large sample sizes or numbers of replicates	Key conceptual or empirical evaluations of power and mapping resolution
Population genomic scans	Usually <i>low</i> , but possibly <i>moderate</i> when (a) individuals are sampled from different habitats many times independently and (b) the population is panmictic across habitats or habitat variation doesn't strongly correlate with population structure ^b	Replicating interhabitat 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 linkage disequilibrium (LD) and 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 the 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	Kofler & Schlötterer 2014, Franssen et al. 2015, Levy et al. 2015, Terhorst et al. 2015
In vitro functional genomics (e.g., genome editing)	<i>High</i> , but only interrogates a single locus chosen a priori rather than the whole genome	N/A	Turner 2014, Li et al. 2015

Abbreviation: N/A, not applicable.

^aWe 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 2012).

^bA caveat: If fitness is highly polygenic (such that mutations affecting fitness arise at a high rate) or has high genetic redundancy, different alleles will respond to SVS in different populations (Kelly 2006, Yeaman 2015), greatly reducing a study's power to detect small-effect loci.

regions responding to specific selective agents (Schlötterer et al. 2015). Experiments can range in duration from single to many generations and can be conducted in the lab (Egan et al. 2015) or in nature (Soria-Carrasco et al. 2014). In vivo functional genomics are increasingly feasible in nonmodel systems thanks to rapid advances in genome-editing technology (Groen & Whiteman 2016). 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 (clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9).

6.3. Limitations to Methods for Detecting Variation Maintained by SVS

With the exception of in vivo functional genomics, the above approaches 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 can also impede the identification of loci with relatively small effects on fitness (**Table 1**). Accordingly, in vivo functional genomic techniques are necessary to validate candidate loci identified through other approaches and understand how much functional genetic variation is maintained by SVS. For example, Chakraborty & Fry (2015) used genome editing to demonstrate a habitat-dependent fitness trade-off at two alleles of a detoxification enzyme in *D. melanogaster*. The trade-off had been predicted on the basis of 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 that the magnitude of this trade-off 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. One example that may be used is 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 of characterizing the genetic architecture of traits impacted by SVS are illustrated through two recent GWAS studies. Comeault et al. (2014) explored the genetic basis of 10 traits divergently selected across host plant species in *T. cristinae*, whereas 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 single nucleotide polymorphisms (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 narrowly mapping loci involved in 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 remain at a relatively intermediate equilibrium frequency. Second, LD should decay quickly around these loci (Charlesworth 2006). These two patterns are ideal because techniques to narrowly map loci influencing fitness, such as GWAS (assuming high marker density) and evolve and resequence approaches, are most effective for detecting alleles that are at intermediate frequency and have low LD with noncausative loci. The promise of narrowly mapping individual loci affecting fitness in panmictic populations experiencing SVS contrasts with cases of host race formation, in which reduced interhabitat gene flow is expected to facilitate LD among coselected alleles at different loci (Feder et al. 2014).

To leverage these advantages, we recommend identifying genetic variation maintained by SVS in panmictic populations through the following approach: (a) Use mapping techniques, such as GWAS and the evolve and resequence approach, to identify candidate loci underlying host-associated fitness trade-offs. (b) Identify loci showing parallel host-associated divergence across many pairs of natural populations. (c) For loci shared between the first two approaches, use genome editing, if possible, to validate fitness trade-offs. (d) Using estimated selection coefficients for each locus (Gompert et al. 2014) and (optionally) information about the abundance of each host species, apply models such as 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.

SUMMARY POINTS

1. Theory predicts that habitat variation, such as variation among host species, can maintain genetic variation within and between herbivorous insect populations.
2. Genomic studies of host races and locally adapted populations, which involve low rates of gene flow among habitats, generally support this prediction.
3. 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.
4. 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.

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