THE IMPORTANCE OF THE ONTOGENETIC NICHE IN RESOURCE-ASSOCIATED DIVERGENCE: EVIDENCE FROM A GENERALIST GRASSHOPPER

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Abstract.—Geographic variation in resource use can produce locally adapted populations that exhibit genetic and phenotypic divergence. In the bird-winged grasshopper (Schistocerca emarginata = [lineata]), we investigate whether genetic data exist in accordance with geographic variation in resource (host) use and coloration. In Texas, juvenile grasshoppers feed almost exclusively on one of two host plants, Rubus trivialis (Rosaceae) or Ptelea trifoliata (Rutaceae), whereas adults of both forms are dietary generalists and consume many plants from unrelated families. Along with differences in juvenile feeding, differences in a density-dependent color polyphenism are concordant with genetic (mitochondrial DNA) variation among eight populations of the bird-winged grasshopper. Forms feeding on R. trivialis and those feeding on P. trifoliata represent monophyletic lineages according to phylogenetic analysis and maximum-likelihood tests of two alternative phylogeographic hypotheses for geographic variation in host use. Character-state optimization of host-plant acceptability on a phylogeny containing S. emarginata and outgroup taxa indicates that populations consuming R. trivialis gave rise to populations consuming P. trifoliata. Juvenile grasshoppers that consume P. trifoliata acquire deterrence against predation, suggesting that enemy-free space facilitated this host shift. In extant populations, adaptations stemming from alternative resource use during ontogeny present possible barriers to gene exchange. This study represents the first demonstration of resource-associated divergence in an otherwise generalist insect that exhibits temporal variation in resource use, characterized as developmental changes in host specialization. Our findings suggest that exploitation of different resources may have unexplored significance for generalist species that compartmentalize specialization to particular life stages.

Key words.—Divergence, generalist, genetic, host plant, ontogeny, Schistocerca, specialist.

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The evolution of reproductive isolation as a consequence of divergent selection in alternative environments has been a central tenet in models of speciation (e.g., Mayr 1942; Schluter 1996, 1998). In herbivorous insects, host-plant specialization plays a distinct role in the evolution of reproductive barriers because geographic variation in resource association influences the environmental context of selection. Host-plant specialization limits gene exchange if populations that exploit alternative plant species exhibit phenotypic differentiation arising from differences in host-plant use and if such adaptations directly or indirectly lead to assortative mating or the production of inferior hybrids (Bush 1994; Feder 1998).

Parasitic insects, which require the use of a single host individual for normal growth and development, are excellent candidates for studying host-associated divergence, primarily because their life history necessitates extreme specialization. Moreover, their relative immobility during periods of dietary specialization (e.g., during holometabolous development) facilitates detection of population-level differences in specificity and, consequently, the historical effects of host-associated reductions in gene flow. When also considering the abundance of parasite organisms (Bush 1975a), it is not surprising that recent investigations of host-associated divergence have been dominated by host specialists with parasitic lifestyles (e.g., moths, Menken et al. 1981; treehoppers, Wood and Guttman 1983; apple maggot flies, Feder et al. 1988; ball-gallmaker flies, Craig et al. 1997; soapberry bugs, Carroll and Boyd 1992; sawflies, Roininen et al. 1993; leaf beetles, Funk 1998; aphids, Via 1999). However, the real pattern of host-associated divergence may extend beyond herbivorous insects with parasitic life histories. If true, such a taxonomic bias in the type of species studied would give the interaction inappropriate significance as a mechanism that generates biological diversity. If parasitic insects have been emphasized in investigations of host-associated divergence because their life history favors extreme specialization, then it is the presumed uniqueness of their developmental program that has deemphasized the study of other herbivorous insects.

The grazing lifestyle is commonly expected to be associated with polyphagy (Thompson 1994). That is, species whose members feed on multiple individuals also tend to feed on many species from unrelated families. This relationship likely exists because the advantages of polyphagy—improved growth from diet mixing (Bernays and Bright 1993) and associative learning (Bernays and Chapman 2000)—are facilitated by high mobility (Chapman 1990). Probably no other insect group epitomizes the grazing-polyphagy relationship more than the herbivorous Orthoptera, specifically the grasshoppers (Chapman and Sword 1997). However, even grazing organisms can exhibit resource specialization for all or part of their life history.

The idea that the pattern of an organism’s resource or habitat use changes as it increases in size, from birth to its maximum, is called the ontogenetic niche (reviewed in Werner and Gilliam 1984). Although this concept has received much attention from ecologists, particularly with those studying aquatic communities, it seems to have been largely over-
looked by many evolutionary biologists interested in specialization and, more generally, in adaptation. This is unfortunate because the ontogenetic niche offers a temporal perspective of variation in habitat or resource use that can direct investigation toward life stages in which specialization and the propensity for resource-associated adaptation is most likely. In one common ontogenetic pattern, the average number of used resources increases through ontogeny, with resource breadth changing from narrow early in life to broad later in life. If resource composition were to vary between populations during this “ontogeny of specialization” (Thompson 1994), then divergent adaptations could evolve that influence gene flow in much the same way as in parasitic lineages.

The concept of the ontogenetic niche has been particularly useful for studying the bird-winged grasshopper, *Schistocerca emarginata* (= *lineata*; Hubbell 1960) (Orthoptera: Acrididae). Although initial investigation of adult populations in Texas suggested that the bird-winged grasshopper exhibited the typical grazing-polyphagous relationship, further inspection showed a clear developmental shift in resource breadth (Sword and Dopman 1999). Juvenile *S. emarginata* exhibit a narrow diet breadth consisting primarily of a single species of host plant, despite maintaining a high level of mobility within their habitat. Moreover, juveniles are highly discriminative in their host-plant choice, with Texas populations feeding on either *Ptelea trifoliata* (Rutaceae; hereafter referred to as *Ptelea*) or *Rubus trivialis* (Rosaceae; hereafter referred to as *Rubus*). These findings underscore two patterns that are common in other herbivorous insect groups: that diet is not an invariant property of species and that even grazing insects may exist as local host-specific populations (Fox and Morrow 1981). Recognizing local host-plant specificity in *S. emarginata*, however, required knowledge of the ontogenetic niche with specialization and generalization occurring at endpoints along a developmental continuum, a pattern never before demonstrated in Orthoptera.

If temporal changes in host-plant specialization create an ontogenetic niche in *S. emarginata*, whereby resource use and the associated biotic and abiotic interactions scale with periods of life history, then populations specializing on *P. trifoliata* and those specializing on *R. trivialis* should exhibit host-associated adaptations. Reciprocal survivorship experiments suggest a level of host-plant adaptation, with *Rubus*-feeding juveniles being unable to survive on a diet of *Ptelea*, whereas *Ptelea*-feeding juveniles survive on *Rubus* (Sword and Dopman 1999). Moreover, *Ptelea*-feeding juveniles express density-dependent warning coloration and are unpalatable to predators when feeding on *Ptelea* (Sword 1999, 2001). *Rubus*, however, is an ineffective deterrent, and *Rubus*-feeding juveniles are much less responsive to changes in density. If such host-plant adaptations incidentally create a barrier to gene exchange between populations specializing on *Ptelea* and *Rubus* following a historical host shift, then genetic differentiation at neutral loci should exist, with grasshoppers sharing a common host also sharing a common history. Conversely, if a single, highly polymorphic lineage has colonized a second host plant and gene exchange is unaffected by observed variation, then no dependent relationship should exist between diet, coloration, and neutral genetic variation. We test these hypotheses through a parametric bootstrapping technique under maximum likelihood, which assesses the significance of phylogeographic topologies expected under each hypothesis.

**Materials and Methods**

**The Study System**

*Schistocerca emarginata* is a polyphagous, highly mobile, grazing insect that lives throughout central North America (Hubbell 1960). The ecology of host-plant use has been studied in both juvenile and adult populations at localities in Texas (Fig. 1; Sword and Dopman 1999). Juveniles from BBSP, ALT, and LWSP are dietary specialists on *Rubus*, and *Rubus* comprises 100%, 95%, and 80% of the respective population-level diets. Juveniles from BFL, PFSP, and KSSP specialize on *Ptelea*, which constitutes 93%, 97%, and 95% of the population-level diets, respectively. *Ptelea* does not live at BBSP, ALT, and LWSP, and *Rubus* does not live at BFL, PFSP, and KSSP. At the LHCP site both host-plants grow, and juveniles are dietary specialists feeding on either *Rubus* or *Ptelea* at 93% and 92% of the population-level diets, respectively. Although diet was not tracked through ontogeny in these populations, the proportion of adults with more than one type of plant in their diet is significantly greater than in juveniles, and the numbers of plants in the adult population diet is also significantly greater (Sword and Dopman 1999). These data suggest that the increase in population-level generalization is due to an increase in individual generalization, instead of an increase in individual specialization on different host species. In this study, we use specimens from these populations.

**Specimen Collection**

In spite of a systematic search of all plants at each locality, juvenile *S. emarginata* were only discovered on or within the proximity of *Rubus* or *Ptelea* host plants, with 17 (BBSP) to 61 (LHCP) juveniles being collected per site (Sword and Dopman 1999). Population-level diet was determined by microscopic fecal analysis, and plant availability was determined from 30-m point transects traversing the collection area (Sword and Dopman 1999). Following diet analysis, we randomly chose two individuals for the genetic analysis from each site collection (e.g., BBSP A and B). Four juvenile grasshoppers were chosen from the mixed locality at LHCP, two from each host plant; LHCP A and B were *Rubus*-feeders and LHCP C and D were *Ptelea*-feeders. We included three congeneric grasshoppers in the genetic analysis to serve as outgroup taxa: *Schistocerca obscura*, *S. americana*, and *S. nitens*.

**Mitochondrial DNA Sequencing**

We extracted genomic DNA from leg muscle in a 5% Chel-ex (Biorad, Hercules, CA) solution by vortexing for 20 sec and heating at 95°C for 15 min. We amplified a region of mitochondrial DNA (mtDNA) encompassing a portion of the 16S rRNA gene, valine, and a portion of the 12S rRNA gene. Our oligonucleotide primers were designed from Simon et al. (1994) and amplified a region of approximately 858 bp: 16Sa, LR-J-13417 (5’-ATGTTTTGATAAACAGGCG-3’).
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Fig. 1. Approximate distribution of Rubus trivialis (Rutaceae), Ptelea trifoliata (Rosaceae), and sampled Schistocerca emarginata localities in Texas. Details may be found in Sword and Dopman (1999). LHCP contained juveniles that consumed both P. trifoliata and R. trivialis. Juveniles at PFSP, BFL, and KSSP all consumed P. trifoliata, and R. trivialis was absent from these localities. Juveniles at LWSP, ALT, and BBSP all consumed R. trivialis, and P. trifoliata was absent from these localities.

and 12Sc, SR-N-14275 (5'-AAGGTGGATTGATAGT AAT-3').

Polymerase chain reactions (PCRs) used 2 µl Chelex extracted template in a final volume of 100 µl with a final concentration of 100 µM each of primers 16Sa and 12Sc, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 0.001% gelatin (Sigma, St. Louis, MO), 0.12 mM dNTPs, and 0.5 units of Taq polymerase. PCR cycling conditions were: initial denaturing for 5 min at 94°C; 35 cycles of 1 min at 94°C, 1 min at 48°C, and 1 min at 72°C; and a final extension for 5 min at 72°C. Following amplification, PCR products were purified using Wizard PCR Preps DNA Purification System (Promega, Madison, WI) and the 16Sa primer was used to create single-stranded DNA that was sequenced using an ABI 377 automated sequencer (Perkin Elmer, Foster City, CA). Automated dye-terminator based chemistry yielded approximately 700 bp per sample.

Alignment and Phylogenetic Analysis

We checked each sequence against the ABI trace file for errors and then trimmed by eye in EDITSEQ (DNASTAR, Madison, WI). Sequence data for all 19 taxa were then entered into the sequence alignment program, MEGALIGN (DNASTAR), and aligned with a gap and gap length penalty of 10. Phylogenetic inference was carried out using PAUP* phylogenetic analysis program using either maximum-parsimony (MP) or maximum-likelihood (ML) optimality criteria (Swofford 2000). For our first analysis under MP, we employed an exhaustive search algorithm, in which gaps were treated as a fifth base and haplotypes representing outgroup taxa were transferred to the outgroup category. Our second MP analysis used these same conditions, but a nonparametric bootstrap with a heuristic search algorithm was used to assess support for clades. For bootstrapping, the starting trees were obtained using stepwise addition, the addition sequence was random, the Multrees option was in effect, and TBR was the branchswapping algorithm. Our ML analysis also employed a heuristic search, but outgroup taxa were excluded. We chose F81 as our model of base substitution with rate heterogeneity (λ), which was assumed to follow a discrete gamma distribution with four rate categories. ML options also included estimating starting branch lengths using Rogers-Swofford approximation, the addition sequence was as is, the base frequencies were empirical, no sites were invariable, the Multrees option was in effect, and TBR was our branch-swapping algorithm.

Test of Tree Topologies

We obtained estimates of phylogenetic support using non-parametric and parametric approaches. A nonparametric bootstrap analysis (Felsenstein 1985), which allows a determination of support for particular nodes, was performed on our data with 10,000 pseudoreplicates. However, such an analysis is ineffective for testing specific a priori evolutionary hypotheses about the whole tree topology (see Hillis et al. 1996, pp. 523–526). Conversely, parametric bootstrapping (i.e., Monte Carlo simulation) under ML allows statistical testing of alternative phylogenetic topologies by simulating under null hypotheses (e.g., Hillis and Huelsenbeck 1994;

We used Monte Carlo simulation under ML to test two phylogeographic hypotheses for Texas populations of *S. emarginata*. Our first hypothesis states that geographic variation in juvenile host-plant use and coloration is due to genetic variation within a single, highly polymorphic lineage. If this is true, we expect host use and coloration to be independent of haplotype sharing in a phylogeny created from markers unlinked to these traits (e.g., mtDNA). Thus, a pattern of isolation-by-distance (IBD) may accurately reflect genetic differences between populations at neutral loci. However, because geographic distance seems correlated with host-plant use (Fig. 1), and the true influence of geographic distance on gene flow is unknown, a conservative estimate of an IBD pattern for the purposes of establishing the presence of a single lineage would require that individuals from the mixed LHCP locality share a common history, to the exclusion of insects from other populations that share a common host. Haplotype sharing for much of the genome should exist in accord with juvenile host-plant use and coloration across all populations. Again, because the influence of geographic distance on gene flow is unknown, haplotypes within host groups are free to vary: host divergence hypothesis (T_H) = ((Ptelea-feeding grasshoppers), (Rubus-feeding grasshoppers)).

These hypotheses are extremes on a continuum, and both factors could influence the real topology. However, the real tree will likely have a structure that is more similar to one or the other of these null topologies. The topologies represented by the genetic variation (T_C) and host divergence (T_H) hypotheses were used as monophyly constraints in two ML analyses in PAUP* with all *S. emarginata* taxa (outgroup taxa omitted). When using a monophyly constraint in PAUP*, a hypothesised tree is consistent with the constraint if it contains the specified relationship of the taxa in the constraint. Relationships not explicitly represented in the constraint are free to vary in their connection to the tree.

We noted ML scores from the constrained searches and used the best tree consistent with each hypothesis as a model for simulation (genetic variation model = T_C; host divergence model = T_H). Branch lengths and the tree topology from each tree were entered into EVOLVER, an application in the PAML package (Yang 2000), and 100 datasets for each hypothesis were simulated under the same conditions used in the ML analysis in PAUP*. A null distribution was generated from differences in ML scores for each simulated dataset under the null hypothesis (T_C or T_H) and the alternative hypothesis, which was the tree maximized under ML. Test statistics were created from the difference in ML scores between the tree maximized from the original dataset (T_M) and null trees (T_C, T_H), which were also from the original data. These statistics were tested against their null distributions, using an α = 0.05 for statistical significance (one-sided). Phylogenetic estimation in PAUP* and simulation in PAML used the F81 + λ (discrete) model of base substitution.
with no invariant sites and empirical base frequencies. Additional ML parameters in PAUP* searches included: starting branch lengths using Rogers-Swofford approximation, addition sequence as is, Multrees option was in effect, and TBR was the branch-swapping algorithm.

**RESULTS**

Following trimming, we deposited each 609-bp sequence in GenBank under accession numbers AF155548–AF155566. Excluding outgroup taxa, five different haplotypes were found from the 609-bp mtDNA fragments. Our exhaustive search under MP yielded one most-parsimonious reconstruction (tree length = 51, CI = 0.96, RI = 0.93, HI = 0.04; Fig. 2). Including outgroups, there were 42 variable sites, 21 of which were parsimony informative (Table 1). Six of the parsimony-informative sites distinguished insects with a juvenile diet of *Ptelea* or *Rubus*, and separated the *S. emarginata* populations into two sister clades (three transitions, two transversions, one indel; Fig. 2; Table 1). Additional clades existed within the *Rubus* group, but no additional parsimony-informative characters existed within the *Ptelea* group.

Our unconstrained ML analysis, which included all *S.*
Schistocerca emarginata and no outgroups, reconstructed a tree with ML score of \(-733.84\) (\(T_{\text{MAX}}\); Fig. 3). The ML reconstruction confirmed the exclusive relationship between populations that were feeding on different juvenile host plants. The branch-length structure within the Rubus-hosting clade was more than within the Ptelea-hosting clade ((LHCP A, LHCP B); 0.0016); ((LHCP A, LHCP B), ALT B; 0.0016); vs. zero in Ptelea clade). The ML score obtained by using F81 + \(\lambda\) and other simple parameters (e.g., empirical frequencies) did not substantially differ from scores obtained by using more complex parameters (e.g., HKY + \(\lambda\)), thus we chose the less complex model to reduce computation time for Monte Carlo simulation.

Tests of Tree Topologies

Nonparametric bootstrapping with all the data yielded high support for the node leading to S. emarginata at 84% and nodes for Ptelea and Rubus host forms at 70% and 97%, respectively (Fig. 2). These groups are considered well supported because proportions of 70% or more often indicate high probability that a given phylogenetic inference is real (Hillis and Bull 1993). Estimates of nodal support were weaker for (LHCP A, LHCP B, ALT B) and (LHCP A, LHCP B) at 63% and 63%, respectively.

Our constrained ML analyses using all S. emarginata haplotypes and no outgroups found the best trees consistent with our two null hypotheses (Figs. 3, 4). The ML score for the genetic variation hypothesis was \(T_{G}^{M} = -767.03\), and the ML score for the host divergence hypothesis was \(T_{H}^{M} = -733.84\). The topology of \(T_{G}^{M}\) was substantially different from \(T_{\text{MAX}}\), the best-unconstrained tree, but \(T_{H}^{M}\) was concordant with this topology.

The best trees consistent with the null hypotheses (Figs. 3, 4) served as models for simulation using EVOLVER (Yang 2000). Conditions for simulation were identical to conditions used in PAUP* to reconstruct the null and alternative hypotheses. Each simulated dataset consisted of 16 sequences of 609 bp. One hundred datasets for each null hypothesis were generated. Following simulation, each replicate dataset was analyzed using ML under the null (\(T_{H}^{M}\) or \(T_{G}^{M}\)) and alternative (ML maximized) hypotheses. Estimated parameters from each simulated dataset were fixed from the null hypotheses for all ML analyses under the alternative hypothesis, as they are stable over different topologies (e.g., Yang 1997).

Null distributions (one-sided) were plotted to test the significance of each null hypothesis. This distribution consisted of differences in ML scores between trees maximized (\(T_{\text{MAX}}\)) and consistent with the null hypothesis (\(T_{H}^{M}\) or \(T_{G}^{M}\)) for each of the simulated datasets (100 per hypothesis). The test
statistics, the differences in ML score between the maximized tree from the original data (T_{\text{Max}}) and the best tree consistent with the null hypotheses (T_{null}), were plotted against their null distributions. The differences in ML scores between the maximized tree (T_{\text{Max}}) and the constraint tree (T_{\text{null}}) were all less than 5.0 for the 100 simulated datasets, whereas the difference in this test statistic for the actual data was 33.19 ($P \ll 0.01$; Fig. 5). Conversely, the null hypothesis of host divergence could not be rejected because the ML score of the statistic did not fall outside of the expected distribution. This result was expected because the topologies and likelihood scores were identical for the alternative and null hypotheses (Fig. 3).

**DISCUSSION**

Resource-associated divergence describes the correspondence of phenotypic and neutral genetic differentiation to alternative resource or habitat use. Although this differentiation could arise because of random drift among isolated populations, selection plays a critical role if adaptations stemming from the use of different resources limit gene exchange. Resource-associated divergence has been well documented in parasitic insects adapted to different host species, but it has never been demonstrated in a generalist species with a grazing life history. This is probably because their high mobility makes detecting differences between populations difficult and because a common assumption is that grazing individuals do not specialize on any particular resource.

In the bird-winged grasshopper, a polyphagous species that exhibits a grazing life history, host-associated divergence was investigated by using Monte Carlo simulation, which permitted testing of two possible hypotheses for observed variation in resource use and adaptive coloration. One process that could account for our observations of phenotypic differentiation between juvenile S. emarginata feeding on Rubus and Ptelea is if a single lineage colonized a second host plant. If gene exchange is unaffected by host-specific adaptations and diet, then mating should be random among individuals from the mixed LHCP locality (at least with respect to juvenile host-plant use), in which case they may be more closely related to each other than they are to individuals from allopatric populations (T_{null} above). Conversely, populations having evolved adaptations corresponding to differences in the environments represented by Rubus and Ptelea could have incidentally evolved barriers to gene exchange. If so, a dependent relationship may exist between coloration, resource use, and much of the neutral genetic variation that occurs within the S. emarginata genome. Specifically, genetic variation could be separated into two host-associated clades, independent of geography (T_{null} above). Parametric bootstrapping using mtDNA sequence data rejects a single lineage expressing genetic variation as a viable explanation (Figs. 4, 5), but the presence of two divergent lineages cannot be rejected because the tree representing this hypothesis is a perfect fit of our data (Fig. 3). Other patterns may provide insight into the historical process that gave rise to these lineages. Specifically, a host-shifting mode of divergence (reviewed in Bush 1994) may produce predictable differences in relative acceptability of the ancestral and the derived host plant and may allow us to identify the ancestral and derived populations of S. emarginata by association.

Assessing the identity of the derived host in S. emarginata can be accomplished through a comparative approach that maps the acceptability of Ptelea and Rubus as suitable hosts onto our phylogeny that includes S. obscura, S. nitens, and S. americana (Fig. 2). Sword and Dopman (1999) and Otte (1975) showed that Ptelea is accepted by the Ptelea host form of S. emarginata, but not by other Schistocerca species or the Rubus host form. Both S. emarginata host forms, however, readily accept Rubus. Although Rubus was not tested against the outgroup taxa, the acceptability of the plant as a host would be placed at or below the branch leading to both S. emarginata host forms, whereas the ability to consume Ptelea would be a derived state on the branch leading to Ptelea-feeding populations of S. emarginata (Fig. 2). This character-state optimization suggests that populations consuming Rubus gave rise to populations consuming Ptelea.
A Host Shift in Schistocerca emarginata via Enemy-Free Space

Successful completion of a host shift to a novel plant may be a rare occurrence for insects because it requires change in both behavioral preference for a novel host and physiological tolerance of a novel host’s chemistry and phenology (Futuyma 1983). One model of host shifting proposes that populations of dietary specialists initially undergo behavioral preference changes for a new host plant, followed by physiological performance changes (Bush 1975b; Futuyma 1983). If preference alleles cause individuals to recognize a former deterrent as an attractant, novel host-plant chemistry and phenology may be overcome by individuals who also harbor rare alleles for physiological tolerance or high performance (Futuyma 1983). Although a reasonable possibility, this model requires us to account for the spread of preference alleles for a new host when initial performance costs may exist. Considering the substantial role that plant chemistry may play in determining host use (Ehrlich and Raven 1964), this conceptual difficulty becomes compounded when accounting for shifts to unrelated plants that differ substantially in their chemical properties.

One promising resolution of this apparent preference-performance paradox results if a reduction in deleterious ecological interactions occurs when shifting to a novel host plant. These reductions would minimize initial fitness costs and increase the net benefit of novel host-plant use (Price et al. 1980; Jeffries and Lawton 1984; Gratton and Welther 1999). A new host plant may offer refuge from habitat-specific predators, parasites, and competitors associated with a previously used host plant (e.g., Brown et al. 1995; Feder et al. 1995; Gratton and Welther 1999). Moreover, although generalist natural enemies may act as a major selective force in limiting diet (Bernays 1989), they may also facilitate novel plant use when the new host provides superior enemy-free space through chemical or mechanical protection (Jeffries and Lawton 1984). This, in addition to spatial refuge from habitat-specialist enemies associated with the previous host, would ease the physiological cost of novel host-plant use. Novel preference alleles may then be advantageous relative to ancestral alleles, favoring fixation in a population and completing the host shift.

The importance of enemy-free space for the host shift in S. emarginata can be investigated by taking advantage of the ability of juveniles from Ptelea-feeding populations to consume both Ptelea and Rubus. When controlling for juvenile coloration in palatability assays with a common generalist predator, it was discovered that Ptelea-fed juveniles survived attacks, whereas juveniles that were fed Rubus never survived (Sword 1999, 2001). These data imply that preference for Ptelea may have been favored if the benefits of Ptelea-conferring unpalatability to generalist predators and escape from Rubus-associated enemies outweighed the presumed benefits of Rubus-feeding and costs of Ptelea-feeding. Physiological tolerance of Ptelea, which contains cytotoxins (Petit et al. 1993), could have evolved at any time following the shift. In extant populations, there is no obvious nutritional advantage for feeding on Rubus, which is a congener of blackberries, suggesting that any nutritional costs associated with the shift to Ptelea have since disappeared (Sword and Dopman 1999).

Adaptations during the Ontogenetic Niche as Barriers to Gene Exchange in Schistocerca emarginata

As a survival strategy against visually hunting predators, when conspecific densities are high unpalatable prey should be conspicuously colored to augment avoidance behavior in predators (Gittleman and Harvey 1980) and when densities are low cryptically colored to avoid discovery (Mallet and Singer 1987). Juvenile grasshoppers of Ptelea-feeding S. emarginata illustrate this phenomenon by expressing density-dependent warning coloration and by deriving unpalatability to predators due to the presence of noxious host-plant material (Ptelea) in their digestive tracts (Sword 1999, 2001). The plastic color polyphenism expressed by these juveniles produces insects that can be either a cryptic green or a conspicuous yellow and black in response to low and high rearing densities, respectively. Juvenile Ptelea-feeding S. emarginata in the field commonly express the high-density warning coloration because of congregation on their host plant. Adult coloration is also striking as Hubbell (1960) noted a “brilliantly colored south Texas type.” Although Hubbell lacked sufficient data to link his observations with host-use ecology, Ptelea often comprises over 50% of the adult diet (compared to 93–97% in juvenile populations; Sword and Dopman 1999), indicating that color may serve the same warning function as it does in juveniles. Conversely, Rubus does not confer chemical protection from predators, but like many plant species in the family Rosaceae, it possesses thorns on its vegetative surface that may also offer enemy-free space via mechanical protection (Jeffries and Lawton 1984). Juvenile coloration from Rubus-feeding individuals responds less to changes in population density, and juveniles show only minor changes from their normal cryptic coloration when reared at high density (Sword 1998). In the field, juvenile Rubus-feeding grasshoppers are commonly green, whereas adults are commonly tan or brown. Taken together, differences in color and juvenile specialization on Ptelea and Rubus seem to be divergent evolutionary responses to predation in different ontogenetic niches (Werner and Gilliam 1984).

Body patterning likely plays an additional role for males of a congeneric grasshopper, Schistocerca gregaria. Reproductive males actively scan the visual field when searching for mates (Wallace 1982) and search for females hidden from view, but mating propensity is unaffected when acoustic signaling of females is suppressed (Inayatullah et al. 1994). These findings suggest that S. gregaria combines visual and long-range chemical cues, but not acoustic, for mate location and recognition. If mate communication in S. emarginata is similar to its congener, Ptelea- and Rubus-feeding grasshoppers also use visual signals for mate location and recognition. Sexual isolation arising from differences in adult body patterning then becomes an obvious mechanism for incidental host-associated reproductive isolation.

Barriers in the form of hybrid incompatibility or decreased fitness would also influence gene exchange in S. emarginata. No data exist regarding this possibility because controlled mating failed in the laboratory, despite numerous attempts.
However, a very small number of potential hybrid individuals were observed in the field at the mixed LHCP locality. These juvenile grasshoppers consumed *Rubus*, but expressed the warning coloration normally associated with *Ptelea*-feeding (Sword and Dopman 1999). If these individuals represent hybrids, then they are likely experiencing incidental fitness costs associated with their expression of host-plant adaptations in the incorrect environment (e.g., Hatfield and Schluter 1999). Specifically, their conspicuous coloration and lack of chemical defense from *Ptelea* ingestion makes them palatable and visually distinctive prey for birds and lizards, two common predators in *S. emarginata* habitat. Identifying precise reproductive barriers and determining their function clearly warrants additional field and laboratory work.

**Conclusions**

In spite of the limitations of mtDNA in delimiting species boundaries (e.g., Moritz et al. 1992), the genetic discontinuity that is maintained in sympatry suggests that gene exchange has been restricted between host-specific *S. emarginata* for some time (Figs. 1–3). Considering this genetic evidence and the phenotypic differentiation between populations feeding on *Ptelea* and *Rubus*, host-associated populations merit sister-species status. Although genetic divergence among host-associated populations appears to be relatively common in parasitic herbivorous insects, such divergence has never been demonstrated in an otherwise generalist species that exhibits specialization during ontogeny. The presence of both phenotypic and genetic divergence between *Rubus-* and *Ptelea*-associated lineages of *S. emarginata* supports the notion that an ontogeny of specialization (Thompson 1994) and the ontogenetic niche (Werner and Gilliam 1984) facilitates habitat-attached lineages of *S. emarginata* facilitating a central theme in the host shifting mode of speciation (Bush 1994; Feder 1998). It seems reasonable that this pattern may be found in other stereotypical generalists, in which case the perspective of the ontogenetic niche offers promise in directing future research.

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