

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

ERIK B. DOPMAN,^{1,2,3} GREGORY A. SWORD,^{1,3,4} AND DAVID M. HILLIS^{1,5}

¹Section of Integrative Biology, School of Biological Sciences, University of Texas, Austin, Texas 78712

³Brackenridge Field Laboratory, University of Texas, Austin, Texas 78712

⁵E-mail: dhillis@mail.utexas.edu

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 parasitic 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-

² Present address: Department of Ecology and Evolutionary Biology, Cornell University, Corson Hall, Ithaca, New York 14853; E-mail: ebd5@cornell.edu.

⁴ Present address: USDA/ARS, 1500 North Central Avenue, Sidney, Montana 59270; E-mail: gsword@sidney.ars.usda.gov.

looked by many evolutionary biologists interested in speciation 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-polyphagy 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 end-points 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 created 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% Chellex (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'-ATGTTTTTGATAAACAGGCG-3')

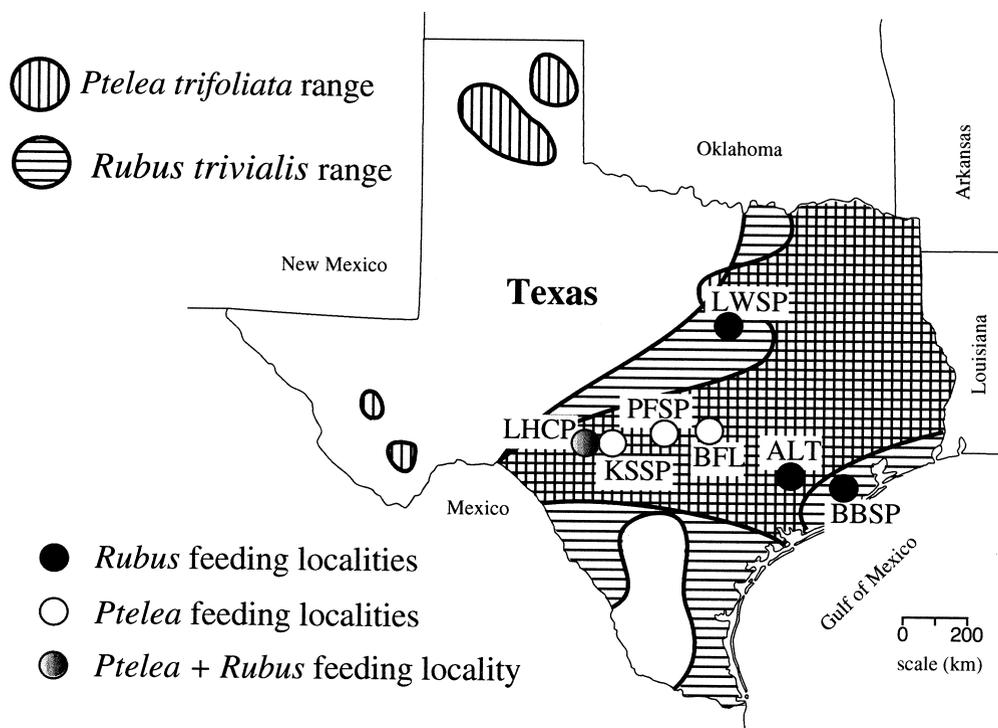


FIG. 1. Approximate distribution of *Rubus trivialis* (Rutaceae), *Ptelea trifoliata* (Rosaceae), and sampled *Schistocerca emarginata* localities in Texas. Details may be found in Sword and Dopman (1999). LHCP contained juveniles that consumed both *P. trifoliata* and *R. trivialis*. Juveniles at PFSP, 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'-AAGGTGGATTTGATAGT AAT-3').

Polymerase chain reactions (PCRs) used 2 μ l Chelex extracted template in a final volume of 100 μ l with a final concentration of 100 pM 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 branch-swapping 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 nonparametric 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;

TABLE 1. Variable nucleotide position for *Ptelea trifoliata*-feeding and *Rubus trivialis*-feeding *Schistocerca emarginata*, *S. americana*, *S. obscura*, and *S. nitens*. An asterisk indicates variable nucleotide positions informative for host-form monophyly; α , *Ptelea trifoliata*-feeding juvenile *S. emarginata*; β , *Rubus trivialis*-feeding juvenile *S. emarginata*.

Taxa		Variable nucleotide position																			
		*															*				
		1	2	3	4	8	9	9	1	1	1	1	1	1	2	2	2	2	2	2	
LHCP C	α	A	C	A	A	T	A	A	T	T	A	G	T	T	T	A	A	C	G	T	A
LHCP D	α
BFL A	α
BFL B ¹	α
KSSP A	α
KSSP B	α
PFSP A	α
PFSP B	α
ALT A	β	T	.	.	G
ALT B	β	T	.	.	G	.	.	.	C
BBSP A	β	T	.	.	G
BBSP B	β	T	.	.	G
LHCP A	β	T	.	.	G	.	.	.	C	.	.	.	A
LHCP B	β	T	.	.	G	.	.	.	C	.	.	.	A
LWSP A	β	T	.	.	G
LWSP B	β	T	.	.	G
<i>S. americana</i>	.	.	A	.	.	A	T	T	.	A	T	A	.	.	T
<i>S. obscura</i>	.	.	.	T	.	.	C	.	.	.	T	T	C	.	.	.
<i>S. nitens</i>	T	T	.	A	T	A	.	C	A	T	.	.	.	C	G	.

¹ The sequence ends at position 466.

Huelsenbeck et al. 1996; Huelsenbeck and Crandall 1997; Goldman et al. 2000).

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 diet. This expectation can be represented by the following topology: genetic variation hypothesis (T_G) = ((sympatric LHCP grasshoppers), others).

Our second hypothesis states that geographic variation in host-plant use in *S. emarginata* is due to a historical host-plant shift. If differences in specialization incidentally created reproductive barriers between *Rubus*- and *Ptelea*-associated populations, we expect the presence of two genetically differentiated lineages. If this is true, exclusive 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_G) 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 hypothetical 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_G^M ; host divergence model = T_H^M). 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_G 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_{Max}) and null trees (T_G^M , T_H^M), which were also from the original data. These statistics were tested against their null distributions, using an $\alpha = 0.05$ for statistical significance (one-sided). Phylogenetic estimation in PAUP* and simulation in PAML used the F81 + λ (discrete) model of base substitution

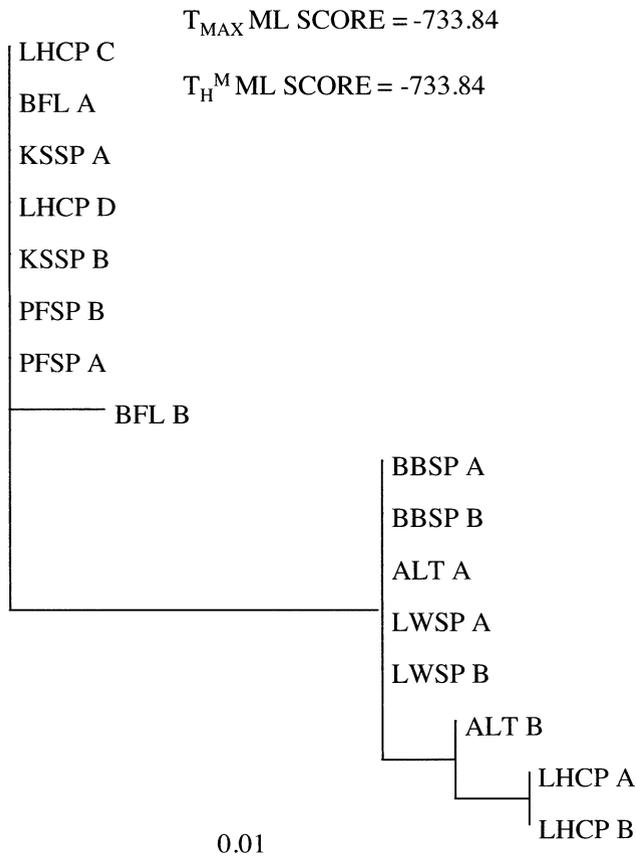


FIG. 3. Maximized unconstrained maximum-likelihood (ML) analysis of all *Schistocerca emarginata* sequence data and no outgroups using PAUP* (Swofford 2000). The best tree consistent with the host divergence hypothesis was not significantly different from this tree topology.

emarginata and no outgroups, reconstructed a tree with ML score of -733.84 (T_{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*-feeding clade was more than within the *Ptelea*-feeding 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 + λ and other simple parameters (e.g., empirical frequencies) did not substantially differ from scores obtained by using more complex parameters (e.g., HKY + λ), 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.

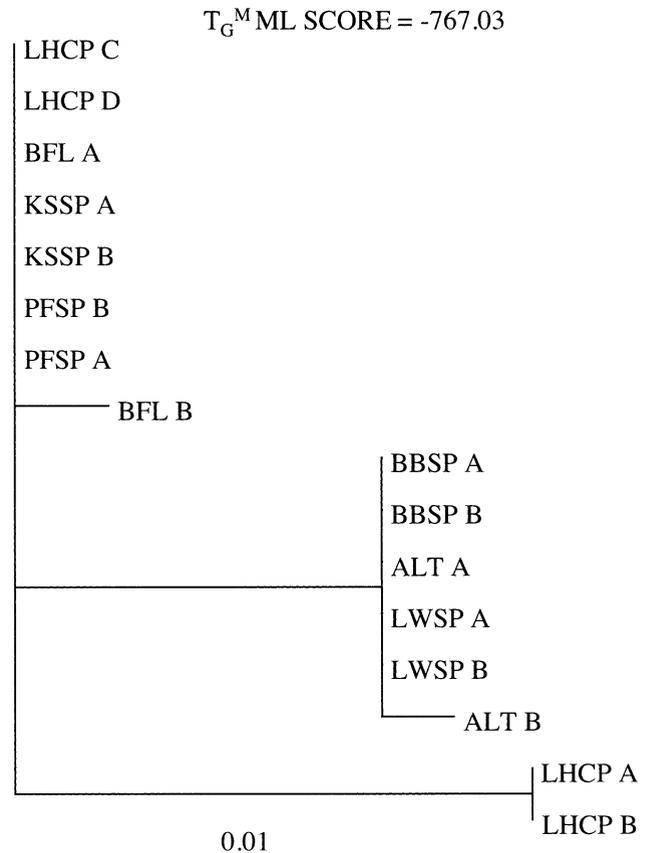


FIG. 4. The best tree consistent with the genetic variation hypothesis. This tree served as a model for simulation using EVOLVER (Yang 2000) under the same conditions used for T_{Max} in PAUP* (Swofford 2000).

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_{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 or T_G) 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_{Max}) and consistent with the null hypothesis (T_H or T_G) for each of the simulated datasets (100 per hypothesis). The test

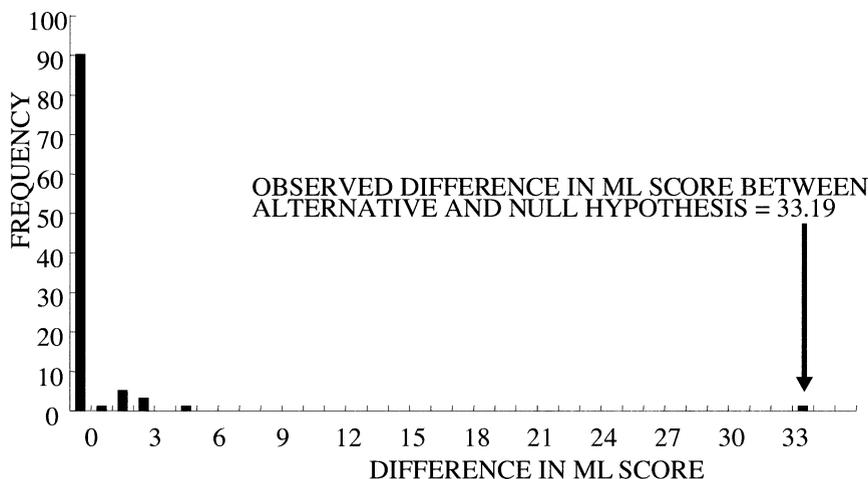


FIG. 5. Null distribution (one-sided) plotted to test the significance of the T_G null hypothesis. The test statistic measures the difference in maximum-likelihood (ML) score between the maximized tree from the original data (T_{Max}) and the best tree consistent with the null hypotheses (T_G^M). Differences this great would be expected to occur less than 1% of the time.

statistics, the differences in ML score between the maximized tree from the original data (T_{Max}) and the best tree consistent with the null hypotheses (T_G^M or T_H^M), were plotted against their null distributions. The differences in ML scores between the maximized tree (T_{Max}) and the constraint tree (T_G^M) 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 ju-

venile host-plant use), in which case they may be more closely related to each other than they are to individuals from allopatric populations (T_G 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_H 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 Welter 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 Welter 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*-conferred 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- or resource-dependent selection, 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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LITERATURE CITED

Bernays, E. A. 1989. Host range in phytophagous insects: the potential role of generalist predators. *Evol. Ecol.* 3:299–312.

- Bernays, E. A., and K. L. Bright. 1993. Dietary mixing in grasshoppers: a review. *Comp. Biochem. Physiol. A* 104:125–131.
- Bernays, E. A., and R. F. Chapman. 2000. Plant secondary compounds and grasshoppers: beyond plant defenses. *J. Chem. Ecol.* 26:1773–1794.
- Brown, J. M., W. G. Abrahamson, R. A. Packer, and P. A. Way. 1995. The role of natural-enemy escape in a gallmaker host-plant shift. *Oecologia* 104:52–60.
- Bush, G. L. 1975a. Modes of animal speciation. *Annu. Rev. Ecol. Syst.* 6:339–357.
- . 1975b. Sympatric speciation in phytophagous parasitic insects. Pp. 187–206 in P. W. Price, ed. *Evolutionary strategies of parasitic insects and mites*. Plenum Press, New York.
- . 1994. Sympatric speciation in animals: new wine in old bottles. *Trends Ecol. Evol.* 9:285–288.
- Carroll, S. P., and C. Boyd. 1992. Host race radiation in the soapberry bug: natural history with the history. *Evolution* 46:1052–1069.
- Chapman, R. F. 1990. Food selection. Pp. 39–72 in R. F. Chapman and A. Journ, eds. *Biology of grasshoppers*. Wiley, New York.
- Chapman, R. F., and G. A. Sword. 1997. Polyphagy in the Acridomorpha. Pp. 183–195 in S. K. Gangwere, M. C. Muralirangan, and M. Muralirangan, eds. *Bionomics of the Orthopteroids*. CABI, Wallingford, U.K.
- Craig, T. P., J. D. Horner, and J. K. Itami. 1997. Hybridization studies on the host races of *Eurosta solidaginis*: implications for sympatric speciation. *Evolution* 51:1552–1560.
- Ehrlich, P. R., and P. H. Raven. 1964. Butterflies and plants: a study in coevolution. *Evolution* 18:586–608.
- Feder, J. L. 1998. The apple maggot fly, *Rhagoletis pomonella*: flies in the face of conventional wisdom about speciation? Pp. 130–144 in D. J. Howard and S. H. Berlocher, eds. *Endless forms: species and speciation*. Oxford Univ. Press, New York.
- Feder, J. L., C. A. Chilcote, and G. L. Bush. 1988. Genetic differentiation between sympatric host races of the apple maggot fly *Rhagoletis pomonella*. *Nature* 336:61–64.
- Feder, J. L., K. Reynolds, W. Go, and E. C. Wang. 1995. Intra- and interspecific competition and host race formation in the apple maggot fly, *Rhagoletis pomonella* (Diptera: Tephritidae). *Oecologia* 101:416–425.
- Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39:783–791.
- Fox, L. R., and P. A. Morrow. 1981. Specialization species property or local phenomenon? *Science* 211:887–893.
- Funk, D. J. 1998. Isolating a role for natural selection in speciation: host adaptation and sexual isolation in *Neochlamisus bebbianae* leaf beetles. *Evolution* 52:1744–1759.
- Futuyma, D. 1983. Selective factors in the evolution of host choice by phytophagous insects. Pp. 227–245 in S. Ahmad, ed. *Herbivorous insects: host-seeking behavior and mechanisms*. Academic Press, New York.
- Gittleman, J. L., and P. H. Harvey. 1980. Why are distasteful prey not cryptic? *Nature* 286:149–150.
- Goldman, N., J. P. Anderson, and A. G. Rodrigo. 2000. Likelihood-based tests of topologies in phylogenetics. *Syst. Biol.* 49:652–670.
- Gratton, C., and S. C. Welter. 1999. Does ‘‘enemy-free space’’ exist? Experimental host shifts of an herbivorous fly. *Ecology* 80:773–785.
- Hatfield, T., and D. Schluter. 1999. Ecological speciation in sticklebacks: environment-dependent hybrid fitness. *Evolution* 53:866–873.
- Hillis, D. M., and J. J. Bull. 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Syst. Biol.* 42:182–192.
- Hillis, D. M., and J. P. Huelsenbeck. 1994. Support for dental HIV transmission. *Nature* 369:25.
- Hillis, D. M., B. K. Mable, and C. Moritz. 1996. Applications of molecular systematics: the state of the field and a look to the future. Pp. 515–543 in D. M. Hillis, C. Moritz, and B. K. Mable, eds. *Molecular systematics*. Sinauer, Sunderland, MA.
- Hubbell, T. H. 1960. The sibling species of the Alutacea group of the bird-locust genus *Schistocerca* (Orthoptera, Acrididae, Cyr-

- tacanthacridinae). Miscellaneous Publication of the Museum of Zoology at the University of Michigan no. 116.
- Huelsenbeck, J. P., and K. A. Crandall. 1997. Phylogeny estimation and hypothesis testing using maximum likelihood. *Annu. Rev. Ecol. Syst.* 28:437–466.
- Huelsenbeck, J. P., D. M. Hillis, and R. Nielsen. 1996. A likelihood-ratio test of monophyly. *Syst. Biol.* 45:546–558.
- Inayatullah, C., S. El Bashir, and A. Hassanali. 1994. Sexual behavior and communication in the desert locust, *Schistocerca gregaria* (Orthoptera: Acrididae): sex pheromone in solitaria. *Environ. Entomol.* 23:1544–1551.
- Jeffries, M. J., and J. H. Lawton. 1984. Enemy-free space and the structure of ecological communities. *Biol. J. Linn. Soc.* 23:269–286.
- Mallet, J., and M. C. Singer. 1987. Individual selection, kin selection, and the shifting balance in the evolution of warning colors: the evidence from butterflies. *Biol. J. Linn. Soc.* 32:337–350.
- Mayr, E. 1942. *Systematics and the origin of species*. Columbia Univ. Press, New York.
- Menken, S. B. J., T. K. Wood, and S. I. Guttman. 1981. Host races and sympatric speciation in small ermine moths, *Yponomeuta padellus* (Yponomeutidae). *Entomol. Exp. Appl.* 30:280–292.
- Moritz, C., C. J. Schneider, and D. B. Wake. 1992. Evolutionary relationships within the *Ensatina eschscholtzii* complex confirm the ring species interpretation. *Syst. Biol.* 41:273–291.
- Otte, D. 1975. Plant preference and plant succession a consideration of evolution of plant preference in *Schistocerca*. *Oecologia* 18:129–144.
- Petit, P. G., M. Montagu, G. J. Tremouillaux, J. C. Chenieux, and M. Rideau. 1993. *Ptelea trifoliata* (quinine tree, hop tree): in vitro culture and the production of alkaloids and medicinal compounds. Pp. 280–295 in Y. P. S. Bajaj, ed. *Biotechnology in agriculture and forestry*. Vol. 21. Medicinal and aromatic plants. Springer, Berlin.
- Price, P. W., C. E. Bouton, P. Gross, B. A. McPheron, J. N. Thompson, and E. A. Weis. 1980. Interactions among three trophic levels: influence of plants on interactions between insect herbivores and natural enemies. *Annu. Rev. Ecol. Syst.* 11:41–66.
- Roininen, H., J. Vuorinen, J. Tahvanainen, and R. T. Julkunen. 1993. Host preference and allozyme differentiation in shoot galling sawfly, *Euura atra*. *Evolution* 47:300–308.
- Schluter, D. 1996. Ecological speciation in postglacial fishes. *Phil. Trans. R. Soc. B Biol. Sci.* 351:804–814.
- . 1998. Ecological causes of speciation. Pp. 114–129 in D. J. Howard and S. H. Berlocher, eds. *Endless forms: species and speciation*. Oxford Univ. Press, New York.
- Simon, C., F. Frati, A. Beckenbach, B. Crespi, H. Lui, and P. Flook. 1994. Evolution, weighting, and phylogenetic utility of conserved mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Ann. Entomol. Soc. Am.* 86:651–701.
- Swofford, D. L. 2000. *PAUP*: phylogenetic analysis using parsimony (*and other methods)*. Sinauer, Sunderland, MA.
- Sword, G. A. 1998. Host-plant use and density dependent aposematism in *Schistocerca* (Orthoptera: Acrididae). Ph.D. diss., University of Texas, Austin, TX.
- . 1999. Density-dependent warning coloration. *Nature* 397:217.
- . 2001. Tasty on the outside, but toxic in the middle: grasshopper regurgitation and host-plant mediated toxicity to a vertebrate predator. *Oecologia* 128:416–421.
- Sword, G. A., and E. B. Dopman. 1999. Developmental specialization and geographic structure of host plant use in a polyphagous grasshopper, *Schistocerca emarginata* (= *lineata*) (Orthoptera: Acrididae). *Oecologia* 120:437–445.
- Thompson, J. N. 1994. *The coevolutionary process*. Univ. of Chicago Press, Chicago, IL.
- Via, S. 1999. Reproductive isolation between sympatric races of pea aphids. I. Gene flow restriction and habitat choice. *Evolution* 53:1446–1457.
- Wallace, D. W. 1982. Visual scanning in the desert locust, *Schistocerca gregaria* Forskal. *J. Exp. Biol.* 36:512–525.
- Werner, E. E., and J. F. Gilliam. 1984. The ontogenetic niche and species interactions in size-structured populations. *Annu. Rev. Ecol. Syst.* 15:393–426.
- Wood, T. K., and S. I. Guttman. 1983. *Enchenopa binotata* complex: sympatric speciation? *Science* 220:310–312.
- Yang, Z. 1997. PAML: a program package for phylogenetic analysis by maximum likelihood. *Cabios* 13:555–556.
- . 2000. *Phylogenetic analysis by maximum likelihood (PAML)*. University College, London.

Corresponding Editor: K. Shaw