Adaptation without Specialization Early in a Host Shift

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ABSTRACT: Students of speciation debate the role of performance trade-offs across different environments early in speciation. We tested for early performance trade-offs with a host shift experiment using a member of the *Enchenopa binotata* species complex of tree-hoppers (Hemiptera: Membracidae). In this clade of plant-feeding insects, different species live on different host plants and exhibit strong behavioral and physiological host specialization. After five generations, the experimental host shifts resulted either in no adaptation or in adaptation without specialization. The latter result was more likely in sympatry; in allopatry, populations on novel host plants were more likely to become extinct. We conclude that in the early stages of speciation, adaptation to novel host plants does not necessarily bring about performance trade-offs on ancestral environments. Adaptation may be facilitated rather than hindered by gene flow, which prevents extinction. Additional causes of specialization and assortative mating may be required if colonization of novel environments is to result in speciation.

Keywords: adaptation, novel environment, performance trade-off, specialization, speciation with gene flow, sympatric speciation.

Introduction

Speciation often involves divergence in a broad range of traits. When we examine closely related species, we find they often look different, behave differently, live in different environments, use different resources, and differ across their genomes (Mayr 1942; Coyne and Orr 2004; Michel et al. 2010; Sobel et al. 2010; Seehausen et al. 2014; Riesch et al. 2017). How are these distinct trait packages assembled? In the simplest case, change in a single trait may suffice—for example, if the use of different environments or resources also changes the site of mating. Such "magic traits" offer a straightforward solution to the problem (Servedio et al. 2011; Smadja and Butlin 2011; Nosil 2012; Kopp et al. 2018). But speciation often involves divergence in suites of polygenic traits (e.g., Michel et al. 2010; Seehausen et al. 2014; Riesch et al. 2017; McGee et al. 2020). Such cases require the establishment of linkage disequilibrium, perhaps often with gene flow, as in sympatric speciation or in allopatric speciation with secondary contact before reproductive isolation is complete (Kirkpatrick and Ravigné 2002; Coyne and Orr 2004; Kopp et al. 2018).

The ecological speciation hypothesis offers an explanation for how divergent trait combinations arise (Schluter 2001, 2009; Coyne and Orr 2004; Price 2008; van Doorn et al. 2009; Sobel et al. 2010; Servedio et al. 2011; Smadja and Butlin 2011; Nosil 2012; Kopp et al. 2018). This hypothesis posits that when populations colonize and adapt to different environments, the adaptations that arise in each environment may be mutually incompatible. As populations become locally adapted, there may arise trade-offs in the adaptations favored in the different environments, leading to specialization (Futuyma and Moreno 1988; Jaenicke 1990; Fry 1996, 2003; Kawecki and Ebert 2004; Savolainen et al. 2013). Specialization, in turn, selects for assortative mating to avoid producing maladaptive hybrids, because of correlations between ecological and reproductive traits or because of relationships between environmental differences and the development or expression of sexual ornaments and mating decisions.

Thus, in ecological speciation the initiating event is colonization of novel environments across which the diverging populations face performance trade-offs. Testing the

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ecological speciation hypothesis therefore requires testing for trade-offs at the early stages of the process, that is, concomitant with adaptation to the environments. A focus on early stages is also necessary because as speciation approaches completion more and more traits are likely to be involved (Nosil et al. 2009; Kulmuni et al. 2020), and divergence may continue after speciation is complete (Sobel et al. 2010). Thus, at late stages of speciation it is difficult to untangle and assess the importance of the contribution of different traits and processes. A lack of early trade-offs would suggest that different or additional traits and processes are required to initiate speciation (Kopp et al. 2018)—for example, early assortative mating caused by divergence in sexual ornaments and mate preferences due to sexual selection (West-Eberhard 1983; Coyne and Orr 2004). Testing for early trade-offs is thus key to understand the processes that initiate speciation. Few studies have the required resolution along the speciation continuum, however.

Here, we report on a quasi-natural selection experiment (Fry 2003) that we used to test for trade-offs early in a speciation-initiating event. We worked with a plant-feeding insect, a member of the *Enchenopa binotata* complex of treehoppers (Hemiptera: Membracidae). Speciation in plant-feeding insects is strongly associated with shifts to novel host plant species (Jaenicke 1990; Wood 1993; Berlocher and Feder 2002; Drès and Mallet 2002; Cocroft et al. 2008; Forbes et al. 2017), as is the case in the *E. binotata* complex (Wood and Guttman 1983; Wood 1993; Cocroft et al. 2008; Hsu et al. 2018).

We experimentally shifted *E. binotata* treehoppers from one natural population on one host plant to three different novel host plant species. Thus, there were three independent host shift experiments. We conducted each shift under conditions of allopatry (only one host species present, ancestral or novel) and sympatry (ancestral and novel hosts present). The shift under allopatry to the ancestral host constitutes a control (Fry 2003) that allows testing for changes unrelated to adaptation to the novel hosts.

The trade-off hypothesis predicts different changes in performance between insects shifted to the ancestral host (henceforth, "ancestral-line insects") and insects shifted to a novel host (henceforth, "novel-line insects"; fig. 1). These predictions can be stated in terms of whether ancestral and novel hosts vary in quality for the insects, whether the insects adapt to those differences, and whether adaptation brings trade-offs.

An initial requirement for testing the predictions is for ancestral and novel hosts to differ in quality: ancestral-line insects should have lower performance on novel hosts than on the ancestral host (black lines in fig. 1B–1D). If ancestral-line insects were to have equal performance on the ancestral and novel hosts (black line in fig. 1A), the test
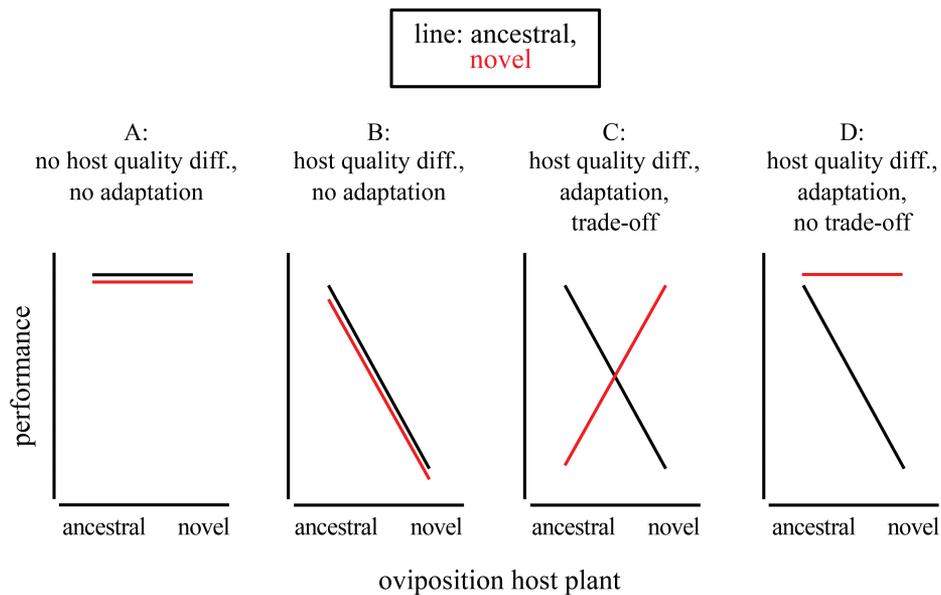

Figure 1: Scenarios for change in fecundity in a host shift (ancestral and novel lines are shown in black and red, respectively) and reciprocal transplant rearing experiment (X-axis: ancestral and novel hosts). Scenarios for changes in fecundity are as follows: the hosts do not differ in quality for the insects (A), the hosts differ in quality but the host shift does not result in adaptation to the novel host (B), the hosts differ in quality and the host shift results in adaptation to the novel host with a performance trade-off on the ancestral host (C), and the hosts differ in quality and the host shift results in adaptation to the novel host with no performance trade-off back on the ancestral host (D).

would not be possible. A further requirement is for novel-line insects to have adapted to the novel host; if their performance on novel hosts was similar to that of ancestral-line insects (red and black lines in fig. 1*B*), the test would also not be possible.

Given these requirements, the trade-off hypothesis predicts that while ancestral-line insects should have low performance on novel hosts, novel-line insects should have improved performance on the novel host as well as lowered performance back on the ancestral host (fig. 1*C*; cf. Kawecki and Ebert 2004). The hypothesis would be rejected if novel-line insects had adapted to the novel host with no loss of performance on the ancestral host (fig. 1*D*). Other scenarios are possible (e.g., intermediates between fig. 1*C*, 1*D*; Kawecki and Ebert 2004; and see below). The crucial point for the trade-off hypothesis is that adaptation to a novel environment should come at a cost to performance back on the ancestral environment.

Our emphasis was on testing for trade-offs at an early stage of the process initiated by the experimental host shifts while allowing enough time for some evolution to occur. To strike this balance, we investigated the results of the experiment five generations into the host shifts.

Our allopatric versus sympatric comparisons further allow for testing the role of gene flow in speciation. Gene flow is broadly expected to oppose divergence unless selection is strong or robust mechanisms of assortative mating are already in place (Coyne and Orr 2004; Kopp et al. 2018). This hypothesis predicts that adaptation will be more likely under allopatry in the experimental host shifts. However, gene flow may instead promote divergent adaptation through a rescue-like effect, supplementing genetic variation on which selection may act (Holt and Barfield 2011; Eriksson et al. 2014; Tomasini and Peischl 2020). This hypothesis predicts that adaptation will be more likely under sympatry.

The *E. binotata* complex is a clade of phloem-feeding insects with more than 11 species (most yet to be described), each specializing on a different host plant species and occurring in close sympatry across eastern North America (Wood 1993; Lin and Wood 2002; Cocroft et al. 2008; Hsu et al. 2018). Host shifts in the complex can lead to reproductive isolation in several ways. Reproductive isolation may arise immediately if the ancestral and novel hosts differ in phenology, as the treehoppers lay their eggs on the plants and embryo development is triggered by the flow of sap in the spring. Consequently, host plants that differ in phenology hold *E. binotata* populations that differ in the timing of their mating season (Wood and Keese 1990). Additionally, the treehoppers are host specialists, surviving poorly on the host plants of other species in the complex and showing strong behavioral preferences for their own host (Wood et al. 1999). Furthermore, there is divergence in their systems of plant-borne vibrational communication, with

species-specific male advertisement signals, female response signals, and female mate preferences (Rodríguez et al. 2004, 2006; Rodríguez and Cocroft 2006; Cocroft et al. 2010).

We took individuals from a natural *E. binotata* population on the host plant *Viburnum lentago* (Adoxaceae; the ancestral source population and host, respectively) and shifted them to three novel host plant species: *V. lantana*, *V. prunifolium*, and *V. utile*. Two of these plants (*V. lantana* and *V. utile*) are not used by any other member of the *E. binotata* complex. The other (*V. prunifolium*) is used by our study species (Cocroft et al. 2010; Hsu et al. 2018) but not at the locality of the source population; this species is also more closely related to the ancestral host (*V. lentago*) than the other novel hosts (Clement et al. 2014; fig. A1).

Thus, our experimental host shifts varied in the relatedness between the novel and ancestral hosts and hence potentially in the distance in resource space. We consider *V. prunifolium* to be the most similar to *V. lentago* and either *V. utile* or *V. lantana* to be the most distinct, on phylogenetic (fig. A1) and phenotypic grounds (R. B. Cocroft, personal observation). Distance in resource space can influence the likelihood of colonization and speciation. Successful colonization may be most likely with the most similar novel host, whereas speciation may be most likely across hosts that differ to intermediate degrees (according to the intermediate distance hypothesis; Nyman 2010) or to even larger degrees (Muschick et al. 2020). In the *E. binotata* complex, shifts across widely unrelated hosts, often belonging to different orders, have been predominant (Hsu et al. 2018). Thus, our shifts do not represent an *E. binotata*-typical long-distance shift. However, some shifts among closely related hosts have occurred in the complex (Hsu et al. 2018), and using much more phylogenetically unrelated novel hosts would reduce the likelihood of initial colonization, impeding the experiment.

A prior study conducted with individuals from the ancestral source population (collected from the source locality) reared on the above-described ancestral and novel hosts found broad-sense heritability in several traits related to performance on the hosts (male and female longevity, nymph survival, and female development time; Tilmon et al. 1998). This heritable variation in performance traits suggests that the ancestral source population likely had the capability to respond to the selection generated by our host shifts. Additionally, there was genotype × environment interaction in several of these traits (female longevity and fecundity, nymph survival, and male and female development time), and positive genetic correlations between performance-related traits occurred mostly between only two of the hosts (*V. lentago* and *V. prunifolium*). Accordingly, different genotypes are likely to do well on different host plant species (Tilmon et al. 1998).

Methods

Quasi-Natural Host Shift Selection Experiment

We established the host shifts in 1995. We collected a large sample of mated females in late August, shortly after the end of the mating season, from a natural population on *Viburnum lentago*. The collecting site was in Frederick County, Virginia (Virginia Welcome Center Clear Brook, mile 320 on Route 81, near Winchester: 39°14′33.16″N, 78°6′57.13″W).

We set up the experiment in 28 screened enclosures (each 2.4 m tall, 1.8 m wide, and 9.1 m long) at an outdoor facility at the University of Delaware (fig. A2). Each enclosure contained two trees 7.3 m apart. Placing ancestral and novel hosts at this distance within the same enclosure ensured that the trees were within the insects' "cruising range" (sensu Mayr 1942) but far enough away that the insects' high host fidelity would allow for persistence of populations on individual plants (see below).

The combinations of trees in each enclosure defined the experimental conditions. In sympatry, the combinations were as follows: shift 1, *V. lentago–V. lantana*; shift 2, *V. lentago–V. prunifolium*; and shift 3, *V. lentago–V. utile*. In allopatry, each enclosure had two trees of the same species: on the ancestral host plant, *V. lentago–V. lentago*; shift 1, *V. lantana–V. lantana*; shift 2, *V. prunifolium–V. prunifolium*; or shift 3, *V. utile–V. utile*. There were four enclosures (i.e., replicates) for each of the seven possible tree/treatment combinations (fig. A2). In all shifts, all of the plants were clones propagated from suckers obtained from a single initial plant from the field in order to minimize variation between conspecific trees.

At the start of the host shifts, we released 500 mated females onto each tree in each enclosure (500 females/tree and 1,000 females/enclosure, for a total of 28,000 females across the 28 enclosures). We released females in the enclosures within 24 h of capture in the field. Before release, we kept them on cut *V. lentago* branches in resealable plastic bags.

The treehoppers established sustainable populations in the enclosures with only one exception: all allopatric shift 3 (*V. utile–V. utile*) populations (i.e., all four enclosures) became extinct within four generations.

As noted above, the two trees in each enclosure were well within the treehoppers' cruising range (fig. A2). However, in a study conducted with a subset of the same experimental populations 8 years after founding, the treehoppers showed a strong tendency to remain on their natal host plant to mate and oviposit, even when the two plants in the enclosure were the same species (Stearns et al. 2013). We therefore consider that the experiment achieved actual host shifts because of the treehoppers' fidelity to the host on which they developed. While move-

ment of treehoppers between trees in sympatric conditions was certainly possible, we are unable to say whether this involved mating between treehoppers from ancestral and novel lines; that is, we cannot distinguish actual gene flow from mere dispersal between hosts in sympatry. Our analyses below therefore focus on the contrast between sympatric conditions where gene flow between hosts was possible and allopatric conditions where gene flow was impossible. Any differences in adaptation/specialization between sympatry and allopatry test the effect of gene flow.

As noted above, to focus on the early stages of evolution after host shifts, we assessed the results 5 years after the start of the host shifts. *Enchenopa binotata* have one generation per year (eclosing from eggs in the spring, mating season over the summer, egg laying throughout the fall; Cocroft et al. 2008). Thus, we assessed the results after five generations, with the offspring of the field-collected females constituting the first generation.

Reciprocal Transplant Rearing Experiment

To test for adaptation and trade-offs among treehoppers on different host plants, we conducted a reciprocal transplant rearing experiment in which we manipulated the oviposition host for females (fig. 2 outlines the steps involved in the experimental host shifts and the reciprocal transplants for oviposition). We started the reciprocal transplant rearing experiment in 2001. We lost one or two enclosures per treatment as a result of sources of attrition unrelated to adaptation—for example, death of trees due to flooding or the collapse of enclosure roofs in a winter storm. We used treehoppers from all remaining enclosures: two enclosures each for sympatric shifts 1 and 2, three enclosures for sympatric shift 3, and two enclosures for each of the allopatric combinations (note that treehoppers in allopatry were reared only on the host plant species on which they had been for the five generations, whether ancestral or novel). We collected a sample of mated females from each tree in each enclosure at the beginning of the oviposition season in the fall and assigned them either to the tree on which they were collected or to the other tree in that enclosure. Individual sleeve cages confined each female to her stem throughout the oviposition season. Sample sizes were 10–30 females per enclosure. Thus, for example, in each sympatric shift 2 (*V. lentago–V. prunifolium*) enclosure, there was a 2 × 2 reciprocal transplant with females collected on *V. lentago* sleeved onto *V. lentago* and *V. prunifolium* and with females collected on *V. prunifolium* sleeved onto *V. prunifolium* and *V. lentago*. And in each allopatric shift 2 (*V. prunifolium–V. prunifolium*) enclosure, there was a 2 × 2 transplant with females collected on either of the *V.*
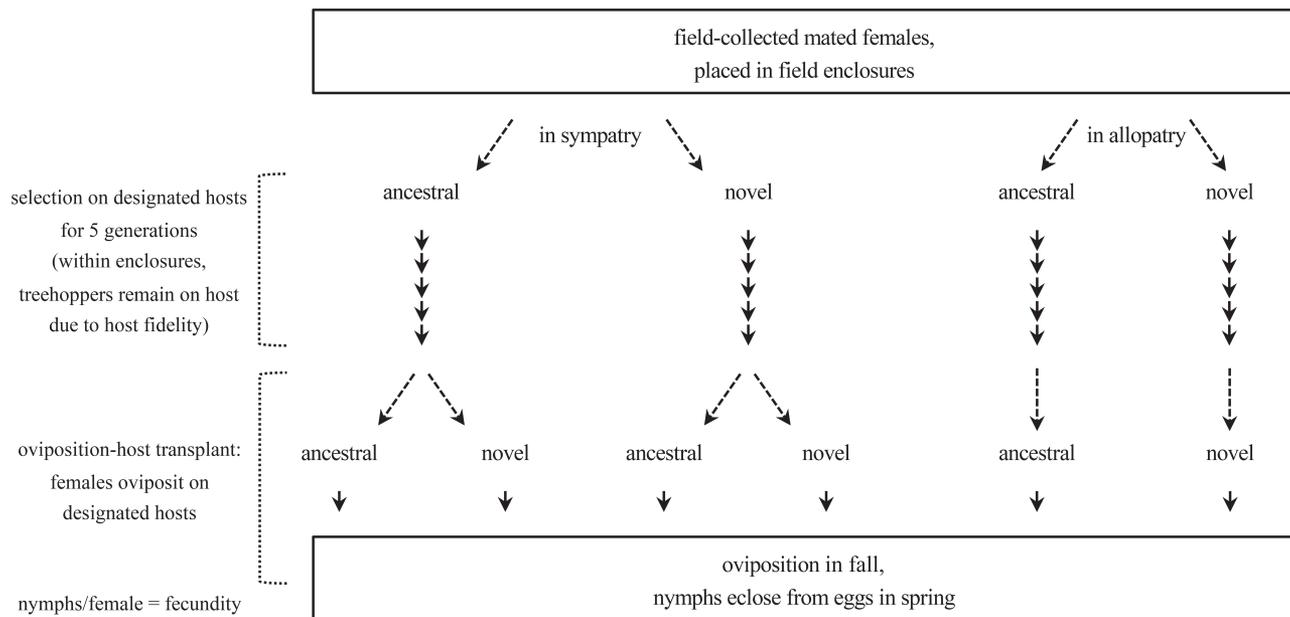
Figure 2: Outline of the host shift experiment. We collected a large sample of mated females and shifted them to novel hosts in conditions of sympatry (*left*) or allopatry (*right*). After five generations of selection, we conducted a rearing experiment involving reciprocal transplantation for oviposition. Note that in allopatry, the oviposition host was the host on which the treehoppers had been on for the five generations of the host shift.

prunifolium trees sleeved onto the same or the other tree. We removed the sleeve cages when females died at the end of the fall (in late November), to prevent the growth of mold on the stems and eggs. We tagged the stems and monitored them during the spring until the nymphs started to hatch.

We measured performance as female fecundity (number of nymphs that eclosed from the eggs laid by each female; fig. 2). With this estimate, we sought to strike a balance between counting viable offspring (as opposed to eggs that did not hatch) and representing the females' fitness rather than their offspring's, which may confound selection analyses (Wolf and Wade 2001).

Statistical Analysis

In the models described below, the dependent variable was female fecundity, which had the bimodal distribution often seen in fitness data (see below; Shaw and Geyer 2010). We therefore used generalized linear mixed models with negative binomial distributions (glmmTMB package in R; Brooks et al. 2017; R Core Team 2021).

We consider each host shift to be an independent experiment. Whether the treehoppers adapt or specialize to the novel host in one shift (e.g., *V. lentago–V. lantana*) is independent of whether the other populations in other shifts adapt or specialize to another novel host (e.g., *V. lentago–V. prunifolium*). We therefore ran the models detailed below separately for each shift, considering that this does not constitute repeated testing of the same data set and consequently that our analyses are not at elevated risk of returning spurious significance (cf. Rice 1989).

Do Adaptation and Specialization Arise in Sympatry? To test for adaptation and specialization in each host shift in sympatry, we used a model with the following explanatory variables: (i) the line of the females (ancestral, novel), (ii) the host plant on which the females were placed for oviposition (henceforth, "egg host," "ancestral," and "novel"), (iii) the line × egg host interaction, and (iv) random terms for enclosure identity and the position of the rearing tree in the enclosure (fig. A2). The main term for female line tests for overall differences in fecundity between ancestral- and novel-line treehoppers, regardless of the egg host. The main term for egg host tests for overall differences in fecundity between ancestral and novel oviposition hosts, regardless of the treehopper line. The line × egg host interaction tests for trade-offs in fecundity across treehopper lines shifted to different hosts.

Does the Effectiveness of Adaptation Differ between Sympatry and Allopatry? We asked whether the effectiveness of adaptation differs between sympatry and allopatry in

two ways, running the tests separately for each host shift. First, we compared the effectiveness of adaptation to novel hosts between conditions of sympatry and allopatry. We ran a model including only data from novel-line females that were raised on, and then oviposited on, novel hosts. The model had the following explanatory variables: (i) condition (allopatry/sympatry) and (ii) random terms for enclosure identity and the position in the cage of the host plant trees (fig. A2). We excluded the *V. utile* shift from this test because the population in allopatry became extinct, so that this comparison would entail a single value of zero for the allopatric condition.

Second, we tested for negative consequences for ancestral lines arising from gene flow from novel lines. We compared fecundity on the ancestral host across the allopatric and sympatric shifts (including only data from ancestral-line females that were raised on, and then oviposited on, the ancestral host). The model had the following explanatory variables: (i) host shift and (ii) a random term for enclosure identity (we did not include the term for the position in the cage of the host plant trees because this test included only one tree for each enclosure; fig. A2).

Does the Risk of Extinction Differ between Sympatry and Allopatry? To investigate whether the risk of extinction differs between sympatry and allopatry, we counted the number of extinctions per host shift, excluding those extinctions attributable to sources of attrition unrelated to adaptation (e.g., death of trees due to flooding; see

"Methods"). We then compared the likelihood of extinction across conditions of sympatry and allopatry with a two-tailed Fisher's test.

Results

Adaptation Was Rare in Sympatry and Arose without Specialization

Two of the three sympatric host shifts resulted in no adaptation and no trade-offs (shifts 1 and 2; *Viburnum lentago–V. lantana* and *V. lentago–V. prunifolium*). In these shifts, regardless of line, treehopper females tended to have lower fecundity when they oviposited on the novel host, significantly so for shift 1 (table 1; fig. 3; compare black vs. red histograms in sympatry for these shifts). Data underlying figure 3 have been deposited in the Dryad Digital Repository (https://datadryad.org/stash/dataset/doi:10.5061/dryad.mkkwh710k; Rodríguez et al. 2021). These results thus correspond to scenario B in figure 1, with host quality differences and no adaptation.

By contrast, in shift 3 (*V. lentago–V. utile*), the terms for line and egg host were both significant (after removing the nonsignificant line × egg host interaction; table 1): novel host-line treehoppers had higher fecundity on both the novel and ancestral hosts (fig. 3; compare black vs. red histograms in sympatry for this shift). This corresponds to a variant of the scenario in figure 1D: host quality differences, adaptation without trade-off, and (remarkably) improved performance of the shifted line back in the ancestral host.

Table 1: Analysis of variation in female fecundity for each of the three *Enchenopa* host shifts in sympatry
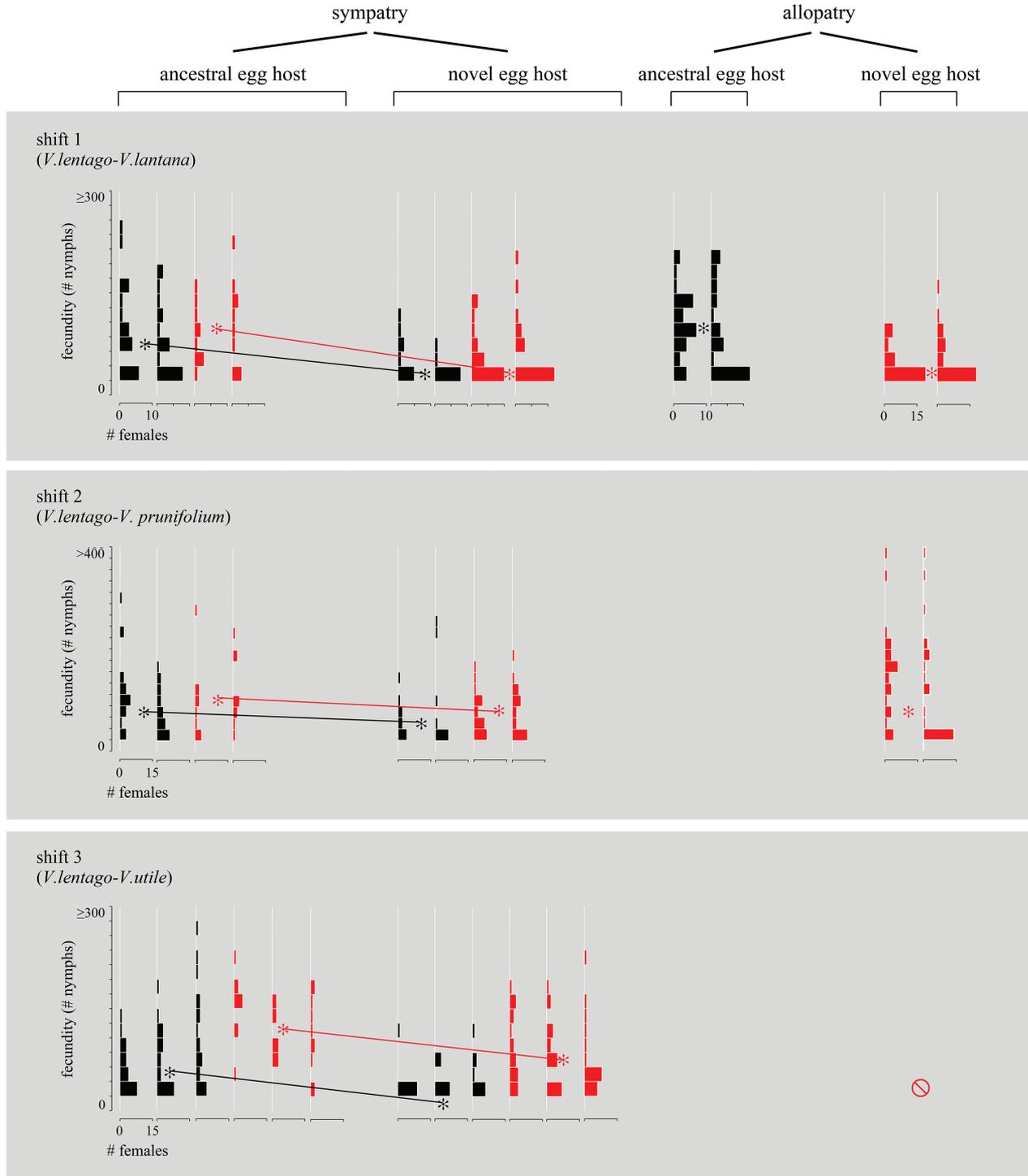
| Source | Host shifts in sympatry, χ_1^2, P | | |
	Shift 1 (*Viburnum lentago–Viburnum lantana*)	Shift 2 (*Viburnum lentago–Viburnum prunifolium*)	Shift 3 (*Viburnum lentago–Viburnum utile*)
Line	.07, .80	.09, .76	2.53, .11
Egg host	**6.25, .012**	1.01, .32	**8.94, .003**
Line × egg host	.52, .47[a]	.01, .92[b]	1.10, .29[c]
Enclosure	1.0e−09 ± 3.2e−05	1.9e−09 ± 4.3e−05	3.4e−09 ± 5.8e−05
Tree position	1.5e−09 ± 3.8e−05	9.3e−11 ± 9.6e−06	9.5e−10 ± 3.1e−05

Note: The term for female treehopper line (ancestral vs. novel) indicates the host plant species onto which the treehoppers were shifted. Egg host indicates the host plant (ancestral vs. novel) on which females were placed for oviposition. The main term for line tests for overall differences in fecundity between ancestral- and novel-line treehoppers regardless of the egg host (tests for overall adaptation). The main term for egg host tests for overall differences in fecundity between ancestral and novel oviposition hosts regardless of the treehopper line. The line × egg host interaction tests for trade-offs due to adaptation to novel hosts (tests for specialization). The terms for enclosure and tree position within enclosures were random effects. For the fixed terms, we report type III Wald χ^2 values generated by the generalized linear mixed models with negative binomial distributions (see "Statistical Analysis"). For the random terms, we report the estimated variance component and its standard deviation. Significant terms are shown in boldface. In the footnotes we report the results for the main terms after removing the (always nonsignificant) interaction terms.

[a] With interaction term removed, $\chi^2 = 1.06$ and $P = .30$ for line, $\chi^2 = 7.18$ and $P = .0074$ for egg host.
[b] With interaction term removed, $\chi^2 = 0.11$ and $P = 0.74$ for line, $\chi^2 = 2.35$ and $P = .13$ for egg host.
[c] With interaction term removed, $\chi^2 = 9.61$ and $P = .0019$ for line, $\chi^2 = 9.02$ and $P = .0027$ for egg host.

Figure 3: Variation in female fecundity in *Enchenopa* across the host shifts and reciprocal transplant rearing experiments. We show histograms (all: 25 nymphs per bin) for each replicate (enclosure); stars indicate median values. There are no data for shift 3 in allopatry (on *Viburnum utile*) because this shift became extinct before the fifth generation (see text).

Table 2: Analysis of variation in fecundity for *Enchenopa* females shifted to novel host plants in conditions of allopatry versus sympatry

	Novel hosts, χ_1^2, P		
Source	Shift 1 (on *Viburnum lantana*)	Shift 2 (on *Viburnum prunifolium*)	Shift 3 (on *Viburnum utile*)
Condition (allopatry/sympatry)	.69, .41	**4.85, .028**	The population on this host became extinct in allopatry
Enclosure	2.7e−09 ± 5.2e−05	3.4e−09 ± 5.9e−05	
Tree position	3.5e−11 ± 5.9e−06	1.1e−15 ± 3.3e−08	

Note: This test includes only novel-line females that were raised on, and then oviposited on, novel hosts. The terms for enclosure and tree position within enclosures were random effects. For the fixed term, we report type III Wald χ^2 values generated by the generalized linear mixed models with negative binomial distributions (see "Statistical Analysis"). For the random terms, we report the estimated variance component and its standard deviation. Significant terms are shown in boldface.

Adaptation Occurred under Either Allopatry or Sympatry in Different Shifts

The effectiveness of adaptation to novel hosts between conditions of sympatry and allopatry differed between shifts. In shift 1 (on *V. lantana*), novel-line females that were raised on, and then oviposited on, the novel host did not differ in fecundity between sympatry and allopatry (table 2; fig. 3; compare red histograms between sympatry and allopatry for this shift). However, in shift 2 (on *V. prunifolium*), novel-line females that were raised on, and oviposited on, the novel host had higher fecundity in allopatry (table 2; fig. 3; compare red histograms between sympatry and allopatry for this shift)—and, in fact, there was no detectable adaptation to the novel host in sympatry in this shift (table 1; fig. 3). Because of the larger variance in allopatry in this comparison (fig. 3), we also ran this test with a Welch ANOVA allowing for unequal variances and obtained the same result of higher fecundity in allopatry for shift 2 ($F_{1, 85.853} = 14.1$, $P = .0003$). Finally, in shift 3 (on *V. utile*), the treehoppers became extinct in allopatry (fig. 3), whereas sympatry for this shift was the only case where adaptation occurred (see above).

When we tested for negative consequences for ancestral lines due to gene flow from novel lines, we found that ancestral-line females that were raised on, and then oviposited on, the ancestral host did not differ appreciably in fecundity between allopatry and sympatry (table 3; fig. 3; compare black histograms between allopatry and sympatry on the ancestral host). Note that the lack of differences between the ancestral lines in sympatry and the control line (the shift to the ancestral host in allopatry) reveals no changes independent of adaptation to novel hosts.

Extinction Was More Likely in Allopatry than in Sympatry

There were no population extinctions in shifts 1 and 2 (except those arising from causes unrelated to host plant adaptation; see above). In shift 3, all sympatric enclos-ures persisted, but all allopatric enclosures became extinct. This represents a significantly higher risk of extinction in allopatry than in sympatry (Fisher's exact test: $P = .029$).

Discussion

We examined the processes involved in the early stages of speciation with a quasi-natural selection experiment (Fry 2003). We shifted a host specialist plant-feeding insect, a member of the *Enchenopa binotata* complex of treehoppers, to different host plant species. We analyzed the results five generations into the host shifts with a reciprocal transplant rearing experiment. We focused on whether local adaptation to novel environments brings early trade-offs and on whether gene flow influences the result.

The treehoppers successfully colonized (established persisting populations on) all of the novel hosts in sympatry and on all but one in allopatry. However, adaptation to novel hosts was rare: it occurred in only one sympatric and one allopatric case. When adaptation to a novel host did occur in sympatry, it brought no trade-offs with performance back on the ancestral host. These results suggest that performance trade-offs do not necessarily arise early on during the process of local adaptation. Consequently, local adaptation does not necessarily lead to early selection favoring assortative mating—in fact, as treehoppers adapted to the novel host had higher performance back on the ancestral host than did treehoppers that had

Table 3: Analysis of variation in fecundity for *Enchenopa* females shifted to the ancestral host (*Viburnum lentago*) in conditions of allopatry versus sympatry

Source	χ_3^2, P
Host shift	1.01, .80
Enclosure	3.2e−09 ± 5.6e−05

Note: For the fixed term, we report type III Wald χ^2 values generated by the generalized linear mixed models with negative binomial distributions (see "Statistical Analysis"). For the random term, we report the estimated variance component and its standard deviation.

remained on the ancestral host, the latter might be selected to hybridize with the former rather than avoid hybridization. This is not to say that specialization due to trade-offs never arises during speciation. For instance, the *E. binotata* complex shows strong host specialization, with decreased performance on hosts of other species in the complex (Wood et al. 1999). Our results suggest, however, that trade-offs may not necessarily be the cause of selection favoring assortative mating at the early stages.

To the extent that our results are typical of the early stages of speciation (and more experiments like ours will be required to assess this), the origin of ecological divergence and assortative mating may involve traits and processes additional to local adaptation and trade-offs. Even with processes such as genome hitchhiking facilitated by genomic and chromosomal structure (Flaxman et al. 2013, 2014), the absence of trade-offs would not initiate ecological specialization or select for assortative mating. Possible additional factors include developmental plasticity in ecological and reproductive traits and sexual selection on reproductive traits (West-Eberhard 1983, 2003; Coyne and Orr 2004). In a forthcoming article, we analyze the role of developmental plasticity in mating signals in the host shifts.

Adaptation was equally likely in sympatry and allopatry—it occurred in one of the three shifts in each condition, although on different novel hosts. Extinction, by contrast, was more likely in allopatry. These results support a rescue-like effect for gene flow, not hindering local adaptation but instead facilitating establishment on novel environments (Holt and Barfield 2011; Eriksson et al. 2014; Tomasini and Peischl 2020).

Successful colonization did not depend on the similarity between the novel and ancestral host, and adaptation occurred in sympatry only on the novel host (*Viburnum utile*) that was most distinct from the ancestral host (*V. lentago*; fig. A1). These results suggest that adaptation to novel environments and speciation may be favored by larger (rather than intermediate) differences between ancestral and novel environments (Muschick et al. 2020; cf. Nyman 2010). However, our sample of novel hosts was small (only three plant species), all much more closely related than the typical host differences observed across the *E. binotata* complex (Hsu et al. 2018).

Caveats with Experimental Evolution Studies of Early Speciation

Drift. We seek to interpret our results in terms of the response to selection in the experimental host shifts. One important concern, however, is the degree to which the results may be influenced by drift. The starting number of replicates (enclosures) for each treatment was not high (four per treatment), and the final number was lower (two

or three per treatment) because of sources of attrition unrelated to adaptation (except in the case of one shift in allopatry; see above). These are not unusual levels of replication in artificial selection experiments, but the concern remains. The founding sample was large (28,000 females) and fits well with the goal of collecting from a wild population to represent the genetic variation present in the population (Fry 2003). Although we did not keep a close tally of population sizes in each replicate throughout the generations, we can qualitatively say that they were always well above 50 breeding adults per replicate, the suggested minimum to reduce the influence of drift (Fry 2003). Our analyses provide some indication that drift was not a serious factor. Its effects could be revealed in variation between replicates (enclosures) within treatments. This component of variation was always very small (tables 1–3). Furthermore, our analyses included replicate in the model (see "Methods") to account for between-replicate variation. Nevertheless, we temper our interpretation because of the potential role of drift, perhaps more so for the cases where no adaptation or extinction occurred rather than for the cases in which adaptation did occur.

Lack of Genetic Variation in the Source Population. Another potential concern is whether the source population contained sufficient genetic variation to respond to the selection experiment. For instance, strong divergent selection across host species due to trade-offs could erode genetic variation within any one population, reducing the capability of the host shift experiment to detect the trade-offs. Two observations suggest that this was not a serious problem in our experiment. First, the treehoppers successfully colonized the novel hosts in most cases. Second, a prior reciprocal transplant experiment with treehoppers from the ancestral source population and with the same ancestral and novel hosts detected broad-sense heritability in several performance-related traits (male and female longevity, nymph survival, and female development time) and genotype × environment interaction in several of these traits (female longevity, female fecundity, nymph survival, and male and female development time; Tilmon et al. 1998). We therefore consider it likely that the source population had some genetic variation to respond to the selection generated by our host shifts and that different genotypes would be selected on different hosts. Low genetic variation might explain why adaptation was rare in the experiment, however.

Gene Flow versus Demographic Rescue in Sympatry. As noted above, we do not have observations that can distinguish between gene flow and dispersal between hosts in sympatry. We therefore focused on contrasting sympatric conditions (where gene flow between hosts was possible) and allopatric conditions (where gene flow was impossible).

Specifically, the result in the *V. utile* host shift (adaptation and improved performance back on the ancestral host in sympatry vs. extinction in allopatry) points to an actual effect of gene flow—demographic rescue through dispersal alone would not produce better-adapted treehoppers on both the novel and the ancestral hosts.

Limited Differences in the Novel Hosts Used in the Host Shifts. As noted above, the three host shifts varied in the likely size of the ecological differences between the novel and ancestral hosts (fig. A1). However, the hosts we used were much more similar to each other than the different host plant species typically used by different members of the *E. binotata* complex (although they do approximate some of the shifts that have occurred; Hsu et al. 2018). This increased the likelihood that the shifted populations would successfully colonize the novel hosts but may have limited our ability to detect trade-offs. Experiments with more distinct novel hosts would be interesting, although perhaps more difficult.

Statistical Power. Our test for trade-offs relies on distinguishing scenarios like those in figure 1*A* and 1*B* from scenarios like those in figure 1*C* and 1*D*—that is, it relies on testing for an interaction between treehopper line and oviposition host. In the case where we detected adaptation to the novel host without trade-offs back on the ancestral host (significant line term but nonsignificant interaction term; fig. 3; table 1), there might be a concern that the lack of significance for the interaction term is due to low power. However, we consider this not to be a problem in our study. The sample size for this host shift was large ($n = 180$ females, with three replicates per treatment). Furthermore, inspection of the data shows no indication of an interaction (i.e., no indication of a change in slope; see the red line in fig. 3) rather than a statistical failure to detect it.

Conclusion

With an unusual level of detail about the processes at play at the beginning of speciation, our host shift exper-

iment suggests that performance trade-offs do not necessarily arise in the early stages of speciation involving colonization of novel environments but may instead arise later in the process or after speciation is complete (cf. Sobel et al. 2010). To the extent that our results are typical of the early stages of speciation (and we are cautious in this suggestion), they posit a challenge for theoretical and empirical research: How common in nature are the more complex speciation scenarios that establish linkage disequilibrium between broad suites of polygenic traits, and what additional processes besides local adaptation and trade-offs are involved at the early stages (Kopp et al. 2018)?

Statement of Authorship

T.K.W. conceived the study. T.K.W., F.W.S., R.L.S., K.J.T., and M.S.C. conducted the experiment. R.L.R. and R.B.C. analyzed the data and wrote the manuscript. All authors read the manuscript and contributed to revisions.

Data and Code Availability

Data for the analyses in this study have been deposited in the Dryad Digital Repository (https://datadryad.org/stash/dataset/doi:10.5061/dryad.mkkwh710k; Rodríguez et al. 2021). We did not use any special code.
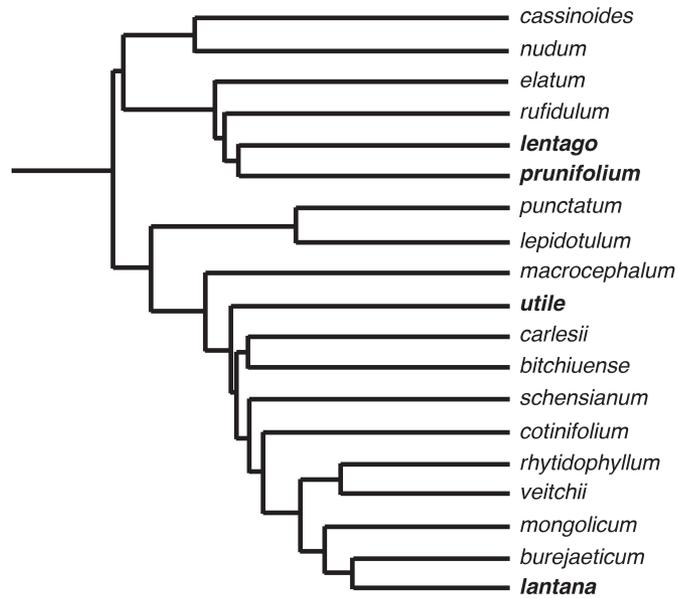
APPENDIX

Figure A1: Phylogenetic relationship between the plant species used in the *Enchenopa* host shift experiment (indicated in boldface). One of the novel hosts (*Viburnum prunifolium*) is the sister species of the ancestral host of the population of treehoppers from which we collected, and it is used by other populations of our study species (Cocroft et al. 2010; Hsu et al. 2018). The other novel hosts (*V. utile* and *V. lantana*) are more distantly related to the ancestral host. Figure modified from Clement et al. (2014).
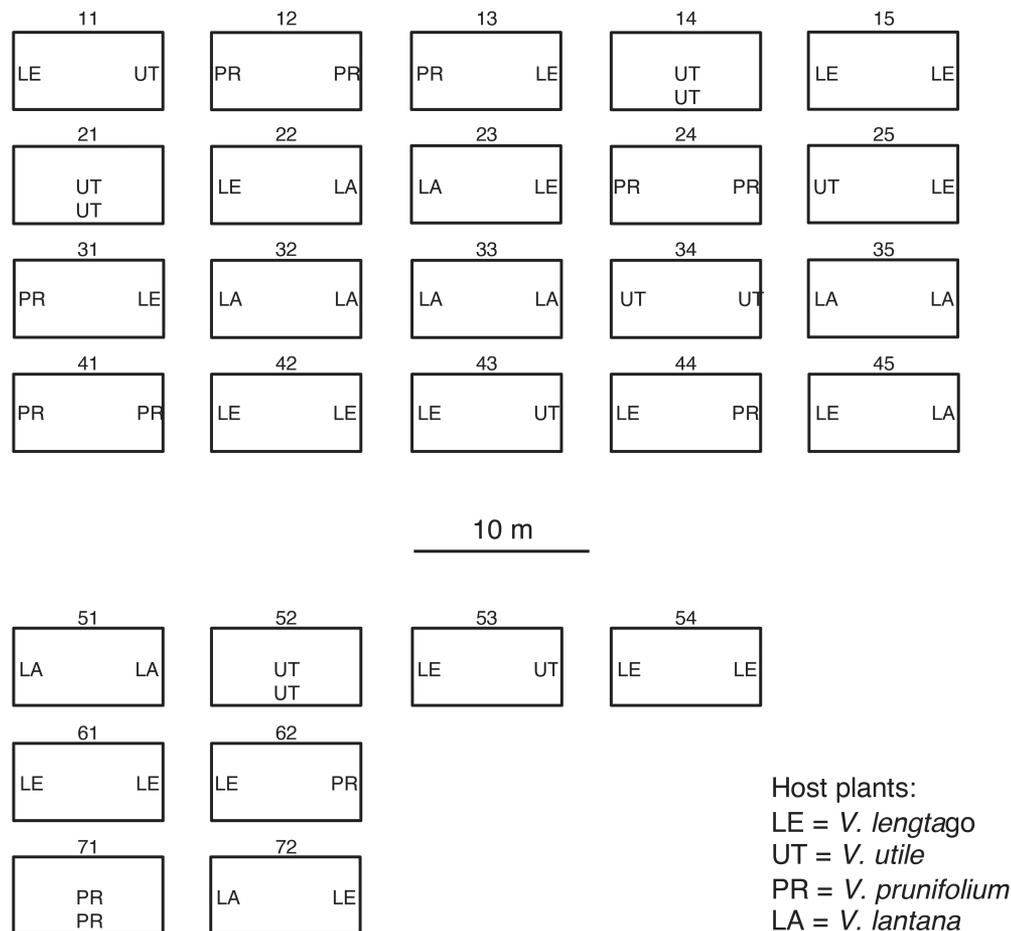
Figure A2: Physical layout of the enclosures used in the *Enchenopa* host shift experiment. Each enclosure was 2.4 m tall, 9.1 m long, and 1.8 m wide. Inside each enclosure, the two trees were 7.3 m apart.

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The members of the *Enchenopa binotata* complex are host plant specialists. Shown are nymphs of three different species, each on its own host plant. From left to right: on *Robinia pseudoacacia*, on *Ptelea trifoliata*, and on *Viburnum rufidulum* (being tended by an ant). Photos by R. B. Cocroft.