# Social Plasticity Enhances Signal-Preference Codivergence

Camille Desjonquères,[1,2,3,*] Bretta Speck,[1,4] Sara Seidita,[1] Lauren A. Cirino,[1] Ignacio Escalante,[1,5] Clinton Sergi,[1] Jak Maliszewski,[1] Christine Wiese,[1] Gerlinde Hoebel,[1] Nathan W. Bailey,[2] and Rafael L. Rodríguez[1]

1. Behavioral and Molecular Ecology Group, Department of Biological Sciences, University of Wisconsin–Milwaukee, Milwaukee, Wisconsin 53211;   2. School of Biology, University of St Andrews, St Andrews, Fife KY16 9TH, United Kingdom;   3. Université Grenoble Alpes, Université Savoie Mont Blanc, CNRS, LECA, Grenoble, France;   4. Department of Life and Earth Sciences, Concordia University Wisconsin, Mequon, Wisconsin 53097;   5. Department of Biological Sciences, University of Illinois–Chicago, Chicago, Illinois 60607

ABSTRACT: The social environment is often the most dynamic and fitness-relevant environment animals experience. Here we tested whether plasticity arising from variation in social environments can promote signal-preference divergence—a key prediction of recent speciation theory but one that has proven difficult to test in natural systems. Interactions in mixed social aggregations could reduce, create, or enhance signal-preference differences. In the latter case, social plasticity could establish or increase assortative mating. We tested this by rearing two recently diverged species of *Enchenopa* treehoppers—sap-feeding insects that communicate with plant-borne vibrational signals—in treatments consisting of mixed-species versus own-species aggregations. Social experience with heterospecifics (in the mixed-species treatment) resulted in enhanced signal-preference species differences. For one of the two species, we tested but found no differences in the plastic response between sympatric and allopatric sites, suggesting the absence of reinforcement in the signals and preferences and their plastic response. Our results support the hypothesis that social plasticity can create or enhance signal-preference differences and that this might occur in the absence of long-term selection against hybridization on plastic responses themselves. Such social plasticity may facilitate rapid bursts of diversification.

*Keywords:* indirect genetic effects, mating preference, courtship signal, Membracidae, vibrational communication.
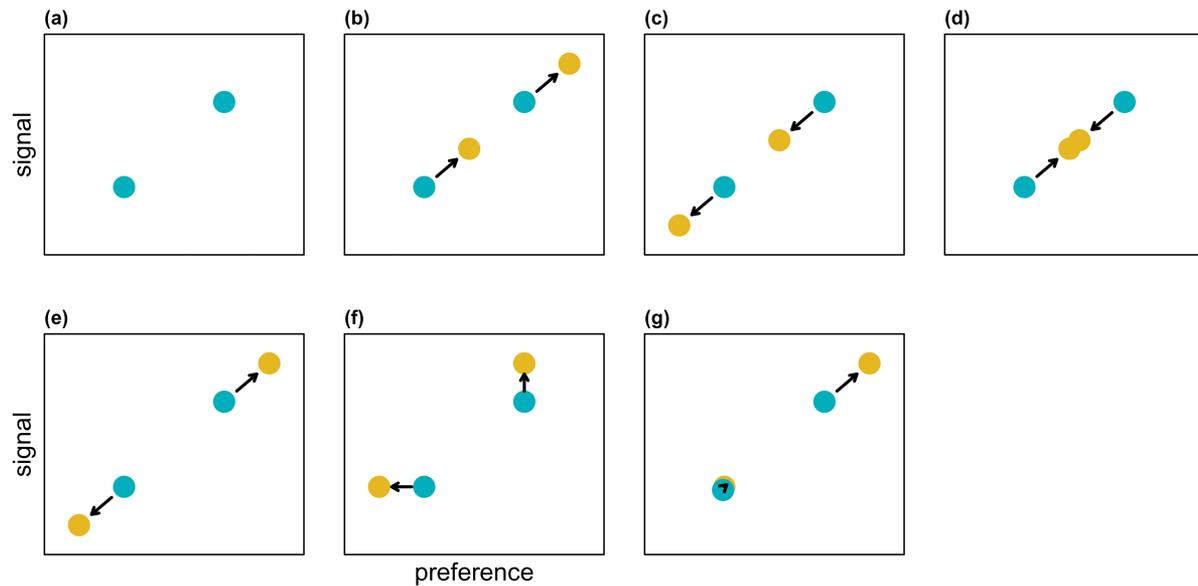
## Introduction

Phenotypic plasticity in sexual traits, such as advertisement signals and mate preferences, may have important consequences for speciation. Speciation involves the establishment of linkage disequilibrium in polygenic suites of ecological and sexual traits (Kirkpatrick and Ravigné 2002; Coyne and Orr 2004; van Doorn et al. 2009; Michel et al. 2010; Nosil 2012; Riesch et al. 2017; Kopp et al. 2018). Factors that contribute to linkage disequilibrium include geographic structure, habitat or mate preferences, genetic architecture, and "magic traits" that produce both ecological divergence and assortative mating (Kirkpatrick and Ravigné 2002; Servedio et al. 2011; Flaxman et al. 2013, 2014; Nonaka et al. 2015; Kopp et al. 2018; Mendelson and Safran 2021). Social plasticity is an additional factor that may promote signal-preference codivergence (see Bailey and Moore 2012; Rebar and Rodríguez 2015).

Here we focus on plasticity arising from interactions in mixed aggregations of diverging populations or recently diverged species, as in sympatric speciation or in allopatric speciation on secondary contact with incomplete reproductive isolation. In such cases, plasticity in mate preferences or signals could arise from learning owing to prior positive or negative experiences with potential mate types (e.g. Dukas 2004; Dukas et al. 2006; Rather et al. 2022), imprinting (Servedio et al. 2009; Hebets and Sullivan-Beckers 2010; Verzijden et al. 2012), or other effects of the social environment, such as habituation or exposure to differing degrees of sexual trait variability (Bailey 2011; Bailey and Moore 2012; Fowler-Finn and Rodríguez 2012a, 2012b; Rodríguez et al. 2013c; Marie-Orleach et al. 2019, 2020). The diverging populations might show various forms of plasticity in response to the different social environment caused by that contact (fig. 1). They might respond in the same way so that their phenotypes shift in similar directions and magnitudes (fig. 1b, 1c). Alternatively, they might respond differently. The populations might become more similar to each other (e.g., if they were to imprint on each other; fig. 1d). Such

* Corresponding author; email: cdesjonqu@gmail.com.

ORCIDs: Desjonquères, https://orcid.org/0000-0002-6150-3264; Speck, https://orcid.org/0000-0002-3939-5332; Cirino, https://orcid.org/0000-0002-3399-6946; Escalante, https://orcid.org/0000-0003-1919-4303; Bailey, https://orcid.org/0000-0003-3531-7756; Rodríguez, https://orcid.org/0000-0003-0262-0839.

**Figure 1:** Sketch of possible effects of social plasticity on signal-preference differences. When two populations or closely related species first meet (*a*), interactions may increase or decrease the signal-preference values of both populations or species (*b* or *c*, respectively), make each population or species more similar to the other (*d*), or create/enhance signal-preference differences (*e–g*). There are many other possible scenarios combining species and sex differences in the plastic response (64 possible scenarios if we consider that each species' trait values could shift in eight different directions). In the interest of space, we illustrate only the major categories of scenarios.

effects would be interesting, perhaps promoting the establishment of novel signals (see Broder et al. 2021; Tibbetts and Snell-Rood 2021), but they would not enhance assortative mating. Here we note that the converse is also possible, however: the populations could become more different from each other (fig. 1*e*), establishing or enhancing assortative mating. To our knowledge, this is a possibility that has not been explored. However, it arises intuitively from consideration of variation in the "sign" of the effects inducing plasticity. Additionally, there could be population and/or sex differences in the magnitude and direction of plasticity (fig. 1*f*, 1*g*), with one population or sex lacking plasticity altogether but plasticity still contributing to assortative mating.

Here we test the hypothesis that plasticity in response to interactions in mixed-species aggregations creates or enhances signal-preference codivergence (fig. 1*e*–1*g*). To do this, we tested for plasticity in signals and preferences generated by interactions between two members of the *Enchenopa binotata* species complex of treehoppers (Hemiptera: Membracidae). Species in this clade of host specialist sap-feeding insects show remarkable signal-preference coevolution (Rodríguez et al. 2006; Cocroft et al. 2008, 2010; Rodríguez et al. 2013*c*). As many plant-feeding insects, *Enchenopa* communicate with plant-borne vibrational signals, both as juveniles and as adults (Cocroft and Rodríguez 2005; Cocroft et al. 2008; Hill 2008; Hill and Wessel 2016; Rodríguez et al. 2018; Rodríguez and

Desjonquères 2019). These interactions are an important cause of plasticity in adult signals and preferences, with inputs from the social environment and development at different life stages having varying effects (Fowler-Finn and Rodríguez 2012*a*, 2012*b*; Rebar and Rodríguez 2013, 2014*a*, 2014*b*, 2015; Fowler-Finn et al. 2017; Desjonquères et al. 2019*a*, 2019*b*, 2021; Desjonquères and Rodríguez 2023). The strongest social effects on signals and preferences appear to result from the amount and nature of signalling interactions in the juvenile stage (Desjonquères et al. 2021; Desjonquères and Rodríguez 2023). Our two study species differ in their rate of signalling as juveniles (Rodríguez et al. 2018). Here we examine the overall effect of lifelong interactions by rearing individuals from nymphs to adults in treatments of mixed-species versus own-species aggregations. We tested the effect of these treatments on the adult treehoppers' male advertisement signals, female preferred signal values (peak preferences), and the strength of those preferences (Kilmer et al. 2017).

Our two species likely diverged from each other within the past 130,000–60,000 years (Hsu et al. 2018). There is strong support for sympatric speciation across the *E. binotata* complex, driven by colonization of novel host plants and signal-preference coevolution (Wood 1993; Cocroft et al. 2008). For *E. binotata* populations on *Viburnum* host plants, however, there is evidence suggestive of a role for isolation by distance and/or secondary colonization from

other hosts (Cocroft et al. 2010; Hsu et al. 2018). Thus, our two study species likely represent a case of recent secondary encounter on *Viburnum lentago* in some of our study sites. Our geographic sampling (see below) also allows testing for reinforcement in signals and preferences and reinforcement in their plastic response to the treatment for the species that occurs both in sympatry and in allopatry at our sites, as there has been an opportunity for selection against hybridization between our two study species at the sites where they co-occur.

## Material and Methods

### Field Collection

Most of the species in the *Enchenopa binotata* complex survive poorly on hosts used by other members of the complex (Wood and Guttman 1983; Cocroft et al. 2008). Reproductive isolation between species in the complex arises from multiple causes—phenological differences between host plants, physiological host specialization, behavioral host preferences, and behavioral mate preferences for species-specific advertisement signals (Wood 1993; Cocroft et al. 2008). Nevertheless, there are some sites throughout the range of the complex across North America where different *E. binotata* species live on the same host (Cocroft et al. 2010; R. B. Cocroft, personal communication). We took advantage of one such case in Wisconsin, where two members of the complex occur on *Viburnum lentago* (Adoxaceae) plants at some sites (Rodríguez et al. 2018). Only one of

our study species occurs at most sites in our study area, with the exception of two sites where both species co-occur reliably in distributions that have remained stable since we first observed them in 2012 (microareas of the order of a few square meters within each site where either species occurs alone and microareas where both occur side by side, sometimes on the same plant; D. Rebar and R. L. Rodríguez, unpublished data). Despite extensive searching, we have never found allopatric sites with the other species, so all observations of it are from the two sympatric sites (see below).

Most members of the *E. binotata* complex have not been formally described (Hamilton and Cocroft 2009). However, they can be readily distinguished by their host plant species, nymph coloration, and adult signal frequencies (Pratt and Wood 1992; Cocroft et al. 2008). For shorthand, here we refer to our study species as $sp_{low}$ and $sp_{high}$ because of their distinctive male signal frequencies (~165 and 275 Hz, respectively) and corresponding female mate preferences (peak preferences at ~185 and 295 Hz, respectively; Rodríguez et al. 2013*b*, 2018; Rebar and Rodríguez 2015).

We conducted the experiment over the summers of 2018–2020. Each June, we collected third-instar nymphs (the earliest stage at which the species can be distinguished by their different nymph coloration; fig. 2; Rodríguez et al. 2018) from five populations (fig. S1; figs. S1, S2 available online). These sites include three allopatric sites with only $sp_{low}$ (BOG, OLT, and PNV) and two sympatric sites with both $sp_{low}$ and $sp_{high}$ (FST and FGC). We collected more than

(a) own, $sp_{low}$    (b) mixed, $sp_{low}$ and $sp_{high}$    (c) own, $sp_{high}$
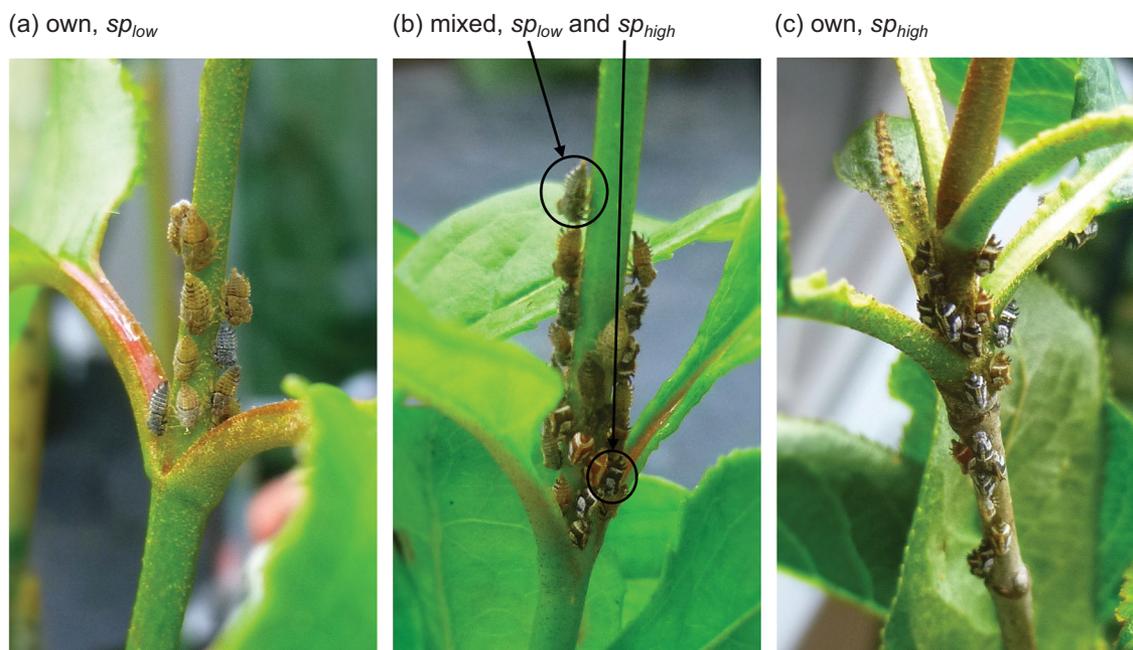


**Figure 2:** Examples of own- and mixed-species rearing aggregations that constituted the treatments in our experiment.

3,200 individuals during those 3 years (table S1) that were subsequently installed on rearing plants at the University of Wisconsin–Milwaukee greenhouse (total of 80 plants over the 3 years).

### Rearing Aggregation Treatments

We created treatments consisting of own- or mixed-species aggregations (figs. 2, 3). We randomly assigned individuals from each of the above sites to one or the other treatment. To assemble an aggregation, we placed 40 nymphs on a 40–60-cm-tall potted *V. lentago* host plant. In the own-species treatment, each rearing plant/aggregation contained either 40 $sp_{low}$ or 40 $sp_{high}$ nymphs from a single site. In the mixed-species treatment, each rearing plant/aggregation had 20 $sp_{low}$ and 20 $sp_{high}$ nymphs (fig. 3). Within species, all individuals were from the same site, but the two sets of 20 individuals in the mixed-species treatment could be from either the same or different sites.

Although species in the *E. binotata* complex can be distinguished as nymphs by their distinctive coloration pat-terns ($sp_{low}$ nymphs are gray, while $sp_{high}$ nymphs are dark brown with white stripes; fig. 2; Rodríguez et al. 2018), the adults are very similar morphologically (Pratt and Wood 1992; Cocroft et al. 2008). To distinguish the adults in our experiment, we marked them once they molted with white or red nontoxic acrylic paint (Apple Barrel matte acrylic paint 21469E flag red and 20503E white; Plaid Norcross, Peachtree Corners, GA). This required a brief separation of late-instar nymphs in the mixed-species groups (fig. 3). When the very first adults molted in the mixed-species groups, we separated the remaining nymphs from each rep-licate onto two separate plants—one for each species (we excluded those first few adults that molted from the exper-iment, as we had no way of knowing their species identity). Then, as new adults molted, we marked them and reas-sembled the experimental aggregations. We applied exactly the same manipulation to own-species aggregations (sep-aration and marking) to avoid confounding effects. Thus, individuals spent $7.1 \pm 3.8$ days (mean $\pm$ SD) out of their treatments. At that time in the life of these insects, there is little signalling by nymphs (Desjonquères et al. 2019*a*),
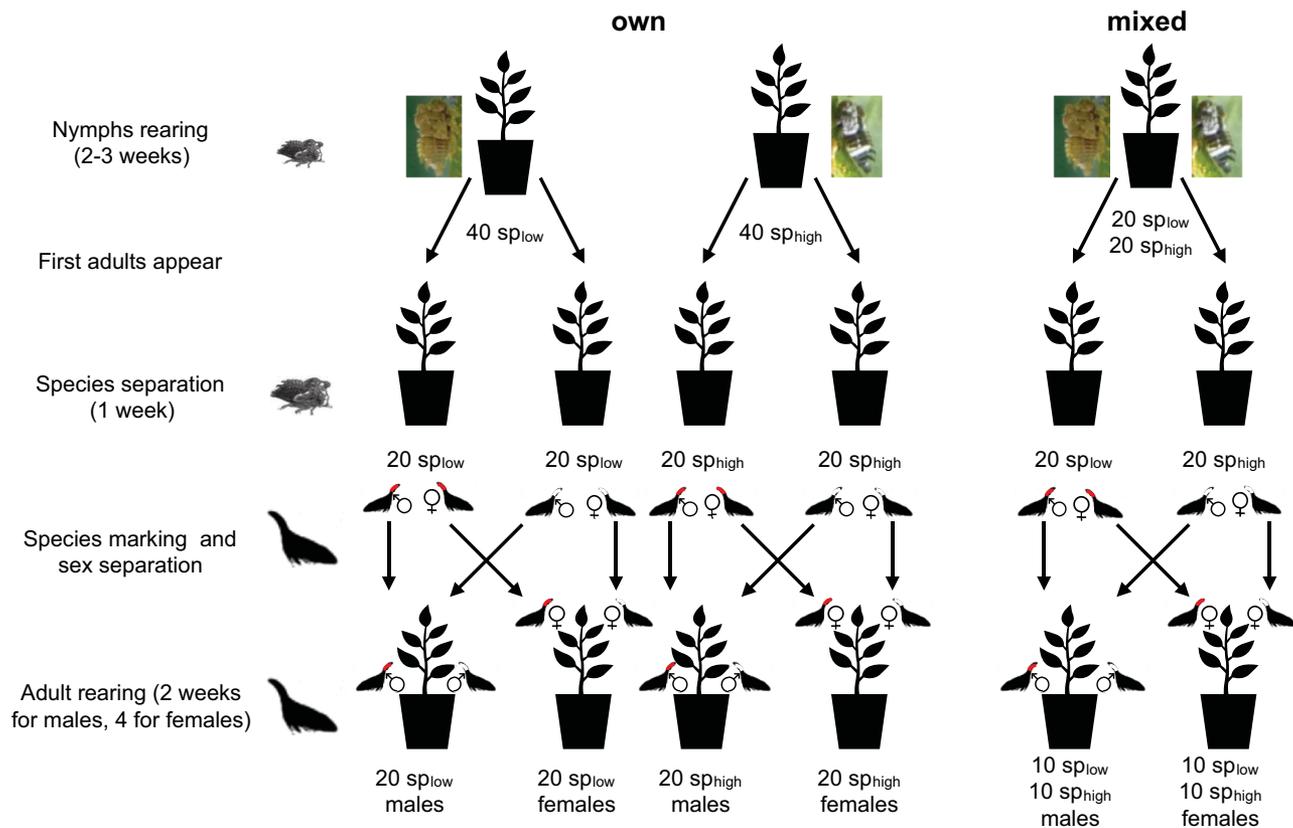


**Figure 3:** Flowchart of the experimental design to rear individuals in own- or mixed-species treatments. Nymphs were brought into a green-house and installed on host plants as own- or mixed-species aggregations. When the first adults appeared, we separated the two species onto two rearing plants (for the own-species treatment, aggregations were just split onto two new rearing plants to follow the same procedure as for mixed-species treatment). As adults continued to appear, we marked them according to their rearing plant and separated males and females.

and adult signalling has not yet begun (see below), so this represented little interruption in the experimental manipulation. In the reassembled aggregations of adults, we also separated the sexes onto different plants to prevent females from mating and becoming unresponsive to playbacks (see below). We randomly switched the color for species across plant replicates to avoid confounding effects of color. For own-species replicates, we randomly assigned a color to each plant aggregation such that about half were white and half were red.

### Adult Signals Recording and Analysis

We recorded adult male and female vibrational signals with laser vibrometry. We focused a portable laser Doppler vibrometer (Polytec PDV-100, Polytec, Auburn, MA) on a piece of adhesive reflective tape on the stem of the recording plant (a potted *V. lentago* plant). The signal was band pass filtered between 40 and 3,000 Hz with an electronic variable filter (model 3202, Krohn-Hite, Brockton, MA) and transferred to an iMac computer (MacBook Pro, Apple, Cupertino, CA) with a USB audio interface (Edirol USB Audio Capture UA-25, Roland, Hamamatsu, Japan). We recorded the output on the iMac with the program Audacity (ver. 2.1.2; http://audacity.sourceforge.net/) at a sampling rate of 44.1 Hz. We used two digital thermometers (Fisher Scientific, Pittsburgh, PA; Extech Instruments SDL500, Nashua, NH) to monitor room temperature during signal and preference recordings.

Sexually active *Enchenopa* males signal spontaneously when placed on a stem of their host plant (starting approximately 2 weeks after the adult molt). We placed each male on the recording plant, and if the male did not signal after 1 min, we primed the male with a playback of a recorded male followed by a female response (see below for vibrational playback method). To avoid making assumptions about the signal phenotypes resulting from the experiment, we primed all males with both $sp_{low}$ and $sp_{high}$ playbacks, emitting one primer of each species separated by 30 s of silent interval for a total of two primers in random order. If a male did not signal within 10 min, we placed him back on his replicate plant and tried again every 2–4 days or until he died. Males that signalled did so within $2.8 \pm 1.6$ tries (mean $\pm$ SD). From the resulting 150 male recordings (table S1; tables S1–S6 are available online), we measured the dominant frequency of male signals using Audacity and core functions in R (ver. 3.0.6; R Core Team 2015).

### Female Mate Preference Description

Sexually receptive *Enchenopa* females (starting approximately 4 weeks after the adult molt) duet with the signals of males that they find attractive, providing a realistic, natural, and convenient means for assessing their mate preferences for male signal traits (Rodríguez et al. 2004, 2006, 2012; Cocroft et al. 2008). To describe female preferences for signal frequency, we presented vibrational playback stimuli through a piezoelectric stack coupled to the stem of the plant with soft wax, driven by a piezoelectric controller (Thorlabs, Newton, NJ). We recorded female signals and playbacks with the laser vibrometer, as described above. The amplitude of playback stimuli was calibrated to 0.15 mm/s using an oscilloscope. We placed each female on a potted plant, allowing her to settle for 30 s, and we then tested whether the female was receptive with a maximum of six primer playbacks of recorded male signals from both species. The $sp_{low}$ and $sp_{high}$ primer playbacks were emitted in alternation and separated by 15 s of silent intervals. If a female did not respond to any of the six primers, we returned her to her replicate plant and tried again every 2–4 days or until she died. If the female responded to a $sp_{low}$ (or $sp_{high}$) primer, we gave her a full preference sequence. The 374 females (table S1) that responded did so within $1.9 \pm 1.4$ tries (mean $\pm$ SD).

To obtain female preference functions, we used vibrational playback sequences composed of synthetic stimuli varying in frequency, with all other features set to the population mean of each species (e.g., $sp_{low}$ males produce signals with four pulses/signal, so each of our stimuli had four pulses/signal, and so on; see table S2 for details on the stimuli features). We exposed each female to a randomized sequence of 18 playback stimuli. To capture the full shape of the preference functions, the range of stimuli frequencies varied from 100 to 440 Hz in 20-Hz increments, exceeding the range of signal frequency values in the two species (Kilmer et al. 2017). Each playback stimulus was a bout with four signals with that frequency, each separated from the next by 1.9 or 2.5 s (for $sp_{low}$ and $sp_{high}$, respectively, based on average population values) of silence. Each playback bout was separated from the next by 15 s of silence. We assayed female preference with the number of responses (between zero if she did not respond to any signal and four if she responded to all the signals in the synthetic bout) that each female produced in response to each of the 18 stimuli. A score of four responses for a stimulus thus indicates maximum attractiveness, and a score of zero indicates the lowest attractiveness.

We fitted cubic spline regressions to the response data for each female and generated individual preference function curves using the program PFunc (ver. 1.0.0; https://github.com/Joccalor/PFunc and https://hub.docker.com/r/joccalor/pfunc/; Kilmer et al. 2017). This approach allows any shape for the preference functions with a certain

level of smoothness that is determined empirically (Schluter 1988; Kilmer et al. 2017). PFunc fits curves using the gam function in the mgcv R package (Wood and Wood 2015). We used the default smoothing parameter values calculated by PFunc for all of our curves, setting the range of smoothing values between 0.005 and 0.5. This means that females could vary in smoothing values set for their curve (see table S3 for all smoothing values). Additionally, we checked all curves and slightly changed the smoothing value for females with curves that strongly deviated from the raw data (fig. S2). Smoothing values did not differ significantly between species and treatment (linear model with smoothing as a response variable and species, treatment, and their interaction as test variables; $P$ value > .45 for the three terms). We then analyzed variation in the individual preference functions using the preference peak and preference strength metrics implemented in PFunc (Kilmer et al. 2017). Preference peak is preferred display trait value, measured as the signal frequency with the highest response likelihood on the preference function. Preference strength is the degree to which attractiveness falls away from peak preference, calculated as $(\mathrm{SD[response\ values]}/\mathrm{mean[response\ values]})^2$, where SD is standard deviation. These two traits are significantly repeatable (peak preference: $r = 0.28$; preference strength: $r = 0.74$; Cirino et al. 2023).

### Statistical Analysis

We conducted all analyses using the lmer function of the R package lme4 (ver. 1.1-25; Bates et al. 2014). We built linear mixed models (described below) in which the error structure was Gaussian. We checked the assumptions of normality and homoscedasticity of residuals by visually examining a quantile-quantile plot and the residuals against the fitted values, both indicating no deviation from these assumptions. We assessed model stability by excluding data points one at a time from the data, fitting the model, and collecting the parameter estimations. If the range of parameter estimations over all of these iterations included zero, the model and variable estimation were considered unstable. To test for collinearity between fixed effects, we derived variance inflation factors (Field 2009) using the function vif of the R package car (ver. 2.1-4; Fox and Weisberg 2011), and they revealed the absence of collinearity between fixed effects (maximum value of 1.5; collinearity issues usually indicated by values higher than four).

*Testing for Plasticity due to Rearing in Own-Species versus Mixed-Species Aggregations.* To test for an effect of the rearing treatments on male signal frequency and female peak preference, we built a linear mixed model in which we used a reaction norm approach with one dependent vari-

able that represented both male signal frequency and female peak preference (see Fowler-Finn et al. 2015; Rebar and Rodríguez 2015). This approach allowed us to analyze the relationship between the effects of the treatments on both preferences and signals with a single model. The model had the following explanatory variables: treatment (mixed or own), species ($\mathrm{sp_{low}}$ or $\mathrm{sp_{high}}$), sex (male or female), year (categorical variable with three levels: 2018, 2019, or 2020), and recording temperature. Recording temperature and year were included as control variables. Typically, year would be included as a random factor, but because it had fewer than five categorical levels, we included it as a fixed effect (Arnqvist 2020). The model also included all two- and three-way interactions between treatment, species, and sex. These interaction terms test for species and sex differences in the plastic response to the rearing treatments. For instance, the best-case scenario for plasticity arising from interactions in mixed-species aggregations to contribute to assortative mating would require that the signals and preferences of each species become more distinct in the mixed treatment (fig. 1*e*). This would be indicated by a significant species × treatment interaction (with visual inspection to distinguish between the scenarios in fig. 1*d* vs. 1*e*). Other scenarios would be indicated as follows: no significant effects (fig. 1*a*), only treatment significant (fig. 1*b*, 1*c*), and significant sex × treatment interaction and species × treatment and/or three-way interactions (fig. 1*f*, 1*g*). As there were several individuals on each rearing plant/aggregation, the model also included rearing plant/aggregation identity as a random term. We initially included collection site as a term, but it was never significant ($P > .07$ in all cases), so we removed it from our analyses. We used a second similar model to test for an effect of the treatments on female preference strength, with preference strength as the dependent variable.

*Testing for Reinforcement in $\mathrm{sp_{low}}$.* We focused this analysis on $\mathrm{sp_{low}}$, for which we had both sympatric and allopatric populations (we were unable to find allopatric sites for $\mathrm{sp_{high}}$, despite considerable efforts; see above). We built a linear mixed model with frequency (of male signals or female preference) as the dependent variable. The explanatory variables were treatment, population type (allopatric or sympatric), sex, year, and recording temperature. We included an interaction between treatment and sex to keep the model as similar to the previous one as possible to keep them comparable. We also included an interaction between treatment and population type to test for differences in the effect of treatment in different population types. The model included rearing plant/aggregation identity as a random term. We used a similar model to test for geographic variation in the form of plasticity for female preference strength in $\mathrm{sp_{low}}$.

## Results

### Plasticity due to Rearing in Own- versus Mixed-Species Aggregations

Our study species responded differently to the rearing treatments of own- versus mixed-species aggregations (significant species × treatment and species × treatment × sex interactions; table 1), supporting a scenario similar to figure 1*f* or 1*g*. The $sp_{high}$ individuals reared in mixed-species aggregations differed more (by 11% on average) from $sp_{low}$ in male advertisement signals and female mate preferences than individuals reared in own-species aggregations (figs. 4, 5, 6*a*, 6*b*). By contrast, $sp_{low}$ exhibited little plasticity in signals or preferences in response to the rearing treatments (figs. 4, 5, 6*a*, 6*b*). Note, however, that $sp_{low}$ and $sp_{high}$ showed comparable overall plasticity in signals and preferences due to within-treatment variation in developmental and social environments (significant random terms for rearing plant/aggregation; table 1). Interestingly, the variance in male signals did not differ between treatments, while the variance in female peak preference was higher in own-species treatments than in mixed-species treatments for both species (table S4).

The rearing treatments also tended to affect female preference strength differently in the two species (marginally significant species × treatment interaction; table 2). The $sp_{high}$ females reared in mixed-species aggregations tended to have stronger preferences than females reared in own-species aggregations (fig. 6*c*). By contrast, $sp_{low}$ females exhibited little plasticity in preference strength according to the rearing treatments but tended to express constitutively higher strength than that of $sp_{high}$ females in own-species aggregations (fig. 6*c*). Nevertheless, as above, both species showed comparable plasticity due to within-treatment variation in the developmental and social environment (significant random terms for rearing plant/aggregation; table 2).

### No Reinforcement in $sp_{low}$

There was no difference between sympatric and allopatric populations in signals and preferences within treatment (nonsignificant population type; table S5; figs. 4, 5, 6*a*, 6*b*) and in the form of the plastic response to the rearing treatments in signals or preferences (nonsignificant population type × treatment interaction; table S5; figs. 4, 5, 6*a*, 6*b*).

There was also no difference between sympatric and allopatric populations in female preference strength within treatment (nonsignificant population type; table S6; fig. 6*c*) and in the form of the plastic response in female preference strength (nonsignificant effect of population type × treatment interaction; table S6; fig. 6*c*).

## Discussion

Here we propose a heuristic model whereby plasticity due to interactions in mixed aggregations of diverging populations or recently diverged species may create or enhance signal-preference differences and promote assortative mating (fig. 1). We also present a proof of concept test of this hypothesis with two recently diverged species in the *Enchenopa binotata* complex of treehoppers that differ in signals and preferences but not ecologically. We found that social plasticity enhances signal-preference differences between two closely related species of *Enchenopa* treehoppers mediated via the plastic response of one species. When reared in mixed-species aggregations, males of $sp_{high}$ had higher frequency signals, and females had preferences for higher signal frequencies than when reared in own-species aggregations. The other species ($sp_{low}$) did not show plasticity in response to these rearing treatments.

Social plasticity enhanced signal-preference differences between these two species by about 10%. This is a relatively small but important increase in the signal-preference species differences. *Enchenopa binotata* females have strong preferences for signal frequency, and a 10% deviation from peak preference typically decreases signal attractiveness by ~50% (Rodríguez et al. 2006, 2013*a*). Furthermore, the combined effect through signals and preferences further enhances that effect. Despite a wide frequency gap between the two species, the range of variation in the population, especially in female preferences (fig. 4), points to some risk of hybridization. Indeed, some females appear potentially willing to accept males of the other species (e.g., female 12, 25, 33, 260, 289 or 291 in fig. S2). Hence, we consider that the observed plastic response is likely to increase assortative mating in a biologically relevant way between the two species. These results support a key component of the scenario
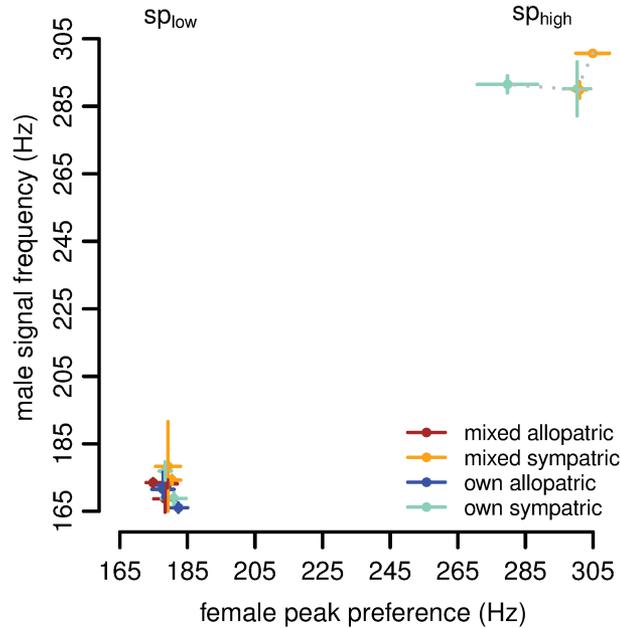
**Table 1:** Variation in male signal frequency and female peak preference in two *Enchenopa* species according to own- versus mixed-species rearing treatments

| Term | $\chi^2$ | df | $P$ |
|---|---|---|---|
| Fixed effects: | | | |
| Species | 4,520.22 | 1 | **<.0001** |
| Sex | 22.79 | 1 | **<.0001** |
| Treatment | 2.01 | 1 | .16 |
| Year | 9.61 | 2 | **.009** |
| Temperature | 14.67 | 1 | **.00013** |
| Species × sex | .57 | 1 | .45 |
| Species × treatment | 5.10 | 1 | **.024** |
| Sex × treatment | .14 | 1 | .71 |
| Species × sex × treatment | 5.70 | 1 | **.017** |
| Random effect: | | | |
| Rearing plant/aggregation | 4.11 | 1 | **.043** |

Note: Significant and marginally significant effects are shown in bold.
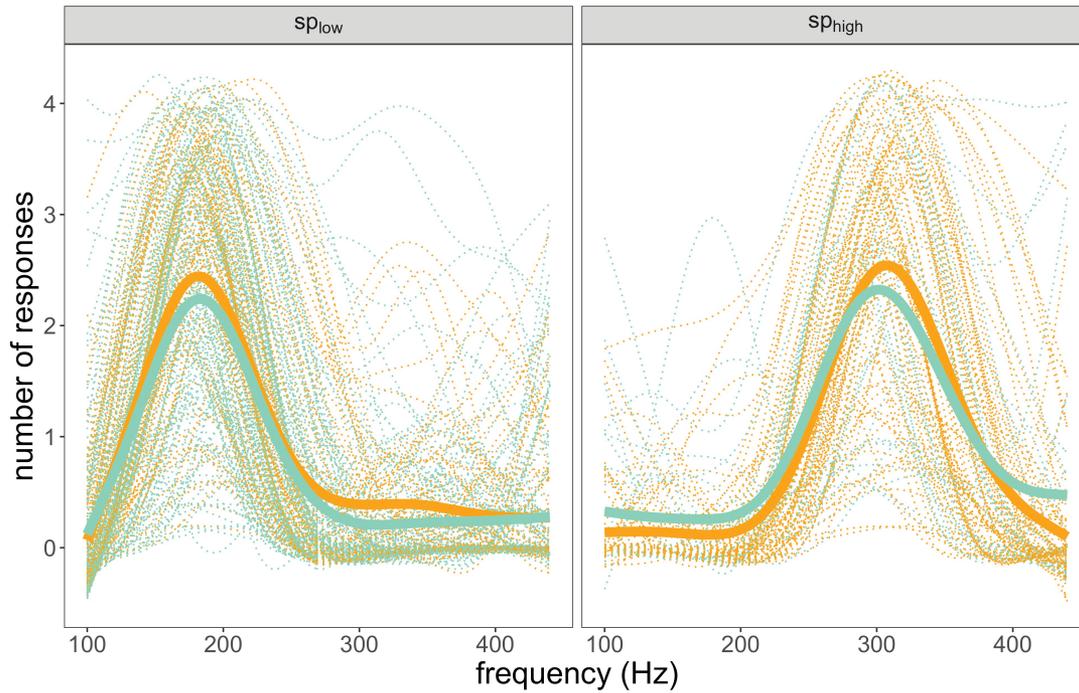
**Figure 4:** Variation in female preference curves in *Enchenopa* according to species and own- versus mixed-species rearing treatments. Dotted lines present individual-level preference curves. Solid lines present group-level preference curves for each treatment-species combination. Light blue indicates females reared in own-species treatments, and orange indicates those reared in mixed-species treatments. The left panel shows $sp_{low}$, and the right panel shows $sp_{high}$.
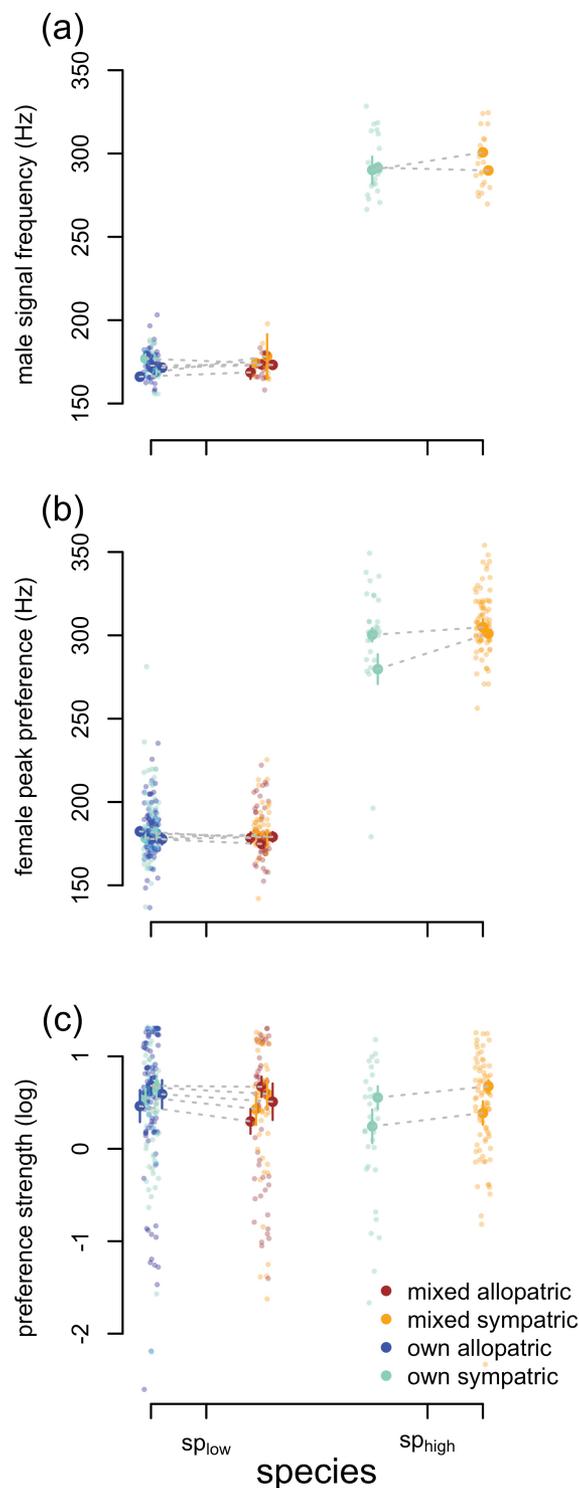


outlined in figure 1*g*: a species difference in social plasticity with plasticity of the predicted sign in one species and no plasticity in the other species.

Mate preferences can differ in preferred signal value but also in strength, and the two can have distinct evolutionary consequences (Bailey 2008; Rodríguez et al. 2013*a*; Bailey and Macleod 2014; Kilmer et al. 2017). Our rearing treatment influenced not only the phenotypic values for male signals and female peak preferences but also the strength of those preferences: $sp_{high}$ females reared in mixed-species aggregations had stronger preferences than those reared in own-species aggregations. This compounded effect

error for each rearing treatment–site–species combination (data corrected for the effects of temperature, year, and plant replicate using model predictions). For reference, data from $sp_{low}$ and $sp_{high}$ species occupy the lower-left and upper-right portions of the plot, respectively. Dark blue and light blue indicate means of individuals reared in own-species treatments, and red and orange indicate means of individuals reared in mixed-species aggregations. Lighter colors (orange and light blue) indicate individuals from sympatric populations, while darker colors (red and dark blue) indicate individuals from allopatric populations. There were no allopatric populations for $sp_{high}$; thus, there are no dark colors for that species.

**Figure 5:** Effect of own- versus mixed-species treatments on male signal frequency and female peak preference in two *Enchenopa* species. Points and associated bars show the mean and standard

**Figure 6:** Effect of own- versus mixed-species treatments on male signal frequency (*a*), female peak preference (*b*), and preference strength (*c*) in two *Enchenopa* species according to own- versus mixed-species rearing treatments. Opaque points and associated bars show the mean and standard error for each rearing site–treatment–species combination (data corrected for the effects of temperature, year, and plant replicate using model predictions). Transparent points show the individual data. Dark blue and light blue indicate means for individuals reared in own-species treatments. Red and orange indicate means for mixed-species aggregations. Lighter colors (orange and light blue) indicate individuals from sympatric populations, while darker colors (red and dark blue) indicate individuals from allopatric populations. Note that there were no allopatric $sp_{high}$ populations; thus, there are no dark colors for that species. Dashed gray lines show the reaction norm for each site (two sites for $sp_{high}$ and five sites for $sp_{low}$).

could further increase assortative mating when the two species are in contact, as females have not only a preference for higher frequencies but a stronger preference for higher frequencies. The $sp_{low}$ female preference strength was not plastic in response to our treatments but constitutively higher than that of $sp_{high}$ females in own-species aggregations. The combined effect of preference peak and strength thus results in a reduction of $sp_{low}$ signal attractiveness for $sp_{high}$ females, likely further enhancing assortative mating.

Although we did not attempt to identify the specific inputs into trait expression that may be responsible for the observed plasticity, several aspects of social interactions may have been at play. The manipulation in the social environment that we applied resulted in lifelong changes in the interactions experienced by individuals. As juveniles, $sp_{low}$ have higher signalling rates than $sp_{high}$ (Rodríguez et al. 2018). Thus, the mixed treatment likely presented a change for nymphs of both species, with higher than usual signalling rates for $sp_{high}$ and lower than usual rates for $sp_{low}$. Males of the two species differ in their advertisement signals (mainly in dominant frequency; see above), and that would offer strong differences in experience between the treatments. However, prior work found that such differences in male adult experience alone do not change male signal frequency (Rebar and Rodríguez 2016). Furthermore, although the strongest effects found were on female mate preferences, females were not exposed to male signals during the treatments and would not themselves signal until later in life and then mainly in response to males, so our treatments likely varied little at this stage for females. Consequently, we consider that our results likely arise from the effects of inputs that occurred during the juvenile stage, which prior work has shown to be important (Desjonquères et al. 2019*a*, 2019*b*, 2021).

The observed divergence-enhancing plasticity could arise in two ways. It could occur on first encounter—without prior selection against hybridization—and immediately establish or strengthen assortative mating. Alternatively, it may arise from selection against hybridization (i.e., reinforcement; Servedio and Noor 2003). Reinforcement could act not only on signals or preferences themselves but also on their plastic response (see Lesna and Sabelis

**Table 2:** Variation in female preference strength in two *Enchenopa* species according to own- versus mixed-species rearing treatments

| Term | $\chi^2$ | df | $P$ |
|---|---|---|---|
| Fixed effects: | | | |
|   Species | .10 | 1 | .76 |
|   Treatment | .17 | 1 | .68 |
|   Year | 3.34 | 2 | .19 |
|   Temperature | .54 | 1 | .46 |
|   Species × treatment | 3.23 | 1 | **.073** |
| Random effects: | | | |
|   Rearing aggregation | 4.30 | 1 | **.038** |

Note: Significant and marginally significant effects are shown in bold.

1999; Pfennig 2007; Chaine and Lyon 2008). The "first encounter" and "reinforcement of plasticity" scenarios may be contrasted by testing for geographic variation in the form of plasticity and measuring the fitness of hybrids. Under the reinforcement of plasticity scenario, plasticity due to interactions in mixed-species aggregations would create or enhance signal-preference differences only in individuals from sympatric populations where both populations or species have a history of coexistence and not in individuals from allopatric sites where only one species occurs. We occasionally find treehoppers with intermediate signals and preferences (a few individuals out of hundreds collected each year; K. D. Fowler-Finn and R. L. Rodríguez, unpublished data), suggesting that these species may hybridize at low rates in the field. However, hybrids are unlikely to mate, as their intermediate signals and preferences will fail to be attractive to (or be attracted by) either parental species. We found no differences in the plastic response between sympatric and allopatric sites for $sp_{low}$, but we were unable to conduct a similar test for $sp_{high}$. Future work would profit from a renewed population-sampling effort to confidently test a first encounter versus reinforcement scenario.

Our results support the hypothesis that social plasticity can create or enhance signal-preference differences and promote assortative mating. Specifically, we suggest that a change in the social environment can enhance phenotypic differences in mating signals and mate preferences, promoting reproductive isolation. This process might represent an underappreciated cause of assortative mating and signal-preference divergence in the early stages of speciation. Once present, new or enhanced signal-preference differences expressed because of social plasticity would not only promote assortative mating but also facilitate further codivergence through subsequent evolution of signals, preferences, and/or their plastic response (the latter potentially involving genetic accommodation or assimilation; West-Eberhard 2003, 2005). Such subsequent evolution may lead to genetic change

in signals, preferences, and/or the machinery involved in their development, as well as genetic change in the elements of the social environment responsible for the plasticity-inducing inputs—change in the indirect genetic components of signals, preferences, and their developmental regulation (see Bailey and Moore 2012; Rebar and Rodríguez 2015). The importance of this process for speciation will depend on how common, how strong, and of what sign the first encounter effects of social plasticity are. Furthermore, reinforcement of the plastic response is an interesting and potentially important outcome that should be explored further with experimental research. Such early unselected, first encounter plasticity may even contribute to that reinforcement through genetic accommodation of the plastic response. Comparative work to answer these questions and test these hypotheses will be illuminating.

## Statement of Authorship

C.D. and R.L.R. obtained the funding to run the experiments. C.D., G.H., N.W.B., and R.L.R. developed the hypotheses. C.D., B.S., S.S., C.S., J.M., and C.W. ran the experiments under the supervision of C.D. and R.L.R. C.D., L.A.C., and I.E. extracted the data from the recordings. C.D. and R.L.R. worked on the representation of the data and wrote the original draft of the manuscript. All authors contributed critically to the drafts and gave final approval for submission.

## Data and Code Availability

The data related to this article are publicly available on Zenodo (https://doi.org/10.5281/zenodo.7844980; Desjonqueres 2023).

## Literature Cited

Arnqvist, G. 2020. Mixed models offer no freedom from degrees of freedom. Trends in Ecology and Evolution 35:329–335.

Bailey, N. W. 2008. Love will tear you apart: different components of female choice exert contrasting selection pressures on male field crickets. Behavioral Ecology 19:960–966.

———. 2011. Mate choice plasticity in the field cricket *Teleogryllus oceanicus*: effects of social experience in multiple modalities. Behavioral Ecology and Sociobiology 65:2269–2278.

Bailey, N. W., and E. Macleod. 2014. Socially flexible female choice and premating isolation in field crickets (*T. eleogryllus* spp.). Journal of Evolutionary Biology 27:170–180.

Bailey, N. W., and A. J. Moore. 2012. Runaway sexual selection without genetic correlations: social environments and flexible mate choice initiate and enhance the fisher process. Evolution 66:2674–2684.

Bates, D., M. Maechler, B. Bolker, and S. Walker. 2014. lme4: linear mixed-effects models using Eigen and S4. R package version 1.1-7. http://CRAN.R-project.org/package=lme4.

Broder, E. D., D. O. Elias, R. L. Rodríguez, G. G. Rosenthal, B. M. Seymoure, and R. M. Tinghitella. 2021. Evolutionary novelty in communication between the sexes. Biology Letters 17:20200733.

Chaine, A. S., and B. E. Lyon. 2008. Adaptive plasticity in female mate choice dampens sexual selection on male ornaments in the lark bunting. Science 319:459–462.

Cirino, L. A., I. D. Gallagher, C. Desjonquères, and R. L. Rodríguez. 2023. Robust mate preferences despite means and opportunity for mate choice copying in an insect. Animal Behaviour 200:137–146.

Cocroft, R. B., and R. L. Rodríguez. 2005. The behavioral ecology of insect vibrational communication. Bioscience 55:323–334.

Cocroft, R. B., R. L. Rodríguez, and R. E. Hunt. 2008. Host shifts, the evolution of communication, and speciation in the *Enchenopa binotata* species complex of treehoppers. Pages 88–100 *in* K. Tilmon, ed. Specialization, speciation, and radiation: the evolutionary biology of herbivorous insects. University of California Press, Berkeley.

———. 2010. Host shifts and signal divergence: mating signals covary with host use in a complex of specialized plant-feeding insects. Biological Journal of the Linnean Society 99:60–72.

Coyne, J. A., and H. A. Orr. 2004. Speciation. Vol. 37. Sinauer, Sunderland, MA.

Desjonqueres, C. 2023. Data from: Social plasticity enhances signal-preference codivergence. Version 1. American Naturalist, Zenodo, https://doi.org/10.5281/zenodo.7844980.

Desjonquères, C., J. Maliszewski, E. N. Lewandowski, B. Speck, and R. L. Rodríguez. 2019*a*. Social ontogeny in the communication system of an insect. Animal Behaviour 148:93–103.

Desjonquères, C., J. Maliszewski, and R. L. Rodríguez. 2021. Juvenile social experience and practice have a switch-like influence on adult mate preferences in an insect. Evolution 5:1106–1116.

Desjonquères, C., and R. L. Rodríguez. 2023. The direction and strength of social plasticity in mating signals and mate preferences vary with the life stage of induction. Animal Behaviour 200:255–261.

Desjonquères, C., B. Speck, and R. L. Rodríguez. 2019*b*. Signalling interactions during ontogeny are a cause of social plasticity in *Enchenopa* treehoppers (Hemiptera: Membracidae). Behavioural Processes 166:103887.

Dukas, R. 2004. Male fruit flies learn to avoid interspecific courtship. Behavioral Ecology 15:695–698.

Dukas, R., C. W. Clark, and K. Abbott. 2006. Courtship strategies of male insects: when is learning advantageous? Animal Behaviour 72:1395–1404.

Field, A. 2009. Discovering statistics using SPSS. Sage, Thousand Oaks, CA.

Flaxman, S. M., J. L. Feder, and P. Nosil. 2013. Genetic hitchhiking and the dynamic buildup of genomic divergence during speciation with gene flow. Evolution 67:2577–2591.

Flaxman, S. M., A. C. Wacholder, J. L. Feder, and P. Nosil. 2014. Theoretical models of the influence of genomic architecture on the dynamics of speciation. Molecular Ecology 23:4074–4088.

Fowler-Finn, K. D., D. C. Cruz, and R. L. Rodríguez. 2017. Local population density and group composition influence the signal-preference relationship in *Enchenopa* treehoppers (Hemiptera: Membracidae). Journal of Evolutionary Biology 30:13–25.

Fowler-Finn, K. D., J. T. Kilmer, A. C. Hallett, and R. L. Rodríguez. 2015. Variation in signal-preference genetic correlations in *Enchenopa* treehoppers (Hemiptera: Membracidae). Ecology and Evolution 5:2774–2786.

Fowler-Finn, K. D., and R. L. Rodríguez. 2012*a*. The evolution of experience-mediated plasticity in mate preferences. Journal of Evolutionary Biology 25:1855–1863.

———. 2012*b*. Experience-mediated plasticity in mate preferences: mating assurance in a variable environment. Evolution 66:459–468.

Fox, J., and S. Weisberg. 2011. An R companion to applied regression. 2nd ed. Sage, Thousand Oaks CA.

Hamilton, K. G. A., and R. B. Cocroft. 2009. Establishing the identity of existing names in the North American *Enchenopa binotata* species complex of treehoppers (Hemiptera: Membracidae). Entomological News 120:554–565.

Hebets, E., and L. Sullivan-Beckers. 2010. Mate choice and learning. Pages 389–393 *in* M. D. Breed and J. Moore, eds. Encyclopedia of animal behavior. Academic Press, London.

Hill, P. S. 2008. Vibrational communication in animals. Harvard University Press, Cambridge, MA.

Hill, P. S. M., and A. Wessel. 2016. Biotremology. Current Biology 26:R187–R191.

Hsu, Y.-H., R. B. Cocroft, R. L. Snyder, and C.-P. Lin. 2018. You stay, but I hop: host shifting near and far co-dominated the evolution of *Enchenopa* treehoppers. Ecology and Evolution 8:1954–1965.

Kilmer, J. T., K. D. Fowler-Finn, D. A. Gray, G. Höbel, D. Rebar, M. S. Reichert, and R. L. Rodríguez. 2017. Describing mate preference functions and other function-valued traits. Journal of Evolutionary Biology 30:1658–1673.

Kirkpatrick, M., and V. Ravigné. 2002. Speciation by natural and sexual selection: models and experiments. American Naturalist 159(suppl.):S22–S35.

Kopp, M., M. R. Servedio, T. C. Mendelson, R. J. Safran, R. L. Rodríguez, M. E. Hauber, E. C. Scordato, et al. 2018. Mechanisms of assortative mating in speciation with gene flow: connecting theory and empirical research. American Naturalist 191:1–20.

Lesna, I., and M. W. Sabelis. 1999. Diet-dependent female choice for males with "good genes" in a soil predatory mite. Nature 401:581–584.

Marie-Orleach, L., N. W. Bailey, and M. G. Ritchie. 2019. Social effects on fruit fly courtship song. Ecology and Evolution 9:410–416.

Marie-Orleach, L., A. M. Sanz, N. W. Bailey, and M. G. Ritchie. 2020. Does the response of *D. melanogaster* males to intrasexual

competitors influence sexual isolation? Behavioral Ecology 31:487–492.

Mendelson, T. C., and R. J. Safran. 2021. Speciation by sexual selection: 20 years of progress. Trends in Ecology and Evolution 36:1153–1163.

Michel, A. P., S. Sim, T. H. Powell, M. S. Taylor, P. Nosil, and J. L. Feder. 2010. Widespread genomic divergence during sympatric speciation. Proceedings of the National Academy of Sciences of the USA 107:9724–9729.

Nonaka, E., R. Svanbäck, X. Thibert-Plante, G. Englund, and Å. Brännström. 2015. Mechanisms by which phenotypic plasticity affects adaptive divergence and ecological speciation. American Naturalist 186:E126–E143.

Nosil, P. 2012. Ecological speciation. Oxford University Press, Oxford.

Pfennig, K. S. 2007. Facultative mate choice drives adaptive hybridization. Science 318:965–967.

Pratt, G., and T. K. Wood. 1992. A phylogenetic analysis of the *Enchenopa* binotata species complex (Homoptera: Membracidae) using nymphal characters. Systematic Entomology 17:351–357.

Rather, P. A., A. E. Herzog, D. A. Ernst, and E. L. Westerman. 2022. Effect of experience on mating behaviour in male *Heliconius melpomene* butterflies. Animal Behaviour 183:139–149.

R Core Team. 2015. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna.

Rebar, D., and R. L. Rodríguez. 2013. Genetic variation in social influence on mate preferences. Proceedings of the Royal Society B 280:20130803.

———. 2014a. Genetic variation in host plants influences the mate preferences of a plant-feeding insect. American Naturalist 184:489–499.

———. 2014b. Trees to treehoppers: genetic variation in host plants contributes to variation in the mating signals of a plant-feeding insect. Ecology Letters 17:203–210.

———. 2015. Insect mating signal and mate preference phenotypes covary among host plant genotypes. Evolution 69:602–610.

———. 2016. Males adjust their signalling behaviour according to experience of male signals and male-female signal duets. Journal of Evolutionary Biology 29:766–776.

Riesch, R., M. Muschick, D. Lindtke, R. Villoutreix, A. A. Comeault, T. E. Farkas, K. Lucek, et al. 2017. Transitions between phases of genomic differentiation during stick-insect speciation. Nature Ecology and Evolution 1:1–13.

Rodríguez, R. L., J. W. Boughman, D. A. Gray, E. A. Hebets, G. Höbel, and L. B. Symes. 2013a. Diversification under sexual selection: the relative roles of mate preference strength and the degree of divergence in mate preferences. Ecology Letters 16:964–974.

Rodríguez, R. L., and C. Desjonquères. 2019. Vibrational signals: sounds transmitted through solids. Pages 508–517 in J. C. Choe, ed. Encyclopedia of animal behaviour. 2nd ed. Academic Press, London.

Rodríguez, R. L., C. Haen, R. B. Cocroft, and K. D. Fowler-Finn. 2012. Males adjust signaling effort based on female mate-preference cues. Behavioral Ecology 23:1218–1225.

Rodríguez, R. L., A. C. Hallett, J. T. Kilmer, and K. D. Fowler-Finn. 2013b. Curves as traits: genetic and environmental variation in mate preference functions. Journal of Evolutionary Biology 26:434–442.

Rodríguez, R. L., K. Ramaswamy, and R. B. Cocroft. 2006. Evidence that female preferences have shaped male signal evolution in a clade of specialized plant-feeding insects. Proceedings of the Royal Society B 273:2585–2593.

Rodríguez, R. L., D. Rebar, and K. D. Fowler-Finn. 2013c. The evolution and evolutionary consequences of social plasticity in mate preferences. Animal Behaviour 85:1041–1047.

Rodríguez, R. L., L. E. Sullivan, and R. B. Cocroft. 2004. Vibrational communication and reproductive isolation in the *Enchenopa binotata* species complex of treehoppers (Hemiptera: Membracidae). Evolution 58:571.

Rodríguez, R. L., J. E. Wojcinski, and J. Maliszewski. 2018. Between-group variation in *Enchenopa* treehopper juvenile signaling (Hemiptera Membracidae). Ethology Ecology and Evolution 30:245–255.

Schluter, D. 1988. Estimating the form of natural selection on a quantitative trait. Evolution 42:849–861.

Servedio, M. R., G. S. V. Doorn, M. Kopp, A. M. Frame, and P. Nosil. 2011. Magic traits in speciation: "magic" but not rare? Trends in Ecology and Evolution 26:389–397.

Servedio, M. R., and M. A. Noor. 2003. The role of reinforcement in speciation: theory and data. Annual Review of Ecology, Evolution, and Systematics 34:339–364.

Servedio, M. R., S. A. Sæther, and G.-P. Sætre. 2009. Reinforcement and learning. Evolutionary Ecology 23:109–123.

Tibbetts, E. A., and E. C. Snell-Rood. 2021. Reciprocal plasticity and the diversification of communication systems. Animal Behaviour 179:297–306.

van Doorn, G. S., P. Edelaar, and F. J. Weissing. 2009. On the origin of species by natural and sexual selection. Science 326:5.

Verzijden, M. N., C. ten Cate, M. R. Servedio, G. M. Kozak, J. W. Boughman, and E. I. Svensson. 2012. The impact of learning on sexual selection and speciation. Trends in Ecology and Evolution 27:511–519.

West-Eberhard, M. J. 2003. Developmental plasticity and evolution. Oxford University Press, Oxford.

———. 2005. Developmental plasticity and the origin of species differences. Proceedings of the National Academy of Sciences of the USA 102:6543–6549.

Wood, S., and M. S. Wood. 2015. mgcv: mixed GAM computation vehicle with automatic smoothness estimation. R package version 1(29), 729. https://CRAN.R-project.org/package=mgcv.

Wood, T. K. 1993. Speciation of the *Enchenopa binotata* complex (Insecta: Homoptera: Membracidae). Evolutionary Patterns and Processes 14:299–317.

Wood, T. K., and S. I. Guttman. 1983. *Enchenopa binotata* complex: sympatric speciation? Science 220:310–312.