Uniformity in premating reproductive isolation along an intraspecific cline

Jeanne Marie Robertson\textsuperscript{a,b,*}, Roman Nava\textsuperscript{a,c}, Andrés Vega\textsuperscript{d}, and Kristine Kaiser\textsuperscript{a,b}

\textsuperscript{a}Department of Biology, California State University, Northridge, 18111 Nordhoff Street, Northridge, CA 91330-8303, USA, \textsuperscript{b}Natural History Museum of Los Angeles County, 900 Exposition Boulevard, Los Angeles, CA, 90007, USA, \textsuperscript{c}Department of Biology, San Diego State University, 5500 Campanile Drive, San Diego, CA 92116, USA, and \textsuperscript{d}AMBICOR, 400 E., 75 S., 75 E. de la Municipalidad de Tibas, Tibas, Costa Rica

*Address correspondence to Jeanne Marie Robertson. E-mail: jeanne.robertson@csun.edu.

Received on 3 August 2017; accepted on 10 November 2017

Abstract

Premating reproductive isolation (RI) may reduce gene flow across populations that have differentiated in traits important for mate choice. Examining RI across genetic and phenotypic clines can inform the fundamental evolutionary processes that underlie population and lineage differentiation. We conducted female mate-choice studies across an intraspecific red-eyed treefrog cline in Costa Rica and Panama with 2 specific aims: (1) to characterize RI across the cline and examine the relationship between premating RI and genetic and phenotypic distance and (2) to evaluate our results within a broader evolutionary and taxonomic perspective through examination of other RI studies. We found that female red-eyed treefrogs prefer local males relative to non-local males, indicating that some premating RI has evolved in this system, but that preference strength is not associated with phenotypic or geographic distance. Our analysis of 65 other studies revealed no clear pattern between the strength of RI and geographic distribution (allopatry, parapatry, cline) or phenotypic distance, but revealed extreme variation and overlap in levels of intra- and interspecific levels of RI. This work contributes to a growing body of literature that examines intraspecific RI across a cline to understand the selective processes that shape evolutionary patterns at the earliest stages of divergence.

Key words: advertisement call, anuran, color pattern, courtship signals, mate choice.

Behavioral reproductive isolation (RI) is accelerated when traits that are important for mate discrimination and choice diverge among populations and lineages (Rundle et al. 2005; Svensson et al. 2006; Colliard et al. 2010; Hoskin and Higie 2010; Richards-Zawacki and Cummings 2010; Selz et al. 2016). When social signals vary along an ecological cline, these evolved differences may reduce gene flow among neighboring populations. Clines are thus a classical system for examining the balance between strong selection on an ecological/social signal and the homogenizing effects of gene flow that could bring potentially maladaptive alleles into a population (Slatkin 1973; Endler 1980, 1982; Rosenblum 2006; Mullen and Hoekstra 2008).

Because the extent to which patterns of genetic and phenotypic diversity are concordant can provide insight into the strength of selection in a system, the integration of such studies along a cline has greatly contributed to an understanding of the mechanisms that mediate patterns of diversification (Barton and Hewitt 1985; Arnold et al. 2012; De La Torre et al. 2014; Walsh et al. 2016). However, predicting the evolution of RI based on patterns of genetic, phenotypic, and geographic distance is complicated and challenging. In part, this is because theoretical expectations of how populations diverge and diversify—whether in contact (e.g., along a cline) or in isolation (Coyne and Orr 1989, 1997; West-Eberhard 1983)—are not always supported by empirical studies. For example, although...
strong RI is expected to evolve for isolated populations (Coyne and Orr 1989), several studies have found phenotypically differentiated but genetically connected populations exhibit higher levels of premating RI than do allopatric populations (Prohl et al. 2006; Seehausen et al. 1997; Prohl et al. 2006; Tobler et al. 2009; Gabirot et al. 2013; Hughes et al. 2013), presumably due to strong selection to prevent hybridization (West-Eberhard 1983). There are also numerous examples of weak association between genetic isolation and RI (Tilley et al. 1990; Prohl et al. 2006). These conflicting examples demonstrate the extreme variability of premating RI. This is especially true for estimating premating RI at the intraspecific level where populations are in the earliest stages of lineage divergence (Tregenza 2002).

Here, we focus on premating isolation across a genotypic and phenotypic cline where populations vary in multiple social signals. We had 2 specific aims: first, we used female mate-choice tests to infer the strength of premating RI along an intraspecific genetic and phenotypic cline. Based on cline theory, we predicted that premating RI would (i) be detectable but incomplete along the cline, and (ii) vary with genetic, geographic, and/or phenotypic distance. Second, we conducted a literature review to evaluate the relative strength of assortative mating for clinal populations of red-eyed treefrogs and to contextualize the findings of our study of RI. We calculated premating RI from data presented in mate-choice studies of inter- and intraspecific clinal populations to provide a standardized metric (Ramsey et al. 2003; Martin and Mendelson 2016) and then compared levels of RI across other taxa. We integrate these 2 aims to further illuminate the processes that shape evolutionary patterns at the earliest stages of divergence (Tregenza 2002; Lemmon 2009; Selz et al. 2016; Stewart et al. 2016).

Materials and Methods

Study species

The red-eyed treefrog *Agalychnis callidryas* (Cope 1862) is a broadly distributed Neotropical frog ranging from southern México to Colombia with a nearly continuous distribution along the Caribbean versant of the Talamanca mountains (Savage 2002). Populations from northeastern Costa Rica to central Panamá exhibit population divergence in multiple traits, including color pattern (Robertson and Vega 2011), genotype (Robertson et al. 2009), body size (Robertson and Robertson 2008), male advertisement call (Akopyan et al. 2017), and antimicrobial skin peptides (Davis et al. 2016). We focus on color pattern and male advertisement call in this study as they are well established social signals important in communication in anurans (Ryan and Rand 1993; Summers et al. 1999; Ryan 2001; Taylor et al. 2007; Gomez et al. 2009).

We chose 3 populations for this study and refer to them according to relative location along the cline: north, central, and south (Figure 1). Across these 3 focal populations, color pattern, and call show discordant patterns of diversity (Figures 1 and 2): color pattern changes abruptly between the northern and central populations, with a more gradual change between central and southern populations (Robertson and Robertson 2008; Robertson and Vega 2011), while male advertisement call exhibits a relatively small change between northern and central populations, but large differences between central and southern populations (Akopyan et al. 2017).

Previous analyses on these populations reveal some gene flow among phenotypically divergent populations (Robertson et al. 2009). Specifically, mtDNA haplotypes from divergent populations are contained in the same clades, indicating historical levels of gene flow (Robertson and Zamudio 2009). Estimates of genetic distance ($F_{ST}$) based on microsatellite loci show weak differentiation among phenotypically distinct populations. This finding was supported by Bayesian assignment analyses that reveal admixture of differentiated populations. A strong pattern of genetic isolation by distance is observed along the cline (Robertson and Zamudio 2009; Robertson and Vega 2011), with no evidence that geographic barriers serve to isolate phenotypically differentiated populations. Combined, these findings indicate that selection could contribute, in part, to trait differentiation along the cline (Robertson et al. 2009). Behavioral studies show that female red-eyed treefrogs choose local males over males from an allopatric population that exhibit high levels of phenotypic and genetic differentiation (Jacobs et al. 2016), suggesting that premating barriers have begun to evolve among isolated populations.

Aim 1. Stimulus preparation

We used models for visual stimulus accompanied by acoustic playback. Models rather than live males provide inherent experimental and logistical advantages (Taylor et al. 2008): from an experimental perspective, the use of models controls for the confounding effects of male behavior (e.g., calling, postural displays) as well as population differences in male body size, all of which can affect female
choice (Briggs 2008; Taylor et al. 2008). Logistically, transporting live males among sites and across country borders is prohibited by Costa Rican and Panamanian governmental agencies due to the threat of accidental introductions of non-local species and/or disease.

We hand-sculpted plasticine models and painted them to resemble the color and stripe patterns of focal populations (Supplementary Material S1). To match each focal population we quantified the hue, saturation, and brightness (HSB) of the flank/leg color of a color-corrected digital photograph of a representative male from each population (Robertson and Robertson 2008) using Adobe Photoshop CC (2015). We then mixed paint by eye and quantified HSB of paint samples as described above. We adjusted the paint color and repeated the HSB measurements until we achieved a match. We placed models on a small, rotating platform attached to a motor (Vex Robotics, Vex IQ, Greenville, TX, USA) and programmed the motors to move using the Modkit software for Vex Robotics with the following looping program: repeat twice at a speed of 100° s⁻¹: (spin −20°, wait 20 s, spin 20°, wait 20 s); spin 45° at 50° s⁻¹; repeat twice at 50° s⁻¹: (spin −40°, wait 25 s, spin 40°, wait 25 s); spin 90° at 50° s⁻¹, wait 30 s, spin −90° at 50° s⁻¹, wait 60 s. This animation provided a visual stimulus (Paluh et al. 2014) but was not intended to mimic the natural movement of adult frogs. In order to minimize potential acoustic and vibrational interference from the motor, we surrounded it with cotton and foam baffling and put it in a plastic container.

We created acoustic stimuli in Audacity (v. 2.0.6) using previously collected recordings of male A. callidryas advertisement calls. We imported each recording and isolated a single-note call. We replicated that call at pseudorandom intervals at a rate of 2.75 calls per minute, approximating natural call rates (unpublished data). We used the Match Volume function in Adobe Audition (Adobe Creative Suite v. 5.5) to equalize the total RMS power in each playback. We created one stimulus from each individual male recording.

Figure 2. Variation in 2 social signals for Agalychnis callidryas. A. Leg coloration: percent of leg that is red with standard error. B. Male advertisement call, dominant frequency, in Hz with standard error. C. Male advertisement call, bandwidth, in Hz with standard error. Phenotypes show anti-parallel change. The geographic distance (km) between north, central, and south populations provided.
We broadcast vocalization playbacks from speakers (Pignose 7-1000, Las Vegas, NV, USA) positioned immediately behind models. Playbacks were broadcast at approximately 65 dB SPL at 1 m, similar to natural calls of this species (unpublished data), and were calibrated with a sound-level meter (Radio Shack 33-2055, Fort Worth, TX, USA). Local and non-local male models and their associated calls were randomly assigned to the right or left side/speaker. We used a different call for each female within each site but used the same set of stimuli across sites.

**Experimental chamber and mate-choice trials**

This study was conducted from June to August 2015. We used a 2-choice design in a partitioned Y-maze enclosure, consisting of a 1.2 × 1.2 × 1.0 m metal frame with fine metal mesh on all but one side. The final side was covered with coarse plastic mesh to allow us to visualize trials. A piece of black tarp extended 50 cm forward from the back of the enclosure, creating the “Y.” A speaker (Pignose 7-1000) was suspended above the enclosure from which ambient noise was broadcast during trials (Supplementary Material S2).

Females from each population were tested against male stimuli from all 3 populations, but each female was used in only one trial. We used wild-caught, gravid females from each population. Trials were conducted the night that females were captured. Focal females were provided with a choice between a local stimulus (local male color pattern and local advertisement call) and a non-local stimulus. At the start of each trial, the female was acclimated to the experimental enclosure under an opaque container for 2 min. During this time, the speaker above the enclosure broadcast a recording of a natural chorus (60 dB SPL at 1 m) that included conspecifics. After 2 min, the conspecific call playback from speakers behind the models started, and the speaker above the enclosure broadcast a recording of a chorus that did not include conspecific calls. This background stimulus was used to avoid possible inadvertent cues suggesting danger: an absence of chorus noise may indicate the presence of a predator, thus making females less likely to choose a mate (Dapper et al. 2011). Finally, the acclimation chamber was removed and the trial began. Each trial lasted 10 min and was visualized with an infrared video camera (Bell and Howell DNV16HDZ Night Vision Camcorder, Wheeling, IL, USA). A choice was scored when a female displayed mating behavior to a male stimulus (Akopyan et al. 2017). Mating displays included flank displays, where a female approached the male and turned 90° to extend her flank to the male, and/or back displays, where a female turned 180° and presented her back to the male (Akopyan et al. 2017). Females that did not make a choice were excluded from analysis.

To be sure we did not inadvertently resample females in a given experiment, we photographed each female for individual identification (EOS Rebel T3, Canon, Melville, NY, USA) and manually checked photographs. Frogs were released at the point of capture.

**Statistical Analyses**

We calculated a standardized metric of premating behavioral RI using the following formula (Martin and Mendelson 2016):

\[
RI = \frac{\text{Choice}_{\text{local}} - \text{Choice}_{\text{non-local}}}{\text{Choice}_{\text{local}} + \text{Choice}_{\text{non-local}}}
\]

RI ranges from −1 to +1, with negative values demonstrating an increase in gene flow among populations and positive values indicating decreased gene flow. A value +1 indicates complete RI (100% choice of the local stimulus).

In addition to calculating premating RI, we used 3 distinct but complementary statistical approaches to test for assortative mating and to test for an effect of the cline on choice. First, we performed a Chi-square analysis using all trials for which we observed female choice to determine whether there was local-male preference. Second, we used a log-linear model (StataIC v. 10.1, College Station, TX, USA) to determine the factors that best predict female choice among 3 fixed-effects variables that included male chosen (local vs. non-local), female natal population (north, central, south), and chosen male population (north, central, south). “Female natal population” tests for whether a single population drives a pattern of choice, or whether all females choose local males, regardless of their natal population. “Chosen male population” tests for a “super male”—that is, a population that was preferentially chosen, regardless of whether that male population was a local or non-local stimulus. Within the glm function, we set the distribution to Poisson to allow for categorical variables, and the link identity to log (Agresti 2007). We calculated the incident rate ratio (IRR) from the final model. The IRR is a measure of the frequency with which events occur. We used this to determine the likelihood that a female would choose a male based on the final set of model parameters using the eform command in Stata. We performed stepwise model reduction, using \( P = 0.05 \) as a criterion for retention in the model. We used Akaike’s Information Criterion (AIC) to assess model performance.

Third, we used JMATING v. 1.08 (Carvajal-Rodriguez and Rolan-Alvarez 2006) to test for RI across the cline, using the pairwise total isolation (PTI) metric. PTI is the most appropriate measure for a mate-choice study involving models because only female choice contributes to the measure (Rolan-Alvarez E, personal communication). In addition, PTI provides an estimate of assortative mating for each pair separately, which allows for evaluation of patterns of assortative mating across the cline. PTI values >1 indicate evidence for assortative mating, while lower PTI values (<1) indicate disassortative mating (Rolan-Alvarez et al. 2012). Thus, if the cline has an effect on mating patterns, then measures of PTI should differ between the 2 non-local males, with stronger estimates of disassortative mating for one male relative to the other, but with PTI for local male being the highest. In contrast, if there is an overall pattern of assortative mating without a cline effect, then the PTI should resemble these results except that the PTI for the 2 non-local males should be similar. Significance was determined for PTI for each pairwise population comparison based on 10,000 bootstrapping replicates and a G-test.

**Does RI vary with phenotypic, genetic, and geographic distance along the cline?**

We tested the association between RI and indices of phenotypic, genetic, and geographic distance using Mantel tests. We constructed Euclidean-distance matrices of leg coloration based on both hue and the proportion of the leg that is red for each population (Robertson and Vega 2011). The protocol for these color measurements is detailed previously (Robertson and Robertson 2008; Robertson and Vega 2011); briefly, digital photographs were color corrected in Adobe Photoshop CC (2015) using a standard gray-scale card in the background of each photograph. Photos were imported into ImageJ Ver. 1.50h (Abramoff et al. 2004) for measurements. The Euclidean-distance matrix for male advertisement call was based on the dominant frequency and bandwidth of 12–22 males from each population (Akopyan et al. 2017). We used 2 measures of pairwise population genetic distance: the first based on 6 microsatellite loci and the second estimated from mtDNA NADH1 haplotypes.
Robertson et al. · Reproductive isolation among intraspecific populations

Table 1. The number of mate choice trials for each population pair \( (n_{\text{total}}) \) and those that resulted choice \( (n_{\text{choice}}) \) for 3 focal populations of *Agalychnis calidryas*

| Female site | Male natal population | \( n_{\text{total}} \) | \( n_{\text{choice}} \) | Local male | RI |
|-------------|-----------------------|------------------------|------------------------|------------|----|
| North       | Central               | 23                     | 19                     | 12 (63%)   | 0.26|
|             | South                 | 24                     | 20                     | 13 (65%)   | 0.30|
| Central     | North                 | 32                     | 18                     | 12 (60%)   | 0.20|
|             | South                 | 33                     | 20                     | 12 (60%)   | 0.20|
| South       | Central               | 26                     | 20                     | 13 (65%)   | 0.30|
| Totals      |                       | 163                    | 117                    | 73         |    |

Note: The number (and percentage) of trials that resulted local male choice are provided, along with estimates of RI for each population comparison (see text for calculation of RI).

(Robertson et al. 2009). We measured geographic distance (kilometer) as straight-line distance between sites in ArcGIS (Robertson et al. 2009). We conducted 10,000 permutations for each pairwise Mantel test in R (v. 3.2.1).

Aim 2. Examining patterns and predictors of premating RI across taxa

We conducted a Web of Science search on 24–26 April 2016 using keywords mate choice, cline, RI, intraspecific. We included only publications that 1) described a trait relevant to mate choice and 2) reported mate preference/choice so that we could calculate RI. We calculated RI as above to test whether RI varies with respect to categorical estimates of lineage divergence (intraspecific or interspecific), geographic isolation (cline, allopatry, sympatry, parapatry [non-clinal but geographically disjunct as defined by the authors]) and/or social trait that characterized the differentiated populations (e.g., call, color pattern, body size, pheromone, or combinations of several traits). In all cases, RI was measured from raw mate-choice data, a standard approach for examining behavioral isolation among diverging taxa. We also used the literature review to compare our estimates of premating RI across the red-eyed treefrog clade with other studies of RI in interspecific and intraspecific mate-choice clines.

Results

Aim 1. Premating RI along the red-eyed treefrog cline

We conducted 163 female-choice trials across the 3 sites, 117 (72% of trials) of which resulted in a choice (Table 1). When considering all trials combined, we found a local-male advantage: females chose a local male in 73/117 (62%) of trials (\( z^2 = 7.180, P = 0.007 \)). Estimates of premating RI were uniformly greater than zero, indicating assortative mating and ranged from 0.2 to 0.3 (Table 1).

Log-linear models revealed that female natal population did not drive choice of local or non-local male (\( z = 0.24, P = 0.81, \text{IRR} \ 1.03 \)); that is, females from all populations behaved similarly, with a slight preference for local males over non-local males. Male population of origin also did not predict choice (\( z = -0.31, P = 0.76, \text{IRR} \ 0.96 \)), indicating no advantage of a single phenotype across populations. Therefore, both female and male population of origin were dropped from the model in a stepwise manner (Supplementary Material S3). The final model included only whether male stimuli were local or non-local for predicting female choice (\( z = -6.27, P < 0.01 \)): the IRR shows that females were 3.32 times more likely to choose a local male than a non-local male.

Aim 2. Examining patterns and predictors of premating RI across taxa

The Web of Science search resulted in a total of 65 studies that met our search criteria. Not surprisingly, there was no single social signal that best predicted premating RI along intraspecific clines (Figure 4). Because differences in life history, ecology, and social signal function could explain possible taxonomic differences in RI, we also examined each major taxonomic lineage separately; as expected, this yielded no striking insights, largely due to the taxonomic biases. For example, for frog studies, call and color dominated the literature, while bird studies were largely based on color as a social signal.

![Figure 3. Premating RI in red-eyed treefrogs does not vary geographic distance. See text for calculation of RI.](https://academic.oup.com/cz/advance-article-abstract/doi/10.1093/cz/zox066/4630269)
Although many studies use RI as a measure for divergence, to our knowledge, this is the first review of RI across clinal populations and species and thus gives important context for such data.

We calculated estimates of inter- and intraspecific premating RI across taxa. As expected, average premating RI was higher for interspecific than for intraspecific comparisons, but RI is highly variable for both and estimates overlapped substantially (interspecific comparisons: mean = 0.42; SD = 0.44; range = 0.6–1.0; 104 estimates from 31 studies examined; intraspecific: mean = 0.28; SD = 0.44; range = 1.0–1.0; 98 estimates from 33 studies examined; Table 3 and Figure 4).

Discussion

Divergent populations of red-eyed treefrogs exhibit a general pattern of female choice for local males, with estimates of premating RI that are similar to other intraspecific and interspecific studies. However, our results departed from expectations that clinal populations would show concordant patterns of assortative mating in 3 ways: (1) we found relative uniformity in assortative mate choice and RI rather than a correlative relationship between RI and geographic distance between sites (Figure 3). Only one population, central, avoided the non-local male stimulus, although there was no difference in negative preference for the non-local populations; (2) we found no relationship between phenotypic distance and RI; and (3) we detected no pattern of increased RI with increased genetic distance.

Understanding non-clinal RI along a cline

In this study, estimates of premating RI were uniform along the cline, and did not vary with geographic, genetic, or phenotypic distance. Despite a strong signal of genetic isolation with geographic distance across Costa Rican and Panamanian populations, as well as high estimates of pairwise genetic distances between focal sites (Robertson and Vega 2011), we found that neutral levels of gene flow are not correlated with premating isolation. An examination of RI at multiple points along the cline (that increases the number of...
Table 3. Estimates of premating RI for major taxonomic groups summarized from 65 published papers

| Taxon                                      | Taxonomic level | Geographic distribution | Divergent phenotype studied | RI average | Range          | References                  |
|--------------------------------------------|-----------------|-------------------------|-----------------------------|------------|----------------|-----------------------------|
| Insects                                    |                 |                         |                             |            |                |                             |
| *Carabus lewisiatus*, *C. albrechti*       | Interspecific   | NA                      | Body size                   | 0.13       | 0.11–0.14      | Takami and Suzuki (2005)    |
| *Chauliognathus pennsylvanicus*            | Intraspecific   | Clinal                  | Body size                   | 0.71       | 0.43–1         | McLain (1985)               |
| *Colletes cumuliculus*                     | Interspecific   | Allopatric              | Pheromone                   | –0.51      | −0.58 to −0.43 | Vercken et al. (2006)       |
| *Heliconius erato*                         | Interspecific   | Allopatric              | Color pattern               | 0.15       | NA             | Finkbeiner et al. (2014)    |
| *Leptidea sinapis*, *L. reali*, *L. juvernica* | Interspecific   | NA                      | Color pattern               | 1          | NA             | Dinca et al. (2013)         |
| *Fishes*                                   |                 |                         |                             |            |                |                             |
| *Campylomormyrus compressirostris*, *C. rhynchophorus*, *C. tamandua* | Interspecific | NA                      | Electric discharge          | 0.19       | 0.06–0.32      | Feulner et al. (2009)       |
| *Cichlasoma citrinellum*, *C. zahioum*     | Interspecific   | NA                      | Ecomorph                    | 1          | NA             | Baylis (1976)               |
| *Cyprinella venusta*, *C. lutrens*         | Interspecific   | NA                      | Pheromone                   | 0.58       | 0.51–0.64      | Ward and Blum (2012)        |
| *Cyprinodon beltrani*, *C. labioso*, *C. maya* | Interspecific   | NA                      | Body size                   | 0.61       | −0.2–1.0       | Strecke and Kodric-Brown (2000) |
| *Cyprinodon pecosensis*, *C. Variegatus*   | Interspecific   | NA                      | Color pattern               | 0.04       | −0.27–0.35     | Rosenfield and Kodric-Brown (2003) |
| *Cyprinodon variegatus variegatus*, *C. v. hubbsi* | Interspecific | NA                      | Pheromone                   | 0.13       | 0.07–0.18      | Brix and Grosell (2013)     |
| *Etheostoma barrense*, *E. zonale*         | Interspecific   | NA                      | Color pattern               | 0.97       | 0.95–1.0       | Williams and Mendelson (2010) |
| *Etheostoma smithi*, *E. nigripinne*       | Interspecific   | NA                      | Color pattern               | 0.63       | 0.45–0.82      | Williams and Mendelson (2011) |
| *Gambusia affinis*, *G. geiseri*           | Interspecific   | NA                      | Body size                   | 0.63       | 0.6–0.66       | Espinoza et al. (2010)      |
| *Gambusia affinis*, *G. nobilis*           | Interspecific   | NA                      | Pheromone                   | 0.2        | 0.1–0.3        | Swenton (2011)              |
| *Gasterosteus aculeatus*                   | Intraspecific   | Sympatric               | Ecomorph                    | 0.38       | −0.25–1.0      | Albert (2005)               |
| *Gasterosteus aculeatus*                   | Intraspecific   | Allopatric              | Ecomorph                    | 0.74       | NA             | Vines and Schluter (2006)   |
| *Gasterosteus aculeatus*                   | Intraspecific   | Allopatric              | Ecomorph                    | 0.1        | 0.05–0.14      | Hughes et al. (2013)        |
| *Gasterosteus aculeatus*                   | Intraspecific   | Allopatric              | Body size                   | 0.52       | −0.17–1.0      | Ziegler and Zotin (1995)    |
| *Gasterosteus wheatlanti*, *G. aculeatus*  | Interspecific   | NA                      | Ecomorph                    | 0.17       | −0.43–1.0      | Jones et al. (2008)         |
| *Girardinichthys multiradiatus*            | Intraspecific   | Allopatric              | Body size                   | 0.74       | NA             | Zuurh and Garcia (2006)     |
| *Haplochromis nyererei*                    | Intraspecific   | Clinal                  | Color pattern               | 0.64       | 0.56–0.71      | Seelhausen et al. (1997)    |
| *Labeotrophus fuelleborni*                 | Intraspecific   | Allopatric              | Color pattern, pheromone    | 0.68       | 0.5–0.87       | Pauers et al. (2010)        |
| *Maylandia zebra*, *M. benetos*            | Interspecific   | NA                      | Color pattern               | 1          | NA             | Ding et al. (2014)          |
| *Nothobranchius furzeri*, *N. orthoponos*  | Interspecific   | NA                      | Body size                   | 0.33       | −0.2–0.71      | Polacik and Reichard (2011) |
| *Nothobranchius korthausae*                | Intraspecific   | Allopatric              | Body size                   | 0.29       | 0.05–0.53      | Reichard and Polacik (2010) |
| *Poecilia latipinna*, *P. formosa*         | Interspecific   | NA                      | Pheromones                  | 0.43       | NA             | Gabor and Grober (2010)     |
| *Poecilia mexicana*                        | Intraspecific   | Allopatric              | Color pattern               | 0.06       | −0.23–0.23     | Bierbach et al. (2013)      |
| *Poecilia Mexicana*, *P. sulphuraria*      | Intraspecific   | Allopatric              | Ecomorph                    | 0.13       | −0.04–0.22     | Tobler et al. (2009)        |
| *Poecilia reticulata*                      | Intraspecific   | Allopatric              | Color pattern               | 0.03       | −0.26–0.36     | Plath et al. (2010)         |

(Continued)
| Taxon                          | Taxonomic level | Geographic distribution | Divergent phenotype studied | RI average | Range       | References                      |
|-------------------------------|-----------------|-------------------------|------------------------------|------------|-------------|--------------------------------|
| Poecilia reticulata, *P.* picta | Interspecific    | NA                      | Pheromone                    | 0.37       | 0.02–0.9    | Magurran and Ramnarine (2004)   |
| Poeciliopsis occidentalis, *P.* sonorenensis | Interspecific    | NA                      | Behavior                     | 0.46       | 0.02–0.9    | Hurt et al. (2004)              |
| Pseudotropheus zebra, *P.* emmiltos | Interspecific    | NA                      | Color pattern                | 0.63       | −0.17–1     | Blais et al. (2009)             |
| Pandanima nyereei           | Intraspecific   | Sympatric               | Color pattern                | 0.15       | 0.14–0.16   | Maan et al. (2004)              |
| Taeniopygia guttata guttata, *T. g.* castanotis | Interspecific    | NA                      | Body size                    | 0.5        | 0.35–0.7    | Clayton (1990)                  |
| Tropeus sp.                  | Interspecific   | Allopatric              | Color pattern                | 0.44       | −0.6–1      | Sefc et al. (2015)              |
| Tropeus moorii               | Interspecific   | Allopatric              | Color pattern                | 0.87       | 0.74–1      | Egger et al. (2008)             |
| Uraeginthus angolensis, *U.* bengalus, *U.* cyanocephalus | Interspecific   | NA                      | Color pattern                | 0.54       | 0.28–0.8    | Collins and Luddem (2002)       |
| Xiphophorus birchmanni, *X.* malinche | Interspecific   | NA                      | Pheromone                    | 0.14       | −0.47–0.74  | Wong et al. (2005)              |
| Agalychnis callidryas        | Intraspecific   | Cline                   | Color, call                  | 0.25       | 0.2–0.3     | This study                      |
| Agalychnis callidryas        | Intraspecific   | Allopatric              | Color, call                  | 0.45       | 0.3–0.6     | Jacobs et al. (2016)            |
| Dendrobates pumilio          | Intraspecific   | Allopatric              | Color pattern                | 0.02       | −0.22–0.11  | Richards-Zawacki and Cummings (2010) |
| Dendrobates pumilio          | Intraspecific   | Allopatric              | Color pattern                | 0.7        | 0.5–0.89    | Reynolds and Fitzpatrick (2007) |
| Litoria gemmaculata          | Intraspecific   | Allopatric              | Color pattern                | 0.67       | 0.62–0.74   | Summers et al. (1999)           |
| Notopelphalmus virdescens, *N.* v. dorsalis | Intraspecific   | NA                      | Call                         | 0.38       | −0.2–1      | Hoskin et al. (2005)            |
| Physalaemus pastulosus       | Intraspecific   | Cline                   | Call                         | 0.33       | −0.07–0.55  | Prohl et al. (2006)             |
| Physalaemus pastulosisus, *P.* enesefae | Interspecific | NA                      | Call                         | 0.7        | NA          | Bonachea and Ryan (2011)        |
| Plethodon cinereus           | Intraspecific   | Sympatric               | Color pattern                | 0.45       | NA          | Anthony et al. (2008)           |
| Ranitomeya imitator          | Intraspecific   | Clinal                  | Call                         | 0.17       | −0.09–0.5   | Twomey et al. (2016)            |
| Mus musculus domesticus, *M.* m. musculus | Interspecific | NA                      | Pheromone                    | −0.03      | −0.41–0.06  | Smajda and Ganem (2002)         |
| Mus musculus musculus, *M.* m. domesticus | Interspecific | NA                      | Pheromone                    | 0.33       | NA          | Zinck and Lima (2013)           |
| Neotoma lepida, *N.* bryanti | Interspecific   | NA                      | Pheromone                    | 0.4        | −0.2–1      | Shurtliff et al. (2013)         |
| Otomys noratus               | Intraspecific   | Allopatric              | Color pattern                | 0.45       | 0.32–0.58   | Pillay et al. (1995)            |
| Rhabdomys pumilio            | Intraspecific   | Allopatric              | Color pattern                | 0.37       | 0.13–0.67   | Pillay (2000)                   |
| Aves                          | Interspecific   | Allopatric              | Color pattern                | −0.01      | −0.6–0.45   | Moore (1987)                    |
| Columbid sp.                 | Interspecific   | NA                      | Color pattern                | 0.06       | 0–0.12      | Burley (1981)                   |
| Ficedula hypoleuca, *F.* albicollis, *F.* semitorquata | Interspecific | NA                      | Color pattern                | 0.75       | 0.67–0.82   | Saetre et al. (1997)            |
| Larus argentatus, *L.* cachinnans | Interspecific | NA                      | Body size                    | 0.45       | 0.43–0.48   | Neubauer et al. (2009)          |
| Sula leucogaster             | Interspecific   | Allopatric              | Color pattern                | 0.38       | −0.01–0.76  | Lopez-Rull et al. (2016)        |
| Podarcis hispanica           | Intraspecific   | Parapatric              | Pheromone                    | −0.03      | −0.1–0.04   | Gabirot et al. (2012)           |
| Podarcis muralis             | Intraspecific   | Sympatric               | Color pattern                | −0.17      | −0.39–0.37  | Sacchi et al. (2015)            |
| Sceloporus undulatus         | Intraspecific   | Parapatric              | Color pattern                | 0.12       | −0.4–0.6    | Hardwick et al. (2013)          |
| Sceloporus graciosus         | Intraspecific   | Clinal                  | Behavior                     | 0.5        | 0.32–0.76   | Bissell and Martins (2006)      |

Notes: For each study, we include the taxonomic level (interspecific/intraspecific), geographic distribution (cline, allopatric, parapatric), the trait studied, average RI, and range. RI calculated as in text. NA, not available.
replicate estimates of RI) would provide insight into fine-scale pre-
mating RI along a genetic cline. Similarly, we found that neither 

color pattern nor call correlated with RI. Two possible scenarios 
might account for our finding that premating isolation did not 

increase with either measure of phenotypic divergence. One expla-
nation is that neither trait evolves through strong selection. However, 
given the importance of call and/or color in mediating social interac-
tions for most anurans (Rand 2001; Ryan 2001; Gomez et al. 2009), 
it is more likely that, instead, both traits act as relevant cues in this 
species: previous work demonstrates that females choose visually 
displaying mates in the absence of male calling (Jacobs et al. 2016), 
indicating that color pattern is likely an important mate-choice cue.

The observation of anti-parallel patterns of divergence in red-
eyed treefrog social traits (Figure 2) could have important repercus-
sions for premating isolation. This discordant pattern of differen-
tiation presents a particularly interesting opportunity to understand 

how phenotypic divergence affects mate choice: in population com-
binations where call shows shallow differentiation, color divergence 
is steep, while the converse is true for other population comparisons 
(Figure 2). It is possible that divergence in either trait may suffice to 
maintain preference for local males. That is, it is possible that once a 
threshold is reached where social traits have sufficiently diverged to 
result in a mate preference for local phenotype, greater phenotypic 
divergence among populations encodes relatively little additional 
relevant information. As a result, increased divergence would not 
necessarily result in a stronger, correlative increase in local prefer-
ence until the signal shifts to become unrecognizable as a conspecific 
signal and behavioral RI is complete. We thus propose that red-eyed 

(treefrog females use information encoded in at least 2 signals, result-
ing in partial assortative mating. The relatively uniform estimates of 

RI across populations support this interpretation. Further investiga-
tion of the mechanisms of assortative mating will be the focus of 

future studies.

**Evolutionary consequences of uniform premating isolation along a cline**

The strength of premating isolation along a phenotypic cline can lend insight into the evolutionary processes mediating cline dynamics 
and lineage diversification (Seehausen 1996; Bissell and Martins 
2006; Selz et al. 2014; Stewart et al. 2016). A few general patterns 
emerged from the review of intra- and interspecific studies of RI 
(Table 3). As expected, average premating RI is higher for interspe-
cific rather than intraspecific comparisons. In addition, premating 

RI does not differ among allopatric, clinal, and sympatric compari-
sions, regardless of whether we combined all taxonomic groups or 
examined each major lineage independently. Finally, both inter- and 
intraspecific RI are highly variable, with ranges overlapping sub-
stantially (Figure 4), suggesting that the rate and extent of RI cannot 
solely be predicted based on the time since divergence.

We compared our estimates of premating RI along the red-eyed 
treefrog cline with other studies of RI in intraspecific mate-choice experiments. Estimates of premating RI for red-eyed treefrogs in our 
study (RI = 0.2–0.3) are similar to the average intraspecific RI com-
parison (Figure 4). In addition, allopatric populations of red-eyed 
treefrogs show higher levels of premating RI (Jacobs et al. 2016), 
suggesting that RI can be asymmetric and potentially more pro-
nounced for allopatric populations.

There are 3 caveats to our interpretations from the literature 
review. First, the potential for sampling bias in publications should 
be considered: researchers are less likely to study premating isolation 
for intraspecific populations that do not exhibit apparent differences 
in social signals (Gleason and Ritchie 1998). Therefore, publication 
results are biased toward a higher estimate of RI for intraspecific 
comparisons. Similarly, the traits most commonly accepted to be 
important in communication in a taxonomic group tend to be the 
traits that are studied. Our analyses of RI studies by taxonomic line-
age show that fish studies are most diverse in terms of the number of 
modalities tested for premating RI (e.g., color pattern, behavior, 
body size, ecomorph, electric discharge, pheromone), while most 

bird and frog studies focus on color pattern and/or call as important 

mate-choice cues. We do not interpret this as evidence that diverse 
modalities are more important in fish than other taxonomic groups; 
indeed, recent research has focused on the importance of multiple 

communication modes in a variety of taxa. Rather, we point out 
that there remains a testing bias to the traits examined in RI studies. 
As studies begin to incorporate mechanistic studies of the relative 
role of multiple signaling modalities, we will better understand how 

they interact to affect mate choice. The final caveat is that data 
reported in the literature may lead to an oversimplified interpreta-
tion that premating RI does not vary among social signals. Out of 
necessity, studies generally focus on a single or small number of 
traits, but mate choice is complex and receivers of mating signals 
may make decisions based on numerous traits, including those 

researchers do not directly test. For example, red-eyed treefrog pop-
ulations also exhibit strong differentiation in other traits that may 
be relevant in mate choice, including body size, other aspects of 

color pattern variation (Robertson and Robertson 2008) and antim-
icrobial skin peptides (Davis et al. 2016); it is likely that this is also 
the case for other divergent populations and species.

In summary, speciation theory predicts that disruptive sexual 
selection for traits important in population and species-recognition 
mediates lineage divergence and speciation (Boul et al. 2007; 
Hoskin and Higgin 2010): our analyses suggest this could be true for 
red-eyed treefrog populations along the Caribbean cline. Although 
we found a signature of female choice for local males, the extent of 

premating RI was far from complete, but consistent with findings from other studies of RI for intraspecific comparisons. Overall, our 
results indicate that incomplete premating isolation has evolved 
through female mate choice, that premating RI likely plays a role 

population diversification, and that multiple social signals should be 
evaluated for understanding mechanisms of lineage diversification.

**Author Contributions**

All authors contributed to fieldwork/mate-choice trials and manu-
script preparation. In addition, J.M.R., A.V., and K.K. designed the 
study and J.M.R., R.N.-L., and K.K. contributed to data analyses.

**Acknowledgments**

We thank the Autoridad Nacional del Ambiente from the Republic of 
Panamá and Ministerio de Ambiente y Energía of Costa Rica for granting per-
miss to conduct research. We thank the Organizational for Tropical Studies, 
La Selva Biological Research Station, Smithsonian Tropical Research 
Institute, Gamboa Field Station, and Cabinas Kániki for logistical support 
and permission to conduct research. D.M. Spear, C. Hitchcock, J. Kołowski, 
and V. Hermann provided field assistance. C. Hitchcock sculpted and 
painted the male models. Thank you to E. Rolan for help with JMATING and 
the interpretation of the data. We thank S. Fitzpatrick, R. Espín:oza, and 3 
anonymous reviewers for comments that greatly improved the manuscript. 
We thank M. Caldwell for providing frog recordings from Gamboa, Panamá 
for use in trials. This research was funded by California State University,
Northridge and the National Geographic Society Waitt Grant (W377-15) to J.M.R., A.V., and K.K.

Supplementary Material

Supplementary material can be found at https://academic.oup.com/cz.

References

Abramoff MD, Magalhaes PJ, Ram SJ, 2004. Image processing with ImageJ. Bioprotocol Int 11:36–42.
Agresti A, 2007. An Introduction to Categorical Data Analysis. Hoboken (NJ): Wiley.
Akopian M, Kaiser K, Savant NG, Owen CY, Vega A et al., 2017. Melodic males and flashy females: geographic variation in male and female reproductive behavior in red-eyed treefrogs Agalychnis callidryas. *Etology*. doi: 10.1111/eth.12705.
Albert AYK, 2005. Mate choice, sexual imprinting, and speciation: A test of a one-allele isolating mechanism in sympatric sticklebacks. *Evolution* 59:927–931.
Anthony CD, Venesky MD, Hickerson CAM, 2008. Ecological separation in a polymorphic terrestrial salamander. *J Anim Ecol* 77:646–653.
Arnold ML, Ballerini ES, Brothers AN, 2012. Hybrid fitness, adaptation and evolutionary diversification: lessons learned from Louisiana irises. *Heredity* 108:159–166.
Barton NH, Hewitt GM, 1985. Analysis of hybrid zones. *Ann Rev Ecol Syst* 16:113–148.
Baube CL, 2008. Body size and the maintenance of reproductive isolation in stickleback, genus Gasterosteus. *Etology* 114:1122–1134.
Bayls JR, 1976. Quantitative study of long-term courtship. 1. Ethological isolation among sympatric populations of midas cichlid, Cichlasoma cimilum, and arrow cichlid, C. zelosum. *Behaviour* 59:59–69.
Bierbach D, Penshorn M, Hamfler S, Herbert DB, Appel J et al., 2013. Gradient evolution of body colouration in surface- and cave-dwelling Poecilia mexicana and the role of phenotype- assortative female mate choice. *Biomed Res Int*. doi: 10.1155/2013/148348.
Bissell AN, Martins EP, 2006. Male approach and female avoidance as mechanisms of population discrimination in sagebrush lizards. *Behav Ecol Sociobiol* 60:655–662.
Blais J, Pledgerlein M, Risco C, Taylor MI, Seehausen O et al., 2009. Assortative mating among Lake Malawi cichlid fish populations is not simply predictable from male nuptial colour. *BMC Evol Biol* 9. doi: 10.1186/1471-2148-9-53.
Bonachea LA, Ryan MJ, 2011. Predation risk increases permissiveness for heterospecific advertisement calls in tangles frogs, *Physalaemus pustulosus*. *Anim Behav* 82:347–352.
Boul KE, Funk WC, Darst CR, Cannatella DC, Ryan MJ, 2007. Sexual selection drives speciation in an Amazonian frog. *Proc R Soc Lond Ser B Biol Sci* 274:399–406.
Briggs VS, 2008. Mating patterns of red-eyed treefrogs, *Agalychnis callidryas* and *A. moreletii*. *Etology* 114:489–498.
Brix KV, Grosell M, 2013. Evaluation of pre- and post-zygotic mating barriers, hybrid fitness and phylogenetic relationship between *Cyprinodon variegatus* variegatus and *Cyprinodon variegatus hubbsi* (Cyprinodontiformes, Teleostei). *J Evol Biol* 26:854–866.
Burley N, 1981. Male choice by multiple criteria in a monogamous species. *Am Nat* 117:515–528.
Carvalho-Rodriguez A, Rolan-Alvarez E, 2006. JMATING: a software for the analysis of sexual selection and sexual isolation effects from mating frequency data. *BMC Evol Biol* 6; doi: 10.1186/1471-2148-6-40.
Clayton NS, 1990. Assortative mating in zebra finch subspecies, *Taeniopygia guttata guttata* and *T. g. castanostis*. *Philos Trans R Soc Lond B Biol Sci* 330:351–370.
Colliard C, Sicilia A, Turrisi GF, Arculeo M, Perrin N et al., 2010. Strong reproductive barriers in a narrow hybrid zone of West-Mediterranean green toads (*Bufo viridis* subgroup) with Pleo-Pleistocene divergence. *BMC Evol Biol* 10:232; doi: 10.1186/1471-2148-10-232.
Collins SA, Luddem ST, 2002. Degree of male ornamentation affects female preference for conspecific versus heterospecific males. *Proc R Soc Lond B Biol Sci* 269:111–117.
Cope ED, 1862. Catalogue of the reptiles obtained during the explorations of the Parana, Paraguay, Vermejo, and Uruguay Rivers, by Captain Thos. J. Page, U.S.N., and of those procured by Lieut. N. Michler, U.S. Top. Eng., Commander of the expedition conducting the survey. *Proc Acad Nat Sci Philadelphia* 14:346–359.
Coyne JA, Orr HA, 1989. Two rules of speciation. In: Otte D, Endler JA, editors. *Speciation and Its Consequences*. Sunderland (MA): Sinauer, 180–207.
Coyne JA, Orr HA, 1997. “Patterns of speciation in Drosophila” revisited. *Evolution* 51:295–303.
Dapper AL, Baugh AT, Ryan MJ, 2011. The sounds of silence as an alarm cue in tangles frogs, *Physalaemus pustulosus*. *Biotropica* 43:6.
Davis LR, Klonski K, Rutschow HL, Van Wijk KJ, Sun Q et al., 2016. Host defense skin peptides vary with color pattern in the highly polymorphic red-eyed treefrog. *Front Ecol Evol* 4. doi: 10.3389/fevo.2016.00097.
De La Torre AR, Wang TL, Jaquish B, Atkin SN, 2014. Adaptation and exogogenous selection in a *Picea glauca × Picea engelmannii* hybrid zone: implications for forest management under climate change. *New Phytol* 201:687–699.
Dinca V, Wiklund C, Lukhanov VA, Kodandaramaiah U, Noren K et al., 2013. Reproductive isolation and patterns of genetic differentiation in a cryptic butterfly species complex. *J Evol Biol* 26:2095–2106.
Ding B, Daugherthy DW, Husmann M, Chen M, Howe AE et al., 2014. Quantitative genetic analyses of male color pattern and female mate choice in a pair of cichlid fishes of Lake Malawi, East Africa. *PLoS ONE* 9. doi: 10.1371/journal.pone.0114798.
Egger B, Mattersdorfer K, Seif KM, 2010. Variable discrimination and asymmetric preferences in laboratory tests of reproductive isolation between cichlid colour morphs. *J Evol Biol* 23:433–439.
Egger B, Obermuller B, Eigner E, Sturmbauer C, Seif KM, 2008. Assortative mating preferences between colour morphs of the endemic Lake Tanganyika cichlid genus Trophes. *Hydrobiologia* 615:37–48.
Endler JA, 1980. Natural selection on color patterns in *Poecilia reticulata*. *Evolution* 34:76–91.
Endler JA, 1982. Convergent and divergent effects of natural selection on color patterns in two fish faunas. *Evolution* 36:178–188.
Espinozo CM, Gabor CR, Asbury AS, 2010. Males, but not females, contribute to sexual isolation between two sympatric species of Gambusia. *Evol Ecol* 24:865–878.
Feulner PGD, Plath M, Engelmann J, Kirschbaum F, Tiedemann R, 2009. Electrifying love: electric fish use species-specific discharge for mate recognition. *Behav Ecol* 20:225–228.
Finkbeiner SD, Briscoe AD, Redd RD, 2014. Warning signals are seductive: Relative contributions of color and pattern to predator avoidance and mate attraction in *Heliconius* butterflies. *Evolution* 68:3410–3420.
Gabriot M, Lopez P, Martin J, 2012. Interpopulation variation in chemosensory responses to selected sterols from femoral secretions of male lizards, *Podarcis hispanica*, mirrors population differences in chemical signals. *Chemoecology* 22:65–73.
Gabriot M, Lopez P, Martin J, 2013. Female mate choice based on pheromone content may inhibit reproductive isolation between distinct populations of Iberian wall lizards. *Carr Zool* 59:210–220.
Gabor CR, Grober MS, 2010. A potential role of male and female androgen in species recognition in a unisexual-bisexual mating complex. *Horm Behav* 57:427–433.
Glegg JM, Ritchie MG, 1998. Evolution of courtship song and reproductive isolation in the *Drosophila willistoni* species complex: do sexual signals diverge the most quickly? *Evolution* 52:1493–1500.
Gomez D, Richardson C, Lengagne T, Plenet S, Joly P et al., 2009. The role of nocturnal vision in mate choice: females prefer conspicuous males in the European tree frog *Hyla arborea*. *Proc R Soc Lond B Biol Sci* 276:2351–2358.
Hardwick KM, Robertson JM, Rosenblum EB. 2013. Asymmetrical mate preference in recently adapted White Sands and black lava populations of Scoloporus undulatus. *Carr Zool* 59:20–30.

Hoskin CJ, Higgin M. 2010. Speciation via species interactions: the divergence of mating traits within species. *Ecol Lett* 13:409–420.

Hoskin CJ, Higgin M, McDonald KR, Montiz C. 2005. Reinforcement drives rapid allopatric speciation. *Nature* 437:1333–1336.

Hughes LC, Foster SA, Baker JA. 2013. Can ecotopic differences in male courtship behaviour be explained by visual cues provided by female three-fin stickleback? *Evol Ecol Res* 15:437–451.

Hurt CR, Stares-Ellis S, Hughes KA, Hedrick PW. 2004. Mating behaviour in the endangered Sonoran toadstoolpom: speciation in action. *Annu Behav* 67:343–351.

Jacobs L, Vega A, Dudgeon S, Kaiser K, Robertson JM. 2016. Local not vocal: assortative female choice in divergent populations of red-eyed treefrogs *Agalychnis callidryas* (Hyliidae: Phyllomedusinae). *Biol J Linn Soc* 120:171–178.

Jones FC, Brown C, Braithwaite VA. 2008. Lack of assortative mating between incipient species of stickleback from a hybrid zone. *Behaviour* 145:463–484.

Lemmon EM. 2009. Diversification of conspecific signals in sympathy: geographic overlap drives multidimensional reproductive character displacement in frogs. *Evolution* 63:1155–1170.

Lopez-Rull I, Litshitz N, Macias Garcia C, Graves JA, Torres R. 2016. Females of a polymorphic seahorse dislike foreign-looking males. *Annu Behav* 113:31–38.

Maan ME, Seehausen O, Soderberg I, Johnson L, Ripmeester EAP et al., 2004. Intraspecific sexual selection on a speciation trait, male coloration, in the Lake Victoria cichlid *Pundamilia nyererei*. *Proc R Soc Lond Ser B Biol Sci* 271:2445–2452.

Magramun AE, Paxton CGM, Seghers BH, Shaw PW, Carvalho GR. 1996. Genetic divergence: female choice and male mating success in Trinidadian guppies. *Behaviour* 133:503–517.

Magramun AE, Rammarine IW. 2004. Learned mate recognition and reproductive isolation in guppies. *Annu Behav* 67:1077–1082.

Martin MD, Mendelson TC. 2016. The accumulation of reproductive isolation in early stages of divergence supports a role for sexual selection. *J Evol Biol* 29:676–689.

McLain DK. 1985. Clinical variation in morphology and assortative mating in the soldier beetle, *Chauliognathus pennsylvanicus* (Coleoptera, Cantharidae). *Biol J Linn Soc* 25:103–117.

Moore WS. 1987. Random mating in the northern flicker hybrid zone – implications for the evolution of bright and contrasting plumage patterns in birds. *Evolution* 41:539–546.

Mullen LM, Hockstra HE. 2008. Natural selection along an environmental gradient: a classic cline in mouse pigmentation. *Evolution* 62:1535–1569.

Neubauer G, Zagalska-Neubauer MM, Pons J-M, Crochet P-A, Chylarecki P et al., 2009. Assortative mating without complete reproductive isolation in a zone of recent secondary contact between herring gulls (*Larus argentatus*) and caspian gulls (*L. cachinnans*). *Auk* 126:409–419.

O’Rourke CF, Mendelson TC. 2010. Male and female preference for conspecifics in a fish with male parental care (*Percidae: Catonotus*). *Behav Processes* 85:157–162.

Paluh DJ, Hantak MM, Saporito RA. 2014. A test of aposematism in the dendrobatid poison frog *Oophaga pumilio*: the importance of movement in clay model experiments. *J Herpetol* 48:249–254.

Pauers MJ, Ehlinger TJ, McKinnon JS. 2010. Female and visually based mate preferences are consistent with reproductive isolation between populations of the Lake Malawi endemic Labeotropheus fuelleborni. *Carr Zool* 56:653–72.

Pillay N. 2000. Female mate preference and reproductive isolation in populations of the striped moust Haplochromis pumilio. *Behaviour* 137:1431–1441.

Pillay N, Willan K, Meester J, Cooke J. 1995. Evidence of pre-mating reproductive isolation in 2 allopatric populations of the vlei rat, *Otomys irroration*. *Ethology* 100:61–71.

Plath M, Riesch R, Oranth A, Dzienko J, Karau N et al., 2010. Complementary effect of natural and sexual selection against immigrants maintains differentiation between locally adapted fish. *Naturwissenschaften* 97:769–774.

Polack M, Reichard M. 2011. Asymmetric reproductive isolation between two sympatric annual killifish with extremely short lifespans. *PLoS ONE* 6: doi: 10.1371/journal.pone.0022684.

Prohl H, Koshy RA, Mueller U, Rand AS, Ryan MJ. 2006. Geographic variation of genetic and behavioral traits in northern and southern Tungara frogs. *Evolution* 60:1669–1679.

Ramsey J, Bradshaw H, Schemske DW. 2003. Components of reproductive isolation between the monkeyflowers *Mimulus lewisii* and *M. cardinals* (*Phrymaceae*). *Evolution* 57:1520–1534.

Rand SA. 2001. A history of frog call studies from 405 B.C. to 1980. In: Ryan MJ, editor. *Annu Communication*. Washington (DC): Smithsonian Institution Press, 23–35.

Reichard M, Polack M. 2010. Reproductive isolating barriers between colour-differentiated populations of an African annual killifish, *Notrobranchus korthausiae* (*Cynodontiformes*). *Biol J Linn Soc* 100:62–72.

Reynolds RG, Fitzpatrick BM. 2007. Assortative mating in poison-dart frogs based on an ecologically important trait. *Evolution* 61:2253–2259.

Richards-Zawacki CL, Cummings ME. 2010. Intraspecific reproductive character displacement in a polymorphic poison dart frog *Dendrobates pumilio*. *Evolution* 65:259–267.

Robertson J, Robertson A. 2008. Spatial and temporal patterns of phenotypic variation in a Neotropical frog. *J Biogeog* 35:830–843.

Robertson JM, Dursey MC, Zumadio KR. 2009. Discordant patterns of evolutionary differentiation in two Neotropical treefrogs. *Mol Ecol* 18:1375–1395.

Robertson JM, Vega A. 2011. Genetic and phenotypic variation in a colourful treefrog across five geographic barriers. *J Biogeog* 35:2122–2135.

Robertson JM, Zumadio KR. 2009. Genetic diversification, vicariance, and selection in a polytypic frog. *J Hered* 100:715–731.

Rolán-Alvarez E, Saura M, Díaz AP, Rivera MJ, Alvarez M et al., 2012. Can sexual selection and disassortative mating contribute to the maintenance of a shell color polymorphism in an intertidal marine snail? *Carr Zool* 58:463–474.

Rosenblum EB. 2006. Convergent evolution and divergent selection: lizards at the White Sands ecotone. *Am Nat* 167:1–15.

Rosenfield JA, Kodric-Brown A. 2003. Sexual selection promotes hybridization between Pecos pupfish, *Cyprinodon pecosensis* and sheepshead minnow, *C. variegatus*. *J Evol Biol* 16:595–606.

Rundle HD, Chenoweth SF, Dougherty P, Blows MW. 2005. Divergent selection and the evolution of signal traits and mating preferences. *PLoS Biol* 3:1988–1995.

Ryan MJ. 2001. *Amurian Communication*. Washington (DC): Smithsonian Institution Press.

Ryan MJ, Rand AS. 1993. Species recognition and sexual selection as a unitary problem in animal communication. *Evolution* 47:647–657.

Sacchi R, Ghitti M, Scali S, Mangiacotti M, Zuffi MAL et al., 2015. Common Wall Lizard females *Podarcis muralis* do not actively choose males based on their colour morph. *Ethology* 121:1145–1153.

Saetre GP, Moum T, Bares S, Kral M, Adamjan M et al., 1997. A sexually selected character displacement in flycatchers reinforces premating isolation. *Nature* 387:589–592.

Savage JM. 2002. *The Amphibians and Reptiles of Costa Rica: a Herptofauna between Two Continents, between Two Seas*. Chicago (IL): The University of Chicago Press.

Seehausen O. 1996. Distribution and of reproductive isolation among color-morphs of a rock-dwelling Lake Victoria cichlid *Haplochromis nyererei*. *Ecol Freshw Fish* 5:1195–1202.

Seehausen O, van Alphen JMJ, Witte F. 1997. Cichlid fish diversity threatened by eutrophication that curbs sexual selection. *Science* 277:1808–1811.

Sect KM, Herrmann CM, Steinwender B, Brindl H, Zimmermann H et al., 2015. Asymmetric dominance and asymmetric mate choice oppose premating isolation after allopatric divergence. *Evol Ecol* 5:1549–1562.

Selz OM, Thommen R, Pierotti MER, Anayarojas JM, Seehausen O. 2016. Differences in male coloration are predicted by divergent sexual selection between populations of a cichlid fish, *Proc R Soc Lond Ser B Biol Sci* 283: doi:10.1098/rspb.2016.0172.
Selz OM, Thommen R, Maan ME, Seehausen O, 2014. Behavioural isolation may facilitate homoploid hybrid speciation in cichlid fish. *J Evol Biol* 27:275–289.
Shurtleff QR, Murphy PJ, Yetter JD, Matocq MD, 2013. Experimental evidence for asymmetric mate preference and aggression: behavioral interactions in a woodrat *Neotoma* hybrid zone. *BMC Evol Biol* 13:1–13.
Slatkin M, 1973. Gene flow and selection in a cline. *Genetics* 75:733–756.
Smadja C, Ganem G, 2002. Subspecies recognition in the house mouse: a study of two populations from the border of a hybrid zone. *Behav Ecol* 13:312–320.
Stewart KA, Austin JD, Zamudio KR, Lougheed SC, 2016. Contact zone dynamics during early stages of speciation in a chorus frog *Pseudacris crucifer*. *Heredity* 116:239–247.
Strecker U, Kodric-Brown A, 2000. Mating preferences in a species flock of Mexican pupfishes (*Cyprinodon*, Teleostei). *Biol J Linn Soc* 71:677–687.
Summers K, Symula R, Clough M, Cronin T, 1999. Visual mate choice in poison frogs. *Proc R Soc Lond Ser B Biol Sci* 266:2141–2145.
Svensson EI, Eroukhmanoff F, Friberg M, 2006. Effects of natural and sexual selection on adaptive population divergence and premating isolation in a damselfly. *Evolution* 60:1242–1253.
Swenton DM, 2011. Sex differences in mate preference between two hybridizing species of poecilid fish. *Ecolology* 117:208–216.
Takahashi MK, Takahashi YY, Parris MJ, 2010. On the role of sexual selection in ecological divergence: a test of body-size assortative mating in the eastern newt *Notophthalmus viridescens*. *Biol J Linn Soc* 101:884–897.
Takami Y, Suzuki H, 2005. Morphological, genetic and behavioural analyses of a hybrid zone between the ground beetles *Carabus lewisi* and *C. albrechti* (*Coleoptera*, *Carabidae*): asymmetrical introgression caused by movement of the zone? *Biol J Linn Soc* 86:79–94.
Taylor RC, Buchanan BW, Doherty JL, 2007. Sexual selection in the squirrel treefrog *Hyla squirella*: the role of multimodal cue assessment in female choice. *Anim Behav* 74:1753–1763.
Taylor RC, Klein BA, Stein J, Ryan MJ, 2008. Faux frogs: multimodal signaling and the value of robotics in animal behaviour. *Anim Behav* 76:1089–1097.
Tilley SG, Verrell PA, Arnold SJ, 1990. Correspondence between sexual isolation and allozyme differentiation: a test in the salamander *Desmognathus ochrophaeus*. *Proc Natl Acad Sci USA* 87:2715–2719.
Tobler M, Risch R, Tobler CM, Schulz-Mirbach T, Plath M, 2009. Natural and sexual selection against immigrants maintains differentiation among micro-allopatric populations. *J Evol Biol* 22:2298–2304.
Tregenza T, 2002. Divergence and reproductive isolation in the early stages of speciation. *Genetica* 116:291–300.
Towner E, Vestergaard JS, Venegas PJ, Summers K, 2016. Mimetic divergence and the speciation continuum in the mimic poison frog *Ranitomeya imitator*. *Am Nat* 187:205–224.
Vercken E, Massot M, Smerco B, Clobert J, 2006. Colour variation and alternative reproductive strategies in females of the common lizard *Lacerta vivipera*. *J Evol Biol* 20:11.
Vines TH, Schluter D, 2006. Strong assortative mating between allopatric sticklebacks as a by-product of adaptation to different environments. *Proc R Soc Lond, Ser B: Biol Sci* 273:911–916.
Walsh J, Shriver WG, Olsen BJ, Kovach AI, 2016. Differential introgression and the maintenance of species boundaries in an advanced generation avian hybrid zone. *BMC Evol Biol* 16:65; doi: 10.1186/s12862-016-0635-y.
Ward JL, Blum MJ, 2012. Exposure to an environmental estrogen breaks down sexual isolation between native and invasive species. *Evol Appl* 5:901–912.
West-Eberhard MJ, 1983. Sexual selection, social competition, and speciation. *Q Rev Biol* 58:153–183.
Williams TH, Mendelson TC, 2010. Behavioral isolation based on visual signals in a sympatric pair of darter species. *Ethology* 116:1038–1049.
Williams TH, Mendelson TC, 2011. Female preference for male coloration may explain behavioural isolation in sympatric darters. *Anim Behav* 82:683–689.
Wong BBM, Fisher HS, Rosenthal GG, 2005. Species recognition by male swordtails via chemical cues. *Behav Ecol* 16:818–822.
Zinck L, Lima SQ, 2013. Mate choice in *Mus musculus* is relative and dependnt on the estrous state. *PLoS ONE* 8, doi: 10.1371/journal.pone.0066064.
Zuganov VV, Zotin AA, 1995. Pelvic girdle polymorphism and reproductive barriers in the nine-spine stickleback *Pungitius pungitius* (L) from northwest Russia. *Behaviour* 132:1095–1105.
Zuurth CG, Garcia CM, 2006. Phenotypic differentiation and pre-mating isolation between allopatric populations of *Girardinichthys multiradiatus*. *Proc R Soc Lond Ser B Biol Sci* 273:301–307.