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## Article

# Uniformity in premating reproductive isolation along an intraspecific cline

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## 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 Higgie 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 (Pröhl et al. 2006; Seehausen et al. 1997; Pröhl 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; Pröhl 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
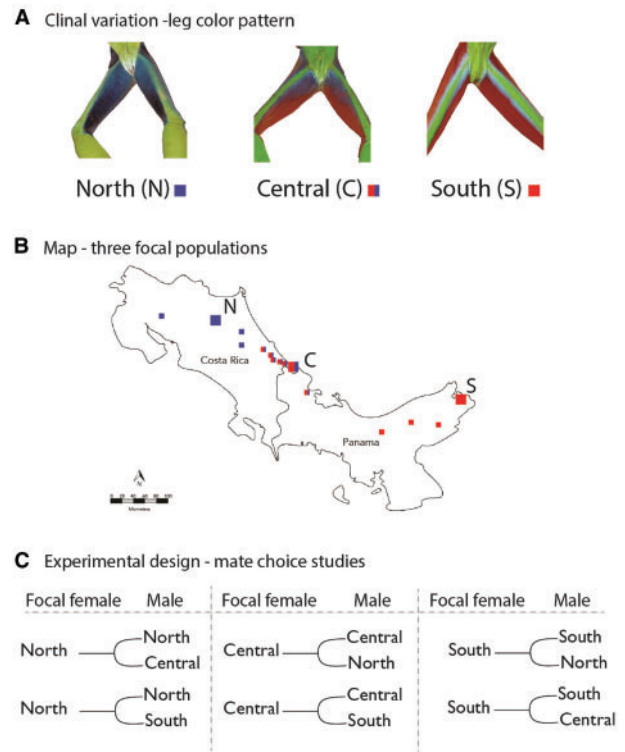


**Figure 1.** Color variation and 3 focal populations of red-eyed treefrog and experimental design. **A**. Photographs of the dorsal aspect of legs show differences in leg color pattern among 3 focal sites along the cline. **B**. We chose 3 focal populations along a nearly continuous distribution of red-eyed treefrogs to represent each of the primary color forms from northeastern Costa Rica to southern Panama: North: La Selva Biological Station, Heredia, Costa Rica, 10.432990, −84.002959; Central: Playa Gandoca, Limón, Costa Rica, 9.597799, −82.606251; and South: Gamboa, Colón, Panamá, 9.115688, −79.696573. **C**. The design of behavioral experiments for each focal female population. For each trial, a female was provided a choice between a local and one non-local stimulus male.

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
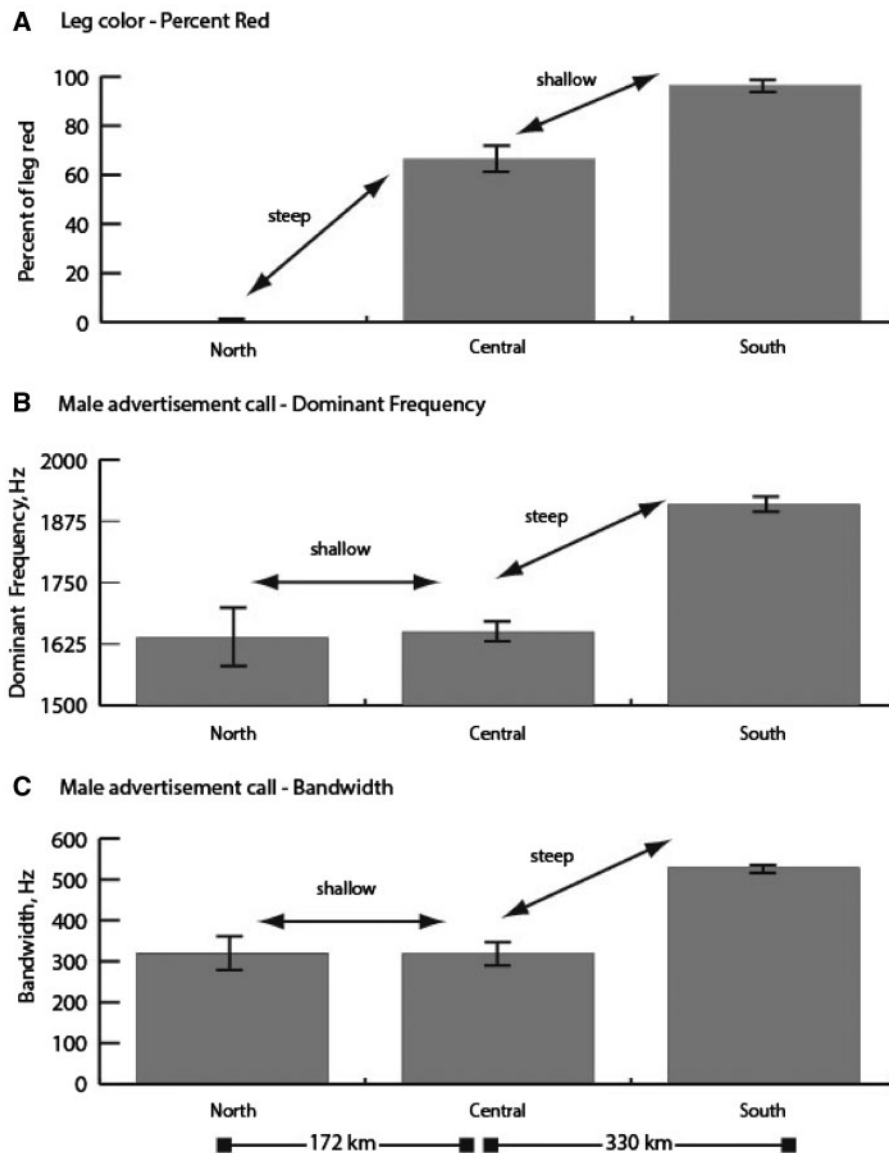
**Figure 2.** Variation in 2 social signals for *Agalychnis callidryas*. **A.** Leg coloration: percent of leg that is red with standard error. **B.** Male advertisement call, dominant frequency, in Hz with standard error. **C.** Male advertisement call, bandwidth, in Hz with standard error. Phenotypes show anti-parallel change. The geographic distance (km) between north, central, and south populations provided.

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.

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 $1.2 \times 1.2 \times 1.0$ 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):

$$RI = \frac{Choice_{local} - Choice_{non-local}}{Choice_{local} + Choice_{non-local}}$$

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 complimentary 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 instead 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. 1.50i (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

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

| Female site | Male natal population | $n_{total}$ | $n_{choice}$ | Local male | RI |
|---|---|---|---|---|---|
| North | Central | 23 | 19 | 12 (63%) | 0.26 |
| | South | 24 | 20 | 13 (65%) | 0.30 |
| Central | North | 32 | 20 | 12 (60%) | 0.20 |
| | South | 25 | 18 | 11 (61%) | 0.22 |
| South | North | 33 | 20 | 12 (60%) | 0.20 |
| | 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 along the red-eyed treefrog cline with other studies of RI in interspecific and intraspecific mate-choice experiments.

## 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 ($\chi_1^2 = 7.180$, $P = 0.007$). 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 ($z = 0.24$, $P = 0.81$, 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 ($z = -0.31$, $P = 0.76$, 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.

**Table 2.** Measures of reproductive isolation (PTI) show uniform estimates of assortative mating for local males across the cline

| Population | North | Central | South |
|---|---|---|---|
| North | 1.8197* | 0.6181 | 0.5516 |
| Central | 0.5392* | 1.883* | 0.5323* |
| South | 0.5867 | 0.6045 | 1.8613* |

*Notes*: The central population shows significant preference for local males and a significant avoidance of non-local males. The north and south populations only show preference for local males. PTI values >1 indicate evidence for assortative mating, while lower PTI values (<1) indicate non-assortative mating. PTI estimates from generated in JMATING. Significance (*$P < 0.05$) estimated from 9,999 bootstrapping replicates.
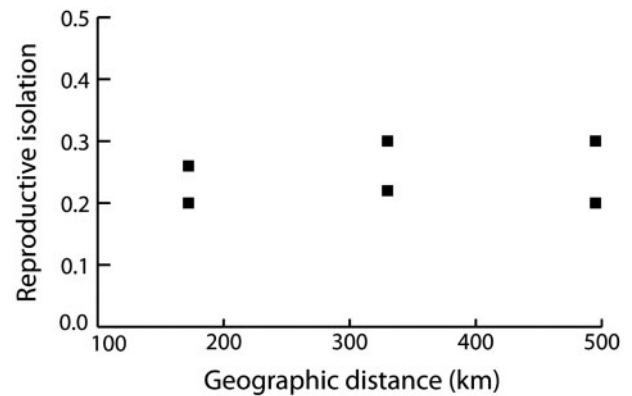


**Figure 3.** Premating RI in red-eyed treefrogs does not vary geographic distance. See text for calculation of RI.

Results from JMATING were aligned with the log-linear model analyses, with each pairwise test resulting in PTI scores greater than 1, demonstrating that females from each population preferred local males. However, we found no effect of the cline: females from the north and south populations responded to the 2 non-local males equally, with PTI < 1. The central population also responded equally to the 2 non-local males. For this population, the negative association was significant, yet similar, among the 2 non-local populations. Thus, the central population shows both strong preference for local males and an equally strong negative preference for both non-local males (Table 2).

Mantel tests showed that across the red-eyed treefrog cline, RI was not associated with phenotypic, genetic (Supplemental Material S4), or geographic distance: color ($R^2 = 0.92$, $P = 0.15$), call ($R^2 = -0.06$, $P = 0.67$), genetic distance (nuclear microsatellites: $R^2 = 0.90$, $P = 0.169$; mtDNA: $R^2 = 0.10$, $P = 0.535$), and geographic distance ($R^2 = 0.51$, $P = 0.34$; Figure 3).

### 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.
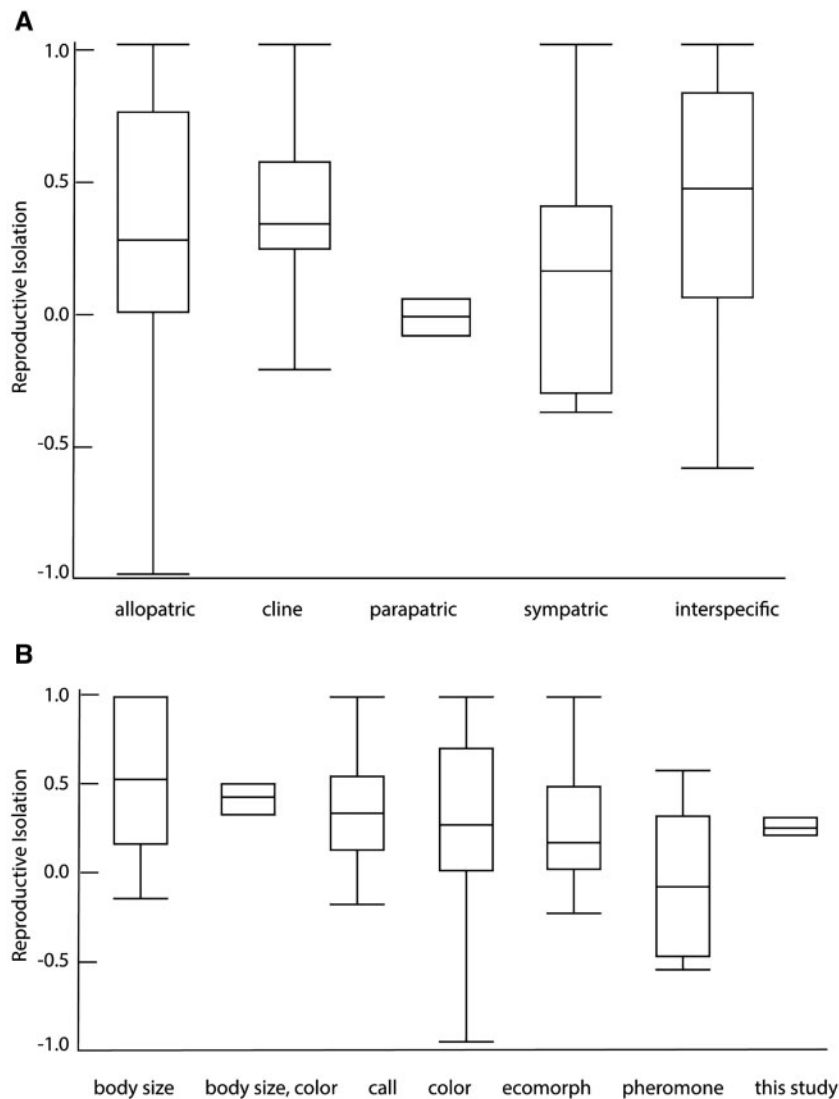
**Figure 4**. Premating RI estimates from a literature review of 65 studies of interspecific and intraspecific comparisons. We standardized measures of RI as a metric that ranges from −1.0 (disassortative mating) to 0 (random mating) to 1 (assortative mating). See Table 3 for references. **A**. RI does not vary between interspecific and intraspecific studies, nor does RI vary among geographic mode of isolation for intraspecific studies (allopatry, parapatry, cline, sympatry). **B**. RI does not vary among phenotypic trait under study. The results of this study are provided for comparison.

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 distance 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 lewisianus*, *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 cunicularius* | Intraspecific | Allopatric | Pheromone | −0.51 | −0.58 to −0.43 | Vercken et al. (2006) |
| *Heliconius erato* | Intraspecific | 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. zaliosum* | Interspecific | NA | Ecomorph | 1 | NA | Baylis (1976) |
| *Cyprinella venusta*, *C. lutrensis* | Interspecific | NA | Pheromone | 0.58 | 0.51–0.64 | Ward and Blum (2012) |
| *Cyprinodon beltrani*, *C.labiosus*, *C. maya* | Interspecific | NA | Body size | 0.61 | −0.2–1.0 | Strecker 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 barrenense*, *E. zonale* | Interspecific | NA | Color pattern | 0.97 | 0.95–1.0 | Williams and Mendelson (2010) |
| *Etheostoma barrenense*, *E. zonale* | Interspecific | NA | Color pattern | 0.63 | 0.45–0.82 | Williams and Mendelson (2011) |
| *Etheostoma smithi*, *E. nigripinne* | Interspecific | NA | Color pattern | 0.88 | NA | O'Rourke and Mendelson (2010) |
| *Gambusia affinis*, *G. geiseri* | Interspecific | NA | Body size | 0.63 | 0.6–0.66 | Espinedo 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 | Ziuganov and Zotin (1995) |
| *Gasterosteus aculeatus* | Interspecific | NA | Ecomorph | 0.17 | −0.43–1.0 | Jones et al. (2008) |
| *Gasterosteus wheatlandi*, *G. aculeatus* | Interspecific | NA | Body size | 1 | NA | Baube (2008) |
| *Girardinichthys multiradiatus* | Intraspecific | Allopatric | Body size | 0.74 | NA | Zuarth and Garcia (2006) |
| *Haplochromis nyererei?* | Intraspecific | Clinal | Color pattern | 0.64 | 0.56–0.71 | Seehausen et al. (1997) |
| *Labeotropheus 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. orthonotus* | 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 | Clinal | Color pattern | 0.06 | −0.23–0.23 | Bierbach et al. (2013) |
| *Poecilia mexicana* | Intraspecific | Allopatric | Ecomorph | 0.13 | −0.04–0.22 | Tobler et al. (2009) |
| *Poecilia Mexicana*, *P. sulphuraria* | Interspecific | NA | Body size | 0.03 | −0.26–0.36 | Plath et al. (2010) |
| *Poecilia reticulata* | Intraspecific | Allopatric | Color pattern | 0 | −1–1.0 | Magurran et al. (1996) |

(Continued)

**Table 3.** 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. sonoriensis* | 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) |
| *Pundamilia nyererei* | 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) |
| *Tropheus* sp. | Intraspecific | Allopatric | Color pattern | 0.44 | −0.6–1 | Sefc et al. (2015) |
| *Tropheus moorii* | Intraspecific | Allopatric | Color pattern | 0.87 | 0.74–1 | Egger et al. (2008) |
| *Tropheus moorii* | Intraspecific | Allopatric | Color pattern | 0.22 | −0.6–1 | Egger et al. (2010) |
| *Uraeginthus angolens, 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) |
| **Amphibians** | | | | | | |
| *Agalychnis callidryas* | Intraspecific | Clinal | Color, call | 0.25 | 0.2–0.3 | This study |
| *Agalychnis callidryas* | Intraspecific | Allopatric | Color, call, body size | 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) |
| *Dendrobates pumilio* | Intraspecific | Allopatric | Color pattern | 0.67 | 0.62–0.74 | Summers et al. (1999) |
| *Litoria genimaculata* | Intraspecific | Allopatric | Call | 0.38 | −0.2–1 | Hoskin et al. (2005) |
| *Notophthalmus viridescens viridescens, N. v. dorsalis* | Interspecific | NA | Body size | −0.06 | −0.09 to − 0.03 | Takahashi et al. (2010) |
| *Physalaemus pustulosus* | Intraspecific | Clinal | Call | 0.33 | −0.07–0.55 | Pröhl et al. (2006) |
| *Physalaemus pustulosus, 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 | Allopatric | Color pattern | 0.17 | −0.09–0.5 | Twomey et al. (2016) |
| **Mammals** | | | | | | |
| *Mus musculus domesticus, M. m. musculus* | Interspecific | NA | Pheromone | −0.03 | −0.41–0.06 | Smadja 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 iworatus* | Intraspecific | Allopatric | Pheromone | 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** | | | | | | |
| *Colaptes auratus, C. a. auratus* | Interspecific | NA | 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 argentaus, L. cachinnans* | Interspecific | NA | Body size | 0.45 | 0.43–0.48 | Neubauer et al. (2009) |
| *Sula leucogaster* | Intraspecific | Allopatric | Color pattern | 0.38 | −0.01–0.76 | Lopez-Rull et al. (2016) |
| **Lizards** | | | | | | |
| *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 explanation is that neither trait evolves through strong selection. However, given the importance of call and/or color in mediating social interactions 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 repercussions for premating isolation. This discordant pattern of differentiation presents a particularly interesting opportunity to understand how phenotypic divergence affects mate choice: in population combinations 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 preference 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, resulting in partial assortative mating. The relatively uniform estimates of RI across populations support this interpretation. Further investigation 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 interspecific rather than intraspecific comparisons. In addition, premating RI does not differ among allopatric, clinal, and sympatric comparisons, 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 substantially (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 comparison (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 pronounced 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 lineage 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 interpretation 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 populations 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 antimicrobial 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 Higgie 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 manuscript 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.

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## Supplementary Material

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

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