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Brief Communication

Character displacement of egg colors during tinamou speciation

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Abstract

The divergence of reproductive traits frequently underpins the evolution of reproductive isolation. Here we investigated whether tinamou (Tinamidae) egg colorations function as mating signals that diverged as character displacement (mating signal character displacement hypothesis). We tested three evolutionary predictions behind the hypothesis: (a) egg colors coevolve with known mating signals; (b) signal divergence is associated with divergent habitat adaptation; and (c) sympatric tinamou species with similar songs have different egg colors as character displacement during speciation. We found support for all three predictions. In particular, egg colors coevolved with songs; songs and egg colors coevolved with habitat partitioning; and tinamou species that were likely sympatric with similar songs tended to have different egg colors. In conclusion, the mating signal character displacement hypothesis is well supported in which egg colors serve as mating signals that undergo character displacement during tinamou speciation.

Keywords: character displacement, egg color, Tinamou, speciation, mating signal, coevolution, song

Introduction

Reproductive trait divergence is crucial for speciation because reproductive traits frequently form barriers of gene flow at the onset of speciation (Koski & Ashman, 2016; Pfennig, 2016). A puzzling reproductive trait divergence exists in tinamou egg colorations, where the ground nesting tinamous lay bright, divergently colored eggs among different species, ranging from brilliant magenta to pink, purple, turquoise, and olive green (Figure 1; Cabot, 1992). Tinamiformes (common name: tinamou) is the most species-rich order of Palaeognathae, containing 48 extant species (Figure 1; Cabot, 1992; Davies, 2002). The evolutionary mechanism underlying tinamou egg color divergence remains unclear due to the cryptic and elusive plumage and behavior of the tinamous (Cabot, 1992; Davies, 2002).

To explain the color variation of tinamou eggs, two hypotheses have been proposed. The "aposematism hypothesis" (Swynnerton, 1916) states that bright egg colors could warn predators of their distastefulness. However, this aposematic function has been questioned because egg predators, such as nocturnal mammals or reptiles, tend to prioritize chemical cues over visual cues (Brennan, 2010; Cabot, 1992; Skutch, 1966). The other hypothesis is the "mating signal hypothesis," which suggests that egg color stimulates the reproductive investment of the incubating sex (Brennan, 2009, 2010; Hanley et al., 2010; Weeks, 1973). It has been reported in different tinamous species that males collect and incubate eggs laid by multiple females (Cabot, 1992; Davies, 2002). The "mating signal hypothesis" predicts that the salient egg colors could stimulate male incubation and reproductive investment (Hanley et al., 2013).

The "mating signal hypothesis" effectively explains the egg color brightness within species but does not fully explain the divergence of egg colors among tinamou species. We further expanded the "mating signal hypothesis" into the "mating signal character displacement hypothesis," and we propose that egg colors serve as mating signals for mate attraction and recognition, and reinforcement could drive egg color divergence as character displacement that reduces hybridization among closely related species in sympatry or parapatry. Typically, an initial female tinamou is attracted to a male-guarded nest by distinctive songs and courtship display, lays eggs, and leaves before other females come and lay more eggs in the same nest (Brennan, 2010; Cabot, 1992; Davies, 2002). We suspect that egg colors can provide subsequent females with mate-choice copying and species-recognition cues that prevent costly hybridization. The egg colors could also signal species identity to the incubating male and prevent parental investment in eggs from heterospecific mating. As an alternative mating signal channel, the egg color signal might be especially favored when the plumage is adapted for camouflage (Cabot, 1992; Davies, 2002). Since the birds themselves (instead of the eggs) tend to attract nest predation (Brennan, 2010), egg coloration could be employed to fulfill signal multimodality, complementing the constraints of plumage camouflages. If so, the egg color signal should have coadapted with other mating signals in tinamous. Songs of tinamous are highly divergent mating signals among species (Bertelli & Tubaro, 2002; Boesman et al., 2018; Cabot, 1992; Laverde-R and Cadena, 2014). Furthermore, multimodal mating signals would be under divergent selection as tinamou species adapted to divergent

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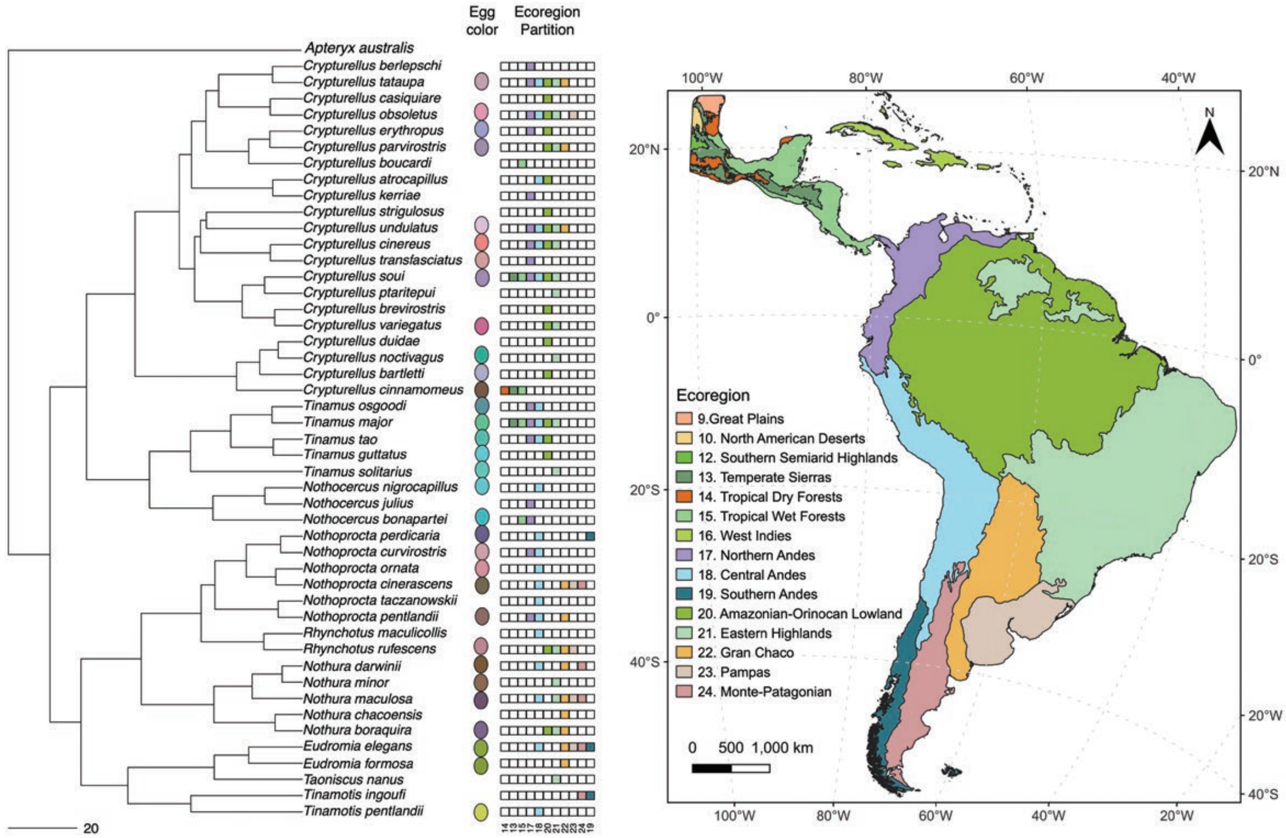

Figure 1. Egg colors and ecoregion partitions across the Tinamidae phylogeny. Tinamou ecoregion and song space copartitioning are significantly associated with egg colors among species, with greater color divergence among species that partition similar ecoregions after controlling for song variation (partial mantel test, $p < .05$). The Tinamidae phylogeny (with *Apteryx australis* as the outgroup species) was inferred from OneZoom Explorer (http://www.onezoom.org). Filled squares depict tinamou occupancy across available ecoregions, with the same color scheme as the map. The Lambert azimuthal equal-area projection was applied to the ecoregion map.

habitats (sensory drive hypothesis; Endler, 1992). If egg colors of different tinamou species are adapted for mate recognition, reinforcement could have driven egg color divergence among different tinamou species at sympatry (Dobzhansky, 1937; Liou & Price, 1994; Mayr, 1942; Servedio, 2000). The divergence of other signaling modalities, such as songs, that contribute to reproductive isolation could dampen the selection of egg color divergence. Therefore, reinforcement-driven character displacement in egg colors could be especially strong in sympatric tinamou species with similar songs.

Here we test three predictions from the “mating signal character displacement hypothesis.” If tinamou egg colors promote mate attraction and serve as species-recognition signals, (a) egg colors would have coevolved with other mate-recognition signals, for example, species-specific songs (multimodal signal coevolution prediction); (b) this sensory signal might be divergently adapted to local habitats (“sensory driven” prediction); and (c) species with a more sympatric history and similar songs (alternative divergent signals) should have more divergent egg colors (character displacement prediction).

Methods

We tested the three predictions of the mating signal character displacement hypothesis by examining the association among songs, egg colors, and ecoregion copartitions among tinamou species.

Tinamou egg color

Tinamou egg coloration data were extracted from a book (Schläpfer, 2018), in which egg coloration of 32 tinamou species is RGB quantified based on nest photos from citizen submissions. Although avian color signals are usually quantified in the tetrahedron color space, RGB color is likely more suitable to the tinamou visual system, as an earlier study suggests that tinamous might not have UV perception since neither the brushland tinamou (*Nothoprocta cinerascens*) nor the Chilean tinamou (*Nothoprocta perdicaria*) has short wavelength sensitive type1 cones (Mullen & Pohland, 2008). Notably, this does not rule out the possibility of undetected UV sensitivity and/or UV perception in other tinamou species. RGB color metrics are highly predictive of avian perception ($R^2 = 0.837$; Bergeron & Fuller, 2018), thus providing an effective and practical way to quantify egg color variations among tinamou species given the challenges in egg-searching, curation, and color preservation.

Since tinamou egg colors are known to decay over the course of the museum storage (Hanley et al., 2013), field nest photos are preferred to represent the functional egg colors among species (Schläpfer, 2018). Ideally, egg colors would be quantified with a color standard to correct for ambient lighting. However, due to the difficulty in tinamou nest detection, Schläpfer (2018) made use of 60 citizen science nest photos of 29 species and the museum specimens of an additional three species (*Crypturellus cinnamomeus*, *Nothura darwii*, *Nothura minor*) to quantify egg colors. For each species, the egg coloration reflected in RGB color space was quantified (Schläpfer, 2018).

A caveat of using citizen science nest photos without a color standard is the lack of control of the ambient light. To evaluate the reliability of the between-species egg color difference, we quantified and compared egg colors of the nest photo with the museum egg specimens within and between species (see below). To evaluate egg color variations among species, we conducted a Fisher discriminant analysis of the RGB color space with color values centered around zero and scaled to unit variance. Museum egg specimens include four tinamou species: *Crypturellus undulatus*, *Eudromia elegans*, *Nothura boraquira*, and *Tinamus major*. Photos of egg collections from the Field Museum of Natural History were taken with a standard color reference chart by a camera (Canon EOS 5D Mark IV; Supplementary Figure S1B). All images were set to the RGB 16bit mode and white-normalized by defining the brightest white patch of the color standard as white in Photoshop (function "set white point"). Central areas 3 mm away from the edge of each egg were selected to avoid shadows, and all reflections, cracked slots, and labels were excluded from further quantification. Colors of the remaining central areas were averaged to generate one RGB reading for each egg. Up to four eggs at the most left and top positions were quantified if more than four eggs from a clutch were photographed in one image. To evaluate the representativeness of the measurement by Schläpfer (2018), we examined whether the nest photo RGB scores would cluster with museum photo RGB scores within species and whether these color clusters are distinct between species.

Tinamou songs

Song data were acquired from a previous study (Bertelli & Tubaro, 2002) to quantify four song variables from 40 tinamou species: maximum frequency (Hz), minimum frequency (Hz), emphasized frequency (frequency of the note with the highest amplitude in the song, Hz), and bandwidth (the difference between maximum and minimum frequency, Hz). Because these variables are correlated (Supplementary Figure S2), we used the principal component analysis (PCA) with scaled values to generate PC1 that captures 83% of the variation in the song variation.

Likelihood of ancestral sympatry

There have been drastic changes in fauna and flora distributions in Latin America over the time span of the tinamou diversification (Vuilleumier, 2012). Therefore, we cannot use the current range overlap to infer ancestral sympatry over tinamou speciation. Instead, we inferred the likelihood of ancestral sympatry with ecoregion co-partitioning, which widely incorporates both the ecological and geographical distribution of tinamou species. Species ranges were generated according to their occurrences. Geo-referenced occurrences were downloaded from GBIF (GBIF.org, accessed on 30 September 2021, https://doi.org/10.15468/dl.fqnhrk) for all tinamou species. Occurrences with geo-resolution better than 10 km were kept and further filtered to remove redundant localities within a 1-km^2 grid cell via R package *ecospat* (Cola et al., 2017) after Lambert azimuthal equal-area projection (ranging 9–9,413, median = 469). Ecoregion designation for each species was determined by intersecting occurrences with the ecoregion map of America. Ecoregion maps were downloaded separately for North America (https://www.epa.gov) and South America (http://ecologicalregions.info) at level I. Then they were combined to include 11 ecoregions (of 15 possible ecoregions) that exhibited any tinamou occurrences. For each species, ecoregions with occurrences greater than 10% (or >50 if the total number was >1,000) were included in the species' ecoregion distribution.

To determine the likelihood of ancestral sympatry for each pair of species during speciation, we calculated the pairwise probability of ecoregion copartitioning among tinamou species. For each pair of species, the probability of ecoregion copartitioning is the sum (across all ecoregions) of the multiplication of the probability of species-specific partitioning within each ecoregion. For each species, the probability of partitioning within an ecoregion is the number of points of occurrence within the ecoregion over the total points of occurrence for the species. If the probability of ecoregion copartitioning is an effective indicator of sympatry or parapatry in the course of tinamou speciation, more closely related species are more likely to copartition ecoregions, given the limited dispersal of tinamous (Davies, 2002). We employed the Mantel Pearson's correlation test with the function *mantel.test* in R to test whether the species distance matrix is correlated with the ecoregion copartitioning probability matrix.

Statistical tests

We tested four macroevolutionary associations: (A1) whether egg colors are correlated with songs; (A2) whether songs are associated with habitat types; (A3) whether egg colors are predicted by habitat types; and (A4) whether egg color displacement is predicted by ecoregion copartitioning and song similarities. Species-specific habitat type is a variable with "open," "mixed," and "close forest" habitat categories acquired from a previous study (Bertelli & Tubaro, 2002). For (A1)–(A3), we used *gls* with *corPagel* function in the *ape* package R to account for phylogenetic signals (Paradis & Schliep, 2019). For egg color and song variables, we used PC axes that cumulatively explain over 80% of the variation, which lead to PC1 (54%) and PC2 (38%) for egg color PCA and only PC1 (83%) of song PCA.

To account for phylogenetic uncertainty, we downloaded a subset of 500 random trees with Hackett backbone for tinamou species from VertLife (http://vertlife.org/phylosubsets; Jetz et al., 2012). Additionally, we generated a representative phylogeny based on OneZoom Explorer with branch length as divergence time for visualization (http://www.onezoom.org). To correct for multiple hypotheses testing, we conducted the false discovery rate correction (Benjamini & Hochberg, 1995).

We examined whether tinamou egg colors are character displacement traits by testing whether egg color differences between species are more likely to share ecoregions and/or have similar songs (A4). Since songs may also be character displacement traits, we controlled for song similarity matrix when we evaluated egg color distance and ecoregion copartitioning between species. We first computed the distance matrices of egg color PC1 and song PC1, respectively, with the *dist* function in R. To examine the correlation between the distance matrix of egg color PC1 and the probability matrix of ecoregion copartitioning, we controlled for song PC1 distance matrix among species and used the one-tailed Partial Mantel Pearson's correlation test (10,000 iterations) with the *mantel.partial* function. A positive association would imply a greater egg color distance at potential sympatry during speciation.

Results

We identified significant evidence for the mating signal character displacement hypothesis. First, we compared and validated egg color RGB data (Schläpfer, 2018) with museum specimens of the same species. We found that between-species egg color variations are more prominent than within-species variations (Supplementary Figure S1), indicating the validity of Schläpfer (2018) data for between-species comparative analyses. Then, we tested the three predictions that correspond to four evolutionary associations (A1–A4) under the mating signal character displacement hypothesis. Specifically, we found significant associations between three trait pairs, including songs and egg colors (A1), songs and habitats (A2), as well as egg colors and habitats (A3) among tinamou species (Figure 2). We further examined the evolutionary association between the likelihood of sympatry and egg color divergence during tinamou speciation (A4) and found that ecoregion similarity predicts egg color distance among species after normalizing song variations among tinamou species (Figure 1). This result supports the character displacement prediction.

Egg color covaries with song and habitat variations across 32 tinamou species (Figure 2). PC1 (54% of the variation) reflects the color gradient from red to green/blue, and PC2 (38% of the variation) corresponds to egg brightness (Supplementary Figure S3A; Supplementary Table S1). In contrast, Song PC1 (83% of the variation) corresponds to greater song frequencies (Supplementary Table S1), which are positively associated with the openness of the habitat (A2, Figure 2B). Interestingly, there is a significant association between egg color PC2 and song PC1 (A1, Table 1; Figure 2A and C), where species with higher song pitches lay brighter-colored eggs. The relationship is unlikely confounded by habitat types among tinamou species, as egg color PC1, but not egg color PC2, is significantly associated with habitat types (A3, Table 1; Figure 2D). On the other hand, the open-field tinamou species tend to lay warm-colored eggs, whereas there is a broader variation in egg colors (from pink to greenish blue) among the mixed and forested tinamou species (Figure 2D). These results support the predictions of multimodality signal coevolution and sensory drive.

Ecoregion copartitioning is an effective indicator of parapatry or sympatry in the history of tinamou speciation because closely related species tend to copartition ecoregions (Mantel Z = 1530.38, p = .001; Figure 1). Specifically, we observed stronger egg color divergence between species that copartition ecoregions after normalizing song variation

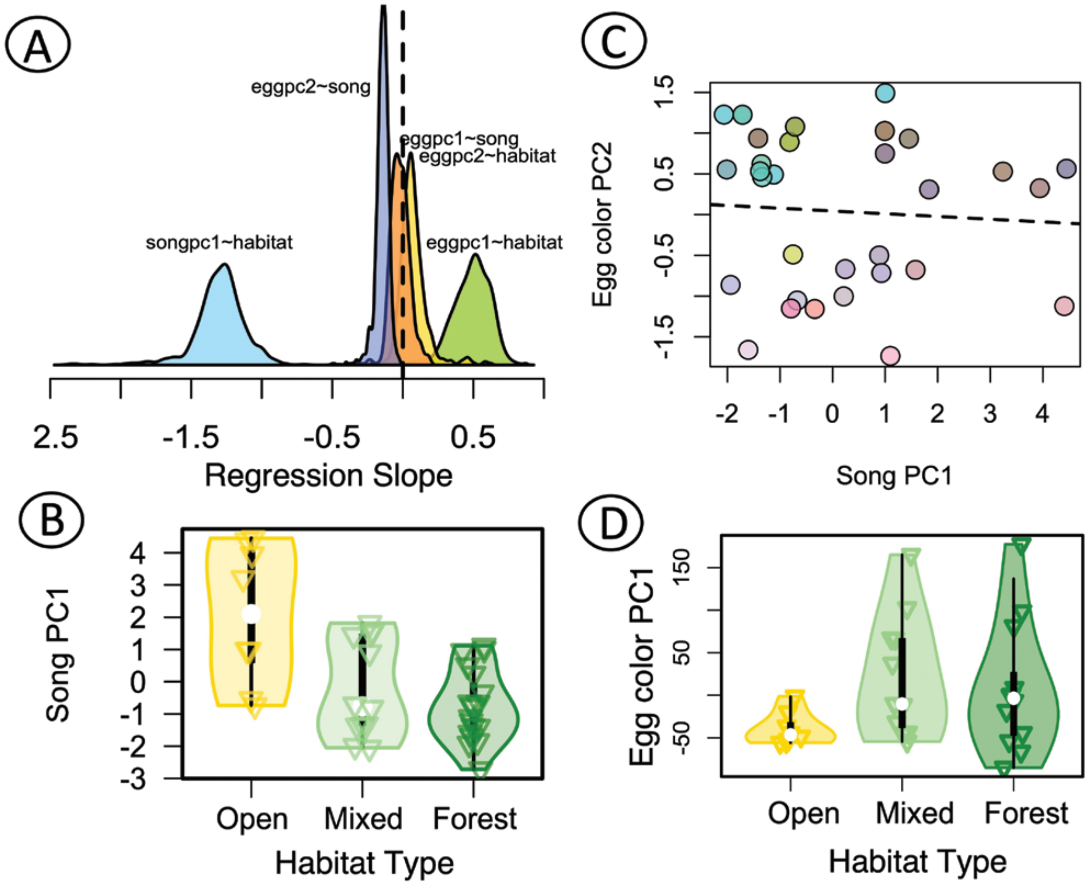

Figure 2. Association of habitat types, songs, and egg colors across tinamou species. (A) The distribution of regression coefficients in phylogenetic regression models (response variable ~ predicting variable) accounts for phylogenetic uncertainty (Table 1). Three distributions of regression slopes significantly deviate from zero: (1) between egg color PC2 and song PC1 (purple, also see B); (2) between song PC1 and habitat types (blue, also see C), and (3) between egg color PC1 and habitat types (green, also see D). (B) Egg color PC2 is significantly associated with song PC1: The dot color represents the egg RGB color of each species. (C) Phylogenetic analysis reveals a significant association between song PC1 and habitat types among tinamou species, consistent with a previous study (Bertelli & Tubaro, 2002). (D) Egg color PC1 is significantly associated with habitat types among tinamou species.

Table 1. Phylogenetic regression models indicate associations among egg colors, songs, and habitat types across tinamou species

Response variable	Predictor	Mean b_1	95% CI b_1	Mean b_0	95% CI b_0	R^2
Egg color PC1	Song PC1	−0.017	(−0.118, 0.128)	−0.007	(−0.274, 0.265)	0.008
	Habitat	**0.483**	(0.151, 0.693)	**−1.074**	(−1.546, −0.353)	**0.119**
Egg color PC2	Song PC1	**−0.144**	(−0.244, −0.072)	**0.157**	(0.034, 0.278)	**0.069**
	Habitat	0.071	(−0.090, 0.392)	−0.060	(−0.750, 0.259)	0.010
Song PC1	Habitat	**−1.297**	(−1.729, −0.990)	**2.929**	(2.175, 3.861)	**0.383**

Note. For each pair of variables, a phylogenetic regression model was run with each of the 500 tinamou phylogenies to account for phylogenetic uncertainty. Significant (0 falls out of the 95% confidence interval, CI) intercepts (b_0) and slopes (b_1) are in bold.

among tinamou species. The egg color divergence is represented by PC1, which explains 54% variation in the RGB color space among species. The distance of egg color PC1 is positively associated with the ecoregion copartitioning among species controlling for song distance among tinamou species (partial Mantel $r = 0.066$, $p = .048$; Figure 1). This association between egg color distance and ecoregion copartitioning is consistent with character displacement prediction.

Discussion

We found support for the "mating signal character displacement hypothesis." There is significant evidence that tinamou egg coloration is an alternative mating signal (Brennan, 2010; Weeks, 1973) that coevolved with songs and habitat partitioning and was divergently selected at potential sympatry. Egg colors and songs could be divergently selected as multimodal mate-recognition signals among tinamou species with a similar appearance that partition similar ecoregions. When the plumage modality is dull for camouflage, egg coloration could be opportunistically adopted for species recognition in sympatry. This study sheds light on the evolution of multimodal sexual signals that complement natural selection for plumage camouflage in the most species-rich family of Palaeognathae.

Mating signal evolution

Egg coloration could be both a pre- and/or postmating signal in tinamous. In many other bird species, egg colors are postmating signals indicating female quality, thus influencing male incubation and promiscuity (English, 2009; Soler et al., 2005). This is consistent with the mating signal prediction that egg colors could stimulate tinamou male incubation and parental care (Brennan, 2010; Weeks, 1973). In addition, egg colors can serve as premating mate-recognition signals among females that mate with the same male if the signals enhance the offspring's fitness by protecting their reproductive investment from costly heterospecific mating interference (Gröning & Hochkirch, 2008). Although understudied, tinamou males are known to guard and incubate the eggs laid by multiple females with mating system records (Cabot, 1992). The coloration of existing eggs in the nest could be a mating signal received by subsequent females. As premating signals, egg colors could reflect species identity and mate quality (Sætre et al., 1997; Secondi et al., 2015; Servedio & Noor, 2003) and also stimulate female mate choice copying (Dugatkin, 1992; Gibson & Höglund, 1992). The coevolution of egg color and song (Figure 2) sheds light on all these possibilities. The future behavioral study could parse out the specific signaling targets and functions of egg colors in tinamous.

We propose that egg colors are complementary mating signals to songs. Songs are important mating signals involved in tinamou mating partners duetting (Bertelli & Tubaro, 2002; Boesman et al., 2018). However, acoustic modality is usually insufficient in complex environments (Partan & Marler, 1999; Rowe, 1999; Secondi et al., 2015; Uy et al., 2008). Tinamous appear to be constrained in plumage coloration potentially because of antipredatory camouflage (Cabot, 1992; Davies, 2002), unlike many other birds (Seddon et al., 2013; Uy et al., 2008, 2009). Egg coloration, thus, can be an alternative modality of mate signaling in tinamous towards mate-searching refinement, resolving the conflict between natural and sexual selection (Heinen-Kay et al., 2015). To refine and enhance mate searching and recognition, specificities of signal modalities are expected to coadapt and coevolve, and indeed we observed that egg color brightness coevolves with song variation among species (Table 1, Figure 2A and B).

Speciation history and egg color divergence

Reinforcement may have driven egg color and song divergence among sympatric or parapatric tinamou species. Many closely related tinamou species demonstrate a contemporary ecoregion overlap (Cabot, 1992; Figure 1), which indicates potential sympatry/parapatry in their speciation history. Ecological and/or intrinsic incompatibility among diverged lineages leads to reduced hybrid fitness, which promotes premating signal divergence that avoids costly hybridization (Dobzhansky, 1940; Liou & Price, 1994; Mayr, 1942; Servedio, 2000). Mating signal multimodality is needed to ensure mate recognition in complex heterospecific environment (Secondi et al., 2015; Uy et al., 2008). The divergence of tinamou songs and egg colorations could thus jointly reduce heterospecific reproductive efforts among sympatric tinamou species. The codivergence of mating signals and habitat partitioning (Figure 2) supports the presence of divergent selection on signal evolution.

Closely related tinamou species that are currently allopatric could still harbor footprints of historical character displacement of egg colors formed at ancestral sympatry/parapatry. Notably, the species with a greater likelihood of ecoregion co-occurrence tend to display divergent egg colors (Figure 1). For example, the closely related species *Crypturellus noctivagus*, *C. bartletti*, and *C. variegatus* still partition similar ecoregions and demonstrate distinct egg coloration, which may have been driven by reinforcement at historical sympatry (Figure 3). Although the speciation events among tinamou species have long passed (>7 million years), the likelihood of ecoregion copartitioning is potentially an effective indicator of historical sympatry/parapatry in the history of tinamou speciation as closely related species tend to copartition

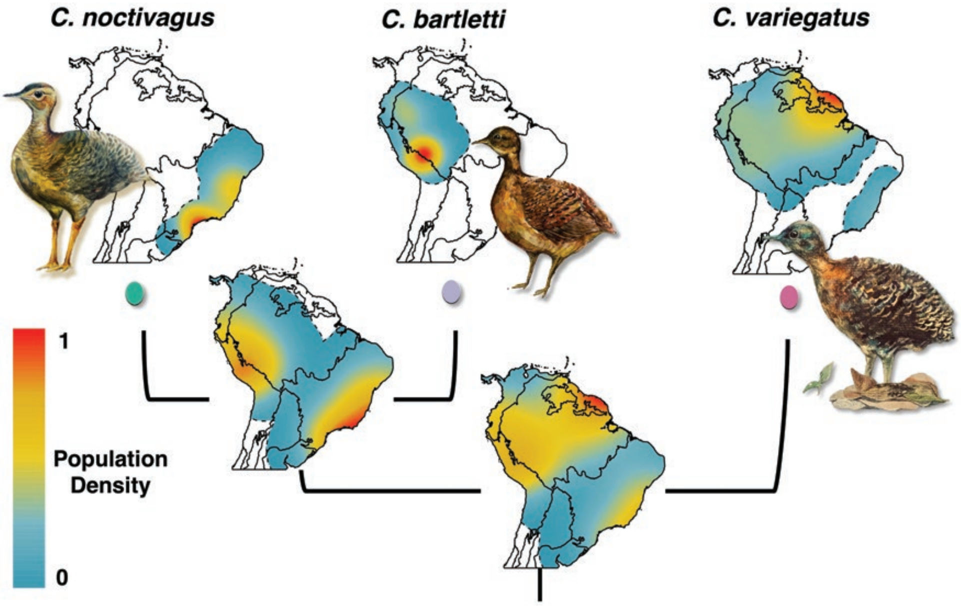

Figure 3. Reconstructed ancestral distribution of three *Crypturellus* species. Black lines depict the boundaries of ecoregions in Figure 1, and dashed lines depict the boundaries of species distribution ranges. Range maps are density polygons that were generated via R package *rangemap* (Cobos et al., 2021), with a hierarchical clustering method. Kernel densities were estimated via R package *spatstat* (Adrian & Ege Rubak, 2015) to capture the spatial configuration of the occurrences. Distributions of internal nodes were generated by the same method with an intermediate step of rescaling densities (at a 100-km resolution) of all descendants to offset the effect of variation in abundance. Blue-to-red colors depict the relative scale of kernel densities from low to high (ranging from 0 to 1).

ecoregions. The association of egg colors and habitat types among tinamou species (Table 1, Figure 2A–D) recapitulates divergent selection on egg colors in the speciation history.

Caveat

Although we found significant evidence that supports the three predictions of the mating signal character displacement hypothesis, the percentage of variance in egg color explained by the model (r) was small. This indicates that the egg color measurements could be noisy and/or other latent factors could be at play. One caveat of using citizen science nest photos is the lack of control of ambient light, which could especially influence the association with habitats and egg colors (A3). However, we tested this effect by comparing the nest photo color measurements to the museum specimens of the same species with the light control and found that the effect does contribute to intraspecific variations but does not disrupt interspecific distinction (Supplementary Figure S1). Different egg color axes support different macroevolutionary predictions: egg color hue (PC1) coevolved with songs (A1) and egg color brightness (PC2) coevolved with habitat partitioning (A3 and A4). This indicates that tinamou egg color divergence is complex and fine-tuned, and other latent evolutionary forces may contribute. The relative contribution of song and egg color as mating signals could be highly variable among tinamou species. Besides the local tinamou species assembly, other complex aspects of abiotic and biotic interactions, such as sexual conflicts, predation, and parasitism, might contribute to the egg color signal diversity. Future studies should investigate mating signal performance under specific ecological and evolutionary forces at multispecies sympatry.

Signal genetics

The genetic mechanism of such song and egg color association is still being determined. The simplest genetic mechanism is pleiotropy, in which one gene affects multiple traits (Fisher, 1930; Williams, 1957). For example, the pleiotropic *foraging* gene underpins the association between social behaviors and life-history traits in natural populations (de Belle et al., 1989; Mery et al., 2007; Wang & Sokolowski, 2017). If so, a wide array of traits of such pleiotropy are expected to coevolve. However, the association between song and egg color of tinamous is specific: only the brightness of egg colors (egg color PC2) is associated with tinamou songs (Table 1, Figure 2A and B). Such specificity in the song–egg–color association is consistent with mating signal multimodality, in which specific signal features are coupled among modalities (Gilliard, 1956; Hebets & Papaj, 2005; Partan & Marler, 1999). Different genes can underpin various signal modalities and be selected to coevolve as multimodal signals. Future investigation of the genetic underpinnings of tinamou songs and egg colors would shed light on this interesting codivergence.

Conclusion and implication

In sum, the results presented herein are concordant with the mating signal character displacement hypothesis that explains the color divergence of tinamou eggs. This is a rare example in which a postmating character could be opportunistically employed as a signal of reproductive isolation.

Supplementary material

Supplementary material is available online at *Evolution*.

Data availability

All the variables involved in the study have been deposited on Dryad (https://doi.org/10.5061/dryad.rfj6q577g).

Author contribution

S.W. designed the study and conducted the majority of data curation and analysis in the study. Q.L. conducted the geographic analysis with S.W. Q.L. photographed museum egg collections, and D.C. conducted pigment analysis. S.W. wrote the initial manuscript and worked with Q.L. for earlier versions. All authors contributed to the later and final version of the manuscript.

Conflict of interest: The authors declare that they have no conflict of interest.

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