Male competition drives song divergence along an ecological gradient in an avian ring species

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Sexual selection operates via female choice and male competition, which can act independently, in concert, or in opposition. Female choice is typically considered the stronger selective force, but how these two processes interact to shape phenotypic divergence is poorly understood. I tested the hypothesis that variation in sexual selection in different habitats drives song divergence in the greenish warbler ring species. I evaluated the strength, direction, and targets of female choice and male competition in three populations spanning 2400 km of latitude. Average song length increased with latitude, concomitant with a decline in population density. Within populations, males sang longer songs when females were fertile and shorter songs during territory establishment. Females consistently preferred males with longer songs and larger song repertoires. By contrast, playback experiments showed that males used short songs in territory defense. Songs were shortest at high densities, and in the highest density population only, song traits preferred by females correlated with male territoriality. Stronger male competition at high population densities likely constrains maximum song length, whereas weaker competition at low densities allows expression of female choice for long songs. Interactions between male competition and ecology may be a crucial but oft-overlooked component of phenotypic divergence and speciation.

Sexual selection has traditionally been separated into two related processes: female choice, whereby certain males are preferred as mates by females; and male competition, whereby males compete with each other for access to mates or resources necessary for attracting mates (Darwin 1859; Andersson 1994). Sexual selection can lead to rapid trait divergence between populations, generating the geographic variation necessary for the initial stages of speciation (Hoekstra et al. 2001; Panhuis et al. 2001; Coyne and Orr 2004; Safran et al. 2013; Servedio and Boughman 2017). However, the causes of variation in female choice and male competition among populations, and the relative contributions of these two processes to phenotypic divergence, remain largely unexplored. There is a consequent a gap in our understanding of the mechanisms driving population divergence and speciation (Qvarnström et al. 2012; Tinghitella et al. 2018). Here, I examined how variation in the strength, direction, and targets of both female choice and male competition contributes to song divergence in an avian ring species, the greenish warbler (*Phylloscopus trochiloides*).

Sexually selected traits evolve to advertise information about male quality to potential mates and competitors (Zahavi 1975; Andersson 1994). This information can include direct benefits that increase female fitness, such as paternal care and territory quality (Price et al. 1993; Johnstone 1995), indirect benefits that result in attractive or healthy offspring (Sheldon and Verhulst 1996; Houle and Kondrashov 2002; Neff and Pitcher 2005), and advertisement of competitive ability and territory boundaries (Emlen and Oring 1977; Yasukawa et al. 1980; Gosling and Roberts 2001). Because sexually selected traits should evolve to be both condition dependent (Nur and Hasson 1984; Grafen 1990) and clearly detectable

in a particular habitat (Endler and Basolo 1998; Boughman 2002), an important prediction of sexual selection models is that the information content of a sexual signal is tied to the environment (Proulx 2001; Vergara et al. 2012; Giery and Layman 2015). Female choice and male competition should thus be directed toward the traits (and corresponding aspects of male quality) that most increase individual fitness in a particular ecological or social context (Schluter and Price 1993; Jia and Greenfield 1997; Welch 2003; Miller and Svensson 2014).

However, female choice and male competition may not always operate in the same direction or on the same traits. For example, females in poor quality habitats may prefer mates that defend high quality resources. Males then compete for resources, the best competitors achieve the highest reproductive success, and female choice and male competition act in the same direction on male phenotype (e.g., Doutrelant and McGregor 2000; Hunt et al. 2009). By contrast, females in a high-quality habitat may choose mates based on traits that advertise aspects of male quality unrelated to territory quality or competitive ability (e.g., Mountjoy and Lemon 1996; Buchanan et al. 2003; McGhee et al. 2007), but males may still compete with each other for access to females. This can lead to conflict between inter- and intrasexual selection on male phenotype and phenotypic divergence (Qvarnström and Forsgren 1998; Moore and Moore 1999; Wong and Candolin 2005).

Because sexually selected traits are typically costly to maintain (Zahavi 1975; Schluter and Price 1993; Rowe and Houle 1996), male allocation of resources to traits used in competition versus female attraction will ultimately depend on the relative fitness costs and benefits associated with investment in these traits. When female choice and male competition act synergistically on the same traits, resource allocation is straightforward. However, if male competition and female choice act in opposition, males can pursue two nonmutually exclusive strategies. First, they can express multiple sexually selected traits, with different traits targeted at different receivers (e.g., certain traits advertise competitive ability and others are attractive to females, Marchetti 1998, Pryke et al. 2001; Andersson et al. 2002; Candolin 2003; Taff et al. 2012). Second, trait expression can be labile, with males expressing different traits in competitive versus mate-attraction contexts (e.g., by varying acoustic signals or behavioral displays, Trillo and Vehrencamp 2005; Benedict et al. 2012; Fang et al. 2014; Geberzahn and Aubin 2014). Within populations, the strength, direction, and targets of female choice and male competition, whether these two processes act in synergy or in opposition, and how males allocate resources to sexual signals will therefore depend on environmental quality and signal information content.

Bird song is a well-studied trait that is used in both female choice and male competition (Catchpole and Slater 2008) and fre-
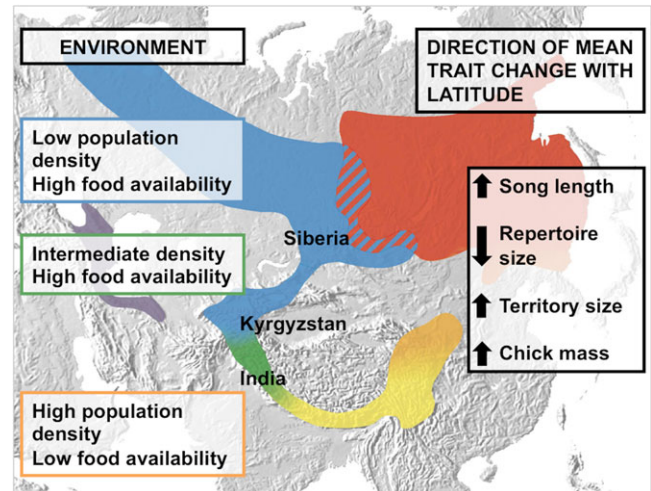

Figure 1. Greenish warbler breeding range, with locations of study populations noted. Ecological characteristics of each site are summarized to the left, with colored box outlines denoting colors used for each population in all figures. Directions of trait changes with latitude found in this study are summarized to the right. Figure adapted from Irwin et al. (2001).

quently acts as a premating reproductive barrier (Irwin et al. 2001; Price 2008; Lipshutz et al. 2017). Conflicts between female choice and male competition are readily apparent in bird song. In many species, females prefer males that sing long, complex songs (e.g., Catchpole and Slater 2008; Collins et al. 2009; but see Byers and Kroodsma 2009; Cardoso and Hu 2011). However, short, simple songs are often used in competitive contexts, likely because short songs facilitate the call-and-response interactions with neighbors required for territory maintenance (Catchpole 1982; Hasselquist and Bensch 1991; Mountjoy and Lemon 1996; Catchpole and Slater 2008). Males can resolve conflicts between female choice and male competition by using different songs for different groups of receivers (e.g., Moller and Pomiankowski 1993; Candolin 2003; Leedale et al. 2015). However, the two selective pressures may conflict in species with limited song lability. Geographic variation in song length and complexity may therefore reflect the relative strength of male competition versus female choice in different habitats.

The greenish warbler is an ideal system in which to study the selective pressures shaping song variation: it is a ring species wherein two reproductively isolated forms that coexist in central Siberia are connected by a chain of genetically and phenotypically intergrading populations encircling the Tibetan Plateau (Irwin 2005; Alcaide et al. 2014, Fig. 1). Population divergence occurs along an ecological as well as latitudinal gradient (Irwin 2000; Scordato 2017, Fig. 1). Songs are short and simple in the southern part of the range, where population density is high and food is scarce. Songs increase in length and internal complexity

along both the eastern and western arms of the ring, concomitant with decreased population density and increased food abundance (Irwin 2000, Fig. 1). Song is a premating barrier in Siberia, where sympatric males do not respond to playbacks of heterotypic song (Irwin et al. 2001). Phenotypic differences persist between populations despite inferred gene flow (Irwin et al. 2005; Alcaide et al. 2014), and may therefore reflect adaptation to local selective pressures.

Based on the decrease in population density and increase in food abundance with latitude, Irwin (2000) suggested that strong male competition in low-quality southern habitats favors short songs, whereas weak male competition in high-quality northern habitats allows songs to become longer in response to female preferences. This hypothesis posits geographic variation in the strength of different selective pressures acting on song; that is, selection for short songs is stronger in southern environments than northern environments, resulting in shorter songs farther south. However, the observed pattern of song divergence is consistent with two additional explanations. First, the targets of female choice or male competition may vary in different habitats. Changes in the targets of selection would occur if, for example, females preferred long songs in one population and large song repertoires in another. Song length and repertoire size would then vary concomitant with female preferences. Second, there may be shifts in the direction of selection on song. Changes in the direction of selection occur if the same trait is under positive selection in one population and negative selection in another, as would occur if females preferred long songs in one population and short songs in another. Differentiating these alternative hypotheses requires assessing the strength, direction, and targets of both male competition and female choice in different habitats.

I tested these three hypotheses in three greenish warbler populations spanning 2400 km of latitude along the western arm of the ring (Fig. 1). Habitats were characteristic of each latitudinal band, and the large distance between sites maximized differences in both song structure and habitat among populations. In each population, I (1) measured associations between male song and fitness, as a proxy for female mate choice; and (2) experimentally manipulated songs to evaluate their role in male competition. I used song playback experiments to measure male response to different song components, and interpreted a stronger aggressive response to mean that song component was used in competitive interactions (Benedict et al. 2012; Greig et al. 2015). I analyzed songs throughout the entire breeding season in each population to determine song lability across different social and breeding contexts. This combination of season-long observational and experimental approaches allowed me to test for changes in the strength, targets, and direction of sexual selection on song in different habitats.

Methods

I studied three populations of *P. trochiloides* in three different years: Keylong, India, in 2008 (3300 m, 32.50086°N, 76.97841°E); Ala Archa Park, Kyrgyzstan, in 2009 (2600 m, 42.54669°N, 74.48806°E); and Tigirek Nature Reserve, Siberia, Russia, in 2010 (2100 m, 51.123353°N, 83.037105°E). These populations were chosen because they represent a continuous gradient of divergence: the population in Kyrgyzstan is geographically, phenotypically, and ecologically intermediate between India and Siberia (Fig. 1). Early analysis using AFLPs (Irwin et al 2005) and subsequent work with next-generation sequence data (Alcaide et al. 2014) indicate a pattern of isolation-by-distance between these three populations. However, genome-wide data also show that Siberia and Kyrgyzstan belong to the same genetic cluster, whereas the population in India is part of an intergrade between two genetic clusters (Alcaide et al. 2014). Kyrgyzstan remains genetically intermediate between India and Siberia, although it is slightly more closely related to Siberia than to India (Alcaide et al. 2014). Extensive interbreeding in India after an inferred period of allopatry has homogenized phenotypes, and signatures of past isolation are only detectable via high-resolution genomic analyses (Alcaide et al 2014).

STUDY SPECIES

P. trochiloides is a small (∼8 g), insectivorous warbler that breeds in Asia and Europe and overwinters in peninsular India and southeast Asia. Greenish warblers are socially monogamous. Males arrive on the breeding grounds in late May and establish territories. Females arrive 1–3 weeks after males, choose a mate, and build a nest on his territory. Chicks fledge 12–14 days after hatching. Pairs have one brood per season.

My field assistants and I captured male birds when they arrived on the breeding grounds using a combination of passive and targeted mist netting, and banded each bird with a numbered aluminum leg band and a unique combination of color bands. Females were typically captured while feeding chicks at their nests, and chicks were banded on day 10 posthatching. We measured wing length (flattened against a standard wing rule) and mass of each bird. For measurement of extra-pair paternity, we collected a small blood sample via brachial veinpuncture and blotted it onto EDTA-treated filter paper, then added an additional drop of EDTA to the fresh blood sample (Petren 1998).

P. trochiloides is sexually monomorphic. Females were identified by presence of a brood patch, and birds observed singing and defending territories were identified as males. Chicks and any ambiguously assigned adults were molecularly sexed (Griffiths et al. 1998). We monitored territories every 2–3 days throughout the breeding season to determine pairing status, and, when possible, the lay date of the first egg, hatching date, clutch size, and

number of chicks. We recorded a GPS point for each sighting location of color-banded birds. I calculated territory size using minimum convex polygons implemented in the R package adehabitat HR (Calenge 2006). Home range estimates excluded the 1% of GPS points located farthest from the barycenter of the home range so that instances of birds observed far from their regular territories did not affect territory size estimates.

SONG VARIATION

Irwin (2000) found that song rate, average song length, and song complexity varied among *P. trochiloides* populations, and I therefore measured these features for each color-banded male. I also measured song unit repertoire size, which is an important target of sexual selection in many species (Searcy 1992) but had not been previously studied in *P. trochiloides*.

Song rate: Song rate was defined as the number of songs a male produced in a 15-minute period. We measured song rate by visiting territories of each color-banded male between 0500 and 1230. If the focal male was singing when an observer arrived, we counted the number of times he sang in 15 minutes. If the male was silent when we arrived, we spent a maximum of 5 minutes searching for him, and then began the 15-minute trial. If the male began singing during those 15 minutes, the number of times he sang in the remaining time was recorded, and song rate was calculated from the beginning of the trial. Males that were undetected for the entire trial were scored as producing zero songs. This method provided an estimate of a male's vocal output unbiased by whether he was singing when the observer arrived. We attempted to measure song rate three times for each male (mean $\pm$ SD = 3.33 $\pm$ 1.77), with observations conducted during the nest building, incubation, and chick rearing stages. Because song output may be influenced by both transient (e.g., presence of a predator) and temporal factors (e.g. decreased singing corresponding to chick hatching), observations were averaged to produce a single measure of vocal output for each male.

Song length and complexity: We recorded song bouts throughout the season from each color-banded bird on a Marantz PMD-660 solid-state recorder attached to a Sennheiser ME67 shotgun microphone. Recordings were made 5–10 m from singing birds in 16-bit PCM WAV format and sampled at 44.1 kHz. I analyzed at least 30 songs per individual (mean = 40) collected on at least two different days (mean = 2.4) using Raven v 1.3 (Cornell Lab of Ornithology). *P. trochiloides* songs are formed from a series of discrete "units" or "syllables" (Irwin 2000, Fig. 2). Song units consist of several "elements" (continuous vocal sweeps, Fig. 2). An individual song comprises 1–6 different song unit types, each repeated 1–16 times. To quantify song complexity, I counted the number of elements, the number of units, and the number of unit types per song for each song in the dataset. I calculated song length using the onscreen highlighting tool in Raven.

Song repertoire size: To determine an individual's repertoire size, I visually matched song units across all individuals in each population. The structure of song units was consistent within and among individuals (as noted by Irwin (2000)), and there were few cases of ambiguous classification. I defined a male's repertoire size as the total number of unique song unit types produced across all analyzed songs. I also counted the number of unique songs (i.e., each unique combination of unit types) to account for new songs produced by rearrangement of song units (Lynch and Baker 1993). Counts of song types from a finite number of songs can underestimate true repertoire size due to incomplete sampling. However, simulation studies have shown that for relatively small repertoire sizes (< 200 song types; *P. trochiloides* mean is 22 unit types and 30 unique song types), counting a set number of songs is an equivalent or superior method for estimating repertoire size than projection methods (e.g., curve fitting and capture-recapture Botero et al. 2008). I found no significant correlation between number of songs counted and individual repertoire size in my dataset (India, $n = 21$ birds, mean 54 songs per bird, $r = 0.10$, $P = 0.65$; Kyrgyzstan, $n = 33$ birds, mean = 31 songs per bird, $r = 0.25$, $P = 0.15$; Siberia, $n = 28$ birds, mean = 42 songs per bird, $r = -0.04$, $P = 0.82$), and therefore considered the number of unique song units to be representative of a male's true repertoire size.

FEMALE CHOICE: SEXUAL SELECTION ON MALE PHENOTYPE

I measured the following components of male fitness as proxies for female choice: pairing success (paired or unpaired), breeding date, the proportion of extra-pair offspring in a nest, and offspring condition.

Pairing success: Pairing success is frequently used as a proxy for female choice (e.g., Hill 1990; Webster et al. 1995). We visited territories of color-banded males every 1–3 days to look for evidence of pairing and search for nests. Males were considered paired if they were observed foraging or in close proximity to a conspecific without interacting aggressively at least twice. I considered males that were never seen with a female and had no behavioral indicators of pairing (e.g., feeding chicks, food carrying, decreased singing corresponding to chick hatching times) to be unpaired. Territory ownership was an apparent prerequisite for pairing.

Breeding date: Breeding date is a widely used proxy for female choice because the most attractive males are typically the first to obtain mates and thus breed earlier in the season (Kirkpatrick et al. 1990). I used chick banding date as a proxy for breeding date. Sample sizes were very small in Siberia ($n = 5$) due to the difficulty of finding nests. However, territories of color-banded males were searched daily, and banded males whose nests were not located were observed feeding fledglings.
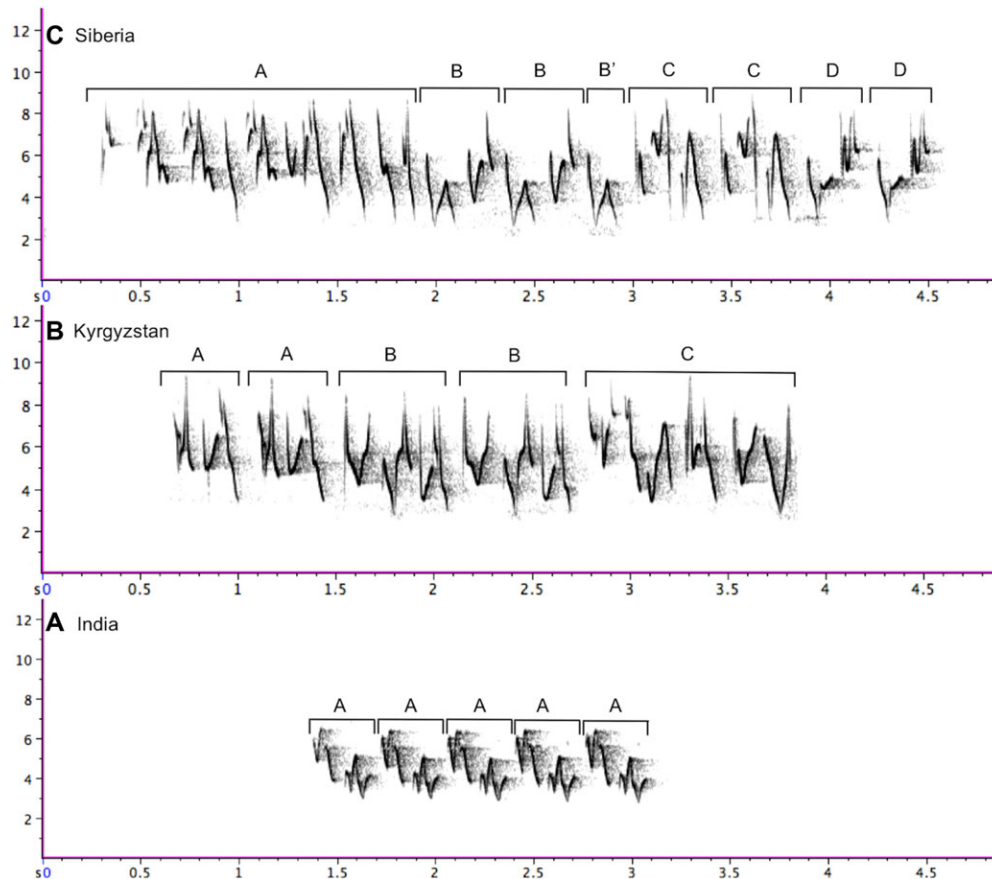
Figure 2. Representative *P. trochiloides* songs from (A) India, (B) Kyrgyzstan, and C) Siberia. Each song is made up of discrete song units (labeled A–D), which are largely invariant within and between individuals. Each song unit is a sequence of several elements (continuous vocal sweeps). In India, song units contain few elements, and each song is comprised of few unit types repeated several times. In Kyrgyzstan, units contain more elements, and songs are comprised of several unit types repeated several times. Songs are on average longest and most complex in Siberia: units are comprised of many elements, and each song contains several different unit types.

I therefore assigned those males ($n = 7$) a conservative fledging date of four days prior to the date fledglings were first sighted.

Chick condition and extra-pair paternity: High quality males may produce heavier chicks (Velando et al. 2001) and females paired to more attractive males may invest more resources in offspring (Limbourg et al. 2004). I measured mass at day 10 as an indicator of chick condition. I also determined the proportion of offspring in a nest that were fathered by an extra-pair male, as females paired to less attractive males may seek more extra-pair copulations (Kempenaers et al. 1997). All parents and chicks were genotyped at eight microsatellite loci to assign paternity (Scordato and Kardish 2014). Paternity analysis was conducted using Cervus v. 3.0 (Kalinowski et al. 2007). Details of microsatellite typing and paternity analysis are in the Supplemental Material and Scordato and Kardish (2014). Three out of five nests in Siberia were predated shortly after chicks hatched, and it was not possible to capture fledglings. I therefore only analyzed chick condition and extra-pair paternity for India and Kyrgyzstan.

MEASUREMENT OF MALE COMPETITION: PLAYBACK EXPERIMENTS

I used playbacks that simulated a territorial intruder to assay male competition in two different ways. First, I played natural (unmanipulated) songs early in the breeding season, when males were establishing territories. I calculated an aggressiveness score for each male from the response to this playback (see below). Second, I used playbacks of manipulated song files to determine whether the intensity of territorial behavior varied in response to differences in song length and repertoire size.

Constructing playback files: I used recordings of local songs made prior to onset of incubation to construct a set of playback stimulus files for each population. For playbacks of natural song, each stimulus file consisted of 10 songs recorded from a singing bout from the same male. I used the first 10 high quality songs for the playback file, standardized intersong intervals to 10 s, and filtered out background noise below 3000 Hz. Each file began with 10 songs, followed by 2 minutes of quiet. The first 5 songs were then repeated, followed by 1 minute of quiet, and the second

5 songs were repeated, followed by 3 minutes of quiet. I created 10–15 natural playback files in each population.

In Kyrgyzstan and Siberia (the second and third years of the study), I created four sets of playback files manipulated along the two main axes of song variation: song length and repertoire size (see Results). This experiment allowed me to ask how different song components are used in competitive interactions. To construct these files, I recorded songs from 10 different local males early in the breeding season in each population. I measured the length of the first 10 high-quality songs from each male. I sorted the resulting 100 songs by length, and used the 25 longest to create "long song" files and the 25 shortest to create "short song" files. Within each length category, I created files that varied by repertoire size. "Large repertoire" files consisted of 10 songs, of which 8–10 were comprised of unique combinations of song units (songs were otherwise randomly assigned). "Small repertoire" files also consisted of 10 songs, but were comprised of only two unique songs, which were alternated five times. This method of randomization ensured that none of the manipulated files contained only songs from a single bird, and thus controlled for possible effects of individual identity on receiver response.

I created 10 different files for each of four treatment groups: long songs, small repertoire; long songs, large repertoire; short songs, small repertoire; and shorts songs, large repertoire. Most files were played to only one bird, and no files were played to more than two birds to control for simple pseudoreplication (Kroodsma 1990; Kroodsma et al. 2001). No male heard playbacks of his own song or the songs of his close neighbors to control for the effects of familiarity on aggressive response. All playbacks were standardized to the same amplitude ($\sim$80–90 dB, the loud end of the natural *P. trochiloides* singing range) with a handheld sound meter 3 m from the speaker. Each male was the subject of no more than two manipulated playback experiments, and playback stimulus files were randomly assigned to focal birds. Playbacks to individual birds were separated by at least 3 days to minimize habituation. All playback experiments were conducted during the nest building and incubation stages of the breeding season ($n = 135$ experiments) because male responsiveness to playback decreases after chicks hatch (Scordato 2017).

Playback protocol: Details of the playback protocol are in Scordato (2017). Briefly, I conducted a 5-minute pre-trial in each male's territory to collect baseline behavioral data. I then played a stimulus file through a Mineroff AFS playback speaker at a central location in the territory. Each trial lasted for 10 minutes, was videotaped and narrated, and was later scored for rates of eight different behaviors: singing, calling, fly-overs, wing flicks, soft songs, and approach within 5, 10, and 20 m. Videos were scored by an observer blind to the type of stimulus file being played. I calculated the difference in the rate of each behavior between the pretrial and playback periods. I then ran a principal

component analysis (PCA) on the correlation matrix of these rate differences. The most aggressive display traits (fly-overs, calls, close approaches, and wing flicks) loaded most strongly on PC1, and PC1 score was therefore used as a measure of aggressive response in subsequent analyses (Table S1).

Statistical Analysis

TEMPORAL VARIATION IN SONG FEATURES

If song is labile and used in both female choice and male competition, song features preferred by females might be produced most frequently when females are fertile, and those that are targets of male competition produced most frequently during territory establishment (e.g., Riters et al. 2000; Leitner et al. 2001). I therefore identified candidate song features that may be targets of sexual selection by testing for temporal variation in song structure over the breeding season in each population. For each song recording from an individual male, I calculated means for song length, number of unit types per song, number of units per song, and number of unique songs per recording (mean $\pm$ SD $= 2.4 \pm 1$ recordings per bird, 14 ± 8 songs per recording). I then averaged the means for each song feature across all recordings made on a particular day (range 2–5 different males) to examine population-wide patterns of song variation.

I assessed the relationship between population mean song values and date using generalized additive models (GAMs) implemented in the R package *mgcv* (Wood 2012). GAMs make no assumptions about the relationship between explanatory and response variables and are therefore useful for analyzing nonlinear variation. I fit two models to seasonal song variation in each population. The first model fit a single nonparametric cubic regression spline smoother to all three populations, with the smoother allowed to vary by a constant. The second model fit a separate smoother to each population, which allowed temporal variation in song structure to differ among populations (Zuur et al. 2009). All models included population as a parametric factor. I compared model fits using AIC. This analysis allowed me to compare patterns of variation in song structure between populations.

Within each population I compared song structure during periods of female fertility with the rest of the season using *t*-tests. To determine the population-wide fertile period, I counted backwards from the first and last chick fledging dates in each population, assuming 12-day incubation and 12-day nestling periods. Females were assumed to be fertile for 4 days prior to laying the first egg (Kempenaers 1993).

STRENGTH, DIRECTION, AND TARGETS OF FEMALE CHOICE

I next examined the strength, direction, and targets of proxies for female choice. Song features were highly correlated, so I

Table 1. Principal component loadings of song features.

Song variable	PC1	PC2
Average song length	**0.57**	0.15
Average unit types per song	**0.57**	0.15
Average number of elements per song	**0.55**	–0.06
Song unit repertoire size	–0.05	**0.61**
Unique song repertoire	–0.21	**0.55**
Average number of units per song	0.01	**0.52**
Proportion variance	0.44	0.34
Cumulative variance	0.44	0.78

Loadings greater than 0.5 are highlighted in bold. PC1 accounts for 44% of the total variance and can be interpreted as a measure of song length and within-song complexity: songs that are longer, have more unit types, and more elements load positively onto PC1. PC2 is a measure of repertoire size: individuals with large song unit and song type repertoires have larger values of PC2.

performed a PCA on the correlation matrix of song features across all males ($n = 82$). The first PC is primarily a measure of song length and internal song complexity (Table 1). The second PC is primarily a measure of song repertoire size (Table 1). The first two PCs explain 78% of the variance in song structure, and I used PC1 and PC2 to describe song variation in the following analyses.

To identify traits associated with male pairing status (paired or unpaired), standardized breeding date, and paternity loss, I built maximal models that included song PC1 (song length), song PC2 (repertoire size), song rate, territory size, male body mass, and aggressiveness score as explanatory variables. Population was included as a random effect. In models of chick condition (mean mass of all chicks in a nest), I included male song PCs, nest fledging date, and number of chicks per nest as explanatory variables, with population as a random effect. Stepwise model selection was performed for each maximal model by deleting the least significant term and re-running the model until all terms were significant (Zuur et al. 2009). Traits significantly associated with male fitness were considered targets of female choice. There were not enough degrees of freedom to include interactions between population and each explanatory variable in the maximal models. Therefore, to assess variation in the direction of the association between a male trait and fitness (the direction of female choice) and the strength of selection on a trait (strength of female choice), I ran separate linear models for each significant explanatory variable that included only an interaction between that variable and population. I compared models with interactions to those without interactions. All territorial birds were paired in Siberia, and sample sizes were very small for extra pair young and chick mass ($N = 5$ nests), so I was only able to analyze breeding date at this site.

STRENGTH, DIRECTION, AND TARGETS OF MALE COMPETITION

I assessed the strength, direction, and targets of male competition by examining aggressive response to experimentally manipulated playback files in Kyrgyzstan and Siberia. I used a linear mixed model with experimental treatment, breeding stage, and population as factors, and individual bird as a random effect. Model selection for fixed effects proceeded as described above, starting with the three-way interaction between factors. Residuals were not normally distributed, so I also tested the following error variance structures: homogeneous variance; separate variances for population, breeding stage, and stimulus type; all two-way interactions between population, breeding stage, and stimulus type; and the three-way interaction. I chose the error structure with the lowest AIC in the maximal model (Zuur et al. 2009). Differences among treatment groups were tested using Tukey tests on the contrast matrix with the R package *multcomp* (Hothorn et al. 2016).

Results
TEMPORAL VARIATION IN SONG FEATURES: TARGETS OF MALE COMPETITION AND FEMALE CHOICE

Seasonal variation in song features was strikingly similar across populations despite significant geographic variation in population means. Daily means of song features varied significantly nonlinearly across the breeding season in each population (Fig. 3, Table 2). The only trait that did not exhibit significant temporal variation was the number of unique songs per bout in India and Kyrgyzstan (Table 2). In all populations, males sang significantly shorter, simpler songs when they were competing with other males to establish territories, and produced significantly longer and more complex songs when females were fertile (Fig. 3, Table 2). Population-level variation suggests that short, simple songs may be targets of male competition, whereas long, complex songs may be targets of female choice across all populations.

FEMALE CHOICE: CONSISTENT STRENGTH, TARGETS, AND DIRECTION OF SELECTION

Song PCs are used in the analyses of female choice. For ease of interpretation, I refer to PC1 as "song length" and PC2 as "repertoire size" (Table 1). Longer, more internally complex songs (PC1) and larger song repertoires (PC2) were both significantly associated with pairing success in India and Kyrgyzstan; all birds were paired in Siberia (Fig. 4A, B, generalized linear-mixed model with binomial errors: song length, $\beta = 0.92 \pm 0.4$, $z = 2.30$, $P = 0.02$; repertoire size, $\beta = 0.48 \pm 0.25$, $z = 1.9$, $P = 0.05$). No other male traits were significantly associated with pairing success.
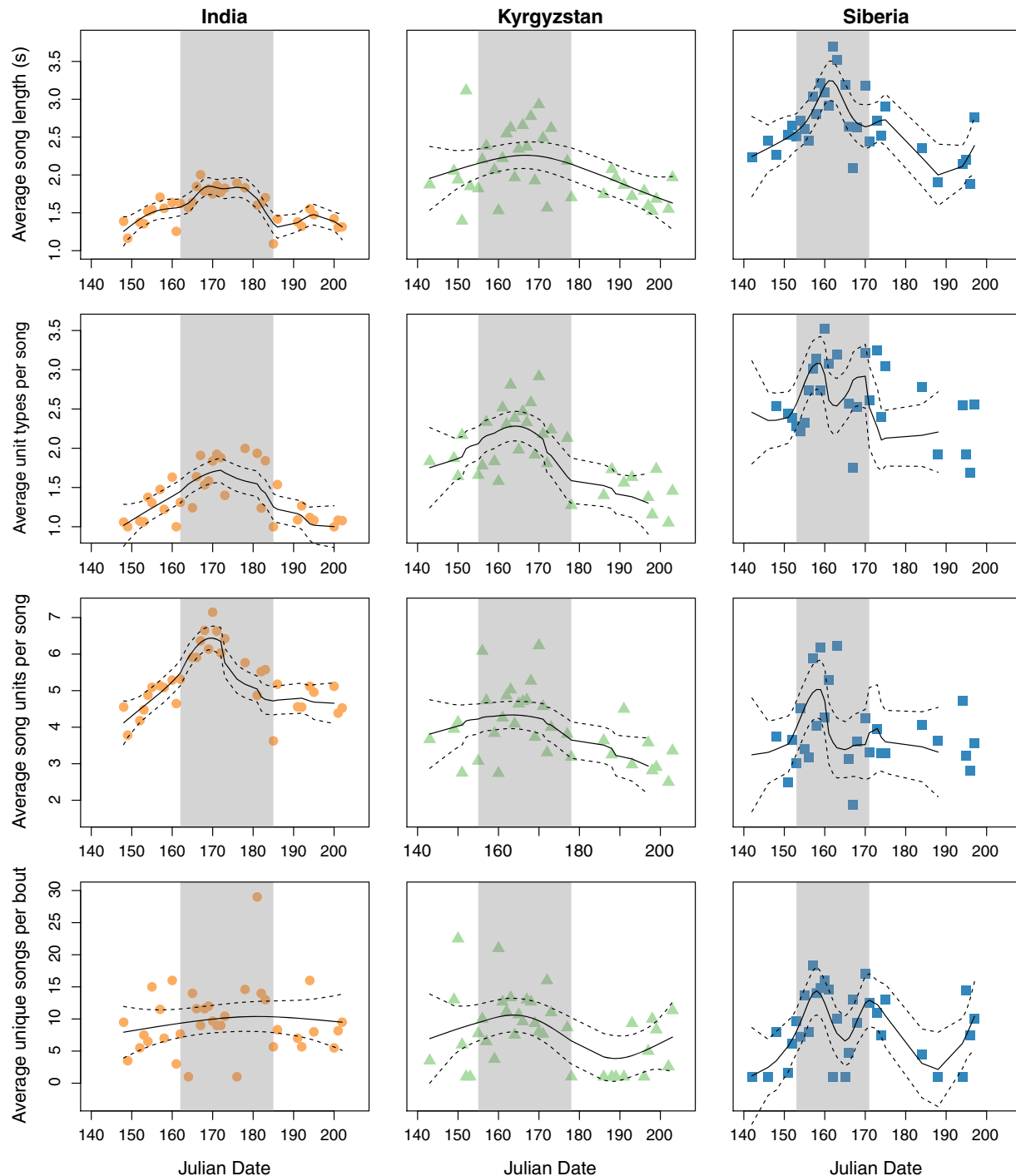
Figure 3. Variation in daily means of song length, number of unit types per song, number of units per song and number of unique songs per bout in India, Kyrgyzstan, and Siberia. Shaded areas correspond to periods of peak female fertility in each population. Mean trait values in the fertile period are significantly larger than the nonfertile period in all cases except number of unit types per song in Siberia (2nd row) and unique songs per bout in India and Kyrgyzstan (bottom row). For illustrative purposes, curves ± standard errors show fitted values from generalized additive models that fit a cubic spline smoother to each population individually. Statistics in Table 2 are from a model that fits a separate smoother to each population while including population as a parametric factor; curves in both sets of models are significantly nonlinear.

Table 2. Seasonal variation in daily means of song length, number of song unit types per song, number of units per song, and number of unique songs per bout.

Song characteristic				Fertile vs nonfertile period			
Song length		df	F	P			
Population		2	7.53	<0.001			
Smooth terms	edf	Ref.df	F	P	t	df	P
India	3.16	3.77	2.43	0.06	4.00	76.1	<0.001
Kyrgyzstan	3.16	3.86	6.17	<0.001	5.33,	94.3	<0.001
Siberia	7.20	8.09	5.06	<0.001	2.63	48.6	0.01
Adj. $R^2 = 0.74$, Deviance explained = 77.7%							
Song unit type		df	F	p			
Population		2	1.44	0.24			
Smooth terms	edf	Ref.df	F	P	t	df	P
India	3.28	3.91	4.77	0.002	5.81	55.7	<0.001
Kyrgyzstan	4.68	5.68	7.01	<0.001	6.50	75.6	<0.001
Siberia	6.89	7.82	3.48	0.002	3.45	50.6	0.001
Adj. $R^2 = 0.77$, Deviance explained = 80.6%							
Units per song		df	F	P			
Population		2	1.29	0.28			
Smooth terms	edf	Ref.df	F	P	t	df	P
India	4.60	5.48	5.49	<0.001	8.62	70	<0.001
Kyrgyzstan	2.71	3.28	4.07	0.008	4.03	72.4	0.001
Siberia	6.57	7.52	2.64	0.01	3.31	44.9	0.001
Adj. $R^2 = 0.57$, Deviance explained = 63.9%							
Unique songs		df	F	P			
Population		2	0.51	0.95			
Smooth terms	edf	Ref.df	F	P	t	df	P
India	2.243	2.62	0.82	0.392	1.75	88.0	0.2
Kyrgyzstan	3.855	4.70	1.94	0.078	2.11	85.2	0.1
Siberia	5.716	6.71	1.64	0.106	5.22	85.9	<0.001
Adj. $R^2 = 0.15$, Deviance explained = 25.4%							

Left side of table shows results from GAMs that include population as a parametric factor and fit a separate cubic spline smoother to each population. Models that fit a separate smoother were all > 2 AIC units lower than models that fit a single smoother, with the exception of unique songs per bout, for which the separate smoother model was only one AIC unit lower than the single smoother model. Right side of table shows *t*-test comparing song characteristics in the fertile and nonfertile periods for each site, with *P*-values adjusted for multiple tests.

Among paired males, those with longer songs and larger repertoires bred significantly earlier in all three populations (Fig. 4C, D, song length alone: $\beta = -0.41 \pm 0.15$, $F_{1,33} = 7.88$, $P = 0.008$; repertoire size alone: $\beta = -0.32 \pm 0.09$, $F_{1,33} = 11.6$, $P = 0.002$). Song length and repertoire size were significantly positively correlated within Kyrgyzstan and Siberia but not in India (India: $n = 21$, $r = 0.27$, $P = 0.22$; Kyrgyzstan: $n = 32$, $r = 0.54$, $P = 0.001$; Siberia: $n = 28$, $r = 0.47$, $P = 0.01$). When both song length and repertoire size were included in the model, only repertoire size remained significant (repertoire size $\beta = -0.31 \pm 0.01$, $F_{1,32} = 10.12$, $P = 0.003$; song length $\beta = -0.05 \pm 0.1$, $F_{1,32} = 1.38$, $P = 0.23$). As with models of pairing success, no other male traits were significantly associated with early breeding. Song length and repertoire size are thus consistent targets of female choice in all populations.

Because song length and repertoire size (PC1 and PC2) were the only traits significantly associated with fitness, I examined variation in the strength and direction of selection on these traits among populations. I standardized song PCs and breeding date within each population to have zero mean and unit variance (pairing success was binomial). I then compared models that included an interaction between population and song trait to models without the interaction using an ANOVA. Significant interactions indicate a change in the strength of selection (the slope of the association between traits and fitness). Variation in the sign of the slope indicates a change in the direction of selection. There were no significant interactions in any model, no changes in the sign of the slope, and models with interactions did not fit the data significantly better than models without interactions (Fig. 4, Table S2). Proxies of female choice therefore indicate consistent strength
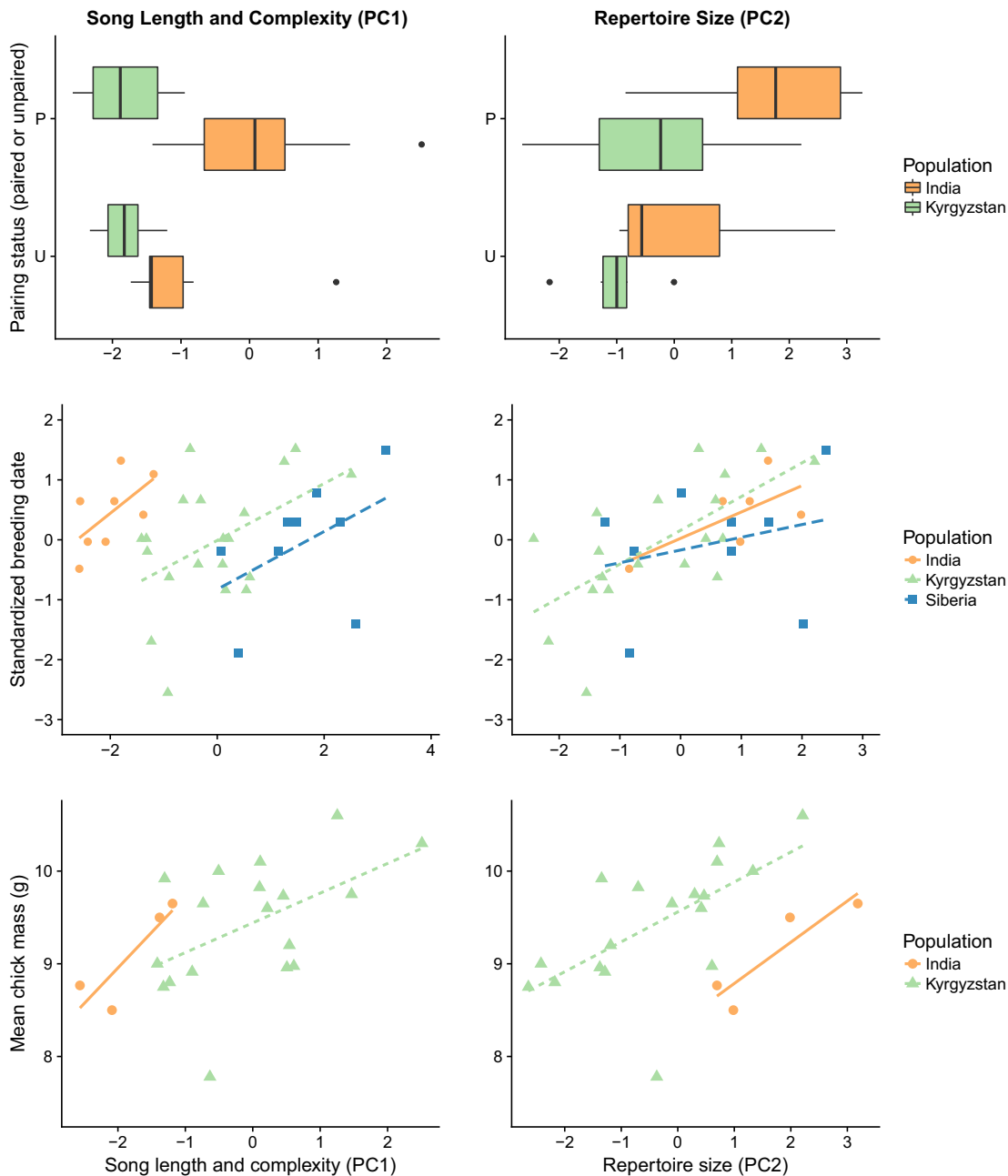
Figure 4. Relationship between song PCs and pairing success, standardized breeding date, and mean chick fledging mass in each population. Top row: males with longer songs (PC1) and larger repertoires (PC2) had higher pairing success in India and Kyrgyzstan (P = paired, U = unpaired). Middle row: in all three populations, males with longer songs and larger repertoires bred earlier in the season. Breeding dates have been multiplied by -1 to make relationship easier to interpret, such that earlier breeding dates have larger numbers on the y-axis, and longer songs/larger repertoires have larger values on the x-axis. Bottom row: males with longer songs and larger repertoires reared larger chicks, an effect primarily driven by Kyrgyzstan.

and direction of selection for males with large song repertoires and long, complex songs within all populations, despite significant variation in mean values for these traits among populations.

Males with longer songs and larger repertoires fledged larger chicks in India and Kyrgyzstan (Fig. 4E, F). As in the analysis of breeding date, repertoire size (PC2) was significant

in a multivariate model with song length (song length, $\beta = 0.20 \pm 0.12$, $F_{1,18} = 2.62$, $P = 0.14$; repertoire size, $\beta = 0.21 \pm 0.10$, $F_{1,18} = 4.63$, $P = 0.05$). Song length was significant only when repertoire size was removed from the model (song length alone, $\beta = 0.29 \pm 0.1$, $F_{1,19} = 8.27$, $P = 0.01$; repertoire size alone, $\beta = 0.32 \pm 0.09$, $F_{1,19} = 13.74$, $P = 0.002$). Sample sizes
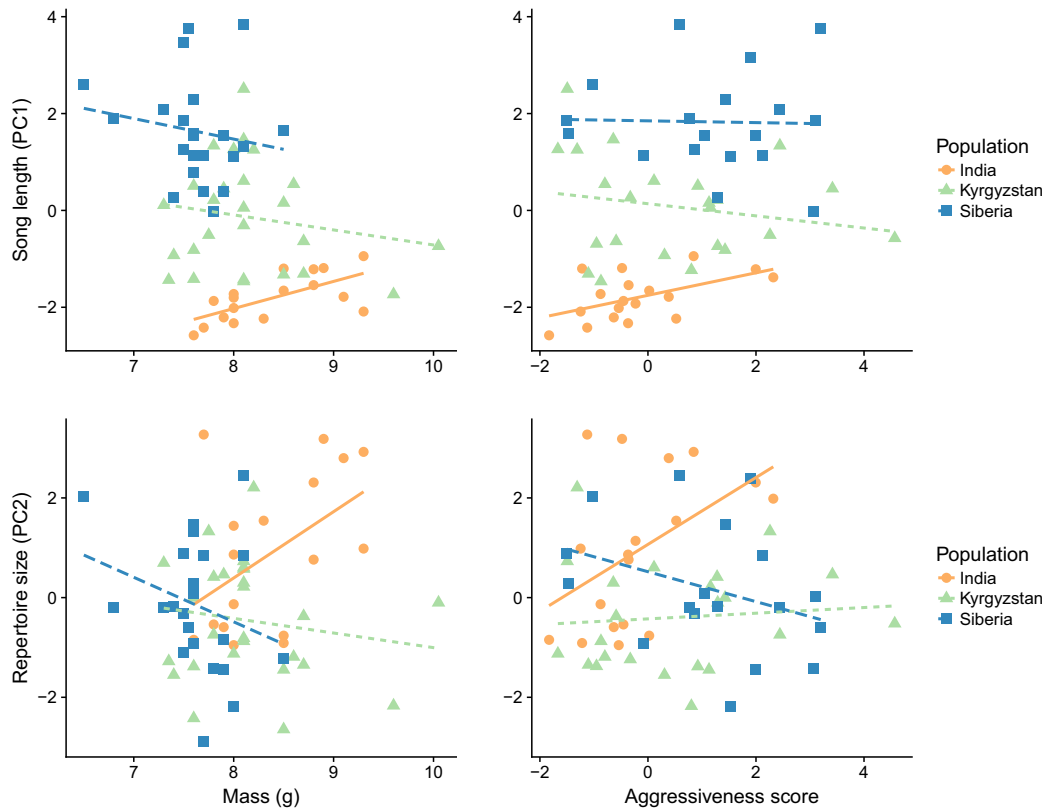
Figure 5. Correlations between song PCs and male body mass and aggressiveness score in each population. Top row: song length (PC1) and repertoire size (PC2) are significantly correlated with male body mass in India, but not in Kyrgyzstan or Siberia. Bottom row: male aggressiveness score is positively correlated with song length and repertoire size in India, but not in Kyrgyzstan or Siberia. In India only, males that are heavier and more aggressive sing longer songs and have larger song repertoires.

for chick mass in India were small due to high predation rates, so this result is mainly driven by data from Kyrgyzstan. No other traits were significantly associated with chick mass.

The previous results pertain to social fathers, but these males were often cuckolded (43% of chicks were extra-pair in India, 32% in Kyrgyzstan). I measured paternity loss using the proportion of extra-pair young in a nest. There was a significant interaction between song length and body mass ($z = 2.51$, $P = 0.012$). However, this result was driven by one large male whose entire nest was cuckolded, likely reflecting a genotyping error or other misassignment (see Supplemental Material). Removal of this male results in no significant relationship between any male trait and paternity loss. It was not possible to identify extra-pair fathers, and I was therefore unable to examine female choice of social versus extra pair mates.

FEMALE CHOICE: SONGS AS INDICATORS OF MALE QUALITY

The previous analyses indicate that females in all populations preferred males with long, complex songs and large repertoires; that is, there was no variation in the strength, direction, or targets of selection among populations. To examine what information

about male quality might be advertised by male song, I examined whether song length and repertoire size were correlated with other male traits (song rate, body mass, territory size, and aggressiveness score).

In India, song length (PC1) and repertoire size (PC2) were both positively correlated with male body mass (Fig. 5, song length: $n = 16$, $r = 0.66$, $P = 0.004$; repertoire size: $r = 0.48$, $P = 0.05$). Both song traits also correlated positively with early-season aggressiveness (Fig. 5, song length: $n = 16$, $r = 0.52$, $P = 0.02$; repertoire size: $r = 0.57$, $P = 0.04$). Thus, in India, males with on-average larger song repertoires and longer songs were larger and more aggressive. Males with longer songs had smaller territories ($n = 15$, $r = -0.56$, $P = 0.02$; repertoire size was not significantly correlated with territory size), but were nonetheless preferred by females (Fig. 4). These males may defend favorable areas where density is especially high, or some unmeasured aspect of territory quality rather than territory size per se may be a target of female choice.

In contrast to India, there were no significant correlations between song and other male traits in Kyrgyzstan and Siberia (Fig. 5). Moreover, three out of four correlations between mass, aggressiveness, and song PCs were significantly larger in
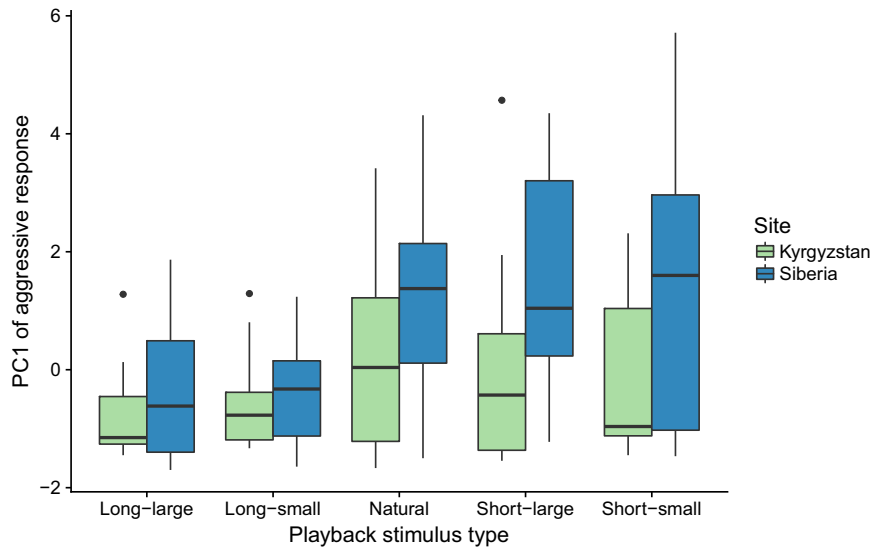
Figure 6. Male aggressive response to different playback stimuli. Males responded more aggressively to playbacks of short songs and natural songs than to long songs. Males responded slightly more aggressively to natural songs compared to playbacks with larger repertoires, but there were no other differences in responsiveness based on repertoire size of the playback file.

India than in Kyrgyzstan and Siberia combined (Fisher's r to z transformation; mass and song length, $z = 3.82$, $P < 0.001$; mass and repertoire size, $z = 2.4$, $P = 0.01$; aggressiveness and song length, $z = 1.79$, $P = 0.07$; aggressiveness and repertoire size, $z = 2.97$, $P = 0.003$). Song length and repertoire size are thus honest indicators of male body size and aggressiveness in the high-density, low-food environment of India, but not in the low-density, high-food Kyrgyz and Siberian populations.

MALE COMPETITION: AGGRESSIVE RESPONSE TO DIFFERENT SONG TYPES

Finally, I examined variation in the strength, direction, and targets of male competition by playing manipulated song files to male territory owners. The best model predicting male response to playback included significant effects of population and treatment group. Individual was included as a random effect, and I fit a different error variance for each population to reduce heterogeneity in the residuals (Table S3). Male territory owners in both Kyrgyzstan and Siberia responded more aggressively to playbacks of natural and short songs than to long songs (Fig. 6, Tukey post-hoc tests: short songs vs long songs, $P = 0.008$, natural songs vs long songs, $P = 0.001$, short songs vs natural songs, $P = 0.65$). Males responded more aggressively to natural songs compared to playbacks with large repertoires ($P = 0.05$), but there was otherwise no significant effect of repertoire size on aggressive response to playback (Tukey post-hoc tests: large vs small repertoires, $P = 0.89$, small repertoires vs natural songs, $P = 0.15$). Responses were stronger overall in Siberia than in Kyrgyzstan ($P = 0.004$, Fig. 6, Table S3). There were

no significant interactions in the model, indicating no changes in the direction of aggressive response toward different song types. Short songs, but not repertoire size, thus appear to be a consistent target of male competition in both *P. trochiloides* populations.

Discussion

In this study, I asked if song variation in the greenish warbler ring species was associated with changes in the strength, direction, or targets of female choice and male competition in different habitats. Despite significant geographic variation in song structure, I found no corresponding evidence for variation in the strength, direction or targets of female choice. Furthermore, males in the two northern populations responded more aggressively to short songs than to long songs, suggesting consistent targets and direction of male competition. Average song length was shortest in the southernmost population (India), where population density was highest. These results complement a previous study showing that male territorial strategies varied among populations, with birds defending their territories throughout the entire breeding season in India, but primarily guarding mates rather than territories in the two northern populations (Scordato 2017). Together, these findings support Irwin's (2000) hypothesis that weaker competition for territories at higher latitudes allows greater expression of female preferences for long, complex songs, whereas strong male competition and the need to defend territories throughout the breeding season limits maximum song length in the south. Variation in the strength of male competition in different environments, rather than female choice, thus likely drives song divergence in this system.

CAUSES OF GEOGRAPHIC VARIATION IN SONG STRUCTURE: A BALANCE BETWEEN FEMALE CHOICE AND MALE COMPETITION

Male competition and female choice appeared to act in opposing directions on *P. trochiloides* song. Males resolved this conflict via two strategies. First, they exhibited labile singing behavior (e.g., Celis-Murillo et al. 2016; Zhang et al. 2016). In all populations, songs were comparatively short and simple early in the season, suggesting short songs are important to territory establishment. Males produced long, complex songs when females were fertile, and resumed singing short songs once most females had mated (Fig. 3). The seasonal peaks in song length and complexity may advertise male quality to prospective females. This interpretation was supported by the finding that in all populations, males that sang (on average) longer, more complex songs bred earlier, and in India and Kyrgyzstan, had higher pairing success and reared larger chicks. Males thus appeared to adjust the length and complexity of their songs depending on breeding stage and intended receiver. The extent to which interactions between female choice and male competition generate seasonal variation in song structure in other species warrants further examination.

Second, males resolved conflicts between female choice and male competition by aiming different song components at different receivers. In addition to song length, large song repertoires were associated with earlier breeding, higher pairing success, and larger chicks. Repertoire size may indeed be a more important component of female choice than song length. In models of breeding date including both song length and repertoire size, only repertoire size remained significant, although correlations between song traits within populations made it challenging to separate these two variables. Unlike song length, however, song repertoire size (measured as the number of unique songs produced per singing bout) varied comparatively little over the breeding season and among populations. Males also did not adjust aggressive response to playbacks that varied in repertoire size. These results imply that song repertoire is targeted primarily at female receivers, as has been found in several other species (Buchanan and Catchpole 1997; Reid et al. 2004). The absence of conflicts with male competition may make repertoire size a more consistently reliable signal of male quality for female receivers (Candolin 2003).

Although male *P. trochiloides* seemed to balance the conflict between male competition and female choice by exhibiting labile singing behavior and aiming different song components at different receivers, there was nonetheless significant geographic variation in song length and complexity among populations. The shortest, simplest songs were in India and the longest, most complex songs in Siberia. This pattern is likely due to variation in the strength of male competition and male competitive strategies in different environments. There was a near-perfect negative correlation between population density and average song length across

sites ($r = -0.99$, $n = 3$, $P = 0.02$). Moreover, in the high-density, short-song Indian population, males primarily sang in response to playbacks, and defended their territories via singing throughout the entire breeding season (Scordato 2017). In the two more northerly populations, low population density and denser forests may interfere with a singer's ability to listen to neighbors (Catchpole 1982), and males exhibited close-range aggressive displays more frequently in these populations (Scordato 2017). Furthermore, males in Kyrgyzstan and Siberia did not defend their territories throughout the season. Instead, they exhibited a burst of aggressive behavior early in the season, consistent with mate guarding when females were fertile, and ceased exhibiting territorial behavior once most females had paired (Scordato 2017). This early burst of aggressive behavior was stronger across all playback types in Siberia than in Kyrgyzstan (Scordato 2017, Fig. 6). A shift in territorial strategies and in the way signals are used in competitive interactions supports a more important role for short songs in male territory defense in India, and less importance of territoriality overall in Kyrgyzstan and Siberia.

Interpreting responses to playback experiments is challenging, because birds may respond less aggressively to songs from higher quality males (de Kort et al. 2008; Searcy and Beecher 2009). Furthermore, I was only able to play manipulated playbacks in Kyrgyzstan and Siberia, and thus it is possible that male birds in India use different aggressive signals. However, concordance between natural, seasonal variation in song length in all populations (short songs produced early in the season) and response to experimental playbacks (stronger aggressive response to short songs) strongly suggests that short songs are consistently used in territorial interactions, as has been reported in many other species (Catchpole and Slater 2008). Taken together, my results suggest that the benefits males in India gain from allocating resources to season-long territory defense maintained by short songs are great enough to counteract female choice for long songs, and thus constrain mean song length in this population.

GEOGRAPHIC VARIATION IN INFORMATION CONTENT OF SONGS: THE SAME TRAITS SIGNAL DIFFERENT ASPECTS OF MALE QUALITY

Given that the strength of male competition varied across habitats, it was surprising that female choice remained consistent. Previous sexual selection studies have shown that females select for different aspects of male quality under different ecological conditions (Bro-Jørgensen 2010; Cornwallis and Uller 2010; Botero and Rubenstein 2012), social contexts (Kwiatkowski and Sullivan 2002; Kasumovic and Andrade 2009), and over time (Chaine and Lyon 2008). However, meta-analyses of selection gradients also show that, despite examples of context-dependent mate choice, sexual selection switches direction less frequently than

natural selection (Siepielski et al. 2011), and selection in general varies in strength more frequently than direction (Siepielski et al. 2013). A possible resolution to this paradox is that the same signals can advertise different aspects of male quality in different environments.

In India, repertoire size and song length correlated with male aggressiveness and body mass, suggesting that song traits preferred by females were associated with male competitive traits. This is intuitive: density is higher and food is scarcer in India than in the other populations (Scordato 2017). Access to a good-quality territory is therefore likely important to female fitness, and female choice and male competition may be expected to act synergistically in this poor-quality environment (Qvarnström and Forsgren 1998). By contrast, there were no significant correlations between song and competitive traits in the high-quality habitats of Kyrgyzstan and Siberia. However, males with larger repertoires and longer songs still had higher fitness in these populations, suggesting that song advertises some unmeasured feature of male quality (Alatalo et al. 1986; Mountjoy and Lemon 1996; Gil and Slater 2000). Future work focused on female choice of extra-pair mates could shed additional light on the aspects of male quality advertised by different song traits. Simulation (Higginson and Reader 2009) and empirical (Narango and Rodewald 2018) studies have shown that environmental heterogeneity can compromise the honesty of sexual signals as indicators of male quality. My data imply that the same song features can become associated with different aspects of male quality in different habitats, even in populations connected by gene flow. The extent to which the information content of sexual signals is dynamic across environments is an exciting avenue for future studies of sexual selection and phenotypic divergence.

SEXUAL SELECTION, ECOLOGY, AND SPECIATION

Ecological and social context influence divergence in female preferences (Maan and Seehausen 2011; Scordato et al. 2014) and male competition (Jirotkul 1999; Knell 2009; Lackey and Boughman 2013), but interactions between sexual selection and ecology have rarely been compared across populations (Qvarnström et al. 2012). Studies that do examine these interactions tend to focus on signal detectability (e.g., Boughman 2002). For example, song structure often varies to optimize transmission in dense forests (Patten et al. 2004; Price 2008; Tobias et al. 2010) or minimize interference from other species (Singh and Price 2015). However, Irwin (2000) assessed and ruled out signal detectability as a primary driver of song divergence in *P. trochiloides*, and I found no evidence for sexual selection on song frequency (data not shown). Tietze et al. (2015) also found little evidence for acoustic adaptation across the *Phylloscopus* genus. The interactions between ecology and sexual selection in *P. trochiloides* are thus different from those that arise due to variation in the transmission envi-

ronment. Instead, geographic variation in population density and food availability likely leads to variation in the strength of male competition and competitive strategies, as well as variation in the information females use to evaluate mates.

Female choice is typically considered a stronger selective force than male competition because, although males compete for access to females, females usually control mating decisions (Andersson 1994). The contribution of male competition to population divergence has thus often been overlooked (Qvarnström et al. 2012; Tinghitella et al. 2018). However, studies from a variety of taxa show that aggressive behavior and territorial defense vary geographically (Newman et al. 2006; Apfelbeck and Goymann 2011; Bastianelli et al. 2015), with ecological factors such as population density (Gosden and Svensson 2008), resource distribution (Taff et al. 2013), sex ratio (Kokko and Rankin 2006), and breeding synchrony (Thusius et al. 2001), and may be heritable targets of selection (Sih et al. 2004). Moreover, there is increasing evidence for a direct role of male competition in diversification: color divergence in darters (*Etheostoma*) has been attributed to male competition rather than female choice (Martin and Mendelson 2016), and male competition based on throat color may enhance ecological divergence in sticklebacks (*Gasterosteus*, Tinghitella et al. 2015).

Most examples of male competition contributing to speciation focus on the maintenance of reproductive barriers in secondary contact, and show that male competition can drive character displacement (Seehausen and Schluter 2004; Drury and Grether 2014; Rybinski et al. 2016), lead to differential introgression of competitive traits (While et al. 2015), and limit immigration (Bensch et al. 1998; Slabbekoorn and Smith 2002). The present study differs by focusing on an earlier stage of speciation. Although *P. trochiloides* forms are reproductively isolated in secondary contact in Siberia (Irwin et al. 2001; Irwin 2005), the populations studied here are diverging in parapatry (Alcaide et al. 2014), and offer insight into the processes operating in the early stages of divergence. Although female greenish warblers seem to prefer long, complex songs, in poor quality habitats, male competition is strong enough to limit expression of female choice. These interactions between male competition, female choice, and habitat have cascading effects that lead to song divergence and ultimately contribute to the formation of premating reproductive barriers. Future studies of sexual selection and phenotypic divergence should consider how interactions between male competition and female choice vary across different ecological contexts. A stronger influence of male competition on sexual signals in poor quality habitats, but stronger effects of female choice in good quality habitats, may be a reasonable, general hypothesis for understanding geographic variation and subsequent divergence of sexual signals in species with socially monogamous mating systems.

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Associate Editor: Gerlinde Höbel
Handling Editor: Mohamed A. F. Noor

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. PC loadings for PCA of aggressive behavior based on response to playbacks of natural song.
Table S2. Selection differentials for song PC1 and PC2.
Table S3. Linear mixed model of aggressive response to different playback treatments.