

SONG VARIATION IN AN AVIAN RING SPECIES

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Abstract.—Divergence of mating signals can occur rapidly and be of prime importance in causing reproductive isolation and speciation. A ring species, in which two reproductively isolated taxa are connected by a chain of intergrading populations, provides a rare opportunity to use spatial variation to reconstruct the history of divergence. I use geographic variation in the song of a likely ring species, the greenish warbler (*Phylloscopus trochiloides*) to reconstruct the microevolutionary steps that occurred during divergence of a trait that is often important in speciation in birds. Populations of a western Siberian (*P. t. viridanus*) and an eastern Siberian (*P. t. plumbeitarsus*) form of the greenish warbler meet, but do not interbreed in central Siberia; these forms are connected by a chain of interbreeding populations extending in a ring to the south around the treeless Tibetan Plateau. I show that: (1) song structure differs greatly between the two Siberian forms, which share the same habitat; (2) song structure changes gradually around the ring; (3) singing behavior is relatively simple in the Himalayas, but becomes increasingly complex to the north, both to the west and east of the Tibetan Plateau; and (4) song varies along independent axes of complexity in the western and eastern south-north clines. By comparing geographic variation in singing behavior and ecological variables, I distinguish among possible causes of song divergence, including selection based on the acoustic environment, stochastic effects of sexual selection, and selection for species recognition. I suggest that parallel south-to-north ecological gradients have caused a greater intensity of sexual selection on song in northern populations and that the stochastic effects of sexual selection have led to divergence in song structure.

Key words.—Complexity, greenish warbler, *Phylloscopus trochiloides*, *plumbeitarsus*, ring species, sexual selection, song, speciation.

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The evolutionary divergence of mating signals is important in speciation. Two populations are essentially different species if they have diverged sufficiently so that members of one do not recognize members of the other as potential mates. Among closely related species, mating signals are often more divergent than morphological, genetic, or other behavioral traits (Miller 1996; Jones 1997; Wells and Henry 1998; Irwin et al. 2001). Across a variety of taxa experiments have demonstrated that females discriminate between conspecific and heterospecific male signals (e.g., Uetz and Stratton 1982; Verrell and Arnold 1989; Clayton 1990; Ryan and Rand 1993; Gerhardt 1994; Wells and Henry 1998). These observations suggest that divergence in mating signals occurs rapidly and can quickly generate reproductive isolation.

The causes of mating signal divergence are often not clear. Debated issues include the relative importance of natural and sexual selection, the role of sympatric interactions, and the importance of ecological divergence in causing signal divergence (West-Eberhard 1983; Andersson 1994; Butlin 1995; Jones 1997; Wells and Henry 1998; Price 1999). Distinguishing among possible causes requires knowledge of both the microevolutionary changes that occurred during divergence of taxa under study and the correspondence between changes in mating signals and in ecological factors. In most systems, however, we are presented with only products of divergence—two species—and can only speculate about traits of their common ancestor or evolutionary steps that produced present differences between those species.

A rare opportunity to study divergence during speciation arises when two coexisting and reproductively isolated taxa are connected by a long chain of intergrading populations. Such systems, called “ring species,” were described by Mayr (1942, p. 180) as the “perfect demonstration of speciation,” and are ideally suited for studies of the steps involved in the

divergence of two species from one (e.g., color patterns of *Ensatina* salamanders, Stebbins 1949; Wake 1997). Variation in space suggests how evolution occurred in time. The Greenish Warbler (*Phylloscopus trochiloides*) provides such a system. Based on a study of morphological variation in museum specimens, Ticehurst (1938) concluded that two forms of the greenish warbler, *P. t. viridanus* in the west and *P. t. plumbeitarsus* in the east, coexist in central Siberia without interbreeding; these forms are connected by a chain of intergrading populations to the south through the Himalayas (Fig. 1). The ring of populations encircles the Tibetan Plateau and other arid areas in central Asia.

In this paper, I use geographic variation in the song of the greenish warbler to infer microevolutionary steps that have led to mating signal divergence between different parts of the range. Male song is often used in mate choice and species recognition within birds (Catchpole and Slater 1995), particularly within the genus *Phylloscopus* (Martens 1980; Salomon 1989; Irwin et al. 2001). Studies of interspecific song variation have revealed that bird songs are rapidly evolving and show little imprint of phylogeny compared to other traits (Badyaev and Leaf 1997; Van Buskirk 1997; Irwin et al. 2001). The greenish warbler complex therefore provides a powerful system in which to study the evolutionary divergence of a rapidly evolving mating signal that often plays a role in speciation.

An additional strength of the greenish warbler system is that song variation can be interpreted in terms of a reasonable historical scenario. Three lines of evidence imply that the ancestral range of the greenish warbler lay in the Himalayas (as suggested by Ticehurst 1938). First, the Himalayas is the site of maximum species diversity of the genus *Phylloscopus* (Martens 1980; Price et al. 1997). Second, a mitochondrial gene tree has its root in the Himalayas (Price et al. 1997; D.

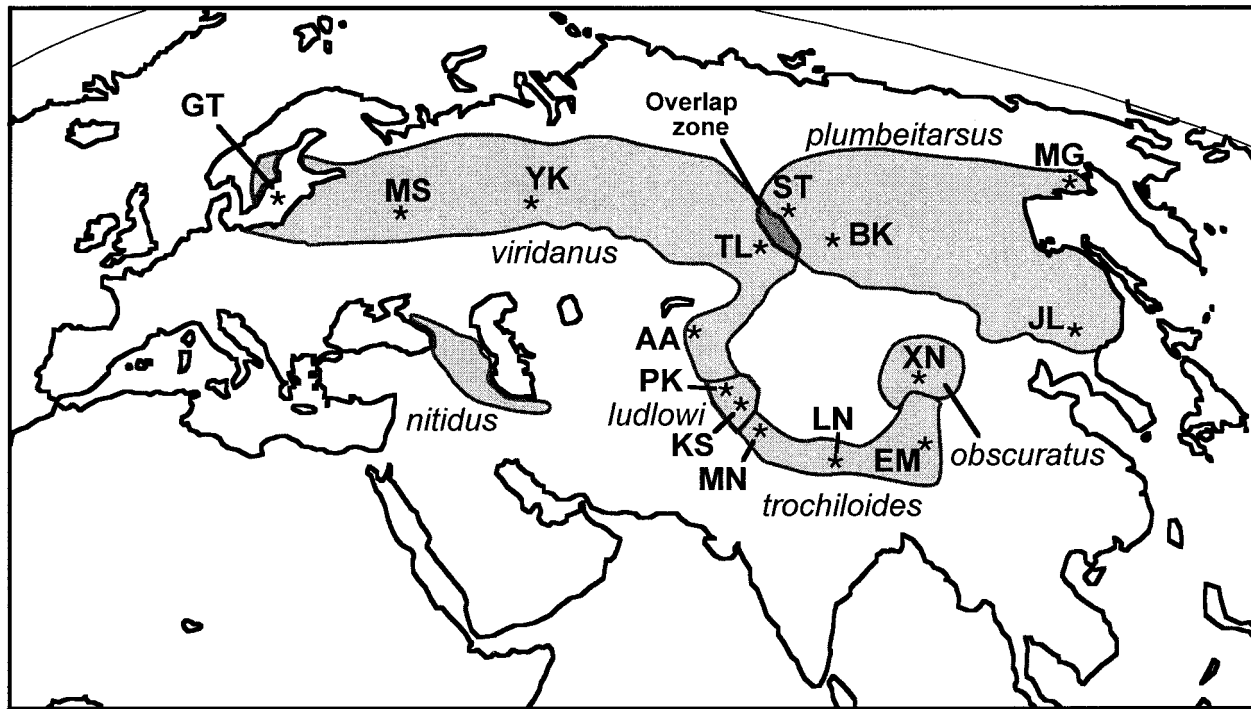


FIG. 1. Map showing ranges during the breeding season of the six taxa in the greenish warbler species complex. Locations where song recordings were obtained are shown. See Table 1 for names and information on the locations. Note the overlap zone between *Phylloscopus trochiloides viridanus* and *P. t. plumbeitarsus* in central Siberia, where the two forms do not interbreed. *Phylloscopus t. ludlowi* is a transitional form between *P. t. viridanus* and *P. t. trochiloides*, and *P. t. obscuratus* is a transitional form between *P. t. trochiloides* and *P. t. plumbeitarsus*. The geographically disjunct *P. (t.) nitidus* is not studied in this paper.

E. Irwin, pers. obs.). Third, climatic history indicates that more northerly areas have regularly been inhospitable for warblers (Price et al. 1997). Variation in song can thus be interpreted dynamically in terms of northward spread out of the Himalayas along two pathways, to the west and east of the Tibetan Plateau.

As I show in this paper, ecological factors differ strongly between southern and northern populations of greenish warblers, a result of the strong latitudinal gradient in climate. In contrast, ecological factors differ relatively little between western and eastern populations at the same latitude. Thus, different causes of song divergence should result in different spatial patterns of song variation. The ecological patterns, together with the historical understanding, allow discrimination among four possible causes of mating signal divergence. The four hypotheses and predicted patterns of song variation are as follows.

(1) *Ecological differences cause different natural selection optima, leading deterministically to signal divergence.*—Such signal divergence has been discussed by Paterson (1985), Vrba (1995), and Jones (1997). For example, different frequencies of sound travel best in different environments (Morton 1975; Hunter and Krebs 1979; Wiley and Richards 1982; Wiley 1991). Under natural selection, we expect strong south-to-north difference in Greenish Warbler song, but little west-to-east difference. Because the subspecies share similar habitat, there should be little difference between *viridanus* and *plumbeitarsus* in central Siberia.

(2) *Sexual selection leads to signal divergence, even in the*

absence of ecological differences.—Studies by Darwin (1871), Fisher (1930), West-Eberhard (1983), Kirkpatrick (1987), Andersson (1994), Iwasa and Pomiankowski (1995) have emphasized the role of sexual selection in causing signal divergence. This is because the absence of a well-defined optimum, the attractiveness of novelty, and the potential for runaway change give sexual selection a highly stochastic quality. In the greenish warbler, differences in song should increase with geographic distance through the breeding range, independent of direction and ecology. We expect a large difference between *viridanus* and *plumbeitarsus* because of different histories and isolation by distance.

(3) *Ecological differences influence the balance between sexual and natural selection, leading to divergence.*—There is often a balance between sexual selection for elaboration of a signal and natural selection opposing elaboration (Darwin 1871; Kirkpatrick 1987; Price 1999). Ecological factors can influence the strength and form of sexual selection or opposing natural selection, and thus shift the equilibrium level of signal elaboration (Catchpole 1980, 1982; Endler 1992; Read and Weary 1992; Schluter and Price 1993; Hill 1994; Møller et al. 1995). In bird song, these interactions influence traits such as song length, repertoire size, and within-song complexity. Females of many species have been shown to prefer greater song complexity and repertoire size (Howard 1974; Kroodsma 1976; Catchpole 1987; Bensch and Hasselquist 1992; Hasselquist et al. 1996; Searcy and Yasukawa 1996; Vehrencamp 1999). In the greenish warbler, we expect parallel south-to-north clines in these traits, both

west and east of the Tibetan Plateau, but little west-to-east change in these traits. There should be abrupt differences between *viridanus* and *plumbeitarsus* in other song traits due to the stochastic effects of sexual selection.

(4) *Selection for species recognition leads to signal divergence.*—When two forms occur sympatrically, reinforcement or reproductive character displacement can lead to signal divergence (Dobzhansky 1940; Blair 1955; Howard 1993). A west-to-east transect across Eurasia may reveal a pattern of reproductive character displacement in which songs of *viridanus* and *plumbeitarsus* differ more in sympatry than in allopatry.

To test these hypotheses, I compare observed patterns of song structure, song rate, and repertoire size to the predicted patterns. I conclude that patterns of song variation in the greenish warbler meet the predictions only of hypothesis 3. There are large differences in song structure between *viridanus* and *plumbeitarsus* in central Siberia and a cline between these forms through the populations to the south. Gradual change in song is associated primarily with the two parts of the range that extend from south to north, and in both cases songs change from short and simple in the south to long and complex in the north. These results suggest that whereas parallel ecological shifts have increased the role of sexual selection on song in two northward expansions, the stochastic nature of sexual selection has resulted in divergence of song structure.

MATERIALS AND METHODS

The Study Organism

In his monograph of the *Phylloscopus*, Ticehurst (1938) described the greenish warbler *P. trochiloides* as consisting of six subspecies: *viridanus*, *ludlowi*, *trochiloides*, *obscuratus*, *plumbeitarsus*, and *nitidus* (see Fig. 1 for breeding distributions). I did not examine *nitidus* in this study, because it is geographically disjunct from the other taxa and not part of the ring surrounding the Tibetan Plateau. Based on variation in morphology (including size measurements, wing formulae, and plumage color), Ticehurst (1938) argued that *ludlowi* is an intergrading form between *viridanus* and *trochiloides* and that *obscuratus* is an intergrading form between *trochiloides* and *plumbeitarsus*. There is no evidence for intergradation between *viridanus* and *plumbeitarsus* where they come into contact in central Siberia, and these taxa can be distinguished by differences in the sizes of pale color patches on the wings.

Ecological Influences on Song

Various ecological factors can influence song both directly (through the acoustic environment, hypothesis 1 above) and indirectly (through modifying the strength of sexual selection, hypothesis 3 above). Using field observation and a literature review, I collected information on geographic variation in the ecological factors listed below. Each of these factors leads to specific predictions for how singing behavior should vary.

Forest density.—The consensus from many studies (Morton 1975; Sorjonen 1986; Handford and Lougheed 1991; Wiley 1991; Badyaev and Leaf 1997; Van Buskirk 1997) is that

the acoustic environment of denser forests selects for smaller frequency ranges and less rapid frequency modulation (Catchpole and Slater 1995). In addition to these direct effects of habitat on song, there are possible indirect effects. *Phylloscopus* warblers use multiple cues in mate choice (Marchetti 1998), and habitat variables probably influence the relative importance of different mating signals. Because of the greater difficulty of using vision, denser forests may lead to greater reliance on song in finding a mate, and thus greater intersexual selection on song. Therefore, we predict that populations in denser forests should have smaller frequency ranges, but more intense and complex singing behavior. I measured tree cover of greenish warbler territories at four sites (YK, AA, LN, and ST, see Fig. 1 for locations) as follows. In each of at least five male territories at each site, I set up two line transects with randomized endpoints (the only constraint being that the ends of each transect must be in the territory), and established points spaced 8 m apart along each transect. At each point I recorded the approximate height and species of any tree cover directly above that point. I recorded the cover as “open” if there was no vegetation above 2 m in height.

Length of breeding season.—Catchpole (1980, 1982) proposed that there is greater intersexual selection on song in migratory species because females must choose mates quickly after arriving on the breeding grounds, and song is a signal that can be assessed quickly. This hypothesis was supported by Read and Weary (1992), who conducted a broad survey of passerine species and found that migrants have larger song and syllable repertoires. Under this hypothesis, we predict that singing behavior is more intense and complex in the north, where populations migrate further to their wintering grounds and spend less time on the breeding grounds.

Population density.—Slater (1981) and Catchpole (1982) proposed that intersexual and intrasexual selection pressures on song oppose each other. Whereas female choice leads to greater length and complexity, male-male competition leads to shorter and simpler songs. This is because males need to listen for replies from their competitors (Slater 1981) and must have a limited repertoire so that they are recognized by their neighbors (Horn and Falls 1996). Under this hypothesis, greater density of greenish warblers should lead to greater male-male competition and shorter and simpler songs. The density of greenish warbler populations was measured through territory mapping. I first color-banded all of the singing males within an area of known size, and then noted the locations of individual singing males over a period of several days or weeks.

Food availability.—One cost of singing behavior is the time spent not doing other activities, such as feeding (Reid 1987; Cuthill and MacDonald 1990; Nyström 1997). Greater food availability in some populations of greenish warblers should lead to higher song rates, which could indirectly cause greater sexual selection on song. Data on caterpillar abundance (a major food source for the greenish warbler) was provided by T. Price, who conducted surveys by counting the number of caterpillars that fell into a 20-cm by 40-cm pan after beating a tree branch with a stick (Price 1991). At least 50 beats were done at each of four sites in May and

TABLE 1. Locations where recordings of greenish warblers were obtained.

Location	Map symbol	Latitude	Longitude	Elevation (m)	Number of individuals
Gotland	GT	57°N	18°E	20	1
Moscow	MS	56°N	38°E	100	1 ¹
Yekaterinburg	YK	57°N	60°E	260	5
Teletsk	TL	52°N	88°E	610	5
Ala Archa	AA	43°N	74°E	2000	5
Pakistan	PK	35°N	74°E	3300	5 ²
Kashmir	KS	34°N	75°E	3550	2 ³
Manali	MN	32°N	77°E	3900	5 ⁴
Langtang	LN	28°N	85°E	3700	5
Emeishan	EM	30°N	103°E	2900	3 ⁵
Xining	XN	37°N	102°E	2700	1 ⁵
Jilin	JL	42°N	128°E	1000	1 ⁵
Baikal	BK	52°N	105°E	460	4 ⁶
Stolbi	ST	56°N	93°E	650	5
Magadan	MG	60°N	151°E	50	1 ³

¹ Recording obtained by B. Veprintsev.

² Recordings obtained by S. Gross and T. Price.

³ Recordings obtained by K. Marchetti and T. Price.

⁴ Recordings obtained by Z. Benowitz-Fredericks and K. Marchetti.

⁵ Recordings obtained by P. Alström and U. Olsson.

⁶ One of the recordings was obtained by P. Alström.

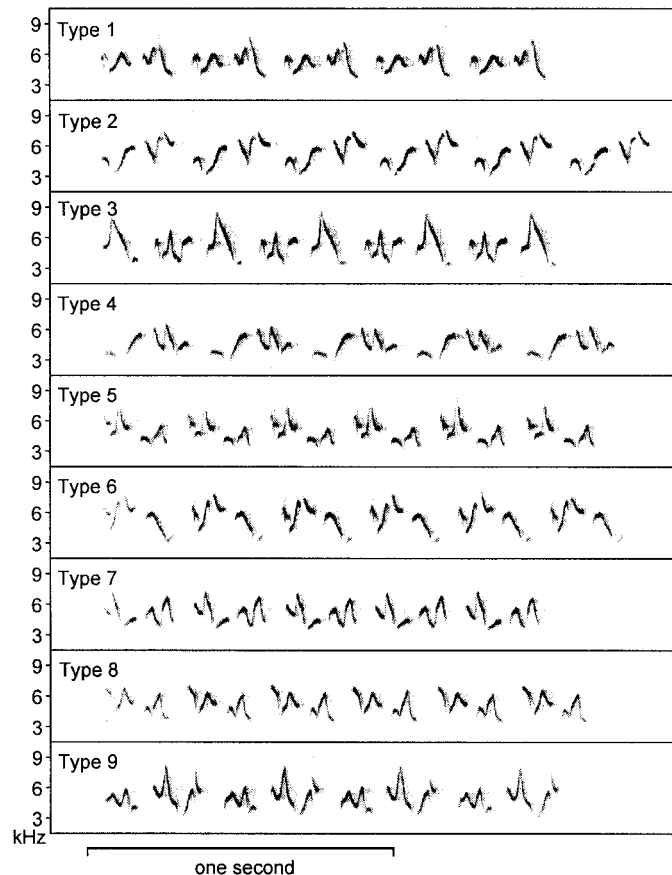


FIG. 2. The nine song types of a single greenish warbler at Manali (site MN, Fig. 1). Each song type consists of four to six repetitions of a distinct song unit.

June of various years (site KS in 1986 and 1987, MG in 1992, MN in 1994 and 1996, and TL in 1995).

Recordings and Spectrograms

I studied the singing behavior of greenish warblers at eight sites distributed throughout their range (Table 1 and Fig. 1, sites GT, YK, TL, AA, MN, LN, BK, and ST). Songs were recorded in the morning, when the warblers are most active. I used an Audio-Technica (Stowe, OH) 815a shotgun microphone and a Sony TCD-D7 DAT recorder at all of these sites. To broaden the study, I obtained recordings made by other researchers from seven additional sites (Table 1). Spectrograms were made using the program Canary 1.2 (Mitchell et al. 1995) on default settings.

The Concept of Song Units

Greenish warbler song has an unusual structure. The birds apparently remember song in groups of syllables, and syllables within each group are always sung in the same order. When constructing songs, birds can repeat one group of syllables several times or string different groups together. I call these groups of syllables "song units." They are highly distinct and provide a straightforward way to measure the temporal structure of song.

Much of this study is based on the reality of song units and my ability to visually identify and classify them. To test whether visual categorization is reliable, I compared visual categorization with an objective categorization of spectrograms by a computer. First, I visually examined spectrograms of 57 consecutive songs of a single greenish warbler recorded at Manali (site MN, Fig. 1). Birds at this site sing relatively simple songs; their songs typically consist of four to six repetitions of a single unit. I categorized the 57 songs into nine song types, which I labeled 1–9 (Fig. 2). Different songs of the same song type are extremely similar (Fig. 3). Second, I used the cross-correlator function of the program Canary 1.2 to calculate similarities between each of the 57 spectrograms with each of the others, resulting in a similarity matrix. This function in effect slides two spectrograms past each other in time, calculating a maximum correlation value for the similarity of the two spectrograms (Charif et al. 1995). I then used the program NJ in PHYLIP 3.5c (Felsenstein 1993) to construct a neighbor-joining network from the similarity matrix. This procedure grouped the 57 spectrograms into nine major categories corresponding to the nine song types that I had grouped the songs into using visual categorization (Fig. 4). In every case, visual and computer categorization were consistent, demonstrating the reality of discrete song unit types and the reliability of visual categorization. This entire procedure was repeated on a sample of 50 consecutive songs from another individual at Manali, and again there was complete concordance between visual and computer categorization. For that individual, both methods identified 11 song types.

Song Structure

Spectrograms from up to five individuals per site were measured. Greenish warblers generally switch song types be-

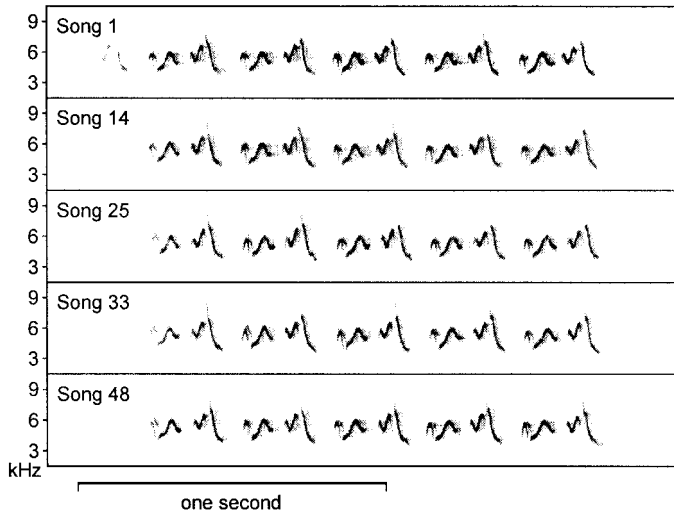


FIG. 3. Five renditions by a single greenish warbler at Manali (site MN, Fig. 1) of its song type 1 (see Fig. 2). The song numbers indicate the order in which the songs were sung out of a recording of 57 consecutive songs.

tween consecutive songs. For each song, I measured the following variables: song length (L), maximum frequency (MxF), minimum frequency (MnF), number of song units (U), and number of unit types (UT). I used the measurement panel of Canary 1.2 to measure the first three variables from on-screen spectrograms. Before making a spectrogram, the amplitude of each song was adjusted to a standard level so that measurements would be independent of recording volume. Before measuring the number of song units and unit types, I examined at least 30 song spectrograms per individual, when possible, to determine the appearance of the units that each individual can sing. For each individual, means of each variable were calculated using measurements from 10 consecutive songs. Individual means were then used to calculate population means and standard errors with individuals as replicates. A principal components analysis on the correlation matrix of individual means was used to identify two variables (PC1 and PC2) that are linear functions of the above five song variables (L , MxF , MnF , U , UT). PC1 is the function that explains the most variation in the five song variables, and PC2 is the function that explains the most remaining variation while being orthogonal to PC1.

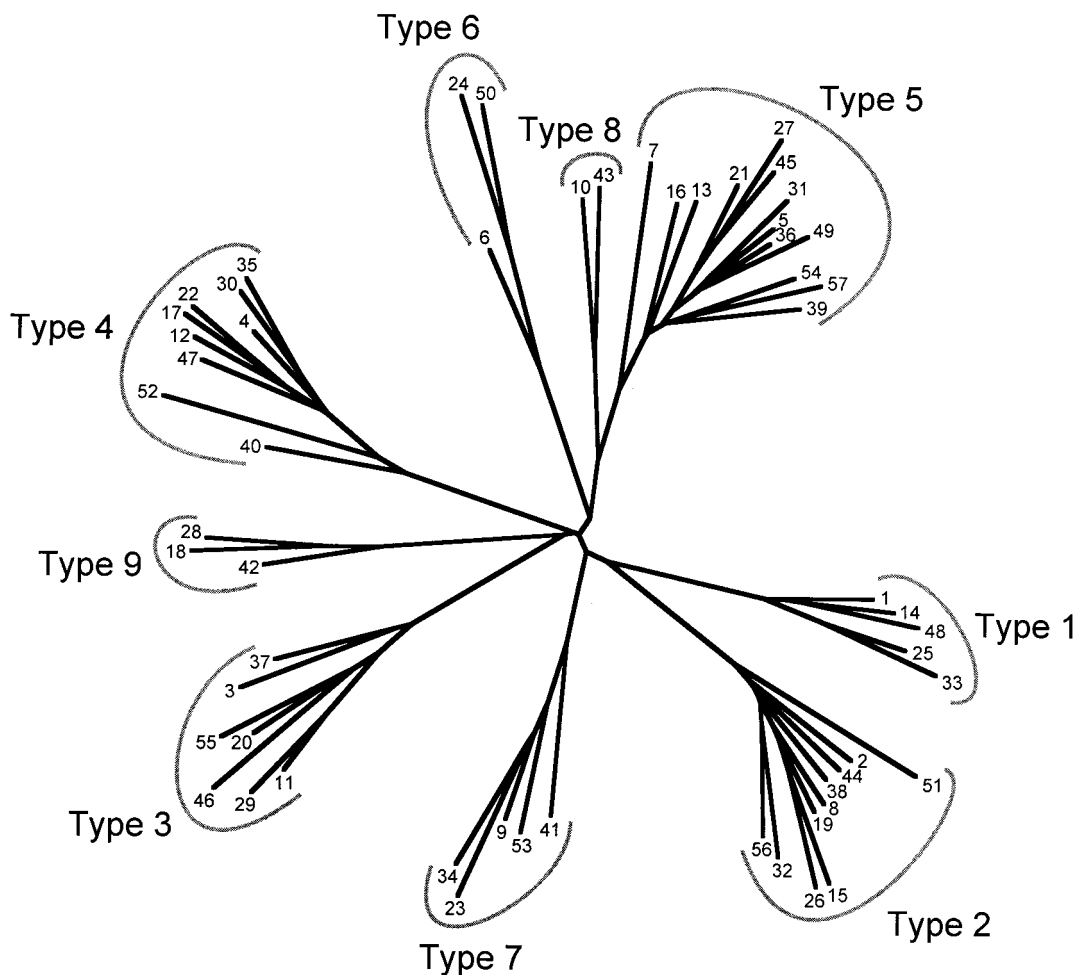


FIG. 4. Song similarity network for 57 consecutive songs sung by a single individual in Manali (see also Figs. 2, 3). The network was produced through pairwise cross-correlation of spectrograms followed by neighbor-joining (see Methods for details). Previously assigned song types based on visual classification are shown. Small numbers at the tips of the network indicate the order of each song in the sample of 57 songs (1 was sung first, 57 last).

Repertoire Size and Length

To estimate the number of unique song units each bird can sing (the song unit repertoire size), I examined samples of 12–91 song spectrograms per individual, depending on the length of the recordings. I counted the number of distinct song unit types in the sample (n), the number of unit types that occurred only once in the sample (S), and the total number of independently occurring units in the sample (N). I considered a unit to be independently occurring if it did not immediately follow a unit of the identical type. The song unit repertoire size (R) for each bird was estimated by using the formula $R = n/[1 - S/N]$ (after Kroodsmas and Canady 1985). I also estimated the total length in time of all of the song units in an individual's repertoire, or the repertoire length. This was done by multiplying the song unit repertoire size (R) of each individual by the mean length per song unit (L/U) of 10 songs from that individual.

Song Rate

I measured song rates of up to five individuals per population by calculating the average number of songs per minute from tape recordings. The song rates calculated by this method apply only to active song bouts. Whenever possible, I measured the amount of time it took the individual to sing 20 songs, but for some individuals, recordings were shorter than 20 songs (one bird at YK, one at TL, two at KS, three at EM, one at XN, one at JL, one at BK, and two at ST). The fraction of time spent singing during song bouts was calculated using the song rate and the mean song length of each bird.

RESULTS

Ecological Factors

Ecological variables differ strongly between southern and northern populations of Greenish Warblers, but not between western and eastern populations of similar latitude. Vegetation surveys in four populations as well as observations at other sites indicate that northern populations inhabit taller and denser forests (Fig. 5) at lower elevations (Table 1) than southern populations. In the Himalayas, greenish warblers are typically found in sparsely wooded areas near treeline (Price 1991; Martens and Eck 1995), where vegetation is dominated by *Rhododendron* bushes, birch (*Betula*), and scattered juniper (*Juniperus*) and fir (*Abies*). Populations in both western and eastern Siberia inhabit dense, lowland forest consisting of a mixture of broad-leaved trees, such as birch and poplar (*Populus*), and coniferous trees, such as pine (*Pinus*), larch (*Larix*), spruce (*Picea*), and fir (see also Cramp 1992). At an intermediate latitude, populations in central Asia (e.g., site AA) occupy open stands of fir and birch at intermediate elevations. These different habitats differ in the abundance of caterpillars, a major food source for greenish warblers, which is greater in the north (Fig. 5).

Population densities also differ latitudinally, with southern populations having greater density in the appropriate breeding habitat (Fig. 5). Whereas population densities in Nepal have been estimated as exceeding 100 pairs/km² (Diesselhorst 1968b in Cramp 1992), the highest population density

reported of 15 studies of western Siberian and European *viridanus* was 28 birds/km² (Vartapetov 1984 in Cramp 1992; the mean was 6.2 birds/km²). Compared to the latitudinal variation, densities vary little between west and east Siberia.

More northerly populations uniformly migrate greater distances to their wintering grounds and spend less time on the breeding grounds. Populations in western Siberia and Europe (*viridanus*) migrate to southern India through central Asia (Ticehurst 1938; Cramp 1992). Eastern Siberian populations (*plumbeitarsus*) migrate to Indochina through eastern China (Ticehurst 1938; Williamson 1962). All of these northern populations spend three to four months (late May to August or September) on the breeding grounds (Dementev and Gladkov 1968). Himalayan populations (*trochiloides*) migrate shorter distances and appear to spend more time (April to October) on or near the breeding grounds (Williamson 1962; Martens and Eck 1995).

Song Structure

Song structure varies greatly throughout the range of the greenish warbler. For example, in western Siberia (*viridanus*) songs are long, have a large frequency range, and are constructed of long song units that are rarely repeated within a song (Fig. 6a). Eastern Siberian (*plumbeitarsus*) songs are very long, have a small frequency range, and consist of many small song unit types, each of which is usually repeated a number of times (Fig. 6b). Songs in the Himalayas (*trochiloides*) have a simpler structure than either Siberian form; they are short, have a small frequency range, and usually consist of a single song unit that is repeated four to five times (Fig. 2).

These patterns are quantified in Figure 7, which illustrates that songs change clinally throughout most of the range. The populations represented in this figure are arranged along each horizontal axis in geographic order (see Fig. 1) according to the ring species interpretation, beginning at the western edge of the range, moving toward site TL (*viridanus*) in central Siberia, then southward to the Himalayas, then eastward and northward to site ST (*plumbeitarsus*), and finally out to the eastern edge of the range. Populations geographically intermediate to two sites usually have intermediate song characteristics. Figure 8 illustrates this clinal variation using representative song spectrograms.

The pattern of song variation is clarified by principal components analysis, which was used to summarize the variation in the basic five variables (Fig. 9, Table 2). PC1, which increases primarily with song length, number of units per song, and number of unit types per song, is high in eastern Siberia and lower elsewhere. PC2, which increases primarily with frequency range (i.e., high maximum frequency, low minimum frequency) and length per unit (i.e., long song length, low number of units per song), is high in western Siberia and lower elsewhere. Both of these variables are measures of complexity, and Himalayan song, the simplest form, has low values of both PC1 and PC2. There is a rough geographic ordering of populations on the PC chart (e.g., around the ring: TL-AA-PK-KS-MN-LN-EM-XN-JL-ST, Fig. 9), once again indicating clinal variation in song.

Although song variation throughout most of the range of

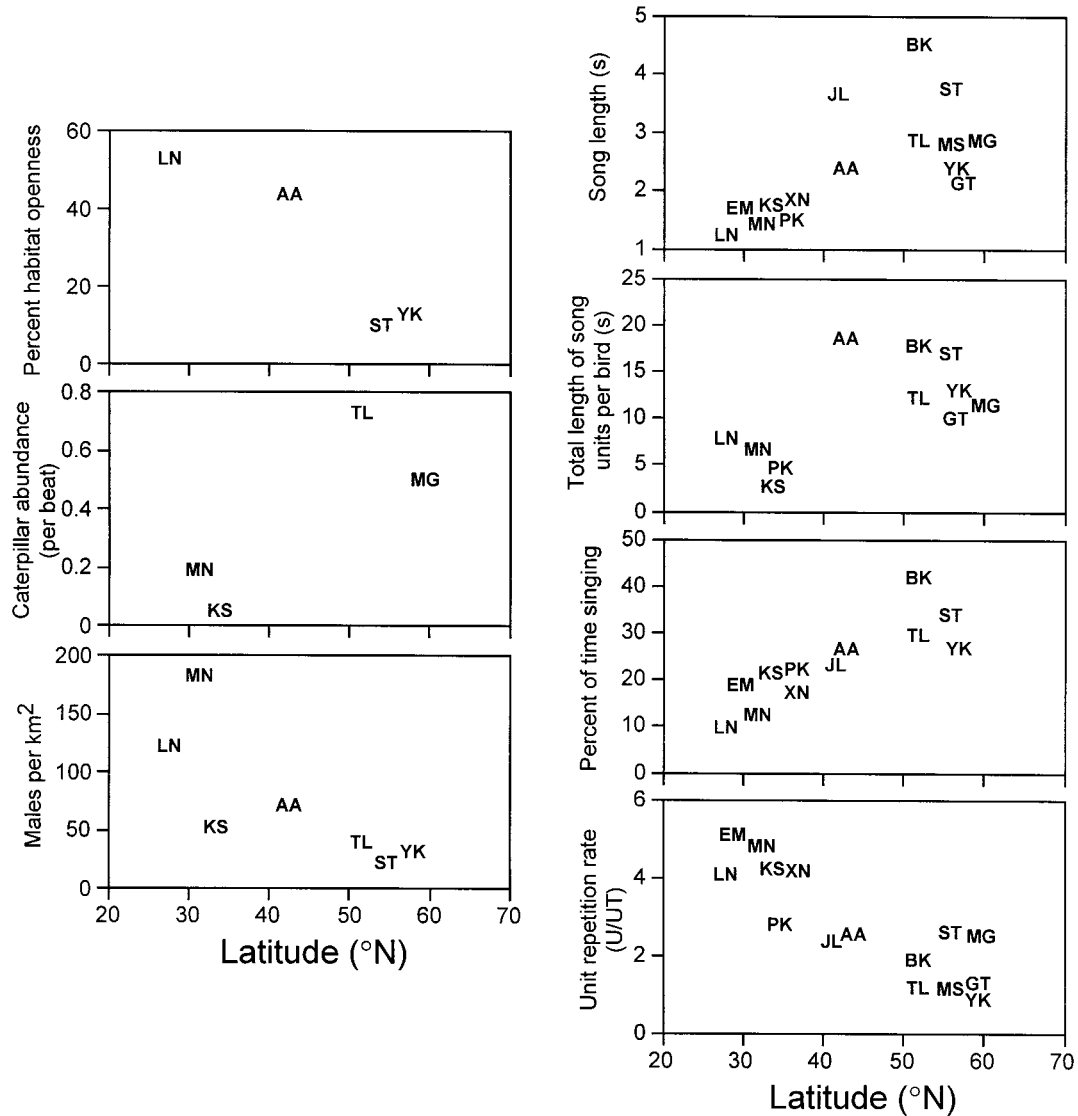


FIG. 5. Latitudinal variation among greenish warbler populations in (left) three ecological variables and (right) four aspects of singing behavior. Each population is represented by its two-letter designation (Fig. 1, Table 1). Left: Moving northward, habitat openness decreases ($R^2 = 0.91$, $F_{1,2} = 20.71$, $P < 0.05$), there is a trend toward increasing caterpillar abundance ($R^2 = 0.67$, $F_{1,2} = 4.11$, ns), and population density decreases ($R^2 = 0.62$, $F_{1,5} = 8.08$, $P < 0.05$). Right: Moving northward, song length increases ($R^2 = 0.42$, $F_{1,13} = 9.34$, $P < 0.01$), repertoire length increases ($R^2 = 0.39$, $F_{1,9} = 5.824$, $P < 0.05$), the fraction of time singing during song bouts increases ($R^2 = 0.70$, $F_{1,10} = 22.94$, $P < 0.001$), and the unit repetition rate decreases ($R^2 = 0.71$, $F_{1,13} = 32.395$, $P < 0.0001$).

the greenish warbler is gradual, there is an abrupt change between the subspecies *viridanus* at site TL and *plumbeitarsus* at site ST in central Siberia (Figs. 6–10). A graph of geographic distance versus song PC distance between adjacent sites (Fig. 10) shows that although the geographic distance between sites TL and ST is one of the shortest, the song PC distance is the largest. Between these sites there are differences in maximum frequency, units per song, and unit types per song (Fig. 7). Whereas songs at site TL are composed of long units with almost no repetition of units within a song, songs at site ST are made of many small units that are often repeated. The differences in the songs of *viridanus* and *plumbeitarsus* are easily heard in the field and can be used to distinguish the taxa in the overlap zone (Fig. 1), where the

two forms are sometimes found living within the same patch of forest (D. E. Irwin, pers. obs.). Except for this abrupt change, relatively little change in song occurs over the three parts of the breeding distribution that are oriented in a west-east direction (western Eurasia, sites GT, MS, YK, TL; the Himalayas, sites MN, LN, EM; and east Siberia, sites JL, BK, ST, MG).

In contrast, two parts of the breeding distribution (see Fig. 1) that are oriented in a south-to-north direction show large changes in song. These are central Asia, between sites MN and TL, over which PC2 changes, and central China, between sites EM and JL, over which PC1 changes (Fig. 9). Thus, song complexity changes with latitude, but not longitude. Like complexity, song length increases gradually with lati-

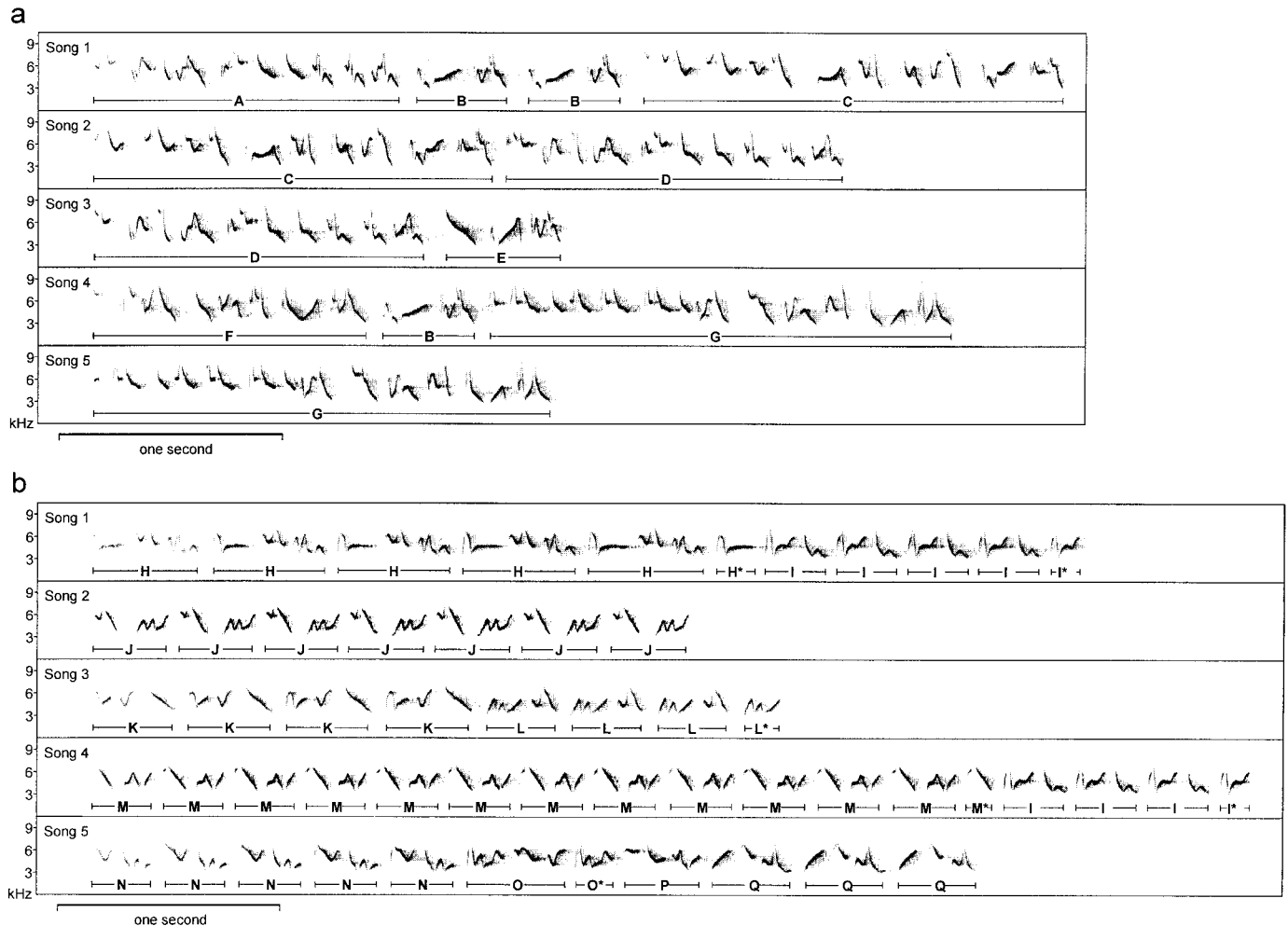


FIG. 6. Song spectrograms of two Siberian forms of the greenish warbler. Unit designations are shown below each spectrogram. (a) Five consecutive songs of a single *Phylloscopus trochiloides viridanus* at site TL. Songs of *P. t. viridanus* consist of long units with high frequency ranges. Units are rarely repeated within a song. (b) Five consecutive songs of a single *P. t. plumbeitarsus* at site ST. Songs of *P. t. plumbeitarsus* consist of short units with low frequency range. Units are repeated often within songs. Occasionally, units are not sung in their entirety; these incomplete units are marked with an asterisk.

tude (Fig. 5). The unit repetition rate, or the average number of times each song unit is repeated within a song, decreases with latitude (Fig. 5).

Repertoire Size and Length

Another measure of song complexity, repertoire length, also changes with latitude. The number of different song units sung by individual birds, or the unit repertoire size, is low in western Siberia (mean \pm SD: 8.5 ± 3.8), intermediate in the Himalayas (21.8 ± 21.1), and high in eastern Siberia (37.5 ± 8.0). Because the length of song units varies between sites, a more meaningful measure of song repertoire is repertoire length, the total length in time of the units in an individual's repertoire. This increases with latitude in both the western and eastern parts of the range (Fig. 5).

Song Rate

Like song complexity, the fraction of time spent singing during song bouts is greater in the north than in the south

(Fig. 5). There is no association between song rates and latitude ($R^2 = 0.001$, $F_{1,11} = 0.006$, ns), but because of the variation in song length, there is significant latitudinal variation in the percent of time spent singing during song bouts. This ranges from about 10% in the Himalayas to 25–30% in western Siberia and 25–45% in eastern Siberia.

Reproductive Character Displacement

Reproductive character displacement is the pattern of greater differences between traits of sympatric than of allopatric populations. In a ring species, such a pattern is confounded with the gradient in traits around the ring. The range of the greenish warbler, however, has regions that extend far outside of the ring, to the west in *viridanus* and to the east in *plumbeitarsus*. If a process of reinforcement were occurring in central Siberia, it might generate a pattern of reproductive character displacement in a west-to-east transect across Siberia.

There is little, if any, support for a pattern of reproductive

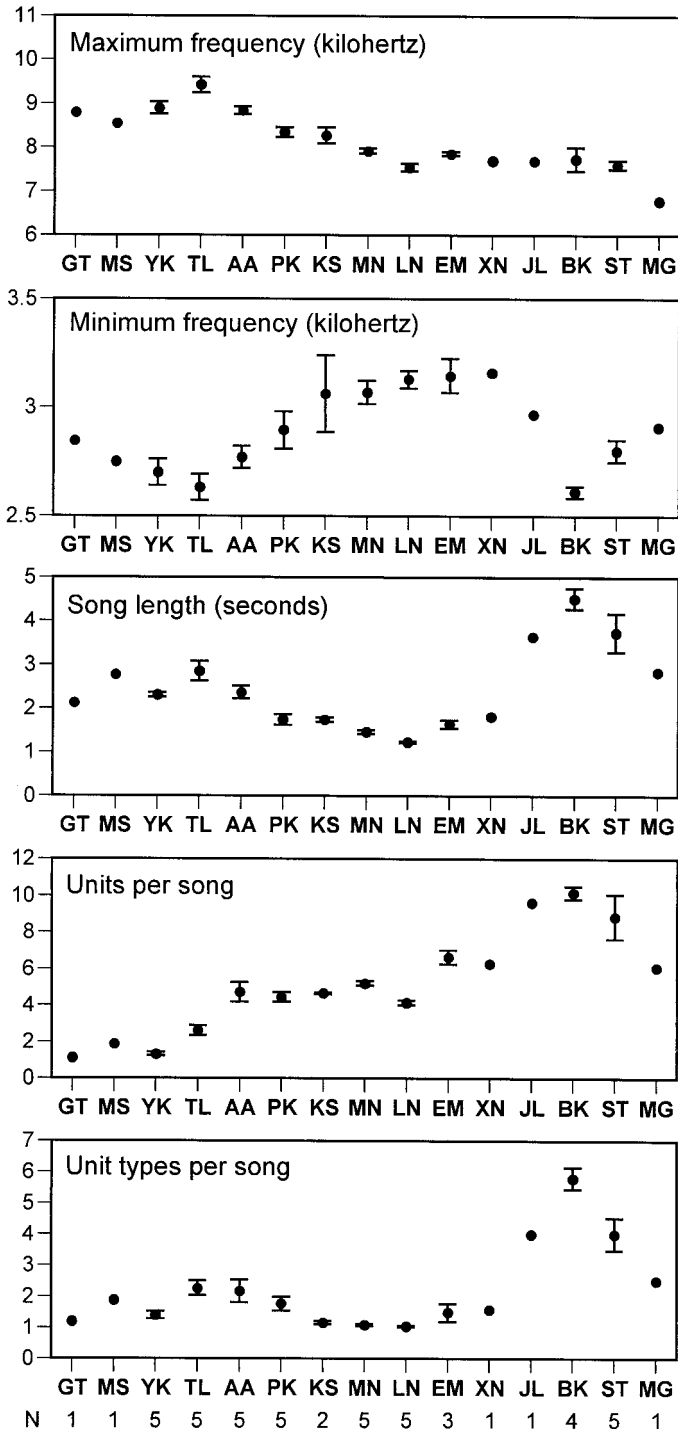


FIG. 7. Clinal geographic variation in five basic song variables. See Figure 1 for locations of sites. The sample size (number of individuals) for each site is shown at the bottom of the figure. Error bars show standard errors, using individuals as replicates. Note that at some sites only one individual was measured; thus there is no error bar. There is statistically significant geographic variation in all five variables (Kruskal-Wallis test: maximum frequency, $H_{14} = 42.41$; minimum frequency, $H_{14} = 36.72$; length, $H_{14} = 45.00$; units, $H_{14} = 43.76$; unit types, $H_{14} = 40.42$; for each analysis, $P < 0.001$).

character displacement between *viridanus* and *plumbeitarsus*. Populations of the two taxa on either side of the central Siberian zone of contact (TL and ST) do not differ significantly more in song traits than populations far to the west (GT, MS, and YK) and east (BK and MG; see Figs. 7 and 9).

DISCUSSION

A striking result from this study is the large difference in song structure between two closely related taxa within the same habitat in central Siberia. Both *viridanus* and *plumbeitarsus* sing long, complex songs, but they use different rules to build their songs out of different fundamental units. Without the ring of populations connecting them to the south, we would have little understanding of how the songs of these taxa diverged. The spatial variation in these populations, together with the likely history of two range expansions northward out of the Himalayas, provides a likely scenario. The pattern suggests that the western and eastern Siberian songs each evolved from the much shorter and simpler Himalayan songs. Although some of the evolution in the two northward expansions was parallel (e.g., length and unit repetition rate), much was divergent (e.g., song unit length and temporal arrangement of units).

By comparing the geographic pattern of song variation to variation in ecological variables, we can infer which of the possible causes of song divergence has produced the differences between *viridanus* and *plumbeitarsus*. Of the hypotheses listed in the introduction, the data support only hypothesis 3, which states that ecological differences influence the balance between sexual and natural selection, thus leading to divergence. Song length, song complexity, the percent of time singing, and repertoire length are all greater in the north and the unit repetition rate is lower; there is relatively little west-to-east differentiation in these traits. This pattern is consistent with south-to-north increases in the intensity of sexual selection or decreases in the opposing natural selection on song. Natural selection alone (hypothesis 1) should not result in such a large difference in song structure between *viridanus* and *plumbeitarsus* in central Siberia. Sexual selection alone (hypothesis 2) should result in as much west-to-east differentiation as north-to-south and would not necessarily lead to the parallel evolution of longer and more complex songs in the north. Species recognition (hypothesis 4) is also not supported, due to the lack of a strong pattern of reproductive character displacement.

I attribute the greater intensity and complexity of singing behavior in the north to latitudinal variation in a number of ecological factors. These factors can influence song by affecting the strength of both intersexual selection (female choice) and intrasexual selection (male-male territorial interactions). Whereas intersexual selection favors long, complex songs, intrasexual selection leads to short, stereotyped songs (Catchpole 1980, 1982). Intersexual selection likely has greater influence on song in the north because greater forest density, lower population density, and shorter breeding seasons in the north increase the importance of song in mate attraction and because greater food abundance in northerly populations decreases the cost of singing. In contrast, in-

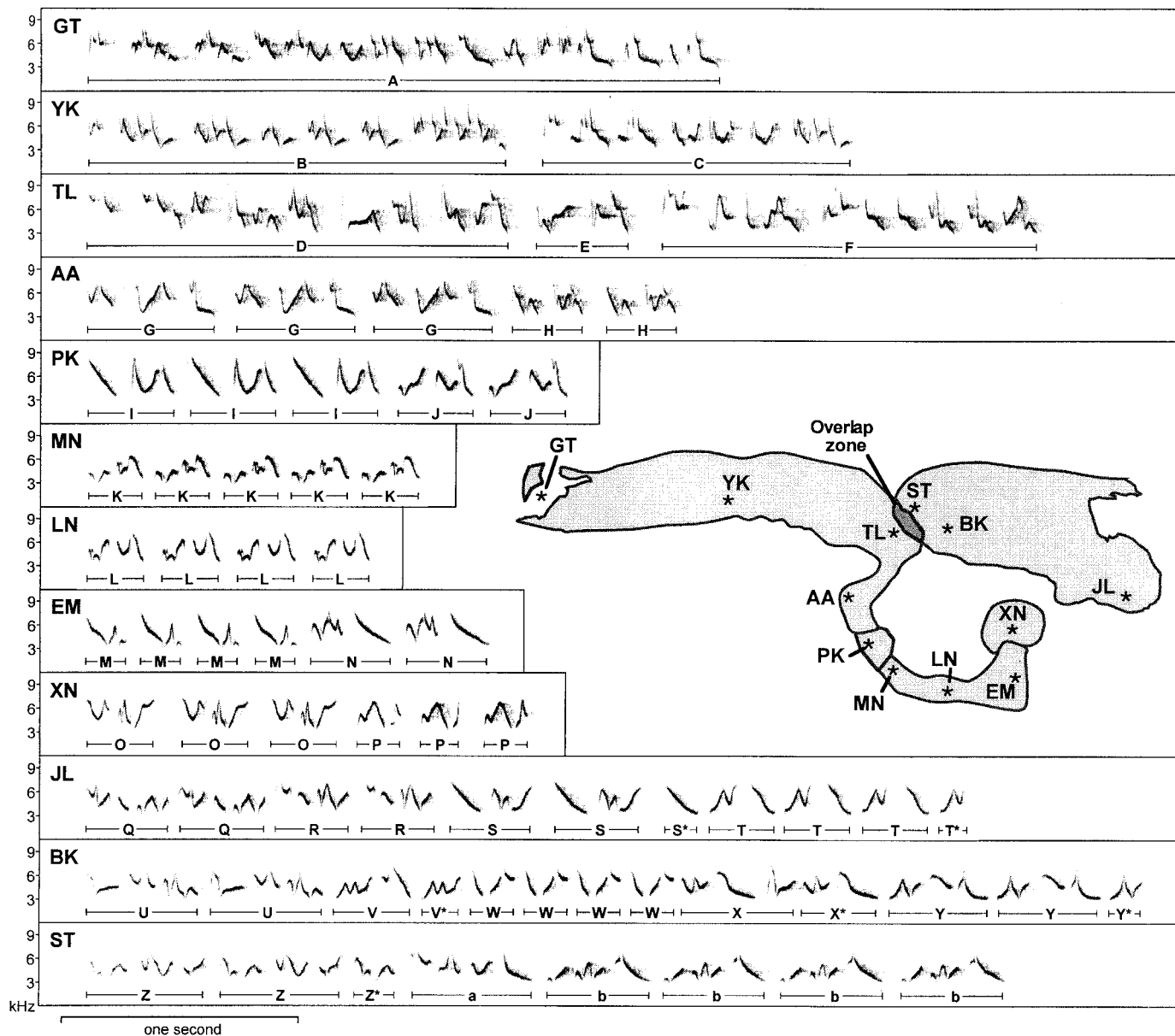


FIG. 8. Representative song spectrograms from 12 populations of greenish warblers. Spectrograms are arranged in geographic order according to the ring species configuration, starting in western Siberia and moving south, then east, then north around the ring. Each spectrogram in this figure was chosen because its length, number of units, and number of unit types are close to the averages in its population. Song unit designations (different than other figures) are shown below each spectrogram. Incomplete units are marked with an asterisk. Note the gradual change in song length and syntax around the ring.

trasexual selection is likely greater in the south because of greater population densities and lower food abundance. These factors should lead to long, complex songs in the north and short, stereotyped songs in the south.

Although there is strong support for habitat influencing song indirectly by affecting the strength of sexual selection (hypothesis 3), there is little support for a direct effect of habitat on song (hypothesis 1). Because vegetation tends to interfere with the transmission of higher frequencies (Morton 1975), we might expect greenish warblers in more forested habitats to use lower frequencies overall. The minimum frequency used in songs is lower in northern than in southern

populations. However, the maximum frequency shows a more complicated pattern; in western Siberia it is much higher than in southern populations, and in eastern Siberia it differs little from southern populations. The greater frequency ranges in the northern populations might not be a result of direct selection by the acoustic environment, but rather an effect of the general increase in the complexity of singing behavior in the north. With a greater frequency range, a bird can make a greater variety of sounds.

Much of the debate about speciation centers around the question of whether ecological divergence is important in starting the process of speciation. Whereas some stress the

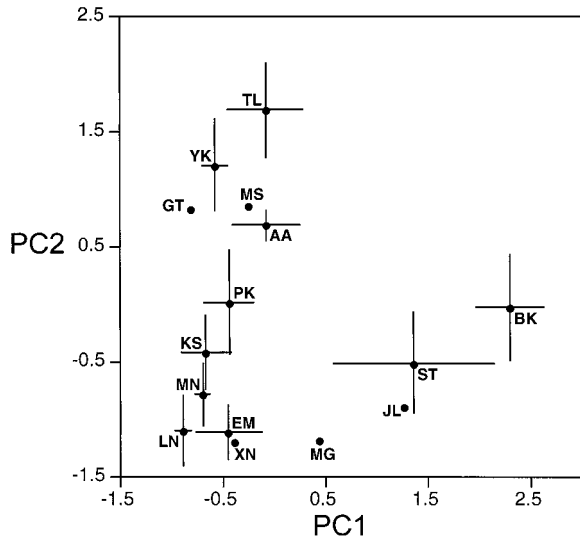


FIG. 9. Bivariate plot of the first two principal components from an analysis of song variation of greenish warblers. Population means are shown (see Fig. 1 for locations) and error bars show standard deviations. At some sites there are no error bars because only one bird was recorded. PC1 explains 55.9% of the variation, and PC2 accounts for 35.5%. See Table 2 for correlations between song variables and each principal component.

stochastic effects of sexual selection and argue that ecological change is not necessary to get changes in mating signals (Fisher 1930; West-Eberhard 1983; Iwasa and Pomiankowski 1995), others argue that ecological divergence is important (Schluter and Price 1993) or even required (Vrba 1995) to get signal divergence. The song of the greenish warbler suggests another possibility, that parallel ecological changes can cause mating signal divergence. The fact that *viridanus* and *plumbeitarsus* have converged ecologically and now share the same habitat might imply that ecology has had little role in the divergence of their songs. However, the song divergence was likely accelerated by the parallel south-to-north shifts in the intensity of sexual selection, and these shifts were a result of ecological changes. If so, the divergence of song was ultimately a result of parallel ecological changes, rather than divergent ones.

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TABLE 2. Correlations between five song variables and the first two principal components.

	PC1	PC2
Maximum frequency	-0.242	0.888
Minimum frequency	-0.478	-0.793
Length	0.941	0.222
Number of units	0.809	-0.523
Number of unit types	0.976	0.050

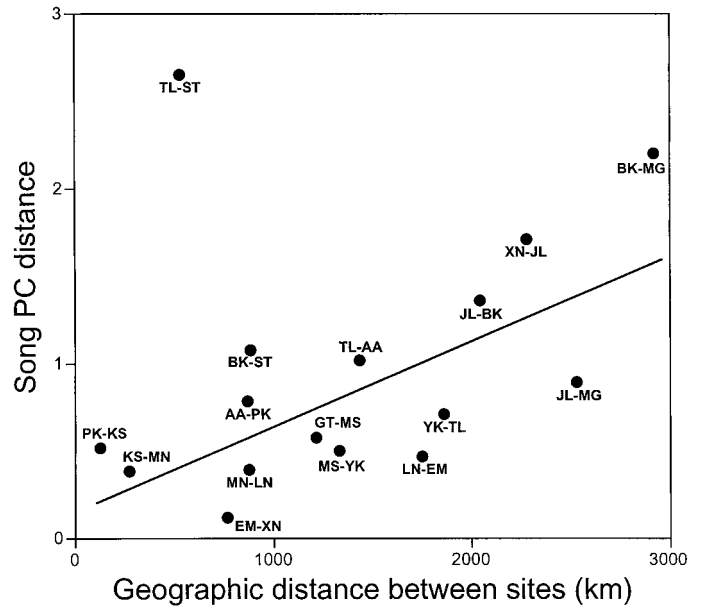


FIG. 10. Bivariate plot of pairwise geographic distances versus song principal component distances between geographically adjacent research sites (see Fig. 1). Song principal component distance was measured as the Euclidean distance between the population means of the two sites on Figure 9. The line is the regression of song principal component distance on geographic distance, excluding the TL-ST comparison, which crosses the putative species barrier in central Siberia. Song differences increase with geographic distance ($R^2 = 0.53$, $F_{1,13} = 14.86$, $P < 0.01$). Sites TL and ST are separated by one of the shortest geographic distances, but differ in song more than all other pairs of geographically adjacent sites.

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