HABITAT SELECTION, ACOUSTIC ADAPTATION, AND THE EVOLUTION OF REPRODUCTIVE ISOLATION

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Abstract.—We examined barriers to gene flow in a hybrid zone of two subspecies of the song sparrow (*Melospiza melodia*). We focused on how mating signals and mate choice changed along an environmental gradient and gathered data on the morphology, genetics, ecology, and behavior across the zone. *Melospiza m. heermanni* of the Pacific slope of California and *M. m. fallax* of the Sonoran Desert, each distinct in plumage, meet across a steep environmental gradient in southeastern California. Although both subspecies occur in riparian habitat, their occupied habitat differs structurally, the former subspecies occurring in areas with denser understory and greater vertical heterogeneity. Song elements varied concomitantly, as predicted by the acoustic adaptation hypothesis, with *heermanni* having lower-pitched, more widely spaced elements. Females of both subspecies responded more strongly to homotypic than heterotypic song, and addition of subspecific plumage cues caused increased response if song was homotypic but not if heterotypic. Females thus assess multiple male traits, weighing song more heavily. Males of both subspecies showed significantly greater agonistic response to homotypic song. Microsatellite variation is correlated significantly with plumage variation across the zone and suggests limited gene flow between the taxa. The association of song and plumage with the environment and in turn with assortative mating suggests a means by which reproductive isolation may evolve or be maintained in hybrid zones.

Key words.—Ecotone, gene flow, hybrid zone, mate choice, *Melospiza melodia*, signaling, song sparrow, speciation.

Understanding the evolution of barriers to gene flow is tantamount to understanding speciation. This understanding is especially important in hybrid zones, areas where the ranges of distinct populations meet and where mixed offspring of those populations are produced (Harrison 1993; Arnold 1997). A key issue in the study of hybrid zones is determining how lineages remain distinct in the face of on-going hybridization and introgression (Endler 1977; Barton and Hewitt 1989). Despite a considerable amount of work on the genetics and structure of hybrid zones (see reviews in Barton and Hewitt 1985; Harrison 1993; Arnold 1997), there has been far less work on the ecology of these zones, particularly for animals (Freeman et al. 1999; cf. Rand and Harrison 1989; Gee 2003). Variation in phenotype has been described for many hybrid zones, but a link between phenotypic divergence and the evolution of barriers to gene flow remains murky, although it seems likely that reproductive isolation may evolve as a byproduct of ecological selection (Rice and Hostert 1993; Schluter 2001; Smith et al. 2001).

It has been suggested that a fundamental step in the formation of a hybrid zone is the formation or existence of an environmental cline or ecotone (e.g., Howard 1986; Rand and Harrison 1989; Moore and Price 1993; Arnold 1997), a region across which ecology, geography, and climate change gradually or abruptly over a relatively short distance (Endler 1977; Moore 1977; Barton and Hewitt 1985, 1989). Local adaptation across the ecotone yields a region of morphological or genetic intermediacy bracketed by the parental populations. Moreover, strong divergent natural selection across a sufficiently steep ecotone may result in the evolution of

barriers to gene flow (T. B. Smith et al. 1997, 2001; Schneider et al. 1999). Yet important questions remain. For example, how do barriers to gene flow evolve in hybrid zones and how are they maintained? What prevents the interbreeding populations from homogenizing to such an extent that heritable differences between them disappear? Also, what role does behavioral ecology play in the evolution of reproductive isolation? In particular, to what extent does habitat affect mating signals and mate choice and in turn affect gene flow between parapatric species?

We present multifarious data from a hybrid zone of two subspecies of the song sparrow (*Melospiza melodia*) that provides insight into barriers to gene flow in these zones. We attempted to present a comprehensive view of this hybrid zone. As a result, we not only describe the extent of morphological and genetic variation across the zone but also elucidate the roles ecology and behavior play in mediating gene flow, including an examination of the relationship between song structure and habitat physiognomy and measurements of responses to mating and territorial signals. We had a specific chain of reasoning and associated questions. First, for a hybrid zone to be examined easily, morphological differences between hybridizing taxa must be sharp, with overlap minimal or absent. In our system, (1) are the subspecies diagnosable (sensu Patten and Unitt 2002) and are hybrids identifiable readily? Second, differences in the physical and/ or biotic environment must exist before related divergence of mating signals can be explored, therefore (2) does the physiognomy of the habitats occupied by each subspecies differ? If mating signals are shaped by environmental constraints, they should be associated with differences in habitat structure. Thus, (3) does song differ between subspecies and are any differences related to habitat physiognomy? We addressed this question via the acoustic adaptation hypothesis

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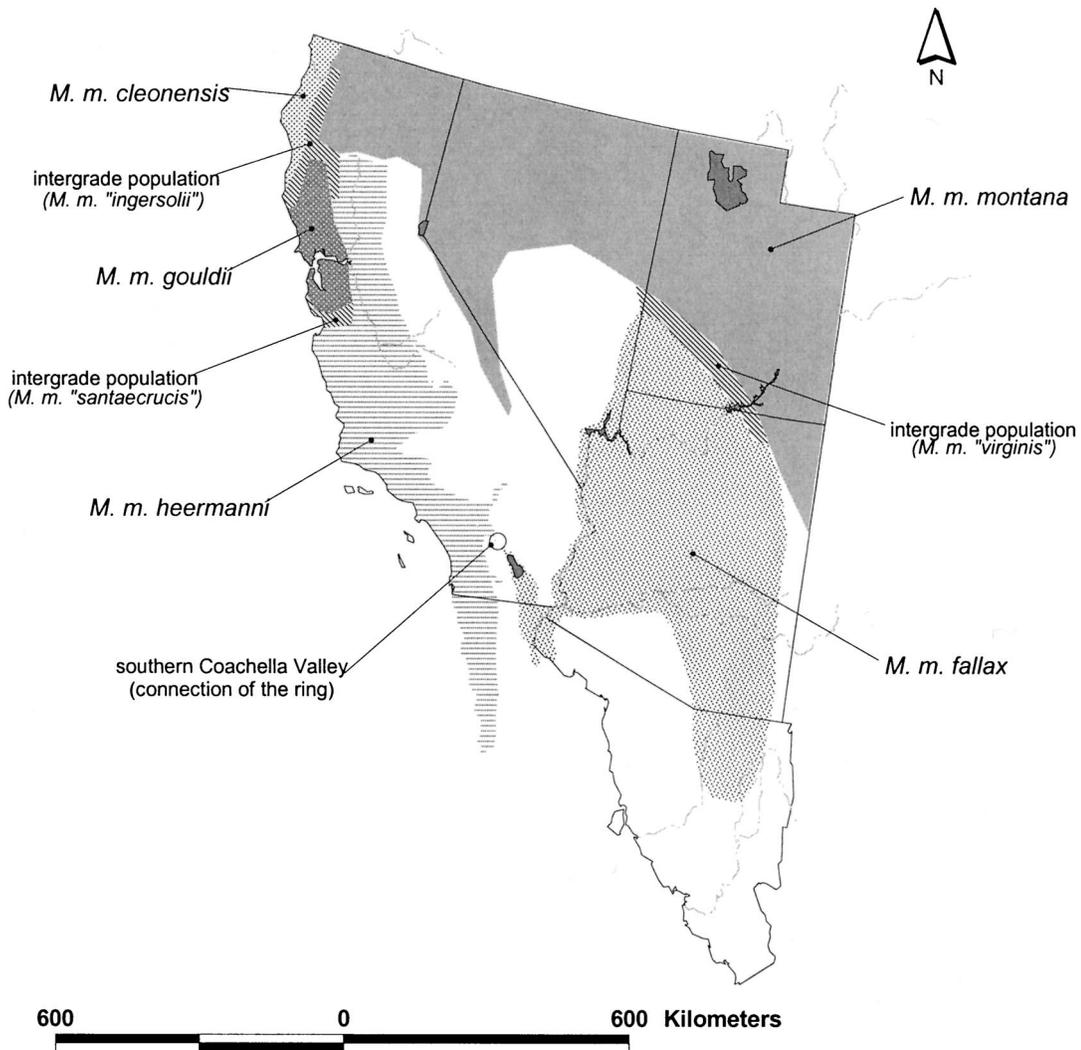
FIG. 1. Subspecific breeding ranges, clinal variation, and general variation trends in the song sparrow ring in western North America. Crossing hatching represents zones of hybridization. See the Discussion for more information.

(Morton 1975), which predicts particular associations between signals and the environment in which they are transmitted. If song has diverged, females ought to prefer like males. Accordingly, (4) do females exhibit assortative mate preference for like males on the basis of song and plumage? Along these same lines, males should defend territories only against like males, else waste energy defending against irrelevant individuals; thus, (5) do males exhibit divergent agonistic behavior, responding more strongly to like males? Finally, if subspecies in the hybrid zone mate assortatively, we expect significant subdivision among microsatellite loci, therefore (6) are the subspecies differentiated genetically? And (7) is genetic differentiation correlated with morphological differentiation?

METHODS

Study System

The song sparrow hybrid zone we studied involves two subspecies, *M. m. heermanni* of the Pacific slope and Central Valley of California, a dark gray bird with black streaks, and *M. m. fallax* of the Sonoran Desert, a pale gray bird with rufous streaks (taxonomy per Arcese et al. 2002). Their contact zone lies in southeastern California near the base of a steep environmental gradient (Patten et al. 2003): in only 50 km, through San Gorgonio Pass to the southern Coachella Valley, elevation drops from 750 m above sea level to 10 m below, rainfall drops from 30 cm/yr to <6 cm/yr, and plant life changes from semimesic riparian forest and surrounding coastal sage scrub to xeric riparian scrub and surrounding alkaline scrub. This zone may be the connecting point of a ring of song sparrow subspecies in western North America (Fig. 1; see Discussion). This same region is the setting for various other avian hybrid zones, whether between sister species (e.g., Gee 2003, 2004) or subspecies (see Patten et al. 2003, 2004).

Morphological Divergence

We scored 463 specimens and live birds from across the ranges of *heermanni* (n = 236) and *fallax* (219), including

eight putative hybrids, on nine plumage traits: ventral streak color (ordinal score of 0–4), dorsal streak color (0–5), ground color of undertail coverts (0–2), malar streak color (0–2), ground color of sides of neck and nape (0–3), crown color (0–3), shape of the tip of the patterned innermost secondaries or ''tertials'' (0–3), and color pattern of the fringe of the innermost secondaries (0–3). We chose a standard specimen for each score from the collection at the San Diego Natural History Museum. Each trait was polarized so that *fallax* had the low score. After obtaining a repeatable system (no changes in scores in >95% of retrials), we matched the color of a particular trait on each standard specimen to a Munsell (GeriagMacbeth, Regersdorf, Switzerland; www.munsell. com) color standard, allowing us to assess specimens and live birds without needing the standard specimens at hand. We obtained all scores in strong, clear light. The final score is the sum of each trait score, such that extreme *fallax* = 0 and extreme *heermanni* = 27. We judged birds to be putative hybrids if they had intermediate sum scores (9–16) or if plumage appeared to consist of a hodgepodge of characters (e.g., malar matching *fallax* but breast streaks matching *heermanni*). We found no evidence of foxing, postmortem color changes that pale and redden plumage, with year of collection accounting for <2.5% of variation in paleness ($r_s < 0.15$, ns). We therefore did not correct statistically for specimen age.

Habitat Structure

We measured structural and abiotic habitat on 10×10 m plots centered on the shrub used as a song perch for each of 68 males whose songs we recorded (see below). Features estimated on each plot were width (m) of the riparian tract at the central shrub, number of trees with a diameter at breast height > 3 cm, percent bare ground, open water, rock, tree, shrub, forb, and grass, and whether water was running or still. We estimated percent cover by species for every tree and shrub. We categorized each tree species into a height class: 1 = <0.33 m, 2 = 0.33–1 m, 3 = 1–3 m, 4 = 3–9 m, and 5 = >9 m. We also estimated mean height for each shrub species and ranked the five dominant grass and forb species on each plot (1 = highest cover, 5 = lowest cover). We conducted point quarter estimates on each plot, centered on the shrub used as a song perch, recording the species, height (dm), and (average) radius (dm) of the central shrub. We tallied the numbers of live stems and of dead stems touching a pole (''hits'') inserted horizontally at the center of the shrub along both a north-south axis and an east-west axis. We recorded distance to and species, height, and radius of the nearest shrub in each of four quadrats around the central shrub. We created two habitat structure variables: canopy cover (a circle defined by shrub/tree radius) and shrub/tree volume (a cylinder defined by the product of canopy cover and shrub/ tree height).

Song Structure

From March to July 1998 we recorded songs from territorial males across the southern California range of *heermanni* ($n = 34$) and westernmost range of *fallax* ($n = 33$); we also obtained data for one hybrid. We recorded a 3–5 min random sample of songs from each male using a Nagra IV-D reel-to-reel tape recorder (Kudelski Group, Cheseaux, Switzerland), a Sennheiser ME62 omnidirectional microphone (Sennheiser, Wedemark, Germany), and a parabolic reflector (23″ Telinga Pro Universal, Telinga, Tobo, Switzerland). All songs were recorded when singing activity was high (mid-March to early July) to avoid seasonal differences in song stereotypy (G. T. Smith et al. 1997). We used Canary sound analysis software (http://birds.cornell.edu/brp/canaryInfo.html) to create sonograms for 3–12 songs from every male recorded, yielding a total of 339 sonograms. Despite great variation both within and between individuals, song sparrow songs can be described as a series of short introductory notes, a central trill, and a closing flourish of two-note phrases, often including a characteristic buzz (the ''main buzz''). Using these divisions, we quantified nine characteristics for each sonogram representing a different song type for that male: (1) total duration (sec) of song, (2) average frequency (kHz) of introductory notes, (3) cadence (sec)—the mean amount of space between notes in a phrase (i.e., the internote interval)—of introductory notes, (4) average frequency (kHz) of central trill, (5) cadence (sec) of this trill, (6) total duration (sec) of this trill, (7) average frequency (kHz) of main buzz, (8) total duration (sec) of this buzz, and (9) frequency (kHz) of maximal amplitude— the mean frequency of the phrase containing the song's maximal energy. To avoid pseudoreplication, we represented a male by his mean for each of these nine values, with that mean taken across the multiple song types sampled from him (range: 3–6 types).

Relationship of Habitat and Song

Because some song and habitat variables did not lend themselves to interpretation in the framework of the acoustic adaptation hypothesis, we removed them from this analysis even if these variables differed significantly between the subspecies. For example, width of occupied riparian corridors was significantly higher in *heermanni* (*t*-test: $P < 0.05$), but it is not clear how this habitat feature influences song structure. We included only song variables of average frequency for parts of the song, specifically the introductory notes, central trill, and buzz, and of cadence of the introductory notes and trill. We only included habitat variables for the amount of open space, cover of forbs, shrubs, and trees, and measures of shrub/tree height mean, variability, and density. We evaluated the relationship between the sets of 15 habitat and six song variables using canonical correlation analysis (CC), then used redundancy analysis of the CC to determine percent variance of song variables explained by habitat. For both habitat and song data, we used canonical discriminant function analyses (DFA) to determine the strength of relationships between the raw variables and linear combinations (i.e., factor loadings) necessarily created in any general linear model procedure (e.g., CC). We did so only to aid in interpretation of the composite axes. If factor loadings were small (<|0.25|), we did not interpret the composite axis in terms of that raw variable.

Female Mate Preference

We used a randomized block factorial design to test the pattern of female mate preference. Treatments were male

plumage and male song. Each treatment had two levels: homotypic and heterotypic. Six females, three *heermanni* and three *fallax*, were tested with four pairs of stimuli: (1) *heermanni* mount and *heermanni* song, (2) *heermanni* mount and *fallax* song, (3) *fallax* mount and *heermanni* song, and (4) *fallax* mount and *fallax* song. Females were wild caught in June and July 2000 and made reproductively receptive via subcutaneous injection of 0.5 mg β-oestradiol (1,3,5[10]-Estratriene-3, 17(-diol) suspended in corn oil (Searcy 1992). They were tested 48–60 h after injection. In each trial, we presented a mount of a male song sparrow, posed in singing posture, to a female and played a sparrow song over a speaker mounted above her side of the test box. Commercial seed and water were provided ad libitum and 2–4 h elapsed after the female was placed in the test box and the first trial began. Two mounts each of *heermanni* and *fallax* were used (each for three females). The mount was placed in profile close to a dividing glass. We used the same songs within trials, such that a given female heard the same set of *heermanni* songs twice and the same set of *fallax* songs twice, each time paired with different mounts. We used different song exemplars between trials, thus different females heard a different set of songs, but we held repertoire size constant (three per taxon). All songs were recorded throughout the range of each subspecies and chosen for similarity to the mean song characteristics for each subspecies. Treatment tapes were created by the Library of Natural Sounds, Cornell University, Ithaca, New York, with constant amplitude across songs. Each song was repeated at 10-sec intervals for 3 min; this 3-min period constituted the trial period. One hour elapsed between each trial, during which time females fed and preened. Song sets were presented to each female randomly, half starting with *heermanni*, half with *fallax*; choice of the initial mount was likewise randomized. Females were monitored from behind a blind. We used a solicitation assay (O'Loghlen and Beecher 1997) to score female response at 10-sec intervals (i.e., her position every 10 sec). We used the total number of solicitations regardless of intensity as the dependent variable (Searcy et al. 1997). We analyzed the data using a randomized block factorial ANOVA, a repeated-measures design, with female blocked and song and plumage (and their interaction) as treatments.

Male Agonistic Behavior

We created known differences between treatment levels by using multiple songs (both within and between males) from both *heermanni* and *fallax* near the means for their respective subspecies. We used songs with predetermined differences and similar signal-to-noise ratios (from our set of 339 songs). In June 1999, we conducted playback experiments before one thousand twenty hours PDT using an Aiwa CA-DW235 (Aiwa, San Diego, CA) with the volume level constant. Treatments for each subspecies consisted of three song types, each from a male not a neighbor of the other two males whose songs were used. Each of the final six (three *heermanni*, three *fallax*) song types varied from 2.7–2.9 sec, with equivalent duty cycles. For the playback, individual songs within each type plus spaces between them lasted 10 sec; amplitude was held constant. Each song type was repeated nine times, so

playback time for each type was 90 sec. The sequence was repeated upon completion of playback of the three song types for a total playback time of 9 min per trial. Initial subspecific song used for testing was chosen in a stratified random manner, on the condition that half of each of the four test groups heard *heermanni* songs first. All males were retested 3–4 days after initial testing with the song they did not hear during the initial trial. Ultimately each male was tested for 18 min: 9 min with its own subspecies' song, 9 min with the other's song. Subjects were 40 territorial males, 20 of each subspecies. Half of each subspecies sampled were males at the edge of the hybrid zone and half were in an allopatric area >50 km from the zone.

We tallied distance from the subject to the speaker at 5-sec intervals during the 9-min playback. We recorded distance data in five classes (Searcy et al. 1997): 0–2 m, 2–4 m, 4–8 m, 8–16 m, and >16 m. We also recorded presence of agonistic behaviors: countersinging, alarm calls, wing fluttering, diving, and attacking. We classified dives as weak (<5 times overall, approaches to the speaker >5 dm) and strong (>10 times overall, approaches to the speaker <2 dm). Mean distance to the speaker was defined as the mean distance class across the 108 samples taken during the 9-min playback. Paired comparisons were made for every male song sparrow tested. We assessed differences in response, as measured by the average distance from the speaker, using a Wilcoxon matched-pairs, signed-ranks test. We used median two-sample tests on difference scores of distance to heterotypic song minus homotypic to compare response levels both between subspecies and between males in allopatry and at the edge of the hybrid zone. Agonistic data were dichotomous and form a repeated measure, so we analyzed them using the McNemar test. Tests were within allopatry or within hybrid zone males and determined if response to homotypic versus heterotypic song differed. We used an extension of McNemar test (Maxwell 1970) for diving behavior, which had three levels. Males in allopatry and those at the edge of the hybrid zone were compared using *n*-sample McNemar tests (Marascuilo and Serlin 1979; Feuer and Kessler 1989).

Microsatellite Data

We obtained blood samples from 72 wild-caught birds (37 *heermanni*, 35 *fallax*). We obtained allelic data (distance moved on a gel) from the ESCU 1 (Hanotte et al. 1994), GF 2.35 (Petren 1998), and MME 1, MME 2, MME 3, MME 7, and MME 12 (Jeffery et al. 2001) loci for 51–55 sparrows, split between the subspecies; see Chan and Arcese (2002) for details of extraction, amplification, and electrophoretic techniques. For our analyses, we calculated the frequency of individuals with private alleles (i.e., alleles unique to one subspecies). We constructed two matrices using the 39 individuals for which we obtained microsatellite data at all seven loci; one matrix was of morphological similarity, calculated as an absolute difference between individuals at each of the nine plumage scores, the other matrix was of genetic similarity, calculated similarly and assuming a single-step mutation model, such that alleles closer to one another on the gel were assumed to be more similar genetically (i.e., fewer mutations separate them). We used a Mantel test to

TABLE 1. A posteriori classification matrix from a discriminant function analysis of nine plumage colors and patterns of song sparrow specimens across the *Melospiza melodia heermanni/M. m. fallax* hybrid zone. A priori classification of species based on collection locality. Eight putative hybrids, identified a priori by a mixture characters and an intermediate plumage score, were treated as a third group in the analysis. A total of 97.8% of all specimens ($n = 463$) were correctly classified by the function.

Original classification	A posteriori classification		
	M. m. fallax	*M. m. heermanni*	Hybrid
M. m. fallax	215	0	4
	98.2%	0.0%	1.8%
M. m. heermanni	0	231	5
	0.0%	97.9%	2.1%
Hybrid	0	1	7
	0.0%	12.5%	87.5%

examine the relationship between morphology and genetics. We further examined this relationship by plotting individual morphological scores against individual genetic scores, that latter following Pritchard et al.'s (2000) method and associated software (Structure 2.0, 2002, available at: http://pritch.bsd.uchicago.edu) and assuming uncorrelated alleles (they show Mendelian inheritance; Jeffery et al. 2001). We determined genetic assignment using either an admixture model or prior population information (i.e., plumage) to assist clustering. We calculated hierarchical F-statistics to determine level of population substructure and used the method of private alleles (Slatkin 1985) to estimate the number of migrants per generation (Nm). Analogous R-statistics designed for microsatellite data produce overestimates of gene flow and genetic distance (Balloux et al. 2000), particularly when the number of loci scored is <20 and sample sizes are <50. Under these conditions, F-statistics perform better, although gene flow still tends to be overestimated (Balloux et al. 2000). Statistical significance of the Mantel statistic and F_{ST} were estimated by randomization, the first by Monte Carlo reshuffling of one matrix while holding the other fixed, the second by jackknifing. Data for the microsatellites, plumage, songs, habitat, and playback are available online only at: http://dx.doi.org/10.1554/04-276.1.s1.

RESULTS

Plumage Variation

Unlike all other adjacent pairs of song sparrow subspecies in western North America, the contact zone between *heermanni* and *fallax* is abrupt, with limited evidence of hybridization (Patten et al. 2003). Apart from *fallax* averaging a longer tail (*fallax* = 67.0 ± 3.0 mm, *heermanni* = 62.7 ± 3.0 mm; $n = 28$ live males of each, $t = 5.34$, $P < 0.001$), it does not differ mensurally from *heermanni* (Aldrich 1984). Their plumage is strikingly dissimilar (Table 1, Fig. 2). *Melospiza m. fallax* is clay-gray streaked rich rufous-red, spotted ochreous on the tail coverts, and generally pallid. By contrast, *heermanni* is olivaceous mouse-gray streaked fuscous-black, spotted black on the tail coverts, and generally dark. Plumage scores between these subspecies do not overlap, typical *fallax* scoring 0–6 (mean = 2.6 ± 1.8 SD) and typical *heermanni* scoring 20–27 (mean = 23.6 ± 2.0 SD). The association of pale plumage with arid areas (*fallax*) and dark plumage with wetter ones (*heermanni*) is consistent with Gloger's rule.

Of eight sparrow specimens with apparent mixed plumage, one scored 7, so is best considered *fallax*, and one scored 18, so is best considered *heermanni*; each of the other six scored between 9 and 13 and are thus intermediate. Four of these birds are from the Coachella Valley, where the subspecies come into contact as breeders (Figs. 1, 2), but two are from the southwestern edge of the Salton Sea, to where some *heermanni* (and presumably also their hybrids) migrate in winter (Patten et al. 2003).

Habitat and Song Variation

Although both subspecies occupy riparian habitats, *heermanni*, a coastal-slope taxon, occupies more mesic gallery riparian forest dominated by willows (*Salix* spp.), whereas *fallax*, a Sonoran Desert taxon, occupies more xeric riparian scrub dominated by nonnative saltcedar (*Tamarix ramosissima*). Habitat physiognomy occupied by territorial males differs significantly between subspecies (MANOVA: Wilks' $\Lambda = 0.42$, $P < 0.001$). *Melospiza m. heermanni* habitat is characterized by higher cover of forbs, taller and bigger shrubs, greater vertical heterogeneity, and wider spacing between trees/shrubs. *Melospiza m. fallax* habitat is characterized by more open area (bare ground or water), smaller trees/shrubs that are more tightly packed, and more uniform vertical structure (Table 2).

Song structure of each subspecies also differs significantly (MANOVA: Wilks' $\Lambda = 0.56$, $P < 0.01$). The song of *heermanni* is characterized by greater cadence of introductory notes, trill length, trill cadence, and buzz length, whereas *fallax* song has a higher frequency of introductory notes and larger number of introductory notes (Table 3). The song of *fallax* is thus more tightly spaced and higher in pitch. Habitat structure is a good predictor of song structure (Fig. 3; canonical correlation analysis: $r_c = 0.82$, Wilks' $\Lambda = 0.09$, $P < 0.05$), in the directions predicted by the acoustic adaptation hypothesis (Morton 1975; Wiley and Richards 1978): *Melospiza m. heermanni*, the subspecies inhabiting more cluttered riparian vegetation, sings lower in pitch with individual notes spaced more widely. The frequency of introductory notes, cadence of introductory notes, and cadence within the main trill showed the strongest associations with habitat structure. The set of habitat variables accounted for 33.3% of the variation in the set of song variables (redundancy analysis). Increased vegetation cover, shrub height, and variation in shrub height and shrub spacing accounted for high levels
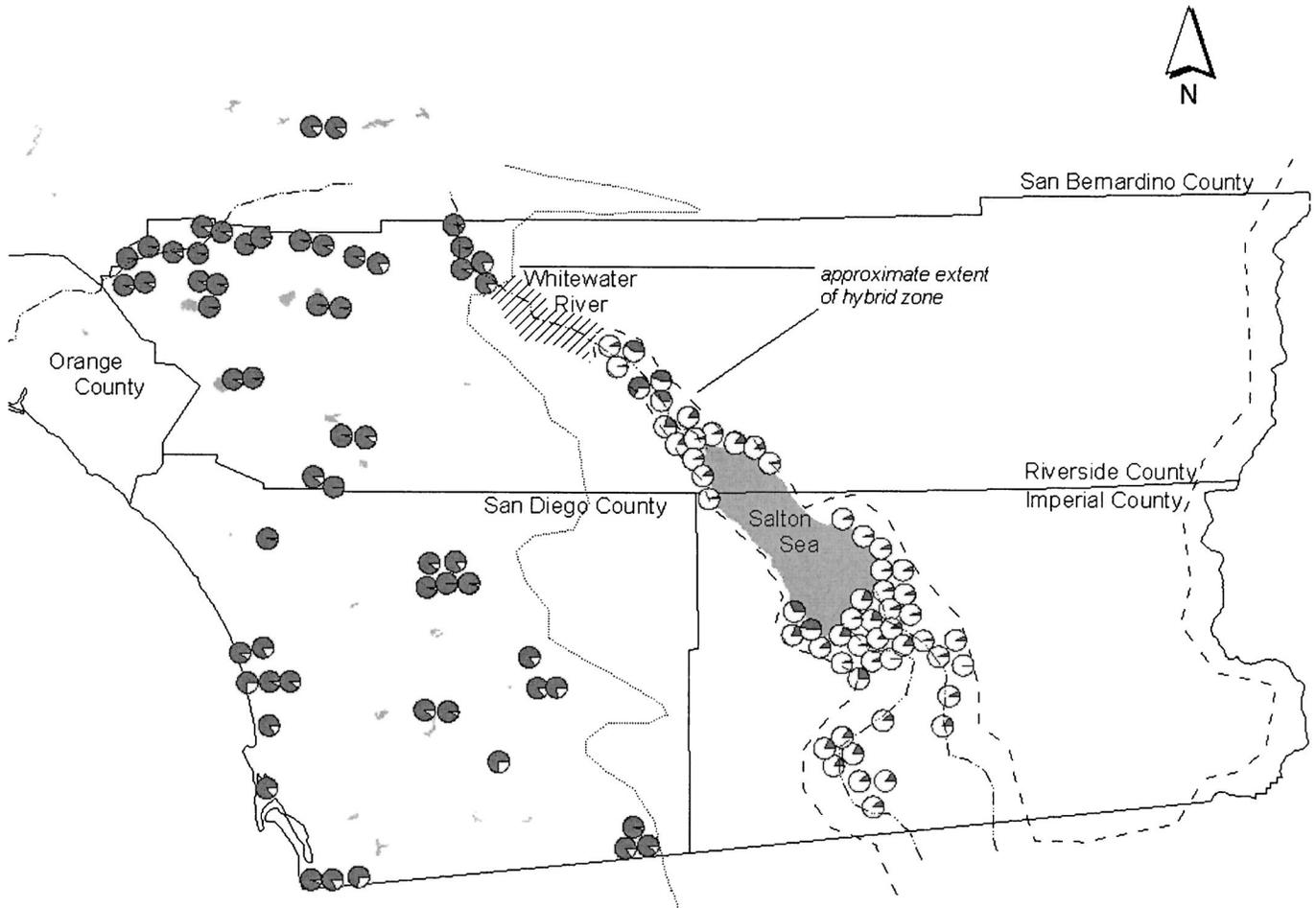
FIG. 2. Recent plumage variation across the *Melospiza melodia heermanni/M. m. fallax* hybrid zone in southern California. Each pie represents a proportion of the maximal plumage score (see text) for an individual song sparrow collected since 1980 or banded by M. A. Patten in 1999–2000 ($n = 113$). All gray signifies ''maximal'' *M. m. heermanni* plumage (score = 27), and all white signifies ''maximal'' *M. m. fallax* plumage (score = 0). The dotted line represents the eastern limit of the range of *heermanni*, the dashed line the western limit of the range of *fallax*. The crosshatched area is the northern Coachella Valley, through which the Whitewater River flows before emptying into the Salton Sea. Much of the hatched area, including along the river, is developed as private resorts or country clubs, hampering the sampling of specimens.

of variation in frequency of the introductory notes ($R^2 = 0.29$) and cadence of the main trill ($R^2 = 0.25$).

Female Preference

Female song sparrows showed a significant preference for like males (Fig. 4). Both song and plumage elicited significant responses (Table 4). Song was particularly important, with females preferring the song of their own subspecies but still responding strongly even if the plumage was incorrect. When presented with a heterotypic song, females responded equally weakly, regardless of male plumage. Thus, song appears to be critical in mate preference, but it shows a significant interaction with plumage, such that the correct song coupled with the correct plumage elicits the strongest response. Females of both subspecies showed similar responses to combinations of song and plumage (Table 4; individual effect, $P > 0.05$).

Male Behavior

Males responded significantly more strongly to homotypic song (Fig. 5; mean $\pm$ SE = 3.77 $\pm$ 0.03 m) than to heterotypic song (4.46 $\pm$ 0.04 m). A stronger response to homotypic song was evident in both *heermanni* (Wilcoxon matched-pairs signed-rank test: $T_s = 28$, $n = 20$, $P < 0.01$) and *fallax* ($T_s = 35$, $n = 20$, $P < 0.01$), but neither subspecies responded more strongly than the other (median two-sample test: $S = 10$, $n = 40$, ns). Behavioral data show the same patterns, with males responding more strongly to homotypic song in incidence of alarm calls (McNemar test: $\chi^2 = 12.36$, df = 1, $P < 0.001$), attacks ($\chi^2 = 6.17$, $P = 0.01$), and, particularly, wing fluttering ($\chi^2 = 27.72$, $P \ll 0.0001$). Males equally countersang ($\chi^2 = 1.39$, ns) and dived ($\chi^2 = 3.03$, df = 2, ns). Regardless of subspecies, males in allopatry had a strong tendency to respond more strongly to homotypic song (relative to heterotypic song) than did males at the edge of the hybrid zone ($S = 13$, $n = 40$, $P = 0.06$). Males in allopatry

TABLE 2. Factor loadings (correlations between composite axis and raw variables) from a discriminant function analysis (DFA) of habitat structure occupied by each song sparrow subspecies, with mean values by subspecies for each habitat feature. We interpreted loadings exceeding |0.25| (i.e., ±8% overlapping variance). The axis was oriented so *Melospiza melodia heermanni* had positive values.

Raw variables	DFA loading	*heermanni* mean	*fallax* mean
Width of riparian area (m)	−0.01	92.5	93.9
% open area	−0.20	4.28	8.27
% grass cover	0.05	3.65	2.76
% forb cover	0.48	26.44	2.15
% shrub cover	0.10	20.2	14.8
% tree cover	−0.23	49.8	67.6
Mean shrub height (dm)	0.22	20.1	13.8
Mean tree height (class)	−0.10	3.69	3.96
Height of central shrub/tree (dm)	−0.09	47.7	51.6
Density of central shrub/tree (''hits'')	−0.49	13.4	21.3
Height range of shrub/tree (dm)	0.20	35.4	26.6
Minimum shrub spacing (dm)	0.18	10.85	8.24
Maximum shrub spacing (dm)	0.06	34.4	29.6
SD of shrub/tree height (dm)	0.24	15.3	10.7
SD of shrub/tree radius (dm)	0.28	8.60	5.51
SD of shrub/tree canopy (dm^2)	0.27	1251.2	627.6
SD of shrub/tree volume (dm^3)	0.25	87813	40269

also exhibited more wing fluttering in response to homotypic song than did males at the edge of the hybrid zone (two-sample McNemar test: $Z = -3.11$, $n = 40$, $P < 0.001$). There were no differences in the incidence of alarm calls ($Z = -0.44$, ns), attacks ($Z = -0.65$, ns), countersinging ($Z = 1.45$, ns), or diving (*n*-sample McNemar test: ($\chi^2 = 3.90$, 4 df, ns).

Genetic Variation

As expected for microsatellite loci, allelic variation and heterozygosity were high (Table 5), although the latter was lower in *heermanni* for some alleles (MME3, MME7), in *fallax* for others (ESCU1, MME2). Analyses of genetic variation in microsatellite loci across the hybrid zone indicate limited gene flow between *heermanni* and *fallax*. The proportion of individuals with private alleles averaged ±0.10 per locus (Table 5), yielding $Nm \approx 0.7$ per the method of Slatkin (1985). We also found significant subdivision between subspecies for five of the seven loci. Predominantly negative values of F_{IS} (Table 5) imply sex-biased dispersal (Hartl and Clark 1989); female-biased dispersal is the rule in passerines (Clarke et al. 1997). Plumage scores and microsatellite variation are correlated (Mantel standardized $r =$

0.07, $P < 0.03$). Genetic scores vary substantially among individuals if we make no prior assumptions about population assignment (Fig. 6, top); however, if we allow prior assignment, on the basis of plumage, to assist genetic assignment, the subspecies are as distinct genetically as morphologically (Fig. 6, bottom).

DISCUSSION

Population Differentiation

Most song sparrows in the western Sonoran Desert are typical of the subspecies *M. m. fallax*, showing no signs of intergradation with *M. m. heermanni* (Patten et al. 2003). Likewise, sparrows from the northern edge of the Coachella Valley (Whitewater Canyon, Palm Springs, CA) are typical of *heermanni* (Patten et al. 2003), thus the break in plumage color and pattern between these two subspecies is abrupt (Fig. 2). Indeed, plumage differences are so striking that the two taxa can be diagnosed easily in the field. The most parsimonious conclusion from these data is that there exists a narrow hybrid zone where *heermanni* meets *fallax* in the southern Coachella Valley of southeastern California, with resultant hybrids occasionally dispersing southward around

TABLE 3. Factor loadings from a discriminant function analysis (DFA) of song structure of each song sparrow subspecies, with mean values by subspecies for each measure. See Table 2 for details about loadings. The axis was oriented so *Melospiza melodia heermanni* had positive values.

| Raw variables	DFA loading	*heermanni* mean	*fallax* mean
Maximum frequency (kHz)	0.13	9.05	8.90
Minimum frequency (kHz)	−0.01	1.77	1.77
Introductory note number	−0.25	2.89	3.50
Introductory note frequency (kHz)	−0.29	4.69	5.06
Introductory note cadence (s)	0.28	0.33	0.29
Main trill frequency (kHz)	0.07	5.23	5.14
Main trill length (s)	0.32	0.55	0.44
Main trill cadence (s)	0.56	0.08	0.03
Frequency of maximal amplitude (kHz)	0.02	5.29	5.22
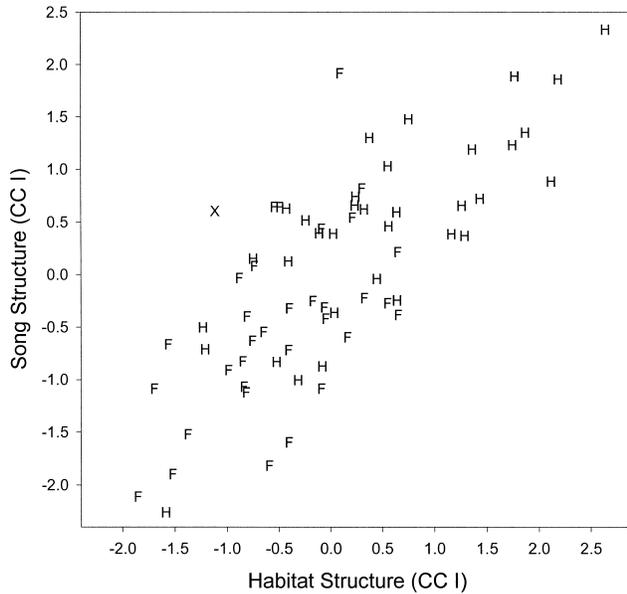
FIG. 3. The relationship between habitat structure and song structure on the first canonical correlation (CC) axis. Even though but one axis is displayed, note the strong relationship between habitat and song ($y = 0.75x$, $r^2 = 0.56$, $P < 0.0001$) and the good segregation of subspecies (H = *Melospiza melodia heermanni*, F = *M. m. fallax*, X = hybrid). Higher vegetation density is associated with lower-pitched, more widely spaced song elements.

FIG. 4. Number of copulation solicitation displays (mean ± SE) given by six female song sparrows to presentations of combinations of homotypic and heterotypic song and plumage, and their interaction. Females responded most strongly to a presentation of a combination of homotypic song and plumage and least strongly to the heterotypic song regardless of the plumage presented. The interaction was also significant ($F_{1,15} = 39.5$, $P < 0.001$).

the Salton Sea. Plumage differences fit predictions of Gloger's rule (Zink and Remsen 1986): *fallax*, a taxon of a hotter, drier region, is markedly paler and redder than *heermanni*, a taxon of a cooler, wetter region that is markedly darker and blacker.

Previous studies of genetic variation across the range of the song sparrow detected no concordance between mitochondrial DNA (mtDNA) and morphology and an intermediate level of mtDNA differentiation but no geographic substructure in mtDNA (Zink and Dittmann 1993; Fry and Zink 1998). However, genes evolving more rapidly (e.g., microsatellites) should provide a more accurate picture of the genetic substructure of the various subspecies of the song sparrow, better reflecting patterns of gene flow and population divergence. In our study the proportion of individuals with private alleles averaged ±0.10 per locus (Table 4), implying limited gene flow between *heermanni* and *fallax* (Slatkin 1985). By comparison the frequency of individuals with private alleles was lower in the four San Francisco Bay subspecies, averaging <0.02 per locus (Chan and Arcese 2002).
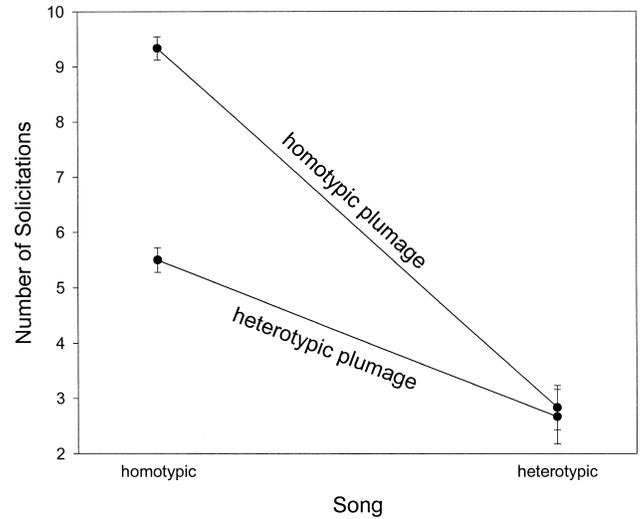
We also found significant subdivision between subspecies for five of the seven loci (Table 4), yielding estimated rates of gene flow on the order of $Nm = 0.7$, an exchange of <1 bird per generation; rates are 10 × higher between San Francisco Bay subspecies (Chan and Arcese 2002). Nonetheless, ongoing hybridization may suggest no postmating isolation between *heermanni* and *fallax*. The high philopatry of individual song sparrows, with dispersal distances estimated at 300 m from demographic data and 6.1 km from mtDNA data (Zink and Dittmann 1993), suggests that the hybrid zone is stable. Because *fallax* is effectively sedentary but *heermanni* exhibits some winter dispersal or partial migration, the hybrid zone may be maintained by annual colonization by a few individuals of the latter subspecies. Regardless of how the hybrid zone is maintained, morphology and genetic variation in the two subspecies are correlated, although it is clear that plumage has diverged to a much greater degree than have the microsatellites (Fig. 6, top). The large difference in plumage relative to the small difference in microsatellites implies either that hybridization is ongoing or divergence is recent and rapid. The former scenario is likely true in light of the

TABLE 4. Results from a randomized block factorial ANOVA analysis of female mate choice data (n = six females). Song, plumage, and their interaction all showed significant effects on female response. The large effect size ($d = 3.0$) accounts for the significant difference despite the small sample size.

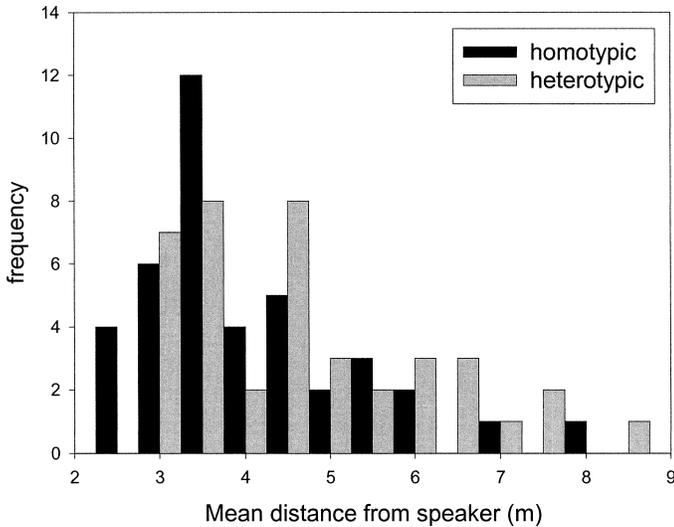| Source	SS	df	MS	F	P
Females	7.33	5	1.47	2.87	>0.05
Treatment	174.83	3	58.28		
Plumage	24.00	1	24.00	46.96	<0.001
Song	130.67	1	130.67	255.65	<0.0001
Plumage × song	20.17	1	20.17	39.46	<0.001
Residual	7.67	15	0.51		
Total	189.83	23			

FIG. 5. Responses of individual male song sparrows to homotypic and heterotypic song, measured as average closest approach to the speaker. Note the tendency for birds to respond more strongly to homotypic song (Wilcoxon matched-pairs signed-ranks test: $T_s = 119$, $N = 40$, $P < 0.01$, $d = 1.6$).

recent hybrids we documented (Table 1), although we did not document successful backcrossing.

Mate Choice and Agonistic Behavior

Our data show that both females and males respond assortatively to signals, enough so that *heermanni* and *fallax* have diverged to a substantial degree behaviorally as well as morphologically and genetically. Male sparrows responded more strongly to homotypic than heterotypic song, as measured by proximity to the playback unit and agonistic behaviors (Fig. 5). This result is consistent with those from numerous other studies of passerines, including the song sparrow (e.g., Searcy et al. 1997, 2003). Response was higher in allopatry than at the edge of the hybrid zone, suggesting that males of either subspecies defend territories against any song sparrow, an important point given that competition between males affects hybridization rates (Bronson et al. 2003).

Females showed at least a threefold increase in solicitation displays to presentations of homotypic song relative to heterotypic song, a fourfold increase if male plumage was also homotypic (Table 4, Fig. 4). This pattern was like that re-

ported for female choice in the indigo (*Passerina cyanea*) and lazuli (*P. amoena*) buntings, although females of those species weighed plumage more highly than song (Baker and Baker 1988, 1990). In either case females assess multiple cues when choosing a mate, a theoretical expectation with empirical support (e.g., Borgia 1995; Uetz and Roberts 2002). Barriers to gene flow are more likely to evolve if each cue has diverged. In birds, visual signals may be as prevalent as audible ones, for ''plumage colour among birds is more strongly influenced by the signalling environment than by the risk of hybridization'' (McNaught and Owens 2002). Eventually mate recognition should act in concert with signal optimization, strengthening the barriers. For our song sparrows, we expect that female preference has evolved in response both to song, which differs in association with physiognomy of occupied habitat (see below), and to plumage, which differs as a result of forces underlying Gloger's rule.

Habitat and Signaling

Geographic variation in vocalization has been documented in numerous species of songbirds, despite predicted stabilizing selection on song (Martens 1996). Recognition that signal transmission may be shaped by environmental constraints may resolve this apparent paradox. If both signaling and perception vary concomitantly with the environment, then regional differences may be evolutionarily meaningful and may arise even along a sharp environmental cline across which gene flow is maintained (Endler 1977). Considering the preponderance of environmental gradients across the globe, this mechanism of population divergence may be ubiquitous in nature (Smith et al. 2001; Slabbekoorn and Smith 2002).

Even though the song sparrow learns the principal mating signal we examined (song), learning itself may evolve—individuals of a particular taxon may prefer to learn homotypic songs (Nelson 2000; Wright and Dorin 2001). Young song sparrows preferentially learn songs from neighbors, facilitating communication between neighbors once a youngster establishes a territory (Beecher et al. 1994). Because the environment shapes song learning within the constraints of acoustic adaptation (Hansen 1979), we expect songs to match habitat from the outset, as soon as they are learned. Failure to learn a song that transmits well in the habitat may hinder a male's ability to attract a mate and to defend a territory (Hansen 1979; Morton 1986).

Physiognomy of habitat occupied by *heermanni* differs

TABLE 5. Sample sizes and hierarchical F-statistics for seven microsatellite loci across the hybrid zone of *Melospiza melodia heermanni* (H) and *M. m. fallax* (F). The ''% private'' column is the percentage of alleles found in one subspecies but not the other. Values of F_{ST} in bold are significant at $P < 0.05$, with ''S'' being the subspecific level.

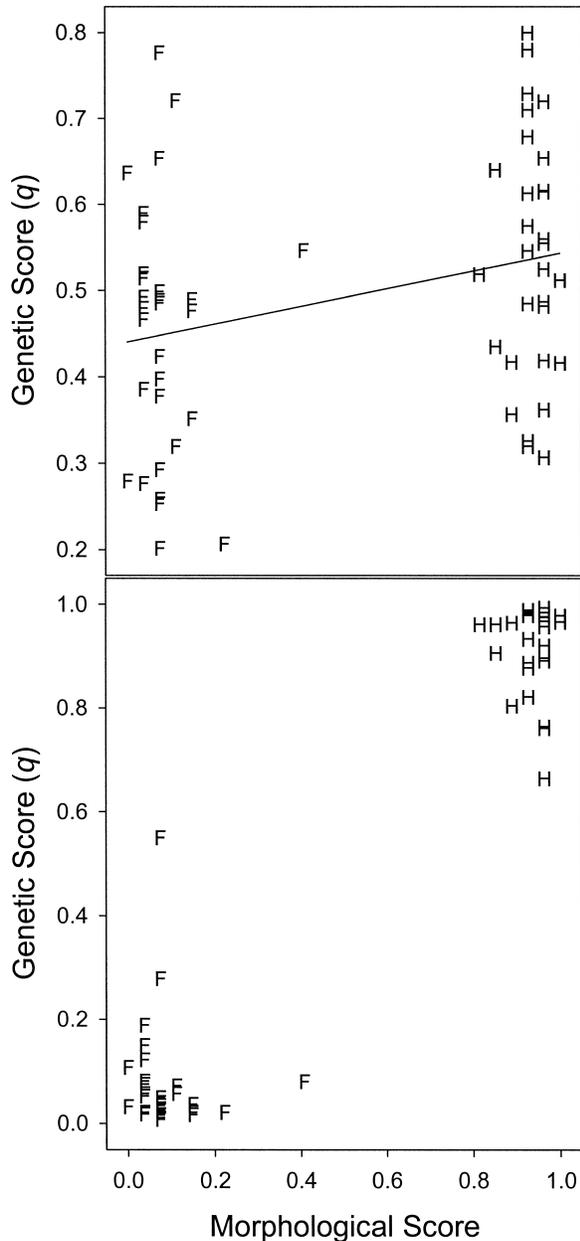| Locus	alleles	Sample sizes		Heterozygosity		% private	F_{IS}	F_{ST}	F_{IT}
		H	F	H	F				
ESCU 1	13	26	27	0.81	0.67	7.5	0.068	0.009	0.076
GF 2.35	9	24	27	0.79	0.82	2.9	−0.076	**0.018**	−0.057
MME 1	17	24	28	0.92	0.97	17.6	−0.046	**0.023**	−0.022
MME 2	9	25	29	0.83	0.70	6.7	−0.088	**0.024**	−0.062
MME 3	11	24	27	0.73	0.86	4.6	−0.033	0.007	−0.026
MME 7	11	26	29	0.72	0.85	10.8	0.033	**0.019**	0.052
MME 12	23	25	26	1.00	0.96	19.6	−0.050	**0.023**	−0.057

FIG. 6. Relationships between morphology and genetics (H = *Melospiza melodia heermanni*, F = *M. m. fallax*). Scales on both the abscissa and ordinate are a probability of being classified as *heermanni*, the morphological score being an individual's score divided by the maximum possible score, the genetic score being the proportion of an individual's genome associated with that subspecies, assuming allelic frequencies are uncorrelated (Pritchard et al. 2000). In the top figure, individual assignment to subspecies is based solely on microsatellite data. Despite the scatter of genetic assignment, the correlation between plumage and microsatellites is significant ($r = 0.30$, $P < 0.05$). In the bottom figure, individual assignment on the basis of microsatellites was aided by morphology. Given prior knowledge of plumage, genetics can classify >95% of individuals correctly.

from that occupied by *fallax*, and song structure differs between the subspecies. We detected a significant relationship between habitat and song ($r_c = 0.82$; Fig. 3), with a full third of the variation in song structure attributable to habitat phys-

iognomy. We interpreted our results within the framework of the acoustic adaptation hypothesis, which is well supported (Morton 1975; Wiley and Richards 1978; Forrest 1994; Catchpole and Slater 1995; Brown and Handford 1996; Bradbury and Vehrencamp 1998), though support is not universal (e.g., Daniel and Blumstein 1998). This hypothesis predicts that a taxon occupying more cluttered habitat (*heermanni*, in our case) will have a song that is pitched significantly lower with elements spaced more widely. Our observations fit this prediction.

We would be remiss to argue that all variation in song is shaped by variation in habitat, but our data suggest an important role for habitat physiognomy even if a suite or combination of factors—among them drift, cultural transmission, and body condition (Slater 1986; Lynch 1996; Nowicki et al. 1998)—also influence song learning. Although our results conform to expectations of acoustic adaptation, this hypothesis could be evaluated further by quantifying propagation of individual song components in the different habitats. Still, song recognition is complex, depending on more than a simple matching of particular phonological cues (Ryan et al. 2003; Searcy et al. 2003), so it may be impossible to identify how a specific component is shaped by the environment or to determine its function in the overall signal.

Evolutionary History

Females and males of both *M. m. heermanni* and *M. m. fallax* tend to behave as good biological species where their ranges meet; indeed, it might be argued that these taxa are in fact distinct species, yet such a conclusion is dispelled by an examination of geographic variation. The evolutionary history of the song sparrow requires further investigation, but mtDNA evidence is consistent with the species' spread in the wake of glacial retreat (Zink and Dittmann 1993).

On the basis of plumage, song sparrow subspecies in southwestern North America form what could be called an "embedded ring": just a portion of the component subspecies constitute a ring (i.e., the ring is embedded within only a portion of the species' range). A valid ring species must satisfy three criteria (Irwin and Irwin 2002): (1) geographic contact between neighboring subspecies must be clear, (2) except for the endpoints, transitions between neighbors must be smooth, with clear intergradation, and (3) the endpoints must behave like good biological species where they meet. In this ring, *heermanni* and *fallax* are the endpoints, and subspecies connecting them grade into each other broadly and continuously (Fig. 1). Specifically, *montana* is darker than *fallax* but is more like that taxon than either is to *heermanni*, *gouldii* is redder than *heermanni* but is more like that taxon than either is to *fallax*, and *cleonensis* lies roughly midway between *montana* and *gouldii*. Intermediates granted trinomials but not in fact diagnosable as subspecies (sensu Patten and Unitt 2002) occupy narrow zones wherever ranges meet (Fig. 1); this intermediacy suggests broad intergradation. We posit that such areas are also hybrid zones, as each appears to reside in an area with a smooth environmental gradient. For example, *fallax* and *montana* meet at the transition from the warmer Mojave Desert to the colder Great Basin Desert, and *heermanni* and *gouldii* meet at the transition from the

relatively xeric coastal sage scrub to the more mesic coastal chaparral. The most abrupt ecotone lies at the base of the San Gorgonio Pass, where *fallax* and *heermanni* meet. This pass is the site of a drastic change in climate and vegetation, marking the transition from coastal to desert bioregions (Welsh 1994), including the avifauna (see Patten et al. 2003, 2004).

We lack genetic and behavioral data from these additional hybrid zones, which limits our ability to draw inferences about the species' evolutionary history. Geographic ranges of the component subspecies, with their broad contact zones (Fig. 1), argue against allopatric differentiation. We are left with two alternatives: (1) differentiation in a ring, with *heermanni* and *fallax* meeting in secondary contact (the species' postglacial spread was southward, our taxa being in contact the briefest time), or (2) differentiation in parapatry, with *heermanni* and *fallax* meeting in primary contact (postglacial spread was northward, our taxa being in contact the longest time). Determining whether contact between two taxa is primary or secondary is a seemingly intransigent problem in evolutionary biology, largely because mechanics of clinal and allopatric speciation are similar (Saloman 2001) and isolation by distance can be as effective as a simple physical barrier (Barton 1988). Our data cannot distinguish between these alternatives, but the system does suggest a potentially fruitful avenue for future research.

Exogenous selection, as mediated by habitat selection and its relationship to female preference and male competition, best accounts for the structure of the song sparrow hybrid zone we studied. Previous studies have shown relationships between mating patterns and habitat (e.g., Rand and Harrison 1989) and between habitat and signaling (e.g., Morton 1975). Our study provides evidence that both processes can occur in the same system and demonstrates that the consequences may be a reduction of gene flow. We also suggest that song sparrows in southwestern North America form a ring species. If true, our study underscores the potentially important link between local adaptation and speciation (Irwin et al. 2001).

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