

Nonlocal male mountain white-crowned sparrows have lower paternity and higher parasite loads than males singing local dialect

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Bird song often varies geographically, and when this geographic variation has distinct boundaries, the shared song types are referred to as song dialects. We investigated the role of song dialect in male mating success in a wild breeding population of mountain white-crowned sparrows (*Zonotrichia leucophrys oriantha*). In 2 of 3 years, males singing unusual songs (“nonlocal” males) had lower total fertilization success (measured by microsatellite paternity analysis) than did males singing the local dialect (“local” males). Similarly, females produced disproportionately more young with local than with nonlocal males. However, dialect was not a significant predictor of male mating success when controlling for other factors that might affect paternity. Instead, the low mating success of nonlocal males was apparently due to an interaction between song dialect and parasite load. Nonlocal males were more severely infected by bloodborne *Haemoproteus* than were local males, although they did not differ in any other measured aspect of quality. Immigrant birds may be immunologically disadvantaged, possibly due to a lack of previous experience with the local parasite fauna, resulting in low mating success. *Key words:* *Haemoproteus*, parasite load, parasite resistance, paternity, song dialect, white-crowned sparrows, *Zonotrichia leucophrys oriantha*. [*Behav Ecol* 13:682–689 (2002)]

Among nonhuman animals, the best studied example of a stable, culturally transmitted trait is bird song. Oscine birds (suborder Passeri) learn their songs, with young birds generally learning to produce species-typical songs by copying conspecific adults (Catchpole and Slater, 1995). As a result of vocal learning, bird song often varies geographically within a species. This geographic variation is thought to be due to the combined effects of imperfect song copying and philopatry (Baker and Cunningham, 1985). When the geographic variation in song has well-defined boundaries, so that variation between groups is greater than that within groups, the shared song types are referred to as dialects (Marler and Tamura, 1962, 1964). Song dialects have been described in many songbird species (Lemon, 1966; Mundinger, 1975; Nottebohm, 1969; Payne et al., 1981; Rothstein and Fleischer, 1987). Vocal dialects in general have been documented in such diverse vertebrate taxa as yellow-naped amazon parrots (*Amazona auro-palliata*; Wright, 1996), sperm whales (*Physeter macrocephalus*; Whitehead et al., 1998), and humans. It has been proposed that song dialects facilitate assortative mating, and thereby restrict gene flow among songbird populations (Marler and Tamura, 1962, 1964). Thus, song dialects may represent a behavioral mechanism whereby a culturally transmitted trait affects the genetic structure of populations.

Three major hypotheses have been proposed to explain the adaptive significance (if any) of song dialects. The genetic adaptation hypothesis (Marler and Tamura, 1962, 1964; Not-

tebohm, 1969) proposes that dialects serve as behavioral isolating mechanisms and restrict gene flow among populations. According to this hypothesis, young birds learn to produce or recognize song early in life, while still in their natal region, and adult birds use song as a cue for assortative mating. The social adaptation hypothesis (Payne, 1981) also proposes that song dialect affects social and sexual interactions, but asserts that males can modify their songs to match those of a new dialect area into which they disperse as juveniles or to match those of their neighbors during their first breeding season. By learning to produce the predominant local dialect and thus disguising their nonlocal origins, immigrant males might benefit through enhanced attractiveness to local females and/or greater ability to defend a territory through song. Thus, the genetic and social adaptation hypotheses differ with respect to the relative timing of song learning and dispersal. The epiphenomenon hypothesis (Andrew, 1962) suggests that song dialects are mere byproducts of vocal learning and are not behaviorally salient.

These three hypotheses can be distinguished primarily by the predictions they make regarding whether song dialect affects individual fitness and/or the genetic structure of a population. Specifically, the genetic adaptation hypothesis predicts that dialect causally affects both mating success and population genetic structure. The social adaptation hypothesis predicts that dialect affects the fitness of individual males, but not population genetic structure (because song does not necessarily indicate place of origin). The epiphenomenon hypothesis proposes that song dialect does not affect mating success but may be correlated with population genetic structure.

The present study addressed a long-standing problem: the relationship between song dialect and mating success in a wild population. Bird song generally serves a dual purpose of attracting mates and deterring competitors, so if there is a mating advantage associated with producing the local song dialect, it might be mediated by female preference, male aggression, or both (Baker and Cunningham, 1985). Thus, distinguishing among the genetic adaptation, social adaptation, and epiphenomenon hypotheses requires first determining wheth-

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er song dialect affects paternity and then identifying the mechanism(s) driving this effect. Although the effect of song dialect on male mating success has been the subject of much speculation, previous research has focused on the proximate mechanisms by which paternity might be affected (in particular the song preferences of captive females), without first ascertaining whether singing the local song actually confers a mating advantage in the wild. Furthermore, little consensus has been reached even within a single species. The studies reviewed below examine song preferences and mating behavior in white-crowned sparrows, *Zonotrichia leucophrys*.

The effect of song dialect on male mating success is ideally studied in the field, as captive studies may have only limited applicability to the behavior of free-living individuals (Chilton and Lein, 1996) unless combined with field studies. The evidence that wild songbirds pair assortatively based on song dialect is mixed. The presence or absence of assortative mating in the wild is sometimes inferred by treating females with testosterone to induce song and comparing the females' songs to those of their social mates. Using this technique to infer assortative mating relies on the assumption that females treated with testosterone will sing their natal song type, but testosterone-treated females may instead sing the song of their first mate (Chilton and Lein, 1996). Female *Z. l. nuttalli* treated with testosterone did produce songs similar to those of their social mates, suggesting that these females had mated assortatively (Tomback and Baker, 1984). However, other studies conducted on *Z. l. nuttalli* and *Z. l. oriantha* found no consistent similarity between the songs induced from females and those of their social mates (Baptista and Morton, 1982; Petrinovich and Baptista, 1984). In a mixed-dialect population of *Z. l. gambelii* and *Z. l. oriantha*, the mates of individual females in successive years were not more likely to be of the same dialect than predicted by chance (Chilton et al., 1990). Thus, there is little consensus even within this species whether song dialect is likely to affect male mating success. In any case, looking only at social mating success (whether a male can attract a social mate) is unlikely to prove very informative in species where extrapair mating is frequent, as it is in mountain white-crowned sparrows (Sherman and Morton, 1988). The ideal way to compare the fitness of local and nonlocal males is to measure mating success directly, using genetic markers to assess paternity in a wild population.

In this study we addressed the relationship between song dialect and genetic mating success in a wild population where approximately 90% of males in any given year sing the local dialect and where previous estimates indicated that about 40% of nestlings were extrapair young (Sherman and Morton, 1988). First, we compared the mating success of male mountain white-crowned sparrows singing local and nonlocal song dialects to test the hypothesis that local males father more young than do nonlocal males. Second, we controlled for other potential determinants of paternity to determine whether the observed difference in paternity was attributable to song dialect or to other differences in quality between local and nonlocal males. In addition to song dialect, the other potential determinants of paternity we investigated included age, body size, mass, territory quality, and bloodborne parasite load. Differences between local and nonlocal males in any of these factors might suggest possible proximate mechanisms for a mating advantage to local males.

METHODS

Study site and population

We investigated the role of song dialect in male mating success in a migratory population of mountain white-crowned spar-

rows (*Z. l. oriantha*) breeding at and around Tioga Pass, California, USA, from May to August 1997, 1998, and 1999. Long-term studies of this population indicate high natal and adult philopatry in general, with males being more philopatric than females (Morton, 1992). Mountain white-crowned sparrows are socially monogamous, although extrapair matings are frequent (Sherman and Morton, 1988). This population, particularly the 20–30 pairs of adults breeding on an area of about 50 ha called Tioga Pass Meadow, has been studied from 1968–1997 by M. L. Morton and colleagues, and by us since 1995. These birds are ideal for a study of parentage because many variables that may influence paternity (such as age and territory quality) are known and can be controlled for statistically.

Patterns of geographic variation in song and the maintenance of song dialects have been well studied in Sierra Nevada *Z. l. oriantha*. The song typically consists of an introductory whistle, a buzz, or whistle-buzz, a highly variable note complex, a variable trill of simple syllables, and a terminal vibrato (Nelson et al., 1995; Orejuela and Morton, 1975). Song is generally similar within geographic areas, with sudden discontinuities, indicative of song dialects (Nottingham, 1999; Orejuela and Morton, 1975). These dialects appear to be temporally stable, having changed little over at least 26 years (Harbison et al., 1999). Although there is no strict consensus in the literature on what constitutes a dialect versus variation in song type within a dialect, our research team uses both note complex and trill elements, the two most variable elements of the song, to define dialect membership (see "Song recording and analysis," below). Of the birds breeding at Tioga Pass, about 60% produce song type T1 of the local dialect, and 20–30% produce song type T2 of the local dialect (see Harbison et al., 1999, for detailed descriptions and exemplars). From 10% to 20% of males sing nonlocal songs characteristic of other regions in the Sierra Nevada or elsewhere (Harbison et al., 1999; Nottingham, 1999; Orejuela and Morton, 1975).

Field techniques

In the spring of 1997, 1998, and 1999, we captured adult mountain white-crowned sparrows arriving at Tioga Pass in a trapline of seed-baited Potter traps. We followed Morton (1992) in considering any unbanded adults arriving at the study area ("adult recruits") to be yearlings, for several reasons. First, an intensive long-term band and recapture study of this population indicated that return rates are much higher for adults of both sexes (> 50%) than for juveniles (Morton, 1992; Morton et al., 1972), suggesting near-total adult philopatry. Second, a great effort is made each year to capture and band any unbanded adults and juveniles in the study area, so that adult recruits are presumably visiting the site for the first time. Finally, adult recruits generally had one or more brown feathers among the black feathers at the base of bill or in the crown (typical of known yearlings) and shorter wings than birds known to be 2 years or older (MacDougall-Shackleton et al., personal observations; Morton et al., 1990).

From each newly arrived individual, we collected morphological measurements (body mass upon arrival at the breeding grounds and wing chord, tarsus length, and keel length). We marked each bird with a U.S. Fish and Wildlife Service leg band (banding permit no. 22712) and a unique combination of color bands to allow individual identification from a distance. To avoid an effect of trapping on paternity, we discontinued the trapline once females were judged to be depositing yolk for eggs (as determined by increased body mass). We located almost all of the nests initiated on Tioga Pass and the surrounding area, then checked each nest every few days until the nestlings fledged or the nest failed. In 1999 extra field

hands were available and the study area was expanded to include areas a few kilometers to the north of Tioga Pass.

From each breeding adult, we collected a small blood sample (<50 μ L) by brachial venipuncture, then blotted these samples onto individual filter papers (Whatman) saturated with 0.5 M EDTA. The samples were allowed to dry and stored desiccated at ambient temperature for several months before laboratory analysis. We collected small blood samples from all nestlings (using femoral venipuncture), usually on day 1 after hatch. We collected and froze eggs that remained unhatched several days after their nest mates had hatched. We stored eggs at -20°C for several months until the embryos could be dissected for genetic analysis (see below).

In 1999 we prepared blood smears from all adults to check for the presence of hematozoa, bloodborne parasites transmitted by biting Diptera. Most smears were collected within a month of the birds' arrival, shortly before nesting began. To prepare slides, we touched a 50 μ L capillary tube against a glass slide to deliver a small drop of blood, then used a clean slide to push the blood across the slide as a thin layer. Slides were air dried and fixed in 100% methanol for 30 s, within 24 h of sampling. The slides were kept at ambient temperature for several months until they could be stained and analyzed. For each slide we examined 10 fields of view, each containing approximately 1000 erythrocytes, under oil immersion at $\times 500$ or $\times 1000$ magnification, and noted the number of hematozoa (if any) from genus *Haemoproteus*. Hematozoa were identified with reference to Campbell (1995).

Song recording and analysis

We recorded songs from all males at the study area as early as possible after their arrival on the breeding grounds, using a Sony TCM-5000EV recorder and Sennheiser ME66 directional microphone. At least 10 songs were recorded from each individual; mountain white-crowned sparrow song is highly stereotyped, and approximately 95% of Tioga Pass individuals produce only a single song type (Harbison et al., 1999). We used the sound analysis program Canary 1.2.4 (Cornell Laboratory of Ornithology) to generate sound spectrograms of digitized songs. We categorized songs as local or nonlocal dialect based on the nature of the note complex and the trill (Nottingham, 1999). In most cases nonlocal songs were easily assigned to other nearby dialect areas in the Sierra Nevada (Harbison et al., 1999; MacDougall-Shackleton and MacDougall-Shackleton, 2001; Nottingham, 1999). Thus, we are confident that a nonlocal song probably indicates that a male was reared outside the local dialect area, rather than representing an improvisation or an error in song imitation.

Genetic analysis

We used a guanidine-based method (Ausubel et al., 1988) to extract genomic DNA from blood samples and estimated DNA concentration by agarose gel electrophoresis. Unhatched eggs were thawed and opened, and embryos were homogenized for DNA extraction with Chelex (Biorad). Genotyping was performed in a 96-well format. The polymerase chain reactions (PCRs; total volume 10 μ L) contained approximately 25 ng DNA, 50 mM KCl, 10 mM Tris-HCl pH 8.3, 2.5 mM MgCl_2 , 0.2 mM of each dNTP, 0.5 μ M of each primer (12.5% of one primer end-labeled with ^{32}P -ATP) and 0.5 units Taq polymerase. Thermocycling conditions included a step of 94°C for 3 min, a cycle of 94°C for 40 s, 50°C for 40 s, and 72°C for 40 s repeated 33 times, and a final extension step of 72°C for 5 min. We ran the PCR products on 6% acrylamide gels (90 individuals per gel) along with cycle-sequenced pVZ plasmid

size-standards. Gels were dried, then exposed overnight to X-ray film to visualize the PCR products.

We scored the developed films manually, with reference to the pVZ size standards. At each locus, the genotypes of individual nestlings were compared to those of their putative parents. We characterized each allele as consistent or inconsistent with their social parents' genotypes. In a few cases a single mismatch of one repeat unit (2 base pairs) was noted and attributed to mutation. If a nestling was mismatched at more than one locus, or by more than one repeat unit, the nestling was categorized as extrapair. To determine whether such nestlings resulted from extrapair mating by the female at the nest or from intraspecific brood parasitism (egg dumping), we assessed allele sharing between the nestlings and their social mothers. In all cases, extrapair nestlings were determined to result from extrapair mating.

We determined the genotype of each individual at eight microsatellite loci, all developed for use in other Emberizid species (Hanotte et al., 1994; Jeffery et al., 2001; Petren, 1998). All the loci were polymorphic, with a mean observed heterozygosity of 0.592 (range 0.321–0.881). Analysis of genotype frequencies revealed no linkage disequilibrium (χ^2 analysis, $p > .05$ for all locus pairs), indicating that these loci are inherited independently. The loci used had a combined exclusion probability (likelihood of correctly identifying a nestling as extrapair) of greater than 0.9999, and a combined identification probability (likelihood of misassigning an extrapair nestling to an unrelated male) of 3.47×10^{-7} .

To identify the genetic fathers of extrapair young, we compared the nestlings' genotypes with those of their mothers and identified the paternally inherited alleles. In 122 of 141 cases, only 1 of the males sampled could have been the genetic father of a certain nestling, and the nestling was ascribed to that male's extrapair mating success. An additional 14 nestlings could have been sired by either one of a father-son pair of males breeding on the study site who had similar genotypes. Each of these nestlings came from territories immediately adjacent to one or the other of these two related males and was assigned to that individual. The remaining five nestlings were not assigned to a male parent; these nestlings were found in nests near the periphery of the study area and had evidently been sired by males from whom blood samples were not obtained.

Total mating success (also referred to here as fertilization success or paternity) was measured as the number of young in the nest on day 1 that a male had sired with his social mate, plus those sired with other females on the study site. We used this metric (fertilization success) rather than number of fledglings produced, as we were primarily interested in how song dialect affects a male's ability to obtain successful copulations.

RESULTS

Breeding conditions and extrapair mating

The 3 years of the study had quite disparate breeding phenologies. The first year, 1997, was characterized by approximately average snowpack and breeding density of *Z. l. oriantha* (Morton ML, personal communication). A heavy snowpack in 1998 was followed by a low breeding density for that year. In 1999 the snowpack was again near average, with higher than average breeding density. One male in 1997 and three males in 1999, all of whom were yearlings singing nonlocal song types, defended territories but did not attract social mates. The numbers of males singing local versus nonlocal song, and their ages, are summarized in Table 1. Extrapair paternity was common throughout all 3 years of the study. Forty-five percent to 68% of females produced at least one

Table 1
Age classes of male *Z. l. oriantha* singing local versus nonlocal dialect in 1997, 1998, and 1999

	Yearlings		Two years or older	
	Local	Nonlocal	Local	Nonlocal
1997	7	4	19	3
1998	9	0	14	5
1999	27	4	15	5

The yearling category includes both known yearlings and newly arrived adults that were considered to be yearlings (see text).

extrapair nestling (17/25, 16/24 and 21/47 females in 1997, 1998, and 1999, respectively). Overall, 30–56% of nestlings were fathered by a male other than their social father (45/90, 45/81, and 51/171 nestlings in 1997, 1998, and 1999, respectively).

Song dialect and paternity

In 2 of 3 years, males singing the local song dialect (hereafter termed “local” males) had greater fertilization success than did males singing nonlocal dialects (“nonlocal” males; Table 2). Total fertilization success (within-pair + extrapair young) was greater for local than for nonlocal males in 1997 and 1999 (Mann-Whitney *U* test; 1997: $Z = 2.674, p = .0075, N = 20$ local, 7 nonlocal; 1999: $Z = 1.996, p = .0459, N = 36$ local, 9 nonlocal), but not in 1998 ($Z = .266, p = .7900, N = 18$ local, 5 nonlocal). Similarly, local males had greater paternity with their social mates than did nonlocal males in 1997 and 1999 (1997: $Z = 2.136, p = .0327, N = 19$ local, 7 nonlocal; 1999: $Z = 2.125, p = .0336, N = 36$ local, 9 nonlocal), but not in 1998 ($Z = 0.502, p = .6155, N = 18$ local, 5 nonlocal). Nonlocal males had reduced extrapair mating success in 1997 ($Z = 2.294, p = .0218, N = 26$ local, 7 nonlocal), but not in 1998 or 1999 (1998: $Z = 0.360, p = .7189, N = 23$ local, 5 nonlocal; 1999: $Z = 1.047, p = .2949, N = 40$ local, 9 nonlocal).

To determine whether local males fathered a disproportionate number of nestlings during all 3 years of the study, we analyzed the mating history of each female that bred at least once on the study site and whose immediate neighbors were known. For each female we compared the observed and expected number of young fathered by local males. Expected values were calculated by multiplying the total number of young produced by a female in a given year, by the proportion of males that sang local dialect in that female’s immediate

Table 2
Within-pair, extrapair, and total fertilization success of males singing local versus nonlocal dialect in 1997, 1998, and 1999

	Within-pair young fathered		Extrapair young fathered		Total young fathered	
	Local	Nonlocal	Local	Nonlocal	Local	Nonlocal
1997	1.95*	0.71*	0.77*	0*	2.93*	0.71*
	(1.30–2.60)	(0–2.10)	(0.39–1.15)	(0.0–0.0)	(2.23–3.63)	(0–2.10)
1998	1.44	2.00	1.44	1.00	2.33	3.00
	(0.69–2.19)	(0–4.48)	(0.44–2.44)	(0–3.15)	(1.20–3.46)	(0–7.12)
1999	3.14*	1.67*	0.91	0.67	3.92*	2.33*
	(2.59–3.69)	(0.66–2.68)	(0.48–1.34)	(0–1.53)	(3.12–4.72)	(0.61–4.05)

Values shown are mean number of day 1 nestlings sired per male, with 95% confidence limits in parentheses.

* Significant difference between local and nonlocal singers.

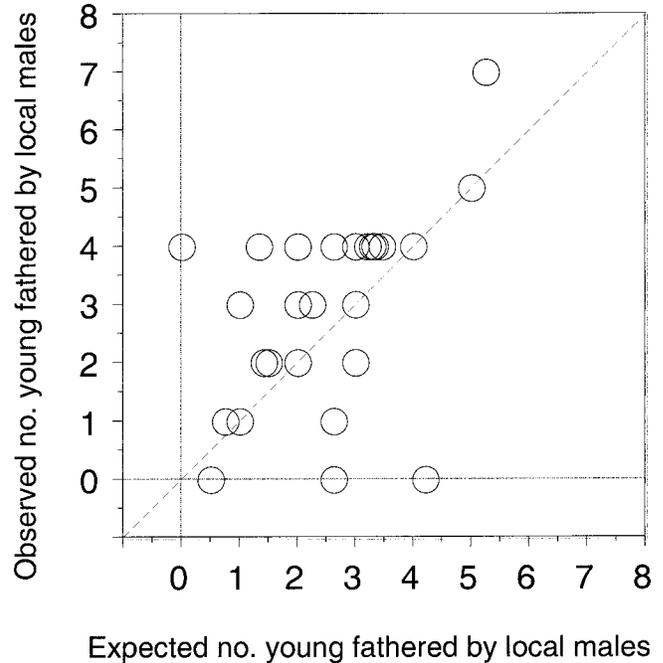


Figure 1
Observed versus expected numbers of nestlings fathered by local males, for each of 52 female *Z. l. oriantha* breeding at Tioga Pass from 1997 to 1999.

area (social mate plus males on adjacent territories). Expected values thus assume that females mate randomly within their immediate areas, which is probably not strictly true, but the high incidence of extrapair fertilization suggests that this assumption is not completely unreasonable. For females that returned to breed in multiple years, we summed the annual values for observed and expected young produced by local males. Females produced disproportionately more young fathered by local than by nonlocal males (Wilcoxon signed-rank, $Z = 2.512, p = .012$; Figure 1). Thus, the number of young produced by nonlocal males is lower than that expected if females mate randomly with respect to song type.

Nests attended by local males produced more fledglings than those attended by nonlocals in 1999 ($Z = 2.015, p = .0439$, mean = 3.49 local, 1.86 nonlocal, $N = 36$ local, 7 nonlocal), although not in the 2 previous years (1997 $Z = 0.575, p = .5654$, mean = 3.25 local, 3.00 nonlocal, $N = 18$ local, 4

Table 3
Variables, identified by stepwise regression, that predicted total reproductive success of male *Z. l. oriantha* in 1999

Step no.	Variable	Cumulative r^2	p
1	Tarsus + keel (mm)	.289	.019
2	<i>Haemoproteus</i> per 10,000 cells	.415	.012
3	Age (years)	.598	.003

Song dialect, mass, and territory quality were entered into the regression but did not contribute significantly to the predictive power of the model.

nonlocal; 1998 $Z = 0$, $p = .999$, mean = 2.67 local, 2.75 nonlocal, $N = 18$ local, 4 nonlocal).

Determinants of paternity

To investigate the relationship between song dialect and male mating success while controlling for other potential determinants of paternity, we ran a stepwise regression (Statview 5.0, SAS Institute). We used song dialect, age, mass, body size, parasite load, and territory quality as independent variables and measured their ability to explain total fertilization success in 1999 (the year in which the most complete data were collected). As an index of body (skeletal) size, we summed the lengths of tarsus and keel for each male. We used the number of *Haemoproteus* per 10,000 cells as an index of parasite load. To estimate territory quality, we used the number of nests that had been initiated in a given 100 × 100 m area over the last 30 years (Morton ML, personal communication). We plotted the distributions of all continuous variables to check for normality and used natural log or square-root transformations to normalize distributions where needed. The stepwise regression indicated that the three most useful variables in explaining fertilization success are (in decreasing order of importance) body size, parasite load, and age. These three factors combined explained about 60% of the variation in fertilization success (Table 3); specifically, larger, less heavily parasitized, and older males had higher total fertilization success. Song dialect, mass, and territory quality did not contribute significantly to the predictive value of the model when these other factors were controlled. A reverse stepwise regression confirmed that song did not contribute to the model's predictive value (data not shown). Thus, song dialect was probably not excluded inappropriately via intercorrelation of the independent variables (Crawley, 1993).

We ran similar stepwise regressions on the 1997 and 1998 data sets, for which only data on song dialect, body size, mass, and age were available. In 1997, song type was the only significant predictor of fertilization success ($r^2 = .209$, $p = .0372$,

$N = 17$), whereas in 1998, the only predictive factor was body mass ($r^2 = 0.134$, $p = .0572$, $N = 21$). Reverse stepwise regressions (data not shown) confirmed that the other factors were not inappropriately excluded.

Because nonlocal singers had low mating success relative to local singers (Table 2), but song dialect itself appears not to drive this difference in success, it is likely that local and nonlocal singers might differ in some other determinant of mating success. To test this possibility, we ran a series of unpaired t tests comparing local and nonlocal males' age, mass, body size, parasite load, and territory quality. Local and nonlocal males did not differ in mean age, mass, body size, or territory quality, but nonlocal males had significantly higher parasite loads than local males (Table 4). The likelihood of being parasitized at all was not significantly different between local and nonlocal males (10/42 locals vs. 4/8 nonlocals, Fisher's Exact $p = .1966$).

DISCUSSION

Determinants of paternity

Extrapair mating represented an important part of the breeding strategy for mountain white-crowned sparrows at Tioga Pass, with 30–56% of nestlings each year resulting from extrapair fertilizations. This proportion is consistent with a previous estimate that 38–40% of nestlings in this population are extrapair (Sherman and Morton, 1988). Extrapair paternity was less common in 1999, when breeding density was highest, in contrast to patterns reported for other songbirds (e.g., Bullock's oriole, *Icterus galbula bullockii*; Richardson and Burke, 2001). It is not yet clear what factors might drive this among-year variation in *Z. l. oriantha*.

A major finding of this study is that males singing local song enjoyed higher total fertilization success in 2 of the 3 years examined. Similarly, females produced disproportionately more young fathered by local than by nonlocal males. In contrast, previous studies of wild mountain white-crowned sparrows found no assortative mating based on song similarity between mated pairs and no apparent advantage to local males in attracting social mates (Baptista and Morton, 1982). Given that extrapair mating is widespread among songbirds in general (Birkhead and Møller, 1992) and among mountain white-crowned sparrows in particular (Sherman and Morton, 1988; this study), it is important to measure actual paternity to detect the reduced mating success of males singing nonlocal song dialects.

Although nonlocal males suffered reduced mating success, song dialect in itself did not explain a significant amount of variation in paternity. Instead, body size, parasite load, and age predicted most of the variation in male fertilization success. The low mating success of nonlocal males appears to be due to an interaction between song dialect and parasite load.

Table 4
Comparison of males singing local versus nonlocal dialect in 1999 in terms of body size, mass, age, territory quality, and parasite load

	Local males	Nonlocal males	df	t	p
Tarsus + keel (mm)	48.2 (47.9–48.6)	48.5 (47.8–49.1)	50	0.55	.583
Mass (g)	27.9 (27.3–28.6)	28.4 (27.6–29.2)	49	0.65	.516
Age (years)	1.6 (1.3–1.9)	2.2 (1.3–3.1)	56	1.73	.089
Territory quality	24.4 (14.5–34.3)	32.0 (0–209.9)	17	0.76	.460
<i>Haemoproteus</i> per 10,000 cells	3.45 (0.54–6.36)	114.63 (0–298.24)	48	3.51	.001*

Values shown are means, with 95% confidence limits in parentheses.

* Significant difference between local and nonlocal singers.

That is, nonlocal males were more heavily parasitized by *Haemoproteus* and, possibly as a result, fathered fewer offspring than did local males. This finding should, however, be viewed with some caution until it can be confirmed by experimental studies addressing the relationships between song, immune function, and parentage.

We found considerable interyear variation in terms of which variables best predicted male mating success. In 1997, the apparent importance of song dialect in predicting paternity may have been due to a link between song and parasite load (which was not measured that year), rather than to a behavioral advantage to the local dialect as such. Conversely, in 1998 (the coldest of the three summers), parasitic infection by *Haemoproteus* may have been a relatively weak selective force, as biting flies (vectors for hematozoa) likely emerged later and/or in lower numbers. Furthermore, in 1997 and 1999 most of the nonlocal singers were yearlings, whereas in 1998 all nonlocal singers were 2 years or older and had bred (or attempted to breed) at Tioga the previous year (Table 1). Presumably, then, in 1998 all nonlocal males had prior experience with the local parasite fauna and may have been less immunologically challenged. The possible importance of prior exposure to the local parasites is discussed below. This population routinely experiences strikingly different breeding environments from year to year. For example, the onset of nesting can vary by a month or more depending on the residual snowpack (Morton, 1978). Such variability in breeding conditions may well result in differences in the strength of sexual selection and in the traits favored among years.

Song dialects and parasite load

Why should nonlocal males have heavier parasite loads than males singing the local dialect? There are several potential explanations for this finding. First, if bloodborne parasites or their vectors are more abundant in other dialect regions than in our study area, then males singing nonlocal dialect might show higher parasite loads due to previous infection in their natal regions. Similarly, birds may have been infected on the wintering grounds or during migration. *Haemoproteus* may be detected in the blood as early as 6 days after infection (Shutler et al., 1995), which is well within our 1-month time frame during which most blood smears were collected. However, this latency period can be as long as 21 days for some songbird hosts (Kirkpatrick and Suthers, 1988). Although studies of other northern temperate birds have shown that *Haemoproteus* and other hematozoa are transmitted primarily during the breeding season (Atkinson and Van Riper, 1991), making broad generalizations about transmission across species and habitats is problematic. Thus, we cannot exclude the possibility that some of the infections we sampled may have been acquired before arrival at the breeding grounds. We are currently collecting data on the age at which juvenile *Z. l. orientalis* first display evidence of parasitic infections, which should cast some light on the timing of infection relative to detection in this population.

A second possible explanation is that males that disperse out of their natal dialect regions might be lower in quality than more philopatric males. If so, dispersing males might have less effective immune systems and hence inferior ability to deal with parasitic infection. However, the fact that local and nonlocal singers differed only in parasite load but not in body size, age, or any other measured aspect of quality strongly suggests that nonlocal males are disadvantaged specifically in terms of their immune response to local parasites.

A third potential explanation for their increased parasite load is that nonlocal birds are poorly adapted genetically to the selective pressures posed by local parasite fauna. That is,

populations characterized by different dialects might be evolutionarily adapted to different local conditions (Nottebohm, 1969). Such local conditions were originally thought to involve habitat structure, but a similar argument could be made for parasites or other pathogens as selective forces. In humans, for example, populations of different ethnicities differ in rates of infection by the malaria-causing hematozoan *Plasmodium falciparum*; this difference appears to be genetically based (Modiano et al., 1996). However, despite the low mating success of nonlocal males shown here, mountain white-crowned sparrows exhibit considerable gene flow across dialect borders (although dialects are associated with some reduction in gene exchange; MacDougall-Shackleton and MacDougall-Shackleton, 2001). Gene flow among dialects might be sufficient to prevent local populations from evolving resistance to the local suite of parasites.

A fourth possibility is that the immunological disadvantage to nonlocal males is based on experience rather than on genetics. That is, nonlocal males may be more susceptible to hematozoan parasites because they have not been previously exposed to the local parasite strains. Among humans, unfamiliar strains of *P. falciparum* do appear to be more destructive than previously encountered strains (Gupta et al., 1994). In mountain white-crowned sparrows, previous infection with *Haemoproteus* might similarly induce some degree of strain-specific immunity. Consistent with this possibility is the fact that nonlocal males returning for a third or fourth breeding season were less heavily parasitized than were first- and second-year nonlocals (although this comparison reduces sample sizes to the point where inferential statistics are inappropriate). Morton (1992) noted that previous familiarity with the study area may enhance male reproductive success, as yearling males that had been banded as juveniles on the study area were more likely to breed than adult recruits. One mechanism for this effect of previous familiarity could be experience with local strains of parasites.

Dialects, parasites, and mate choice

Parasitic infection is thought to affect fitness in many songbird species. For example, secondary sexual characteristics of affected individuals may be compromised (e.g., Buchanan et al., 1999; Hamilton and Zuk, 1982; Møller, 1990; Thompson et al., 1997), presumably resulting in decreased attractiveness to potential mates. In mountain white-crowned sparrows, song dialect appears to be a fairly reliable index of male parasite load and may thus represent a useful cue with which females can select mates. That is, by mating preferentially with local singers, females may avoid mating with parasitized or parasite-susceptible males. Our findings neither support nor conclusively contradict this possibility. Dialect did not predict male mating success when parasite load and other variables were controlled for. However, given the correlation between song and parasite load and the relatively low power of this analysis to detect an effect of song (song varies discretely rather than continuously, and there were relatively few nonlocal males in the analysis), we cannot yet dismiss the possibility that dialect affects paternity.

If females do use song dialect as a cue to avoid mating with parasitized or parasite-susceptible males, they would presumably benefit by enhanced offspring survival. In this population, nests at which both parents are free of hematozoan infection are more likely to fledge young successfully than are nests at which one or both parents are parasitized (Derryberry, 2000; Richardson, 1997). Furthermore, if the difference in parasite load between local and nonlocal males reflects genetic differences in parasite resistance, offspring sired by local males may be better adapted to the local parasite fauna. In

bluethroats (*Luscinia svecica*), extrapair nestlings are more immunocompetent than their half-sibs, suggesting that at least in this species, females use extrapair mating to enhance offspring immunocompetence (Johnsen et al., 2000).

Conversely, song dialect may not be directly involved in mating success except as a correlate of parasite load. As a consequence of being heavily parasitized, nonlocal males may be less able than locals to attract mates or defend territories. We found no difference between locals and nonlocals in age, body size, mass, or territory quality, but parasite load might affect more immediate behavioral measures such as song rate or ability to patrol a territory, which could in turn influence male mating success. Severe parasitic infection might also compromise sperm viability (by inducing fever, for example) and thereby reduce paternity.

Conclusions

Our findings suggest that the relationship between song dialect and fitness is more complex than previously supposed. As predicted by both the genetic and social adaptation hypotheses, males singing the local dialect enjoyed enhanced fertilization success relative to nonlocal singers. However, this effect appears to be due to an interaction between song dialect and parasite load rather than to the effects of dialect per se. Furthermore, it remains unclear whether the low parasite loads of local singers result from genetic adaptations to the local parasite fauna or from previous exposure to these parasites (in which case males of any genotype might eventually be able to develop resistance). Thus, the adaptations apparently favoring local males might be experiential rather than genetic. Conversely, nonlocal (dispersing) males might have inherently poor immune systems relative to local (nondispersing) males, rather than a specific inability to deal with local parasites. Conclusively addressing this possibility will require experimental manipulations with familiar and novel pathogens. In any case, parasitic resistance and susceptibility may provide a mechanism to explain the relationship between song dialects and population genetic structure found in these birds (MacDougall-Shackleton and MacDougall-Shackleton, 2001). This study has taken a novel approach in using molecular techniques to identify parentage and measuring multiple variables that may contribute to fertilization success. This new approach provides direction for future research that may further clarify whether the genetic adaptation, social adaptation, epiphenomenon, or some other hypothesis best explains the evolution and maintenance of song dialects in these, and perhaps in other, songbirds.

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