Temporal and Geographic Homogeneity of Gene Frequencies in the Fox Sparrow (*Passerella iliaca*)

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Protein electrophoresis has been used frequently in the study of genetic variation within and among natural populations (Lewontin 1974, Avise and Aquadro 1982, Nevo et al. 1984). A goal of protein electrophoretic studies is to describe patterns of genetic variation and deduce historical processes (such as fragmentation of populations and gene flow) that have produced such patterns (Felsenstein 1982). A second, but relatively little-studied, aspect of the geography of genetic variation is the temporal stability of patterns of gene frequencies. There are both systematic and evolutionary implications of temporal variation in allozymic frequencies. If samples are taken from different sites in different years, temporal variation might confound systematic interpretation of geographic patterns (Zink 1983). Many investigators have implicated natural selection in the maintenance of enzyme polymorphisms (Mitton and Grant 1984, Mueller et al. 1985, Barker et al. 1986; but see Zink et al. 1985). A correlate of genetic polymorphisms influenced by selection is temporal change in gene frequencies at a site, resulting from directional natural selection. Thus, it is of interest to examine the temporal stability of gene frequencies.

Previous studies of temporal variation in gene frequencies have either considered relatively few generations, limited geographic areas, or few loci (Baker and Fox 1978, Gyllensten 1985, Henderson 1977, McCauley et al. 1988, Redfield 1974). In this study, we assessed temporal and geographic variation in gene frequencies in population samples of the Fox Sparrow (*Passerella iliaca*). A previous allozymic survey (Zink 1986), based on samples taken in 1978–1980 in California, Oregon, and Nevada, revealed essentially no geographic differentiation in gene frequencies; the F_{ST} value (derived from 31 samples of individuals, 38 presumptive genetic loci, and a total of 619 individuals) was 0.0135 ± 0.0033 (SE). At some of these sites, Zink (1983) detected morphometric differentiation between samples taken in the mid-1920s and 1978–1980, a span of 50 years. We used protein electrophoresis to compare levels and patterns of genetic variation in seven samples (from California and Nevada) of Fox Sparrows taken in 1988 with those taken in 1978–1980, a span of 8–10 generations. In addition, we sampled two new sites (Table 1) geographically distant from the original sample sites to assess geographic differentiation on a larger scale.

Approximately one half of our aqueous tissue extracts were obtained during preparation of tissues for mitochondrial DNA (mtDNA) analysis (see Avise and Zink 1988), and the others were prepared as outlined by Zink (1986). Methods for protein electrophoresis are given in Zink (1986). For the samples collected in 1988, we surveyed only the loci found to be polymorphic in the earlier study (Table 1). Because sample sizes for our recent samples are relatively small (11–20) and because Zink (1986) showed that the same allele was the common allele at all sites for each locus, we compared only the frequencies of common alleles among sites and across generations; noncommon alleles were pooled. We performed 2 × 2 *G*-tests (Sokal and Rohlf 1981) for each locus and site by tallying the number of common and noncommon alleles in the "old" (1978–1980) and "new" (1988) samples. Such multiple testing requires adjustment of significance values. We followed Rice's (1989) suggestion and divided the alpha level of 0.05 by the number of *G*-tests (72) to get a significance level (0.0007) appropriate for our multiple *G*-tests. The 19 sites at which the common allele was fixed in samples taken in both years were not tested, which explains why we tested 72 values and not 91 (13 loci × 7 sites). Rice (1989) noted that, in a series of tests such as ours, if the smallest probability value is not significant, then all entries in the table are judged nonsignificant, regardless of the significance levels of individual tests. We recognize that this is a conservative test, and one that requires perhaps larger samples than we have to detect significant effects. Therefore, we computed a *G*-value by summing all 72 tests to test for a table-wide effect of temporal variation. We report the observed direct-count heterozygosity value for each sample (see Zink 1986). We also computed separate F_{ST} values (each corrected for sampling a finite number of individuals (Wright 1978)) for the old samples, new samples, and the new samples plus those from Wyoming and Colorado to evaluate temporal and geographic variation.

Temporal variation.—*G*-tests revealed no significant values (at the corrected *P*-value) in the occurrence of common alleles between old and new samples (Table 1). We found that 7 of the 72 (10%) tests produced significant *G*-values at the 0.05 level when each test was evaluated independently; hence, correction for multiple testing influences interpretation (Rice 1989). We also tested for locus and site effects by summing *G*-values across loci and across sites, and found only one significant value, that for the locus Lgg (Table 1). We noted during our study that gel patterns at Lgg were difficult to interpret for individuals prepared for mtDNA analysis relative to standard preparations, an apparent artifact of the mtDNA buffer. It is unclear, therefore, if the marginally significant value for Lgg deserves special explanation, both because of possible

TABLE 1. Frequencies of the most common allele for each locus at each site in old (1978–1980) and recent (1988) samples of Fox Sparrows (sample sizes in parentheses). *G*-values for sites and loci are the sums of individual *G*-values. H = direct count heterozygosity.

	Locality[1]							
	1		2		3		4	
Locus	1980	1988	1979	1988	1979	1988	1978/79	1988
Lgg	0.956 (23)	0.750 (10)	0.854 (24)	0.794 (17)	0.914 (29)	0.727 (11)	0.800 (20)	0.781 (16)
La-1	1.000 (23)	1.000 (9)	1.000 (24)	0.941 (17)	1.000 (29)	0.909 (11)	1.000 (20)	1.000 (16)
La-2	0.696 (23)	0.773 (11)	0.875 (24)	0.950 (20)	0.845 (29)	0.818 (11)	0.850 (20)	0.825 (20)
Mpi	1.000 (23)	1.000 (3)	1.000 (24)	0.958 (12)	1.000 (29)	0.750 (2)	1.000 (20)	1.000 (12)
Ada	0.891 (23)	0.909 (11)	0.833 (24)	0.750 (20)	0.845 (29)	0.909 (11)	0.800 (20)	0.925 (20)
Np	0.978 (23)	1.000 (11)	0.937 (24)	0.925 (20)	1.000 (29)	0.955 (11)	0.800 (20)	0.825 (20)
6-Pgd	0.956 (23)	0.955 (11)	0.979 (24)	1.000 (20)	1.000 (29)	1.000 (11)	1.000 (20)	1.000 (20)
Gpi	0.956 (23)	0.909 (11)	0.958 (24)	0.975 (20)	0.993 (29)	0.955 (11)	1.000 (20)	0.975 (20)
Icd-1	0.957 (23)	1.000 (11)	0.979 (24)	1.000 (20)	1.000 (29)	0.955 (11)	0.975 (20)	0.900 (20)
Eap	0.913 (23)	1.000 (10)	0.979 (24)	1.000 (19)	0.965 (29)	1.000 (10)	0.950 (20)	0.947 (19)
Est-D	0.978 (23)	0.909 (11)	0.854 (24)	0.850 (20)	0.948 (29)	0.909 (11)	0.900 (20)	0.850 (20)
Pgm	0.978 (23)	0.955 (11)	0.938 (24)	0.975 (20)	1.000 (29)	1.000 (11)	0.900 (20)	0.950 (20)
α-Gpd	1.000 (23)	1.000 (10)	1.000 (24)	0.941 (17)	0.983 (29)	1.000 (7)	0.975 (20)	0.972 (18)
H	3.2	2.9	3.3	3.6	3.4	4.3	5.1	4.6
G	11.66		11.10		13.32		6.72	

[1] Localities: 1 = California, San Bernardino Co., 1.5 km N, 3.0 km E Butler Peak; 2 = California, Fresno Co., 7.2 km W Hume Lake; 3 = Nevada, Esmeralda Co., Trail Canyon; 4 = California, Placer Co., 6.4 km S, 3.2 km E Ward Peak; 5 = California, Trinity Co., 12.9 km N, 9.7 km W North Yolla Bolly Mtn.; 6 = California, Shasta Co., 10.4 km N, 11.2 km E Lassen Peak; 7 = Nevada, Elko Co., Lamoille Canyon, Ruby Mtns.; 8 = Wyoming, Lincoln Co., Grey's River, 5.6 km N, 12.1 km E Mt. Wagner; 9 = Colorado, Gunnison Co., 4.8 km N, 2.0 km W Ohio Peak.

[2] Does not include localities 8 and 9; * = significant at the adjusted alpha level (see text).

methodological artifacts at that locus and because only 1 in 14 loci exhibited a temporal change. The *G*-value computed over loci and sites, 86.6 (df = 72), was insignificant ($P > 0.05$). We conclude that there is a general pattern of temporal stability in the common allele frequencies. Clearly, there could be changes in noncommon alleles that are biologically significant, but our sample sizes are insufficient to detect such an effect. The heterozygosity estimates (Table 1) for the recent samples are consistent (within 1 SE) with those for the old samples.

Waples (1989) noted that most published tests of temporal change in gene frequency were unable to distinguish between the effects of genetic drift and natural selection. In particular, Waples noted that standard Chi-square or *G*-tests do not account for the increased variance in allelic frequencies over time caused by genetic drift. In our analysis, because of the general pattern of temporal stability, observed differences were due to sampling error (i.e. our data suggest that sampling over 10 yr was no different than taking two samples from the same year). Because Waples' (1989) test for temporal heterogeneity results in lower *G*-values, his method need not be used for our data because our values are already nonsignificant. If we had rejected temporal stability, then Waples' test would be required to distinguish between the effects of drift and natural selection. It might be possible to determine if there was less difference over the 8–10-yr sampling period than expected by sampling error plus genetic drift. However, Waples' (1989) method requires estimation of effective population size, which we cannot do accurately for the Fox Sparrow.

The F_{ST} value for the old samples was 0.0099 ±

TABLE 1. Continued.

Locality[1]								
5		6		7		8	9	
1978	1988	1978/80	1988	1979	1988	1988	1988	G^2
0.848	1.000	0.902	0.708	0.962	0.850	0.808	0.583	21.05*
(23)	(14)	(51)	(12)	(13)	(10)	(13)	(12)	
1.000	0.929	0.970	1.000	0.923	0.900	0.962	0.875	9.37
(23)	(14)	(51)	(12)	(13)	(10)	(13)	(12)	
0.783	0.794	0.794	0.893	0.846	0.833	0.706	0.667	3.79
(23)	(17)	(51)	(14)	(13)	(12)	(17)	(12)	
1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	2.22
(23)	(11)	(51)	(6)	(13)	(8)	(10)	(8)	
0.891	0.676	0.902	0.893	0.846	0.750	0.853	0.875	10.67
(23)	(17)	(51)	(14)	(13)	(12)	(17)	(12)	
0.978	0.853	0.902	0.786	0.962	0.917	1.000	1.000	9.07
(23)	(17)	(51)	(14)	(13)	(12)	(17)	(12)	
1.000	0.971	0.980	1.000	1.000	1.000	1.000	1.000	1.31
(23)	(17)	(51)	(14)	(13)	(12)	(17)	(12)	
0.957	0.971	0.990	0.964	0.962	0.875	0.794	1.000	3.98
(23)	(17)	(51)	(14)	(13)	(12)	(17)	(12)	
0.988	0.971	0.971	0.821	1.000	1.000	1.000	0.958	11.55
(23)	(17)	(51)	(14)	(13)	(12)	(17)	(12)	
0.978	1.000	0.971	0.893	1.000	1.000	1.000	1.000	3.69
(23)	(17)	(51)	(14)	(13)	(11)	(16)	(11)	
0.891	0.941	0.952	0.964	1.000	1.000	1.000	0.958	3.13
(23)	(17)	(51)	(14)	(13)	(12)	(17)	(12)	
1.000	1.000	0.942	1.000	1.000	1.000	0.971	0.958	4.21
(23)	(17)	(51)	(14)	(13)	(12)	(17)	(12)	
0.988	1.000	1.000	1.000	1.000	1.000	1.000	1.000	2.56
(23)	(16)	(51)	(14)	(13)	(11)	(13)	(9)	
4.2	3.7	3.6	5.1	2.6	3.1	3.1	4.7	
19.96		19.51		4.37				

0.0042 (SE) and for the new samples (not including Wyoming and Colorado) 0.0129 ± 0.0044, which suggests (given the large SEs) temporal stability in the amount of genetic variation partitioned among sites.

Several authors report that natural selection influences gene frequencies (e.g. Mitton and Grant 1984, Szymura and Barton 1986, Pemberton et al. 1988). Barrowclough et al. (1985) compared the distribution of allelic polymorphisms within demes to that expected from a simple model of selective neutrality (the "Infinite alleles-Constant mutation rate" model; Chakraborty et al. 1980) and found that in 23 of 24 avian species tested, patterns of polymorphisms were consistent with neutralist expectations. Similar consistency has been documented elsewhere (Zink and Winkler 1983, Zink et al. 1985, Zink and Watt 1987). Results of the present study reveal no evidence of strong directional selection. Of course, weak selection would not be detected by our analysis.

Several studies have reported fluctuating gene frequencies across few generations (months in some fruit flies, *Drosophila*), due to drift (Lynch 1987) and natural selection (Mueller et al. 1985). Our samples would not detect year-to-year changes in gene frequencies, but it would be difficult to envision the importance of natural selection acting yearly when we observed temporal stability over 8–10 generations. Selection for heterozygotes, an alternative to directional selection (Mitton and Grant 1984), should lead to equalization of allelic frequencies at 0.50 (for adaptively important loci) to maximize heterozygosity, assuming that the homozygous classes have equal fitness. These particular conditions seem not to hold in our population samples of the Fox Sparrow (Table 1). If there is selection for heterozygotes, the homozygous classes have unequal fitness values.

In summary, we advocate multilocus, multisite studies of temporal variation to avoid interpretations of natural selection and adaptation based on spurious results (see Rice 1989).

Geographic differentiation.—Considerable attention has focused on levels of intraspecific genetic differentiation in vertebrates (Avise and Aquadro 1982). Many studies have documented low levels ($F_{ST} \leq 0.05$) of allozymic differentiation among avian populations (Barrowclough 1983, Corbin 1987). Using our estimates of the frequency of common alleles, we computed an F_{ST}-value of 0.019 ± 0.0046 for all new samples (including Wyoming and Colorado samples), which means that <2% of the variance in allelic frequencies is explained by the site at which the samples were taken. Our results extend Zink's (1986) study and reveal genetic homogeneity among populations of Fox Sparrows from the Sierra Nevada-Cascade axis, Transverse Range (southern California), Great Basin, and Rocky Mountains. This is one of the most geographically extensive intraspecific surveys of avian genetic variation (see also Grudzien et al. 1987, Johnson and Zink 1983, Johnson and Marten 1988) and corroborates the observation that temperate, migratory avian populations are an order of magnitude less differentiated in allozymes than populations of other vertebrates (Avise and Aquadro 1982).

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Literature Cited

Avise, J. C., & C. F. Aquadro. 1982. A comparative summary of genetic distances in the vertebrates. Pp. 151–185 *in* Evolutionary biology, vol. 15 (M. Hecht, B. Wallace, and R. Praus, Eds.). New York, Plenum Publ. Co.

———, & R. M. Zink. 1988. Molecular genetic divergence between avian sibling species: King and Clapper rails, Long-billed and Short-billed dowitchers, Boat-tailed and Great-tailed grackles, and Tufted and Black-crested titmice. Auk 105: 516–528.

Baker, M. C., & S. F. Fox. 1978. Dominance, survival, and enzyme polymorphism in Dark-eyed Juncos, *Junco hyemalis*. Evolution 32: 697–711.

Barker, J. S. F., P. D. East, & B. S. Weir. 1986. Temporal and microgeographic variation in allozyme frequencies in a natural population of *Drosophila buzzatii*. Genetics 112: 577–611.

Barrowclough, G. F. 1983. Biochemical studies of microevolutionary processes. Pp. 223–261 *in* Perspectives in ornithology (A. H. Brush and G. A. Clark Jr., Eds.). New York, Cambridge Univ. Press.

———, N. K. Johnson, & R. M. Zink. 1985. On the nature of genic variation in birds. Pp. 135–154 *in* Current ornithology, vol. 2 (R. F. Johnston, Ed.). New York, Plenum Press.

Chakraborty, R., P. A. Fuerst, & M. Nei. 1980. Statistical studies on protein polymorphism in natural populations. III. Distribution of allele frequencies and number of alleles per locus. Genetics 94: 1039–1063.

Corbin, K. W. 1987. Geographic variation and speciation. Pp. 321–353 *in* Avian genetics (F. Cooke and P. A. Buckley, Eds). New York, Academic Press.

Felsenstein, J. 1982. Numerical methods for inferring evolutionary trees. Q. Rev. Biol. 57: 379–404.

Grudzien, T. A., W. S. Moore, J. R. Cook, & D. Tagle. 1987. Genic population structure and gene flow in the Northern Flicker (*Colaptes auratus*) hybrid zone. Auk 104: 654–664.

Gyllensten, U. 1985. Temporal allozyme frequency changes in density fluctuating populations of Willow Grouse (*Lagopus lagopus* L.). Evolution 39: 115–121.

Henderson, B. A. 1977. The genetics and demography of a high and low density of Red Grouse *Lagopus scitucys* L. J. Anim. Ecol. 46: 581–592.

Johnson, N. K., & J. A. Marten. 1988. Evolutionary genetics of flycatchers. II. Differentiation in the *Empidonax difficilis* complex. Auk 105: 177–191.

———, & R. M. Zink. 1983. Speciation in sapsuckers (*Sphyrapicus*): I. Genetic differentiation. Auk 100: 871–884.

Lewontin, R. C. 1974. The genetic basis of evolutionary change. New York, Columbia Univ. Press.

Lynch, M. 1987. The consequences of fluctuating selection for isozyme polymorphisms in *Daphnia*. Genetics 115: 657–669.

McCauley, D. E., M. J. Wade, F. J. Breden, & M. Wohltman. 1988. Spatial and temporal variation in group relatedness: evidence from the imported willow leaf beetle. Evolution 42: 184–192.

Mitton, J. B., & M. C. Grant. 1984. Associations among protein heterozygosity, growth rate, and developmental homeostasis. Annu. Rev. Ecol. Syst. 15: 479–499.

Mueller, L. D., L. G. Barr, & F. J. Ayala. 1985. Natural selection vs. random drift: evidence from temporal variation in allele frequencies in nature. Genetics 111: 517–554.

Nevo, E., A. Beiles, & R. Ben-Shlomo. 1984. The evolutionary significance of genetic diversity: ecology, demographic and life history correlates. Lect. Notes Biomath. 53: 13–213.

Pemberton, J. M., S. D. Albon, F. E. Guinness, T. H. Clutton-Brock, & R. J. Berry. 1988. Genetic variation and juvenile survival in red deer. Evolution 42: 921–934.

REDFIELD, J. A. 1974. Genetics and selection at the *Ng* locus in Blue Grouse (*Dendragapus obscurus*). Heredity 36: 69–78.

RICE, W. R. 1989. Analyzing tables of statistical tests. Evolution 43: 223–226.

SOKAL, R. R., & F. J. ROHLF. 1981. Biometry. New York, W. H. Freeman and Co.

SZYMURA, J. M., & N. H. BARTON. 1986. Genetic analysis of a hybrid zone between the fire-bellied toads, *Bombina bombina* and *B. variegata*, near Cracow in southern Poland. Evolution 40: 1141–1159.

WAPLES, R. S. 1989. Temporal variation in allele frequencies: testing the right hypothesis. Evolution 43: 1236–1251.

WRIGHT, S. 1978. Evolution and the genetics of natural populations: IV. Variability within and among natural populations. Chicago, Univ. Chicago Press.

ZINK, R. M. 1983. Evolutionary and systematic significance of temporal variation in the Fox Sparrow. Syst. Zool. 32: 223–238.

———. 1986. Patterns and evolutionary significance of geographic variation in the Schistacea group of the Fox Sparrow (*Passerella iliaca*). Ornithol. Monogr. 40.

———, M. F. SMITH, & J. L. PATTON. 1985. Associations between heterozygosity and morphological variance. J. Heredity 76: 415–420.

———, & D. J. WATT. 1987. Allozymic correlates of dominance rank in sparrows. Auk 104: 1–10.

———, & D. W. WINKLER. 1983. Genetic and morphologic similarity of two California Gull populations with different life history traits. Biochem. Syst. Ecol. 11: 397–403.

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Evidence for Redetermination of Migratory Direction Following Wind Displacement

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Nocturnal passerine migrants are capable of *selecting* a direction with reference to a variety of environmental cues (Emlen 1975, Able 1980b, Able and Cherry 1986, Moore 1987). How well they *maintain* that direction is difficult to evaluate. Although free-ranging migrants are seldom observed to be disoriented, flight in seemingly inappropriate directions is not an uncommon observation (Griffin 1973; Richardson 1978; Able 1980a, 1982; Alerstam 1979, 1981). Besides the problem of maintaining a predetermined heading, orientation errors occur, especially among young, inexperienced migrants (Herbert 1970, Ralph 1978, McLaren 1981, Gauthreaux 1982, DeSante 1983, Moore 1984).

For small passerines, displacement by wind is a real possibility. Whether migrants correct for displacement or the extent to which they correct is difficult to determine (Evans 1968; Alerstam 1979, 1981; Richardson 1982; Bingman et al. 1982). Migrants may "correct" while aloft (Myres 1964, Richardson 1978, Cochran and Kjos 1985), or they may redetermine directions soon after landing or before their next departure (Evans 1968, Gauthreaux 1978). If migrants select a direction at the time of takeoff, the next morning would be a convenient time to reorient if displaced during a night's flight (Vleugel 1954, Lowery and Newman 1955, Moore 1987).

During a study of migrants after they migrated across the Gulf of Mexico, natural variation in wind conditions over five days provided an opportunity to investigate the orientation of migrants in response to presumed wind displacement. South-southwesterly winds (3–4 m/s) prevailed on 14 and 15 April 1985, but they shifted to moderately strong easterly winds (7–8 m/s) by 17 April after the passage of a weak front. Winds returned to southerly by midafternoon on 17 April. The pattern of change in prevailing winds as migrants arrived over the northern Gulf Coast represents a natural analog of a pre-test/post-test experimental design for the treatment effect (wind change). Most trans-Gulf migrants arrived on 17 April between 1000 and 1200 CST after experiencing easterly winds over the northern Gulf of Mexico. If they drifted from their preferred heading, which is likely when migrating over water (Lack 1959, Alerstam and Pettersson 1976), migrants might compensate for the displacement and reorient their activity the night of their arrival or the following morning. Differences in orientation should exist among migrants sampled during the 5-day period if they redetermine direction in response to wind conditions experienced during migration.

I conducted cage-orientation experiments with migrating warblers (Parulinae) that had stopped over at an isolated woodland (29°45'N, 93°33'W) along the northern coast of the Gulf of Mexico following trans-Gulf migration (see Moore 1986, Moore and Kerlinger 1987). Birds were captured in mist nets on the day of arrival, held overnight, and placed in funnel-shaped orientation cages (Emlen and Emlen 1966) the next morning near the beginning of civil twilight. Activity was recorded for 90 min; then I released the birds.