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Seasonal Variation in Gene Frequencies in the House Sparrow (*Passer domesticus*)

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Differential survivorship within a population is usually considered to be a result of natural selection. It is, however, difficult to identify specific causes of selection and the levels at which they operate (Endler 1986, Grant 1986). Several avian studies have examined genetic changes in allozymic frequencies in populations across seasons, one period when selection could operate (Fleischer 1983a, b, Burns and Zink 1990, Retzlaff 1989). For allozymes, the question of whether gene frequencies vary seasonally is relevant because of the controversy over whether or not allozymic variation is primarily neutral (Fuerst et al. 1977, Baker and Fox 1978, Barrowclough et al. 1985, Nei 1987, Zink and Watt 1987).

The House Sparrow (*Passer domesticus*) has been analyzed for geographic variation in both allozymes and morphology (reviewed by Parkin 1987). Seasonal variation in morphology attributed to natural selection has been reported by Fleischer and Johnston (1982) for House Sparrows in Manhattan, Kansas. However, there was no seasonal variation in allozymes in the House Sparrow populations from Kansas (Fleischer 1983a, b), and none was found in a similar study on House Sparrows collected in North Dakota (Retzlaff 1989). During 1987-1988, we studied a population of House Sparrows in Baton Rouge, Louisiana, a site with considerably milder winters than Kansas or North Dakota. Baton Rouge winters consist of short cold periods usually lasting for only a few days, and freezing temperatures are recorded only a few times each winter. Temperatures for the 1987-1988 winter followed this pattern, although the mean daily minimum temperature for January 1988 was 2.5°C, which is 2° colder than the long-term monthly mean (N.O.A.A. 1987, 1988). We tested the null hypothesis that recruitment and over-winter survival in this House Sparrow population was independent of an individual's genotype at 29 allozyme loci.

We used mist nets and traps to collect 186 House Sparrows from several sites around the campus of Louisiana State University (LSU), Baton Rouge. All

individuals were collected between September 1987 and June 1988 and prepared as skeletons, which are housed at the Museum of Natural Science, LSU. From each individual, samples of liver, heart and breast muscle were collected and stored at -76°C. Protein electrophoresis followed standard techniques (Selander et al. 1971, Harris and Hopkinson 1976, Zink 1986). During electrophoretic analyses, no attempt was made to sort individuals by date of collection, sex, or age classes, to prevent biased interpretation of the data. In an initial screening, 28 individuals were surveyed for 30 loci, and the following loci (acronyms follow Gerwin and Zink 1989) had little or no variability (frequency of the common allele > 95%): ACON1, ACON2, ADH, ALD, EAP, ESTD, FUMH, GOT1, GOT2, αGPD, GPT, GR, Hb, HK, LDH1, LDH2, ME, MDH1, MDH2, MPI, SDH, SOD1, SOD2. All 186 individuals were surveyed for the six loci most variable: sLDH, mLDH, PGM, 6PGD, and peptidases LA1 and LGG. An additional locus, purine nucleoside phosphorylase (NP), appeared to show substantial variation; however, after a large series of reruns on all individuals, scoring was too inconsistent and the locus was excluded from the analysis.

For analysis, specimens were combined from the several collecting sites (located up to 8 km apart) because no significant differences in heterozygosity or gene frequencies could be detected among sites for any locus. Specimens, divided by sex and age, were divided into three temporal categories: fall (September to 14 December 1987, $n = 64$); winter (24 January to 13 February 1988, $n = 43$); and summer (May and June 1988, $n = 79$). All birds in which the skull was less than 90% ossified were considered immatures. By the end of December, hatching-year (HY) birds probably have completely ossified skulls (Pyle et al. 1987). All birds in the winter sample had ossified skulls, so these groups likely include some HY birds.

Heterozygosities (H) were calculated by direct count for each locus and summed over all loci for each sample category. G -tests were performed on genotype

frequencies across each locus for all age classes and temporal groupings using the computer program GENESYS written by K. W. Corbin. We tested the distribution of allele frequencies for the 28 individuals surveyed for 29 loci (excluding NP) for fit to predictions of the mutation-drift theory (infinite alleles-constant mutation model, IC; Fuerst et al. 1977, Chakraborty et al. 1980) using a computer program written by G. F. Barrowclough (Barrowclough et al. 1985). A Kolmogorov-Smirnov test was used to examine the null hypothesis that the observed distribution of alleles was consistent with the distribution predicted by the IC model (Barrowclough et al. 1985). Failure to reject the null hypothesis suggests that mutation and genetic drift are sufficient to explain the maintenance of genetic variation within populations.

All groups exhibited a predominant allele at each locus (Table 1). Of the polymorphic loci, mIDH and sIDH exhibited two alleles, and the other four loci exhibited a rare third allele, which was pooled with the other uncommon allele. For the 28 individuals surveyed for 29 loci, $H = 0.04$, a typical value for birds (Corbin 1987). In females, overall H for the six variable loci increased slightly through the winter, although only variation at LGG was responsible for this trend (and variation at LGG was not statistically significant; $G = 20.9$, $df = 18$, $P > 0.05$). There is no trend for H in males across loci. Genotypic frequencies for each age and sex group over the three seasonal periods for the six variable loci exhibit some variation (Table 1). However, only variation at mIDH was significant ($G = 29.6$, $df = 18$, $P < 0.05$; Table 1). Winter males, spring adult males, and winter females exhibited proportionately fewer heterozygotes for mIDH than observed in other classes. The distribution of alleles in the 28 individuals surveyed for 29 loci did not differ significantly from that predicted by the mutation-drift (IC) model (Kolmogorov-Smirnov $D_{\max} = 0.10$, $P > 0.05$).

To examine further the nature of variation at mIDH, we calculated observed and expected gene frequencies for juvenile birds from spring 1988 and the potential parents of the spring 1988 juveniles, including all fall and winter birds from 1987 (first-year House Sparrows often breed [Summers-Smith 1963]). Observed and expected genotype frequencies did not differ significantly ($X^2 = 0.93$, $P = 0.628$; Table 2). Genotype proportions for the mIDH locus for both classes appear to be in Hardy-Weinberg equilibrium.

Fleischer et al. (1983) found that heterozygosity at several loci, including mIDH, was correlated negatively with morphological variability in Kansas House Sparrows. They suggested that heterozygosity per se may be adaptive because the presence of two alleles buffers an individual against environmental extremes (Lerner 1954). If a buffering effect due to heterozygosity at mIDH were occurring in House Sparrows, one would predict that heterozygosity would increase over the course of the winter. However, three studies

(Fleischer 1983a, Retzlaff 1989, this study) did not find significant over-winter increases in heterozygosity. Heterozygosity per se is not directly heritable. Therefore, the fitness effect of H on survival is indirect, if an effect occurs at all. The conformance of our data, and those for other avian species, to predictions of the IC model indicates that stochastic factors (e.g. genetic drift) are sufficient to explain the maintenance of genetic variation at enzyme-coding loci (Barrowclough et al. 1985). We suggest that allozymic heterozygosity per se is of little or no adaptive value in House Sparrows, as has been suggested for other species (Zink et al. 1985, Zink and Watt 1987).

Given the mild winter climate of Louisiana relative to Kansas and North Dakota, perhaps seasonal variation in allozyme frequencies should not be expected in the population we studied. Although no studies of geographical variation in cold tolerance in House Sparrows have been undertaken, seasonal acclimatization has been found within some populations (Barnett 1970, Blem 1980). If higher heterozygosity enhances fitness, heterozygosity should increase in more harsh environments (such as populations from North Dakota, England and New Zealand compared to southwestern Europe and Australia; Retzlaff 1989, Parkin and Cole 1984), but it does not. However, low levels of natural selection might not be detected by our survey, and we encourage other such studies to test the generality of our conclusion with larger sample sizes.

Burke (1984) established Mendelian inheritance of alleles at mIDH in House Sparrows breeding in England. Our results show significant variation in heterozygosity at mIDH between the classes studied; however, we find no pattern in the variation that implicates natural selection. The lowest heterozygosity for either sex was observed in winter birds collected after the colder-than-usual January, contrary to the prediction of enhanced survival due to heterozygosity. Segregating data by age class also allowed us to evaluate the notion that selection operates across the winter. If natural selection favored heterozygosity at mIDH (e.g. overdominance), the winter sample should have shown departures from Hardy-Weinberg equilibrium, which it did not. Although one generation of random mating would restore Hardy-Weinberg equilibrium in the offspring, both potential parents and juveniles show similar heterozygosities, gene frequencies, and conformance to predictions of Hardy-Weinberg equilibrium (Table 2). Genotypes of the juvenile cohort were a random sample of those in the breeding population, indicating no effects of selection in early development.

Our study and others on House Sparrow populations elsewhere (Australia, Manwell and Baker 1975; New Zealand, Parkin and Cole 1985; England, Parkin and Cole 1984; North America, Fleischer 1983a, Retzlaff 1989) have revealed that heterozygosity at the mIDH locus is unusually high ($H = 0.40$) compared

TABLE 1. Genotype frequencies and heterozygosity estimates for six variable loci in three seasonal samples of House Sparrows separated into sex and age categories. Sample sizes for each group shown in parentheses. G-test scores (df = 18) indicate amount of heterogeneity between samples for given locus.

Genotype	sIDH	mIDH	PGM	6PGD	LA	LGG	H ^a
Fall adult males (22)							
AA	0.82	0.33	0.91	1.00	0.91	0.95	
BB	0.00	0.08	0.00	0.00	0.00	0.00	
AB	0.18	0.59	0.09	0.00	0.09	0.05	0.167
Fall juvenile males (19)							
AA	0.95	0.21	0.89	1.00	0.89	0.95	
BB	0.00	0.16	0.00	0.00	0.00	0.00	
AB	0.05	0.63	0.11	0.00	0.11	0.05	0.158
Winter males (24)							
AA	1.00	0.58	0.88	0.83	0.96	1.00	
BB	0.00	0.09	0.04	0.04	0.00	0.00	
AB	0.00	0.33	0.08	0.13	0.04	0.00	0.167
Summer adult males (14)							
AA	0.86	0.71	0.86	0.93	0.93	1.00	
BB	0.00	0.08	0.00	0.00	0.00	0.00	
AB	0.14	0.21	0.14	0.07	0.07	0.00	0.107
Summer juvenile males (28)							
AA	0.86	0.43	0.83 ^b	0.86	1.00	1.00	
BB	0.00	0.00	0.00	0.04	0.00	0.00	
AB	0.14	0.57	0.17	0.10	0.00	0.00	0.168
Fall adult females (9)							
AA	1.00	0.55	1.00	1.00	0.89	1.00	
BB	0.00	0.00	0.00	0.00	0.00	0.00	
AB	0.00	0.45	0.00	0.00	0.11	0.00	0.093
Fall juvenile females (14)							
AA	1.00	0.38 ^b	0.93	1.00	1.00	1.00	
BB	0.00	0.08	0.00	0.00	0.00	0.00	
AB	0.00	0.54	0.07	0.00	0.00	0.00	0.096
Winter females (19)							
AA	0.84	0.74	0.74	0.95	0.84	0.89	
BB	0.00	0.05	0.11	0.00	0.00	0.00	
AB	0.16	0.21	0.15	0.05	0.16	0.11	0.140
Summer adult females (12)							
AA	0.83	0.25	0.91 ^b	0.92	0.83	0.67	
BB	0.00	0.25	0.00	0.00	0.00	0.00	
AB	0.17	0.50	0.09	0.08	0.17	0.33	0.211
Summer juvenile females (25)							
AA	0.84	0.46 ^b	0.96 ^b	0.88	0.88	0.88	
BB	0.04	0.12	0.00	0.00	0.00	0.00	
AB	0.12	0.42	0.04	0.12	0.12	0.12	0.158
H ^c	0.10	0.42	0.10	0.06	0.08	0.05	0.140 ^d
G	19.205	29.561 ^e	14.689	18.465	10.565	20.923	

^a Heterozygosity across loci within sex/age classes.

^b Genotype of one individual in sample could not be determined for locus.

^c Heterozygosity per locus across sex/age classes.

^d Heterozygosity across all loci and sex/age classes.

^e Significant at $P < 0.05$.

to this or any other locus investigated in traditional avian studies (not employing isoelectric focusing). St. Louis and Barlow (1988) found heterozygosities at mIDH of 0.3 in five of six populations of a presumably

closely related congener to the House Sparrow, the Eurasian Tree Sparrow (*Passer montanus*). This suggests that, within *Passer*, there is a phylogenetic component to this high heterozygosity, because Evans

TABLE 2. Genotypes for mIDH locus separated into potential parents (see text) and summer juveniles. Expected values for juveniles calculated from potential parents assuming random mating.

Genotype	Potential parents	Summer 1988 juveniles	
		Expected	Observed
AA	65	21.7	23
AB	57	25.7	26
BB	11	4.6	3

(1987) reported only 19% (12 of 63) of studies of mIDH in birds found polymorphism at the locus. When a locus that is not generally variable in most bird species is highly heterozygous in one species, it merits study. The high levels of mIDH polymorphism in House Sparrows might simply be a random result of the distribution of heterozygosity across loci. Possibly age-dependent effects influence mIDH gel patterns (and mimic a pattern of inheritance), but this remains to be tested. We suggest that natural selection is an unlikely influence on this locus.

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Experimental Evidence for Importance of Male Parental Care in Monogamous House Wrens

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One hypothesis for the predominance of monogamous pair bonds among birds is that biparental care is essential if individuals are to raise any young to independence (Wittenberger and Tilson 1980). To test this hypothesis and to assess the importance of male care in determining reproductive success, researchers have removed males from breeding pairs in a number of monogamous bird species (reviews in Wolf et al. 1988, Bart and Tornes 1989, Dunn and Hannon 1989). In one such study on an Ohio population of House Wrens (*Troglodytes aedon*), Bart and Tornes (1989) removed males from territories approximately one-third of the way through the nestling stage. Under normal breeding conditions, "widowed" females raised as many young as control females from intact pairs. Widowed females experienced reduced fledging success only during one unusually cold, wet spring.

Because Bart and Tornes (1989) did not remove males until several days after eggs hatched, they could not assess the importance of the male's presence during the earliest part of the nestling stage. However, male feeding assistance may be most critical at this time because females must provide the heterothermic young with heat during extended periods of brooding. Nestling House Wrens cannot thermoregulate as a brood until sometime between the fifth and tenth day of the nestling stage, depending on brood size (Dunn 1976). Until this time, the need to brood young may limit a female's ability to compensate fully for a lack of male parental effort (Wittenberger and Tilson 1980, Clark and Ricklefs 1988). Here we assess the importance of having male care during the early part of the nestling stage by examining the reproductive success of female House Wrens whose mates were removed two to four days before eggs hatched.

We conducted this study in 1990 on cattle ranches near Big Horn, Sheridan Co., Wyoming (44°40'N, 106°56'W). All wrens studied nested in wooden boxes mounted on greased poles to reduce nest predation.

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