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Relationships among allozyme heterozygosity, morphology and lipid levels in house sparrows during winter

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(With 1 figure in the text)

House sparrows (*Passer domesticus*) were collected during cold (43 individuals) and warm (31 individuals) periods in February 1982 from farms near Lawrence, Kansas. Fat scores (from flank, rump and furcula) and body mass were measured from the carcasses, which were then reduced to skeletons. Heterozygosity was determined by electrophoresis of three polymorphic allozymes. Pectoral lipid content was determined by petroleum ether extraction. Principal component (PC) analysis was conducted on 14 skeletal measurements. Summed fat score, body mass and pectoral lipid content served as dependent variables in three multiple regressions. Sex, period (cold versus warm), number of heterozygous loci (0–3), and scores of PC1, PC2 and PC3 were independent variables. Each of the dependent variables was also included as an independent variable in regressions in which they were not the dependent variable. Pectoral lipid content was significantly related only to the number of heterozygous loci ($R^2 = 0{\cdot}18$, $P = 0{\cdot}008$). Summed fat score differed between sexes and periods ($P = 0{\cdot}012$ and $0{\cdot}001$, respectively) when body mass was included in the regression ($P < 0{\cdot}001$). Body mass was related to summed fat score as above, and also to body size (PC1, $P < 0{\cdot}0001$) and period ($P = 0{\cdot}014$). Females exhibited a positive regression between body size and summed fat score ($P = 0{\cdot}007$). Body size (PC1) was greater for both sexes in the sample from the warm period ($P = 0{\cdot}022$), suggesting that selection for increased body size may have occurred. Increased metabolic efficiency of heterozygous enzymes or individuals is suggested as an explanation for the observed relationship between heterozygosity and intramuscular lipid level.

Contents

Introduction

Species of birds that overwinter in cold climates often exhibit a number of adaptations to the rigors of winter. These include the *ability* to make physiological adjustments, such as increased thermogenic capacity (West, 1972; Marsh & Dawson, 1988) and deposition of fat (Blem & Shelor, 1986; Dawson & Marsh, 1986); the ability to make behavioural adjustments, such as caching food, flocking, or huddling (see Haftorn, 1988 for review); and morphological adaptations, such as modifications in body size or core-to-limb ratios (Johnston & Selander, 1964, 1971; James, 1970; Fleischer & Johnston, 1982; Zink & Remsen, 1986). Two other aspects whose roles in winter survival of birds are relatively unstudied are genetic and biochemical variability.

Studies of house sparrows (*Passer domesticus*) have addressed a number of these potential adaptations to cold winter environments. House sparrows clearly increase fat stores during winter (Barnett, 1970; Blem, 1980; but see Palokangas, Nuuja & Koivusaari, 1975), but not to the degree seen in many other ground foraging passerines (Helms & Drury, 1960; Dawson & Marsh, 1986). Likewise, Barnett (1970) and Blem (1973) have shown that winter acclimatized sparrows have greater cold tolerance than summer acclimatized individuals. Barnett (1970) also demonstrated that the thermogenic capacity of sparrow muscle and brain tissue *in vitro* was higher in winter acclimatized birds. Behavioural responses to winter conditions include decreased individual distance and increased dominance interactions as temperatures drop (Cink, 1977).

Morphological adaptations of house sparrows to winter climate have been inferred from analyses of geographic variation (Johnston & Selander, 1964, 1971, 1973) and overwinter mortality (Bumpus, 1899; Rising, 1973; Johnston & Fleischer, 1981; Fleischer & Johnston, 1982, 1984; Retzlaff, 1989). Results of nearly all the studies cited above suggest that *severe* winter conditions select for large-sized males. In contrast, the direction of selection on female body size is less clear, with some studies providing evidence of only stabilizing or no selection (Bumpus, 1899; Rising, 1973; Retzlaff, 1989) and others indicating directional selection for small size (Johnston & Fleischer, 1981; Fleischer & Johnston, 1982, 1984).

Studies of overwinter changes in genetic or biochemical variation in birds have only been conducted by Fleischer (1983*a*) and Retzlaff (1989), who assessed modifications in allozyme allele frequencies and heterozygosity in house sparrow populations in Kansas and North Dakota, respectively. Neither found significant changes in spite of large sample sizes. However, Fleischer, Johnston & Klitz (1983) found that heterozygosity was negatively correlated with morphological variability in sparrows, suggesting that heterozygosity of enzymes or individuals may buffer against environmental extremes (*sensu* Lerner, 1954; Mitton & Grant, 1984; Allendorf & Leary, 1986). Additional studies addressing this issue are needed.

In the study reported here, we take an integrated approach and attempt to identify the environmental and individual factors responsible for variation in body condition in house sparrows in Kansas. Fat has been considered a good indicator of overall condition in wintering birds (Blem & Shelor, 1986; Dawson & Marsh, 1986). We thus measured lipid levels, morphological characters and allozyme heterozygosity for individuals collected during a cold and a warm period during February 1982 to identify which traits were most closely related to fat levels and, thus, perhaps, to survival.

Methods

Field sites and collections

House sparrows were collected during February 1982 from 3 closely located farms in Leavenworth County, Kansas (Skeet, Swearingen and Hemphill; see Fleischer *et al.*, 1984 and Lowther, 1979 for maps and details on the farms). All sparrows were collected within the last hour of daylight during 2 periods: a cold period (2–8 February 1982) and a warm period (17–27 February 1982). During the cold period daily highs averaged $-3{\cdot}5$ °C (S.D. $=5{\cdot}91$, $n=7$ days) and daily lows averaged $-15{\cdot}2$ °C (S.D. $=5{\cdot}84$, $n=7$ days). During the warm period daily highs averaged 13·3 °C (S.D. $=7{\cdot}49$, $n=11$) and daily lows averaged 0·7 °C (S.D. $=4{\cdot}01$, $n=11$). About 15 cm of snow covered the ground during the last 4 days of the cold period and no snowcover was present during the warm period. Over the entire winter, conditions were close to average, with December means about 2 °C higher, and January and February means about 2 °C lower than average (NOAA, 1982). Records showed that the cold collection period was the coldest 7-day period over the winter.

House sparrows were collected by placing mist-nets near areas where they tended to concentrate (e.g. hedgerows, barns, chicken coops). Birds were killed, weighed and returned to the museum where they remained frozen (-20 °C) until they were processed.

Specimen preparation and measurement

Specimens were thawed, skinned and scored for fat. Fat scores were estimated for furcula, flank and rump on a scale of 0–3, with 0 indicating no fat, and 3 indicating that fat overflowed the region. All fat scores were estimated by one individual (MTM). The 3 fat scores were summed for each bird for subsequent analyses. Tissues were removed from each bird: livers and kidneys were placed in tubes and stored frozen at -30 °C until electrophoresis; the right *pectoralis* and *supracoracoideus* muscles were removed and stored at -20 °C for eventual lipid extraction. Carcasses were dried and macerated in water with detergent at the Kansas University Museum of Natural History until most or all tissue had been removed from the bone. Skeletons were bleached in 5% ammonia and air-dried.

Skeletal measurements were made with steel callipers to the nearest 0·1 mm. A suite of 14 skeletal elements were measured, including 5 head elements, 4 body core elements, and 5 limb elements (Table I; see Johnston & Selander, 1971 for precise description of the skeletal measurements). All measurements were taken by one individual (RCF). Specimens are currently housed in the Kansas University Museum of Natural History (accession number 7227).

Allozyme electrophoresis

We used starch gel electrophoresis to identify electromorphs at 3 loci: esterase-1 (EST-1), isocitrate dehydrogenase-2 (IDH-2) and isocitrate dehydrogenase-3 (IDH-3). These 3 were found to be polymorphic in a previous study from the same study area (Fleischer, 1983*a*, *b*). Protocols for the preparation of tissue extracts, recipes for buffers and protein stains, and electrophoresis protocols for these 3 loci can be found in Fleischer (1983*a*: Appendix 1). All electromorphs were scored by one individual (RCF). Electromorphs were interpreted as genotypes: such an interpretation has been verified via examination of crosses for IDH-2 in house sparrows by Burke (1984), and is likely for the other 2 loci.

Allele frequencies were computed from the genotype frequencies and comparisons were made between the actual genotype frequencies and those expected under Hardy-Weinberg equilibrium. We found no significant deviations from Hardy-Weinberg. Allele frequencies were compared to those documented for the same sites in 1978–79 (Fleischer, 1983*a*, *b*) by contingency table analysis. Again, no differences existed between our samples and those collected in 1978–79. Individual heterozygosity was calculated simply as the number of heterozygous loci of the three.

Lipid extraction

The excised flight muscles were oven-dried to constant mass and pulverized roughly with mortar and pestle. Lipids were then extracted from the pulverized muscle using petroleum ether in a modified Soxhlet procedure (by MTM). The lipid extracted samples were then dried, weighed, and subjected to additional extractions until constant mass was obtained. The mass difference between dry and lipid-free (=lean) dry samples was the ether-extractable muscle lipid content. We defined pectoral lipid content as the percentage of the dry muscle mass composed of ether-extractable lipid (lipid mass/dry muscle mass).

Data analysis

All statistical analyses were conducted using SAS (SAS Institute, 1987). Skeletal measurements were log transformed before principal component analysis. PC scores for PC1, PC2 and PC3 (PC1–3) were computed for each individual and used in subsequent analyses. We initially compared the means of summed fat score, body mass, pectoral lipid content and PC1–3 scores between the sexes and periods (warm versus cold) using 2-way analysis of variance. Summed fat score, body mass and pectoral lipid content then served as dependent variables in 3 multiple regressions. Sex, period, number of heterozygous loci (0–3), and scores of PC1–3 were used as independent variables in these regressions. In addition, the dependent variables were included as independent variables in regressions in which they were not the dependent variable. We used the backward elimination procedure in each multiple regression to find the subset of independent variables that contributed significantly ($P<0{\cdot}05$) to variation in the dependent variable in question.

Results

Principal component analysis

Eigenvectors and the proportion of the total variation explained for each of the first three principal components are shown in Table I. Eigenvalues for the first three axes all exceeded 1·5 and together accounted for about 75% of the variation in the original suite of measurements. Each PC

TABLE I

The eigenvectors and percentage of total variance in skeletal measurements explained by the first three principal components

Variable	PC1	PC2	PC3
Premaxilla length	0·199	0·478	−0·042
Skull width	0·237	0·084	0·229
Skull length	0·272	0·351	−0·121
Mandible length	0·259	0·391	−0·054
Dentary length	0·201	0·442	−0·016
Coracoid length	0·308	−0·186	0·091
Sternum length	0·252	−0·129	0·383
Keel length	0·166	−0·164	0·555
Sternum depth	0·195	0·128	0·471
Humerus length	0·334	−0·232	−0·099
Tibiotarsus length	0·324	−0·183	−0·263
Ulna length	0·317	−0·250	−0·078
Femur length	0·288	−0·121	−0·343
Tarsometatarsus length	0·314	−0·183	−0·199
Proportion of variance explained	0·492	0·144	0·108

axis was interpreted on the basis of the sign and magnitude of the eigenvectors for each variable. Because of the positive and similar-sized eigenvectors (Table I), we interpret PC1 as a variable summarizing overall individual size. PC2 has high positive eigenvectors for skull measurements and negative eigenvectors for most of the core and limb measurements (Table I). Thus we interpret PC2 as a variable indicating relative head size. PC3 has high positive eigenvectors on core measurements and skull width, and negative eigenvectors primarily on leg measurements (Table I). Thus we interpret PC3 as a variable reflecting a body core to leg ratio. These results generally conform to those documented in previous studies of house sparrow skeletal morphology (Johnston & Selander, 1971; Fleischer & Johnston, 1984).

Differences between the sexes and periods

Two-way analysis of variance of pectoral lipid content, summed fat score, and body mass revealed significant variation between periods only for summed fat score (Table II). Fat scores in both sexes were higher during the warm period. Pectoral lipid content did not vary significantly between sexes or periods, and averaged about 8·5% of dry pectoral muscle mass (Table II). While the two-way ANOVAs did not reveal statistically significant effects of sex on any of the variables, we did find a significant effect of sex on summed fat score, and of period on body mass, when other variables were controlled using multiple regressions (see below).

Two-way analysis of variance revealed significant differences in PC1 and PC2 (Table II). Males had significantly higher PC1 scores than females, as expected, and in both sexes, PC1 scores were

TABLE II

Means, standard deviations, and results of analyses of variance comparing summed fat score, pectoral lipid content, body mass and PC1-3 between sexes and periods of collection

Sex	Period	N	Fat score	Lipid content	Body mass
Male	Cold	19	5·34 ± 0·85	8·26 ± 1·69	31·6 ± 2·28
Male	Warm	20	6·33 ± 1·03	8·70 ± 1·35	31·2 ± 1·88
Female	Cold	23	5·95 ± 1·07	8·41 ± 1·50	30·8 ± 1·93
Female	Warm	11	6·77 ± 1·01	8·15 ± 1·02	31·5 ± 1·50
Two-way ANOVA:					
Model F			5·14**	0·45, n.s.	0·62, n.s.
Sex			2·07, n.s.	0·22, n.s.	0·58, n.s.
Period			13·24***	0·15, n.s.	0·01, n.s.
Sex/period			0·10, n.s.	0·97, n.s.	1·27, n.s.
Sex	Period	N	PC1	PC2	PC3
Male	Cold	19	−0·04 ± 2·28	−0·72 ± 1·27	0·14 ± 1·05
Male	Warm	20	1·53 ± 2·90	−0·23 ± 1·45	0·33 ± 1·03
Female	Cold	23	−1·25 ± 2·53	0·73 ± 1·39	−0·08 ± 1·51
Female	Warm	11	−0·07 ± 1·52	0·17 ± 1·05	−0·66 ± 1·06
Two-way ANOVA:					
Model F			4·40**	4·29**	1·68, n.s.
Sex			7·56**	10·30**	3·13, n.s.
Period			5·53*	0·01, n.s.	0·26, n.s.
Sex/period			0·11, n.s.	2·55, n.s.	1·64, n.s.

* $P < 0·05$; ** $P < 0·01$; *** $P < 0·001$

larger during the warm (later) period. PC2 scores differed among the sexes, indicating that males had relatively smaller heads in comparison to their body cores than did females.

Multiple regressions of body condition

Using the backward elimination procedure we found that from one to three variables contributed significantly to variation in the three dependent variables (Table III). We specifically address each regression below:

Pectoral lipid content. In accordance with our previous results, sex and period did not influence the variation in pectoral lipid content. In addition, pectoral lipid content was not correlated with summed fat score, contrary to some previous findings for other species (e.g. Blem & Shelor, 1986). Only one variable, the number of heterozygous loci, contributed significantly to the regression, but a second, PC3, was close to significance, and is included in Table III. The two variables together accounted for about 18% of the variation in pectoral lipid content. Lipid content was related positively to heterozygosity (Table III), showing a similar relationship in both warm and cold periods (Fig. 1). Lipid content exhibited a negative relationship to PC3 (Table III). Thus birds with relatively small body cores and long legs tended to have higher pectoral lipid contents.

Summed fat score. The multiple regression indicated that both sex and period significantly influenced variation in summed fat score (Table III). As indicated in Table II, fat scores were highest during warm periods and in females. The former is in contrast to results of virtually all previous studies of overwintering birds (Dawson & Marsh, 1986). In addition, body mass (g) was positively and significantly related to summed fat score. The three variables together explained about 34% of the variance in summed fat score. The significant sex effect was contrary to the results of the two-way analysis of variance (Table II), and appeared to be related to the positive relationship between summed fat score and body mass. Once body mass was controlled in the regression model the difference between the sexes in summed fat score became significant.

Because of the significant effect of sex on summed fat score, we conducted additional multiple regression by sex. For males, three variables contributed significantly to the variation in summed fat score ($R^2 = 0{\cdot}575$, $P < 0{\cdot}0001$); period ($P = 0{\cdot}002$); number of heterozygous loci ($P = 0{\cdot}008$); and

TABLE III

Results of the multiple regression analyses for pectoral lipid content, summed fat score and body mass

Variable	Regression coefficient	F-value	*P*
(a) Pectoral lipid content: $R^2 = 0{\cdot}181$; *d.f.* = 2, 55; $P = 0{\cdot}004$			
No. heterozygous loci	0·639	7·67	0·008
PC3	−0·277	3·80	0·056
(b) Summed fat score: $R^2 = 0{\cdot}338$; *d.f.* = 3, 54; $P = 0{\cdot}0001$			
Sex	0·640	8·35	0·012
Period	0·825	11·30	0·001
Body mass	17·33	13·99	0·0004
(c) Body mass: $R^2 = 0{\cdot}479$; *d.f.* = 3, 54; $P = 0{\cdot}0001$			
Period	−0·014	6·42	0·014
PC1	0·006	32·86	0·0001
Summed fat score	0·007	8·56	0·005

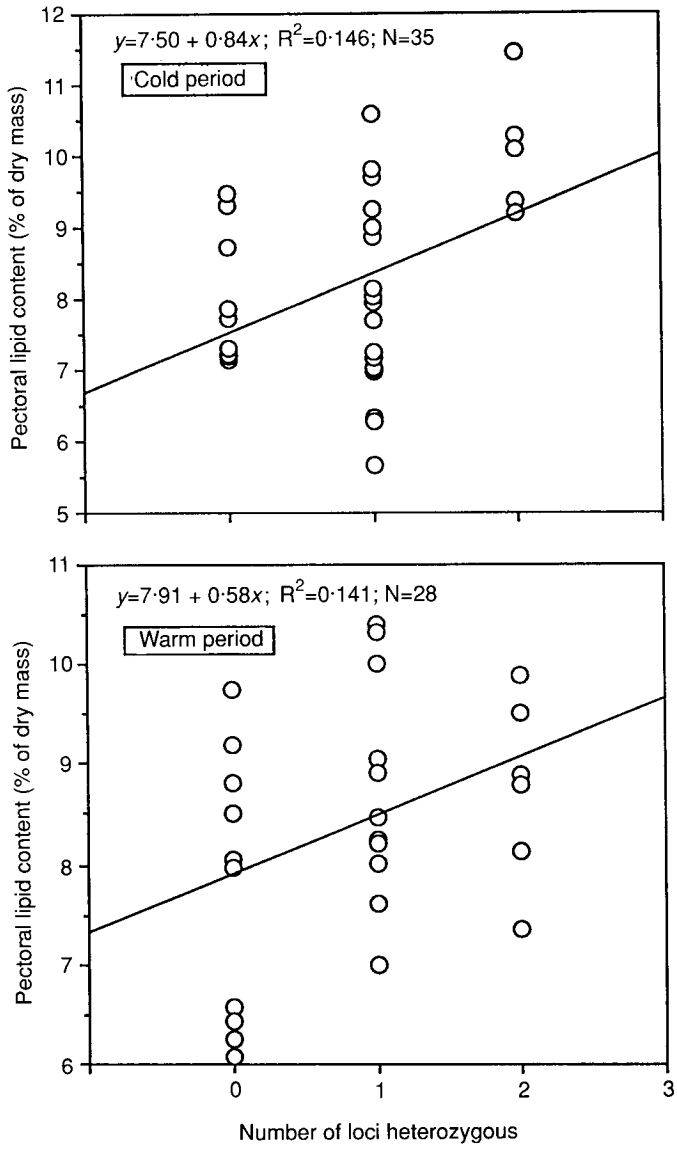

FIG. 1. Relationship between pectoral lipid content and the number of heterozygous loci for each individual in (a) the cold and (b) the warm periods of collection.

body mass ($P=0{\cdot}0004$). Highly heterozygous, large males collected during the warm period had higher values of summed fat score. For females, only PC1 ($P=0{\cdot}007$) and PC2 ($P=0{\cdot}039$) significantly explained variation in summed fat score ($R^2=0{\cdot}327$, $P=0{\cdot}007$). In this case, females of larger overall body size (PC1) with relatively small heads (PC2) had higher fat scores.

Body mass. About 48% of the variation in body mass was explained by three variables. Of these, PC1 (i.e. body size) was the most important (Table III). As we found in the regression of summed

fat score above, body mass was also significantly related to summed fat score. Lastly, birds were heavier during the warm period, which is reflected by their larger fat and PC1 scores during that period.

Discussion

Previous studies of house sparrows in Kansas have suggested that overwinter selection can be important in shaping sparrow morphology (Johnston & Fleischer, 1981; Fleischer & Johnston, 1984). We initiated this study to examine three general hypotheses concerning physiological condition (as assessed by fat level) in house sparrows and its relationships to winter stress, body size and allozyme heterozygosity. Ultimately we hoped to relate our findings to overwinter survival in house sparrows in an attempt to understand better the basis for overwinter selection.

The first of these was to examine the relationship between environmental conditions and endogenous energy storage. Our prediction was that if food is limiting during winter then lipid storage should be depressed during energetically stressful periods. This was verified. Our second prediction, which was based on a finding of apparent overwinter selection against large female and small male sparrows (Johnston & Fleischer, 1981; Fleischer & Johnston, 1982, 1984), was that lipid stores should be highest among small females and large males. We found no support for this hypothesis, and, in fact, patterns in females ran opposite to our predictions. Finally, we examined the possible role of allozyme heterozygosity as a factor in winter energy storage or metabolism. Based on the assumption that highly heterozygous individuals are better buffered against varying environments (Lerner, 1954), or that allozymes in a heterozygous state are more efficient at processing substrate (Trehan & Gill, 1987), we predicted a positive relationship between allozyme heterozygosity and fat levels. In general, our findings support this hypothesis.

Fat and winter stress

The storage of large quantities of energy in the form of fat is one of the most important adaptive responses to energy stress displayed by birds that inhabit cold, winter environments (Blem, 1976; Marsh & Dawson, 1988). In nearly all species examined, the period of maximum lipid storage corresponds to the period of the year when days are shortest and/or environmental temperatures are lowest (Blem & Shelor, 1986; Dawson & Marsh, 1986; Marsh & Dawson, 1988). Dawson & Marsh (1986) have interpreted this as an evolved response to a consistently high probability of energy stress during cold winter periods, proximately cued by photoperiod. Levels of storage vary among species, and this has been interpreted to reflect a balance between the need to store energy yet also minimize wing loading and thus manoeuvrability in response to predator pressure (Blem & Shelor, 1986; Lima, 1986). Ground foragers, because their food supplies can be covered by snow, are predicted to carry the greatest fat reserves, and patterns among species conform to this prediction (Rogers, 1987).

Barnett (1970), Palokangas *et al.* (1975) and Blem (1980) found significant variation in fat reserves over the annual cycle in house sparrows, but as Blem (1980) emphasized, the increase during the winter months was much lower than that found for other species. He related this to their use of "altered microclimates around man's buildings and artificial food sources". Alternatively, house sparrows may lack the physiological mechanisms to store large amounts of lipid because in the subtropical regions, where they evolved relatively recently (Summers-Smith, 1988), such mechanisms were not necessary.

Within the winter season some north temperate ground-foraging species exhibit an inverse correlation between temperature and fat reserves (Blem & Shelor, 1986; Dawson & Marsh, 1986; Murphy & Bakken, MS). We found, however, that house sparrows had significantly higher fat scores during the warm period. These differences occurred in spite of the collection periods being only 10 days apart, emphasizing the immediate impact of weather on fat metabolism. We do not know, however, whether the lower fat scores were a result of the lower temperatures or the snowcover which occurred during the cold periods (four of seven days with 15 cm of snow on the ground). In either case, these findings are significant because they indicate that house sparrows are energy stressed under winter conditions. It may be that their failure to put on large mid-winter supplies of fat reflects an inability to adjust physiologically to winter conditions or that food resources are not as easily obtained by house sparrows as others have suggested (e.g. Blem & Shelor, 1986).

Body size and fat levels

Studies of overwinter change in house sparrow morphology by Johnston & Fleischer (1981; Fleischer & Johnston, 1982, 1984) suggested that large males and small females had a survival advantage over small males and large females during extremely cold winters. On the other hand, during relatively mild winters, little or no evidence for such selection was found (Rising, 1973; Fleischer & Johnston, 1984; Retzlaff, 1989). If condition reflects the probability of surviving overwinter, and if fat levels are indicative of overall condition, then the prediction follows that larger male and smaller female sparrows should have higher fat levels when winter conditions are severe.

We found, however, that *larger* female sparrows had higher fat scores than smaller ones regardless of the period of collection (i.e. severity of the weather). Unexpectedly, we also found that body size increased significantly in both males and females over the 10-day period between the collection of the cold and warm samples (but sample size for females in the warm period was only 11). The increase in size, if real, may have been caused by natural selection in the severe weather period.

Heterozygosity and lipid metabolism

Perhaps our most intriguing results were the positive relationships between heterozygosity at the three loci and pectoral lipid content and summed fat score for males. A few previous studies have found relationships between metabolic efficiency and heterozygosity (e.g. Koehn & Shumway, 1982; Garton, 1984; Garton, Koehn & Scott, 1984; Mitton, Carey & Kocher, 1986), but none that we know of has found differences in the use or storage of lipids. Furthermore, all of the other studies have concerned ectotherms.

As in the above studies relating heterozygosity to physiological measurements, and others on growth rates, morphological variance or asymmetry (see Mitton & Grant, 1984; Allendorf & Leary, 1986 for reviews), we do not know what may have caused the relationship. Higher metabolic efficiency could be caused by the enzymes themselves. There are some indications that when two copies of an enzyme exist, each with different optima of reaction (e.g. temperature, pH, etc.), heterozygotes may be most efficient at processing substrate into product (Trehan & Gill, 1987), and thus cause overdominance. This activity difference could ultimately be translated into increased lipid production or decreased lipid use (hence higher lipid stores). Isocitrate dehydrogenases are directly involved in energy metabolism, as are many esterases.

Alternatively, the relationship may be caused by loci to which the allozyme loci are linked, or to a general level of heterozygosity that these three loci indicate (Mitton & Grant, 1984). It does not seem likely, however, that three loci would be able to indicate genome-wide heterozygosity (Mitton & Pierce, 1980; Chakraborty, 1981; Chakraborty & Ryman, 1983), even given a high degree of linkage.

Assuming that lipid content is important for overwinter survival, then whichever causal explanation applies, allozyme heterozygosity should increase overwinter. The two studies which have addressed this issue in house sparrows (Fleischer, 1983*a*; Retzlaff, 1989) have not found such an increase. Further field studies are needed, but perhaps the only way to confirm these relationships and to identify their causes may be to conduct controlled laboratory experiments on individuals of known genotype (as in Vrijenhoek & Wetherington, MS).

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