

A test of the good-genes-as-heterozygosity hypothesis using red-winged blackbirds

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Brown recently proposed that the “good genes” that females pursue when choosing mates may be individual heterozygosity because more heterozygous mates sire offspring with higher fitness. Further, because heterozygosity might enhance developmental stability, males with more heterozygosity are recognized by the reduced fluctuating asymmetry (FA) of their bilaterally paired traits. We used a point sample of 67 male red-winged blackbirds (*Agelaius phoeniceus*) to test two predictions of this hypothesis: (1) males with more heterozygosity have higher fitness, and (2) males with more heterozygosity have lower FA. We identified 7 polymorphic loci from an initial screening of 16 enzymes; 32 individuals were completely homozygous, and 35 individuals were heterozygous at at least 1 locus. Larger and older males realized higher mating success in this population, but neither size nor age was related to heterozygosity. Heterozygous males were not in better condition than homozygous males, nor were they less infected by hematozoa, lice, or mites. Among 1-year old males, epaulet length did not differ between homozygotes and heterozygotes, but among older males, heterozygotes did have longer epaulets. Homozygotes and heterozygotes did not differ in their mean FA scores for nine individual characters. Although the two groups of males did differ in composite FA, heterozygous males were less symmetrical. Interestingly, this difference was attributable to a single allele at the PGM-3 locus. Combined with previous results showing that FA was generally unrelated to male health, viability, parental care, social dominance, or mating success, the present results indicate that Brown's hypothesis does not explain mate choice or male quality in this population of red-winged blackbirds. *Key words*: fitness, fluctuating asymmetry, good genes, heterozygosity, isozymes, red-winged blackbirds. [*Behav Ecol* 10:619–625 (1999)]

The idea that female animals might choose males to enhance the genetic quality of their offspring has a long history in evolutionary theory, although for much of that history the idea has not been generally accepted (Andersson, 1994). However, the combination of both theoretical (e.g., Pomiankowski and Møller, 1995) and empirical (e.g., Petrie, 1994) support have helped move “good genes” ideas into the mainstream of evolutionary theory. Recently, Brown (1997) suggested that it was time for empiricists to shift their focus from asking whether females select males for their genetic quality, to determining the nature of that genetic quality. Furthermore, Brown (1997) gave direction to that focus by proposing that genetic quality might equate with genetic diversity, such that the traits preferred in males are predictive of heterozygosity. Here we test several predictions of this heterozygosity hypothesis using data collected from male red-winged blackbirds (*Agelaius phoeniceus*).

In principle, Brown's (1997) heterozygosity hypothesis is a clear departure from the general notion that male genetic quality is some absolute value, whereby each male in a population can be ranked according to that quality (Pomiankowski, 1990). If genetic diversification of offspring is the function of mate choice, then male quality ceases to be absolute. Rather, what constitutes the best mate for a given female will depend on her own genetic makeup, and thus will differ among females. In practice, however, Brown (1997) points out that for many animals, females may be unable to choose mates according to specific genetic markers. In this situation females should prefer more heterozygous males over less heterozygous males because the progeny they sire will be

genetically more diverse. Thus, males can be ranked along a heterozygosity continuum that is functionally equivalent to a continuum of absolute genetic quality, even though the quality that is valued is genetic diversity rather than specific genetic traits.

Brown (1997) linked his good-genes-as-heterozygosity hypothesis to another idea of burgeoning interest in behavioral ecology by suggesting that a preference for more heterozygous males could explain why females of a number of species appear to prefer more symmetrical males as mates (e.g., Harvey and Walsh, 1993; McLachlan and Cant, 1995; Møller, 1994, 1996; Simmons, 1995; Thornhill, 1992). Increased heterozygosity appears to be associated with increased developmental homeostasis, which in turn results in more symmetrical development of bilaterally paired traits (Palmer and Strobeck, 1986; Mitton, 1993). Furthermore, increased heterozygosity has been linked to higher viability (Allendorf and Leary, 1986; Lynch and Walsh, 1998). Therefore, by preferentially mating with more symmetrical males, females are likely to be mating with more heterozygous males, thereby producing more heterozygous, and thus more fit, offspring.

We used an existing sample of male red-winged blackbirds (Dufour and Weatherhead, 1996) to test several of the assumptions and predictions of Brown's (1997) hypothesis. First, we determined whether larger males are more heterozygous, based on the evidence that larger male red-winged blackbirds attract more mates in this socially polygynous species, resulting in higher reproductive success (Weatherhead and Boag, 1995), and larger males survive better between breeding seasons (Weatherhead and Clark, 1994). Second, we determined whether heterozygosity differs between the two identifiable age classes in our sample, second year (SY) and after second year (ASY) males. ASY males include individuals that have survived at least 2 and potentially up to 9 years in our study population (Weatherhead and Boag, 1997), whereas SY males have survived for only 1 year. If heterozygosity is

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Received 7 August 1998; revised 22 February 1999; accepted 7 March 1999.

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associated with enhanced viability, ASY males should be more heterozygous on average. Furthermore, age contributes positively to male extrapair mating success (Weatherhead and Boag, 1995), providing an additional reason to predict greater heterozygosity among older males according to Brown's (1997) hypothesis. Third, we assessed the relation between heterozygosity and two indices of male health: condition (mass corrected for body size) and infection by parasites (hematozoa and two types of ectoparasites). Finally, we determined whether the extent of fluctuating asymmetry (FA; random deviations from symmetry in paired characters) is negatively related to heterozygosity, using allozymes to quantify heterozygosity. We consider FA in both nonornamental traits and in an ornamental (secondary sexual) trait, as well as using a composite index of asymmetry that combines information across multiple characters.

We should make clear at the outset that results of an intensive investigation of correlates of FA in this population of red-winged blackbirds might argue against this species, or at least this population, being an appropriate subject for current purposes. We found no evidence that more symmetrical males were either healthier or more likely to survive the nonbreeding season (Dufour and Weatherhead, 1998a), that they were more successful in competing for territories, social mates, or extrapair mates (Dufour and Weatherhead, 1998c), or that they were more successful in competing for resources outside the breeding context (Dufour and Weatherhead, 1998b). Thus, in this population of red-winged blackbirds, FA does not appear to reveal anything about male quality or to be involved in mate choice. Nonetheless, there are also strong arguments for using red-winged blackbirds to test the heterozygosity hypothesis. First, Brown (1997) proposed the hypothesis as a general model of mate choice, thereby making all species in which mate choice seems likely to occur appropriate subjects to investigate. Second, our breadth of knowledge regarding FA and factors affecting fitness in this population will inform our interpretation of any patterns we discover. Finally, any of the various possible outcomes we might obtain (e.g., heterozygosity related to FA but not to fitness-related traits; heterozygosity related to fitness-related traits but not to FA; heterozygosity, FA, and fitness all unrelated) would provide important insights into the generality of Brown's (1997) hypothesis.

METHODS

The sample of birds we used in this study is the same sample Dufour and Weatherhead (1996) used to demonstrate that there was significant concordance in FA measured on different bilateral traits within individuals, and that they subsequently used as part of their analysis to assess the relation between FA and various measures of health and viability (Dufour and Weatherhead, 1998a). Given that detailed methods are described in those papers, we repeat only the relevant aspects of our methods in abbreviated form here.

Sampling birds

We collected the sample of 67 male red-winged blackbirds by capturing the birds in mist nets as they entered a prebreeding spring roost on three evenings in the week beginning 23 April 1993. The roost was approximately 10 km west of the Queen's University Biological Station in eastern Ontario (44°34' N, 76°20' W). Captured birds were transported immediately to the biological station, where we determined their age (SY or ASY), weighed them (± 0.5 g), took a blood smear for quantifying hematozoa, and then placed the bird for 5 min in a polyethylene bag containing a cotton bag soaked with chloroform. The chloroform killed the birds, whose carcasses were

required for skeletal analysis, and also caused ectoparasitic mites and lice to detach, allowing us to identify and count them (Martin, 1977). The birds were then frozen at -20°C . We sent the blood smears to the International Reference Centre for Avian Haematozoa, Memorial University, Newfoundland, for analysis.

We thawed the frozen birds and measured four external paired characters: tarsus length, wing chord, tail length, and epaulet length. We removed and refroze a sample of approximately 500 mg of heart, liver, and skeletal muscle tissue from each bird for subsequent allozyme analysis (see below). Carcasses were then sent to the Royal Ontario Museum to have the soft tissue removed from the skeletons by dermestid beetles. Lengths of the coracoids, femurs, tibiotarsis, humeri, and ulnas were then measured for each skeleton (see Dufour and Weatherhead, 1996, for complete descriptions of all characters). K.W.D. made all the measurements. For both the external and internal characters, the complete set of characters was measured once, and then a second complete set of measurements was made. We used the mean value of the two replicate measures of each character in all analyses to reduce the effects of measurement error (Yezerinac et al., 1992). Damaged skeletal elements and worn feathers were not measured, so sample sizes vary slightly among analyses.

Quantifying FA, size, and condition

As detailed previously (Dufour and Weatherhead, 1996), all characters showed measurable departures from perfect symmetry (i.e., significant among-individual variability), with character-specific levels of measurement error in signed asymmetry scores (left minus right) ranging from very low to moderate (0.9–20.8%). Although distributions of these measurements of asymmetry generally conformed to patterns expected for FA (i.e., normally distributed with a mean of zero), there was evidence of subtle directionality of asymmetry for some characters (Dufour and Weatherhead, 1996). Thus, we adjusted distributions of left minus right values for each character to a mean of zero before converting these asymmetry measures to absolute values. We then transformed absolute asymmetry scores to square roots because the distributions of the absolute values are strongly positively skewed. Within characters, absolute FA was unrelated to character size (Dufour and Weatherhead, 1996).

For analyses examining the relations between FA and other variables, we used both FA for individual characters and a composite measure of FA. Although there is significant concordance in FA among characters, it is quite weak, indicating that a composite measure of FA is necessary to characterize an individual's overall FA (Dufour and Weatherhead, 1996). Furthermore, in a previous analysis, the composite index had revealed relations between FA and ectoparasite infestations that were not apparent from the single character analyses (Dufour and Weatherhead, 1998a). For each individual we computed the composite asymmetry score as the sum of the ranks of its FA scores for all individual characters.

We used principal components analysis (PCA) to derive a measure of body size from the individual characters. The first principal component (PC1) described positive covariation among skeletal characters, while PC2 described positive covariation between feather traits (details provided in Dufour and Weatherhead, 1998a). We used PC1 scores as our index of body size. Three individuals were excluded from the PCA because they were missing measurements for a particular character (e.g., a broken feather precluded measuring tail length). We estimated their PC1 scores from a multiple linear regression model relating PC1 to five individual characters for which all individuals had measurements ($R^2 = 0.98$; Dufour and

Table 1
Enzymes assayed for allozyme variability in a roosting population of red-winged blackbirds

Enzyme	Acronym	E.C. no. ^a	Buffer system ^b	Tissue ^c	No. of loci ^d
Acid phosphatase	ACP	3.1.3.2	—	L	0
Aconitate hydratase	ACO	4.2.1.3	—	L	0
Alcohol dehydrogenase	ADH	1.1.1.1	—	L	0
Aspartate aminotransferase	AAT	2.6.1.1	MC	L	2
Creatine kinase	CK	2.7.3.2	—	M	0
Glucose-phosphate isomerase	GPI	5.3.1.9	HC	L	1
Glutamate dehydrogenase	GDH	1.4.1.3	MC	L	1
Isocitrate dehydrogenase	IDH	2.7.1.1	MC	L	2
Lactate dehydrogenase	LDH	1.1.1.27	MC	M+H	2
Malate dehydrogenase	MDH	1.1.1.37	MC	L	2
Malic enzyme	ME	1.1.1.40	—	L	0
Mannose-phosphate isomerase	MPI	5.3.1.8	MC	L	1
Peptidase	PEP	3.4.11 or 13	—	L	0
Phosphoglucosmutase	PGM	2.7.5.1	MC	L	2
6-Phosphogluconate dehydrogenase	6PGD	1.1.1.44	MC	L	1
Purine nucleoside phosphorylase	NP	2.4.2.1	—	L	—

^a Enzyme Commission numbers follow the International Union of Biochemistry (1984).

^b MC = morpholine citrate, HC = histidine citrate (Murphy et al., 1990).

^c Tissues used as a source for the assayed enzyme: L, liver; M, skeletal muscle; H, heart muscle.

^d Those enzymes for which no loci were scored were either unresolvable or could not be scored with confidence for all samples.

Weatherhead, 1998a). We used residuals from simple linear regression of mass on body size (PC1) as our measure of condition (i.e., mass corrected for size).

Quantifying heterozygosity

We assayed allozyme variation using horizontal starch-gel electrophoresis following Bogart (1982), with modifications outlined by Loughheed and Handford (1992). Tissue samples were homogenized in an equal volume of grinding buffer (10 mM Tris, 1 mM EDTA (disodium.2H₂O), 50 mM NADP, pH 6.8). Before electrophoresis samples were spun at 6000 rpm for 5 min. Supernatant was absorbed on Whatman 3MM chromatography paper wicks, which were inserted into 450 ml 12% (w/v) starch gels measuring 19 × 14 cm. Samples were run under standard voltage and current at 4°C until indicator dye (Club House™ green food coloring) had migrated about 9 cm. Enzymes were stained following either Murphy et al. (1990) or Richardson et al. (1986).

We initially screened 16 enzymes, from which we chose 9 (representing 14 isozymes) that could be clearly resolved for all individuals (Table 1). For each individual and locus assayed, we recorded the electrophoretic genotype, designating the most common allozyme as “B,” the more anodally migrating allozyme as “A,” and more cathodally migrating allozymes as “C” or “D.”

Data analysis

Our general analytical approach was to use general linear modeling procedures (McCullagh and Nelder, 1989) to assess relations between two variables of interest (e.g., FA and heterozygosity), controlling for additional variables as necessary. Where interactions between variables were not significant, reduced models without interactions were used and reported below. All analyses were performed using the statistical routines in JMP (version 3.1; SAS Institute, 1994).

RESULTS

Of the nine clearly resolved enzymes, polymorphism was detected at one locus for five (GDH, GPI, LDH, MPI, 6PGD) and at two loci for one (PGM). Across these 7 loci, 32 individuals were homozygous, 28 individuals were heterozygous at 1 locus, 3 individuals were heterozygous at 2 loci, and 4 individuals were heterozygous at 3 loci. Because few birds were heterozygous at more than one locus, for most analyses we compare two classes of males: those that were homozygous at all loci and those that were heterozygous at one or more loci.

Age

Contrary to prediction, older males were not significantly more heterozygous than younger males. Nineteen of 33 ASY males were heterozygous, compared to 16 of 34 SY males (2 × 2 contingency table, $G = 0.74$, $df = 1$, $p = .39$).

Secondary sexual traits

The reduced two-way ANOVA with body size as the response variable and male age and heterozygosity as predictor variables was significant ($F = 3.26$, $df = 2$, 64 , $p = .04$) and indicated that SY males were smaller than ASY males ($F = 6.51$, $df = 1$, 64 , $p = .01$) but that there was no difference in size between homozygous and heterozygous males ($F = 0.02$, $df = 1$, 64 , $p = .90$, Table 2).

In the initial analysis of epaulet length, two-way ANOVA revealed a significant interaction between age and heterozygosity ($F = 7.13$, $df = 1$, 62 , $p = .01$). Analyses conducted separately by age indicated that, among SY males, homozygotes had longer epaulets than heterozygotes ($t = 2.0$, $df = 32$, $p = .05$; Figure 1), whereas among ASY males, heterozygotes tended to have longer epaulets than homozygotes ($t = 1.9$, $df = 30$, $p = .07$; Figure 1). The pattern for ASY males is consistent with what we predicted, but the result for SY males is opposite to what we predicted. Further exploration of these data indicated that this difference between age classes in the

Table 2

Body size of male red-winged blackbirds classified according to age (SY: second year; ASY: after second year) and heterozygosity status

Age	Status	PC1		
		<i>n</i>	Mean	SE
SY	Heterozygous	16	-0.70	0.449
	Homozygous	18	-0.46	0.529
ASY	Heterozygous	19	0.76	0.557
	Homozygous	14	0.64	0.422

Heterozygous: ≥ 1 heterozygous loci detected; homozygous: no heterozygous loci detected. Body size estimates are scores along the first principal component (PC1) obtained from a correlation-based principal components analysis of size measures made on three external and five skeletal characters.

relation between heterozygosity and epaulet length was largely a consequence of one locus. When we reclassified males as heterozygous or homozygous excluding PGM-3, then for SY males, mean epaulet length did not differ between homozygotes (39.05 ± 0.56) and heterozygotes (39.52 ± 1.11 ; $t = 0.4$, $df = 32$, $p = .71$), and heterozygous ASY males still had longer epaulets (45.31 ± 0.53) than homozygous ASY males (43.78 ± 0.38 ; $t = 2.4$, $df = 30$, $p = .03$).

Health

A two-way ANOVA with condition as the response variable and male age and heterozygosity as predictor variables indicated that SY males were in poorer condition (-1.35 ± 0.46) than ASY males (1.39 ± 0.47 ; $F = 16.57$, $df = 1, 64$, $p = .0001$), but did not detect a difference in condition between homozygotes (-0.37 ± 0.53) and heterozygotes (0.34 ± 0.51 ; $F = 0.43$, $df = 1, 64$, $p = .51$).

For analyses of parasite infection, we treated lice, mites and hematozoa separately. Details of the species included in each of these groups can be found in Dufour and Weatherhead (1998a). Because most individuals had some lice and mites, we analyzed variation in intensity of infestation by these two types of parasites. For hematozoa the more appropriate approach was to analyze presence or absence of infection because a substantial number of males had no detectable hematozoa. A two-way ANOVA evaluating the effects of male age and heterozygosity on lice infestation indicated a highly significant effect of male age ($F = 7.66$, $df = 1, 64$, $p = .007$), with younger males more heavily infested than older males, but no significant effect of heterozygosity ($F = 0.30$, $df = 1, 64$, $p = .59$; Table 3). Results were similar for mites, although the age difference was no longer significant ($F = 2.8$, $df = 1, 64$, $p = 0.10$), while the effect of heterozygosity remained non-significant ($F = 0.6$, $df = 1, 64$, $p = .43$; Table 3). Because the proportion of SY and ASY males infected with hematozoa was not different (44% and 36%, respectively; $G = 0.42$, $df = 1$, $p = .52$), we pooled age classes for further analysis. The proportion of males parasitized by hematozoa did not differ between heterozygotes (43%) and homozygotes (38%; $G = 0.20$, $df = 1$, $p = .66$).

Asymmetry

Our analysis of fluctuating asymmetry involved several steps, including determining that the asymmetry observed was primarily true asymmetry rather than measurement error, and confirming that distributions of symmetry values are approximately normal with a mean of zero. All of these issues are dealt with in detail in Dufour and Weatherhead (1996). Analy-

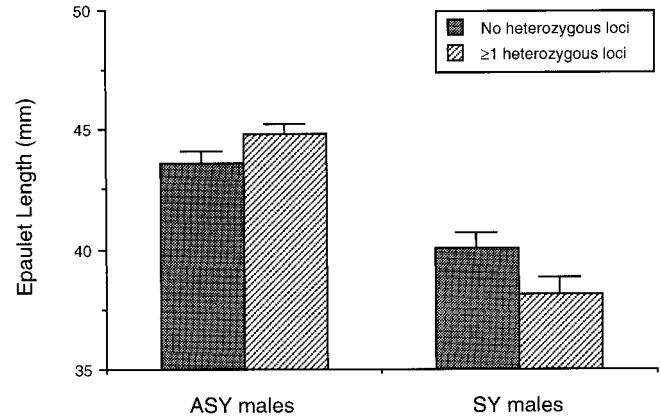


Figure 1

Variation in epaulet length with age and allozyme heterozygosity in male red-winged blackbirds. Males were classified as either second year (SY) or after second year (ASY). Bars represent means +1 SE.

ses presented in that paper also indicated there was no difference in asymmetry between SY and ASY males. Thus, because we detected no difference in heterozygosity between age classes, we pooled all males to assess whether heterozygotes were more symmetrical than homozygotes. For each of the nine characters we measured, there was no difference in asymmetry between heterozygotes and homozygotes (Table 4). However, in our analysis of composite asymmetry, there was a trend for heterozygotes to be more asymmetrical (301.06 ± 11.12) than homozygotes (273.63 ± 11.66 ; $t = 1.70$, $df = 61$, $p = .09$). Closer inspection of the data again revealed that variation at PGM-3 was responsible for this trend. When PGM-3 was excluded, there was no difference in composite asymmetry between heterozygotes (280.71 ± 15.82) and homozygotes (290.70 ± 9.61 ; $t = 0.54$, $df = 61$, $p = .59$). However, for PGM-3 alone, heterozygotes were more asymmetrical (317.40 ± 13.50) than homozygotes (273.30 ± 9.54 ; $t = 2.67$, $df = 61$, $p = .01$). At the PGM-3 locus there were 36 males with the BB genotype, 20 with the BC genotype, 6 with the CC genotype, and a single individual with the CD genotype. Excluding the individual with the unique genotype and comparing the composite symmetry of males with the three more common genotypes revealed that the symmetry difference was a consequence of a single allele. Individuals bearing the C allele (BC and CC) were less symmetrical than individuals without the C allele (one-way ANOVA: $F = 4.9$, $df = 2, 59$, $p = .01$; Figure 2). Multiple comparisons (Tukey-Kramer HSD tests; Sokal and Rohlf, 1981) indicated that mean composite asymmetry scores differed between genotypes BB and BC ($p < .05$). Although the mean score for CC individuals was vir-

Table 3

Ectoparasite burdens of individual male red-winged blackbirds classified according to age (SY: second year; ASY: after second year) and heterozygosity status

Age	Status	<i>n</i>	Louse burden		Mite burden	
			Mean	SE	Mean	SE
SY	Heterozygous	16	6.3	1.10	3.8	0.72
	Homozygous	18	10.2	2.06	4.7	0.98
ASY	Heterozygous	19	5.9	1.95	2.6	0.53
	Homozygous	14	3.9	1.22	3.3	0.79

Heterozygous: ≥ 1 heterozygous loci detected; Homozygous: no heterozygous loci detected.

Table 4

Levels of character asymmetry exhibited by males heterozygous for ≥ 1 loci (heterozygotes) and those homozygous for all loci surveyed (homozygotes)

Character	Asymmetry (mm)						t^a	p
	Heterozygotes			Homozygotes				
	Mean	SE	n	Mean	SE	n		
Tarsus	0.26	0.041	35	0.21	0.034	32	0.64	.526
Wing	1.06	0.112	35	0.86	0.119	32	1.41	.162
Tail	0.74	0.169	35	0.84	0.206	31	0.25	.806
Epaulet	1.21	0.147	34	1.70	0.301	32	0.90	.369
Coracoid	0.11	0.015	34	0.09	0.013	32	1.17	.247
Femur	0.07	0.009	34	0.06	0.009	31	0.37	.715
Tibiotarsus	0.14	0.021	35	0.10	0.017	32	1.50	.139
Humerus	0.07	0.008	35	0.06	0.011	32	0.77	.447
Ulna	0.08	0.009	35	0.08	0.010	32	0.29	.773

Asymmetry for each character is computed as the absolute value of the difference between the size of the left and right elements.

^a Pooled variance t test (two-tailed) based on square root-transformed asymmetry values.

tually identical to that for BC individuals, it was not significantly different from that for BB birds because the sample size was small.

DISCUSSION

Brown (1997) proposed that when animals select mates on the basis of genetic quality, the quality they prefer is heterozygosity. Mates that are more heterozygous will sire offspring that are more heterozygous on average, which may increase the viability of those offspring (Allendorf and Leary, 1986). Finally, because heterozygosity may also be associated with increased developmental stability (Mitton, 1993), Brown (1997) proposed that greater heterozygosity is recognized in potential mates through the higher symmetry of their bilateral traits. Thus, the key assumptions of this hypothesis are that viability, heterozygosity, and bilateral symmetry are all positively related.

We tested these assumptions using a sample of male red-winged blackbirds collected from a spring roost. From previous analyses of asymmetry in this population, we already knew that asymmetry was not related to mating success or viability (Dufour and Weatherhead, 1998a,c). However, the relations between heterozygosity and viability and between heterozy-

gosity and asymmetry were unknown. Overall, our results did not support either of these assumptions of the heterozygosity hypothesis. Older males were not more heterozygous than younger males, and heterozygosity was generally unrelated to body condition or to infection by mites, lice, or hematozoa. Heterozygosity was also generally unrelated to asymmetry in nine bilateral characters. Finally, because heterozygosity was unrelated to both the age and size of males, which are the two traits known to correlate positively with mating success in this population (Weatherhead and Boag, 1995), it seems unlikely that heterozygosity was related to female choice in this population of red-winged blackbirds.

Before concluding that our study does not support Brown's (1997) hypothesis, two issues must be addressed. First, although the weight of evidence did not support any relation between heterozygosity and morphology, age, or parasite infection, two significant but contrasting patterns were detected. As predicted, ASY heterozygotes had longer epaulets than ASY homozygotes. Moreover, epaulet size appears to be positively related to nest-defense behavior and dominance rank in this population of red-winged blackbirds (Eckert and Weatherhead, 1987a,b). Although it is tempting to provide an optimistic interpretation of this result in terms of Brown's (1997) hypothesis, data from additional samples are required to confirm that this lone correlation is more than just the expected type I error in a study involving many statistical tests.

The other significant pattern in our data involves an unexpected positive relation between heterozygosity and composite FA, due almost entirely to a pronounced difference in FA between PGM-3 genotypes. Individuals possessing allele C had FA scores 18.4% greater than those of BB homozygotes. There are two possible interpretations of this result. The first is that the pattern is simply an artifact of sampling or type I error (see above). The alternative, genetic interpretation is that allele C is associated with decreased developmental stability. Alternate genotypes of major glycolytic isozymes have been shown to have clear fitness consequences for a few taxa: *Colias* butterflies (PGI and PGM; Watt et al., 1996), fire ants (PGM; Ross and Keller, 1995), killifish (LDH; Schulte et al., 1997), and humans (PGM; Bottini et al., 1994). However, there is little general evidence for phenotypic differences among individuals bearing alternative electrophoretic alleles at such major housekeeping genes (Lynch and Walsh, 1998). Thus, it is more likely that the association between allele C at PGM-3 and asymmetry would be caused by disequilibrium be-

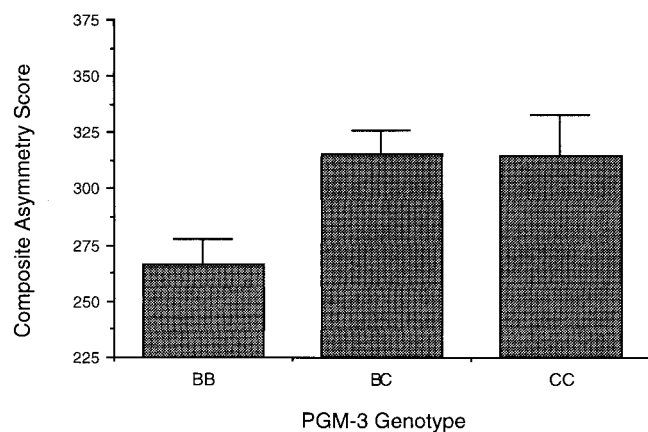


Figure 2
Variation in composite asymmetry between genotypes at the PGM-3 locus among male red-winged blackbirds. Bars represent means +1 SE.

tween it and alleles at other loci that directly influence development.

An apparent association between allele C at PGM-3 and FA might occur if the roost we sampled included birds from different populations that covaried in allele frequencies at PGM-3 and average levels of FA. This possibility seems unlikely for several reasons. When we collected the sample of birds from the roost in late April, male red-winged blackbirds in this area had already been back from migration for about a month (Greenwood and Weatherhead, 1982), and females had already begun to settle on territories (Muma and Weatherhead, 1989). Thus, it seems most likely that the occupants of the roost at the time we sampled were local residents, and patterns of natal dispersal in this population indicate that the population is highly outbred (Weatherhead and Montgomerie, 1995). Furthermore, because differences in FA should be more readily detected between individuals from different populations than between individuals from the same population, the contribution of more than one breeding population to the roost we sampled should have increased the likelihood of detecting positive correlations between heterozygosity and other fitness components (Lynch and Walsh, 1998; Palmer and Strobeck, 1986). We found no such correlations.

The second caveat in interpreting our results in terms of Brown's (1997) hypothesis is that our measure of heterozygosity was based on a relatively small number of enzymes. In historically large, randomly mating populations, it is unlikely that a sample of 14 loci will accurately reflect genome-wide heterozygosity (Chakraborty, 1981; Palmer and Strobeck, 1986). Error in measuring overall heterozygosity will weaken observed correlations with FA or fitness components. Small samples of enzyme loci are expected to index overall heterozygosity more accurately if linkage disequilibrium among loci is generally high or if the population is somewhat inbred (Lynch and Walsh, 1998; Palmer and Strobeck, 1986). This might be likely if the roosting population of blackbirds we sampled was genetically subdivided to some extent, or if there was nonrandom mating within breeding colonies. For the reasons given above, it seems most reasonable to assume that our sample of birds was drawn from a large, panmictic population at equilibrium, so heterozygosity at the small number of loci we sampled may not be representative of overall heterozygosity.

Although our ability to interpret some of the more intriguing results from this study is limited by the uncertainties discussed above, our data, taken together, do not provide any compelling support for the assumptions or predictions of Brown's (1997) hypothesis as applied to red-winged blackbirds. Instead, the results of this study add to the emerging picture of widely inconsistent and unpredictable relations between heterozygosity, fitness, and fluctuating asymmetry (Clarke, 1993; Lynch and Walsh, 1998; Palmer and Strobeck, 1986; Roff, 1997).

We thank Kit Muma, Kevin Teather, and Stephen Yezerinac for help in the field, Kit Muma for helping with specimen preparation, Gordon Bennett and associates for hematozoa identification, Bert Nesbitt for identifying ectoparasites, the Royal Ontario Museum for use of their dermestid colonies, Queen's University for use of the Biological Station, and the Natural Sciences and Engineering Research Council of Canada for financial support.

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