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Heterozygosity-Fitness Correlations in Adult and Juvenile Zenaida Dove, *Zenaida aurita*

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Abstract

Understanding how fitness is related to genetic variation is of crucial importance in both evolutionary ecology and conservation biology. We report a study of heterozygosity-fitness correlations in a wild, noninbred population of Zenaida Doves, *Zenaida aurita*, based on a sample comprising 489 individuals (382 adults and 107 juveniles) typed at 13 microsatellite loci, resulting in a data set comprising 5793 genotypes. In both adults and juveniles, and irrespective of sex, no evidence was found for an effect of either multilocus or single-locus heterozygosity on traits potentially related to fitness such as foraging tactic, competitive ability, and fluctuating asymmetry. In contrast, a significant negative correlation between body condition and multilocus heterozygosity, indicative of outbreeding depression, was found in juveniles, whereas no such trend was observed in adults. However, the frequency distribution of heterozygosity did not differ between the two age classes, suggesting compensatory growth by heterozygous juveniles. We discuss our results in relation to some practical limitations associated with studies of heterozygosity-fitness correlations, and suggest that tropical bird species with allopatric divergence between island populations may provide a good biological model for the detection of outbreeding depression.

Key words: *body condition, island population, microsatellite markers, multilocus heterozygosity, outbreeding depression*

Understanding to what extent individual genetic variation affects Darwinian fitness remains a key question in both evolutionary ecology and conservation biology. One way to address the question is to look for heterozygosity-fitness correlations (HFCs; David 1998; Coltman and Slate 2003; Chapman et al. 2009; Szulkin et al. 2010), considering multilocus heterozygosity and, depending on studies, single-locus heterozygosity. The application of HFCs to various organisms has so far resulted in contrasted results, possibly because several HFC studies suffered from low statistical power, particularly when considering populations with a low level of inbreeding (Chapman et al. 2009; Szulkin et al. 2010). Overall, the magnitude of the correlation is generally weak (about 1% of the variance in phenotypic characters explained), as indicated by a recent meta-analysis (Chapman et al. 2009). This is however not surprising, as theoretical considerations do predict a weak effect of heterozygosity on fitness (Chapman et al. 2009).

Capture–mark–recapture models have recently proved to be an efficient tool to directly assess the influence of individual heterozygosity on various demographic traits, such as adult and juvenile survival or recruitment (Brouwer et al. 2007; Banks et al. 2010), but their use remains contingent on the availability of rather large data sets derived from long-term studies (Lebreton et al. 1992). Not surprisingly, then, a majority of HFC studies has examined particular fitness traits, such as, for instance, reproductive success (Neff 2004; Seddon et al. 2004; Lieutenant-Gosselin and Bernatchez 2006; Kempenaers 2007), survival (Coulson et al. 1998, 1999; da Silva et al. 2008; Mainguy et al. 2009), developmental instability (Vøllestad et al. 1999; Neff 2004; Vangestel et al. 2011), or competitive ability (Höglund et al. 2002; Tiira et al. 2006; Välimaki et al. 2007). A few studies have directly compared the levels of individual heterozygosity between survivors and nonsurvivors (Ferguson and Drahushchack

1990; Kretzmann et al. 2006), or between different age classes (Diehl and Koehn 1985; Patarnello et al. 1991; Cohas et al. 2009). However, the two approaches have rarely been combined in a single study (but see Fessehaye et al. 2007).

Here, we combine different approaches to examine the influence of heterozygosity on fitness in a tropical bird species, the Zenaida Dove, *Zenaida aurita*, using a large number of individuals and a reasonable number of polymorphic microsatellite markers (Monceau et al. 2009). Since 2007, monitoring of a banded population of Zenaida Doves in Barbados has provided detailed information on interindividual variation in morphometrics (Dechaume-Moncharmont et al. 2011) and foraging tactics (Monceau et al. 2011; see also Sol et al. 2005), making possible an indirect evaluation of the influence of heterozygosity on some fitness-related traits. For instance, comparing heterozygosity between juvenile and adult Zenaida Doves can provide an indirect estimate of its effects on survival, as disadvantaged phenotypes (presumably more homozygous individuals) are expected to disappear quickly in the first steps of their development (Koehn and Gaffney 1984; David 1998; Cohas et al. 2009). In addition, some evidence suggests that body condition can vary with heterozygosity in birds (Fleischer and Murphy 1992; Townsend et al. 2010). Heterozygous individuals may have also an advantage over homozygous ones in obtaining and defending a territory as observed, for example, in Black grouses, *Tetrao tetrix* (Höglund et al. 2002). Part of the Barbados population of Zenaida Doves exhibits year-round territory defense (Quinard and Cézilly 2012), whereas the other part feeds in flock at seed storage facilities with little, if any, agonistic behavior (Carlier and Lefebvre 1996; Dolman et al. 1996; Sol et al. 2005; Monceau et al. 2011). It has been suggested that birds foraging in flocks are inferior competitors, unable to acquire and defend a territory (Sol et al. 2005; Monceau et al. 2011). Territory acquisition and defense in Zenaida Doves (see Quinard and Cézilly 2012) are assumed to be linked to individual competitive abilities, which may potentially reflect genetic quality through heterozygosity. Furthermore, Zenaida Dove territorial defense includes a conspicuous ritualistic behavior consisting of wing-raising (Lefebvre et al. 1996; Quinard and Cézilly 2012), and Monceau et al. (2011) recently showed that territorial birds have longer wings than flock-feeding ones. As some evidence suggests that genetic heterozygosity can have a significant influence on competitive ability in vertebrate species (Seddon et al. 2004; Tiira et al. 2006; Välimäki et al. 2007), the relationship between wing length and heterozygosity in Zenaida Doves was also examined. Finally, because heterozygous individuals are supposed to be less susceptible to developmental instability, we investigated the relationship between fluctuating asymmetry (FA; Palmer and Strobeck 1992; Palmer 1994) and heterozygosity in Zenaida Doves (Clarke 1993; Leamy and Klingenberg 2005).

Materials and Methods

The Zenaida Dove is a socially monogamous columbid species that breeds throughout the Caribbean region and the south of the Yucatan peninsula (Raffaele et al. 1998). Our study population was located on the island of Barbados (West Indies; 13°10'N, 59°32'W), a geologically young island about 600 000–700 000 years old (Buckley et al. 2009), where the species is both abundant and ubiquitous (Buckley et al. 2009). No formal population estimates are available, but given the size of the island (430 km^2) and considering, based on observations in different habitats, an average density of 1 bird per ha, we estimate the population size to be of the order of 40 000 individuals.

The founders of avian lineages now present on Barbados probably arrived on the island long after its emergence, and contemporary gene flow with other islands is supposed to be moderate or weak (Lovette et al. 1999). This is to a certain extent confirmed by the fact that the population of Zenaida Doves from Barbados shows significant levels of genetic and morphological differentiation with some populations from other islands in the Lesser Antilles (although no significant isolation by distance was observed; Monceau K, Wattier R, Cézilly F, unpublished data). However, on several islands the species is very tame and can be observed near docks, such that the sporadic arrival in Barbados of birds from neighboring islands via cruise-ships is feasible. Occasional dispersal may also occur as a consequence of hurricanes or tropical storms (Wunderle 2005).

All birds were captured using walk-in baited traps and single-catch closing net bird traps. Territorial individuals were caught in two different locations, the Folkestone park area and the Sunset Crest area near Holetown (Saint James Parish), whereas individuals feeding in flocks were captured at two different grain-storage facilities, Roberts manufacturing and the flour mill compound in the Bridgetown Harbour (see Monceau et al. 2011 for details). All captures were made between February and May, each year from 2007 to 2010. On capture, birds were banded with a unique combination of color plastic bands (A.C. Hughes Ltd., Hampton Hill, UK) and one numbered aluminum ring from the Muséum National d'Histoire Naturelle de Paris. Each banded bird was then measured twice for tarsus length (with a digital caliper, accuracy: ±0.2 mm) and wing chord (with a ruler, accuracy: ±1 mm). Birds were weighed (Pesola digital pocket scale MS 500, accuracy: ±0.1 g) and blood sampled (see Monceau et al. 2011 for details). Juveniles were easily differentiated from adults based on the absence of iridescent patches on each side of the neck, the presence of grayish first feathers, and high-pitched vocalizations (Sol et al. 2005; Monceau et al. 2011). All birds were then released where they had been caught. A total of 489 birds (382 adults and 107 juveniles) captured during this period were used in the present study. However, five juveniles were excluded from morphometric analyses due to missing data.

Genetic Analyses

DNA Extraction and Molecular Sex Identification

Zenaida Doves show very little sexual dimorphism (Dechaume-Moncharmont et al. 2011), such that sex identification from external appearance is not reliable. We therefore relied on molecular sexing to assign a sex to each captured

individual. DNA was extracted with standard phenol–chloroform method. Sex was then identified using 2550F/2718R primer pair, standard PCR and electrophoresis conditions (Fridolfsson and Ellegren 1999; Monceau et al. 2011).

Microsatellite Genotyping

All individuals (juveniles and adults) used for this study were genotyped for 13 microsatellite markers developed specially for the Zenaida Dove (Monceau et al. 2009). Genetic data for the different combinations of age classes (adult vs. juvenile) and foraging tactics (territorial vs. flocking) are summarized in Supplementary Appendix A. Typing of the 489 individuals at the 13 microsatellite markers resulted in a data set comprising 5793 genotypes. Both single- and multilocus Hardy–Weinberg equilibrium (HWE, 104 000 randomizations) and pairwise loci linkage disequilibrium (12 480 permutations) were tested in each group using FSTAT (v. 2.9.3.2; Goudet 1995). In order to control for the false discovery rate, 5% nominal significance levels were adjusted, using the Benjamini–Yekutieli step-up procedure (Benjamini and Yekutieli 2001; Narum 2006). Loci deviating from HWE were explored for possible genotyping error source with the software MICROCHECKER (van Oosterhout et al 2004). Microsatellite neutrality was tested in the four populations using LOSITAN based on both infinite allele and stepwise mutation model (50 000 randomizations; Beaumont and Nichols 1996; Antao et al. 2008). All loci could be considered as neutral (Figure 1). Inbreeding in the overall sample was estimated with F_{IS} but also compared between territorial and flock-feeding individuals using the comparison between groups procedure with 10 000 permutations within FSTAT.

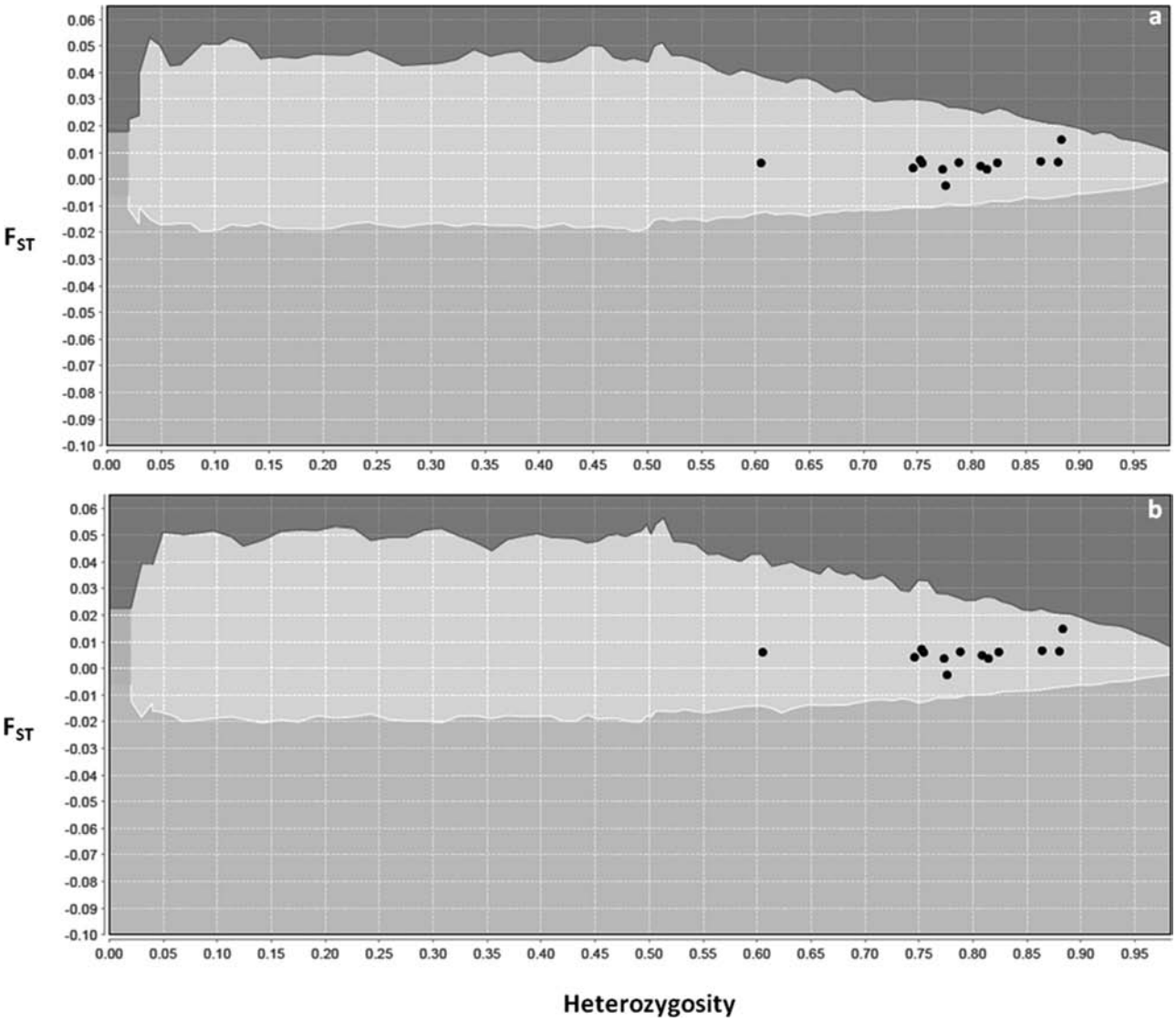

Figure 1. F_{ST} outlier tests for microsatellite neutrality based on (a) the infinite allele model and (b) the stepwise mutation model. All 13 microsatellite markers (black dots) are located in the neutral section delimitated by 95% confidence intervals.

Heterozygosity Scoring

Several indices have been developed to assess heterozygosity (see Coulson et al. 1998; Coltman et al. 1999; Amos et al. 2001; Hedrick et al. 2001; Aparicio et al. 2006; Chapman et al. 2009). Following Aparicio et al. (2006), we used the homozygosity per locus (HL) to provide estimate genome-wide heterozygosity. The HL index, however, represents homozygosity and not heterozygosity *per se*, meaning that it is negatively correlated with heterozygosity.

Although in most studies only linear relations between fitness traits and heterozygosity have been tested (implying directional selection on heterozygosity), heterozygosity can be under stabilizing selection, with highest fitness corresponding to intermediate values of heterozygosity (see for example Neff 2004). Therefore, we included HL (linear effect) and HL^2 (quadratic effect) in our heterozygosity-fitness analyses.

Tests for Correlation in Heterozygosity Among Markers

Correlation in heterozygosity and/or homozygosity across loci, known as identity disequilibrium, is considered to be the main cause of HFC (Szulkin et al. 2010). Identity disequilibrium can be measured as the excess of double heterozygous at two loci relative to the expectation of random association (i.e. covariance in heterozygosity) standardized by average heterozygosity (Szulkin et al. 2010). This measure is provided by parameter g_2, which is constant for any pair of loci considered and only depends on the mean and variance of inbreeding in the population (David et al. 2007). We therefore relied on the method proposed by David et al. (2007) and used the RMES (Robust Multilocus Estimate of Selfing) software (available at http://www.cefe.cnrs.fr/en/genetique-et-ecologie-evolutive/patrice-david, last accessed Sept. 3, 2012) was used to calculate g_2, and test whether this parameter differed significantly from zero.

Statistical Analyses

Prior to morphometric analyses, the repeatability of measurements was checked using Linear Mixed-effects Model-based repeatability estimates, with restricted maximum likelihood for estimating unbiased variance components following Nakagawa and Schielzeth (2010). Significant variance of the random effects was tested using likelihood ratio tests with a 95% confidence interval for both tarsus and wing lengths. Because repeatability was significant ($0.943 < R < 0.994$; $P < 0.0001$ in all cases) and measurement error (ME) was moderate ($0.50 < \text{ME} < 5.50$), the means of double measurements were used in the analyses. Moreover, as correlations between left and right side were high (Spearman's correlation test, tarsus length: $\varrho = 0.86$, $P < 0.0001$; wing chord: $\varrho = 0.92$, $P < 0.0001$), means of the left and right sides were used.

Individual Heterozygosity, Foraging Tactic, Age, and Sex

Heterozygosity differences between territorial and flock-feeding Zenaida Doves, with a control for potential sex effect, were tested with a Generalized Linear Model (GLM) considering capture sites as a nested effect within foraging tactic. Potential effect of differential mortality between juveniles and adults was assessed in comparing the mean and the frequency distribution of HL between adult and juvenile Zenaida Doves using a *t*-test and a two-sample Kolmogorov–Smirnov test, respectively.

Individual Heterozygosity and Competitive Ability

Relation between competitive abilities and heterozygosity was investigated through analyzing variation in wing length (controlled for body size with tarsus length) in relation to HL and HL^2 (quadratic effect) using GLMs in adults and juveniles separately. As a previous study showed differences in wing length in relation to foraging tactic and sex in adults (Monceau et al. 2011), the two variables were included in the model. In juveniles, only HL and HL^2 were included in the model as wing length has been previously shown to be independent of foraging tactic and sex in that age class (Monceau et al. 2011).

Individual Heterozygosity and Body Condition

Relation between heterozygosity and body condition was investigated through analyzing the scaled mass (SM) index. Briefly, SM was computed for each *i* individuals as follow (Peig and Green 2009):

$$\text{SM}_i = M_i \times (L_0/L_i)^b$$

where M_i and L_i are, respectively, the body mass and the tarsus length for the individual *i*, L_0, the mean tarsus length for the population (L_0 adults = 26.21 mm and L_0 juveniles = 25.67 mm), and *b* the slope of type II regression analysis of log(body mass) on log(tarsus length) following the standard major axis method (*b* adults = 2.64 and *b* juveniles = 3.42). Then, body condition (SM) variations were analyzed according to HL and HL^2, using GLM in adults and juveniles separately. Foraging tactic and sex effects were not included in the models as body condition has been previously shown to be independent of both effects in adults and juveniles (Monceau et al. 2011).

Individual Heterozygosity and Asymmetry for Tarsus and Wing Length

FA is defined as subtle deviation from bilateral symmetry. FA should reflect the capacity of an individual to produce the closest perfect symmetrical phenotype whatever the stress encountered during its development (Palmer and Strobeck 1992; Clarke 1993; Palmer 1994; van Dongen and Lens 2000; Debat and David 2001). Therefore, this analysis was only performed on adults, as the morphological development of juveniles was not achieved at the time they were measured.

Before analyzing the relationship between FA and heterozygosity, we first verified that ME did not exceed FA, to avoid any bias in FA estimation. To that end, we used an analysis of variance to compare variations between side and repeated measurements (Palmer 1994). We then used the mean of repeated measurement for subsequent analyses. We also checked for two other potential sources of asymmetry which could bias the detection and/or interpretation of

true FA (Palmer and Strobeck 1986): antisymmetry (AS) and directional asymmetry (DA). For AS, deviations are randomly distributed on one side or the other of the distribution, whereas for DA deviations are always produced on the same side (Palmer and Strobeck 1986, 1992, 2003; Palmer 1994). To test the occurrence of AS, we performed Shapiro–Wilk tests on the distribution of the differences between left and right sides for both tarsus length and wing chord. DA detection was realized in comparing left and right mean characters with a Student *t*-test. The presence of AS and DA was checked for all adults, and then within each sex separately. Groups exhibiting AS and/or DA were excluded from FA tests. Following Palmer (1994), two FA indices were retained: FA1 (absolute difference between left and right side) and FA4 (difference between left and right side). Before using them to test the relation with heterozygosity, independence of the absolute difference of left and ride sides was tested in a regression with the mean of the character. If a significant relationship was detected, FA indices should be corrected for body size variations. Relation between FA indices, HL, and HL^2 was then tested using a GLM, controlling for foraging tactic.

All GLMs used identity log-link function, and the statistical significance of each parameter was assessed with an analysis of deviance based on *F*-statistics.

Tests for Single-Locus Effects

Following David (1997) and Szulkin et al. (2010), the presence of single-locus (SL) effects for the various fitness-related traits was estimated from the difference in variance between a model incorporating all SL effects and a model incorporating the multilocus effect, using an *F*-test. A larger variance explained by the SL model as compared with the HL one would be indicative of the existence of SL effects (Szulkin et al. 2010). Single-locus heterozygosity was encoded as 0 for heterozygous individuals and 1 for homozygous ones when computing HL. Missing data were replaced by the mean value for the locus (Szulkin et al. 2010).

All statistical analyses were performed with R 2.12.1 (R Development Core Team 2008) implemented with *mutoss* (Benjamini–Yekutieli's correction), *Rhh* (HL computation), *lmodel2* (type II regression analysis) and *rpt*R packages (linear mixed-effects model-based repeatability).

Results

Only 3 out of 104 single-loci tests (13 loci by eight groups, e.g. four sites with two age classes per site) showed deviation from HWE (excess of homozygous individuals) for ZaC11 and ZaC12 in adults from Harbour and for ZaD121 in juveniles from Sunset Crest (Supplementary Appendix A). Such deficit in heterozygote could be ascribed by MICROCHECKER to either null allele or stuttering. Nevertheless, such deviations are minor ones, as multilocus tests showed no deviation from HWE for each of the eight groups (Supplementary Appendix A). No linkage disequilibrium was detected. Level of inbreeding estimated with 13 microsatellite markers was low in the overall sample (F_{IS} = 0.023, N = 489), and did not differ between foraging tactics (territorial birds: F_{IS} = 0.022, N = 344, and flock-feeders: F_{IS} = 0.024, N = 145; Permutation test, 10 000 permutations, P = 0.82). In addition, we found no evidence for a correlation in heterozygosity between our 13 microsatellite markers as g_2 did not differ from zero (g_2 = −0,002, P = 0.18, N = 489, 10 000 iterations), suggesting limited statistical power to detect inbreeding if presents.

Individual Heterozygosity, Foraging Tactic, Age, and Sex

Mean heterozygosity did not differ between sexes (GLM: $F_{1,\ 483}$ = 1.34, P = 0.25), foraging tactics ($F_{1,\ 483}$ = 0.17, P = 0.68), and sites ($F_{2,\ 483}$ = 0.50, P = 0.61). Interaction between sex and foraging tactic was not significant ($F_{1,\ 483}$ = 0.01, P = 0.94). In addition, neither the frequency distribution of HL (two-sample Kolmogorov–Smirnov test: D = 0.11, P = 0.24, Figure 2) nor the mean HL (*t* test: t = 1.05, P = 0.29) differed between juveniles and adults (Figure 2).

Individual Heterozygosity and Competitive Ability

Overall, competitive ability, as reflected by wing length, was independent of heterozygosity in both age classes. In juveniles, wing length only varied with body size (GLM: $F_{1,\ 98}$ = 23.05, P < 0.0001), with no effect of heterozygosity (HL: $F_{1,\ 98}$ = 1.63, P = 0.20 and HL^2: $F_{1,\ 98}$ = 0.01, P = 0.90). In adults, wing length was independent of heterozygosity (HL: $F_{1,\ 376}$ = 0.48, P = 0.49 and HL^2: $F_{1,\ 376}$ = 1.49, P = 0.22), and differed between foraging tactics ($F_{1,\ 376}$ = 105.39, P < 0.0001) and sexes ($F_{1,\ 376}$ = 94.07, P < 0.0001). Irrespective of the overall body size ($F_{1,\ 376}$ = 67.87, P < 0.0001), males and

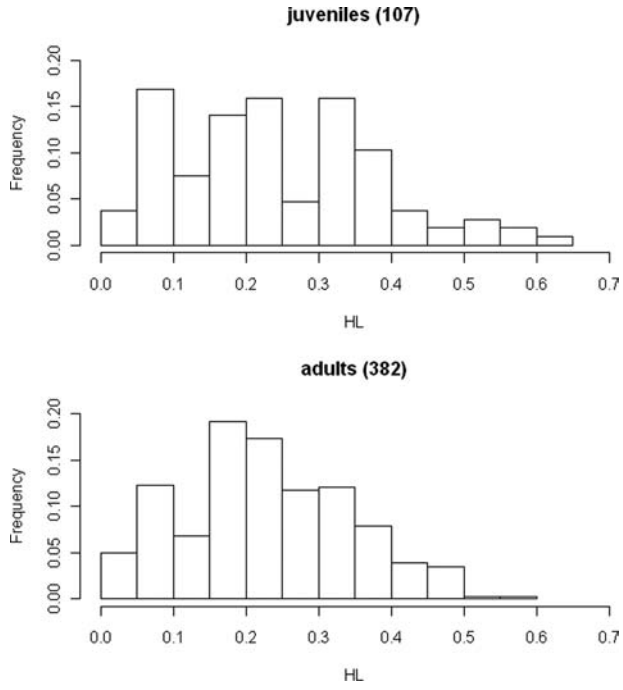

Figure 2. Histograms of HL frequencies distribution in juveniles and adults.

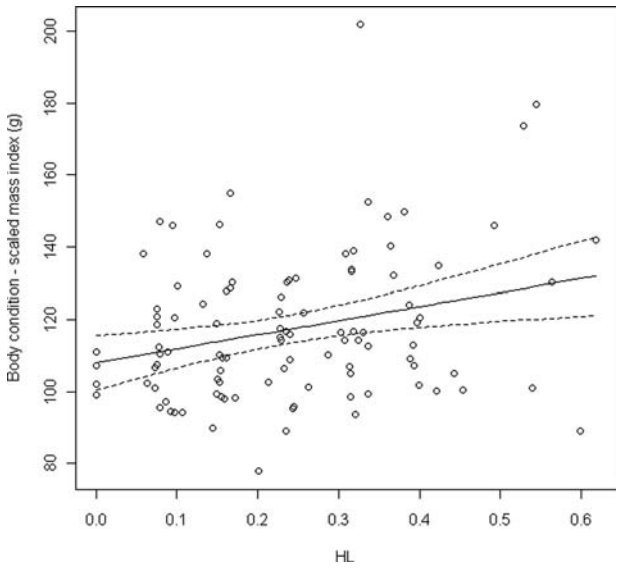

Figure 3. Relation between body condition (scaled mass index) and HL. Plain line represents predicted values fitted from GLM model (plain line) assorted with 95% confidence interval (dash lines).

territorial doves had, respectively, longer wings than females and flock-feeding individuals.

Individual Heterozygosity and Body Condition

In juveniles, variation in body condition was positively related to variation in HL (GLM: $F_{1, 99} = 7.85$, $P < 0.01$), but not with variation in HL^2 ($F_{1, 99} = 0.11$, $P = 0.74$). Body condition thus decreased with increasing heterozygosity in juveniles (Figure 3), with homozygous individuals having a better body condition than heterozygous ones. In contrast, no effect of heterozygosity was evidenced in adults (HL: $F_{1, 379} = 0.25$, $P = 0.62$ and HL^2: $F_{1, 379} = 0.45$, $P = 0.50$).

Individual Heterozygosity and Asymmetry for Tarsus and Wing Length

The interaction between side and repeated measurements was significant for both tarsus length and wing chord measurements (analysis of variance, respectively: $F_{381, 764} = 5.20$, $P < 0.0001$ and $F_{381, 764} = 7.24$, $P < 0.0001$), thus indicating that ME did not exceed FA. Deviation from normal distribution was detected in both adults and females for tarsus length and wing chord (Table 1). In males, differences between left and right sides deviated from normality, whereas no such effect was detected for tarsus length differences (Table 1). Consequently, subsequent analyses focused on male tarsus length ($N = 177$). No difference was detected between left and right male tarsus length (*t* test: $t = -0.14$, $P = 0.89$). Because a significant relationship was detected (linear regression: $R^2 = 0.07$, $F_{1, 175} = 13.72$, $P < 0.001$), FA1 and FA4 were corrected for body size variations through dividing them by the mean of tarsus length. FA1 and FA4 were both independent of foraging tactic (GLM, FA1: $F_{1, 173} = 0.56$, $P = 0.46$, and FA4: $F_{1, 173} = 0.005$, $P = 0.94$), and heterozygosity (FA1 – HL: $F_{1, 173} = 0.08$, $P = 0.78$, FA1 – HL^2: $F_{1, 173} = 2.67$, $P = 0.10$, FA4 – HL: $F_{1, 173} = 0.08$, $P = 0.78$, and FA4 – HL^2: $F_{1, 173} = 0.02$, $P = 0.88$).

Table 1 Detection of antisymmetry (AS) in testing the distribution of the differences between left and right sides for both tarsus length and wing chord with Shapiro–Wilk test in overall sample and for males and females separately

		Tarsus length		Wing chord	
Groups	*N*	*W*	*P*	*W*	*P*
Overall	382	0.97	<0.0001	0.83	<0.0001
Males	177	0.99	0.40	0.74	<0.0001
Females	205	0.95	<0.0001	0.98	<0.01

N, sample size; *W*, Shapiro–Wilk test statistical value.

Table 2 Comparison between a model including HL and a model including all SL effects for three different fitness-related traits and two age classes

Fitness trait	Group	*dfs*	*F*	*P*
Competitive ability	Adults	12, 367	1.28	0.23
	Juveniles	12, 87	0.96	0.49
Body condition	Adults	12, 368	1.36	0.18
	Juveniles	12, 88	0.89	0.56
Fluctuating asymmetry	FA1 – adults males	12, 162	0.33	0.98
	FA4 – adults males	12, 162	1.30	0.22

Tests for SL Effects

Finally, for each of the three fitness characters, we found no difference between a model including HL and a model including all SL, both in adults and juveniles (Table 2).

Discussion

A recent meta-analysis of HFC showed that only 24% of 481 effect sizes were significant, most of them tending to be relatively weak (Coltman and Slate 2003; Chapman et al. 2009). Accordingly, we failed to evidence any significant effect of HL on several traits potentially related to fitness in the Barbados population of Zenaida Doves, except for a negative relationship between body condition and heterozygosity in juveniles, suggestive of outbreeding depression (see below).

Positive HFCs are expected when more inbred individuals are both more homozygous for their marker loci and less fit due to inbreeding depression (Tsitrone et al. 2001; Hansson and Westerberg 2002; Harrison et al. 2011). However, as emphasized by Szulkin et al. (2010), estimates of HFC are bound to be imprecise when inbreeding is weak, as it seems to be the case in our study population. In addition, limitations of HFC studies are associated with sample size and the use of microsatellites to estimate heterozygosity. Concerning the sample size, most of the studies reviewed in Chapman et al. (2009) were conducted on less than 400 individuals

(range: 7–1055), with a number of markers ranging between 5 and 20 (range: 3–101, see Figures 2 and 3 in Chapman et al. 2009). In the present study, a relatively large number of individuals were genotyped for a reasonable (according to previous published studies) set of molecular markers, thus placing our study in favorable conditions.

The absence of significant HFCs in the present study could, however, result from some limitations associated with the use of microsatellites to estimate heterozygosity. In particular, genetic diversity estimated with microsatellite markers may not necessarily reflect genome-wide heterozygosity (Balloux et al. 2004; Slate et al. 2004; David et al. 2007; Väli et al. 2008). Indeed, our calculations using parameter g_2 indicate that our set of 13 markers was probably not powerful enough to reflect accurately the actual level of genome-wide heterozygosity within individuals (see David et al. 2007). Similarly, Chapman and Sheldon (2011) recently concluded that low power to infer genome-wide heterozygosity from 26 microsatellite markers was largely explaining the absence of evidence for either multi- or single-locus HFC in several morphological and fitness traits in a large outbreed population of great tits, *Parus major*.

In the present study, HL, as estimated from 13 neutral microsatellite markers, had a positive effect on juvenile body condition, suggestive of outbreeding depression. This is not contradictory with our failure to evidence correlation in heterozygosity among our 13 markers. This is because traits potentially related to fitness might be influenced by a much larger number of loci than the number of markers typed (Szulkin et al. 2010), making outbreeding easier to detect through its phenotypic effect than through heterozygosity at a reduced number of loci. In addition, empirical evidence shows that outbreeding depression in vertebrates is most commonly detected in juvenile organisms (Marshall and Spalton 2000; Sagvik et al. 2005; Granier et al. 2011; Houde et al. 2011; see also Szulkin and David 2011). We therefore considered that the observed positive correlation between juvenile body condition and HL might be a true biological effect that deserves some interpretation. The Barbados population of Zenaida Doves is morphologically and genetically differentiated from other Lesser Antillean populations (Monceau K, Wattier R, Cézilly F, unpublished data). The genetic composition of the Barbados population may then have diverged from those of neighboring islands due to a combination of random drift and local adaptation. In particular, Barbados is located east of the Lesser Antillean arc and its climate markedly differs from that of neighboring islands characterized by a typical West Indian humid tropical forest climate (Buckley et al. 2009). In addition, all native forest was rapidly cleared after the colonization of Barbados by Europeans in 1627 (Buckley et al. 2009). However, evidence exists for low gene flow between Barbados and neighboring islands, with two to five effective migrants per generation (Monceau K, Wattier R, Cézilly F, unpublished data). Some effective migrants coming from other islands may reach Barbados either through transportation by human vessels, particularly large cruise ships, or following hurricanes and tropical storms. Indeed, Fleming and Murray (2009) have shown that hurricane-aided dispersal can affect the genetic composition of Phyllostomid bats on Caribbean islands. A certain proportion of the more heterozygous individuals in our sample may then possibly consist of crosses between individuals from genetically differentiated populations. Their lower body condition as compared with that of crosses between individuals from the Barbados population might be due to a breakup of coadapted gene complexes or favorable epistatic interactions (Lynch 1991; see also Marr et al. 2002). Indeed, differences between environmental conditions may select from different growth trajectories in chicks (Gebhardt-Henrich and Richner 1998; Ardia 2006), and there is some evidence that outbreeding depression can negatively affect growth in vertebrates (Tymchuk et al. 2007). Under such a scenario, negative or quadratic effects of heterozygosity on fitness are expected (e.g. Marshall and Spalton 2000; Neff 2004), as observed in the present study.

However, despite the fact that variation in body condition of juveniles is known to affect individual fitness in birds (Magrath 1991; Christensen 1999; Steinen and Breeninkmeijer 2002; Braasch et al. 2009), we found no evidence for a filter effect (Koehn and Gaffney 1984; David 1998), as the distribution of HL did not differ between juveniles and adults. One possibility, then, is that the initial disadvantage of more heterozygous juveniles in terms of body condition was later cancelled through compensatory growth (Hegyi and Török 2007), with no long-term penalty (Kersten and Benninkmeijer 1995). Interestingly, Miller (2011) recently found no evidence for a cost of compensatory growth in the closely related *Zenaida macroura*. On the other hand, individuals with higher levels of heterozygosity might have been purged earlier, for instance through hatching failure, as most juveniles considered in the present study were caught after fledging. Although the amount of available data is very limited, it has been suggested that outbreeding depression might be more common in vertebrates than generally supposed (Marshall and Spalton 2000). More recently, it has been proposed that reduced gene flow between two populations of the same species having evolved in different habitats may increase the probability of outbreeding depression (Frankham et al. 2011). Our results suggest that the examination of HFCs in species of birds with allopatric divergence between island populations, as observed in the avifauna of the Lesser Antilles (Ricklefs and Bermingham 2008), might prove useful to test such ideas.

Supplementary Material

Supplementary material can be found at http://www.jhered.oxfordjournals.org/.

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