

Heterozygosity–fitness correlations in a bottlenecked island species: a case study on the Seychelles warbler

L. BROUWER,* J. KOMDEUR* and D. S. RICHARDSON†

*Animal Ecology Group, Centre for Ecological and Evolutionary Studies, University of Groningen, PO Box 14, 9750 AA Haren, The Netherlands, †Centre for Ecology, Evolution and Conservation, School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ, UK

Abstract

We used capture–mark–recapture models to investigate the effects of both individual and parental heterozygosity, measured at microsatellite loci on the survival of Seychelles warblers (*Acrocephalus sechellensis*), an endemic island species which went through a severe population bottleneck in the middle of the last century. We found that an individual's survival was not correlated with multilocus heterozygosity, or with heterozygosity at any specific locus. However, maternal, but not paternal, multilocus heterozygosity was positively associated with offspring survival, but only in years with low survival probabilities. A nestling cross-fostering experiment showed that this was a direct maternal effect as there was an effect of the genetic mother's, but not of the social mother's, heterozygosity. Heterozygosity–fitness correlations at microsatellite markers were generally assumed to reflect genome-wide effects. Although this might be true in partially inbred populations, such correlations may also arise as a result of local effects with specific markers being closely linked to genes which determine fitness. However, heterozygosity at the individual microsatellite loci was not correlated and therefore does not seem to reflect genome-wide heterozygosity. This suggests that even in a small bottlenecked population, heterozygosity–fitness correlations may not be caused by genome-wide effects. Support for the local effects hypothesis was also equivocal; although three specific loci were associated with offspring survival, including all single-locus heterozygosities as independent predictors for the variation in survival was not supported by the data. Furthermore, in contrast to the local effects hypothesis, the loci which contributed most to the heterozygosity–survival relationship were not more polymorphic than the other loci. This study highlights the difficulties in distinguishing between the two hypotheses.

Keywords: heterozygosity, capture–mark–recapture, Seychelles warbler, survival

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Introduction

Inbreeding between closely related individuals will result in decreased heterozygosity in offspring which may lead to reduced fitness (Hartl & Clark 1997). Several studies have shown direct relationships between heterozygosity measured across multiple microsatellite loci and components of fitness, e.g. birth weight (Coltman *et al.* 1998), survival (Bean *et al.* 2004), recruitment (Hansson *et al.* 2001), reproductive success (Slate *et al.* 2000; Amos *et al.* 2001) and sperm quality (Gage *et al.* 2006). Variation in multilocus heterozygosity

has also recently been shown to be associated with variation in sexually selected fitness traits, such as territory size (Seddon *et al.* 2004), plumage ornamentation (Foerster *et al.* 2003) and song repertoire size (Marshall *et al.* 2003). However, it is still difficult to assess the generality and importance of heterozygosity–fitness correlations in natural populations as null results are likely to be under-represented in the literature (Coltman & Slate 2003; but see: Whitlock 1993; Duarte *et al.* 2003; Overall *et al.* 2005). Furthermore, the underlying mechanisms which are normally responsible for the heterozygosity–fitness correlations observed in natural populations are still unresolved.

Heterozygosity–fitness correlations can arise in different ways (reviewed by Hansson & Westerberg 2002). First,

Correspondence: L. Brouwer, Fax: +31 (0) 50 3635205; E-mail: L.Brouwer@rug.nl

heterozygous individuals may have higher fitness as a result of the scored loci which themselves influence fitness in an overdominant manner, the 'direct effect' hypothesis (Mitton 1997; David 1998). However, this does not easily explain correlations between fitness and heterozygosity at noncoding markers, such as microsatellite loci, as these are considered to be selectively neutral (Queller *et al.* 1993). Second, heterozygosity across multiple microsatellite markers could reflect genome-wide heterozygosity, the 'general effect' hypothesis (e.g. Lynch & Walsh 1998). This idea was initially favoured by studies investigating heterozygosity–fitness correlations (Coltman *et al.* 1998; Coulson *et al.* 1998, 1999; Slate *et al.* 2000). However, heterozygosity at microsatellite markers may only reflect genome-wide heterozygosity in partially inbred populations, and when very large numbers of markers are analysed, which is often not the case (Balloux *et al.* 2004; Slate *et al.* 2004). Third, heterozygosity–fitness correlations can be explained by the 'local effect' hypothesis, in which the heterozygote advantage is a result of a genetic association between a neutral marker and a locus under selection (David 1998; Lynch & Walsh 1998). This requires linkage disequilibria – the nonrandom associations of alleles at different loci – which is expected in small or recently bottlenecked populations (Hartl & Clark 1997; Lynch & Walsh 1998). Until recently, few studies had considered the local effect hypothesis as an appropriate explanation for heterozygosity–fitness correlations, as linkage disequilibria were only considered to occur over a limited area of the genome, i.e. a few kilobases. However, recent empirical studies have shown that high levels of linkage disequilibrium may occur in natural populations (Yan *et al.* 1999; Reich *et al.* 2001; Dawson *et al.* 2002; Sinervo & Clobert 2003). Consequently, linkage disequilibrium might be an important factor causing heterozygosity–fitness correlations in natural populations. Indeed, in several studies some loci seem to contribute more than others to (associated measures of) heterozygosity–fitness correlations (Merilä *et al.* 2003; Bean *et al.* 2004; Hansson *et al.* 2004; Acevedo-Whitehouse *et al.* 2006).

It is also possible that heterozygosity may not only affect the fitness of the individual but also of its offspring. Offspring fitness could be affected directly by the parents via somatic effects or hormones (direct parental effects) (e.g. Eising *et al.* 2001), or indirectly through the environment created by parents, e.g. via provisioning or parental care (environmental parental effects) (e.g. Hakkarainen & Korpimäki 1994). Several studies have shown effects of inbreeding on egg hatchability (van Noordwijk & Scharloo 1981; Bensch *et al.* 1994; Keller 1998; Westemeier *et al.* 1998; Kruuk *et al.* 2002; Hansson 2004) and/or offspring fledging success (van Noordwijk & Scharloo 1981; Kruuk *et al.* 2002; Jamieson *et al.* 2003). Nevertheless, evidence that parental heterozygosity–offspring fitness correlations occur is currently lacking.

Here we investigate heterozygosity–fitness correlations in a population of the Seychelles warbler (*Acrocephalus sechellensis*), an endemic species restricted to a few islands in the Indian Ocean. Genome-wide inbreeding effects could be important in this species as the last remaining population on Cousin Island went through a severe bottleneck. Only *c.* 30 individuals survived between 1920 and 1968 due to habitat deterioration as result of the planting of coconuts (*Cocos nucifera*) in the 1910s (Vesey-Fitzgerald 1940; Crook 1960; Loustau-Lalanne 1968). Since then, the Cousin Island population has expanded and has been stable since 1982 with *c.* 320 individuals (Komdeur 1992). Importantly, these conditions could also generate considerable linkage disequilibrium (Nei 1975; Reich *et al.* 2001). In this population, close inbreeding – resulting in decreased offspring multilocus heterozygosity – has been shown to occur for over 5% of offspring (Richardson *et al.* 2004). An earlier study has suggested that low multilocus heterozygosity of the genetic mother is linked to reduced first-year survival of the offspring (Richardson *et al.* 2004). However, this study did not investigate the effect of multilocus vs. loci specific heterozygosity nor did it investigate the effects of heterozygosity on adult survival. Furthermore, the survival analysis did not use capture–mark–recapture analysis which incorporates resighting probabilities and is a more robust technique than logistic regression (Lebreton *et al.* 1992).

In the present study, we investigate the effect of both the individual's own heterozygosity, and that of its parents – measured across 14 microsatellite loci – upon future survival using capture–mark–recapture analyses. First, we investigate the role of parental multilocus heterozygosity on offspring survival after testing for individual multilocus heterozygosity. Second, we use data from a cross-fostering experiment to disentangle direct parental and environmental parental effects by testing the effect of both the social and genetic parents' multilocus heterozygosity on offspring survival probabilities. Third, as close inbreeding occurs in this population, we investigate whether heterozygosity is correlated across loci and therefore could reflect general effects. Fourth, we investigate whether all single-locus heterozygosities explain variation in survival better than multilocus heterozygosity, which would indicate local effects. This is, to our knowledge, the first time that capture–mark–recapture analyses have been used to investigate heterozygosity–fitness correlations.

Methods

Study area and data collection

Data were collected from *Acrocephalus sechellensis* offspring which hatched between 1997 and 1999 and which were subsequently monitored up to 2004 as part of the long-term

Table 1 Design of data sets used for testing the effects of individual, paternal and maternal heterozygosity on Seychelles warbler survival probabilities. G, genetic parent; F, foster parent

Design			Effect of heterozygosity		
Offspring	Father	Mother	Individual and direct parental	Environmental paternal	Environmental maternal
Within-group	G = F	G = F	+	-	-
Extra-group	G ≠ F	G = F	+	+	-
Cross-fostered	G ≠ F	G ≠ F	+	+	+

study of the Seychelles warbler population on Cousin Island (04°20'S, 55°40'E). During the main breeding season (July to September), and the minor breeding peak (January to March) each territory was checked for breeding activity at least once every 2 weeks by following the resident female for 30 min (Komdeur 1992). Nests were observed throughout the breeding cycle. Most Seychelles warblers produce one clutch per season (Komdeur 1996) and this normally consists of just one egg, but about 20% of nests contain two or three eggs (Richardson *et al.* 2001). Nestlings were ringed with a unique combination of three ultraviolet light-resistant colour rings and a British Trust for Ornithology ring at approximately 12 days of age. Blood samples (*c.* 15 µL) were collected by brachial venipuncture and then diluted in 800 µL of 100% ethanol in a 2.0-mL screw-cap microfuge tube and stored at room temperature. If a nest could not be reached, the nestling was caught with mistnets after fledging but while still dependent upon its parents within the natal territory. We attempted to sample all offspring produced in each breeding season. Although a large proportion of nests could not be reached, it is hard to imagine that birds nesting higher also have a different heterozygosity as there is large variation in selection of the height of the nest sites within individuals (personal observation). Offspring that were found dead in the egg ($n = 8$) or nest ($n = 10$) were included in the survival analysis. These early deaths, and an additional seven eggs and 16 nestlings sampled in 1995 and 1996, were used to investigate whether there was any relation between multilocus heterozygosity and early mortality. Molecular sexing using the PCR method was used to determine the sex of each individual (Griffiths *et al.* 1998).

Genotypes were identified for all individuals in the population using 14 polymorphic microsatellite markers previously isolated in the Seychelles warbler (Richardson *et al.* 2000). Multilocus heterozygosity was calculated, for offspring and their parents, as the number of heterozygous loci divided by the number of loci examined. Parentage was assigned with > 80% confidence (Richardson *et al.* 2001) for 181 offspring sampled between 1997 and 1999. This is the same data set as used in Richardson *et al.* (2004),

except that we did not exclude the 1997 cohort in which mortality was high due to severe weather, but included year effects in our model (see below). These offspring were used to investigate whether the offspring's own multilocus heterozygosity or the multilocus heterozygosity of the genetic parents was associated with their juvenile and adult survival probabilities (Table 1). The degree of linkage disequilibrium was estimated in ARLEQUIN 2.0 (Schneider *et al.* 2001). Gametic phase was unknown and pairwise tests for linkage disequilibrium were therefore performed using a likelihood-ratio test whose empirical distribution was obtained by a permutation procedure (Slatkin & Excoffier 1996). In this test, the haplotype frequencies were estimated using the expectation-maximization (EM) algorithm to obtain the likelihood of the data not assuming linkage equilibrium. The EM algorithm has a very high ability of inferring correct haplotypes when sample sizes are large (> 100 individuals) as is the case in this investigation (Excoffier & Slatkin 1995).

To disentangle direct parental and environmental parental effects on survival probabilities, same age (± 2 -day difference) nestlings between 0 and 6 days of age were cross-fostered between nests on different territories. 45 cases of the noncross-fostered offspring had extra-group paternity (EGP, sired by a male outside the group, Richardson *et al.* 2001). Consequently, environmental parental effects — i.e. the multilocus heterozygosity of the social mother ($n = 58$) and the multilocus heterozygosity of the social father (cross-foster + EGP, $n = 103$) — as well as direct parental effects on offspring survival probabilities could be analysed (Table 1).

Data analyses

Two resighting periods were defined; the first from 1 July to 1 September, the second from 1 January to 1 March; however, most birds were resighted within the first 2 weeks of each fieldwork period. Individuals were recorded as present if observed at least once within this period, or absent if not. Data for January–March were not available for 2000–2002, for these years dummy variables were created by including zero's in the encounter history, with

the survival parameter (Φ) set equal to 1 and the resighting parameter (recapture, ρ) set equal to 0. The resighting history files were used as input files in the program MARK (White & Burnham 1999). Akaike's information criterion corrected for sample size (AIC_c) was used to select the most parsimonious model, with better fitting models resulting in lower AIC_c values (Akaike 1973; Burnham & Anderson 2002). For one model to be selected above another, the AIC_c should decrease with at least 2 (Burnham & Anderson 2002). Additionally, we report the normalized Akaike weights to assess the relative likelihood of competing models.

To investigate survival and resighting probabilities, we employed an a priori approach in which a set of candidate models was created based on biological reasoning. Previous analyses showed that the survival of Seychelles warblers was lower in their first year of life (juvenile survival) than for older birds (Brouwer *et al.* 2006). Furthermore, survival varied between years, with similar variation between the years for both age classes. Resighting probabilities varied across the age classes, with a lower

resighting probability for individuals in their first two years of life and higher probabilities for older birds (Brouwer *et al.* 2006). There was no variation in survival nor in resighting probability between the sexes, and neither territory quality (measured according to Komdeur (1992)), the number of nest mates or the number of nonhelping subordinates in the natal territory were associated with survival probabilities (Brouwer *et al.* 2006; L. Brouwer, D. S. Richardson, J. Komdeur unpublished data). However, the number of helpers in the natal territory had a positive effect on offspring survival (L. Brouwer *et al.* unpublished data) and the local density, measured as the average group size that an individual lived in, had a negative effect on survival (Brouwer *et al.* 2006). However, the number of helpers and the average group size an individual lived in were not correlated with individual or parental multilocus heterozygosity (all: $-0.09 < r < 0.09$, $P > 0.24$). Consequently, we started our analyses on a global model with time-dependent juvenile and adult survival and two age classes in the resighting probability (Table 2a, model 12). We checked the

No.	Model	No. par.	ΔAIC_c	Deviance	AIC_c weights	$B \pm SE$
(a)						
1	$\Phi_{(sq \times gm + a1, 2+)}$	8	0.0	749.8	0.46	-0.03 ± 0.12 0.90 ± 0.35
2	$\Phi_{(sq + a1, 2+)}$	6	4.0	758.0	0.06	
3	$\Phi_{(sq \times gf + a1, 2+)}$	8	4.1	753.9	0.06	-0.12 ± 0.12 0.66 ± 0.34
4	$\Phi_{(sq + gm \times a1, 2+)}$	8	4.1	754.0	0.06	0.32 ± 0.17 -0.42 ± 0.17
5	$\Phi_{(sq + gm + a1, 2+)}$	7	5.3	757.2	0.03	0.10 ± 0.11
6	$\Phi_{(sq + hz^2 + a1, 2+)}$	7	5.7	757.6	0.03	-0.08 ± 0.12
7	$\Phi_{(sq + hz + a1, 2+)}$	7	5.9	757.8	0.02	-0.04 ± 0.10
8	$\Phi_{(sq + gf + a1, 2+)}$	7	6.0	757.9	0.02	-0.03 ± 0.11
9	$\Phi_{(sq \times hz + a1, 2+)}$	8	6.8	756.7	0.02	-0.10 ± 0.12 0.27 ± 0.26
10	$\Phi_{(sq + hz \times a1, 2+)}$	8	7.9	757.7	0.01	-0.01 ± 0.15 -0.06 ± 0.21
11	$\Phi_{(sq + gf \times a1, 2+)}$	8	8.0	757.9	0.01	-0.01 ± 0.17 -0.04 ± 0.23
12	$\Phi_{(t + a1, 2+)}$	14	16.6	754.0	0.00	
(b)						
13	$\Phi_{(sq + sm + a1, 2+)}$	7	3.2	755.1	0.09	-0.35 ± 0.22
14	$\Phi_{(sq \times sm + a1, 2+)}$	8	4.6	754.5	0.05	-0.41 ± 0.23 0.59 ± 0.75
15	$\Phi_{(sq + sm \times a1, 2+)}$	8	5.3	755.1	0.03	-0.34 ± 0.30
16	$\Phi_{(sq + sf + a1, 2+)}$	7	5.9	757.8	0.02	-0.07 ± 0.13
17	$\Phi_{(sq \times sf + a1, 2+)}$	8	7.0	756.8	0.01	-0.001 ± 0.15 -0.32 ± 0.34
18	$\Phi_{(sq + sf \times a1, 2+)}$	8	7.8	757.7	0.01	-0.12 ± 0.18 0.10 ± 0.27

Table 2 Summary of model selection statistics for the effects of: (a) the individual's and genetic parents' multilocus heterozygosity, and (b) the social parents' multilocus heterozygosity on survival probabilities of the Seychelles warbler. Survival was dependent on the following (individual) covariates: (t), year (a1,2+), two age classes: first year and older (sq), season quality; (hz), individual heterozygosity; (gm), heterozygosity of genetic mother; (gf), heterozygosity of genetic father; (sm), heterozygosity of social mother; and (sf), heterozygosity of social father. Estimates of effect sizes (B , main effect (upper) and interaction (lower)) of individual covariates are given on the logit scale

The resighting probability was similar for all models with a lower resighting probability for individuals in their first 2 years of life and a higher probability for older birds. Models were ranked according to their AIC_c value, with the best supported model on top. ΔAIC_c being the difference between the AIC_c of the best supported model and the model considered.

goodness-of-fit of this model by using parametric bootstrap procedures implemented in the program MARK, which suggested that our global model fitted the data well (bootstrap goodness-of-fit, $n = 1000$, $P = 0.18$). The global model was then used to investigate the effects of individual and parental heterozygosity on survival. These effects were also tested in an interaction with age and year, with the main effects always included in the model when testing for an interaction. A model including heterozygosities at all 14 loci was then compared to the models with multilocus heterozygosity to investigate whether such a model would fit the data better and consequently whether correlations arise as a result of local effects. Including individual multilocus heterozygosity with parental heterozygosity in the same model did not change the results, but for clarity we present each effect separately.

Heterozygosity was included as an individual covariate in the analyses. As direct parental and environmental parental effects can only be differentiated using the cross-fostered/EGP offspring, two groups were created in which the covariates were only included for the group for which this data was available (see section 'Study area and data collection'). In this way, the complete data set could still be used to estimate the common survival and resighting parameters. The individual covariates were analysed by using the logit link function, with values of the individual covariates standardized to Z-scores (by subtraction of the mean and division by the standard deviation). Model notation was followed according to Lebreton *et al.* (1992).

Results

Early mortality

There was no indication that individual multilocus heterozygosity influenced mortality in the early stages of life. First, the multilocus heterozygosity of eggs that did hatch did not differ from that of eggs which did not hatch (hatched vs. nonhatched: mean \pm S.E. = 0.50 ± 0.01 , $n = 173$ vs. 0.56 ± 0.05 , $n = 15$, Mann-Whitney: $U = 1207.0$, $P = 0.58$). Second, the multilocus heterozygosity of fledglings did not differ from that of nestlings which did not fledge (fledged vs. nonfledged: mean \pm S.E. = 0.49 ± 0.01 , $n = 163$ vs. 0.52 ± 0.03 , $n = 26$, Mann-Whitney: $U = 1847.0$, $P = 0.29$).

Juvenile and adult survival

Our initial model structure (Table 2a, model 12) showed a clear division in survival between seasons. Juvenile survival in the first two seasons (average: 0.30 ± 0.06) was lower than in the remaining seasons (average: 0.68 ± 0.05); however, adult survival was relatively constant (0.88 ± 0.02). Creating a model with constant adult survival and two groups of seasons for juvenile survival was better

supported by the data than the fully time-dependent model (Table 2a, model 2 vs. model 12). Resighting probability was lower in the first 2 years of life (0.92 ± 0.02) and higher for older birds (0.96 ± 0.01). Model 2 (Table 2a) was used as a starting model to test for effects of parental and individual heterozygosity on survival.

Individual multilocus heterozygosity was not associated with survival; a model including individual multilocus heterozygosity was not supported by the data (Table 2a, model 7 vs. model 2). Furthermore, individual multilocus heterozygosity did not affect the age classes differently (Table 2a, model 10 vs. model 2). Even under adverse conditions, an association between juvenile survival and multilocus heterozygosity did not become apparent; a model including an interaction between multilocus heterozygosity and season quality did not provide a better alternative to the starting model (Table 2a, model 9 vs. model 2). The survival of individuals with extremely high or low multilocus heterozygosity did not differ from individuals with intermediate multilocus heterozygosity; including multilocus heterozygosity as a squared effect was not supported by the data (Table 2a, model 6 vs. model 2).

No direct effect of paternal multilocus heterozygosity on offspring survival was found; including multilocus heterozygosity of the genetic father was not supported by the data (Table 2a, model 8 vs. model 2). Furthermore, multilocus heterozygosity of the genetic father did not affect the age classes differently (Table 2a, model 11 vs. model 2), nor was it associated with survival in low quality seasons (Table 2a, model 3 vs. model 2). Multilocus heterozygosity of the genetic mother was not directly associated with offspring survival (Table 2a, model 5 vs. model 2), nor did it affect the age classes differently (Table 2a, model 4 vs. model 2), but it was positively associated with juvenile survival in low quality seasons (Table 2a, model 1 vs. model 2; Fig. 1). This model was almost eight times better supported by the data than was the model without these effects (Table 2a, model 1 vs. model 2).

In order to resolve between direct and environmental parental effects on offspring survival, the heterozygosity of the social parents was also investigated. However, multilocus heterozygosity of the social parents was not associated with offspring survival (Table 2b, models 13 and 16 vs. model 2), and there were no effects of the social parents' multilocus heterozygosity in an interaction with season quality or age (Table 2b, models 14, 15, 17 and 18 vs. model 2).

General vs. local effects

For multilocus heterozygosity to reflect genome-wide heterozygosity, heterozygosity at the individual loci should be correlated. We estimated heterozygosity twice for each individual, once using seven randomly sampled markers and then again based on the heterozygosity of

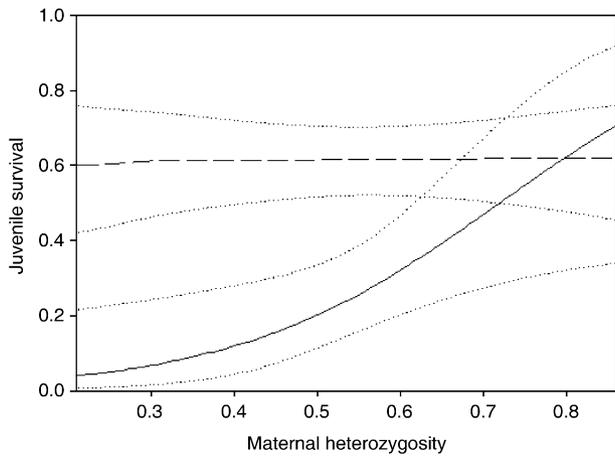


Fig. 1 The relation between juvenile survival and maternal multilocus heterozygosity in low (·) and high (—) quality seasons with the 95% confidence interval based on model 1 (Table 2a).

the remaining seven markers (Balloux *et al.* 2004). A regression on both estimates indicates that heterozygosity at the individual loci was not correlated as $r^2 < 1\%$. In addition, we performed an analysis based on the sum of covariances in heterozygosity between every pair of loci (for details see: Slate *et al.* 2004). This yielded $\sigma = 0.26$. To determine the statistical significance, individual heterozygosity was randomized across individuals at each locus and the sum of covariances was recalculated. Repeating this 10 000 times also showed that heterozygosity at the individual loci was not correlated, as our test statistic was not significantly different from random ($P = 0.38$). Similarly, such correlations were not significant in the mothers of the individuals born in the low quality years ($N = 49$, $r^2 < 1\%$).

Analyses of linkage disequilibrium showed that 24 out of 91 pairs of loci were in significant linkage disequilibrium, which was much higher than would be expected by chance (4.6 cases at $\alpha = 0.05$). After sequential Bonferroni correction, still eight pairs of loci showed significant linkage disequilibrium (*Ase*: 48-37, 9-37, 48-13, 42-13, 37-13, 10-

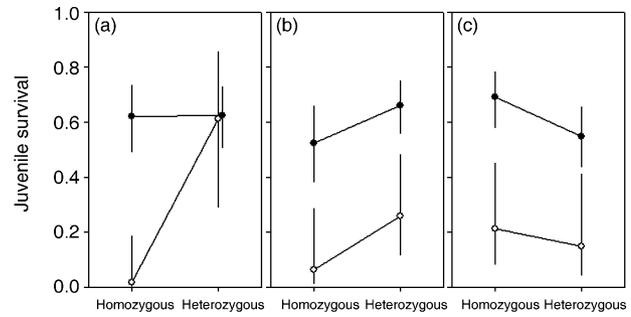


Fig. 2 The relation between juvenile survival and maternal homozygosity and heterozygosity at; (a) locus *Ase48* (b) locus *Ase35* and (c) locus *Ase9* in low (○) and high (●) quality seasons with the 95% confidence interval based on model 3 (Table 3).

35, 48-6, 37-6, 13-6), indicating that local effects could be important in this study.

To investigate whether the effect of mother's multilocus heterozygosity on offspring survival in low quality seasons was caused by local effects at specific loci, we included all single-locus heterozygosities as independent predictors in one model. However, this model was not better supported by the data than the model including multilocus heterozygosity, not surprisingly as the number of parameters increased with 26 (Table 3, model 3 vs. model 1). Investigating the estimates of the effect sizes of maternal heterozygosity at each locus on offspring survival showed that locus *Ase48* was associated with juvenile offspring survival in low-quality seasons and loci *Ase35* and *Ase9* were associated with offspring survival in all years, with a positive effect of *Ase35* and a negative effect of *Ase9* on offspring survival (Table 4, Fig. 2). These effects remained when these loci were tested separately (not shown). Furthermore, loci *Ase56*, *Ase6* and *Ase37* show similar effects to locus *Ase48*, but locus *Ase37* is in the opposite direction (Table 4). When testing these loci separately, the effects of loci *Ase56* and *Ase6* remain similar, but their confidence intervals overlap with zero (not shown). However, the

Table 3 Summary of model selection statistics for the effect of heterozygosity at maternal loci and an individuals own loci on survival probabilities of the Seychelles warbler. Survival was dependent on the following (individual) covariates: (sq), season quality; (a1,2 +), two age classes: first year and older (gm), multilocus heterozygosity of genetic mother; (gmloci), heterozygosity at each locus of genetic mother; and (loci), heterozygosity at each locus

No	Model	No. par.	ΔAIC_c	Deviance	AIC_c weights
1	$\Phi_{(sq \times gm + a1,2+)}$	8	0.0	749.8	0.88
2	$\Phi_{(sq + a1,2+)}$	6	4.1	758.0	0.11
3	$\Phi_{(sq \times gmloci + a1,2+)}$	34	9.2	703.1	0.01
4	$\Phi_{(sq \times loci + a1,2+)}$	34	17.7	722.5	0.00

The resighting probability was similar for all models with a lower resighting probability for individuals in their first 2 years of life and a higher probability for older birds. Models were ranked according to their AIC_c value, with the best supported model on top. ΔAIC_c being the difference between the AIC_c of the best supported model and the model considered.

Locus	Allele sizes (bp)	H_O	H_E	$B \pm SE$
<i>Ase48</i>	272 (278 + 279), 284	0.56	0.65	0.02 ± 0.16 2.33 ± 1.02
<i>Ase9</i>	(130 + 131), 133, 138	0.42	0.44	-0.32 ± 0.13 0.04 ± 0.49
<i>Ase10</i>	122, 130, 143	0.48	0.50	-0.16 ± 0.15 -0.39 ± 0.75
<i>Ase42</i>	249, 253	0.30	0.28	-0.10 ± 0.13 0.01 ± 0.47
<i>Ase37</i>	237, 241, 247	0.42	0.49	0.22 ± 0.15 -2.57 ± 1.23
<i>Ase18</i>	184, 188, 196	0.47	0.49	-0.09 ± 0.15 -0.05 ± 0.51
<i>Ase58</i>	283, 293, 299, 305, 310	0.73	0.72	-0.08 ± 0.15 1.12 ± 0.70
<i>Ase25</i>	173, 197, 202, 206, 209, 213	0.64	0.73	-0.04 ± 0.16 1.62 ± 0.89
<i>Ase27</i>	184, 209, 213, 217, 222, 225, 230	0.64	0.67	-0.20 ± 0.13 0.34 ± 0.48
<i>Ase13</i>	140, 147, 154	0.49	0.53	0.06 ± 0.15 0.32 ± 0.55
<i>Ase35</i>	230, 232, 234	0.54	0.60	0.30 ± 0.14 0.48 ± 0.50
<i>Ase4</i>	106, 108	0.45	0.42	0.09 ± 0.14 -0.53 ± 0.58
<i>Ase56</i>	299, 301, 305	0.31	0.39	0.01 ± 0.14 1.55 ± 0.67
<i>Ase6</i>	117, 119, 125, 129	0.69	0.69	-0.08 ± 0.14 1.31 ± 0.50

Alleles in brackets were lumped due to difficulties in resolving alleles with only one base pair difference (see Richardson *et al.* 2001).

effect of locus *Ase37* disappears and was apparently caused by its strong linkage disequilibrium.

To test whether the single loci that were more associated with offspring survival were also more polymorphic than the other loci, we analysed the strength of the heterozygosity–survival correlation for each locus measured by its AIC_c value in relation to the expected heterozygosity and the number of alleles at each locus. However, the loci which were more associated with offspring survival were not more polymorphic than the other loci (expected heterozygosity: $r_s = -0.29$, $n = 14$, $P = 0.32$; no. of alleles: $r_s = -0.10$, $n = 14$, $P = 0.74$).

As correlations among pairs of loci might have masked an effect of multilocus heterozygosity on individual survival, we then went back and tested the effect of heterozygosity at each locus in one model. However, a model including heterozygosity at each locus was not supported by the data (Table 3, model 4 vs. model 2). Although two loci seem to be associated with survival in low-quality seasons, locus *Ase48* ($B \pm SE = 1.00 \pm 0.48$) and *Ase13* ($B \pm SE = -1.79 \pm 0.71$), their effects are in the opposite direction and disappear when testing them separately, apparently caused by their strong linkage disequilibrium.

Table 4 Locus names with their allele sizes, observed (H_O) and expected (H_E) heterozygosity and estimate of effect size (B) of maternal single-locus heterozygosity on offspring survival as a main effect (upper) and in interaction with season quality (lower) (on logit scale), based on model 3 (Table 3)

Discussion

General vs. local effects

Despite the fact that a previous study showed that offspring heterozygosity decreased with increasing parental relatedness, i.e. with inbreeding (Richardson *et al.* 2004), we found that neither multilocus nor single locus heterozygosity was associated with an individual's own survival. However, the correlation between offspring heterozygosity and parental relatedness might be circular as parentage analyses and parental relatedness were based on genotypic similarity at the same microsatellite loci. The lack of heterozygosity–survival correlations is not surprising given that the heterozygosities of the microsatellite loci were not correlated either. Although there seemed to be high levels of linkage disequilibrium in the Seychelles warbler, none of the specific loci appeared to be linked to genes that were associated with an individual's own survival. High levels of linkage disequilibrium might have reduced the degree of information of the microsatellite loci, as they are not independent. Alternatively, the lack of an association between single-loci heterozygosity of an

individual and its survival might be explained by the fact that any linked loci are not overdominant or that the variation in fitness is only revealed under adverse conditions. In our study, survival in two seasons was very low, but even under these conditions individuals with lower heterozygosity did not, themselves, have decreased survival. The similar levels of heterozygosity in eggs that did or did not hatch, and nestlings that did or did not fledge, indicate that heterozygosity-linked mortality did not take place at an earlier stage of life. Another possibility is that any recessive deleterious alleles that had an effect directly upon individual survival have been purged. Although recent work suggests that purging will not substantially reduce inbreeding depression for most field populations (Keller & Waller 2002), the population we studied does meet the conditions where purging is likely to be most effective; the population went through a severe bottleneck (26–29 individuals) which occurred for several generations. As purging is likely to be most effective on alleles subject to strong selection (Hedrick 1994), the bottleneck in our population might have led to purging of the deleterious alleles affecting an individual's own survival directly, but not those alleles affecting offspring survival.

Many studies have investigated heterozygosity–fitness correlations (for review see Hansson & Westerberg 2002); however, only a few have considered the local effect hypothesis as an appropriate explanation. Some studies have shown that one or a few loci contribute more to a heterozygosity–fitness correlation than others (Merilä *et al.* 2003; Bean *et al.* 2004; Acevedo-Whitehouse *et al.* 2006). However, this is expected by chance even under the general effect hypothesis. Comparing a model with multilocus heterozygosity to a model including heterozygosity at each locus would be the best way to distinguish between the hypotheses (David *et al.* 1997). However, due to the increase in the number of parameters the power of such analysis will decrease with an increasing number of loci tested. Ideally, corrections for genome-wide inbreeding effects should be made, as for example in the great reed warbler (*Acrocephalus arundinaceus*) study by Hansson *et al.* (2004) where full siblings with different heterozygosity values at microsatellite loci are compared. In that study, the heterozygosity–survival correlation was especially pronounced at four loci. However, as complete pedigree data is often not available, it is possible that local effects, rather than general effects, may explain the heterozygosity–fitness correlations observed in many other studies (see also: Hansson & Westerberg 2002; Balloux *et al.* 2004; Slate *et al.* 2004). Part of the reason for this may be that the procedure used to select microsatellites for use in molecular studies on populations is biased. For instance, for studies that include parentage analyses (as in this present study), only the most polymorphic loci are selected for use. The

higher variance at these loci might be a result of the longer coalescence time between the alleles, but could also be an artefact of either nonstepwise mutation processes or high mutation rates, resulting in stronger linkage disequilibrium (Slate & Pemberton 2002). Hansson *et al.* (2004) showed that the more polymorphic loci were related to survival. This is not the case in our study. However, with only 14 microsatellites and the low variance in the number of alleles (Richardson *et al.* 2000), our power to detect such a relationship was low.

Parental heterozygosity

We found evidence that the multilocus heterozygosity of the genetic mothers was positively associated with juvenile survival of their offspring, however, only in low-quality seasons. The local effects hypothesis was not supported as maternal multilocus heterozygosity explained the variation in survival better than including the single locus heterozygosities. However, we found that the maternal heterozygosity–survival correlation in low quality seasons was mainly due to locus *Ase48*, but loci *Ase56* and *Ase6* show similar effects. Loci *Ase9* and *Ase35* were also associated with offspring survival in all seasons but to a much lesser degree. Surprisingly, higher heterozygosity at locus *Ase9* resulted in lower offspring survival. Why this should be the case is unknown, but one explanation might be that a rare deleterious allele causes higher mortality and that selection cannot act on this allele because it is fixed or linked to another locus.

Heterozygosity of both the genetic and the social father was not associated with offspring survival. This is in contrast to the earlier study by Richardson *et al.* (2004), where heterozygosity of the social father was positively associated with offspring survival. However, in that study the direct parental and environmental parental effects were not completely separated as heterozygosity of the social parents was analysed on both cross-fostered and noncross-fostered offspring. Furthermore, year and age effects were not accurately corrected for, as only cohort effects were taken into account. Nevertheless, the study of Richardson *et al.* (2004) did show the negative trend between maternal heterozygosity and juvenile survival.

Maternal effects

Our cross-foster experiment shows that there is only an association between offspring survival and heterozygosity of the genetic mother, and not of the foster mother. Consequently, the genes involved in the heterozygosity–survival association must affect fitness through direct maternal effects and not via maternal feeding or care. It is known that maternal effects may profoundly influence the

survival of offspring (Mousseau & Fox 1998; Wolf *et al.* 1998) and that they can occur through differential investment in eggs, e.g. differences in egg size (Potti 1999), hormone levels (Eising *et al.* 2001) or antibodies (Heeb *et al.* 1998). It is possible that, in the Seychelles warbler, these fitness genes are related to one or more of the above factors and that being heterozygous at these loci provides an advantage. Unfortunately we do not have any data on egg size or hormone composition of this data set. Two other studies, one on the song sparrow (*Melospiza melodia*, Keller 1998) and the other on the takahe (*Porphyrio hochstetteri*, Jamieson *et al.* 2003) have shown that the mother's, but not the father's, level of inbreeding affects offspring fitness. However, in these studies reduced offspring fitness may have been due to decreased heterozygosity in the offspring itself and not to the mother's level of inbreeding per se. To our knowledge, the present study is the first to show a direct relationship between maternal heterozygosity and offspring fitness in a natural population, although only under adverse conditions.

Seasonal selection events

Maternal heterozygosity is only associated with juvenile offspring survival in seasons with low survival probabilities. During the almost 20 years that the population of Seychelles warblers on Cousin Island has been studied, such bad years (with below 50% juvenile survival) have occurred only twice, in 1997–1998 and in 1986 (Brouwer *et al.* 2006). These 2 years were characterized by extreme weather conditions, specifically by either very high (1997) or very low (1986) levels of rainfall (Brouwer *et al.* 2006). With an average lifespan of 4 years after surviving their first year (average survival probability = 84%, Brouwer *et al.* 2006), many birds will never face such a selection event. Furthermore, even during such events selection is not acting directly on the mother, but indirectly on her offspring; consequently, the mother will face more opportunities to reproduce (in high quality seasons) and pass on her genes. Consequently, selection against any deleterious alleles exposed through homozygosity must be relatively weak, and this may explain how these alleles have persisted in the small bottlenecked population of the Seychelles warbler. Our results support the idea that genetic effects should not be considered independently of environmental effects (Bijlsma *et al.* 1999), as has been shown through a number of other studies on wild populations. For example, in the inbred population of song sparrows on Mandarte Island, outbred individuals survived better than inbred individuals under environmental stress (Keller *et al.* 1994). In Darwin's finches (*Geospizinae*), oscillating selection acts on morphological characters depending on the food supply (Gibbs & Grant 1987). While in the inbred takahe (*Porphyrio hochstetteri*), newly established

island populations had a higher level of reproductive failure than the population living in the natural habitat (Jamieson *et al.* 2003).

The identity of associated genes

Several of the microsatellite markers isolated from the Seychelles warbler (Richardson *et al.* 2000) were also used in a study of the great reed warbler (*Acrocephalus arundinaceus*, Hansson *et al.* 2004). Intriguingly, one locus (*Ase58*), used in both studies had a positive significant effect on survival in the great reed warblers but not in the Seychelles warbler. As the environment these species live in is completely different, it might be that the gene linked to *Ase58* is of lesser importance in the Seychelles warbler than in the great reed warbler. Alternatively, as this locus appeared to have a direct effect on the survival of great reed warblers any deleterious alleles at this locus may have been purged in the Seychelles warbler. Whether the loci *Ase48*, *Ase9* and *Ase35* associated with offspring survival in the Seychelles warbler are also associated with offspring survival in the great reed warbler has not yet been investigated.

A predicted, passerine genome map of microsatellites shows that one of the loci associated to the maternal effect in the Seychelles warbler (locus *Ase9*) is located on chromosome 3, while another (*Ase48*) is located on chromosome 5 of the chicken genome (Dawson *et al.* 2006). Unfortunately, the three *Ase* loci associated with survival in the great reed warbler study were not successfully mapped. Future research taking more loci into account and comparing which loci affect fitness across a range of populations of the same or closely related species should make it possible to detect the location and even the function of the genes causing the heterozygosity–fitness correlation, and by this get a better understanding of the underlying processes involved. Furthermore, complete pedigrees of populations will help us to investigate how known levels of *f* relate to heterozygosity and/or fitness.

Conclusion

Our study shows that heterozygosity–fitness correlations can occur in bottlenecked populations, although such correlations might be very limited, in our case only heterozygosity of the mother was associated with offspring survival in low-quality seasons. Our results support the idea that even in a small inbred population, heterozygosity measured at microsatellites might not reflect genome-wide heterozygosity or therefore individual inbreeding. However, support for local effects caused by linkage disequilibrium between specific neutral markers and fitness genes was also equivocal, highlighting the difficulty in distinguishing between the two hypotheses.

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Lyanne Brouwer is a PhD student at the University of Groningen working on population regulation and cooperative breeding behaviour in the Seychelles warbler. Jan Komdeur has a chair in evolutionary ecology and his research focuses on behavioural adaptations in relation to ecological and social conditions. Members of his group work on a wide range of animals including insects fish, but mainly birds. His own work focuses on two long term studies: of Seychelles warblers in Seychelles; and of blue tits in The Netherlands. David Richardson heads a research group at UEA that focuses on the use of molecular tools to resolve evolutionary and ecological questions, such as the role of the MHC in sexual selection and the evolution of cooperative breeding, using model avian systems.
