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MHC-dependent survival in a wild population: evidence for hidden genetic benefits gained through extra-pair fertilizations

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Abstract

Females should prefer to be fertilized by males that increase the genetic quality of their offspring. In vertebrates, genes of the major histocompatibility complex (MHC) play a key role in the acquired immune response and have been shown to affect mating preferences. They are therefore important candidates for the link between mate choice and indirect genetic benefits. Higher MHC diversity may be advantageous because this allows a wider range of pathogens to be detected and combated. Furthermore, individuals harbouring rare MHC alleles might better resist pathogen variants that have evolved to evade common MHC alleles. In the Seychelles warbler, females paired with low MHC-diversity males elevate the MHC diversity of their offspring to levels comparable to the population mean by gaining extra-pair fertilizations. Here, we investigate whether increased MHC diversity results in higher life expectancy and whether there are any additional benefits of extra-pair fertilizations. Our 10-year study found a positive association between MHC diversity and juvenile survival, but no additional survival advantage of extra-pair fertilizations. In addition, offspring with a specific allele (*Ase-ua4*) had a fivefold longer life expectancy than offspring without this allele. Consequently, the interacting effects of sexual selection and pathogen-mediated viability selection appear to be important for maintaining MHC variation in the Seychelles warbler. Our study supports the prediction that MHC-dependent extra-pair fertilizations result in genetic benefits for offspring in natural populations. However, such genetic benefits might be hidden and not necessarily apparent in the widely used fitness comparison of extra- and within-pair offspring.

Keywords: mark-recapture survival analysis, mate choice, MHC class I, pathogens, Seychelles warbler, viability selection

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Introduction

The idea that individuals may be able to gain indirect genetic benefits through mate choice has long appealed to biologists (Searcy 1982; Jennions & Petrie 2000; Charmantier & Sheldon 2006), not least because it could explain mate choice by females in situations where direct benefits do not appear to be gained, such as in socially monogamous species where females pursue extra-pair fertilizations (EPFs) (Andersson 1994). Theory suggests that females may gain indirect genetic benefits

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either through the acquisition of 'good' paternal genes (Hamilton & Zuk 1982), increased genetic diversity (Brown 1997) or the enhanced genetic compatibility of maternal and paternal genomes (Zeh & Zeh 1996). However, the existence and nature of such genetic benefits remain controversial (Jennions & Petrie 2000; Griffith *et al.* 2002; Westneat & Stewart 2003; Arnqvist & Kirkpatrick 2005; Akçay & Roughgarden 2007; Griffith 2007). Several studies have provided evidence that mate choice may increase offspring fitness through genetic benefits (Petrie 1994; Hasselquist *et al.* 1996; Sheldon *et al.* 1997; Kempenaers *et al.* 1999; Johnsen *et al.* 2000; Fossøy *et al.* 2008), but identifying the genes which link both mate choice and fitness variation has proven to be difficult (but see Eizaguirre *et al.* 2009).

The genes of the major histocompatibility complex (MHC) have received a great deal of attention as candidates for providing genetic benefits (von Schantz *et al.* 1996; Grob *et al.* 1998; Jordan & Bruford 1998; Penn & Potts 1999; Ekblom *et al.* 2004; Consuegra & Garcia de Leaniz 2008). Not only because the MHC is an important component of the vertebrate immune system that determines which antigens trigger an adaptive immune response (Hughes & Yeager 1998), but also because it appears to affect mating preferences (for a review see: Penn 2002). The extraordinary levels of variation that occur within the MHC are thought to be maintained by pathogen-mediated selection (Jeffery & Bangham 2000; Bernatchez & Landry 2003), through mechanisms such as heterozygote advantage (Doherty & Zinkernagel 1975), rare-allele advantage (Slade & McCallum 1992) and fluctuating selection (Hedrick 2002) or a combination of these mechanisms (for a review see: Spurgin & Richardson 2010). Females may choose among males to acquire the best genes for their offspring, either by gaining 'good' MHC haplotypes associated with improved resistance to pathogens, or by avoiding 'bad' MHC haplotypes associated with deleterious effects (Applanalp *et al.* 1992; Wedekind *et al.* 1996; Rülicke *et al.* 1998). Alternatively, females may be seeking the best combination of maternal and paternal MHC genes to improve offspring MHC characteristics: females may prefer to mate with males with the most dissimilar set of MHC genes so as to maximize offspring MHC diversity (Brown 1997) or they may choose males with which they share an intermediate number of MHC alleles so as to optimize offspring MHC diversity at an intermediate level (Nowak *et al.* 1992).

Evidence for associations between MHC genotypes and pathogen resistance is overwhelming and comes from both laboratory/semi-natural (Carrington *et al.* 1999; Senseney *et al.* 2000; Langefors *et al.* 2001; Lohm *et al.* 2002; Penn *et al.* 2002; Worley *et al.* 2010) and natural populations (Paterson *et al.* 1998; Meyer-Lucht & Sommer 2005; Schad *et al.* 2005; Westerdahl *et al.* 2005; Bonneaud *et al.* 2006). However, the fitness consequences of MHC-conferred pathogen resistance are often not clear. First, many studies have tested host resistance using assays that do not directly assess the impact of the pathogens on fitness. Second, laboratory studies have often tested host resistance using single strains of pathogens (but see Penn *et al.* 2002), whereas under natural conditions multiple infections might have to be combated simultaneously (Hedrick 2002). Third, in wild populations the individuals in which pathogens are detected might actually be the survivors of infection, rather than the diseased (Westerdahl *et al.* 2005). Unequivocal evidence for the idea that MHC-dependent patterns of mate choice can confer indirect genetic benefits requires that the fitness consequences of MHC diversity/specific alleles are studied in a natural population and that individuals are compared within birth cohorts over most of their life.

In the Seychelles warbler (*Acrocephalus sechellensis*; a tropical passerine), evidence suggests that balancing selection has occurred within the MHC class I loci of this species (Richardson & Westerdahl 2003) and has maintained MHC variation in the face of a recent population bottleneck (Hansson & Richardson 2005; Richardson *et al.* 2005). EPFs were found to be common (40% of offspring; Richardson *et al.* 2001) and MHC dependent: females were more likely to obtain EPFs when their social mate had low MHC diversity (below the population average as measured across four duplicated loci; Richardson *et al.* 2005). No effect of MHC similarity between the social male and female was found. Importantly, the extra-pair males had higher MHC diversity than the cuckolded pair male, but not higher than the population average (Richardson *et al.* 2005). Because the MHC diversity of offspring depends on the diversity of the genetic parents, the gaining of EPFs by the females resulted in offspring with higher MHC diversity than would have been the case if the female had mated with the pair male, but not higher than the population level of diversity for within-pair offspring (Richardson *et al.* 2005). However, for this to translate into a genetic benefit of EPFs the elevated MHC diversity resulting from EPFs would have to confer a fitness advantage.

The Seychelles warbler population, like other island populations (MacArthur & Wilson 1967; Poulin 1997), appears to harbour a relatively depauperate pathogen community. For example, there is no evidence of diseases such as avian pox (*Poxvirus avium*) or avian mycoplasmosis. Nonetheless, important pathogens such as a *heamoprotus* strain of avian malaria have been detected in ca 40% of individuals (Hutchings 2009). Given the lack of predators and low variability in climatic conditions on this island, it is likely that such pathogens are

a major source of mortality in this population. Furthermore, this species exhibits cooperative breeding, in which habitat saturation causes offspring to delay dispersal and remain as subordinates in a territory, often for several years (Komdeur 1992, 1996; Richardson *et al.* 2002). Consequently, many individuals never obtain a breeding position and survival (lifespan) will be the main cause of variation in lifetime reproductive success in this long-lived species (Clutton-Brock 1988). These two factors mean that in the Seychelles warbler fitness differences because of MHC diversity are most likely to occur through variation in survival.

Here, we use capture-mark-recapture analyses on multiple cohorts of fledglings that were followed for 10 years in a closed island population to investigate the role of MHC class I exon 3 variation on survival and lifespan. We first test whether extra-pair offspring have a survival advantage compared to within-pair offspring *per se*. Subsequently, we test whether there are genetic benefits to the patterns of MHC-dependent EPFs observed in this species. The reduced MHC variation observed in the bottlenecked Seychelles warbler population (Richardson & Westerdahl 2003) also provides us with a powerful opportunity to test for allele-specific survival associations; an opportunity typically lacking in more MHC-diverse species (i.e. high MHC diversity means that specific alleles occur in only few individuals which reduces the statistical power to detect fitness associations).

Methods

Study area and data collection

The Seychelles warbler went through a severe bottleneck from 1920 to 1968 with the last ~30 individuals of this species remaining on the tiny island of Cousin (29 ha; 04°20'S, 55°40'E; Crook 1960, Loustau-Lalanne 1968). After regeneration of the original vegetation, the population recovered and has stabilized at ~320 adults since the 1980s (Bathe & Bathe 1982; Brouwer *et al.* 2009a). Here, we monitor the fate of fledglings of the 1997–1999 cohorts ($n = 160$) until 2007 as part of an intensive long-term study on the Seychelles warbler (Richardson *et al.* 2004; Brouwer *et al.* 2009a). Between 1997 and 1999, over 97% of adults were ringed and blood sampled, and all breeding attempts were followed. During the main breeding season (July–September), and the minor breeding peak (January–March), each territory was checked regularly for breeding activity. Most Seychelles warblers produce one clutch per season, which normally consists of a single egg, but 20% of nests contain two to three eggs (Richardson *et al.* 2001). Nestlings were ringed with a unique combination of three colour rings and a British Trust for Ornithology ring at ~12 days of age (just before fledging). If a nest could not be reached, the nestling was mist-netted shortly after fledging while still dependent upon its parents within the natal territory.

Blood samples (ca. 25 μL) were collected by brachial venipuncture, diluted in 800 μL of 100% ethanol in a 2.0-mL microfuge tube and stored at room temperature. For parentage analyses, individuals were genotyped using 14 polymorphic microsatellite markers isolated in the Seychelles warbler (Richardson *et al.* 2000). Parentage had previously been determined using program Cervus (Richardson *et al.* 2001) and has been confirmed using MasterBayes (Hadfield *et al.* 2006).

MHC screening

We screened variation at exon 3 of MHC class I using the primers and methodology outlined in Richardson & Westerdahl (2003), but using Reference Strand Conformation Analysis (RSCA) to separate sequence variants within individuals (Drake *et al.* 2004; Worley *et al.* 2008). Exon 3 encodes the peptide-binding region of the MHC class I genes and therefore is crucial for antigen recognition and adaptive immunity against intra-cellular pathogens (Hughes & Yeager 1998). We failed to screen 33 offspring because their blood samples had been depleted in previous analyses (e.g. Richardson *et al.* 2001). We assumed the missing data were random and unbiased with respect to MHC diversity, and we found no evidence that survival differed between individuals that were screened for MHC ($n = 127$) and those that were not ($n = 33$; mark-recapture analysis, likelihood ratio test: $\chi^2_{\text{d.f.}=1} = 1.83$, $P = 0.18$). Successful screening of 127 offspring detected 2–7 MHC sequence variants per individual (median = 4) of 9 variants in the population in total, each of which encoded a different set of amino acids (Richardson & Westerdahl 2003). The screening method is not locus specific and therefore does not allow specific sequence variants to be assigned as alleles at specific loci. Notwithstanding this limitation, individuals that have more MHC variants are expected to be heterozygous at more loci than individuals with fewer MHC variants. The number of MHC variants within an individual (MHC diversity) will therefore be an indicator of heterozygosity and provide a broad estimate of overall diversity across the four duplicated MHC class I loci. Henceforth, the exon 3 sequence variants will be termed 'alleles' for reasons of simplicity.

Survival analyses

Dispersal from the island is virtually absent (Komdeur *et al.* 2004), meaning that – in contrast to most other

(nonclosed) studies – undetected dispersal does not bias our survival estimates (Tinbergen 2005). Two re-sighting periods were defined: the main breeding season (1 July–1 September) and the minor breeding peak (1 January–1 March). Individuals were recorded as present if observed in the last 2 weeks of each breeding season. The biannual re-sighting periods allow us to estimate survival over two 6-month periods for each year. From 2000 to 2003 and in 2006, we did not have a re-sighting period in the minor breeding season, thus for these years we could only calculate survival over the whole year. Ten birds that were part of a translocation in 2004 (Brouwer *et al.* 2009b) were removed from the dataset from that moment on (i.e. treated as right censored). Two groups were created in the analyses: one for which MHC data were available and one for which data were missing. Individuals with missing MHC data were included in the analyses to improve parameter estimation of MHC-independent variation in survival and re-sighting rates.

Individuals' re-sighting histories were used as input files for survival analyses in the program MARK (White & Burnham 1999). We adopted the basic model structure of a previous study (Brouwer *et al.* 2007 with virtually the same dataset) as a starting (null) model for our analyses. This, together with previous studies, showed that: (i) juvenile (first-year) survival was lower than adult survival (survival is constant from the second year on) (Brouwer *et al.* 2006), (ii) re-sighting rate was lower in the first 2 years of life than at older ages (Brouwer *et al.* 2006), (iii) survival did not differ between the sexes (Brouwer *et al.* 2006), (iv) juvenile survival varied between high- and low-quality seasons, but adult survival was relatively constant over time (Brouwer *et al.* 2007) and (v) the heterozygosity of the genetic mother (measured across a suite of 14 neutral microsatellite markers, Richardson *et al.* 2000, 2001) has been shown to positively affect survival in low-quality seasons (Brouwer *et al.* 2007). By accounting for the previous effects in all our models, we reduce the unexplained variance in survival and thereby increase the power to detect effects of MHC and extra-pair fertilizations. Estimating the amount of overdispersion using the median ĉ-procedure implemented in program MARK (Cooch & White 2004) suggested our null model fitted the data well (variance inflation factor $\hat{c} = 0.99 \pm 0.02$).

We first tested whether extra-pair offspring have a survival advantage compared to within-pair offspring. We then investigated whether both MHC diversity and/or the presence of specific MHC alleles were associated with survival by including these variables as individual covariates in the analyses (using a logit link function). As most MHC alleles are inter-correlated (covariance matrix, in 16 of 36 covariances the mean ± 2 SE does not overlap with zero), all alleles were included in the same model simultaneously to estimate their effect on survival. The presence of allele *Ase-ua1* was highly correlated with allele *Ase-ua10* ($r = 0.98$), and therefore we did not consider the association between survival and allele *Ase-ua10*. Model selection was based on Akaike's information criterion corrected for sample size (AIC_c; Akaike 1973). Models that are better supported by the data result in lower AIC_c values, but models with $\Delta AIC_c < 2$ are considered to be approximately equally well supported. Additionally, we report the normalized Akaike weights to assess the relative support for competing models (Burnham & Anderson 2002). Because re-sighting probabilities are close to one in this study (0.92 ± 0.02 in the first 2 years of life and 0.98 ± 0.01 for older birds), time of death can be determined with high confidence, therefore Kaplan–Meier curves were used to illustrate how survival differences translate into differences in lifespan of Seychelles warblers (Statistica 7.0; StatSoft Inc.).

Results

Survival of extra- vs. within-pair offspring

Extra-pair offspring did not have higher survival compared to within-pair offspring. A model that included extra-pair fertilization (yes/no) as a covariate of juvenile and/or adult survival was not better supported by the data than a reduced model (Table 1, model 12 & 13 vs. model 11). Even after accounting for effects of MHC diversity and specific MHC alleles (see next sections), there was no evidence that extra-pair offspring had different survival than within-pair offspring (Fig. 1a, Table 1, model 1 vs. 2). There also was no evidence that extra-pair and within-pair offspring primarily differed in survival in specific seasons (i.e. in either high- or low-quality seasons; Table 1, model 14 vs. 11). Consequently, on average extra-pair and within-pair offspring do not differ systematically in their life expectancy (Fig. 1b).

MHC diversity and survival

Individuals with higher MHC diversity had higher juvenile survival, but adult survival did not covary with MHC diversity (Fig. 1c). A model whereby the association between survival and MHC diversity was allowed to vary between adults and juveniles was better supported by the data than a model with a similar effect for juveniles and adults (Table 1, model 4 vs. 15; model 4 was also better supported than the null

Table 1 Summary of model selection statistics for the effect of extra-pair fertilization (EPF), MHC diversity and specific MHC alleles on survival probabilities of the Seychelles warbler

No.	Model description	No. parameters	ΔAIC_c	Deviance	AIC_c weights
1	Log(MHC diversity) × Age + Allele *Ase-ua4*	11	0	822.7	0.594
2	Log(MHC diversity) × Age + Allele *Ase-ua4* + EPF × Age	13	0.9	819.4	0.381
3	Log(MHC diversity) × Age	10	7.7	832.5	0.013
4	MHC diversity × Age	10	9.7	834.5	4.6×10^{-3}
5	Allele *Ase-ua4* × Age	10	10.9	835.7	2.6×10^{-3}
6	Allele *Ase-ua4*	9	10.9	837.7	2.5×10^{-3}
7	Allele *Ase-ua4* × Season quality	10	12.6	837.3	1.1×10^{-3}
8	$(\text{MHC diversity})^2 \times$ Age	10	14.2	838.9	5.0×10^{-4}
9	MHC diversity × Season quality	10	16.6	841.3	1.5×10^{-4}
10	All alleles × Age	24	17.8	813.2	8.3×10^{-5}
11	Null model	8	18.2	847.1	6.5×10^{-5}
12	EPF × Age	10	18.5	843.2	5.9×10^{-5}
13	EPF	9	19.0	845.8	4.4×10^{-5}
14	EPF × Season quality	10	19.4	844.2	3.6×10^{-5}
15	MHC diversity	9	19.6	846.4	3.3×10^{-5}
16	All alleles × Season quality	24	22.2	817.7	8.8×10^{-6}
17	All alleles	16	22.8	835.2	6.5×10^{-6}

The re-sighting probability was similar for all models with a lower re-sighting 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. In addition to the two age-specific re-sighting parameters, the null model includes the following survival parameters: two age-specific and two time-specific survival parameters and an effect of heterozygosity of the genetic mother in an interaction with the season quality. The number of parameters of other models equals the eight parameters from the null model plus the parameters used to estimate the effects given in the model description.

model 11). The positive association between MHC diversity and survival appeared to show diminishing returns with increasing diversity, with individuals with two alleles having lower survival than all the remaining individuals (Fig. 1c). In fact, a model where survival was fitted as a logarithmic function of MHC diversity was better supported by the data than a model in which a linear effect of MHC diversity was fitted (Table 1, model 3 vs. 4). In disagreement with the optimal MHC diversity hypothesis (Nowak *et al.* 1992), we found no evidence that an intermediate number of alleles was associated with the highest survival in juveniles (i.e. no stabilizing viability selection). More specifically, the inclusion of MHC diversity as a quadratic effect was not better supported by the data than a logarithmic effect of MHC diversity (Table 1, model 8 vs. 3). Furthermore, there was no evidence that viability selection on MHC diversity fluctuated between high- and low-quality seasons (Table 1, model 9 vs. 3). To illustrate the severe consequence of the positive association between MHC diversity and juvenile survival, Fig. 1d shows that the median life expectancy of fledglings with at least four (=median) MHC alleles was 2.5 times higher than that of individuals with less than four MHC alleles (1.0 vs. 2.5 years).

The association between MHC diversity and survival does not appear to be linked to genome-wide heterozygosity/inbreeding. Parents of offspring with fewer MHC alleles did not have higher pair-wise relatedness (calculated across 30 microsatellite loci in program KINGROUP, Konovalov & Heg 2008) than parents of offspring with more MHC alleles (Pearson $r_s = 0.10$, $n = 125$, $P = 0.26$). Furthermore, survival was not associated with heterozygosity measured at 14 microsatellite loci (Brouwer *et al.* 2007), nor when this analysis was repeated with 30 microsatellite loci (effect heterozygosity: $\beta = -0.76 \pm 1.68$, interaction age × heterozygosity, $\beta = 0.03 \pm 2.24$). In fact, against predictions based on the idea that low MHC individuals would be inbred and have low heterozygosity, MHC diversity covaried negatively with heterozygosity measured at 30 microsatellite loci ($r = -0.22$, $n = 127$, $P = 0.01$).

Individual MHC alleles and survival

A model that included the effects of the presence/absence of each individual allele simultaneously on survival was not better supported than the null model (Table 1, model 17 vs. 11). Neither when this was tested in an interaction with age or season quality (Table 1, model 10 and 16 vs. model 11). Nevertheless,

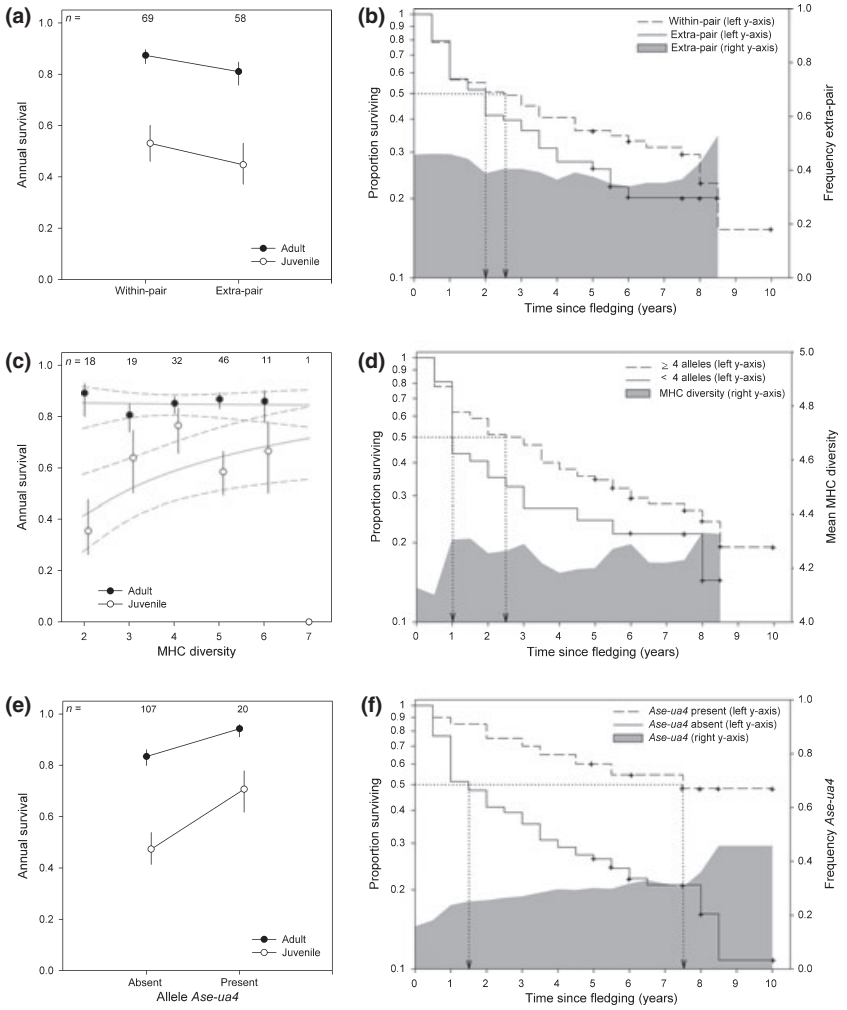

Fig. 1 Effects of (a, b) extra-pair paternity, (c, d) MHC diversity and (e, f) a specific MHC allele on annual survival (left panels) and lifespan (right panels) of Seychelles warbler fledglings. In the left-hand panels, annual juvenile and adult survival are shown in relation to (a) extra-pair and within-pair paternity, (c) MHC diversity (number of alleles) and (e) presence or absence of allele *Ase-ua4*. These maximum likelihood survival estimates are based on model 2 in Table 1, which included all three above mentioned covariates. Numbers on top indicate number of offspring followed; error bars are based on SE. Lines in (c) show predictions (±95% CI) of the effect of MHC diversity on survival. In the right-hand panels, the proportion surviving since fledging (on log scale; left *y*-axis) is shown for individuals with (b) extra-pair or within-pair paternity, (d) a high or low MHC diversity (i.e. ≥4 or <4 alleles) and (f) allele *Ase-ua4* present or absent. The dotted lines point to the median life expectancy of fledglings (i.e. the age at which 50% of the cohort has died). The grey surfaces depict the changes in frequency of (b) extra-pair paternity, (d) mean MHC diversity and (f) frequency of *Ase-ua4* over time in the cohorts (right *y*-axis). Note that sample sizes decrease over time, and consequently stochasticity causes small fluctuations to be more pronounced. Translocated individuals and individuals alive at the end of the study and were treated as right censored (indicated with the symbol '+').

investigating the estimates of the effect sizes for each allele separately showed that *Ase-ua4* positively affected survival (Table 2). Indeed, a model that included *Ase-ua4* was 38 times better supported by the data than the null model without this allele (Table 1, AIC_c of model 6 with *Ase-ua4*/AIC_c weight of model 11 without *Ase-ua4* = $2.5\ 10^{-3}/6.5 \times 10^{-5} = 38$). Formal hypothesis testing suggested the positive effect of allele *Ase-ua4* on survival was significant (likelihood ratio test: $\chi^2_{\text{d.f.}=1} = 9.4$, $P = 0.002$) even after sequential Bonferroni correction for the testing of multiple alleles (threshold value is α/no. tests = 0.05/8 = 0.006).

There was no evidence that the association between survival and *Ase-ua4* fluctuated between high- and low-quality seasons (Table 1, model 7 vs. 6). Likewise, there was no strong evidence that *Ase-ua4* was differentially associated with the survival of juveniles and adults (Table 1, model 5 vs. 6; slope age × *Ase-ua4*: $\beta = 1.25 \pm 0.92$ SE). However, this comparison of slopes concerns survival on the (relative) logit scale.

Table 2 Estimates of effect size (ß) of each MHC allele on juvenile and adult survival of Seychelles warblers. Estimates are on the logit scale derived from model 17 (Table 1). Term shown in bold is significantly different from zero

Allele	Effect size ß ± SE
Ase-ua1	0.12 ± 0.45
Ase-ua2	0.50 ± 0.75
Ase-ua3	−0.12 ± 0.33
Ase-ua4	**1.18 ± 0.46**
Ase-ua5	−0.19 ± 0.32
Ase-ua6	0.34 ± 0.35
Ase-ua7	0.37 ± 0.54
Ase-ua9	0.00 ± 0.47

Consequently, the absolute effect of *Ase-ua4* on survival seemed three fold larger for juveniles (Fig. 1e). A consequence of the positive association between *Ase-ua4* and both juvenile and adult survival is that the median life expectancy of an individual with *Ase-ua4* is five times higher than an individual without this allele (7.5 vs. 1.5 years, Fig. 1f).

The positive association between survival and MHC diversity was not caused by the presence of the *Ase-ua4* allele (or vice versa), because individuals with *Ase-ua4* did not have a significantly higher mean MHC diversity than individuals without (+*Ase-ua4*: 4.3 ± 0.12 SE; −*Ase-ua4*: 4.1 ± 0.23 SE; $t_{1,125} = -0.49$, $P = 0.62$). Furthermore, removing allele *Ase-ua4* from the measure of MHC diversity or analysing the effect of MHC diversity only in individuals without allele *Ase-ua4* did not change the association between MHC diversity and survival (original model: $\beta = 2.91 \pm 0.82$ SE; excluding allele *Ase-ua4* from MHC diversity: $\beta = 2.83 \pm 0.82$ SE; only individuals without allele *Ase-ua4*: $\beta = 2.94 \pm 0.84$ SE). In fact, a model that included the effects of both *Ase-ua4* and log[MHC diversity] explained variation in survival much better than a model that included each effect separately (Table 1, model 1 vs. 3 and 6).

MHC trait dynamics resulting from viability selection

Because of the increased survival of juveniles with a higher number of MHC alleles, the mean MHC diversity in the cohorts increased in the first year after fledging but not thereafter (Fig. 1d). In contrast, the *Ase-ua4* allele increased in frequency in the cohorts steadily throughout both the juvenile and adult life stages. At fledging *Ase-ua4* was a relatively rare allele that occurred in only 16% of the individuals but the strong viability selection caused this proportion to increase in these cohorts over time to 45% 10 years later (Fig. 1f).

Discussion

MHC and Genetic benefits of EPFs

Female Seychelles warblers have already been shown to be more likely to gain EPFs when paired with a social mate with low MHC diversity. Furthermore, they gained these EPFs from males with significantly higher MHC diversity than their cuckolded mates, resulting in offspring with higher MHC diversity than would have been the case if they had remained faithful to the pair male (Richardson *et al.* 2005). We have now shown that a higher than median MHC diversity resulted in a more than twofold longer lifespan. We did not find any evidence for genetic benefits of extra-pair fertilizations *per se*, as on average extra- and within-pair offspring survived equally well.

At first sight, our results seem to contradict each other. However, they can be reconciled by the fact that females gained EPFs from males with higher MHC diversity than their cuckolded pair male, but not higher than the population average (Richardson *et al.* 2005). Consequently, the extra-pair offspring's MHC diversity will increase to levels comparable to within-pair offspring. To put it another way, by gaining EPFs, females paired with low MHC-diversity males are ensuring that their offspring do not end up with below average levels of MHC diversity and therefore lower survival. In hindsight, the result that, on average, extra-pair and within-pair offspring in the population had similar fitness may, therefore, not be surprising, because EPFs result in genetic benefits for specific individuals only. However, it does point out that 'hidden' benefits as observed in our study are difficult to disentangle directly, which may explain why many other studies failed to detect population-wide genetic benefits of EPFs (e.g. Lubjuhn *et al.* 1999; Whittingham & Dunn 2001; Kleven & Lifjeld 2004).

Potentially, the benefits of EPFs can be studied by comparing the survival of half-sibs within a brood, i.e. the within- and extra-pair offspring of the same social pair. The typical single egg clutches (Richardson *et al.* 2001), low nesting success and replacement of breeders over the seasons make such a comparison impossible in the Seychelles warbler. However, we emphasize that comparing half-sibs could also be misleading. The problem being females paired with low MHC-diverse males are more likely to gain EPFs (Richardson *et al.* 2005) and, as a consequence, the pairs that produce both within-pair and extra-pair half-sibs are likely to be biased towards pairs with MHC-diverse males. Offspring from MHC-diverse males are expected to gain little from EPFs, and therefore the genetic benefits of MHC-dependent mate choice may also remain hidden

in a half-sib comparison of extra-pair and within-pair offspring.

A previous study found that female Seychelles warblers paired with low MHC-diverse males did not gain EPFs with the highest MHC diversity males in the population (Richardson *et al.* 2005). Our new results may now help us understand why females do not always prefer the most MHC-diverse males. We have shown that the association between survival and MHC diversity levelled off with increasing diversity, thus choosing males with above average MHC diversity would not have resulted in any additional fitness benefits for the offspring. A thing that remains unknown, however, is what mechanism drives the patterns of MHC-dependent extra-pair mate choice. Experiments are needed to determine whether females actively choose more diverse MHC males or whether other factors like male–male competition or sperm competition play a role.

We found that birds with one specific allele (*Ase-ua4*) had a survival advantage compared to birds without this allele; however, it was unknown whether allele *Ase-ua4* was associated with extra-pair mate choice in a similar way as MHC diversity (Richardson *et al.* 2005). Post-hoc analysis of the Richardson *et al.* (2005) data, including both MHC diversity and presence/absence of *Ase-ua4*, shows that females tended to be more likely to gain EPFs when paired with a male without allele *Ase-ua4*, although this was not statistically significant (Logistic regression: *Ase-ua4*: $\beta = -1.20 \pm 0.73$, Wald: 2.73, $P = 0.10$, MHC diversity: $\beta = -0.66 \pm 0.27$, Wald: 5.93, $P = 0.02$; total $R^2 = 0.14$). Because *Ase-ua4* was a relatively rare allele and the frequency in the population is expected to increase, further investigation with a larger data set in the future is required to see if this pattern is consistent.

MHC and survival

Associations between MHC traits and resistance to pathogens have often been found (for review see: Bernatchez & Landry 2003), but associations with survival mainly come from laboratory or semi-natural situations (Lohm *et al.* 2002; Penn *et al.* 2002; Pitcher & Neff 2006; Wegner *et al.* 2008). However, a study on wild Soay sheep (*Ovis aries* L.) found that specific alleles (but not diversity) were associated with both parasite resistance and juvenile survival (Paterson *et al.* 1998), while a study on wild great reed warblers (*Acrocephalus arundinaceus*) showed that there was only a nonsignificant tendency for MHC diverse birds to have higher survival (Hansson *et al.* 2004). Although associations between MHC diversity and survival are predicted given the implicit role of MHC in the immune system (Hughes & Yeager 1998), our results are the first evidence for an association between MHC diversity and lifespan from a natural population.

The survival advantage for birds with higher MHC diversity is consistent with the heterozygote advantage hypothesis (Doherty & Zinkernagel 1975). Apparently, Seychelles warblers with higher MHC diversity (which will reflect higher levels of heterozygosity across the four MHC loci detected in this species) are able to detect and combat a wider range of pathogens than less diverse individuals. However, without being able to assign alleles to specific loci it is not possible to determine whether the benefit of increased MHC diversity is caused by overdominance (Spurgin & Richardson 2010). Why MHC diversity negatively covaried with heterozygosity measured at microsatellite markers remains unknown, but the development of an extensive pedigree will allow us to investigate this in the future.

The absence of an association between MHC diversity and survival during the adult life stage may have resulted from the selective disappearance of individuals with low MHC diversity during their first year of life (see Fig. 1d). In contrast, the association between one specific allele (*Ase-ua4)* and survival was apparent during both the juvenile stage and adulthood. Clearly despite the strong effect on juvenile survival, the rarity of *Ase-ua4* meant that also individuals without *Ase-ua4* survived into adulthood, and thus selection for *Ase-ua4* operates in this age class too.

The survival advantage of Seychelles warblers with *Ase-ua4* suggests that individuals with this allele are better at combating a specific pathogen in the environment. Further studies are now needed to identify the specific pathogens involved. The *Ase-ua4* allele was initially at a low frequency in the cohorts followed (16%) but increased to a frequency of 45% in the surviving individuals after 10 years. The allele's initial rareness and strong viability selection advantage suggests that it has only recently come to confer resistance to a specific pathogen, which would imply that a rare-allele advantage/negative frequency-dependent selection is involved (Takahata & Nei 1990; Jeffery & Bangham 2000; Hedrick 2002). Alternatively, it may be that the pathogen that is interacting with *Ase-ua4* has recently been introduced or increased in prevalence within the population (i.e. fluctuating selection hypothesis; Hedrick 2002). As it is the MHC class I that we are screening here it is mainly intra-cellular pathogens that we would expect to be exerting the detected selection pressure. However, we have no evidence of any new pathogens (intra- or extracellular) invading, or increasing in frequency, within the limited and stable pathogen fauna detected in the long-term screening of this population; no gastrointestinal parasites, only one blood pathogen detected (avian malaria), and no evidence of fungal or virus pathogens

has been found (DS. Richardson unpublished data), though we realize that our screening is not exhaustive.

Further work is needed to screen the MHC allele frequencies in the current population, more than a decade after the initial screening took place, to determine whether the allele *Ase-ua4* is now much more common among the new generations (as expected given the survival advantage and resulting larger probability of being transferred to the next generations). Only by tracking the selective advantage of the *Ase-ua4* allele over many years, and investigating whether it is linked to fluctuations in the island's pathogen fauna, we will be able to investigate whether selection is driven by a rare-allele advantage or fluctuating selection. Discerning between the pathogen-mediated selection mechanisms that interact with MHC variation is extremely difficult (see review in: Spurgin & Richardson 2010); however, the Seychelles warbler may provide a unique opportunity to do so. The new populations of warblers set up through translocations (Brouwer *et al.* 2009a), two of which no longer contain avian malaria (DS. Richardson unpublished data), may also facilitate this investigation.

MHC, pathogens and extinction risk

Evidence has now accumulated showing that genetic factors contribute significantly to extinction risk in the wild (Saccheri *et al.* 1998; Spielman *et al.* 2004; Frankham 2005). Key functional immunogenetic loci, such as the MHC, may be particularly important in this as lower levels of variation at these loci will reduce a populations' ability to detect and combat pathogens. It is not surprising then that pathogens are considered a major threat to endangered species, which, almost by definition, are characterized by low levels of genetic variation. For example, in the crested ibis (*Nipponia nippon),* where a severe population bottleneck is thought to be responsible for the low observed levels of MHC diversity (Zhang *et al.* 2006), up to 40% of mortality was attributed to diseases (Zhou *et al.* 2001). The spread of avian malaria and pox has also been implicated in the decline and extinction of several bottlenecked endemic Hawaiian bird species (van Riper *et al.* 1986; Atkinson *et al.* 1995, 2001; Jarvi *et al.* 2001).

MHC diversity in the Seychelles warbler is low compared to outbred congeneric species (Hansson & Richardson 2005), and this species may therefore be susceptible to pathogens. However, island species normally harbour less parasites than their mainland relatives (MacArthur & Wilson 1967; Poulin 1997), and the isolated location, small island size and virtual absence of between-island dispersal by Seychelles warblers (Komdeur *et al.* 2004) may limit the introduction of novel pathogens into the populations (MacArthur & Wilson 1967; Poulin 1997; Lindström *et al.* 2004). Indeed there is, as yet, no evidence of the introduced epidemics such as avian pox *(Poxvirus avium)* or avian mycoplasmosis that have plagued other bird populations (DS. Richardson unpublished data). This may be seen as a good thing in the short term; however, the depauperate pathogen fauna may mean that compared to other species, pathogen-mediated selection on MHC diversity might be weak in the Seychelles warbler. Consequently, although predicted to be a rare event, the introduction of novel pathogens into the system, e.g. by transmission via other species (Wikelski *et al.* 2004), remains a very real threat over the long-term, which may pose a serious extinction risk to the Seychelles warbler (Diamond 1994). The maintenance of MHC variation is therefore crucial and, in this light, it may be important that the nine identified alleles of the Seychelles warbler's MHC code for different amino acids sequences; a fact that suggests that there is still adaptive potential to respond to new pathogens in this species.

Conclusion

Our study showed that although genetic benefits of EPFs were not detected by directly comparing within- and extra-pair offspring, hidden benefits might still exist and act through a more complex mechanism. We showed that a sexually selection mechanism – the gaining of EPFs – can promote MHC diversity and consequently survival of offspring in the Seychelles warbler. This combination of natural and sexual selection on MHC diversity may explain how a relatively high level of MHC diversity compared to neutral variation has been maintained in this species (Richardson *et al.* 2000; Richardson & Westerdahl 2003). Ironically, it may actually be the persistence, rather than the absence, of pathogens and the selective pressure they exert (balancing selection) that helps to maintain MHC diversity through genetic bottlenecks.

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