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Risk of ectoparasitism and genetic diversity in a wild lesser kestrel population

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Abstract

Parasites and infectious diseases are major determinants of population dynamics and adaptive processes, imposing fitness costs to their hosts and promoting genetic variation in natural populations. In the present study, we evaluate the role of individual genetic diversity on risk of parasitism by feather lice *Degeeriella rufa* in a wild lesser kestrel population (*Falco naumanni*). Genetic diversity at 11 microsatellite loci was associated with risk of parasitism by feather lice, with more heterozygous individuals being less likely to be parasitized, and this effect was statistically independent of other nongenetic parameters (colony size, sex, location, and year) which were also associated with lice prevalence. This relationship was nonlinear, with low and consistent prevalences among individuals showing high levels of genetic diversity that increased markedly at low levels of individual heterozygosity. This result appeared to reflect a genome-wide effect, with no single locus contributing disproportionably to the observed effect. Thus, overall genetic variation, rather than linkage of markers to genes experiencing single-locus heterosis, seems to be the underlying mechanism determining the association between risk of parasitism and individual genetic diversity in the study host–parasite system. However, feather lice burden was not affected by individual heterozygosity; what suggest that differences in susceptibility, rather than variation in defences once the parasite has been established, may shape the observed pattern. Overall, our results highlight the role of individual genetic diversity on risk of parasitism in wild populations, what has both important evolutionary implications and major consequences for conservation research on the light of emerging infectious diseases that may endanger genetically depauperated populations.

Keywords: ectoparasites, *Falco naumanni*, heterozygosity, lesser kestrel, microsatellites

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Introduction

Parasites and infectious diseases play a major role on population dynamics and have a major influence on host ecology and evolution (Hamilton & Zuk 1982; O'Brien & Evermann 1988; Hamilton *et al*. 1990; Møller 1997; Castro & Bolker 2005). Accumulating evidence suggests that pathogens increase host genetic diversity by exerting a negative frequency-dependent selection, favouring rare alleles and selecting against common ones (Clarke & Partridge 1988). Within a population, higher individual host genetic diversity is likely to increase parasite resistance if such variability comprises a higher variety of resistance alleles or a repertoire of inducible defences. Most studies providing conclusive data on the association between risk of parasitism and individual genetic diversity are based on the analyses of polymorphism at the major histocompatibility complex (MHC), a genetic region involved in immune recognition (Hedrick *et al*. 2001; Penn *et al*. 2002; McClelland *et al*. 2003; Westerdahl *et al*. 2005). Nevertheless, whether risk of parasitism can be influenced by overall genetic variation in natural populations, rather than just by variability at specific loci implicated in immune response, is still controversial (Coltman *et al*. 1999; Poulin *et al*. 2000a, b; Spielman *et al*. 2004; Luong *et al*. 2007). Genome-wide heterozygosity may

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have an important influence on risk of parasitism because genetically diverse individuals not only present a higher chance of carrying adaptive alleles to fight off parasites but they also show a reduced chance of expression of deleterious recessive alleles and gain heterozygote advantage for other traits controlled by genes experiencing some form of balancing selection (Coltman *et al*. 1999; Cassinello *et al*. 2001). This is likely to make them metabolically more efficient what could confer important benefits under changed or stressful conditions such as imposed by parasites (Keller *et al*. 2002; Armbruster & Reed 2005; Luong *et al*. 2007). Given that several physiological and behavioural mechanisms are generally implemented to respond to disease, genetically more diverse individuals would be the best able to cope with parasites (Luong *et al*. 2007). Thus, determining whether parasite-mediated selection promotes variability across the genome has important implications for species conservation (Altizer *et al*. 2003; Pearman & Garner 2005) and to comprehend the maintenance of the high levels of genetic diversity generally found in natural populations ('the paradox of variation'; Lewontin 1974).

One approach to test the influence of reduced overall heterozygosity on risk of parasitism is using neutral polymorphic markers, such as microsatellites, to estimate genome-wide heterozygosity (Balloux *et al*. 2004; Aparicio *et al*. 2006; Aparicio *et al*. 2007). Many recent studies have found associations between risk of parasitism and heterozygosity measured at a handful of neutral markers (Coltman *et al*. 1999; Acevedo-Whitehouse *et al*. 2003; Acevedo-Whitehouse *et al*. 2005; Hawley *et al*. 2005; MacDougall-Shackleton *et al*. 2005; Acevedo-Whitehouse *et al*. 2006), but others have found no such relationship (Côte *et al*. 2005; Westerdahl *et al*. 2005). Recent theoretical and empirical studies point out that the small number of neutral markers generally employed may be a poor estimate of genome-wide heterozygosity in large, outbred populations so that it has been suggested that the general prevalence of positive results reported in most studies may have been exaggerated by publication biases (Balloux *et al*. 2004; Slate *et al*. 2004). In other cases, it is not clear whether the obtained correlation reflects a genome-wide effect, the general effect hypothesis (Weir & Cockerham 1973; David 1998) or, otherwise, is the result from a linkage disequilibrium between a single or few neutral markers and functional genes under balancing selection (local effect hypothesis) (David 1998; Hansson & Westerberg 2002; Balloux *et al*. 2004). Thus, the link between genetic diversity and parasite prevalence is unclear and more case studies are necessary to better understand their underlying mechanisms.

The pervasiveness of the parasitic lifestyle has selected for a wide variety of defences in animal hosts, including innate and adaptive immunity, antiparasite behaviour, mechanical defences, and barriers to infection (Hart 1990). In spite of this variety of responses to defend against pathogens and diseases, most studies analysing the role of host genetic diversity on parasite resistance have focused efforts on host–parasite systems where the main antiparasite defence has an immunological nature (e.g. Acevedo-Whitehouse *et al*. 2003; Acevedo-Whitehouse *et al*. 2005; Côte *et al*. 2005; Hawley *et al*. 2005; MacDougall-Shackleton *et al*. 2005; Westerdahl *et al*. 2005; Acevedo-Whitehouse *et al*. 2006; see, however, Luong *et al*. 2007). In birds, an interesting case study to test whether mechanisms other than immune defences are influenced by host genetic variability is analysing the influence of individual genetic diversity on risk of parasitism by feather-feeding lice (e.g. Hart 1997; Whiteman *et al*. 2006), parasites that have no direct contact with living host tissues and are not impacted by the immune system (Moyer *et al*. 2002; Møller & Rózsa 2005). Feather lice are permanent parasites whose transmission depends on body contact between hosts. They complete their entire life cycle on the host feeding on feathers and they are known to have a direct effect on host fitness by damaging feathers, which compromises thermoregulatory ability (Booth *et al*. 1993), affects flight behaviour (Barbosa *et al*. 2002) and reduces survivorship (Clayton *et al*. 1999) and male-mating success (Clayton 1990; Kose & Møller 1999; Kose *et al*. 1999). Birds mainly control feather lice by preening, a costly behaviour that can constrain investment in other life-history components such as feeding, courtship and vigilance (Redpath 1988; Croll & McLaren 1993). Thus, these parasites are likely to be also an important selective agent for the evolution of efficient behavioural and physical host defences that may be sensitive to host genetic diversity (e.g. Whiteman *et al*. 2006; Luong *et al*. 2007).

In the present study, we evaluate whether genetic diversity, measured through 11 highly polymorphic microsatellite markers, influences the risk of parasitism by the feather lice *Degeeriella rufa* (Phthiraptera: Ischnocera) in the lesser kestrel (*Falco naumanni*), a colonial small size bird of prey. The lesser kestrel suffered a sharp population decline in the middle of the last century (Biber 1990; González & Merino 1990), what may have reduced their genetic variability and increased the intensity of inbreeding depression and susceptibility to diseases (e.g. O'Brien & Evermann 1988; Whiteman *et al*. 2006). Thus, this species is a good candidate to analyse the association between parasite occurrence and genetic diversity. Apart from including information on host genetic characteristics, we analyse other parameters that may influence ectoparasite burdens and prevalence, such as colony size, sex and host condition (Potti & Merino 1995; Hoi *et al*. 1998; Whiteman & Parker 2004). Finally, we also explored whether any locus in particular contributed disproportionately to the association between lice parasitism and heterozygosity (e.g. Bean *et al*. 2004; Acevedo-Whitehouse *et al*. 2006; Ortego *et al*. 2007).

Methods

Study population and field procedures

The study was conducted in La Mancha, central Spain (600–800 m above sea level), in an area covering approximately 1000 km² (see Ortego *et al*. 2007 for a detailed description). We studied 22 lesser kestrel colonies clustered in two subpopulations separated by 30 km: 'Villacañas' subpopulation (39°30′N, 3°20′W; 16 colonies) and 'Consuegra' subpopulation (39°35′N, 3°40′W; 6 colonies). However, in spite of the low exchange of individuals between both subpopulations, a preliminary analysis suggests that they are not genetically differentiated (Ortego *et al*. 2007).

Kestrels normally arrive to the study area from their winter quarters in Africa in mid-February or the beginning of March, depending on the year. During 2005 and 2006 breeding seasons, adult lesser kestrels were trapped with a noose carpet or by hand during incubation, measured and individually marked with metallic and coloured plastic rings for further identification. Blood samples (100 μL) were obtained by venipuncture of the brachial vein and preserved in ~1200 μL ethanol 96% at –20 °C. We also used pectoral thickness as an estimator of body condition (Aparicio 1997; Aparicio & Cordero 2001). This trait has been used in previous studies as a measure of body condition in several bird species (Bolton *et al*. 1991; Newton 1993), and has been considered a more reliable measure of condition than residuals of body mass on tarsus length (Gosler & Harper 2000). Moreover, it is easy to measure accurately on live birds by using a portable ultrasonic metre, in this case a Krautkrämer USM22F (accuracy 0.1 mm), especially designed to measure animal tissues. We knew the exact age of around a third of individuals that were ringed as fledglings. For all other birds, we considered that individuals captured for the first time were in their first year if they presented yearling plumage or in their second year if they presented adult plumage (e.g. Aparicio & Cordero 2001; Foerster *et al*. 2003; Ortego *et al*. 2007). The relationship between lice prevalence/burden and age did not differ between individuals with known and estimated ages (data not shown), and so we pooled all data for the analyses. We found a single species of feather lice *Degeeriella rufa* parasitizing lesser kestrels. One of us (J.O.) determined the occurrence of this feather lice by direct visual examination of all rectrix (i.e. stiff tail feathers) and primary feathers (the plumage part where this parasite mainly concentrates), without using special methods such as fumigation (e.g. Szczykutowicz *et al*. 2006). The number of feather lice was recorded for each primary and rectrix feather, and the total number of lice found was used as an estimate of feather lice abundance (we will use interchangeably the terms intensity of infestation and parasite burden, e.g. Potti & Merino 1995). We will mean by prevalence as the presence of *D. rufa* during this examination (e.g. Hoi *et al*. 1998; Valera *et al*. 2003; Szczykutowicz *et al*. 2006). We manipulated and banded lesser kestrels under license from the Spanish institutional authorities (Environmental Agency of Junta de Comunidades de Castilla-La Mancha and the Ringing Office of the Ministry of Environment) and we followed general ethical guidelines for animal welfare and nature conservation.

Microsatellite genotyping and estimation of genetic diversity

We quantified multilocus heterozygosity in 181 lesser kestrels across 11 highly polymorphic microsatellite markers: Fp5, Fp13, Fp31, Fp46-1, Fp79-4, Fp86-2, Fp89 (Nesje *et al*. 2000), Fu1, Fu2 (J. Wetton, unpublished), Fn1-11, and Fn2-14 (Ortego *et al*. in press). All individuals were genotyped at all these 11 microsatellite markers. We used QIAamp DNA Blood Mini Kits (QIAGEN) to extract and purify genomic DNA from the blood samples. Approximately 5 ng of template DNA was amplified in 10-μL reaction volumes containing 1× reaction buffer [67 mM Tris-HCl, pH 8.3, 16 mM $(NH_4)_2SO_4$, 0.01% Tween 20, EcoStart Reaction Buffer, Ecogen], 2 mM $MgCl_2$, 0.2 mM of each dNTP, 0.15 μM of each dye-labelled primer (FAM, HEX or NED) and 0.1 U of *Taq* DNA EcoStart Polymerase (Ecogen). All reactions were carried out on a Mastercycler EpgradientS (Eppendorf) thermal cycler. The polymerase chain reaction (PCR) programme used was 9 min denaturing at 95 °C followed by 30 cycles of 30 s at 94 °C, 45 s at the annealing temperature and 45 s at 72 °C, ending with a 5-min final elongation stage at 72 °C. Amplification products were electrophoresed using an ABI PRISM 310 Genetic Analyser (Applied Biosystems) and genotypes were scored using GENESCAN 3.7 (Applied Biosystems).

Statistical analyses

Tests for deviation from Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium between markers were calculated following Weir (1996). In the present study, we use homozygosity by loci (*HL*) to estimate individual genetic diversity, a microsatellite derived measure that improves heterozygosity estimates in open populations by weighting the contribution of each locus to the homozygosity value depending on their allelic variability (Aparicio *et al*. 2006; Ortego *et al*. 2007).

We analyse the relationship between *HL* and both feather lice prevalence and burden using generalized linear mixed models (GLMM) implemented with the GLIMIX macro of SAS (SAS Institute 2004). GLMMs allow analyses of data where the response variable is determined by both random and fixed effects. Prevalence (229 data from 181 independent individuals) was analysed using a binomial

error structure and logit link. Since the distribution of ectoparasites usually follows a negative binomial distribution (Rekasi *et al*. 1997), we used the following natural logarithmic transformation to analyse ectoparasite burden: $y = \ln(x + 0.5k)$, where k is the exponent of the negative binomial distribution. The exponent k was estimated using the formula: $k = m^2(s^2 - m)^{-1}$, where m is the mean and s^2 is the variance of the distribution (Bliss & Fisher 1953). We analysed ectoparasite burden both including (229 data from 181 independent individuals) and excluding individuals with zero values of feather lice (140 data from 119 independent individuals). GLMMs were constructed by fitting H_L as explanatory variable together with nongenetic terms (covariates: day of capture, pectoral thickness, age, and colony size; fixed factors: locality, year, sex) that could potentially influence feather lice prevalence (e.g. Potti & Merino 1995; Hoi *et al*. 1998). No pair of these independent variables was strongly correlated (all $r < 0.35$) so we initially included all of them in our analyses (Green 1979). The identities of colonies and cohort were included as random effects to control for the potential nonindependence of parasite prevalence and burden within colonies and cohorts, in the manner of a randomized complete block design to avoid pseudoreplication (Krackow & Tkadlec 2001). Given that some individuals were monitored in both years of study, we also included individual identity nested within colony identity (i.e. higher-level factor; for the rationale of the model, see Singer 1998) as random effect.

Initially, each GLMM was constructed with all explanatory terms fitted, including first-order interactions and quadratic effects to account for potential nonlinear relationships. Final models were selected following a backward procedure, by progressively eliminating nonsignificant variables. The significance of the remaining variables was tested again until no additional variable reached significance. The result is the minimal most adequate model for explaining the variability in the response variable, where only the significant explanatory variables are retained. All tests were performed using the residual degrees of freedom (SAS Institute 2004). Hypotheses were tested using *F*-statistics and all *P* values refer to two-tailed tests.

Finally, we assessed evidence for the contribution of single locus to heterozygosity–fitness associations that might result from linkage between marker loci and genes influencing fitness. For this purpose, we re-analysed our data including into the models heterozygosity of each locus as a binary variable and *HL* calculated without including the locus being considered.

Results

The mean number of alleles per locus was 25.7, and ranged from 3 to 128. After applying sequential Bonferroni corrections to compensate for multiple statistical tests, four loci deviated from HWE (Fp13, Fp86-2, Fu2, Fn2-14). The probability of departure from HWE had no effect on the contribution of each particular locus to the obtained correlation between prevalence of feather lice and *HL* ($F_{1,9} = 0.04$, $P = 0.851$; see results below and Tables 1, 2; see Lieutenant-Gosselin & Bernatchez 2006 and Ortego *et al*. 2007 for a similar analysis). There was no evidence of genotypic linkage disequilibrium at any pair of loci and subpopulation (all $P > 0.05$).

Table 1 GLMMs for (a) feather lice prevalence (binomial error and logit link function) and burden (normal error and identity link function) (b) including and (c) excluding nonparasitized individuals in relation to *HL* and nongenetic terms (covariates: day of capture, pectoral thickness, age, and colony size; fixed factors: locality, year, sex). Only variables included in the models are indicated. Parameter estimates and S.E. for the levels of fixed factors were calculated considering a reference value of zero for the male level in the variable 'sex', for Villacañas subpopulation level in the variable 'locality', and for 2006 breeding season level in the variable 'year'

	Estimate	± S.E.	d.f.	*F*	*P*
(a) Parasite prevalence					
Intercept	–0.534	0.686	1, 222		
HL^2	6.094	2.249	1, 222	7.34	0.007
Colony size	0.119	0.050	1, 222	5.75	0.017
Colony size^2	–0.003	0.001	1, 222	9.35	0.002
Sex	0.677	0.344	1, 222	3.86	0.051
Year	–1.515	0.395	1, 222	14.72	< 0.001
Locality	1.351	0.409	1, 222	10.88	0.001
Covariance parameter estimates:					
	Estimate ± S.E.	*Z*	*P*		
Individual identity	0	—	—		
Colony identity	0	—	—		
Cohort	0	—	—		
(b) Parasite burden (including nonparasitized individuals)					
Intercept					
Colony size	0.061	0.030	1, 223	4.27	0.040
Colony size^2	–0.002	0.001	1, 223	8.44	0.004
Sex	0.696	0.201	1, 223	12.04	0.001
Year	–1.238	0.209	1, 223	34.99	< 0.001
Locality	0.753	0.214	1, 223	12.37	< 0.001
Covariance parameter estimates:					
	Estimate ± S.E.	*Z*	*P*		
Individual identity	0	—	—		
Colony identity	0	—	—		
Cohort	0.037 ± 0.111	0.34	0.368		
(c) Parasite burden (excluding nonparasitized individuals)					
Intercept	1.599	0.147	1, 137		
Sex	0.565	0.152	1, 137	13.78	< 0.001
Year	–0.672	0.152	1, 137	19.46	< 0.001
Covariance parameter estimates:					
	Estimate ± S.E.	*Z*	*P*		
Individual identity	0	—	—		
Colony identity	0.017 ± 0.043	0.39	0.348		
Cohort	0	—	—		

Table 2 Test for the effects of single locus heterozygosity and general (all loci, measured as squared *HL* excluding the locus being considered) heterozygosity on feather lice prevalence. Table shows *P* values

Locus	Single locus	General
Fp5	0.278	0.022
Fp13	0.170	0.027
Fp31	0.860	0.004
Fp46-1	0.169	0.019
Fp79-4	0.742	0.003
Fp86-2	0.302	0.010
Fp89	0.060	0.016
Fu1	0.663	0.005
Fu2	0.237	0.011
Fn1-11	0.375	0.020
Fn2-14	0.784	0.015

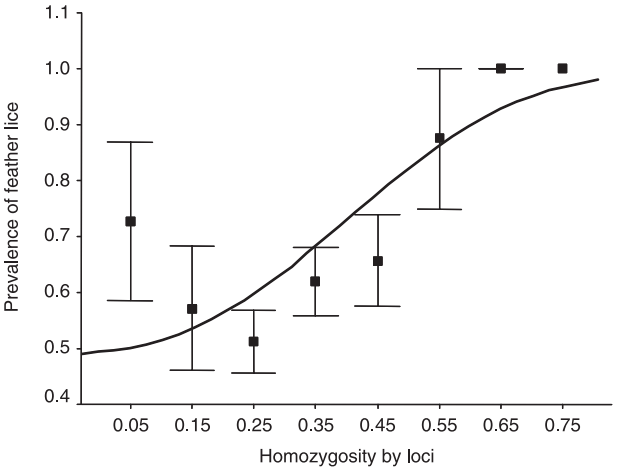

Fig. 1 Mean +1 SE. feather lice prevalence in relation to homozygosity by loci. Solid line shows predicted values from the GLMM.

The mean prevalence of *Degeeriella rufa* was 53% in 2005 and 80% in 2006. Considering only parasitized individuals, average parasite burden was 4.95 (S.E. = 5.71; range 1–28) in 2005 and 10.50 (S.E. = 11.55; range 1–61) in 2006. Prevalence of feather lice was positively associated with squared *HL* (Table 1; Fig. 1), indicating that more homozygous individuals were more likely of being parasitized. The linear term of *HL* was not significant when it was included together with squared *HL* into the model ($F_{1,221} = 1.39$, $P = 0.239$). However, after excluding squared *HL* from the model, linear term of *HL* became positively and significantly associated with parasite prevalence ($F_{1,222} = 6.69$, $P = 0.010$). In any case, the model that included the quadratic term of *HL* (presented in Table 1a; explained deviance: 11.52%) provided a better fit to the data than did the model including the linear term of *HL* (explained deviance: 10.52%). Feather lice prevalence increased and then declined significantly with colony size and females were more frequently parasitized than males (Table 1a). Lice prevalence was higher in 2006 breeding season and in Consuegra subpopulation (Table 1a). However, age ($F_{1,221} = 0.11$, $P = 0.744$), pectoral thickness ($F_{1,221} = 1.55$, $P = 0.215$), and day of capture ($F_{1,221} = 0.09$, $P = 0.769$) had no effect on lice prevalence. The interactions between genetic diversity and sex (*HL*: $F_{1,221} = 0.84$, $P = 0.360$; Squared *HL*: $F_{1,221} = 1.64$, $P = 0.202$), year (*HL*: $F_{1,221} = 0.80$, $P = 0.373$; Squared *HL*: $F_{1,221} = 1.22$, $P = 0.271$) and subpopulation (*HL*: $F_{1,221} = 1.09$, $P = 0.298$; Squared *HL*: $F_{1,221} = 0.64$, $P = 0.426$) were all not significant, indicating that the association between genetic diversity and parasite prevalence was consistent between sexes, years and localities. Other quadratic terms and interactions between independent variables were not significant in this analysis ($P > 0.1$ in all cases). The relationship between the quadratic and linear terms of *HL* and lice prevalence were also highly significant when these parameters were included alone into the models (*HL*: $F_{1,227} = 5.81$, $P = 0.017$; squared *HL*: $F_{1,227} = 7.73$, $P = 0.006$), so that the effect we found was not due to interactions among independent variables. We also explored whether a particular locus significantly influenced the effect of heterozygosity on ectoparasite prevalence. After removing any single locus from the calculation of *HL*, the relationship between genetic diversity and feather lice prevalence remained always significant (Table 2). Of the 11 markers used, none revealed an individual significant association with ectoparasite prevalence (Table 2).

When nonparasitized birds were considered in the analyses, we found that parasite burden increased and then declined significantly with colony size and females were more frequently parasitized than males (Table 1b). Parasite burden was higher in 2006 breeding season and in Consuegra subpopulation (Table 1b). However, *HL* ($F_{1,222} = 2.54$, $P = 0.112$) or its squared term ($F_{1,222} = 3.01$, $P = 0.084$) had no effect. The interactions between genetic diversity and sex (*HL*: $F_{1,221} = 0.55$, $P = 0.458$; Squared *HL*: $F_{1,221} = 1.01$, $P = 0.316$), year (*HL*: $F_{1,221} = 0.02$, $P = 0.889$; squared *HL*: $F_{1,221} = 0.11$, $P = 0.743$) and subpopulation (*HL*: $F_{1,221} = 1.88$, $P = 0.172$; Squared *HL*: $F_{1,221} = 1.50$, $P = 0.222$) were all not significant in this model. Including nonparasitized individuals, parasite burden was not associated with age ($F_{1,222} = 0.05$, $P = 0.825$), pectoral thickness ($F_{1,222} = 1.64$, $P = 0.202$), or day of capture ($F_{1,222} = 2.50$, $P = 0.116$). Quadratic terms and other interactions between independent variables were not significant in this analysis ($P > 0.1$ in all cases). This GLMM accounted for 20.24% of the original deviance.

Considering only the parasitized kestrels, we found that parasite burden was only influenced by sex and year but not by homozygosity ($F_{1,136} = 1.01$, $P = 0.316$) or its squared term ($F_{1,136} = 0.66$, $P = 0.419$; Table 1c). Parasite burden

was higher in females and in 2006 breeding season. The interactions between genetic diversity and sex (*HL*: $F_{1,135} = 0.66$, $P = 0.517$; squared *HL*: $F_{1,135} = 0.15$, $P = 0.702$), year (*HL*: $F_{1,135} = 2.14$, $P = 0.121$; squared *HL*: $F_{1,135} = 2.24$, $P = 0.110$) and subpopulation (*HL*: $F_{1,135} = 0.79$, $P = 0.454$; squared *HL*: $F_{1,135} = 0.51$, $P = 0.476$) were all not significant in this model. Parasite burden did not differ between subpopulations ($F_{1,136} = 0.55$, $P = 0.460$) and was not associated with age ($F_{1,136} = 1.15$, $P = 0.286$), pectoral thickness ($F_{1,136} = 0.72$, $P = 0.397$), or day of capture ($F_{1,136} = 0.48$, $P = 0.492$). Quadratic terms and other interactions between independent variables were not significant in this analysis ($P > 0.1$ in all cases). This GLMM accounted for 20.87% of the original deviance.

Discussion

We found that genetic diversity increases the risk of parasitism by the feather lice *Degeeriella rufa* in a wild lesser kestrel population, an effect that was independent of other individual or ecological factors also affecting ectoparasite prevalence. Interestingly, parasite prevalence was better explained by a nonlinear relationship with genetic diversity. The positive effect of squared *HL* indicates that parasite prevalence was low and consistent among individuals showing high levels of genetic diversity and then it increased markedly at low levels of individual heterozygosity. Thus, feather lice prevalence reached a plateau in individuals showing the highest heterozygosity values, suggesting that the effects of genetic diversity on risk of parasitism follows a saturation curve. Similar nonlinear associations have been obtained for MHC diversity in relation with blood parasite prevalence (Westerdahl *et al*. 2005) as well as between different marker-based measures of genetic variability and other components of fitness (Fu & Ritland 1996; Hansson 2004). All these results could be consequence of epistatic effects between loci that may reinforce the negative effects of reduced genetic diversity and result in a nonlinear decline of fitness with homozygosity (Crow & Kimura 1970; Fu & Ritland 1996; Dudash *et al*. 1997; Lynch & Walsh 1998).

The correlation between lice prevalence and heterozygosity appeared to reflect a genome-wide effect, with no single locus contributing disproportionably to the observed effect. Thus, overall genetic variation, rather than linkage of markers to genes experiencing single-locus heterosis, seems to be the underlying mechanism determining the association between risk of parasitism and individual genetic diversity in the studied host–parasite system. Some intrinsic characteristics of both the study species and population may be favouring the detection of such relationship. First, the sharp population decline suffered by lesser kestrels in the Iberian Peninsula in the last century could have reduced the species genetic variability, making risk of parasitism more sensitive to individual heterozygosity in comparison with other populations which have not suffered a large reduction in size (e.g. Pearman & Garner 2005). Apart from the population inbreeding history, other factors such as the admixture of genetically differentiated individuals during the actual expansion of the lesser kestrel in the study area (J.M. Aparicio, unpublished data) as well as the philopatric behaviour of the species, that may favour crosses between close relatives, could be generating enough inbreeding variance to be reflected in neutral markers (Tsitrone *et al*. 2001; Balloux *et al*. 2004). Thus, the obtained relationship between feather lice prevalence and genetic diversity, as well as the positive effect of individual heterozygosity on the species fecundity found in a previous study (Ortego *et al*. 2007), may be indicative that some of the factors above suggested could be favouring the detection of heterozygosity fitness correlations in the study population.

The result obtained suggests that parasites also contribute to maintain overall genetic diversity rather than only just variability at specific loci directly involved on pathogen resistance. Further, the studied parasite is not impacted by the immune system, indicating that other than immune defence mechanisms to fight off parasites may be also modulated by genetic variability (Whiteman *et al*. 2006). Thus, parasite-mediated selection would maintain variation throughout the genome not only acting on host genotype at some genes involved in immune response, but also exerting selection against homozygous individuals that may suffer from the expression of deleterious recessive alleles or do not benefit from heterozygosity advantage at genes under balancing selection involved in several other physiological, biochemical or behavioural mechanisms influencing risk of parasitism (Luong *et al*. 2007). In the studied host–parasite system, reduced genetic diversity could directly affect mechanical host defences and more heterozygous individuals would be also better able to deal with energetic and time cost of preening, resources that otherwise could be allocated to other life-history components (Sheldon & Verhulst 1996; Norris & Evans 2000). A recent experimental study has demonstrated that increased host homozygosity reduces host stamina what results in increasing susceptibility to ectoparasites (Luong *et al*. 2007). Thus, the observed negative association between individual genetic diversity and risk of parasitism may be caused by the inability of homozygous hosts to sustain energetically expensive defensive behaviours.

Apart from genetic diversity, other factors influenced the prevalence of feather lice in lesser kestrels, including colony size, year of study, sex and locality. Colony size is likely to affect positively parasite prevalence if a higher population density in larger colonies increases the chance of contact between hosts and favours horizontal parasite transmission (Anderson & May 1978; May & Anderson

1978). This would be especially important for feather lice that depend on direct contact among host for their transmission (Rozsa *et al*. 1996). Some studies have found a positive association between a host social behaviour/colony size and lice abundance or prevalence (Rozsa *et al*. 1996; Rekasi *et al*. 1997; Hoi *et al*. 1998; Whiteman & Parker 2004). However, we found that parasitism by feather lice first increased and then decreased with colony size, indicating that individuals from the largest colonies suffered lower parasitism than those from intermediate colony sizes. Previous studies on the lesser kestrel ecology have found that larger colonies are generally of better quality and experience reduced predation, what attracts more individuals that must compete for limited nest sites and mates (Serrano *et al*. 2005). So, larger colonies may be over-represented by high quality individuals that could effectively fight off feather lice in other ways not analysed here. As found in a previous study, lice prevalence and abundance was female-biased (Potti & Merino 1995). This may be associated with differences in susceptibility between sexes or could reflect a greater selection of feather lice on males that may reduce the mean and variance of ectoparasite burdens (Potti & Merino 1995). Also, males generally devote more time to grooming and other kinds of maintenance than do females, what could effectively contribute to the observed sex-biased pattern of parasitism (Cotgreave & Clayton 1994). Finally, we found that parasitism by feather lice varied between years and localities. However, we did not obtain any significant interaction between heterozygosity and year/locality, indicating that the strength of the correlation did not depend on parasite pressure as would have been expected if the impact of reduced genetic diversity on fitness components depends on environmental conditions (Keller *et al*. 2002; Armbruster & Reed 2005; Lesbarreres *et al*. 2005; but see, Kruuk *et al*. 2002).

We found no effect of individual genetic diversity on parasite load, indicating that heterozygosity may not confer an advantage in controlling louse proliferation once an individual has become parasitized. Thus, differences in the risk of parasite establishment rather than in defences once the parasite has been established would underlie the observed association between genetic diversity and parasite prevalence. In any case, the correlative nature of the present study does not allow determining whether unparasitized individuals have coped with infestations or if the use of different behavioural mechanisms has prevented them from becoming parasitized. Thus, further studies based on experimental infestations may help to get a better understanding on the underlying mechanism generating the association between feather lice prevalence and genetic diversity (e.g. Hawley *et al*. 2005; Luong *et al*. 2007).

On the whole, the results obtained suggest that ectoparasites, not only endoparasites, may contribute to maintaining overall genetic variation in natural populations, and this has important implications for conservation research. Genetically depauperate populations may be more often affected by disease outbreaks, which in turn might lead to a subsequent reduction in population size, depleting genetic variability even more, leading to a self-perpetuating cycle towards population extinction (Altizer *et al*. 2003; Castro & Bolker 2005, e.g. Pearman & Garner 2005; Whiteman *et al*. 2006). Possible events of emerging infectious diseases linked with climate change and habitat alterations make it necessary to preserve genetically diverse populations capable of dealing with the potential impact of parasites and pathogens (O'Brien & Evermann 1988; Daszak *et al*. 2000).

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