J Ornithol (2008) 149:337–343
DOI 10.1007/s10336-008-0287-9

ORIGINAL ARTICLE

Consequences of chronic infections with three different avian malaria lineages on reproductive performance of Lesser Kestrels (*Falco naumanni*)

Joaquín Ortego · Pedro J. Cordero · José Miguel Aparicio · Gustau Calabuig

Received: 14 November 2007 / Revised: 9 January 2008 / Accepted: 28 January 2008 / Published online: 15 February 2008
© Dt. Ornithologen-Gesellschaft e.V. 2008

Abstract We studied the consequences of chronic infections by three different lineages of avian malaria, two *Plasmodium* (RTSR1, LK6) and one *Haemoproteus* (LK2), on reproductive performance of Lesser Kestrels (*Falco naumanni*). Malaria infections in male and female parents had no effect on clutch size, hatching success or nesting success. However, when only successful nests were considered, we found that males parasitized by LK6 raised a lower number of fledglings, suggesting that the level of parental effort by males may be limited by this particular lineage of *Plasmodium*. This effect was not evident in females, probably due to the higher investment of males during the chick rearing period in this species. Overall, we have found that chronic stages of specific malaria lineages have certain negative consequences on host reproductive performance, highlighting the importance of considering genetic differences among malaria parasites to study their consequences on natural bird populations.

Keywords Avian malaria · Disease · *Falco naumanni* · *Haemoproteus* · *Plasmodium*

Introduction

Infectious diseases have a major influence on host population dynamics and evolution, promoting host genetic variation and influencing co-evolutionary processes (Hamilton and Zuk 1982; Poulin et al. 2000). The study of the interaction between avian malaria parasites and their hosts has greatly contributed to the understanding of several aspects of host/parasite ecology and evolution (Hamilton and Zuk 1982; Poulin et al. 2000; Bensch et al. 2000). Further, avian malaria parasites are responsible for widespread declines in Hawaiian native forest birds (van Riper et al. 1986), and several negative effects, such as increased mortality and reduced reproductive outputs, have been described in a number of bird species (Atkinson et al. 2000; Dawson and Bortolotti 2000; Merino et al. 2000; Sol et al. 2003). For this reason, researchers have devoted much effort to studying these parasites and their consequences on natural bird populations.

In recent years, DNA sequencing has revealed several cases of cryptic speciation of avian malaria, indicating that the number of species in this parasite group is much higher than previously thought (Bensch et al. 2000, 2004; Fallon et al. 2005; Ricklefs et al. 2005). Thus, several species of avian malaria, originally defined based on host taxonomy or morphological criteria, have been found to be composed of a number of independent evolutionary units (Bensch et al. 2000, 2004; Beadell et al. 2006). After the application of molecular techniques, the study of avian malaria has switched from considering variation at the level of genera to analyzing specific lineages defined on the basis of molecular information (Bensch et al. 2004, 2007). This has important applied and theoretical implications because different lineages may have evolved different adaptations and affect host differentially (Westerdahl et al. 2005). Previous studies analyzing data obtained on the basis of visual examination of blood smears are likely to have disregarded and/or confounded different and potentially pathological effects of cryptic lineages. Furthermore,

Communicated by F. Bairlein.

J. Ortego (✉) · P. J. Cordero · J. M. Aparicio · G. Calabuig
Grupo de Investigación de la Biodiversidad Genética y Cultural, Instituto de Investigación en Recursos Cinegéticos, IREC (CSIC, UCLM, JCCM), Ronda de Toledo s/n, 13005 Ciudad Real, Spain
e-mail: joaquin.ortego@uclm.es

recent studies have shown that several malaria lineages are undetected by traditional visual blood smear analyses, indicating that the prevalence of malaria has been severely underestimated (Waldenström et al. 2004; Ortego et al. 2007a). However, although molecular techniques have now been widely applied to the study of avian malaria parasites, to the best of our knowledge only a single study has analyzed the consequences on host fitness of different molecularly typed lineages in a wild bird population (Bensch et al. 2007).

The Lesser Kestrel (*Falco naumanni*) is a colonial and migratory falcon. This species was once one of the most abundant birds of prey in southern Europe, but suffered a sharp population decline in its Western Paleartic breeding range in the second half of the twentieth century that led to complete extinction in several countries and to strong declines in others (Biber 1990). Thus, it is of great interest to analyze the potential negative consequences of avian malaria parasites on this species. A previous study has reported that infections by *Haemoproteus* parasites had no effect on clutch size in this species, but the potential consequences of malaria parasites on other parameters related to the species reproductive performance have not so far been analysed (Tella et al. 1996). Also, that study was based on visual examinations of blood smears, and parasites of the genera *Plasmodium*, supposedly the most pathogenic ones (Bensch et al. 2000; Atkinson et al. 2000), probably went undetected because they occurred at intensities of infection that are hard to detect in blood smears (Ortego et al. 2007a).

Molecular techniques have now revealed that Lesser Kestrels are parasitized by at least six different avian malaria lineages, with two *Plasmodium* (RTSR1, LK6) and one *Haemoproteus* (LK2) lineages being the most common in our study population from Central Spain (Ortego et al. 2007a). The mean prevalence of these three lineages in the study population is 3.4% for RTSR1, 4.6% for LK6 and 4.1% for LK2; no mixed infections have been detected (Ortego et al. 2007a). According to the obtained sequence divergences (RTSR1–LK6: 6.9%; RTSR1–LK2: 12.8%; LK2–LK6: 14.9%), these lineages seem to correspond to three well-differentiated species (Ortego et al. 2007a), and so they may have evolved different adaptations and affect hosts differentially (Bensch et al. 2004). At least one of these lineages (RTSR1) has active transmission in Africa as it has been also detected in African resident birds coexisting with the Lesser Kestrel in its wintering area (Waldenström et al. 2004; Ortego et al. 2007a). On the other hand, there is no evidence of transmission of these parasites in the European breeding quarters as they have not been found infecting fledgling Lesser Kestrels sampled in northeastern ($n = 85$; Tella et al. 1996) and central Spain ($n = 279$; J. Ortego et al., unpublished). Infection intensities of these lineages in breeding Lesser Kestrels are generally below the limit of detection by methods based on microscopy examinations of blood smears (Ortego et al. 2007a), indicating low-intensity peripheral parasitemia characteristic of chronic infections (Jarvi et al. 2003). Thus, avian malaria lineages parasitizing adult Lesser Kestrels on breeding grounds probably circulate in the blood stream as chronic infections. In the present study, we used blood parasite data reported in a previous study (Ortego et al. 2007a) to investigate the consequences of chronic infections by these malaria lineages on the reproductive performance of Lesser Kestrels.

Methods

Study population and field procedures

The study was conducted in La Mancha, central Spain (600–800 m asl), in an area covering approximately 1,000 km^2. We studied 30 Lesser Kestrel colonies clustered in two subpopulations separated by 30 km: "Villacañas" subpopulation (39°30′N, 3°20′W; 24 colonies) and "Consuegra" subpopulation (39°35′N, 3°40′W; 6 colonies). The climate is meso-Mediterranean with mean temperatures ranging from 24–26°C in July to 4–6°C in January, and with 300–400 mm of rainfall mainly concentrated in spring and autumn.

In our study area, the Lesser Kestrel mainly breeds in abandoned farmhouses, both under tiled roofs and inside holes in walls. Kestrels normally arrive in this area from their winter quarters in Africa in mid-February or the beginning of March, depending on the year. Egg laying lasts from the end of April to the first week of June (Aparicio and Bonal 2002). Kestrels are mainly monogamous, and lay only one clutch per season with the exception of rare replacement clutches (ca. 0.5%). During the 2001–2006 breeding seasons, we located nest-sites before the onset of egg laying. Each potential nest was monitored every 6 days from the middle of April to find the first eggs, and then every 2 days until the clutch was finished. Thus, since females usually lay four or five eggs with mean intervals of 2 days, we were able to find the maximum number of unfinished clutches with minimum disturbance of the colonies. Laying date was defined as the date the first egg was laid (Aparicio and Bonal 2002). Near the expected hatching dates, we inspected the nests regularly to ascertain the exact hatching pattern of the eggs. We defined as unhatched eggs those that survived the entire incubation period but failed to hatch. Young were marked at hatching with a waterproof felt-tip pen, and they were banded 5–7 days later (for more details on field methods, see Aparicio 1997).

Adults were trapped with a noose carpet or by hand during incubation, measured and individually marked with metallic and colored plastic rings. Blood samples (100 μl) were obtained by venipuncture of the brachial vein and preserved in ~1,200 μl ethanol 96% at −20°C. We used pectoral thickness as an estimator of adult body condition (Aparicio 1997; Aparicio and 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 and Harper 2000). Moreover, it is easy to measure accurately on live birds by using a portable ultrasonic meter, in this case a Krautkrämer USM22F (accuracy 0.1 mm), specially 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 and Cordero 2001; Foerster et al. 2003). This approach is not likely to bias age estimates because modal life-span in Lesser Kestrels is only 4 years and almost all nestlings and ~70% of breeding birds are captured and banded every year in the study area (Ortego et al. 2007b).

Screening for avian malarial infections

We used QIAamp DNA Blood Mini Kits (QIAGEN) to extract and purify DNA from the blood samples. Each bird was screened for malaria infection using a highly efficient nested PCR protocol that amplifies a 524-bp fragment (including primers) of the mitochondrial cytochrome *b* gene of both *Plasmodium* and *Haemoproteous* parasites (Waldenström et al. 2004). This method consists of two rounds, an initial 20 cycles of PCR using the primers HAEMNF and HAEMNR2 and a final 35 cycles of PCR using the internally nested primers HAEMF and HAEMR2 (Waldenström et al. 2004). For the second PCR, 1 μl of the PCR product from the initial PCR was used as template. All PCR reactions were performed in 25-μl volumes and we routinely used positive (i.e., DNA from individuals with known malarial infections) and negative (i.e., samples with ddH_2O instead of genomic DNA as template) controls to ascertain that the outcome of each PCR run was not affected by contamination (Waldenström et al. 2004). Further, negative infections were confirmed by repeated PCR. The PCR programme, thermal profile, and reagent proportions were as described by Waldenström et al. (2004), with the exception of using a 9-min denaturing at 95°C rather than 3 min at 94°C because we used a hot-start polymerase (EcoStart; Ecogen). All reactions were carried out on a Mastercycler EpgradientS (Eppendorf) thermal cycler. In a previous study including a subset of the samples used here ($n = 288$), we found that all infections detected by visual screening of blood smears gave positive PCR amplifications while several PCR detected infections could not be determined using visual examinations (Ortego et al. 2007a). As found in several studies, this indicates that the nested PCR protocol is much more sensitive than the traditional microscopy-based examinations of blood smears (Waldenström et al. 2004). Positive or negative second round PCR products (i.e., birds having or not gametocytes or merozoites in their blood stream) were scored by electrophoresis on 2% agarose gels stained with ethidium bromide and determining the presence/absence of a band of the expected size under UV light. PCR products from positive samples were purified using NucleoSpin Extract II (MACHEREY-NAGEL) kits and bidirectionally sequenced on an ABI 310 Genetic Analyser (Applied Biosystems). Sequences were edited and aligned using the program BioEdit (Hall 1999) and are available through GenBank (accession numbers for the three most common lineages analyzed here: RTSR1: AF495568; LK2: EF564175; LK6: EF564179).

Statistical analyses

We analyzed the relationship between reproductive performance and malaria presence using Generalized Linear Mixed Models (GLMMs) 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. Parameters related with the species reproductive performance were included as response variables in different GLMMs. We considered clutch size (Poisson error and log ling function; males, $n = 247$; females, $n = 280$), hatching success (binomial error, logit link function; males, $n = 206$; females, $n = 229$), nesting success/failure (binomial error, logit link function; males, $n = 240$; females, $n = 267$), and number of fledglings per successful nest that survived at day 30 (Poisson error and log ling function; males, $n = 174$; females, $n = 189$) as dependent variables. GLMMs were constructed by fitting presence/absence of the three most common avian malaria lineages (RTSR1, LK2, LK6; Ortego et al. 2007a) parasitizing Lesser Kestrels in the study population as fixed factors together with other terms (covariates: laying date, parental pectoral thickness, parental age; fixed factor: locality) that could potentially influence reproductive performance in this species (e.g., Ortego et al. 2007c). As done in other studies, we developed separate models for males ($n = 277$) and females ($n = 307$) because the detrimental consequences of blood parasites on reproductive performance may differ between

sexes (Korpimäki et al. 1993; Dawson and Bortolotti 2001). 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, cohort, and breeding season were included as random effects to control for the potential non-independence of reproductive parameters within colonies, cohorts, and years in the manner of a randomized complete block design to avoid pseudoreplication (Krackow and Tkadlec 2001).

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 non-significant variables. Terms were deleted from the model if their removal caused a non-significant change in deviance ($P > 0.05$). Thus, the variables retained in the models were those that provided unique explanatory power. 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.

Results

Predictably, all studied parameters related with Lesser Kestrel reproductive performance were negatively associated with laying date (Table 1; Perrins 1970). As found in other bird species, clutch size increased and then declined significantly with both male and female age (Table 1; e.g., Kruuk et al. 2002; Reid et al. 2003). Nesting success increased with male age (Table 1). Males parasitized by lineage LK6 raised a lower number of fledglings (Table 1; Fig. 1a). We re-analyzed our data including into the models total prevalence (i.e., all lineages pooled) as a binary variable rather than the presence/absence of each particular avian malaria lineage. Total prevalence did not influence clutch size (males: $F_{1,242} = 1.70$, $P = 0.193$; females: $F_{1,275} = 1.82$, $P = 0.178$), hatching success (males: $F_{1,204} = 1.82$, $P = 0.179$; females: $F_{1,226} = 0.17$, $P = 0.677$), or nesting success (males: $F_{1,236} = 0.08$, $P = 0.772$; females: $F_{1,264} = 0.20$, $P = 0.652$). However, parasitized males raised a lower number of fledglings ($F_{1,171} = 5.40$, $P = 0.021$) and, once again, we found no effect of female parasitic status ($F_{1,186} = 2.75$, $P = 0.099$). Quadratic terms, with the exception of age in clutch size model, and interactions between independent variables were not significant in any analysis ($P > 0.1$ in all cases).

Discussion

Here, we analyze the consequences of three different avian malaria lineages on reproductive performance of Lesser Kestrels. Malaria infections in male and female parents had no apparent effects on clutch size, hatching success or nesting success. However, when only successful nests were considered, we found that males parasitized by LK6 raised a lower number of fledglings, suggesting that the level of parental effort by males may be limited by this particular *Plasmodium* lineage. Such an effect was not detected in females, probably due to a higher proportion of parental investment of males during the chick rearing period as observed by extensive video-tape recording of prey deliveries in several colonies from the study population (G. Calabuig, unpublished data). Similar differences between sexes in the effects of avian malaria parasites or trade-offs between parasitism and reproduction have also been reported in previous studies in relation to differential parental investment (Dawson and Bortolotti 2001; Korpimäki et al. 1993). This result suggests that males suffering from avian malaria may be less efficient foragers, and this could have resulted in a reduced number of raised fledglings. Medication experiments in birds maintaining chronic infections have found that the effects of infection are usually paid for by nestlings, suggesting that the level of parental effort may be limited by malaria parasites as a result of a trade-off between reproductive effort and investment in immune/physiological anti-parasite defence to avoid relapses of chronic infections acquired in previous years (Merino et al. 2000; Tomás et al. 2005). Thus, non-parasitized male Lesser Kestrels could increase the amount of resources devoted to parental care through a reduced allocation to immune response (Merino et al. 2000).

Although we used a relatively large sample size, the effects we found were weak, with a complete absence of negative consequences of the studied avian malaria lineages on most reproductive parameters. The Lesser Kestrel is predominantly infected by only three parasite lineages, a number much smaller than reported for other bird species (e.g., Hellgren 2005; Bensch et al. 2007; Perez-Tris et al. 2007). Further, the low prevalence observed would have led to a low probability of mixed infections (Bensch et al. 2007). Accordingly, no mixed infections have been detected in the study population of Lesser Kestrels (Ortego et al. 2007a), although this may be partially due to the difficulty of detecting concomitant infections because of PCR competition among lineages with different levels of parasitemia (Bensch et al. 2007). In any case, this scarce parasite diversity may have reduced within-host competition between genetically distinct parasites and resulted in low virulence of the studied lineages (Frank 1996). Alternatively, the scarce effects observed may also be a

Table 1 GLMMs for different components of reproductive performance in relation to laying date, age, pectoral thickness, locality, and presence/absence of the three most common malaria lineages (*Plasmodium*: RTSR1, LK6; *Haemoproteus*: LK2) parasitizing Lesser Kestrels (*Falco naumanni*)

	Males			Females		
	Estimate ± SE	Test	*P*	Estimate ± SE	Test	*P*
Clutch size						
Intercept	1.877 ± 0.157			2.307 ± 0.164		
Laying date	−0.006 ± 0.001	$F_{1,243} = 32.28$	<0.001	−0.008 ± 0.001	$F_{1,276} = 53.67$	<0.001
Age	0.176 ± 0.047	$F_{1,243} = 14.26$	<0.001	0.116 ± 0.034	$F_{1,276} = 11.79$	<0.001
Age^2	−0.024 ± 0.008	$F_{1,243} = 10.28$	0.002	−0.013 ± 0.005	$F_{1,276} = 8.35$	0.004
Pectoral thickness		$F_{1,242} = 2.03$	0.156		$F_{1,275} = 0.01$	0.945
Locality		$F_{1,242} = 0.01$	0.911		$F_{1,275} = 0.64$	0.424
Presence of RTSR1		$F_{1,242} = 0.01$	0.928		$F_{1,275} = 0.01$	0.928
Presence of LK6		$F_{1,242} = 0.22$	0.640		$F_{1,275} = 1.19$	0.276
Presence of LK2		$F_{1,242} = 2.23$	0.136		$F_{1,275} = 0.08$	0.775
Hatching success						
Intercept				5.439 ± 1.573		
Laying date		$F_{1,204} = 2.69$	0.102	−0.028 ± 0.012	$F_{1,227} = 5.28$	0.022
Age		$F_{1,204} = 3.13$	0.078		$F_{1,226} = 0.40$	0.529
Pectoral thickness		$F_{1,204} = 0.18$	0.668		$F_{1,226} = 2.13$	0.146
Locality		$F_{1,204} = 0.22$	0.641		$F_{1,226} = 0.01$	0.984
Presence of RTSR1		$F_{1,204} = 1.63$	0.203		$F_{1,226} = 0.01$	0.912
Presence of LK6		$F_{1,204} = 1.42$	0.235		$F_{1,226} = 0.01$	0.906
Presence of LK2		$F_{1,204} = 2.39$	0.124		$F_{1,226} = 0.06$	0.800
Nesting success/failure						
Intercept	8.032 ± 3.303			9.399 ± 2.432		
Laying date	−0.063 ± 0.023	$F_{1,237} = 7.56$	0.006	−0.063 ± 0.018	$F_{1,265} = 11.90$	<0.001
Age	0.749 ± 0.352	$F_{1,237} = 4.53$	0.034		$F_{1,264} = 2.11$	0.147
Pectoral thickness		$F_{1,236} = 0.90$	0.343		$F_{1,264} = 3.38$	0.067
Locality		$F_{1,236} = 0.46$	0.496		$F_{1,264} = 0.21$	0.650
Presence of RTSR1		$F_{1,236} = 0.39$	0.531		$F_{1,264} = 0.04$	0.833
Presence of LK6		$F_{1,236} = 0.82$	0.367		$F_{1,264} = 0.97$	0.327
Presence of LK2		$F_{1,236} = 0.01$	0.963		$F_{1,264} = 0.01$	0.993
Number of fledglings per successful nest						
Intercept	2.067 ± 0.483	$F_{1,171} = 7.86$	0.006	2.537 ± 0.425		
Laying date	−0.010 ± 0.003	$F_{1,171} = 7.86$	0.006	−0.011 ± 0.003	$F_{1,187} = 11.67$	<0.001
Presence of LK6	0.321 ± 0.146	$F_{1,171} = 4.83$	0.029		$F_{1,186} = 2.77$	0.098
Age		$F_{1,170} = 1.69$	0.195		$F_{1,186} = 0.01$	0.997
Pectoral thickness		$F_{1,170} = 1.61$	0.207		$F_{1,186} = 0.13$	0.723
Locality		$F_{1,170} = 0.19$	0.660		$F_{1,186} = 0.88$	0.349
Presence of RTSR1		$F_{1,170} = 0.18$	0.669		$F_{1,186} = 0.16$	0.689
Presence of LK2		$F_{1,170} = 3.31$	0.071		$F_{1,186} = 0.54$	0.464

Colony identity, cohort, and year were included as random effects in all these analyses. Parameter estimates ± SE were calculated considering a reference value of zero for the parasitized level in the variable "*Presence of LK6*"

consequence of the correlative nature of this study (Bensch et al. 2007; Ortego et al. 2007d). Thus, the results obtained do not necessarily mean that malaria parasites are harmless for other fitness-related traits (Bensch et al. 2007). In fact, the severest fitness consequences of avian malaria generally occur during the acute infection just after the first exposure to the parasite (Atkinson et al. 2000), whereas individuals surviving the primary infection generally clear the infection or carry chronic stages with supposedly limited consequences on host fitness (Bensch et al. 2007). On the other hand, despite the nested PCR approach being much more efficient than the traditional visual examination of blood

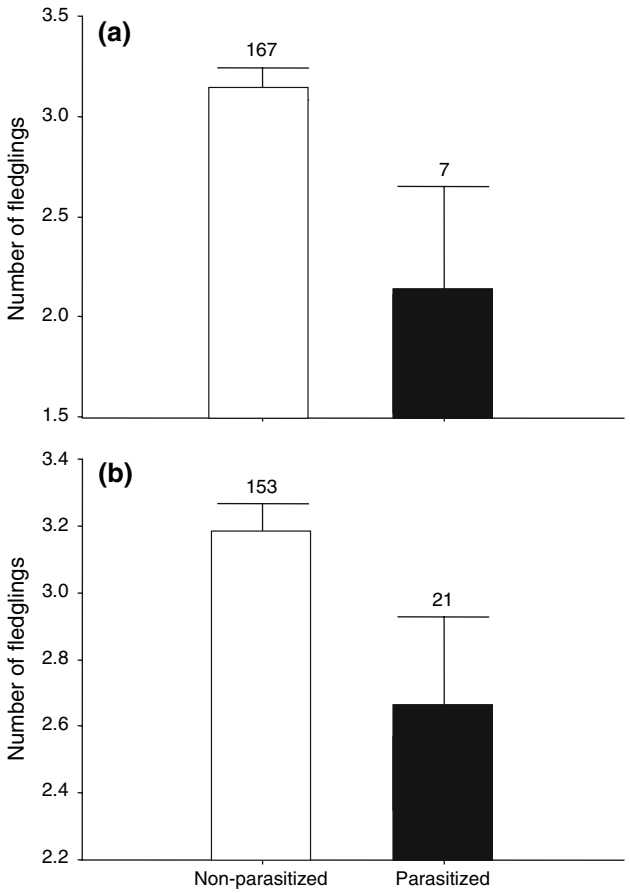

Fig. 1 Mean (±SE) number of fledglings per successful nest in relation with **a** parasitism by lineage LK6 and **b** total prevalence in male Lesser Kestrels (*Falco naumanni*). *Figures* above *bars* indicate sample size in each group

smears, parasite prevalence observed in Lesser Kestrels was very low, and this may have reduced the effective power of our statistical analyses in spite of the number of birds screened being relatively high (Ortego et al. 2007d; see also Tella et al. 1996). Further, some birds could be chronically infected without stages of malaria parasites being present in the blood, and these infections may have gone undetected by nested PCR (Jarvi et al. 2002).

Overall, we have found that chronic stages of specific malaria lineages have certain negative consequences on host reproductive performance. This highlights the importance of considering genetic variability among malaria parasites when studying their consequences on natural bird populations. Future studies should consider analyzing intensities of infections of molecularly typed lineages, which could be reliably assessed using real-time PCR techniques (e.g., de Roode et al. 2005). Also, experimental approaches, including medication experiments (Merino et al. 2000; Tomás et al. 2005) or experimental infections (Atkinson et al. 2000; Garvin et al. 2003), could also help to get a better understanding on the consequences of malaria parasites on bird hosts.

Zusammenfassung

Die Folgen chronischer Infektionen mit drei unterschiedlichen Vogelmalaria-Linien für die reproduktive Leistung von Rötelfalken (*Falco naumanni*)

Wir haben die Folgen chronischer Infektionen mit drei unterschiedlichen Linien der Vogelmalaria, zwei *Plasmodium* (RTSR1, LK6) und einer *Haemoproteus* (LK2), für die reproduktive Leistung von Rötelfalken (*Falco naumanni*) untersucht. Malariainfektionen bei männlichen und weiblichen Eltern hatten keinen Effekt auf Gelegegröße, Schlupferfolg oder Nisterfolg. Wurden jedoch lediglich erfolgreiche Nester berücksichtigt, fanden wir, dass von LK6 parasitierte Männchen weniger flügge Junge aufzogen, was darauf hindeutet, dass das Ausmaß männlicher Brutfürsorge durch diese bestimmte Linie von *Plasmodium* eingeschränkt sein könnte. Dieser Effekt war bei Weibchen nicht zu beobachten, wahrscheinlich weil Männchen bei dieser Art mehr in die Jungenaufzucht investieren. Insgesamt haben wir herausgefunden, dass chronische Stadien spezifischer Malarialinien gewisse negative Konsequenzen für die reproduktive Leistung des Wirts haben, was hervorhebt, dass es von Bedeutung ist, genetische Unterschiede zwischen Malariaparasiten bei der Untersuchung ihrer Folgen für natürliche Vogelpopulationen in Betracht zu ziehen.

Acknowledgments This manuscript was greatly improved by the comments of Robert E. Ricklefs and an anonymous reviewer. This work received financial support from the projects: CGL2005-05611-C02-02/BOS (Ministerio de Educación Ciencia) and PAI05-053 (Junta de Comunidades de Castilla-La Mancha). During this work J.O and G.C. were supported by predoctoral fellowships from the Junta de Comunidades de Castilla-La Mancha and the European Social Fund. We performed all the laboratory work at the Laboratory of Genetics of the IREC and sequencing was performed by the Centro de Investigaciones Biológicas (CSIC) of Madrid. We manipulated and banded Lesser Kestrels under license from the Spanish institutional authorities (Environmental Agency of the Community of Castilla-La Mancha and the Ringing Office of the Ministry of Environment) and we followed general ethical guidelines for animal welfare and nature conservation.

References

Aparicio JM (1997) Costs and benefits of surplus offspring in the lesser kestrel (*Falco naumanni*). Behav Ecol Sociobiol 41:129–137

Aparicio JM, Bonal R (2002) Effects of food supplementation and habitat selection on timing of lesser kestrel breeding. Ecology 83:873–877

Aparicio JM, Cordero PJ (2001) The effects of the minimum threshold condition for breeding on offspring sex-ratio adjustment in the lesser kestrel. Evolution 55:1188–1197

Atkinson CT, Dusek RJ, Woods KL, Iko WM (2000) Pathogenicity of avian malaria in experimentally-infected Hawaii amakihi. J Wildl Dis 36:197–204

Beadell JS, Ishtiaq F, Covas R, Melo M, Warren BH, Atkinson CT, Bensch S, Graves GR, JhalaYV, Peirce MA, Rahmani AR, Fonseca DM, Fleischer RC (2006) Global phylogeographic limits of Hawaii's avian malaria. Proc R Soc Lond B 273:2935–2944

Bensch S, Perez-Tris J, Waldenstrom J, Hellgren O (2004) Linkage between nuclear and mitochondrial DNA sequences in avian malaria parasites: multiple cases of cryptic speciation? Evolution 58:1617–1621

Bensch S, Stjernman M, Hasselquist D et al (2000) Host specificity in avian blood parasites: a study of *Plasmodium* and *Haemoproteus* mitochondrial DNA amplified from birds. Proc R Soc Lond B 267:1583–1589

Bensch S, Waldenstrom J, Jonzen N et al (2007) Temporal dynamics and diversity of avian malaria parasites in a single host species. J Anim Ecol 76:112–122

Biber JP (1990) Action plan for the conservation of western lesser kestrel *Falco naumanni* populations. ICBP, Cambridge

Bolton M, Monaghan P, Houston DC (1991) An improved technique for estimating pectoral muscle protein condition from body measurements of live gulls. Ibis 133:264–270

Dawson RD, Bortolotti GR (2000) Effects of hematozoan parasites on condition and return rates of American kestrels. Auk 117:373–380

Dawson RD, Bortolotti GR (2001) Sex-specific associations between reproductive output and hematozoan parasites of American kestrels. Oecologia 126:193–200

de Roode JC, Helinski MEH, Anwar MA, Read AF (2005) Dynamics of multiple infection and within-host competition in genetically diverse malaria infections. Am Nat 166:531–542

Fallon SM, Bermingham E, Ricklefs RE (2005) Host specialization and geographic localization of avian malaria parasites: a regional analysis in the Lesser Antilles. Am Nat 165:466–480

Foerster K, Delhey K, Johnsen A, Lifjeld JT, Kempenaers B (2003) Females increase offspring heterozygosity and fitness through extra-pair matings. Nature 425:714–717

Frank SA (1996) Models of parasite virulence. Q Rev Biol 71:37–78

Garvin MC, Homer BL, Greiner EC (2003) Pathogenicity of Haemoproteus danilewskyi, Kruse, 1890, in blue jays (*Cyanocitta cristata*). J Wildl Dis 39:161–169

Gosler AG, Harper D (2000) Assessing the heritability of body condition in birds: a challenge exemplified by the great tit *Parus major* L. (Aves). Biol J Linn Soc 71:103–117

Green RH (1979) Sampling design and statistical methods for environmental biologists. Wiley, New York

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41:95–98

Hamilton WD, Zuk M (1982) Heritable true fitness and bright birds—a role for parasites. Science 218:384–387

Hellgren O (2005) The occurrence of haemosporidian parasites in the Fennoscandian bluethroat (*Luscinia svecica*) population. J Ornithol 146:55–60

Jarvi SI, Schultz JJ, Atkinson CT (2002) PCR diagnostics underestimate the prevalence of avian malaria (*Plasmodium relictum*) in experimentally-infected passerines. J Parasitol 88:153–158

Jarvi SI, Farias MEM, Baker H, Freifeld HB, Baker PE, Van Gelder E, Massey JG, Atkinson CT (2003) Detection of avian malaria (*Plasmodium* spp.) in native land birds of American Samoa. Conserv Gen 4:629–637

Korpimäki E, Hakkarainen H, Bennett GF (1993) Blood parasites and reproductive success of tengmalm owls—detrimental effects on females but not on males. Funct Ecol 7:420–426

Krackow S, Tkadlec E (2001) Analysis of brood sex ratios: implications of offspring clustering. Behav Ecol Sociobiol 50:293–301

Kruuk LEB, Sheldon BC, Merila J (2002) Severe inbreeding depression in collared flycatchers (*Ficedula albicollis*). Proc R Soc Lond B 269:1581–1589

Merino S, Moreno J, Sanz JJ, Arriero E (2000) Are avian blood parasites pathogenic in the wild? A medication experiment in blue tits (*Parus caeruleus*). Proc R Soc Lond B 267:2507–2510

Newton SF (1993) Body condition of a small passerine bird—ultrasonic assessment and significance in overwinter survival. J Zool 229:561–580

Ortego J, Calabuig G, Cordero PJ, Aparicio JM (2007a) Genetic characterization of avian malaria (Protozoa) in the endangered lesser kestrel, *Falco naumanni*. Parasitol Res 101:1153–1156

Ortego J, Calabuig G, Cordero PJ, Aparicio JM (2007b) Egg production and individual genetic diversity in lesser kestrels. Mol Ecol 16:2383–2392

Ortego J, Cordero PJ, Aparicio JM, Calabuig G (2007c) No relationship between individual genetic diversity and prevalence of avian malaria in a migratory kestrel. Mol Ecol 16:4858–4866

Ortego J, Aparicio JM, Calabuig G, Cordero PJ (2007d) Increase of heterozygosity in a growing population of lesser kestrels. Biol Lett 3:585–588

Pérez-Tris J, Hellgren O, Križanauskienė A, Waldenström J, Secondi J, et al. (2007) Within-host speciation of malaria parasites. PLoS ONE 2(2):e235. doi:10.1371/journal.pone.0000235

Perrins CM (1970) The timing of birds' breeding seasons. Ibis 112:242–255

Poulin R, Marshall LJ, Spencer HG (2000) Genetic variation and prevalence of blood parasites do not correlate among bird species. J Zool 252:381–388

Reid JM, Arcese P, Keller LF (2003) Inbreeding depresses immune response in song sparrows (*Melospiza melodia*): direct and inter-generational effects. Proc R Soc Lond B 270:2151–2157

Ricklefs RE, Swanson BL, Fallon SM, Martínez-Abraín A, Scheuerlein A, Gray J, Latta SC (2005) Community relationships of avian malaria parasites in Southern Missouri. Ecol Mon 75:543–559

SAS Institute (2004) SAS/STAT 9.1 user's guide. SAS Institute, Cary

Sol D, Jovani R, Torres J (2003) Parasite mediated mortality and host immune response explain age-related differences in blood parasitism in birds. Oecologia 135:542–547

Tella JL, Forero MG, Gajon A, Hiraldo F, Donazar JA (1996) Absence of blood-parasitization effects on lesser kestrel fitness. Auk 113:253–256

Tomás G, Merino S, Martinez J, Moreno J, Sanz JJ (2005) Stress protein levels and blood parasite infection in blue tits (*Parus caeruleus*): a medication field experiment. Ann Zool Fenn 42:45–56

Van Riper C, Vanriper SG, Goff ML, Laird M (1986) The epizootiology and ecological significance of malaria in hawaiian land birds. Ecol Mon 56:327–344

Waldenström J, Bensch S, Hasselquist D, Ostman O (2004) A new nested polymerase chain reaction method very efficient in detecting *Plasmodium* and *Haemoproteus* infections from avian blood. J Parasitol 90:191–194

Westerdahl H, Waldenstrom J, Hansson B et al (2005) Associations between malaria and MHC genes in a migratory songbird. Proc R Soc Lond B 272:1511–1518