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**Juan José Sanz · Elena Arriero · Juan Moreno
Santiago Merino**

Interactions between hemoparasite status and female age in the primary reproductive output of pied flycatchers

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Abstract The relationship between hemoparasite infection (measured just after egg laying) and primary reproductive output (laying date, clutch size and egg volume) was studied in female pied flycatchers, *Ficedula hypoleuca*, of different ages (2 years old vs. 4 or more years old). The hemoparasite (*Haemoproteus balmorali* and *Trypanosoma* spp.) prevalence increased with advancing female age. *H. balmorali*-infected females initiated egg laying earlier and laid larger clutches. There was no evidence that infected females laid smaller eggs or had a lower body mass after egg laying than non-infected ones. Only for *H. balmorali*-infected females was there an increase in clutch size between age groups. The present results suggest that primary reproductive output of females will depend on their health-dependent residual reproductive value. Only old females may be able to control a chronic or latent infection by hemoparasites and thereby invest more heavily in reproduction than younger but experienced females. Alternatively, high hemoparasite prevalence may be the cause of increased female primary reproductive output in old females.

Keywords Blood parasites · *Ficedula hypoleuca* · *Haemoproteus* · Residual reproductive value · *Trypanosoma*

Introduction

In iteroparous vertebrates, increased parental investment in the current reproductive attempt can negatively affect future survival and reproductive success (Williams 1966; Stearns 1992). If the number of offspring that an individual can expect to produce after the current reproduction (residual reproductive value; Stearns 1992) declines with age, the theory predicts that reproductive effort should increase with advancing age until the benefit of current reproduction no longer outweighs future costs (Williams 1966; Pianka and Parker 1975; Charlesworth 1980; Clutton-Brock 1984; Pärt et al. 1992; Stearns 1992). In order to maximise their lifetime reproductive success, individuals are expected to optimally allocate resources to reproduction at each age (Williams 1966; Charlesworth 1980; Minchella and Loverde 1981; Clutton-Brock 1984). High levels of reproductive effort are predicted when extrinsic adult mortality is high (Hirschfield and Tinkle 1975), that may result from factors such as predation or parasitism.

In cases where survival declines with age, we might expect that old individuals (with reduced residual reproductive value) will have a higher reproductive output than young ones, for a given parasite load (Forbes 1993). In birds, one problem for testing this hypothesis is that a high reproductive effort has been shown to increase parasite loads both for natural clutch sizes (Korpimäki et al. 1993; Allander and Bennett 1995; Dufva 1996; Oppliger et al. 1997; Ilmonen et al. 1999) and for experimentally manipulated clutch sizes (Norris et al. 1994; Richner et al. 1995; Ots and Hõrak 1996; Allander 1997; Siikamäki et al. 1997; Wiehn and Korpimäki 1998; Wiehn et al. 1999; but see Merino et al. 1996). Therefore, studies looking for effects of hemoparasites on reproductive output in natural populations should ideally sample birds before the onset of breeding (Appleby et al. 1999).

Hemoparasites, such as *Haemoproteus* or *Trypanosoma*, can have severe effects on their hosts (Atkinson and van Riper 1991). Birds experimentally infected with *Haemoproteus* developed myopathy, had reduced growth, and some of them died (Atkinson and van Riper 1991). Parasites that enter the erythrocytes probably cause additional physiological stress to the host by destroying the cells and consuming haemoglobin (Atkinson and van Riper 1991). However, negative effects may not be easy to document, especially in free living birds, without manipulating parasite load (Bennett et al. 1993).

J.J. Sanz (✉) · E. Arriero · J. Moreno · S. Merino
Departamento de Ecología Evolutiva,
Museo Nacional de Ciencias Naturales (CSIC),
José Gutiérrez Abascal 2, 28006 Madrid, Spain
e-mail: sanz@mncn.csic.es
Tel.: +34-1-914111328, Fax: +34-1-915645078

Studies of hemoparasites in temperate climates have shown that most transmission occurs during the breeding season when vector populations are increasing with the onset of warmer weather, and when individuals with chronic, relapsing infections are available as sources of infection (Atkinson and van Riper 1991).

Here, we examine the association between hemoparasite prevalence (*Haemoproteus balmorali* and *Trypanosoma* spp.) and primary reproductive output by using laying date, clutch size and egg size as estimates. Female pied flycatchers, *Ficedula hypoleuca*, were sampled just after they had laid their clutches in two breeding seasons (1998–1999). If degree of infection influences primary reproductive output, we predict that infected females will breed earlier and lay larger clutches than non-infected ones since their expectation of future reproduction is reduced. If degree of hemoparasite infection is the cause of reproductive effort, the same prediction will be expected. However, if hemoparasites are pathogenic, infected females will breed later and lay smaller clutches than non-infected ones, reflecting a cost of parasitism. On the other hand, a fraction of female pied flycatchers were infected with both hemoparasite species. We examine whether there was a higher cost of having multiple infections than having just one hemoparasite species.

In the present study population, no decline in reproductive performance was detected before the age of 5 years, and even after that age, the evidence for reproductive senescence was weak (Sanz and Moreno 2000). In addition, the evidence for a decline in residual reproductive value for old females in this study was weak (Sanz and Moreno 2000). However, in our previous study the effect of parasitism on primary reproductive output was not controlled. It is possible that there may exist an interaction between infection status and age leading to a decline in reproductive prospects only for aged females in poor health. Thus, in the present non-experimental study, we investigated the relationships (1) between hemoparasite infection status of female pied flycatchers and their age, and (2) between hemoparasite infection status of young but experienced females (2 years old) or old females (4 or more years old) and their primary reproductive output (laying date, clutch size and egg volume).

Material and methods

Study species and study area

The pied flycatcher is a small (12–13 g), migratory, sexually dichromatic and hole-nesting passerine of European woodlands (Lundberg and Alatalo 1992). Egg laying in the population being studied typically begins in late May, and clutch size ranges from 2 to 8 eggs with a mode of 6 eggs (Sanz 1997). The female incubates alone and receives part of her food from her mate, and both sexes feed the young that fledge within 15–18 days of hatching (Lundberg and Alatalo 1992).

The study was conducted during the breeding seasons of 1998 and 1999 in a deciduous forest of Pyrenean oak, *Quercus pyrenaica*, at 1200 m above sea level in the vicinity of La Granja, central Spain (40°54′N, 04°01′W). Sanz (1995) describes the study area in detail. Frequent checks of nest-boxes provided data on the date of clutch initiation and clutch size for all breeding pairs. On the day after clutch completion, eggs were measured with a caliper to the nearest 0.1 mm, and egg volume was calculated from the formula given by Kern and Cowie (1996). On the same day, females were captured, aged as yearling or older (Karlsson et al. 1986; Potti and Montalvo 1991a), banded with numbered rings and measured. All females were weighed to the nearest 0.1 g and their tarsus length was measured to the nearest 0.01 mm (Svensson 1992). Immigrant (unringed) females aged as adults at first capture were given a minimum age of 2 years. This is a reasonable assumption as, especially in females, few birds breed for the first time at ages older than this (Potti and Montalvo 1991b). For the analyses of parasite prevalence or intensity in relation to host age, females older than 5 years were grouped to increase sample sizes of older birds. To test whether old female pied flycatchers have a larger primary reproductive output (measured as laying date, clutch size and egg volume), we compared these breeding parameters between young but experienced females (2 years old) and old females (4 or more years old). Partner age did not differ between these female groups (mean±SD, 2-year-old females: 1.83±0.38, n=42; 4 or more years old: 1.96±0.21, n=23, χ^2=2.09, df=1, P=0.15). Subsequently, clutches were manipulated for other purposes.

Blood sampling

A drop of blood from the left brachial vein was collected in a microcapillary tube and transferred to a glass slide, smeared, air-dried and fixed in absolute ethanol some hours later. Smears were Giemsa-stained for 45 min to assess their infection by haematozoa (Merino and Potti 1995; Merino et al. 1997). E. A., who had no prior knowledge of the reproductive effort of the females involved, examined all blood smears. Smears were microscopically examined for parasites under 200× magnification objective in search of large extraerythrocytic haematozoa (i.e. *Trypanosoma*). To prevent the possibility that the symmetry of the blood smear might cause a non-random distribution of parasites (Godfray et al. 1987), one-half of each smear was entirely scanned (about 300 fields scanned, one-half being chosen at random; Merino and Potti 1995). We quantified the number of *Trypanosoma* spp. by counting the number of fields scanned and transforming the number of parasites observed to parasites per 100 fields (Merino and Potti 1995). Subsequently, intraerythrocytic haematozoa (i.e., *Haemoproteus*) were detected and quantified under oil immersion (1,000× magnification) by counting the number of parasites per 2,000 erythrocytes (Godfray et al. 1987) in the other half of the smear. When the infection by *H. balmorali* was very slight (i.e. less than one parasite per 10,000 erythrocytes), we assigned an infection intensity of 0.1 parasites per 2,000 erythrocytes, that is, just half of one parasite per 10,000 erythrocytes. Prevalence was defined as the proportion of infected females in the sample.

Statistical procedures

For females that had been sampled in both years, we randomly picked one observation to be included in the analyses to avoid pseudoreplication. Thus, each bird was only entered once in the analysis. The effect of blood parasites on primary reproductive output (laying date, clutch size and egg volume) was analysed by using ANOVA and entering the respective breeding parameters as the dependent variables. To test the effect of hemoparasites, we classified females for each parasite (*H. balmorali* and *Trypanosoma* spp.) in two categories, infected and non-infected. As intensities of hemoparasite infection distributions were highly skewed, we $\log_{10}(x+1)$ transformed these variables before analyses. Because we expected differences in the breeding parameters between female age classes and between study years we also entered these as categorical variables in our analyses. The same type of analyses were done to test the effect of the number of hemoparasite species on primary reproductive output. All statistical analyses were performed using SPSS for Windows software (Norusis 1993), and are two-tailed.

Results

Hemoparasite prevalence and female age

H. balmorali was the commonest blood parasite in our breeding population; the prevalence of infection was 35% (*n*=167). Moreover, about 16% of females sampled were infected with *Trypanosoma* spp. (*n*=167). The mean *H. balmorali* intensity for infected females was 14.7 parasites per 2,000 erythrocytes (SD=24.2, *n*=58). The mean *Trypanosoma* spp. intensity for infected females was 0.89 parasites per 100 fields (SD=0.73, *n*=26).

H. balmorali prevalence significantly increased with female age and was not dependent on study year (Fig. 1a; Table 1), while *Trypanosoma* spp. prevalence significantly increased with female age and significantly differed between study year (Fig. 1b; Table 1). Among hemoparasite-infected females, we analysed whether mean intensity of infection varied between female age groups and study years. Analysis shows no significant relationship between mean intensity of *H. balmorali*-infection and female age, when study year was included as factor (ANCOVA, model: $F_{2,51}$=0.86, *P*=0.43; study year: $F_{1,49}$=0.04, *P*=0.84; female age: $F_{1,49}$=1.72, *P*=0.20). Analysis shows no significant relationship between mean intensity of *Trypanosoma* spp. infection and female age, when study year was included as factor (ANCOVA, model: $F_{2,22}$=0.43, *P*=0.66; study year: $F_{1,20}$=0.18, *P*=0.68; female age: $F_{1,20}$=0.78, *P*=0.39).

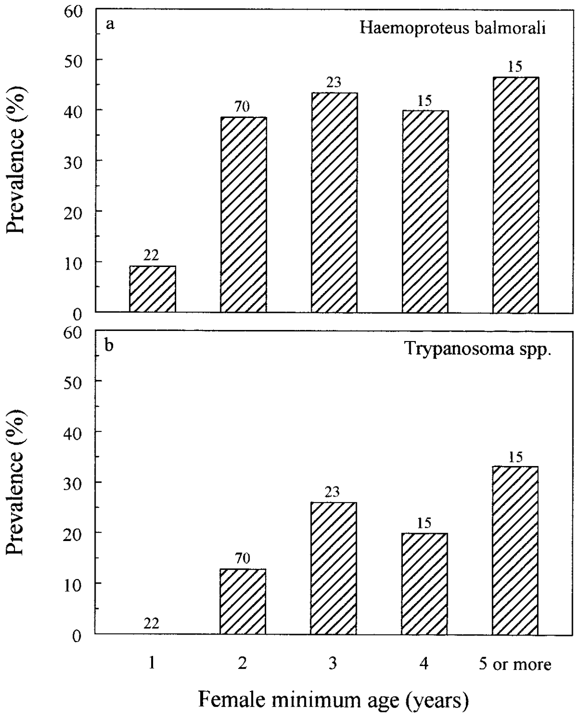

Fig. 1 Prevalence (% birds infected) of **a** *Haemoproteus balmorali* and **b** *Trypanosoma* spp. in female pied flycatchers in relation to minimum age. *Sample sizes* are given above the *bars*

Association between hemoparasite infection and primary reproductive output

Body mass did not differ between *H. balmorali*-infected females and non-infected ones (ANOVA, $F_{1,93}$=0.43, *P*=0.52), and between females with or without *Trypanosoma* spp. infection ($F_{1,93}$=0.13, *P*=0.72). The same analysis showed that there was no difference in female body mass between female age groups (2 years old vs. 4 or more years old; $F_{1,93}$=0.17, *P*=0.68) or study years ($F_{1,93}$=0.32, *P*=0.57).

Analysis of variance reveals that *H. balmorali*-infected females start to lay eggs earlier than non-infected females (Fig. 2a; ANOVA, $F_{1,94}$=5.69, *P*=0.019). There was no difference in laying date between females with or without *Trypanosoma* spp. infection ($F_{1,94}$=1.54, *P*=0.22), and between female age groups ($F_{1,94}$=1.34, *P*=0.25). Females laid significantly later their clutches in the second study year ($F_{1,94}$=7.32, *P*=0.008). The interaction term between *H. balmorali* infection and female age group was nonsignificant ($F_{1,94}$=0.52, *P*=0.47), showing that differences in laying date of infected females and those non-infected for both age groups were in the same direction (Fig. 2a). We found no relationship between the number of hemoparasite species (parasite-free, one or two) in females and their laying date (ANOVA, $F_{2,95}$=1.40, *P*=0.25), when female age group ($F_{1,95}$=0.73, *P*=0.39) and study year ($F_{1,95}$=6.10, *P*=0.015) were taken into account.

It is well known that clutch size and egg size of most birds is negatively associated with laying date, and therefore laying date was included as a covariate when analysing possible effect of hemoparasites on clutch size and egg size. Analysis reveals that clutch size was larger for *H. balmorali*-infected females than non-infected females, when laying date was included as a covariate (Fig. 2b; ANCOVA, *H. balmorali* prevalence: $F_{1,93}$=4.63, *P*=0.034; laying date: $F_{1,93}$=2.23, *P*=0.14), and there was no difference in clutch size between females with or without *Trypanosoma* spp. infection

Table 1 Logistic regression analyses with hemoparasite (*Haemoproteus balmorali* or *Trypanosoma* spp.) prevalence as the dependent variable, and female age and study year as explanatory variables

Variable	Estimate	Wald statistic	*df*	*P*
H. balmorali (*n*=167)				
Female age	0.1393	5.089	1	0.024
Study year	–0.1875	1.217	1	0.270
Trypanosoma spp. (*n*=167)				
Female age	0.5086	36.071	1	<0.001
Study year	–06051	8.174	1	0.004

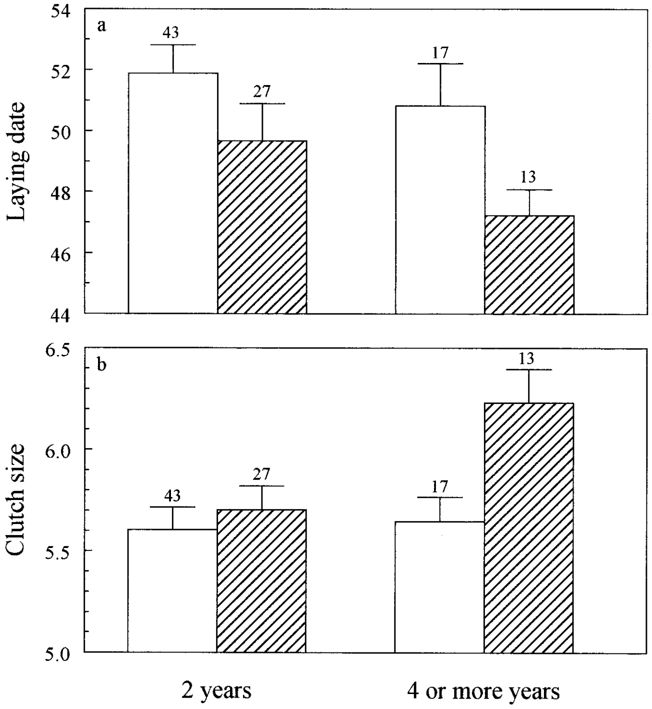

Fig. 2 Mean (±SE) **a** laying date (1=1 of April) and **b** clutch size according to female age groups (2 years old vs. 4 or more years old) and female infection by *H. balmorali* (*open bars* non-infected females, *shaded bars* infected females). *Sample sizes* are given above the *bars*

($F_{1,93}=0.76$, $P=0.38$). There was no difference in clutch size between female age groups ($F_{1,93}=2.17$, $P=0.14$), and females laid significantly larger clutches in the first study year ($F_{1,93}=11.85$, $P=0.001$). The interaction term between *H. balmorali* infection and female age group was significant ($F_{1,93}=3.97$, $P=0.049$), suggesting that mainly for infected females was there an increase in clutch size with age (Fig. 2b). We found no relationship between the number of hemoparasite species (parasite-free, one or two) in females and their clutch size, when laying date was included as a covariate (ANCOVA, number of hemoparasites: $F_{2,94}=0.35$, $P=0.71$; laying date: $F_{1,94}=4.33$, $P=0.04$), and female age group ($F_{1,94}=0.99$, $P=0.32$) and study year ($F_{1,94}=9.47$, $P=0.003$) were taken into account.

Finally, there were no differences in egg volume between females with or without *H. balmorali* infection ($F_{1,88}=2.02$, $P=0.16$), between females with or without *Trypanosoma* spp. infection ($F_{1,88}=0.00$, $P=0.96$), between female age groups ($F_{1,88}=0.13$, $P=0.72$) and between study years ($F_{1,88}=3.52$, $P=0.06$), when laying date was included as a covariate ($F_{1,88}=4.27$, $P=0.042$). We found no relationship between the number of hemoparasite species (parasite-free, one or two) in females and their egg volume, when laying date was included as a covariate (ANCOVA, number of hemoparasites: $F_{2,88}=0.90$, $P=0.41$; laying date: $F_{1,88}=5.12$, $P=0.026$), and female age group ($F_{1,88}=0.08$, $P=0.78$) and study year ($F_{1,88}=4.01$, $P=0.048$) were taken into account.

Discussion

The overall prevalence of hemoparasites in females in our study population is similar to that reported in other studies performed with pied flycatchers (Rätti et al. 1993; Bennett et al. 1995; Merino and Potti 1995; Dale et al. 1996; Merino et al. 1996; Siikamäki et al. 1997). Hemoparasites are most readily detected at a time when the intensity of parasitism is high. The onset of breeding induces hormonal changes which together with the stress of oviposition, may lead to a recrudescence of a chronic infection previously acquired, making diagnosis of the hemoparasite infection relatively easy at this time (Norris et al. 1994). Latent hemoparasite infections are known to relapse in spring (Bennett and Cameron 1974; Kirkpatrick and Suthers 1988).

We show that the hemoparasite prevalence increases with advancing age in female pied flycatchers, as previously reported for other passerine species (Weatherhead and Bennett 1991; Allander and Bennett 1994; Norris et al. 1994; but see Potti and Merino 1996). This argument assumes that in a cohort of females, older ones should have higher prevalence of parasite infection. However, our data set may not be appropriated to test this assumption. Møller and de Lope (1999) provided such a test in a long-term population study of the barn swallow *Hirundo rustica*. The intensity of infestations with two species of ectoparasites increased among old barn swallows (Møller and de Lope 1999). Hemoparasite prevalence may increase with advancing age as birds have had a longer exposure to vectors (Davidar and Morton 1993; Allander and Bennett 1994). Alternatively, old infected birds may develop a chronic or latent infection in which the immune system of the host controls the parasite, and it may relapse under conditions of physiological stress or environmental stress. Old females may be able to afford a relatively higher reproductive investment and still be able to sustain acceptable resistance to hemoparasite infections (Nordling et al. 1998). Whether the fact that old birds tended to suffer from hemoparasites more often than younger ones is due to differences in exposure or immune defence remains unknown.

There are few studies of positive associations between primary reproductive output and parasite loads in natural populations (Minchella and Loverde 1981; Festa-Bianchet 1989; Sorci et al. 1994), and many more of negative or non-significant associations (Møller et al. 1990; Møller 1993; Norris et al. 1994 and references therein). Any causal relationship between parasitism and host reproduction could go in either direction (Norris et al. 1994; Merino et al. 1996; Oppliger et al. 1997). Our study shows that *H. balmorali*-infected females initiated egg-laying earlier and laid larger clutches than non-infected females. Thus, infected female pied flycatchers in the population under study might not suffer from reduced fitness since early breeders produce more recruits than late-breeding individuals in this species (Lundberg and Alatalo 1992). Differences in laying date between infected and non-infected females did not explain observed differences in clutch size. Hemoparasites could reduce the general condition of females, and this might be reflected by their body mass. Nevertheless, there was no evidence that infected females had lower body masses after egg laying than non-infected females. There were not additive effects in primary reproductive output of both hemoparasites (*H. balmorali* and *Trypanosoma* spp.) together. Our results suggest that only *H. balmorali* have a pronounced impact on reproductive performance in this breeding population, but why only *H. balmorali* caused the observed findings? One reason may be that different hemoparasite species may have different impacts on their hosts (Bennett et al. 1993) and the impact may depend on the overlap between the different phases of the hemoparasite life-cycle and the host breeding-cycle. Moreover, *Haemoproteus* complete part of their complex life cycle within circulating erythrocytes and probably may cause additional physiological stress to the host by cause anemia (Atkinson and van Riper 1991). We should not expect similar effects of diferent hemoparasites since life-history strategies of different parasites and immune reactions of hosts may vary (Toft and Karter 1990).

Only a few studies have reported data that suggest that individuals will increase their reproductive effort late in life (Clutton-Brock 1984; Pärt et al. 1992). We showed that only infected female pied flycatchers increase their primary reproductive output (clutch size) late in life, when the probability of further successful reproduction is probably becoming low. To our knowledge, our study is the first to find that old infected females may make a terminal reproductive investment. However, this terminal reproductive investment may not be associated to a higher reproductive performance, as has been shown in the red deer *Cervus elaphus* (Clutton-Brock 1984) and the collared flycatcher *Ficedula albicollis* (Pärt et al. 1992). It may be that infected females occupy better territories and/or mate with higher-quality males, which could easily explain differences in laying dates between infected and non-infected females.

A larger reproductive investment by old infected individuals would lead one to expect an accelerated reproductive senescence for these individuals. Møller and de Lope (1999) have showed this in a non-experimental study with barn swallow *Hirundo rustica*. They found that some measures of reproductive performance deteriorated and that the frequency of ectoparasitism increased among old barn swallows (Møller and de Lope 1999). In our study population, no reproductive senescence measured as changes in annual reproductive output and survival probabilty, was discernible at the female age of 5 years (Sanz and Moreno 2000). Even after that age, the evidence for female reproductive senescence was weak (Sanz and Moreno 2000). In the present study, we show that mainly infected old females increased their primary reproductive output. The present results suggest that female primary reproductive output will depend on their health-dependent residual reproductive value. Whether this effect on primary reproductive output is due to parasitism remains to be explored.

Alternatively, if reproductive effort increases the susceptibility to hemoparasite infection or the likelihood that latent, chronic infections may relapse, parasitism may be a consequence of reproductive effort (Oppliger et al. 1997). As a consequence of life-history trade-offs (Stearns 1992), the females that invest more in reproduction may be less resistant to parasites (Deerenberg et al. 1997; Nordling et al. 1998; Moreno et al. 1999). Thus, according to this interpretation this study supports the hypothesis that egg production in birds might be costly in terms of health (Partridge and Harvey 1985; Stearns 1992; Monaghan et al. 1995). We cannot exclude this alternative hypothesis in the present non-experimental study. Clearly, more detailed studies are needed to reveal the effect of hemoparasites on the ecology of free-living hosts.

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