Resource levels, reproduction and resistance to haematozoan infections

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Compromise of immune function during reproduction may form a link between parental effort and the cost of reproduction, but the role of environmental variation in structuring intra-individual life-history trade-offs has been poorly investigated. We manipulated the need for parental effort in Eurasian kestrels, *Falco tinnunculus*, by food-supplementing broods for three nestling periods, during which the natural main food supply (voles) varied, and found that parental parasitaemia was inversely related to yearly vole densities. The level of parasitaemia in females was, however, reduced by food supplements. No effect on males was expected, as earlier work has shown that only females responded to the supplements by changing their behaviour. We show directly that the likelihood of female parasitaemia was diminished by spending less time in flight-hunting, which was related to reproduction during a good vole year, to our supplementary feeding, and to being mated to a male with high parental effort. Our results represent a novel direct benefit for females in resource-providing species, linked to female as well as offspring, well-being, and they provide insight into why the appearance of reproductive costs may be linked to gender or environmental conditions.

Keywords: cost of reproduction; direct benefits; *Falco tinnunculus*; haematozoa; life-history trade-offs; parasitic infections

1. INTRODUCTION

That reproduction can entail a cost has become clear (Lessells 1991). Recently, reduced immunocompetence as a consequence of increased reproductive effort has been seen as an important pathway for the cost of reproduction (see Sheldon & Verhulst (1996) for a review). However, the role of environmental variability in the context of intra-individual trade-offs has been poorly studied. The connection between high current reproductive effort and low residual reproductive value assumes the ratio of investment between reproduction and functions that are likely to increase future survival or fecundity to be always of same magnitude (Tuomi *et al.* 1983). This might not always be the case, as environments usually vary in quality over time or space, and hence individuals breeding in good-quality environments might not face as large allocation trade-offs as those breeding in poor-quality environments.

To study the role environmental variability can have in structuring intra-individual life-history trade-offs, we conducted a supplementary feeding experiment on Eurasian kestrels, *Falco tinnunculus*, (hereafter kestrel) breeding in Finland, in a highly variable environment with respect to the yearly supply of their main food, voles (Korpimäki 1985). Natural-sized kestrel broods were food-supplemented for the whole nestling period over three years in which the abundance of voles varied, and the prevalence of parental intracellular *Haemoproteus* and extracellular *Trypansoma* haematozoa was determined. These haema-

tozoa have experimentally been shown to cause histopathological changes in hosts (Molyneux *et al.* 1983; Atkinson *et al.* 1988), and, in particular, have potentially been shown to reduce the fitness of kestrels (Korpimäki *et al.* 1995). In a previous study we showed that females of food-supplemented nests decreased their hunting effort and provisioning of their nests, whereas males continued with unchanged levels of hunting and provisioning. Similarly, females hunted little if their mates were active in provisioning the brood (Wiehn & Korpimäki 1997). Therefore, in addition, to study the effects of resource manipulation, we directly assess the relationship between hunting effort and parasitism in this study, and evaluate the consequences of reduced male provisioning for female susceptibility to parasitaemia.

2. METHODS

We studied nestbox-breeding kestrels in an agricultural area of western Finland (62°59'–63°10' N, 22°50'–23°20' E), where migrant kestrels arrive from late March to early May (Palokangas *et al.* 1992) and exhibit low breeding site tenacity (yearly mean turnover of breeding males = 75%, and of females = 92%, during 1983–1992 (Korpimäki 1988; J. Wiehn & E. Korpimäki, unpublished data)).

The kestrel is a widespread open-country raptor, which shows a clear sexual division of labour; males provide most of the food between the pair-formation and mid-nestling phase, and females incubate and brood the young exclusively and start to hunt along with their mates only when nestlings are around two weeks old. Their main hunting modes are wind-hovering, soaring and perching (Village 1990).

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Figure 1. Prevalence (% individuals infected) of *Haemoproteus* spp. (a, c) and *Trypanosoma* spp. (b, d) among male (a, b) and female (c, d) parents rearing young in years of low (1993), intermediate (1992) and high (1995) natural food (vole) supply in nests that served as controls (open circles) or were supplemented (filled circles) with rooster chicks for the period of nestling time. Numbers are sample sizes. Significant terms: *Haemoproteus*, year (LR, $\chi^2=17.39$, d.f.=2, $p=0.0002$); treatment $\times$ year (LR, $\chi^2=8.44$, d.f.=2, $p=0.0147$); gender $\times$ treatment $\times$ year (LR, $\chi^2=7.55$, d.f.=2, $p=0.0230$); *Trypanosoma*, year (LR, $\chi^2=12.25$, d.f.=1, $p=0.0005$); treatment (LR, $\chi^2=5.27$, d.f.=1, $p=0.0217$); gender $\times$ treatment (LR, $\chi^2=4.03$, d.f.=1, $p=0.0488$). Means ($\pm$ binomial s.e.) are shown.

(a) Feeding experiments

Nests were food-supplemented as described earlier (Wiehn & Korpimäki 1997). Briefly, we supplemented nests every other day for the whole duration of the nestling period by providing them with dead rooster chicks (ca. 30 g); first with one (young kestrels ≤ 1 week) and later with two (young > 1 week) per kestrel nestling, thus covering approximately half of the daily requirement of nestlings (Masman *et al.* 1989). Nests with matched clutch sizes and hatching dates were identified as controls. Experiments were conducted for three years, which differed with respect to the natural abundance of voles; based on the snap-trapping indices of voles, these years can be regarded as 'low', 'intermediate' or 'high' with respect to relative vole abundance during breeding seasons 1993, 1992 and 1995, respectively (for detailed indices, see fig. 1 in Wiehn & Korpimäki (1997)). This variation in food conditions is a general pattern at the study site, directly reflecting clutch and brood sizes, which are, on average, 18% and 30% larger in years of high rather than of low vole abundance (J. Wiehn & E. Korpimäki, unpublished data).

Experimental nests were also subject to behavioural observations. Based on 6–8 h time budgets per pair during mid-nestling time (the young were aged 13–16 d), we calculated individual hunting effort (proportion of time spent in energetically expensive (Masman & Klaassen 1987) flight-hunting, i.e. directional flight, wind-hovering, soaring and gliding) and prey-delivery rates to nests (prey items per hour).

(b) Blood sampling

Male and female parents were captured with a swing-door trap attached to the nestbox when the nestlings were about two weeks old. Blood samples from parents were taken by brachial venipuncture. Two taxon-specific methods were applied to determine the prevalence (% individuals infected) of extracellular *Trypanosoma* spp. (*T. avium* complex) and intracellular *Haemo-*

proteus spp. (*H. tinnunculi* and *H. brachiatu*s) infections. (No other infections were found.) For determining haemoproteids, a drop of blood was collected in a microcapillary tube, transferred to a clean glass slide, smeared, air-dried, and fixed in absolute ethanol some hours later (Bennett 1970). Slides labelled only with a ring number were sent to the International Reference Centre of Avian Haematozoa, Canada, where they were subsequently inspected by G. F. Bennett by screening 100 fields under oil, using $\times 1000$ magnification. From 1993, flagellated trypanosomes, which divide and circulate in the bloodstream, were also quantified by means of a haematocrit centrifuge method (Woo 1970). For this method, blood was collected in 60 μ l haematocrit tubes, which were subsequently stored at ambient temperature. Within 12 h of sampling, the tubes were centrifuged for 5 min at 5000 r.p.m. Immediately thereafter, trypanosomes were screened by inspecting each tube for 5 min under a light microscope at $\times 400$ magnification. In 1993, the tubes were inspected by I. Pen, and in 1995 by both Ido Pen and J.W., and the repeatability between screeners was good: all the individuals were determined as either infected or non-infected by both the screeners.

(c) Statistical analyses

As the infections were treated as binary variables (non-infected or infected), logistic regression was used to estimate infection probabilities of kestrels. Year and treatment (supplementation or control) were treated as categorical variables, with indicator (dummy) coding, and hunting effort and prey-delivery rate as continuous covariates. We entered the main effects and their interactions into analyses, and chose the model with best fit using backward (stepwise) model selection. The significance of the models was estimated using the likelihood ratio (LR) method, with the logistic regression procedure in SPSS for Windows software (Norusis 1993). We report only two-tailed significant terms.

3. RESULTS AND DISCUSSION

We found 27% of the birds to be infected with *Haemoproteus* and 28% with *Trypanosoma*. Seventy-four per cent had either both of these parasites or none of them ($\chi^2=8.90$, d.f.=1, $p=0.0029$) and 26% were infected with one of the parasites but not the other. Within a given pair, the infection status would differ; for both of the parasites, in most cases where females were infected, males were not infected (Fisher's exact probability tests, $p=0.25$ (*Haemoproteus*), $p=1.0$ (*Trypanosoma* spp.)).

(a) Parasites, food supply and parental effort

Whereas the parasitic prevalence of males followed inversely yearly food conditions (figure 1a–b), a similar relationship in females was modified by food supplements, resulting in 81% lower prevalence of *Haemoproteus* in the year of low food supply (1993) (LR, $\chi^2=6.49$, d.f.=1, $p=0.0108$, figure 1c) and 84% lower *Trypanosoma* prevalence in the supplemented nests when years were combined (LR, $\chi^2=5.67$, d.f.=1, $p=0.0173$, figure 1d). By giving supplemental food, we could decrease females' hunting times (Wiehn & Korpimäki 1997), and increased time in energetically expensive (Masman & Klaassen 1987) flight-hunting predicted elevated parasitaemia in females, but not in males (figure 2). Again, this relationship in females was modified by year, in that flight-hunting predicted parasitaemia in years other than the high food year, when females had both very low levels of infection (figure 1) and reduced hunting times (figure 2).

These results support findings that poor nutrition is often associated with impaired immunological defence and disease (Chandra & Newberne 1977), and that energetically expensive (Demas *et al.* 1997) immune response is impaired by increased parental effort (Richner *et al.* 1995; Deerenberg *et al.* 1997). However, we believe that our study provides the first direct evidence for hunting-investment-related parasitaemia in nature, further showing that the magnitude of the trade-off between conflicting demands, such as parental investment and immunofunction, is likely to be modified by environmental conditions. But, why was this seen only in females? If the connection between hunting for nestlings and parasitaemia was straightforward, hunting time should also have predicted male parasitaemia, even if the hunting of males was not changed by food supplements (Wiehn & Korpimäki 1997). Therefore, it seems as if hunting, which in females only begins during the nestling phase, makes females susceptible to infection, whereas in males, the main providers for the family, the parasitaemia is perhaps influenced by cumulative investment effects during the entire breeding cycle. The negative relationship, however, between yearly food supply and parasitaemia in males also indicates that the same amount of investment in hunting over the years (Wiehn & Korpimäki 1997) leads to decreased ability to invest in parasite defence with declining environmental conditions.

Parasitaemia in adults could arise during the nestling period in two different ways. First, migrant birds might arrive at the breeding areas without detectable infections and, as a result of either a relapse of an old infection (Atkinson & van Riper 1991), or a newly acquired infection, show increased levels of parasitaemia during

Figure 2. Probability of (a) *Haemoproteus* spp. and (b) *Trypanosoma* spp. infection in female (black dotted lines) and male (grey solid lines) parents as a function of hunting effort (% of time spent in flight-hunting). The categories of low, intermediate and high refer to the yearly natural food (vole) supply in 1993, 1992 and 1995, respectively. (a) Hunting effort $\times$ year (LR, $\chi^2=9.31$, d.f.=2, $p=0.0095$) for females; no significant relationship for male hunting effort (LR, $\chi^2=2.56$, d.f.=1, $p=0.11$). (b) Hunting effort $\times$ year (LR, $\chi^2=10.71$, d.f.=1, $p=0.001$) for females; no significant relationship with male hunting effort (LR, $\chi^2=0.09$, d.f.=1, $p=0.77$). Lines depict the predictions of regression models; the estimates of hunting effort represent the range of observed flight-hunting times. Note that the x-axis is offset slightly below zero of the y-axis to avoid overlapping of the x-axis and prediction lines.

the nestling-rearing period. Hard-working, in nature, might cause an infection (relapse or new) through reduced immunocompetence or increased exposure to vectors, or both. In our study, vector densities could have varied, or microhabitat use in hunting (and thus the probability of encountering vectors) could have differed from year to year. Whether the parasitaemia arose from only the energy allocation trade-off or with different vector pressure accompanying it remains unclear, but our results clearly suggest that the trade-off between two competing processes, reproductive effort and immune response, is most pronounced during unfavourable breeding conditions. During years of better food conditions this trade-off is apparently less important.

(b) Male parental investment and direct benefits for females

If hunting has such a pronounced effect on female susceptibility, why should they hunt, especially given the males' main role as provider? Within pairs, provisioning

Figure 3. Probability of (a) *Haemoproteus* spp. and (b) *Trypanosoma* spp. infection among females as a function of male prey-delivery rate (items brought to nest per hour). Significant terms are: (i) male prey-delivery rate (LR, $\chi^2=7.18$, d.f.=1, $p=0.0074$) and treatment (LR, $\chi^2=4.00$, d.f.=1, $p=0.0455$), (ii) male prey-delivery rate (LR, $\chi^2=4.37$, d.f.=1, $p=0.0366$) and the male delivery rate $\times$ treatment interaction (LR, $\chi^2=5.61$, d.f.=1, $p=0.0178$). The relationships do not change qualitatively if year (categorical variable) is added to the model. For simplicity of presentation, year is omitted. Lines depict the predictions of regression models; the delivery rates represent the range of observed rates. Note that the x-axis is offset slightly below zero of the y-axis to avoid overlapping of the x-axis and prediction lines.

rates of males and females were negatively correlated, which suggests that females had to increase their own hunting to compensate for their mates' poor provisioning (Wiehn & Korpimäki 1997). Consequently, low provisioning of males increased the likelihood of parasitaemia in females (figure 3). This relationship was further modified by food supplements, in that females of supplemented nests had a lower probability of infection on a given male provisioning rate than females of control nests (figure 3). In effect, the supplementary feeding simulated increased male provisioning. From a female's point of view, this raises mate choice considerations, and suggests that kestrel females are likely to gain direct benefits by choosing a 'good parent' (Palokangas *et al.* 1994; Wiehn 1997; Wiehn *et al.* 1997). Traditionally, the direct benefits for females choosing a male with high parenting contribution have been understood as gains for the well-being of females' offspring (Andersson 1994). This is, to our

knowledge, the first study showing that the benefit may come through the well-being of the female, which may then enhance her own residual reproductive value.

Our results also pinpoint a potential defect of many observational studies reporting reduced reproductive output of individuals with high parasitic levels (e.g. Møller *et al.* 1990; Loye & Zuk 1991; Korpimäki *et al.* 1993, 1995). These negative associations can arise when individuals differ in genotypic quality or as a consequence of nutritional effects creating covariance between fitness and parasite prevalence, without any direct causal connection between the two. This is clearly seen in our experiment: kestrel nests that were food-supplemented fledged more young each year than control nests (Wiehn & Korpimäki 1997) and females attending these supplemented nests also showed lower prevalence of parasites than those of control nests. Therefore, without experimental verification, the causality in relationships between reproductive output and parasitaemia should be interpreted with caution.

We have experimentally demonstrated, with unique manipulation of resources within years of naturally different food conditions, the role that environmental variation plays in structuring life-history trade-offs. Moreover, we have demonstrated a potential cost of being paired to an individual that makes a low investment in parental care. Ultimately, these results suggest a mechanism for the trade-off between reproduction and survival demonstrated experimentally in the kestrel (Daan *et al.* 1996), and they aid in understanding why the appearance of trade-offs may be linked to gender or environmental conditions (Korpimäki & Rita 1996).

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