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Reproductive effort influences the prevalence of haematozoan parasites in great tits

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Summary

1. The influence of reproductive effort on host susceptibility to parasitism was examined in great tits, *Parus major*, by comparing the prevalence of haematozoan parasites with respect to clutch size in male and female parents.

2. Observational and experimental studies were conducted. Observational studies documented the relationship between clutch size and parasite prevalence in males and females in unmanipulated nests. Reproductive effort was manipulated by exchanging complete clutches between pairs of nests during incubation. Parents experienced a maximum manipulation of ± 5 eggs.

3. Observational studies showed that the prevalence of parasites was higher in females than males. The prevalence of parasites in males increased with both increasing clutch size and increasing age. There was no evidence of similar effects in females.

4. Experimental manipulation of clutch sizes showed that males were more likely to be infected if they had naturally large clutches or when their clutch size was artificially increased. There was no evidence of such effects on female infection probability. Reproductive effort thus increases susceptibility of males to parasites throughout the breeding period.

5. The mechanism which relates parasitism to reproductive effort in males is discussed. Reproductive effort might result in greater exposure, decreased ability to control chronic infections, or both. Nevertheless, reproductive effort increases susceptibility to haematozoan infection; whether this represents a cost of reproduction depends on the virulence of these parasites. It may also provide an explanation of previously reported patterns in haematozoan prevalence across bird species.

Key-words: blood parasites, cost of reproduction, *Leucocytozoon*.

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Introduction

Does reproductive effort increase susceptibility to parasitism? Anti-parasite adaptations like cell-mediated immunity, preening and non-specific responses, are probably energetically costly (e.g. Brindley & Dobson 1981; reviewed Keymer & Read 1991) and reproductive effort certainly can be (Lessells 1991). Consequently, there is a potential conflict between a host's level of reproductive effort and its ability to mitigate parasitic infection. This conflict may be exacerbated where reproductive behaviour is mediated by immunosuppressive hormones (Zuk 1991; Folstad & Karter 1992) and/or increases exposure to parasites (e.g. Hausfater & Watson 1976).

There are, however, relatively few reports of positive associations between reproductive output and parasite loads in natural populations (e.g. Festa-Bianchet 1989), and many more of negative or non-significant associations (e.g. Schall 1983; Gill & Mock 1985; Bennett, Caines & Bishop 1988; Rubenstein & Hohmann 1989; Weatherhead 1990; Forbes & Baker 1991; Loye & Zuk 1991; Hudson, Newborn & Dobson 1992b). Such varied outcomes are perhaps not surprising: any causal relationship between parasitism and host reproduction could go in either or both directions, and the variables can be indirectly related both positively and negatively by the effects of third variables such as host viability or nest site. Experimental manipulations go some way towards untangling these

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relationships. Many investigators of parasite virulence have appreciated this, and it is now abundantly clear from manipulations of parasite burdens that parasites can have a negative impact on host reproduction in natural populations (see reviews by Møller, Allander & Dufva 1990; Read 1990; Loye & Zuk 1991). However, experimental manipulations of parasites neither address nor exclude the possibility that reproduction increases susceptibility to parasitism, even where parasites do reduce host fitness.

In mammals, parasite responses to reproductive hormones, often in the context of vertical transmission (Shoop 1991), or host-mediated immunosuppression associated with gestation and lactation (McGregor & Wilson 1988; Behnke 1990) can increase parasite burdens. For example, in big-horn sheep (*Ovis canadensis*) pregnant ewes had higher larval counts of prenatally transmitted nematodes than did non-pregnant ewes (Festa-Bianchet 1989; Shoop 1991); in humans, malaria parasitemia is frequently exacerbated in pregnant women (McGregor 1988). Clearly, such phenomena demonstrate that reproduction can increase susceptibility to infections, but because they may be caused by factors peculiar to mammalian reproduction, they shed little light on whether reproduction *per se* affects susceptibility. The only data we know of which are indicative that general reproductive effort increases susceptibility come from big horn sheep (Festa-Bianchet 1989). For example, big-horn ewes which first lactated at 2 years of age suffered increased mortality during a subsequent pneumonia epizootic than did non-lactating ewes of similar age (Festa-Bianchet 1989).

However, an unambiguous demonstration that reproductive effort *per se* causes increased susceptibility to parasitism requires manipulations of reproductive effort; we are aware of no such experiments in wild populations. Here we use data on natural and manipulated clutch sizes to examine whether reproductive effort in great tits (*Parus major* L.) influences the prevalence of haematozoan parasites. We use the term susceptibility to cover both risk of exposure to parasites and/or decreased ability to control chronic infections; we return to this distinction in the discussion.

Costs of extra reproduction can be paid by one or both parents, and at any time during the nesting cycle. We manipulated reproductive effort by exchanging whole clutches between nests during late incubation. This design means that the timing of any cost of parasitism can be identified from the form of the relationship between the clutch size manipulation and parasite prevalence (Fig. 1). Consider the following. Increasing or decreasing clutch size should influence the effort parents have to expend while raising nestlings. This is the conventional cost associated with raising a large clutch (Drent & Daan 1980), and if this cost alone influences susceptibility to parasites then the size of the manipulation should be positively correlated with

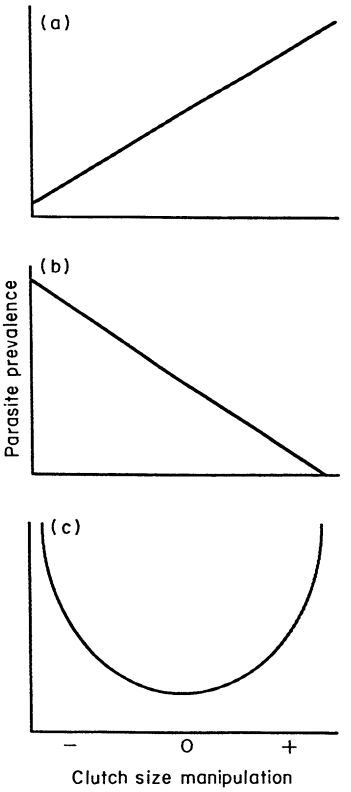

Fig. 1. Hypothetical relationships between the prevalence of haematozoa and the clutch size manipulation if there is increase susceptibility to infection (**a**) prior to or during egg laying, i.e. before clutches were exchanged; (**b**) during the nestling period; or (**c**) both during both egg laying and the nestling period.

prevalence (Fig. 1a). However, a consequence of exchanging whole clutches is that parents with a small clutch can only receive a similar sized or larger clutch in the manipulations, and *vice versa* for parents with large natural clutches. If reproductive effort influences parasitism only prior to clutch exchange (e.g. during nest building or egg laying), then prevalence should be negatively correlated with the size of the manipulation (Fig. 1b), since parents who laid large clutches were likely to receive a small clutch if exchanges were random. Finally, larger clutches might increase susceptibility to infection both prior to egg laying and during the nestling period. Under these circumstances, parasite prevalence would have a U-shaped relationship with manipulation (Fig. 1c): reductions originally had large clutches and would have higher prevalence due to costs incurred during egg laying; enlargements would have higher prevalence due to the costs of parental care.

Study area and methods

The study was carried out during the breeding seasons of 1990 to 1992 inclusive on populations of great tits nesting in Bagley and Wytham Woods, near Oxford,

UK. The nest box population in Bagley ($n = 180$ boxes) occupies an area of approximately 100 ha of the 230 ha wood. Studies in Wytham were conducted in an area known as Marley Plantation (*c.* 70 boxes). Both woodlands are deciduous, dominated by mature oak (*Quercus robur* L.).

The general methods used to collect breeding data were similar in each study area. Nest boxes were visited at weekly intervals in order to determine clutch size, hatching date, the number of young which hatched and the number which subsequently fledged. Laying date was calculated assuming that one egg was laid per day (Perrins 1979). Hatching date was established by daily visits to the nest box. Nestlings were weighed on day 7 (day of hatching = 1) and again on day 15 when they were ringed. Parents were captured while feeding nestlings after their chicks had reached a minimum age of 9 days, ringed and blood samples taken. Adults were sexed and aged (as yearlings or older) according to Svensson (1984), and wing-length, tarsus-length and body mass recorded.

Parents were sampled for haematozoan parasites by taking a thin smear with blood from the brachial vein (Bennett 1970). Smears were air-dried and fixed with absolute methanol in the field and subsequently stained with Giemsa stain. The entire film was screened first by conventional light microscopy ($\times 400$), then under oil emersion ($\times 1000$). A minimum of 400 fields per slide were searched. Blood smears were screened for parasites by M.A. who had no prior knowledge of the reproductive effort of the birds involved. We use prevalence throughout to mean the proportion of birds from which we were able to detect haematozoa. It is well known that infections can persist at densities below the limit of detectability in blood smears (Atkinson & van Riper 1991). Intensity was not assayed because such counts are usually made relative to red or white blood cell density, both of which are themselves a function of parasite density. Furthermore, even if meaningful intensity data (such as parasites/μl) were possible, the effects of time since inoculation and current immune status are likely to be confounded in single point estimates. Parasite nomenclature and authorities are discussed by Peirce (1981) and references therein.

Observations on the prevalence of parasites with respect to the reproductive effort of the breeding adults were conducted during the 1990 and 1991 breeding seasons. During these years complete, partially incubated clutches were exchanged between nest pairs. Nests were paired for similar laying dates and clutch sizes such that the fostered brood actually raised by a pair was similar in size and hatched on a similar date to their own clutch in its foster nest (Norris 1993).

Reproductive effort was experimentally manipulated by performing a similar clutch exchange experiment during the 1992 breeding season. Great tit nests were paired in order to induce a change in subsequent brood size and complete, partially incubated clutches exchanged to alter the number of chicks parents subsequently had to raise. Clutches were exchanged on average 7 days after the last egg in a clutch had been laid (range 0–16). Using this method parents experienced a maximum manipulation of ± 5 eggs, but all parents experienced clutch and subsequent brood sizes within the natural range in the study area. In ten nests, clutch sizes were not altered; in eight of these clutches were actually exchanged. In the other two, clutches were not exchanged; removing these from the analysis does not alter the conclusions. Samples sizes are variable because of missing values which arise where we were unable to catch parents, or accurately age them or because of nest failures at various stages during the nesting cycle.

G-tests with William's correction (Sokal and Rohlf 1981, p. 737) were used to analyse 2×2 frequency tables. More complex models were analysed using conventional multiple regression, or where the dependent variable was binary (infected, uninfected; yearling, >1 year old), logistic regression models fitted to the data using the GLIM statistical package (Numerical Algorithms Group 1987) according to the model $\ln[p/(1-p)] = a + b$ (clutch size), where p = the binomial probability, a and b are constants generated by the logistic regression model, and clutch size is either natural clutch size (1990 to 1991 data) or the manipulation in clutch size (1992 data). Significance tests are given by the change in deviance (denoted ΔD) which corresponds approximately to a chi-squared distribution with corresponding degrees of freedom (Armitage & Berry 1987). Two-tailed *P*-values are reported throughout.

Results

OBSERVATIONAL STUDY

During the 1990 and 1991 breeding seasons, when clutch sizes were not manipulated, haematozoan infection was detected in 36·3% (41/113) of breeding birds sampled. All parasites were from the genus *Leucocytozoon* Ziemann, 1898. Prevalence did not differ significantly between years ($G = 1{\cdot}88$, df = 1, $P = 0{\cdot}17$). 23% (12/53) of breeding males and 48% (29/60) of breeding females were infected with haematozoa, a significant difference ($G = 8{\cdot}11$, df = 1, $P = 0{\cdot}004$). There was no relationship between the infection status of a bird and that of its mate ($G = 0{\cdot}01$, df = 1, $P = 0{\cdot}91$). The age structure (proportion of yearlings) and sex ratio of birds sampled did not differ between years ($G = 1{\cdot}07$, df = 1, $P = 0{\cdot}30$; $G = 0{\cdot}04$, df = 1, $P = 0{\cdot}84$, respectively).

If reproductive effort influences susceptibility to parasitism, then the prevalence of parasites should increase with increasing clutch size. Such a relationship was found amongst males, but not females

(Fig. 2). This suggests that reproductive effort may increase susceptibility to parasitism, but that this effect is predominantly restricted to males. This was tested by constructing a logistic regression model including data from all breeding birds, with sex as a factor. If reproductive effort involves a cost of parasitism specific to males than there should be a significant interaction between clutch size and sex. This interaction was marginally significant ($\Delta D = 3{\cdot}99$, df = 1, $0.05 < P < 0{\cdot}10$), indicating that although the relationship between prevalence and clutch size is significantly different from zero for males, but not females, the evidence that the sexes differ from each other is not compelling.

Age-dependent probability of infection may confound the relationship between prevalence and clutch size in males. In both sexes, older birds had larger clutches (relationship between clutch size and the proportion of birds aged more than 1 year: males, $\Delta D = 5{\cdot}85$, df = 1, $P < 0{\cdot}025$; females, $\Delta D = 4{\cdot}568$, df = 1, $P < 0{\cdot}05$). Older males also had a higher prevalence of infection than first year males (10/31 compared to 2/22, respectively; $G = 4{\cdot}31$, df = 1, $P = 0{\cdot}038$). Therefore, the relationship between prevalence and clutch size in males could arise because older birds are more likely to be both infected and have larger clutches. To exclude this possibility, logistic regression models were constructed including the proportion of adults in each clutch size as a covariate (Table 1). This analysis showed that males associated with large clutches had a higher prevalence even when the effects of age were controlled in the analysis. Prevalence did not differ with age in females (first-years: 22/40; older females: 7/20; $G = 2{\cdot}16$, df = 1, $P = 0{\cdot}15$), and there was still no significant correlation between clutch size and parasitism in females when age was controlled for (Table 1).

Fourteen birds sampled in 1990 were recaptured in 1991. Six of these were female, and of these, two were still positive in the second year, two were still negative, one became positive and the other became negative. Eight males were recaptured. Six remained negative, one remained positive and one became negative. Overall, the likelihood of being infected in the second year was independent of infection in the previous year, but only marginally so (Fisher's exact $P = 0{\cdot}095$). To cover the possibility that the use of the same bird in both years might bias the conclusions about the effect of clutch size, the analysis was repeated with one duplicate reading removed. This does not affect the conclusions regarding females. However, the association between prevalence and clutch size for males (Fig. 2) becomes marginally significant ($\Delta D = 3{\cdot}54$, df = 1, $0{\cdot}05 < P < 0{\cdot}10$), even when age is controlled for as above ($\Delta D = 3{\cdot}28$, df = 1, $0{\cdot}05 < P < 0{\cdot}10$). Whilst not formally significant, these correlational results are, nonetheless, highly suggestive and emphasize the need for experimental manipulations.

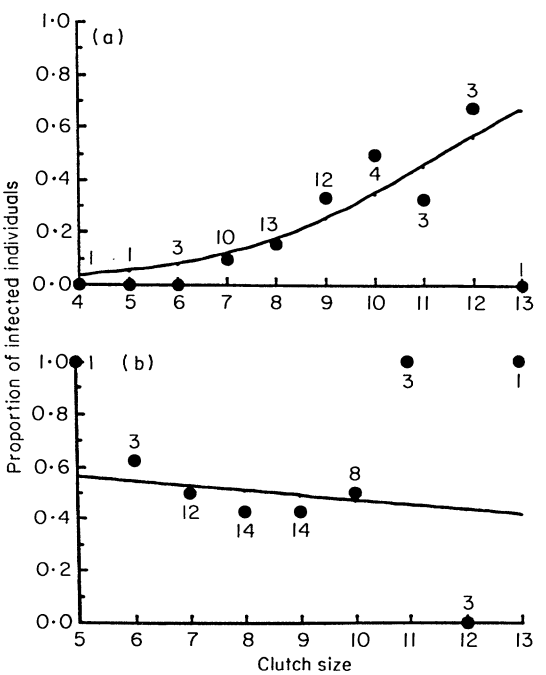

Fig. 2. The relationship between unmanipulated clutch size and the prevalence of haematozoa in (a) male parents and (b) female parents. Sample sizes are shown on the figure. The fitted lines correspond to the relationship predicted by logistic regression (see Methods). The relationship was significant for males ($\Delta D = 5{\cdot}43$, df = 1, $P < 0{\cdot}025$), but not for females ($\Delta D = 0{\cdot}259$, df = 1, $P > 0{\cdot}5$).

MANIPULATION OF REPRODUCTIVE EFFORT

During the 1992 breeding season, when the clutch exchange experiment was performed, the most common parasite in both sexes was *Leucocytozoon sp.*, but three other species were also recorded (Table 2). Prevalence of haematozoa in breeding males was more than twice that of the previous two years (59% [32/54]), a significant difference ($G = 15{\cdot}0$, df = 1, $P = 0{\cdot}0001$). Prevalence in females was unchanged (57% [30/53], $G = 0{\cdot}76$, df = 1, $P = 0{\cdot}38$). Because of the increase in prevalence in males, there was no sex difference in prevalence ($G = 0{\cdot}08$, df = 1, $P = 0{\cdot}78$), in contrast to that found in the previous 2 years. Similarly, the effect of age on prevalence in males was no longer apparent (57% in first-years and 63% in adults; $G = 0{\cdot}18$, df = 1, $P = 0{\cdot}67$). There was still no effect of host age on female prevalence (56 and 57%, respectively; $G < 0{\cdot}01$, df = 1, $P = 0{\cdot}95$).

It seems likely that the increased prevalence in males over that found in the previous 2 years is not a consequence of a natural between-year effect, but rather is due to our clutch size manipulation. As part of the experimental design, clutch size was unaltered in 10 nests in 1992. Infection was detected in only two of the ten males caught at these nests. This prevalence is comparable to that found in males in the two previous years ($G = 0{\cdot}04$, df = 1, $P = 0{\cdot}86$), but is significantly

Table 1. Logistic regression analysis of the relationship between the prevalence of haematozoa and clutch size controlling for the proportion of adult parents raising different sized clutches. The null model had a deviance value of 9·53 with df = 9 for males and 12·17 with df = 8 for females

Predictor variable	Δ Deviance	Parameter estimates
Males		
Intercept		−5·83±2·27
Proportion of adult breeders	2·05	−2·00±2·95
Clutch size	3·88*	0·67±0·391
Females		
Intercept		−0·34±1·46
Proportion of adult breeders	1·77	−2·44±1·84
Clutch size	0·37	0·13±0·21

*$P < 0·05$.

Table 2. The diversity of haematozoa present in blood samples taken from parents during the 1992 clutch exchange experiment. The cells of the table refer to the number of parents captured which had a parasite belonging to that particular group of parasites

	Sex	
Parasite	Males	Females
Leucocytozoon	25	27
Plasmodium	3	1
Trypanosoma	1	0
Other haematozoa	9	10
Number sampled	54	53

different from the prevalence in males at manipulated nests in 1992 (30/44, $G = 7·54$, df = 1, $P = 0·006$). Furthermore, prevalence amongst males at nests with experimentally reduced clutch sizes in 1992 (i.e. those with naturally large clutches, see Introduction) was not significantly different from the prevalence amongst males at nests with large natural clutch sizes in the two previous years ($G = 0·74$, df = 1, $P = 0·39$, where the comparable prevalence for 1990 and 1991 was calculated by averaging prevalence across clutch size classes, with each class weighted by its relative frequency amongst experimentally reduced clutches in 1992).

Clutch exchange experiments could alter reproductive effort in two ways. First, since clutches were swapped during incubation, parents could experience an energetic cost of incubating a larger clutch. There is evidence that larger clutches can take longer to incubate (Coleman & Whittall 1988; Smith 1989). This possibility was tested by using the number of days a female incubated her new clutch before the nestlings hatched as a measure of incubation costs. Since clutch exchanges were not all conducted at the same time during incubation, this quantity would depend on how soon clutches were exchanged after the female had laid the last egg of her original clutch. Furthermore, the timing of egg laying is also likely to be important. This is because females laying late in the season would tend to be given new clutches which had been incubated longer than their own, so reducing the number of days the clutch required incubating prior to hatching. The opposite effect would influence incubation in early laying females. As a result, a multiple regression model was constructed, with incubation period as the dependent variable, and three independent variables: the size of the clutch size manipulation, the timing of the clutch exchange after the last egg of the original clutch was laid, and the day the last egg in the original clutch was laid. All four variables in the model are approximately normally distributed. The three independent variables were together significantly related to incubation period ($F_{3,52} = 5·45$, $P = 0·0026$), but there was no evidence that the magnitude of the clutch size manipulation on its own increased the length of the incubation period (partial correlation of clutch size manipulation on incubation period, controlling for lay date and time after laying clutches exchanged: $r = 0·17$, $P = 0·22$).

The second way clutch size manipulations could affect reproductive effort is by altering the number of offspring parents subsequently raise. The data show that, as expected, parents which experienced an increase in clutch size subsequently raised a larger brood than parents which experienced a reduction (correlation between clutch size manipulation and number of chicks 6 days after hatching: $r = 0·52$, $n = 57$, $P = 0·0001$). Similarly, parents whose clutch size was artifically increased fledged a greater total biomass of chicks (correlation between clutch size manipulation and total mass of brood 14 days after hatching: $r = 0·46$, $n = 54$, $P = 0·0005$). Thus, the clutch size manipulation influenced costs parents incurred while raising nestlings.

As expected in clutch exchange experiments (see Introduction), the clutch manipulation experienced by parents was negatively correlated with their original clutch size ($r^2 = 0·426$, $P = 0·0001$) (note that there was no significant correlation between hatching date and manipulation: $r^2 = 0·01$, $P = 0·97$ showing the effects of manipulation are not confounded by date). This means the form of the relationship between the size of the manipulation and prevalence can be used to examine the effects of reproductive effort before and after manipulation (Fig. 1).

The prevalence of all parasites in both males and females in relation to the manipulation of clutch size is shown for each sex in Fig. 3. There was a significant relationship in males which was non-linear, with high prevalence associated with both artificially reduced and enlarged clutch sizes. There was no significant relationship in females. To test whether the relationship was significantly different between the sexes, a logistic regression model, similar to that used to test the same issue in the observational data from 1990 and 1991 (above), was used. Although the interaction term between sex and manipulation was not significant, the trend suggests that the effects of the manipulation were more pronounced in males than in females (Table 3).

There was no evidence to suggest that these relationships were influenced by the age of parents. As noted above, prevalence was not related to bird age in the year the manipulations were performed. Furthermore, the proportion of yearlings did not vary significantly with the clutch size manipulation in either sex (males: $\Delta D = 0{\cdot}87$, df = 1, $P > 0{\cdot}25$; females: $\Delta D = 2{\cdot}667$, df = 1, $P > 0{\cdot}10$). Therefore, the results for males are consistent with the pattern of Fig. 1c, suggesting that large clutches both prior to and following the clutch exchange lead to increased susceptibility to parasitism.

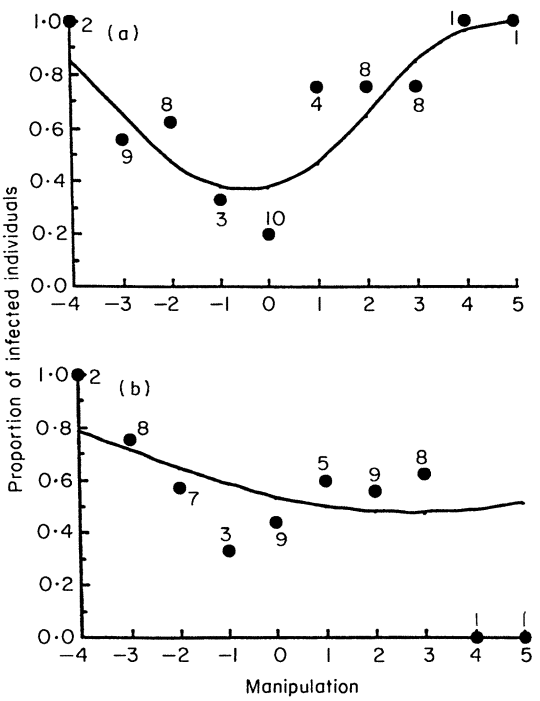

Fig. 3. The relationship between the clutch size manipulation and the prevalence of haematozoa in (**a**) male parents and (**b**) female parents. Sample sizes are shown on the figure. The fitted lines correspond to the relationship predicted by logistic regression. For both sexes the model can be described as $\ln[p/(1-p)] = a + b\,(\text{manipulation}) + c\,(\text{manipulation})^2$, where p = the binomial probability, and a, b and c are constants generated by the logistic regression model. The linear and quadratic terms were significant for males ($\Delta D = 8{\cdot}129$, df = 2, $P < 0{\cdot}025$; linear term: $\Delta D = 1{\cdot}038$, df = 1, $P > 0{\cdot}10$, quadratic term: $\Delta D = 7{\cdot}091$, df = 1, $P < 0{\cdot}01$), but not for females ($\Delta D = 1{\cdot}999$, df = 2, $P > 0{\cdot}10$; linear term: $\Delta D = 1{\cdot}718$, df = 1, $P > 0{\cdot}10$, quadratic term: $\Delta D = 0{\cdot}281$, df = 1, $P > 0{\cdot}50$).

Discussion

REPRODUCTIVE EFFORT AND PARASITISM

Our results show that increased reproductive effort increases the detectability of haematozoa parasites in the peripheral blood of male great tits throughout the breeding period. An increase in prevalence of haematozoa could come about in two ways, which are not mutually exclusive: reproductive effort may increase exposure to haematozoan vectors, or it may increase the stress on the immune system increasing the likelihood that latent, chronic infections may relapse or allow new infections to more easily establish.

Infections with haematozoa usually enter a chronic phase in which the immune system of the host controls the parasite (Atkinson & van Riper 1991). Under these circumstances the infection may relapse under conditions of both physiological or environmental stress. Physiological stresses include the suppressive effects of sex hormones on the immune system of the host (Zuk 1990; Folstad & Karter 1992). A potentially important environmental stress could be the time and energy costs parents incur during breeding. The energetic costs of raising large broods are well documented (Drent & Daan 1980) and could account for any relapse in infection in males during this period. During egg laying, males provide food for their female (Perrins 1979), as well as performing mate guarding behaviours to protect their paternity (Bjorklund & Westman 1986; Birkhead & Møller 1992). There is some evidence that increased feeding by the male prior to and during egg laying may increase the subsequent clutch size in certain species (Carlson 1989). Also, more mate guarding by males must be involved with larger clutches, since an egg is laid each day, and a female is fertile prior to the laying of each egg. Mate guarding is energetically expensive to males (Birkhead & Møller 1989) and, in addition, may be mediated by increased concentrations of immunosuppressive corticosteroids.

An alternative explanation for the effects of reproductive effort on prevalence concerns exposure to vectors carrying the parasites. Males may spend more time in areas of high vector density and/or have less time available for anti-vector behaviours when the female is laying and rearing a large clutch. Haematozoa are transmitted by ornithophyllic insect vectors (e.g. blackflies, mosquitos, midges), which infect the avian host when the vector takes a blood meal. Vectors are often distributed in areas of high avian density, which are usually locations in which the birds

Table 3. Logistic regression analysis of the relationship between the prevalence of haematozoa and the clutch size manipulation compared between the sexes. Sex is included in the model as a factor with 2 levels (1 = male, 2 = female). The null model had a deviance value of 21·93, with df = 19

Predictor variable	Δ Deviance	Parameter estimates
Sex	0·08	−0·11 ± 0·40
Manipulation	0·04	−0·03 ± 0·090
Manipulation^2	4·20*	0·09 ± 0·044
Sex* manipulation	3·15	
Sex* manipulation^2	2·73	

*$P < 0·05$.

feed or roost, and transmission is thought to be at a peak during breeding (Atkinson & van Riper 1991; Lehane 1991). If greater reproductive effort increases the time males must spend in these locations, then risk of exposure may increase. Furthermore, many avian species display behaviours (e.g. preening) designed to reduce the foraging success of haematophagous insects (van Riper *et al.* 1986). Reproductive effort may reduce the time available for such behaviours, and increase the likelihood that a biting insect gains a blood meal and so transmits any haematozoa it is carrying. Time budget data frequently shows reduced preening by males during the breeding period (e.g. Read 1988).

Relapse of existing infections or increased likelihood of acquiring new infections could account for the relationship between reproductive effort and parasite prevalence in male great tits, but begs the question of why females did not show a similar response. In the years where we did not manipulate reproductive effort, prevalence was higher in females than males. This suggests that females normally incur greater stress during reproduction increasing relapses of existing infections, or incur greater risks of exposure than males. These risks are only comparable in males with a high investment in reproductive effort, a position supported by our experimental manipulation of reproductive effort in 1992. This implies that females generally make a greater investment in terms of time and energy in reproduction than males, or spend more time in areas of higher vector density. These results also suggest that the manipulation of reproductive effort disproportionately influences either stress or exposure in male parents. This is not unreasonable since the male incurs costs of feeding and guarding the female during egg laying, but also relative male care is high early in the nestling period (*c.* 80% of feeding visits are made by the male when the nestlings are 4 days old; Royama 1966). Therefore, it seems likely that males would be more likely to increase their reproductive effort as a result of the manipulation than females during the nestling period immediately prior to sampling for haematozoa (parents were trapped on or after the nestlings reached 9 days old). Although the results suggest that males were more affected by the manipulation than females, the possibility remains that the prevalence of haematozoa increased in females subsequent to sampling, when they provide an equivalent amount of parental care to males. Furthemore, we note that our results formally demonstrate an absence of any effects of reproductive effort on females, rather than the presence of any sex differences.

The results of the experimental study also suggest that age-related changes in reproductive effort may be responsible, at least in part, for age-related changes in prevalence. Under natural conditions male great tits in their first year as breeders had lower prevalence of haematozoa than older birds. Weatherhead and Bennett (1991) report similar patterns for haematozoan infections in red-winged blackbirds (*Agelaius phoenicurus*). Since it is relatively common for reproductive effort to increase with increasing age in birds (Clutton-Brock 1988), it is interesting to speculate that age-related changes in parasite prevalence may be at least in part caused by these changes.

Although our results are primarily of intraspecific significance, the finding that reproductive effort increases the prevalence of haematozoa does have wider implications. Interspecific differences in haematozoan prevalences have been subject to little study, but three striking patterns have been recently reported (Read 1991; Ricklefs 1992). First, monogamous passerine species have a higher prevalence of haematozoa than do polygynous species. Secondly, amongst non-raptorial altricial land birds, incubation periods have been reported to be shorter for a given egg size in species with higher prevalences. Thirdly, amongst those same birds, prevalence in temperate regions was more than twice that in tropical regions. A number of explanations have been proposed to explain these patterns, but there are few supporting data. Our results suggest an alternative explanation which may underpin all these patterns: species differences in reproductive effort per breeding attempt.

If, on average, reproductive effort is higher in monogamous species, either because more males in a population are involved in rearing young or perhaps because monogamy allows larger broods to be raised, average susceptibility to haematozoa may be higher (either through greater exposure, or relapses of latent infections). Mating system was still correlated with

prevalence across species when the effects of sex differences in parental care were controlled (Read 1991), but differences in parental care at each stage of the nesting cycle was categorized into just three groups: only female, largely female and both sexes. We note that the variation in parental effort that we induced in our experiments would have been placed in the same category in that analysis (i.e. both sexes). If greater parental effort does explain why monogamous populations have higher prevalence than polygynous populations, the immunosuppressive effects of hormones and stress presumably associated with greater levels of polygyny must be outweighed by the increased susceptibility resulting from parental effort (cf. Zuk 1990).

Ricklefs (1992) hypothesized that incubation periods were shorter in taxa characterized by higher prevalences because in such taxa, the immune system is less developed prior to exposure to disease at hatching. Assuming that the pattern being explained holds after the confounding effects of phylogenetic non-independence, and variables such as mating system and nest type are statistically removed (for example, differences in both incubation period and exposure are likely associated with hole and open nesting), our results suggest an alternative explanation. Across species, shorter incubation periods are associated with larger clutch sizes (Trevelyan 1991). Our experiments show that larger clutches increase average infection probability within a species. If this translates into differences across species, an association between incubation period and prevalence would be generated by species differences in clutch size. We note too, that differences in clutch size could explain Ricklefs' (1992) unexpected finding that tropical birds have *lower* disease prevalences than temperate birds: it is well known that clutch sizes are lower in the tropics (Lack 1968; Trevelyan 1991). In contrast, the immune-development hypothesis actually predicts the opposite pattern: prevalence should be higher in the tropics, where incubation per egg is shorter. Egg sizes do not differ between tropical and temperate birds (Trevelyan 1991). Clearly, further comparative work could provide an important link between intra- and inter-specific studies of the relationship between reproductive effort and parasitism in birds.

PARASITISM AS A COST OF REPRODUCTION

Life-history theory rests on the premise that investment in current reproduction is made at the expense of investment in future reproduction. Although intuitive, the empirical demonstration of reproductive costs has been contentious (Reznick 1985; Bell & Koufopanou 1986; Partridge & Harvey 1985, 1988; Partridge 1989; Lessells 1991). Experimental manipulations of reproductive effort have established that current reproduction can be costly (Nur 1988, 1990; Lindén & Møller 1989), resulting in reduced offspring survival (Cronmiller & Thompson 1980; Gustafsson & Sutherland 1988; Pettifor, Perrins & McCleery 1988; Tinbergen & Daan 1990), reduced adult survival (Askenmo 1979; Reid 1987), or reduced future fecundity (Røskaft 1985; Gustafsson & Sutherland 1988). However, several studies have failed to document any costs (e.g. Finke, Milinkovich & Thompson 1987; Korpimäki 1988).

Although experimental studies have been particularly valuable in determining the type and magnitude of reproductive costs, there are limitations. One problem concerns the power of experiments to reveal effects of evolutionary importance (DeSteven 1980; Nur 1988, 1990). This is particularly relevant to the detection of survival differences of parents in relation to reproductive effort; statistically indistinguishable effects may be sufficiently large to have important evolutionary effects on the optimal clutch-size (Nur 1988, 1990). Furthermore, survival costs may only be apparent under severe environmental conditions (Festa-Bianchet 1989; Lindén & Møller 1989).

Our results suggest that parastism is a potential cost of reproductive effort for male great tits. However, demonstrating that it is requires evidence that patent haematozoa infections reduce host fitness. This apparently simple prediction is relatively difficult to test empirically and we know of no evidence directly relevant to great tits. Simply showing that males which had high reproductive effort have a higher prevalence of parasites and are more likely to subsequently die, shows that reproductive effort involves a mortality cost, but not that this cost was mediated by parasitism. Experimental infections and drug treatment of adult birds would be the most profitable approach to documenting the mortality cost of being parasitized. Even then, it is frequently difficult to statistically distinguish mortality costs of evolutionary significance (De Steven 1980; Nur 1988, 1990), especially where these are not consistent between breeding seasons, as is often the case for parasite-induced mortality (Loye & Zuk 1991). A number of studies have used brood-size manipulations to experimentally investigate the costs of reproductive effort in great tits (Pettifor *et al.* 1988; Tinbergen & Daan 1990), without demonstrating any survivorship costs to male or female parents.

Nevertheless, there is now considerable evidence that parasites can affect host fitness in natural bird populations (Møller *et al.* 1990; Loye & Zuk 1991). Frequently, this is because parasitism increases the susceptibility of the host to other mortality risks, such as predation (Kenward 1978; Temple 1987, Hudson, Dobson & Newborn 1992a) and even collisions with motor vehicles (van Riper *et al.* 1986). Haematozoa are known to cause substantial pathology and mortality in waterfowl and domestic poultry (Atkinson & van Riper 1991), and introduced *Plasmodium* has catastrophic effects on endemic birds in Hawaii (van Riper *et al.* 1986). The situation in older passerine-haematozoa interactions is unclear. Several studies

have failed to find correlations between components of host fitness and prevalence and/or intensity of haematozoan infections (Bennett *et al.* 1988; Weatherhead 1990, Weatherhead & Bennett 1992, Norris, Anwar & Read, unpublished), but the interpretation of correlations between parasites and host fitness is notoriously difficult (see Introduction). That haematozoan infections are effectively controlled by the host's immune system (Atkinson & van Riper 1991) implies that the parasites are capable of causing mortality in the host, but are prevented from doing so under normal circumstances.

The data from our experimental manipulations suggests that a majority of males are exposed to haematozoa, either because of vector transmission during breeding or because they have chronic, latent infections which can relapse. Because of this, haematozoan infections in great tits could be a potentially important influence on the evolution of life histories, and of clutch sizes in particular (Lessells 1991; Forbes, in press). Whether they are, depends on the potential virulence of the parasites and how modifications in reproductive effort affect susceptibility. In any case, reproductive effort by hosts clearly has the potential to influence disease epidemiology.

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