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Benefits of induced host responses against an ectoparasite

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As a consequence of the deleterious effects of parasites on host fitness, hosts have evolved responses to minimize the negative impact of parasite infection. Facultative parasite-induced responses are favoured when the risk of infection is unpredictable and host responses are costly. In vertebrates, induced responses are generally viewed as being adaptive, although evidence for fitness benefits arising from these responses in natural host populations is lacking. Here we provide experimental evidence for direct reproductive benefits in flea-infested great tit nests arising from exposure during egg production to fleas. In the experiment we exposed a group of birds to fleas during egg laying (the exposed group), thereby allowing for induced responses, and kept another group free of parasites (the unexposed group) over the same time period. At the start of incubation, we killed the parasites in both groups and all nests were reinfested with fleas. If induced responses occur and are adaptive, we expect that birds of the exposed group mount earlier responses and achieve higher current reproductive success than birds in the unexposed group. In agreement with this prediction, our results show that birds with nests infested during egg laying have (i) fewer breeding failures and raise a higher proportion of hatchlings to fledging age; (ii) offspring that reach greater body mass, grow longer feathers, and fledge earlier, and (iii) a higher number of recruits and first-year grandchildren than unexposed birds. Flea reproduction and survival did not differ significantly between the two treatments. These results provide the first evidence for the occurrence and the adaptiveness of induced responses against a common ectoparasite in a wild population of vertebrates.

Keywords: birds, fleas, host–parasite coevolution, host resistance, induced responses, virulence

1. INTRODUCTION

Parasites are ubiquitous, and many organisms face them at some stage in their life cycles. By definition, parasites draw the resources required for reproduction and survival from their hosts (Price 1980). When parasite infestation results in the reduction of host fitness (reviews on ectoparasites: Møller *et al.* 1990; Lehmann 1993), the degree of reduction in host fitness is taken as a measure of their virulence (Read 1994; Ebert 1998). Natural selection will favour hosts that are able to mount responses to minimize their effect (Hamilton *et al.* 1990; Keymer & Read 1991; Ebert & Hamilton 1996). In domesticated or captive vertebrates, host responses have been shown to reduce food intake, reproductive rate, and survival of ectoparasites (Devaney & Augustine 1988; Allen 1994; Randolph 1994; Wikel 1996).

If host responses are costly, they should be mounted only if a host becomes infested with parasites (Keymer & Read 1991). Inducible defences against parasites (or predators), are most beneficial if the risk of facing natural enemies is unpredictable and the host (or prey) can use reliable cues for their presence (Lively 1986; Harvell 1990; Clark & Harvell 1992), a common property of many host–parasite systems (Keymer & Read 1991; Lively & Apanius 1995). Examples of inducible responses are the acquired immunity of vertebrates, which generally refers to the antibody-mediated responses to the invasion of foreign antigens (Mitchison 1990; Wakelin 1996; Roitt *et al.* 1996), but can also include behavioural changes triggered by parasites (Hart 1990; Clayton 1991; Christe *et al.* 1994, 1996*a*,*b*; Keymer & Read 1991). In a recent model, it was suggested that for parasites that have the potential to reduce current reproduction (e.g. fleas), the optimal response by the hosts will be an increase in reproductive effort (Perrin *et al.* 1996). It remains undetermined whether induced responses, as shown in captive and domesticated vertebrates, are of functional significance in natural populations (Read *et al.* 1995; Goater & Holmes 1997).

The great tit, *Parus major*, is a small passerine bird common in woodlands, parks and gardens (Gosler 1993). During the breeding season, monogamous pairs defend a breeding territory and nest in cavities which often contain hen fleas, *Ceratophyllus gallinae* (Harper *et al.* 1992; Heeb *et al.* 1996; Tripet & Richner 1997). Hen fleas reduce the reproductive success of great tits by decreasing the number and quality of young fledged (Richner *et al.* 1993).

In this study we examine the benefits of induced responses by great tits to hen flea infestations. We predict that great tits exposed to fleas during egg laying start mounting responses at an earlier time, raise young in better conditions, and achieve higher current reproductive success than birds unexposed to fleas during egg laying. We

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also test whether host-induced responses reduce flea survival and reproduction.

2. MATERIALS AND METHODS

The experiment was carried out in spring 1995 on a great tit population breeding in nest boxes in the Bremgartenwald, near Bern, Switzerland. On the day the birds laid their second egg, all nests ($n=46$) were heat-treated in a microwave appliance to eliminate nest-based ectoparasites (Richner *et al.* 1993). Nests were then assigned randomly to a group either left free of parasites or to a group infested with 40 adult hen fleas obtained from naturally infested nests. When the whole clutch (ranging from 4–12 eggs) had been laid and incubation started, all nests were heat-treated for a second time and infested anew with 40 adult fleas. Thus, the experimental design resulted in one group of 23 breeding pairs with nests infested by 40 adult fleas from the laying of the second egg onwards (the exposed group) and another group of 23 pairs with nests infested only from the first day of incubation onwards (the unexposed group), hereafter refered to as exposed and unexposed groups respectively. Birds in the exposed group were in the presence of flea-infested nests for a mean of 10.4 ± 0.6 d; $\pm$s.e. before the start of incubation. The birds *in both treatments raised their young in flea-infested nests* and did not differ significantly in laying date (Mann–Whitney U-test, $U=260$, $p=0.94$).

Eleven days after the start of incubation, the nests were visited daily to determine the day of hatching. Hatching success was calculated as the proportion of eggs in the clutch that hatched. Fledging success was calculated as the proportion of eggs that hatched which resulted in fledged young. Adults were captured when nestlings were 14 days old. There were no significant differences between the two groups in tarsus length, body mass and wing length for both adult males and females ($p>0.40$ for all tests). The proportion of first year or older birds did not differ between the two treatments ($p>0.50$ for both sexes). Nestling body mass was measured on days 1, 5, 9, 14, and 16 after hatching to a precision of 0.1 g with a Sartorius electronic balance. Numbered aluminium rings were placed on the tarsus of nine-day-old nestlings. When the chicks were 14 days old, the tarsus length was measured with calipers to the nearest 0.1 mm and the length of the third primary to the nearest 1 mm. On the same day, 20 µl of blood from the brachial vein of three chicks in each brood (the lightest, the heaviest, and an intermediate one) were collected, and the mean hematocrit level of the brood determined (the proportion of blood volume occupied by packed red blood cells). Fledging time of the brood is defined as the number of days between the hatching of the first egg to the day when the last young in the brood left the nest box.

The young recruited into the breeding population of 1996 were recorded and the total number of fledglings raised by these recruits determined. Thus, for the pairs of the 1995 experiment which recruited young, we assessed their number of first-year grandchildren. The number of grandchildren raised is a strongly fitness-correlated measure of current reproductive success, because it combines survival and fertility of the offspring (Clutton-Brock 1988).

Nine days post-hatching, a dummy camera box was installed to accustom the birds to the presence of the original camera box the following day. Ten days post-hatching, we filmed the feeding visits of adults to the nest by use of a video camera equipped with an infrared light source. Great tits usually resume normal feeding within less than 15 min after a nest visit by a human (I. Werner, personal observations). We discarded the first 15 min of filming, recorded the total feeding frequency during the subsequent 130 min for a total of 32 nests, and calculated hourly feeding rates. The observer of the video had no knowledge of the infestation treatments when analysing the films.

We collected the nests on the day the last nestling fledged, and measured fresh mass of the nests before freezing them to $-20\,^{\circ}$C. At a later date, the number of adult fleas, larvae, and cocoons were counted by carefully searching through the nest material (Heeb *et al.* 1996).

Directed tests were used whenever the expected order of means across experimental groups was specified by our hypothesis (Rice & Gaines 1994). The program Systat (Wilkinson 1989) and the Statistix statistical package (Analytical software 1989) were used for the analyses. All tests are two-tailed.

3. RESULTS

(a) *Effects of brood size and clutch size*

Birds in the unexposed group tended to lay larger clutches and had larger brood sizes at hatching (table 1). In order to test whether variations in brood size affected our results, the effect of brood size on the breeding parameters of the adults and the measurements of nestlings was analysed. None of the analyses shows significant brood size effects ($p>0.25$ for all tests). Inclusion of brood size in the statistical model does not affect the observed significance values. Thus, brood size is not included hereafter as a factor in the analysis of the breeding parameters of the adults and the measurements of nestlings. The same holds for clutch size.

(b) *Breeding failures and fledging success*

The hatching success of the birds in the two groups did not differ significantly between the two treatments (table 1). The number of young fledged by the breeding pairs did not differ significantly between the two treatments; however, birds in the exposed group had a higher fledging success (table 1). Fewer breeding pairs in the exposed group failed to raise at least one fledgling than pairs in the unexposed group (one pair versus eight pairs; Fisher exact test, $p_{\mathrm{dir}}=0.015$), five pairs failed during incubation, and four pairs failed when raising their young.

(c) *Nestling growth, fledging times and parental feeding rates*

The mean mass of nestlings on the first day after hatching did not differ between the two treatments ($t_{37}=0.85$, $p_{\mathrm{dir}}=0.25$). However, 16 days after hatching, nestlings of the exposed group were significantly heavier than nestlings of the unexposed group (t-test: $t_{37}=3.42$, $p_{\mathrm{dir}}=0.0012$; figure 1). Nestlings in the exposed group also grew longer feathers and tended to have longer tarsi than chicks in the unexposed group (table 1). The mean hematocrit level of the nestlings in the two groups did not differ significantly (table 1). The power to detect a significant difference in hematocrit levels in an undirected test was of 22% ($\alpha=0.05$, effect size $d=0.43$; Buchner *et al.* 1996), suggesting that caution is necessary when accepting the null hypothesis. The fledging time of exposed broods was significantly shorter than that of unexposed broods (table 1).

No significant differences in the number of feeding visits to ten-day-old nestlings were observed both for males

Table 1. *Breeding parameters of adult great tits and nestling measurements*

(Birds in the exposed group had their nests infested by fleas during egg laying, birds in the unexposed group had no fleas in their nests during egg laying. Thereafter, birds in both groups incubated their eggs and raised their young in flea-infested nests. p_{dir} shows the statistic for directed tests. U=Mann–Whitney U-test, medians and ranges shown; t = t-tests, means ±s.e. shown. n=sample sizes.)

	exposed	unexposed	statistic	
breeding parameters:				
clutch size	7 (5–12) $n=23$	9 (4–10) $n=23$	$U=334$	$p=0.11$
brood size at hatching	7 (3–9) $n=22$	7 (4–10) $n=19$	$U=281$	$p=0.05$
hatching success	100 (0–100) $n=23$	100 (0–100) $n=23$	$U=279$	$p_{dir}=0.45$
number young fledged	6 (1–8)	6 (0–10)	$U=216$	$p_{dir}=0.47$
fledging success	100 (14–100) $n=22$	78 (0–100) $n=19$	$U=147$	$p_{dir}=0.05$
nestling measurements:				
third primary length	34.2±0.4	32.8±0.6	$t=1.90$	$p_{dir}=0.04$
tarsus length	22.5±0.1	22.3±0.1	$t=1.75$	$p_{dir}=0.055$
haematocrit levels	46.1±0.9 $n=22$	44.3±1.0 $n=16$	$t=1.31$	$p_{dir}=0.12$
fledging time	19 (17–21) $n=22$	20 (17–21) $n=15$	$U=242$	$p=0.015$

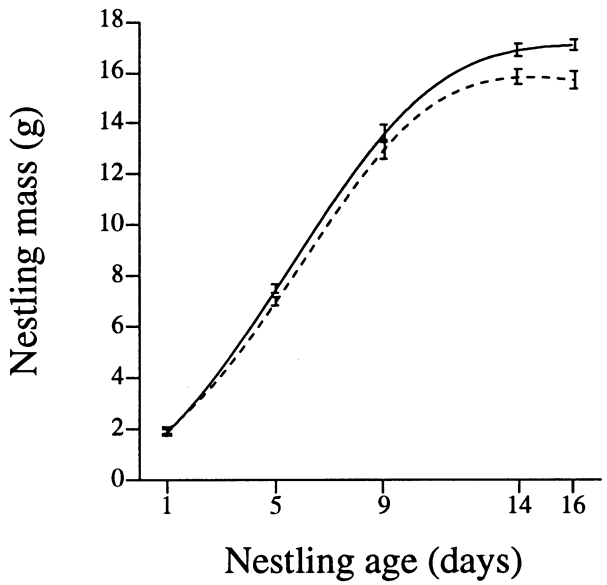

Figure 1. Growth of great tit nestlings in flea-infested nests following exposure or non-exposure of parents to fleas during egg laying. Nestlings from birds exposed to fleas during egg laying (solid line) reached heavier fledging mass than nestlings from birds unexposed to fleas during egg laying (dashed line). Means±s.e.

(exposed, 21.5±1.9, feeds h^{-1}; unexposed, 21.1±2.2; $t_{29}=-0.14$, $p_{dir}=0.56$) and females (exposed, 23.5±2.0; unexposed, 20.6±3.0; $t_{30}=-0.79$, $p_{dir}=0.27$), suggesting that the depressed growth of nestlings in the unexposed group was not caused by detectable differences in parental feeding rates.

(d) *Local recruitment and number of grandchildren*

In 1995, a total of 94 young fledged from nests in the unexposed control group, and 13 (13.8%) were recaptured as recruits in 1996. From nests in the exposed group, a total of 121 young fledged of which 24 (19.8%) were recaptured in 1996. To test for differences between treatments, the number of recruits was included as the dependent variable in a Poisson regression with treatment as a factor and brood size as a covariate. There was a significant effect of treatment on the number of young recruited (change in deviance, 3.39, $p_{dir}=0.041$, d.f.=1), and the effect of brood size was not significant ($p=0.12$). Among birds which recruited young, pairs from the exposed group recruited a median of two young (range, 1–4, $n=12$) whilst pairs in the unexposed group recruited a median of one young (range, 1–3, $n=9$).

The number of grandchildren was not analysed in a Poisson regression model since data for grandchildren was overdispersed. A Mann–Whitney U-test showed that the number of grandchildren raised in 1996 by the recruits was higher for birds in the exposed group in 1995 than for birds of the unexposed group ($p_{dir}=0.05$; figure 2). It may be noted that the number of grandchildren raised is not independent of the number of young recruited, since both are strongly correlated ($r_s=0.82$, $p>0.001$, $n=21$).

(e) *Parasite survival and reproduction*

The number of adult fleas and larvae in the two groups of nests did not differ significantly (adult fleas, $t_{38}=0.43$, $p_{dir}=0.42$; flea larvae + cocoons, $t_{38}=1.22$, $p_{dir}=0.14$). The mean number of larvae and cocoons was slightly higher in the nests of unexposed birds (figure 3). The power to reject the null hypothesis in an undirected test was 21% ($\alpha=0.05$, effect size $d=0.39$; Buchner *et al.* 1996), so the null hypothesis may be accepted with caution only.

4. DISCUSSION

This experimental study shows that exposure to ectoparasites during egg laying induces host responses which result in (i) fewer breeding failures and a higher proportion of hatchlings reaching fledging age; (ii) nestlings reaching higher body mass, longer feathers and earlier fledging; and (iii) a greater number of recruits and first-

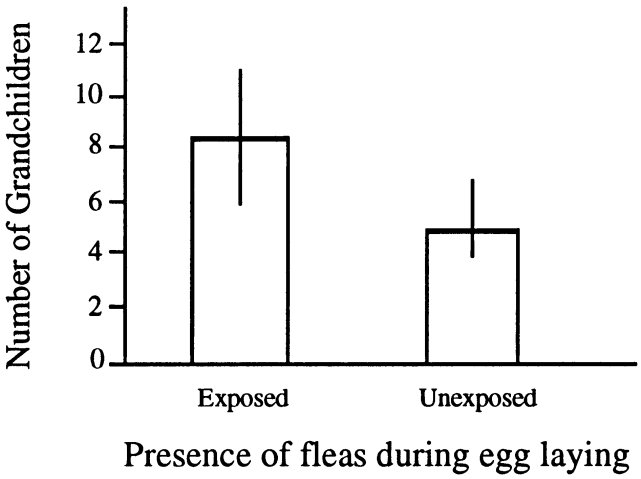

Figure 2. Number of first-year grandchildren in relation to exposure of parent great tits to fleas during egg laying. Birds of the group exposed in 1995 had significantly more first-year grandchildren in 1996 than birds of the unexposed group. Median ± interquartile ranges.

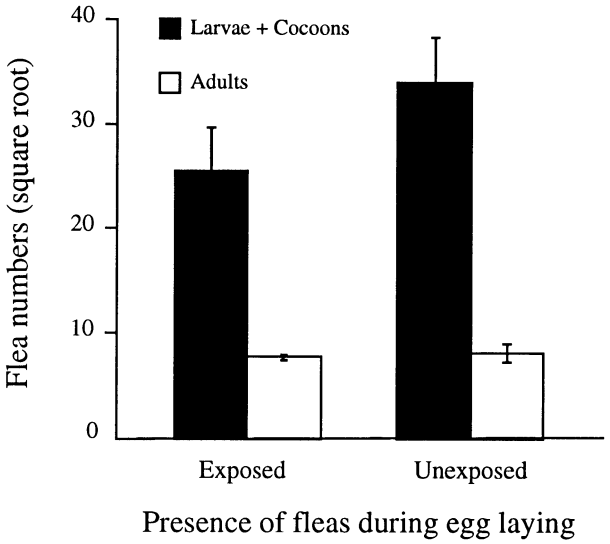

Figure 3. Flea numbers at fledging of nestlings in relation to exposure of parent great tits during egg laying. The number of fleas did not differ significantly between nests of exposed and unexposed birds. Mean ± s.e.

year grandchildren. As far as we are aware, these results provide the first evidence for adaptive induced responses serving to minimize the negative effect of a common ectoparasite in a wild population of vertebrates.

The virulence of a parasite depends on its interaction with its host, and consists usually of a decrease in some components of host fitness (Read 1994; Ebert 1998). Our results show that the parasite-induced response enhances the fitness of parasitized hosts and may therefore represent an alternative mechanism to the evolution of fixed and costly resistance (Minchella 1985; Keymer & Read 1991; Lively & Apanius 1995). In contrast to the highly significant effect of induced responses on nestling growth, there was a weak tendency only for an effect of host resistance on the number of flea larvae and cocoons. This shows that the precise relationship between parasite infestation intensity and host fitness may be nonlinear (Clayton *et al.* 1992). Our study suggests that the benefits of induced responses arose through an increase in flea tolerance by the nestlings, but does not exclude that host resistance is also at work.

Immunocompetence can be modified by levels of food intake (Gershwin *et al.* 1985) and also, as shown in bobwhite chicks (Lochmiller *et al.* 1993), low protein intake leads to a decrease in immunocompetence. In our study, feeding rates of the parents did not differ significantly between treatments, suggesting that increases in flea tolerance were not caused by differing rates of food delivery by the parents. In birds, high hematocrit levels are required for efficient oxygen uptake and transfer by the bloodstream (Gessaman *et al.* 1986). In another study on great tits, flea infestations were shown to decrease the hematocrit level of nestlings (Richner *et al.* 1993). In contrast to the strong effect on nestling growth, mean hematocrit levels of the brood did not differ significantly between treatments.

Modifications in the flea tolerance of nestlings or build-up of resistance seem to have been induced during the egg-laying period. Since blood-feeding by fleas is known to induce host immune reactions (Allen 1994; Jones 1996) in response to flea bites, female great tits could have produced antibodies and transferred them into the eggs. There is growing evidence that female birds transfer antibodies into their eggs as a response to parasites (Graczyk *et al.* 1994; Smith *et al.* 1994). The presence of egg-based antibodies could have reduced the stress of nestlings for mounting an immune response against flea immunogens (Klasing *et al.* 1987, 1991). Thus, female great tits exposed to fleas during egg laying could have transferred more antibodies into their eggs, leading to greater flea tolerance or resistance of their nestlings. For a given level of food intake, nestlings hatching from eggs of unexposed females could have faced a greater immunologically mediated stress (Klasing *et al.* 1987, 1991). Acquired tolerance of nestlings could have reduced the energetic constraints caused by the immunologically mediated stress, leading to depressed growth and lower survival. There may be a trade-off between maintaining suitable hematocrit levels and growth. An increase in immunologically mediated stress caused by parasites might alter the resource allocation of nestlings between the two processes. Resource allocation to growth is important since body mass at fledging usually correlates with post-fledging mortality (Tinbergen & Boerlijst 1990; Magrath 1991), but maintaining a threshold hematocrit level for oxygen transport might be of even higher importance.

Future experiments should determine whether female birds transfer antibodies in their eggs as a result of early exposure to ectoparasites. This possibility raises the interesting prediction that, when the risk of parasite infestation during breeding is high, female birds should expose themselves as early as possible to the parasites, thus promoting the development of immunological responses (Hart 1990). It may also provide an explanation as to why many great tit females use nest boxes as night roosts shortly before breeding (P. Heeb, personal observations).

More generally, it remains to be determined to what degree the development of tolerant or resistant phenotypes affects the dynamics of host–parasite coevolution (Lively

& Apanius 1995; Read *et al*. 1995). Genotype–environment interactions are likely to be important factors in the benefits of mounting inducible responses (Keymer & Read 1991; Lively & Apanius 1995). Also, non-genetic maternal effects within populations could lead to an overestimation of genetic variation for parasite susceptibility (Sorci *et al*. 1997). Further theoretical and empirical studies on the role played by epigenetic processes in host–parasite interactions may help us to understand the evolution of host tolerance and resistance towards parasites.

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