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Parasite-induced maternal response in a natural bird population

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Summary

1. The timing and mechanism of the maternal response to ectoparasites is investigated in a host–parasite system consisting of great tits and a haematophageous ectoparasite, the hen flea. It has been demonstrated previously that a maternal response to this parasite enhances survival and fertility of the offspring. This may have arisen by either a maternally transferred protection via the egg, or by a parental response affecting the common rearing environment after hatching. Two experiments aimed to differentiate between the two possibilities are reported here.

2. First, mothers were either exposed to or kept free of ectoparasite during egg production, and subsequently the newborn nestlings were cross-fostered between the two treatments. The experimental design discriminates as to whether the maternal effect arises before or after hatching. Within the same nest, the nestlings originating from previously exposed mothers grew faster than nestlings of unexposed mothers.

3. Secondly, we tested for the transfer of parasite-induced immunoglobulins (IgG) via the egg. Mothers were kept free of ectoparasites until they had laid the first egg and were then either exposed to or kept free of ectoparasites to the end of laying. The IgG-concentration significantly increased from the first to the eighth egg of exposed mothers, but not in eggs of unexposed ones.

4. In summary, the first experiment shows that ectoparasites can induce a beneficial maternal response at egg laying, and the second experiment suggests that the maternal effect is due to immunoglobulins transferred via the egg. Maternal responses to other parasites, e.g. blood parasites, are known for chicken in captivity. In natural populations of birds both the timing and mechanism of the response are poorly understood, despite their relevance for behavioural and population ecology.

Key-words: host–parasite interaction, IgG × transfer, maternal response, parasite-induced immunoglobulins, *Parus major*.

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Introduction

Since parasites are often harmful, natural selection favours hosts with efficient physiological, behavioural or immunological responses aimed at reducing the parasites' direct impact on hosts. Many parasite species, and in particular the highly mobile ectoparasites of vertebrates, are most common during the reproductive cycle of their hosts, and are thereby potentially transmitted to the hosts' offspring. Thus, in addition to selection for responses which alleviate the direct impact of parasites onto the infected individual, selection will also favour parental responses where protection is extended to newborn young. An important category of such responses are the parasite-induced maternal effects, which have been observed in domestic and captive bird and mammal species (Brambell 1970; Rose & Orlans 1981; Liu & Higgins 1990; Ritchie *et al*. 1992; Allen 1994; Graczyk *et al*. 1994; Carlier & Truyens 1995). A recent study (Heeb *et al*. 1998) on the great tit, *Parus major* L., has for the first time demonstrated the adaptive significance of such maternal responses to

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ectoparasites in a natural bird population. Offspring of ectoparasite-exposed mothers suffered less from parasitaemia, grew faster and had a higher chance to survive and reproduce the following year. Conclusions of this experiment regarding the mechanism involved are limited since the result could have arisen by both a maternally transferred protection via the egg, or by the common rearing environment after hatching, e.g. through an increase in parental nest cleaning or feeding. Thus, a physiological, behavioural or immunological mechanism could be at the origin of the found fitness benefits. Here we investigate, by means of two experiments, the timing and nature of the parasite-induced maternal response.

In a first experiment on the timing of the parasite-induced maternal response, female great tits were either exposed to ectoparasites, *Ceratophyllus gallinae*, shortly before egg laying or kept parasite-free, and nestlings exchanged at hatching between the two treatments. If the maternal response occurs before hatching, we predict that within nests the true offspring of the exposed mothers will grow faster than the true offspring of the unexposed mothers. If the response arises post-hatching through an effect of prelaying exposure on parental care, we predict no difference between the nestlings of the two origins.

In a second experiment on the nature of the parasite-induced maternal response, females were either exposed to ectoparasites after having laid the first egg, or were kept unexposed. Then the immunoglobulin (IgG) concentration of the first, fifth and eighth egg was determined. Blood-sucking ectoparasites produce small wounds with their mouthparts and introduce saliva containing anticoagulant factors, histolytic enzymes, vasoactive amines or toxins. The proteins present in the saliva are potent immunogens and elicit strong immune responses (Allen & Nelson 1982; Baron & Weintraub 1987; Wikel & Bergman 1997). Studies on domestic chicken parasitized by ticks have shown that an effective and long-lasting immunity develops rapidly, being apparent within a week after initial infection (Wakelin 1996). Therefore we predict an increase in IgG-concentration from the first to the eight egg of exposed females, but not in eggs of unexposed ones.

Methods

TIMING OF THE RESPONSE

The experiment was performed in a population of great tits near Bern (The Forst, Switzerland), breeding in nestboxes in a mixed forest predominated by beech trees. For this experiment newly bought, empty nestboxes were set up in November 1995 and used for nest-building by great tits the following spring. At the beginning of March 1996, after territory acquisition by great tits (Gosler 1993) and before the start of nest building, all nestboxes within the study area were lined with 20 g of fresh moss as a natural substrate for fleas. Then a random sample of nestboxes (experimental nests) was infested with 60 hen fleas while another sample was left uninfested (control nests). Fleas can immigrate but the numbers immigrating (5·8 fleas per nest per season ±1 SE, $n = 40$ nests; Heeb *et al*. 1996) are naturally far below the chosen infestation levels here, and most probably random with respect to our treatment groups. Under natural conditions the fleas persist in large numbers at the cocoon stage from one year to the next inside nestboxes, and great tits do accept nestboxes containing fleas. Thus an experimental infestation with 60 fleas lays within the natural range. Ectoparasitic hen fleas used for the inoculation of experimental nests were extracted from great tit nests collected within the study area at the end of the previous breeding season. Experimental nests were reinfested with an additional 20 fleas in the second half of March. On the day the female birds had laid their second egg, all nests were deparasitized in a microwave oven which kills adult fleas, flea larvae and flea eggs (Richner, Oppliger & Christe 1993). Nest height was then standardized to a height of 7 cm. All 23 experimental and 23 control nests were then infested with 40 fleas.

For cross-fostering, from each nest one-half of the nestlings which hatched the first day were selected randomly from both an experimental and a control nest with identical hatching date, then marked for later identification and exchanged between nests. Nestlings were exchanged only among broods with a similar number (±1) of nestlings. At hatching, there was no significant difference between mean body mass of exchanged vs. remaining hatchlings (paired t-test: $t_{45} = 0{\cdot}47$, $P = 0{\cdot}64$) within nests. Nine days after hatching, nestlings were marked on the head with a minute spot of red paint (Kölliker *et al*. 1998) and filmed with an infra-red sensitive video camera inside the nestbox. Recordings at experimental and control nests were synchronized in time, i.e. started within 30 min (t-test: $t_{34} = 0{\cdot}06$, $P = 0{\cdot}95$). From the films, we analysed parental feeding visits and the number of feedings each nestling received during 1 h, i.e. between 0·5 h and 1·5 h after setting the camera. One hour of filming provides a reliable estimate of feeding rates as we had previously found a high correlation ($r = 0{\cdot}826$, $n = 65$ broods) between feeding rates calculated from 0·75 h and 2·15 h of filming, respectively.

Thirteen days post-hatching both parents of all nests were caught, measured and then released. There was no significant difference in body size among both male and female parents from experimental vs. control nests (all P-values > 0·3), showing that parents had been randomized over the treatments. Fifteen days post-hatching the nestlings were measured. The statistical analysis compares cross-fostered nestlings with nestlings that remained in their own nest, and among these two types of nestlings is restricted to nestlings which hatched the same day, i.e. were of the same age, and came from broods with a similar number (±1) of nestlings. A total of 23 pairs of broods ($n = 46$ broods) were

successfully manipulated according to the above criteria. In a repeated-measures ANOVA the mean weight of nestlings originating from exposed mothers is compared to the mean weight of nestlings originating from unexposed mothers (= repeats) within each nest, where the pair of nests among which nestlings had been exchanged is defined as a factor.

MECHANISM OF THE RESPONSE

New, empty nestboxes were set up in November 1996 in a part of the same large forest and used for nest-building by great tits the following spring. Fleas hibernate in old nests during the winter months and thus do not migrate between boxes; therefore, nestboxes were still free of fleas at the start of the breeding season in 1997. After females had laid the first egg, 49 such nests were heat-treated (see above). After the heat-treatment the nests were assigned randomly to either the group that remained uninfested (n = 24 nests) or the group which was then infested with 60 adult fleas (n = 25 nests). In all nests, the first, fifth and eighth egg in the laying sequence was removed, weighed and replaced by an artificial egg resembling a great tit egg. Twenty-one females of the uninfested group and 20 females of the infested group laid at least eight eggs and are thus retained in the analysis. In the laboratory, the egg yolk of the removed eggs was separated from the egg white on the day of egg-collection and washed thoroughly in a jet of water to remove the adhering albumen. Albumen was not sampled because of its very low antibody content (Rose, Orlans & Buttress 1974). The collected yolks were then weighed, placed in sample cups and stored at –20 °C until further use. Later, each yolk was prediluted 1 : 5 in PBS buffer (PBS-Tween +0·02% NaN3) and, since antibodies are laid down in the yolk in a series of concentric circles (Brambell 1970), mixed thoroughly with a sterile syringe to obtain a homogenous sample. This sample was then diluted to 1 : 100 giving a final dilution of 1 : 500. A sandwich enzyme-linked immunosorbent assay (ELISA) technique was used to determine IgG concentration in egg yolks. ELISA was performed as described previously (Gottstein *et al*. 1993), unless otherwise stated. As a solid support, microtitre plates (Nunc-Immuno Module MaxiSorp) were used. Each well was coated with 100 µL of a 5-µg/mL concentration of rabbit antichicken IgG (affinity purified, BIO-SCIENCE/CH) in coating-buffer (pH 9·6) and incubated overnight in a moist chamber at 4 °C. The plates were then washed three times with PBS-Tween 20 (0·3%) buffer (PTB) prior to addition of 100 µL aliquots of PTB containing 0·5% bovine haemoglobin. After incubation for 30 min at 37 °C the solution was discarded and the plates were incubated with yolk samples (50 µL) diluted 1: 50 and 1 : 500 in PTB for 30 min at 37 °C. The plates were washed three times with PBS. Subsequently, 50 µL of alkaline phosphatase-conjugated rabbit antichicken IgG (affinity purified, Bio-Science, Switzerland), diluted 1 : 500 in PTB were added to each well. After incubation at 37 °C for 30 min, plates were again washed three times with PBS, followed by addition of 50 µL of freshly prepared substrate solution (1 mg/mL Sigma 104 phosphatase substrate in substrate-buffer). The reaction was continued for 20 min. Each plate included controls for diluents, conjugates and buffer solutions to determine non-specific background values of absorbance. As a positive reference, a purified (Gottstein & Hemmeler 1985) chicken IgY (0·02 µg/mL) was used in serial dilutions to calculate relative antibody concentrations in test yolk samples. Absorbance was read at 405 nm in an automatic micro-ELISA photometer. ELISA values were corrected for intertest variation with the corresponding negative and positive controls of each plate (test performances were validated when inter- and intratest variations of controls were = 10%) and multiplied by 100 to obtain integers. Data presented correspond to the 1 : 500 dilution of the test sample. The same results were obtained with the 1 : 50 dilution. The specificity of the primary and the conjugated antichicken IgG antibody for *P. major* IgG had been assessed previously by Western-blotting, showing that cross-reactivity of the antibodies were monospecific to *P. major* IgG heavy chain at M_r 68 K.

Results

TIMING OF THE RESPONSE

Within nests of growth the nestlings which hatched from eggs of exposed mothers (experimental nests) were significantly heavier 15 days post-hatching (Fig. 1) than nestlings of unexposed mothers (control nests), demonstrating that prelaying exposure to ectoparasites affects postnatal growth (repeated-measures ANOVA with nestlings from exposed vs. unexposed mothers as repeats and nest pair as a factor: prelaying exposure to parasites: F = 8·31, d.f. = 1, P = 0·008; pair of nests among which hatchlings were exchanged: F = 2·40,

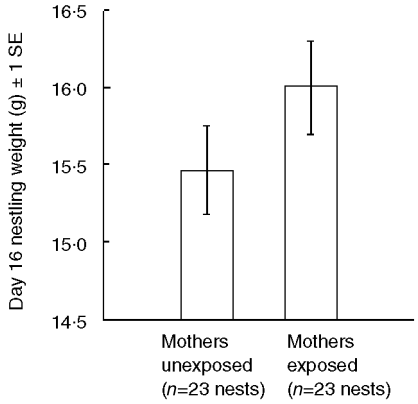

Fig. 1. Paired comparison of mean body mass 16 days after hatching of nestlings growing up in the same nest but originating from mothers exposed (n = 23) or unexposed (n = 23) to fleas during egg laying.

d.f. = 22, $P = 0{\cdot}021$). The rate of food provisioning by parents did not differ significantly (*t*-test: $t = 0{\cdot}19$, d.f. = 30, $P = 0{\cdot}85$) among control and experimental nests and, within the nests, the nestlings originating from exposed mothers received a similar number of feedings as nestlings from unexposed mothers (repeated-measures ANOVA: prelaying exposure to parasites: $F = 0{\cdot}29$, $P = 0{\cdot}60$; pair of nests among which hatchlings were exchanged: $F = 2{\cdot}26$, d.f. = 16, $P = 0{\cdot}066$).

MECHANISM OF THE RESPONSE

The hypothesis that mothers increase the IgG-concentration in the eggs as a consequence of ectoparasite exposure predicts an increase in IgG from egg one to eight in exposed mothers, but not in unexposed ones. Thus, in a statistical analysis with egg sequence as the repeated measure within individual mothers, a significant statistical interaction term between prelaying parasite treatment and egg sequence is predicted. The interaction should arise because mothers of both treatments had not been exposed to fleas prior to laying the first egg in this experiment and the first eggs in both treatment groups should therefore show similar antibody concentrations, but at least the eighth egg in the laying sequence of exposed mothers should show elevated IgG-concentrations, as compared to the eighth egg of unexposed mothers. For the fifth egg a difference is unlikely since the mother's response of producing antibodies takes several days. As predicted, the interaction between prelaying exposure to ectoparasites and egg sequence is significant (repeated-measures ANOVA, within-subjects: interaction term = egg sequence × prelaying exposure $F = 3{\cdot}31$, d.f. = 2, $P = 0{\cdot}042$, egg sequence $F = 4{\cdot}66$, d.f. = 2, $P = 0{\cdot}012$; between-subjects: prelaying exposure $F = 0{\cdot}05$, d.f. = 1, $P = 0{\cdot}82$. The increase in the IgG-concentration from the first to the eighth egg (Fig. 2) was $4{\cdot}22 \pm 1{\cdot}58$ (optical density measured at 405 nm, multiplied by 100 ± 1 SE) for exposed mothers, and $-0{\cdot}78 \pm 1{\cdot}55$ for unexposed mothers (*t*-test: $t = 2{\cdot}26$, $n = 41$, $P = 0{\cdot}029$).

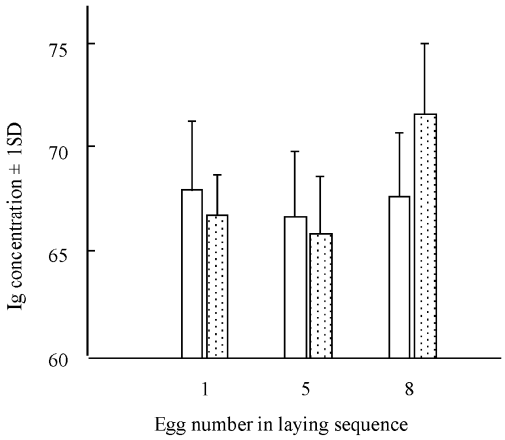

Fig. 2. Immunoglobulin (IgG) concentration of the first, fifth and eighth eggs in laying sequence from mothers exposed (hatched bars, $n = 20$ clutches) or unexposed (open bars, $n = 21$ clutches) to fleas after having laid the first egg. IgG concentration is expressed as the optical density measured at 405 nm, multiplied by 100. Data shown refer to a 1 : 500 dilution of test samples.

Discussion

The first experiment was designed to investigate the timing of the maternal response induced through exposure to ectoparasites shortly before egg laying. Within nests of growth, an adaptive response before hatching predicts better growth of hatchlings originating from exposed mothers than from unexposed ones, as shown here. A difference in body mass as the one observed here may have substantial consequences for later survival (e.g. Tinbergen & Boerlijst 1990). In the study by Heeb *et al.* (1998) nestlings with an average day 14 body mass of 15·9 g had a 13·8% chance of recruiting into the local population the following year, the nestlings with an average of 16·6 g a chance of 19·8%. A response after hatching would have predicted that, independently of their origin, nestlings in the nest of exposed mothers grow faster than nestlings in the nest of unexposed mothers. This was not the case (*t*-test: $t = 0{\cdot}40$, $n = 46$, $P = 0{\cdot}69$) as mean nestling weight in nests of exposed mothers ($15{\cdot}8$ g $\pm$ 1·8 SD) was similar to mean nestling weight in nests of unexposed ones ($15{\cdot}6$ g $\pm$ 1·9 SD). The alternative possibility that nestlings of exposed mothers receive more feedings can also be ruled out since monitoring of feeding frequencies of individual nestlings showed no such effect. The results therefore suggest the induction of a maternal response at clutch formation, which is then transmitted to nestlings via the egg. A probable mechanism is the transfer of antibodies from mother to offspring via eggs.

Maternal or parental antibody passage in birds can be achieved through egg yolk or crop-milk (Rose & Orlans 1981). In the former, the antibody transfer occurs largely during the 4–5 days preceding ovulation, when the growth of the ovum is most rapid. Serum IgG is accumulated in the egg yolk, whereas other antibody isotypes, IgA and IgM, are transferred only to the egg white (Malkinson 1965; Rose & Orlans 1981; Gottstein & Hemmeler 1985). During the following egg development, the transfer of maternal antibodies from the egg contents to the developing avian embryo occurs via the vitelline and hepatic portal circulation. Most of these antibodies pass from the yolk sac to the embryo during the last 5–6 days of embryonic development (Rose & Orlans 1981). Studies on maternal–fetal antibody transfer in birds have been described in captive birds such as chicken (Brambell 1970), mallard ducklings (Liu & Higgins 1990), cockatoos and parrots (Ritchie *et al.* 1992), pigeons (Rose & Orlans 1981) and penguins (Graczyk *et al.* 1994). Maternal antibodies protected chicken against clinical haemorrhagic enteritis (Fadly & Nazerian 1989), against avian leukosis virus (Fadly & Smith 1991) and against infectious bursal disease (Komine 1989; Homer *et al.* 1992).

The vast majority of investigations have focused on ticks, with little attention given to other blood-feeding arthropods. The interaction of ticks and naive hosts is characterized by a complex array of immunological responses (Brown 1985; Wikel 1996; Wikel & Bergman 1997), but the time-scale of this reaction to an initial bite is too slow to inconvenience the ticks seriously, which can complete their feeding normally. However, in an immune host with primed T-cells and preformed antibodies the response is very rapid and may cause ticks to stop salivating and feeding and then to detach directly (Allen 1989). Host reactions against fleas appear to be similar (Benjamini *et al*. 1963; Larrivee *et al*. 1964; Dryden & Blakemore 1989). Previous work has demonstrated that the oral secretions of the flea contain components responsible for producing the hypersensitivity reactions (Benjamini, Feingold & Kartman 1960). In chicken, the parasite-specific IgG levels in egg yolk or sera of young chicks correlate with immunity to a particular parasite (Smith *et al*. 1994). The result of our second experiment, designed to detect changes in IgG-levels in the yolk of eggs of exposed mothers, shows a significant increase of antibody concentration with egg sequence. It suggests therefore that this IgG-increase provides the protection of newborn nestlings, as observed in the first experiment, in this host–ectoparasite system. Future work should be focused upon the identification of specific immunogens and the analysis of the specificity of the immunological response.

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