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Origin-related, environmental, sex, and age determinants of immunocompetence, susceptibility to ectoparasites, and disease symptoms in the barn owl

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Knowledge of the role of origin-related, environmental, sex, and age factors on host defence mechanisms is important to understand variation in parasite intensity. Because alternative components of parasite defence may be differently sensitive to various factors, they may not necessarily covary. Many components should therefore be considered to tackle the evolution of host–parasite interactions. In a population of barn owls (*Tyto alba*), we investigated the role of origin-related, environmental (i.e. year, season, nest of rearing, and body condition), sex, and age factors on 12 traits linked to immune responses [humoral immune responses towards sheep red blood cells (SRBC), human serum albumin (HSA) and toxoid toxin TT, T-cell mediated immune response towards the mitogen phytohemagglutinin (PHA)], susceptibility to ectoparasites (number and fecundity of *Carnus haemapterus*, number of *Ixodes ricinus*), and disease symptoms (size of the bursa of Fabricius and spleen, proportion of proteins that are immunoglobulins, haematocrit and blood concentration in leucocytes). Cross-fostering experiments allowed us to detect a heritable component of variation in only four out of nine immune and parasitic parameters (i.e. SRBC- and HSA-responses, haematocrit, and number of *C. haemapterus*). However, because nestlings were not always cross-fostered just after hatching, the finding that 44% of the immune and parasitic parameters were heritable is probably an overestimation. These experiments also showed that five out of these nine parameters were sensitive to the nest environment (i.e. SRBC- and PHA-responses, number of *C. haemapterus*, haematocrit and blood concentration in leucocytes). Female nestlings were more infested by the blood-sucking fly *C. haemapterus* than their male nestmates, and their blood was less concentrated in leucocytes. The effect of year, season, age (i.e. reflecting the degree of maturation of the immune system), brood size, position in the within-brood age hierarchy, and body mass strongly differed between the 12 parameters. Different components of host defence mechanisms are therefore not equally heritable and sensitive to environmental, sex, and age factors, potentially explaining why most of these components did not covary.

ADDITIONAL KEYWORDS: *Carnus haemapterus* – cellular-mediated immune system – humoral immune system – parasite – pathogen – *Tyto alba*.

INTRODUCTION

Although evolutionary biologists have shown that genetic, environmental, sex, and age factors (Brinkhof *et al*., 1999; Christe *et al*., 2000; Alonso-Alvarez & Tella, 2001; Fargallo *et al*., 2002; Soler, Moreno & Potti, 2003) can determine the magnitude of immune responses, it is largely unknown whether each component of parasite resistance is differentially sensitive to each particular factor (El-Leythey, Huber-Eicher & Jungi, 2003). Evidence is accumulating for the hypothesis that immune responses are not similarly costly (Bonneaud *et al*., 2003; Martin, Scheuerelein & Wikelski, 2003), implying that the optimal expression of

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each single host defence mechanism may rely on alternative resources. Immune responses may therefore be traded-off against different life-history traits such as reproductive rate, developmental homeostasis, growth rate, body size, or age at maturity (Soler *et al*., 2002; Ardia, Schat & Winkler, 2003; Bize, Roulin & Richner, 2004), but also against each other if the full expression of one component is impaired by investment in another one. In other situations, an efficient resistance to parasitism may require several immune reactions potentially leading to positive covariations between them. It may therefore be difficult to predict how different host defence components vary and covary. Due to specific genetic, environmental, sex, and age constraints, various components may display different dynamics following an infection, and on its own each component may be a poor indicator of host–parasite interaction. As a result, alternative components of host defence mechanisms may evolve at different rates and not necessarily always towards a higher effectiveness. This may promote the evolution of complementary defence mechanisms that would permit hosts to resist parasites under many environmental situations. In the field of ecological immunology, knowledge of the role of genetic, environmental, sex, and age factors on the expression of many components of host defence may therefore provide important insights into the evolution of host–parasite interactions.

Using the barn owl as a model organism, we investigated variation and covariation in 12 traits involved in host–parasite interactions. Our aim is to determine the role of origin-related (i.e. genetic and maternal effects confounded), environmental (i.e. body condition, year, season and nest of rearing), and sex and age (i.e. reflecting the degree of maturation of the immune system) factors in immunocompetence, susceptibility to ectoparasites and disease symptoms. To distinguish between origin-related and environmental components of resisting parasites, we carried out cross-fostering experiments where one-half of hatchlings were exchanged between pairs of nests. We assessed humoral and T-cell mediated immunocompetence by vaccinating nestlings with three antigens [sheep red blood cells (SRBC, human serum albumin (HAS), tetanus toxoid (TT)] and the mitogen phytohemagglutinin (PHA), respectively. Susceptibility to ectoparasites was given by the number of ectoparasitic flies *Carnus haemapterus* and European castor-bean ticks *Ixodes ricinus* on the nestling body but also by the fecundity of *C. haemapterus*. We measured five disease symptoms, the size of two immune organs (bursa of Fabricius and spleen), haematocrit, blood concentration in leucocytes and the proportion of proteins that are immunoglobulins. These traits may reflect both the degree to which parasites have already invaded a host and the host ability of fending off disease. Following an infection, the immune organs bursa of Fabricius and spleen increase in size to produce more immune cells (Glick, 1983; John, 1994), hosts produce more leucocytes (Roitt, Brostoff & Male, 1996) and immunoglobulins (Bos *et al*., 1987; Freitas *et al*., 1991), and ectoparasites, such as *C. haemapterus*, that feed upon erythrocytes (Kirkpatrick & Colvin, 1989), can reduce haematocrit (Richner, Oppliger & Christe, 1993).

MATERIAL AND METHODS

Model organisms

The barn owl is a medium-sized bird with a body mass in the range 241–380 g in males and 263–478 g in females. It mainly preys upon small mammals and breeds in holes located in houses, churches, castles, and nest-boxes. In our Swiss population, mean laying date is 30 April (range 5 March to 30 July), mean clutch size 6.0 (range 2–11), and mean brood size at fledging 4.1 (range 1–9). Eggs are laid every 2.5 days and, because incubation starts when the first egg is laid, a pronounced age hierarchy is established among the siblings. Nestlings fledge at approximately 56 days of age and return to the nest until they reach 11–14 weeks of age (all information in Roulin, 2002). In our population, blood parasites seem to be rare at least in nestlings because not a single one could be detected in blood smears of 81 nestlings, possibly because infections had no time to develop (S. Merino, pers. comm.). Although, in the barn owl, numerous endoparasites have been identified, including trematodes, cestodes, and nematodes (Roulin, 2002), we did not consider them in the present study because of the difficulty of quantifying them.

In the barn owl, the most abundant ectoparasite is the two millimetres long blood-sucking fly *C. haemapterus* (Roulin, 1998). The level of infestation increases from birth to 25 days of age, then declines and, at fledging, nestlings are virtually free of parasites. When ectoparasites are still rare in the few days after hatching, *C. haemapterus* are mainly located on the first-hatched nestling hosts but, as nestlings grow up, parasite intensity increases and the last-hatched nestlings become the most infested ones (Roulin, 1998; Roulin *et al*., 2003). The blood-sucking European castor-bean tick (*I. ricinus*) is randomly distributed with respect to hatching ranks of their nestling hosts (Roulin *et al*., 2003).

General method

The study was carried out in western Switzerland (46°49′N, 06°56′E) between 1996 and 2003 in an area covering 190 km^2 located at an altitude ranging

between 430 and 520 m a.s.l. We monitored barn owls breeding in nest-boxes ($1.0 \times 0.6 \times 0.5$ m) that were fastened to barn walls in 1987. Nests, composed of turf and pellets, were not cleaned to allow *C. haemapterus* pupae to overwinter in the nest of their host. To determine the relationship between wing length and age, this trait was measured on several occasions in 187 nestlings of known age (in total, 1300 measures; Roulin, 2004). Wing length allows estimation of age accurately, at least early in life, because this trait is weakly sensitive to body condition (Durant & Handrich, 1998). Partial cross-fostering experiments were carried out in 1996, 1998, 2001, and 2002 by exchanging approximately one-half of the hatchlings between 'pairs of nests' with the criterion that broods had a similar hatching date (Pearson correlation: $r = 0.98$, $N = 88$, $P < 0.0001$). The nest where nestlings were born is called the 'nest of origin', and the nest where they were reared is called the 'nest of rearing'. Per nest, on average 1.9 ± 0.6 cross-fostered chicks fledged successfully. Because we did not necessarily wait until all eggs hatched to cross-foster hatchlings (we could predict how many eggs would hatch by mirroring them), mean hatching ranks (i.e. rank 1 for the first hatched chick, rank 2 for the second hatched, and so on) of cross-fostered and noncross-fostered siblings was similar (paired t-test: $t_{174} = 0.74$, $P = 0.46$); in approximately one-half of the cases, we cross-fostered the first-hatched chicks and in the other half the last-hatched ones. Our cross-fostering experiment is therefore not biased by hatching-rank related variation in egg quality. Furthermore, chicks positioned at a different position in the within-brood age hierarchy (i.e. nestmates were ranked by age starting with rank 1 for the oldest individual) in the foster nest were cross-fostered at a similar age [age at which chicks were cross-fostered as dependent variable in a two-way analysis of variance (ANOVA) based on mean sibling values, rank in the within-brood age hierarchy as first independent variable: $F_{1,179} = 2.31$, $P = 0.13$; brood size as second variable: $F_{1,179} = 0.55$, $P = 0.46$]. However, because we cross-fostered birds at a mean age of 4.0 ± 2.2 days, heritability estimates may be inflated by the fact that siblings were raised in the nest of origin during for an average of 4 days. This drawback (nests being distant sometimes by more than 40 km, cross-fostering experiments in the barn owl are particularly fastidious) forces us to cautiously interpret results based on cross-fostering experiments. Before being ringed, individuals were marked with a nontoxic colour paint to recognize their identity. In 1997 and 2000, no cross-fostering experiment was carried out whereas, in 1999 and 2003, a full cross-fostering experiment was performed by swapping clutches between pairs of nests (Roulin *et al*., 2001). The latter experiment does not allow to partition origin-related from environmental components of traits measured in nestlings. A blood sample was taken from nestlings to determine their sex from blood cell DNA using sex-specific molecular markers (Roulin, Ducrest & Dijkstra, 1999).

Nestlings were not all measured at the same age and, hence, to derive a nestling body condition index, we first extracted residuals from a second-order curve of body mass on wing length ($\text{mass} = -2.50 + 3.84 \times \text{wing length} - 0.01 \times \text{wing length}^2$; $F_{2,1389} = 3935.82$, $P < 0.0001$; each chick appears only once in this analysis). We then removed variation in these residuals explained by sex (two-way ANOVA: $F_{1,1385} = 35.80$, $P < 0.0001$; females are heavier than males) and hour of the day when chicks were measured ($F_{1,1385} = 161.88$, $P < 0.0001$; chicks are heavier in the morning than in the evening). The latter residuals were our condition index and used in statistical analyses. We measured nestlings before they naturally lose body mass at an age of 40 days (so-called body mass recession). This is important because food supply alters mainly body condition before the start of body mass recession rather than at fledging (Durant & Handrich, 1998).

The assessment of immunocompetence

Humoral immune responses

Titres of antibodies against three antigens used to challenge the humoral immune system were determined in 1998. We immunized 79 male and 84 female nestlings from 38 broods with SRBC, HSA, and TT. When the oldest nestling was approximately 40 days, all nestmates were injected subcutaneously in the neck with a cocktail of antigens (0.25 mL per bird, containing 30 mg HSA and 8 IU TT). Because of hatching asynchrony, there was ample variation in age at injection (mean 36 days, range 14–45 days). A second vaccination was simultaneously carried out in all individuals with 0.1 mL of 20% of SRBC (10% v/v in phosphate-buffered saline (PBS), with 10 mM phosphate, pH 7.4). To measure antibodies specifically directed towards the three antigens, we took five 100-μL blood samples from the brachial vein of all 163 individuals before immunization, and 3, 8, 13, and 18 days later. Methods to determine antibody titres are explained in Roulin *et al*. (2000) and El-Leythey *et al*. (2003). For statistical analyses, we log-transformed antibody titres of days 3, 8, 13, and 18, and then calculated the mean value.

T-cell-mediated immune response

To determine a parameter reflecting T-cell-mediated immunity, we challenged nestlings with an injection of the mitogen PHA using the simplified skin testing technique (Smits, Bortolotti & Tella, 1999). This

method assays the cutaneous basophil hypersensitivity reaction that represents a special form of T-cell-mediated immunity (McCorkle, Olah & Glick, 1980; Corrier & DeLoach, 1990). T-cell mediated immunity as measured with PHA has been demonstrated to be a reliable indicator of viability of hosts (Saino, Calza & Møller, 1997) without imposing high stress levels in nestling birds (Merino *et al*., 1999). In 2002, 145 males and 125 females from 67 broods received, subcutaneously into one wing, 0.1 mg of PHA, dissolved in 0.02 mL of PBS. Birds were aged 12–39 days (mean 26 days). The thickness difference of the wing web prior to injection and 24 h (range ±1) later was referred to as 'PHA-response'. Because individuals varied in the wing web prior to injection, we controlled for this factor in the statistical analyses. A Vernier calliper was used to avoid pressing too strongly on the soft tissue where the injection was made. Two successive measurements were taken on all chicks in two rounds (i.e. all nestlings were measured once and again 5 min later blindly with respect to the previous measurement). Within individuals, the two measures were significantly correlated (repeatability $r = 0.97 \pm 0.01$, $F_{270,271} = 61.80$, $P < 0.0001$), and the first and second measures did not differ (paired t-test, $t_{270} = -0.80$, $P = 0.42$). Just before injecting nestlings and 24 h later, wing length and body mass were measured to the nearest mm and g, respectively. The values proved to be normally distributed.

In 2003, PHA-response was determined in 181 nestlings aged 12–37 days (mean 28 days) from 39 broods. Using mean sibling values, we investigated seasonal, age, brood size, and body condition effects on PHA-response.

The assessment of susceptibility to ectoparasites

Number of Carnus haemapterus (Diptera: Carnidae)
A single person screened the body of 680 male and 709 female nestlings aged 5–48 days (mean 24 days) with the exception of places where flies were not visible such as the openings of the ears. For each nest, parasites were counted on all nestmates on a single nest visit. The method of counting flies is reliable (Roulin, 1998). Flies were counted in 63 nests in 1996, 29 in 1997, 46 in 1998, 39 in 1999, 44 in 2000, 44 in 2001, and 64 in 2002.

Clutch size of C. haemapterus
Gravid female *C. haemapterus* are easily identified by their white abdomen, and hence could be easily sampled on nestling barn owls. In 1998, 1458 gravid females were collected in 36 nests on 77 male and 80 female nestlings aged 11–31 days (mean 23 days). In each nest, eight to 73 gravid flies were sampled on a single nest visit. Each fly was put separately in a 1.5 mL Eppendorf tube during 24 h at 37 °C, a temperature close to the bird body temperature (Wehner & Gehring, 1990). After that time, all ectoparasites were dead, and eggs laid on the tube surface were counted. Because it is unclear why 58% of the females did not lay eggs, we considered only those that laid at least one egg. More information can be found in Roulin *et al*. (2001). The number of eggs was Box-Cox transformed to normalize the data set.

In 1999, *C. haemapterus* were collected in 31 nests used in an experiment where clutches were swapped between pairs of nests (Roulin *et al*., 2001). *Carnus haemapterus* were also collected in four other nests not used in this experiment. As soon as the older nestling ($N = 64$ males and 68 females) was 20 days of age, up to five gravid female *C. haemapterus* were collected on each nestling every 5 days up to fledging. Per nest, the mean number of visits with capture of flies was 4.8 (range 3–7) and, in total, 12–141 flies were collected per nest, giving 2282 flies. The fecundity of each *C. haemapterus* was determined as explained above. Using only females that laid at least one egg (69% of the collected flies), for each nest-box visit, we calculated the mean number of eggs for each nestling, and then mean sibling values. Values found at all visits were averaged providing a mean overall value that was used in the statistical analyses.

Number of European castor-bean tick Ixodes ricinus
From 1996 to 2002, the bodies of 1401 nestlings from 330 nests were screened for ticks. We checked chicks on several occasions, and considered that a nest is infested by ticks if at least one parasite was found on at least one nest visit.

The assessment of disease symptoms

Size of the bursa of Fabricius in dead birds
In nestling birds, the organ called 'bursa of Fabricius' produces B cells and is located near the cloaca. To investigate sex and condition-dependent effects on the size of this organ, 136 males and 140 females were collected dead along roads in the regions Bourgogne and Champagne in France between 11 July 1996 and 18 March 2001. In 1996, 75 individuals were measured; in 1997, 35 were measured; in 1998, 47 were measured; in 1999, 53 were measured; and, in 2000, 66 were measured. Owls stayed less than a day along the roads (the 'Société des Autoroutes Paris-Rhin-Rhône' collects systematically dead animals three times daily) before being collected and put in a freezer until dissection for sex determination and measurement of immune organs. Bodies were weighed after having removed stomach content. Most

bursae of Fabricius were weighed to the nearest g, and, for all of them, length and width were measured to the nearest 0.1 mm with a Vernier calliper. The square-root transformed product of these two values (i.e. surface) was used as an index of the size of this organ; fresh mass is strongly correlated with surface as shown with a subsample of the birds ($r = 0.90$, $N = 204$, $P < 0.0001$). Because this organ regresses prior to sexual maturity (17 barn owls with a bursa of Fabricius were aged 115–257 days and 17 birds without a bursa were 143–2078 days; data from the Natural History Museum of Basel), we statistically controlled for date of the collection of dead bodies. For this purpose, we defined 30 June as day 0, an arbitrary reference for birth date. Adults have no bursa of Fabricius and, hence, to be sure that we consider only individuals in their first-year of life, we used only birds in which this organ was found. To test for allometric relationship between the size of this immune organ and body size, we measured bill length to the nearest 0.1 mm, a trait that reliably reflects overall body size (Roulin *et al*., 2001).

Spleen size in dead birds

The avian spleen, located along the digestive tube, produces lymphocytes responsible for humoral and T-cell-mediated immune responses. This organ was weighed to the nearest g in 45 male and 57 female dead bodies collected between 28 August 1996 and 14 July 2000 in the same French region. Five individuals were measured in 1996, one in 1997, 50 in 1998, 45 in 1999, and one in 2000. The data was $\log_{10}$-transformed to normalize its distribution. Birds with a bursa of Fabricius were denoted 'juveniles' and those without were considered 'adults'.

Proportion of proteins that are immunoglobulins

In 2002, we assayed the proportion of proteins that are immunoglobulins (Ig) in 139 male and 112 female nestlings from 59 broods. These broods were the same as the ones used to study PHA-response. A 100-μL blood sample was taken from the brachial vein when nestlings were 10–39 days of age (mean 26 days). Blood samples were centrifuged, and sera collected and stored at −20 °C for 5 months. After electrophoretic separation of proteins on agarose gels (Paragon SPE Kit, Beckman), Ig were quantified by densitometric analysis using a computer image analysis procedure (Gelanalyst, Eidosoft). The relative titre of Ig was expressed as the ratio between the area of the densitometric profile corresponding to the immunoglobulin region and the total area of the densitometric profile (for additional information, see Christe *et al*., 2000). Ig-titre is sensitive to any variation in the blood concentration in Ig but also in other proteins. Ig-titre was normally distributed.

Haematocrit and blood concentration in leucocytes

In 1998, haematocrit and blood counts of leucocytes were assessed in 78 male and 84 female nestlings from 36 broods. These broods were the same as the ones used to assay humoral immunity towards SRBC, HSA, and TT. For each of the 162 nestlings aged 15–45 days (mean 27 days), a blood sample was taken in a capillary tube after puncturing the brachial vein. Blood samples were immediately centrifuged for 10 min at 11 500 r.p.m. The amount of erythrocytes, leucocytes, and plasma was measured with a Vernier calliper to the nearest 0.1 mm, and expressed as a percentage of the total blood sample. Variation in haematocrit vs. blood concentration in leucocytes can be due either to variation in the amount of erythrocytes vs. leucocytes or in blood liquid. To obtain normal distributions, we Box-Cox transformed haematocrit values and square-root transformed leucocyte values.

In 1999, erythrocytes and leucocytes were quantified using a 'Neubauer chamber'. This method allows measurement of number of cells per mL of blood. Fifty-eight male and 55 females from 31 broods were tested at an average age of 56 days (range 47–65 days). We used this method because it allows a more precise quantification of leucocytes than the one used in 1998. The data were Box-Cox transformed to normalize their distribution.

Statistical analyses

All analyses were performed using the statistical package JMP (Sall & Lehman, 1996). Heritabilities (h^2) were computed with the software S-Plus 2000 (MathSoft Inc.). We made three major types of analyses. First, to test whether immune and parasitic parameters were associated with diverse factors, including, for example, year, brood size, nestling body condition, age, and date (number of days to the first of January), we calculated mean nestmate values. In cases where there was significant seasonal variation in immune or parasitic parameters, we tested whether this effect was due to variation in air temperature. Mean daily air temperature (based on 36 measurements) was measured in Payerne located in the centre of our study area. When analysing factors explaining the presence or absence of ticks in barn owl nests, we applied a stepwise backward procedure of a logistic regression with a binomial error distribution. We fitted a full model including all the explanatory variables (i.e. date, year, brood size, and body condition). The significance of variables was tested using the chi-squared distributed change in both deviance and number of degrees of freedom when the variable was dropped from the full model, including significant and nonsignificant terms. Second, to test for sex effects, we compared mean values of male and female nestmates.

Third, to partition variation in immune and ectoparasitic parameters into origin-related and environmental components, we performed nested ANOVAs. The term pair of nests (random effect) accounts for any difference in phenotypic variation among pairs of nests, most likely attributable to seasonal effects. The terms nest of origin (random effect) and nest of rearing (random effect) were nested within pairs of nests. We considered only nests where at least one own and one foreign young survived up to fledging in both nests of the pair. This restriction explains disparities in sample sizes with previous studies. Covariates such as nestling age, body condition, sex, and place in the within-brood age hierarchy were introduced only if they explained a significant part of the variation.

Although the present study is based on data collected over 8 years, field data are homogenous because a single person measured each immune and parasitic parameter. When the data could not be normalized without any transformation, we carried out nonparametric analyses. Standardized regression coefficients β were given when relationships were significant. All statistical analyses are two-tailed and $P < 0.05$ is considered statistically significant. Data are provided as the mean ± SD.

RESULTS

IMMUNOCOMPETENCE

Humoral immune responses

With respect to SRBC, the nontransformed agglutination titres at days 0, 3, 8, 13, and 18 were 1.6, 4.3, 25.0, 21.7, and 13.3, respectively. Therefore, antibody production increased by a factor 15.6 between days 0–8 (paired *t*-test: $t_{37} = 17.03$, $P < 0.0001$). Using mean nestmate values, the amount of antibodies specifically directed against SRBC was larger in older nestlings (four-way ANOVA: $F_{1,33} = 5.97$, $P = 0.02$; $\beta = 0.42$; the relationship is linear, data not shown) and in larger broods ($F_{1,33} = 5.60$, $P = 0.024$; $\beta = 0.33$). In the same model, date ($F_{1,33} = 2.73$, $P = 0.11$) and body condition ($F_{1,33} = 0.007$, $P = 0.93$) did not explain variation in SRBC antibody response. Within nests, male and female nestlings did not differ in the magnitude of the humoral immune response towards SRBC (paired *t*-test: $t_{35} = 0.62$, $P = 0.54$). A nested ANOVA showed that siblings raised in different nests ($h^2 = 0.11$), but that also unrelated nestlings raised in the same nest mounted a similar antibody response (Table 1). Within broods, later-hatched chicks mounted a less intense immune response (Table 1).

The nontransformed HSA-antibody titres at days 0, 3, 8, 13, and 18 were 48, 56, 91, 87, and 63, respectively. Antibody production between day 0 and maximal response at day 8 therefore increased by only 1.9-fold ($t_{37} = 13.37$, $P < 0.0001$). The amount of antibodies specifically directed against HSA increased with age at which this antigen was injected (four-way ANOVA: $F_{1,33} = 4.31$, $P = 0.046$; $\beta = 0.44$; the relationship is linear, data not shown) but was not significantly associated with date ($F_{1,33} = 3.02$, $P = 0.09$), body condition ($F_{1,33} = 0.12$, $P = 0.73$), and brood size ($F_{1,33} = 0.03$, $P = 0.85$). Male and female nestmates mounted a similar immune response towards HSA ($t_{35} = 1.48$, $P = 0.15$). A nested ANOVA revealed a significant effect of the nest of origin on the immune response towards HSA ($h^2 = 0.27$; Table 1). In the same model, rank in the within-brood age hierarchy proved not to be significant and hence was removed.

Unlike antibody responses towards SRBC and HSA showing a peak at day 8, the response towards TT was maximal at day 18 (nontransformed antibody titres at days 0, 3, 8, 13, and 18 were 346, 444, 465, 535, and 598, respectively). The high titre values suggest that there were pre-existing antibodies against TT and, hence, antibody response towards TT may be a recall response. As for HSA, the increase in the amount of antibodies between day 0 and maximal response was low (1.7-fold; $t_{37} = 8.80$, $P < 0.0001$). The antibody response towards TT was not associated with nestling age (four-way ANOVA: $F_{1,33} = 1.67$, $P = 0.21$), date ($F_{1,33} = 0.01$, $P = 0.90$), body condition ($F_{1,33} = 0.07$, $P = 0.79$), and brood size ($F_{1,33} = 0.61$, $P = 0.44$). Within nests, male and female nestlings did not differ in the magnitude of the immune response towards TT ($t_{35} = 0.93$, $P = 0.36$). We did not find evidence that antibody production against TT was related to the factors pair of nests, and the nests of rearing and origin ($h^2 = 0.03$; Table 1). Later-hatched nestlings tended to mount a less intense immune response (Table 1).

T-cell-mediated immune response

In 2002, the mean PHA-response was 1.76 ± 0.55 mm (range 0.18–3.70 mm). Using mean nestmate values, and after controlling for skin thickness measured just before the injection (six-way ANOVA: $F_{1,56} = 6.51$, $P = 0.014$; $\beta = -0.39$), PHA-response increased along the season ($F_{1,56} = 20.55$, $P < 0.0001$; $\beta = 0.51$), was greater in nests in which individuals were in better condition ($F_{1,56} = 3.71$, $P = 0.059$; $\beta = 0.23$) and increased in mass more between the time of PHA-injection and 24 h later ($F_{1,56} = 4.65$, $P = 0.035$; $\beta = 0.24$), but was not associated with age ($F_{1,56} = 1.34$, $P = 0.25$) and brood size ($F_{1,56} = 1.12$, $P = 0.29$). Seasonal effect on PHA-response was not due to an increase in air temperature along the season (two-way ANOVA with PHA-response as dependent variable, date as first independent variable: $F_{1,60} = 14.90$, $P = 0.0003$; $\beta = 0.48$; temperature as second variable: $F_{1,60} = 0.08$, $P = 0.78$). Within nests, male and female nestlings mounted a similar PHA-response ($t_{56} = 0.71$, $P = 0.65$). A nested ANOVA showed that unrelated

Table 1. Mixed-model nested analysis of variance on body condition, immunocompetence, susceptibility to parasites and disease symptoms in barn owl chicks

Parameter (years when measured)	Source of variation	d.f.	*F*	*P*
Body condition (1996, 1998, 2001, 2002)	Pair of nests	85, 497	0.90	0.69
	Nest of rearing (pair of nests)	86, 497	3.93	< 0.0001*
	Nest of origin (pair of nests)	86, 497	1.77	< 0.0001*
	Rank in within-brood age hierarchy	1, 497	30.91	< 0.0001*
Sheep red blood cells (1998)	Pair of nests	18, 105	1.12	0.40
	Nest of rearing (pair of nests)	19, 105	2.07	0.011*
	Nest of origin (pair of nests)	19, 105	2.08	0.01*
	Rank in within-brood age hierarchy	1, 105	17.31	< 0.0001*
Human serum albumin (1998)	Pair of nests	18, 106	1.06	0.47
	Nest of rearing (pair of nests)	19, 106	0.88	0.61
	Nest of origin (pair of nests)	19, 106	1.93	0.019*
Tetanus toxoid (1998)	Pair of nests	18, 105	2.02	0.14
	Nest of rearing (pair of nests)	19, 105	1.22	0.26
	Nest of origin (pair of nests)	19, 105	0.86	0.63
	Rank in within-brood age hierarchy	1, 105	3.60	0.06
Phytohemagglutinin (2002)	Pair of nests	29, 177	1.26	0.28
	Nest of rearing (pair of nests)	30, 177	1.74	0.015*
	Nest of origin (pair of nests)	30, 177	1.32	0.14
	Skin thickness before injection	1, 177	21.32	< 0.0001*
	Rank in within-brood age hierarchy	1, 177	6.11	0.014*
	Body condition	1, 177	4.83	0.029*
Number of *Carnus haemapterus* (1996, 1998, 2001, 2002)	Pair of nests	86, 501	1.23	0.15
	Nest of rearing (pair of nests)	87, 501	6.18	< 0.0001*
	Nest of origin (pair of nests)	87, 501	1.97	< 0.0001*
	Rank in within-brood age hierarchy	1, 501	3.31	0.07
	Nestling age	1, 501	11.31	0.0008*
Clutch size of ectoparasites (1998)	Pair of nests	17, 102	1.91	0.13
	Nest of rearing (pair of nests)	18, 102	1.09	0.38
	Nest of origin (pair of nests)	18, 102	1.53	0.09
	Rank in within-brood age hierarchy	1, 102	7.11	0.01*
Immunoglobulins (2002)	Pair of nests	29, 162	4.48	0.0008*
	Nest of rearing (pair of nests)	30, 162	1.39	0.10
	Nest of origin (pair of nests)	30, 162	1.00	0.47
Hematocrit (1998)	Pair of nests	17, 103	1.42	0.22
	Nest of rearing (pair of nests)	18, 103	3.25	< 0.0001*
	Nest of origin (pair of nests)	18, 103	2.21	0.007*
	Rank in within-brood age hierarchy	1, 103	6.27	0.014*
Leukocyte (1998)	Pair of nests	17, 104	4.64	0.009*
	Nest of rearing (pair of nests)	18, 104	1.80	0.034*
	Nest of origin (pair of nests)	18, 104	0.67	0.83

In these models, the term 'pair of nests' was the main effect, whereas the 'nests of rearing' and 'nests of origin' were nested in the main effect as indicated by parenthesis. Covariates including nestling age, rank in within-brood age hierarchy, body condition, sex, body mass change, and skin thickness were introduced when these effects were known to affect immune or parasitic variables. However, in the final models presented here, we included these covariates only if they explained a significant part of the variation.
Significant values ($P < 0.05$) are indicated by an asterisk.
d.f., degrees of freedom.

individuals raised in the same nest mounted a similar PHA-response, and that later-hatched chicks responded to a lower extent (Table 1). The heritability estimate is 0.008.

In 2003, PHA-response was 1.60 ± 0.45 mm (range 0.60–3.00 mm). Using mean nestmate values, mean PHA-responses measured in 2002 and 2003 were not significantly different from each other (Student's *t*-test:

$t_{102} = 1.58$, $P = 0.12$). Within nests, male and female nestlings mounted a similar PHA-response (paired *t*-test: $t_{33} = 0.43$, $P = 0.57$). The use of mean nestmate values showed that none of the variables introduced in a six-way ANOVA was significant (date: $F_{1,32} = 0.97$, $P = 0.33$; brood size: $F_{1,32} = 0.58$, $P = 0.45$; skin thickness measured just before the injection: $F_{1,32} = 0.08$, $P = 0.78$; age: $F_{1,32} = 0.05$, $P = 0.83$; body condition: $F_{1,32} = 1.12$, $P = 0.30$; body mass change between the time of PHA-injection and 24 h later: $F_{1,32} = 0.14$, $P = 0.71$). Within nests, PHA-response was not associated with hatching ranks ($F_{1,141} = 0.10$, $P = 0.75$ after controlling for nest site: $F_{1,141} = 2.30$, $P = 0.0002$).

Susceptibility to ectoparasites

Number of C. haemapterus

Carnus haemapterus prevalence was high with 318 of 329 nests (97%), with chicks having at least one ectoparasite on their body. Mean number of *C. haemapterus* per nestling per nest was 39 ± 40 (range 0–208). Maximal number of *C. haemapterus* recorded on a single nestling was 383. Parasite intensity differed significantly between years (six-way ANOVA: $F_{6,314} = 3.18$, $P = 0.005$), was greater in nests occupied than unoccupied by a barn owl brood the year before ($F_{1,314} = 12.86$, $P = 0.0004$, $\beta = 0.19$), decreased along the season ($F_{1,314} = 31.84$, $P < 0.0001$, $\beta = -0.32$), tended to decrease with nestlings age ($F_{1,314} = 2.90$, $P = 0.09$, $\beta = -0.10$), but was neither associated with mean nestling body condition ($F_{1,314} = 0.11$, $P = 0.74$) nor with brood size ($F_{1,314} = 1.84$, $P = 0.18$). Seasonal effect on parasite intensity was due both to a decrease in number of flies with date (two-way ANOVA with parasite intensity as dependent variable, date as first independent variable: $F_{1,323} = 65.93$, $P < 0.0001$; $\beta = -0.42$) and to a positive relationship between number of flies and air temperature ($F_{1,323} = 13.88$, $P = 0.0002$; $\beta = 0.19$). Female nestlings harboured more *C. haemapterus* than their male nestmates ($t_{273} = 2.08$, $P = 0.039$; mean: 43.9 vs. 37.5). This result is not inflated by hatching asynchrony, because within nests there was no sex-difference in mean rank in the within brood age hierarchy ($z = 0.48$, $N = 274$, $P = 0.63$). Within nests, parasites were less abundant on earlier-hatched nestlings (four-way ANOVA: $F_{1,1060} = 12.82$, $P = 0.0004$, controlling for nest, sex, and age). In a nested ANOVA, the amount of *C. haemapterus* counted on each nestling was related to the factors pair of nests, nest of rearing and origin ($h^2 = 0.16$) after controlling for age and rank in the within-brood age hierarchy (Table 1).

Clutch size of C. haemapterus

In 1998, *C. haemapterus* laid between 0 and 109 eggs. Considering only females having laid at least one egg, mean number of eggs laid per *C. haemapterus* per nest was 44 ± 7.0 (range 27–55). Mean number of eggs laid by *C. haemapterus* increased with date when parasites were collected (four-way ANOVA: $F_{1,31} = 3.94$, $P = 0.05$; $\beta = 0.35$), but was not associated with mean nestling age at which flies were collected ($F_{1,31} = 0.01$, $P = 0.91$), body condition ($F_{1,31} = 0.0003$, $P = 0.99$), and brood size ($F_{1,31} = 0.48$, $P = 0.49$). Seasonal effect on *C. haemapterus* fecundity was probably due to an increase in air temperature along the season (two-way ANOVA with *C. haemapterus* fecundity as dependent variable, date as first independent variable: $F_{1,33} = 0.04$, $P = 0.84$; temperature as second variable: $F_{1,33} = 7.49$, $P = 0.01$, $\beta = 0.52$). Within nests, fecundity of *C. haemapterus* was not associated with the sex of their host (paired *t*-test: $t_{32} = 0.49$, $P = 0.63$). The terms 'pair of nests', 'nest of origin' ($h^2 = 0$), and 'nest of rearing' did not predict fly fecundity (Table 1) perhaps because flies fed upon several chicks. In this model, *C. haemapterus* laid more eggs when collected on later-hatched nestlings.

In 1999, *C. haemapterus* laid between 0 and 118 eggs. Considering only females having laid at least one egg, mean number of eggs laid per *C. haemapterus* per nest was 42 ± 6.7 (range 14–53). Using mean nestmate values, fecundity of ectoparasites measured in 1998 and 1999 was not significantly different (Student's *t*-test: $t_{69} = 1.38$, $P = 0.17$). Mean number of eggs laid by *C. haemapterus* was not associated with hatching date ($F_{1,27} = 0.04$, $P = 0.84$), brood size ($F_{1,27} = 1.32$, $P = 0.26$), and mean nestling body condition ($F_{1,27} = 0.67$, $P = 0.42$). After controlling for nest (three-way ANOVA, $F_{1,95} = 2.65$, $P = 0.0001$), *C. haemapterus* fecundity was not associated with sex ($F_{1,95} = 0.20$, $P = 0.66$) and rank in the within-brood age hierarchy ($F_{1,95} = 0.93$, $P = 0.34$) of the chicks on which ectoparasites were collected.

Number of European castor-bean tick I. ricinus

One to five ticks were found in 62 out of 330 nests (19%). In nests with ticks, 1.7 ± 1.0 ticks were discovered on average. A logistic regression showed that ticks were more frequently found earlier in the season (ΔDev = 10.07, d.f. = 1, $P = 0.0015$), but year, brood size, and mean nestling body condition did not explain the presence or absence of ticks. Within nests, male nestlings were infested by a similar number of ticks as female nestmates (Wilcoxon matched-pair signed-rank: $z = 1.58$, $N = 41$ nests with at least one tick, $P = 0.12$).

Disease symptoms

Size of the bursa of Fabricius in dead birds

The mean surface of bursa of Fabricius was 106 ± 61.1 mm^2 (ranging 15–315 mm^2). After control-

ling for date (five-way ANOVA: $F_{1,267} = 150.84$, $P < 0.0001$; $\beta = -0.60$; this seasonal effect was revealed to be due to a decline with age) and year ($F_{4,267} = 5.81$, $P = 0.0002$), the size of bursa of Fabricius was greater in heavier individuals ($F_{1,267} = 4.28$, $P = 0.04$; $\beta = 0.10$). In the same statistical model, we did not detect any sex effect ($F_{1,267} = 1.90$, $P = 0.17$) and allometric relationship between this immune organ and bill length ($F_{1,267} = 0.03$, $P = 0.86$).

Spleen size in dead birds

Mean spleen weight was 16 ± 12 g (range 3–79 g). Spleens measured in 1998 and 1999 did not differ in mass (Student's t-test: $t_{93} = 0.90$, $P = 0.37$) and, hence, we did not control for this factor in subsequent statistical analyses. Juveniles had heavier spleens than adults ($t_{103} = 2.29$, $P = 0.024$) and, for this reason, we made separate analyses for these two age classes. In juveniles, spleen weight decreased from the 30 June to the next spring (four-way ANOVA: $F_{1,82} = 12.65$, $P = 0.0006$; $\beta = -0.36$) and was greater in heavier birds ($F_{1,82} = 6.97$, $P = 0.001$; $\beta = 0.29$). In the same model, the factors sex ($F_{1,82} = 0.87$, $P = 0.35$) and bill length ($F_{1,82} = 0.26$, $P = 0.61$) did not explain any significant part of the variation. In adults, spleen weight was not significantly related to sex (three-way ANOVA: $F_{1,11} = 0.97$, $P = 0.35$), body mass ($F_{1,11} = 0.45$, $P = 0.52$), and bill length ($F_{1,11} = 1.10$, $P = 0.32$). Because, in this analysis, the sample size was small ($N = 15$), the results have to be interpreted cautiously.

Proportion of proteins that are immunoglobulins

The mean proportion of serum proteins that were Ig was $6.6 \pm 2.7\%$ (range 0–27%). Among nests, the amount of Ig increased along the season (four-way ANOVA: $F_{1,59} = 24.13$, $P < 0.0001$; $\beta = 0.50$) and was greater when nestlings were in better condition ($F_{1,59} = 12.58$, $P = 0.0008$; $\beta = 0.36$). In the same model, the variables mean nestling age ($F_{1,59} = 0.67$, $P = 0.42$) and brood size ($F_{1,59} = 0.11$, $P = 0.74$) were not significant. Seasonal effect on Ig-production was not due to an increase in air temperature along the season (two-way ANOVA with Ig as dependent variable, date as first independent variable: $F_{1,60} = 26.67$, $P < 0.0001$; $\beta = 0.61$; temperature as second variable: $F_{1,60} = 0.72$, $P = 0.40$). Within nests, the amount of Ig did not differ between the sexes ($t_{56} = 0.09$, $P = 0.93$). In a nested ANOVA, only the factor pair of nests (i.e. seasonal effect) explained a significant part of the variation in Ig (Table 1). The heritability estimate is 0.007.

Haematocrit and blood concentration in leucocytes

In 1998, mean haematocrit and blood concentration in leucocytes were $34.4 \pm 3.0\%$ (range 29.8–41.5%) and $1.40 \pm 0.54\%$ (range 0.45–2.94%), respectively. Haematocrit was lower in nestlings in better condition (four-way ANOVA: $F_{1,31} = 4.62$, $P = 0.04$; $\beta = -0.37$) but was not associated with mean nestling age ($F_{1,31} = 0.18$, $P = 0.67$), date ($F_{1,31} = 0.04$, $P = 0.85$) and brood size ($F_{1,31} = 0.53$, $P = 0.47$). Blood concentration in leucocytes decreased with age (four-way ANOVA: $F_{1,31} = 5.95$, $P = 0.02$; $\beta = -0.40$) and tended to increase with date (ANOVA: $F_{1,31} = 3.33$, $P = 0.078$; $\beta = 0.32$). In the same model, body condition ($F_{1,31} = 0.05$, $P = 0.82$) and brood size ($F_{1,31} = 0.88$, $P = 0.35$) were not significant. Seasonal effect on blood concentration in leucocytes was not due to an increase in air temperature along the season (two-way ANOVA with leucocyte as dependent variable, date as first independent variable: $F_{1,33} = 5.08$, $P = 0.03$, $\beta = 0.41$; temperature as second variable: $F_{1,33} = 1.81$, $P = 0.19$). Male and female nestmates had similar haematocrit ($t_{33} = 1.16$, $P = 0.25$) and blood concentration in leucocytes ($t_{33} = 0.48$, $P = 0.63$). Nested-ANOVAs showed that haematocrit values were significantly related to the nests of rearing and origin ($h^2 = 0.26$), whereas blood concentration in leucocytes was associated with the group of nests (i.e. seasonal effect) and the nest of rearing ($h^2 = 0.09$; Table 1). Within nests, earlier-hatched nestlings had higher haematocrit values (Table 1).

In 1999, using a Neubauer chamber, counts of erythrocytes and leucocytes were $3.76 \pm 0.69 \times 10^{12}$ (range $1.17–9.74 \times 10^{12}$) and $3.5 \pm 0.06 \times 10^9$ cells L^{-1} of blood (range $2.7–5.8 \times 10^9$), respectively. Counts of erythrocytes increased along the season (four-way ANOVA: $F_{1,26} = 22.93$, $P < 0.0001$; $\beta = 0.68$), but were not associated with mean nestling body condition ($F_{1,26} = 0.58$, $P = 0.45$), age ($F_{1,26} = 0.002$, $P = 0.97$), and brood size ($F_{1,26} = 0.59$, $P = 0.45$). Seasonal effect on counts of erythrocytes was not due to an increase in air temperature along the season (two-way ANOVA with erythrocytes as dependent variable, date as first independent variable: $F_{1,28} = 24.37$, $P < 0.0001$, $\beta = 0.68$; temperature as second variable: $F_{1,28} = 0.27$, $P = 0.61$). Blood concentration in leucocytes did not covary with date (four-way ANOVA: $F_{1,26} = 0.03$, $P = 0.86$), mean nestling body condition ($F_{1,26} = 3.23$, $P = 0.08$), age ($F_{1,26} = 0.32$, $P = 0.57$), and brood size ($F_{1,26} = 0.17$, $P = 0.68$). Within nests, there was no sex difference in counts of erythrocytes ($t_{26} = 0.43$, $P = 0.67$). By contrast, male nestlings produced more leucocytes than their female nestmates ($t_{26} = 2.21$, $P = 0.036$). Because male and female nestlings were raised at a similar rank in the within-brood age hierarchy ($t_{26} = 1.22$, $P = 0.23$), the latter result cannot be explained by this factor. Within-brood age hierarchy did not predict blood concentration in erythrocytes (three-way ANOVA, rank hierarchy: $F_{1,80} = 0.38$, $P = 0.54$; nest: $F_{1,80} = 4.60$, $P < 0.0001$; age: $F_{1,80} = 3.02$, $P = 0.09$) but leucocytes with earlier-hatched nestlings producing more cells (two-way ANOVA, rank hierarchy:

$F_{1,80} = 8.13$, $P = 0.006$, $\beta = -0.28$; nest: $F_{1,80} = 1.46$, $P = 0.09$; age: $F_{1,80} = 1.59$, $P = 0.21$).

Covariation between parameters linked to immunocompetence, susceptibility to parasites, and disease symptoms

To correlate immune and ectoparasitic parameters, for each nest we calculated mean values. In 1998, out of 21 correlations only three were significant (Table 2). The relationship between SRBC- and HSA-antibody production remained positively correlated after including age at which nestlings were vaccinated in a two-way ANOVA (SRBC-response as dependent variable, HSA-response as first independent variable: $F_{1,35} = 4.34$, $P = 0.045$; age as second variable: $F_{1,35} = 12.56$, $P = 0.0011$). By contrast, the relationship between HSA- and TT-antibody production was no more significant in a similar model (HSA-response as dependent variable, TT-response as first independent variable: $F_{1,35} = 3.57$, $P = 0.07$; age as second variable: $F_{1,35} = 0.47$, $P = 0.50$). The relationship between mean number of eggs laid by *C. haemapterus* and blood concentration in leucocytes disappeared after including date (number of eggs as dependent variable, leucocytes as first independent variable: $F_{1,33} = 1.10$, $P = 0.30$; date as second variable: $F_{1,33} = 4.80$, $P = 0.036$). In 1999, all six covariations were not significant (Table 3). In 2002, there was a positive correlation between the proportion of proteins that were Ig and PHA-response (Table 4). This relationship was explained by the fact that the two immune parameters increased along the season (quantity of Ig as dependent variable, date as a first independent variable:

Table 2. Covariation between immunological and ectoparasitic parameters in 1998

1998	SRBC-response	HSA-response	TT-response	Hematocrit	Leukocyte	*Carnus haemapterus* fecundity
HSA-response	$r = 0.38$, $N = 38$, $P = 0.018$*					
TT-response	$r = 0.07$, $N = 38$, $P = 0.70$	$r = 0.34$, $N = 38$, $P = 0.03$*				
Hematocrit	$r = 0.21$, $N = 36$, $P = 0.23$	$r = 0.18$, $N = 36$, $P = 0.29$	$r = -0.06$, $N = 36$, $P = 0.71$			
Leukocyte	$r = -0.06$, $N = 36$, $P = 0.72$	$r = 0.04$, $N = 36$, $P = 0.80$	$r = -0.05$, $N = 36$, $P = 0.78$	$r = -0.15$, $N = 36$, $P = 0.38$		
Carnus haemapterus fecundity	$r = -0.19$, $N = 36$, $P = 0.26$	$r = -0.22$, $N = 36$, $P = 0.19$	$r = -0.11$, $N = 36$, $P = 0.53$	$r = -0.14$, $N = 36$, $P = 0.42$	$r = 0.34$, $N = 36$, $P = 0.04$*	
Carnus haemapterus	$r = -0.03$, $N = 38$, $P = 0.85$	$r = 0.01$, $N = 38$, $P = 0.93$	$r = -0.06$, $N = 38$, $P = 0.71$	$r = -0.20$, $N = 36$, $P = 0.23$	$r = 0.04$, $N = 36$, $P = 0.80$	$r = 0.05$, $N = 36$, $P = 0.76$

Pearson correlations are presented. Significant values ($P < 0.05$) are are indicated by an asterisk.
SRBC, sheep red blood cells; HSA, human serum albumin; TT, toxoid toxin.

Table 3. Covariation between immunological and ectoparasitic parameters in 1999

1999	Hematocrit	Leukocyte	Fecundity
Leukocyte	$r = 0.24$, $N = 31$, $P = 0.19$		
Fecundity	$r = 0.02$, $N = 31$, $P = 0.90$	$r = 0.13$, $N = 31$, $P = 0.50$	
Carnus haemapterus	$r = -0.12$, $N = 31$, $P = 0.53$	$r = 0.16$, $N = 31$, $P = 0.40$	$r = 0.12$, $N = 35$, $P = 0.51$

Table 4. Covariation between immunological and ectoparasitic parameters in 2002

2002	*Carnus haemapterus*	Immunoglobulin
Immunoglobulin	$r = -0.15$, $N = 60$, $P = 0.26$	
PHA-response	$r = -0.16$, $N = 60$, $P = 0.23$	$r = 0.35$, $N = 60$, $P = 0.007$*

Pearson correlations are presented. Significant values ($P < 0.05$) are are indicated by an asterisk.
PHA, phytohemagglutinin.

$F_{1,57} = 14.88$, $P = 0.0003$; PHA-response as second variable: $F_{1,57} = 0.86$, $P = 0.36$). In sum, out of 30 covariations, only one (3%) was significant, namely antibody production towards SRBC and HSA.

DISCUSSION

Origin-related variation in immunocompetence, susceptibility to parasites, and disease symptoms

Parasitism is a major ecological force shaping life-history traits of their hosts, promoting the evolution of potent defence mechanisms to resist parasite attacks. Host defence mechanisms are therefore hypothesized to be under intense selection and, consequently, to show low additive genetic variance (Mousseau & Roff, 1987; Price & Schluter, 1991). Even if there is strong evidence that parasites entail a significant reproductive cost to their hosts (Clayton & Moore, 1997), the efficiency of natural selection at depleting genetic variation is likely to be hampered by trade-offs between investment in host defence and other life-history traits (Bonneaud *et al*., 2003), the risk of contracting autoimmune diseases (Råberg *et al*., 1998), and the arm race between hosts and parasites favouring host genetic solutions that reduce the likelihood that a novel parasite offence strategy will spread (Hamilton & Zuk, 1982). A literature survey of cross-fostering experiments carried out in wild birds revealed that, in ten out of 22 cases (45%; Table 5) traits closely linked to host defence mechanisms were significantly heritable. Assuming that maternal effects are weak (but see also Gasparini *et al*., 2001; Soler, Moreno & Potti, 2003; Müller *et al*., 2005), in many cases, genetic variation is maintained, and hence coevolution between parasite offence and host defence is not arrested. From a qualitative point of view, hosts can potentially evolve further towards greater or lower mean levels of antiparasite defence mechanisms. However, we have to keep in mind that nonsignificant studies are likely to be under-represented in our review (Table 5) because researchers tend to publish preferentially significant over nonsignificant results. It is therefore difficult to provide a general statement about the extent to which coevolution between hosts and parasites is a moving process. Moreover, because our results show all but two measures of immunocompetence, susceptibility to parasites and disease symptoms were uncorrelated, implying that a single measurement of immunocompetence may not necessarily reflect host resistance to prevailing parasites. This conclusion pleads for the consideration of as many immune parameters as possible if one wishes to draw a clear picture of host–parasite interactions. The aim of the present study was to make a modest move into this direction.

In the barn owl, we found significant heritabilities in the humoral immune response towards injection of two of three antigens, in haematocrit and number of ectoparasitic flies *C. haemapterus* (Tables 1, 6). By contrast, we did not find any statistical evidence for an origin-related component in the proportion of proteins that were immunoglobulins, in leucocyte blood concentration, PHA-response, and fecundity of *C. haemapterus* (Tables 1, 6). The absence of significant heritabilities in four parameters suggests either that statistical power was not sufficiently high, that genetic variation is maintained for a subset of immune and parasitic parameters, or that significant heritabilities cannot be detected every year depending on specific environmental conditions (Hoffmann & Merilä, 1999). Ideally, cross-fostering experiments should be repeated in several years to gain confidence in whether a particular component of host defence is heritable or not, and under what circumstances. As previously mentioned, we have to keep in mind that nestlings were not all cross-fostered just after hatching implying that cross-fostered and noncross-fostered siblings shared the same nest during a couple of days, which could have inflated our heritability estimates.

Environmental effects on immunocompetence, susceptibility to parasites, and disease symptoms

Condition-dependent effects

Immune responses are reported to be costly (Bonneaud *et al*., 2003; Martin, Scheuerlein & Wikelski, 2003; Schmid-Hempel, 2003), and hence are expected to strongly rely on host condition. Condition-dependent effects on immune and parasitic parameters are usually assessed by introducing in statistical models residuals from the regression of body mass on body size (Brinkhof *et al*., 1999). This method can be problematic for two reasons. First, correlation is not causality because individuals in good condition can be simultaneously immune responsive but for other rea-

Table 5. Review of studies that investigated origin-related variation in immune and parasitic parameters in wild birds via cross-fostering experiments

Parameter	Number of broods	Number of individuals	Statistics	*P*	Species	Author
SRBC-response	38	163	$h^2 = 0.11$	0.01	Barn owl	Present study
HSA-response	38	163	$h^2 = 0.27$	0.02	Barn owl	Present study
TT-response	38	163	$h^2 = 0.03$	0.65	Barn owl	Present study
	28	135	$h^2 = 0.56$	< 0.010	Great tit	Kilpimaa *et al*. (2005)
Diphteria	28	135	$h^2 = 0.79$	< 0.006	Great tit	Kilpimaa *et al*. (2005)
PHA-response	34	288	?	0.004	Barn swallow	Saino *et al.* (1997)
	59	340	$V = 15\%$	< 0.001	Great tit	Brinkhof *et al*. (1999)
	28	135	$h^2 = 0.07$	0.15	Great tit	Kilpimaa *et al*. (2005)
	68	273	$h^2 = 0.007$	0.59	House martin	Christe *et al.* (2000)
	44	131	$V = 4\%$	0.16	American kestrel	Tella *et al*. (2000)
	16	72	$V = 8.73\%$	0.23	Pied flycatcher	Soler *et al.* (2003)
	67	270	$h^2 = 0.008$	0.17	Barn owl	Present study
Number of ectoparasites	48	?	$r = 0.48$	< 0.001	Barn swallow	Møller (1990)
	176	776	$h^2 = 0.16$	< 0.001	Barn owl	Present study
Fecundity of ectoparasites	36	157	$h^2 = 0$	0.10	Barn owl	Present study
Ectoparasite induced change in body condition	59	340	?	0.22	Great tit	Brinkhof *et al*. (1999)
Immunoglobulin	68	273	$h^2 = 0.051$	0.27	House martin	Christe *et al.* (2000)
	59	251	$h^2 = 0.007$	0.34	Barn owl	Present study
Hematocrit	68	273	$h^2 = 0.14$	0.031	House martin	Christe *et al.* (2000)
	36	162	$h^2 = 0.26$	0.017	Barn owl	Present study
Leukocyte	68	273	$h^2 = 0.059$	0.65	House martin	Christe *et al.* (2000)
	36	162	$h^2 = 0.09$	0.85	Barn owl	Present study

SRBC, sheep red blood cells; HSA, human serum albumin; TT, toxoid toxin.; PHA, phytohemagglutinin; *V*, proportion of origin-related variation in immune or parasitic parameters; h^2, heritability, *r*, Pearson correlation coefficient.

sons. To circumvent this problem, experiments were carried out in wild populations using various methods including manipulation of food supply (Alonso-Alvarez & Tella, 2001), the amount of proteins in the diet (Saino *et al.*, 1997), and brood size (Saino *et al.*, 1997; Ilmonen *et al*., 2003). These experiments were able to separate the effect of body condition from other variables that can also influence immunocompetence and hence blur any existing relationship. In the barn owl, the only experiment (i.e brood size manipulation) that significantly altered nestling body mass did not affect the number of *C. haemapterus* per nestling (Roulin, 1998). This experiment indicates that body condition plays no or a weak role in parasite counts. Second, the absence of any relationship between residual body mass and immunocompetence does not necessarily imply that body condition is a minor factor. Indeed, we found that later-hatched nestlings mounted a lower immune response towards SRBC, and parasites were more abundant and more fecund on later-hatched nestlings. There might be a threshold above which increase in food intake and body mass does not further enhance host defence mechanisms (Christe *et al*., 2003). The hypothesis of such a threshold may explain why body condition was associated with only five out of 16 measurements (i.e. PHA-response, bursa of Fabricius, spleen, proportion of proteins that were Ig, and haematocrit; Table 6). More experiments are needed to further investigate the role of body condition in immune and parasitic parameters.

Seasonal effects

Seasonal changes in the magnitude of host defence mechanisms, and the severity in parasite attacks are predicted to covary because peak in host reproduction should coincide with peak in parasitism (Christe, Arlettaz & Vogel, 2000; Møller, Erritzøe & Saino, 2003). However, it is difficult to provide a clear prediction regarding temporal variation in immunocompetence, parasite intensity, and disease symptoms. Indeed, different patterns of seasonal changes can occur depending on a plethora of factors. For example, parasite lifestyle, life history, and virulence are crucial parameters with different species of parasites being

Table 6. Summary of the results reported in the present study

Immune or parasitic paramete (year when measured)	Year	Season	Age	Rank	Brood size	Body condition	Sex	Heritability	Rearing nest
SRBC-response (1998)	?	No	+	–	+	No	No	Yes	Yes
HSA-response (1998)	?	No	+	No	No	No	No	Yes	No
TT-response (1998)	?	No	No	No	No	No	No	No	No
PHA-response (2002)	No	+	No	–	No	+	No	No	Yes
PHA-response (2003)		No	No	No	No	No	No	?	?
Number of *Carnus haemapterus* (1996, 1998, 2001, 2002)	Yes	–	–	+	No	No	Yes	Yes	Yes
Fecundity of *Carnus haemapterus* (1998)	No	+	No	+	No	No	No	No	No
Fecundity of *Carnus haemapterus* (1999)		No	?	No	No	No	No	?	?
Number of tick (1996–2002)	No	–	?	?	No	No	No	?	?
Bursa of Fabricius (1996–2001)	Yes	?	–	?	?	+	No	?	?
Spleen (1996–2000)	No	?	–	?	?	+	No	?	?
Immunoglobulin (2002)	?	+	No	No	No	+	No	No	No
Hematocrit (1998)	?	No	No	–	No	–	No	Yes	Yes
Hematocrit (1999)		+	No	No	No	No	No	?	?
Leukocyte (1998)	?	+	–	No	No	No	No	No	Yes
Leukocyte (1999)		No	No	–	No	No	Yes	?	?

No, no association was found with this factor on immune or parasitic parameter; Yes, an association was significant; +, positively correlated; –, negatively correlated; ?, factor not investigated. Rank denotes 'rank in within-brood age hierarchy'. Absence of an effect of hatching asynchrony on number of ticks was determined by Roulin *et al*. (2003).
SRBC, sheep red blood cells; HSA, human serum albumin; TT, toxoid toxin; PHA, phytohemagglutinin.

more abundant early or late in the season, either because they exploit alternative ecological niches or because they produce a different number of generations within a single season. Seasonal changes in humoral and T-cell mediated immune responses may also occur because they are triggered by parasites, and hence should closely mirror temporal variation in the degree of parasite offence (Christe *et al*., 2001). Furthermore, key ecological factors influencing population dynamics of hosts and parasites can vary from one year to another, generating variation in seasonal changes both in host immunocompetence, abundance, and virulence of parasites. Finally, seasonal changes in immunocompetence and the general ability to resist parasites may take place if high quality individuals can afford to be in sufficient good condition to breed before parasites are abundant.

The present study shows that, although the abundance of the most prevalent ectoparasites *C. haemapterus* and *I. ricinus* decreased along the breeding season, temporal variation in immunocompetence, fecundity of *C. haemapterus* and disease symptoms varied from one year to the next. We found no date effect on antibody responses, whereas PHA-response, the proportion of proteins that were Ig, and blood concentration in leucocytes increased with date (Table 6). The finding that PHA-response, haematocrit, and fecundity of ectoparasites were associated with date in only one of 2 years shows that seasonal effects on immune and parasitic parameters can vary annually if the environmental factors that generate such seasonal changes are not stable across years. This is the case with respect to air temperature, a factor that explained variation in *C. haemapterus* fecundity but not in PHA-response (but see also Lifjeld, Dunn & Whittingham, 2002), the proportion of proteins that are Ig, haematocrit, and blood concentration in leucocytes.

Sex effects on immunocompetence, susceptibility to parasites, and disease symptoms

From a proximate point of view, sex-differences in immunocompetence and susceptibility to pathogens should appear whenever males and females differ in the circulation of sex-specific hormones, such as testosterone, that impair the ability to defend themselves against pathogens and parasites (Moore & Wilson, 2002). Therefore, in monogamous species in which sex-difference in immunosuppressive hormones can be weak, males and females may be equally immunocompetent (Zuk & McKean, 1996), or females may even be more susceptible to pathogens and parasites (Bize *et al*., 2005). In the barn owl, male and female nestlings do not differ in the amount of circulating testosterone (Roulin *et al*., 2004). It is therefore difficult to predict which sex should be more susceptible to

parasites. Accordingly, among 12 measurements, we only found that females were more infested by *C. haemapterus* than males and that, in one year, blood concentration in leucocytes was greater in males (Table 6). Male and female nestlings may therefore be susceptible to different parasites and pathogens or, alternatively, they may show different levels of susceptibility depending on specific environmental conditions.

CONCLUSIONS

The use of several immune and parasitic parameters measured in several years reveals that studies performed on a single parameter measured in a single year may oversimplify our understanding of host–parasite interactions. We hope that the present paper will stimulate researchers to carry out long-term studies of host–parasite interactions and to publish significant and nonsignificant results.

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