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Interactions between a brood parasite and its host in relation to parasitism and immune defence

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ABSTRACT

Nestlings of many brood parasites are reared together with those of their hosts, but still manage to monopolize a disproportionate share of the food delivered by adult hosts. We hypothesized that: (1) the low levels of host-specific pathogens of such brood parasite nestlings provide them with an advantage in competition for limited food; (2) a higher provisioning rate and the resulting better body condition of brood-parasite nestlings compared with that of host nestlings may increase the efficiency of their immune defence; (3) brood parasites may decrease the risk of parasitism and improve the immune response level of their offpring by selecting hosts with a more efficient immune defence (low pathogens load) and by selecting high-quality hosts, respectively. This effect should provide brood-parasite nestlings with an advantage in their competition with host nestlings. This hypothesis was tested in the magpie *Pica pica*, which is the main European host of the great spotted cuckoo *Clamator glandarius*. Cuckoo nestlings are reared together with magpie nestlings, but the former usually manage to outcompete the latter. The results of our experiment were as follows. Magpie and great spotted cuckoo nestlings had similar prevalence of a generalist, directly transmitted haematophagous Diptera of the family Carnidae. Magpie nestlings had considerably higher prevalence and intensity of a haematozoan parasite of the genus *Leucocytozoon* than nestlings of the great spotted cuckoo. Great spotted cuckoo nestlings had greater immune responses, measured in terms of T-cell response to an injection with phytohaemagglutinin, sedimentation rate of erythrocytes and colour of the plasma than magpie nestlings. Heavier magpie nestlings had greater immune responses than lighter conspecifics. We found no support for the third hypothesis, however. Therefore, brood-parasite nestlings may outcompete host nestlings because of pathogen specificity, and because of their efficient immune system mediated by their higher rate of food intake.

Keywords: brood parasitism, *Clamator glandarius*, competition, haematocrit, leukocytes, *Leucocytozoon*, *Pica pica*, T-cell response.

INTRODUCTION

Parasite infections are widespread owing to the ubiquity of parasites; more than half of all organisms are estimated to be parasites in one way or another (Price, 1980). Host

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individuals with a more efficient immune response are expected to enjoy higher reproductive success and survival (fitness) compared with individuals with a less efficient immune response (e.g. Zuk, 1994). Conversely, parasites have the potential of imposing severe selective pressures on their hosts (Haldane, 1949; Clarke, 1979; Hamilton, 1980; Price, 1980; Møller *et al.*, 1990; Lehmann, 1993; Møller, 1996), and are thus likely to be important in ecological and evolutionary processes.

Recently, the immune system has been suggested to play an important mediating role in host–parasite interactions because individuals with superior immune responses may gain an advantage over parasites (Zuk, 1994). For example, the efficiency of the immune system may significantly influence host sexual selection (Møller and Saino, 1994; Zuk, 1994), and females choosing males with well-developed ornaments may obtain genes of parasite resistance for their offspring (Hamilton and Zuk, 1982; Hamilton, 1990; Zuk, 1991).

An efficient immune defence is beneficial, although energetically costly. Defence costs can be inferred indirectly by the increased virulence of parasites when their hosts undergo physiological stress (de Lope *et al.*, 1993). Short-term defence costs include energy and resources (de Lope *et al.*, 1993; Møller and Saino, 1994; Ho *et al.*, 1995; König and Schmid-Hempel, 1995; Wei *et al.*, 1995; Saino and Møller, 1996), while long-term evolutionary costs involve antagonistic pleiotropic effects that reduce fitness as a result of parasite resistance (Minchella and LoVerde, 1983; Lenski, 1988).

Avian brood parasitism is a reproductive strategy arising from individuals of one species (the brood parasite) laying eggs in the nests of individuals of another species (the host), the host then caring for the offspring of the brood parasite. Aspects of brood parasitism that have received scientific attention include host responses to brood parasitism (e.g. evolution of brood parasite egg and chick recognition, nest defence by hosts against brood parasites, recognition of adult brood parasites) and parasite responses to host defences (e.g. egg and nestling mimicry, adult mimicry of potential host predators; for reviews, see Payne, 1977; Rothstein, 1990). A number of physiological characteristics of brood parasites provide them with an advantage over hosts: a reduction in the duration of laying (Brooker and Brooker, 1991; Sealy *et al.*, 1995), a reduction in the duration of the incubation period (Briskie and Sealy, 1990; Soler and Soler, 1991), a faster growth rate than that of host nestlings (Soler and Soler, 1991), and a thicker eggshell than that of the host (Spaw and Rohwer, 1987; Rahn *et al.*, 1988; Brooker and Brooker, 1989, 1991; Soler, 1990).

A rather neglected aspect of the interaction between brood parasites and their hosts concerns the possible differential susceptibility to parasites. Brood parasites may enjoy an advantage over hosts in terms of resistance to parasites because host species generally differ in immune function (van Riper *et al.*, 1986). The immune response can be influenced by either genetic or phenotypic factors, such as nutrition; in this regard, differences between brood parasites and their hosts may have important ecological and evolutionary implications. There are several reasons why brood parasites may benefit from having their offpring raised by another species:

Hypothesis 1: Nestling brood parasites experience restricted vertical transmission of pathogens because brood-parasite nestlings have little or no contact with adult brood parasites, at least compared with other bird species (Fig. 1) (Payne, 1977; Rothstein, 1990). In addition, many pathogens are highly host-specific (Brooks and McLennan, 1993), largely freeing brood-parasite nestlings from the transmission of specialist pathogens (Fig. 1). Although an absence of selection for a well-developed immune response in brood-parasite

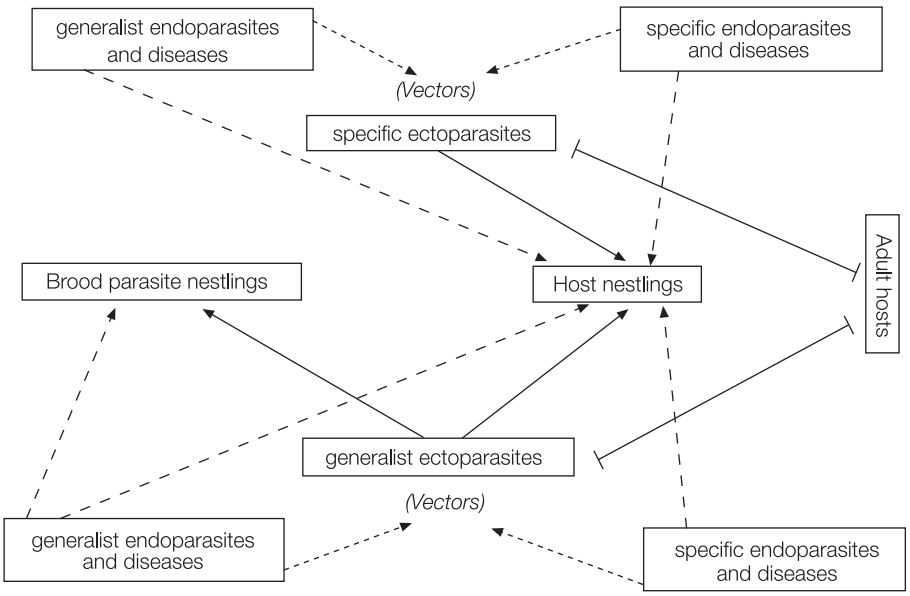

Fig. 1. Risk of direct transmission of pathogens in host and brood-parasite nestlings. Solid arrows represent direct transmission from adult hosts; dotted arrows represent transmission mediated by ectoparasite vectors. Bars represent ectoparasites on adult hosts.

nestlings – because the immune system is developed early in life – and its proper early development is critical for the immune system of the adult brood parasite (Kramer and Good, 1978), we would not expect an immune system of the brood-parasite nestling that is weaker than that of the host. Therefore, we predict a reduced prevalence and intensity of host-specific pathogens in brood parasite nestlings compared with those of the host (prediction 1a), while we predict no difference between brood parasite and host nestlings with respect to intensity of generalist pathogens (prediction 1b).

Hypothesis 2: A protein-rich diet boosts the efficiency of the immune response in many organisms, including birds (Chandra and Newberne, 1977; Gershwin *et al*., 1985), and some passerine species, although feeding on vegetable matter, provide their nestlings with insects. Cuckoos, honeyguides and cowbirds parasitize hosts that feed insects to their nestlings (Payne, 1977); this feeding habit is a very important determinant of host selection (Soler *et al*., in press, provide information on host selection by the European cuckoo, *Cuculus canorus*). When host and parasite nestlings are reared in the same nest and thus compete for food, brood-parasite nestlings are preferentially fed over those of the host (e.g. great spotted cuckoo, *Clamator glandarius*: M. Soler *et al.*, 1995; brown-headed cowbird, *Molothrus ater*: Payne, 1977). On the other hand, when brood-parasite nestlings evict host nestlings, and are therefore reared alone in the nest, they receive all parental care from their foster parent while host nestlings have to share parental care with siblings in unparasitized nests. Thus, this should result in the largest amount of food being received by brood-parasite nestlings compared with what is received by host nestlings. This difference in food intake between host and brood-parasite nestlings could seriously affect the efficiency of their immune system. Brood parasites may have a more efficient immune response than their

hosts due to their receipt of more parental care from their foster parents than their hosts. Therefore, we should expect a stronger immune response in brood-parasite fledglings than host fledglings (prediction 2).

Hypothesis 3: Brood parasites may decrease the risk of parasitism (by generalist pathogens) by selecting hosts with a more efficient immune defence and hence a low pathogen load. Ornaments and other secondary sexual displays may reveal the ability of individual hosts to resist pathogens, characteristics that have been shown to be important in mate choice (Møller and Saino, 1994). Brood parasites could use the same traits that females use as cues in mate choice to select a suitable host, and could thereby decrease the likelihood of their offspring being infected by generalist pathogens. Moreover, brood parasites could also select hosts based on their territory or parental quality, which are likely to be related to nest size in passerines (Soler *et al.*, 1998). Such host selection would result in better fed nestlings compared with those reared in randomly chosen host nests. Therefore, we predict that nestlings reared in nests selected by the cuckoo (parasitized nests) should suffer less from pathogens (prediction 3a) and have a stronger immune response than those from non-selected nests (prediction 3b).

The ecological and evolutionary importance of a reduced prevalence and intensity of pathogens in brood parasites has never been considered as a potential advantage for brood parasites in their interactions with hosts. Similarly, an improved immune response in brood parasites compared with that of the host has never been emphasized. In this study, we tested these ideas concerning parasitism of brood parasites and their hosts and examined the importance of differences in immune responses of hosts and brood parasites that share the same nest environment. This was done by using the great spotted cuckoo and its magpie (*Pica pica*) host as a model system. Great spotted cuckoo nestlings are reared together with host nestlings and, therefore, many of the ecological factors affecting development of the immune system, such as parent feeding ability, number of nestlings and food availability, were automatically controlled.

MATERIALS AND METHODS

Study area

The population of brood parasites and hosts is located in Hoya de Guadix, southern Spain (37°18′N, 3°11′W), a cereal-producing, high-altitude plain, approximately 1000 m above sea level. The vegetation is scarce with many groves of almond trees (*Prunus dulcis*) in which magpies nest at high density. A detailed description of the study area and the cuckoo and magpie populations is given in Soler (1990).

Natural history of the brood parasite and its host

We studied the brood-parasitic great spotted cuckoo and its main European host, the magpie, because: (1) Neither adult great spotted cuckoos nor nestlings completely destroy or eject host eggs or nestlings, and therefore magpie and great spotted cuckoo nestlings are reared together in the same nest (Cramp, 1985; Soler, 1990). (2) During this period, host and parasite nestlings compete for food, with brood-parasite nestlings

outcompeting host nestlings. Superior food monopolization by the brood-parasite offspring may strongly enhance the development of an efficient immune system (Chandra and Newberne, 1977; Gershwin *et al.*, 1985). (3) Brood parasites develop more rapidly than their magpie hosts, both during the incubation (Soler, 1990) and the nestling period (Soler and Soler, 1991). Moreover, in this host–parasite system, it has been shown that the great spotted cuckoo selects magpie hosts according to nest size, which is related to territory quality (J.J. Soler *et al.*, 1995).

Adult magpies preferentially allocate food to great spotted cuckoo nestlings rather than their own nestlings (M. Soler *et al.*, 1995). Great spotted cuckoo nestlings leave the nest when 16 days old and magpie nestlings when 21 days old (Soler and Soler, 1991).

General methods

We experimentally eliminated the great spotted cuckoo's advantage of hatching earlier than the magpie by cross-fostering cuckoo eggs and synchronizing the hatching of cuckoo and magpie eggs. In this way, we reduced the possible confounding factors of differential hatching time and growth rate of great spotted cuckoos and magpies. These interspecific differences in developmental rate could greatly sway the outcome of interspecific competition for food and therefore for the development of an efficient immune system (Chandra and Newberne, 1977; Gershwin *et al.*, 1985).

Assessment of parasite loads of cuckoo and magpie nestlings

Great spotted cuckoo and magpie nestlings were parasitized by a generalist haematophagous fly (Diptera: Carnidae), which can seriously damage their hosts in terms of blood loss (J.J. Soler, personal observation). Although both great spotted cuckoo and magpie nestlings are also parasitized by other Diptera, such as hippoboscid flies, and mallophagans, we studied the carnid fly because it is easily detectable – adult flies lose their wings after reaching a host and produce highly conspicuous haematomas and dry blood spots. We recorded the presence of this parasite among great spotted cuckoo and magpie nestlings in experimentally manipulated nests in 1994 by inspecting the skin of great spotted cuckoo and magpie nestlings when 13 and 19 days old, respectively. In 1995, we also examined magpie nestlings when 13 days old.

During 1994, we recorded haematozoan prevalence and intensity as a measure of internal parasites. We took one blood smear from the brachial vein of all great spotted cuckoo and magpie nestlings when 13 and 19 days old, respectively. This smear was subsequently air-dried and fixed in absolute ethanol for 3 min. The blood smears were then sent to the International Reference Centre for Avian Haematozoa (Memorial University of Newfoundland, Canada), where G.F. Bennett investigated the smears for haematozoa. Blood smears were dyed with a Giemsa stain and subsequently investigated for protozoan parasites using oil-immersion. The intensity of parasitism was estimated by counting the number of parasites in 100 microscopic fields.

Measurement of immune responses

The immune system of birds consists of three major components: phagocytosis, T-cell-mediated immunity and antibody responses (Cheng and Lamont, 1988). Genetic control of

these three components of the immune system appears to be independent (Cheng and Lamont, 1988). In combination, these facets of immunity enable an individual to resist parasite infection.

In this study, we analysed only the T-cell-mediated immune response, while indirect information was collected for the other two components. We estimated immune responses when the oldest magpie nestling in magpie nests with or without great spotted cuckoo nestlings was 13 ± 2 days old. The following measurements were recorded:

1. *T-cell-mediated immunity*. We used an injection of phytohaemagglutinin-P (PHA-P) to evaluate the *in-vivo* T-cell-mediated immune response of great spotted cuckoo and magpie nestlings following the protocol described by Lochmiller *et al.* (1993). Nestlings were injected intradermically in the centre of the right wing patagial with 0.5 mg of PHA-P (Sigma Chemical Co., St. Louis, MO) in 0.1 ml of saline solution (Bausch and Lomb Co.). The left wing patagial was used as a control by injecting 0.1 ml of saline solution. The thickness of each wing patagial was measured with a digital calliper (to the nearest 0.01 mm) at the injection site before and 24 ± 2 h post-injection. The cell-mediated immune response was simply estimated as the change in thickness of the right wing patagial from the day of injection with PHA-P until the following day minus the change in thickness of the left wing patagial from the day of injection with saline water until the following day. We usually measured the thickness of the patagial of the same wing three times and we used the mean value in the subsequent analyses. These repeated measurements allowed us to assess repeatability (Becker, 1984; Falconer, 1989) of patagial thickness (Table 1).

To avoid the potentially confounding influences of age differences, the number of nestlings in the nest and nestling body mass, we first ran a multiple-regression model with T-cell response as the dependent variable, and nestling age, number of nestlings and nestling mass as independent variables (Table 2). The corrected T-cell response was simply the residual from this regression model for nestlings and for the mean values of nestlings in a nest. When we tested the differences between brood-parasite and host nestlings reared in the same nest, we used absolute values of the T-cell response because the three independent variables were almost identical (number of nestlings, to some extent nestling age), or they were the result of host competition with the brood parasite (to some extent nestling age, nestling body mass).

Table 1. Repeatability of immune response variables between measurements (T-cell response) or capillary tubes (other responses)

Measurement	No. of measurements	Repeatability	*F*	d.f.	*P*
T-cell response	3	0.94	51.63	100,202	<0.0001
Sedimentation rate	2	0.94	31.69	57,58	<0.0001
Red cells	2	0.88	16.14	49,50	<0.0001
White cells	2	0.87	14.02	49,50	<0.0001
Plasma	2	0.88	16.13	49,50	<0.0001
Plasma colour	2	0.90	18.85	62,63	<0.0001

Table 2. Multiple-regression analysis for magpie and great spotted cuckoo nestlings between T-cell response (dependent variable) and the number of nestlings (magpie and great spotted cuckoo nestlings), the age of nestlings and their body mass (independent variables)

	Magpie nestlings				Great spotted cuckoo nestlings			
	r	Tests	d.f.	*P*	*r*	Tests	d.f.	*P*
Nestlings as observations								
Model	0.24	$F = 1.51$	3,71	0.22	0.30	$F = 0.78$	3,23	0.52
No. of nestlings	–0.04	$t = 0.33$	72	0.74	–0.17	$t = 0.78$	23	0.45
Age	0.00	$t = 0.04$	72	0.96	–0.12	$t = 0.57$	23	0.57
Body mass	–0.20	$t = 1.68$	72	0.10	–0.26	$t = 1.13$	23	0.27
Mean values per nest								
Model	0.34	$F = 1.07$	3,24	0.38	0.23	$F = 0.27$	3,15	0.84
No. of nestlings	–0.09	$t = 0.46$	25	0.65	–0.13	$t = 0.49$	16	0.63
Age	–0.09	$t = 0.43$	25	0.67	–0.20	$t = 0.79$	16	0.44
Body mass	–0.20	$t = 0.98$	25	0.34	–0.02	$t = 0.07$	16	0.95

We also measured the degree of erythema of the patagial at the point of injection by subjectively assigning a numerical score as described by Kramer and Good (1978), where 0 = none, 1 = slight, 2 = moderate and 3 = intense. This was also done 24 h post-injection.

2. *Sedimentation rate of red blood cells* was estimated from a blood sample drawn from the brachial vein into a capillary tube when the nestlings were 13 days old and injected with PHA-P. Capillaries were placed vertically for 2.5 ± 0.5 h in a portable refrigerator, and the length of the capillary containing plasma and total length of the capillary were measured under a magnifying glass (to the nearest 0.1 mm). At the same time, we usually took two blood samples to calculate the repeatability of the trait. Sedimentation rate was calculated as the length of the capillary tube containing blood plasma. Since the sedimentation rate is slower when the blood contains more erythrocytes, we regressed the sedimentation rate on haematocrit (see below) and used residuals from this regression model as a direct estimate of relative sedimentation rate corrected for the amount of red blood cells. The repeatability of the sedimentation rate was statistically significant (Table 1). Variation in sedimentation rate is due to any humoral disequilibrium affecting plasma proteins. Sedimentation rate, being slower when the blood contains a larger amount of fibrinogen or globulins (Bacells, 1978), can be used as an indirect index of the amount of immunoglobulins in the blood.

3. *Haematocrit*. After measurement of the sedimentation rate, the heparinized micro-capillary tubes (32 mm, 9 μl) were centrifuged in a portable haematocrit centrifuge (Ames Microspin 6500-200) for 3 min, and the volumes of plasma and red and white blood cells were measured to the nearest 0.1 mm. The repeatability of these measurements was shown to be statistically significant (Table 1). The haematocrit was used as an index of possible anaemic diseases.

4. *Plasma colour*. After centrifugation of the micro-capillary tubes, we estimated the yellow colour of the plasma according to a colour atlas (Küppers, 1979). We quantitatively scored the hue of the yellow colour of the plasma using p. 45, column A00 in Küppers (1979). The colour of the plasma was used as an index of the amount of immunoglobulins in the blood (Gustafsson *et al*., 1994).

Experimental manipulation of brood parasitism

Great spotted cuckoos are known to select magpies of superior phenotypic quality as hosts (J.J. Soler *et al*., 1995). To determine whether any differences in parasitism rate and immune defence occurred between selected and not-selected hosts, we experimentally parasitized non-selected magpie nests and removed great spotted cuckoo eggs from parasitized nests during the incubation period. These treatments were randomly assigned to nests and other parasitized and non-parasitized magpie nests were kept as controls. Thus, we had four different treatments: (1) parasitized control nests (PC), (2) experimentally parasitized control nests (PE), (3) non-parasitized control nests (NC) and (4) experimentally non-parasitized nests (NE). All nests were manipulated and, if possible, we kept the number of eggs constant at four (four magpie eggs in non-parasitized nests, or two magpie and two great spotted cuckoo eggs in parasitized nests). Most of the experimental and control nests with cuckoo nestlings were manipulated to reduce hatching asynchrony and thereby increase the probability of survival of magpie and cuckoo chicks used for the immune response tests, the manipulations being dependent on the availability of other magpie nests with similar laying date. Nevertheless, some magpie nestlings died before the measurement of immune responses. Magpie nestlings survived in only one of 11 control parasitized nests until the immunological tests were performed (2 magpie and 15 great spotted cuckoo nestlings). We checked 10 experimentally parasitized nests, of which 7 contained both species when we measured the immune response (6 magpie and 15 great spotted cuckoo nestlings). We tested the immune response in 15 non-parasitized control nests containing 45 magpie nestlings and in 5 experimentally non-parasitized magpie nests containing 15 magpie chicks.

Statistical analysis

All continuous variables used in the analyses were normally distributed (Lilliefors' test, $P > 0.2$), and we followed Sokal and Rohlf (1981) for the statistical tests. When sample sizes were small, or variables were discrete, we used non-parametric tests following Siegel and Castellan (1988). The values are reported as the mean ± standard error (S.E.).

Magpie and cuckoo nestlings sharing the same nest cannot be considered statistically independent observations. However, most nestlings were experimentally exchanged between different nests and, therefore, the genetic similarity between nestlings as well as between nestlings and foster parents was greatly reduced. Therefore, we performed our calculations in two ways: (1) using each nestling as an independent observation and (2) using mean values of the nests as independent observations.

RESULTS

Parasite loads of cuckoo and host nestlings

Haematozoa loads

We analysed only Haematozoa from the blood smears taken in 1994. Haematozoa were found in 28.0% of the magpie nestlings ($n = 82$), while only one great spotted cuckoo nestling (4.6%) appeared to be infected ($n = 22$). In line with prediction 1a (hypothesis 1), this resulted in a highly significant difference in the probability of infection of brood-parasite and host nestlings ($G^2 = 6.91$, d.f. = 1, $P = 0.0086$). This difference was even more marked when we took into account that the Haematozoa found in magpie nestlings belonged to the species *Leucocytozoon sakharoffi* (intensity: 53.6 ± 15.0 Haematozoa per microscope field per individual host, $n = 23$) and those found in the brood-parasite nestlings were *L. centropi* (only one cuckoo nestling infected with five haematozoan parasites). Although this result cannot be evaluated without knowing the life cycles of the haematozoan species, the difference is remarkable.

An analysis based on nests as statistically independent observations gave a similar result, with 42.3% ($n = 26$) of the nests with magpie nestlings being infected, while only 5.6% ($n = 18$) of the nests containing great spotted cuckoo nestlings were infected ($G^2 = 8.41$, d.f. = 1, $P = 0.0037$).

We also analysed the possible relationship between the probability of any of the magpie nestlings being infected and the number of nestlings in the nest and nestling body mass, respectively. However, neither brood size nor body mass was able to explain a significant proportion of the variance ($n = 26$, brood size: maximum likelihood chi-square = 0.76, d.f. = 1, $P = 0.38$; nestling body mass: maximum likelihood chi-square = 2.90, d.f. = 1, $P = 0.09$).

Ectoparasite loads

In agreement with prediction 1b (hypothesis 1), we did not find a statistically significant difference between the percentage of cuckoo and magpie nestlings with generalist haematophagous Diptera when both bird species were of the same age (1995: 16.9% of 77 magpie nestlings and 13.3% of 30 great spotted cuckoo nestlings had ectoparasites; $G^2 = 0.21$, d.f. = 1, $P > 0.60$). These differences were also non-significant when comparing individuals with similar feather development (great spotted cuckoo nestlings 13 days old with magpies 19 days old) in both years (in 1994, 14.6% of 82 magpie nestlings and 13.0% of 23 great spotted cuckoo nestlings had ectoparasites: $G^2 = 0.04$, d.f. = 1, $P > 0.80$; in 1995, 26.4% of 53 magpie nestlings and 13.3% of 30 great spotted cuckoo nestlings had ectoparasites: $G^2 = 2.04$, d.f. = 1, $P > 0.15$).

A common mode of ectoparasite transmission in host species with parental care is vertical transmission from adults to nestlings (Marshall, 1981), and the infestation of individual nestlings is thus not independent of the nest in which they are reared. Therefore, we analysed the possible differences between magpie and great spotted cuckoo nestlings reared in the same nest. In 1994, 4 of 11 nests contained both species of birds, in 3 of which the brood-parasite nestlings were infested. We obtained similar results in 1995: in two of eight nests with both species of birds, some of the nestlings were infested with haematophagous Diptera; brood-parasite and host nestlings were

infested in two nests. Therefore, the difference in Diptera prevalence was not significantly different when data from the 2 years were pooled (chi-square = 0.27, d.f. = 1, $P > 0.60$).

The probability of a nest being infested by ectoparasites could be affected by brood size. However, a logistic regression revealed that, in 1994, the number of nestlings explained only 3.0% of the variation in ectoparasite prevalence at the nest level ($n = 34$, maximum likelihood chi-square = 1.50, d.f. = 1, $P = 0.22$). In 1995, brood size explained 13.1% of the variance in whether a nest was infested with ectoparasites ($n = 28$, maximum likelihood chi-square = 1.98, d.f. = 1, $P = 0.16$). Hence, brood size was not a key determinant of ectoparasite infestation.

Immune defence of cuckoo and magpie nestlings

T-cell immune responses

In accordance with the second prediction (hypothesis 2), there was a highly significant difference in the intensity of the relative T-cell immune response corrected for nestling age, number of nestlings and nestling body mass between great spotted cuckoo and magpie nestlings, with cuckoos having a higher level of immune response ($t_{100} = 4.43$, $P = 0.000024$; Table 3). A similar conclusion was reached when we used the mean response for each species reared in the same nest (difference in thickness of the skin of magpie nestlings: 0.792 ± 0.07 mm; difference in thickness of the skin of great spotted cuckoo nestlings: 1.193 ± 0.12 mm; Wilcoxon matched pairs test, $n = 7$, $z = 2.36$, $P = 0.018$).

The degree of erythema as a response to the injection with phytohaemagglutinin-P in the patagial was also greater in great spotted cuckoo nestlings than in magpie nestlings (Mann-Whitney *U*-test, $z = 3.04$, $P = 0.002$; Table 3). However, this difference was not statistically significant when we compared the responses of nestlings of the two species reared in the same nest (magpie nestlings: 1.32 ± 0.18; great spotted cuckoo nestlings: 1.57 ± 0.13; Wilcoxon matched pairs test, $n = 7$, $z = 0.94$, $P = 0.35$).

Table 3. Immune responses of magpie and great spotted cuckoo nestlings[a]

	Magpie		Great spotted cuckoo	
	Mean ± S.E.	*n*	Mean ± S.E.	*n*
T-cell immune response	–10.22 ± 4.24	75	28.38 ± 8.52	27
Degree of erythema	1.19 ± 0.06	75	1.52 ± 0.08	27
Sedimentation rate	1.72 ± 1.07	75	–4.48 ± 0.82	27
Plasma colour	45.14 ± 0.99	75	52.80 ± 1.58	27

[a] T-cell immune response values are differences in relative skin thickness between the right and left wing following injection with phytohaemagglutinin-P after controlling for the number of nestlings, nestling age and the mean body mass of nestlings (see 'Materials and methods' for further details). Degree of erythema values are differences in erythema between the right and left wing following injection with phytoheamagglutinin-P (erythema was scored on a 4-point scale according to Kramer and Good, 1978). Values of relative sedimentation rate of erythrocytes in blood samples are corrected for heamatocrit. Values of colour of the plasma were estimated from a colour atlas (Küppers, 1979).

Sedimentation rate

The sedimentation rate of red blood cells was faster in magpie nestlings than in great spotted cuckoo nestlings ($t_{101} = 3.50$, $P = 0.00069$; Table 3). This conclusion was upheld using the mean response for each species reared in the same nest (magpie nestlings: 22.07 ± 3.07; great spotted cuckoo nestlings: 14.17 ± 1.62; Wilcoxon matched pairs test, $n = 8$, $z = 2.24$, $P = 0.025$). Therefore, in accordance with prediction 2 (hypothesis 2), the blood of the great spotted cuckoos contained more immunoglobulins than that of the magpies.

Plasma colour

In line with the differences in sedimentation rate – which, like plasma colour, is an indirect index of the amount of immunoglobulins in the blood – the plasma of great spotted cuckoo nestlings was significantly yellower than that of magpie nestlings (Mann-Whitney *U*-test, $z = 3.40$, $P = 0.00069$; Table 3). This was also the case when we analysed the mean response for each species when reared in the same nest (magpie nestlings: 43.84 ± 2.92; great spotted cuckoo nestlings: 55.63 ± 3.95; Wilcoxon matched pairs test, $n = 8$, $z = 2.03$, $P = 0.042$).

Haematocrit and leukocytes

Great spotted cuckoo nestlings had a significantly higher haematocrit level than magpie nestlings ($t_{101} = 3.63$, $P = 0.0005$; Fig. 2). This difference was still statistically significant when comparing the haematocrit of magpie and cuckoo nestlings reared in the same nest (magpie nestlings: 43.56 ± 2.66; great spotted cuckoo nestlings: 52.37 ± 2.41; Wilcoxon matched pairs test, $n = 8$, $z = 2.52$, $P = 0.012$). Therefore, great spotted cuckoo nestlings suffered less than magpie nestlings from possible anaemic diseases.

Great spotted cuckoo nestlings had a higher level of leukocytes than magpie nestlings ($t_{101} = 3.4$, $P = 0.001$; Fig. 2). However, when we compared cuckoo and magpie nestlings

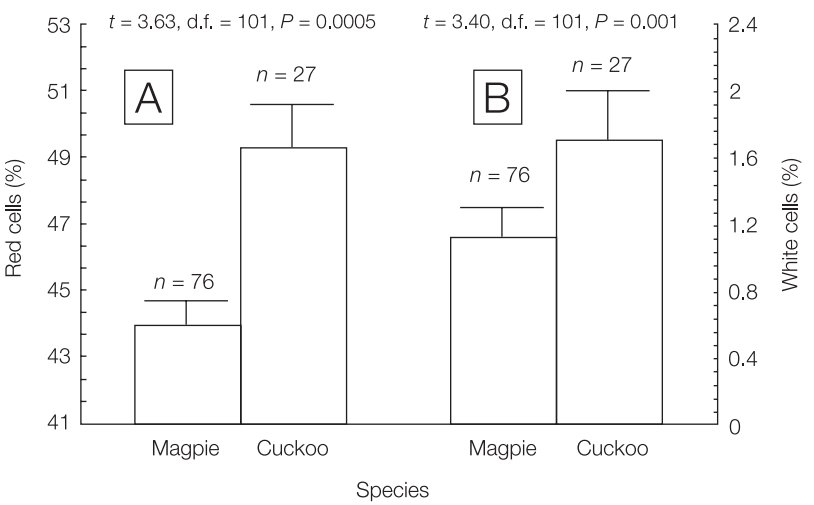

Fig. 2. (A) Haematocrit (%) and (B) relative amount of leukocytes (%) in blood samples from magpie and great spotted cuckoo nestlings. Values are the mean ± standard error.

reared in the same nest, this difference was no longer significant (magpie nestlings: 1.50 ± 0.29%; great spotted cuckoo nestlings: 1.70 ± 0.21%; Wilcoxon matched pairs test, $n = 8$, $z = 0.98$, $P = 0.33$).

Repeatability of immune responses

If the amount of food received by magpie nestlings is more similar among siblings than between magpies reared in different nests, because of differences in the quality of parental care among nests, then we should expect the immune response of siblings to be more similar than that of magpies reared in different nests. We calculated the repeatability of immune responses of magpie nestlings reared in the same nest, and found the repeatability to be high for most immune response parameters, the only exception being leukocyte concentration (Table 4). This similarity in immune response disappeared when we compared the response of magpie nestlings reared in neighbouring nests (Table 4), indicating that there was indeed a difference in response caused by variance in parental quality.

When magpie nestlings were reared together with a cuckoo nestling, the intensity of food competition increased (Soler and Soler, 1991), and the similarity in immune response

Table 4. Repeatability of immune response variables among magpie nestlings reared in the same nest and in neighbouring nests, and among magpie and cuckoo nestlings reared in the same nest[a]

Measurement	Repeatability	*F*	d.f.	*P*
Among magpie nestlings reared in the same nest				
T-cell response	0.46	3.76	22,48	<0.0001
Sedimentation rate	0.74	10.39	22,48	<0.0001
Red cells	0.48	3.96	22,48	<0.0001
White cells	0.08	1.23	22,48	0.27
Plasma	0.49	3.97	22,49	<0.0001
Plasma colour	0.25	2.05	22,49	0.018
Among magpie nestlings reared in neighbouring nests				
T-cell response	0.27	1.75	12,13	0.164
Sedimentation rate	0.08	1.17	12,13	0.390
Red cells	0.06	1.13	12,13	0.414
White cells	0.08	1.16	12,13	0.393
Plasma	0.06	1.12	12,13	0.421
Plasma colour	0.0001	1.00	12,13	0.497
Among magpie and cuckoo nestlings in the same nest				
T-cell response	–0.21	0.65	7,8	0.71
Sedimentation rate	0.32	1.94	7,8	0.19
Red cells	0.34	2.01	7,8	0.17
White cells	0.65	4.64	7,8	0.023
Plasma	0.57	3.63	7,8	0.06
Plasma colour	0.18	1.45	7,8	0.31

[a] Repeatability for magpies and for cuckoos was calculated by subtracting the mean value from the values of each species. Similar results were obtained when the calculations were based on absolute values.

between magpies and cuckoos should therefore have been low. This was shown to be the case by repeatability analyses (Table 4); only the leukocyte concentration was significantly repeatable (Table 4).

Relationship between nestling body mass and immunological response

We hypothesized that the relatively well-fed nestlings would have a more intense immune response than the less well-fed nestlings. We found no relationship between the body mass of 13-day-old nestlings (magpie or great spotted cuckoo) and their immunological response (Table 2). However, the immunological response to the PHA-P injection depended on the level of infection of the individual (Farreras, 1988) and, therefore, on the level of activity of their immune response (whether or not T-cell production was already activated at injection). Magpie nestlings aged 5–15 days have a high rate of mortality (J.J. Soler, personal observation), possibly because of parasite infections, and such variability in infection level would mask the expected relationship between physical condition (body mass) and immune response. In line with this, we found a positive relationship between the mean level of immune system activity (measured as sedimentation rate, white cells and colour of the plasma) of magpie nestlings and parasitism by Haematozoa (logistic regression, $r = 0.52$, $n = 24$, maximum likelihood chi square = 7.80, d.f. = 3, $P = 0.05$). When magpie nestlings are 19 days old, all nestling mortality has occurred and nearly 100% of the nestlings fledge. Since the infection status decreases for nestlings at this age, the relationship between magpie nestling immune response (T-cell response) and their body mass became significantly positive (Fig. 3). This relationship remained significant when the mean immune response per nest was used as an independent observation ($r = 0.51$, $n = 20$, $P = 0.021$). We were unable to test for a relationship in the great spotted cuckoo at this age because they fledge when 16 days old (Soler and Soler, 1991).

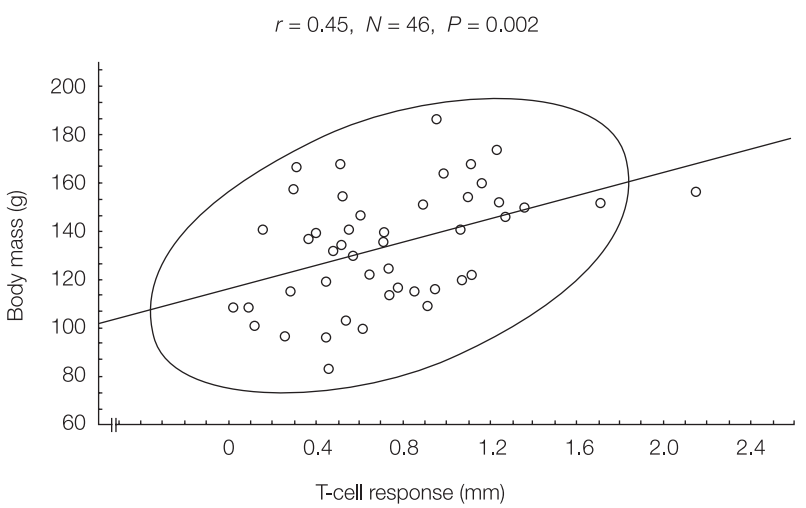

Fig. 3. Relationship between body mass of 19-day-old magpie nestlings and their T-cell-mediated immune response. The ellipse is the 99% confidence ellipse.

Parasite load and experimental brood parasitism

Differences in the prevalence of ectoparasites in magpie chicks between the four kinds of experimental magpie nests were non-significant for Diptera: 20% of the PC nests ($n = 6$), 50% of the PE nests ($n = 6$), 11% of the NC nests ($n = 9$) and 11% of the NE nests ($n = 6$) had ectoparasites in 1994 ($G^2 = 5.86$, d.f. = 3, $P = 0.12$), whereas 0% of the PC nests ($n = 1$), 29% of the PE nests ($n = 7$), 7% of the NC nests ($n = 15$) and 20% of the NE nests ($n = 5$) had ectoparasites in 1995 ($G^2 = 2.24$, d.f. = 3, $P = 0.54$). Similarly, haematozoan parasite loads were also non-significant: 20% of the PC nests ($n = 5$), 33% of the PE nests ($n = 6$), 56% of the NC nests ($n = 9$) and 0% of the NE nests ($n = 6$) had haematozoan parasites ($G^2 = 5.86$, d.f. = 3, $P = 0.12$). These results do not support hypothesis 3 (prediction 3a).

When we compared nests selected (NE and PC magpie nests) and those not selected (PE and NC nests) by the great spotted cuckoo, there were non-significant differences in ectoparasite loads: in 1994, 9% of the selected nests contained magpie nestlings with ectoparasites ($n = 11$), whereas 27% of the non-selected nests ($n = 15$) contained magpie nestlings with ectoparasites ($G^2 = 1.36$, d.f. = 1, $P = 0.24$); in 1995, 17% of the selected nests ($n = 6$) contained magpie nestlings with ectoparasites, whereas 14% of the non-selected nests ($n = 22$) contained magpie nestlings with ectoparasites ($G^2 = 0.03$, d.f. = 1, $P = 85$). For Haematozoa, the differences were also non-significant: 36% of the selected nests ($n = 11$) contained magpie nestlings with Haematozoa, whereas 47% of the nestlings in non-selected nests ($n = 15$) had Haematozoa ($G^2 = 0.28$, d.f. = 1, $P = 0.60$).

The results for the parasite load of great spotted cuckoo nestlings were not significant (Table 5). Therefore, there were no major differences in parasite load based on whether a magpie nest had been parasitized or not by the great spotted cuckoo. No support was therefore found for hypothesis 3.

Immune response and experimental brood parasitism

The immune response of great spotted cuckoo and magpie nestlings may depend on the nest in which they are reared. Great spotted cuckoos prefer to lay their eggs in the nests of magpies of high phenotypic quality (J.J. Soler *et al.*, 1995), and nestlings reared in such nests may have a more efficient immune system than nestlings reared in a randomly chosen magpie nest. To test whether this was the case, we compared the immune response of magpie and cuckoo nestlings reared in PC, PE, NC and NE nests. For magpie nestlings, we found statistically significant differences in sedimentation rate, haematocrit and T-cell response (Table 5). However, magpie nestlings survived only in one naturally parasitized nest (PC), which is the group that showed a large difference from the others (Table 5). However, any difference caused by this group may have been due to the very small sample size. We analysed the data without the PC group and found differences in haematocrit only to be statistically significant.

When we analysed the mean values of magpie nestling variables for each nest as independent observations (without the naturally parasitized control nest, because sample size was one nest), all significant differences disappeared ($P > 0.14$). Therefore, there was no support for differences in the immune response of magpie nestlings based on whether a magpie nest was parasitized or not by the great spotted cuckoo (prediction 3b, hypothesis 3).

For great spotted cuckoo nestlings, there were statistically significant differences for sedimentation rate only. In accordance with hypothesis 3 (prediction 3b), sedimentation rate was higher in experimentally parasitized nests than in naturally parasitized nests, both when we used nestlings and nests as independent observations (Table 5). Therefore, some differences in the immune system of great spotted cuckoo nestlings appear to depend on whether a magpie nest is selected by the great spotted cuckoo.

DISCUSSION

Parasitism of brood parasites and their hosts

Parasites impose a high fitness cost on their hosts (Connors and Nickol, 1991; de Lope *et al*., 1993; Møller, 1993) and immunocompetence has been proposed as a factor regulating vertebrate populations (Lochmiller, 1996). Brood parasites might avoid high fitness costs from host-specific ecto- and endoparasites by laying their eggs in the nests of other species which differ from brood parasites in specialist parasite fauna (hypothesis 1). Thus, direct transmission of host-specific parasites to nestling brood parasites is low (Fig. 1), occurring primarily after fledging, when juvenile brood parasites encounter conspecific adults. There is anecdotal evidence to suggest that European cuckoos are not infested with ectoparasites of their passerine hosts when young, but they have been found to carry cuckoo lice when adults (Dogiel, 1964).

In line with the predictions of hypothesis 1, we have shown that the prevalence of a generalist haematophagous parasitic Diptera was similar in great spotted cuckoo nestlings and those of their magpie hosts, independent of whether they were reared in the same nest. However, the infection by specialist *Leucocytozoon* blood parasites differed between cuckoo and magpie nestlings, the latter showing a considerably higher rate and intensity of infection. Host-specific pathogens may thus be generally more abundant in hosts than in brood parasites reared in the same environment, as in the great spotted cuckoo and the magpie. In conclusion, great spotted cuckoo nestlings have a reduced risk of being infected by parasites compared with magpie nestlings, because of host specificity or differences in epidemiology. This might be a general pattern in brood parasite–host systems.

Immune defence of brood parasites and their hosts

There is an extensive literature on the relationship between immune defence and nutrition based on medical and veterinarian research. The general consensus is that the quality and quantity of food play an important role in developing and fostering an efficient immune response (see reviews in Chandra and Newberne, 1977; Gershwin *et al*., 1985). These findings have important implications for the ability of free-living animals to deal with parasites. Cuckoo eggs hatch earlier than magpie eggs (by an average of 2 days; Soler, 1990) and the larger size of a cuckoo nestling provides it with a considerable competitive advantage in terms of access to food. Moreover, adult magpies preferentially feed great spotted cuckoo rather than magpie nestlings (M. Soler *et al*., 1995). The number of host fledglings in parasitized nests is therefore reduced by almost half compared with the number of fledglings in unparasitized nests (Soler, 1990). Furthermore, magpie nestlings reared together with a great spotted cuckoo nestling have considerably lower body mass than magpies reared in unparasitized nests (Soler, 1990). Acute and chronic malnutrition has

Table 5. Mean, standard error, sample size and the results of one-way analysis of variance comparing parameters of the immune system of magpie and great spotted cuckoo nestlings in parasitized control nests (PC), experimentally parasitized nests (PE), non-parasitized control nests (NC) and experimentally non-parasitized nests (NE)

	Magpie								Great spotted cuckoo							
	Mean value per nest				Nestlings as observation				Mean value per nest				Nestlings as observation			
	PC	PE	NC	NE	PC	PE	NC	NE	PC	PE	NC	NE	PC	PE	NC	NE
Plasma colour																
Mean	30.00	45.82	45.96	46.25	30.00	46.56	45.23	46.33	54.50	53.67			53.85	52.00		
S.E.	—	2.67	1.71	2.65	0.00	2.25	1.27	2.29	1.66	3.50			1.46	2.52		
n	1	7	15	5	2	16	44	15	10	10			13	15		
Tests	$F_{3,24} = 2.09$, $P = 0.13$				$F_{3,72} = 2.41$, $P = 0.07$				$F_{1,18} = 0.05$, $P = 0.82$				$F_{1,26} = 0.40$, $P = 0.53$			
Sedimentation rate																
Mean	−17.07	2.17	3.32	4.85	−18.39	−0.42	3.07	2.80	−6.02	−1.20			−7.29	−2.88		
S.E.	—	2.97	2.45	3.45	1.02	1.80	1.53	1.79	1.61	0.88			1.29	0.86		
n	1	7	15	5	2	16	44	14	10	10			12	15		
Tests	$F_{3,24} = 2.00$, $P = 0.14$				$F_{3,72} = 4.19$, $P < 0.01$				$F_{1,18} = 7.65$, $P = 0.01$				$F_{1,25} = 9.39$, $P < 0.01$			
% of red cells																
Mean	32.65	45.12	44.60	39.99	32.65	45.34	45.11	40.14	48.45	52.01			48.06	50.28		
S.E.	—	2.05	1.38	0.72	5.93	1.55	1.02	1.05	2.26	2.05			2.01	1.84		
n	1	7	15	5	2	16	44	14	10	10			12	15		
Tests	$F_{3,24} = 3.25$, $P = 0.04$				$F_{3,72} = 4.88$, $P < 0.01$				$F_{1,18} = 1.51$, $P = 0.23$				$F_{1,25} = 0.71$, $P = 0.41$			

% of white cells												
Mean	1.17	1.55	1.17	0.91	1.17	1.33	1.12	0.94	1.67	1.75	1.62	1.77
S.E.	—	0.36	0.13	0.21	0.06	0.22	0.11	0.20	0.32	0.20	0.28	0.18
n	1	7	15	5	2	16	44	14	10	10	12	15
Tests	$F_{3,24} = 1.16$, $P = 0.35$				$F_{3,72} = 0.67$, $P = 0.57$				$F_{1,18} = 0.05$, $P = 0.82$		$F_{1,25} = 0.24$, $P = 0.63$	
Corrected T-cell response												
Mean	–13.29	–9.59	–10.38	–36.59	32.92	–3.45	–8.80	–26.80	17.25	25.50	24.77	31.26
S.E.	—	8.95	8.48	19.94	39.92	8.79	5.63	9.64	16.07	12.63	13.05	12.40
n	1	7	15	5	2	15	43	15	9	10	12	15
Tests	$F_{3,24} = 1.21$, $P = 0.33$				$F_{3,71} = 2.25$, $P = 0.02$				$F_{1,17} = 0.19$, $P = 0.67$		$F_{1,25} = 1.00$, $P = 0.71$	
Degree of erythema												
Mean	2.00	1.32	1.30	0.98	2.00	1.22	1.22	0.97	1.36	1.53	1.30	1.50
S.E.	—	0.18	0.12	0.12	0.00	0.12	0.07	0.09	0.18	0.11	0.17	0.11
n	1	7	15	5	2	16	44	15	11	10	15	15
Tests	$F_{3,24} = 1.17$, $P = 0.19$				$F_{3,73} = 3.43$, $P = 0.09$				$F_{1,19} = 0.58$, $P = 0.46$		$F_{1,28} = 0.14$, $P = 0.33$	

been shown to reduce the efficiency of the humoral and the cell-mediated immune response (Bell *et al.*, 1976; Glick *et al.*, 1981, 1983; Willis and Baker, 1981; Tsiagbe *et al.*, 1987; Klasing, 1988; Wan *et al.*, 1989). We would therefore expect similar effects when investigating the difference in the immune response of great spotted cuckoo and magpie nestlings. In line with this, we found a positive relationship between the mean level of immune system activity (measured as sedimentation rate, white cells and colour of the plasma, but not measured as T-cell response) of magpie nestlings and parasitism by Haematozoa. Moreover, when magpie nestlings were 19 days old, the relationship between magpie nestling immune response (T-cell response) and their body mass became significantly positive (Fig. 3).

We hypothesized that the relatively well-fed nestlings of the great spotted cuckoo would show a more intense immune response than the less well-fed host nestlings. We estimated the T-cell-mediated immune response directly, as a measure of immunocompetence, by injection of phytohaemagglutinin-P in the wing of magpie and great spotted cuckoo nestlings. There was a considerable difference in the T-cell response, with cuckoo nestlings having a greater response than magpie nestlings (Fig. 2). There was a more than 50% difference in response when we compared the mean T-cell response of magpie and cuckoo nestlings reared together in the same nest. Such a large difference in immune response is likely to have a significant effect on the survival prospects of cuckoo nestlings compared with magpie nestlings. The PHA-P reaction, which depends on a T-lymphocyte response, has been demonstrated to reliably reflect *in-vivo* cellular immunity in chickens (Goto *et al.*, 1978; McCorkle *et al.*, 1980).

The T-cell response of 19-day-old magpie nestlings was positively related to nestling body mass (see above). The greater response of cuckoos compared with magpie nestlings of the same age, even when taking into account that some nestlings already had an activated immune system, is likely to be mediated through the differential acquisition of protein-rich food by the large cuckoo nestlings. However, further work needs to be conducted before a definitive conclusion can be reached.

We indirectly investigated other aspects of the immune system of great spotted cuckoo and magpie nestlings. The relative sedimentation rate corrected for haematocrit was significantly slower in the great spotted cuckoo than in the magpie (Table 3); this was the case regardless of whether individual nestlings or nests were used as statistically independent observations. The relative sedimentation rate is slow when the blood contains a large amount of plasma proteins such as fibrinogens and globulins (Bacells, 1978). A slow sedimentation rate thus indicates a high immunoglobulin content in the blood. This interpretation is also supported by a statistically significant difference in plasma colour between great spotted cuckoos and magpies (Table 3). Differences in the colour of the plasma are presumed to reflect the amount of protein, including immunoglobulins, in the blood (e.g. Gustafsson *et al.*, 1994).

Finally, we estimated the relative amount of erythrocytes and leukocytes in the blood. A low level of haematocrit partially reflects anaemic diseases (Sturkie, 1986). Great spotted cuckoo nestlings had higher levels of haematocrit than magpie nestlings (Fig. 2), on average 20% higher for nestlings reared together in the same nest. Leukocytes are part of the immune system and leukocyte numbers respond quickly to stress, disease and parasitism (Wintrobe, 1974; Fox and Solomon, 1981; Sturkie, 1986). The relative amount of leukocytes was slightly, but not significantly, larger in great spotted cuckoos than in magpies (Fig. 2).

In conclusion, in line with hypothesis 2, great spotted cuckoo nestlings had on average better immune defences than magpie nestlings, even when comparisons were restricted to nestlings reared in the same nest. This may imply that great spotted cuckoos have a competitive advantage.

Should brood parasites choose host individuals with a weak or a strong immune response?

The answer to this question depends on the costs and benefits of parasitizing a host with an efficient immune system. Immunocompetent host individuals are likely to suffer infrequently from the negative effects of parasitism; such hosts should therefore be better able to provide food for parasitic offspring, but they should also be able to defend their nest better against brood parasites. Another potential cost of choosing a host with an efficient immune response is that host offspring may be better competitors for food. However, this effect is unlikely to be important because brood parasites generally hatch well ahead of the offspring of their hosts, providing them with an advantage in terms of competition for food. Therefore, although there is no evidence of a cost for brood parasites in terms of risking pathogen infections arising from brood parasitism of high- versus low-quality hosts, brood parasites should choose hosts with the most efficient immune system. In accordance with hypothesis 3, we found that great spotted cuckoo nestlings reared in magpie nests selected by adult cuckoos had a slower sedimentation rate and a higher level of immunoglobulins than cuckoo chicks reared in non-selected magpie nests. We also found a higher percentage of infection in non-selected magpie nests than in nests selected by the great spotted cuckoo. However, this difference in ectoparasite load was not statistically significant.

In conclusion, great spotted cuckoo nestlings had fewer host-specific haematozoan parasites than their magpie hosts, and their immune responses were generally more intense than those of host nestlings. These differences in parasitism rate and anti-parasite immune response are likely to provide brood parasites with an important advantage in their exploitation of host populations. However, the species studied here are of different orders, and we cannot rule out the possibility that the phylogenetic constraints were different in terms of the immune response. To reach general conclusions, other studies are required comparing immunological responses of a brood parasite and its host either from the same genus (e.g. *Molothrus*) or from the same family (e.g. Ploceidae: Vidua), or studies comparing immunological responses of brood-parasite and non-brood-parasite species from the same family or genus. This paper describes a novel aspect of the cuckoo–host relationship, but the hypotheses and predictions formulated are applicable to a general parasite–host relationship or species without parental care, due to these species having a reduced risk of infection compared to free living species. We hope that our results will stimulate research of a more diverse nature.

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