# Ectoparasite load is linked to ontogeny and cell-mediated immunity in an avian host system with pronounced hatching asynchrony

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Several contrasting hypotheses have been proposed to account for host age-biased parasite distribution, with some of them suggesting a key role of ectoparasites in the evolution and maintenance of weight hierarchies within broods. We examined parasite distribution among individual hosts across the whole period of host exposure to the parasite in a host system that shows distinct within-brood differences in age and age-related mortality. By contrast to previous hypotheses, we found that the abundance of a haematophagous, mobile ectoparasite *Carnus haemapterus* on nestling European rollers (*Coracias garrulus*) was highest approximately during the mid-nestling stage of their host, coinciding with the inflection point of the host growth phase. Parasite load increased neither with absolute resource availability (i.e. body size), nor body condition index. By contrast to previous evidence, higher parasite load under natural conditions was associated with a stronger cell-mediated immune response. However, this association was moderated by low parasite densities, as well as a better brood body condition index. Overall, although we revealed remarkable host ontogenetic effects on parasite distribution, the present study suggests that a highly mobile ectoparasite generally prefers healthier hosts. We propose that, in host systems with a marked asynchrony of hatching and background mortality within the brood, parasites favour persistence rather than nutritional attractiveness of the host. © 2008 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2008, **94**, 463–473.

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## INTRODUCTION

Knowledge of host-choice strategies by parasites is basic to understanding the evolution of host–parasite associations, the epidemiological significance of such associations, and the fitness consequences for hosts (Christe, Moller & de Lope, 1998; Lively, 2001; Galvani, 2003; Giorgi *et al.*, 2004). Whereas recent work reports host preferences by a variety of parasite systems (Roulin *et al.*, 2003; Mikheev, Pasternak & Valtonen, 2004; Hawlena, Abramsky & Krasnov, 2005; Krasnov *et al.*, 2005), establishing the general rules of host-choice strategies by parasites has proved

to be difficult (Roulin *et al.*, 2003; Hawlena *et al.*, 2005). One such area of controversy exists over the host preference by the parasite in relation to host age.

Several hypotheses have been proposed to account for host age-biased parasite distribution (Table 1). Evidence in favour and against some of the predictions and/or assumptions of these hypotheses has been reported (Descamps *et al.*, 2002; Roulin *et al.*, 2003; Simon *et al.*, 2003; Valera *et al.*, 2004). The contradictory evidence obtained to date could be explained by particular aspects of the host–parasite systems studied (Roulin *et al.*, 2003; Krasnov, Stanko & Morand, 2006), by the effect of confounding variables (e.g. host size: Valera *et al.*, 2004; parasite density: Hawlena *et al.*, 2005; seasonal effects: Roulin *et al.*, 2007) or because the basic assumptions of the hypotheses were not fulfilled. The results obtained hitherto suggest that parasite host-selection criteria

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**Table 1.** Hypotheses on host age-dependent variation in parasitism intensity

| Hypothesis | Assumption | Prediction | Reference |
|---|---|---|---|
| Tasty chick | Junior nestlings are less immunocompetent than their senior siblings | Within broods parasites prefer junior rather than senior nestlings | Christe *et al.* (1998) |
| Well-fed host | Older hosts represent better nutritional resources than younger hosts | Parasites prefer older (well-fed) rather than younger (poorly fed) hosts | Christe *et al.* (2003) |
| Context-dependent | Quality of adult and young hosts is relative | Parasites change their preference for adult (well-fed) and juvenile (poorly fed) hosts depending on host, parasite, and environment-related factors | Hawlena *et al.* (2005) |

are more complex and dynamic than previously thought and our approaches to the problem may have been insufficient. Several approaches have been followed to advance knowledge with respect to this issue. Some studies have focused on individual species (Christe *et al.*, 1998, 2003; Descamps *et al.*, 2002; Simon *et al.*, 2003; Valera *et al.*, 2004), whereas others have addressed the problem using a multi-species approach (Roulin *et al.*, 2003) or a novel host–parasite system (Hawlena *et al.*, 2005).

Although parasite distribution has become increasingly studied across different host–parasite systems, surprisingly little information exists about the key assumptions that are necessary to establish predictions of parasite distribution (e.g. ontogenetic variation in host immunity and body condition; Bize *et al.*, 2003), the applicability of predictions to different host systems (e.g. host systems with a variable predictability of background mortality), or the flexibility of parasite decision criteria in relation to changing ecological factors (Hawlena *et al.*, 2005). Thus, a detailed examination of age-biased parasite distribution models under natural conditions, with careful consideration of underlying assumptions and confounding variables, would be opportune to further advance our understanding of parasite distribution.

In the present study, we examined current age-biased parasitism hypotheses (Table 1) using a new study system involving an ectoparasitic fly *Carnus haemapterus* (Nitzsch, 1818; Diptera: Carnidae) and the nestlings of an avian host, the European roller (*Coracias garrulus*, Linnaeus, 1758). This system is relevant for an investigation into age-biased parasite distribution because *C. haemapterus* only parasitizes nestlings (Grimaldi, 1997), it does not need a host for transmission (Grimaldi, 1997), it is haematophagous and therefore likely to be affected by host immune defence (Wakelin & Apanius, 1997), and nestling rollers show distinct within-brood differences in age

and age-related mortality. Thus, considering their good chemosensory and locomotory abilities (Grimaldi, 1997) and the close spatial proximity of their potential hosts, *Carnus* flies are excellent candidates for exhibiting host preferences at an individual level.

## MATERIAL AND METHODS

### STUDY AREA AND SPECIES

The study area was located at the Desert of Tabernas (Almería, South-east Spain, 37°05′N, 2°21′W). The landscape mostly consists of badlands and wadis with olive and almond groves interspersed among numerous dry riverbeds. The climate in this area is semiarid with long, hot summers and high annual and seasonal variability of rainfall (mean annual rainfall = 218 mm). There were strong interannual differences in weather during the 2-year study period (2005 and 2006), with the winter in 2005 being the coldest one in the last 20 years (range of minimum temperatures from 1 January to 31 March: 2005 = −7.7 °C to 13.4 °C; 2006 = −0.7 °C to 10.0 °C). This, together with an extremely dry breeding season in 2005 (total precipitation from 1 April to 15 July: 2005 = 6.6 mm; 2006 = 129.8 mm), reduced primary and secondary productivity in 2005 and likely accounted for the poorer breeding performance of rollers. Both clutch size and brood size were significantly lower in 2005 than in 2006 (clutch size ± SE, 2005 = 4.28 ± 0.17, $N$ = 39; 2006 = 5.13 ± 0.12, $N$ = 45; separate variance $t$ = 4.15, $P$ < 0.001; brood size ± SE at the time of *Carnus* parasitism assessment: 2005 = 3.35 ± 0.35, $N$ = 23; 2006 = 4.52 ± 0.20, $N$ = 25; $t$-test: $t$ = 2.98, $P$ < 0.005).

The European roller (hereafter roller) is a common avian breeder in the study area, occupying natural holes excavated in sandy cliffs as well as cavities in human constructions. Because laying occurs at 2-day

intervals and incubation begins before the clutch is complete, egg hatching in rollers is distinctly asynchronous (within-brood nestling ages are in the range 2–10 days; R. Václav, unpubl. data). We detected prominent age-biased mortality in nestling rollers. In particular, the mortality of the youngest and second youngest nestlings amounted to 82% (51/62 nests) and 39% (24/62 nests), respectively. The actual mortality rates of junior nestlings are likely to be even higher because we estimated mortality during the mid-nestling stage. Nestlings are naked at hatching but, by the age of 13 days, their body is almost completely covered with closed feather sheaths. The sheaths open from around 15–17 days, with flight feathers appearing first, followed by the feathers of the throat, belly, and rump (Cramp, 1998; R. Václav, pers. observ.). Nestling rollers fledge approximately 20–22 days after hatching (R. Václav, pers. observ.).

*Carnus haemapterus* (hereafter *Carnus*) is a 2-mm long, highly mobile ectoparasitic fly that colonizes nestling birds (Grimaldi, 1997), usually concentrating on a few specific regions of the host, such as inguinal and axillary areas and the skin at the base of wing axillae (Marshall, 1981). This haematophagous parasite (Kirkpatrick & Colvin, 1989; Dawson & Bortolotti, 1997) can have detrimental effects on nestling health (Whitworth, 1976; Cannings, 1986; Soler *et al.*, 1999), even though some studies found no evidence that *Carnus* infestation adversely affects hosts (Kirkpatrick & Colvin, 1989; Dawson & Bortolotti, 1997; Liker *et al.*, 2001). Adult flies have a winged and wingless phase. After their emergence, adults are initially winged, but lose their wings as soon as they locate a suitable host (Roulin, 1998). Carnid flies do not need a host for transmission because they actively colonize hosts' nests during the winged phase of their life cycle (Grimaldi, 1997). Therefore, it is unlikely that the need for successful transmission could influence *Carnus* host choice. *Carnus* emergence is usually synchronized with the occurrence of the host and persists continuously throughout the whole nestling period of the host (Valera, Casas-Crivillé & Hoi, 2003). To our knowledge, other aspects of the life cycle of this parasite, such as the life span of adult flies, or the rate of feeding, remain unknown.

## FIELD METHODS

Fieldwork was carried out in 2005 and 2006. From the first observations of rollers in early April, a population holding approximately 40 pairs was visited at least five times per week. After sighting first copulations, potential nest cavities were inspected every other day until the day of hatching of the last chick of the study population. During regular nest inspections, we monitored progress in egg laying and hatch-

ing. We took measurements of wing length (maximum flattened chord measured to the nearest 1 mm with a wing rule), body mass (with 0.1 g accuracy), and tarsus length (with 0.1 mm accuracy) after all chicks in a brood had hatched and subsequently always on both days of the nestling immunity test (see below).

The relationship between wing length and body mass is based on the values taken at the day of phytohemagglutinin (PHA) administration for all the nestlings involved in our analyses ($N$ = 180). Based on the logistic term including wing length and body mass, we calculated residual body mass, which served as a body condition index (Schulte-Hostedde *et al.*, 2005). Within-nest (brood) body condition refers to the mean body condition index of all nestlings in the brood.

To estimate the size of habitat patch available for *Carnus* flies, we calculated nestling body surface area by raising nestling body mass to the 2/3 power (Heusner, 1985). In 2006, nestling body temperature (cloacal) for 22 broods was measured with a thermometer immediately before PHA administration. As body temperature gradually increased with nestling age (see below) in the analysis of *Carnus* parasitism, we used the residuals of body temperature from the linear regression between body temperature and wing length.

## HOST IMMUNITY ASSESSMENT

We estimated nestling cell-mediated immunity (CMI) by administering to each nestling 0.4 mg of PHA (PHA-P; Sigma L8754) in 40 µL of phosphate-buffered saline, injecting PHA solution subcutaneously into the nestling's left wing patagium. PHA is a mitogen stimulating the proliferation of T-lymphocytes, but also other leucocyte types, with a resulting inflammatory response and a swelling at the site of PHA inoculation (Elgert, 1996). Wing web thickness was measured five times at the injection site with a digital thickness gauge (Mitutoyo 7/547) before and 24 h after PHA administration. In the analyses, we used mean values of wing web thickness. The PHA skin-swelling response was estimated as the difference between mean initial and final left wing web thickness (Smits, Bortolotti & Tella, 1999; Martin *et al.*, 2006). PHA treatment was usually carried out when the oldest nestlings in the brood reached 8–12 days of age. This period was chosen because it coincided with the most intensive brood infestation by *Carnus* flies. All nestlings in the nest were injected with PHA solution at the same day and only once during the nestling period.

## CARNUS HAEMAPTERUS SAMPLING

Within-brood variation in *Carnus* load was studied in 2005 (23 nests) and 2006 (25 nests) at the day of PHA

administration. Roller broods were carefully taken from the nest and placed in a cotton bag. Subsequently, each nestling was separated and placed in an individual cotton bag and the number of carnid flies on the body surface of each chick was counted twice. This visual census method has been found to be reliable (Roulin, 1998). The number of flies recorded in both counts for 195 nestlings was highly repeatable ($r_1 = 0.98$, d.f. = 194, 195, $P < 0.001$). Thus, we used means calculated from the two counts. Every nest was sampled once. Additionally, to study the relationship between the occurrence of the flies on a nestling and actual *Carnus* parasitism, we recorded the occurrence of bites by *Carnus* flies (conspicuous bloody marks distributed mainly under the wings and along feather sheaths; Soler *et al.*, 1999) for all the nestlings sampled at the day of PHA administration in 2006 ($N = 114$). We found a significant positive association between the occurrence of flies and the occurrence of skin lesions (of 101 nestlings with *Carnus* flies, 101 nestlings had wounds, whereas, of 13 nestlings without *Carnus* flies, five had wounds and eight did not have wounds; Yates corrected $\chi^2 = 57.75$, d.f. = 1, $P < 0.001$), suggesting that *Carnus* flies used the nestlings on which they were found for feeding. In most cases, the only ectoparasite found on nestling rollers was *Carnus*. Exceptionally, two roller nests were infested by *Ixodes* ticks and haematophagous mites in 2005, and four nests had haematophagous mites in 2006.

## DATA ANALYSIS

We studied *Carnus* parasitism across all developmental stages of nestling rollers. Therefore, the first step was to scale nestling *Carnus* load intensity, immune response, body temperature, and body mass to a measure of nestling age. We used wing length as a measure of nestling age (for a similar avian system, see Lessells & Avery, 1989). The relationships between the studied variables and wing length were examined with quadratic (parasite load; see Results), linear [body temperature (°C) = 37.99 + 0.25 (wing length); $r^2 = 0.19$, $F_{1,93} = 21.83$, $P < 0.001$], sigmoidal (logistic: body mass $= a/[1 + be^{-r(\text{wing length})}]$; Weibull, Gompertz, and Richards) and rational models (including polynomials; rational: CMI response; see Results). The most successful model (explaining most of observed variance) for each relationship was used to calculate residuals.

In 2006, 20 nestlings from four nests showed more than three-fold higher *Carnus* infestation rates than usual for nestlings of their age. Also, unlike for nestlings from other nests, the nestlings in the four nests were clearly apathetic and lethargic. Therefore, to avoid skewing the regression computation toward the

20 nestlings, we established the regression curve between *Carnus* load and wing length without the 20 nestlings. However, we calculated residual *Carnus* load and examined *Carnus* parasitism for all nestlings. Including the 20 nestlings in the analysis on *Carnus* load does not change the main results.

The predictors of *Carnus* infestation intensity were examined with general linear models; we checked the assumptions of normality (Shapiro–Wilks *W*-test), homoscedascity (Cochran test), and linearity (residual plots inspections). In addition, we tested the assumption that there is no interaction between categorical factors and continuous predictors with homogeneity-of-slopes generalized linear model (GLM). To overcome the problem of intercorrelation between continuous predictors, we included in the model the interaction term between significantly correlated predictors (Neter *et al.*, 1996). To achieve normality, *Carnus* load data (counts) and nestling body surface area were square-root and square-transformed, respectively, before analysis.

Because we detected significant interannual differences in CMI, body condition index, and *Carnus* load intensity (see Results), we controlled for the effect of year by calculating residuals from a linear regression of a respective variable on year, entering year as a dummy variable.

We studied host–parasite relationships at nestling and brood levels. To avoid the problem of pseudo-replication when using nestlings as data points and to control for parental effects (e.g. laying date), we used general linear mixed models with nest as a random effect. Based on colour ringing, we estimate that approximately 30% (14/46) of adults bred in the study population in both years. To determine whether pseudo-replication could affect our results, we randomly subsampled (ten times) the original data set with a sampling rate of 70%. Because subsampling did not affect our main findings, the complete data set was used for analyses. Unless otherwise stated, data are presented as the mean ± SE.

## RESULTS

### PATTERNS OF *CARNUS* PARASITISM

Infestation of nestling rollers by carnid flies was widespread among nests in both study years, with 100% prevalence of *Carnus* parasitism found both in 2005 (range: 5–120 flies per nest, $N = 23$) and 2006 (3–458 flies per nest, $N = 25$). The *Carnus* load of examined nests was considerable in both study periods (2005: 14.42 ± 4.28 carnid flies per nest, $N = 23$; 2006: 29.62 ± 4.11, $N = 25$), but it was significantly higher in 2006 than 2005 (separate variance $t = 2.54$, d.f. = 40.9, $P = 0.015$). *Carnus* load varied
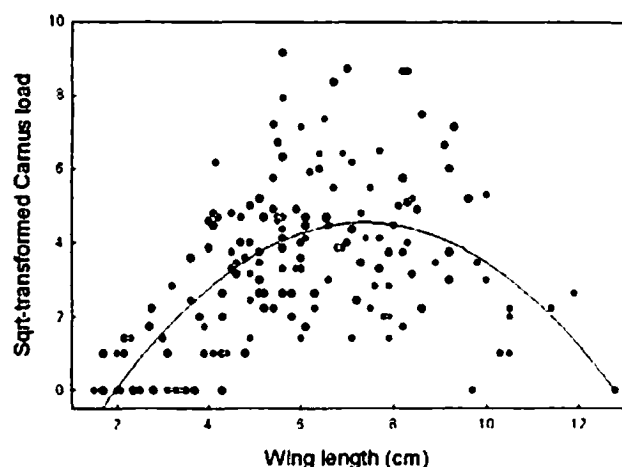
**Figure 1.** Relationship between nestling wing length (cm) and *Carnus* load. Except for four nests with extremely *Carnus*-infested nestlings ($N$ = 20 chicks), the *Carnus* plot shows data for both study years (2005, grey circles; 2006, black circles).

extensively within nests (2005, range: 0–63 flies per nestling, $N$ = 72; 2006: 0–179 flies per nestling, $N$ = 108). Of 14 (of 180) nestlings showing no *Carnus* infestation, nine nestlings were youngest and five second-youngest in the brood age hierarchy.

Overall, there was a quadratic relationship between *Carnus* load and roller wing length [square-root-transformed *Carnus* load = –3.88 + 2.29(wing length) – 0.16(wing length)$^2$; $F_{3,157}$ = 239.44, $P$ < 0.001], so that the former increased sharply until the nestling's wing length reached 7–8 cm (approximately 13–14 days of age), after which it declined rapidly (Fig. 1). *Carnus* load was not influenced by habitat availability (i.e. nestling body surface area) because the relationship between the nestling's body surface area and *Carnus* load was quadratic [square-root-transformed *Carnus* load = –2.51 + 0.02(square-transformed body surface) – 0.000022(square-transformed body surface)$^2$, $F_{3,157}$ = 207.51, $P$ < 0.001]. That is to say, nestlings weighing approximately 100–120 g carried more flies than smaller and bigger nestlings.

### PATTERNS OF HOST BODY CONDITION AND IMMUNOCOMPETENCE

The relationship between body mass and wing length followed a logistic function [body mass (g) = 138.58/(1 + 5.61e$^{-0.46(\text{wing length})}$), $F_{3,177}$ = 5441.85, $P$ < 0.001; Fig. 2]. Nestlings and broods in 2006 showed higher body condition index than in 2005 (nestlings: separate variance $t$ = 4.05, $P$ < 0.001, $N$ = 180; broods: $t$-test: $t$ = 2.27, $P$ = 0.028, $N$ = 48). A body condition index did not show any clear relationship with wing length (linear model: $r^2$ = –0, $F_{1,178}$ < 0.01, $P$ = –1).
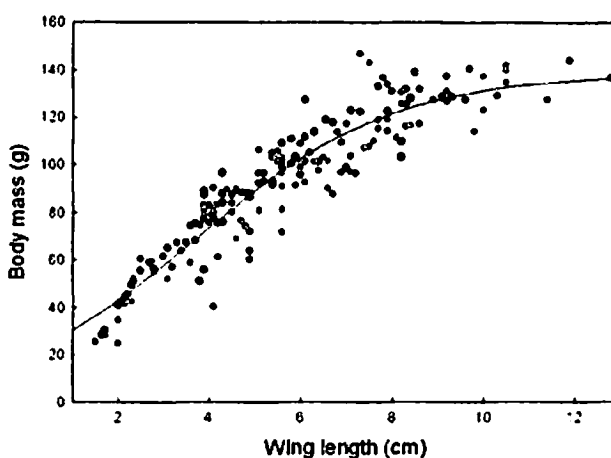


**Figure 2.** Relationship between nestling wing length (cm) and body mass (g). The graph includes data for both years (2005, grey circles; 2006, black circles).
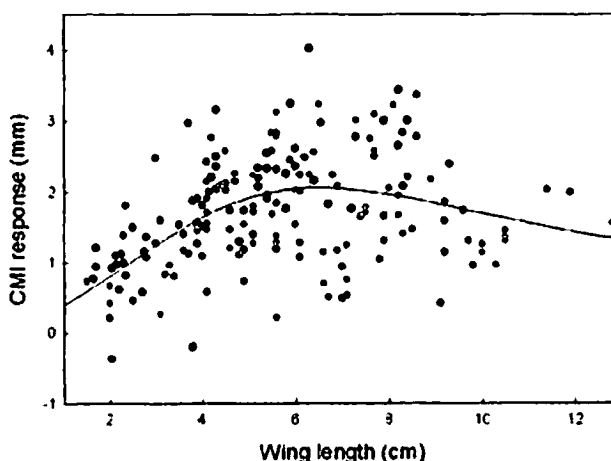


**Figure 3.** Relationship between nestling wing length (cm) and cell-mediated immunity (CMI) response (mm). The graph includes data for both years (2005, grey circles; 2006, black circles).

The CMI response peaked when the nestling's wing length reached 6–7 cm (i.e. approximately 12 days of age), and a gradual fall continued thereafter until the fledging date [rational function, CMI response (mm) = (0.07 + 0.27[wing length])/(1–0.17[wing length] + 0.02[wing length]$^2$), $F_{4,176}$ = 294.87, $P$ < 0.001; Fig. 3]. The relationship between CMI and wing length was not confounded by *Carnus* load [CMI response corrected for *Carnus* load = (0.80 + 0.35[wing length])/(1–0.06[wing length] + 0.01[wing length]$^2$), $F_{4,176}$ = 516.05, $P$ < 0.001]. The CMI response was higher in 2006 compared to 2005 both at an individual ($t$-test: $t$ = 2.85, $P$ < 0.005, $N$ = 180) and, marginally, at a brood ($t$-test: $t$ = 1.82, $P$ = 0.075, $N$ = 48) level.

**Table 2.** Predictors of nestling *Carnus haemapterus* load

| | SS | d.f. | F | P |
|---|---|---|---|---|
| Nest | 42.83 | 47 | 6.79 | 0.001 |
| CMI response | 0.60 | 1 | 4.49 | 0.036 |
| Body condition index | 0.27 | 1 | 2.00 | 0.160 |
| CMI response × Body condition index | 0.52 | 1 | 3.84 | 0.052 |
| Error | 17.32 | 129 | | |

The nestling's cell-mediated immunity (CMI) response was standardized for wing length through nonlinear (rational) regression. The nestling's body condition index was calculated by scaling body mass to wing length using a logistic regression model. Both predictors were corrected for year. Nest was entered as a random factor.

## GENERAL PREDICTORS OF *CARNUS* PARASITISM

The nestling's CMI response was a significant predictor of *Carnus* load (Table 2); *Carnus* load was positively related to the CMI response (slope = 0.13, $t_{129} = 2.12$, $P = 0.036$), although this relationship seemed to have been mediated through interaction with body condition (Table 2).

When comparing the pairs of siblings showing similar wing lengths but contrasting CMI responses (wing length: high-CMI response chicks = 6.31 ± 0.31 cm; low-CMI response chicks = 6.03 ± 0.33 cm; paired *t*-test, $t = 1.71$, $P = 0.094$, $N = 45$ sibling pairs; wing length- and year-corrected CMI response: high-CMI response chicks = 0.58 ± 0.09 mm; low-CMI response chicks = -0.46 ± 0.08 mm; $t = 10.23$, $P < 0.001$, $N = 45$), we found that siblings with a stronger CMI response showed heavier *Carnus* infestation than their siblings with a lower CMI response ($t = 2.37$, $P = 0.022$, $N = 45$ pairs; Fig. 4).

## CONTEXT-DEPENDENT *CARNUS* PARASITISM

Examining *Carnus* parasitism at a brood level, we found that there was a significant interaction effect of the within-nest CMI response and year on nest *Carnus* load (homogeneity of slopes GLM: year × within-nest CMI response, $F_{1,40} = 5.61$, $P = 0.023$). Consequently, analysing both years separately, we found that *Carnus* parasitism within nests was a positive function of the within-nest CMI response in 2005 (multiple linear regression: $r^2 = 0.29$, $F_{2,20} = 4.06$, $P = 0.033$; within-nest CMI response, slope = 0.55, $t_{20} = 2.83$, $P = 0.010$, within-nest body condition index, slope = -0.19, $t_{20} = -1.00$, $P = 0.33$), but not in 2006 ($r^2 = 0.05$, $F_{2,22} = 0.57$, $P = 0.57$; within-nest CMI response, slope = -0.18, $t_{22} = -0.85$, $P = 0.40$; within-nest body condition index, slope = 0.15, $t_{22} = 0.73$, $P = 0.47$).
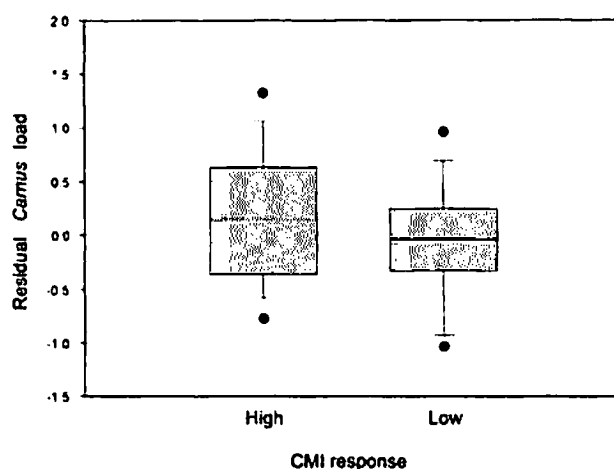


**Figure 4.** Difference in *Carnus* load between pairs of siblings showing a contrasting cell-mediated immunity (CMI) response but comparable size. *Carnus* load was square-root-transformed, wing length-corrected and then corrected for interannual differences. The plot shows means (solid line), lower and upper quartiles (boxes), 10th and 90th percentiles (whiskers), and outliers (dots).

Similarly, as for the within-nest *Carnus* load, we found a tendency for the interaction effect of year and nestling CMI response (homogeneity of slopes GLM, including year, nestling CMI response, and body condition index: $F_{1,172} = 3.81$, $P = 0.052$) but also year, nestling CMI response, and body condition index ($F_{1,172} = 3.55$, $P = 0.061$) on nestling *Carnus* load. Year effects could be attributed to the interannual differences in brood body condition and *Carnus* load (see above). Thus, to test whether there is a relationship between a nestling's *Carnus* parasitic load and its CMI response when controlling for the year effects, we examined the interaction effects of the nestling's CMI response and brood body condition index and brood *Carnus* abundance on nestling *Carnus* loads (Table 3). We found that, when brood condition was low, the nestlings with the strongest CMI response were most highly parasitized but, if brood condition was high, the link betwen the nestling's CMI response and *Carnus* load was largely absent (Fig. 5A). In turn, if broods were more highly parasitized, the most highly parasitized chicks were those with the highest CMI response but, if brood *Carnus* abundance was low, the association between CMI and *Carnus* load disappeared (Fig. 5B).

## PHENOTYPIC CUES TO HOST IMMUNOCOMPETENCE

Apart from ontogenetic effects (Fig. 3), nestling CMI could also covary with body condition or body temperature. There was no interaction effect of year and body condition index on CMI response (homogeneity
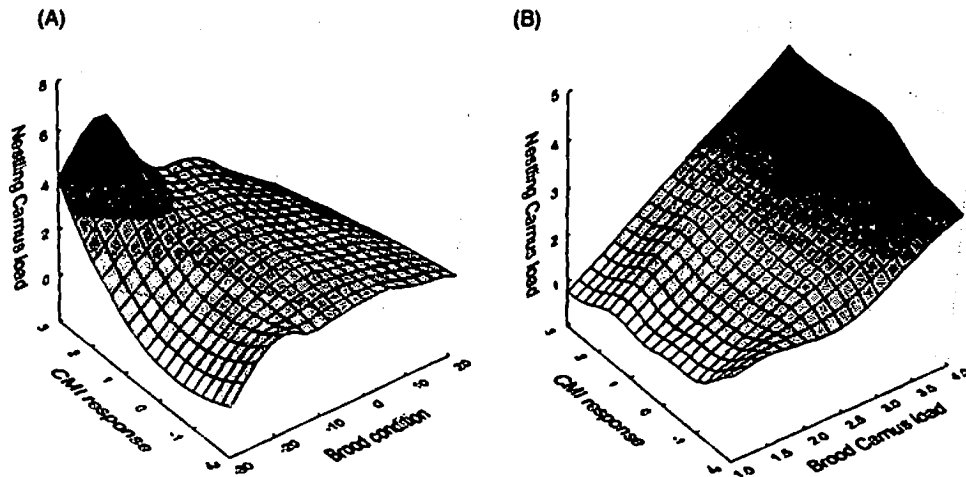
(A)                                    (B)



**Figure 5.** Relationship between nestling cell-mediated immunity (CMI) response, *Carnus* load and brood (A) body condition index and (B) *Carnus* load. Brood body condition was calculated by averaging nestling body condition indexes within broods. Overall, brood *Carnus* load was calculated by averaging wing length-corrected and square-root-transformed nestling *Carnus* loads within broods. Nestling *Carnus* load and phytohemagglutinin response were corrected for wing length.

**Table 3.** Predictors of nestling *Carnus haemapterus* load, assuming that the form of the relationship between the nestling's cell-mediated immunity (CMI) response and *Carnus* load is dependent on the within-nest body condition index and *Carnus* load (i.e. the two traits that differed between years)

|  | SS | d.f. | F | P |
|---|---|---|---|---|
| Nest | 48.92 | 47 | 7.96 | 0.001 |
| Nestling CMI response | 0.28 | 1 | 2.10 | 0.149 |
| Brood condition × Nestling CMI response | 0.66 | 1 | 5.02 | 0.027 |
| Brood *Carnus* load × Nestling CMI response | 0.52 | 1 | 3.94 | 0.049 |
| Error | 16.88 | 129 | | |

The nestling's CMI response was standardized for wing length through nonlinear (rational) regression. Nest was entered as a random factor.

of slopes GLM: year × body condition index, $F_{1,176} = 0.11$, $P = 0.74$). Consequently, utilizing pooled data from both years, we found that the nestling's CMI response was weakly, but significantly related to body condition index (linear regression: $r^2 = 0.06$, slope = 0.25, $t_{178} = 3.41$, $P < 0.001$). After examining nestling body temperature data from 2006, wing-length corrected body temperature turned out to be a more sensitive predictor of nestling CMI than body condition index (multiple linear regression: $r^2 = 0.10$, $F_{2,92} = 4.92$, $P < 0.01$; size-corrected body temperature, slope = –0.23, $t_{92} = -2.33$, $P = 0.022$; body condition

**Table 4.** Predictors of nestling *Carnus haemapterus* load including body temperature

|  | SS | d.f. | F | P |
|---|---|---|---|---|
| Nest | 453.91 | 21 | 6.95 | 0.001 |
| CMI response | 13.91 | 1 | 4.44 | 0.039 |
| Body condition index | 0.13 | 1 | 0.04 | 0.84 |
| Body temperature | 0.42 | 1 | 0.14 | 0.71 |
| CMI response × Body condition index | 8.63 | 1 | 2.77 | 0.10 |
| CMI response × Body temperature | 0.58 | 1 | 0.19 | 0.67 |
| Error | 211.60 | 68 | | |

The cell-mediated immunity (CMI) response and body temperature were standardized for wing length through nonlinear (rational) and linear regression, respectively. A body condition index was calculated by scaling body mass to wing length using a logistic regression model. Nest was entered as a random factor. The results are based only on data from 2006.

index, slope = 0.19, $t_{92} = 1.92$, $P = 0.059$). Despite this, the nestling's CMI response remains the most important predictor of *Carnus* load (Table 4).

## DISCUSSION

ONTOGENETIC PATTERNS OF *CARNUS* PARASITISM AND HOST IMMUNITY AND CONDITION

Under natural conditions, *Carnus* loads on nestling rollers peaked approximately during the mid-nestling

stage when nestlings were just at the inflection point of their growth phase. *Carnus* load did not increase with increasing absolute resource availability because host body mass increased sinusoidally during the nestling period. *Carnus* flies did not have a propensity to aggregate on older or younger hatchlings, regardless of their position in the brood hierarchy.

The immune activity of nestling rollers was nonlinearly related to a measure of age; the immune response to a T-cell mitogen (PHA) increased sharply until approximately 12 days of age when it started to decline; see also Tella, Scheuerlein & Ricklefs (2002). Because the inclusion of nestlings of the same age but with a variable position in the brood age hierarchy did not distort the sigmoidal CMI–wing length relationship, the ontogeny of the immune system appears to play a vital role in variation in the immune function of nestling rollers, with mid-aged nestlings showing a relatively stronger CMI response compared with that of older and, particularly, younger nestlings.

By contrast to nestling immunity or *Carnus* load, an index of body condition did not clearly change during the nestling phase. Nevertheless, a body condition index still represents a relevant biological measure for nestling rollers because it correlated positively with CMI.

## WITHIN-BROOD *CARNUS* DISTRIBUTION

Three hypotheses were proposed about how parasites should distribute themselves among available hosts in relation to host age. The present study does lend direct support to any of them but instead stresses the importance of the time at which parasite load is estimated. Given that the parasite infestation of an individual host changes with its age, parasite loads for the same brood could be higher in senior or junior siblings (i.e. in older or younger half of a brood) depending on the time of parasite load estimation. For example, Valera *et al.* (2004) showed that *Carnus* flies preferred bigger hosts in a similar host system (European bee-eaters, *Merops apiaster*) to that used in the present study. However, although Valera *et al.* (2004) reported that the highest *Carnus* load occurs in bee-eater nestlings at the age of 15 days (note that bee-eaters fledge at approximately 30 days of age), Valera *et al.* (2004) only examined the parasite choice for 5–15-day-old nestlings. We propose that the ontogenetic pattern of parasite infestation intensity and host immunity be known before making any inference about the host exploitation strategy in relation to host age.

We found that, irrespective of hatching order, nestling rollers until approximately 5 days of age were free or carried minimal numbers of *Carnus* flies. After controlling for year and nest-related effects and for host age and body condition index, the highest

*Carnus* infestation rates were consistently found on the nestlings mounting the strongest CMI. Thus, the results of the present study disagree with the idea of Christe *et al.* (1998) that parasites should distribute themselves on the most vulnerable host within a brood. Roulin *et al.* (2003) suggested that parasites might not show a consistent preference for the least resistant host in all host systems due to the low nutritional profitability of 'vulnerable' hosts or due to the high costs of sampling and switching among potential hosts. The present study supports the findings of Roulin *et al.* (2003) demonstrating that *Carnus* ectoparasites avoid certain host phenotypes, particularly feathered nestlings. There is evidence that *C. haemapterus* feeds on the basis of growing feathers (Marshall, 1981), and thus the avoidance of older chicks by *Carnus* flies may be due to the retraction of blood vessels nourishing feathers as well as the keratinization of feather sheaths.

The present study is apparently in agreement with the 'well-fed host strategy' proposed by Christe *et al.* (2003) because we found that the highest *Carnus* infestation coincided with the time when both nestling growth and body mass culminated. Nevertheless, when examined together, nestling CMI turned out to explain significantly more of the variance in *Carnus* infestation intensity than body condition. Nutritional status and immunity are usually tightly positively correlated (for birds, see Alonso-Alvarez & Tella, 2001). Nevertheless, these two parameters may provide different cues to host quality. Although nestling body condition can be a proxy for the quantity and/or quality of food resources, nestling immune function also is a reliable predictor of nestling survival prospects (Christe *et al.*, 1998; Gonzalez *et al.*, 1999; Christe *et al.*, 2001; Møller & Saino, 2004). Moreover, the development of an immune function can be traded off with other important functions such as growth (Saino, Calza & Moller, 1998; Norris & Evans, 2000). Therefore, immunity and condition may not always covary. For example, Dubiec & Cichoń (2005) showed that, although condition in late and early hatched nestling great tits *Parus major* did not differ, the nestlings hatching later developed a less competent immune system. We propose that, in host systems with age-biased background mortality, parasites preferring fitter nestlings might not seek abundant but persistent food resources (Jovani & Serrano, 2002).

The present study suggests that, under high brood conditions, *Carnus* flies moderated their preference for immunocompetent nestling rollers. Christe *et al.* (2003) proposed that parasites can exploit either vulnerable or well-fed hosts depending on between-host differences in physiological status. In particular, it was suggested that parasites switch between adult and juvenile hosts depending on differences in host

nutritional status. However, adults and juveniles differ profoundly in more than just nutritional status. For example, juveniles commonly have lower survival prospects than adults (Gaillard, Festa-Bianchet & Yoccoz, 1998), which has been attributed to the lower capacity of the immune system in juveniles (Gonzalez et al., 1999; Tella et al., 2000).

Independent of host-related effects, Hawlena et al. (2005) found that the switch in parasite preference occurs with changing parasite density. The present study supports the result of Hawlena et al. (2005) that, under high intraspecific parasite density, parasites prefer a less vulnerable host. The Hawlena et al. (2005) were not able to discriminate whether the switch occurred due to differences in host mortality or nutritional status. By contrast, the results of the present study suggest that parasites prefer a less vulnerable host due to its better survival prospects rather than nutritional quality because, under high parasite densities, parasites appeared to enhance their preference for the immunocompetent host. Overall, we revealed a high degree of flexibility in host-selection criteria with respect to host- (ontogenetic) and parasite-related (socio-ecological) factors.

## WHY SHOULD HOST IMMUNITY BE IMPORTANT?

Immune function is an important life history trait that has evolved to increase fitness through longevity; yet immune function is continuously traded off with other bodily functions that are immediately more important (Sheldon & Verhulst, 1996). Thus, immunocompetence can be a sensitive marker for the physiological burden placed upon an animal, indicating whether the animal trades survival in immediately unfavourable conditions with a less-well developed immune defence mechanism (Lochmiller & Deerenberg, 2000). The positive and negative relationships of CMI with body condition index and size-corrected body temperature, respectively, imply that the PHA-induced immune response did not simply follow trends in parasite loads but, instead, reflected health status because nestling rollers mounting a weaker CMI response had lower body mass index and elevated internal body temperature (Roulin et al., 2007).

Nestling rollers represent a host system with differential within-brood mortality where last-hatched nestlings face very small chances of survival until fledging. Therefore, ectoparasites infesting nestling rollers, such as Carnus flies, could be under selection to aggregate on a more persistent host because host recolonization might imply costs related to switching to lower quality hosts that are likely to perish or to higher quality individuals that are likely already settled by conspecifics. Provided that intraspecific competition among unrelated parasites further increases parasite virulence and host mortality (Frank, 1996; Galvani, 2003), the preference for a less vulnerable host should be particularly important under high parasite densities. Our results stress the fact that Carnus flies favoured the most immunocompetent hosts consistently across broods of all ages. However, under closer examination, the preference for healthy nestlings was found to be strongest particularly under conditions that heighten the risk of host mortality (i.e. low brood condition and high parasite density). Therefore, we propose that host mortality can affect the preference for relatively healthy hosts in systems with high host background mortality.

Experimental work has shown that parasitization usually negatively affects host CMI (Christe et al., 2000; Martin et al., 2006). The apparent lethargy and apathy of heavily infested broods of rollers suggests that high Carnus abundance can be detrimental to nestling rollers. However, under natural conditions, more infested nestling rollers showed better CMI than their less infested siblings. Therefore, the present study implies that Carnus flies may flexibly switch among hosts and select those with immediately better health status.

In summary, after taking into account strong ontogenetic effects on parasite distribution, the present study strongly suggests that, in a host system with heterogeneous within-brood background mortality, a haematophagous ectoparasite preferred healthier hosts. This implies, on the one hand, outstanding discrimination abilities of the parasite because host health changes dynamically during host ontogeny. On the other hand, the ability of Carnus to moderate its preference according to ecological circumstances implies some degree of flexibility in host selection criteria. Finally, the present study suggests that the host's health may not always be a consequence of parasite abundance, but that parasites might cue on hosts' health status and colonize them accordingly.

## ACKNOWLEDGEMENTS

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