Palatability of passerines to parasites: within-brood variation in nestling responses to experimental parasite removal and carotenoid supplementation

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Asynchronous hatching of eggs in avian clutches produces a size hierarchy among nestlings that may lead to variation within broods in resistance to pathogens or parasites. In this study, we tested several predictions regarding variation in immunocompetence and distribution of parasites within avian broods by combining parasite removal and carotenoid supplementation treatments in nests of mountain bluebirds Sialia currucoides. Last-hatched nestlings were less likely to invest carotenoids in an induced cell-mediated immune response, suggesting they may be more susceptible to parasites; however, parasite removal disproportionately benefited middle-ranked nestlings. This supports the hypothesis that some avian ectoparasites balance host resistance against nutritional benefits by preferentially parasitizing nestlings of intermediate quality and immunocompetence. We found no evidence that males positioned last in the hatching sequence were differentially affected by ectoparasites, and, contrary to some previous studies in other passerines, last-hatched nestlings in asynchronously hatching broods were not less immunocompetent than their nest mates. In fact, junior nestlings exhibited weaker immune responses than their siblings in more synchronously hatching broods, and we suggest this may reflect environment-dependent maternal effects that warrant further investigation. Overall, our results highlight the importance of understanding the feeding and host selection behaviour of ectoparasites, as well as the fitness consequences thereof, since many predictions related to within-brood distribution of parasites require that parasites are able to discern the relative quality of available hosts.
general explanation for the evolution and maintenance of avian HA in the presence of ectoparasites therefore remains uncertain.

According to the TCH, more asynchronously hatching clutches should exhibit greater variation in within-brood immunocompetence, and last-hatched nestlings in particular should be less immunocompetent relative to their nest mates as the degree of HA increases. However, maternal allocation of resources to eggs within a clutch often varies predictably over the laying sequence, and last-hatched nestlings may exhibit physiological differences relative to earlier-hatched nest mates as a result of these differential investments, independent of their position in the size hierarchy of the brood (Parsons 1975, Badyaev et al. 2002). Yolk androgens have been shown to enhance growth but reduce T-cell mediated immunocompetence of nestling birds (Navara et al. 2005, but see Navara et al. 2006), whereas antioxidants generally have immune stimulating effects (Saino et al. 2003, Biard et al. 2007). Females of many bird species allocate more androgens (e.g. testosterone), and may simultaneously reduce investment of antioxidants, such as carotenoids, in yolk of eggs positioned late in the laying sequence (Royle et al. 2001, Groothuis and Schwabl 2002), and under these conditions last-hatched nestlings exhibit reduced immunity relative to their senior nest mates (Müller et al. 2003). This pattern of within-brood variation in immunocompetence is consistent with predictions of the TCH, and may lead to the aggregation of parasites on last-hatched nestlings, but does not necessarily depend on the degree of HA or variation in body condition within the brood. Such a pattern can therefore not be taken as evidence supporting the TCH, unless a specific influence of HA on relative immunity of last-hatched nestlings is also demonstrated.

Maternal effects due to yolk androgen investment may be compounded in male offspring by endogenous hormone production and/or increased sensitivity to androgens (e.g. via higher receptor density on target tissues, Sockman et al. 2007). In some birds, male nestlings are more severely affected by parasites, potentially due to these hormonal effects (Potti and Merino 1996). Given that yolk androgens tend to increase with laying order in birds (Royle et al. 2001, Groothuis and Schwabl 2002), sons may be particularly influenced by maternal androgens if they are positioned last in the laying sequence (Royle et al. 2001, Groothuis and Schwabl 2002), sons may be particularly influenced by maternal androgens if they are positioned last in the laying sequence. The relative performance of last-hatched nestlings in parasitized broods may therefore not only be a consequence of the degree of HA, as suggested by the TCH, but also of the sex of the last-hatched nestling (hereafter referred to as the ‘tasty son hypothesis’, TSH). Although a number of studies have examined sex differences in performance of nestling passerines in relation to their hatching order (Badyaev et al. 2002, Arnold and Griffiths 2003) and degree of hatching asynchrony (Tilgar and Mánd 2006), few have specifically considered the role of parasites in mediating these relationships.

The TCH states that parasites should focus their feeding efforts on the smallest and least immunocompetent nestling in a brood, and the TSH modifies this to state that parasites should particularly focus their feeding activities on the smallest, last-hatched nestling in the brood if it is male. However, fecundity of parasites such as avian fleas is greater when they feed on hosts in good condition (Tschirren et al. 2007); thus, even though nestlings in good condition may be capable of mounting a stronger immune response, there may nonetheless be a selective advantage for parasites to risk the potential costs of feeding on high-quality hosts to secure these fitness benefits (Bize et al. 2008). If parasites face a tradeoff between host quality and immune defence, however, the optimal resolution of this tradeoff may result in middle-ranked nestlings being the most heavily parasitized, since they provide moderate resource quality but may mount a less robust immune response compared to their largest nest mates (Bize et al. 2008).

The goals of this study were: (1) to examine the influence of nest-dwelling ectoparasites on the growth and size of nestlings over the hatching sequence in a passerine exhibiting a moderate degree of HA, and (2) to investigate whether within-brood variation in susceptibility to parasites is influenced by differences in immune function among nest mates. By combining ectoparasite removal and carotenoid supplementation treatments and analyzing within-brood variation in nestling performance, we were able to test three competing predictions related to within-brood patterns of parasite aggregation on nestlings: first, according to the TCH, last-hatched nestlings in asynchronously hatching broods should disproportionately benefit from either parasite removal or immune enhancement with supplemental carotenoids. Alternatively, according to the TSH, last-hatched male nestlings, particularly in asynchronously hatching broods, will be expected to show greater benefits from parasite removal or carotenoid supplementation than either males hatching last in relatively synchronous broods, or last-hatched female nestlings. Finally, if ectoparasites forage optimally within avian broods by balancing nutritional benefit against the cost of host immune response, removal of parasites or provision of supplemental carotenoids should disproportionately benefit nestlings positioned in the middle of the hatching sequence.

**Methods**

**Study area and species**

We studied mountain bluebirds *Siala currucoides* breeding in nest boxes in central British Columbia, Canada (51°N, 122°W) in 2006. The study area consists of arid grassland with patches of mature Douglas-fir. Seventy-two nest box pairs were mounted on fence posts approximately 5 m apart, with successive pairs separated by approximately 200 m. Bluebirds return to the site as early as late March, and initiate clutches beginning in late April. Hatching is moderately asynchronous, with up to two days between hatching of the first and last egg in a clutch (O’Brien unpubl.). Nestlings in this population are parasitized predominantly by nest-dwelling larval blow flies *Protocalliphora* spp., and among broods, the experimental removal of ectoparasites from nests significantly enhanced growth of nestlings (O’Brien and Dawson 2008). Adults are dimorphic in plumage colouration as well as length of primary flight feathers and rectrices, however, nestlings do not exhibit similar sexual dimorphism in feather length prior to fledging, despite some differences in plumage colouration (below, Power and Lombardo 2007).
1996). Neither adults nor nestlings are dimorphic in body mass (Power and Lombardo 1996).

General field procedures

Nest boxes were visited daily beginning in mid-April to determine clutch size and initiation date, and again beginning 12 days after clutch completion to document date of hatch (designated nesting day 0). On day 1, nestlings were weighed to the nearest 0.01 g using an electronic scale. Difference in mass between the largest and smallest chick (range 0.4–3.8 g) was used as an estimate of HA, since this measure obtained early in the nestling period in passerines has been shown to reflect total hatching time for the clutch (Slagsvold 1986, Magrath 1992). In some cases the last egg had not hatched by day 1, and in these nests difference in mass at day 2 was used as an estimate of HA. We then classified broods as either synchronous or asynchronous relative to the mean HA estimated for all first broods (mean difference in mass = 1.4 g), and used this bivariate term in all subsequent analyses. In broods classified as synchronous, the mean difference in mass between the largest and smallest chick was 0.9 g, whereas asynchronous broods differed by an average of 1.9 g. Each nestling in a brood was marked on its tarsi with nontoxic ink to allow for individual identification throughout the nestling period. Although it was not possible to determine exact hatching order of all nestlings according to size rank at day 1 or 2, the last-hatched nestling was identifiable at this early stage as the smallest relative to its nest mates, and is usually from the last egg in the laying sequence in this species, as with most passerines (O’Brien and Dawson unpubl., see Magrath 1992 for an example in another species). All other size ranks approximate laying and hatching order; i.e. first- and second-ranked nestlings emerge from eggs produced early in the laying sequence, and are similarly positioned early in the hatching sequence (O’Brien and Dawson unpubl.).

We measured nestling mass (nearest 0.125 g, with a spring balance) and tarsus length (nearest 0.01 mm, using digital calipers) every two days, from day 3–15, and measured length of the eighth primary feather (nearest 0.5 mm, with a ruler) at the same intervals once flight feathers had emerged, from day 7–15. Each nestling was banded with a numbered aluminum band on day 13. Male bluebirds exhibit bright blue primary feathers as nestlings, whereas flight feathers of females are slate grey (Power and Lombardo 1996); we used this difference in plumage colour to assign sex of nestlings at day 15. Of 19 nestlings (seven females and 12 males) that recruited locally as second-year breeders in 2007, all had been correctly sexed according to these plumage characteristics. Once nestlings had fledged, nests were collected and stored in sealed bags for 14 days. We then sifted the material to extract all blow fly pupae and puparia as an estimate of the minimum level of infestation (Dawson et al. 2005a).

Parasite treatment and carotenoid supplementation

We combined parasite removal and carotenoid supplementation treatments in a $2 \times 2$ design, yielding four treatment combinations. Details of the methods are described in O’Brien and Dawson (2008). Briefly, nest-dwelling ectoparasites were reduced by heating experimental nests in a portable microwave appliance every two days, from day 1 until day 13. Control nests were not removed from nest boxes so that natural levels of parasite infestation were maintained, but control broods were visited at the same frequency as broods in heated nests. To enhance the immune response of nestlings within a subset of broods, we provided carotenoid supplements throughout the nestling period. Supplements consisted of beadlets containing 5% lutein and 0.2% zeaxanthin, suspended in sunflower oil; each nestling in supplemented broods received 750 µg of beadlets in 0.05 ml oil, while control broods received 0.05 ml of oil only (Biard et al. 2007). Mock (1991) reported that nestling western bluebirds Sialia mexicana require 15 g of food per day during peak growth; as the diet of nestling bluebirds consists primarily of lepidopterans and orthopterans, which are estimated to contain 520 mg kg$^{-1}$ of carotenoids (Olson 2006), each nestling consumes approximately 8 mg of carotenoids per day. A dosage of 750 µg was therefore biologically relevant, as it represented approximately 10% of the maximum daily carotenoid intake of nestling bluebirds. Supplements were delivered to nestlings with a 1-ml syringe every two days, from day 1 until day 13. Adults did not alter their provisioning behaviour in response to either the parasite removal or carotenoid supplementation treatment (O’Brien and Dawson 2008). In combination, these treatments allowed us to examine the specific influence of host immune response on susceptibility to parasites.

Immune function assessment

We assessed cell-mediated immunity (CMI) of eight-day-old nestlings using the phytohaemagglutinin (PHA) skin test, which measures the ability of birds to respond to a novel mitogen (Smits et al. 1999). This response is complex and may involve several classes of immune cells, including T-lymphocytes, basophils and macrophages (Martin et al. 2006); however, PHA response has been shown to be associated with resistance of nestlings to ectoparasites (Tschirren et al. 2007), and within-brood variation in this measure may therefore reflect differences in the relative vulnerability of individual nestlings to parasitism. We used a thickness gauge to measure the thickness (nearest 0.01 mm) of the right wing web immediately before and 24.40 h ($\pm 0.05$ SE, $n = 241$) after intradermal injection of 30 µl of 2 mg ml$^{-1}$ PHA-P (Sigma) in phosphate-buffered saline. To control for growth-related changes in wing web thickness, we also measured the un.injected left wing web over the same interval and subtracted this difference from the initial CMI value (O’Brien and Dawson 2008).

Statistical methods

Female birds may allocate resources differently to eggs in first clutches compared to replacement or second clutches within the same breeding season, with potential consequences for the relative performance of last-hatched nestlings (Tobler et al. 2007). To ensure that our analyses only
included first breeding attempts, we therefore considered only those clutches that were initiated prior to known second broods or re-nests (after a failed first attempt). Seven of 57 first broods failed, and an additional 12 experienced partial brood reduction. The last-hatched nestling died in only four of these 12 nests with partial mortality; due to this small sample size, we did not investigate factors influencing survival of last-hatched nestlings. All nests with brood reduction were excluded from the analysis to ensure our within-brood repeated measures were consistent among nests. The remaining 38 nests that did not experience brood reduction consisted of nine control heat/control supplement, eight control heat/carotenoid supplement, 10 heated/control supplement and 11 heated/carotenoid supplement. Growth rates for morphological measures were calculated as described in Dawson et al. (2005b), using a logistic model for growth of mass, Gompertz model for growth of tarsus and a linear model for growth of eighth primary feathers. Body condition of nestlings on the day of PHA injection (day 8) was calculated as the residuals of the linear regression of body mass on tarsus length, since these variables were highly correlated (n = 197, R = 0.65, p < 0.001).

Since we were interested in examining the immune response, growth and size of last-hatched nestlings compared to all other nestlings in the brood, we used repeated measures analysis of variance (ANOVA) to examine the performance of the last-hatched nestling relative to its senior nest mates. To account for our inability to assign precise hatching positions to all senior nestlings, we classified first- and second-ranked nestlings as ‘early’, and third- and fourth-ranked nestlings as ‘middle’ in the hatching order. Second- and third-ranked nestlings differed in mass by 0.24 g (0.16–0.32 g, 95% CI), and there were no cases in which their masses were equivalent; we are therefore confident that these nestlings were correctly assigned to ‘early’ and ‘middle’ groups. Similarly, fourth- and last-ranked nestlings differed by 0.66 g (0.44–0.89 g, 95% CI), and were never equivalent in mass at day 1. Mean values were then calculated for nestlings in ‘early’ and ‘middle’ hatching rank groups. Our analyses therefore included three repeated measures for ‘early’, ‘middle’ and ‘last’ nestlings in each brood. Broods classified as first breeding attempts contained either five or six nestlings; in broods of six, fifth-ranked nestlings were not included to ensure the ‘middle’ within-subjects level represented only the third- and fourth-ranked nestlings for all comparisons. In cases where the sphericity assumption was violated, we applied the Huynh–Feldt correction. The heating and supplementation treatments were included in all ANOVA models as between-subjects factors; all initial models also included sex of last-hatched nestling (25 male, 13 female) and HA (synchronous or asynchronous) as between-subjects factors, as well as all first order interactions. The second-order interaction of heat × sex of last-hatched × asynchrony was also included to test the TSH prediction that last-hatched sons in asynchronously hatching nests would be disproportionately benefited by parasite removal. Non-significant terms were removed using a backward stepwise procedure, and significant terms in the final models were further explored using paired comparisons. Statistical tests were two-tailed, and the significance level was p = 0.05.

Analyses were performed with SPSS (Norusis 2000). Mean values are presented ± 1 SE.

Results

The experimental manipulation was effective at removing nest-dwelling ectoparasites: control nests contained 23.2 ± 4.3 blow fly pupae, while the average infestation of heated nests was 0.6 ± 0.4 pupae (range: 0–9; Mann–Whitney U = 15.0, p < 0.001, n = 38 nests). Only four of 21 heated nests had any detectable blow fly pupae (range: 1–9), whereas all but one control nest (16/17) contained blow flies (range: 5–68).

We used PHA injection to assess the immune response of nestlings in 42 first broods in our study population in 2006; based on mean values for these nestlings, neither mass nor body condition of nestlings was correlated with PHA response (mass: r = −0.05, p = 0.77; condition: r = −0.14, p = 0.36). There were no consistent differences in immune response to PHA within broods (F1.7,49 = 2.03, p = 0.15); however, within-brood variation in immune response depended both on the carotenoid supplement and degree of asynchrony (within-subjects immune response × carotenoids: F1.7,49 = 3.38, p = 0.05; within-subjects immune response × asynchrony: F1.7,49 = 5.94, p < 0.01). In broods that did not receive supplemental carotenoids, PHA response did not differ among nest mates (Fig. 1a; all p > 0.38). In contrast, in supplemented broods, the magnitude of the immune response was greater for nestlings positioned earlier in the hatching sequence, and both early and middle nestlings exceeded the response of their last-hatched nest mate (Fig. 1b; early–middle: p < 0.01; early–last: p < 0.01; middle–last: p = 0.04). This suggests that both middle- and last-hatched nestlings were less likely to invest supplemental carotenoids in immune function than their senior siblings, and have the potential to be more attractive to ectoparasites. In relatively synchronous broods, early- and middle-positioned nestlings did not differ in their immune response (Fig. 2a; p = 0.71); however, early-hatched nestlings tended to exhibit a stronger response than their last-hatched nest mate (Fig. 2a; early–last: p = 0.06), and the response of middle nestlings to PHA was significantly stronger than that of their junior sibling (Fig. 2a; middle–last: p < 0.01). Thus, in synchronously hatching broods, last-hatched nestlings were generally less immunocompetent than all of their senior siblings. In contrast, early-hatched nestlings in asynchronously hatching broods showed a stronger response to the PHA injection compared to their middle-hatched siblings (Fig. 2b; early–middle: p = 0.03), but, contrary to the prediction of the TCH, the immune response of last-hatched nestlings did not differ from any of their siblings when they hatched asynchronously (Fig. 2b; p > 0.18). Although last-hatched nestlings were more likely to be male (25/38; χ² = 3.79, DF = 1, p = 0.05), sex of the junior nestling did not predict their relative immune response under any conditions (F1.7,49 = 0.02, p = 0.97). These within-brood patterns were not driven by differences in average immune response for the brood, since mean PHA response for asynchronously hatching broods did not differ
from that of synchronously hatching broods (independent samples t = 0.127, DF = 31, p = 0.90).

Early-hatched nestlings grew their eighth primary feathers faster than both middle- and last-hatched nest mates, as shown by a significant within-subjects effect (F1.6,57 = 4.59, p = 0.02; early-middle, mean difference in growth rate: 0.07 ± 0.03, p = 0.02; early-last, mean difference: 0.15 ± 0.05, p = 0.01). Middle-ranked nestlings did not, however, differ from their last-hatched sibling (mean difference: 0.07 ± 0.05, p = 0.20), and this overall pattern was irrespective of the degree of HA, sex of the last-hatched nestling, presence of parasites or carotenoid supplementation (all p > 0.13). The within-brood analysis of tarsus growth rate similarly showed early-hatched nestlings exceeded all of their junior nest mates, but middle-ranked nestlings also grew their tarsus faster than their last-hatched sibling (F1.7,59 = 35.68, p < 0.001; early-middle, mean difference in growth rate: 0.01 ± 0.003, p = 0.001; early-last: 0.04 ± 0.005, p < 0.001; middle-last: 0.02 ± 0.005, p < 0.001). This pattern was similarly evident regardless of HA, last-hatched sex, parasites or supplemental carotenoids (all p > 0.10). Rate of body mass growth did not consistently vary with position in the hatching sequence under any conditions (all p > 0.10).

Consistent with their faster rate of feather growth, early-hatched nestlings had longer eighth primary feathers prior to fledging compared to all of their siblings (within-subjects effect: F1.8,64 = 23.0, p < 0.001); mean difference in feather length, early–middle: 0.77 ± 0.33 mm, p = 0.03; early–last: 2.79 ± 0.50 mm, p < 0.001); however, middle nestlings also exceeded last-hatched nest mates in primary feather length (mean difference, middle–last: 2.02 ± 0.43 mm, p < 0.001). This was not further influenced by sex of the last-hatched nestling, degree of HA or experimental conditions (all
p > 0.22). Despite significant within-brood differences in rate of tarsus growth, final size attained varied only marginally according to relative hatching position (F2,70 = 2.81, p = 0.07). Paired comparisons showed that early-hatched nestlings were larger than their last-hatched nest mate (mean difference: 0.20 ± 0.9 mm; p = 0.03), but did not differ from middle-ranked nestlings (mean difference: 0.04 ± 0.08 mm; p = 0.65). Tarsus length of middle nestlings also did not differ from that of the last-hatched nest mate (mean difference: 0.16 ± 0.10 mm; p = 0.11). Thus, tarsus and feather growth and size at fledging exclusively reflected the position of a nestling in the hatching sequence, regardless of the degree of hatching asynchrony, immune enhancement or presence of parasites. In contrast, within-brood variation in mass at day 15 depended on the presence of parasites, indicated by a significant interaction between the heat treatment and within-subjects factor (F2,70 = 3.66, p = 0.03). In parasitized nests, body mass of nest mates did not consistently differ (Fig. 3a; all paired comparisons, p > 0.14); however, in nests that had parasites removed by heating, middle-ranked nestlings performed better than both their early- and last-hatched siblings, suggesting that removal of nest-dwelling ectoparasites disproportionately benefited nestlings positioned in the middle of the hatching sequence (Fig. 3b; early–middle: p = 0.03; early–last: p = 0.64; middle–last: p < 0.01).

Discussion

Across all broods, last-hatched nestling mountain bluebirds were less likely to invest supplemental carotenoids in immune function compared to their siblings (Fig. 1). This suggests that last-hatched nestlings may prioritize other developmental processes such as somatic growth over immune function, or are less able to absorb and assimilate dietary carotenoids; in either case, this supports the hypothesis that these smallest nestlings may, under at least some conditions, represent a low-cost resource to parasites. However, within-brood patterns of immune response in relation to HA were opposite to the prediction of the TCH: last-hatched nestlings had a less robust immune response compared to their siblings in relatively synchronous broods, but did not differ from their senior siblings in asynchronously hatching broods (Fig. 2). Since PHA response has been shown to be related to the resistance of altricial nestlings to ectoparasites (Tschirren et al. 2007), based on this evidence alone, last-hatched nesting bluebirds in synchronous broods would be expected to be more heavily parasitized than in asynchronous broods. Consequently, these results do not indicate a potential role of HA in allowing female birds to maximize their reproductive success within the ecological context of ectoparasitism.

Despite these within-brood differences in immune response, last-hatched nestlings did not show a disproportionate benefit from removal of nest-dwelling ectoparasites or carotenoid supplementation in any measure of growth or size at fledging, indicating that parasites do not feed preferentially on the smallest nestlings in this host-parasite system, regardless of the degree of asynchrony or relative immunity alone. In contrast, middle-ranked nestlings were heavier at fledging compared to all of their siblings in nests that had parasites experimentally removed (Fig. 1b). This supports the hypothesis that parasites balance host quality against host resistance, leading to greater parasite pressure on nestlings positioned in the middle of the hatching sequence. Indeed, these nestlings were intermediate in most measures of growth and size, and thus likely of intermediate resource quality for parasites, and under some conditions were less immunocompetent than their larger, presumably higher-quality, siblings (Fig. 1b, 2b). Studies of bird-parasite systems are increasingly incorporating optimal host-choice strategies for parasites that take into consideration not only cost avoidance (Christe et al. 1998), but also maximization of energy gain (Bize et al. 2008, Václav et al. 2008); this approach may be of particular value for studies of nest-dwelling avian ectoparasites that feed intermittently, and are therefore readily able to sample multiple hosts when foraging.
Nestlings hatching last in our study population were more likely to be male, however, the relative performance of last-hatched nestlings was unrelated to their sex, regardless of the degree of HA, or whether nests were parasitized. This indicates that male bluebirds positioned last in the egg-laying and hatching sequence, particularly those at a substantial initial size disadvantage due to HA, are not disproportionately affected by parasites. In fact, for sexually dimorphic species that exhibit sex differences in growth patterns, males may perform better under parasite exposure when they hatch later relative to their nest mates. For example, within broods of house finches *Carpodacus mexicanus*, later hatching positions were biased toward sons when females were exposed to parasitic mites during egg-laying (Badyaev et al. 2006). This was suggested to be an adaptive maternal response because late-hatched sons were more likely to survive in parasitized nests compared to sons positioned early in the hatching sequence (Badyaev et al. 2006). Since female finches were exposed to mites during egg laying, however, these observed patterns of nestling growth and survival may have been partly a result of parasite-induced physiological responses of females that affected offspring differentially depending on hatching order and sex (e.g. variation in investment of immunoglobulins in egg yolk, Gallizi and Richner 2008; or variation in yolk androgen investment across the laying sequence, Navara et al. 2006, Tobler and Sandell 2009). Since blow flies are the most abundant parasites affecting nestlings in our study system, and these parasites neither feed on adult birds nor are present in nests during egg laying (Sabrosky et al. 1989), such parasite-induced maternal responses would not be expected. Despite the sex ratio bias of this hatching position, our lack of evidence for sex differences in parasite susceptibility of late-hatching nestling bluebirds, combined with the contrasting results of Badyaev et al. (2006) when female finches were exposed to parasites during egg laying, suggest the role of maternal effects in within-brood comparisons such as these may be considerable. Future studies should therefore examine the influence of interactions between pre-hatching parasite-induced patterns of maternal investment and sex-specific physiology of nestling birds on susceptibility to ectoparasites.

Immune function in vertebrates represents a complex outcome of interactions among nutritional, endocrine and genetic components (Casto et al. 2001, Brzék and Konarzewski 2007, Owen et al. 2008), and altricial young in particular encounter significant tradeoffs between rapid somatic growth and development of the immune system (Soler et al. 2003). In birds, HA may generate variation in the magnitude of such physiological tradeoffs experienced by individual nestlings within broods (Martin-Vivaldi et al. 2006); however, the degree of HA may itself be mediated by several factors. Within-brood variation in timing of hatching can be influenced by embryo development time, particularly between embryos of different sexes (Cook and Monaghan 2004). Factors influencing timing of incubation onset by female birds have received the greatest research attention as predictors of HA, since incubation initiated prior to clutch completion allows early-laid eggs to begin development sooner, and thus to hatch earlier, than later-laid eggs. For example, in several bird species, females provided with supplemental food initiated incubation earlier and thereby produced more asynchronously hatching broods, suggesting that incubation represents an energetic constraint (Nilsson 1993, Eikenaar et al. 2003, but see Wiebe and Bortolotti 1994 for the opposite pattern in American kestrels, *Falco sparverius*). In this study, we show that last-hatched nestling mountain bluebirds are less immunocompetent than their larger siblings in synchronously hatching broods, but not in broods that hatch asynchronously (Fig. 2). This suggests that resources may have been distributed more evenly among nestlings (either pre- or post-hatching) in asynchronously hatching broods, allowing last-hatched nestlings to maintain both immunity and growth despite their significant size disadvantage at hatching. This pattern may be explained by differences in food availability: as shown by Nilsson (1993) and Eikenaar et al. (2003) in other bird species, female bluebirds with access to more food may have initiated incubation earlier (prior to clutch completion) while also investing resources more equitably among all eggs or nestlings. We do not have data on food availability for this population; however, we suggest that the proximate influence of food availability early in the breeding season on female incubation behaviour requires further investigation in a greater range of bird species, since it may have important consequences for hatching patterns and within-brood variation in nestling performance.

Preferences of ectoparasites for specific hosts may vary depending on the ecology and life history traits of the parasite (Roulin et al. 2003). Our results suggest that, at least for host–parasite systems involving intermittently-feeding, nest-dwelling ectoparasites such as blow flies, optimality models may inform predictions related to host selection, since middle-ranked nestlings showed disproportionate benefits from experimental parasite removal. Little is known, however, about the feeding and host selection behaviour of ectoparasites, or the fitness consequences thereof; we suggest this represents an important area for future research (Tschirren et al. 2007, Gallizi and Richner 2008), since the relevance of optimality models to questions concerning host selection by parasites depends on a greater understanding of these behaviours.


