Immunocompetence and its costs during development: an experimental study in blue tit nestlings

Jon E. Brommer

Bird Ecology Unit, Department of Ecology and Systematics,
PO Box 65 (Viikinkaari 1), University of Helsinki, FIN 00014
Helsinki, Finland (jon.brommer@helsinki.fi)

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The allocation into T-cell-mediated immunocompetence was experimentally increased in 68 out of 139 nestlings by supplementary feeding methionine to half of the nestlings in 15 blue tit nests. Methionine-supplemented nestlings had an increased T-cell-mediated immunocompetence, but a reduced growth compared with control siblings. Nestlings that had low initial weights and nestlings that were supplemented with methionine had an increased mortality risk. The investment that nestling blue tits make in immunocompetence appears to be tightly controlled by survival costs paid through a trade-off between immunocompetence and growth.

Keywords: ecological immunology; trade-off; phytohaemagglutinate assay; methionine; life history

1. INTRODUCTION

Immunocompetence (the ability to withstand disease and parasites) is a flexible physiological component in developing vertebrates (Klasing & Leshchinsky 1999; Janeway *et al.* 2001). Understanding the functional aspects behind such a physiological trade-off can have ecological consequences because conditions during early development may have long-term effects on individual performance (Lindström 1999). Consequently, offspring immunocompetence has received increasing attention from ecologists interested in linking initial conditions to ecological and evolutionary scenarios (Norris & Evans 2000).

Offspring immunocompetence is thought to be an inducible defence against parasitism (Szép & Møller 1999). In wild bird nestlings, manipulation of parasite load has shown that nestling immunocompetence and growth are negatively correlated traits (Saino *et al.* 1998; Szép & Møller 1999). An increased investment in immunocompetence may therefore result in a size reduction or an increase in developmental time that both could lead to an increased mortality risk (Martin *et al.* 2001). Both immunocompetence and survival are condition-dependent traits (Sheldon & Verhulst 1996). Therefore, the investment in immunocompetence needs to be manipulated directly to study whether such investments indeed show a trade-off with other fitness components and what are the costs of such a trade-off

(Lochmiller & Deerenberg 2000; Norris & Evans 2000; Soler *et al.* 2003).

Dietary methionine supplementation induces increased T-cell-mediated immunocompetence in broiler chickens, *Gallus domesticus* (Tsiagbe *et al.* 1987; Swain & Johri 2000). Recently, Soler *et al.* (2003) showed that supplementing methionine to passerine (magpie, *Pica pica*) nestlings induces a higher T-cell-mediated immunocompetence and reduces growth. Methionine, a sulphur amino acid, appears to be required in higher amounts than endogenous sources supply (Grimble & Grimble 1998). Insufficiency of sulphur amino acids compromises the synthesis of glutathione, a substance that augments the activation of T cells (Dröge *et al.* 1986). In addition, sulphur amino acids themselves may directly affect T-cell production (Kinscherf *et al.* 1994). Methionine supplementation hence changes the priority rules (*sensu* Zera & Harshman 2001) of resource allocation (Soler *et al.* 2003). Here, I study costs (in terms of a reduction in growth and survival) of an experimentally induced exaggerated allocation into T-cell-mediated immunocompetence in blue tit (*Parus caeruleus*) nestlings.

2. MATERIAL AND METHODS

(a) Study area and species

Blue tits were studied in a study area of 6 km² in southern Finland in 2003. All pairs bred in nest-boxes. The laying date of the first egg was established by finding the incomplete clutch through weekly checks of the nest-boxes. Nests were checked daily around the estimated time of hatching. Hatching dates were set at the first day on which at least one chick had hatched.

(b) Experimental protocol

Nestlings were induced to allocate more resources to their T-cell-mediated immune defence by supplementing them with DL-methionine (Sigma code M-9500). When the brood was 5 days old, the feet of all chicks were individually coloured using felt-tip waterproof markers and all nestlings were weighed (accuracy of ± 0.05 g). Nestlings were ordered by their weight and the treatment of the heaviest chick was decided at random. The treatment of all other chicks then alternated down the size hierarchy (see also Soler *et al.* 2003). The individual markings on the feet of the nestlings were remade at every consecutive visit until chicks could be ringed (at around days 8–10). Methionine-supplemented nestlings received a specific volume of 0.1 g methionine suspended in 1 ml water for 5 consecutive days, starting when the brood was 5 days old (day 5). Control nestlings received the same amount of water during this period. Chicks were administered 100 μ l of fluid (containing either methionine or not) at days 5 and 6, 150 μ l at day 7 and 200 μ l at days 8 and 9. The volume was increased during the 5 day period of methionine supplementation because nestlings increase by ca. 1 g in body mass daily. Dosage was based on administering ca. 2 mg methionine per 1 g of chick body weight.

(c) Immunocompetence

Twelve days after hatching, chicks' T-cell-mediated immune response was measured in a modification of the phytohaemagglutinate assay protocol described by Smits *et al.* (1991). Nestlings were weighed and three nestlings (with heaviest, lightest and average weights) from each treatment were chosen for further measurement. Small feathers on the left patagium (wingweb) of these birds were removed and the thickness of a spot marked with a waterproof marker was measured using a thickness gauge (AlPa, Italy). The spring of this gauge had been removed and instead a 15 g weight was attached. The thickness of the patagium was measured again 24 h (± 0.5 h) after injecting into the patagium 0.04 ml of a solution of 5 mg of lectin (Sigma code L-8754) per 1 ml of isotonic water (9 mg NaCl ml⁻¹, Fresenius Kabi Norway). The swelling was measured twice (pairwise correlation $r = 0.93$, $n = 90$, $p < 0.001$). T-cell-mediated immunocompetence was calculated as the average of the two post-injection measurements minus the pre-injection measurement. A higher response indicated a stronger T-cell-mediated immunocompetence.

(d) Analysis

The protocol was performed in 15 nests. All analyses were done on this dataset within the framework of a generalized linear mixed model (GLMM) including 'nest' as a random factor and all other explanatory variables as fixed effects. For the analysis of mortality risk, binomial errors and a logit link were used. Nestlings that died before fledging were scored as '1' and survivors as '0' and one nest was excluded because it was predated just before fledging. The relationship between growth and immunocompetence on the individual level was studied by comparing pairs of methionine-supplemented and control nestlings. In each nest, one methionine-supplemented (M) and one control (C) nestling with the smallest difference in their weight on day 5 was chosen for this comparison (average weight at day 5 of these nestlings: 5.0 ± 0.1 g ($n = 30$), average difference (M - C): -0.02 g [range of -0.1 to 0.1 g], test for difference from zero: $t = -0.9$, d.f. = 14, $p = 0.4$). Analyses were performed in S-PLUS (Insightful Corporation, USA).

3. RESULTS

Methionine supplementation increased the T-cell-mediated immunocompetence of nestlings (figure 1a, table 1a). In addition, T-cell-mediated immunocompetence was condition-dependent: nestlings that were heavier at day 12 had a higher immunocompetence (figure 1a, table 1a). Methionine supplementation led to a lower increase in weight between day 5 and day 12, but nestlings increased equally in weight independent of their initial weight (figure 1b, table 1b). Hence, methionine-treated nestlings lost rank within the brood's size-hierarchy.

Methionine treatment had a correlated effect in that it both reduced an individual's expected size at day 12 (figure 1b, table 1b) and the expected T-cell-mediated immunocompetence was dependent on the weight at day 12 (figure 1a, table 1a). To visualize the direction of the trade-off between growth and immunocompetence, the difference in growth and immunocompetence was compared for initially equal-sized pairs of methionine-supplemented and control siblings (one pair per nest). For 11 of these 15 pairs, the methionine-supplemented nestling had a lower weight on day 12 than its control sibling (figure 2). The average weight of these nestlings on day 12 was 10.0 ± 0.2 g and the difference between methionine-treated and control nestlings was therefore in some cases substantial (figure 2, average difference in weight at day 12: -0.6 ± 0.2 g). Notwithstanding their lower weight, methionine-treated nestlings still tended to have a higher immunocompetence than their control siblings (figure 2, $F_{1,13} = 4.0$, $p = 0.07$), which is consistent with individuals trading-off immunocompetence against their growth.

Owing to overall poor weather, the nestling mortality was substantial in 2003. Nestlings with a low initial weight were more likely to suffer mortality (table 1c). In addition, an exaggerated allocation into immunocompetence increased mortality probability (table 1c) and 23% (15 out of 64) of methionine-treated nestlings died versus 12% (8 out of 67) of control nestlings.

4. DISCUSSION

Supplementary feeding of methionine induces blue tit nestlings to allocate more resources into their T-cell-mediated immunocompetence. These nestlings suffer a reduction in their growth, causing an individual to lose rank in the within-brood size hierarchy and—probably coupled to this—an increased mortality risk. An exaggerated allocation to T-cell-mediated immunocompetence

Figure 1. The trade-off between growth and immunocompetence. Plotted are the individual data points with methionine-supplemented (filled circles) and control nestlings (open circles). (a) T-cell-mediated immunocompetence as a function of treatment and nestling weight at the time of measuring the immunocompetence. (b) Growth (increase in weight between days 5 and 12) plotted against initial weight for both methionine-supplemented (filled circles) and control nestlings (open circles). See table 1 for sample sizes and statistics.

therefore trades-off with growth and has clear (survival) costs in blue tits nestlings.

In the only other study to date that has directly manipulated allocation to immunocompetence in the wild, Soler *et al.* (2003) found that growth in magpies was initially reduced while T-cell-mediated immunocompetence was increased. However, neither fledging condition nor survival to fledging was affected (Soler *et al.* 2003). Nevertheless, Soler *et al.* (2003) supplemented methionine relatively early during development and measured T-cell-

Table 1. T-cell-mediated immunocompetence, growth and mortality of blue tit nestlings explained by condition (weight) and methionine supplementation (treatment).

(Data on 15 nests were used, but one nest was excluded for the mortality analysis owing to predation. Presented are the results of GLMMs. (a) Phytohaemagglutinate assay (PHA) response on three methionine-supplemented and three control nestlings per nest ($n = 90$). (b) Growth measured as the increase in weight between day 5 and day 12 of 118 nestlings that survived to day 12. (c) Probability of mortality before fledgling for 5-day-old nestlings ($n = 131$). Coefficients for fixed effects are presented with their standard errors. Nest was fitted as a random effect and differed significantly from zero in all three models. Interactions were always insignificant and the constant was always included (not reported).)

variable	coefficient ($\pm$ s.e.)	test statistic	<i>p</i>
(a) PHA response			
weight at day 12	0.03 ± 0.01	$F_{1,73} = 6.3$	0.01
treatment	0.06 ± 0.02	$F_{1,73} = 7.2$	0.009
(b) growth			
weight at day 5	-0.04 ± 0.08	$F_{1,101} = 0.96$	0.33
treatment	-0.65 ± 0.11	$F_{1,101} = 35.0$	< 0.001
(c) mortality			
weight at day 5	-1.0 ± 0.2	$F_{1,115} = 11.5$	< 0.001
treatment	0.6 ± 0.2	$F_{1,115} = 4.6$	0.03

Figure 2. The trade-off between immunocompetence and weight for individual nestlings. Plotted is the difference in PHA response against the difference in weight at day 12 between methionine-supplemented (M) and control (C) siblings. A positive difference indicates a higher value for the methionine-supplemented nestling. Data are for 15 nests with one methionine-supplemented and one control sibling each, where the siblings had about equal weights before methionine treatment (at day 5).

mediated immunocompetence and fledgling condition proportionally later (10 days after the period of methionine administration) than was done in this study (2 days). Nestlings in their study therefore had more time during the late nestling period to compensate for the methionine-induced reduction in their growth. Both descriptive and experimental studies have found that growth and immunocompetence are mediated by parasite abundance (Szép & Møller 1999; Møller *et al.* 1999; Merino *et al.* 2000;

Møller *et al.* 2003). Hence, combined manipulation of immunocompetence and parasite load is needed further to explore the fitness consequences of immunocompetence and growth during the nestling period.

Ecological immunology is increasingly focussing on developmental processes, because such processes may have long-term effects on individual performance (Klasing & Leshchinsky 1999; Lindström 1999; Norris & Evans 2000). Whereas the importance of body weight for future recruitment is well documented in birds (Amundsen & Slagsvold 1998), it is currently unclear what the selective advantages of an increased immunocompetence are for post-fledgling survival. The putative fitness benefits of an increased offspring immunocompetence have been invoked to explain, for example, parental care (Saino *et al.* 1997) and selective mate choice (Johnsen *et al.* 2000). However, evidence linking offspring immunocompetence to their long-term performance is currently correlative and hampered by the positive condition dependence of both immunocompetence and other traits promoting survival (Sheldon & Verhulst 1996; Lochmiller & Deerenberg 2000; Norris & Evans 2000). Studying the long-term consequences of an experimentally altered allocation into immunocompetence is needed to understand not only the costs but also the ultimate benefits of investments in immunocompetence that blue tits make during their nestling period.

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