Effects of a Dipteran Ectoparasite on Immune Response and Growth Trade-Offs in Barn Swallow, Hirundo rustica, Nestlings

Author(s): Nicola Saino, Stefano Calza and Anders Pape Møller

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Parasites can have a profound effect on biology and evolution of the hosts, which are expected to have evolved physiological and developmental mechanisms that allow them to minimise the costs imposed by parasites. In this study we analyse the effects of a dipteran ectoparasite on barn swallow (*Hirundo rustica*) nestling biology including rate of somatic growth, plasma protein concentration, blood cell sedimentation rate, hematocrit, concentration of leukocytes in peripheral blood, and T-lymphocyte cell-mediated immunocompetence. In a natural population, intensity of parasite infestation was positively correlated with growth of feathers. Nestlings in heavily infested nests may decide to allocate more resources to feather growth thus fledging early. To test this hypothesis, the detrimental effects of parasites on nestlings, and the existence of trade-offs between competing growth processes, we inoculated some nests with additional flies. Nestlings exposed to increased infestation had larger rate of feather growth but were in poorer condition than unmanipulated controls. Parasite inoculation resulted in larger concentrations of eosinophils and lymphocytes. Among siblings of broods inoculated with parasites, those that had the largest rate of feather growth had the lowest rate of increase in tarsus length and body mass. We conclude that louse flies depress barn swallow nestling condition and influence their immune profile. However, they also enhance growth of a morphological character that may allow nestlings to reduce the impact of parasites. Nestlings apparently experience a trade-off between the competing demands for growing feathers and other somatic characters.

N. Saino and S. Calza, Dipartimento di Biologia, Sezione di Zoologica Scienze Naturali, Università di Milano, Via Celoria 26, I-20133 Milano, Italy (n.saino@imiucca.csi.unimi.it). – A. P. Møller, Laboratoire d'Ecologie, CNRS URA 258, Université Pierre et Marie Curie, 7 quai St. Bernard, Case 237, F-75252 Paris Cedex 05, France.

Parasites are likely to have an influence on many aspects of the evolutionary biology of birds (Price 1980). Although theoretical and experimental studies recently have indicated the relevance of interactions between parasites and their avian hosts for a variety of processes, ranging from development to reproduction and sexual selection (e.g. Loye and Zuk 1991), there is a general lack of comprehensive information on the effects of parasites on physiology and immunology of their hosts under natural conditions.

A number of observational and manipulative studies at the individual host level have demonstrated effects of parasites on dispersal (Brown and Brown 1992), choice and desertion of the nesting site (Duffy 1983, Mappes et al. 1994) colony occupation (Emlen 1986, Chapman and George 1991), sexual displays (Møller 1990a, 1991a, Clayton 1991), timing of breeding and optimal clutch size (Møller 1991b), and hatching and fledging success (Merino and Potti 1995). Studies of the effect of ectoparasites on growth of nidicolous offspring have

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reached quite variable conclusions, either showing negative or no correlations between intensity of infestation and somatic growth (e.g. Moss and Camin 1970, Møller 1990a, Johnson and Albrecht 1993). Similarly, in a few studies where physiology of nestlings has been analysed, either negative or no relationships have been found with intensity of parasite infestation (Shields and Crook 1987, Whitworth and Bennett 1992, Johnson and Albrecht 1993).

By definition, parasites subtract energy or materials from their hosts and, other things being equal, parasite infestation is expected to result in some degree of depression of host fitness compared to a condition of no infestation. Life history theory predicts that natural selection favours the evolution of physiological mechanisms that ensure optimal allocation of limited resources by individuals to competing activities (Stearns 1992). When infested by parasites, hosts are expected to invest more in those activities that will ensure the maximum reward in terms of countering infestation by parasites or its fitness effects. When the current resource budget is limited, hosts experience a trade-off between energy demanding activities, and increased investment in parasite defence will be to the detriment of other functions.

In the present study, our main aims were to experimentally analyse 1) the effect of infestation by a dipteran ectoparasite (the hippoboscid fly *Ornithomyia biloba* Dufour 1827) on several aspects of morphology and physiology of nestling barn swallows (*Hirundo rustica*), and 2) the effect of physiological trade-offs, under experimentally increased parasite infestation, on growth and physiology of nestlings while controlling for genetic and environmental effects.

Our general predictions were that parasite infestation depressed host condition. However, if the effect of parasites was not very severe, nestlings exposed to high levels of parasitism might have shown enhanced growth or immune functions most likely to counter the effect of parasites.

In the first study year we analysed the relationships between intensity of louse fly infestation and nestling growth and physiology under natural conditions. In the second year we experimentally increased the level of parasite infestation in some nests, and analysed the effect of parasite manipulation on nestlings using an extended set of physiological and immunological variables compared to the previous year. We measured rectrix length indicating feather development, tarsus length indicating body size, and body mass. As serological measures of condition we considered plasma protein content, blood cell sedimentation rate, and hematocrit. Avian species are known to respond to parasitism and infectious diseases by increasing concentrations of leukocytes, mostly heterophils and lymphocytes (Rose et al. 1979, Davis 1981, Hawkey et al. 1983, Averbeck 1992). Increase of lymphocytes and polymorphonuclear leukocytes, for example, has been suggested to reflect inflammatory processes and infection determined by parasite infestation (Lucas and Jamroz 1961). We therefore analysed the immunological profile of nestlings by estimating the concentration of five different types of leukocytes (basophils, eosinophils, heterophils, lymphocytes and monocytes) in relation to red blood cells.

The ability to mount a T-lymphocyte cell-mediated immune response can be evaluated, in vivo, by an experimental challenge with phytohemagglutinin, a lectin, injected intradermally (Cheng and Lamont 1988). The physiological events associated with the response to PHA have been described by Goto et al. (1978) and McCorkle et al. (1980). Macroscopically, injection in the wing web of birds results in erythema, induration and thickening. Increase of wing web thickness 24 h post-injection has been considered a measure of the intensity of the cellular immunity in poultry (Goto et al. 1978, McCorkle et al. 1980).

The host

The barn swallow is a migratory, socially monogamous, colonial passerine which feeds on the wing on flying insects. In southern European regions, adults arrive to the breeding areas in February–March. Breeding pairs usually settle in rural buildings, mainly cow stables, forming colonies of few to several tens of pairs which either refurbish nests from previous years or build new ones. Nests are often very close to each other, the modal nearest-neighbour distance being 2 m, and some nests can be 20 cm apart. Only females incubate 1–3 clutches of 1–7 eggs that hatch usually after 14 d of incubation. Offspring are fed by both parents during the nestling period (17–21 d) and also for some days after fledging. Barn swallow are infested by a variety of ectoparasites, including mites (*Ornithonyssus bursa*), Mallophaga (*Machaerilaemus malleus*, *Myrsidea rustica*), and Diptera (*Protocalliphora hirundo*, *Stenepteryx hirundinis*, *Ornithomyia biloba*). Mites and blow flies have been shown to negatively affect nestling condition and survival (Shields and Crook 1987, Møller 1994).

The parasite

Information reported below is obtained from Bequaert (1953), Kemper (1951) and references therein, and from personal observations. Hippoboscid flies (Diptera) are obligate blood-sucking ectoparasites of birds and mammals. The louse flies we studied belong to a species, *Ornithomyia biloba*, which is a specialised parasite of barn swallows. It is a medium-sized (body length: 6–7 mm) adenotrophic, viviparous holometabolous fly. In the swallow breeding areas, adult parasites are found

on hosts and in their nests already a few days after arrival, and infestation in most years increases as the season progresses. As far as we are aware, the life cycle of this species has not been examined in detail. In other hippoboscid species, larvae have intrauterine development during which they undergo two moults. In *Melophagus ovinus*, for example, a single non-feeding motionless prepuparium is voided per reproductive cycle and adults take 19–23 d to emerge. The first parturition occurs 14–15 d after emergence of the female from the puparium, so that generation time is 33–38 d. Generation time in other related species is similar, suggesting that also *Ornithomyia biloba* might have a similar life cycle. Parturitions of *Ornithomyia biloba* usually occur weekly, the prepuparium being deposited in the swallow nest. Overwintering puparia, produced in the autumn, are known to develop in adults in early spring. The information about the frequency and size of the meals is inadequate to accurately calculate the total amount of blood imbibed during the average life cycle of *Ornithomyia biloba* as well as other species. However, from the data available for a related species, *Crataerina pallida*, it can be estimated that each adult fly may take up, during the nestling period, more than 8% of the total blood volume of an average, 12 d old nestling (see also Lee and Clayton 1995).

Methods

The study was conducted during the barn swallow breeding season of 1995 and 1996 in one colony located in a single stable a few kilometres east of Milano (Northern Italy). Swallows were caught at sunrise by mist nets starting on 28 March at maximum intervals of seven days until all the individuals had been captured. Each individual was marked with a metal ring on one leg, a plastic colour ring on the other, and a unique combination of colours on breast and belly feathers. At first capture, individuals were sexed according to the shape of the cloacal protuberance and by inspection for presence (female) or absence (male) of an incubation patch. Assignment was confirmed at later recaptures by inspection of the incubation patch and by observation of sexual and breeding behaviour. Males and females were assigned to individual territories and nests located under the stable roof by close observation of colour rings and markings. Nests were inspected usually every second day to record reproductive events, and every day during egg laying and around the estimated hatching date, 14 d after laying of the last egg. To allow identification of individual nestlings, in the season of 1996 each nestling was given a standard numbered metal ring six days after hatching, when morphology and body mass were recorded for the first time (see below).

During the first study year we did 2–3 capture sessions every week to count the parasites on adults.

Parasite counts and inoculation

Louse flies can readily be seen in the plumage of barn swallows. To extract the parasites from the plumage, we used an apparatus consisting of a glass container in which chloroform vapour could be insufflated. The swallow was kept with the head outside the container while the body was exposed to chloroform vapour for approximately 60 s. Anaesthetised louse flies usually fell to the bottom of the container, but we also carefully inspected the whole plumage to check for any parasites left. After each count, louse flies were allowed to recover from the effects of chloroform by leaving them in a vial for approximately 0.5 h while their original host was left in a bag. Fully recovered louse flies were then put in the bag containing their original host a few minutes before release.

To count louse flies in the nests, we removed all the nest material and immediately put it in a hermetic plastic bag. Chloroform was then added to the bag and the nest material carefully inspected by placing it in the same glass container used for extraction of parasites from adults. We also checked for the presence of louse flies left in the nest. The nest material was then put back in the nest together with the parasites. In the first study year we counted parasites in the nests only when nestlings were 12 d old. In 28 control nests of the second study year we repeated this procedure 6, 9, and 12 d after hatching. In 15 nests in the second study year we inoculated the same number of louse flies we found at the time of inspection of the nest material, so that the number of parasites post-inoculation in the nest was twice the number we found at the time of inspection. Louse flies due to be inoculated were extracted on the same day of inoculation from nests that were not included in the experiment. We also inspected the nests two days after that nestlings had fledged to estimate abundance of mites by placing one hand on the nest rim and estimating the number of mites on the hand after 10 s. The abundance of mites was then expressed in five categories: no mites (0), 1–10 (1), 11–100 (2), 101–999 (3), 1000 or more (4). This method has been shown to give estimates of mite numbers strongly and positively correlated with the number of mites that can be extracted from the nest (Møller 1991a).

Nestling measurements

During both study years we measured length of the left innermost rectrix with a ruler with a precision of 0.5 mm, length of the right tarsus with a calliper with a precision of 0.05 mm, and body mass with a spring

Table 1. Kendall's τ simple correlation coefficients between louse fly count in the nest and number of eggs laid, number of eggs at hatching and number of fledglings. Sample size is 36 for 1995 and 28 for 1996.

Year	Eggs laid	Clutch size at hatching	Number of fledglings
1995	$\tau = -0.26$	$\tau = -0.44$	$\tau = -0.36$
	$p = 0.06$	$p < 0.001$	$p < 0.01$
1996	$\tau = 0.14$	$\tau = 0.07$	$\tau = 0.08$
	n.s.	n.s.	n.s.

balance with a precision of 0.1 g. During the second study year, however, we also recorded these measures 6 d after hatching, i.e. at the time of parasite load manipulation in the nests. In the second study year, change of morphology and body mass between days 6 and 12 was expressed as the difference between the measures recorded on day 12 and those recorded on day 6.

Blood sampling and measurement of serological variables

When nestlings were 12 d old we took a blood smear and a blood sample sized, on average, 60 µl in heparinised hematocrit capillaries. To measure blood cell sedimentation rate, capillary tubes containing blood samples were put in vertical position for 4 h in a refrigerated room (4°C) after having been stored horizontally in a cool bag in the field. Sedimentation rate (proportion of blood sedimented/h) was expressed as volume of the part of the capillary occupied by plasma × (blood volume in the capillary)$^{-1}$ × 0.25. Blood samples were then centrifuged for 10 min (at 4000 rpm in the first year and at 11 500 in the second year) and hematocrit was expressed as volume of the part of the capillary occupied by blood cells × (blood volume in the capillary)$^{-1}$. Plasma was then stored at −30°C. Protein assay was done by spectrophotometric analysis of opportunely diluted small amounts of plasma following Bio-Rad protein micro-assay procedure (1990), and was expressed as µg/ml of plasma.

Leukocyte counts and concentrations

Leukocytes and red blood cells were counted by an experienced person after blood smears had been air dried and stained by the May-Grunwald-Giemsa staining method. Blood smears were scanned at 630× magnification following standard routines. In each microscopic field we counted red blood cells and leukocytes classified as lymphocytes, monocytes, eosinophils, heterophils, and basophils. In each smear we counted 130 to 170 leukocytes, and the corresponding red blood cells. This allowed us to calculate the number of leukocytes of the different types per 10 000 red blood cells. Even when fewer leukocytes were counted, this method has been shown to give significantly repeatable within-blood smear measures of leukocyte concentration (Saino et al. 1995).

Immunocompetence test

The thickness of the left and right wing webs of nestlings was measured 12 d after hatching by a spessimeter (Alpa S.p.A., Milano, cod. SM112) with an accuracy of 0.01 mm, and the measurement site on both wings was marked with a pen (see also Saino et al. 1997). The right wing web was injected with 0.2 mg of PHA (Sigma, L-8754) in 0.04 ml of phosphate buffered saline (PBS). Twenty-four h later we re-measured the thickness of wing webs at the inoculation sites. To express the reaction to PHA while controlling for the effect of injection per se and thickening due to PBS inoculation, we computed the difference between change in thickness of the right PHA-inoculated wing web (thickness 24 h after inoculation minus thickness just before inoculation) and the change in thickness of the left wing web that only was inoculated with PBS (Lochmiller et al. 1993). Following Lochmiller et al. (1993) values of this index were assumed to be proportional to the intensity of T-lymphocyte cell mediated immunocompetence.

Feeding rate recordings

Feeding rates of male and female parents were recorded during 1 h to 1.25 h daily (excluding Sundays) observation sessions between 06:00 and 10:00 from the day of hatching to the day after PHA treatment. Per capita feeding rates were expressed as number of feeding trips × brood size^{-1} × observation hours^{-1}.

Feeding rates of parents, expressed as (total number of feedings)/(number of observation hours), estimated

Table 2. Kendall's τ simple correlation coefficients between mean within-brood values of left innermost rectrix and tarsus length, body mass, blood cell sedimentation rate, and hematocrit, respectively, and louse fly counts in the nests. Sample size is 35 in all cases.

	Tarsus length	Rectrix length	Body mass	Sedimentation rate	Hematocrit
Louse fly count	−0.05	0.37	−0.01	0.05	0.01
	n.s.	$p = 0.003$	n.s.	n.s.	n.s.

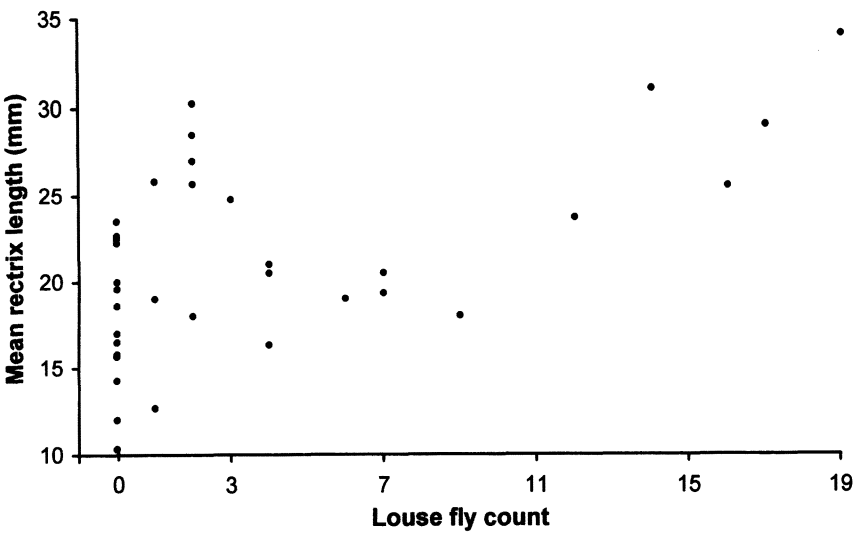

Fig. 1. Mean within-brood left innermost rectrix length in relation to louse fly count in 35 unmanipulated nests on day 12 after hatching. The correlation was positive and statistically significant ($\tau = 0.37$, $n = 35$, $p = 0.003$).

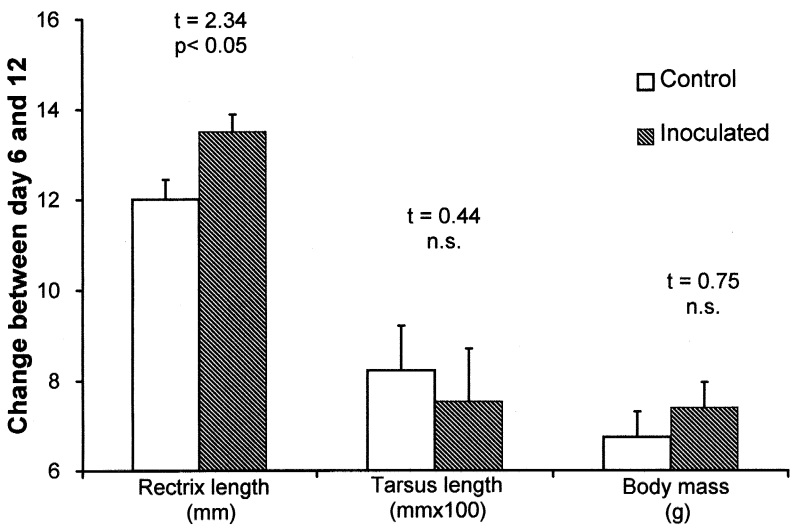

Fig. 2. Mean (+SE) within-brood increase in morphological measures in 25 control broods and 15 broods inoculated with louse flies. Student's t and associated significance values are also shown.

during a part of a given day are known to be positively correlated with feeding rates in other parts of the same day (see Saino et al. 1997). This proportionality allowed us to assume that feeding rates recorded during the morning provided reliable estimates of parental feeding effort of each pair relative to other pairs.

Mean values reported in the Results section are followed by the standard error of the mean.

Results

In the first study year we considered a sample of 36 first broods. During the second year, we considered a total of 43 nests, 15 of which were randomly chosen and inoculated with parasites while the remaining ones were controls. Due to a variety of reasons, in some cases we could not measure one or more variables for a whole brood or one nestling in a brood. In the data analysis, however, we always used the maximum sample size available.

Importantly, during the first study year, when we could capture parents of broods in our sample quite frequently, we found a significant positive association between the number of louse flies in the nest and the total number of louse flies on the two parents (mean for parents 4.98 ± 0.82; correlation with counts in the nest: $\tau = 0.37$, $n = 34$, $p = 0.006$). Moreover, the number of louse flies on a sample of adults was found to be significantly repeatable in two different counts separated by 14 or more days, indicating that individuals that were heavily infested at a given count tended to be heavily infested also two or more weeks later (ANOVA; females: $F = 7.66$, df = 21,22, $p < 0.0001$; males: $F = 2.53$, df = 37,38, $p = 0.003$; repeatabilities computed according to Falconer (1989) were 0.77 for females and

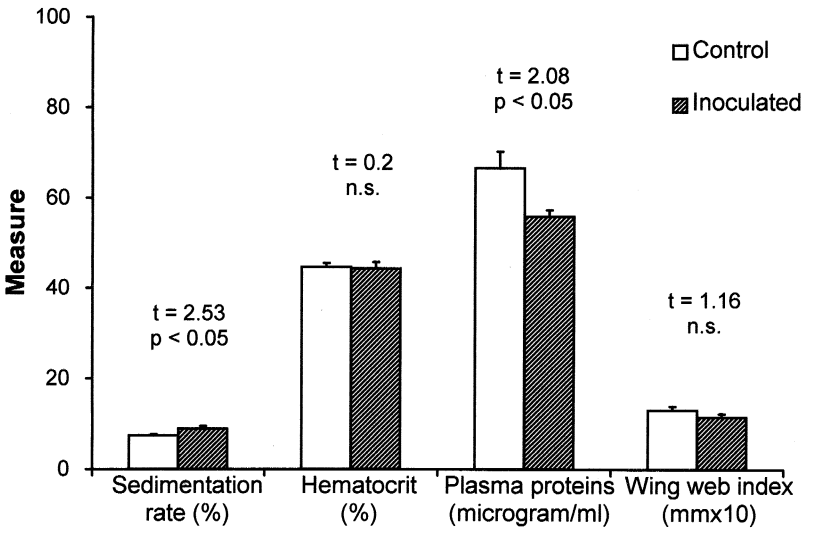

Fig. 3. Mean (+SE) within-brood values of serological variables and an index of T-lymphocyte cell mediated immunocompetence in 28 control broods ($n = 27$ for plasma proteins) and 15 broods inoculated with louse flies. Student's t and associated significance values are also shown.

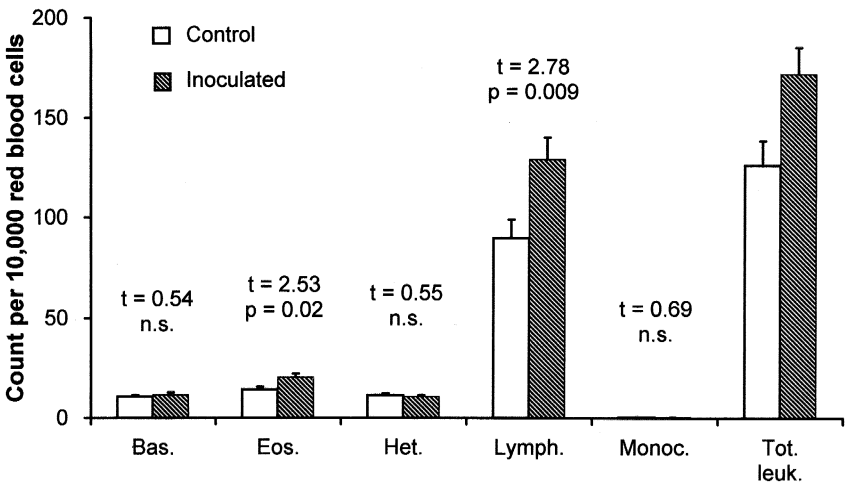

Fig. 4. Mean (+SE) within-brood values of concentration of five types of leukocytes (basophils, eosinophils, heterophils, lymphocytes and monocytes) in 19 control broods and 15 broods inoculated with louse flies. Student's t and associated significance values are also shown.

0.43 for males). These findings suggest that parasite counts on adults provide reliable estimates of intensity of parasite infestation, and counts in the nests were consistently proportional to the total number of parasites infesting each family. Since in the second study year, owing to the intense effort spent on experiments, we could not capture adults sufficiently frequently to allow parasite counts during the nestling period, in all the analyses reported here only parasite counts in the nests have been considered.

Parasite counts and reproduction in the natural population

Prevalence (proportion of infested nests/number of nests inspected) at day 12 in 1995 was similar to that observed in control nest of 1996 (1995: 0.61, 1996: 0.75). However, when we considered the proportion of nests that were found to be infested in any of the inspections of the nest in 1996, the prevalence increased to 0.93.

In the nests of the first study year and in control nests of the second year, parasite counts on day 12 were weakly, but not significantly correlated with breeding date as indexed by day of hatching (1995: $\tau = 0.08$, $n = 36$, n.s.; 1996: $\tau = 0.06$, $n = 28$, n.s.). In the first year we found significant negative correlations between louse fly count on day 12 and the number of eggs that were left in the nest immediately before hatching, and number of fledglings, whereas the correlation with the number of eggs laid was negative and non-significant (Table 1). Post-hatching mortality was very low, and only 2 nestlings out of the 122 hatched in the 36 broods in 1995 died before day 12. Observations of 20 nests revealed that no nestlings died later on before fledging. Hence, the negative correlation between the number of fledglings and intensity of parasite infestation was

Table 3. Analysis of covariance of change in rectrix length, tarsus length, body mass between day 6 and day 12 of age, and plasma protein content, sedimentation rate, hematocrit, and immunocompetence measured on day 12, respectively, in relation to parasite manipulation (parasite inoculation versus control). Cumulative louse fly count in the nest was entered as a covariate. Since a positive correlation was found between rate of feather growth and hatching date in the sample of unmanipulated nests, we also included hatching date as a covariate in the analysis of feather growth.

	Mean sum of squares	F	df	p	Sign of covariation
Rectrix length ($n = 40$)					
Treatment	28.1	10.31	1	<0.005	
Louse fly count	5.0	1.83	1	n.s.	
Hatching date	46.9	17.16	1	<0.001	+
Tarsus length ($n = 40$)					
Treatment	0.00	0.00	1	n.s.	
Louse fly count	0.29	1.26	1	n.s.	
Body mass ($n = 40$)					
Treatment	3.55	0.50	1	n.s.	
Louse fly count	0.01	0.00	1	n.s.	
Plasma proteins ($n = 41$)					
Treatment	502.6	2.29		1	n.s.
Louse fly count	1144.5	5.15	1	<0.05	−
Sedimentation rate ($n = 42$)					
Treatment	252.1	4.60	1	<0.05	
Louse fly count	28.2	0.52	1	n.s.	
Hematocrit ($n = 43$)					
Treatment	1.4	0.05	1	n.s.	
Louse fly count	44.6	1.67	1	n.s.	
Immunocompetence index ($n = 43$)					
Treatment	1564.8	0.85	1		n.s.
Louse fly count	516.3	0.28	1	n.s.	

Table 4. Nested analysis of covariance of increase in morphological variables, serological variables and immunocompetence index (dependent variables), respectively, in relation to increase in rectrix length of nestlings in broods inoculated with additional louse flies. The effect of the covariate (increase of rectrix length) was nested in that of brood (classification factor).

	Mean sum of squares	F	df	p
Body mass				
Brood	4.11	3.05	14	<0.005
Rectrix length	4.23	4.23	15	<0.001
Tarsus length				
Brood	0.23	2.20	14	<0.05
Rectrix length	0.46	4.40	15	<0.001
Plasma proteins				
Brood	90.45	0.58	14	n.s.
Rectrix length	68.27	0.44	15	n.s.
Sedimentation rate				
Brood	29.08	0.73	13	n.s.
Rectrix length	21.00	0.50	14	n.s.
Hematocrit				
Brood	9.73	0.27	14	n.s.
Rectrix length	9.66	0.23	15	n.s.
Immunocompetence index				
Brood	3181	3.01	14	<0.006
Rectrix length	1407	2.78	15	<0.008

clearly not caused by post-hatching mortality. Ten eggs disappeared from seven nests in which, therefore, clutch size at hatching was found to be smaller than the number of eggs laid. Mean louse fly count in nests in which one or more eggs disappeared during the incubation period was significantly larger than in nests in which no egg loss occurred (count in nests with no egg loss: 3.31 ± 1.09, $n = 29$; nests in which one or more eggs disappeared $= 8.71 \pm 3.51$, $n = 7$; $t = 2.18$, 34 df, $p = 0.036$).

In 1996 only 2 nestlings out of 111 hatched in the 28 control broods died before age 12 and no nestlings died afterwards, before fledging in a sample of 22 nests. However, there were neither significant correlations nor negative trends between variables describing breeding performance and louse fly counts (Table 1). Some of

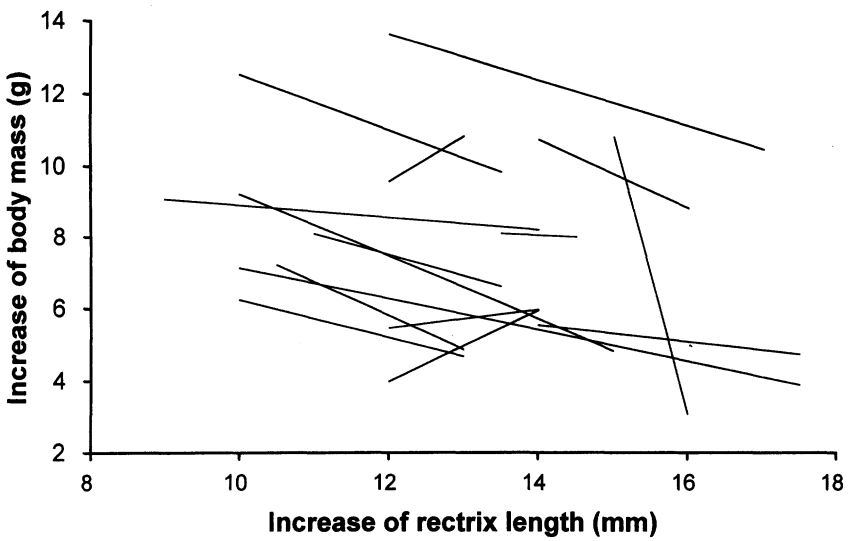

Fig. 5. Within-brood regression lines of increase in body mass as a function of increase in rectrix length in 15 broods inoculated with additional louse flies.

the eggs (18 in 13 nests) disappeared before hatching also during the second year. There was no significant difference in louse fly counts between nests in which no eggs disappeared (mean: 3.00 ± 1.13, $n = 15$) and those in which at least one egg was lost (1.92 ± 1.92, $n = 13$; $t = 0.82$, 26 df, $p = 0.42$). These differences between study years were not due to inter-annual variation in intensity of parasite infestation since mean louse fly count on day 12 during 1995 was not significantly larger than in control nests of 1996 (mean count in 1995: 4.36 ± 1.03; 1996: 2.50 ± 0.65; $t = 1.43$, 62 df, $p = 0.16$). The correlations reported in Table 1 were qualitatively identical, in terms of statistical significance, also after controlling for the effect of breeding date by Kendall partial correlation analysis.

Nestling morphology, serology, immune profile and immunocompetence in relation to louse fly counts and manipulation

In the first year, we found a significant positive correlation between louse fly counts and mean within-brood rectrix length, whereas the correlations with tarsus length, body mass, sedimentation rate or hematocrit were not significant (Table 2; Fig. 1).

To experimentally test for effects of louse flies on nestlings, in the second year we inoculated some nests with a number of parasites proportional to the count recorded in the nest. Manipulation of numbers of vagile nest parasites is generally not an easy task. By individually marking a large number of parasites (Saino, Calza and Møller unpubl.) we found that louse flies can move rapidly from the nest to the parents, and can be horizontally transmitted between mates, between an adult and its extra-pair copulation partner, and, thus, to the extra-pair nest, and between individuals involved in close aggressive interactions. We tried to control for the effect of transmission by restricting our analyses to the effect of parasites on a small time scale and inoculated the nests with parasites twice at short (3 d) intervals.

Nests of the inoculated group had significantly larger post-manipulation parasite loads compared to the control group (manipulation on day 6: inoculated nests 6.73 ± 1.13; control nests 3.5 ± 0.83; $t = 5.29$, 41 df, $p < 0.05$; manipulation on day 9: inoculated nests 6.73 ± 1.21; control nests 2.11 ± 0.47; $t = 4.25$, 41 df, $p < 0.001$). Ranking of nests with respect to post-manipulation parasite counts on day 6 was similar to pre-manipulation counts on day 9 ($\tau = 0.39$, $n = 43$, $p < 0.001$). Similarly, ranking of nests on day 9 was similar to that on day 12 ($\tau = 0.27$, $n = 43$, $p < 0.05$). These findings together with the positive correlation between parasite numbers in the nests and on parents suggest that our procedure was effective in increasing the number of parasites with which nestlings came into contact in inoculated nests as compared to the control nests. In the analyses reported below where we control for the effect of louse fly counts in the nests, we considered the sum of individual parasites recorded post-manipulation on day 6, pre- and post-manipulation on day 9, and on day 12. Brood size of inoculated broods was not significantly different from that of control broods (mean of control broods 3.89 ± 0.2, mean of inoculated broods 4.2 ± 0.22, $t = 0.98$, 41 df, n.s.).

Increase in rectrix length was larger in broods with increased numbers of parasites than in controls, while no significant effect of parasite infestation on tarsus growth and body mass was observed (Fig. 2). Sedimentation rate was significantly larger and plasma protein content significantly smaller in inoculated nests than in controls (Fig. 3). Total leukocyte concentrations appeared to be larger for nestlings in broods inoculated

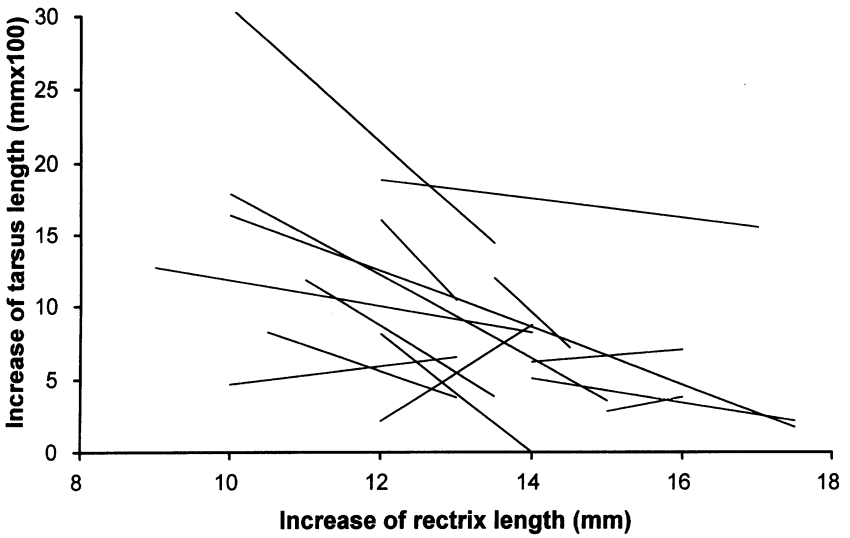

Fig. 6. Within-brood regression lines of increase in tarsus length as function of increase in rectrix length in 15 broods inoculated with additional louse flies.

with parasites. In particular, concentration of eosinophils and lymphocytes were significantly larger than in control broods (Fig. 4). However, the effects of manipulation on hematocrit and immunocompetence index were statistically not significant (Fig. 3).

When we checked for an effect of hatching date on change in morphology of control broods, a significant positive relationship emerged for rectrix length with later broods increasing more in size ($F_{1,23} = 14.74$, $p < 0.001$), whereas no significant relationships were found for change of tarsus length or body mass (tarsus length: $F_{1,23} = 0.59$, n.s.; body mass: $F_{1,23} = 2.59$, n.s.). To control for the effect of hatching date and cumulative louse fly counts on feather growth, we ran an ANCOVA with hatching date and parasite count as covariates. This confirmed the significant effect of parasite inoculation on rectrix growth (Table 3). The results of covariance analysis of change in tarsus length or body mass, sedimentation rate, plasma protein content, hematocrit, and immunocompetence in which treatment was the factor and louse fly counts the only covariate qualitatively confirmed those obtained by simple comparison between group means. Sedimentation rate was significantly different and larger in inoculated than in control nests (Table 3). In addition, a significant negative covariation between plasma protein content and louse fly counts emerged while controlling for the effect of treatment. Lymphocyte and eosinophil concentration were also significantly larger in inoculated broods but no significant covariation was observed between cumulative louse fly counts and concentration of any leukocyte type while controlling for treatment effect (not shown).

Intensity of mite infestation was not significantly correlated with louse fly counts in the first study year ($\tau = 0.01$, $n = 27$, n.s.) or in unmanipulated nests of the second study year ($\tau = 0.19$, $n = 23$, n.s.). In addition, we found no significant correlations between intensity of mite infestation and morphological, serological and immunological variables during both study years (not shown).

Per capita feeding rates

Mean per capita feeding rate to nestlings in control broods did not differ from that to nestlings in inoculated broods (control broods: 6.64 ± 0.31, $n = 25$; inoculated broods: 6.84 ± 0.58, $n = 12$; $t = 0.33$, 35 df, n.s.).

Trade-offs in nestling growth

If nestling growth was limited by resources available, then the effect of parasite inoculation in enhancing feather growth rate should have been reflected also in a detrimental effect on other functions. This prediction was confirmed by a nested analysis of covariance, in which change of body mass or tarsus length were the dependent variables, brood was the factor and change of rectrix length was a covariate whose effect was nested in that of brood. This analysis allowed us to analyse the covariation between increase in body mass or tarsus length, respectively, and increase in rectrix length within each brood while controlling for the effect of variability among broods. In parasite inoculated broods both increase in body mass and tarsus length significantly covaried with increase in rectrix length (Table 4). The sign of the within-brood regression coefficients on increase in rectrix length was negative in 12 out of 15 cases for increase in body mass ($\chi^2 = 5.4$, 1 df, $p = 0.02$), and in 11 out of 15 cases ($\chi^2 = 3.27$, 1 df, $p = 0.07$) for increase in tarsus length (Figs 5 and 6). The mean of within-brood regression coefficients of increase of body mass on increase in rectrix length was

smaller than 0 though not significantly so (Wilcoxon test, $z = -1.65$, $n = 15$, $p = 0.1$). However, the corresponding mean for increase in tarsus length was significantly smaller than 0 (Wilcoxon test, $z = -2.33$, $n = 15$, $p = 0.02$). Taken together, these results suggest that enhanced feather development under conditions of increased parasite infestation was to the detriment of increase of body mass and tarsus growth, also when the effect of genetic and environmental factors were partly controlled by comparing siblings. However, in control broods, neither increase of body mass nor increase in tarsus length significantly covaried with increase in rectrix length (not shown).

In analyses of covariance with the same model, plasma protein concentration, sedimentation rate, and hematocrit did not significantly covary with increase of rectrix length in inoculated (Tables 4) or control broods (not shown), whereas a significant covariation emerged for immunocompetence index only in inoculated broods. However, this result was equivocal since in 11 out of 15 broods the within-brood correlation coefficient was positive ($\chi^2 = 3.27$, 1 df, $p = 0.07$), but mean of within-brood regression coefficients was negative, though not significantly so (mean -0.77 ± 0.98; $t = 0.79$, 14 df, $p = 0.44$).

Discussion

Parasite infestation and breeding success

During the first study year (1995), nests that were found to be heavily infested produced the smallest number of fledglings. Since nestling mortality in our study population was very low, ectoparasites are unlikely to be a major determinant of fledging success. The results from the first study year suggested that larger egg loss in heavily infested nests is one of the causes of the negative relationship between parasite infestation and fledging success. One possible explanation for the effect of parasites on egg loss is that females and their mates had to spend longer time foraging to compensate for the detrimental effect of parasites on condition. Reduced parental care, in terms of guarding the nest for egg removal by extra-pair males or house sparrows (*Passer domesticus*), would have resulted in a larger number of eggs disappearing from nests with large parasite numbers. This interpretation, however, relies on the assumption that parasite counts during the nestling period were consistently and directly proportional to the number of parasites on adults or in the nest during incubation. This might have been the case because the ranking of adults with respect to intensity of louse fly infestation is consistent in different stages of the breeding cycle, and parasite counts on adults are proportional to those in the nest. These results are similar to those reported for the haematophagous mite *Ornithonyssus bursa* (Møller 1990b).

The pattern of correlation between breeding performance and parasite infestation was markedly different during the second year (1996), the level of parasite infestation being lower but not significantly different from that observed in the first year. At present, we have no specific explanation for this, since any differences in the ecological conditions in the wintering areas, during migration and soon after arrival in the breeding quarters could have determined this discrepancy. However, we could observe no significant differences in female body mass or condition, or in physiological variables during the pre-laying period between the two study years (Saino, Calza and Møller unpubl.).

Effect of parasite manipulation on nestling development and condition

We interpreted the positive correlation between parasite count in the nest and rectrix length during the first study year as the result of nestlings investing more in feather growth in order to reduce the duration of the nestling period. It has been reported that nestlings of the barn swallow and also other passerine species fledged earlier when their nest was heavily infested by ectoparasites (Møller 1990b, Chapman and George 1991). Faster plumage development of nestlings in heavily infested nests during the first study year, and in nests inoculated with parasites, might have been an adaptive response to reduce the time of exposure to the detrimental effects of large parasite numbers in the nest. We might also speculate that louse flies do not parasitize offspring after fledging because they leave their natal colony as soon as they are independent. Louse flies that stayed on fledglings would therefore not reproduce given that parturitions are very likely to occur in the nests. Moreover, fledging early might allow nestlings to be exposed to fewer generations of parasites.

Were nestlings paying any costs as a consequence of intense parasitism? The net cost might have been absent if parents were compensating by food provisioning for resources removed by parasites. In this case, parents would have paid for their offspring. However, we found no evidence for parents of inoculated nests to feed more frequently than parents of control broods. This might have occurred for several reasons including, for example, parents 1) being unable to compensate because they were already working at their maximum possible intensity, 2) trading current reproductive effort against their own survival or future reproduction, 3) themselves being affected by parasites, or 4) having no cue to assess increased needs of nestlings.

We could show no significant effect of parasites on tarsus growth and body mass either in the natural

population or in the parasite manipulation experiment. However, nestlings from inoculated broods appeared to be in poorer condition than those from control broods, since they had lower plasma protein content and larger blood cell sedimentation rate. Lower plasma protein content of nestlings in experimentally infested broods might have been the result of more intense resource allocation to feather development or, alternatively, an effect of parasites on nestling physiology independent from the effect on feather development (see also below). Large blood cell sedimentation rate in heavily infested broods may have resulted from altered plasma protein content, which may suggest that rapid blood cell sedimentation is associated with intense parasitism in birds. Leukocytosis is commonly observed in response to parasite infestation in birds. The increase in total leukocyte concentration in peripheral blood observed in inoculated broods was apparently due lymphocytes and eosinophils. This result is consistent with a study on cliff swallows (*Hirundo pyrrhonota*; Chapman and George 1991) in which a significantly larger concentration of lymphocytes, eosinophils, but also basophils was observed in offspring from unmanipulated nests as compared to nests that had been sprayed to remove ecto-parasites.

Trade-offs in nestling development

The way in which trade-offs should be measured is still a debated and controversial issue. In particular, it has been emphasised that major confounding factors such as genotype by environment interactions and phenotypic variation among individuals may affect any measurement of a trade-off (Stearns 1992). In this study, both environmental and genetic effects were partly controlled because genetically similar individuals developing in the same nest and being fed by the same adults were considered.

Consistent with the hypothesis of a trade-off between competing functions, nestlings that grew their feathers at the largest rate had the smallest increase in body size (as indexed by tarsus length) and body mass, possibly reflecting both anabolism of muscular tissues and fat reserve accumulation. From our results, however, it is not immediately clear which kind of cost parasitism was imposing on nestlings, given that nestlings in inoculated broods did not experience lowered rates of increase in tarsus length or body mass, as predicted by a trade-off with feather growth. Low plasma protein content and large blood cell sedimentation rates are presumably not costs per se but, rather, are means by which other functions are depressed. These results suggest that in order to raise the rate of feather development without compromising somatic growth and reserve accumulation, nestlings exposed to experimentally elevated levels of parasitism may have reduced their level of plasma protein concentration thus diverting resources from other activities which, however, remained unidentified in the present study.

We conclude that parasites can depress condition of barn swallow nestlings as indicated by their effect on plasma protein content and blood cell sedimentation rate, and determine an increase in concentration of leukocytes in peripheral blood. Nestlings under heavy parasite infestation invest more in growth processes likely to prevent prolonged exposure to parasites, and this results in a trade-off between growth of different characters.

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