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Costs of incubation and immunocompetence in the collared flycatcher

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Abstract This paper investigates the costs of incubation in terms of reduced reproductive success and investigates whether incubation competes with immune function for resources. I performed a clutch size manipulation experiment in which two eggs were either removed from or added to the nests of collared flycatchers, *Ficedula albicollis*, for 1 week during incubation and subsequently returned to their original nests before hatching. To induce immune response, the females were challenged with sheep red blood cells. While the duration of incubation, hatching success and fledgling number did not differ between experimental groups, fledgling condition was significantly lower in broods that had been enlarged during incubation. Neither the females' condition nor their ability to respond to a novel antigen differed between treatments. The relationship between antibody production and female condition was significantly positive, but only among females incubating reduced clutches. I conclude that the costs of incubation in the collared flycatcher are not negligible and are manifested only at the chick-rearing phase.

Key words Reproductive costs · State-dependent strategies · Antibody production · Body condition · Haemagglutination

Introduction

In life history theory, the costs of reproduction are commonly regarded as important, as are the strategies of resource partitioning between reproductive activities and other functions (Roff 1992; Stearns 1992). A general question concerns identifying the limits to reproduction and the reproductive stages at which these limits are

most strict. Since an increase in current reproduction may entail costs against residual reproductive value, this by itself may constitute a limit on reproduction. Investments in current reproduction may affect components of residual reproductive value by, for example, decreasing future survival prospects (survival costs) and/or future fecundity (fecundity costs). Assuming the existence of such reproductive costs, organisms are expected to optimise present reproductive output, taking into account future prospects of survival and fecundity (Williams 1966). However, limits on reproduction can operate within a single reproductive event. While studies of reproductive costs have concentrated on variation between breeding attempts, the trade-offs operating within breeding cycles, though acknowledged, have been little investigated (e.g. Lessells 1991).

Studies of birds generally have been successful in revealing the costs of reproduction. Since the avian breeding cycle consists of distinct phases, birds are very useful in studies of the relative impact of different reproductive phases upon overall reproductive investment. Nestling provisioning has drawn most of the attention of researchers, and been shown to impose very high resource requirements (Lindén and Møller 1989; Stearns 1992). The incubation phase has been perceived as rather undemanding (e.g. King 1973; Grant 1984), though some workers have pointed out that egg production and incubation costs are not negligible in the overall reproductive budget and can play a significant role in determining avian clutch size (see Monaghan and Nager 1997 for a review). Some studies have shown that incubation is costly: incubating extra eggs may negatively affect female condition (Jones 1987; Moreno and Carlson 1989), the duration of incubation (e.g. Moreno and Carlson 1989; Siikamäki 1995), hatching success (Moreno et al. 1991; Siikamäki 1995), hatching asynchrony (Moreno and Carlson 1989) and fledgling condition (Heaney and Monaghan 1996). Clutch size enlargement is associated with higher energy expenditure in incubating females (Moreno et al. 1991; Tatner and Bryant 1993; Moreno and Sanz 1994; see Thomson et al. 1998 for a review).

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The costs of incubation have not always been demonstrable, for example because of the effect of favourable ambient temperature (e.g. Moreno et al. 1991). Most studies have not properly distinguished between the incubation and chick-rearing phases since manipulations of reproductive effort affected both. The only study of the common tern (*Sterna hirundo* L.) manipulated only the incubation phase and demonstrated costs of enhanced incubation effort in terms of reduced growth rate and fledgling mass of the second-hatched chick (Heaney and Monaghan 1996). Hence, brood-rearing costs as well as incubation costs can potentially constitute a constraint in clutch size optimisation.

In the present study, I have investigated the reproductive consequences of altered incubation effort in a small altricial bird species, the collared flycatcher (*Ficedula albicollis*). This species relies primarily on immediate resource income for its breeding, and females incubate alone. I also investigated whether incubation of enlarged clutches is associated with reduced immunocompetence: if incubation drains resources from immune function, increased incubation effort should lead to immune suppression. The immune system is an important self-maintenance mechanism, presumably important to an individual's survival probabilities. Thus, investments in immune defence potentially affect life history strategies and can be a component in individual optimisation. The ability of an organism to undergo state-dependent modulation of the immune response is beginning to draw the attention of researchers (Sheldon and Verhulst 1996; Norris and Evans 2000). Impairment of immune functioning, due to resource depletion associated with reproduction and consequent susceptibility to infection, has been suggested to mediate the costs of reproduction (Gustafsson et al. 1994; Deerenberg et al. 1997; Nordling 1998; Nordling et al. 1998; Moreno et al. 1999). To study the possibility that incubation may compete with immune function for resources, I manipulated the clutch size of collared flycatchers and at the same time induced a specific immune response in incubating females.

Materials and methods

The study was conducted in a collared flycatcher population breeding in nestboxes on the island of Gotland, Sweden in 1998 (for details about the study area see Gustafsson 1989). The collared flycatcher is a small (≈ 13 g) migratory passerine bird species breeding mainly in eastern and central Europe and wintering in southern and central Africa. Flycatchers arrive at the breeding grounds on Gotland in the beginning of May and the females start laying around 20 May. The clutches consist of four to eight eggs, with a modal size of six. Incubation starts after clutch completion and lasts 14 days. The female incubates alone, but the male provisions her with food during this period. Both parents feed nestlings.

To study the costs of incubation, I altered the clutch size of the incubating females for 6 days during the second half of the incubation period. In pairs of clutches matched in terms of egg number and laying date, two eggs were moved from a randomly chosen nest to the paired nest on day 5 of incubation. Only clutches of six and seven, initiated between 18 and 23 May, were included in the experiment. The experiment did not include a control group with unchanged clutch size, because the number of available nesting birds was limited. To identify the box of origin, the eggs were

marked with waterproof pen. At the same time, the incubating females were caught in the nestbox, weighed, and injected intraperitoneally with 0.1 ml of a 2% suspension of sheep red blood cells (SRBCs) to induce the specific immune response, then immediately released. The suspension contained 5×10^7 SRBCs in 100 μ l sterile phosphate-buffered saline (PBS). SRBCs are a standard complex antigen commonly used in immunological studies (Hay and Hudson 1989). After 6 days (the day before expected hatching), the females were recaptured while incubating. They were weighed and ~ 100 μ l of blood was taken into haematocrit capillaries for analyses of antibody concentration against SRBCs. At the same time, the alien eggs were moved back to their original nests.

The microcapillaries were stored vertically in a cooled box for several hours while in the field, and centrifuged at 11,000 rpm for 12 min after reaching the laboratory. Capillaries were then cut above the leucocyte layer and the plasma removed into Eppendorf tubes. The plasma was stored in a freezer at -20°C until analysed. A standard haemagglutination test was employed to quantify the specific antibody concentration against SRBCs. The plasma was heat-inactivated at 56°C for 30 min. For each bird, 10 μ l of plasma was diluted 1:1 in PBS and then serially diluted in 12-well U-type microtitre plates. Ten microlitres of 2% suspension of SRBCs was added to each well and incubated at 37°C for 60 min. The presence of antibodies is associated with haemagglutination of erythrocytes. In a positive reaction, erythrocytes settle as a characteristic carpet over the base of the well, while in a negative reaction, the cells form a small, easily recognisable pellet. The number of titres showing positive haemagglutination represents the antibody concentration and is based on a log scale (Hay and Hudson 1989). The test was performed twice for each bird and the average titre number was used in the subsequent analyses.

The experimental nests were inspected daily from the day of expected hatching (date of clutch completion plus 12) until most of the eggs had hatched. The parents were trapped in the nestboxes while feeding 5- to 10-day chicks and measured. The nestlings were measured for tarsus length and weighed at fledging (13 days after hatching). To correct for individual differences in weight due to structural size, the residuals from a linear regression of body weight on tarsus length were used as an index of body condition, or tarsus length was included in the ANCOVA as a covariate.

In total, 17 pairs of clutches were manipulated, but not all the experimental nests were successful. Two females deserted their clutches before the second capture, and six other broods were deserted before fledging. One experimental clutch contained unfertilised eggs. The probability of desertion was not associated with the experimental treatment ($\chi^2=0.14$, $df=1$, $P>0.7$).

Results

Experimental reduction and enlargement of clutches during incubation failed to affect hatching date or hatching success (Table 1). However, females incubating extra eggs raised fledglings in poorer condition than those whose clutches were reduced during incubation. This was not, however, the result of differential hatchability (i.e. clutches enlarged during incubation containing a larger number of nestlings than the reduced ones; Table 1).

Incubation of clutches experimentally altered in size did not affect female condition (reduced: mean= 15.22 ± 0.87 SD; enlarged: mean= 15.21 ± 1.2 ; ANCOVA, $F_{1,18}=0.18$, $P=0.67$), calculated as female weight at the end of incubation corrected on female weight at the time of clutch alteration, and on tarsus length (the two latter variables are covariates). No effect of experimental treatment on specific antibody production was found among incubating females (reduced:

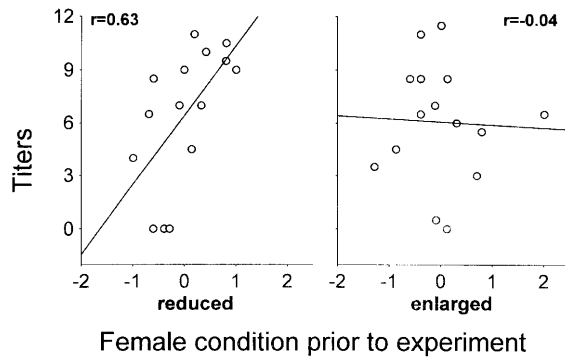


Fig. 1 Relationship between female condition prior to the experiment and specific antibody production (number of titres showing positive haemagglutination) against sheep red blood cells, separately for females incubating reduced and enlarged clutches (r denotes Pearson correlation coefficient)

Table 1 Effects of experimental treatment on the difference between observed and expected hatching date, hatching success (%), fledgling number and fledgling weight, 13 days after hatching in collared flycatchers. t represents the t -test statistic, F ANCOVA, and z the Mann-Whitney statistic. The difference between experimental groups in fledgling weight was tested using ANCOVA in which nestbox was nested in the experimental treatment, and tarsus length was introduced as a covariate accounting for individual differences in structural size

	Reduced (mean \pm SD)	Enlarged (mean \pm SD)	Statistic		P
Hatching date	1.75 \pm 0.75	2.15 \pm 1.14	$t=-1.03$	$df=23$	0.31
Hatching success	0.98 \pm 0.08	0.96 \pm 0.09	$z=-0.31$	$n=26$	0.76
Fledgling number	5.00 \pm 1.84	4.67 \pm 1.30	$t=0.50$	$df=21$	0.62
Fledgling weight	15.24 \pm 1.2	14.22 \pm 1.87	$F=38.96$	$df=1,88$	<0.01

mean=6.03 \pm 4.08; enlarged: mean=6.07 \pm 3.37; t -test, $t_{29}=-0.03$, $P=0.98$).

Immune system functioning may be condition dependent. To account for differences in female body condition, I performed an analysis of variance, testing for effects of experimental treatment (independent factor) and female condition prior to the experiment (covariate) on antibody production. Neither of the effects was significant (ANCOVA, experimental treatment: $F_{1,28}=0.000$, $P=0.99$; covariate: $\beta=0.28$, $F_{1,28}=2.47$, $P=0.12$). However, the relationship between female condition and immune response was significantly non-parallel in the experimental groups (ANCOVA test for parallelism; $F_{1,28}=5.75$, $P=0.024$; Fig. 1). Closer inspection of the data revealed a significant positive relationship among females incubating reduced clutches ($r=0.63$, $n=16$, $P=0.01$), but no relationship at all among females incubating enlarged clutches ($r=-0.04$, $n=15$, $P=0.89$).

If poorer fledgling condition is a penalty not only for enhanced incubation effort but also for mounting an im-

mune response, a negative relationship between specific antibody production and fledgling condition would be expected, and this relationship should be more pronounced among females incubating enlarged clutches. The expected negative relationship was, however, statistically non-significant (ANCOVA accounting for experimental treatment; $\beta=-0.31$, $F_{1,18}=1.92$, $P=0.18$; the level of immune response was introduced as a covariate), and there was no significant interaction between experimental groups (ANCOVA; $F_{1,17}=0.001$, $P=0.98$).

Discussion

The present results indicate that there are indeed costs associated with incubation for the collared flycatcher. Poorer fledgling condition seemed to be the most important penalty for incubating extra eggs. The lack of a significant effect of experimental treatment on hatching date, hatching success and number of fledged young may indicate that females forced to incubate larger clutches were maximising hatching success at the expense of their capacity to sustain offspring growth. The poorer condition of fledglings of females forced to incubate an enlarged clutch could have been caused by a number of factors, for example, slower embryonic development in enlarged clutches with a subsequent effect on nestling growth (Sanz 1997), or the female's reduced ability to provision her nestlings, or a combination of both. Unfortunately, individual nestlings within the brood could not be identified, so direct comparisons were not possible. If this effect is mediated by slower embryonic development, one would expect higher variance in fledgling condition among nestlings of females whose clutches were reduced during incubation, because some of their eggs developed in enlarged clutches and some in reduced ones; however, the tendency was in the opposite direction and the difference in variance was not statistically significant (Levene's test for homogeneity of variance, $P>0.1$).

In the only study manipulating incubation effort and nothing else, the addition of an extra egg to two-egg clutches during incubation in the common tern resulted in lower growth rate of the second-hatched chick (Heaney and Monaghan 1996). The present work supports this result because it shows the incubation costs in a small passerine bird with a totally different life history strategy (altricial income breeding with uniparental incubation). The costs of reproduction related to provisioning of additional nestlings have been previously demonstrated in this population of collared flycatchers (Gustafsson and Sutherland 1988; Gustafsson and Pärt 1990; Gustafsson et al. 1995; Cichoń et al. 1998; Nordling 1998), but my results suggest that incubation might also impose considerable costs in this species.

Impairment of immune functioning due to resource depletion for reproduction has been suggested to mediate the costs of reproduction (Gustafsson et al. 1994). Experimental enlargement of brood size may indeed

have negative consequences for the functioning of the immune system, as shown by a number of studies (Gustafsson et al. 1994; Deerenbeg et al. 1997; Nordling et al. 1998; Moreno et al. 1999). In the present study, I focused on whether incubation effort might affect immune responsiveness. Females incubating reduced and enlarged clutches did not differ in their specific antibody production after being challenged with non-pathogenic standard antigen (SRBCs), though the response was relatively high. This may suggest that incubation and immune functions do not compete for limited resources. The resources required by immune defence and incubation may differ, since the former involves cell proliferation and production of diverse proteins. Possibly, other aspects of immune defence not studied here were affected, or the sample size was too small to provide a significant result. Only one type of immune activity was measured, while immune defence involves a complex array of mechanisms. Production of specific antibodies seems to be a sensitive measure of immunocompetence, but the negative result is inconclusive, especially since the power of the test was low (0.50).

Life history decisions are usually state dependent (McNamara and Houston 1996), which means that a female's response to clutch size manipulation can be dependent on variables describing her state. Immune response was positively related to female condition at the start of the experiment, but only among females incubating reduced clutches. This may indicate that the females incubating enlarged clutches were in fact beyond the range of their phenotypic plasticity and were not acting optimally. They were also possibly responding strongly regardless of their state at the expense of other functions, hence delaying the cost of incubation until later. Females incubating enlarged clutches raised fledglings in worse condition, but there was no relationship between fledgling condition and immune response. Nor was there any interaction between experimental groups. The lack of such a relationship may mean that females responding strongly to the antigen were not sacrificing their nestlings, provisioning in favour of self-maintenance. However, the non-significance of this relationship could be due to partner compensation or the low power of the test.

In conclusion, experimentally increased incubation effort negatively affected the condition of the fledglings; incubation seems to entail costs in collared flycatchers, and these may constitute a limit on reproduction. In this population, costs of rearing extra chicks have been previously established but the present results suggest that incubation costs may also be of considerable importance. This is an important finding, since the existence of incubation costs may help explain why clutch sizes observed in nature are often smaller than the calculated optimum (e.g. Godfray et al. 1991). I failed to find any direct indication of a trade-off between incubation effort and immune functioning, and thus no evidence that the costs associated with incubation may be mediated by impairment of immune function.

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