

Individual quality mediates trade-offs between reproductive effort and immune function in tree swallows

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Summary

1. Individual variation in the trade-off between self-maintenance and offspring quality was assessed in tree swallows, *Tachycineta bicolor*, by manipulating reproductive effort while simultaneously immunochallenging breeding females.

2. An experimental manipulation of parental effort was conducted by creating broods of, on average, three, five and eight nestlings. Breeding females were immunochallenged to mount a humoral immune response to sheep red blood cells (SRBC) and a cell-mediated response to phytohaemagglutinin (PHA). The consequences of adult decisions on offspring quality were assessed through immune responses to PHA, growth rates and residual body mass of nestlings.

3. Clutch initiation date, a strong measure of individual quality in tree swallows, was linked with immune responses, with earlier-nesting, presumably higher quality, females mounting stronger immune responses than did later-nesting birds. Increased reproductive effort led to decreased parental immune responses. There was a significant interaction between individual quality and reproductive effort treatment, with lower-quality individuals showing greater depression of humoral immune response to SRBC while raising enlarged broods, suggesting individual-level variation in trade-offs.

4. Breeding females raising enlarged broods tended to raise offspring of similar quality to control females, with only growth rate decreasing with increasing brood size, but not residual nestling body mass or nestling immunocompetence. This suggests that females are maintaining offspring quality at the cost of their own immune system maintenance.

Key-words: immune system self-maintenance, individual variation, life history trade-offs.

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Introduction

That individuals differ in their ability to survive and reproduce is one of the central tenets of natural selection theory (Darwin 1859; Williams 1966). Differences among individuals should lead to differences in the fundamental trade-off between current and future reproduction because individuals differ in the costs they suffer and the benefits they accrue at a given level of reproductive investment (Stearns 1992). These differences can underlie phenotypic differences in reproductive behaviour; however, few studies explicitly examine trade-offs along a gradient of individual quality (Pettifor, Perrins & McCleery 1988, 2001). This study examines how individual quality, measured by clutch

initiation date of breeding females, influences trade-offs between reproductive effort and reproductive output through an experimental manipulation of offspring demand. An experimental approach to studying life-history evolution is needed in order to break the positive correlations among traits that arise from large differences in quality (Partridge & Harvey 1988; Winkler & Wilkinson 1988).

In many species of birds, timing of breeding is a phenotypic measure linked with annual reproductive output (e.g. Perrins 1970; Pettifor *et al.* 1988; Verhulst, Balen & Tinbergen 1995; Christians, Evanson & Aiken 2001). In tree swallows (*Tachycineta bicolor* Vieillot 1808) clutch initiation date is the most important predictor of reproductive success (Stutchbury & Robertson 1988; Winkler & Allen 1996); individuals breed as early as possible, but the factors that determine when an individual breeds are a complex interaction among ecological constraints, past experience and the intrinsic quality of the individual.

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One important way in which individuals differ is in the strength and responsiveness of the immune system, which can be used to assess reproductive costs, as the ability to fight infection and parasites is important to survival but is also costly (Gustafsson *et al.* 1994; Saino *et al.* 1999; Lochmiller & Deerenberg 2000; Bonneaud *et al.* 2003). When parents are forced experimentally to shift allocation (e.g. energy, nutrients) from immune system self-maintenance to raising offspring, reduced immune function can affect parental survival, thus enforcing a trade-off between the prospects of offspring and parent (Apanius 1998; Norris & Evans 2000). The general approach has been to modify parental effort, either through modifying offspring demand (as in this study) or by handicapping parents. A manipulation of parental effort can lead to changes in either antibody production (Deerenberg *et al.* 1997; Hasselquist, Wasson & Winkler 2001), or parasite levels (Norris, Anwar & Read 1994), as well as leading to decreased reproductive output. This suggests that the cost of mounting an immune response is high (Ilmonen *et al.* 2003), although not all studies have found an effect of reproductive effort on immune responses (Ilmonen, Tarna & Hasselquist 2002; Ilmonen *et al.* 2003). However, because individuals differ in both susceptibility to disease and allocation decisions (Moreno, De Leon & Fargallo 1998; Navarro *et al.* 2003), it is critical to examine differences among individuals in the interaction between reproductive effort and immune function. In work conducted in the same study area as this study, early-breeding tree swallows mounted higher humoral responses to key-hole limpet haemocyanin (Hasselquist *et al.* 2001); the goal of this study was to examine whether individual quality influenced direct reproductive trade-offs between offspring quality and immune responses.

Here, parental effort was modified experimentally to force breeding females to make reproductive allocation decisions with a modified set of costs and benefits. First, if tree swallows make trade-offs between immune function and reproductive effort, one would predict that increased offspring demand would lead to lower immune responses in parents. The critical prediction of this study is that later-laying, presumably lower-quality, individuals are less able to deal with increases in effort and thus will mount weaker immune responses particularly when raising enlarged broods. However, if individual quality does not influence the trade-off between immune response and effort, then one might predict independent effects of both workload and individual quality, but no interaction between their effects.

Examining parental responses to changes in reproductive costs without examining consequences is incomplete. Parental allocation between workload and immune response can be assessed through offspring condition (defined broadly), a key determinant of fitness, as offspring condition reflects probability of survival (Dhondt 1971; Hochachka & Smith 1991; Nager *et al.* 2000; Tella *et al.* 2000; Naef-Daenzer, Widmer & Nuber 2001). Previous work has demon-

strated that parental effort is linked to offspring quality (Wright *et al.* 1998; Moreno *et al.* 1999; Pettifor *et al.* 2001) and immune function (Saino, Calza & Møller 1997; Hõrak *et al.* 1999; Ilmonen, Taarna & Hasselquist 2002; Ilmonen *et al.* 2003). In this study, individual variation in parental response to modifications of parental effort is assessed. If parents bear costs in order to maintain offspring quality, increasing brood size should have a stronger effect on parents than on offspring; conversely, if parents show limited effects of brood manipulations, while offspring raised in enlarged broods are of lower quality, parents may pass costs along to offspring (Golet, Irons & Estes 1998; Saino *et al.* 1999; Hõrak 2003).

Methods

GENERAL FIELD METHODS

Tree swallows (*Tachycineta bicolor*) are a widespread and common cavity-nesting North American migrant member of the family Hirundinidae (Robertson, Stutchbury & Cohen 1992). In this study, tree swallows bred in nestboxes erected for their use in Tompkins County, New York, USA (centred on 42°29' N, 76°27' W) in 1999 and 2000; tree swallows have been breeding at the site since 1988 (Winkler & Allen 1996). Nests were checked daily to determine date of clutch initiation and clutch size. Breeding females were captured on the nest on day 4 of the nestling period (hereafter all days are referred to by days from hatch of first nestling) and weighed to the nearest 0.1 g. In addition, flattened left wing length (carpal joint to the tip of the longest primary) was measured to the nearest mm with a ruler and head-bill length (distance from the back of the skull to the tip of the bill) to the nearest 0.05 mm with callipers. Breeding females were aged as either second year or after second year by plumage (Robertson *et al.* 1992). Offspring were measured on days 4, 6, 10 and 12 of the nestling period. Each individual was uniquely marked using either nail polish or colour bands depending on age and size. During each visit, body mass, flattened left wing length, head-bill length and tarsus length (to the nearest 0.05 with callipers) were recorded.

In 2000, insect availability was recorded using two 2-m aerial insect samplers powered by a Robbins and Myers 1650 r.p.m. (12.95 m s⁻²) 1/12 HP motor (McCarty & Winkler 1999) to collect daily samples of aerial insect abundance during the breeding season. Packed insect volume was measured by centrifuging samples at 5000 r.p.m. (31.4 m s⁻¹) for 75 min after removing seeds and other debris. In 2000, to record nest visitation rates, three 60-min behavioural observations were conducted using video cameras recording from at least 20 m outside the box between days 8–11 for each breeding pair. Sexes were differentiated in video observations by marking females with a small dot of white paint on the back. For each observation, cameras were set with timers to begin recording 15 min after set-up to minimize the effect of human disturbance on provisioning behaviour.

BROOD SIZE MANIPULATIONS

First broods with the same hatching date were assigned randomly to one of three treatments: (1) increased, (2) decreased or (3) control to create broods that were roughly 50% larger or smaller than original clutch size (average number of nestlings on day 4: increased 7.81, control 5.01, decreased 2.97; total number of nestlings moved 167). Chicks were marked individually and swapped for all treatments on day 3 of the nestling period, so that each breeding female raised a mix of nestlings that were her own offspring and nestlings that were the offspring of at least one other female. Females were allocated to treatments balanced for clutch initiation date (comparison of clutch initiation date among treatments $F_{3,128} = 0.08$, $P = 0.97$) and clutch size ($F_{3,128} = 0.66$, $P = 0.58$).

IMMUNOCHALLENGES AND SEROLOGY

Breeding females were captured, weighed and measured on days 4, 6, and 12 of the nestling period. On day 4, blood was drawn from the brachial vein to determine pre-exposure levels of antibodies (from 20 μL plasma) and each bird was injected intraperitoneally with 5×10^7 sheep red blood cells (ICN Biomedicals, Aurora, OH, USA) suspended in 100 μL PBS (Deerenberg *et al.* 1997). All individuals were captured 8 days later to draw blood to determine post-exposure primary antibody titres in plasma. Antibody titres were measured in all blood samples using a base-2 serial dilution haemagglutination test conducted with 20 μL of plasma on 96-well microtitre plates (Roitt, Brostoff & Male 1998). Samples were serially diluted starting with 20 μL PBS and to each well 20 μL of a 2% suspension of sheep red blood cells (SRBC) in phosphate-buffered saline (PBS) was added. Plates were incubated at 37° for 1 h. Titres are given as the \log_2 of the reciprocal of the highest dilution of plasma showing positive haemagglutination. For each plate, known positive and negative control sera were included.

On day 4, 0.15 mg of phytohaemagglutinin (PHA, Sigma-Aldrich) in 30 μL PBS were injected into the patagial wing web of breeding females (Smits & Williams 1999). On day 10, each nestling was injected with 0.1 mg of PHA in 20 μL PBS in the same location. Prior to each injection, the thickness of the wing web was measured three times using a digital micrometer and recorded as the average thickness. While 24 h is the most common period of measurement, individuals were re-measured approximately 48 h after injection to minimize disturbance and nest abandonment, as pilot data found no difference in response between 24 h and 48 h (unpublished data). Wing web thickness was re-measured three times and the average measurement recorded (repeatabilities: pre-exposure $R_i = 0.82$, $F_{464,465} = 1.92$, $P < 0.001$, post-exposure $R_i = 0.79$, $F_{464,465} = 1.75$, $P < 0.001$). Cell-mediated immune response was considered the ratio of postinjection thickness to pre-injection thickness (Hörak *et al.* 1999).

STATISTICAL ANALYSES

Because breeding dates differ slightly from year to year, for all analyses that included clutch initiation date, clutch initiation date was standardized to a mean = 0 for each year. Parental immune responses were examined using a mixed model analysis (SAS 1988) with year and nest box as random factors and the following fixed effects: standardized clutch initiation date, brood manipulation treatment (reduced, control, enlarged), female age (after second year vs. second year), and clutch size. Because nestlings were switched among nests, nestling measures were analysed using a mixed model analysis with nest of hatch and nest of rearing, as well as year, as random factors and the following fixed effects: standardized clutch initiation date, brood manipulation treatment, clutch size in their natal nest and breeding female age. Because there was an a priori prediction, the interaction between clutch initiation date and brood manipulation treatment was included in all models; however, other pairwise interactions were removed when P was > 0.30 .

Nestling quality was characterized using three variables: (1) cell-mediated immune response to PHA, (2) growth rate and (3) residual body mass. Growth rate from day 4 to day 12 was calculated as the growth rate constant K of a logistic growth function (Starck & Ricklefs 1998) for three nestling measures: primary wing feather, tarsus and head–bill measurements, an approach used previously for tree swallows (Zach & Mayoh 1982; McCarty 2001). The following asymptotic values were used to calculate K : primary wing feather (85 mm), tarsus (16.5 mm) and head–bill (28 mm). Because the three nestling measures are highly correlated, K -values were combined in a principal components analysis and the first principal component (which explained 72% of the variation) was used in statistical analyses. Nestling residual body mass on nestling day 12 was calculated as the residual of a regression of body mass against head–bill size, a structural measure of body size.

Results

INSECT AVAILABILITY

Insect availability showed considerable daily variation, but overall prey availability increased as the breeding season progressed (Julian date vs. packed insect volume mm^3 , $\beta = 9.70$, $F_{1,82} = 20.77$, $P < 0.0001$, $R^2 0.20$).

PARENTAL RESPONSES

Breeding females raising enlarged broods made more feeding visits than did control individuals or those females raising reduced broods (Table 1). There were effects of both brood size and individual quality on immune responses. Females raising enlarged broods showed decreased primary immune response to SRBC and cell-mediated immune response to PHA, relative to control and reduced-brood females (Table 1). Early-nesting,

Table 1. Comparison in responses among brood manipulation treatment groups. For each variable, raw data is listed on the first line and least-square means on the second. ^{a,b}Differences among treatments for each variable at $P < 0.05$

	Reduced		Control		Enlarged		P-value
	n	Mean ± SE	n	Mean ± SE	n	Mean ± SE	
Breeding female responses							
Feeding visits/minute	17	0.17 ± 0.01 0.19 ^a ± 0.01	16	0.18 ± 0.02 0.17 ^a ± 0.01	16	0.22 ± 0.02 0.22 ^b ± 0.01	0.001
Primary response to SRBC (titre)	34	6.26 ± 0.21 6.47 ^a ± 0.48	34	6.35 ± 0.20 6.23 ^a ± 0.47	34	3.55 ± 0.41 3.47 ^b ± 0.41	0.0001
Response to PHA	33	1.48 ± 0.03 1.46 ^a ± 0.12	34	1.42 ± 0.04 1.41 ^a ± 0.18	34	1.35 ± 0.03 1.33 ^b ± 0.13	0.03
Nestling quality							
Growth rate	90	0.55 ± 0.14 0.70 ^a ± 0.16	139	0.56 ± 0.06 0.34 ^a ± 0.15	187	-0.11 ± 0.07 0.07 ^a ± 0.16	0.01
Response to PHA	90	1.32 ± 0.02 1.31 ^a ± 0.03	139	1.36 ± 0.02 1.37 ^a ± 0.03	187	1.33 ± 0.02 1.33 ^a ± 0.03	0.29
Residual body mass day 12	90	-0.63 ± 0.41 0.68 ^a ± 0.50	139	0.21 ± 0.21 0.25 ^a ± 0.49	187	-1.73 ± 0.21 1.46 ^a ± 0.51	0.31

higher-quality individuals mounted stronger immune responses to immunochallenge of both SRBC and PHA (Fig. 1, SRBC $F_{1,93} = 57.63$, $P = 0.0001$; PHA $F_{2,93} = 7.12$, $P = 0.009$). There was an interaction between the effect of individual quality and brood manipulation treatment on immune response to SRBC. The magnitude of the difference between individuals raising enlarged broods vs. control broods differed between high- and low-quality individuals, with lower-quality females raising enlarged broods mounting the weakest primary immune responses to SRBC (Fig. 1a, brood manipulation × clutch initiation date $F_{2,93} = 8.60$, $P = 0.0004$). However, there was no interaction between the effect of offspring number and the role of individual quality in breeding female cell-mediated responses to PHA (Fig. 1b, $F_{2,93} = 0.41$, $P = 0.52$). There was no difference between first-time breeding and older females in response to PHA or SRBC (SRBC $F_{1,93} = 0.57$, $P = 0.45$; PHA $F_{1,93} = 1.51$, $P = 0.22$), nor an effect of clutch size (SRBC $F_{1,93} = 1.75$, $P = 0.18$; PHA $F_{1,93} = 0.71$, $P = 0.63$).

The significant effect of brood manipulation treatment on female response to SRBC was not due to the five individuals in the enlarged brood size treatment that failed to produce antibodies, even though lack of antibody responsiveness is a common response to increased reproductive effort (Deerenberg *et al.* 1997). Mixed model analysis conducted removing those individuals who failed to produce antibodies retained a significant effect of both clutch initiation date and treatment, as well as an interaction between the two (clutch initiation date $F_{1,93} = 45.83$, $P = 0.0001$; treatment $F_{1,93} = 44.52$, $P = 0.0001$; interaction $F_{1,93} = 3.85$, $P = 0.02$).

OFFSPRING QUALITY

Breeding females, regardless of their brood manipulation treatment, raised offspring of similar quality

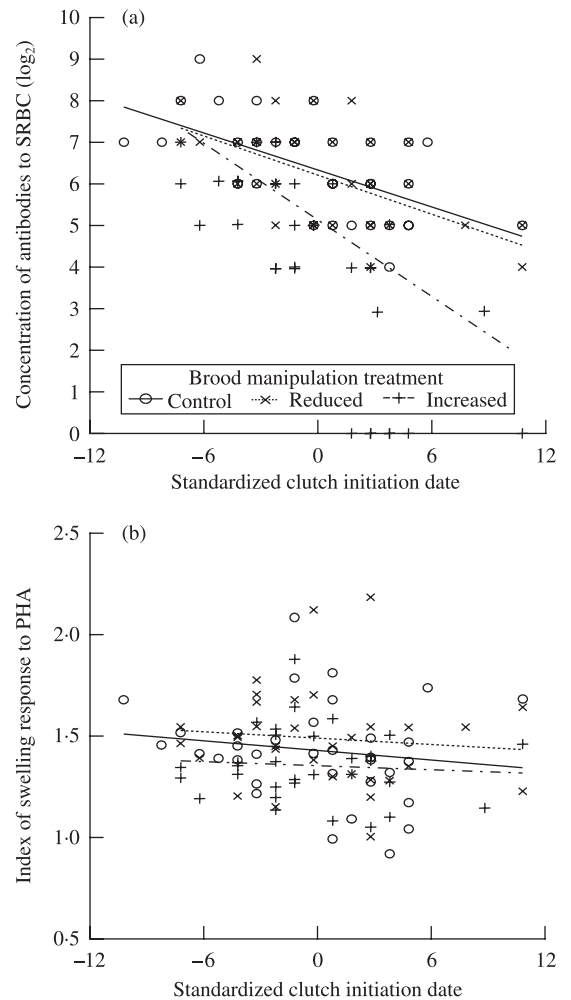


Fig. 1. Breeding female tree swallow immune responses as a function of timing of breeding and brood manipulation treatment ($n = 102$). Date of clutch initiation is standardized within each year to mean = 0. (a) Response to immunochallenge with Sheep red blood cells (SRBC). (b) Response to immunochallenge with phytohaemagglutinin (PHA).

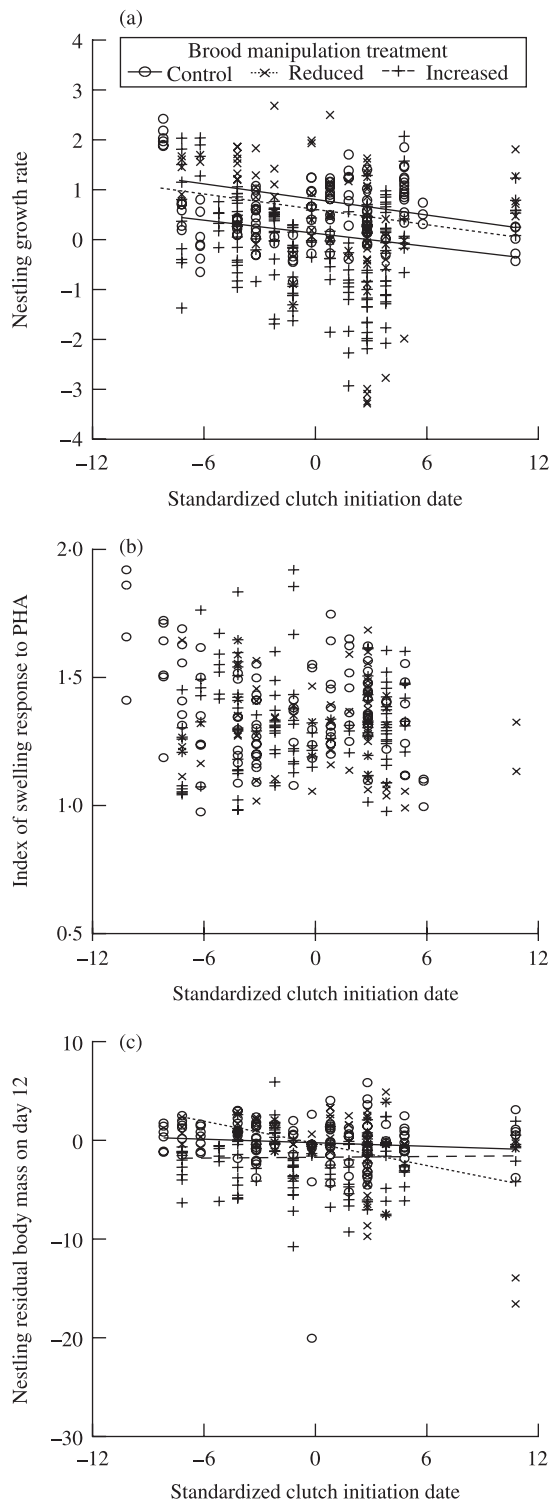


Fig. 2. Quality measures of nestling tree swallows as a function of timing of breeding and brood manipulation treatment ($n = 416$). (a) Nestling growth rate. (b) Immune response to PHA. (c) Nestling residual body mass. No line has been fitted to (b) because there was no central trend in the data.

(Table 1). Females feeding enlarged broods raised chicks that grew slightly slower ($F_{2,186} = 3.45$, $P = 0.06$; Fig. 2a); there was a stronger effect of quality, with lower quality individuals raising nestlings with slower growth rates (clutch initiation date $F_{1,264} = 4.29$, $P = 0.01$). There was no difference among females in the

response of their nestlings to immunochallenge with PHA (effect of treatment $F_{2,186} = 1.84$, $P = 0.43$, clutch initiation date $F_{1,264} = 1.18$, $P = 0.37$; Fig. 2b). Residual body mass of nestlings on day 12 decreased with increasing clutch initiation date ($F_{1,164} = 13.77$, $P = 0.003$; Fig. 2c), but there was no difference among treatments ($F_{2,164} = 1.56$, $P = 0.21$). There was no effect of either female age or clutch size on any of the three offspring quality measures ($F_s < 2$, $P > 0.4$).

Discussion

This study tested whether individuals of different quality differ in the trade-off they make between self-maintenance (reflected in immune function) and offspring quality. There was strong support for the prediction that individual quality, measured by clutch initiation date, mediated the interaction between reproductive effort and immune function. Early-laying individuals mounted stronger cell-mediated responses to PHA and humoral responses to SRBC. Furthermore, individuals differed in their response to increased offspring demand, with later-laying females mounting weaker humoral immune responses when forced to raise additional nestlings than did higher quality individuals also raising enlarged broods. These results suggest that studies examining immune function in the context of life history trade-offs need to consider more closely individual variation in how individuals respond to immunochallenges.

While breeding females differed in immune responses as a function of reproductive effort, there was a limited effect of brood size manipulation on the quality of offspring. Experimentally increased brood size led to slower growth rates, but not to lower residual body mass nor immune response to PHA. That all parents showed a decrease in immune function when raising enlarged broods, while offspring did not, suggests strongly that breeding female tree swallows in Ithaca are not passing along increased costs to offspring. In particular, lower-quality individuals exhibited the greatest decrease in immune response when brood size was increased but were still able to maintain offspring quality. This suggests a strong predilection to invest in offspring at a cost to themselves in self-maintenance and, possibly, yearly survival. For a short-lived migratory bird with yearly adult survival rates of around 50% (Robertson *et al.* 1992), it may be a prudent strategy for low-quality individuals to trade self-maintenance off against offspring quality in periods of increased resource demand, such as probably occurred when raising broods increased by 50%.

Why might lower-quality individuals make stronger trade-offs between immune responses and offspring quality? Individuals may differ in reactivity and the ability to recognize antigens, but these differences may be the proximate underpinnings for differences in strategy and constraint. Individual quality differences could lead to differences in strategy with lower-quality individuals,

which may have the lowest probability of returning to breed, valuing current reproduction the most: thus, an allocation strategy of reducing immune investment in favour of offspring would be favoured. Individual-level differences may also be non-strategic, as immune function can be affected by non-adaptive consequences of increased activity, such as increased metabolic activity (Demas *et al.* 1997; Ots *et al.* 2001) and increased free radical production (Chew 1995; Allen 1997). In addition, lower quality birds may be more likely to exhibit adaptive stress-related immunosuppression to minimize autoimmune cross-reactions (Råberg *et al.* 1998). Lower-quality individuals may have higher circulating levels of stress hormones or may be more at risk of autoimmune disorders. This possibility remains untested.

While there may be limitations to using timing of breeding as a measure of quality, the insect availability data presented here demonstrate that resources increase over the breeding season, therefore lower responses by later-laying individuals are not due to resource limitation. In addition, tree swallows display a suite of correlated life-history traits that appear to be adaptations for breeding early in order to maximize chances of acquiring a nesting cavity, such as high aggressiveness during nest site acquisition and feathering nests to reduce heat loss during cold early season conditions, that suggest strong pressure on individuals to breed early (Winkler 2000). Winkler & Allen (1996) and Winkler (unpublished), studying tree swallows at the same location, found no evidence that conditions improved over the breeding season; conversely, they concluded that later-laying individuals were unable to gather the resources necessary to breed earlier. It is as yet unclear why early-breeding individuals are more successful, although recent work has suggested that individual differences in resource-gathering ability could be underlain by differences in foraging efficiency (Bowlín & Winkler 2004).

There were subtle differences in the cell-mediated and humoral responses of individuals. Both experimentally increased brood size and lower individual quality led to weaker immune responses for both immunochallenges. However, only in humoral responses to SRBC was there an interaction between individual quality and reproductive effort in affecting the response. This suggests limited differences in either strategy or constraint among individuals mounting a cell-mediated immune response. Both cell-mediated and humoral immune responses entail energetic costs (Ots *et al.* 2001; Martin *et al.* 2003), but humoral responses require antigen recognition while the cell-mediated response to the mitogen measured here does not. However, without specific tests it is unclear how the different arms of the immune system respond to stress and somatic resource allocation (Blount *et al.* 2003).

Examining trade-offs between competing life-history demands can reveal evolutionary pressures, and eco-immunology has become an important approach to

consider the role of the immune system (Ricklefs & Wikelski 2002). The results presented here reveal that individuals differ in how they deal with trade-offs leading to individual differences in immune response; however, few studies examine individual differences. Therefore, studies examining eco-immunology of life-history decisions should consider individual variation in immune response and other strategies associated with self-maintenance and reproduction.

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