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Short-term fluctuations in cellular immunity of tree swallows feeding nestlings

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Abstract We examined cellular immunity of adult tree swallows feeding nestlings under variable weather conditions. Birds received an injection of phytohaemagglutinin (PHA), which causes a local swelling, reflecting the strength of T-cell-mediated immunocompetence. There was a negative relationship between the immune response and the number of nestlings in the brood (range 3–6 young) which suggests that parental effort suppresses the immune function. However, there was also a strong effect of ambient temperature and food abundance (aerial insects) on immune response. Parents that received the PHA injection during cold weather and at low food abundance showed a suppressed immune response compared to birds treated during more favourable conditions. They also lost more body mass during the 24 h inoculation period, and their offspring showed reduced growth. When controlling for ambient temperature and food abundance in a multivariate analysis, there was no longer any significant effect of brood size on the parents' immune response. Three of 39 pairs deserted their broods after PHA injection. All three desertions took place when the mean ambient temperature fell below 13°C. The PHA response is known to have both heritable and environmental components; our study emphasizes its condition-dependency. Previous studies of other passerine birds have shown that high levels of parental effort may have an immunosuppressive effect. Our study indicates that weather conditions may override the effects of natural variation in parental effort, and that the PHA response is particularly influenced by short-term fluctuations in energy balance.

Keywords Food abundance · Immunocompetence · Parental care · Phytohaemagglutinin · Weather conditions

Introduction

A central assumption of life history theory is that reproduction entails a cost in terms of future survival or fecundity. Studies of birds, usually involving clutch size or brood size manipulations, have successfully demonstrated a trade-off between current reproductive effort and future viability and reproduction (Røskaft 1985; Gustafsson and Sutherland 1988; Lindén and Møller 1989; Daan et al. 1996). However, the mechanisms by which current reproductive effort can have negative effects in the future are not well understood. Presumably heavy physical exertion causes some form of physiological stress that has long-lasting effects on viability and reproduction. Recently, attention has been directed to the immune system as it offers several possible pathways by which reproductive effort may have long-term effects on future performance (Gustafsson et al. 1994; Sheldon and Verhulst 1996; Råberg et al. 1998). Immunosuppression could be a direct consequence of oxidative stress caused by high metabolic rates (Jenkins 1993). It could also be an adaptive response to resource limitation as the immune system may have energetic or nutritional costs that compete with other costly demands (Lochmiller and Deerenberg 2000). Finally, immunosuppression may prevent damaging autoimmune responses during heavy physical exertion (Råberg et al. 1998). However, we still know very little about any long-term fitness consequences of a suppressed immune system during the reproductive period (Sheldon and Verhulst 1996).

A few studies have documented an immunosuppressive effect of reproductive effort in birds. Deerenberg et al.'s (1997) study on captive zebra finches (*Taeniopygia guttata*), and that of Nordling et al. (1998) on collared flycatchers (*Ficedula collaris*), both found that increased work rate suppressed the humoral immune response.

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Moreno et al. (1999) showed that increased reproductive effort in female pied flycatchers (*F. hypoleuca*) had an adverse effect on a T-lymphocyte-mediated immune response. There is a need for more documentation of the relationship between parental effort and the immune system, as the immunosuppressive effect of parental effort may not be a function of the workload alone. Brood demands will have to be met in a variable environment where the conditions for rearing offspring may fluctuate. Hence, an evaluation of the immunological cost of reproduction should ideally take into account the environmental conditions under which parental effort is expressed.

Here we report the results of an experiment on parent tree swallows (*Tachycineta bicolor*) feeding nestlings. We used the phytohaemagglutinin (PHA) skin testing technique (i.e. the same assay as used by Moreno et al. 1999) to study T-cell mediated immunocompetence in adults during the peak growth period of nestlings. Tree swallows feed on aerial insects, which implies that they may have severe problems meeting the energy demands of their brood in cold and rainy weather (Robertson et al. 1992). In this study we injected parents with PHA during a period of large fluctuations in ambient temperature and food abundance, which created a semi-natural experiment on how short-term energetic stress during brood rearing affects the immune response of the parent bird.

Materials and methods

The study was carried out during May–June 2000 in a box-nesting population of tree swallows at the UWM Field Station near Saukville, Wis., USA (43° 23′N, 88° 01′W). Information about the study area and the spatial distribution of nestboxes can be found in Whittingham and Dunn (2000). The tree swallow is an 18–22 g passerine, with a predominantly socially monogamous mating system, although polygyny occurs among less than 5% of males (Robertson et al. 1992; own observations). Our study included 39 nests, two of which belonged to one polygynous male who provisioned both broods. The study birds constituted roughly the early-breeding half of a population of approximately 80 breeding pairs.

Boxes were inspected daily during the early part of the season to determine the start of egg laying and clutch size, and every 1.5–2 h around the expected time of hatching to determine the exact time of hatching. At the experimental nests, a total of 6 males and 34 females were caught inside nest boxes early in the season, banded, measured and sampled for blood (for a parallel parentage study). On day 5 post-hatch, all experimental adult birds were caught in their box. Unless they had been caught earlier in the season, they were banded, bled and measured. Their body mass was recorded on an electronic balance (Pocket Pro 150-B, Acculab, readability 0.1 g). To test their T-cell-mediated immunocompetence we injected 0.2 mg of phytohaemagglutinin, (PHA-P, Sigma L-8754), in 40 µl of saline subcutaneously into their right wing. PHA causes a local swelling that reflects the strength of T-cell-mediated immunocompetence (Goto et al. 1978; Cheng and Lamont 1988). The injection site was located ventrally between the carpus and metacarpus where there is a thin layer of skin. As a control, we injected 40 µl of pure saline into the left wing. Immediately before injection, and 24±1 h thereafter, we measured the thickness of the wing at the injection site (to the nearest 0.05 mm) using a modified pressure-sensitive micrometer with a rounded tip (Teclock, model SM-112). The device's original spring, which makes a constant, though too strong, pressure on soft tissues, was replaced with a 10-g metal weight mounted on top of the measuring tip to make a more gentle pressure. The pressure could be reduced further with the handle operated by the thumb. This was especially important when measuring the PHA swelling, as too strong a pressure would tend to deflate the swelling. We measured the swelling when the measuring tip barely touched the skin. One person carried out all injections and wing-thickness measurements, and the average of two measurements was recorded each time.

At the 39 experimental nests, we caught and injected all 39 females and 37 of the 38 males. A total of 34 females and 25 males were recaptured the following day and their wing swelling response and body mass recorded. The wing swelling response was expressed as the increase in wing thickness at the injection site of the right wing. At the left wing there was no detectable change in wing thickness as the result of the control injection (mean change =–0.01 mm±0.16 SD; paired *t*-test: t=0.49, df=58, P=0.62). The repeatability (Lessells and Boag 1987) of the measurements of the control site was r=0.53 (F=3.21, df=58, 59, P<0.001). Hence, there was no need to adjust the measurement of the PHA injection site for changes at the control site (see also Smits et al. 1999).

We also monitored the condition of the young in the nest. Nestlings were weighed individually on days 4 and 6 post hatch, using an electronic balance (Scout, Ohaus, readability 0.01 g). The growth rate for each nestling was calculated as the mass increase divided by the time span between the two measurements (range 1.79–2.21 days). A mean nestling growth rate (in g/day) was calculated for each brood.

Data on ambient temperature and rainfall were extracted from a weather station at the field station (30-min sampling intervals). Food abundance was measured by a suction trap, 46 cm in diameter, which collects flying insects in a bottle with 70% ethanol (Southwood 1978: Fig. 4.2). The suction trap was placed in an open area between the two main nest box grids and operated from 0600 to 1900 hours each day. Food abundance was expressed as the total dry biomass of insects collected per day.

Results

The average size of the swelling response due to the injected PHA amounted to 1.53 mm±0.48 SD for females (n=34) and 1.49±0.48 SD for males (n=25). Thus, there was no difference in PHA response between the sexes (t=0.34, P=0.73). Variation in the PHA response was quite large as the measured swellings ranged from 0.40 to 2.35 mm for the two sexes combined.

Parental effort may be expected to vary with the number of young in the nest, as more young will require more food. We therefore analysed the effect of brood size on parental immune response. The PHA response declined with increasing brood sizes (Fig. 1), consistent with an immunosuppressive effect of parental effort. There was no difference in the relationship between PHA response and brood size for the two sexes (ANCOVA: F=0.02, df=1, P=0.88). Brood size declined significantly with date (r^2=0.27, df=32, P=0.0016). When controlling for date in a multiple regression analysis, the PHA response of the parents was still negatively affected by brood size (standardized regression coefficient=–0.288, P=0.05).

However, the immune response was strongly influenced by variation in the weather conditions when individual parents were tested. Figure 2 displays the variation in ambient temperature, rainfall and food abundance (insect biomass/day) during the 13 days of PHA injections. The daily mean temperature varied between

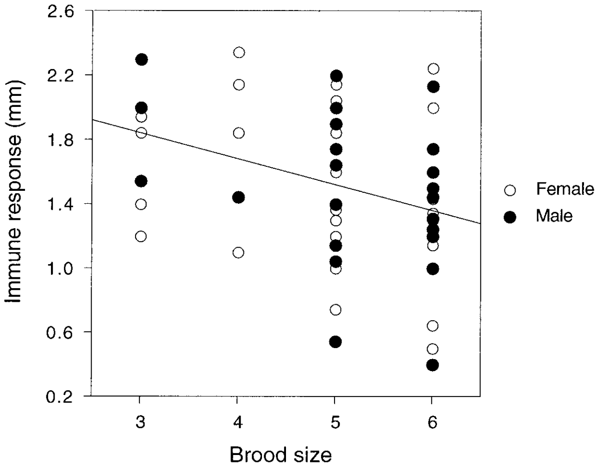

Fig. 1 Relationship between the immune response (thickness of swelling) to injected PHA in tree swallow parents feeding 5-day old nestlings and the number of young in their brood (r^2=0.11, n=59, P=0.010). The linear regression line for both sexes combined is indicated [y=2.325–0.16 (0.06 SE)x]

Table 1 Multiple regression analysis of potential factors influencing the immune response of adult tree swallows. Ambient temperature is expressed as the mean for 30-min intervals during 24 h following PHA injection. Food abundance is expressed as the mean dry biomass of aerial insects collected in the suction trap during the day of PHA injection and the following day

Variable	Standardized regression coefficient	*t*-test	*P*
Ambient temperature	3.76	2.87	0.006
Ambient temperature squared	–3.31	–2.54	0.014
Food abundance	1.40	2.04	0.046
Food abundance squared	–1.40	–2.10	0.040
Date	–0.26	–1.45	0.154
Brood size	–0.20	–1.57	0.122
Intercept	–1.55	–1.52	0.134

Whole model: R^2=0.38, F=5.34, df=6, 52, P<0.001

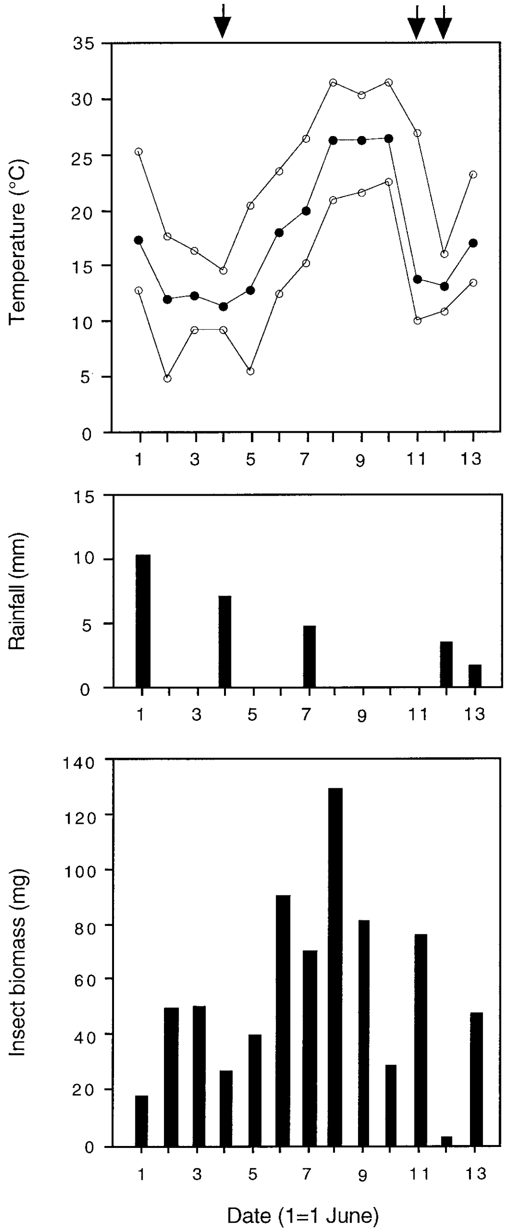

Fig. 2 Ambient temperature, rainfall and food abundance during the period of experimental treatment of adult tree swallows. The three temperature curves denote the maximum, mean and minimum temperatures, respectively. For a given day the values are based on temperature measurements from noon (1200 hours) to noon the following day to better match the exact period of treatment for individual birds. Food abundance is expressed as the total dry biomass of insects collected in the suction trap from 0600 to 1900 hours each day. The *arrows* at the top indicate the days when three brood desertions occurred

11.4°C and 26.5°C, and rainfall occurred predominantly on colder days (Fig. 2). Food abundance correlated positively with mean ambient temperature (r^2=0.34, P=0.037, n=13 days).

The immune response of the adults was related to the mean ambient temperature during the 24 h following PHA injection and to the food abundance averaged for the day of injection and the following day (Fig. 3). The relationships were not strictly linear, as polynomial regressions made a better fit to the data (Fig. 3). Immune responses showed a marked decline at temperatures below 13°C. Although temperature and food abundance were positively correlated, both variables had a significant, independent effect on the immune response as shown in a multiple regression analysis (Table 1). There were no significant effects of brood size or date on the immune response (Table 1). Temperature and food abundance together explained 34% (=R^2) of the variation in the immune response (F=6.85, df=4, 54, P<0.001; brood size and date excluded from the model). Thus, we conclude that environmental conditions, affecting the availability of food, had a significant impact on the immune response of the adults, whereas brood size and date had no such measurable effects.

Most birds lost body mass during the 24 h inoculation period. The average mass loss amounted to 0.48 g

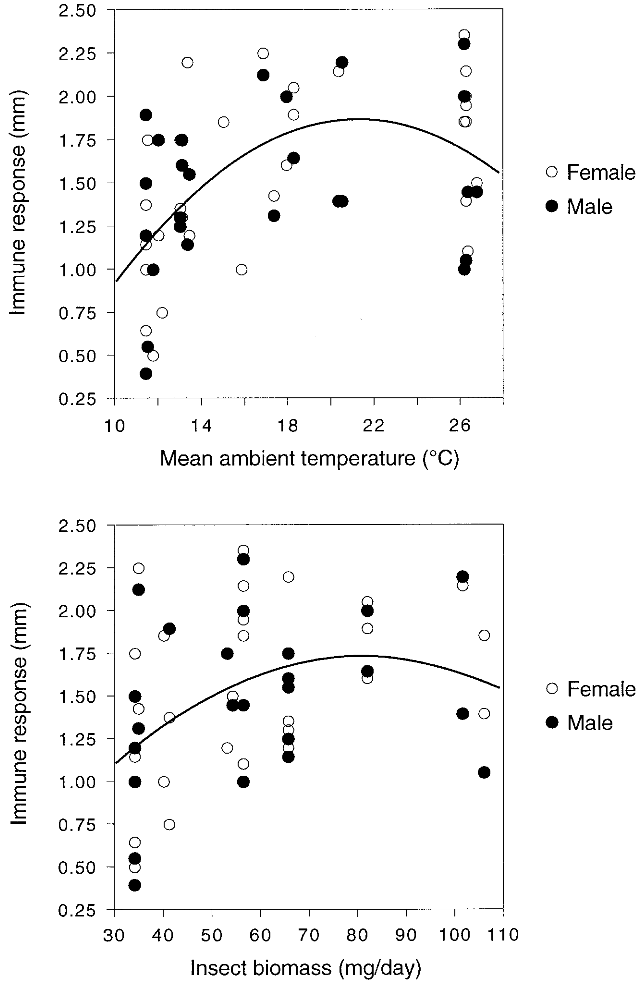

Fig. 3 PHA response in adult tree swallows (n=59) in relation to mean ambient temperature (*upper panel*) and food abundance (*lower panel*). Ambient temperature is expressed as the mean for 30-min intervals during 24 h following the injection. Insect abundance is expressed as the mean dry biomass of aerial insects collected in the suction trap during the day of PHA injection and the following day. Polynomial regression lines are indicated for both sexes combined. *Upper panel*: y=–1.487+0.317 (0.093 SE)x–0.007 (0.002 SE)x^2; R^2=0.28, P<0.0001; linear term P=0.001, quadratic term P=0.004. *Lower panel*: y=0.152+0.039 (0.015 SE)x–0.00024 (0.00011 SE)x^2; R^2=0.18, P=0.004; linear term P=0.011, quadratic term P=0.033

(paired t-test: t=–5.26, df=57, P<0.001). The magnitude of the immune response was positively related to the change in body mass (Fig. 4), which indicates that birds with a poor immune response were also energetically stressed.

Birds with a poor immune response also had nestlings with a reduced growth rate. The mean nestling growth rate from day 4 to day 6 was positively related to the average immune response of the two parents (r^2=0.29, df=32, P=0.0012). This might be a reflection of weather conditions, since nestling growth also correlated strongly with ambient temperature and food abundance (Fig. 5). In a multiple regression analysis, controlling for food abundance and brood size, there was no significant residual effect of the parental immune response on nestling growth rate (Standardized regression coefficient=0.242, P=0.15).

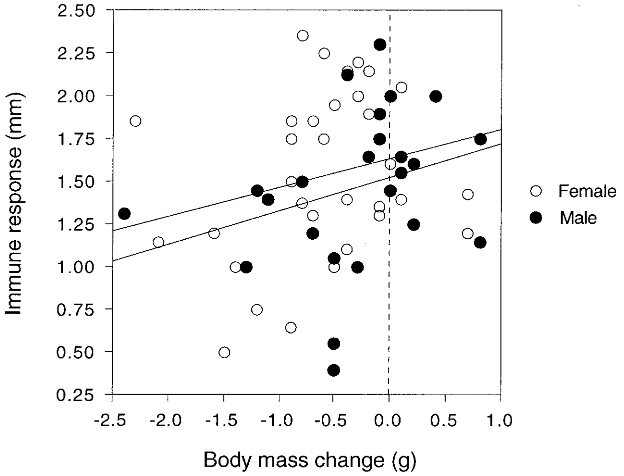

Fig. 4 The relationship between adult immune response and their mass change 24 h after the injection of PHA. Regression lines for each sex are indicated. The immune response was affected significantly by mass change (F=4.10, df=1, 55, P=0.048) in an ANCOVA controlling for sex (F=0.98, df=1, 55, P=0.33)

At 3 of the 39 experimental nests the nestlings died during the night following the injection of the parents, probably because they were not brooded by the female. In all three cases the weather was rather cold (Fig. 2) and the parents tended to have a low initial body mass. In pairs that deserted, females were on average 1.18 g lighter and males 0.90 g lighter than in pairs that continued breeding. The probability of desertion was significantly affected by parental body mass (Logistic regression with sex as a factor: χ^2=4.28, df=1, P=0.039). After the third brood desertion had occurred, we decided to terminate the experiment to avoid any further stress on the remaining breeding birds.

Discussion

The PHA skin test used in the present study was originally developed in the poultry sciences, where it has been shown to be a reliable measure of T-lymphocyte-mediated immunity (Goto et al. 1978; Cheng and Lamont 1988). T-lymphocytes play a major role in antigen recognition and are therefore a key element in the immune defence against pathogens such as bacteria and viruses (Abbas et al. 1994). The test has been used increasingly in ecological studies of free-ranging birds (e.g. Gonzalez et al. 1999; Moreno et al. 1999) and their nestlings (e.g. Saino et al. 1997; Brinkhof et al. 1999; Johnsen et al. 2000). The evidence is strong that this particular immune response is both condition-dependent (Saino et al. 1997; Birkhead et al. 1999; Gonzalez et al. 1999; Moreno et al. 1999) and heritable (Saino et al. 1997; Brinkhof et al. 1999; Johnsen et al. 2000). The

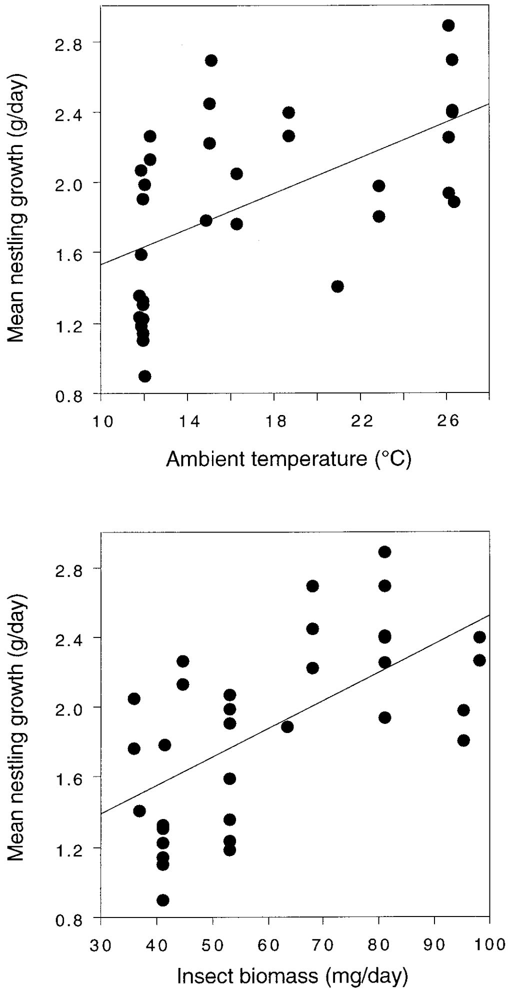

Fig. 5 Mean nestling growth from day 4 to day 6 post-hatch of tree swallow broods (n=33) in relation to the mean ambient temperature (*upper panel*) and mean daily food abundance (*lower panel*) during the same period. Linear regression lines are indicated. *Upper panel*: y=1.024+0.051 (0.013 SE)x; r^2=0.31, P<0.001; *lower panel*: y=0.904+0.016 (0.004 SE)x; r^2=0.39, P<0.001)

present study emphasizes the condition-dependency of the PHA response. We found that the response was significantly suppressed in tree swallows when the weather was bad and the birds lost body mass. This is consistent with the general view that mounting an antigen-induced immune response is energetically costly (Svensson et al. 1998; Lochmiller and Deerenberg 2000).

Our results also illustrate how an aerial insectivore like the tree swallow is susceptible to poor weather conditions, especially during the parental care period. Both the parents and their young suffered from reduced food intake during poor weather conditions, as indicated by the weather-associated changes in body masses and food abundance. Cold and rainy weather reduces the abundance of flying insects significantly, and adult tree swallows have serious problems finding enough food for themselves and their young. Aerially foraging species, like the tree swallow, also have higher energy requirements than other species (Williams 1988). If cold and rainy weather persists for more than a few days, total brood failures become more common in tree swallows (Robertson et al. 1992; own observations). Similar weather susceptibility has been shown for the house martin (*Delichon urbica*), another aerial insectivore with a similar breeding ecology (Bryant 1973, 1978).

The injection of PHA itself may have caused additional stress on the birds, as indicated by the occurrence of three brood desertions, and the fact that most birds lost body mass during the 24-h inoculation period. The PHA skin test may not be as harmless as suggested by Merino et al. (1999), and some caution should be exerted. The amount of injected PHA is apparently not of trivial importance. We injected 0.2 mg per bird, which is an amount similar to what others have used for a bird of similar size (e.g. Saino et al. 1997), but ten times more than that used by Merino et al. (1999). The need for obtaining a measurable swelling response that truly reflects individual responsiveness must be traded against the risk of potentially harmful effects of the treatment. Therefore, we recommend that pilot studies be carried out to determine the minimum amount of PHA needed for an appropriate response in each case.

An alternative to the energetic-stress explanation is that the PHA response is directly affected by heavy workload (Råberg et al. 1998), which may also cause loss of body mass. This possibility seems less likely since studies of energy budgets of house martin parents with dependent young have shown that they reduce, not increase, their metabolic rate under poor weather conditions (Bryant and Westerterp 1980). Thus, reduced food abundance probably leads to reduced foraging activity by the parents, and the two factors together imply a lowered food intake for both parents and their young. In our study, parent tree swallows apparently fed their offspring very little when the weather was cold. This suggests that it is not the energy expenditure itself during offspring rearing that matters for the individual's ability to launch a proper immune response. Rather, it is the total energy balance or physiological condition (which certainly also can be influenced by parental workload) that eventually determines the immunocompetence of the individual. An important implication of our study is that the energy balance of a parent may be more strongly influenced by environmental factors related to food abundance than by variation in the energy demands represented by the number of young in the nest. Therefore, caution should be used in ecological studies when interpreting causal relationships between parental responses and brood demands under fluctuating weather and food conditions. Likewise, observed immune responses may not necessarily reflect inherent quality differences between individuals when environmental conditions vary. Hasselquist et al. (2001) reported a negative association between humoral immunity, measured during nestling provisioning, and breeding date in tree swallow females, which they interpreted as early breeders being of better quality. An alternative interpretation is that the early breeders experienced more favourable environmental conditions during the nestling period than late breeders.

The tight association between the measured PHA responses and the daily fluctuations in environmental conditions indicates a strong short-term plasticity of the PHA response. The cellular immune function is apparently subject to condition-dependent control that may be very fine-tuned and related to the immediate physiological condition of the bird. Other parts of the immune system, e.g. humoral immunity, may have a slower regulation mode and be more strongly affected by physiological stress over a longer time scale. It is therefore questionable whether the PHA skin test is a suitable measure for immunological costs over longer time scales, for example as a measure of an immunological cost of parental effort in a life-history perspective. In the study by Moreno et al. (1999) female pied flycatchers with enlarged broods had a reduced PHA response and a lower body mass than those rearing control and reduced broods. This pattern could simply reflect a short-term difference in current work load between experimental and control birds, rather than a long-term effect of previous effort. Whether such short-term fluctuations in cellular immunity have significant fitness costs in terms of reduced survival or fecundity remains to be studied. More experimental work is certainly needed in this field (Sheldon and Verhulst 1996).

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