

The T-cell-mediated immune response and return rate of fledgling American kestrels are positively correlated with parental clutch size

José L. Tella, Gary R. Bortolotti*, Russell D. Dawson and Manuela G. Forero

Department of Biology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 5E2

Life-history theory predicts that parents face a trade-off between the number and viability of the progeny they produce. We found evidence for an apparent trade-off in a free-living population of American kestrels (*Falco sparverius*), as larger clutches produced more but lighter fledglings. However, while the body mass of fledglings has traditionally been used as a measure of survival prospect, offspring immunocompetence should also play an important role. We thus measured the T-cell-mediated immune response of fledgling kestrels in relation to brood traits and nest-rearing conditions through a cross-fostering experiment. The immune response was positively correlated with the body condition of fledglings, but was also higher in those hatched from five-egg than four-egg clutches. These results were not influenced by other brood traits, nor by current exposure to stressors and infectious agents, as measured by serological variables. Such ability to resist pathogens may account for why the probability of offspring returning to the study area in subsequent years, when controlling for brood size, was higher for five-egg than four-egg clutches. These results suggest an optimal clutch size through maternal effects on offspring immunocompetence rather than a trade-off between the number and quality of the offspring.

Keywords: immunocompetence; clutch size; offspring viability; American kestrel

1. INTRODUCTION

Parents are thought to face a trade-off between the number and quality of the progeny they produce (Stearns 1992). Birds have been used extensively as study subjects in such life-history investigations and compromises have been detected between brood size and the mass of young at independence (Lessells 1991; Stearns 1992). However, finding other, more direct estimates of offspring viability has been a challenge. Whereas body mass and, thus, presumably energy stores may be indicative of the ability of young birds to withstand periods of food deprivation just after leaving the nest (Magrath 1991), other factors may be more important determinants of survival. In particular, immunocompetence, i.e. the ability of an organism to minimize the fitness costs of an infection, may be a better predictor than mass of long-term survival prospects. Recent studies have shown a positive correlation between the ability of the immune system to cope with a novel antigen and further survival in adult birds (Saino *et al.* 1997a; Soler *et al.* 1999). Therefore, the study of offspring immunocompetence could offer new insight into the classic trade-off between the number and quality of progeny (Owens & Wilson 1999).

We investigated the potential trade-offs between the number and viability of young in the American kestrel, a small falcon widely distributed in the New World. First, we assessed the relationships between clutch size, brood size and the mass of fledglings at independence. Then, we challenged the cell-mediated immune response of fledglings, one of the two main branches of the vertebrate acquired immune system (Pastoret *et al.* 1998), through the phytohaemagglutinin skin testing technique (e.g. Smits *et al.* 1999). It is based on the injection of a novel

mitogen (phytohaemagglutinin) into the skin of a bird, typically at the patagium. The mitogen induces a prominent perivascular accumulation of T lymphocytes followed by macrophage infiltration (Goto *et al.* 1978). This technique is considered a useful method of evaluating thymus-dependent immune function (Goto *et al.* 1978), is easily applied in the field and does not provoke potential confounding effects associated with physiological stress (Merino *et al.* 1999). The intensity of skin swelling as a response to the mitogen injection has been proven to correlate with a number of components of fitness in free-living birds (Saino *et al.* 1997b, 1998; Sorci *et al.* 1997; Christe *et al.* 1998; Moreno *et al.* 1998, 1999; Soler *et al.* 1999). In addition, we measured serological parameters (total and differential leucocyte counts and the heterophil:lymphocyte ratio from thin blood smears). These variables can be used as indicators of current exposure to infectious agents and stressors such as food deprivation, inclement weather and psychological disturbance (Maxwell 1993; Moreno *et al.* 1998; Ots *et al.* 1998) which could influence the ability of birds to respond to the mitogen. Finally, we analysed common factors between our immune-related measure of offspring viability and the probability of returning to the natal population in subsequent years.

2. METHODS

(a) *Study area and field procedures*

We studied American kestrels from 1988 to 1997 in the boreal forest of north-central Saskatchewan, Canada (55°N, 106°W) (Gerrard *et al.* 1996). Although cavities were abundant, the kestrels preferred nest-boxes and virtually all of the 150–200 pairs in the area laid in the 380 boxes available each year (Bortolotti 1994; Wiebe & Bortolotti 1995). Our data on their reproductive parameters and return rates are thus complete for

*Author for correspondence (bortolotti@sask.usask.ca).

this population. We determined nesting chronology for all birds by repeated visits to the boxes, usually with an interval of two days, starting before eggs were laid. After the first egg was laid, we waited until the clutch was complete before visiting again to record clutch sizes. The nests were revisited periodically to determine hatching dates and, hence, nestling ages and the number of young at fledging. To evaluate potential trade-offs with clutch size, we used data from 1994 to 1997. For the analysis of the number of offspring produced, we excluded nest failures during incubation which were the result of predation or other hazard casualties. For the analysis of fledgling mass we only used data for nests where measurements were taken at a comparable age (22–23 days old) and where there were no brood manipulations (see §2(b)). For the analyses concerning fledgling mass we computed the within-nest average mass for males and females separately and, in doing so, avoided pseudo-replication and accounted for sexual size dimorphism.

As all fledglings were banded, the subsequent return of these birds to this population was determined by capture. Each year we captured birds using bal-chati traps in the spring during the pre-laying period and later by hand in the nest-boxes during incubation (Bortolotti & Iko 1992). We believe we detected most of the birds which both fledged and eventually returned to our area, given that kestrels are short lived, we captured most of the breeding birds each year and we analysed only those cohorts for which at least five years had elapsed post-fledging (G. R. Bortolotti, unpublished data).

(b) Immune function

In 1997, we performed a cross-fostering experiment where two randomly selected five-day-old nestlings were transferred between nests ($n = 44$) matched by hatching date and clutch size. Nestlings not transferred between nests were removed and transported in the same manner and for the same length of time as their siblings and then put back in their nests to control for the potential effects of handling stress. We identified each nestling as an individual by coloured waterproof marks and later by metal bands. The manipulation helps control for variable nest environments without complicating the clutch size and parental effort effects (see Potti *et al.* 1999). Only clutches of four and five eggs were studied given the rarity or absence of other sizes (see below). We measured the immune function of 142 nestling kestrels at 22 days old (just prior to nest departure). At that age, we weighed the birds (to the nearest gram) and measured the length of the tenth primary (to the nearest millimetre). For the determination of leucocyte differentials, we extracted a drop of blood and smeared it on a microscope slide, which was then air-dried and fixed in absolute ethanol. We later stained these smears with Wright–Giemsa stain and estimated the total white blood cell count (WBC, cells $\times 10^9$ per litre) by taking the average number of leucocytes in five fields observed under a $\times 40$ lens. The WBC was standardized to the number of red blood cells and, to control for variability in the smear preparation, the fields selected were only in an evenly distributed monolayer. The proportions of heterophils and lymphocytes and the heterophil:lymphocyte ratio (H:L) were determined by identifying 100 cells under $\times 40$ power.

To evaluate the T-cell-mediated immune response (CMI), we injected 50 μ l of 1 mg ml⁻¹ phytohaemagglutinin-P (Sigma) in phosphate-buffered saline (PBS) intradermally in the right wing web. The left wing web was injected with an equal quantity of PBS as a control. We measured the thickness of each wing web with a micrometer (to the nearest 0.001 mm) four times just

prior to and 24 h (± 16 min) after injection. As the repeatability of the measurements was high (Smits *et al.* 1999), the mean was used for statistical analysis. The CMI was the difference in wing-web swelling between the mitogen-injected and control sites (Smits *et al.* 1999).

(c) Statistical approach

First, we assessed the relationships between clutch size, brood size and the mass of fledglings at independence by using ANOVA or Kruskal–Wallis ANOVA, depending on whether or not the dependent variables were normally distributed. Second, for multivariate analyses we performed generalized linear models (GLMs) (McCullagh & Nelder 1983) using the GLIM package (Crawley 1993). GLIM allows a simultaneous assessment of the contribution of a number of continuous and categorical explanatory variables and their interactions on a response variable. This statistical procedure also permits the use of other error formulations when the normal error for traditional regression is not applicable. Each explanatory variable is tested for significance in turn following the stepwise branching modelling procedure (e.g. Bustamante 1997). The result is the most adequate model for explaining the variability in the response variable, where only the significant explanatory variables are retained. When data suggest no linear trends, explanatory variables are transformed and fitted again to try and improve their contribution to the model.

We used the normal error and an identity link function (thus approaching a multiple linear regression) for the immune function variables (which were normally distributed) and separate models were performed for each response variable (the CMI, WBC, leucocyte differentials and arcsine H:L). The explanatory variables for each fledgling were laying date, clutch size, ranked hierarchy within the brood at five days old (rank 1 to the eldest nestling and so on), sex, size (tenth primary length), mass, body condition (the residuals from a linear regression of body mass on the cube length of the tenth primary; $r = 0.44$, $F_{1,140} = 15.15$ and $p < 0.001$) and the presence or absence of mortality in the nest at 22 days old. In addition, two categorical variables distinguished birds hatched or reared in the same nest, respectively, to account for potential nest effects.

An improved GLM procedure was used (Crawley 1993; Tella *et al.* 1999) for analysing the probability of fledglings returning to the natal population in relation to clutch size in order to solve the problem of larger clutch sizes producing more returns just by chance because they produce more fledglings. Therefore, instead of using the proportion of returning birds by nest, this GLM procedure used the number of birds returning as the response variable and brood size as the binomial denominator in order to control for differences in brood size. For this kind of binary data (fledglings returning = 1 and fledglings not returning = 0), a binomial error and a logistic link function are adequate, resulting in a GLM which approaches a weighted logistic regression. We analysed the return rate of 1889 fledglings from 505 broods from the cohorts of 1988–1992. Cohort was included as an explanatory variable to investigate year effects.

3. RESULTS

From 1994 to 1997 the modal clutch size of 518 nests was five eggs (67.6%), followed by four eggs (27.4%), three eggs (3.5%), six eggs (1.2%) and two eggs (0.4%). After excluding clutches of two and six eggs because of the small sample, the number of fledglings per brood

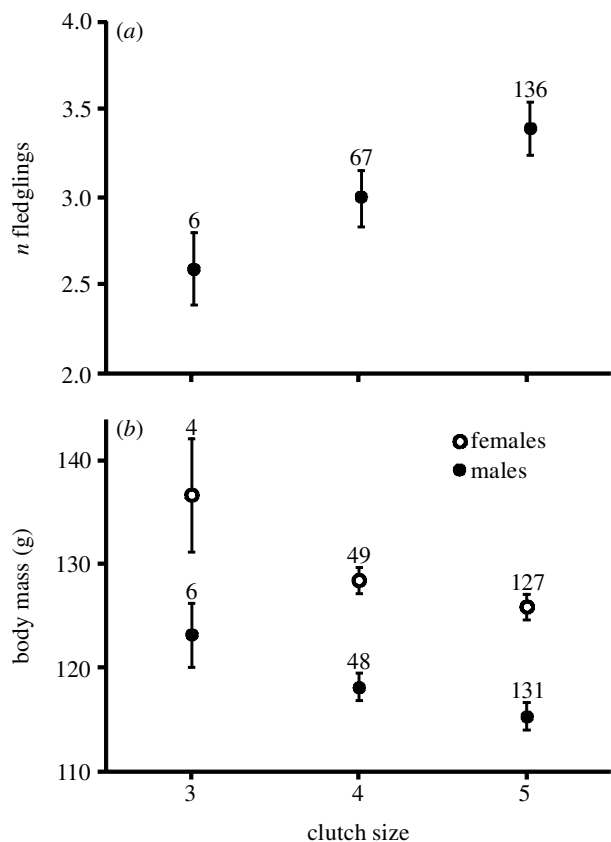


Figure 1. (a) The number and (b) mass of fledglings in relation to clutch size in American kestrels. The values shown are means ± 1 s.e. with the sample sizes of nests above bars.

increased from three- to five-egg clutches (Kruskal–Wallis ANOVA, $\chi^2_2 = 11.52$ and $p = 0.003$) (figure 1a). However, the fledging mass decreased with increasing clutch size (two-way ANOVAs: for males, clutch size $F_{2,180} = 3.16$ and $p = 0.04$, year $F_{3,180} = 3.09$ and $p = 0.03$ and interaction $F_{4,180} = 0.73$ and $p = 0.57$, and for females, clutch size $F_{2,170} = 6.08$ and $p = 0.003$, year $F_{3,170} = 3.15$ and $p = 0.03$ and interaction $F_{4,170} = 1.83$ and $p = 0.13$) (figure 1b).

A GLM model revealed that the CMI was only related to the body condition of the fledglings ($F_{1,141} = 12.96$ and $p < 0.001$) and clutch size ($F_{1,141} = 15.22$ and $p < 0.001$). This model accounted for 17% of the original deviance (i.e. 17% of the variance), which was nearly equally explained by clutch size (54%) and body condition (46%). The immune response was positively correlated with body condition and was higher in kestrels hatched from five-egg than four-egg clutches, as shown by the fitted lines derived from the model (figure 2). No other variables or interactions explained the variance in the CMI. A nearly identical model resulted when mass ($F_{1,141} = 12.77$ and $p < 0.001$) was used instead of body condition. In contrast, the GLM models for the serological parameters showed significant effects for nest of origin and nest of rearing (all $p < 0.001$), but not for any other explanatory variable. Given the complexity of the GLM approach and to remove any question of potential biases because multiple birds per nest were used, we have included two simple statistical tests to support our contention of a clutch-size effect. The within-nest average CMI

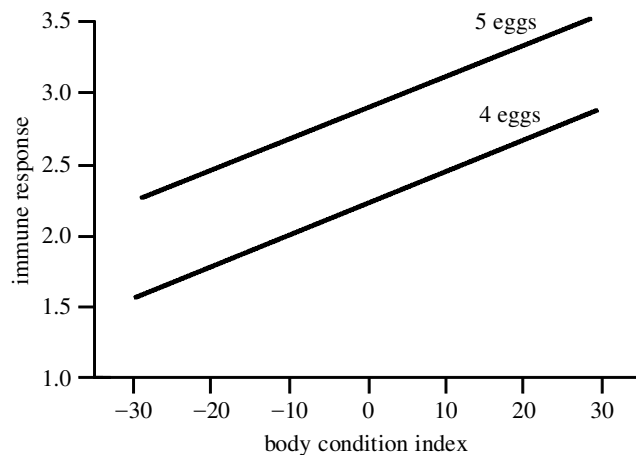


Figure 2. The relationship between the CMI response, clutch size and body condition of 142 fledgling American kestrels. The fitted lines are derived from a GLM model with a normal distribution of errors and identity link.

was higher in birds from five-egg (2.87 ± 0.10 s.e. and $n = 26$ nests) than four-egg clutches (2.21 ± 0.11 s.e. and $n = 8$ nests) (Student's t -test, $t = 3.47$, d.f. = 32 and $p = 0.002$). To control for the influence of body mass on the CMI, we first obtained residuals from the regression of the CMI on body mass ($r = 0.26$, $F_{1,140} = 10.076$ and $p = 0.002$). The within-nest average of these residuals for birds from five-egg clutches (0.21 ± 0.13 s.e.) was also significantly higher than the average from birds from four-egg clutches (-0.68 ± 0.12 s.e.) ($t = 3.53$, d.f. = 32 and $p = 0.001$).

Clutch size also proved to be relevant in explaining the return rate of the kestrels. The GLM obtained showed significant effects of both clutch size ($\chi^2_{4,500} = 12.73$ and $p < 0.025$) and year ($\chi^2_{4,496} = 9.59$ and $p < 0.05$) when controlling for brood size. This model accounted for 8% of the original deviance (57% corresponding to clutch size and 43% to year) and did not show data overdispersion (residual deviance/residual d.f. = 0.54). Birds from five-egg clutches were more likely to return to the study area compared to those from four-egg clutches (figure 3).

4. DISCUSSION

The body mass of nestlings is one of the most commonly measured variables in evaluating avian reproductive performance. Not only is it widely used as an index of the growth and overall quality of the young themselves, but it is also used as a measure of the quality of parental care, habitat and the efficacy of reproductive decisions to name but a few (Tinbergen & Boerlijst 1990; Lessells 1991; Wiebe & Bortolotti 1994). The logic that greater mass equates with higher body reserves at fledging and subsequently higher survival at independence is intuitively appealing. However, empirical evidence has not always supported an association between mass and survival post-fledging (Magrath 1991), nor is it clear how or if mass *per se* is causally responsible.

It is difficult to identify the effects of body mass on survival independently of brood size, feeding conditions and other traits. Studies by Magrath (1991) and Tinbergen & Boerlijst (1990) have supported the idea that mass has a direct effect on survival, but by what

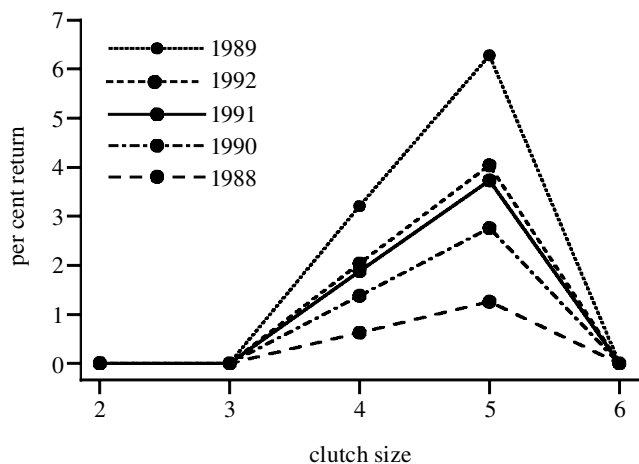


Figure 3. The percentage of nests producing a fledgling which subsequently returned to the study area, controlling for brood size, as derived from a GLM model using binomial error, brood size as binomial denominator and logistic link.

means is unknown. Our work (figure 2) and other recent investigations (Saino *et al.* 1997b; Sorci *et al.* 1997; Christe *et al.* 1998) have suggested a mechanism of explaining survival in that nestlings in good body condition have enhanced immunocompetence. The finding that immune function varies negatively with brood size and positively with feeding rate (Saino *et al.* 1997b) is consistent with Tinbergen & Boerlijst's (1990) hypothesis that mass directly affects survival. In contrast to such immediate environmental effects on immunocompetence, we found that birds from five-egg clutches, regardless of which nest they were reared in and despite being lighter in mass (figure 1), had an enhanced CMI and a substantially higher return rate (figure 3). It is noteworthy that the clutch size effects on return rates were patent in five cohorts exposed to different conditions of weather and food availability (Wiebe & Bortolotti 1994), environmental factors which could obscure potential maternal effects, such as clutch size, on survival (Lindström 1999). We did not detect any effects of laying date or brood size on the CMI as were found by Sorci *et al.* (1997) and Saino *et al.* (1997b), respectively. However, those studies did not take into account clutch size, a trait which typically covaries with laying date and brood size in birds. Our findings were not the result of bias in exposure to infectious agents or stressors because the serological parameters which evaluated health at the time of sampling (Moreno *et al.* 1998; Ots *et al.* 1998) were unrelated to clutch size. Because the return rates we documented were lower than the expected survival rates, a fraction of the birds probably dispersed. However, to our knowledge, the effects of clutch size on dispersal behaviour have not been documented (Forero 1998), so the return rates here probably correlate with survival.

From the more traditional perspective of equating nestling mass with quality (Smith *et al.* 1989), it would appear that parental fitness in American kestrels could be independent of the number of eggs laid (figure 1). However, our immunological results (figure 2) and the analysis of the return rates (figure 3) suggest an optimal clutch size. The failure of six-egg clutches to produce recruits suggests that immunocompetence may not increase linearly with

clutch size. Recent studies have shown that the quality and recruitment probability of the young (Monaghan & Nager 1997) and components of the immune system (Ricklefs 1992) are affected by events early in development. It is known that female American kestrels with large clutches have difficulty in incubating their eggs successfully and that embryogenesis is compromised (Bortolotti & Wiebe 1993; Wiebe & Bortolotti 1993). Otherwise, how young kestrels may have been endowed with a robust CMI is not known. However, as the CMI was explained by clutch size rather than more immediate, proximal factors, such as the number of siblings in the nest, maternal effects on the eggs are implicated. It is known in birds that both nutritional status and the diet of females before laying affect egg size, clutch size and even breeding success (Selman & Houston 1996; Ramsay & Houston 1998). Accordingly, female American kestrels with better pre-laying body condition lay larger eggs and that egg size correlates positively with clutch size (Wiebe & Bortolotti 1995). In other bird species, physiologically active compounds derived from maternal diets are known to be incorporated into their eggs and subsequently enhance the immunocompetence of neonates (Haq *et al.* 1996; Pastoret *et al.* 1998; Surai *et al.* 1998; Royle *et al.* 1999). In addition, as both clutch size (Stearns 1992) and immunocompetence (Pastoret *et al.* 1998) have a genetic basis, these traits could covary through selection for good genes. Therefore, a female may have her biggest impact on the viability of her offspring through her choice of mate. American kestrels pair assortatively with respect to condition (Bortolotti & Iko 1992) and morphological characters thought to be involved in mate choice correlate with parasite resistance (Wiehn 1997; Wiehn *et al.* 1997). Our study suggests a direction for investigating selection avenues for specific resistance to parasites and emphasizes that parents may focus on the viability rather than number of their offspring (Wedekind 1994).

Studies of immune function have provided a new perspective on offspring viability (Saino *et al.* 1997b; Christe *et al.* 1998), while our results add evidence to the recent claim that early development traits, such as clutch size, may play an important role in the survival of birds (Lindström 1999). The degree to which nestling body mass versus brood traits such as clutch size contributes to the immune response may explain the lack of consistency among studies of the effects of mass, brood size and food supply on post-fledging survival (Tinbergen & Boerlijst 1990; Lessells 1991; Magrath 1991). Similarly, manipulations of reproductive parameters in detecting life-history trade-offs or the cost of reproduction should be aware of the potential confounding effects of offspring quality mediated by immune function.

M.G.F. was supported by the Isabel María López Martínez Memorial Scholarship. J.L.T. received a post-doctoral grant from the Spanish Ministry of Education. Funding was provided by the Natural Sciences and Engineering Research Council of Canada (to G.R.B.), the Northern Scientific Training Program and the Collaborative Research Grant Program of NATO. We thank J. Willson and K. Skelton for their invaluable help and D. Shutler, J. E. Smits and K. L. Wiebe for comments on the manuscript. This study was approved by the University of Saskatchewan's Animal Care Committee on behalf of the Canadian Council on Animal Care.

REFERENCES

- Bortolotti, G. R. 1994 Effect of nest-box size on nest-site preference and reproduction in American kestrels. *J. Raptor Res.* **28**, 127–133.
- Bortolotti, G. R. & Iko, W. M. 1992 Non-random pairing in American kestrels: mate choice versus intra-sexual competition. *Anim. Behav.* **44**, 811–821.
- Bortolotti, G. R. & Wiebe, K. L. 1993 Incubation behaviour and hatching patterns in the American kestrel *Falco sparverius*. *Ornis Scand.* **24**, 41–47.
- Bustamante, J. 1997 Predictive models for lesser kestrel *Falco naumanni* distribution, abundance and extinction in southern Spain. *Biol. Conserv.* **80**, 153–160.
- Christe, P., Møller, A. P. & De Lope, F. 1998 Immunocompetence and nestling survival in the house martin: the tasty chick hypothesis. *Oikos* **83**, 175–179.
- Crawley, M. J. 1993 *GLIM for ecologists*. Oxford, UK: Blackwell.
- Forero, M. G. 1998 Dispersal or philopatry? Analyses of causes and consequences in a population of black kites. PhD thesis, University of Sevilla, Spain.
- Gerrard, J. M., Bortolotti, G. R. & Wiebe, K. L. 1996 *The birds of the Besnard Lake area, north-central Saskatchewan, 1968–1994*. Regina Saskatchewan, Canada: Nature Saskatchewan.
- Goto, N., Kodama, H., Okada, K. & Fujimoto, Y. 1978 Suppression of phytohemagglutinin skin response to thymectomised chickens. *Poultry Sci.* **57**, 246–250.
- Haq, A.-U., Bailey, C. A. & Chinnah, A. 1996 Effect of beta-carotene, canthaxanthin, lutein, and vitamin E on neonatal immunity of chicks when supplemented in the broiler breeder diets. *Poultry Sci.* **75**, 1092–1097.
- Lessells, C. M. 1991 The evolution of life histories. In *Behavioral ecology* (ed. J. R. Krebs & N. B. Davies), pp. 32–68. Oxford, UK: Blackwell.
- Lindström, J. 1999 Early development and fitness in birds and mammals. *Trends Ecol. Evol.* **14**, 343–348.
- McCullagh, P. & Nelder, J. A. 1983 *Generalised linear modelling*. London: Chapman & Hall.
- Magrath, R. D. 1991 Nestling weight and juvenile survival in the blackbird, *Turdus merula*. *J. Anim. Ecol.* **60**, 335–351.
- Maxwell, M. H. 1993 Avian blood leucocyte responses to stress. *World Poultry Sci. J.* **49**, 34–43.
- Merino, S., Martínez, J., Møller, A. P., Sanabria, L., De Lope, F., Pérez, J. & Rodríguez-Cabeiro, F. 1999 Phytohaemagglutinin injection assay and physiological stress in nestling house martins. *Anim. Behav.* **58**, 219–222.
- Monaghan, P. & Nager, R. G. 1997 Why don't birds lay more eggs? *Trends Ecol. Evol.* **12**, 270–274.
- Moreno, J., De Leon, A., Fargallo, J. A. & Moreno, E. 1998 Breeding time, health and immune response in the chinstrap penguin *Pygoscelis antarctica*. *Oecologia* **115**, 312–319.
- Moreno, J., Sanz, J. J. & Arriero, E. 1999 Reproductive effort and T-lymphocyte cell-mediated immunocompetence in female pied flycatchers *Ficedula hypoleuca*. *Proc. R. Soc. Lond. B* **266**, 1105–1109.
- Ots, Y., Murumägi, A. & Hörak, P. 1998 Haematological health state indices of reproducing great tits: methodology and sources of natural variation. *Funct. Ecol.* **12**, 700–707.
- Owens, I. P. F. & Wilson, K. 1999 Immunocompetence: a neglected life history trait or conspicuous red herring? *Trends Ecol. Evol.* **14**, 170–172.
- Pastoret, P.-P., Griebel, P., Bazin, H. & Govaerts, A. 1998 *Handbook of vertebrate immunology*. London: Academic Press.
- Potti, J., Moreno, J., Merino, S., Frías, O. & Rodríguez, R. 1999 Environmental and genetic variation in the haematocrit of fledgling pied flycatchers *Ficedula hypoleuca*. *Oecologia* **120**, 1–8.
- Ramsay, S. L. & Houston, D. C. 1998 The effect of dietary amino acid composition on egg production in blue tits. *Proc. R. Soc. Lond. B* **265**, 1401–1405.
- Ricklefs, R. E. 1992 Embryonic development period and the prevalence of avian blood parasites. *Proc. Natl Acad. Sci. USA* **89**, 4722–4725.
- Royle, N. J., Surai, P., McCartney, R. J. & Speake, B. K. 1999 Parental investment and egg yolk lipid composition in gulls. *Funct. Ecol.* **13**, 298–306.
- Saino, N., Calza, S. & Møller, A. P. 1997a Immunocompetence of nestling barn swallows in relation to brood size and parental effort. *J. Anim. Ecol.* **66**, 827–836.
- Saino, N., Bolzern, A. M. & Møller, A. P. 1997b Immunocompetence, ornamentation, and viability of male barn swallows (*Hirundo rustica*). *Proc. Natl Acad. Sci. USA* **94**, 549–552.
- Saino, N., Calza, S. & Møller, A. P. 1998 Effects of a dipteran ectoparasite on immune response and growth trade-offs in barn swallow, *Hirundo rustica*, nestlings. *Oikos* **81**, 217–228.
- Selman, R. G. & Houston, D. C. 1996 The effect of prebreeding diet on reproductive output in zebra finches. *Proc. R. Soc. Lond. B* **263**, 1585–1588.
- Smith, H. G., Källander, H. & Nilsson, J.-A. 1989 The trade-off between offspring number and quality in the great tit *Parus major*. *J. Anim. Ecol.* **58**, 383–401.
- Smits, J. E., Bortolotti, G. R. & Tella, J. L. 1999 Simplifying the phytohemagglutinin skin-testing technique in studies of avian immunocompetence. *Funct. Ecol.* **13**, 567–572.
- Soler, J. J., Martín-Vivaldi, M., Marín, J. M. & Møller, A. P. 1999 Weight lifting and health status in the black wheatear. *Behav. Ecol.* **10**, 281–286.
- Sorci, G., Soler, J. J. & Møller, A. P. 1997 Reduced immunocompetence of nestlings in replacement clutches of the European magpie (*Picapica*). *Proc. R. Soc. Lond. B* **264**, 1593–1598.
- Stearns, S. 1992 *The evolution of life histories*. New York: Oxford University Press.
- Surai, P. F., Ionov, Y. A., Kuklenko, T. V., Kostjuck, I. A., McPherson, A., Speake, B. K., Noble, R. C. & Sparks, N. H. C. 1998 Effects of supplementing the hen's diet with vitamin A in the accumulation of vitamin A and E, ascorbic acid and carotenoids in the egg yolk and in the embryonic liver. *Br. Poultry Sci.* **39**, 257–263.
- Tella, J. L., Blanco, G., Forero, M. G., Gajón, A., Donazar, J. A. & Hiraldo, F. 1999 Habitat, world geographic range, and embryonic development of hosts explain the prevalence of avian hematozoa at small spatial and phylogenetic scales. *Proc. Natl Acad. Sci. USA* **96**, 1785–1789.
- Tinbergen, J. M. & Boerlijst, M. C. 1990 Nestling weight and survival in individual great tits (*Parus major*). *J. Anim. Ecol.* **59**, 1113–1127.
- Wedekind, C. 1994 Mate choice and maternal selection for specific parasite resistances before, during and after fertilization. *Phil. Trans. R. Soc. Lond. B* **346**, 303–311.
- Wiebe, K. L. & Bortolotti, G. R. 1993 Brood patches of American kestrels: an ecological and evolutionary perspective. *Ornis Scand.* **24**, 197–204.
- Wiebe, K. L. & Bortolotti, G. R. 1994 Energetic efficiency of reproduction: the benefits of asynchronous hatching for American kestrels. *J. Anim. Ecol.* **63**, 551–560.
- Wiebe, K. L. & Bortolotti, G. R. 1995 Egg size and clutch size in the reproductive investment of American kestrels. *J. Zool.* **237**, 285–301.
- Wiehn, J. 1997 Plumage characteristics as an indicator of male parental quality in the American kestrel. *J. Avian Biol.* **28**, 47–55.
- Wiehn, J., Korpimäki, E., Bildstein, K. L. & Sorjonen, J. 1997 Mate choice and reproductive success in the American kestrel: a role for parasites? *Ethology* **103**, 304–317.