Longevity differs among sexes but is not affected by repeated immune activation in voles (*Microtus arvalis*)

GODEFROY DEVEVEY*, MICHEL CHAPUISAT and PHILIPPE CHRISTE

Department of Ecology and Evolution, Biophore, University of Lausanne, 1015 Lausanne, Switzerland

Investment of resources in immune defences, despite obvious short-term benefits, may be detrimental to long-term maintenance and thus decrease longevity in absence of parasites. In addition, females and males may differ in immune investment and intrinsic longevity because they are subjected to different degrees of sexual competition and extrinsic mortality. In order to test if sex-specific investment in mounting an immune response reduced longevity, we compared the longevity of captive male and female common voles *Microtus arvalis* regularly challenged with keyhole limpet haemocyanin, an antigen which elicits the production of antibodies, to the longevity of voles injected with the corresponding antigen-free buffer (phosphate-buffered saline). Injections were repeated every 28 days to mimic a chronic infection. The magnitude of immune response did not vary between males and females and did not affect longevity. Overall, females lived longer than males, independently of the immune challenge. Thus, the long-term costs of immunity seem small in voles. The longevity pattern is consistent with the prediction that male-biased predation or parasitism in the wild causes reduced intrinsic lifespan, but this reduction is not mediated by a decrease in male immunity. © 2009 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2009, **97**, 328–333.

ADDITIONAL KEYWORDS: ageing – cost of immunity – intrinsic mortality – keyhole limpet haemocyanin – lifespan – sex-biased longevity.

INTRODUCTION

The immune system plays an essential role in fighting parasites and appears to be one of the major physiological mechanisms regulating host survival (Zuk & Stoehr, 2002). The activation of the immune system generally protects the host, but also entails costs. Indeed, mounting an immune response has been demonstrated to be energetically costly (Demas *et al.*, 1997; Demas, Drazen & Nelson, 2003; Derting & Compton, 2003; Eraud *et al.*, 2005), to induce autoimmune diseases (Janeway *et al.*, 1999) or to decrease fecundity and/or reproductive success (Bonneaud *et al.*, 2003; Faivre *et al.*, 2003; Hanssen, Folstad & Erikstad, 2003; Bertrand *et al.*, 2006; French, Johnston & Moore, 2007; McCallum & Trauth, 2007; but see Shoemaker & Adamo, 2007; Williams *et al.*, 1999 for an absence of costs). The activation of the immune system can reduce reproductive success by affecting secondary sexual traits (Faivre *et al.*, 2003; Jacot, Scheuber & Brinkhof, 2004; Peters *et al.*, 2004), decreasing growth and development time (Soler *et al.*, 2003; Sanz *et al.*, 2004; Prendergast *et al.*, 2004; Uller, Isaksson & Olsson, 2006) or delaying future reproduction (Marzal *et al.*, 2007). As the investment of resources in immune defences may affect the allocation of resources to major life-history traits, it may also decrease longevity even in the absence of infection (Sheldon & Verhulst, 1996; Lochmiller & Deerenberg, 2000).

In contrast to reproductive costs, longevity costs have been little studied. A negative effect of immune activation on survival has been shown in bumblebees *Bombus terrestris* (Moret & Schmid-Hempel, 2000), field crickets *Gryllus campestris* (Jacot *et al.*, 2004) and common eider *Somateria mollissima* (Hanssen *et al.*, 2004; Hanssen, 2006). These survival costs have been detected in stressful conditions such as complete starvation in the laboratory (Moret &

*Corresponding author. E-mail: godefroy.devevey@unil.ch

Schmid-Hempel, 2000) or natural exposition to parasites and predators in the field (Jacot *et al.*, 2004), concomitant with prolonged incubation fasting in the case of eiders (Hanssen *et al.*, 2004; Hanssen, 2006). In mammals, a few studies have assessed the energetic cost of immune defences (Demas *et al.*, 1997, 2003; Derting & Compton, 2003). However the effects of mounting an immune response per se on intrinsic longevity, in the absence of external death factors such as food constraints and predation, has to our knowledge not been studied so far.

Proximately, the trade-off between immune defences and longevity might be mediated by immunopathology (Graham, Allen & Read, 2005). For example, injection of non-pathogenic lipopolysaccharides can be lethal for normal mice because of the release of proinflammatory cytokine tumour necrosis factor (TNF) (Pfeffer *et al.*, 1993). Immune activation also increases susceptibility to oxidative tissue damages (Bertrand *et al.*, 2006), potentially leading to faster senescence and ageing (Kregel & Zhang, 2007). In addition, chronic activation of the immune system is believed to favour autoimmune diseases (Janeway *et al.*, 1999). As a consequence, one could expect that the activation of the immune system would accelerate senescence and thus reduce the longevity of individuals mounting an immune response in the absence of disease.

Sexes might differ in their optimal investment in immunity (Klein & Nelson, 1998; Rolff, 2002) because the fitness of males increases with their mating success, whereas the fitness of females increases with the number of reproductive events. Females may therefore invest more in maintenance and live longer than males do. This prediction applies particularly to species with strong competition among males such as polygynous species (Stoehr & Kokko, 2006) and males are indeed shorter-lived than females in the majority of polygynous species (Clutton-Brock & Isvaran, 2007). Another major factor affecting the evolution of lifespan is the degree of extrinsic mortality, which may lead to sex-specific differences in longevity if one sex experiences a higher extrinsic mortality than the other (Kirkwood & Austad, 2000).

In this study, we investigated the effect of mounting an immune response on the intrinsic longevity of each sex in the common vole (*Microtus arvalis*). This rodent species is polygynous (Heise & Van Acker, 2000) and suffers from male-biased extrinsic mortality (Christe, Keller & Roulin, 2006). We repeatedly stimulated the immune system of male and female captive voles by injecting a non-pathogenic antigen and compared their intrinsic lifespan to control voles injected with the corresponding antigen-free saline buffer. If the activation of the immune system accelerates the ageing process, immune-challenged voles should have a shorter lifespan than controls. Moreover, males should have a shorter lifespan than females in the absence of immune challenge.

MATERIAL AND METHODS

GENERAL PROCEDURE

Common voles *Microtus arvalis* born in captivity from wild-born parents were separated from their mothers when 21 days old. Voles were then housed individually in a polypropylene cage ($36 \times 20 \times 18$ cm) in an animal facility room with a 14-h light : 10 h dark cycle and a constant temperature of 21 ± 1 °C. Cages contained 1 L of sterilized soil and a flowerpot (diameter 14 cm) for roosting. Hay and tap water were available *ad libitum* and animals received apples and seeds regularly throughout the experiment. At weaning, individuals received 4 µL of transcutaneous veterinary Selamectine as an anti-parasitic treatment. When between 27 and 60 days old, voles were weighed and randomly divided in two groups: 41 voles (22 males and 19 females) were subcutaneously injected with 5 mL/kg of sterile phosphate-buffered saline (PBS) and 41 voles (23 males and 18 females) were injected with 5 mL/kg of keyhole limpet haemocyanin (KLH; Sigma, Switzerland) suspended in sterile PBS at a concentration of 1 mg/mL. To mimic the response to a chronic infection, injections were repeated every 28 days until the natural death of the vole. KLH is an innocuous respiratory protein derived from the giant keyhole limpet (*Megathura crenulata*).

In order to control for the production of anti-KLH immunoglobulin type G (IgG) in challenged individuals, we measured the levels of plasmatic anti-KLH IgG twice, the first time 10 days after the third injection and the second time 15 days after the injections 22nd to 25th (590–655 days old). Blood samples (50–100 µL) were drawn by tail-nipping into preheparinized microvettes. Blood samples were centrifuged for 30 min at 440 $\boldsymbol{g}$. Serums were stored at –18 °C until assayed for specific IgG. We measured the anti-KLH IgG concentrations in the vole serum with an enzyme-linked immunosorbent assay (ELISA) as described in Devevey *et al.* (2008).

DATA ANALYSES

Statistical analyses were computed with JMP software 7.0.0. To test for differences in longevity between groups, we used semi-parametric Cox's regressions (Cox, 1972) with sex and immune challenge as principal factors. This model enables the use of body mass at weaning, body mass at first injection, average body mass during adulthood, average variation of body mass during life and anti-KLH IgG level
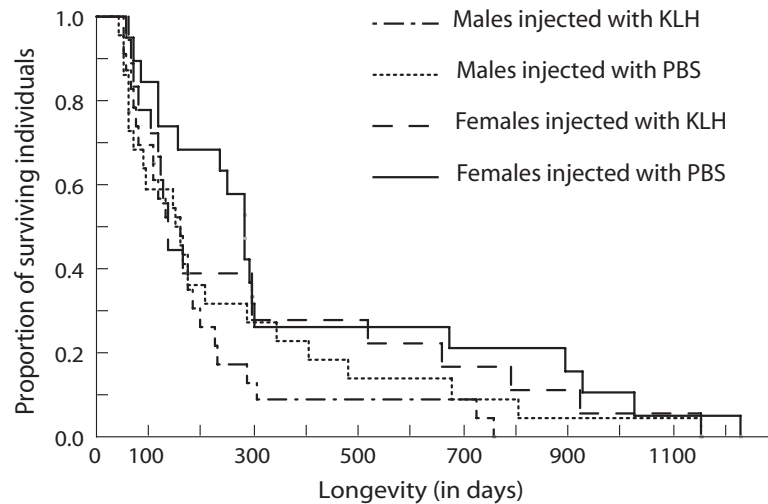
Figure 1. Survival curves of male and female common voles injected with keyhole limpet haemocyanin (KLH) and with antigen free buffer (phosphate-buffered saline; PBS).

after the third injection as covariates. Non-significant interactions and covariates were removed from the model in a backward procedure. We performed power analyses using G*Power version 3 (Faul *et al.*, 2007). Reported values are from post hoc power tests based on degrees of freedom for generic χ^2-tests. There was no difference between challenged and control voles in body mass at weaning or at first or second injection (all $P > 0.35$).

RESULTS

The magnitudes of the immune response were similar between the sexes ($t = -1.558$, d.f. = 22, $P = 0.133$). For the same individual, the 22nd injection with KLH induced a stronger response than did the third one (paired *t*-test: $t = -2.79$, d.f. = 4, $P = 0.049$). When analysing the longevity using the Cox regression, the interaction between sex and immune challenge was not significant ($\chi^2 = 0.01$, d.f. = 1, $P = 0.935$). We observed no significant effect of lifelong immune activation on cumulative mortality (Fig. 1; $\chi^2 = 1.74$, d.f. = 1, $P = 0.19$), with median lifespan being 135 days in challenged voles and 207 days in control voles. A power analysis revealed that a 30% difference between the experimental groups would have been detected with a probability of 0.76 (Faul *et al.*, 2007). Nevertheless, there was a sex difference in longevity, with females exhibiting a longer lifespan than males (Fig. 1; $\chi^2 = 3.98$, d.f. = 1, $P = 0.046$), with median lifespan being 161 days in males and 249 days in females. Mean ± SE lifespan was 228 ± 36 and 362 ± 57 days for males and females voles, respectively. All the covariables (including magnitude of immune response) were not significant (all $P > 0.13$).

DISCUSSION

Our experiment shows that mounting and maintaining a humoral immune response, whatever its magnitude, does not significantly reduce intrinsic longevity in captive voles. Voles repeatedly challenged with KLH over their entire lifetime had similar longevity to the one of control voles and did not show signs of immunosenescence. This result contrasts with the few available data on this topic. Our study had somewhat low power, but also had some particularities that could explain these contrasting results. We compared the survival of voles in controlled laboratory conditions, without predation or reproduction, and with access to *ad libitum* food. So far, survival costs of immune system activation have only been shown under stressful conditions in the field (Hanssen *et al.*, 2004; Jacot *et al.*, 2004; Hanssen, 2006) or laboratory (Moret & Schmid-Hempel, 2000). In particular, bumblebee workers inoculated with micro-latex beads and lipopolysaccharides had a short-term survival cost only when subjected to complete starvation, whereas no such cost was observed with unlimited food supply (Moret & Schmid-Hempel, 2000). Similarly, female common eiders that showed reduced survival were immune challenged during the long fasting period of incubation, which lasts for 22 to 27 days (Hanssen *et al.*, 2004; Hanssen, 2006). Many highly stressed and malnourished eider females exhibited complete immunosuppression during this period, suggesting that the immune system is down-regulated when resources are scarce. In fossorial water voles, parasites and spatio-temporal demographic fluctuations affect the efficiency of cellular immune response (Charbonnel *et al.*, 2008) and the

selection on MHC genes (de Bellocq, Charbonnel & Morand, 2008), further suggesting that ecological conditions affect immune responses.

The absence of effect of immune challenge on longevity when resources are plentiful suggests that a compensatory resource intake mechanism can counterbalance the investment in immunity in captive conditions. It may be more difficult to compensate for the energy and antioxidant consumption of immune activation in natural conditions. In the wild, common voles have many parasites (Beaucournu & Launay, 1990) that affect health conditions (Devevey et al., 2008), fitness components (Deter et al., 2007) and population dynamics (Deter, Charbonnel & Morand, 2008), which together probably amplify the costs of immunity. Moreover, other functions affected by immune activation, such as cognition and thus predator avoidance, may also be important for survival in the wild but not in the laboratory (Barnard et al., 2006).

The type of antigen that we used might also explain the absence of negative effects on longevity. KLH is indeed a rather mild immune challenge, without adjuvant, which induces a humoral immune response without making the animal ill (absence of prolonged inflammation, fever, anorexia . . .) (Dixon, Jacot-Guillarmod & McConahey, 1966; Demas et al., 2003). Thus, our experimental design has the advantage to assess the cost of mounting an immune response per se, independently from any pathogenic or toxic effects. Our immune challenge thus contrasts with the three antigens used in the study on common eiders (sheep red blood cells, diphtheria toxoid and tetanus; Hanssen et al., 2004) that, despite being non-pathogenic, might have had negative effects independently of the activation of the immune system.

The activation of immune defences has to be costly in order to generate the observed trade-offs between reproductive parameters and resistance against diseases in the wild (Lochmiller & Deerenberg, 2000). However, these immunity costs will not necessarily affect all types of life-history traits and their effect on fitness-related traits may depend on conditions (Rigby, Hechinger & Stevens, 2002). For example, in poultry the immune system represents only a few per cent of body weight and requires few nutrients in comparison with egg production or growth (Klasing & Leshchinsky, 1998). Our experiment suggests that the costs of antibody production are small in micromammals and may need stressful conditions to become apparent.

Our experiment revealed that males were shorter-lived than females. A similar sex-specific difference in survival probably also occurs in the Virginia meadow vole, even if male-biased mortality is difficult to distinguish from male-biased dispersal in natural conditions (Rose & Dueser, 1980). In contrast, Selman et al. (2008) did not find this pattern in captive Microtus agrestis. Our result highlights that the general pattern of early male mortality in polygynous species of vertebrates (Clutton-Brock & Isvaran, 2007) is not always as a result of low immunity in males (Stoehr & Kokko, 2006; Forbes, 2007) and that many life-history traits can affect sex-specific lifespan. Interestingly, predation upon voles is strongly male-biased in the field (Christe et al., 2006) and males are generally more infested by parasites than females (Klein, 2004; Morand et al., 2004). In these conditions, males can be selected to pursue a 'live fast, die young' reproductive strategy on certain traits (Bonduriansky et al., 2008) and senescence should occur earlier in males (Kirkwood & Austad, 2000; Christe et al., 2006). Our finding that female common voles had a longer intrinsic lifespan than males in captive condition is in accordance with this hypothesis. Overall, this experiment demonstrates that a sex-specific difference in intrinsic longevity in the common vole is not mediated by differential immune investment (which seems innocuous in captive conditions with ad libitum food) and probably comes from sex differences in reproductive traits leading to high extrinsic mortality in males.

ACKNOWLEDGMENTS

REFERENCES

Barnard CJ, Collins SA, Daisley JN, Behnke JM. 2006. Odour learning and immunity costs in mice. *Behavioural Processes* **72:** 74–83.

Beaucournu J-C, Launay H. 1990. *Les puces de France et du Bassin Méditerranéen Occidental*. Fédération Française des Sociétés de Sciences Naturelles.

de Bellocq JG, Charbonnel N, Morand S. 2008. Co-evolutionary relationship between helminth diversity and MHC class II polymorphism in rodents. *Journal of Evolutionary Biology* **21:** 1144–1150.

Bertrand S, Criscuolo F, Faivre B, Sorci G. 2006. Immune activation increases susceptibility to oxidative tissue damage in Zebra Finches. *Functional Ecology* **20:** 1022–1027.

Bonduriansky R, Maklakov A, Zajitschek F, Brooks R. 2008. Sexual selection, sexual conflict and the evolution of ageing and life span. *Functional Ecology* **22:** 443–453.

Bonneaud C, Mazuc J, Gonzalez G, Haussy C, Chastel O, Faivre B, Sorci G. 2003. Assessing the cost of mounting an immune response. *American Naturalist* **161:** 367–379.

Charbonnel N, Chaval Y, Berthier K, Deter J, Morand S, Palme R, Cosson J-F. 2008. Stress and demographic decline: a potential effect mediated by impairment of reproduction and immune function in cyclic vole populations. *Physiological and Biochemical Zoology* **81:** 63–73.

Christe P, Keller L, Roulin A. 2006. The predation cost of being a male: implications for sex-specific rates of ageing. *Oikos* **114:** 381–384.

Clutton-Brock T, Isvaran K. 2007. Sex differences in ageing in natural populations of vertebrates. *Proceedings of the Royal Society Series B* **274:** 3097–3104.

Cox DR. 1972. Regression models and life-tables. *Journal of the Royal Statistical Society, Series B (Methodological)* **34:** 187–220.

Demas GE, Chefer V, Talan MI, Nelson RJ. 1997. Metabolic costs of mounting an antigen-stimulated immune response in adult and aged C57BL/6J mice. *American Journal of Physiology* **273:** R1631–R1637.

Demas GE, Drazen DL, Nelson RJ. 2003. Reductions in total body fat decrease humoral immunity. *Proceedings of the Royal Society Series B* **270:** 905–911.

Derting TL, Compton S. 2003. Immune response, not immune maintenance, is energetically costly in wild white-footed mice (*Peromyscus leucopus*). *Physiological and Biochemical Zoology* **76:** 744–752.

Deter J, Charbonnel N, Cosson J-F, Morand S. 2008. Regulation of vole populations by the nematode *Trichuris arvicolae*: insights from modelling. *European Journal of Wildlife Research* **54:** 60–70.

Deter J, Cosson J-F, Chaval Y, Charbonnel N, Morand S. 2007. The intestinal nematode *Trichuris arvicolae* affects the fecundity of its host, the common vole *Microtus arvalis*. *Parasitological Research* **101:** 1161–1164.

Devevey G, Niculita-Hirzel H, Biollaz F, Yvon C, Chapuisat M, Christe P. 2008. Developmental, metabolic and immunological costs of flea infestation in the common vole. *Functional Ecology* **22:** 1091–1098.

Dixon FJ, Jacot-Guillarmod H, McConahey P. 1966. The antibody responses of rabbits and rats to hemocyanin. *Journal of Immunology* **97:** 350–355.

Eraud C, Faivre B, Duriez O, Chastel O. 2005. The energetic cost of humoral immunity in the Collared Dove, *Streptopelia decaocto*: is the magnitude sufficient to force energy-based trade-offs? *Functional Ecology* **19:** 110–118.

Faivre B, Grégoire A, Préault M, Cézilly F, Sorci G. 2003. Immune activation rapidly mirrored in a secondary sexual trait. *Science* **300:** 103.

Faul F, Erdfelder E, Lang A-G, Buchner A. 2007. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behavior Research Methods* **39:** 175–191.

Forbes MR. 2007. On sex differences in optimal immunity. *Trends in Ecology and Evolution* **22:** 111–113.

French SS, Johnston GIH, Moore MC. 2007. Immune activity suppresses reproduction in food-limited female tree lizards *Urosaurus ornatus*. *Functional Ecology* **21:** 1115–1122.

Graham AL, Allen JE, Read AF. 2005. Evolutionary causes and consequences of immunopathology. *Annual Review of Ecology Evolution and Systematics* **36:** 373–397.

Hanssen SA. 2006. Costs of an immune challenge and terminal investment in a long-lived bird. *Ecology* **87:** 2440–2446.

Hanssen SA, Folstad I, Erikstad KE. 2003. Reduced immunocompetence and cost of reproduction in common eiders. *Oecologia* **136:** 457–464.

Hanssen SA, Hasselquist D, Folstad I, Erikstad KE. 2004. Costs of immunity: immune responsiveness reduces survival in a vertebrate. *Proceedings of the Royal Society Series B* **271:** 925–930.

Heise SR, Van Acker A. 2000. The effect of social environment on the immune response of female common voles in matriarchal laboratory groups. *Physiology and Behavior* **71:** 289–296.

Jacot A, Scheuber H, Brinkhof MWG. 2004. Costs of an induced immune response on sexual display and longevity in field crickets. *Evolution* **58:** 2280–2286.

Janeway CA, Travers P, Walport M, Capra JD. 1999. *Immunobiology: the immune system in health and disease*. London: Taylor & Francis.

Kirkwood TBL, Austad SN. 2000. Why do we age? *Nature* **408:** 233–238.

Klasing KC, Leshchinsky TV. 1998. Functions, costs, and benefits of the immune system during development and growth. In: Adams NJ, Slotow R, eds. *22nd International Ornithological Congress*. Durban: BirdLife South Africa, 2817–2835.

Klein SL. 2004. Hormonal and immunological mechanisms mediating sex differences in parasite infection. *Parasite Immunology* **26:** 247–264.

Klein SL, Nelson RJ. 1998. Adaptive immune responses are linked to the mating system of arvicoline rodents. *American Naturalist* **151:** 59–67.

Kregel KC, Zhang HJ. 2007. An integrated view of oxidative stress in aging: basic mechanisms, functional effects, and pathological considerations. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology* **292:** 18–36.

Lochmiller RL, Deerenberg C. 2000. Trade-offs in evolutionary immunology: just what is the cost of immunity? *Oikos* **88:** 87–98.

McCallum ML, Trauth SE. 2007. Physiological trade-offs between immunity and reproduction in the northern cricket frog (*Acris crepitans*). *Herpetologica* **63:** 269–274.

Marzal A, Reviriego M, de Lope F, Møller AP. 2007. Fitness costs of an immune response in the house martin (*Delichon urbica*). *Behavioral Ecology and Sociobiology* **61:** 1573–1580.

Morand S, de Bellocq JG, Stanko M, Miklisova D. 2004. Is sex-biased ectoparasitism related to sexual size

dimorphism in small mammals of Central Europe? *Parasitology* **129:** 505–510.

Moret Y, Schmid-Hempel P. 2000. Survival for immunity: the price of immune system activation for bumblebee workers. *Science* **290:** 1166–1168.

Peters A, Delhey K, Denk AG, Kempenaers B. 2004. Trade-offs between immune investment and sexual signalling in male mallards. *American Naturalist* **164:** 51–59.

Pfeffer K, Matsuyama T, Kündig TM, Wakeham A, Kishihara K, Shahinian A, Wiegmann K, Ohashi PS, Krönke M, Mak TW. 1993. Mice deficient for the 55-kd tumor necrosis factor receptor are resistant to endotoxic shock yet succumb to *L. monocytogenes* infection. *Cell* **73:** 457–467.

Prendergast BJ, Hotchkiss AK, Bilbo SD, Nelson RJ. 2004. Peripubertal immune challenges attenuate reproductive development in male Siberian hamsters (*Phodopus sungorus*). *Biology of Reproduction* **70:** 813–820.

Rigby MC, Hechinger RF, Stevens L. 2002. Why should parasite resistance be costly? *Trends in Parasitology* **18:** 116–120.

Rolff J. 2002. Bateman's principle and immunity. *Proceedings of the Royal Society Series B* **269:** 867–872.

Rose RK, Dueser RD. 1980. Lifespan of Virginia meadow voles. *Journal of Mammalogy* **61:** 760–763.

Sanz JJ, Moreno J, Merino S, Tomás G. 2004. A trade-off between two resource-demanding functions: post-nuptial moult and immunity during reproduction in male pied flycatchers. *Journal of Animal Ecology* **73:** 441–447.

Selman C, McLaren JS, Collins AR, Duthie GG, Speakman JR. 2008. The impact of experimentally elevated energy expenditure on oxidative stress and lifespan in the short-tailed field vole *Microtus agrestis*. *Proceedings of the Royal Society Series B* **275:** 1907–1916.

Sheldon BC, Verhulst S. 1996. Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. *Trends in Ecology and Evolution* **11:** 317–321.

Shoemaker KL, Adamo SA. 2007. Adult female crickets, *Gryllus texensis*, maintain reproductive output after repeated immune challenges. *Physiological Entomology* **32:** 113–120.

Soler JJ, de Neve L, Pérez-Contreras T, Soler M, Sorci G. 2003. Trade-off between immunocompetence and growth in magpies: an experimental study. *Proceedings of the Royal Society Series B* **270:** 241–248.

Stoehr AM, Kokko H. 2006. Sexual dimorphism in immunocompetence: what does life-history theory predict? *Behavioral Ecology* **17:** 751–756.

Uller T, Isaksson C, Olsson M. 2006. Immune challenge reduces reproductive output and growth in a lizard. *Functional Ecology* **20:** 873–879.

Williams TD, Christians JK, Aiken JJ, Evanson M. 1999. Enhanced immune function does not depress reproductive output. *Proceedings of the Royal Society Series B* **266:** 753–757.

Zuk M, Stoehr AM. 2002. Immune defense and host life history. *American Naturalist* **160** (Suppl.)**:** S9–S22.