

# Family and population effects on disease resistance in a reptile

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Despite its importance in evolutionary biology, studies of the pattern of disease resistance in natural populations are rare. In this paper, we report patterns of infection of a viral eye disease in juvenile Swedish common lizards (*Lacerta vivipara*). Females were sampled at random from natural populations immediately prior to parturition with equal exposure of pathogens for all lizards once in captivity. No causative agents could be found that linked risk of disease to maternal/interfollicular transfer of pathogens. The results show that a major factor influencing offspring susceptibility is family identity, suggesting heritable variation in pathogen resistance. Our interpopulation comparison provides addi-

tional support for a link between genetics and disease resistance. Lizards in northern Sweden were not only more susceptible to the disease but were also more health compromised once infected, with relatively more reduced growth rate and increased mortality than lizards from the south. This scenario suggests that southern lizards have been under selection for resistance to this pathogen, whereas northern lizards have not, or at least not to the same degree. Thus, this study confirms the importance of genetic (family) effects on pathogen resistance with variation in this trait among natural populations.

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## Introduction

The assumption that disease resistance is at least partly determined by genetic factors is central for models of host–parasite interactions (eg, Anderson and May, 1982; Wakelin and Apanius, 1997). Consequently, such effects on patterns of mortality may also influence life-history evolution (Møller, 1997), processes of sexual selection (Hamilton and Zuk, 1982), maintenance of genetic variation (Hamilton, 1982; Coltman *et al.*, 1999), and conservation status by influencing population dynamics (O'Brien and Evermann, 1988; McCallum and Dobson, 1995; Packer *et al.*, 1999). Despite such wide and far-reaching consequences for many disciplines in evolutionary biology, very few studies have been committed to describing patterns of infectious disease in natural populations, that is, identifying which animals in a population are susceptible or resistant to specific pathogens (Read *et al.*, 1995; Sorci *et al.*, 1997). Indeed, most information on variability in disease resistance comes from studies of laboratory or domestic animals (eg, Kloosterman *et al.*, 1992; Kaufman, 2000). A limited number of studies on birds and mammals, however, suggest that there is heritable variation for parasite resistance in natural populations (Møller, 1990; Boulinier *et al.*, 1997; Smith *et al.*, 1999; Brinkhof *et al.*, 1999; Coltman *et al.*, 2001).

Recent correlative studies of disease susceptibility and MHC haplotypes further support the existence of genetic effects on parasite resistance (Paterson *et al.*, 1998; Langefors *et al.*, 2001), suggesting strong effects of maternal and paternal genotype on offspring immunocompetence. In addition to these, family effects on immunocompetence could also result from interfollicular transfer of pathogens and maternal transfer of antibodies and/or pathogens to the developing fetus (Janeway *et al.*, 1999).

In this paper, we report patterns of infection of an eye disease in juvenile common lizards (*Lacerta vivipara*). Gravid females were sampled at random from natural populations and brought into the laboratory before parturition. Subsequent to parturition, and after removing the females from the facilities where the neonates were kept, a pathogen was accidentally introduced with lizards from a natural population as vectors. Since siblings were kept separately, and cage mates were resampled at random on a weekly basis (see Materials and methods), all neonates were equally exposed to the pathogen and should be at equal risk of developing the disease, which contrasts markedly with our epidemiological findings. The analysis in the present paper takes advantage of a passive introduction of a pathogen into a laboratory population comparable to an experimental introduction designed to analyze genetic variation in disease resistance. Furthermore, by contrasting two sampling regions of populations that differ with respect to the presence of a known viral vector (*Ixodus* sp., absent in the north), we confirm the importance of selection history for disease resistance.

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## Materials and methods

The common lizard is a small, 50–70 mm snout-vent length (SVL), 3–5 g, ground-dwelling lizard. It has one of the largest distributions of all reptilian taxa, occurring from northern Spain to northern Scandinavia, and from Ireland throughout Russia. In Sweden, it inhabits a wide range of habitats, from heath land to rocky coasts. Mating begins a couple of weeks subsequent to emergence from hibernation, which takes place in March–April in southern Sweden and May–June in the northern part. In Sweden, the common lizard is live-bearing and has a mean clutch size of 5–7 young.

Lizards of both sexes were caught by noose at four localities in the southwest of Sweden (Öjersjö: N 57°42' E 12°8'; Sandsjöbacka: N 57°32' E 12°2'; Asketunnan: N 57°22' E 11°58'; Hällsundsudde: N 57°21' E 12°0'), and two localities in the north (Markitta: N 67°10' E 21°30'; Mettä-Markitta N 67°12' E 21°28') during May–June 2001 and were transported to the University of Gothenburg. The southern localities are separated by a maximum of 70 km and the northern by a maximum of 3 km. The sampling areas in the north and south are separated by approximately 1300 km. In total, 56 females were caught. All females were mated in the wild as was evident from copulation scars on the belly (inflicted by the male at copulation; Bauwens and Verheyen, 1985). The lizards were kept in cages (500 × 400 × 350 mm) with peat and bark as bottom substrate, bricks and stones as shelter, and a 40 W spotlight for thermoregulation. The cages had wire mesh on opposite sides to increase ventilation and were placed in a three-level rack. Four to five animals were kept in each cage, with males and females in separate cages. The lizards were fed crickets (*Gryllus* sp.) and mealworms (*Tenebrio* sp. larvae) once a day and had water available *ad libitum*. Immediately prior to parturition, the females were transferred to separate cages to ensure accurate scoring of sibship. Cages were checked at least twice daily for hatchlings. At parturition, the neonates were toe-clipped for individual identification, weighed (to the nearest mg) and measured (snout-vent length and total length to the nearest 0.5 mm). The neonates were kept in cages as described for adults. Water and *Drosophila* flies were provided *ad libitum*.

On days 1–3 after parturition the neonates were tested for endurance in a physiological performance trial. Physiological performance was estimated by letting the neonates swim in a 700 × 400 × 350 mm thermally insulated aquarium filled with water. Each juvenile lizard was subjected to three swimming trials, one on each consecutive day, at three different temperatures (24, 30 or 36°C, in random order). When placed in the water, swimming immediately commenced, predominantly by undulating movements of the tail. When the lizard stopped swimming, it was encouraged to continue by a light tap on the body side with a plastic ruler. When it did not resume swimming after three consecutive taps, the trial was interrupted. Experience from earlier studies shows that this method gives a repeatable estimate of endurance in neonate common lizards with much more repeatable scores than, for example, sprint speed and tread mill endurance in this species (Olsson *et al.*, 2002, M. Olsson *et al.*, personal observation).

Subsequent to the swimming trials, the lizards were used in a growth experiment, in which they were kept in

small separate cages in thermal incubators at two different light regimes, 18:6 L:D, and 6:18 L:D. In the L phase the neonates were kept at 30°C, the preferred body temperature for common lizards (Van Damme *et al.*, 1987), and in the D phase they were kept at 15°C: families were split, with half of the young in each treatment (the results of the growth experiment will be published elsewhere). After 15 days, the experiment was interrupted and the lizards were transferred back to the room where the adult lizards were housed. In total, 15–20 juveniles (depending on size) were kept per cage with paper as substrate. The juveniles were fed small crickets (*Gryllus* sp.), *Drosophila* and water *ad libitum*, and were sorted by size once a week to minimize food competition. Because of the differences in size within families as a result of the growth experiment, all families were represented in at least two size categories (sorted into ca. 3–4 mm separate SVL categories), with each size category being split into two–four cages. All individuals were resampled once every week and were assigned to each cage randomly with respect to family and locality with the cages randomly distributed and weekly reorganised in the rack.

None of the adult females showed any signs of infection during or after pregnancy, and had been transferred to cool rooms (5°C) 20 days before the juvenile captive population was exposed to the disease carried by a captured free-ranging lizard from Sandsjöbacka (N 57°32' E 12°2'). The first symptom of the disease in the infected lizard (an adult male) was a clear fluid discharge from the eyes and the same pattern of disease development was evident in all infected animals. In its later stages, the disease caused conjunctivitis and eventually the eyes became completely sealed. For the majority of the infected juveniles, however, this later stage of the disease was never reached. At the first sign of infection, a veterinary examined the lizards, and confirmed the presence of inclusion bodies in multiple lizards suggesting a viral pathogen, with a field-caught specimen being the vector. There was no evidence for bacteriological cause of the disease. Further support for a viral pathogen was the lack of recovery when infected lizards were treated with wide-spectrum antibiotics (Tetracyclin (oral and cream), Flagyl). Similar symptoms have been described in an English laboratory population of lacertid lizards (Cooper *et al.*, 1980), but not from natural populations of common lizards. In the closely related sand lizard (*Lacerta agilis*), however, free ranging individuals have shown similar symptoms (M. Olsson, personal observation). Viral pathogens causing eye infections in reptiles have been described and identified, but information from natural populations is scarce, as for other taxa (see Mader (1996) and Frye (1991) for treatments of reptile diseases in general, and Millichamp *et al.* (1983) for a review of eye diseases in reptiles). Experiments with newly hatched lizards (not included in this experiment) being isolated in separate cages have shown that the disease is airborne and easily move between cages.

At 22 days subsequent to the first signs of the disease, all juvenile lizards were scored for signs of infection (1 = infected, 0 = noninfected), weighed to the nearest milligram, and measured snout-vent and total length to the nearest 0.5 mm. The infected and noninfected juvenile lizards were from then on kept in separate

cages in the same room with random cage and shelf allocation with respect to family and locality. The healthy lizards were continuously monitored for signs of infection, but very few additional infections occurred. At 50 days subsequent to the last measurement and health scoring, the juveniles were again measured and weighed to allow a comparison between infected and noninfected individuals with respect to growth rate.

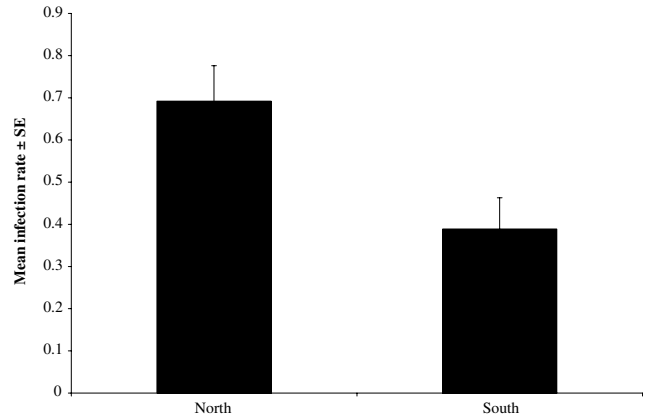
## Results

Of the 56 females caught in the wild, 40 gave birth in the laboratory (mean clutch size 5, range 2–12). Ideally, latitudinal and family effects on infection probability would have been modelled with a logistic regression with latitude and family nested within latitude as factors. As a result of extensive quasi-complete separation of data, however, such an analysis was not feasible within current statistical packages. We therefore ran a likelihood ratio test to look for family effects, with risk of offspring infection differing significantly among females (likelihood ratio test;  $\chi^2 = 91.23$ ,  $P = 0.0001$ ,  $df = 39$ ). To control for the possibility that the family effect was caused by a significant difference between the populations (see below), we also ran separate tests for northern and southern populations. For the Southern population, family proved to be highly significant (likelihood ratio test;  $\chi^2 = 66.52$ ,  $P = 0.0001$ ,  $df = 27$ ), whereas it showed borderline significance for the northern population (likelihood ratio test;  $\chi^2 = 19.39$ ,  $P = 0.054$ ,  $df = 11$ ).

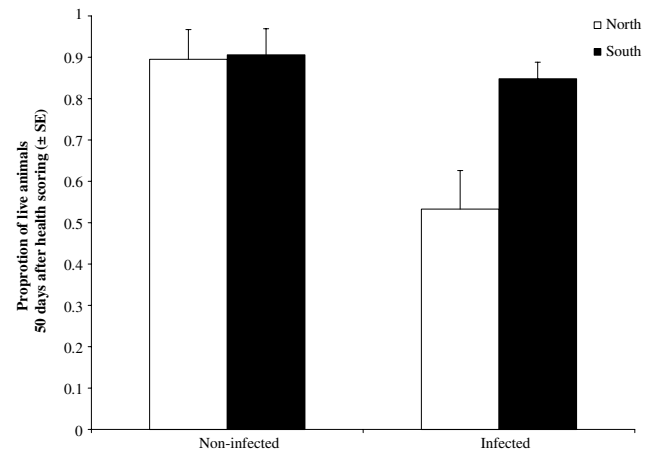
None of the adult females showed any signs of infection, most likely because they were transferred to hibernation cool rooms 20 days before disease transmission from the wild. A paired *t*-test showed that there was no difference in mean birth mass within families between infected and noninfected animals ( $t_{32} = 1.14$ ,  $P = 0.262$ ,  $n = 31$ ), strongly suggesting that developmental processes were unaffected by the pathogen, that females were disease free at parturition, and that, hence, the neonatal infection was postparturient.

Further support for this was the lack of subsequent infection status on offspring performance at parturition. In an ANOVA with endurance at parturition as the dependent variable, and infection risk, family, and the interaction term between infection risk and family as factors, family was significant for each temperature treatment (24°C:  $F_{37,73} = 3.33$ ,  $P < 0.001$ ; 30°C:  $F_{37,73} = 2.37$ ,  $P = 0.0011$ ; 36°C:  $F_{37,75} = 1.95$ ,  $P = 0.0071$ ), whereas neither infection risk nor the interaction term were significant ( $P > 0.5$  in all temperatures). Day length treatment and offspring sex did not significantly influence infection probability (Fischer's exact test;  $P = 0.28$  and 0.72, respectively).

To assess the influence of between-population differences in selection history on disease susceptibility, independently of family effects, we calculated a mean infection score for each family and tested for differences among populations. There was no difference among localities when these were pooled into one data set (Kruskal–Wallis test,  $\chi^2$  approximation;  $\chi^2 = 9.87$ ,  $P = 0.08$ ,  $df = 5$ ), and we therefore split the data set into the two major sampling regions (north and south) and looked for latitudinal effects (inspired by the lack of ticks in the north which serve as vectors for numerous microbes, Reichenbach-Klinke and Elkan, 1965). North-



**Figure 1** Mean infection rates  $\pm$  SE for north and south populations. The difference is statistically significant (see text for test statistics).



**Figure 2** Proportion of live animals in northern and southern populations 50 days after health scoring (see text for test statistics).

ern lizards were significantly more susceptible to the disease than neonates originating from the south (Wilcoxon two-sample test;  $Z = 2.18$ ,  $P = 0.029$ ,  $n_{\text{south}} = 28$ ,  $n_{\text{north}} = 12$ , Figure 1). Furthermore, only 16 out of 28 families included infected offspring in the southern population, whereas all families in the northern population had at least one infected offspring.

In order to investigate other possible secondary fitness consequences of the infection, we compared growth rates of infected versus uninfected juveniles and confirmed a significant three-way interaction between family, infection status, and latitude on growth rate in mass ( $F_{54,52} = 2.18$ ,  $P = 0.0027$ ). In separate ANOVAs with family and infection status nested within family as factors for each latitude, infected animals from the north showed reduced growth rate ( $F_{10,15} = 5.29$ ,  $P = 0.0041$ ), whereas southern lizards showed no significant difference in growth rate between infected and noninfected individuals ( $F_{10,37} = 0.67$ ,  $P = 0.75$ ). Furthermore, lizards from the northern population were more heavily affected by the disease in terms of hard selection, as evident from a significant association between mortality and infection status in the north, but not south (Fisher's exact test;  $P = 0.012$  and 0.300 for north and south, respectively; Figure 2).

## Discussion

Our results clearly demonstrate the negative impact of a pathogen on lizards obtained directly from natural populations. Although the disease was not monitored in free-ranging animals, all lizards were brought haphazardly into captivity from the wild and kept under conditions mimicking a 'disease resistance experiment'. Thus, it can be argued that the patterns of infection reflect those that would have occurred in free-ranging lizards, and are therefore of direct interest for understanding variation in disease susceptibility in the wild. Importantly, our study demonstrates that the major factor influencing risk of infection in juvenile lizards is family identity. Despite its importance for evolutionary biology, evidence for heritable variation in disease resistance in natural populations is rare (Boulinier *et al.*, 1997; Smith *et al.*, 1999), with our present study being the first to report such findings in a reptilian species.

As pointed out in the Introduction, there are at least two potential alternative scenarios that could produce family effects on offspring disease susceptibility. First, in mammals, part of the immune system is known to be acquired from the mother during pregnancy (Janeway *et al.*, 1999), and in birds, antibodies can be transferred from the mother to the chick via the egg yolk or albumen (Buxton, 1952; Kramer and Cho, 1970; Smith *et al.*, 1994). In reptiles, no such mechanism has been demonstrated, but this may be explained by lack of data, and the *L. vivipara* placenta is known to be involved in ion-, water-, and gas-transport (Panigel, 1956; Yaron, 1985). A second, nongenetic, effect causing family-biased infection rate is disease transfer from the mother to the offspring during pregnancy (Janeway *et al.*, 1999). We doubt both immunological and pathogen transfer for the following reasons. First, none of the 40 females showed any symptoms of the disease during or after pregnancy. Thus, this makes it unlikely that the pathogen was transmitted to the offspring from their mothers, and since the females were removed from the facilities before the disease transfer took place, females were never even exposed to the pathogen and, hence, could never have transferred acquired immunity. Second, if the offspring were infected during gestation, this should also have influenced their embryonic growth and development and, hence, birth mass, and the result of their post-parturient physiological performance trials. Since we could test for differences in mass at parturition and physiological performance for neonates that were later scored as infected versus noninfected, we could test this proposition. No such effects could be demonstrated at parturition and we therefore conclude that the offspring became infected after parturition.

Further support for a genetic link to disease resistance comes from our interpopulation comparison. Not only were the lizards from the northern population more susceptible to the disease than those from the southern population (Figure 1), they were also more severely infected, as is evident from the significant interaction term between death rate (dead/alive) and infection status (infected/noninfected) for the northern but not the southern populations (Figure 2). Furthermore, northern lizards showed decreased growth rate when infected, whereas infection did not influence growth rate for lizards from the southern population. The differences

between the populations could presumably arise if the laboratory conditions were closer to optimum for the southern than for the northern population. We find this unlikely, however, since laboratory environments are generally less stressful than conditions in the wild, and there is no reason to believe that the populations should differ in their response to, for example, *ad libitum* food and that this would be reflected in differences in disease susceptibility. One important factor influencing immunobiology in ectotherms is temperature. However, all lizards were given the opportunity to thermoregulate, thereby minimizing the possibility that differences between populations in response to thermal conditions could explain differences in disease susceptibility. Finally, day length treatment did not influence infection probability, even though it significantly affected growth rate and thereby offspring size, indicating that environmental conditions are of minor importance for susceptibility to this disease.

Southern lizards are apparently not affected by the pathogen to the same extent as the northern population, which indicates differences in their selection history for disease resistance to this pathogen in the wild. In the south, ticks often act as an important vector of disease (Reichenbach-Klinke and Elkan, 1965), whereas they are absent in the north (Talleklint and Jaenson, 1998). Where no ticks occur, pathogen transmission between lizards could therefore be reduced compared to where they are present. Thus, differences in epidemiology linked to the presence or absence of common vectors could explain the regional differences in pathogen resistance.

In conclusion, our study provides evidence of genetic heterogeneity in pathogen resistance in natural populations of common lizards. Because populations differed in infection prevalence, selection history is likely to strongly influence pathogen resistance.

## References

- Anderson RM, May RM (1982). Coevolution of hosts and parasites. *Parasitology* **85**: 411–426.
- Bauwens D, Verheyen RF (1985). The timing of reproduction in the lizard *Lacerta vivipara*. Differences between individual females. *J Herp* **19**: 353–364.
- Boulinier T, Sorci G, Monnat JY, Danchin E (1997). Parent offspring regression suggests heritable susceptibility to ectoparasites in a natural population of kittiwake *Rissa tridactyla*. *J Evol Biol* **10**: 77–85.
- Brinkhof MWG, Heeb P, Kölliker M, Richner H (1999). Immunocompetence of nestling great tits in relation to rearing environment and parentage. *Proc R Soc Lond B* **266**: 2315–2322.
- Buxton A (1952). On the transference of bacterial antibodies from the hen to the chick. *J Gen Microbiol* **7**: 268–286.
- Coltman DW, Pilkington JG, Smith JA, Pemberton JM (1999). Parasite-mediated selection against inbred Soay sheep in a free-living, island population. *Evolution* **53**: 1259–1267.
- Coltman DW, Pilkington JG, Kruuk LEB, Wilson K, Pemberton JM (2001). Positive genetic correlation between parasite resistance and body size in a free-living ungulate population. *Evolution* **55**: 2116–2125.
- Cooper JE, McClelland MH, Needham JR (1980). An eye infection in laboratory lizards associated with an *Aeromonas* sp. *Lab Anim* **14**: 149–151.
- Frye FL (1991). *Reptile Care. An Atlas of Diseases and Treatments*. T.F.H. Publications: New Jersey. Vols. 1 and 2.

- Hamilton WD (1982). Pathogens as causes of genetic diversity in their host populations. In: Anderson RM, May RM (eds) *Population Biology of Infectious Diseases*. Springer: Berlin, pp 269–303.
- Hamilton WD, Zuk M (1982). Heritable true fitness and bright birds: a role for parasites? *Science* **218**: 384–387.
- Janeway CA, Travers P, Walport M, Capra JD (1999). *Immunobiology. The Immune System in Health and Disease*, 4th edn. Elsevier Science: London.
- Kaufman J (2000). The simple chicken major histocompatibility complex: life and death in the face of pathogens and vaccines. *Phil Trans R Soc Lond B* **355**: 1077–1084.
- Kloosterman A, Permentier HK, Ploeger HW (1992). Breeding cattle and sheep for resistance to gastrointestinal nematodes. *Parasitol Today* **8**: 330–335.
- Kramer TT, Cho HG (1970). Transfer of immunoglobulins and antibodies in the hen's egg. *Immunology* **19**: 157–167.
- Langefors Å, Lohm J, Grahn M, Andersen Ø, von Schantz T (2001). Association between major histocompatibility complex class IIB alleles and resistance to *Aeromonas salmonicida* in Atlantic salmon. *Proc R Soc Lond B* **268**: 479–485.
- Mader DR (1996). *Reptile Medicine and Surgery*. Saunders: Philadelphia.
- McCallum H, Dobson A (1995). Detecting disease and parasite threats to endangered species and ecosystems. *Trends Ecol Evol* **10**: 190–194.
- Millichamp NJ, Jacobson ER, Wolf ED (1983). Diseases of the eye and ocular adnexae in reptiles. *J Am Vet Med Assoc* **183**: 1205–1212.
- Møller AP (1990). Effects of haematophagous mite on the barn swallow (*Hirundo rustica*): a test of the Hamilton and Zuk hypothesis. *Evolution* **44**: 771–784.
- Møller AP (1997). Parasitism and the evolution of host life history. In: Clayton DH, Moore J (eds) *Host-Parasite Evolution. General Principles and Avian Models*. Oxford University Press: Oxford. pp 105–127.
- O'Brien SJ, Evermann JF (1988). Interactive influence of infectious disease and genetic diversity in natural populations. *Trends Ecol Evol* **3**: 254–259.
- Olsson M, Wapstra E, Olofsson C (2002). Offspring size-number strategies: experimental manipulation of offspring size in a viviparous lizard (*Lacerta vivipara*). *Funct Ecol* **16**: 135–140.
- Packer C, Altizer S, Appel M, Brown E, Martenson J, O'Brien SJ *et al* (1999). Viruses of the Serengeti: patterns of infection and mortality in African lions. *J Anim Ecol* **68**: 1161–1178.
- Panigel M (1956). Contribution à l'étude de l'ovoviviparité chez les reptiles: gestation et parturition chez le lézard vivipare *Zootoca vivipara*. *Ann Des Sci Nat Zool* **11**: 571–668.
- Paterson S, Wilson K, Pemberton JM (1998). Major histocompatibility complex variation associated with juvenile survival and parasite resistance in a large unmanaged ungulate population (*Ovis aries* L.). *Proc Natl Acad Sci USA* **95**: 3714–3719.
- Read AF, Albon SD, Antonovics J, Apanius V, Dwyer G, Holt RD *et al* (1995). Group report: genetics and evolution of infectious diseases in natural populations. In: Grenfell BT, Dobson AP (eds) *Ecology of Infectious Diseases in Natural Population*. Cambridge University Press: Cambridge. pp 450–477.
- Reichenbach-Klinke H, Elkan E (1965). *Diseases of Reptiles*. Academic Press Inc: London.
- Smith NA, Wallach M, Miller CMD, Braun R, Eckert J (1994). Maternal transmission of immunity to *Eimeria maxima*: Western blot analysis of protective antibodies induced by infection. *Infect Immunol* **62**: 4811–4817.
- Smith JA, Wilson K, Pilkington JG, Pemberton JM (1999). Heritable variation in resistance to gastro-intestinal nematodes in an unmanaged mammal population. *Proc R Soc Lond B* **266**: 1283–1290.
- Sorci G, Møller AP, Boulinier T (1997). Genetics of host-parasite resistance. *Trends Ecol Evol* **12**: 196–200.
- Talleklint L, Jaenson TGT (1998). Increasing geographical distribution and density of *Ixodes ricinus* (Acari: Ixodidae) in central and northern Sweden. *J Med Entomol* **35**: 521–526.
- Van Damme R, Bauwens D, Verheyen RF (1987). Thermoregulatory responses to environmental seasonality by the lizard *Lacerta vivipara*. *Herpetologica* **43**: 405–415.
- Wakelin D, Apanius V (1997). Immune defence: genetic control. In: Clayton, DH, Moore J (eds) *Host-Parasite Evolution. General Principles and Avian Models*. Oxford University Press: Oxford. pp 30–58.
- Yaron Z (1985). Reptilian placentation and gestation: structure function, and endocrine control. In: Gans C, Billet F (eds) *Biology of the Reptilia, Vol. 15, Development B*. John Wiley & Sons: New York, pp 527–604.