Functional Ecology

British Ecological Society

Functional Ecology 2008, **22**, 1091–1098 doi: 10.1111/j.1365-2435.2008.01493.x

Developmental, metabolic and immunological costs of flea infestation in the common vole

Godefroy Devevey*, Hélène Niculita-Hirzel, François Biollaz, Candice Yvon, Michel Chapuisat and Philippe Christe

Department of Ecology and Evolution, University of Lausanne, Biophore, Quartier Sorge, CH-1015 Lausanne, Switzerland

Summary

1. Parasites use resources from their hosts, which can indirectly affect a number of host functions because of trade-offs in resource allocation. In order to get a comprehensive view of the costs imposed by blood sucking parasites to their hosts, it is important to monitor multiple components of the development and physiology of parasitized hosts over long time periods.

2. The effect of infestation by fleas on body mass, body length growth, haematocrit, resistance to oxidative stress, resting metabolic rate and humoral immune response were experimentally evaluated. During a 3-month period, male common voles, *Microtus arvalis*, were either parasitized by rat fleas (*Nosopsyllus fasciatus*), which are naturally occurring generalist ectoparasites of voles, or reared without fleas. Then voles were challenged twice by injecting Keyhole Limpet Haemocyanin (KLH) to assess whether the presence of fleas affects the ability of voles to produce antibodies against a novel antigen. During the immune challenge we measured the evolution of body mass, haematocrit, resistance to oxidative stress and antibody production.

3. Flea infestation negatively influenced the growth of voles. Moreover, parasitized voles had reduced haematocrit, higher resting metabolic rate and lower production of antibodies against the KLH. Resistance to oxidative stress was not influenced by the presence of fleas.

4. During the immune challenge with KLH, body mass decreased in both groups, while the resistance to oxidative stress remained stable. In contrast, the haematocrit decreased only in parasitized voles.

5. Our experiment shows that infestation by a haematophageous parasite negatively affects multiple traits like growth, energy consumption and immune response. Fleas may severely reduce the survival probability and reproductive success of their host in natural conditions.

Key-words: body growth, haematocrit, immuno-suppression, *Nosopsyllus fasciatus*, *Microtus arvalis*, resting metabolic rate

Introduction

Life-history theory posits that there are physiological trade-offs between resource-demanding functions within an individual (Stearns 1992). Parasites use resources from their hosts and cause the host to invest in antiparasite-defences (Giorgi *et al*. 2001). These two kinds of costs are likely to have a negative impact on a diversity of morphological, physiological and immunological host traits (van Noordwijk & de Jong 1986; Sheldon & Verhulst 1996; Norris & Evans 2000). In order to fully apprehend the effects of parasites on their hosts, it is therefore important to use an integrative approach jointly targeting multiple host's traits.

Rodents and burrowing mammals are regularly infested by haematophageous ectoparasites. The most abundant of them are fleas, which are widespread in rodent nests (Traub *et al.* 1983). Despite the importance of rodents as reservoir hosts of numerous diseases, only few studies have evaluated the effect of the presence of fleas on fitness related traits and indicators of health status of rodents (Morand *et al.* 2006; Krasnov 2008). Here, we investigate the effects of the generalist flea *Nosopsyllus fasciatus* (Bosc, 1800) on multiple morphological, physiological and immunological parameters of its natural host, the common vole *Microtus arvalis* (Pallas, 1778). Evaluating the effects of parasites at these different levels should permit to identify subtle interactions and potential trade-offs between host responses.

Key morphological parameters often influencing fecundity and survival are body size and body mass (Kawata 1988; Wauters & Dhondt 1989; Crétégny 1997; Murray *et al.* 1997). The effects of ectoparasites on such morphological parameters have been intensively studied in birds (reviewed in Møller

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

1997) but have so far largely been neglected in mammals (Krasnov 2008). Among physiological costs, an important integrative measure is energy consumption, which is reflected in the metabolic rate. An increase in daily metabolic rate has been found in gerbils infested by fleas (Khokhlova *et al*. 2002), which might be due to behavioural modifications such as grooming (Giorgi *et al*. 2001), or to physiological changes in response to parasitism. One way to distinguish between behavioural and physiological responses is to measure the resting metabolic rate (RMR).

Oxidative stress resistance is another major indicator of health status linking various physiological parameters (Lesgards *et al*. 2002) and involved in senescence (Beckman & Ames 1998). Parasites may affect their host oxidative stress resistance in two ways. First, they may increase energy consumption and thus the production of reactive oxygen species. Second they may reduce the level of antioxidant vitamins (Dede *et al*. 2002). Moreover, mounting an immune defence against parasites may also increase the susceptibility to oxidative stress (Bertrand *et al*. 2006).

Because fleas feed on blood, a more specific physiological cost may be a decrease in the haematocrit level, measured as the volume of red blood cells over the total blood volume. A reduction in haematocrit due to the presence of haematophageous ectoparasites has been shown in gerbils and bats (Lehmann 1992; Christe *et al.* 2000a), but not systematically so (Goüy de Bellocq *et al*. 2006).

Finally, fleas could also affect the ability of voles to mount and modulate their immune response. In particular, infestation with ectoparasites may mobilise resources otherwise allocated to other components of immunity (Christe *et al*. 2000a; Goüy de Bellocq *et al*. 2006). Fleas could have either a direct immuno-suppressive effect through injected antigens (Goüy de Bellocq *et al*. 2006) or an indirect effect by reducing body condition (reviewed in Alonso-Alvarez & Tella 2001). A trade-off in the allocation of resources between the different compartments of immune defences may have important consequences for rodent-transmitted diseases and rodent population dynamics.

The aim of the present study was to evaluate the effect of fleas on several indicators of health conditions and to study potential interactions between them. In particular, we investigated the effects of an infestation by ectoparasites on the ability to produce antibodies against a novel antigen. By comparing these different indicators between experimentally infested voles and voles without parasite, we aimed to obtain an integrative view of the costs of fleas on their rodent hosts.

Material and methods

GENERAL PROCEDURE

Subadult male common voles (*M. arvalis*) born in laboratory from deparasitized wild-born voles were housed individually in polypropylene cages (36 × 20 × 18 cm) with a 14 L : 10 D cycle and constant temperature at 21 ± 1 °C. Cages contained one litre of sterilized soil and a flowerpot (diameter 12 cm) as vole roost. Hay and tap water were available *ad libitum* and animals received apple and seeds regularly throughout the experiment.

At day 0, voles originating from 25 litters, were weighed, measured, and blood sampled. Voles were between 45 and 60 days old at the start of the experiment and they reached their adult size at 3 months (Jacob 2003). At day 1, individuals were randomly assigned to the flea-treatment group or non-parasitized control group. If two or more males were present within a litter, they were separated in two groups and distributed between the control and treatment group. Twenty eight individuals were parasitized by fleas (treatment group) and 24 individuals were kept as control. Parasitized voles were exposed to adult and larval rat flea *N. fasciatus* by receiving 15 g of a mix of bedding coming from cages of wild voles which were not deparasitized and where fleas had developed naturally. In four samples of 15 g of bedding, flea loads were comprised between 25 and 64 fleas (average = 44 fleas). The control group received 15 g of a mix of bedding without fleas. Every 49 days, bedding of cages were individually changed and only the nest of the vole was transferred, with fleas and larvae if infested, inducing a reduction of fleas' populations. Fleas were counted in three cages of infested voles after 10 bedding changes, with total numbers of 62, 221 and 108 fleas per cage, respectively. During the experiment, we noticed only one case of very low flea infestation on a vole belonging to the control group, which was immediately deparasitized.

After 98 days of treatment, individuals were weighed, measured, and blood sampled. Then a period of immune challenge began. All individuals were injected with Keyhole Limpet Haemocyanin (KLH) (see details below) at days 99 and 119, and were weighed and blood sampled to measure anti-KLH immunoglobulins at days 119 and 139. RMR was finally measured between days 160 and 185. Because of the short life span of voles, sample sizes differ between days of measure. At the end of the experiment (i.e. when measuring RMR), the sample size for parasitized voles was 10, whereas 15 individuals were still alive in the control group. All manipulations were done under control of the Cantonal Veterinary Authorities of the Canton de Vaud, authorization 1848.

MORPHOLOGICAL MEASUREMENTS

Body mass was measured to the nearest 0·1 g with an electronic balance (Mettler SM-L, Switzerland). Body length was measured with a manual calliper to the nearest millimetre when laying down the vole on a board. Individual nutritional condition at days 0 and 98 were assessed by extracting residuals from the regression of body mass on body length ($r^2 = 0{\cdot}71$, $n = 76$, $P < 0{\cdot}0001$).

BLOOD PARAMETERS

Individuals were blood sampled to measure haematocrit, blood resistance to oxidative stress and anti-KLH immunoglobulin type G (IgG) title. On each sampling day, blood samples (50–100 μL) were drawn from tail-cutting. Blood was collected in pre-heparinized microvette and one pre-heparinized capillary for haematocrit. Haematocrit capillary were centrifuged 10 min with a standard centrifuge (Haematokrit 24, Bioréac SA, Lausanne, Switzerland). The amount of red blood cells relative to the total amount of blood volume was measured with a calliper to the nearest 0·1 mm.

Resistance to oxidative stress was assessed as the ability of red blood cells to resist to a controlled oxidative attack. We used the KRL® test for mammals (Brevet spiral V02023, Couternon, France;

Stocker *et al*. 2003; Alonso-Alvarez *et al*. 2004, 2006; Girard *et al*. 2005). The principle of the test is to submit whole blood to a thermocontrolled oxidative aggression by a chemical reagent: the 2,2′-azobis-(aminodinopropane) hydrochloride [AAPH] (Rojas Wahl *et al*. 1998). The time needed to haemolyze 50% of red blood cells is a good assessor of the resistance to oxidative stress. In this test, all families of free radical scavengers present in blood are mobilized to fight off the oxidant attack (Pieri *et al.* 1996; Lesgards *et al*. 2002). Sixteen micro litres of whole blood were immediately diluted in 584 µL of KRL buffer. Samples were stored at 4 °C before analysis, which occurred within 10 h. Ninety micro litres of KRL®-diluted blood was incubated at 37 °C with 153 µL of a 150-mM solution of AAPH in 96-well microplate. The lyses of red blood cells were monitored with a microplate reader device, which measures the decrease of optical density at the wavelength of 620 nm every 3 min for 225 min.

RESTING METABOLIC RATE

We assessed the RMR of voles by measuring their oxygen (O_2) consumption with a respirometer. We used an open-air flow respirometer containing two independent and identical circuits that enables monitoring of a parasitized individual and a non-parasitized individual at the same time. The respirometer was composed of an atmospheric air pump (WISA D.B.G.M., Model 300, Germany), a debimeter that controls for a constant debit (Model 5878, Krämer AG, CH-6314 Unterägeri), a respirometric chamber and an O_2-probe (4100 Gas Purity Analyzer, Servomex) that measures the decrease in the proportion of oxygen. The probe was connected to an ocillograph (Recorder 320, W + W Electronic Scientific Instrument, Basel, Switzerland) recording the fluctuations in oxygen concentration. The probe was calibrated before each run with respect to a total atmospheric oxygen concentration of 20·930% (± 0·002%). Oxygen consumption measurements took place in a dark and silent environment and the respirometric chambers were submersed in a thermoregulated water bath at 31 °C (± 1) within the voles' thermoneutral zone (Crétégny 1997). Each run lasted 4 h, during which time the vole had no access to food or water. The instantaneous oxygen consumption (VO_2) in mL h^{-1} can be calculated according to Depocas & Hart (1957) as:

$$VO_2 = V_2 \times (F_IO_2 - F_2O_2)/(1 - F_IO_2)$$

where V_2 is the air flow rate measure (mL h^{-1}), F_1O_2 the oxygen concentration prior to the vole's entrance to the chamber and F_2O_2 the oxygen concentration at the time t of the run. RMR was measured as the VO_2 at the instant where oxygen concentration was maximal, that is, when the vole had a minimal activity. Voles were familiarized with the respirometric chamber 4 h per day during the 2 days preceding the experiment. All fleas present on a vole were removed by combing off before the experimental run and care was taken that the manipulation of parasitized animals did not take more time than the manipulation of control animals. Individual body mass was measured prior to entering the respirometric chamber and just after the end of the run.

IMMUNE CHALLENGE

We assessed humoral immune response against a novel antigen, the KLH, by measuring anti-KLH antibodies IgG (Demas*et al.* 2003; Gore *et al*. 2004). KLH was used because it generates a robust antigenic response in rodents, but does not make the animals ill (Drazen *et al.* 2001). Each individual received at days 99 and 119 a subcutaneous injection of KLH (Sigma, Switzerland) without adjuvant diluted in Phosphate Buffer Saline (PBS), proportional to the body mass of the individual (5 µL of KLH solution at 1 g L^{-1} per gram of vole' body mass). Blood was collected before immunization and 20 days post-immunization. Blood sampled before immunization permits to measure the amount of background noise and aspecific antibody binding that are unavoidable in ELISA procedures. These initial values are taken into account in the statistical analysis. Blood samples were centrifuged 30 min at 440 g at 4 °C. Serum aliquots were extracted and stored at –20 °C until assayed.

The anti-KLH IgG concentration in vole serum was measured with the enzyme-linked immunosorbent assay (ELISA) described by Demas *et al*. (2003) with the following modifications. Thawed serum samples were diluted 1 : 10 with PBS-T, and 100 µL of each serum dilution was added in duplicate to the wells of the antigen-coated plates. A serial dilution of an anti-KLH antibody made in mouse (Sigma, Switzerland) was duplicated in each plate as standard. Plates were sealed and incubated with diluted sera for 3 h at room temperature. After three washes with PBS-T, wells were incubated with a 1 : 1000 dilution of the secondary antibody – Anti-Mouse IgG Alkaline Phosphatase Conjugate (Stressgen Bioregeants, Canada) in PBS-T for 1 h at room temperature. After three washes with PBS-T, wells were incubated with 100 µL of p-nitrophenyl phosphate (Chemicon, Switzerland), a phosphatase alkaline substrate. Plates were protected from light during the enzyme–substrate reaction, which was monitored every minute by a plate reader equipped with a 405 nm wavelength filter. Considering the kinetics of the reaction, the measure after 12 min was determined to be the best to compare IgG level between wells and was used to calculate the mean optical density for each set of duplicate wells. To minimize intra-assay variability in statistical analyses, the mean OD for each sample was expressed as a percentage of mouse anti-KLH IgG standards.

STATISTICAL ANALYSIS

The effects of experimental treatment on vole body mass, body length, nutritional condition, haematocrit and resistance to oxidative stress at day 98 of treatment were analysed with ANCOVA using initial measures at day 0 as covariables. Repeated measure ANOVA using Restricted Maximum Likelihood Method were used to compare the dynamics of physiological traits between groups during the immune challenge. Degrees of freedom were corrected using Satterthwaite correction for unequal variances in *t*-tests (Littell *et al.* 2006) and using the Kenward and Roger correction in repeated measure ANOVA (Kenward & Roger 1997). All values reported are mean ± standard error (SE). Non-significant interactions were sequentially removed from models. All tests were two-tailed and were performed with the statistical computer program JMP 6·0·0.

Results

EFFECT OF PARASITISM ON BODY GROWTH

Fleas had a strong negative impact on the growth of voles. At the beginning of the experiment, voles of the parasitized and control groups did not differ significantly in any of the morphological measures ($N_{\text{parasitized}} = 28$, $N_{\text{non-parasitized}} = 24$; Body mass: parasitized: 27·5 ± 1·2 g, non-parasitized: 28·0 ± 0·9 g, $t_{47·5} = 0·33$, $P = 0·74$; Body length: parasitized: 98·4 ± 1·1 mm, non-parasitized: 99·5 ± 1·4 mm, $t_{44·1} = 0·64$, $P = 0·52$; Nutritional condition: parasitized: 0·12 ± 0·78, non-parasitized:

Table 1. Effect of the parasitism treatment (with vs. without fleas) on traits related to growth and physiology, as measured after 98 days of treatment

Trait	Control voles $n = 20$	Parasitized voles $n = 18$				
Effect	LS mean ± SE	LS mean ± SE	df	MS	F	P
Body mass (g)	41·1 ± 1·25	33·8 ± 1·32				
Treatment			1	495·6	15·8	< 0·001
Body Mass at day 0			1	616	19·6	< 0·001
Error			35	31·4		
Body length (mm)	113·6 ± 1·0	108·3 ± 1·0				
Treatment			1	266·1	14·6	< 0·001
Body length at day 0			1	196·1	10·8	0·002
Error			35	18·23		
Nutritional condition	1·43 ± 1·06	–1·66 ± 1·12				
Treatment			1	90·4	4	0·053
Nutritional Status at day 0			1	109·7	4·9	0·034
Error			35	22·5		
Haematocrit	0·53 ± 0·01	0·42 ± 0·01				
Treatment			1	0·105	31·3	< 0·001
Haematocrit at day 0			1	0·001	0·4	0·51
Error			35	0·003		
Oxidative stress resistance (min)	86·4 ± 4·2	88·5 ± 4·4				
Treatment			1	45	0·1	0·72
Oxidative stress resistance at day 0			1	347·7	1	0·33
Error			35	356·1		

–0·15 ± 0·68, $t_{48·9} = 0·26$, $P = 0·80$). After 98 days of treatment, parasitized voles had a smaller body mass and body length than non-parasitized ones (Table 1). Moreover, the nutritional condition tended to be lower in parasitized individuals than in non-parasitized ones (Table 1).

EFFECT OF PARASITISM ON PHYSIOLOGICAL TRAITS AND IMMUNE RESPONSE

The infestation by fleas significantly reduced haematocrit, increased RMR and strongly reduced the ability to mount an immune response towards a novel antigen. Initial values of haematocrit and resistance to oxidative stress did not differ between the two experimental groups ($N_{\text{parasitized}} = 28$, $N_{\text{non-parasitized}} = 24$; Haematocrit: parasitized: 0·50 ± 0·01, non-parasitized: 0·49 ± 0·01, $t_{44·6} = 0·80$, $P = 0·43$; Oxidative Stress resistance: parasitized: 87·08 ± 2·04 min, non-parasitized: 86·52 ± 2·19, $t_{47·5} = 0·18$, $P = 0·85$). After 98 days, the haematocrit of parasitized voles was drastically reduced compared to the one of non-parasitized voles (Table 1). In contrast, the resistance to oxidative stress was similar in the two experimental groups (Table 1).

The RMR corrected for vole body mass was significantly higher in parasitized voles than in non-parasitized ones (parasitized, $n = 10$: 0·233 ± 0·012 mL(O_2) h^{-1} g^{-1}; non-parasitized, $n = 15$: 0·189 ± 0·008 mL(O_2) h^{-1} g^{-1}; t-test: $t_{16·55} = 2·95$, $P = 0·009$). This result indicates that for a similar body mass and when resting, parasitized voles consume more oxygen than non-parasitized ones.

Before immune challenge, ELISA values did not differ between experimental groups (Parasitized: $N = 15$, anti-KLH IgG = 0·143 ± 0·022; non-parasitized: $N = 18$, anti-KLH IgG = 0·189 ± 0·029; $t_{30·1} = 1·26$, $P = 0·21$). During immune challenge, the level of specific IgG increased in both groups but significantly less in parasitized voles than in non-parasitized ones, as indicated by the significant interaction between time and treatment (Fig. 1a, Table 2). The intensity of immune response was independent of body mass ($F_{1,25} = 0·044$, $P = 0·8$).

In both experimental groups, body mass decreased during the period of immune challenge (Fig. 1b; Table 2). As shown by the significant interaction between time and treatment, haematocrit decreased in parasitized voles, but was stable in non-parasitized voles (Fig. 1c; Table 2). The resistance to oxidative stress did not differ between the two groups even during immune challenge (Table 2). The control vole which was found to be parasitized did not qualitatively modify the results (see Appendix S1 and Table S1 for analyses without the incriminated individual).

Discussion

Our experimental manipulation of flea infestation in captive voles shows that fleas have a long-term impact on multiple morphological, physiological and immunological traits of their rodent host. The presence of fleas impaired the growth of voles and caused a general decrease in health status, as shown by the reduction in haematocrit and immune defences in the parasitized group. Moreover, flea infestation was associated with energetic costs, evidenced by the higher oxygen consumption of infested hosts when resting. Among the measured parameters, the resistance to oxidative stress was the only one that was not affected by parasitism. Hereafter we discuss in more details how parasitism by fleas can affect the physiology of their hosts.

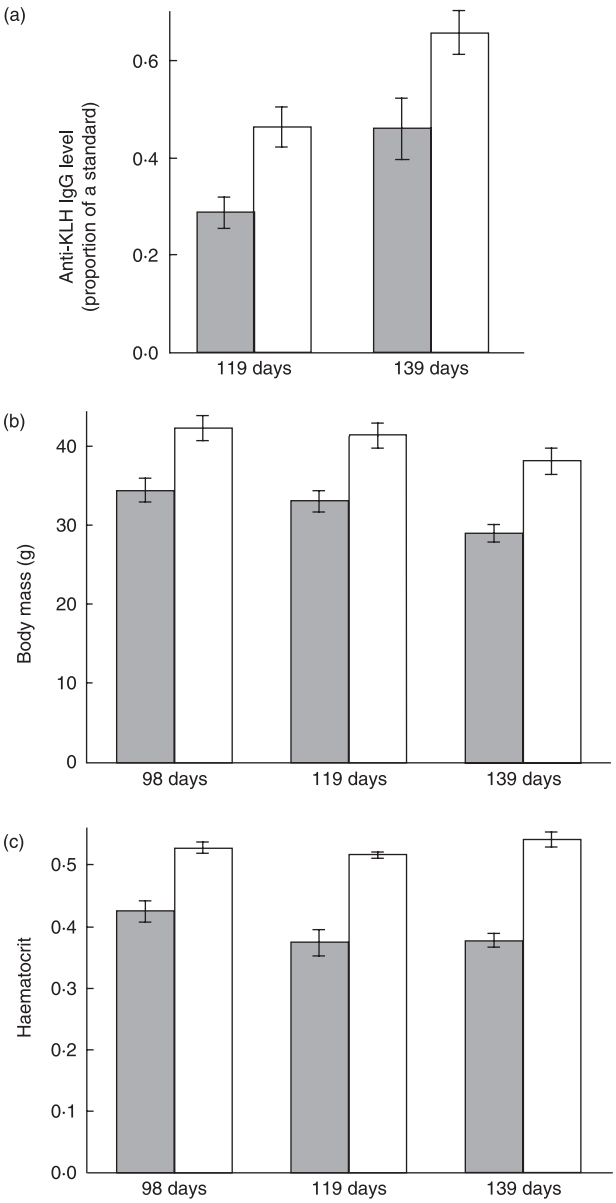

Fig. 1. Change in anti-KLH IgG levels (a), body mass (b) and haematocrit (c) in voles with fleas (filled bars) and without flea (open bars). KLH was injected at days 99 and 119. At days 119 and 139, traits are affected by immune challenge, contrary to day 98 when differences are only due to the parasitic challenge. Bars are means ± SE.

Table 2. Effect of parasitic treatment (with vs. without fleas) on morphological and physiological traits of voles during an immune challenge, as measured at days 98 (before the first injection), 119 (before the second injection) and 139

Effect	*F*	df	*P*
Immune response			
Time	221·1	1, 60·4	< 0·001
Treatment	13·6	1, 31·1	< 0·001
Time × Treatment	14·8	1, 60·4	< 0·001
Body mass			
Time	14·8	1, 66·1	< 0·001
Treatment	12·6	1, 34·4	0·001
Haematocrit			
Time	3·4	1, 67·9	0·070
Treatment	50·5	1, 35·5	< 0·001
Time × Treatment	6·5	1, 67·9	0·013
Oxidative stress resistance			
Time	0·5	1, 68	0·49
Treatment	0·5	1, 35·1	0·47

PARASITISM AND GROWTH

Juvenile voles parasitized by fleas during a 3 months period grew less than individuals kept parasite free. In birds, which grow faster than mammals do, numerous studies have shown that the presence of parasites reduced the body mass, body size and nutritional condition of fledglings (e.g. Chapman & George 1991; Richner *et al.* 1993; Christe *et al.* 1996; Møller 1997; Tschirren *et al.* 2007). In contrast, the very few studies on the effects of parasitism on mammal's growth had variable outcomes. Whereas fleas had no effect on the body mass of free ranging gerbils under natural level of infestation (Hawlena *et al.* 2006a), they negatively affected daily body mass change in juvenile gerbils under laboratory conditions (Hawlena *et al.* 2006b). Experiments on neonates *Mus musculus* infected by the nematode *Heligmosomoides polygyrus* also found mixed results (Kristan, 2002; Kristan, 2004).

Our results show that parasitized voles have a lower body mass mainly because they grew less and reached a smaller body length. In addition, they tended to be in poorer nutritional condition for the same body length. It is worthwhile to note that flea infestation in our experiment occurred when voles were already at least 45-days old, near the end of the growth period of voles, so that the reduction in growth observed in the present study may be weaker than it would have been if voles would have been parasitized since birth. The precise response may depend on the timing and intensities of infestation. For example, the costs of parasitism at an early developmental stage may have long-term negative consequences on survival and reproductive performances (Lindström 1999). However, prenatal exposure of the mothers might also confer some protection to the newborns through adaptive maternal effects (Heeb *et al.* 1998; Grindstaff *et al.* 2003). The voles in our experiment originated from wild caught females that had been deparasitized and had reared their pups in absence of parasitism. It is thus possible that in natural conditions, maternal effects would have attenuated the negative effects of fleas reported in the present study.

PARASITISM AND PHYSIOLOGICAL TRAITS

Parasitized voles had lower haematocrit than non-parasitized ones. A negative relationship between haematocrit and haematophageous parasite load has been found in some bird and mammal studies (Lehmann 1992; Richner *et al.* 1993;

Hurtrez-Boussès *et al*. 1997; Christe *et al*. 2000b). A reduction in haematocrit reveals an incapacity of the host to compensate for the losses due to blood sucking. One or several resources may limit erythropoiesis, the process of division and maturation of haemocytoblastes to erythrocytes in bone marrow, with the consequence of chronic anaemia (Fair, Whitaker & Pearson 2007). In case of anaemia, a natural way to avoid hypoxia and keep a normal input of oxygen is to increase breath and heart output (Blumgart & Atlschule 1948), which in turn may increase RMR.

Previous studies have shown that ectoparasitism can be energetically costly for active hosts (Munger & Karasov 1994; Giorgi *et al*. 2001; Khokhlova *et al*. 2002; Kristan & Hammond 2006). Our study shows that ectoparasitism is energetically costly even while resting. Both groups had been challenged with KLH more than 40 days before we measured RMR, a time lapse that should be long enough to have no more effect of immunization with KLH on metabolic rate (Abbas *et al.* 2000). Moreover, if immune activation is still energetically costly at this time, voles with the higher immune response against KLH (the non-parasitized ones) should consume more energy than the parasitized one, which would make our result conservative.

As suggested above, the higher RMR of parasitized voles might be due to an increase breath and heart output associated with a lower haematocrit caused by the blood feeding of fleas, or to other indirect effects of the parasites. This pattern is consistent with the results found in mice infected by endoparasites (Kristan & Hammond 2000, 2001; Magnanou *et al*. 2006) or rock doves infected by feather-feeding lice (Booth *et al.* 1993). In contrast, the RMR of wild Cape ground squirrel increased after ectoparasite and endoparasite removal which was suggested to be due to an anorexic effect of one particular parasite species or to the limited access to food in the wild (Scantleburry *et al*. 2007).

Higher oxygen consumption, and the resulting higher production of oxidative agents, did not seem to impair the resistance to oxidative stress. The diet of voles might play a role in their high resistance to oxidative stress, as it is rich in antioxidant compounds that are known to confer some protection against oxidative stress (Beckman & Ames 1998).

PARASITISM AND IMMUNITY

Parasitized voles had a reduced ability to mount an immune response against a novel antigen, as compared to parasite-free individuals. This finding is in agreement with the ones of most of the comparable studies. Humoral immune response was reduced in pythons parasitized by blood parasites (Ujvari & Madsen 2006), in house martins infested by the bug *Oeciacus hirundinis* (Christe *et al*. 2000b) and in mice infected by the intestinal nematode *Heligmosomoides polygyrus* (Barnard *et al.* 1998). Moreover, there was a negative relationship between cellular immune response and ectoparasites in the greater mouse eared bat *Myotis myotis* infested with mites (Christe *et al*. 2000a) and in the jird *Meriones crassus* experimentally infested with fleas (Goüy de Bellocq *et al*. 2006).

Several non-mutually exclusive mechanisms could explain the decrease in the anti-KLH response of flea-infested voles. First, the low nutritional condition resulting from the parasite impact during growth may negatively affect the development of organs involved in immune defences (Møller *et al*. 1998). Second, fleas might directly depress the immune system of voles, for example by reducing the proliferation of B-lymphocytes involved in the IgG production. Third, there might be trade-offs in the allocation of resources to different compartments of the immune system, either between immune responses against fleas vs. immune response against KLH, or between the humoral immune function and erythropoiesis. This last trade-off may come from the fact that lymphocytes and erythrocytes derive from haemocytoblasts, which are the stem cells at the very beginning of hematopoiesis (Quesenberry 1990). Thus, a decrease in haematocrit level and pathogen infection may both request haemocytoblasts. The significant interaction between treatment and time for haematocrit during immune response further indicates that parasitized individuals have to deal with a trade-off between haematocrit compensation and immune response which does not affect non-parasitized voles.

CONCLUSION

Parasitism by fleas had a strong and consistent negative impact on vole morphology, health status, metabolism and ability to mount an immune response. These findings highlight the complexity and diversity of interactions between ectoparasites and various physiological responses of their mammal host. More generally these results demonstrate that parasitism by fleas is an important environmental factor that should be integrated in studies of rodent life-history and population dynamics.

Acknowledgement

Authors thank two anonymous referees and the associate editor for helpful comments on a previous version of the manuscript. Thanks to Jérôme Notari for help with vole maintenance. This research was supported by grant 3100A0-104118 and 3100A0-108263 from the Swiss National Science Foundation.

References

Abbas, A.K., Lichtman, A.H. & Pober, J.S. (2000) *Cellular and Molecular Immunology*, 4th edn. W.B. Saunders Company, Philadelphia.

Alonso-Alvarez, C. & Tella, J.L. (2001) Effects of experimental food restriction and body-mass changes on the avian T-cell-mediated immune response. *Canadian Journal of Zoology*, **79**, 101–105.

Alonso-Alvarez, C., Bertrand, S., Devevey, G., Gaillard, M., Prost, J., Faivre, B. & Sorci, G. (2004) An experimental test of the dose-dependent effect of carotenoids and immune activation on sexual signals and antioxidant activity. *The American Naturalist*, **164**, 651–659.

Alonso-Alvarez, C., Bertrand, S., Devevey, G., Prost, J., Faivre, B., Chastel, O. & Sorci, G. (2006) An experimental manipulation of life-history trajectories and resistance to oxidative stress. *Evolution*, **60**, 1913–1924.

Barnard, C.J., Behnke, J.M., Gage, A.R., Brown, H. & Smithurst, P.R. (1998) The role of parasite-induced immunodepression, rank and social environment in the modulation of behaviour and hormone concentration in male laboratory mice (*Mus musculus*). *Proceedings of the Royal Society of London Series B-Biological Sciences*, **265**, 693–701.

Beckman, K.B. & Ames, B.N. (1998) The free radical theory of aging matures. *Physiological Reviews*, **78**, 547–581.

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.

Blumgart, H.L. & Atlschule, M.D. (1948) Clinical significance of cardiac and respiratory adjustments in chronic anaemia. *Blood*, **3**, 329–348.

Booth, D.T., Clayton, D.H. & Block, B.A. (1993) Experimental demonstration of the energetic cost of parasitism in free-ranging hosts. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **253**, 125–129.

Chapman, B.R. & George, J.E. (1991) The effects of ectoparasites on cliff swallow growth and survival. *Bird-Parasite interactions: Ecology, Evolution and Behaviour* (eds J.E. Loye & M. Zuk), pp. 69–92. Oxford University Press, Oxford.

Christe, P., Arlettaz, R. & Vogel, P. (2000a) Variation in intensity of a parasitic mite (*Spinturnix myoti*) in relation to the reproductive cycle and immunocompetence of its bat host (*Myotis myotis*). *Ecology Letters*, **3**, 207–212.

Christe, P., Møller, A.P., Saino, N. & De Lope, F. (2000b) Genetic and environmental components of phenotypic variation in immune response and body size of a colonial bird, *Delichon urbica* (the house martin). *Heredity*, **85**, 75–83.

Christe, P., Richner, H. & Oppliger, A. (1996) Begging, food provisioning, and nestling competition in great tit broods infested with ectoparasites. *Behavioral Ecology*, **7**, 127–131.

Crétégny, C. (1997) Quelques facteurs affectant, durant la lactation, la croissance du campagnol des champs *Microtus arvalis*. Thèse de doctorat, Université de Lausanne.

Dede, S., Deger, Y., Kahraman, T., Deger, S., Alkan, M. & Cemek, M. (2002) Oxidation products of nitric oxide and the concentrations of antioxidant vitamins in parasitized goats. *Acta Veterinaria Brno*, **71**, 341–345.

Demas, G., Drazen, D.L. & Nelson, R.J. (2003) Reductions in total body fat decrease humoral immunity. *Proceedings of the Royal Society of London, Series B*, **270**, 905–911.

Depocas, F. & Hart, S. (1957) Use of the Pauling oxygen analyser for measurement of oxygen consumption of animals in open-circuit systems and a short-lag, closed-circuit apparatus. *Journal of Applied Physiology*, **10**, 388–392.

Drazen, D.L., Demas, G. & Nelson, R.J. (2001) Leptin effects on immune function and energy balance are photoperiod dependent in Siberian Hamsters (*Phodopus sungorus*). *Endocrinology*, **142**, 2768–2775.

Fair, J., Whitaker, S. & Pearson, B. (2007) Sources of variation in haematocrit in birds. *Ibis*, **149**, 535–552.

Giorgi, M.S., Arlettaz, R., Christe, P. & Vogel, P. (2001) The energetic grooming costs imposed by a parasitic mite (*Spinturnix myoti*) upon its bat host (*Myotis myotis*). *Proceedings of the Royal Society of London, Series B*, **268**, 2071–2075.

Girard, A., Madani, S., El Boustani, E.S., Belleville, J. & Prost, J. (2005) Changes in lipid metabolism and antioxidant defense status in spontaneously hypertensive rats and Wistar rats fed a diet enriched with fructose and saturated fatty acids. *Nutrition*, **21**, 240–248.

Gore, E.R., Gower, J., Kurali, E., Sui, J.-L., Bynum, J., Ennulat, D. & Herzyk, D.J. (2004) Primary antibody response to keyhole limpet hemocyanin in rat as a model for immunotoxicity evaluation. *Toxicology*, **197**, 23–35.

Goüy de Bellocq, J., Krasnov, B.R., Khokhlova, I.S., Ghazaryan, L. & Pinshow, B. (2006) Immunocompetence and flea parasitism of a desert rodent. *Functional Ecology*, **20**, 637–646.

Grindstaff, J.L., Brodie, E.D.I. & Ketterson, E.D. (2003) Immune function across generations: integrating mechanism and evolutionary process in maternal antibody transmission. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **270**, 2309–2319.

Hawlena, H., Abramsky, Z. & Krasnov, B.R. (2006a) Ectoparasites and age-dependent survival in a desert rodent. *Oecologia*, **148**, 30–39.

Hawlena, H., Khokhlova, I.S., Abramsky, Z. & Krasnov, B.R. (2006b) Age, intensity of infestation by flea parasites and body mass loos in a rodent host. *Parasitology*, **133**, 187–193.

Heeb, P., Werner, I., Kölliker, M. & Richner, H. (1998) Benefits of induced host responses against an ectoparasite. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **265**, 51–56.

Hurtrez-Boussès, S., Perret, P., Renaud, F. & Blondel, J. (1997) High blowfly parasitic loads affect breeding success in a Mediterranean population of blue tits. *Oecologia*, **112**, 514–517.

Jacob, J. (2003) Body weight dynamics of common voles in agro-ecosystems. *Mammalia*, **67**, 559–566.

Kawata, M. (1988) Mating success, spatial organization, and male characteristics in experimental field populations of the red-backed vole *Clethrionomys rufocanus bedfordidae*. *Journal of Animal Ecology*, **57**, 217–235.

Kenward, M.G. & Roger, J.H. (1997) Small sample inference for fixed effects from restricted maximum likelihood. *Biometrics*, **53**, 983–997.

Khokhlova, I.S., Krasnov, B.R., Kam, M., Burdelova, N.I. & Degen, A.A. (2002) Energy cost of ectoparasitism: the flea *Xenopsylla ramesis* on the desert gerbil *Gerbillus dasyurus*. *Journal of Zoology*, **258**, 349–354.

Krasnov, B.R. (2008) *Functional and Evolutionnary Ecology of Fleas*. Cambridge University Press, Cambridge.

Kristan, D.H. (2002) Maternal and direct effects of the intestinal nematode *Heligmosomoides polygyrus* on offspring growth and susceptibility to infection. *The Journal of Experimental Biology*, **205**, 3967–3977.

Kristan, D.H. (2004) Intestinal nematode infection affects host life history and offspring susceptibility to parasitism. *Journal of Animal Ecology*, **73**, 227–238.

Kristan, D.H. & Hammond, K.A. (2000) Combined effects of cold exposure and sub-lethal intestinal parasites on host morphology and physiology. *The Journal of Experimental Biology*, **203**, 3495–3504.

Kristan, D.H. & Hammond, K.A. (2001) Parasite infection and caloric restriction induce physiological and morphological plasticity. *American Journal of Physiology – Regulatory, Integrative and Comparative Physiology*, **281**, 502–510.

Kristan, D.H. & Hammond, K.A. (2006) Effects of three simultaneous demands on glucose transport, resting metabolism and morphology of laboratory mice. *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology*, **176**, 139–151.

Lehmann, T. (1992) Ectoparasite impacts on *Gerbillus andersoni allenbyi* under natural conditions. *Parasitology*, **104**, 479–488.

Lesgards, J.-F., Durand, P., Lassarre, M., Stocker, P., Lesgards, G., Lanteaume, A., Prost, M. & Lehucher-Michel, M.-P. (2002) Assessment of lifestyle effects on the overall antioxidant capacity of healthy subjects. *Environmental Health Perspectives*, **110**, 479–486.

Lindström, J. (1999) Early development and fitness in birds and mammals. *Trends in Ecology and Evolution*, **14**, 343–348.

Littell, R.C., Milliken, G.A., Stroup, W.W., Wolfinger, R.D. & Schabenberger, O. (2006) *SAS for Mixed Models*. SAS Press, Cary, NC.

Magnanou, E., Fons, R., Feliu, C. & Morand, S. (2006) Physiological responses of insular wild black rat (*Rattus rattus*) to natural infection by the digenean trematode *Fasciola hepatica*. *Parasitological Researches*, **99**, 97–101.

Møller, A.P. (1997) Parasitism and the evolution of host life history. *Host-parasite Evolution, General Principles & Avian Models* (eds D.H. Clayton & J. Moore), pp. 105–127. Oxford University Press, New York.

Møller, A.P., Christe, P., Erritzøe, J. & Mavarez, J. (1998) Condition, disease and immune defence. *Oikos*, **83**, 301–306.

Morand, S., Krasnov, B.R. & Poulin, R. (2006) *Micromammals and Macroparasites. From Evolutionary Ecology to Management*. Springer-Verlag, Tokyo.

Munger, J.C. & Karasov, W.H. (1994) Costs of bot fly infection in white-footed mice: energy and mass flow. *Canadian Journal of Zoology*, **72**, 166–173.

Murray, D.L., Cary, J.R. & Keith, L.B. (1997) Interactive effects of sublethal nematodes and nutritional status on snowshoe hare vulnerability to predation. *Journal of Animal Ecology*, **66**, 250–264.

Norris, K. & Evans, M.R. (2000) Ecological immunology: life history tradeoffs and immune defense in birds. *Behavioral Ecology and SocioBiology*, **11**, 19–26.

Pieri, C., Moroni, F. & Marra, M. (1996) Food restriction increases the protection of erythrocytes against the hemolysis induced by peroxyl radicals. *Mechanisms of Ageing and development*, **87**, 15–23.

Quesenberry, P.J. (1990) Hematopoietic stem cells, progenitor cells, and growth factors. *Hematology* (eds W.J. Williams, E. Beutler, A.J. Erslev & M.A. Lichtman), pp. 129–147. McGraw-Hill Inc, New York.

Richner, H., Oppliger, A. & Christe, P. (1993) Effect of an ectoparasite on reproduction in great tit. *Journal of Animal Ecology*, **62**, 703–710.

Rojas Wahl, R.U., Zeng, L., Stephen, A., Madison, S.A., De Pinto, R.L. & Brian Shay, B.J. (1998) Mechanistic studies on the decomposition of water soluble azo-radical-initiators. *Prekin Transactions 2*, **9**, 2009–2018.

Scantleburry, M., Waterman, J.M., Hillegass, M., Speakman, J.R. & Bennett, N.C. (2007) Energetic costs of parasitism in the Cape ground squirrel *Xerus inauris*. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **274**, 2169–2177.

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

Stearns, S.C. (1992) *The Evolution of Life Histories*. Oxford University Press, Oxford.

Stocker, P., Lesgards, G., Vidal, N., Chalier, F. & Prost, M. (2003) ESR study of a biological assay on whole blood: antioxidant efficiency of various vitamins. *Biochimica et Biophysica Acta*, **1621**, 1–8.

Traub, R., Rotschild M. & Haddow, J.F. (1983) *The Ceratophyllidae: Key to the Genera and Host Relationships*. Cambridge University Press, Cambridge.

Tschirren, B., Fitze, P.S. & Richner, H. (2007) Maternal modulation of natal dispersal in a passerine bird: an adaptive strategy to cope with parasitism? *The American Naturalist*, **169**, 87–93.

Ujvari, B. & Madsen, T. (2006) Age, parasites, and condition affect humoral immune response in tropical pythons. *Behavioral Ecology*, **17**, 20–24.

van Noordwijk, A.J. & de Jong, G. (1986) Acquisition and allocation of resources: their influence on variation in life history tactics. *The American Naturalist*, **128**, 137–142.

Wauters, L. & Dhondt, A.A. (1989) Body weight, longevity and reproductive success in red squirrels (*Sciurus vulgaris*). *Journal of Animal Ecology*, **58**, 637–651.

Received 11 February 2008; accepted 11 September 2008
Handling Editor: Ken Wilson

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Results without parasitized control individual.

Table S1. Change in traits during immune challenge without parasitized control individual.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.