Flea infestation reduces the life span of the common vole

G. DEVEVEY[1,2]* *and* P. CHRISTE[1]

[1] *Department of Ecology & Evolution, University of Lausanne, Biophore, CH-1015 Lausanne, Switzerland*
[2] *Department of Biology, University of Pennsylvania, 433 S. University Avenue, Philadelphia PA 19104, USA*

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SUMMARY

Parasitism is often a source of variation in host's fitness components. Understanding and estimating its relative importance for fitness components of hosts is fundamental from physiological, ecological and evolutionary perspectives. Host-parasite studies have often reported parasite-induced reduction of host fecundity, whereas the effect of parasitism on host survival has been largely neglected. Here, we experimentally investigated the effect of infestation by rat fleas (*Nosopsyllus fasciatus*) on the life span of wild-derived male common voles (*Microtus arvalis*) bred in captivity. We found that the mean life span of parasitized voles was reduced by 36% compared to control voles. Parasitized voles had a smaller body size, but a relatively larger heart and spleen than control voles. These results indicate an effect of flea infestation on host life span and our findings strongly suggest that ectoparasites should be taken into account in the studies of host population dynamics.

Key words: life-history traits, *Microtus arvalis*, *Nosopsyllus fasciatus*, parasitism, survival.

INTRODUCTION

Parasites are often assumed to affect the host's life-history traits in a way that depresses the host's fitness (Møller, 1997). Any depression of the host's fitness occurs through a reduction of the number of reproductive attempts (estimated by the life span and time to first reproduction) and/or a reduction of the fecundity (i.e. the number of produced offspring per reproductive attempt) (Stearns, 1992). Thus parasitism can ultimately result in strong effects on host population dynamics, as suggested by empirical and theoretical studies (Anderson and May, 1978; Dobson and Hudson, 1992; Hudson *et al*. 1992, 1998). Reduction of fecundity due to ectoparasitism has been documented several times (e.g. Deter *et al*. 2007; Møller, 1997; Neuhaus, 2003; Saino *et al*. 1998), whereas evidence of the negative effect of ectoparasites on life span and thus on the reproductive life-time is scarce (but see Brown *et al*. 1995).

Among host-parasite interactions, small mammals and their ectoparasites have been understudied despite their ecological importance in terrestrial ecosystems and their role in epidemiology of several zoonoses (e.g. Chagas' disease: Garcia *et al*. 2007, Lyme disease: Brisson *et al*. 2008). Fleas are widespread parasites of small mammals, vectors of numerous diseases (Medvedev and Krasnov, 2006) and their effects on host's fitness are largely unknown. Some recent studies in gerbils demonstrated that fleas induce body mass loss in adult desert gerbils *Gerbillus dasyurus* (Khokhlova *et al*. 2002), whereas this was not the case in adult Anderson's gerbils *Gerbillus andersoni* (Hawlena *et al*. 2006*a*). Flea infestation may also reduce immune defences (Goüy de Bellocq *et al*. 2006; Devevey *et al*. 2008), which in turn may affect survival. However, evidence of reduced recapture rate has only been provided in parasitized juvenile Anderson's gerbils (Hawlena *et al*. 2006*b*). In the common vole *Microtus arvalis*, flea infestation affects growth and body mass, depresses host immune defences and induces anaemia (Devevey *et al*. 2008), which could severely reduce survival probability, but definitive tests of the effect of flea infestation on life span are still lacking.

In this study, we experimentally tested whether parasitism by fleas reduces the life span of their hosts, the common vole. We monitored the life span of captive males parasitized by fleas or kept non-infested until natural death. We also measured haematocrit and body condition throughout life, and at death we weighed spleen, heart, and testes in order to examine whether life span variations could be due to physiological disorders.

MATERIALS AND METHODS

The stock population consisted of wild adult common voles from meadows surrounding the University of Lausanne (Switzerland). Voles were deparasitized with 4 μl of 120 mg/ml veterinary selamectin (Stronghold®, Pfizer, New-York) deposited beneath the ear at their arrival in the animal room. Topical application of selamectin provides effective

* Corresponding author: Department of Ecology & Evolution, University of Lausanne, Biophore, CH-1015 Lausanne, Switzerland. Tel: +1215 746 1732. Fax: +1215898 8780. E-mail: godefroy.devevey@unil.ch

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protection against external and internal parasites during 4 weeks without negative side-effects on host health (Pipano, 2003). Experimental animals were male common voles born from the stock population. They were aged between 45 and 60 days at the start of the experiment (day 0). Common voles reach their adult size at 3 months (Jacob, 2003). All animals were individually housed in a polypropylene cage (36 cm × 20 cm × 18 cm) in an animal room with a 14 L : 10 D cycle and constant temperature of 21 ± 1 °C. Cages contained 1 litre of sterilized soil and a flowerpot (diameter 14 cm) as a vole roost. Hay and tap water were available *ad libitum* and animals received apples and seeds regularly throughout the experiment.

At day 0, 52 adult males originating from 25 litters were weighed, measured, and blood sampled. At day 1, individuals were randomly assigned to the flea-treatment group or non-parasitized control 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 *Nosopsyllus 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 4 samples of 15 g of bedding, flea loads comprised between 25 and 64 individuals (average = 44 fleas). The control group received 15 g of a mix of bedding without fleas. Voles were housed in the same animal facility and the cages were randomly placed on shelves. See Devevey *et al*. (2008) for more details.

Body mass and haematocrit were measured at the start of the experiment and at days 98, 119, 139, 237, 335, 430, 640, and 742. Sample sizes decrease over time due to this design running until the natural death of every individual.

On each blood sampling day, blood was drawn by tail-cutting into a pre-heparinized capillary for haematocrit. Haematocrit capillaries were centrifuged for 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. Body mass was measured to the nearest 0·1 g. After death, corpse length from the tip of the nose to the base of the tail was measured on a graduated board. Individual body condition at day 98 and the following days was assessed by extracting residuals from the regression of body mass on body length ($r^2 = 0{\cdot}11$, $n = 174$, $P < 0{\cdot}001$). Then the heart, spleen and testes were removed, cleaned from connective tissue and weighed (0·1 mg).

Effect of parasitism on life span of voles was first tested by a Kaplan-Meier survival analysis. The Kaplan-Meier estimator is a product-limit survival estimate from life-time data (Kaplan and Meier, 1958). We analysed change in haematocrit and body condition throughout adulthood (age equal or older than 98 days) with repeated-measure ANOVA over continuous time in backward procedure. Because changes in haematocrit or body condition over time can be due to within-individual changes (improvement, senescence) or to between-individual change (selective disappearance), we added the factor 'age of last measurement' in the ANOVA (van de Pol and Verhulst, 2006). To control for allometric relationships between corpse mass and organs (heart: $R^2 = 0{\cdot}45$, $F_{1,26} = 20{\cdot}9$, $P < 0{\cdot}001$; spleen: $R^2 = 0{\cdot}06$, $F_{1,25} = 1{\cdot}6$, $P = 0{\cdot}2$; testes: $R^2 = 0{\cdot}49$, $F_{1,29} = 28{\cdot}1$, $P < 0{\cdot}001$), we entered corpse mass as a covariate in the analyses of the mass of spleen, heart and testes. Variables were log-transformed if necessary in order to normalise the distribution. Reported values are means ± standard error. All tests were two-tailed and were performed with JMP 7.0.0.

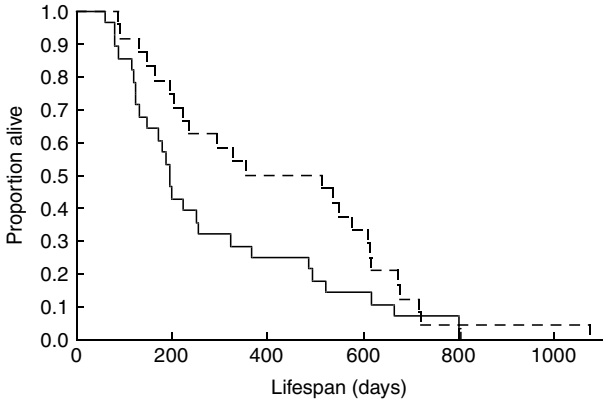

Fig. 1. Life span curves of voles infested by fleas (full line) or non-infested by fleas (dashed line).

RESULTS

The life span of parasitized voles (mean: 293·7 days, median: 194 days, max: 804 days) was reduced compared to control voles (mean: 460 days, median: 538 days, max: 1076 days; $\chi^2 = 6{\cdot}04$, D.F. = 1, $P = 0{\cdot}014$; Fig. 1), even after excluding voles which did not reach 100 days of age (respective medians, 224 and 550 days; $\chi^2 = 6{\cdot}07$, D.F. = 1, $P = 0{\cdot}014$).

The two groups did not differ for body mass and haematocrit at the start of the experiment (*t*-tests, respectively $t_{49} = 0{\cdot}32$, $P = 0{\cdot}8$ and $t_{49} = 0{\cdot}61$, $P = 0{\cdot}5$). The mean haematocrit level of parasitized voles (0·43 ± 0·00) was lower than in control voles (0·52 ± 0·00) throughout life, and in both groups the level decreased as voles aged (Table 1). Body condition tended to be lower in parasitized voles than controls and diminished with age in non-parasitized individuals ($F_{1,98} = 9{\cdot}75$, $P = 0{\cdot}002$; Table 1), whereas that was not the case in parasitized voles ($F_{1,72} = 0{\cdot}53$, $P = 0{\cdot}47$). The non-significant term 'age of last measurement' means that the changes are due to individual variation of trait values and not to selective disappearance (van der Pol and Verhulst, 2006).

Table 1. Results of the repeated measures analyses of variance on haematocrit and body condition throughout the life-time of voles

(The mean haematocrit level of parasitized voles was lower than in control voles, and in both groups the level decreased as voles aged. Body condition tended to be lower in parasitized voles than controls and diminished with age in non-parasitized individuals, whereas that was not the case in parasitized voles.)

Traits / Factors	D.F.	F	*P*
Haematocrit			
Age	1, 142·6	11·98	<0·001
Parasitism	1, 33·6	123·18	<0·001
Age at last measurement	1, 144·5	0·51	0·48
Body condition			
Age	1, 135·7	1·22	0·27
Parasitism	1, 33·1	3·95	0·055
Age at last measurement	1, 168·9	1·41	0·24
Age × Parasitism	1, 146·6	7·13	0·008

At the time of death, parasitized voles were smaller than those of non-parasitized voles (respectively 101·7 ± 2·2 mm and 111·9 ± 2·2 mm, $t_{34} = 3{\cdot}24$, $P = 0{\cdot}003$). After controlling for body mass, parasitized voles had larger spleens (non-parasitized: 31·8 mg ± 1·3; parasitized: 87·4 mg ± 1·4; $F_{1,27} = 5{\cdot}74$, $P = 0{\cdot}024$) and larger hearts (non-parasitized: 130·8 mg ± 1·1; parasitized: 164·7 mg ± 1·1; $F_{1,26} = 6{\cdot}58$, $P = 0{\cdot}016$) than controls, but testes were of similar mass (non-parasitized: 121·0 mg ± 14·8; parasitized: 78·2 mg ± 12·3; $F_{1,26} = 0{\cdot}30$, $P = 0{\cdot}59$).

DISCUSSION

Our study demonstrates that flea infestation by *Nosopsyllus fasciatus* can reduce life span of one of its natural hosts, the common vole. Brown *et al*. (1995) also showed that survival was approximately 12% lower for cliff swallows *Petrochelidon pyrrhonota* reproducing in parasitized nests compared to those from fumigated nests, demonstrating that parasitism experienced during the short period of reproductive effort can result in loss of up to 1 year of life-time reproductive success. We found that the mean life span of parasitized voles decreased by 36%, or 166 days. Theoretically, the life-time reproductive success is correlated with life span in iteroparous species because a long life span may be associated with a higher number of possible breeding attempts and more breeding experience (Clutton-Brock, 1988; Stearns, 1992; Weladji *et al*. 2006). This statement is confirmed by several empirical studies in rodents (Ribble, 1992; Wauters and Dhondt, 1995) as well as in small birds (Schmoll *et al*. 2009) and in large mammals (Pettorelli and Durant, 2007; Weladji *et al*. 2006). In the present study, voles had no access to mates and we have thus no data on life-time reproductive success. However, we can hypothesize that in natural conditions, the decrease of life span induced by parasites or other extrinsic mortality factors leads to a lower number of breeding opportunities and to a lower life-time reproductive success, especially for short-lived species exposed to high extrinsic mortality factors (Christe *et al*. 2006). Moreover, experimental evidence suggests that endoparasitism reduces the reproductive success of breeding common voles (Deter *et al*. 2007).

The exact mechanisms by which flea infestation accelerates death are not yet clear. Nevertheless, this experiment may provide us with some non-mutually exclusive hypotheses, suggesting that flea infestation can trigger complex trade-offs between different functions like the immune system, erythropoiesis and blood circulation. The immune system is chronically in demand and the observed huge spleen relative to the body mass of parasitized voles (on average 274% of the mass of the spleen of the control voles) can be due to intense immune activity (Møller *et al*. 1998) which might favour immune disorder and auto-immune diseases (Sorci and Faivre, 2009). Nevertheless, the immune effort by itself does not shorten the life span of common voles (Devevey *et al*. 2009). Moreover, rodents parasitized by fleas are immuno-depressed (Devevey *et al*. 2008; Goüy de Bellocq *et al*. 2006), and this makes them more sensitive to diseases. Alternatively, the spleen may also act as an erythropoietic organ in microtines (Watkins *et al*. 1991), and thus splenomegaly observed in this study could be due to a combined effect of immune activation and the necessity to produce new red blood cells. This could be one of the morphological changes induced by anaemia. Besides, the hypothesis that parasitized voles have a higher resting metabolic rate due to an increased breath and/or heart output in response to anaemia (Devevey *et al*. 2008) is now corroborated by the finding that parasitized voles have a heavier heart than control voles. The precocious anaemia associated with low body condition early in life and other physiological disorders may explain the early death of parasitized voles. In addition, fleas are known to be vectors of several microorganisms (*Ricketssiae* typhus, trypanosomes …) (Medvedev and Krasnov, 2006) with potentially pathogenic effects on the host. The measured cost of flea infestation is thus the sum of the direct effects of the flea and the indirect consequences of potentially inoculated pathogens.

Overall, these results demonstrate that the presence of fleas affects one important host life-history trait in a way that could depress fitness. It emphasizes the necessity to integrate parasitism, even by seemingly inconspicuous fleas, in studies involving the life-history traits of small mammals. Macroparasites as well as microparasites can affect survival and life

span (Burthe *et al*. 2008) and this may in turn affect population dynamics (Deter *et al*. 2008; Townsend *et al*. 2009).

All manipulations were done under control of the Vaud Veterinary Authorities, authorization 1848.

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