RESEARCH ARTICLE

Flea infestation does not cause a long-term increase in energy metabolism in *Gerbillus nanus*

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SUMMARY

Fleas can increase the metabolic rate of their hosts. It has been suggested that a constitutive response, in which the host constantly maintains a relatively high level of energy metabolism to combat the parasite, is advantageous for hosts with high parasite infestation, while an induced response, in which the host increases energy metabolism in response to a parasite attack, is advantageous with low parasite infestation. As free-living *Gerbillus nanus* show a relatively low flea infestation, we hypothesized that this host uses an induced strategy and, consequently, flea infestation would not impose a long-term effect on energy metabolism. In a previous study in spring, higher field metabolic rate (FMR) was found in free-living parasitized than in non-parasitized *G. nanus*. In this study, *G. nanus* were captured at Hazeva in spring; some had fleas (*N*=14) and some did not (*N*=10). We brought them to the laboratory, removed the fleas from those that were infested and, after 3weeks, measured average daily metabolic rate (ADMR) of all rodents. ADMR averaged $8.68\pm0.95\,\mathrm{kJ\,g^{-0.54}\,day^{-1}}$ for all rodents and was similar between previously parasitized and non-parasitized *G. nanus* while free living. Thus, the hypothesis that flea infestation does not have a long-term effect on energy metabolism was supported, as was the idea of an induced over a constitutive immune response by *G. nanus* in combating parasites.

Key words: host–parasite relationship, fleas, average daily metabolic rate, energy expenditure, ecophysiology, *Gerbillus nanus*.

INTRODUCTION

Parasites may affect potential hosts directly, by reducing the resources of the host, and indirectly, by causing energy-costly behavioural (Cox, 1981; Giorgi et al., 2001; Tripet et al., 2002) and/or immunological (Lochmiller and Deerenberg, 2000; Gallizi et al., 2008) responses. Although infested hosts can increase food intake (Moret and Schmid-Hempel, 2000), most studies have concluded that parasites suppress food consumption (Hunter and Webster, 1974; Kyriazakis et al., 1998; Faro et al., 2000) and foraging behaviour of their hosts (Raveh et al., 2011), which could reduce the energy allocation for reproduction and, consequently, reduce host fitness.

Metabolic rate was found to be higher in parasitized than in non-parasitized rodent hosts under laboratory conditions (average daily metabolic rate, ADMR) (Khokhlova et al., 2002) and, under certain conditions, can be higher in free-living hosts (field metabolic rate, FMR) (Kam et al., 2010). The controlled laboratory experiments concluded that the parasites caused the increase in metabolic rate, perhaps as a result of the costs related to the immune system (Khokhlova et al., 2002). Two immune response strategy options by the host to combat parasite infestation have been defined: (1) constitutive, in which the host constantly maintains a relatively high level of energy metabolism to combat the parasite; and (2) induced, in which the host increases energy metabolism in response to a parasite infestation for a relatively short period. The latter option would be preferred in hosts

experiencing low parasite infestation as this strategy would save energy in the long term, although the host would not be as well prepared to initially combat parasite infestation compared with the former option. Thus, as suggested by Hawlena and colleagues (Hawlena et al., 2006), 'the two strategies are most likely a reflection of interspecific differences in probability of flea attacks'.

A previous study showed that parasitized *G. nanus* have a higher FMR than non-parasitized *G. nanus* at Hazeva in spring (Kam et al., 2010). In the present study, we captured *G. nanus* in their natural habitat in spring at the same site as in the previous study: some were flea infested and some were flea free. We removed the fleas from infested rodents and measured ADMR of all rodents 3weeks after capture. When we compared ADMR of previously infested rodents, that is, rodents that had had their fleas removed, with those that were previously not infested in the field, two scenarios were envisaged: (1) the ADMR of rodents with fleas at capture would be higher than that of rodents without fleas at capture, which would indicate a long-term effect of parasite infestation; or (2) there would be no difference in ADMR between the groups, which would mean a short-term effect. As *G. nanus* have a relatively low rate of flea infestation (Kam et al., 2010), we hypothesized that this host would use an induced immune response strategy and, consequently, we predicted that previously parasitized and non-parasitized hosts would not differ in their ADMR when flea free.

MATERIALS AND METHODS
Animals

The Baluchistan gerbil (*G. nanus*, Blanford 1875) is a nocturnal, granivorous desert rodent. It inhabits burrows in relatively deep soil with abundant vegetation occurring around wadis in desert and semi-desert areas. It is widespread in the western part of the Sahara and Sahel as well as in the Arabian Peninsula and the Middle East through Afghanistan and Pakistan to northwest India (Granjon et al., 2008). Gerbils were captured using Sherman traps at the Sheizaf Nature Reserve located in the Rift Valley (30°45′N, 35°15′E), about 30 km south of the Dead Sea, Israel, at the end of February. Animals were collected from the same site used for measurements of energy expenditure in free-living *G. nanus* and in the same season as before when significant differences in FMR were found between parasitized and non-parasitized hosts (Kam et al., 2010). The traps, baited with millet seeds, were set after sunset and were checked for gerbils in the early morning at first light. Twenty-four *G. nanus*, 10 females and 14 males trapped at our field site, were transported in their individual traps to our laboratory for metabolic rate measurements. Of these rodents, 4 females and 10 males were infested with fleas (Table 1). All fleas were removed manually over a large tub, counted and identified (Krasnov et al., 2006; Kam et al., 2010); no fleas were found off the host and free in the traps. The number of fleas collected from the host was assumed to be a constant proportion of the flea reservoir within rodent burrows, as was reported for other rodent species including gerbils of the same genus, *Gerbillus* (Lehman, 1994; Krasnov, 2008). We identified two species of flea, *Xenopsylla conformis* and *Nosopsyllus pumilionis*, of which *X. conformis* was the most common (Krasnov, 2008), as was found in our earlier study (Kam et al., 2010).

Laboratory measurements

The rodents were maintained at an air temperature of 25±1°C and a photoperiod of 12h:12h L:D in individual metabolic cages (20×10×20cm) with a wire mesh floor that allowed measurement of food intake and faecal output. During the experimental period they were offered *ad libitum* millet seeds and fresh alfalfa leaves in separate food cups attached to the side of the cages; no drinking water was offered as these rodents do not normally drink under free-living conditions (Degen et al., 1997). The animals were allowed 3 weeks for adjustment, and then dry matter intake and energy digestibility were measured over 12 days, during which time metabolizable energy intake (MEI) and body mass changes were measured, and ADMR was calculated. All animals remained healthy throughout the study.

ADMR is the MEI required by a caged animal to maintain constant body energy content, taken as zero change in body mass. Consequently, in this measurement the energy requirements refer to those needed for maintenance under laboratory conditions with minimal animal activity. ADMR includes basal metabolic rate (BMR), heat increment of feeding for maintenance, some minimal activity costs and, possibly, some thermoregulatory costs, and is the closest laboratory measurement to FMR (Degen, 1997; Degen et al., 1998). For these reasons we chose ADMR as a measurement of metabolic rate in this study.

To determine dry matter content, samples of the food offered, daily food remains and the faeces were oven-dried at 100°C to constant mass. These samples were measured for caloric value using a ballistic bomb calorimeter (Gallenkamp, model CBB-370, Loughborough, Leicestershire, UK) with benzoic acid (26.453±3.9 J g^{-1}; BCS-CRM No. 190n, Bureau of Analysed Samples Ltd, Bristol, UK) as standard. Dry matter intake of each

Table 1. Body mass (M_b) and average daily metabolic rate (ADMR) in non-parasitized *Gerbillus nanus*

Sex	Infested	M_b (g)	ADMR (kJ day^{-1})	ADMR (kJ g$^{-0.54}$ day^{-1})
Female	No	28.1	46.30	7.65
Female	No	23.0	48.14	8.86
Female	No	20.2	45.70	9.02
Female	No	22.5	41.87	7.79
Female	No	23.5	44.77	8.14
Female	No	23.6	46.48	8.43
Male	No	26.3	53.16	9.09
Male	No	22.5	55.89	10.41
Male	No	25.1	51.04	8.96
Male	No	22.4	38.72	7.23
Female	Yes	19.8	52.69	10.51
Female	Yes	25.1	48.72	8.55
Female	Yes	14.9	26.99	6.27
Female	Yes	22.1	50.18	9.44
Male	Yes	28.6	54.49	8.91
Male	Yes	27.2	51.98	8.74
Male	Yes	19.7	41.80	8.35
Male	Yes	24.4	49.28	8.77
Male	Yes	26.0	53.21	9.16
Male	Yes	19.2	46.91	9.51
Male	Yes	28.0	48.83	8.08
Male	Yes	27.4	49.76	8.32
Male	Yes	26.4	58.13	9.93
Male	Yes	19.9	41.37	8.23

Included is infestation of the same individuals by fleas in their natural habitat (see Materials and methods for details).

food item was calculated as the difference between dry matter offered and dry matter of the remains (Degen and Kam, 1992). Gross energy intake was calculated from the intake of each item and its calorific value, digestible energy intake was calculated as the difference between gross energy intake and faecal energy output, and metabolizable energy intake was assumed to be 98% of digestible energy intake (Grodziski and Wunder, 1975; Khokhlova et al., 2002).

The animals were weighed (to 0.01 g; Ohaus CT200-S electronic balance, Ohaus Corporation, Pine Brook, NJ, USA) daily before food was offered. A regression analysis of daily body mass change (ΔM_b; g day^{-1}) on MEI (kJ day^{-1}) was done for each gerbil. The equation took the form:

$$\Delta M_b = a + b\,\mathrm{MEI}\,, \qquad (1)$$

where *a* and *b* are constants; MEI at zero daily body mass change was taken as ADMR (Degen, 1997).

For comparison between groups, we calculated ADMR values per body mass to the power of 0.54, which was found to be appropriate for rodents (French et al., 1976; Degen, 1997; Degen et al., 1998; Khokhlova et al., 2002). We used factorial ANOVA (Statistica 7.0) for comparison between sexes and between previously parasitized and non-parasitized gerbils, and accepted $P<0.05$ as the minimal level of significance. Values are presented as means ± s.d.

This study was conducted under permit number 2006/27209 from the Israel Nature and National Parks Protection Authority and approved by the Ben-Gurion University Committee for the Ethical Care and Use of Animals in Experiments.

RESULTS

Of the 24 captured *G. nanus*, 14 were parasitized with fleas and 10 were flea free (Table 1). The number of fleas ranged from 1 to 3
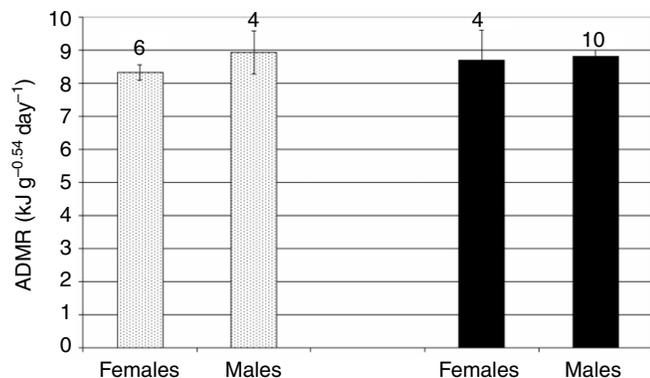
Fig. 1. Average daily metabolic rate (ADMR) of non-parasitized *Gerbillus nanus* that were (filled bars) or were not (open bars) infested in their natural habitat prior to measurement of ADMR. ADMR (±s.e.) of males and females is presented per metabolic mass for comparison (Degen et al., 1998), and numbers above error bars denote sample sizes.

with a mean of 1.50±0.76 fleas per parasitized gerbil. Body mass of female (22.27±3.50 g) and male (24.51±3.22 g) ($F_{1,21}=0.399$, $P=0.534$; parasitized × sex: $F_{1,21}=2.677$, $P=0.117$) and between previously parasitized and non-parasitized ($F_{1,21}=0.078$, $P=0.783$) *G. nanus* did not differ significantly (Table 1).

ADMR, which averaged $8.68\pm0.95\,\mathrm{kJ\,g^{-0.54}\,day^{-1}}$ for all rodents, was similar between sexes ($F_{1,21}=0.242$, $P=0.628$; parasitized × sex: $F_{1,21}=1.922$, $P=0.180$) and between previously parasitized and non-parasitized individuals ($F_{1,21}=0.186$, $P=0.671$) (Fig. 1).

DISCUSSION

Our findings that the ADMR of flea-free *G. nanus* did not differ between individuals that were parasitized 3 weeks previously with fleas in their natural habitat and those that were non-parasitized supported our hypothesis that the effect of flea infestation on energy metabolism is not maintained in the long term and that an induced response strategy is used by this host.

During infection, an increase in host energy expenditure is an indirect result of the flea infestation. Ectoparasites are small in comparison with their hosts and the energy content of the resources they consume from their hosts is a very small proportion of the hosts' energy expenditure (Khokhlova et al., 2002). Consequently, the direct effects on energy balance of the host are negligible (Tschirren and Richner, 2006), as would be the case with fleas and their hosts. For example, when infesting *Gerbillus dasyurus* (mean body mass=29.7 g), 50 fleas of the species *Xenopsylla ramesis* drew 3.68 mg blood, which was only 0.17% of the blood volume of the host (Khokhlova et al., 2002). When infesting *G. nanus*, 50 female and male fleas of *X. ramesis* consumed 2.55 and 1.45 mg of blood, respectively, which would be only 0.15% and 0.08% of the blood volume of *G. nanus* in this study (I.S.K., unpublished data). Nonetheless, fleas, even in small numbers, can cause a significant increase in metabolic rate of the host (Khokhlova et al., 2002; Krasnov, 2008). The increase could be due to behavioural responses, such as an increase in grooming (Scantlebury et al., 2007; Hawlena et al., 2008), and to physiological responses, such as the activation and maintenance of the immune system (Sheldon and Verhulst, 1996; Zuk and Stoehr, 2002).

Our results suggest that the effect of ectoparasite infestation on host energy expenditure lasts a relatively short period. This finding allows us to differentiate between two optional strategies

proposed for the response of the immune system to parasite infestation. In the first, hosts possessing an induced immune response elevate their energy metabolism when parasitized, whereas in the second, hosts possessing a constitutive immune response maintain a constantly high level of energy expenditure against parasites, even when parasites are not present (Khokhlova et al., 2004; Hawlena et al., 2006; Tripet et al., 2008). The latter host strategy is more costly in term of energy expenditure but maintains a high level of immunological 'readiness' to defend against flea infestation.

The pattern of mounting immune responses likely depends on parasite pressure (Combes, 2001), such as the frequency and probability of parasite attacks (Tella et al., 2002). Immunological resistance is costly and thus of little advantage if encounters with the parasite are rare (Poulin et al., 1994). Consequently, if the frequency and/or probability of attacks by parasites is low, then a host can limit its allocation of energy for immune responses by the development of responses only after being attacked by a parasite. If, however, frequency and/or probability of parasitism are high, an advantageous strategy of a host would be a high investment in immune defence and, thus, continuous maintenance of a certain level of immune 'readiness' (Jokela et al., 2000). The rate of encounter of *G. nanus* with fleas is relatively low (Kam et al., 2010) and, therefore, our suggestion that *G. nanus* uses an induced response strategy fits in well with these theories.

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