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Is the feeding and reproductive performance of the flea, *Xenopsylla ramesis*, affected by the gender of its rodent host, *Meriones crassus*?

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SUMMARY

Male-biased parasitism is commonly found in higher vertebrates and is most likely to be a result of higher mobility and lower immunocompetence of male hosts than female hosts. The latter would result in higher fitness of parasites exploiting males rather than females. To test this hypothesis, we investigated foraging and reproductive performance of fleas (*Xenopsylla ramesis*) parasitizing male and female *Meriones crassus*, a gerbilline rodent. We allowed fleas to feed on groom-restricted rodents and predicted that: (1) the size of a blood meal would be greater from a male than a female host and (2) female fleas will produce more eggs when exploiting a male than a female host. There was no effect of host gender on the mass-specific amount of blood consumed by a flea across eight days of feeding. However, on the first day fleas on a male rodent consumed significantly more blood than fleas on a female rodent. Thereafter, the amount of blood consumed from a male host tended to decrease whereas that from a female host tended to increase. A higher proportion of fleas satiated earlier than 60 min when they fed on male rather than on female hosts but this proportion decreased from the first to the last feeding event. Fleas produced significantly more eggs when they fed on male rather than on female hosts for days one to five of oviposition. We concluded that gender difference in immune defence is the mechanism behind male-biased parasitism.

Key words: rodents, fleas, gender, blood meal size, egg production.

INTRODUCTION

The behaviour of an individual is determined, to a great extent, by the evolutionary motivation to maximize lifetime fecundity (Lomnicki, 1988). One of the mechanisms to maximize reproductive success is to select habitats or patches that will lead to the greatest fitness reward (Rosenzweig, 1981). This statement is true for both free-living and parasitic organisms, although the latter are rarely considered as individuals that are able to make decisions regarding their foraging and reproductive success (Sukhdeo, 1994). Nevertheless, recent studies have demonstrated that parasites appear to make choices and decisions that favour environments (=hosts) in which their reproductive benefit is maximized (Krasnov et al., 2002a; Krasnov et al., 2003), although it is still unknown how they make their cost/benefit decisions and by which means they evaluate between-host differences.

The distribution of parasites among hosts is not random. Some host individuals, populations or species are characterized by a higher level of infestation by a parasite than other individuals, populations or species (Combes, 2001; Poulin, 2007). This suggests that parasites prefer some hosts over others and that these preferences are supposedly based on fitness-related decisions. For example, gender-biased differences in infestation by parasites have been recorded in numerous vertebrate hosts and for a variety of parasite taxa (Zuk and McKean, 1996; Poulin, 1996; Schalk and Forbes, 1996; Hughes and Randolph, 2001; Tschirren et al., 2003; Morand et al., 2004; Krasnov et al., 2005; Gorrell and Schulte-Hostedde, 2008). In most higher vertebrate hosts (birds and mammals), parasitism is male-biased, i.e. male hosts are infested more often and/or by more parasites than female hosts, although female-biased parasitism has also been reported (Morales-Montor et al., 2004; Krasnov et al., 2005). This would suggest that in many cases male mammals or birds represent better patches for parasites than females.

A parasite supposedly selects a host that may allow easier encounter and/or more effective resource acquisition (Combes, 2001). The latter includes not only the direct extraction of resources but also the ability of a parasite to cope with the host's defence system. These considerations have led to two main, but not mutually exclusive, hypotheses regarding male-biased parasitism. One hypothesis suggested that the main reason for gender-biased parasitism is gender difference in mobility. Indeed, males of higher vertebrates are usually more mobile than females and, thus, the chances of males to be exposed to a larger variety and number of parasites are greater than those of females (Tinsley, 1989; Lang, 1996). A second hypothesis related male-biased parasitism to differences in immunocompetence between male and female hosts because of the immunosuppressive effect of androgens (Zuk, 1996; Zuk and McKean, 1996). The relative importance of mobility and immunocompetence of hosts in the manifestation of male-biased parasitism is still poorly understood (Morand et al., 2004).

The majority of studies of gender-biased parasitism have been observational and only a few experimental investigations have been carried out. These experiments were aimed mainly to test the immunocompetence hypothesis (for a review, see Klein, 2000) and were host-focused, for example, they considered differential

susceptibilities of males and females to various infections (Klein et al., 1997; Klein, 2000). By contrast, parasite responses to the effect of host gender have been largely neglected. Nevertheless, if host choice by a parasite is important to maximize reproductive success (Lomnicki, 1988), then the study of the parasite's performance in hosts belonging to different genders is crucial for understanding the mechanisms of infestation biases.

One of the reasons for the lack of experimental studies of parasite performance is the difficulty in measuring this performance, although the results of several experiments have been reported (for a review, see Sukhdeo and Sukhdeo, 1994). For example, Sukhdeo measured the number of larvae produced per female *Trichinella spiralis* to test whether the performance of helminths depends on the site of infection (anterior or posterior small intestine of the rat host) (Sukhdeo, 1991). However, such studies require complicated techniques, such as surgery and/or sacrifice of laboratory animals. In contrast to endoparasites, ectoparasites, such as fleas (Siphonaptera), have advantages as laboratory models. These insects are obligatory hematophages feeding mainly on small and medium sized mammals. Flea larvae are not usually parasitic and feed on organic debris and materials found in the nest of the host. In most cases, larval and pupal development is entirely off-host. After emergence from the cocoon, adult fleas locate a host to complete the life cycle. Adults remain as permanent satellites of their mammalian hosts, alternating periods on the host with periods when they occur in the burrow or nest. Thus, it is possible to manipulate flea infestation on living hosts and to monitor changes in an individual flea over time.

In this study, we asked if and how fitness-related parameters of fleas are affected by the gender of their hosts. To answer this question, we investigated foraging and reproductive performance of fleas (*Xenopsylla ramesis*) parasitizing male and female *Meriones crassus*, a gerbilline rodent. *M. crassus* is one of the most common rodent species of southern Israel. It occupies a variety of habitats and is parasitized by several flea species, of which *X. ramesis* is one of the most abundant (Krasnov et al., 1997). In the field, male *M. crassus* have been found to harbor more abundant and more species-rich flea assemblages than females, although this difference was pronounced only in winter (Krasnov et al., 2005). We predicted that fleas will perform better on male than on female hosts in that: (1) the size of a blood meal will be greater when taken from a male rather than a female host and (2) female fleas will produce more eggs when exploiting a male rather than a female host.

MATERIALS AND METHODS

Rodents

We used sexually-naïve, adult male and female *Meriones crassus* Sundevall from our laboratory colony. Progenitors of the colony were captured at the Ramon erosion cirque, Negev Highlands, Israel (30 deg.35′N, 34 deg.45′E) in 1997. The rodents were maintained in plastic cages (60×50×40 cm) (L×W×H) at 25°C with a photoperiod of 12 h:12 h (L:D) and provided with sawdust and dried grass as bedding material. They were offered millet seed and alfalfa (*Medicago* sp.) leaves *ad libitum*. No water was available as the alfalfa supplied enough for their needs. These rodents were never exposed to flea parasitism. Two weeks prior to experiments, each animal was exposed three times to 30 (10 males and 20 females) fleas (*Xenopsylla ramesis* Rothschild) once every two days, for 2 h each time. This was done to allow rodents to develop similar degrees of acquired resistance against fleas (see Khokhlova et al., 2004). The strongest host response to flea bites was reported to occur 7–14 days after flea feeding began (Hudson at al., 1960), although this period can probably be characterized by rapid changes and, therefore, relatively low stability of immune system function. Nevertheless, even a single infestation by ectoparasites was reported to induce acquired resistance (Trager, 1939). In total, we used 32 male and 30 female rodents.

Fleas

Fleas were obtained from our laboratory colony started in 1999 from field-collected specimens on *M. crassus*, using the rearing procedures described elsewhere (Krasnov et al., 2001; Krasnov et al., 2002b), and maintained at 25°C and 75% relative humidity (RH) with a photoperiod of 12 h:12 h (L:D). An individual rodent host was placed in a plastic cage that contained a steel nest box with a wire mesh floor and a pan containing a mixture of sand and dried bovine blood as larvae nutrient medium. Every two weeks, we collected all substrate and bedding material from the nest box and transferred it to an incubator (see below), where the fleas developed at 25°C air temperature and 75% RH. Air temperature was regulated in refrigerated incubators (FOC225E, Velp Scientifica srl, Milano, Italy), humidity was regulated using saturated salt solutions (Winston and Bates, 1960) and both air temperature and humidity were monitored (Fisherbrand Traceable Humidity/Temperature Pen with Memory, Fisher Scientific International, Somerville, NJ, USA). In total, 1240 female and 620 male fleas were used.

Experimental procedures

Fleas used in our experiments were 24–48 h old and did not feed from emergence until experimental treatments. During the period between emergence and experiments, these fleas were maintained in an incubator at 25°C and 75% RH. Each individual rodent was placed in a wire mesh (5×5 mm) tube (15 cm length and 5 cm diameter) that limited movement and did not allow self-grooming. Tubes with rodents were placed in individual white plastic baths. Twenty female and 10 male *X. ramesis* were placed on each rodent. We used more female than male fleas because the time between consecutive matings is much shorter in male than female fleas, so that a single male can copulate with several females during a short period (Marshall, 1981). After feeding on a host for 60 min, fleas were collected by brushing the fur of the rodent with a toothbrush until all of the fleas were recovered. This procedure takes no more than 10 min. To test for the possible confounding effect of shedding excess water from the blood meal on body mass of fleas after feeding, we carried out preliminary measurements in which we weighed fleas (10 males or 10 females by groups in three replicates) immediately after and every 10 min for 60 min post-feeding on an adult male rodent. We analyzed the effect of post-feeding period on body mass change of fleas before and after feeding using repeated-measures analyses of variance (ANOVAs) with body mass change per unit body mass of a starving flea as a dependent variable. Body mass change of fleas during 60 min post-feeding did not show significant differences ($F_{6,12}$=1.27 and $F_{6,12}$=0.83 for male and female fleas, respectively; P>0.34 for both). Fleas from each host were placed in plastic cups (200 ml), of which the bottom of each cup was covered by a thin layer of sand and small pieces of filter paper, then transferred to an incubator and maintained at 25°C air temperature and 92–95% RH. The feeding procedure was repeated for each group of fleas on the same host individual every day for eight consecutive days. On the first, fifth and eighth days of feeding, fleas (males and females separately) were weighed (±0.01 mg, 290 SCS Precisa Balance, Precisa Instruments AG, Dietikon, Switzerland) before and immediately after feeding and the difference in mass was taken as blood consumption. These time intervals were

chosen because our preliminary results demonstrated that the rate of blood consumption by *X. ramesis* decreases substantially after the event and recovers after two to three days (I.S.K. and B.R.K., unpublished data).

Some fleas departed from their hosts before 60 min. We assessed the level of midgut engorgement of fleas under a light microscope (without dissection) and found that in all of them (including those that departed) more than 80% of the midgut was filled with blood. All departed fleas were likely to be those that satiated their appetite earlier than other fleas but consumed approximately the same amount of blood as fleas that did not depart from a host until the end of the experimental bout.

In total, 32 groups of fleas were fed on male rodents and 30 groups on female rodents. From these groups, we randomly selected 10 groups from male hosts and 10 groups from female hosts and measured their egg production. Every day, pieces of filter paper from each plastic cup with fleas were examined under a light microscope, and the day of oviposition from the first feeding event and the number of eggs were recorded.

The experimental design was found to be suitable and to meet requirements of the 1994 Law for the Prevention of Cruelty to Animals (Experiments on Animals) of the State of Israel (Ben-Gurion University Committee for the Ethical Care and Use of Animals in Experiments, License IL-36-9-2007).

Data analysis

Feeding success was evaluated as: (1) the absolute amount of blood consumed per flea, (2) the mass-specific amount of blood consumed by a flea and (3) the proportion of fleas that satiated their appetite in less than 60 min of feeding (see above). We calculated the amount of blood consumed by a flea (=mean blood meal size) per unit body mass as the difference between the total mass of fleas after feeding and the total mass of fleas prior to feeding; this value was divided by the total mass of fleas prior to feeding.

Initially, we calculated the absolute and mass-specific amount of blood consumed and the proportion of early satiated fleas separately for male and female fleas, and tested for flea gender differences in these variables using *t*-tests with flea gender as a grouping factor separately for male and female hosts and for each of the three days of feeding. The absolute amount of blood consumed by a female flea was greater than that of a male flea (on average, 0.066±0.003 and 0.031±0.002 mg, respectively) due to the larger body size of females. However, when between-gender differences in body size were taken into account, males and females consumed similar relative amounts of blood (0.280±0.016 and 0.265±0.020 mg mg^{-1} body mass, respectively, *t*-tests, t=0.31–1.22, P>0.10 for all). The proportion of early satiated individuals was also similar between male and female fleas, all else (host gender and day of feeding) being equal (on average, 0.131±0.009 and 0.129±0.002, respectively; *t*-tests, t=0.55–1.32, P>0.22 for all). Consequently, data on feeding performance of female and male fleas were pooled together.

To evaluate reproductive performance, we calculated the mean number of eggs produced per female flea per day for each group of fleas for five days after the first oviposition event. This event occurred on the fourth day of feeding in all groups of fleas. Reproductive rate was evaluated as: (1) the number of days from the first feeding event to the day with maximal egg output and (2) the number of eggs produced per day during five days of oviposition by a female.

All measurements, except for proportions, were log-transformed prior to analysis. Proportions were arcsin-transformed, which produced distributions that did not deviate significantly from normality (Kolmogorov–Smirnov tests; P>0.20 for all). The only exception was the number of days from the first feeding event to the day with maximal egg output. Frequency distribution of this variable deviated significantly from normality even after log-transformation (Kolmogorov–Smirnov test; P<0.05). Consequently, non-parametric statistics were applied. Time from the first feeding event to the day with maximal egg output was analyzed using a Mann–Whitney *U*-test with host gender as grouping variable.

Because the same group of fleas was fed repeatedly on the same individual rodent, we analyzed the mass-specific amount of blood consumed, the proportion of early satiated fleas and the number of eggs produced per day during five days of oviposition by a female (dependent variables) using repeated-measures ANOVAs with host gender as a between-group factor (categorical predictor) and day of feeding as within-subjects (repeated-measures) factor. Tukey's honest significant difference (HSD) tests were applied for all multiple comparisons. Untransformed data are presented in figures.

RESULTS

Feeding performance

The results of the repeated-measures ANOVAs of the mass-specific amount of blood consumed by a flea and the proportion of early satiated fleas are presented in Table 1. There was no effect of host gender on the mass-specific amount of blood consumed by a flea across the entire period of feeding but the interaction between factors of host gender and day of feeding was significant. This suggested that fleas took similar amounts of blood from male and female hosts on some days but these amounts differed due to host gender on other days. Indeed, when days of feeding were considered separately, fleas consumed significantly more blood from a male rodent than from a female rodent during the first feeding event (Fig. 1) (Tukey's HSD test, P<0.05). During the following days, the amount of blood taken from a male host tended to decrease whereas that taken from a female host tended to increase, so that the difference in the blood

Table 1. Summary of repeated-measures analyses of variance (ANOVAs) of the effect of host gender (HG) and day of feeding (DF) on the mass-specific amount of blood consumed by a flea (blood meal size) and the proportion of early satiated fleas

Dependent variable	Effect	Sum of squares (s.s.)	d.f.	*F*	*P*
Blood meal size	HG	0.02	1	0.1	0.70
	Error	7.01	60		
	DF	0.26	2	0.9	0.41
	HG × DF	1.79	2	6.0	0.003
	Error	0.20	120		
Proportion of early satiated fleas	HG	0.62	1	41.8	<0.0001
	Error	0.89	60		
	DF	0.12	2	6.7	0.001
	HG × DF	0.06	2	3.7	0.02
	Error	0.97	120		

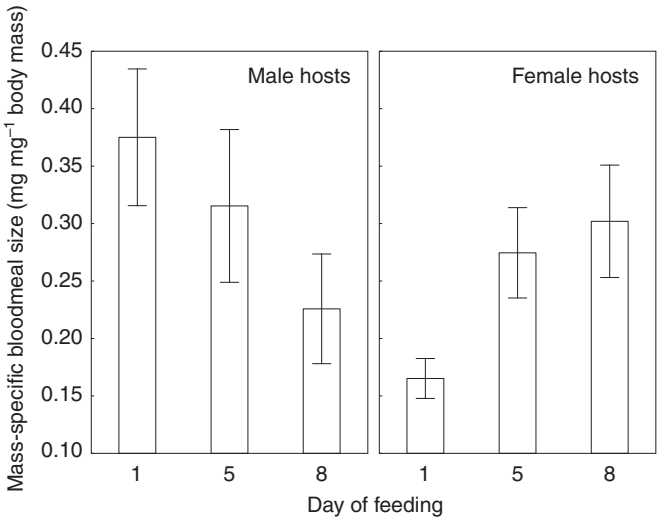

Fig. 1. Amount of blood (mg mg^{-1} body mass of a fasting flea ± s.e.m.) consumed by a flea *Xenopsylla ramesis* from male and female *Meriones crassus* during 60 min on the first, fifth and eighth days of feeding.

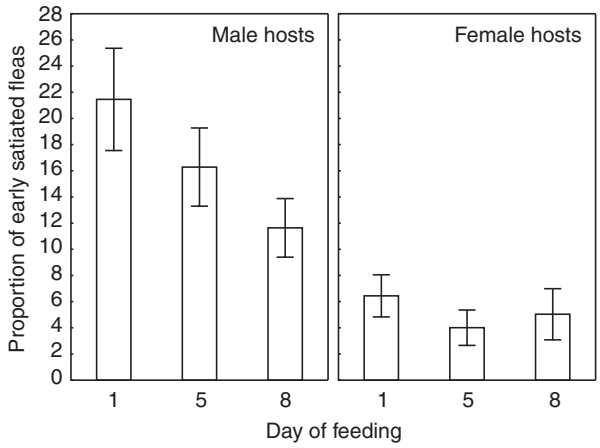

Fig. 2. Proportion (±s.e.m.) of fleas *Xenopsylla ramesis* that satiated their appetite and left male and female *Meriones crassus* in less than 60 min on the first, fifth and eighth days of feeding.

meal size between male and female hosts disappeared by the fifth day of feeding (Fig. 1) (Tukey's HSD tests, $P<0.40$ for all comparisons).

The proportion of early satiated fleas was affected by both host gender, day of feeding and the interaction between these two factors (Table 1). The first effect was manifested in the higher proportion of fleas satiated earlier when they fed on male rather than on female hosts whereas the manifestation of the second effect was that this proportion decreased from the first to the last feeding event (Fig. 2). The latter was true for male hosts (21.4±4.9% *versus* 11.6±2.2% on the first day and eighth day of feeding, respectively; Tukey's HSD test, $P<0.001$) but not for female hosts (6.4±1.6% *versus* 5.0±1.9% on the first day and eighth day of feeding, respectively; Tukey's HSD test, $P>0.90$), which was the reason behind the significance of the interaction factor.

Reproductive performance

Time from the first feeding event until the peak of oviposition per female per day within a group of fleas did not differ between groups of fleas fed on male and female hosts (median time was seven days in both groups; Mann–Whitney *U*-test=45.0, $P=0.74$).

Egg production of female fleas differed significantly between females feeding on male and female hosts and the interaction between factors of host gender and day of oviposition was significant (Table 2). In general, fleas fed on male hosts produced significantly more eggs than those fed on female hosts except for the first and second days of oviposition, i.e. the fourth and fifth days of feeding (Tukey's HSD tests; $P<0.05$ *versus* $P=0.35$–0.99, respectively) (Fig. 3). Egg production within gender was significantly lower on the first day (in fleas fed on female hosts) or the first and second (in fleas fed on male hosts) days of oviposition than later (Tukey's HSD tests; $P<0.05$ for all comparisons) (Fig. 3).

DISCUSSION

In general, the results of our present study supported our predictions in that feeding and reproductive performance of fleas were better on male than on female hosts. Fleas took relatively more blood, satiated their appetite earlier and produced more eggs when they fed on a male than on a female host, although the difference in feeding performance was manifested mainly during the first feeding event. Because our experiments were carried out in the laboratory and because rodents were not permitted to self-groom, these results suggest that gender difference in the immune defence is the proximate mechanism behind male-biased parasitism.

The immune system is the main tool of defence against parasitism. This system is aimed to discriminate between 'self' and 'non-self' and to minimize the consequences of contact with foreign molecules introduced into the host by feeding parasites. Immune defence mechanisms of vertebrates include two components: innate and acquired (adaptive) immunities (Janeway et al., 1999). It is acquired immunity that is believed to play a major role in the host developing resistance to parasites (Wakelin, 1996). In the case of ectoparasites, this is manifested by a decrease in feeding and in the reproduction of ectoparasites exploiting hosts that have been previously repeatedly attacked by this or closely related parasites (Willadsen, 1980; Fielden et al., 1992; Rechav, 1992) [but see Johnston and Brown and Vaughan et al. (Johnston and Brown, 1985; Vaughan et al., 1989)].

Immunocompetence is the general capacity of an organism to mount an immune response against pathogens and parasites (Schmid-Hempel, 2003). Gender differences in immunocompetence have been reported for a variety of homeotherms, with males being generally less immunocompetent than females (Olsen and Kovacs, 1996; Poulin, 1996) supposedly due to higher levels of androgens that suppress the immune system (Folstad and Karter, 1992). However, the relationship between testosterone and immune function is equivocal (Castro et al., 2001; Rolff, 2002; Schmid-Hempel, 2003; Vainikka et al., 2004). For example, Rolff proposed an alternative hypothesis explaining sexual differences in immunocompetence due to a higher investment of females into immune defence (Rolff, 2002). Nevertheless, testosterone injections reduced the resistance of rodents *Myodes glareolus* and *Apodemus sylvaticus* to parasitism of the tick *Ixodes ricinus* (Hughes and Randolph, 2001). Grieves et al. (Grieves et al., 2006) found that testosterone levels in birds *Junco hyemalis* were significantly negatively correlated with immune-related variables, suggesting that elevated testosterone levels may compromise immune function.

Our earlier results suggest that male *M. crassus* are less immunocompetent than conspecific females. Khokhlova et al. found that females of this rodent had higher levels of circulating immune

Table 2. Summary of the repeated-measures analyses of variance (ANOVAs) of the effect of host gender and day of oviposition on the mean number of eggs produced per female flea

Effect	Sum of squares (s.s.)	d.f.	*F*	*P*
Host gender	0.14	1	7.6	0.01
Error	0.33	18		
Day of oviposition	0.14	4	11.7	<0.001
Day of oviposition × host gender	0.03	4	2.9	0.03
Error	0.21	72		

complexes than males (Khokhlova et al., 2004). This indicates a higher synthesis of antibodies and clearance of the antigen through complexation in females. Göuy de Bellocq et al. (Göuy de Bellocq et al., 2006) used the phytohemagglutinin injection assay (PHA test) (Smits et al., 1999) to measure immunocompetence in *M. crassus* by subcutaneous injection of vegetal lectin, a phytohemagglutinin that induces local T-cell stimulation and proliferation that causes swelling. The PHA response was higher in non-parasitized female than in non-parasitized male *M. crassus* but this difference disappeared after the rodents were exposed to parasitism by *X. ramesis*. However, no correlation between the PHA response and egg production and blood consumption of *X. ramesis* was found in this study. The reason for this could be that the PHA test in this previous study was applied after flea infestation trials and the PHA response appeared to be sensitive to flea infestation. Therefore, the strength of the PHA response did not reflect the overall immunocompetence of individuals (Göuy de Bellocq et al., 2006). Consequently, the relationship between performance of fleas and immunocompetence assessed by the PHA response requires further investigation. Future experiments should involve measuring flea performance after applying the PHA test, which would permit the avoidance of the immuno-suppressing effect of flea infestation. However, Göuy de Bellocq et al. (Göuy de Bellocq et al., 2006) found a correlation between changes in rodent leucocyte concentration after 15 days of flea parasitism and flea fitness (egg production and hatching success) and feeding (blood meal size) variables, implying that the host's immune response affected the reproductive physiology of the fleas.

Apart from the present study, other studies have also suggested that gender differences in immunocompetence can cause gender difference in parasite performance. For example, Haas studied survival and feeding of a flea *Xenopsylla vexabilis* parasitizing its rodent host, *Rattus exulans*, and found that the fleas had higher survival and more blood consumption on adult male hosts followed by adult females and juvenile males (Haas, 1965).

The results of our present study strongly advocate that the immediate reason behind male-biased flea parasitism is gender difference in the immune response; however, the effect of differential mobility between males and females on their difference in flea infestation in the field cannot be discounted. Indeed, male *M. crassus* have larger home ranges than females (Daly and Daly, 1975). Consequently, the two mechanisms are not mutually exclusive and both supposedly play a role in gender differences of parasitism pattern. However, during the hot desert summer, when *M. crassus* do not reproduce (Krasnov et al., 1996) and thus testosterone levels in males are supposedly low, males and females were equally parasitized by fleas (Krasnov et al., 2005). This suggests that gender difference in immunocompetence rather than gender difference in mobility may be a more important mechanism for male-biased parasitism, especially given that male and female *M. crassus* do not demonstrate seasonal changes in their home range size (G.I. Shenbrot and B.R.K., unpublished data).

Temporal dynamics of flea responses and host-gender-related differences require some explanation. We found that fleas consumed more blood from a male than from a female host during the first feeding event only. The first feeding event is critically important for fleas as the majority of fleas are able to mate only after feeding. Newly emerged female fleas have underdeveloped ovaries blocked with a follicular plug (Vashchenok, 1966) whereas newly emerged males of many species have a testicular plug that prevents the passage of sperm from the testes to the vas deferens (Dean and Meola, 1997). The first blood meal is a trigger for the development of ovaries in female fleas (Liao and Lin, 1993) and for the dissolution of the testicular plug in males (Kamala Bai and Prasad, 1979). However, after the first blood meal, the relative amount of blood taken from male hosts decreased whereas that taken from female hosts increased. The pattern for male hosts was also supported by the decrease in the proportion of early satiated fleas during the fifth and eighth feeding events as compared with the first feeding event. A possible explanation is that male rodents continued to develop resistance against fleas whereas fleas managed to downregulate the response of female rodents. However, this explanation is highly speculative and requires further investigation.

We also found no host-gender-related differences in the number of eggs produced in the beginning of oviposition. The reason for this may be that first clutches of young fleas are usually small (Vashchenok, 1988; Vashchenok, 1993; Vashchenok, 2001). The rate of egg production then increases, which is followed by a decrease. Vashchenok studied egg production in fleas (*Leptopsylla segnis*) that were allowed continuous access to a host (laboratory mouse) for 40 days (Vashchenok, 2001). Peak egg production occurred when a flea was 6–10 days old. Unimodality of age-related changes in egg production have also been reported for other flea

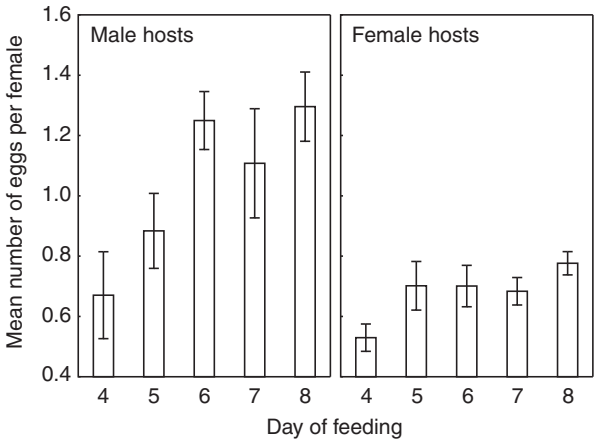

Fig. 3. Mean (±s.e.m.) number of eggs produced per day during five days of oviposition by a female *Xenopsylla ramesis* when feeding on either male or female *Meriones crassus*.

species, such as *Xenopsylla skrjabini*, *Xenopsylla nuttalli*, *Xenopsylla gerbilli* and *Xenopsylla conformis* (for a review, see Krasnov, 2008). Larger blood meals are usually associated with higher egg output in blood-feeding arthropods (Lehane, 2005). However, the generally low rate of first egg laying in fleas coupled with the large amount of blood taken during the first feeding event on male hosts is the likely reason behind an apparent contradiction between the trend of oviposition rate to increase over time (Fig. 3, male hosts) and the trend of blood meal size to decrease over time (Fig. 1, male hosts).

Experimental procedure in our present study included placing the rodents in mesh tubes to minimize the effect of the host's behavioural defence (anti-parasitic grooming). This might cause stress that, in turn, might affect flea feeding. For example, some fleas fed better on unrestrained than on restrained hosts (Bar-Zeev and Sternberg, 1962; Liu et al., 1993). If the levels of stress were gender-specific, this might influence our results *via* gender difference in the level of stress hormones, such as blood cortisol levels. However, we believe that repeated exposures of rodents to fleas prior to experimental treatments (see Materials and methods) decreased the probability of this hypothetical effect because rodents subjected to the restrained conditions every two days were likely to be accustomed to these conditions.

In conclusion, better feeding and reproductive performance of fleas on a male seems to be the mechanism behind male-biased parasitism. However, our present study was restricted to one host species and one flea species. A question that remains is how general is the pattern and to what extent they apply to other parasite–host associations. Studies on other ectoparasite (including other fleas) and host species (including other mammals) would allow us to better understand parasite preference of host gender and whether our finding is a general phenomenon.

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