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Host gender and offspring quality in a flea parasitic on a rodent

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SUMMARY

The quality of offspring produced by parent fleas (*Xenopsylla ramesis*) fed on either male or female rodent hosts (*Meriones crassus*) was studied. The emergence success, duration of development, resistance to starvation upon emergence and body size of the flea offspring were measured. It was predicted that offspring of fleas produced by parents that fed on male hosts (i) will survive better as pre-imago, (ii) will develop faster, (iii) will live longer under starvation after emergence and (iv) will be larger than offspring of fleas fed on female hosts. The emergence success of pre-imaginal fleas was relatively high, ranging from 46.9% to 100.0% and averaging 78.4±3.0%, and was not affected by host gender. The duration of development of pre-imaginal fleas depended on the gender of the host of parents and differed between male and female offspring, with female fleas developing faster. Furthermore, male fleas developed faster if their parents fed on female rather than on male hosts, whereas no difference in the duration of development between host genders was found in female fleas. The time to death under starvation did not depend on the gender of either the flea or the host. A newly emerged flea, on average, lived 31.9±1.0 days without access to food. The relationship between host gender and body size of male flea offspring was the only effect that supported the predictions. An increase in body size in male fleas could increase their mating success and, ultimately, their fitness.

Key words: body size, development, ectoparasitism, fleas, gender, host quality.

INTRODUCTION

The host spectrum of a parasite is thought to be a result of two independent forces described by Combes (Combes, 2001) as the host-encounter and the host-compatibility filters. The former excludes all hosts that a parasite cannot encounter because of ecological or geographic reasons, whereas the latter excludes all hosts in which a parasite cannot survive and develop for morphological, physiological or immunological reasons. Although this concept has initially been proposed to explain among-parasite variation in the number of hosts exploited, it might also be adapted to other aspects and scales of host–parasite relationships. For example, variation among host individuals in their level of infection is well known, suggesting that some individuals might represent better patches for parasites than other individuals (for a review, see Poulin, 2007).

The theory of habitat selection (Rosenzweig, 1981) has been found to be equally applicable to both free-living and parasitic species (Kelly et al., 1996; Krasnov et al., 2003a) (but see Sukhdeo et al., 2002). According to this theory, a selection of a patch by a consumer is based on the among-patch differences in either amount of resources or pattern of resource acquisition or both because exploitation of a patch with a higher amount or more efficient acquisition of resources would provide a higher fitness reward (Rosenzweig, 1981; Morris, 2003). Given that variation among conspecific hosts in the amount of the resources they can provide for a parasite is obviously small, the main among-host differences that affect host selection by a parasite should be frequency of encounter and/or the degree of anti-parasitic defences.

Males of higher vertebrates are usually more mobile than females (e.g. Tew and Macdonald, 1994) and their immunological anti-parasitic defences are usually weaker than those of females owing to immunosuppressive effects of androgens (Folstad and Karter, 1992; Olsen and Kovacs, 1996; Cox and Henry, 2007) (but see Klein et al., 1997). Consequently, male hosts are characterized by a higher probability to encounter a parasite and higher compatibility to a parasite than females. As a result, higher infestation of male than female hosts in terms of abundance, prevalence and species richness of parasites has been reported for a great variety of parasite and host taxa (Zuk and McKean, 1996; Poulin, 1996; Schalk and Forbes, 1997; Hughes and Randolph, 2001; Tschirren et al., 2003; Ferrari et al., 2004; Morand et al., 2004; Krasnov et al., 2005a; Hoby et al., 2006; Gorrell and Schulte-Hostedde, 2008; Matthee et al., 2010), although higher levels of parasite infection have been reported for females in some birds and mammals (McCurdy et al., 1998; Morales-Montor et al., 2004; Krasnov et al., 2005a).

Hypotheses explaining the mechanisms of male-biased parasitism have been tested frequently. The majority of experimental investigations were host-focused (Klein et al., 1997; Klein, 2000; Oppliger et al., 2004), but the parasite responses to the host gender have usually been ignored [the exception is Tschirren et al. (Tschirren et al., 2007)]. However, the effect of host gender on the parasite performance is crucial for understanding the mechanisms of male-biased parasitism.

Recently, we studied feeding and reproductive performance of the flea *Xenopsylla ramesis* Rothschild when exploiting males and females of its rodent host *Meriones crassus* Sundevall (Khokhlova et al., 2009a; Khokhlova et al., 2009b). We found that, in general, fleas fed faster, took relatively more blood and digested it faster when they fed on a male rather than on a female host, although the

host gender-related pattern of blood digestion depended on external conditions (relative humidity). Moreover, fleas exploiting male hosts produced more eggs than fleas exploiting female hosts. These results suggested that the gender difference in immune defence is an important (albeit not the only) mechanism behind male-biased parasitism. However, from an evolutionary perspective, it is the net result of the reproductive effort that matters. The most important outcome in reproduction of a flea is how many second-generation imagos emerge and how many of them produce offspring of the third generation, whereas the egg-productive ability of a parent female is of secondary importance. In other words, host gender might affect not only the quantity but also the quality of the offspring of parasites. This effect has never been tested.

Here, we studied the quality of the offspring of fleas (*X. ramesis*) produced by parents parasitizing either male or female rodents (*M. crassus*). We used emergence success, duration of development, resistance to starvation upon emergence and body size as proxies for the quality of flea offspring. We assumed that a higher emergence success indicates a higher quality of offspring because it mirrors the mortality of pre-imaginal fleas. In addition, the quality of the offspring might be associated with their ability to compete with offspring of other females for larval food (Krasnov et al., 2005b). Furthermore, older flea larvae often cannibalize younger larvae (Lawrence and Foil, 2002), and earlier-emerging fleas probably have a higher probability to find a host. This suggests that a shorter duration of pre-imaginal development of a flea might be an indicator of its higher quality, all else being equal. The resistance to starvation in newly emerged imagos is another proxy of their quality because, when a flea emerges from a cocoon, it possesses energy storage in fat tissue. This energy allows the newly emerged flea to survive until it has an opportunity to attack a host. Thus, the ability of a newly emerged imago to survive unpredictable and sometimes lengthy periods without a blood meal is extremely important. Finally, body size might be considered as an additional indicator of the quality of a flea because larger body size is intraspecifically associated with higher fecundity in insects (Honek, 1993), although this has never been studied in fleas. We predicted that the offspring of fleas produced by parents parasitizing male hosts (i) will survive better as pre-imago, (ii) will develop faster, (iii) will live longer under starvation after emergence and (iv) will be larger than offspring of fleas parasitizing female hosts.

MATERIALS AND METHODS

Rodents and fleas

We used rodents (*Meriones crassus*) and fleas (*Xenopsylla ramesis*) from our laboratory colonies established in 1997 and 1999, respectively. Details on the maintenance and breeding of rodents and fleas have been reported earlier (e.g. Krasnov et al., 2001a; Krasnov et al., 2001b; Krasnov et al., 2002a; Krasnov et al., 2002b; Krasnov et al., 2004; Krasnov et al., 2007; Krasnov et al., 2008a; Khokhlova et al., 2009a; Khokhlova et al., 2009b). In brief, rodents were maintained in plastic cages [60×50×(40/20) cm] and offered millet seed and alfalfa (*Medicago* sp.) leaves *ad libitum*. To obtain fleas, an individual rodent host was placed in a cage that contained a steel nest box with a screen floor and a pan containing a mixture of sand and dried bovine blood. This rodent was infested with 10–15 newly emerged fleas. Every two weeks, we collected all substrate and bedding material from the cage and transferred them into an incubator (FOC225E, Velp Scientifica, Milano, Italy), where flea development and emergence took place at a temperature of 25°C and relative humidity (RH) of 75%.

Experimental procedures

The procedures used for flea feeding and collection of flea eggs have been described in detail by Khokhlova and colleagues (Khokhlova et al., 2009a). A rodent was placed in a wire-mesh tube (5 mm × 5 mm mesh, 15 cm length and 5 cm diameter) that did not allow self-grooming. Thirty newly emerged starving *X. ramesis* (20 females and 10 males) were placed on each rodent and were allowed to feed for 60 min. This procedure was repeated for each group of fleas on the same host individual every day for eight consecutive days. Between feedings, each group of fleas was maintained in plastic cups (200 cm^2) with a bottom covered by a thin layer of sand and small pieces of filter paper at an air temperature of 25°C and RH of 92–95%.

Eggs were obtained from 10 flea groups fed on male hosts and 10 flea groups fed on female hosts. Every day (starting from the third day of feeding and during five consecutive days), pieces of filter paper from each plastic cup with fleas were examined under a light microscope. These eggs were placed individually in 20 ml glass vials that contained a 3 mm layer of sand and larval food medium (94% dry bovine blood, 5% millet flour and 1% ground excrements of *M. crassus*) and were covered by perforated lids. Vials were then maintained at an air temperature of 25°C and RH of 92%. Temperature was regulated in refrigerated incubators (FOC225E, Velp Scientifica, Milano, Italy) and humidity was regulated in 38 cm × 23 cm × 13 cm acrylic humidity chambers using saturated salt solutions. Temperature and humidity were monitored using a Fisherbrand Traceable Humidity/Temperature Pen with Memory (Fisher Scientific International, NJ, USA).

The daily amount of food (larvae medium) required for successful development of flea larvae was determined earlier as 0.07±0.1 mg per individual larva (Krasnov et al., 2005b), while the longest duration of the larval stage in *X. ramesis* at an air temperature of 25°C and RH of 92% was 12 days (Krasnov et al., 2001b). To ensure an excess of food for each larva, the amount of larval medium added to each vial was calculated as the necessary daily amount times the maximum duration of larval stage and then tripled – that is, *ca*. 2.5 mg. The minimal duration of metamorphosis (i.e. from egg to adult) of *X. ramesis* at an air temperature of 25°C and RH of 92% found in our earlier studies was 25 days (Krasnov et al., 2001b). Consequently, starting from the eighteenth day after an egg was produced, we checked each vial twice a day (at 08:00 and at 20:00) until either an adult emerged or for 60 consecutive days. Then, vials with newly emerged adults were checked twice a day until all the adults died. After the death of each imago, we identified its sex by examination of its genitalia using light microscopy.

After the death of adults, we randomly selected 30 male and 30 female fleas produced by parents from either male or female hosts and estimated their body size. We used maximal length of the right hind femur of each dead adult as a measure of its body size. The use of a direct measure of body size (e.g. body length) of a dead adult was not possible because the body shape of a flea could be distorted after starvation and desiccation [see Fielden et al. (Fielden et al., 2002) for details of water balance in *X. ramesis*]. The distortion of the body size of a flea after loss of fat tissue and water arises because the flea thoracic and abdominal segments do not possess posterior walls; consequently, the joints of the thorax and abdomen are highly flexible (Medvedev and Krasnov, 2006). Moreover, the body length of fleas can vary with pressure applied to the specimens when preparing them between slides and cover-slides, resulting in body length being an inaccurate indicator of body size (Tripet et al., 2002). By contrast, morphometrics of the locomotory apparatus in fresh *X. ramesis* demonstrated that femur length is a reliable

indicator of body size because these traits were strongly correlated [R^2=0.51, $F_{1,61}$=64.7, P<0.01; data from Krasnov and colleagues (Krasnov et al., 2003b)]. Femur length was measured on-screen, using a digital microscope camera (Moticam 2000 with the Motic Images Plus 2.0ML program; Motic, Speed Fair, Causeway Bay, Hong Kong), to the nearest 0.01 mm at a magnification of ×40 and calibrated using an object-micrometer.

The experimental design was found to be suitable and to meet the 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

We estimated the emergence success of pre-imaginal fleas as the proportion of eggs that survived until emergence as an imago for each group of 20 female fleas fed simultaneously on a rodent. In addition, we calculated the proportion of emerged female offspring for each group of parent females. The duration of development was calculated for each egg that developed successfully to an imago as the time from oviposition of that egg to emergence. The resistance to starvation of a newly emerged flea was calculated as the time from emergence to death. Before analyses, proportional variables were arcsin transformed, whereas time and size variables were log transformed (figures present untransformed data). Transformed variables did not deviate significantly from normality (Kolmogorov–Smirnov d=0.06–0.08, P>0.20 for all).

We analyzed survival and the proportion of emerged females using ANOVAs, with host gender as an independent variable. To test for deviation of sex ratio of newly emerged fleas from the expected 1:1 ratio, we calculated the odds ratio of proportions of emerged male and female fleas for each group of 20 parent females parasitizing the same rodent individual. Then, to evaluate the general trend of sex ratio, we applied the meta-analyses of the odds ratios across groups of parent fleas. Meta-analyses that used both fixed- and random-effects models produced similar results. Here, we report results of analyses that used the fixed-effects model only. Meta-analyses were performed using the computer program Comprehensive Meta-Analysis 2.2 (Biostat, Englewood, NJ, USA).

Because we fed a group of parent fleas on the same host individual, we needed to account for within-host non-independence of flea offspring. To do this, we initially performed ANOVAs for variables of duration of development, time to death under starvation and femur length size, with the identification number of a rodent as an independent factor. No between-host individual difference was found in any of the parameters ($F_{16,90}$=1.2–1.8, P>0.1 for all) – that is, no block effect for any parameter was found. Consequently, duration of development, time to death under starvation and body size were analyzed using two-way ANCOVAs, with host gender and flea gender as independent variables and egg production effort of a parent female as a covariate. The latter was estimated as the mean number of eggs produced by a flea in a group of 20 parent females.

RESULTS

In total, we obtained 1479 flea eggs, from which 1159 imagos emerged. Emergence success of pre-imaginal fleas was relatively high, ranging between 46.9% and 100.0% and averaging 78.4±3.0%. No effect of host gender on the emergence success of preimaginal fleas was found ($F_{1,18}$=1.1, P=0.31). The sex ratio of newly emerged fleas was highly variable among host individuals, with the proportion of newly emerged females ranging between 21.4% and 71.4%. In general, the ratio deviated significantly, albeit weakly, from unity (meta-analysis; Z=2.77, P=0.006), with the proportion of females averaging 53.0% (confidence limits from 51.0 to 55.9%). The proportion of females among newly emerged fleas did not differ significantly between host genders of parent fleas ($F_{1,18}$=2.4, P=0.13).

A summary of ANCOVAs of duration of development, time of survival under starvation and femur size of male and female flea offspring from parents parasitizing male and female hosts is presented in Table 1. The duration of development of flea offspring depended on the gender of the host on which their parents fed and differed between male and female offspring, with female fleas developing faster (Tukey's honest significant difference tests, P<0.05; Fig. 1). Furthermore, interaction between the two independent factors was significant, indicating a differential effect of host gender on the duration of development between male and female offspring. Indeed, comparison of within-flea gender and between-host gender demonstrates that male fleas developed faster if their parents fed on female rather than on male hosts, whereas no difference in the duration of development between host genders was found in female fleas (Fig. 1). The time to death under starvation conditions did not depend on either flea or host gender, and the interaction between the

Table 1. Summary of ANCOVAs of the effect of host gender (HG) and flea gender (FG) on duration of development, time to death under starvation and length of the right-hind femur of fleas (*X. ramesis*) produced by parents fed on male and female rodent hosts (*M. crassus*)

Dependent variable	Effect	Sum of squares	d.f.	*F*	*P*
Duration of development	RE	0.14	1	34.9	<0.001
	HG	0.04	1	9.1	<0.01
	FG	0.49	1	351.4	<0.001
	HG × FG	0.03	1	64.0	<0.001
	Error	0.36	1149		
Time to death under starvation	RE	0.24	1	9.2	<0.01
	HG	0.01	1	0.13	0.71
	FG	0.02	1	0.8	0.38
	HG × FG	0.06	1	2.39.3	0.12
	Error	2.34	1149		
Femur length	RE	0.001	1	0.62	
	HG	0.005	1	5.1	0.03
	FG	0.12	1	116.5	<0.001
	HG × FG	0.01	1	4.3	0.04
	Error	0.09	115		

Reproductive effort (RE) of parent female was included as a covariate.

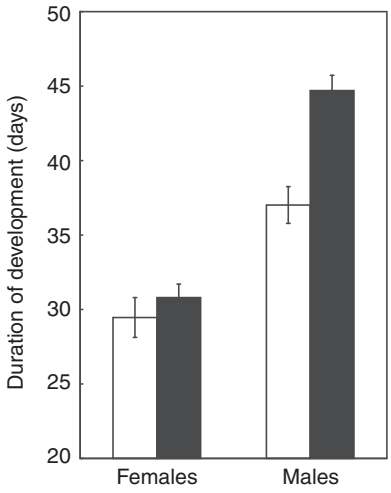

Fig. 1. Means (±s.e.m.) for the duration of development from egg to imago of male and female *X. ramesis* from parents fed on male (black columns) and female (white columns) *M. crassus*. The values are adjusted means after removal of the effect of parent reproductive effort in ANCOVA.

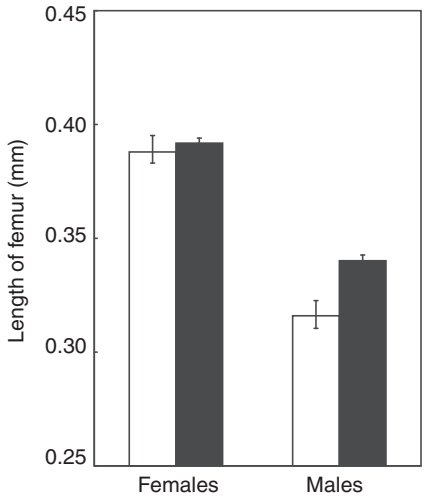

Fig. 2. Means (±s.e.m.) for the length of the hind right-femur of newly emerged male and female *X. ramesis* from parents fed on male (black columns) and female (white columns) *M. crassus*. The values are adjusted means after removal of the effect of parent reproductive effort in ANCOVA.

two factors was non-significant (Table 1). A newly emerged flea, on average, lived 31.9±1.0 days without access to food.

Female fleas from parents parasitizing male and female hosts did not differ significantly in femur length (Fig. 2), whereas the femurs of males from parents parasitizing male hosts were significantly longer than those of males from parents parasitizing female hosts (Fig. 2). In general, female fleas were significantly larger than male fleas based on femur lengths.

DISCUSSION

The relationship between host gender and body size of male flea offspring was the only effect that supported our predictions. However, this effect was not found in female flea offspring. In contradiction to our predictions, fleas from parents parasitizing male hosts developed slower than those from parents parasitizing female hosts. This again was true for male offspring only, whereas no effect of host gender on the duration of development of female offspring was found. The emergence success of fleas as well as their resistance to starvation did not depend on the gender of the hosts parasitized by their parents. In our earlier study, we found that fleas produced more eggs when they exploited male in comparison with female hosts (Khokhlova et al., 2009a). In summary, the benefit experienced by fleas from exploitation of male hosts was manifested as the production of more and larger offspring, although the latter was related to male offspring only. Below, we will discuss the possible causes and consequences of this benefit.

Two not mutually exclusive mechanisms are usually invoked to explain gender-biased parasitism. These are gender-related differences in mobility and defence efforts. The latter include behavioural and immune defences. Males are thought to be less immunocompetent than females owing to higher levels of androgens that suppress male immune function (Folstad and Karter, 1992; Hughes and Randolph, 2001). However, the recent meta-analysis by Roberts and colleagues (Roberts et al., 2004) casts doubt on this hypothesis, especially regarding mammals. Nevertheless, our results suggest that the main cause of the effect of host gender in the present study was most likely gender difference in immunocompetence. Indeed, the higher mobility of male compared with female rodents (e.g. Heske et al., 1995) could be the reason behind the higher abundance of fleas in males, but, first, it is not characteristic for *M. crassus* in our study region (Krasnov et al., 1996) and, second, it cannot explain the quality difference between fleas produced by parents exploiting either male or female hosts. The immunocompetence explanation is also supported by the fact that lower immune abilities in male compared with female *M. crassus* were found in laboratory studies (Khokhlova et al., 2004). In particular, females possessed higher levels of circulating immune complexes than males, indicating higher synthesis of antibodies (Khokhlova et al., 2004). This difference could cause fleas to digest blood from a female host more slowly than blood from a male host (Khokhlova et al., 2009b). In fleas, a faster rate of digestion was found to be associated with lower energy expenditure for digestion (Krasnov et al., 2003c; Sarfati et al., 2005). Consequently, feeding on male blood might allow fleas to allocate more energy for other activities such as egg production (Khokhlova et al., 2009a). However, parasitizing either a male or a female host did not affect the viability of the eggs, as was indicated by the similar emergence success for young fleas.

Nevertheless, male fleas from parents fed on male hosts took longer to develop than those from parents fed on female hosts. On the one hand, this might indicate lower quality of the former due to costs of delayed reproduction or increased risk of intraspecific cannibalism (Zonneveld, 1996; Lawrence and Foil, 2002). However, on the other hand, prolonged development might allow these individuals to attain a larger size when they emerge. The exact physiological mechanism of prolonged development of male pre-imagos from parents fed on male hosts is not clear and requires further investigation.

In insects, longer development is often coupled with a larger size of the emerged imago (Agosta, 2008). Furthermore, it was shown that male size in some insects correlates positively with mating success (Emlen, 1996; Agosta, 2010) (but see Taylor et al., 1998). The relationship between mating success and body size in male fleas has never been studied. Nevertheless, it is known that larger flea individuals might have better locomotory abilities than smaller

individuals (Rothschild et al., 1975). Given that copulation in fleas is sometimes associated with courtship behaviour (Iqbal and Humphries, 1974; Hsu and Wu, 2001), the larger size of a male might be advantageous for locating a female and for successful mating. Females might reject relatively small males when larger ones are in the vicinity, as is the case in some insects (Kumano et al., 2010). This scenario is feasible because fleas are aggregated among their hosts, both on their bodies and in their burrows (Krasnov et al., 2005c). Consequently, an increase in mating success with an increase in body size in fleas could be expected. Thus, from an evolutionary perspective, exploitation of a male host might benefit a flea not only through an increase of its own personal fitness (Khokhlova et al., 2009a) but also through the probable increased fitness of its male offspring. In addition, adult longevity might increase with larger body size (Taylor et al., 1998), allowing a male flea to perform a greater number of matings during its lifetime (Hsu and Wu, 2000).

From an ecological perspective, host gender-related differences in egg production (Khokhlova et al., 2009a) and quality of male offspring in *X. ramesis* might cause not only higher flea loads on male than on female hosts (Krasnov et al., 2005a) but also a higher proportion of male fleas on male hosts. For example, Bursten and colleagues (Bursten et al., 1997) found that juvenile males of the ground squirrel (*Spermophilus beecheyi*) were infested with more fleas (*Oropsylla montana*) than juvenile females and that this disproportionate infestation was due to an excess of male fleas. However, this appeared to be not the case for *X. ramesis* and *M. crassus*. Indeed, the sex ratio of fleas collected from individual rodents did not deviate from unity (Krasnov et al., 2008b).

The effect of host gender on offspring variables was manifested in male but not female offspring. This suggests that male fleas were more sensitive to the gender of the hosts of their parents than were female fleas. In our earlier studies of responses of male and female fleas to a number of factors, we found that gender-bias in the responses varied among factors. For example, female fleas were more sensitive than male fleas to environmental fluctuations during pre-imaginal development (Krasnov et al., 2001b) and to external stimuli (Burdelov et al., 2007). The response to host gender in terms of blood digestion was more pronounced in females than in males (Khokhlova et al., 2009b). By contrast, males responded more strongly than females to starvation (Krasnov et al., 2002a). These differences in responses might be associated with gender-related differences in physiology, such as the activity of salivary enzymes (Ribeiro et al., 1990), metabolic rate (Fielden et al., 2004) and water balance (Fielden et al., 2002). Physiological differences between male and female fleas, in turn, are probably associated with differences in their biological roles as well as with differences in the urgency of a blood meal. The latter is more crucial for females than for males owing to the necessity for feeding before mating (for a review, see Krasnov, 2008).

In conclusion, the results of this study, as well as our earlier reports (Khokhlova et al., 2009a; Khokhlova et al., 2009b), indicate that parasitism on male hosts might be beneficial for fleas not only in terms of their performance but also in terms of body size and, thus, the competitive ability of their male offspring. Nevertheless, the generality of these patterns should be further validated by studies on other parasite–host associations.

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