The Journal of Experimental Biology 215, 1435-1441

doi:10.1242/jeb.061796

RESEARCH ARTICLE

Effects of host diet and thermal state on feeding performance of the flea *Xenopsylla ramesis*

Carmi Korine[1,*], Boris R. Krasnov[1], Irina S. Khokhlova[2] and Berry Pinshow[1]

[1]Mitrani Department of Desert Ecology and [2]Wyler Department of Dryland Agriculture, Jacob Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev, 84990 Midreshet Ben-Gurion, Israel

*Author for correspondence (ckorine@bgu.ac.il)

Accepted 26 December 2011

SUMMARY

We examined feeding performance of the flea *Xenopsylla ramesis* on three different hosts: its natural, granivorous, rodent host, Sundevall's jird (*Meriones crassus*); the frugivorous Egyptian fruit bat (*Rousettus aegyptiacus*); and an insectivorous bat, Kuhl's pipistrelle (*Pipistrellus kuhlii*). Because these fleas are not known to occur on bats, we hypothesized that the fleas' feeding performance (i.e. feeding and digestion rates) would be higher when feeding on their natural host than on either of the bats that they do not naturally parasitize. We found that mass-specific blood-meal size of both male and female fleas was significantly lower when feeding on Kuhl's pipistrelles than on the other two species, but was not different in female fleas feeding on fruit bats or on jirds at all stages of digestion. However, more male fleas achieved higher levels of engorgement if they fed on Sundevall's jirds than if they fed on Egyptian fruit bats. The fleas digested blood of fruit bats and jirds significantly faster than blood of Kuhl's pipistrelle. In addition, after a single blood meal, the survival time of fleas fed on normothermic Kuhl's pipistrelles was significantly shorter than that of fleas fed on Sundevall's jirds and even lower when male fleas fed on Egyptian fruit bats. Thus, our prediction was partially supported: normothermic Kuhl's pipistrelles were inferior hosts for fleas compared with Sandevall's jirds and Egyptian fruit bats. Interestingly, the proportion of engorged fleas that fed on torpid Kuhl's pipistrelles was significantly higher than the proportion of the fleas that fed on normothermic individuals, indicating that becoming torpid might be a liability, rather than an effective defense against parasites.

Key words: bat, blood meal, diet, digestion, flea, herbivory, rodent.

INTRODUCTION

To maximize fitness, individuals of a species select habitats that present them with the resources of the highest available quality, largest quantity and easiest acquisition (Morris, 2003). The habitat used by a parasite, namely its host, provides it with food and a place to live. From this point of view, habitats of parasites do not conceptually differ from habitats of free-living species. However, in contrast with the habitats of free-living species, a host is able to defend itself actively against parasites using specific behavioral, physiological and/or immunological mechanisms, and its anti-parasite responses are often immediate. Thus, the selection of a host by a parasite is determined not only by the quality and quantity of potentially harvestable resources, but also by the host's defensive abilities. Obviously, fitness of a parasite is lower on a host of poorer resource quality and/or better ability to defend itself.

Host species, even closely related ones, often differ in their anti-parasite defense abilities, such as immunocompetence (Klein and Nelson, 1998; Mendes et al., 2006). There are diverse extrinsic causes of interspecific variation in anti-parasite defenses; for example, differences in the probability of attacks by parasites and in parasite diversity (Morand and Poulin, 2000; Lindström et al., 2004; Møller and Rózsa, 2005). There are also intrinsic reasons for interspecific differences in immune ability. For example, carotenoids circulating in plasma may stimulate the immune system (Lozano, 1994; Hughes, 2001; McGraw and Ardea, 2003; Fitze et al., 2007). If this is so, then frugivorous animals, with high concentrations of carotenoids in their diet and blood, might have better immunological resistance to parasites than closely related carnivorous species that eat less carotenoid-containing food. In addition, it has been shown that supplementation of dietary protein improves resistance against parasites within species (see Beisel, 1996) (for a review, see Hoste et al., 2005). If this is the case, then carnivorous species are expected to have better defense abilities than closely related herbivorous ones. Consequently, the composition of a host's diet may well influence the fitness of its parasites.

An outcome of anti-parasite defense by a host is often reduced fitness in the affected parasite. To all intents and purposes, this host-mediated loss of fitness represents host resistance (Poulin, 2007). Accordingly, from an evolutionary perspective, the reproductive success of its parasites should provide a measure of the host's resistance (Combes, 2001). However, it is not always easy to measure the fitness of a parasite, but its feeding performance can serve as a proxy for fitness, as one of the evolutionary tasks of any organism is to translate nutrients and energy extracted from food into offspring.

To examine the relationships between parasite feeding performance and host anti-parasite defense at the level of the host-provided diet, we compared the performance of the same parasite feeding on related host species that differ considerably in their diets. Bats belong to the only order of mammals that contains both purely herbivorous (mainly frugivorous) and purely carnivorous (insectivorous, piscivorous, hematophagous, etc.) species. We used

Egyptian fruit bats [*Rousettus aegyptiacus* (Geoffroy 1810)], an herbivorous species, and Kuhl's pipistrelle (*Pipistrellus kuhlii* Kuhl 1817), an obligate insectivore, as hosts to the flea *Xenopsylla ramesis* Rothschild. In addition, we compared the species' feeding performance on bats with feeding performance on one of its common natural hosts, Sundevall's jird (*Meriones crassus* Sundevall 1842).

Comparison of parasite performance on different host species cannot be done without understanding how morphological, physiological or ecological characteristics of the hosts affect the parasite's fitness. In addition, the confounding effect of common evolutionary history and possible differences in tightness of association between a parasite and its natural host species must be taken into account. Indeed, if a parasite evolved in close association with some, but not other, hosts, the among-host differences in parasite fitness could result from differences in the evolutionarily established relationship of the parasite with the different hosts, rather than from the differences among hosts *per se*. Consequently, a model parasite for such an examination should: (1) be able to survive and reproduce on all the host species being tested, but (2) be equally high or equally low tightness of association with these hosts. If bats are used as a model host taxon, then rodent fleas conform to both these criteria.

Fleas are obligate hematophagous ectoparasites, and are most abundant and diverse on small to medium-sized mammals. Flea larvae are usually not parasitic, and the pre-imaginal development of most fleas occurs off the host. Most flea species that parasitize rodents are not especially host-specific, and usually exploit several host genera of the same family, or several host families in the same order (Krasnov, 2008). Although chiropterans usually host fleas of the family Ischnopsyllidae, which parasitize only bats (Hůrka, 1963; Medvedev, 1989), rodent fleas have often been collected from bats (Lewis, 1967; Lewis, 1990; Lewis et al., 1988). Moreover, rodent fleas, such as *X. ramesis* and *Parapulex chephrenis*, are able to feed on bats and successfully digest their blood (Krasnov et al., 2007). *Xenopsylla ramesis* usually does not parasitize bats. However, Egyptian fruit bats, Kuhl's pipistrelle and the Cairo spiny mouse, *Acomys cahirinus* (which sometimes harbors *X. ramesis*) (Krasnov et al., 1999), often live in close proximity, with spiny mice occupying a rock crevices or cave floors and bats roosting above (C.K., personal observations in the northern part of Israel).

In the present study, we addressed the question of whether the feeding performance of a flea depends on the diet of its host species, and we compared feeding variables of the flea, *X. ramesis*, fed on its natural rodent host, Sundevall's jird, and two bat hosts, the Egyptian fruit bat and Kuhl's pipistrelle. Feeding performance was evaluated by measuring the proportion of fleas that succeeded in filling their midgut with a host's blood, the amount of blood taken by a flea during a single feeding event (blood meal size), the rate of blood digestion, and time of survival of a flea after a single blood meal.

Xenopsylla ramesis has been found on hosts belonging to different rodent families (Krasnov et al., 1999). Nevertheless, it parasitizes mainly members of the subfamily Gerbillinae (Lewis, 1967; Theodor and Costa, 1967) and its preferred host, in terms of abundance and reproductive success is Sundevall's jird (Krasnov et al., 1997; Krasnov et al., 2004a). The Egyptian fruit bat is the only megachiropteran among the 33 species of bat found in Israel. It is common throughout the country, feeding chiefly on fleshy fruit (Korine et al., 1999). Also, it is homeothermic (Noll, 1979). Kuhl's pipistrelle is the most common insectivorous bat in Israel (Yom-Tov and Kadmon, 1988), forages around vegetation, streetlights and over water (Korine and Pinshow, 2004), and regularly enters torpor (A. Muñoz-Garcia, M. Ben-Hamo, B.P., J. P. Williams and C.K., submitted).

We hypothesized that feeding performance of *X. ramesis* would differ on the three chosen host species and tested the prediction that feeding success of fleas is highest on its natural host to which it is adapted, Sundevall's jird. We further hypothesized that the feeding performance of the fleas depends on the thermal state of the bats and predicted that feeding performance of fleas is compromised when feeding on torpid Kuhl's pipistrelles. These experiments were not performed with the Egyptian fruit bat because it does not enter torpor, even at low ambient temperatures (T_a), such as 8°C (Noll, 1979).

MATERIALS AND METHODS

Study species

Five male Sandevall's jirds [average adult body mass (m_b) ~80 g] and five male Egyptian fruit bats (average adult m_b ~150 g) from our laboratory colonies, and four male Kuhl's pipistrelles (average adult m_b ~6 g) captured nearby (see below) were used as hosts. Progenitors of the colony of Sundevall's jird were captured in the Ramon erosion cirque, Negev Highlands, Israel (30°35′N, 34°45′E) in 1996. The jirds were maintained in plastic cages (60×50×40 cm) at 25°C under a 12 h:12 h light:dark photoperiod. Dried grass was supplied as bedding material. Initially, we placed a male and a female in each cage. When the number of individuals in the group exceeded six, young (at 2 months of age) were transferred to new cages. Each new group consisted of a male and female pair with different parents. Animals were fed millet seeds and fresh alfalfa (*Medicago* sp.) *ad libitum*. No water was made available because, from our experience, alfalfa supplies enough water for the animals' needs.

The colony of Egyptian fruit bats was kept in a large (5×4×2.5 m) flight cage at the Blaustein Institutes for Desert Research at Midreshet Ben-Gurion, Israel. The fruit bats originated from a roost in an underground parking lot in Beer Sheva (31°14′N, 34°47′E), where they were captured by mist-netting. Bats were fed an assortment of in-season fruit.

The four male Kuhl's pipistrelles were trapped with mist nets at foraging sites at Midreshet Ben-Gurion (30°52′N, 34°47′E). After the experiments, they were released at the site of capture. This research was done under permits 2883 and 2885 of the Israel Nature and National Parks Protection Authority. All animals used were adult and non-reproductive. Sundevall's jirds from the laboratory colony were never previously infested with fleas, and no fleas were found on any of the captive Egyptian fruit bats or the wild-captured Kuhl's pipistrelles before the experiment.

We used *X. ramesis* from our laboratory colonies, which were founded in 1999 from specimens collected in the field from Sundevall's jirds. Flea rearing procedures are detailed elsewhere (Krasnov et al., 2001; Krasnov et al., 2004a). In the present study we used only 2-day-old fleas that had not fed from emergence until the experimental treatments.

Experimental procedure and measurements

In the first experiment, we randomly drew 175 male and 175 female fleas from the colony and randomly assigned them to one of three host species treatments. Each treatment was replicated five times for Egyptian fruit bats and Sundevall's jirds, and four times for Kuhl's pipistrelles. Each flea and each mammal were used only once. Mammals were placed in wire mesh (5×5 mm) tubes (5×3 cm length×diameter for Kuhl's pipistrelles and 15×5 cm length×diameter for Egyptian fruit bats and Sundevall's jirds),

which limited movement and prevented self-grooming. The mammal-containing tubes were positioned in individual white plastic pans and 10 (five male and five female for Kuhl's pipistrelles) or 30 (15 male and 15 female for Egyptian fruit bats and Sundevall's jirds) fleas were released onto each animal for 1 h. Prior to release on a host, male and female fleas were weighed separately to ±0.01 mg (Precisa Balance, model 290 SCS, Precisa Instruments AG, Dietikon, Switzerland). After 1 h, we collected and counted all fleas, including those that jumped off their hosts during feeding and those that preferred to remain on board. The latter were removed by brushing the hair of the host with a toothbrush or raking it with fine tweezers until all fleas were recovered.

We examined all the fleas under a light microscope, selected those that took a blood meal and assessed the level of their midgut engorgement visually, classifying them as: (1) low/medium (engorged midgut filling less than 75% of the abdominal cavity) or (2) high (engorged midgut filling more than 75% of the abdominal cavity). We calculated the proportion of fleas with highly engorged midguts after 1 h of feeding, and reweighed these fleas as above. Blood-meal size was calculated as the amount of blood taken by a flea per mg of its m_b prior to release on its host. These fleas were then distributed into individual 20 ml glass vials, each covered with a fine nylon mesh screen, placed in incubators (see above) and maintained at 25°C and 92% relative humidity (a vapor pressure of 2.896 kPa). Vapor pressure was controlled using a saturated solution of potassium nitrate (Winston and Bates, 1960). Temperature and humidity were monitored with a tracing humidity/temperature pen with memory (Fisher Scientific International, Hampton, NH, USA).

We examined flea midguts under a light microscope every 4 h and estimated blood digestion status following a modified classification of Ioff (Ioff, 1949) developed by Krasnov et al. (Krasnov et al., 2002a), and measured the duration of each of three stages: (1) early – midgut is stretched and fully filled with light scarlet or dark red blood; (2) middle – the contour of the midgut is jagged and the content is dark brown or black; and (3) late – midgut contains only remnants of digested blood or is empty. To calculate the survival time of fleas after a single blood meal, after digestion was complete we checked the vials with fleas twice a day (at 08:00 and 20:00 h) and the death of each flea was verified by microscopic examination.

In the second experiment, with Kuhl's pipistrelles only, we tested whether the feeding performance of the fleas is affected by the thermal state of the bats, i.e. when the bats are normothermic or torpid. Bats were held either at a T_a of 25°C or at a torpor-inducing low T_a of 10°C, in a controlled-temperature cabinet (Precision 815, Precision Scientific, Chicago, IL, USA) for 24 h prior to feeding them to fleas. The thermal state of a bat was determined by measuring dorsal skin temperature (T_s) using a lacquer-coated 36 g, type-T thermocouple before placing it in the wire mesh restrainer. We considered a bat torpid when its T_s was lower than 25°C (A. Muñoz-Garcia, M. Ben-Hamo, B.P., J. P. Williams and C.K., submitted). We randomly drew 80 female fleas from the colony and randomly assigned them to two treatments. Each treatment was replicated four times for each bat. Each flea and each bat were used only once. Bats and fleas were treated as described above. After 2 h, we collected and counted all fleas and treated them as described above. We measured the proportion of fleas that succeeded in filling their midgut as well as the specific blood-meal size.

Data analysis

Dependent variables were either arcsine- (proportion of fleas with highly engorged midguts after 1 h of feeding) or log-transformed (blood meal size, time of digestion, and time of survival after a single blood meal) prior to analyses. These transformations provided normal distributions (Kolmogorov–Smirnov tests). Non-transformed data are presented in figures. The effects of host species and flea gender on the proportion of fleas with highly engorged midguts and the size of blood meals were analyzed using two-way ANOVA. To ensure that the duration of blood digestion, or time to death when deprived of food, was not affected by individual host, we initially analyzed the effect of host ID on each measured variable within flea gender and host species using one-way ANOVA. No effect of host ID on either variable was found ($F_{1,5–27}$=0.5–1.4, all P>0.1). Consequently, we re-analyzed these data using two-way ANOVA, with flea gender and host species as independent variables. Multiple comparisons of flea variables among host species were performed with Fisher's least significant difference (LSD) test (Sokal and Rohlf, 1995). In analyzing the results of the second experiment, we compared both the proportion of fleas that succeeded in filling their midgut and the blood-meal size between bats in two thermal states using a t-test. For all experiments, we rejected the null hypothesis at P<0.05.

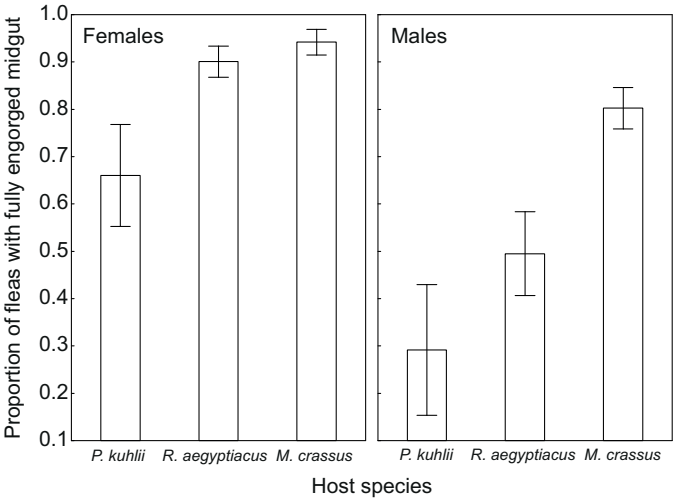

Fig. 1. Proportion (mean ± s.e.m.) of female and male fleas (*Xenopsylla ramesis*) with fully engorged midguts after 1 h of feeding on one of the host species, *Pipistrellus kuhlii* (*N*=4), *Rousettus aegyptiacus* (*N*=5) or *Meriones crassus* (*N*=5).

RESULTS

The proportion of fleas with highly engorged midguts after 1 h of feeding differed significantly between flea genders and among host species (ANOVA, $F_{1,23}$=21.3 and $F_{2,23}$=7.4, respectively, P<0.005 for both), whereas the interaction between host and flea gender was not significant (ANOVA, $F_{2,23}$=0.5, P=0.65). Among fleas that fed on bats, midgut engorgement was higher in females than in males (Fisher's LSD, P<0.05; Fig. 1), but no between-gender difference was found in fleas that fed on rodents (Fisher's LSD, P=0.07). In both male and female fleas, the lowest proportion of fleas that attained high midgut engorgement was observed if they fed on Kuhl's pipistrelle (Fisher's LSD, P=0.05; Fig. 1). Furthermore, the proportion of highly engorged female fleas did not differ between those that fed on Egyptian fruit bats and those that fed on Sundevall's jirds (Fisher's LSD, P=0.37), whereas more male fleas achieved higher levels of engorgement if they fed on Sundevall's jird than if they fed on Egyptian fruit bats (Fisher's LSD, P=0.05).

The mass-specific size of blood meals taken by male and female fleas was not different (ANOVA, $F_{1,23}$=0.3, P=0.61), but differed

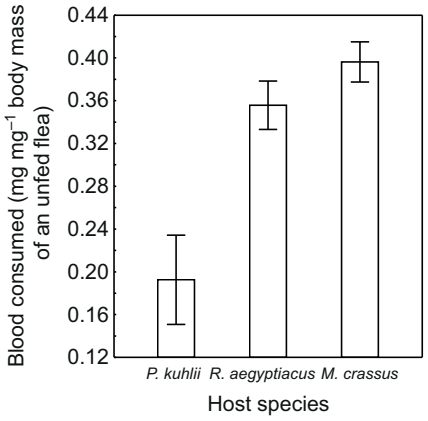

Fig. 2. Body-mass-specific amount of blood (mean ± s.e.m.) consumed by *Xenopsylla ramesis* during 1 h of feeding on one of the host species, *Pipistrellus kuhlii* (*N*=4), *Rousettus aegyptiacus* (*N*=5) or *Meriones crassus* (*N*=5).

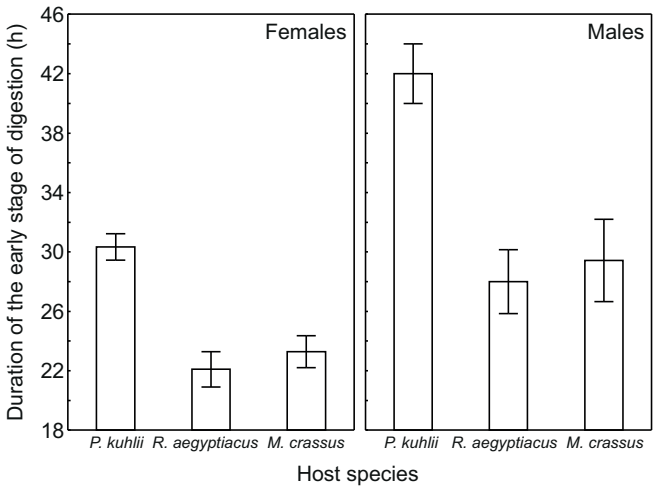

Fig. 3. Duration (mean ± s.e.m.) of the early stage of blood digestion in female and male *Xenopsylla ramesis* after 1 h of feeding on one of the host species, *Pipistrellus kuhlii* (*N*=4), *Rousettus aegyptiacus* (*N*=5) or *Meriones crassus* (*N*=5).

significantly between host species (ANOVA, $F_{2,23}$=5.6, P<0.01). The mass-specific size of blood meals was significantly lower in fleas that fed on Kuhl's pipistrelle (Fisher's LSD, P=0.005), but was not different between fleas that fed on Egyptian fruit bats and those that fed on Sundevall's jird (Fisher's LSD, P=0.71; Fig. 2).

The duration of early, but not middle and late, digestion stages differed significantly between male and female fleas, being longer in males (Fisher's LSD, P<0.05; Table 1, Fig. 3). The effect of host species was also strong (Table 1). At all stages, fleas of both genders digested blood of Egyptian fruit bats and Sundevall's jirds significantly faster than blood of Kuhl's pipistrelles (Fisher's LSD test, P=0.05; Fig. 3), and we found no difference in the time it took for fleas to digest blood of Egyptian fruit bats and Sundevall's jirds (Fisher's LSD, P>0.05).

Time of survival after a single blood meal differed significantly between flea genders; namely, females survived significantly longer than males ($F_{1,69}$=22.7, P<0.001; Fig. 4). We found a strong effect of host species on flea survival. Fleas that fed on Kuhl's pipistrelles survived for less time than fleas that fed on either of the other hosts ($F_{2,69}$=48.2, P<0.0001; Fig. 4). In addition, the interaction between flea gender and host species was significant ($F_{2,69}$=5.0, P<0.01), manifested by the difference in survival time of males that fed on Egyptian fruit bats compared with males that fed on Sundevall's jirds.

At a T_a of 10°C, mean T_s of Kuhl's pipistrelles was significantly lower than at 25°C (15.78±1.23 and 28.27±0.75°C, respectively; t=4.57, P<0.003). The proportion of fleas filling their midgut with blood after 2 h of feeding was significantly higher when they fed on torpid compared with normothermic Kuhl's pipistrelles (0.48±0.28 and 0.18±0.08, respectively; t=3.35, P<0.03). The specific blood-meal size of the fleas that did fill their midgut was not statistically different between those that fed on torpid or normothermic Kuhl's pipistrelles (0.23±0.22 and 0.12±0.08 mg, respectively; P>0.17).

DISCUSSION

Feeding success of male and female fleas

Male and female fleas may differ in some of their feeding variables because of internal and external factors. All else (e.g. feeding time) being equal, because of their larger body size, the absolute amount of blood consumed females is usually greater than that consumed by males (Devi and Prasad, 1985; Vashchenok et al., 1988; Gong et al., 2004). However, when between-sex differences in body size are accounted for, it appears that males and females consume similar mass-specific amounts of blood.

Table 1. Results of ANOVA on the effects of host species (*Pipistrellus kuhlii*, *Rousettus aegyptiacus* and *Meriones crassus*), flea (*Xenopsylla ramesis*) gender and their interaction on the duration of early, middle and late stages of blood digestion

Digestion stage	Factor	d.f.	*F*	*P*
Early	Host species	2	3.8	0.03
	Flea gender	1	6.8	0.01
	Host species × Flea gender	2	0.1	0.9
	Error	69[a]		
Middle	Host species	2	73.9	0.0001
	Flea gender	1	1.1	0.31
	Host species × Flea gender	2	1.7	0.19
	Error	68[a]		
Late	Host species	2	4.2	0.02
	Flea gender	1	0.4	0.54
	Host species × Flea gender	2	5.9	0.005
	Error	40[a]		

[a]The differences in the number of degrees of freedom between digestion stages is due to some fleas dying before they reached the middle and/or late stage of digestion.

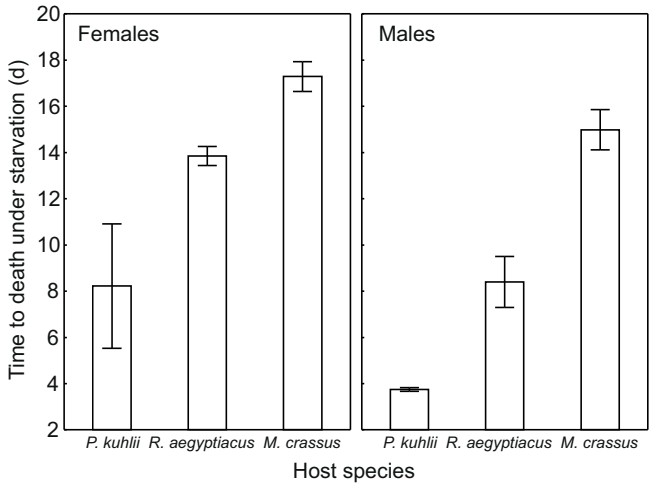

Fig. 4. Survival time (mean ± s.e.m.) of female and male *Xenopsylla ramesis* after 1 h of feeding on one of the host species, *Pipistrellus kuhlii* (*N*=4), *Rousettus aegyptiacus* (*N*=5) or *Meriones crassus* (*N*=5).

We found that female fleas engorged faster than males, although engorgement rate has been shown to be similar in males and females of other flea species (Cadiergues et al., 2001). The gender difference in engorgement may be related to the pattern of association of flea reproduction to feeding on blood that is known to trigger mating behaviour, sperm transfer, egg maturation and oviposition (Rothschild et al., 1970; Kamala Bai and Prasad, 1979; Prasad, 1987; Hsu and Wu, 2001; Dean and Meola, 1997; Dean and Meola, 2002). The necessity of a blood meal for egg maturation in female fleas is well known (Vashchenok, 1988). Male fleas of some species are able to mate after a single blood meal (Iqbal and Humphries, 1976); however, females of most species must feed repeatedly for egg maturation and oviposition to take place (Vashchenok, 1988). Consequently, the urgency of taking a blood meal is more crucial for females than for males and, as a result, females need to have a faster engorgement rate.

Slower digestion of blood in male fleas may be associated with smaller quantities and lower activity of salivary gland lysates (apyrases) in males than in females (Ribeiro et al., 1990). These lysates convert adenosine triphosphate and adenosine diphosphate to adenosine monophosphate and orthophosphate, thus destroying the signal for platelet aggregation. Another, not necessarily alternative, explanation for the faster rate of digestion in female fleas is their higher metabolic rate compared with males (Krasnov et al., 2004b), although manifestation of this gender difference depends on ambient temperature (Fielden et al., 2004).

The shorter survival time of food-deprived male fleas than of females supports our earlier results (Krasnov et al., 2002b), and the higher resistance to starvation in females can be explained, at least in part, by their having larger fat stores than males (Krasnov et al., 2002b). An additional explanation for gender differences in survival of food-deprived fleas may be the higher sensitivity of male fleas to ambient temperature and relative humidity (Krasnov et al., 2001). These two explanations are not mutually exclusive.

Feeding success and host species

In the present study we found that *X. ramesis* performed differently on different host species. Based on our evaluation criteria, the insectivorous Kuhl's pipistrelle was consistently inferior as a source of food to fleas compared with Sundevall's jirds and Egyptian fruit bats. The significant differences that we found in flea fitness between those that fed on Kuhl's pipistrelles and those that fed on Sundevall's jirds and Egyptian fruit bats, which were both superior hosts to the fleas, suggest that there are fundamental differences between these two host groups. One difference may be related to diet, which is nitrogen-rich in pipistrelles (Whitaker, 1988) and nitrogen-poor in jirds and fruit bats (Kam et al., 1997; Korine et al., 1996).

There is evidence that shortage of protein compromises immune defense (Lochmiller et al., 1993; Vestey et al., 1993; Saino et al., 1997). For example, changes in numbers of specific T- and B-lymphocyte subpopulations have been observed in hispid cotton rats, *Sigmodon hispidus*, subjected to dietary protein restriction (Davis et al., 1995). Furthermore, development of the immune system was suppressed in protein-restricted subadult cotton rats, but not in those fed a protein-rich diet (Lochmiller et al., 1998). The manifestation of the effects of protein restriction on the immune system may differ depending on various factors, such as species or age. Indeed, protein restriction resulted in a decrease of the T-cell/B-cell ratio in laboratory rats (Bises et al., 1987), whereas the opposite was found in laboratory mice (Woodward and Miller, 1991).

The immune systems of young animals are more sensitive to protein restriction than those of older individuals [see McMurray et al., and Lochmiller et al. and references therein (McMurray et al., 1981; Lochmiller et al., 1998)]. Nevertheless, the effect of protein shortage on immune function is generally detrimental. To our knowledge, all studies that have tested the effect of diet on anti-parasitic defenses were intraspecific comparisons. We believe that, given the generality of this effect and the likelihood that within-species immunocompetence is subject to natural selection, with protein availability being one of the major factors driving this selection (Lochmiller, 1996), species-specific levels of immunocompetence associated with species-specific dietary habits have probably also evolved.

Another explanation for the poorer performance of *X. ramesis* on Kuhl's pipistrelles than on the two other host species may be among-host differences in metabolic rate. For example, Gregory et al. (Gregory et al., 1996) suggested that host species with high mass-specific basal metabolic rates (BMRs) may increase their susceptibility to parasites because a high mass-specific BMR necessitates a higher feeding rate, requiring the animal to be more active. Mass-specific BMR is higher in the much smaller Kuhl's pipistrelle than in Egyptian fruit bats or Sundevall's jirds [Egyptian fruit bat (Korine and Arad, 1993); Sundevall's jird (Degen et al., 1988); Kuhl's pipistrelle (A. Muñoz-Garcia, M. Ben-Hamo, B.P., J. P. Williams and C.K., submitted)]. Given that mounting an immune response and maintaining a competent immune system is energetically expensive (Moret and Schmid-Hempel, 2000), animals with high mass-specific BMR may allocate relatively less energy to immune response. Indeed, Krasnov et al. (Krasnov et al., 2005) found that food-limited Sundevall's jirds maintained m_b, but significantly reduced their immune defenses against fleas.

Insectivorous bats reduce their body temperature (T_b) and become torpid in response to low T_a as a means of conserving energy and water (Speakman and Thomas, 2004). However, there is a cost associated with becoming torpid, in that the resulting lethargy compromises vigilance and defenses against predators. For example (Humphries et al., 2003). Maintaining relatively low body T_b for long periods during the day may affect feeding performance of fleas on this host. Fleas are known to respond behaviourally to changes in T_a by, for example, changing frequency of blood meals. The proportion of fleas of the species *Citellophilus tesquorum* that fed on a ground squirrel host decreased with decreasing T_a (Gong et

al., 2004). We found that the proportion of fleas that filled their midgut was significantly higher when feeding on torpid compared with normothermic Kuhl's pipistrelles. These results are indirectly supported by observations of flea prevalence in seasonally hibernating rodents (*Marmota baibacina*, *M. sibirica* and *M. camtshatica*) (Suntsov and Suntsova, 2006). The highest prevalence of fleas on these animals was recorded immediately upon termination of hibernation; however, blood-meal size of the fleas did not differ significantly between the two thermal states (Suntsov and Suntsova, 2006).

The results we present indicate that fleas performed as well on one alien host as they performed on their natural host. Fleas successfully fed on and digested the blood of Sandevall's jirds, and better still on Egyptian fruit bats, but were significantly less successful when they imbibed and digested the blood of normothermic Kuhl's pipistrelles. However, fleas feeding on torpid Kuhl's pipistrelles were less successful, indicating that becoming torpid might be a liability, rather than an effective defense against parasites. We cannot, however, rule out other explanations for the feeding performance of *X. ramesis* on the study hosts, such as morphological properties of their skin or physical properties of their blood, and the possible synergistic effects of these factors on the feeding performance of the fleas.

ACKNOWLEDGEMENTS

This is publication number 706 of the Mitrani Department of Desert Ecology.

FUNDING

This study was partly supported by the Israel Science Foundation (grant no. 28/08 to B.R.K. and I.S.K.).

REFERENCES

Beisel, W. R. (1996). Nutrition and immunity: overview. *J. Nutr.* **126**, S2611-S2615.
Bises, G., Mengherai, E. and Gaetani, S. (1987). T-lymphocyte subsets in zinc deficient and protein malnourished rats. *Nutr. Rep. Int.* **36**, 1371-1378.
Cadiergues, M. C., Santamarta, D., Mallet, X. and Franc, M. (2001). First blood meal of *Ctenocephalides canis* (Siphonaptera: Pulicidae) on dogs: time to initiation of feeding and duration. *J. Parasitol.* **87**, 214-215.
Combes, C. (2001). *Parasitism: the Ecology and Evolution of Intimate Interactions*. Chicago, IL: University of Chicago Press.
Davis, R. L., Lochmiller, R. L. and Warde, W. D. (1995). Splenocyte subpopulations of weanling cotton rats (*Sigmodon hispidus*) are influenced by moderate protein intake. *J. Mammal.* **76**, 912-924.
Dean, S. R. and Meola, R. W. (1997). Effect of juvenile hormone and juvenile hormone mimics on sperm transfer from testes of the male cat flea (Siphonatrea: Pulicidae). *J. Med. Entomol.* **34**, 485-488.
Dean, S. R. and Meola, R. W. (2002). Factors influencing sperm transfer and insemination in cat fleas (Siphonaptera: Pulicidae) fed on an artificial membrane system. *J. Med. Entomol.* **39**, 475-479.
Degen, A. A., Kam, M., Khokhlova, I. S., Krasnov, B. R. and Barraclough, T. G. (1998). Average daily metabolic rate of rodents: habitat and dietary comparisons. *Funct. Ecol.* **12**, 63-73.
Devi, S. G. and Prasad, R. S. (1985). Phagostimulants in artificial feeding systems of rat fleas *Xenopsylla cheopis* and *Xenopsylla astia*. *Proc. Indian Natl. Sci. Acad. B* **51**, 566-573.
Fielden, L. J., Krasnov, B. R., Khokhlova, I. S. and Arakelyan, M. S. (2004). Respiratory gas exchange in the desert flea *Xenopsylla ramesis* (Siphonaptera: Pulicidae): response to temperature and blood-feeding. *Comp. Biochem. Physiol.* **137A**, 557-565.
Fitze, P. S., Tschirren, B., Gasparini, J. and Richner, H. (2007). Carotenoid-based plumage colors and immune function: is there a trade-off for rare carotenoids? *Am. Nat.* **169**, 137-144.
Gong, Y.-L., Li, Z.-L. and Ma, L.-M. (2004). Further research of the bloodsucking activities of the flea *Citellophilus tesquorum sungaris*. *Acta Parasitol.* **11**, 47-49.
Gregory, R. D., Keymer, A. E. and Harvey, P. H. (1996). Helminth parasite richness among vertebrates. *Biodiv. Conserv.* **5**, 985-997.
Humphries,. M. M., Thomas, D. W. and Kramer, D. L. (2003). The role of energy availability in mammalian hibernation: a cost-benefit approach. *Physiol. Biochem. Zool.* **76**, 165-179.
Hoste, H., Torres-Acosta, J. F., Paolini Aguilar-Caballero, V. A., Etter, E., Lefrileux, Y., Chartier, C. and Broqua, C. (2005). Interactions between nutrition and gastrointestinal infections with parasitic nematodes in goats. *Small Ruminant Res.* **60**, 141-151.
Hsu, M. H. and Wu, W. J. (2001).Off-host observations of mating and postmating behaviors in the cat flea (Siphonaptera: Pulicidae). *J. Med. Entomol.* **38**, 352-360.
Hughes, D. A. (2001). Dietary carotenoids and human immune function. *Nutrition* **17**, 823-827.
Hůrka, K. (1963). Bat fleas (Aphaniptera), Ischnopsyllidae of Czechoslovakia. Contribution to the distribution, morphology, bionomy, ecology and systematics. Part 1. Subgenus *Ischnopsyllus* Westw. *Acta Faunist. Entomol. Mus. Natl. Prague* **9**, 57-120.
Ioff, I. G. (1949). Aphaniptera of Kyrgyzia, pp. 5-212. Moscow: Academy of Medical Sciences of the USSR.
Iqbal, Q. J. and Humphries, D. A. (1976). Remating of the rat flea, *Nosopsyllus fasciatus* (Bosc.). *Pakistan J. Zool.* **8**, 39-41.
Kam, M., Khokhlova, I. S. and Degen, A. A. (1997). Granivory and plant selection by desert gerbils of different body size. *Ecology* **78**, 2218-2229.
Kamala Bai, M. and Prasad, R. S. (1979). Influence of nutrition on maturation of male rat fleas, *Xenopsylla cheopis* and *X. astia*. *J. Med. Entomol.* **16**, 164-165.
Klein, S. L. and Nelson, R. J. (1998). Adaptive immune responses are linked to the mating system of arvicoline rodents. *Am. Nat.* **151**, 59-67.
Korine, C. and Arad, Z. (1993). Effect of water restriction on temperature regulation of the fruit bat *Rousettus aegyptiacus*. *J. Therm. Biol.* **18**, 61-69.
Korine, C. and Pinshow, B. (2004). Guild structure, foraging space use, and distribution in a community of insectivorous bats in the Negev desert. *J. Zool.* **262**, 187-196.
Korine, C., Arad, Z. and Arieli, A. (1996). Nitrogen and energy balance of the fruit bat, *Rousettus aegyptiacus*, on natural fruit diets. *Physiol. Zool.* **69**, 618-634.
Korine, C., Izhaki, I. and Arad, Z. (1999). Is the Egyptian fruit bat *Rousettus aegyptiacus* a pest in Israel? An analysis of the bat's diet and implications for its conservation. *Biol. Conserv.* **88**, 301-306.
Krasnov, B. R. (2008). *Functional and Evolutionary Ecology of Fleas: a Model for Ecological Parasitology*. Cambridge: Cambridge University Press.
Krasnov, B. R., Shenbrot, G. I., Medvedev, S. G., Vatschenok, V. S. and Khokhlova, I. S. (1997). Host–habitat relation as an important determinant of spatial distribution of flea assemblages (Siphonaptera) on rodents in the Negev Desert. *Parasitology* **114**, 159-173.
Krasnov, B. R., Hastriter, M., Medvedev, S. G., Shenbrot, G. I., Khokhlova, I. S. and Vaschenok, V. S. (1999). Additional records of fleas (Siphonaptera) on wild rodents in the southern part of Israel. *Israel J. Zool.* **45**, 333-340.
Krasnov, B. R., Khokhlova, I. S., Fielden, L. J. and Burdelova, N. V. (2001). The effect of temperature and humidity on the survival of pre-imaginal stages of two flea species (Siphonaptera: Pulicidae). *J. Med. Entomol.* **38**, 629-637.
Krasnov, B. R., Burdelova, N. V., Shenbrot, G. I. and Khokhlova, I. S. (2002a). Annual cycles of four flea species (Siphonaptera) in the central Negev desert. *Med. Vet. Entomol.* **16**, 266-276.
Krasnov, B. R., Khokhlova, I. S., Fielden, L. J. and Burdelova, N. V. (2002b). Time to survival under starvation in two flea species (Siphonaptera: Pulicidae) at different air temperatures and relative humidities. *J. Vector Ecol.* **27**, 70-81.
Krasnov, B. R., Khokhlova, I. S., Burdelova, N. V., Mirzoyan, N. S. and Degen, A. A. (2004a). Fitness consequences of density-dependent host selection in ectoparasites: testing reproductive patterns predicted by isodar theory in fleas parasitizing rodents. *J. Anim. Ecol.* **73**, 815-820.
Krasnov, B. R., Khokhlova, I. S., Burdelov, S. A. and Fielden, L. J. (2004b). Metabolic rate and jumping performance in seven species of desert fleas. *J. Insect Physiol.* **50**, 149-156.
Krasnov, B. R., Khokhlova, I. S., Arakelyan, M. S. and Degen, A. A. (2005). Is a starving host tastier? Reproduction in fleas parasitizing food limited rodents. *Funct. Ecol.* **19**, 625-631.
Krasnov, B. R., Korine, C., Burdelova, N. V., Khokhlova, I. S. and Pinshow, B. (2007). Between-host phylogenetic distance and feeding efficiency in haematophagous ectoparasites: Rodent fleas and a bat host. *Parasitol. Res.* **101**, 365-371.
Lewis, R. E. (1967). The fleas (Siphonaptera) of Egypt. An illustrated and annotated key. *J. Parasitol.* **53**, 863-885.
Lewis, R. E. (1990). An annotated checklist of the fleas (Siphonaptera) of the Middle East. *Fauna Saudi Arabia* **11**, 251-276.
Lewis, R. E., Lewis, J. H. and Maser, C. (1988). *The Fleas of the Pacific Northwest*. Corvallis, OR: Oregon State University Press.
Lindström, K. M., Foufopoulos, J., Pärn, H. and Wikelski, M. (2004). Immunological investments reflect parasite abundance in island populations of Darwin's finches. *Proc. R. Soc. Lond. B* **271**, 1513-1519.
Lochmiller, R. L. (1996). Immunocompetence and animal population regulation. *Oikos* **76**, 594-602.
Lochmiller, R. L., Vestey, M. R. and Boren, J. C. (1993). Relationship between protein nutritional status and immuno-competence in northern bobwhite chicks. *Auk* **110**, 503-510.
Lochmiller, R. L., Sinclair, J. A. and Rafferty, D. P. (1998). Tumoricidal activity of lymphokine-activated killer cells during acute protein restriction in the cotton rat (*Sigmodon hispidus*). *Comp. Biochem. Physiol.* **119C**, 249-255.
Lozano, G. A. (1994). Carotenoids, parasites, and sexual selection. *Oikos* **70**, 309-311.
McGraw, K. J. and Ardia, D. R. (2003). Carotenoids, immunocompetence, and the information content of sexual colors: an experimental test. *Am. Nat.* **162**, 704-712.
McMurray, D. N., Loomis, S. A., Casazza, L. J., Rey, H. and Miranda, R. (1981). Development of impaired cell-mediated immunity in mild and moderate malnutrition. *Am. J. Clin. Nut.* **34**, 68-77.
Medvedev, S. G. (1989). Ecological peculiarities and distribution of the fleas of the family Ischnopsyllidae (Siphonaptera). *Parasitol. Coll.* **36**, 21-43.
Mendes, L., Piersma, T., Hasselquist, D., Matson, K. D. and Ricklefs, R. E. (2006). Variation in the innate and acquired arms of the immune system among five shorebird species. *J. Exp. Biol.* **209**, 284-291.
Møller, A. and Rózsa, L. (2005). Parasite biodiversity and host defenses: chewing lice and immune response of their avian hosts. *Oecologia* **142**, 169-176.

Morand, S. and Poulin, R. (2000). Nematode parasite species richness and the evolution of spleen size in birds. *Can. J. Zool.* **78**, 1356-1360.
Moret, R. and Schmid-Hempel, P. (2000). Survival for immunity: the price of immune system activation for bumblebee workers. *Science* **290**, 1166-1168.
Morris, D. W. (2003). Toward an ecological synthesis: a case for habitat selection. *Oecologia* **136**, 1-13**.**
Noll, U. G. (1979). Body temperature, oxygen consumption, noradrenaline response and cardiovascular adaptations in the flying fox**,** *Rousettus aegyptiacus. Comp. Biochem. Physiol.* **63A**, 79-88.
Poulin, R. (2007). *Evolutionary Ecology of Parasites: From Individuals to Communities*, 2nd edn. Princeton, NJ: Princeton University Press.
Prasad, R. S. (1987). Host dependency among haematophagous insects: a case study on flea-host association. *Proc. Indian Acad. Sci. Anim. Sci.* **96**, 349-360.
Ribeiro, J. M. C., Vaughan, J. A. and Azad, A. F. (1990). Characterization of the salivary apyrase activity of three rodent flea species. *Comp. Biochem. Physiol.* **95B**, 215-218.
Rothschild, M., Ford, B. and Hughes, M. (1970). Maturation of the rabbit flea (*Spilopsyllus cuniculi*) and the oriental flea (*Xenopsylla cheopis*): some effects of mammalian hormones on development and impregnation. *Trans. Zool. Soc. Lond.* **32**, 105-188.
Saino, N., Calza, S. and Møller, A. P. (1997). Immuno-competence of nestling barn swallows in relation to brood size and parental effort. *J. Anim. Ecol.* **66**, 827-836.
Sokal, R. R. and Rohlf, F, J. (1995). *Biometry*, 3rd edn. New York: W. H. Freeman.
Speakman, J. R. and Thomas, D. W. (2004). Physiological ecology and energetics of bats. In *Bat Ecology* (ed. T. H. Kunz and M. B. Fenton), pp. 430-490. Chicago, IL: University of Chicago Press.
Suntsov, V. V. and Suntsova, N. I. (2006). *Plague. Origin and Evolution of Epizootoc System* (*Ecological, Geographical and Social Aspects*). Moscow: KMK Scientific Press.
Theodor, O. and Costa, M. (1967). *A Survey of the Parasites of Wild Mammals and Birds in Israel. (I) Ectoparasites.* Jerusalem: Israel Academy of Science and Humanities.
Vashchenok, V. S. (1988). *Fleas – Vectors of Pathogens Causing Diseases in Humans and Animals.* Leningrad: Nauka.
Vashchenok, V. S., Karandina, R. S. and Bryukhanova, L. V. (1988). Amount of blood consumed by different flea species in the experiments. *Parazitologiya+* **22**, 312-320.
Vestey, M. R., Lochmiller, R. L. and McMurry, S. T. (1993). Influence of dietary protein on selected measures of humoral and cellular immunity in the cotton rat (*Sigmodon hispidus*). *Can. J. Zool.* **71**, 579-586.
Whitaker, J. O., Jr (1988). Food habits analysis of insectivorous bats. In *Ecological and Behavioral Methods for the Study of Bats* (ed. T. H. Kunz), pp. 171-190. Washington, DC: Smithsonian Institution Press.
Winston, P. W. and Bates, D. H. (1960). Saturated solutions for the control of humidity in biological research. *Ecology* **41**, 232-237.
Woodward, B. D. and Miller, R. G. (1991). Depression of the thymus-dependent immunity in wasting protein-energy malnutrition does not depend on an altered ratio of helper ($CD4^+$) to suppressor ($CD8^+$) T-cells or on a disproportionately large atrophy of the T-cell relative to the B-cell pool. *Am. J. Clin. Nutr.* **53**, 1329-1335.
Yom-Tov, Y. and Kadmon, R. (1998). Analysis of the distribution of insectivorous bats in Israel. *Divers. Distrib.* **4**, 63-70.