Reproductive success in two species of desert fleas: density dependence and host effect

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Summary

We tested the hypothesis that a negative fitness–density relationship exists in haematophagous ectoparasites. We studied the effect of flea density on the number of blood meals necessary for starting oviposition and egg production in *Xenopsylla conformis* and *Xenopsylla ramesis* when exploiting two rodent hosts, *Meriones crassus* and *Gerbillus dasyurus*. The number of blood meals taken by a flea prior to first oviposition was similar in both flea species but was dependent on flea density and differed significantly between hosts. When parasitizing *G. dasyurus*, females of both flea species required a similar number of blood meals to start oviposition, independent of density. By contrast, fleas on *M. crassus* at higher densities needed less blood meals than at lower densities. Egg production of female fleas differed significantly between flea and host species and was affected by flea density. *X. ramesis* produced more eggs than *X. conformis*. When parasitizing *G. dasyurus*, density did not affect the number of eggs produced by *X. conformis*, however, when on *M. crassus*, this flea produced significantly less eggs at the highest density. The number of eggs produced by *X. ramesis* at high densities was significantly lower than at low densities when it parasitized either host species. Results of this study demonstrated that reproductive success of fleas was density dependent and, in general, decreased with an increase in density. However, the effect of density on reproductive performance was manifested differently on different host species.

Key words: egg production, density dependence, flea, rodent.

Introduction

Behaviour of an individual is affected by its evolutionary adaptations to maximize fecundity (Lomnicki, 1988). One of the mechanisms to achieve this is to select those habitats (or hosts, in the case of parasites) that provide the greatest reproductive reward. A habitat can be considered as 'the best' in terms of reproductive reward if it presents an exploiter with greater amount of resources and/or greater ease of extraction in comparison with other habitats (Rosenzweig, 1981; Rosenzweig, 1991). This statement conforms to the concept of ideal free distribution (IFD) (Fretwell and Lucas, 1970), predicting that animals competing for resources distribute themselves among habitat patches in such a way that resource use per individual would be equal across patches. In other words, animals are (a) ideal in assessing patch quality and (b) free to enter a habitat and use the resources.

The IFD model of host choice by blood-sucking insects was developed by Kelly and Thompson (Kelly and Thompson, 2000) who suggested that an individual blood-sucking insect should choose a host with a high intrinsic quality, a low defensiveness and a small number of competitors. Suitability of a host is thus assumed to be density dependent with the negative effect of density of parasites on suitability. In other words, this model suggests negative fitness–density relationships given that fitness of a haematophagous parasite is directly related to its feeding success.

However, studies of the relationship between density of haematophagous insects on a host and their reproductive success produced contradictory results. In some studies, negative fitness–density relationships were demonstrated, although evidence for this was sometimes indirect. For example, Gurtler et al. (Gurtler et al., 1997) reported that the proportion of kissing bugs, *Triatoma infestans*, feeding on humans as opposed to chickens and dogs decreased with an increase of the density of bugs on the host. Reproductive success of a flea, *Ceratophyllus gallinae*, breeding in the nest of the blue tit, *Parus caeruleus*, was affected by the number of founder fleas in this nest, although the number of eggs laid per female flea did not decrease with an increase in flea density (Tripet and Richner, 1999). Vashchonok (Vashchonok, 1995) studied egg production of *Leptopsylla segnis* in relation to the number of fleas simultaneously feeding on a host (laboratory mouse). In these experiments, he allowed fleas to stay on a restricted host (prevented from grooming) for at least 4 days

and counted the number of eggs produced per female per day. Egg production decreased slightly but significantly with an increase in flea density from 2–5 to 15–20. However, further increases in density did not result in further decreases in fecundity. By contrast, under very high densities, egg production tended to increase.

One of the reasons for the contradictory responses may be the effect of host species, i.e. density dependence of reproductive success may be manifested differently on different host species. This is because the negative fitness-density relationship stems from intraspecific competition which, in case of imagoes of ectoparasitic insects, seems to be interfering rather than exploitative. Indeed, it does not seem feasible that the blood supply of a host can be a limiting factor (Khokhlova et al., 2002), whereas interference can arise because of competition for those areas of a host body where blood is most readily available, or may be mediated *via* the host. For example, if there is a threshold of host sensitivity to parasite attacks, then its defence systems (behavioural or immune) may be activated once exploiters attain certain abundance (Mooring, 1995; Shudo and Iwasa, 2001). Both these factors can vary among host species (e.g. Khokhlova et al., 2004a). In addition, host body size also can play a role. For example, for the same number of parasites the degree of crowding will be different in small-bodied and large-bodied hosts and, consequently, the effect of density can be manifested in a small host but not in a large host.

In this study, we examined egg production of two flea species (*Xenopsylla conformis* Wagner and *Xenopsylla ramesis* Rothschild) when exploiting two rodent species (*Gerbillus dasyurus* Wagner and *Meriones crassus* Sundevall). Fleas (Siphonaptera) are obligate haematophagous ectoparasites that occur both on the body of their host and in its burrow. In most cases, pre-imaginal development is entirely off-host and the larvae are not parasitic, feeding on organic debris found in the burrow of the host. *X. conformis* and *X. ramesis* are common flea species, and *G. dasyurus* and *M. crassus* are common rodent species in the Negev desert (Krasnov et al., 1996; Krasnov et al., 1997).

We hypothesized that the reproductive success of fleas is (a) density dependent, but (b) manifestation of density dependence varies among host species. We predicted that fleas require less blood meals for successful egg maturation and oviposition and produce more eggs at low than at high densities. Average body mass of an adult *M. crassus* is about 80 g, whereas that of an adult *G. dasyurus* is about 22 g. In addition, *M. crassus* possesses 'pre-invasive' immune responses against fleas and maintains a certain degree of immunological 'readiness' (Khokhlova et al., 2004b), whereas *G. dasyurus* mounts the immune response against fleas only after being attacked (Khokhlova et al., 2004a). Consequently, we predict that negative relationship between reproductive parameters and density will be manifested in *G. dasyurus* more so than in *M. crassus*.

Materials and methods

Fleas

Fleas were obtained from our laboratory colonies started in 1999 from field-collected specimens, using the rearing procedures described elsewhere (Krasnov et al., 2001; Krasnov et al., 2002a). In brief, an individual rodent was maintained in a plastic cage (60×50×40 cm) with a steel nest box. A pan containing a mixture of sand and dried bovine blood (larvae nutrient medium) was placed under the wire mesh floor of the box. The gravid females deposited eggs in the substrate and bedding material in the nest box. Every 2 weeks, we collected all substrate and bedding material from the nest box and transferred it to an incubator (FOC225E, Velp Scientifica srl, Milano, Italy), where flea developed at 25°C air temperature and 75% relative humidity (RH). The newly emerged fleas were collected daily and placed on clean animals. Colonies of fleas were maintained at 25°C and 75% relative humidity with a photoperiod of 12 h:12 h light:dark.

Rodents

We used immune-naïve, adult male *M. crassus* and *G. dasyurus* from our laboratory colonies. The rodents were maintained in plastic cages (60×50×40 cm) with dried grass as bedding material, at 25°C with a photoperiod of 12 h:12 h light:dark. They were offered millet seed and alfalfa (*Medicago* sp.) leaves *ad libitum*. No water was available as the alfalfa supplied enough for their needs. Each individual was used once in an experiment. In total, we used 40 individuals of each rodent species.

Experimental design

We used 900 female and 500 male fleas of each species and assigned them randomly to eight experimental treatments that differed in host species (*G. dasyurus* or *M. crassus*) and number of simultaneously feeding fleas [10 (5 females and 5 males), 15 (10 females and 5 males), 38 (25 females and 13 males) and 75 (50 females and 25 males)]. Each treatment was replicated five times.

Experimental procedures

After emergence and prior to experiments, the fleas were placed in an incubator and maintained at 25°C and 75% RH. Fleas (24- to 48-h old) used in experiments did not feed from emergence until treatments. Rodents were placed in wire mesh (5×5 mm) tubes (10 cm length and 3 cm diameter for *G. dasyurus* and 15 cm length and 5 cm diameter for *M. crassus*) that limited movement and did not allow self-grooming. Tubes with rodents were placed into individual white plastic tubs and *X. conformis* or *X. ramesis* were placed on each rodent for 2 h. We then collected the fleas by brushing the hair of the rodent with a tooth-brush until all fleas were recovered. Both *X. conformis* and *X. ramesis* readily jumped off the host into the tub with the start of the brushing procedure. Then, they were collected from the tub. Time from the start of brushing until all fleas appeared in the tub ranged from 2 to 4 min and did not

Table 1. *Summary of ANOVA of the number of blood meals taken by fleas prior to first oviposition when feeding on different host species at different densities*

Effect	d.f.	MS	F	P
Host species	1	0.52	18.59	<0.0001
Flea species	1	0.03	0.96	0.33
Flea density	3	0.58	20.91	<0.0001
Host species × flea species	1	0.00	0.00	0.95
Host species × flea density	3	0.25	8.90	<0.0001
Flea species × flea density	3	0.01	0.52	0.67
Hosts species × flea species × flea density	3	0.04	1.59	0.20
Error	64	0.03		

Fleas: *Xenopsylla conformis* and *Xenopsylla ramesis*. Hosts: *Meriones crassus* and *Gerbillus dasyurus*.
Flea density: 10, 15, 38, or 75 fleas per host.
MS, mean square.

depend on the number of fleas on a rodent. Therefore, time of uninterrupted staying on a host was similar for fleas from both low and high density treatments. Our previous experiments demonstrated that 2 h of feeding per day is enough for egg maturation and oviposition (Krasnov et al., 2004).

After feeding, fleas from each host were placed in plastic cups (200 cm^2) the bottom of which was covered by a thin layer of sand and small pieces of filter paper. The cups were transferred into an incubator and maintained at 25°C air temperature and 92–95% RH. The second and following feedings of fleas were conducted daily using the procedure described above. Each feeding of a flea was done on a different host of the same species. After each feeding, fleas of the respective treatment were randomly distributed among plastic cups. Female fleas were fed once a day during the 7 days after first oviposition (per group). Pieces of filter paper from each cup were examined thoroughly under light microscope, eggs were counted, and the day of oviposition (from the first feeding) was recorded. The purpose for the maintenance of fleas in groups during experiments was to ensure that they remained in either low or high density conditions during their stay both on and off host. It is known that solitary- and group-maintained fleas demonstrate behavioural differences, for example in activity (Cox et al., 1999).

Air temperature was regulated in refrigerated incubators (see above) and humidity using saturated salt solutions (Winston and Bates, 1960). Temperature and humidity were monitored using Fisherbrand Traceable Humidity/Temperature Pen with Memory (Fisher Scientific International, NJ, USA).

The experimental protocol met the requirements of the 1994 Law for the Prevention of Cruelty to Animals (Experiments on Animals) of the State of Israel and was approved by the Ben-Gurion University Committee for the Ethical Care and Use of Animals in Experiments (License IL-27-9-2003). Details on maintenance of fleas and rodents were published elsewhere (Krasnov et al., 2002a; Krasnov et al., 2003a; Krasnov et al., 2004).

Data analysis

Reproductive success was evaluated as (a) the number of blood meals until first oviposition and (b) the number of eggs produced by a female. Between-replicate, within-treatment variability in the number of blood meals until first oviposition decreased with an increase in density (coefficients of variation in this parameter were 0.17 and 0.31 at densities of 10–15 fleas and 0.00–0.12 at densities of 38–75 fleas). These variables, except for proportions, were log-transformed and then analyzed using three-way ANOVAs with flea and host species and flea density as independent variables. Planned comparisons were further analyzed using univariate tests. Non-transformed data are presented in figures. We avoided an inflated Type I error by performing Bonferroni adjustments of alpha which resulted in an alpha level of 0.025.

Results

The number of blood meals taken by a flea prior to first oviposition was similar in both flea species but was dependent on flea density and differed significantly between hosts (Table 1). In addition, the effect of flea density on first oviposition differed between hosts (significance of the interaction of host species × flea density) (Table 1).

In *X. conformis*, the effect of host species on the number of blood meals prior to oviposition was found at low densities (less blood meals on *G. dasyurus*) but not at high densities ($F_{1,64}$=10.1, P<0.001; Fig. 1). Prior to the first oviposition, *X. ramesis* took more blood meals from *M. crassus* at low densities, but more blood meals from *G. dasyurus* at high densities ($F_{1,64}$=13.2, P<0.001; Fig. 1). When feeding on *G. dasyurus*, females of both species required similar number of blood meals to commence oviposition, independent of density ($F_{1,64}$=1.1, P>0.3; Fig. 1). There was, however, a trend of female *X. conformis* to consume less blood meals prior to oviposition at higher (38 or 75 fleas) than at lower (10 or 15 fleas) densities, although this effect disappeared after Bonferroni adjustment ($F_{1,64}$=3.7, P>0.05; Fig. 1). By contrast, fleas feeding on *M. crassus* at higher densities needed less blood meals than at lower densities ($F_{1,64}$=30.9 for *X. ramesis* and $F_{1,64}$=49.7 for *X. conformis*, P<0.001 for both; Fig. 1).

Egg production of female fleas differed significantly between flea and host species and was affected by flea density (Table 2). The effects of the factors were mainly independent
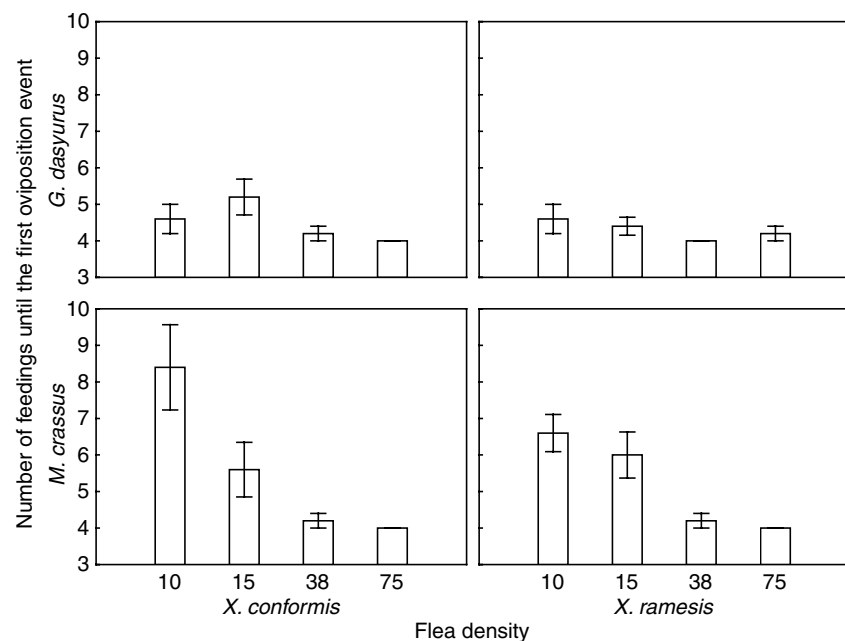
Fig. 1. Number (mean ± s.e.m.) of blood meals taken by *X. conformis* and *X. ramesis* prior to first oviposition when feeding on *G. dasyurus* and *M. crassus*, in relation to flea density.

Discussion

The results partly supported our predictions: (a) reproductive success of fleas was density dependent and, in general, decreased with an increase in density; (b) density dependence was manifested differently in different host species. However, in contrast to our prediction, density dependence of reproductive performance was manifested mainly when fleas parasitized *M. crassus*. In addition, *X. conformis* and *X. ramesis* differed in the pattern of reproductive response to density.

Latency of oviposition and density

Number of blood meals taken by a flea prior to oviposition either decreased with an increase in density (on *M. crassus*) or was density independent (on *G. dasyurus*). These results appear to contradict earlier observations that the amount of blood consumed by haematophagous dipterans is closely related to its fecundity (e.g. Takken et al., 1998). However, it is possible that the number of blood meals necessary for egg maturation is an indirect indicator of the relationship between feeding and reproductive performance. A greater volume of blood taken per meal (Krasnov et al., 2003a) and higher efficiency of blood digestion (Sarfati et al., 2005) can compensate for a smaller number of blood meals. The latter depends mainly on host blood biochemistry (Harrington et al., 2001), whereas the former can depend on flea density either directly or *via* the pattern of host immune response. The density-dependence explanation is more feasible in our study because fleas produced fewer eggs at higher densities (see below) and because the rodents used in our experiments had not been exposed previously to flea parasitism. However, the immune-response explanation cannot be ruled out completely because (a) some degree of immunological readiness against fleas in immune-naïve individuals can result from maternal transfer of immunity, which occurs in *M. crassus* (Khokhlova

as suggested by the non-significance of high-order interactions, although interaction between host species and flea density was on the margin of significance.

In general, *X. ramesis* produced more eggs than *X. conformis* ($F_{1,64}$=7.1–14.4, $P<0.001$; Fig. 2). Although, the effect of host species was weak, there was a difference in response of fleas to different hosts in egg production (Fig. 2). Specifically, *X. conformis* feeding on *G. dasyurus* produced similar number of eggs at all density ($F_{1,64}$=1.0, $P>0.3$), however, when feeding on *M. crassus*, produced significantly less eggs at the highest density ($F_{1,64}$=15.4, $P<0.001$; Fig. 2). The number of eggs produced by *X. ramesis* was significantly lower at higher than at lower densities when feeding on either host species ($F_{1,64}$=17.9 for *G. dasyurus* and $F_{1,64}$=6.2 for *M. crassus*, $P<0.01$ for both; Fig. 2).

Table 2. *Summary of ANOVA of the number of eggs produced by female fleas during 7 days of oviposition when feeding on different host species at different densities*

Effect	d.f.	MS	F	P
Host species	1	0.11	5.41	0.02
Flea species	1	1.73	82.51	<0.0001
Flea density	3	0.25	12.04	<0.0001
Host species × flea species	1	0.00	0.00	0.96
Host species × flea density	3	0.05	2.45	0.07
Flea species × flea density	3	0.01	0.33	0.80
Hosts species × flea species × flea density	3	0.04	1.77	0.16
Error	64	0.02		

See Table 1 for details of independent variables.
MS, mean square.

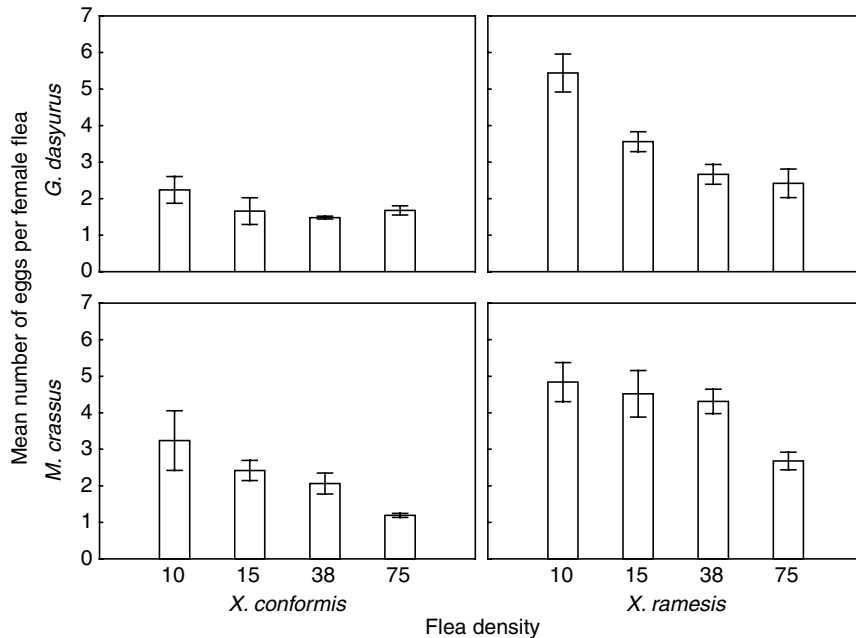Fig. 2. Number (mean ± s.e.m.) of eggs produced per female *X. conformis* and *X. ramesis* during 7 days of oviposition when feeding on *G. dasyurus* and *M. crassus*, in relation to flea density.

et al., 2004b) and (b) the 'post-invasive' immune response against flea parasitism can be quick, and is known to occur in *G. dasyurus* (Khokhlova et al., 2004a). Indeed, if a high number of attackers suppress host immune responses, then the blood intake per flea may increase and, thus, fewer feedings are needed (Randolph, 1994). This can be achieved either by the accumulative effect of anticlotting, antiplatelet and vasodilatory substances contained in saliva of haematophages (for a review, see Wikel, 1996) or by the high cost of immune responses against large number of attackers (Sheldon and Verhulst, 1996; Lochmiller and Deerenberg, 2000; Schmid-Hempel and Ebert, 2003; Krasnov et al., 2005).

Another explanation, not necessarily alternative, can be that fleas at low densities have lower opportunities for mating (Tripet and Richner, 1999) and/or selection for a high quality mate (Crowley et al., 1991), resulting in a delay of oviposition. Furthermore, successful oviposition in some flea species requires multiple matings (Suter, 1964; Humphries, 1967; Iqbal and Humphries, 1976; Tchumakova et al., 1978; Hsu and Wu, 2000) which can be achieved more easily at higher densities. The relationship between density and mating opportunity can also explain why the number of blood meals prior to oviposition was higher when fleas fed on *M. crassus* than on *G. dasyurus*. This finding appears to contradict our previous findings on *X. ramesis* (Krasnov et al., 2004). However, in the present study this difference was manifested at lower rather than at higher densities. *M. crassus* is about four times larger than *G. dasyurus* and consequently, given that the number of fleas on each host was the same, fleas on *M. crassus* are at a lower density than those on *G. dasyurus*. Therefore, the association between density, mating opportunity and the

number of blood meals prior to oviposition can be applied here and explain why at low densities fleas fed on *M. crassus* started to oviposit later.

Reproductive performance and density

At higher densities, flea egg production decreased (except for *X. conformis* on *G. dasyurus*) despite smaller number of blood meals taken prior to oviposition. This provides strong support for the negative fitness-density relationship in fleas.

Density dependence of flea egg production in this study might be an outcome of intraspecific competition for a limited resource (Izraylevich and Gerson, 1995). Although the depletion of host's blood is unlikely for a haematophagous ectoparasite (Tripet and Richner, 1999; Khokhlova et al., 2002), the decreased egg production at higher densities could be due to competition for other limiting resources. For instance, fleas may compete for those areas of a host's body where blood is most readily available (e.g. thinnest skin or closest position of capillary to body surface) or, in case of grooming hosts, for those areas that are least subject to self-grooming. Indeed, Linsdale and Tevis (Linsdale and Tevis, 1951) and Linsdale and Davis (Linsdale and Davis, 1956) reported that the fleas *Orchopeas sexdentatus* and *Anomiopsyllus falsicalifornicus* on the dusky-footed wood rat *Neotoma fuscipes* favoured a relatively small special 'flea spot' in the middle of the chin. Hsu et al. (Hsu et al., 2002) found that the cat flea *Ctenocephalides felis* concentrated on specific areas on the body (head and neck).

An important finding of negative density–fitness relationship in fleas is that assumptions of the ideal free distribution (IFD) theory hold for these insects and, thus, can be applied to explain distribution of fleas within and between host species. For example, the application of the IFD model to flea distribution over host populations explains the aggregation of a parasite population across a host population (Sutherland, 1983; Sutherland, 1996; Kelly and Thompson, 2000). In our previous study, we applied the IFD-based isodar theory (Morris, 1988) to infer mechanisms of host selection by five flea species, each infesting two species of desert rodents (Krasnov et al., 2003b). Results of this application suggested that ectoparasitic insects, like other animals, behaved as if they were able to make choices and decisions that favoured environments in which their reproductive benefit was maximized. Experimental testing of the fitness-related consequences of host selection by two of these five species conformed well to mechanisms revealed by application of the IFD-based theory (Krasnov et al., 2004).

Effect of host species

The effect of host species on the number of blood meals

necessary for oviposition and on the number of eggs produced has been studies in fleas (Hudson and Prince, 1958; Seal and Bhattacharji, 1961; Haas, 1965; Samarina et al., 1968; Prasad, 1969; Krasnov et al., 2002b; Krasnov et al., 2004). For example, the rat fleas *Xenopsylla cheopis* and *Xenopsylla astia* failed to reproduce when they fed on humans (Seal and Bhattacharji, 1961) and fecundity and egg hatchability in *X. cheopis* were higher when the fleas fed on *Rattus rattus* than on *Bandicota bengalensis* (Prasad, 1969). *Parapulex chephrenis* produced more eggs when they fed on *Acomys cahirinus* than on *G. dasyurus*, whereas the opposite was true for *Xenopsylla dipodilli* (Krasnov et al., 2002b).

Krasnov et al. (Krasnov et al., 2004) found that *X. conformis* produced more eggs when exploiting *M. crassus* than *G. dasyurus*, whereas egg production in *X. ramesis* did not differ between host species. This may explain why results of the present study showed between-host difference in the response of reproductive success to flea density. A density-dependent response was found in *X. ramesis* feeding on both hosts and in *X. conformis* feeding on *M. crassus*, but not on *G. dasyurus*. Egg production of *X. conformis* feeding on *G. dasyurus* was generally extremely low, even at low flea densities (see also Krasnov et al., 2004). Consequently, the density dependence of egg production on this host possibly was not detectable.

Results from this study conform well to the difference between natural populations of *X. conformis* and *X. ramesis* in the strategy of choosing between *M. crassus* and *G. dasyurus* (Krasnov et al., 2003b). At low densities, *X. conformis* demonstrated sharp selectivity and parasitized *M. crassus*, and only with an increase in flea population size was *G. dasyurus* also parasitized. By contrast, *X. ramesis* parasitized both hosts equally at low densities and were able to achieve similar maximum fitness under such conditions. However, with an increase of flea population, parasite pressure on *M. crassus* increased at a faster rate than that on *G. dasyurus*, and so at high densities, the fleas showed a preference for *M. crassus*. Our results also support the notion that the effect of density on reproductive success of a forager is greater in those habitats where foraging is less efficient (Morris, 1987a; Morris, 1987b; Morris, 1988).

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