

RESEARCH ARTICLE

Ectoparasite performance when feeding on reproducing mammalian females: an unexpected decrease when on pregnant hosts

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ABSTRACT

Reproduction is an energy-demanding activity in mammalian females, with increased energy requirements during pregnancy and, especially, during lactation. To better understand the interactions between parasitism and host reproduction, we investigated feeding and reproductive performance of fleas (*Xenopsylla ramesis*) parasitizing non-reproducing, pregnant or lactating gerbilline rodents (*Meriones crassus*). Based on energetic considerations, we predicted that feeding and reproductive performance of fleas would be lowest on non-breeding females, moderate on pregnant females and highest on lactating females. We estimated feeding performance of the fleas via absolute and mass-specific bloodmeal size and reproductive performance via egg production and latency to peak oviposition. Host reproductive status had no effect on either absolute or mass-specific bloodmeal size or the day of peak oviposition, but significantly affected the daily number of eggs produced by a female flea. Surprisingly, and contrary to our predictions, egg production of fleas fed on pregnant rodents was significantly lower than that of fleas on non-reproducing and lactating rodents, while no difference in egg production between fleas feeding on non-reproducing and lactating hosts was found. Our results suggest that differences in parasite reproduction when feeding on hosts of different reproductive status are not associated with the different energy requirements of the hosts at non-breeding, pregnancy and lactation but rather with variation in hormonal and/or immune status during these periods.

KEY WORDS: Ectoparasites, Egg production, Lactation, Pregnancy

INTRODUCTION

Reproduction is one of the most energy-demanding activities experienced by mammalian females. To achieve high reproductive output (e.g. production of a large number of offspring), females not only increase the level of resource acquisition (Lee, 1987; Randolph et al., 1995; Speakman, 2008) but also often use their own energy reserves (Degen et al., 2002). Allocation of energy to reproduction is, however, hindered by other energetically demanding activities that occur concomitantly (Kam and Degen, 1993).

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Parasites hijack resources belonging to a host and use these resources for their own maintenance and reproduction. A host, in turn, faces energy costs of parasitism including not only direct loss of energy taken over by parasites but also costs of immunological and/or behavioural anti-parasitic defences. Although anti-parasitic defences are highly demanding energetically (Sheldon and Verhulst, 1996; Lochmiller and Deerenberg, 2000; Zuk and Stoehr, 2002), their costs can be compensated for by reduced feeding and/or reproductive performance of parasites (Fielden et al., 1992), which, in turn, decreases the direct cost of parasitism.

The cost of parasitism is critical in reproducing hosts and is paid by either the parents (reduced mass, survival, future reproductive performance) (Brown et al., 1995; Richner and Tripet, 1999; Fitze et al., 2004; Neuhaus, 2003) or the offspring (reduced mass, growth rate) (Arendt, 1985; Richner et al., 1993; Hollmén et al., 1999; Patterson et al., 2013) or both. In part, the strong negative impacts of parasitism on reproductive hosts have been shown to be due to the reproduction/immunity trade-off, when immune function is suppressed during energetically demanding reproductive periods (Nordling et al., 1998; French et al., 2007; Knowles et al., 2009; Cox et al., 2010). Nevertheless, other studies reported either only a weak (if at all) negative impact of parasitism on reproducing hosts or even an improved reproductive success in parasitized hosts (Kristan, 2002a; Kristan, 2002b; Kristan, 2004; Willis and Poulin, 1999), suggesting that negative effects of parasitism can be compensated for by increased maternal energy intake (Jones et al., 2011), stronger immune response or both (Degen, 2006).

Traditionally, studies on the interactions between parasitism, energy allocation and immunity in reproducing hosts have centred on responses of the hosts (Møller, 1990; Tompkins et al., 1996; Allander, 1998; Murray et al., 1998; Neuhaus, 2003; Ballesteros et al., 2012; Patterson et al., 2013). In contrast, the response of parasites exploiting reproducing hosts has been less studied (but see Vargas-Villavicencio et al., 2005; Gallizzi and Richner, 2008; Tschirren et al., 2009). However, from an evolutionary perspective, the parasite responses to a host are no less important than host responses to a parasite. This is because host defences cause loss of fitness in a parasite, so that this host-mediated loss of fitness in a parasite is a feature of the host that can be measured via the parasite (Combes, 2001; Poulin, 2007), i.e. ‘host virulence against parasites’ (Combes, 2001). Consequently, information about the response of a parasite to a reproductive host will contribute in answering the complicated question about the interactions between parasitism and host reproduction.

In this study, we asked how fleas perform on female hosts in different stages of reproduction. To answer this question, we investigated feeding and reproductive performance of fleas (*Xenopsylla ramesis* Rothschild) parasitizing non-reproducing, pregnant or lactating gerbilline rodents (*Meriones crassus* Sundevall)

Table 1. Summary of ANOVA for the effect of female host reproductive status and/or flea sex on feeding and reproductive performance of fleas

Dependent variable	Effect	Sum of squares	d.f.	F	P
Absolute MBS	RS	<0.01	2	0.07	0.93
	FS	0.005	1	404.4	<0.001
	RS×FS	<0.01	2	1.03	0.36
	Error	<0.01	52		
Mass-specific MBS	RS	<0.01	2	0.12	0.88
	FS	0.007	1	23.04	<0.01
	RS×FS	<0.01	2	1.81	0.17
	Error	<0.01	52		
Egg no.	RS	0.02	2	3.57	0.04
	Error	0.21	26		

RS, reproductive status; FS, flea sex.

Feeding was measured as absolute and mass-specific mean bloodmeal size (MBS); reproductive performance was measured as mean number of eggs produced per female flea per day.

(a typical host of *X. ramesis* in the Negev Desert with almost 100% prevalence) (Krasnov et al., 1997). Fleas (Siphonaptera) represent a convenient laboratory model for studies of parasitism. They are holometabolous obligatory blood-feeding insects that exploit mainly small and medium-sized mammals. Flea larvae are not usually parasitic; instead, they reside in the burrow or nest of their hosts, feeding on a variety of organic debris. In the majority of fleas, larval and pupal development occur off-host. Adult fleas alternate periods when they occur on the body of a host with periods when they occur in the burrow or nest. This life style allows us to manipulate flea infestation on living hosts and to monitor changes in an individual flea or an individual host over time without sacrificing either of them.

Female mammals differ substantially in their energy requirements (Millar, 1977; Degen, 1997; Speakman, 2008) during two main stages of reproduction, namely pregnancy and lactation. In brief, lactation is much more energetically demanding than pregnancy, while the energy expenditure of pregnant females is only slightly higher than that of non-breeding ones, especially in altricial species (Degen, 1997; Speakman, 2008). Fleas have been shown to increase their feeding and reproductive performance when parasitizing energy-deprived hosts (Krasnov et al., 2005a), possibly because the host re-allocates energy from immunity to maintenance. Consequently, and based on energetic considerations, we predicted that feeding and reproductive performances of fleas would be lowest on non-breeding females, moderate on pregnant females and highest on lactating females. We estimated feeding performance of the fleas via (a) absolute and (b) mass-specific bloodmeal size (amount of blood consumed by a flea during a single feeding event) and reproductive performance via (c) egg production (number of eggs produced per female per day) and (d) the day of peak oviposition. We assumed that a higher performance of fleas would be associated with larger bloodmeals, higher egg production and earlier peak oviposition. The last of these is because the offspring of fleas that lay eggs earlier will presumably hatch earlier as larvae and will emerge earlier as new adults. As a result, they are likely to have a competitive advantage over conspecifics that hatch or emerge later because of larval competition and/or cannibalism (Day and Benton, 1980; Krasnov et al., 2005b). Additionally, fleas that hatch or emerge earlier would have better opportunities of locating and attacking a host and producing more new generations during the breeding season.

RESULTS

Host reproductive status had no effect on either absolute or mass-specific size of a flea bloodmeal (Table 1). However, both

measures of feeding performance differed significantly between male and female fleas, with female fleas consuming more blood (Table 1; Fig. 1; univariate tests for planned comparisons; $F=112.4$ – 158.6 for the absolute and $F=6.3$ – 13.4 for the mass-specific mean bloodmeal size – MBS; $P<0.005$ for all), except for the mass-specific MBS taken from pregnant rodents (univariate test for planned comparison, $F=3.6$, $P=0.06$). No pairwise difference was found in the absolute or mass-specific MBS in male

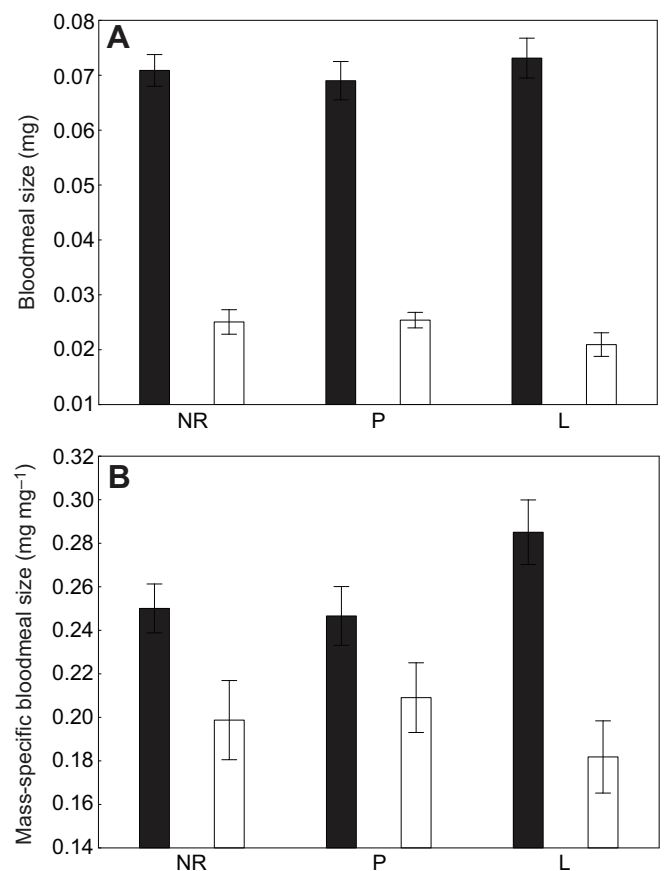


Fig. 1. The effect of host reproductive status on feeding performance of fleas. Mean (\pm s.e.) absolute (A) and mass-specific (B) bloodmeal size of female (black bars) and male (white bars) *Xenopsylla ramesis* fleas when feeding on non-reproducing (NR), pregnant (P) and lactating (L) female *Meriones crassus*.

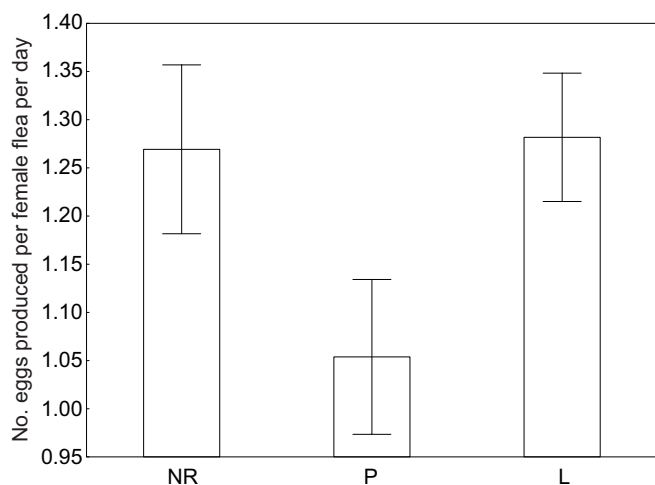


Fig. 2. The effect of host reproductive status on reproductive performance of fleas. Mean (\pm s.e.) number of eggs produced per day by a female *X. ramesis* flea during days 5–8 of the experiment (see Materials and methods for explanation) when feeding on non-reproducing (NR), pregnant (P) and lactating (L) female *M. crassus*.

fleas (univariate tests for planned comparisons; $F=0.01$ – 1.14 for the absolute and $F=0.27$ – 1.26 for the mass-specific MBS; $P>0.26$ for all) or female fleas (univariate tests for planned comparisons; $F=0.2$ – 0.9 for the absolute and $F=0.03$ – 2.37 for the mass-specific MBS; $P<0.12$ for all) feeding on rodents of different reproductive status. In either pregnant or lactating rodents, MBS of fleas did not depend on reproductive effort of a host estimated from either litter size or mass of the newborn pups (Wald statistic= 0.31 – 1.78 for the absolute and Wald statistic= 0.15 – 0.98 for the mass-specific MBS, $P>0.18$ for all).

Host reproductive status significantly affected the daily number of eggs produced by a female flea (Table 1; Fig. 2). Moreover, egg production of fleas fed on pregnant rodents was significantly lower than that of fleas fed on either non-reproducing (univariate test for planned comparisons; $F=5.49$, $P=0.03$) or lactating (univariate test for planned comparisons; $F=4.63$, $P=0.04$) rodents, while no difference in egg production between fleas feeding on non-reproducing and lactating hosts was found (univariate test for planned comparisons; $F=0.03$, $P=0.85$). There was no effect of reproductive effort of a pregnant or lactating host on flea egg production (Wald statistic= 1.49 and Wald statistic= 1.27 for litter size and mass of newborn pups, respectively, $P>0.22$ for both). In addition, there was no difference in the day of peak oviposition between fleas exploiting non-reproducing, pregnant or lactating rodents (Kruskal–Wallis $H=3.81$, $P=0.15$).

DISCUSSION

We found no effect of host reproductive status on flea feeding performance, but flea reproductive performance, in terms of egg production, differed significantly between pregnant hosts and non-reproducing/lactating hosts. Surprisingly, and contrary to our expectations, flea egg production was impaired if they fed on pregnant rodents. This counter-intuitive result suggests that variation in parasite reproduction in hosts of different reproductive status is not associated with differential energetic requirements of a host during non-breeding, pregnancy and lactation, but rather with some other change in a reproducing mammalian female such as hormonal and/or immune status.

Decrease of reproductive performance of fleas on pregnant hosts

Many studies have demonstrated increased parasite infections in pregnant hosts (e.g. Scott and Kaufmann, 1991; Morales et al., 2002; Sharifi et al., 2013), although the opposite has been found in other studies (e.g. Osorio et al., 2008). In general, it is thought that increased infections during pregnancy are the unavoidable side effect of the necessity of maternal tolerance to the fetus, which, in turn, inhibits immune responses against infections and parasites. Among several factors inducing immunosuppression during pregnancy, progesterone has been considered as one of the most important (Arck et al., 2007; Szekeres-Bartho and Polgar, 2010). Blood progesterone level rises during pregnancy (Miyaura and Iwata, 2002), is crucial for maintenance of pregnancy in mammals, and is considered a natural immune suppressor (e.g. Szekeres-Bartho and Polgar, 2010). However, results of studies of the effect of progesterone on parasites are contradictory. For example, establishment, growth and reproduction of the cestode *Taenia crassiceps* in intact laboratory mice increased after progesterone treatment (Vargas-Villavicencio et al., 2005). In contrast, parasite loads decreased substantially in castrated mice treated with progesterone (Vargas-Villavicencio et al., 2006). Furthermore, pregnant laboratory hamsters infected with *Leishmania panamensis* demonstrated a lower parasite burden than non-pregnant control animals (Osorio et al., 2008) because their innate immunity was heightened via hormone-modulated (pregnancy-associated $17\text{-}\beta$ oestradiol and a progesterone metabolite pregnane) nitric oxide production (see also Sacks et al., 1999). Oestradiol is another hormone that may be associated indirectly with changes in immunocompetence during pregnancy. Both maternal and fetal oestradiol levels rise during pregnancy and oestradiol is known to contribute to activation of nitric oxide synthase. In addition to its importance in developing regulation of fetal blood flow (Weiner and Thompson, 1997), nitric oxide may contribute to anti-parasitic mechanisms (Balmer et al., 2000). Independent of reproductive stage, examples of the effects of nitric oxide on parasitic infections have been demonstrated in studies using *Plasmodium* (Balmer et al., 2000; Nahrevanian and Dascombe, 2001), *Babesia* (Goff et al., 2001) and *Leishmania* (Baneth et al., 2008; Osorio et al., 2008). These results suggest that an important interplay between the endocrine and immune systems of a host is involved in protection against parasites. They also suggest that immunosuppression during pregnancy may be not as straightforward as was previously thought.

Furthermore, recent studies suggested that pregnancy should not be considered as a period of immunological weakness (Mor and Cardenas, 2010; Mor et al., 2011). Instead, pregnancy represents a critical period when all protective tools of the mother and the offspring, including their immune systems, should be enhanced (Mor et al., 2011). Mor et al. (Mor et al., 2011) argued that responses of a pregnant female to parasites originate from both the maternal and the fetal–placental immune systems and that pregnancy is ‘a unique immune condition that is modulated but not suppressed’ (see p. 81 of their study). Therefore, higher susceptibility to parasites is not necessarily an inevitable consequence of pregnancy.

Pregnancy is characterized by a shift in investment from Th1-immunity, that is, cellular immunity, to predominantly Th2-immunity, that is, humoral immunity (e.g. Wegmann et al., 1993). Earlier, this concept was applied broadly for the explanation of why a fetus is not rejected by the immune system of a mother but is less accepted in modern reproductive biology (e.g. Saito et al., 2010). In part, this unpopularity is because the composition of immune cells and their products vary during the course of pregnancy from a

predominance of Th1-type cells during implantation and before parturition to a predominance of Th2-type cells during the period of fetal growth and development (Mor and Cardenas, 2010). This period-dependent variation in the balance between Th2-type and Th1-type cells with bias to Th2-type in the middle of pregnancy, i.e. the time when pregnant rodents were exposed to fleas in our experiments, may explain, at least partially, the decreased egg production in fleas fed on pregnant rodents. Indeed, humoral factors may play an important role in host resistance to fleas (Greene et al., 1993; Heath et al., 1994) (but see Khokhlova et al., 2004) because digestion in fleas is intracellular and they lack a peritrophic membrane (Vatshchenok, 1988) that lines the gut of many arthropods, separating ingested food from the gut epithelium and restricting penetration of ingested immune effector components (Eisemann and Binnington, 1994). This explanation is weakened, however, by the fact that anti-inflammatory cytokines produced by Th2-type cells may inhibit activation of a delayed-type hypersensitivity response, thus minimizing host irritability and facilitating ectoparasite feeding (Soares et al., 1998; Lehane, 2005).

No increase of reproductive performance in fleas on lactating hosts

Contrary to our predictions, reproductive performance was not better in fleas exploiting lactating females compared with non-reproducing controls. Although lactating rodents undoubtedly have high energy requirements, they can compensate for energy-demanding lactation by increased food intake, especially when they do not face food limitation (Jones et al., 2011; Ołdakowski et al., 2012). As a result, they can still maintain a high level of both lactation and anti-parasitic defence. Indeed, a trade-off between reproduction and immunity has been shown in field experiments (e.g. Saino et al., 2000). In contrast, under laboratory conditions with food offered *ad libitum*, Brandt voles demonstrated (a) improved rather than reduced immune function in lactating compared with non-breeding voles in terms of total serum immunoglobulin G (IgG) and anti-keyhole limpet haemocyanin (KLH) IgG and (b) no lactation-associated changes in immune function in terms of phytohaemagglutinin response and anti-KLH immunoglobulin M (Xu et al., 2012). As expected, lactating voles had higher food intake and resting metabolic rate than non-reproductive controls. In addition to increased food intake, a maternal transfer of antibodies to protect the offspring from infection could also be a reason for increased total IgG during lactation (Xu et al., 2012). In our studies, both these factors might also play roles given that maternal transfer of anti-parasitic immunity (Khokhlova et al., 2004) and increased food intake during lactation (Kam et al., 2003) have been shown for *M. crassus*. Nevertheless, the level of immunity in lactating *M. crassus* was not dramatically high and did not substantially decrease flea performance relative to non-reproducing females. An additional reason for the lack of increase in flea egg production when exploiting a lactating host could be morphological constraints such as, for example, a species-specific number of ovarioles per ovary (King and Teasley, 1980). In other words, *X. ramesis* are simply not able to produce more eggs. Indeed, the amount of eggs produced by fleas in this study was not higher than that in our earlier experiments when fleas fed on hosts belonging to different species as well as to different sexes and age cohorts (Krasnov et al., 2004; Khokhlova et al., 2009a; Khokhlova et al., 2012; Liberman et al., 2013).

Evolutionary and ecological perspectives

In general, our results demonstrate a relatively weak influence of host reproductive status on flea feeding and reproduction. The only

effect of host reproductive status was a decrease in egg production in fleas exploiting pregnant hosts, while fleas consumed similar amounts of blood from lactating, reproducing and non-reproducing rodents and laid a similar number of eggs from non-reproducing and lactating rodents. One of the reasons for the weak flea response may be the fact that *M. crassus* is a preferable host for *X. ramesis* with almost 100% prevalence of infestation by this flea in the field (Krasnov et al., 1996; Krasnov et al., 1997; Krasnov et al., 1999). From an evolutionary perspective, such 'tight' host-parasite associations often result from prolonged common history and co-evolution [see Krasnov for fleas (Krasnov, 2008)]. During co-evolution of a particular parasite and a particular host, natural selection will favour those parasites that are able to successfully extract resources from hosts while avoiding or not stimulating host defence response (e.g. Vaughan et al., 1989; Khokhlova et al., 2010). This allows prolonged persistence of a parasite population.

From an ecological perspective, the negative effect of host pregnancy on flea reproduction revealed in this study is unlikely to be important because it accounts for only a short portion of the entire annual cycle. Despite a relatively long breeding period, *M. crassus* produce only two to three litters per year (Krasnov et al., 1996). Given 18–22 days of pregnancy, the entire period when fleas might be forced to feed on pregnant females is only about 2 months of the full year during which *X. ramesis* is active (Krasnov et al., 2002a). Moreover, the majority of individual *X. ramesis* prefer to parasitize male rather than female rodents (Krasnov et al., 2005c) and are even able to select male over female hosts presumably using odour cues (Khokhlova et al., 2011). In contrast, the effect of flea parasitism seems to be important for reproducing hosts because it may force pregnant and lactating females to increase foraging activity and thus jeopardize both the mother and the offspring as a result of (a) increased exposure to predation when foraging and (b) longer maternal absence from the nest during lactation. This host-parasite asymmetry suggests that understanding the complicated and intervening relationships and trade-offs between host reproduction, energy demands, hormones and immunity requires not only host-focused but also parasite-focused studies.

MATERIALS AND METHODS

Study animals

We used fleas and rodents from our laboratory colonies started in 1999 and 1997, respectively. Rearing procedures and details of colony maintenance were described elsewhere (e.g. Krasnov et al., 2001; Krasnov et al., 2002b; Khokhlova et al., 2009a; Khokhlova et al., 2009b). In brief, an individual rodent was placed in a plastic cage with a wire mesh floor and a pan containing a mixture of sand and larvae nutrient medium (94% dry bovine blood, 5% millet flour and 1% grind excrement of *M. crassus*). Every 2 weeks, we collected all substrate and bedding material from the pan and transferred it to an incubator (FOC225E, Velp Scientifica srl, Milan, Italy), where the fleas developed at 25°C air temperature and 75% or 90% relative humidity (RH). The rodents were maintained in plastic cages at 25°C with a photoperiod of 12 h:12 h (light:dark), and with sawdust and dried grass as bedding material. They were offered millet seed and alfalfa (*Medicago* sp.) leaves *ad libitum*. All fleas were newly emerged adults (collected 24–48 h after emergence) and did not feed prior to experimental treatments. Rodents were sexually naive males and females, 6–8 months old, that had not been exposed to fleas previously. Each individual rodent and each individual flea were used in the experiment only once. In total, we used 870 female and 580 male fleas and 29 female and 29 male rodents.

Experimental procedures

The experimental procedures complied with the laws of the State of Israel. For mating, 29 pairs of male and female rodents were placed in individual cages for a week, after which time they were separated. We applied fleas to

20 of these females 3–4 days after separation from males, i.e. in approximately the middle of pregnancy. Twelve females gave birth to 3–7 pups per litter and were assigned to the ‘pregnancy’ group (P), while the remaining eight females were assigned to the ‘non-reproducing group 1’ (NR1). Six of the nine females remaining from the original 29 females also gave birth to 3–6 pups per litter and were assigned to the ‘lactation’ group (L), while the other three females were assigned to the ‘non-reproducing group 2’ (NR2). We applied fleas (see below) to L group females at 14 days after parturition. Upon parturition, we counted and weighed the pups daily (± 0.01 g, Precisa BJ410c balance, Precisa Ltd, Milton Keynes, UK).

Experimental procedures for quantifying blood meals and egg production of fleas were as follows. An individual female rodent was placed in a plastic cage (60×50×40 cm) with a floor of 3–5 mm clean sand, which was covered by a wire mesh (5×5 mm). Then, 30 female and 20 male fleas were released into a cage with a rodent for 3 days. Under these conditions, fleas start to oviposit no sooner than on the second day on a host (Khokhlova et al., 2012). Blood feeding is necessary for the dissolution of a testicular plug in newly emerged male fleas and the development of ovaries in newly emerged female fleas (Krasnov, 2008). Three days of uninterrupted access to a host guaranteed that fleas were able to copulate and produce eggs. In *M. crassus*, the oestrous cycle lasts 4–4.5 days (Khokhlova et al., 2000), pregnancy lasts from 18 to 22 days (on average, 20–21 days), and pups start to eat solid food 17 days after birth (Krasnov et al., 1996; Kam et al., 2003; Degen et al., 2011). P females were thus exposed to flea parasitism in the middle of pregnancy, while L females were exposed to fleas at peak lactation. Indeed, backward calculation of the date of conception, based on the date of birth and assuming a 21 day period of pregnancy, meant that rodents were exposed to fleas starting on the 9th to 10th day of pregnancy.

To estimate feeding performance per flea per feeding event and reproductive performance per female flea per day while guaranteed equal feeding chances of fleas, we collected fleas from both the rodent’s body (over a white plastic pan using a toothbrush until no more fleas were recovered) and cage substrate, placed fleas recovered from the same rodent individual in Petri dishes and transferred them to an incubator (see above) at 25°C air temperature and 90% RH for 24 h. The next day, i.e. day 4 of the experiment, we placed each individual rodent in a wire mesh (5×5 mm) tube (15 cm length and 5 cm diameter) that limited movement and did not allow self-grooming. Tubes with rodents were placed in individual white plastic cages. Fleas collected earlier from this individual rodent were weighed (males and females separately; ± 0.01 mg, 290 SCS Precisa Balance, Precisa Instruments AG, Dietikon, Switzerland) and then released onto the animal. After feeding on a host for 2 h, fleas were collected and examined for blood in the midgut under light microscopy (40× magnification). Then, fleas that took a bloodmeal were weighed again (see above) and placed into a Petri dish (males and females together) and transferred again to an incubator for 24 h, after which time we checked the dish and counted newly laid eggs. These procedures were repeated daily over five consecutive days.

Our earlier experiments showed that the confounding effect of water evaporation on body mass of flea body mass after feeding is negligible (Khokhlova et al., 2009a). Estimation of feeding and reproductive performance of fleas during daily 2 h feedings guaranteed that the source of blood for egg production by fleas feeding on L group females was a mother rodent and not both mother and pups.

Estimates of performance and data analyses

Feeding performance of fleas was estimated as the (a) absolute and (b) mass-specific amount of blood consumed per flea. We calculated the amount of blood consumed by a flea (mean bloodmeal size; MBS) as the difference between total mass of fleas after feeding and total mass of fleas prior to feeding; this value was divided by either the number of fleas (for absolute MBS) or the total mass of fleas prior to feeding (for mass-specific MBS). Prior to calculation of mean bloodmeal size, the total mass of fleas after feeding was based only on fleas that took a bloodmeal, while the total mass of fleas prior to feeding was corrected by subtracting the product of mean body mass of a fasting flea and the number of fleas that did not take a bloodmeal. We calculated the absolute and mass-specific MBS separately for male and female fleas for experimental days 4–7 and then averaged daily values for each group of male or female fleas exploiting each female rodent.

To estimate reproductive performance, we calculated the mean number of eggs produced per female flea per day for each group of fleas for days 5–8 of the experiment. Then, we averaged the daily value for each group of female fleas exploiting each female rodent and used them as estimates of egg production effort by a mother flea. In addition, we used the day of peak oviposition (after the start of experimental treatments) for each group of fleas as a supplementary measure of reproductive performance.

Prior to analyses, dependent variables were log- or log+1-transformed (figures present untransformed data). Transformations produced distributions that did not deviate significantly from normality (Kolmogorov–Smirnov $d=0.09$ – 0.10 , $P>0.20$ for all), except for the day of peak oviposition. We tested the effect of host reproductive status on estimates of feeding performance of fleas using 2-way ANOVA with flea sex and host reproductive status as independent variables and the effect of host reproductive status on mean number of produced eggs using 1-way ANOVA. Pairwise comparisons between variables describing flea performance in female hosts of different reproductive status or variables describing feeding performance of male and female fleas within hosts of the same reproductive status were done using univariate tests of significance for planned comparisons. The effect of host reproductive status on the day of peak oviposition was analysed using a Kruskal–Wallis test. Estimates of feeding and reproductive performance did not differ between NR1 and NR2 fleas ($F=0.10$ – 0.55 , $P>0.10$ for all). Consequently, we pooled the data for these groups and named the new group of fleas ‘non-reproductive’ (NR). In addition, to test for the effect of reproductive effort of a reproducing host on flea performance, we applied generalized linear models (GLM) with normal distribution and log-link. In these models, a dependent variable was an estimate of feeding or reproductive performance of fleas (except of the day of peak oviposition), a categorical predictor was the reproductive state of a host (pregnant or lactating), while a continuous predictor was either litter size or litter mass.

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Competing interests

The authors declare no competing financial interests.

Author contributions

B.R.K., I.S.K. and A.A.D. conceived and designed the experiments; E.M.D., C.J.D. and I.S.K. conducted the experiments; E.M.D., B.R.K. and I.S.K. analysed the data; all authors drafted and revised the paper.

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