Reproductive consequences of host age in a desert flea

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SUMMARY

We tested for the effect of age of a rodent host (*Meriones crassus*) on reproductive performance of fleas in terms of number and quality of offspring and predicted that fleas would perform better on juvenile and old than on subadult and adult hosts. The number of flea offspring was evaluated via egg and new imago production, while their quality was estimated via duration of development, resistance to starvation and body size. Although fleas produced more eggs when they exploited adults than when they exploited juvenile, subadult and old hosts, significantly more new imago emerged from fleas fed on juvenile and old hosts than on subadult and adult hosts. Fleas performed better when they fed on juvenile and/or old hosts than on subadult and adult hosts in 2 of 3 measures of offspring quality (duration of development and body size). Nevertheless, when offspring quality was estimated via resistance to starvation of a new imago, fleas demonstrated good performance in young (juvenile and subadult) hosts, while they performed poorly in old hosts. Thus, general reproductive performance of fleas was better when they exploited young and old hosts than when they exploited median age cohorts. However, the effect of host age on flea reproductive performance was manifested somewhat differently between (a) male and female hosts and (b) male and female flea offspring.

Key words: egg production, development, fleas, rodents, survival.

INTRODUCTION

Differential parasite abundance in hosts belonging to different age cohorts has been reported for various host and parasite taxa (Goater and Ward, 1992; Fichet-Calvet *et al*. 2003; Krasnov *et al*. 2006; Alarcos and Timi, 2012). However, the effect of host age on the distribution pattern of parasite abundance differs among different host-parasite associations (Hudson and Dobson, 1995). In some host-parasite associations, parasite abundance increases (linearly or asymptotically) with host age (e.g., Johansen *et al*. 2010; Body *et al*. 2011), while in other associations parasite abundance either increases or decreases in the youngest and oldest hosts compared with median age hosts (Gregory *et al*. 1992; Krasnov *et al*. 2006).

Age-dependent development of defence tools coupled with parasite-dependent host mortality are thought to be the main mechanisms generating these patterns (Pacala and Dobson, 1988; Woolhouse, 1998), although host behaviour and ecology may also be responsible (Krasnov *et al*. 2006). Indeed, the acquired resistance against parasites can be lower in young and/or old hosts than in median age hosts. Young hosts may merely not have enough time to acquire resistance against parasites (Gallie, 1973), while old hosts may lose the capacity to withstand parasites due to immunosenescence (Møller and de Lope, 1999; Gruver *et al*. 2007; Praet *et al*. 2010). As a result, hosts belonging to the youngest and oldest cohorts would represent better habitats for parasites (that is, habitats that allow higher fitness reward), so that the shape of the relationship between parasite abundance and host age would be convex. However, if high parasite burdens cause host mortality, then heavily infested young and old hosts will be lost from the population, transforming the relationship between parasite abundance and host age into a hump-shaped curve (Rousset *et al*. 1996). In addition, if the negative effect of heavy parasite burdens causes mortality mainly in young rather than old hosts, then parasite abundance will increase with an increase in host age. In any of these cases, parasites are expected to perform better on young and old hosts

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(due to their lower defences) than on median age hosts (further referred to as 'adult hosts').

From the ecological and evolutionary perspectives of host-parasite interactions, both responses of hosts to parasites and responses of parasites to hosts are equally important (Combes, 2001). However, the majority of studies of anti-parasitic defences rarely focused on parasites but rather assessed anti-parasitic responses of hosts (but see Jørgensen *et al*. 1998; Sargison *et al*. 2011).

Recently, we tested the effect of age of a rodent host (*Meriones crassus*) on feeding performance of a flea (*Xenopsylla ramesis*) and predicted that fleas would perform better on juvenile and senescent hosts than on subadult and adult hosts (Liberman *et al*. 2011). Fleas are obligatory haematophagous insects that alternate periods when they occur on host body and when they occur in its burrow or nest. In contrast to the imago, flea larvae are not parasitic and usually develop off-host. Upon emergence from a pupa, a new imago flea finds a host because flea reproduction is not possible without blood feeding (Krasnov, 2008). Unequivocal support for our prediction was not found but rather the effect of host age was mediated strongly by the effect of host gender. In particular, from the perspective of resource acquisition (that is, bloodmeal size), a better quality of young and old age cohorts was manifested in female but not in male hosts, while, from the perspective of resource processing (that is, digestion of blood), some trends of age-dependent host quality were found in male but not female hosts. In other words, the results of the study by Liberman *et al*. (2011) suggested that host age could not unequivocally predict whether it is more or less beneficial for a flea. Another reason for inconsistencies in these results could be that feeding performance in hosts belonging to different age cohorts might not necessarily be a good proxy for fitness achieved in these hosts (see also Khokhlova *et al*. 2012). Consequently, investigating mechanisms of the effect of host age on parasite abundance requires experimental measurement of direct fitness-related variables.

Here, the direct effect of host age on parasite fitness was studied using the same model host-parasite association as in our earlier study (Liberman *et al*. 2011), namely a rodent *M. crassus* and a flea *X. ramesis*. Flea fitness was measured in terms of both number and quality of offspring. The number of flea offspring was measured via egg and new imago production, while quality was assessed via duration of development, resistance to starvation and body size. A higher emergence success was assumed to indicate a higher quality of offspring because it mirrors the mortality of pre-imaginal fleas. A shorter duration of pre-imaginal development of a flea may be an indicator of higher quality because (a) earlier-hatched flea larvae often cannibalize later-hatched larvae (Lawrence and Foil, 2002), (b) earlier-hatched flea larvae may have an advantage over later-hatched larvae in competition for food (Krasnov *et al*. 2005), and (c) earlier-emerged fleas likely have a higher probability to find a host than later-emerged fleas. The resistance to starvation in newly emerged imagoes is a measure of their quality because when a flea emerges from a cocoon, it possesses energy storage in the fat tissue. This energy allows the flea to survive until it finds a host. The ability to survive unpredictable and sometimes lengthy periods without a bloodmeal is thus extremely important. Finally, body size may be considered as an additional indicator of a flea's quality because larger body size is intraspecifically associated with higher fecundity in insects (Honek, 1993), although this has never been studied in fleas.

We predicted that fleas would produce more eggs and the emergence success of their offspring would be higher when they exploit juvenile (20–21 days old) and senescent (>4 years old) rodent hosts than subadult (45–60 days old) and adult (6–12 months old) rodent hosts. We also predicted that the offspring of fleas fed on juvenile and old rodents would (a) develop faster, (b) be larger and (c) survive without a bloodmeal longer than those fed on subadult and adult rodents.

MATERIALS AND METHODS

Fleas and rodents

We used fleas and rodents from our laboratory colonies. A detailed description of maintenance of these colonies can be found elsewhere (Khokhlova *et al*. 2009, 2010; Liberman *et al*. 2011). Fleas were maintained on *M. crassus* kept individually in plastic cages with a wire mesh floor over a pan containing a mixture of sand and dried bovine blood. Air temperature was kept at 25 °C and photoperiod at 12:12 (L: D) h. Every 2 weeks, all substrate and bedding material from the rodent's nest box and the pan were collected and transferred to an incubator (FOC225E, Velp Scientifica srl, Milano, Italy; 25 °C air temperature and 75% relative humidity [RH]) where the fleas developed. Newly-emerged fleas were collected from these boxes every 2 weeks. Fleas used in experiments were newly-emerged and were selected randomly from a colony.

Rodents were maintained in plastic cages (60 × 50 × 40 cm), and with sawdust and dried grass as bedding material. They were offered millet seed and fresh alfalfa (*Medicago sp*.) *ad libitum* daily. In this study, we used sexually-naïve juvenile, subadult, adult, and senescent *M. crassus* (107 males and 70 females). Adult rodents (mean body mass 140·2 g and 107·7 g for males and females, respectively) were 6–12 months old and were randomly selected from a laboratory colony. Old rodents (mean body mass 119·2 g and 86·1 g for males and females,

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respectively) were maintained individually from 2 months of age for 4 years. Juvenile rodents (mean body mass 25·8 g for both males and females) were 20–21 days old and pre-weaned. Subadult rodents (mean body mass 65·6 g for both males and females) were 45–60 days old and weaned at 30 days of age. In total, we used 44 juvenile, 57 subadult, 36 adult and 40 old rodents. No sibling rodents were used in the same treatment. For biological reasons, juvenile and subadult rodents were never parasitized by fleas, while adult and old rodents were exposed to fleas on at least 5 occasions previously.

Experimental procedures and design

We used 2 methods for feeding fleas. In the first method, we placed rodents individually in wire mesh (5 × 5 mm) tubes (15 cm length and 4 cm diameter for juvenile rodents and 18 cm length and 6 cm diameter for subadult, adult and old rodents) which prevented movement and self-grooming. Then, the tubes were placed in individual white plastic pans and 10, 20 or 30 fleas (equal number of males and females) were placed on each rodent (juvenile, subadult and adult/old, respectively) for 1 h. We brushed the hair of the rodent several times with soft custom-made forceps until all fleas were recovered. This procedure was repeated for 8 consecutive days. Each group of fleas was fed on the same rodent individual. In the second method, an individual rodent or a female with pre-weaned juveniles were placed in a plastic cage (60 cm by 50 cm by 40 cm) with a floor of 3–5 mm of clean sand covered by a wire mesh (5 mm by 5 mm). Ten (times number of juveniles in the litter), 20 or 30 fleas (equal number of males and females) were released into the cage (see above) and allowed to stay with a rodent for 3 days. Our preliminary observations demonstrated that fleas start to oviposit no sooner than the second day with a host under these conditions. After 3 days of an uninterrupted stay in a rodent's cage, fleas were collected as described above. Then, these fleas were fed daily on the same rodent individual using the first method during 5 days. Between feeding events (1–8 days for the first method and 4–8 days for the second method), fleas of each group (i.e., recovered from the same rodent individual) were placed in 50 ml glass vials and were then transferred to an incubator (see above) at 25 °C air temperature and 90% RH for 24 h. Each day, we collected all fleas from each vial and released them onto a rodent for 1 h. Eggs produced by each group of fleas in each day of oviposition were counted. Only eggs produced during days 6 to 8 from the onset of experiments were taken for subsequent analyses.

Eggs produced in the same day were transferred into new vials. These vials were filled with a 3 mm layer of sand and larval food medium (94% dry bovine blood, 5% millet flour, and 1% grinded excrements of *M. crassus*) and were covered by perforated lids. To ensure excess food for each larva, the amount of larval medium added to each vial was calculated as the necessary daily amount, times the maximum duration of larval stage (see details in Khokhlova *et al.* 2010) times the number of eggs in a vial and then tripled. Vials were than maintained at 25 °C air temperature and 90% RH. Temperature was regulated in refrigerated incubators (see above) and humidity was regulated in 38 × 23 × 13 cm acrylic humidity chambers using saturated salt solutions. Temperature and humidity were monitored using a Fisherbrand Traceable Humidity/Temperature Pen with Memory (Fisher Scientific International, NJ, USA).

Minimal duration of metamorphosis (i.e., from egg to adult) of *X. ramesis* at 25 °C air temperature and 92% RH is 25 days (Krasnov *et al.* 2001). Consequently, starting from the 18th day after an egg was produced, we checked each vial daily until all adults emerged (i.e., the number of emerged adults was equal to the number of eggs) or for 60 consecutive days. We counted new imagos produced by each group of parent fleas. After emergence, each adult was transferred to an Eppendorf vial with a perforated lid and the bottom covered by a thin layer of clean sand and left in the incubator at the same air temperature and RH. Vials with newly-emerged adults were checked daily until all adults died. After the death of each imago, we identified its sex by examination of its genitalia under light microscopy.

After the death of an adult, we measured its body size via maximal length of its right hind femur. The use of direct measure of body size (e.g., body length) of a dead adult was not possible because the body shape of a flea could be distorted after starvation and desiccation. The reason behind this distortion is the high flexibility of the thorax and abdomen because thoracic and abdominal segments do not possess posterior walls (Medvedev and Krasnov, 2006). In addition, body length of fleas may vary with pressure applied to the specimens when preparing them between slides and cover-slides making body length an inaccurate indicator of body size (Tripet *et al.* 2002). In contrast, the length of a femur was shown to be a reliable indicator of body size in fleas because these traits were strongly correlated (Krasnov *et al.* 2003; Khokhlova *et al.* 2010). The length of the femur was measured on a screen using a digital microscope camera Moticam 2000 with the Motic Images Plus 2.0ML program (Motic, Speed Fair Cp., Ltd, Causeway Bay, Honkong) to the nearest 0·01 mm under a magnification of 40X and with calibration using an object-micrometer.

Data analyses

In the analyses of egg and new imago production, a replicate represented a group of fleas feeding simultaneously on a rodent. In total, there were 177 groups

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Table 1. Summary of significant ($P<0{\cdot}05$ for all) effects in 2- and 3-way ANOVAs of flea (*X. ramesis*) reproductive variables as affected by age and gender of a rodent (*Meriones crassus*) host and/or gender of a flea

(Reproductive variables are as follows. EP, egg production (mean number of eggs produced per female flea during 6–8 days of feeding on a host); NIP, new imago production (number of new imago from eggs produced per female flea during 6–8 days of feeding on a host); DD, duration of development (time of development from oviposition till emergence); RS, resistance to starvation (time till death from emergence under starvation), BS, body size (length of right hind femur).)

Reproductive variable	Effect	D.F.	SS	*F*
EP	Host age	3	6·33	23·5
	Host age × Host gender	3	0·96	3·9
NIP	Host age	3	2·68	12·5
	Host gender	1	0·43	6·0
DD	Host age	3	5·04	111·0
	Host gender	1	0·14	9·0
	Flea gender	1	15·15	1003·2
	Host age × Host gender	3	0·54	12·0
	Host age × Flea gender	3	0·56	12·0
	Host gender × Flea gender	1	0·25	17·0
	Host age × Host gender × Flea gender	3	0·59	13·0
RS	Host age	3	27·73	66·8
	Host age × Host gender	3	2·01	4·9
	Host age × Flea gender	3	1·44	3·5
BS	Host age	3	0·003	27·8
	Flea gender	1	0·15	3989·0
	Host age × Host gender	3	0·001	4·8
	Host age × Flea gender	3	0·001	6·8
	Host age × Host gender × Flea gender	3	0·001	3·8

of female fleas that produced eggs. In 24 of these groups, all larvae died prior to pupation due to unknown reasons. These groups were excluded from the analysis. We calculated the number of eggs produced per female flea during 6–8 days of feeding and the number of new imago emerged from these eggs. Initially, these dependent variables were analysed using 3-way ANOVAs with host age, host gender and method of flea feeding as independent variables. No effect of the method of feeding on any of the dependent variables was found ($F_{1,145}=0{\cdot}39$ and $F_{1,145}=0{\cdot}22$; $P>0{\cdot}50$ for both). Consequently, in the final analyses of egg and new imago production, data obtained using the two methods of feeding were pooled, and dependent variables were analysed by 2-way ANOVAs with host age and gender as independent variables. We used univariate tests of significance for planned comparisons to compare dependent variables within host age and gender.

In the remaining analyses, a replicate represented an individual flea. In total, there were 2369 newly emerged fleas (1170 males and 1199 females). Of these, we measured the length of the right femur in 1888 fleas (932 males and 956 females). For each newly emerged flea, we calculated (a) time of development from oviposition till emergence and (b) time from emergence until death under starvation. These data were analysed using 3-way ANOVAs with host age, host gender and flea gender as independent variables. We used univariate tests of significance for planned comparisons to compare dependent variables within host age, host gender and flea gender.

All dependent variables, except for emergence success, were log-transformed prior to analysis. We applied angular transformation to emergence success. Figures represent non-transformed data.

RESULTS

Summary of ANOVAs for the effect of host age and gender and/or gender of a newly emerged flea on number and quality of flea offspring are presented in Table 1. A significant independent effect of host age, but not host gender on flea egg production was found. However, a significant interaction between host age and gender demonstrated that the effect of host age on egg production differed in the response of a parent flea exploiting either a male or female host. In fleas fed on subadult and adult rodents, egg production was significantly higher for those fed on male than on female hosts ($F=5{\cdot}1$ and $F=3{\cdot}9$, respectively; $P<0{\cdot}05$ for both; Fig. 1). However, this was not the case for fleas fed on either juvenile or old hosts ($F=1{\cdot}9$ and $F=1{\cdot}5$, respectively; $P>0{\cdot}16$ for both; Fig. 1). In general, fleas fed on male hosts produced significantly

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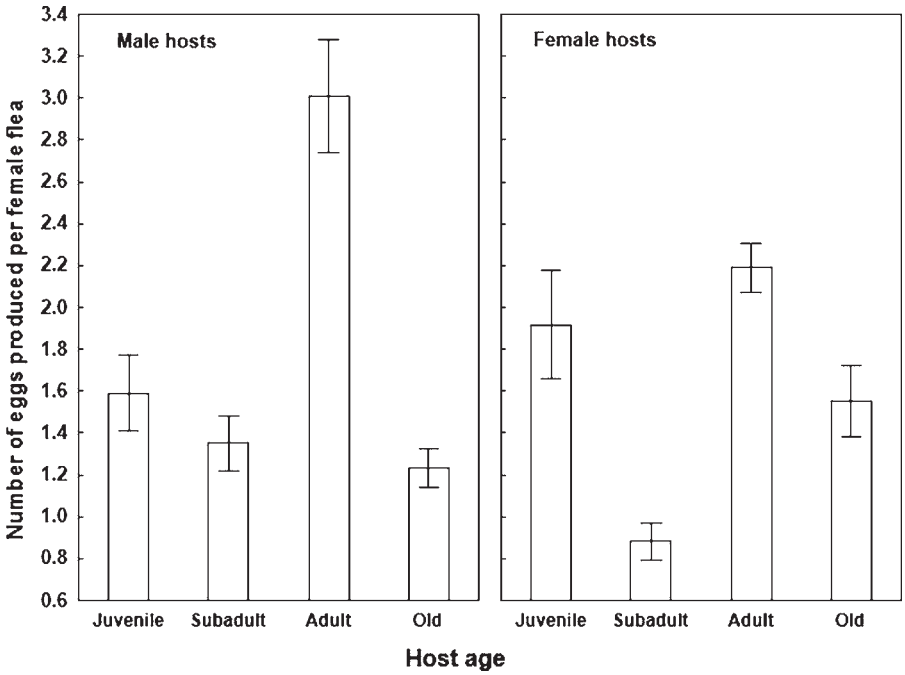

Fig. 1. Mean (±S.E.) number of eggs produced per female *Xenopsylla ramesis* during 6–8 days of feeding on juvenile, subadult, adult and old male and female *Meriones crassus*.

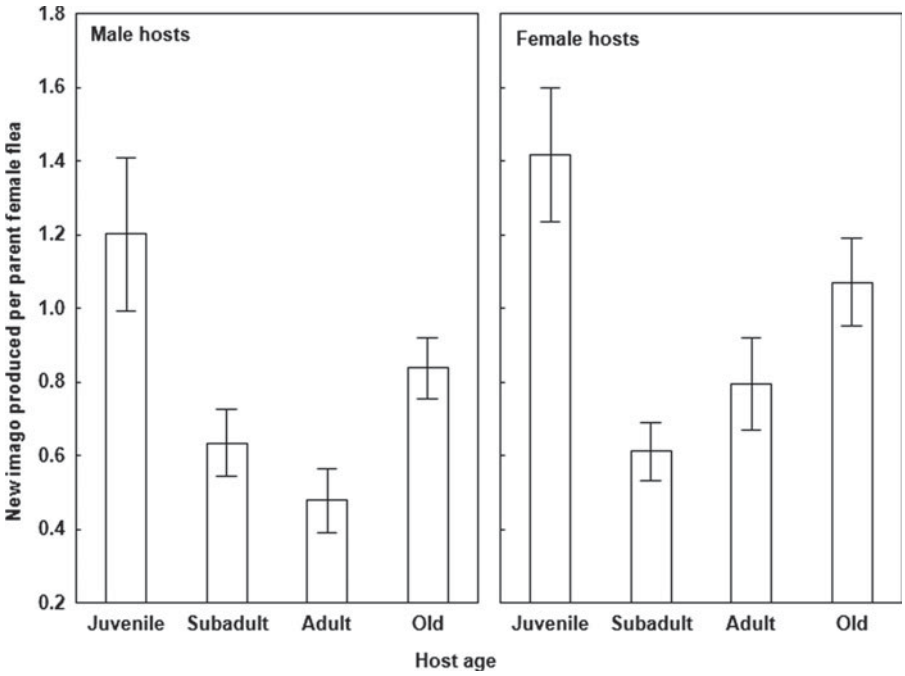

Fig. 2. Mean (±S.E.) number of new imago produced per female *Xenopsylla ramesis* during 6–8 days of feeding on juvenile, subadult, adult and old male and female *Meriones crassus*.

more eggs on adult hosts than the other age groups ($F=49{\cdot}2$, $P<0{\cdot}001$; Fig. 1), which did not differ among themselves ($F=0{\cdot}01$, $P=0{\cdot}9$; Fig. 1). In experiments with female hosts, fleas produced more eggs (a) on juvenile and adult than on subadult and old rodents ($F=20{\cdot}2$, $P<0{\cdot}001$; Fig. 1) and (b) on adult than on subadult rodents ($F=9{\cdot}3$, $P<0{\cdot}01$; Fig. 1).

The number of new imago was affected significantly by host age and gender, while interaction between these factors was not significant (Table 1). Significantly more new imago emerged if a parent flea fed on juvenile and old than on subadult or adult rodents ($F=19{\cdot}0$ for male hosts and $F=14{\cdot}4$ for female hosts; $P<0{\cdot}01$ for both; Fig. 2). Significantly more new imago emerged from eggs laid by females fed on female than on male adult rodents ($F=4{\cdot}0$, $P<0{\cdot}05$; Fig. 2), while female fleas fed on hosts belonging to the 3 remaining cohorts produced

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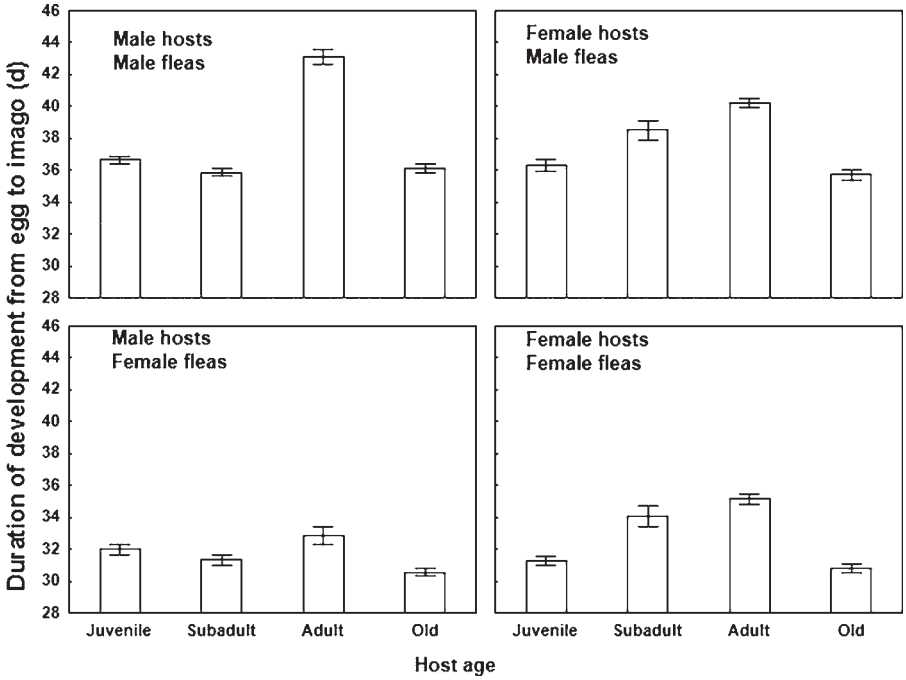

Fig. 3. Mean (±S.E.) duration of development from egg to imago in *Xenopsylla ramesis* from females fed on juvenile, subadult, adult and old male and female *Meriones crassus*.

similar number of new imago, independent of host gender ($F=0{\cdot}01$–$1{\cdot}7$, $P>0{\cdot}19$ for all; Fig. 2).

Duration of development of flea offspring was affected significantly by host age and gender and differed significantly between male and female fleas, while all between-factor interactions were significant (Table 1). It took fleas a significantly longer time to develop if their mothers fed on either adult or both subadult and adult hosts ($F=150{\cdot}8$ for male rodents and $F=128{\cdot}3$ for female rodents; $P<0{\cdot}001$ for both; Fig. 3) than if they fed on hosts belonging to other age cohorts (Fig. 3). Female fleas generally developed faster than male fleas ($F=1003{\cdot}2$, $P<0{\cdot}01$; Fig. 3). Regarding the effect of host gender, female offspring of fleas on subadult and adult male hosts developed faster than those on subadult and adult female hosts ($F=22{\cdot}4$ and $F=38{\cdot}8$, respectively; $P<0{\cdot}01$ for both). The same was true for male offspring of fleas fed on subadult hosts ($F=15{\cdot}4$, $P<0{\cdot}01$; Fig. 3), whereas male offspring developed longer if their parents fed on adult male than female rodents ($F=19{\cdot}5$, $P<0{\cdot}01$; Fig. 3). No effect of host gender on duration of development was found for flea offspring from parents fed on juvenile and old hosts ($F=0{\cdot}16$–$2{\cdot}21$, $P>0{\cdot}13$ for all; Fig. 3).

Among the 3 independent factors, only host age and interactions between host age and either host or flea gender significantly affected time of survival under starvation of flea offspring (Table 1). Fleas from mothers fed on old hosts died under starvation faster than those on juvenile, subadult and adult hosts ($F=150{\cdot}6$, $P<0{\cdot}01$; Fig. 4), except for male offspring of fleas fed on female hosts. The latter survived starvation longer if their hosts belonged to younger (juvenile and subadult) than older (adult and old) age cohorts ($F=25{\cdot}0$, $P<0{\cdot}01$; Fig. 4). The effect of interaction between host age and gender showed that male (but not female) offspring from fleas fed on adult male hosts survived longer than those on adult female hosts ($F=16{\cdot}2$, $P<0{\cdot}01$; Fig. 4), while no difference was found between male and female hosts of other age categories ($F=0{\cdot}3$–$2{\cdot}3$; $P>0{\cdot}31$ for all; Fig. 4). Interaction between host age and flea gender showed significant difference in survival under starvation between male and female flea offspring only for adult female hosts (female fleas survived longer than males; $F=6{\cdot}3$, $P=0{\cdot}01$; Fig. 4).

Body size of flea offspring was affected by both host age and gender and differed significantly between males and females (larger females; Table 1, Fig. 5). In addition, two 2-way and the 3-way interactions were significant (Table 1). Fleas fed on old hosts produced the largest offspring ($F=36{\cdot}4$ for males and $F=45{\cdot}7$ for females; $P<0{\cdot}001$ for both; Fig. 5), while the size of newly emerged females did not differ among the remaining age cohorts ($F=1{\cdot}14$ for male hosts and $F=0{\cdot}45$ for female hosts; $P<0{\cdot}28$ for both; Fig. 5). The same was true for newly emerged males from parents fed on male hosts ($F=0{\cdot}2$, $P=0{\cdot}65$). In contrast, fleas fed on adult female hosts produced the smallest male fleas ($F=18{\cdot}6$, $P<0{\cdot}001$; Fig. 5). The interaction among factors showed a significantly different size of (a) new male imago from fleas fed on male than female adult hosts ($F=13{\cdot}6$, $P<0{\cdot}001$ versus $F=0{\cdot}03$–$2{\cdot}2$, $P>0{\cdot}05$; larger offspring produced from male hosts; Fig. 5) and (b) new female imago from fleas fed on male than female juvenile

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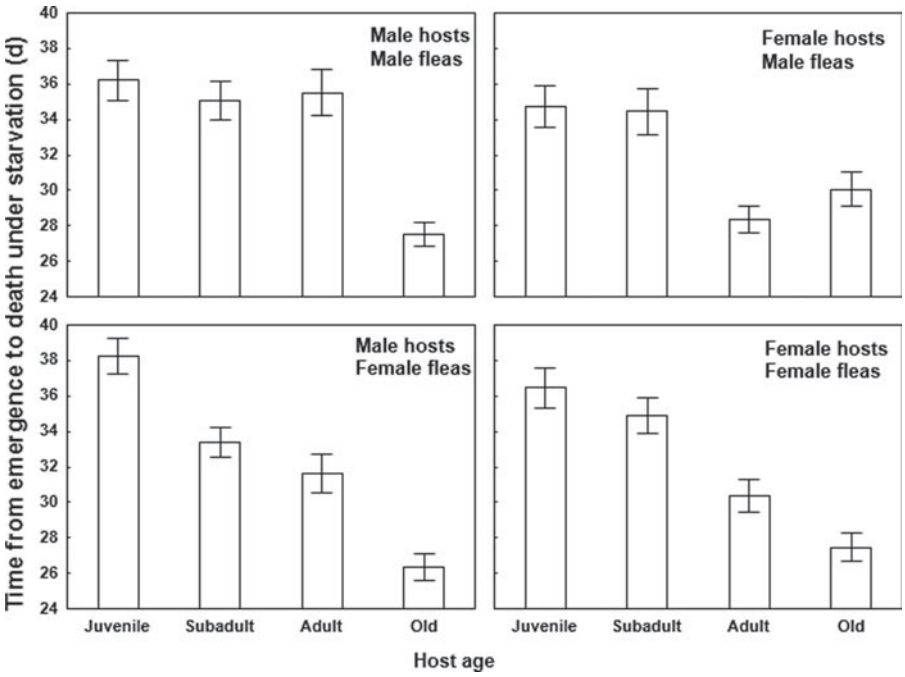

Fig. 4. Mean (±S.E.) time of survival from emergence under starvation in *Xenopsylla ramesis* from females fed on juvenile, subadult, adult and old male and female *Meriones crassus*.

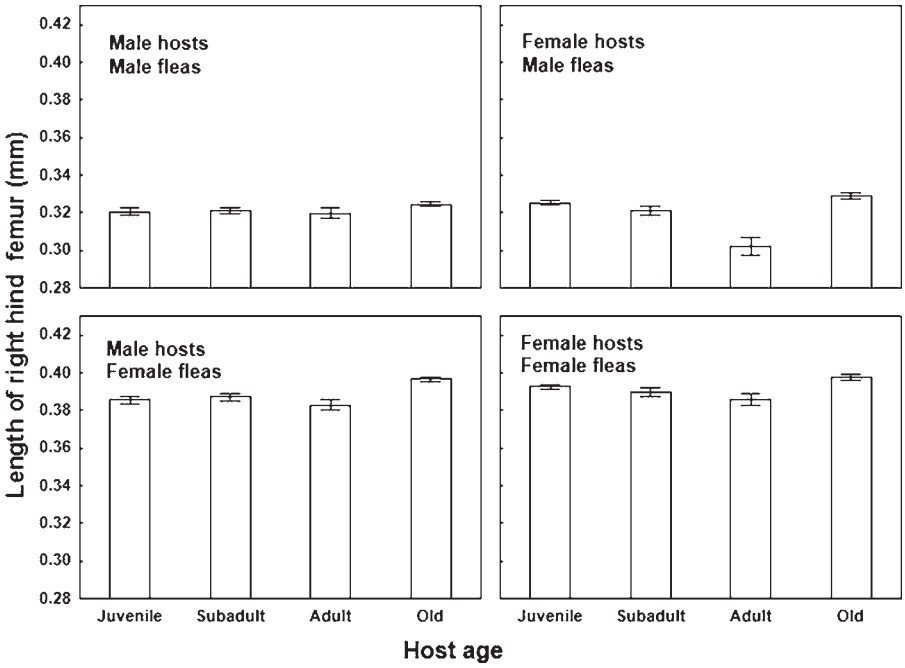

Fig. 5. Mean (±S.E.) length of right hind femur in male and female offspring *Xenopsylla ramesis* produced by females fed on juvenile, subadult, adult and old *Meriones crassus*.

hosts ($F=9{\cdot}5$, $P<0{\cdot}01$ versus $F=0{\cdot}22$–$0{\cdot}99$, $P<0{\cdot}30$; larger offspring produced from female hosts; Fig. 5).

DISCUSSION

Results of this study supported our predictions. Although fleas produced more eggs when they exploited adult hosts than when they exploited juvenile, subadult and old hosts, the net results of their reproduction (that is, number of individuals of new generation) undoubtedly pointed to a better reproductive performance on juvenile and old hosts. The difference between the pattern of egg and new imago production indicated that the quality of eggs produced from juvenile and old hosts was higher than those from subadult and adult hosts because a higher proportion of them attained emergence.

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Furthermore, fleas performed better when they fed on juvenile and/or old hosts in 2 of 3 measures of offspring quality (duration of development and body size). Nevertheless, when offspring quality was estimated via resistance to starvation of a new imago, fleas demonstrated good performance when exploiting young (juvenile and subadult) hosts, while they performed poorly when exploiting old hosts. In general, thus, reproductive performance of fleas was better when they exploited the youngest and the oldest hosts when compared to hosts of median age. However, the effect of host age on flea reproductive performance was manifested somewhat differently (a) when fed on male and female hosts and (b) between male and female flea offspring. In other words, the effect of host age on flea reproduction was mediated by host gender. The effect of host gender on flea reproduction has been discussed in our earlier studies (Khokhlova *et al*. 2009, 2010). Consequently, we focus here mainly on the effect of host age.

Reproductive success of an individual is a net result of quantity of a resource and its quality. Furthermore, the amount of the resource available for a consumer depends not only on the amount of the resource in the surrounding environment (a host, in case of parasites) but can be affected by the pattern of its acquisition. Indeed, it is highly unlikely that the amount of resource varies among hosts belonging to different age cohorts, especially given that fleas usually consume only a small portion of host blood (Khokhlova *et al*. 2002). However, these hosts possess different defence abilities which can affect the amount of blood a flea can obtain. An individual host attacked repeatedly by an ectoparasite develops acquired resistance against this ectoparasite (Willadsen, 1980; Rechav, 1992) which is manifested by decreased feeding and reproduction of the ectoparasite (e.g., Rechav *et al*. 1989; Fielden *et al*. 1992; Khokhlova *et al*. 2008). For example, a study of acquired resistance in guinea pigs showed that repeated infestation of guinea pigs by tick larvae resulted in a sharp reduction in body mass of the larvae (Fielden *et al*. 1992). Thus, higher reproductive outcome and higher quality of offspring in juvenile and, partly, old hosts when compared with subadult and adult hosts can be explained by lower defence abilities of the former, which affects the amount of blood a flea is able to take. Indeed, in our earlier study, we found that fleas took more blood from juvenile and old than from subadult and adult animals (Liberman *et al*. 2011).

The most likely reason behind lower defence abilities of juveniles is their under-developed immune system. Although they can have some protective, albeit not especially effective, immunity transferred from their mothers during pregnancy and lactation (Knopf and Coghlan, 1989; Carlier and Truyens, 1995; Hasselquist and Nilsson, 2009), their skin immunity is functionally immature (Dewar *et al*. 2001). Nevertheless, additional nutrition may provide additional resources to be invested in immune defence which may increase anti-ectoparasite resistance (McCoy *et al*. 2002; Tschirren and Richner 2006). An increase in immunity of nestlings due to additional provisioning by parents has been reported for birds (Saino *et al*. 1997), but an increase in immunity due to increased parent provisional effort (via foraging or lactation) is unlikely to occur in pre-weaned small mammals. This is supported by the fact that milk production and milk fat concentration in female rodents decreases with the age of pre-weaned pups (Kam and Degen 1993; Hinde, 2007) likely because the latter start to feed independently from about the 17th day of age.

Lower defence abilities of old hosts could result from immunosenescence. It is well known that immune defences often deteriorate with age (Tarazona *et al*. 2002; Gruver *et al*. 2007) and, as a result, anti-parasitic defence in old animals is generally weak (Klein, 2004). The decline of anti-parasitic defence with age has been reported for both birds (e.g., Saino *et al*. 2003) and mammals (Pelletier *et al*. 2005; Body *et al*. 2011). The degree of deterioration of the immune function with age can be, however, affected by environmental conditions (e.g., environmental stress; Hayward *et al*. 2009) and may differ between males and females (due to faster aging of males; Clutton-Brock and Isvaran, 2007). However, we did not find any indication of the latter effect in our study.

The pattern of the effect of host age on survival of new imago under starvation differed from that of the 2 other measures of flea offspring quality in this study. Survival of new imago was the shortest if their parents fed on old hosts. This suggested that quality of parasite offspring might be affected not only by the amount of resource (which is relatively high in the case of old hosts; see Liberman *et al*. 2011), but also by the quality of this resource and that nutritional value of blood taken from an old host is likely to be low. The nutritional value of host's blood consumed by a flea can affect its offspring via transfer from a parent as well as in a direct way. As noted above, a newly emerged flea possesses stored energy in fat tissue. It is commonly accepted that the larval stages in holometabolous insects serve to accumulate substrates that allow the imago to emerge with significant fat body stores (Gilbert and Chino, 1974; Anand and Lorenz, 2008). Flea larvae are not parasitic and feed on all kinds of organic debris found in the host's burrow or nest. This debris often includes flea faeces (Silverman and Appel, 1994). Moreover, in some species, females have been shown to expel faecal pellets near the clutch which can later serve as a food source for larvae (see review in Krasnov, 2008). The protein content of flea feces was actually higher than the blood upon which they fed (Hinkle *et al*. 1991). Consequently, flea larvae consume host blood via

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fecal pellets of parent fleas and may be, thus, be directly affected by the quality of this blood. Shortest survival of new imago from parents fed on old hosts hinted on some deterioration of nutritional value of their blood, although resistance to starvation in parent fleas after a direct bloodmeal from a senescent host was not compromised (Liberman *et al*. 2011).

In conclusion, our results suggest that the reproductive performance of a flea is affected by the age of its host and is a trade-off between quantity (determined by defence abilities of a host) and quality (nutritional value) of the resource taken from a host. Furthermore, different manifestation of the host age effect on reproductive performance of parasites (a) due to host gender and (b) in male and female offspring may be the reasons behind variation in age-related patterns of parasite infestation (Krasnov *et al*. 2006).

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