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RESEARCH ARTICLE

Digesting blood of an auxiliary host in fleas: effect of phylogenetic distance from a principal host

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SUMMARY

Fleas are haematophagous ectoparasites that exhibit varying degrees of host specificity. Flea abundance is highest on principal hosts and lower on auxiliary hosts but may vary greatly among auxiliary hosts. We investigated the feeding and energy expenditure for digestion in two flea species *Parapulex chephrenis* and *Xenopsylla ramesis* on a principal host (*Acomys cahirinus* and *Meriones crassus*, respectively) and eight auxiliary host species. We predicted that fleas would perform better – that is (i) a higher proportion of fleas would take a blood meal, (ii) fleas would take larger blood meals and (iii) fleas would spend less energy on digestion – if they fed on (i) a principal host compared with an auxiliary host and (ii) an auxiliary host phylogenetically close to a principal host compared with an auxiliary host phylogenetically distant from a principal host. Energy costs of digestion were estimated using CO_2 emission and represented energy cost during the first stage of blood digestion. Contrary to our predictions, fleas did not always perform better on a principal than on an auxiliary host or on auxiliary hosts phylogenetically closer to the principal host than on auxiliary hosts phylogenetically distant from a principal host. Variation in flea feeding performance may result from the interplay of several factors including co-occurrence between hosts and susceptibility of a host to flea attacks, the species-specific level of immunocompetence of a host and the level of host specificity of a flea. This study describes the first investigation into the metabolic expenditure of parasitism and its relationship to phylogenetic relationships amongst hosts.

Key words: fleas, digestion, energy, rodents.

INTRODUCTION

It is well known that there is large variation in the abundance of a parasite among different host species. The host with the highest abundance of a parasite is commonly defined as the principal host, while hosts with a lower parasite abundance are defined as auxiliary hosts (Dogiel et al., 1961; Marshall, 1981; Poulin, 2005; Poulin, 2007). The principal host may or may not be the species in which the parasite first evolved, but it is currently the one harbouring the majority of individuals of the parasite population (Poulin, 2005). The reasons for differences in abundance of parasites between the principal and any auxiliary host are often associated with the different exploitative and reproductive success of a parasite in a principal host compared with an auxiliary host (e.g. Krasnov et al., 2002; Krasnov et al., 2003). Furthermore, the abundance of a parasite among auxiliary hosts often varies greatly. The causes of this important variation are largely not known. Krasnov and colleagues (Krasnov et al., 2004a) suggested that phylogenetic/taxonomic relatedness between the principal and auxiliary host species may determine what abundance a parasite can achieve on its auxiliary hosts because relatedness should reflect similarities among host species in ecological, physiological and/or immunological characteristics (see also Poulin, 2005). It was found that for fleas (Siphonaptera) parasitic on small mammals, the abundance of a flea on its auxiliary hosts decreased with increasing phylogenetic distance of these hosts from the principal host (Krasnov et al., 2004a). The mechanisms underlying this pattern remain unclear, but are supposedly associated with the differential performance of a flea on auxiliary hosts, which in turn correlates with phylogenetic distance of the auxiliary host from the principal host.

A recent study on feeding performance of fleas on either a principal or an auxiliary host during 2 days of uninterrupted host availability demonstrated that fleas did not always perform better on a principal than on an auxiliary host (Khokhlova et al., 2012). Moreover, in some cases, fleas fed better (e.g. took larger blood meals) on hosts that were phylogenetically distant from their principal host than on hosts that were close. One of the reasons behind these results could be the fact that feeding performance was measured in fleas that were previously fed, while measurement of feeding performance of newly emerging fleas (that is, at the very first feeding in their life) might provide clearer results. This is because previously fed fleas digest blood faster than newly emerged individuals (Vashchenok et al., 1988) as a result of a higher metabolic rate in the former (Fielden et al., 2004) as well as re-distribution of the fat tissue and an increase in activity of a non-specific esterase after the first blood meal (Xun and Qi, 2004; Xun and Qi, 2005). Furthermore, feeding patterns of fleas on different

species might not be the best proxy for comparative assessment of their general performance and evolutionary success on these species as it is not a direct measure of efficiency of resource utilization. Rather, the efficiency of processing the blood meal – specifically, the energy expenditure of a flea for digestion of the host's blood – could be an important reason for a higher abundance of fleas on a principal than on an auxiliary host. The lower energy expenditure of a flea for digestion of blood of a given host would allow allocation of more energy for reproduction and an increase in the number of offspring.

We hypothesized that the feeding performance of newly emerged fleas on an auxiliary host would correlate negatively with phylogenetic distance between this host and the principal host. To test this hypothesis, we examined feeding patterns (willingness to take a blood meal and the size of the first blood meal) and the energy cost of blood digestion (the amount of energy allocated for digestion of this blood meal) in newly emerged fleas of two species (*Parapulex chephrenis* and *Xenopsylla ramesis*) parasitizing a principal and eight auxiliary hosts. We predicted that fleas would perform better – that is, (i) a higher proportion of fleas would take a blood meal, (ii) fleas would take larger blood meals and (iii) fleas would spend less energy on digestion, if they fed on an auxiliary host phylogenetically close to a principal host compared with one that is phylogenetically distant from a principal host. We also predicted that fleas would perform better when they parasitized a principal host rather than any auxiliary host. In addition, we tested whether the relationship between flea performance and phylogenetic distance between an auxiliary host and the principal host would differ between fleas with a different degree of host specificity. We predicted that the effect of phylogenetic distance between the principal and auxiliary host on flea performance on the auxiliary host would be more strongly manifested in the host-specific than in the host-opportunistic flea. Because *P. chephrenis* and *X. ramesis* differ in the pattern of host exploitation (time required for satiation, frequency of feedings, time spent on and off the host body; see below), we analysed the results separately for each flea species and compared performance within rather than between flea species.

MATERIALS AND METHODS

Fleas

Both flea species in this study are common parasites of rodent hosts in the central Negev Desert. *Parapulex chephrenis* (Rothschild, 1903) is a host specialist with the principal host being the Egyptian spiny mouse, *Acomys cahirinus*. This flea species is also found on a congeneric golden spiny mouse, *Acomys russatus*, and occasionally on the gerbils *Meriones crassus* and *Gerbillus dasyrus* (Krasnov et al., 1997; Krasnov et al., 1999). Host-opportunistic *X. ramesis* Rothschild parasitizes a variety of gerbilline species but is the most abundant and prevalent on Sundevall's jird, *Meriones crassus*, which is considered the principal host (Krasnov et al., 1997; Krasnov et al., 1999).

We used fleas from laboratory colonies started in 1999 from field-collected specimens. *Xenopsylla ramesis* were collected from *M. crassus*, *Psammomys obesus* and *G. dasyurus*, while *P. chephrenis* were collected from *A. cahirinus*. In our colonies, fleas were maintained on rodents (*X. ramesis* mainly on *M. crassus* and *P. chephrenis* on *A. cahirinus*) kept individually in plastic cages with a wire mesh floor over a pan with a mixture of sand and dried bovine blood (larvae feeding medium) at 25°C with a photoperiod of 12 h:12 h (L:D). Every 2 weeks, all substrate and bedding material from the rodent's nest box and pan were collected into plastic boxes with perforated lids and transferred to an incubator (FOC225E, Velp Scientifica srl, Milan, Italy; 25°C and 75% relative humidity) where the fleas developed. Once a week, newly emerged fleas were collected from these boxes and were used either for infestation of maintenance rodents or in experiments. Further details on breeding and maintenance of fleas are given elsewhere (e.g. Krasnov et al., 2002; Krasnov et al., 2003; Khokhlova et al., 2009a; Khokhlova et al., 2009b; Khokhlova et al., 2010).

Rodents

We used nine rodent species including five gerbils [*M. crassus* Sundeval 1842, *G. dasyurus* (Wagner 1842), *Gerbillus andersoni* de Winton 1902, *Gerbillus pyramidum* Goeffroy 1803 and *Gerbillus nanus* Blanford 1875), two spiny mice [*A. cahirinus* (É. Geoffrey 1803) and *A. russatus* (Wagner 1840)], a house mouse (*Mus musculus* L.) and a golden hamster (*Mesocricetus auratus* Waterhouse 1839). *Meriones crassus*, *G. dasyurus*, *G. nanus* and both spiny mice were from laboratory colonies started in 1997–1999 and 2009 (*G. nanus*) (for details, see Krasnov et al., 2002; Krasnov et al., 2003; Khokhlova et al., 2009a; Khokhlova et al., 2009b; Khokhlova et al., 2010). *Gerbillus andersoni* and *G. pyramidum* as well as feral house mice were captured in the wild in the Negev Desert. All ectoparasites from wild-caught rodents were removed immediately after capture. These rodents were maintained in a separate room for 2 months prior to experiments. Golden hamsters were available commercially.

Rodents in our colonies were maintained individually or in pairs in plastic cages (60×50×40 cm) at 25°C with a photoperiod of 12 h:12 h (L:D), and with sawdust and dried grass as bedding material. They were offered millet seed and fresh alfalfa (*Medicago* sp.) *ad libitum* daily. No water was available as the alfalfa supplied enough for their needs. *Acomys cahirinus* and *A. russatus* were offered commercial cat chow once a week. In this study, we used adult males that were maintained individually for at least 1 month prior to experiments and had previously been exposed to flea parasitism two to three times. Each individual rodent was used in experiments once only.

Experimental procedures

The experimental procedures complied with the laws of the State of Israel. The procedure that ensured similar age of fleas used in the experiments was as follows. An incubated development box was thoroughly checked for the first time a week after it was placed in an incubator. Fleas collected during the first check were returned to the colony and were not used in experiments. We checked each box every day and fleas collected were used in experiments on the same or the following day. Thus, all fleas used in experiments were newly emerged females (24–48 h after emergence from cocoons) selected randomly from the incubated development boxes. Measurement of feeding performance and CO_2 emission in the same flea species on each of the host species was done on the same day (one measurement for each host species per day).

We measured CO_2 emission in newly emerged females after they fed on a rodent host as well as in newly emerged unfed fleas (24–48 h old). Fleas were fed on rodents placed in a wire mesh (5×5 mm) tube (15 cm length and 5 cm diameter for *M. crassus*, spiny mice and hamsters or 10 cm in length and 2 cm in diameter for other gerbils and house mice) that limited movement and did not allow self-grooming. Each tube with a rodent was placed in an individual white plastic pan and 20 newly emerged fleas were released onto the rodent. We calculated mean body mass of an unfed flea. After allowing fleas to feed for 2 h (*X. ramesis*) or 6 h (*P. chephrenis*), we collected them from a rodent using custom-made forceps and

re-weighed them, taking the difference between body mass of fleas prior to and after feeding as the amount of blood consumed. The difference in flea feeding time is related to between-species differences in the time necessary for the majority of individuals to complete the blood meal successfully. This time was established for *P. chephrenis* and *X. ramesis* from our earlier studies (Sarfati et al., 2005; Khokhlova et al., 2008; Khokhlova et al., 2009a; Khokhlova et al., 2009c). After collecting fleas from a rodent, we examined the midgut of each flea under a light microscope (without dissection) to verify whether a flea took a blood meal and, if yes, to confirm the blood digestion status. Fleas that took a blood meal were found to be at the early stage of digestion (e.g. Khokhlova et al., 2009a). In other words, the status of digestion in *P. chephrenis* after 6 h was similar to that in *X. ramesis* after 2 h. The early stage has been shown to be energetically costly (Sarfati et al., 2005). We counted fleas that took a blood meal and fleas that refused to feed and re-weighed them separately.

Each treatment for each flea species (fleas fed on one of the nine host species or unfed) was replicated six to 13 times. Fleas were assigned to different treatments at random.

Respirometry

A flow-through respirometry system measured CO_2 emission. Incurrent air was first scrubbed of H_2O vapour by Drierite (700 ml volume) and then CO_2 by ascarite (25 ml volume) columns, and then pumped through a respirometer chamber made of Tygon tubing (6.5 mm internal diameter, 3 ml volume) at a flow rate of 50 ml min^{-1}. Flow rate was controlled by a mass flow controller (model FC-260, Tylan, Rancho Dominquez, CA, USA). This dry, CO_2-free air constituted the baseline measurements for all flow-through measurements. CO_2 content (p.p.m.) of air exiting the respirometer chamber, measured by a CO_2 analyser (model 6262, LI-COR, Lincoln, NE, USA) in conjunction with data-acquisition software (ExpeData, Sable Systems, Henderson, NV, USA), was sampled every 2 s. Tygon tubing (3.3 mm i.d.) was used to plumb the system. A stable temperature for the air inside the respirometer tubing (25°C) was regulated by placing the chamber and preceding 6 m of incurrent Tygon tubing into a water bath (model 1013S, Fisher Scientific, Pittsburgh, PA, USA). CO_2 emission for fleas was recorded for 30 min. Baseline measurements, each lasting 5 min, were made before and after each recording to determine zero CO_2 and to correct for any instrument drift. Fleas were measured in groups (N=2–10) as CO_2 emission of an individual flea was only slightly above baseline levels. Fleas from each group were fed at the same feeding bout on the same rodent individual. Variation in sample size of each group reflected the feeding success of fleas placed on different rodents. Only fleas that had taken a blood meal were used in respirometry measurements. Details of the protocol as well as details on the repeatability of the measurements can be found elsewhere (Fielden et al., 2004; Krasnov et al., 2004b; Sarfati et al., 2005; Khokhlova et al., 2009c).

Data analysis

The willingness of fleas to take a blood meal after 2 h of host availability was estimated *via* the proportion of fleas with blood in their midguts. The difference in body mass of fleas before (mean body mass of an unfed flea multiplied by the number of fleas that took blood) and after feeding (total body mass of fleas that took blood) per mg body mass before feeding was considered to be equal to the mass-specific amount of blood consumed.

Measurements of respiratory gas exchange were used to estimate the energy expenditure for blood digestion. All computerized CO_2 emission recordings were converted from p.p.m. to ml CO_2 h^{-1} and processed using the analysis package of ExpeData (see above). We calculated the mass-specific energy cost of a flea for digestion of 1 mg of blood of a given host during 1 h of the early digestion stage as follows. First, we calculated the volume of CO_2 emitted per hour per mg of body mass of a newly emerged unfed female flea on each day of measurement. Then, we used this value to calculate the difference in the mass-specific volume of emitted CO_2 between a digesting flea and an unfed flea for each respirometry measurement carried out on the same day. The quotient of the mass-specific difference in the volume of emitted CO_2 between the digesting and unfed flea and mass-specific amount of consumed blood (see above) was considered as a mass-specific indicator of energy expended for digestion per mg of blood. To convert the rate of CO_2 emission into energy expenditure, we used 24.5 J of energy produced per ml of CO_2 produced (Schmidt-Nielsen, 1990). We assumed a respiratory quotient of 0.8 as was determined previously for unfed female ticks *Amblyomma marmoreum* (Lighton et al., 1993).

We analysed dependent variables using one-way ANOVA with host species as an independent variable separately for *P. chephrenis* and *X. ramesis*. We used Fisher least significant difference (LSD) tests to compare the proportion of fleas that took a blood meal, the mass-specific blood meal size and the energy expenditure of blood digestion among host species.

To test for the relationships between willingness to take a blood meal, size of a blood meal and energy cost of digestion of blood taken from an auxiliary host and the phylogenetic distance between this host and the principal host of a flea, we calculated phylogenetic distances between a principal host (*A. cahirinus* for *P. chephrenis* and *M. crassus* for *X. ramesis*) and each of the remaining eight host species. Phylogenetic distances between hosts were calculated from the branch length of a phylogenetic tree using the package ‘ape’ (Paradis et al., 2004) implemented in the R 2.13.0 software environment (R Development Core Team, 2011). The phylogenetic tree (topology and/or branch length) for nine rodent species was based on previous studies (Jansa and Weksler, 2004; Chevret and Dobigny, 2005; Bininda-Emonds et al., 2007; Abiadh et al., 2010) [see the tree in Khokhlova et al. (Khokhlova et al., 2012)].

Then, we calculated mean values of variables describing flea feeding performance (that is, the proportion of fleas that took a blood meal, the size of the blood meal and the energy cost of digestion) on an auxiliary host and regressed them against the phylogenetic distance of an auxiliary host from a principal host. This last variable included phylogenetic information and, consequently, there was no need for further phylogenetic correction.

In addition, we tested whether willingness to feed on a host correlated with the size of a blood meal taken from this host within the flea species. This was done using Pearson’s product-moment correlations. Prior to analyses, dependent variables were either angular or log+1-transformed. Bar diagrams represent non-transformed data.

RESULTS

Feeding performance

Both the proportion of fleas that took a blood meal and the mean size of the blood meal differed significantly among host species in both flea species (Table 1). For *P. chephrenis*, more individuals took blood when the host was *A. cahirinus* or *A. russatus*, while fewer fleas fed on gerbils, *M. auratus* and *M. musculus* (Fisher LSD tests, P<0.05) (Fig. 1A). Fleas consumed significantly larger amounts of

Table 1. Summary of ANOVA of the effects of host species on dependent variables

Flea species	Dependent variable	SS	*F*	*P*
Parapulex chephrenis	Proportion of fed fleas	1.70	5.79	<0.001
	Size of a bloodmeal	0.02	2.2	0.03
	Energy expenditure for digestion	0.02	2.4	0.02
Xenopsylla conformis	Proportion of fed fleas	4.01	9.8	<0.001
	Size of a bloodmeal	0.22	10.2	<0.001
	Energy expenditure for digestion	0.003	1.7	0.11

Dependent variables: the proportion of newly emerged fleas (*P. chephrenis* and *X. ramesis*) that took bloodmeals during 2 h of feeding; mean mass-specific size of a bloodmeal; and energy expenditure for blood digestion.
SS, sum of squares.
d.f. (effect,error) was 8,88 for *P. chephrenis* and 8,77 for *X. ramesis*.

blood from *A. cahirinus*, *G. andersoni*, *G. nanus*, *M. auratus* and *M. musculus* than from the remaining hosts, including *A. russatus*, which is closely related to the principal host (Fisher LSD tests, $P<0.05$) (Fig. 1B). No relationship between either the proportion of fleas taking blood or the size of a blood meal taken from an auxiliary host and the phylogenetic distance between this host and the principal host (*A. cahirinus*) was found ($r^2=0.05$, $F=0.3$ and $r^2=0.16$, $F=1.2$, respectively; $P>0.05$ for both).

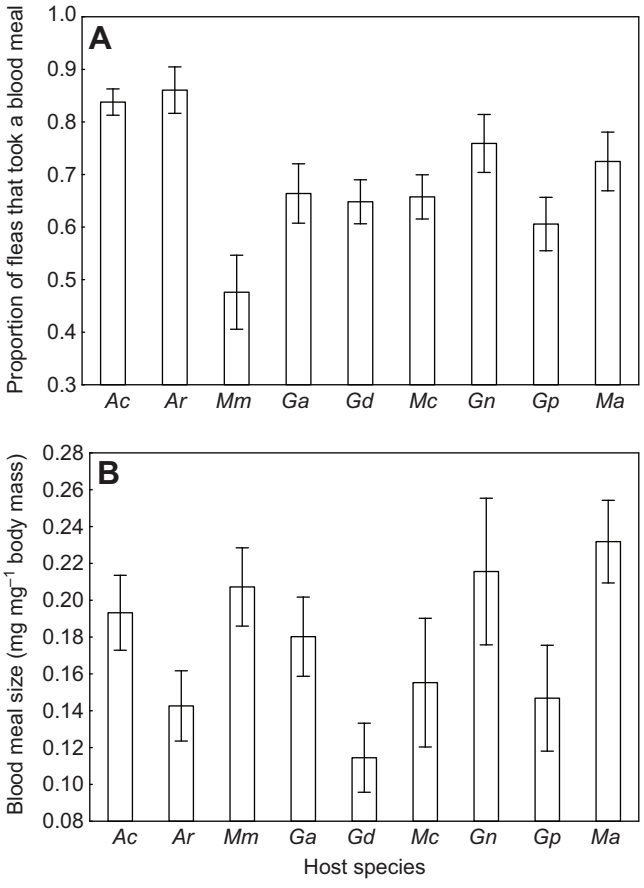

Fig. 1. Mean (±s.e.m.) (A) proportion of *Parapulex chephrenis* that took blood meals during 2 h of feeding and (B) blood meal size when feeding on different host species. Abbreviations of host species names: *Ac*, *Acomys cahirinus*; *Ar*, *Acomys russatus*; *Ga*, *Gerbillus andersoni*; *Gd*, *Gerbillus dasyurus*; *Gn*, *Gerbillus nanus*; *Gp*, *Gerbillus pyramidum*; *Ma*, *Mesocricetus auratus*; *Mc*, *Meriones crassus*; *Mm*, *Mus musculus*. Host species are ordered according to their phylogenetic distance from the principal host (*A. cahirinus*).

The willingness of *X. ramesis* to feed on a host measured by the proportion of individuals that took blood was significantly higher for all gerbilline hosts and *M. auratus* than for both *Acomys* species and *M. musculus* (Fisher LSD tests, $P<0.05$) (Fig. 2A). However, fleas took the largest blood meals from the spiny mice and the hamster and the smallest blood meals from the hosts belonging to the genus *Gerbillus* (Fisher LSD tests, $P<0.05$) (Fig. 2B). As was the case for *P. chephrenis*, the proportion of fleas taking blood from an auxiliary host was not affected by the phylogenetic distance between this host and the principal host (*M. crassus*) ($r^2=0.26$, $F=2.1$, $P>0.05$). However, the size of the blood meal from an auxiliary

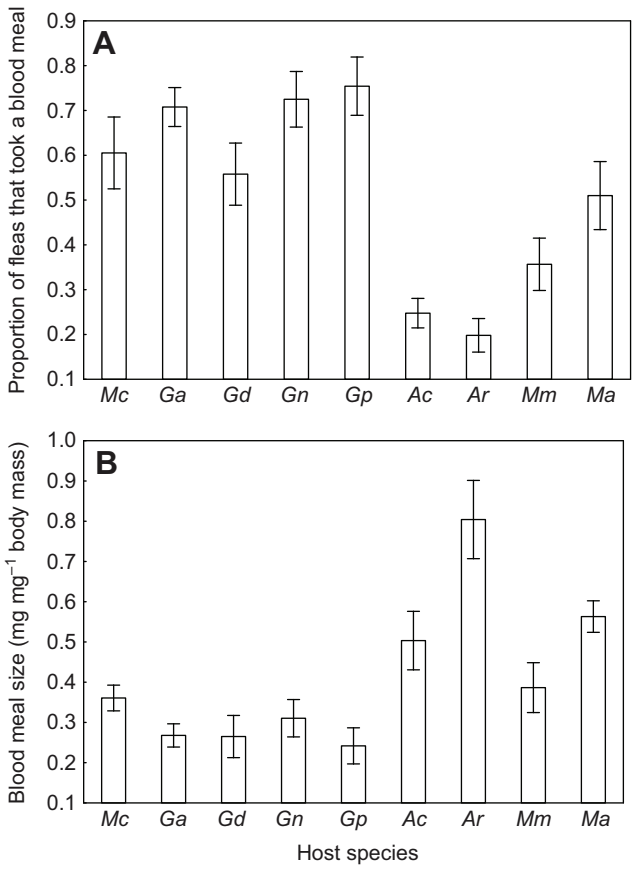

Fig. 2. Mean (±s.e.m.) (A) proportion of *X. ramesis* that took blood meals during 2 h of feeding and (B) blood meal size when feeding on different host species. See Fig. 1 for abbreviations of host species names. Host species are ordered according to their phylogenetic distance from the principal host (*M. crassus*).

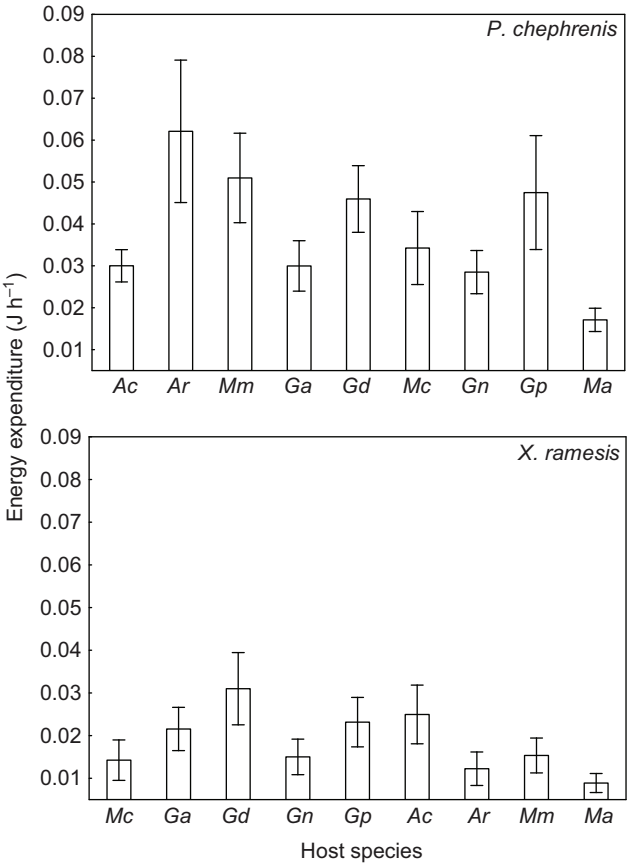

Fig. 3. Mean (±s.e.m.) energy expenditure for digestion per mg of blood taken from different host species in *P. chephrenis* and *X. ramesis*. See Fig. 1 for abbreviations of host species names. Host species are ordered according to their phylogenetic distance from the principal host (*A. cahirinus* for *P. chephrenis* and *M. crassus* for *X. ramesis*).

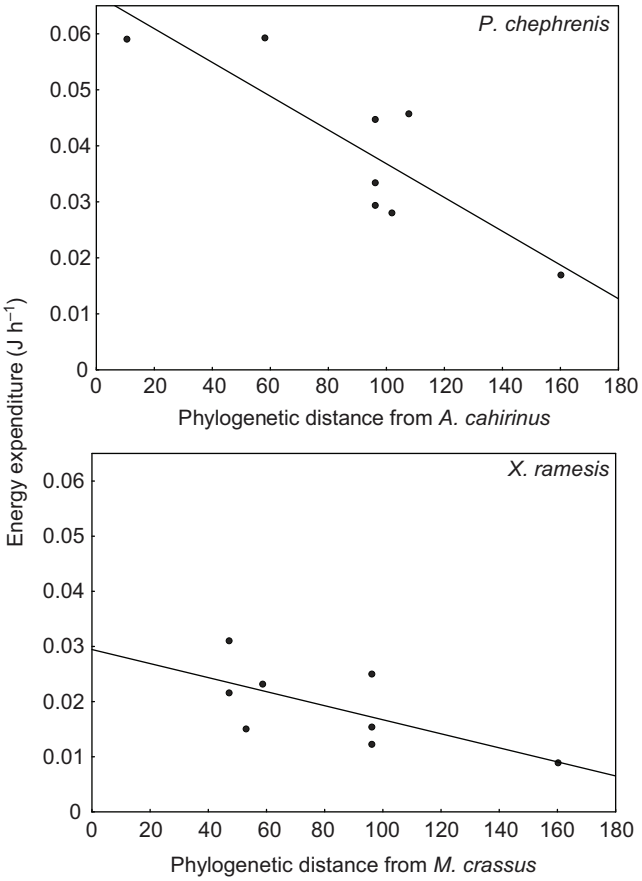

Fig. 4. Relationships between energy expenditure for digestion per mg blood of an auxiliary host and phylogenetic distance between an auxiliary host and the principal host in *P. chephrenis* and *X. ramesis*. Phylogenetic distances among hosts were calculated from branch length of the phylogenetic tree of Bininda-Emonds et al. (Bininda-Emonds et al., 2007). In this tree, branch lengths are proportional to time.

host significantly increased with an increase in phylogenetic distance between this host and *M. crassus* (r^2=0.48, F=5.8, slope=0.001±0.0004, P=0.05).

In *X. ramesis*, the proportion of fleas that took a blood meal correlated negatively with the mean size of a blood meal (r=–0.82; P<0.05) but no significant correlation between these variables was found in *P. chephrenis* (r=0.01, P>0.05).

Energy expenditure for blood digestion

The effect of host species on energy expenditure for blood digestion was significant in *P. chephrenis*, but not in *X. ramesis* (Table 1). The former expended significantly less energy when digesting blood from the principal host (*A. cahirinus*), *G. andersoni*, *G. nanus* and *M. auratus* than from the remaining host species (Fisher LSD tests, P<0.05) (Fig. 3). The highest amount of energy was spent on digestion of blood from *A. russatus* and *M. musculus* (Fig. 3). Although no general effect of host species on the energy cost of digestion was found in *X. ramesis*, pairwise between-host comparisons demonstrated that digestion of blood from *M. auratus* and *A. russatus* was significantly less costly than digestion of blood from the other host species (Fisher LSD tests, P<0.05) (Fig. 3). Furthermore, energy expenditure for digestion of blood taken by *P. chephrenis* from an auxiliary host increased significantly with a decrease in phylogenetic distance between this host and the principal host (*A. cahirinus*) (r^2=0.71, F=15.3, P<0.05) (Fig. 4). The same was true for *X. ramesis* (r^2=0.45, F=15.3, P<0.01) (Fig. 4).

DISCUSSION

In general, the results of this study did not support our predictions that fleas would perform better – that is, more individuals would feed, they would take larger blood meals and would spend less energy on blood digestion – when parasitizing a principal host compared with an auxillary host or that fleas would perform better when parasitizing an auxiliary host that is phylogenetically closer to a principal host compared with a more distant auxiliary host. Although the proportion of fleas that chose to feed was higher on the principal host and closely related auxiliary hosts than on distantly related auxiliary hosts (spiny mice *versus* gerbils in *P. chephrenis* and gerbillines *versus* murines in *X. ramesis*), the size of the blood meal taken from an auxiliary host in *X. ramesis* increased and energy expenditure for digestion of this blood in both fleas decreased with an increase in phylogenetic distance between the auxiliary host and the principal host. In addition, we found a negative correlation between the proportion of fleas that took a blood meal and blood meal size in *X. ramesis* but not in *P. chephrenis*. This suggests that although in some cases only a few individual *X. ramesis* made a decision to take blood from an auxiliary host, they took a large volume of blood, most probably compensating for a supposedly low quality resource.

Physiological perspective

The feeding patterns of fleas taking the first blood meal in their lives are consistent with feeding patterns of the same flea species that took consecutive blood meals (Khokholova et al., 2012), although in our earlier studies we found that the first blood meal was usually larger than any following blood meal (e.g. Khokhlova et al., 2009b). In both this and our previous study (Khokholova et al., 2012), fleas took larger blood meals from auxiliary hosts that were phylogentically distant from the principal host than from those that were closer. Regardless of the size of the blood meal, both flea species decreased metabolic expenditure with an increase in phylogenetic distance of the auxiliary hosts from the principal host. The energy expenditure associated with the processing of food is primarily due to the process of digestion, which in haematophagous insects includes absorption, deamination, lipid synthesis, uric acid synthesis and excretion (Taylor, 1977).

Gas exchange measurements in this study were made on fleas removed from the host after 2 h (for *X. ramesis*) or 6 h (for *P. chephrenis*) of feeding and thus represented the first of three consecutive stages of blood digestion (see Krasnov et al., 2003; Khokhlova et al., 2009a). Therefore, metabolic expenditure most probably represented biochemical processes such as haemolysis and digestion of blood to haematin and not the mechanical aspects of digestion (release of undigested remnants and final products), which are more typical of the later stages of digestion (Sarfati et al., 2005). Metabolic expenditure for the different stages of digestion has been shown to vary according to the host. For example, *P. chephrensis* fed on a non-typical host *G. dasyurus* spend less energy during the earlier stages of digestion but not when they feed on the principal host *A. cahirinus* (Sarfati et al., 2005). Decreased energy expenditure with increasing phylogenetic distance of the auxiliary host from the principal host in this study may be a consequence of only measuring energy expenditure in the first stage of digestion and not at all three stages.

It is interesting to note that the blood meal size in *P. chephrensis* was consistently smaller in all nine host species (0.12–0.22 mg mg^{-1} body mass) than that in *X. ramesis* (0.25–0.80 mg mg^{-1} body mass). However *P. chephrensis* expended almost twofold the amount of energy to digest the blood meal than did *X. ramesis*. These species are approximately the same size but have very different life history strategies as *X. ramesis* spends most of its time off the host while *P. chephrensis* predominantly stays on its host for a long time (Krasnov, 2008). In addition, principal hosts of the two fleas differ in their sociality and space use. *Meriones crassus* is predominantly solitary (Krasnov et al., 1996), whereas *A. cahirinus* tend to nest communally (Shargal et al., 2000). In addition, the majority of individuals in *A. cahirinus* populations reside in their home ranges for a long period (Khokhlova et al., 1994; Khokhlova et al., 2001; Shargal et al., 2000), whereas *M. crassus* continuously change their home ranges and burrows (Krasnov et al., 1996). Consequently, *X. ramesis* may take large blood meals and process them very efficiently to compensate for the relatively low predictability of host availability.

Ecological and evolutionary perspectives

An increase in blood meal size and a decrease in the energy expenditure of fleas feeding on auxiliary hosts with an increase in phylogenetic distance of this host from a principal host is surprising. However, close inspection of Fig. 4 suggests that the pattern was mainly due to contrasting points in the upper left and lower right quadrants. These points correspond to auxiliary hosts that are the closest and the farthest phylogenetically from a principal host, respectively.

Energy expenditure for digestion was highest if a flea fed on a host that was either congeneric (*A. russatus* for *P. chephrenis*) or confamilial (*G. andersoni* and *G. dasyurus* for *X. ramesis*) with a principal host (*A. cahirinus* and *M. crassus*, respectively). Furthermore, despite the same phylogenetic distance between *G. andersoni* and *G. dasyurus* from *M. crassus*, the performance of *X. ramesis* differed between these two hosts. A smaller proportion of fleas took blood from *G. dasyurus* than from *G. andersoni*, but they spent more energy for digestion of blood from the former than from the latter. The main ecological difference between these two rodents in relation to *X. ramesis* is that *G. dasyurus* co-habits with the principal host of this flea and is often parasitized by it in the field, while *G. andersoni* does not co-occur in nature with either *M. crassus* or *X. ramesis* (Krasnov et al., 1999). The two spiny mice are usually found in the same habitat but the prevalence of *P. chephrenis* is much higher on *A. cahirinus* than on *A. russatus* (Krasnov et al., 1997). This suggests that the observed pattern of performance of fleas on auxiliary hosts has not only a phylogenetic but also an ecological component. In particular, there is likely to be an increased probability of flea attacks on auxiliary hosts that co-occur with a principal host. For example, *P. chephrenis* feeds more readily on *A. russatus* than on the majority of the other auxiliary hosts (Fig. 1A).

Investment in anti-parasitic defences has been suggested to depend on the pattern of parasite pressure, such as the frequency and probability of parasite attacks (Combes 2001; Tella et al., 2002), with large investments being of little advantage if encounters with the parasite are rare (Poulin et al., 1994). Consequently, high anti-flea resistance could be selected in *A. russatus* and *G. dasyurus*. This is supported indirectly by the fact that *G. dasyurus* (i) increases its metabolic rate under flea parasitism (Khokhlova et al., 2002) and (ii) mounts immune responses only after being attacked by fleas (Khokhlova et al., 2004). However, the probability of flea attacks on the principal hosts is obviously high, but fleas successfully feed and reproduce on these hosts (Krasnov et al., 2002; Krasnov et al., 2004c). It is possible that fleas were able to evolve some mechanisms that allow them to evade defence efforts of these hosts, while it is unclear why this did not occur with their close relatives. Possible reasons include differences in the species-specific level of immunocompetence, the temporal pattern of mounting immune responses and the energy allocation for immune responses among rodent hosts (Goüy de Bellocq et al., 2006; Hawlena et al., 2006).

The reason behind the best performance of fleas on the most distant relative of the principal host (see Khokhlova et al., 2012) might be the lack of defence of this host against fleas of this species. The most distant relative of both *A. cahirinus* and *M. crassus* in our study was *M. auratus*. This species is a laboratory animal and, as such, has not been exposed to ectoparasites for many generations, so it may have lost its anti-parasitic defence tools. In addition, there are no reports of cricetids parasitized by either *P. chephrenis* or *X. ramesis* because these fleas do not co-occur naturally with these rodents. As mentioned above, natural selection probably does not favour specific anti-parasitic defence of a host if it rarely (if at all) encounters the parasite (Poulin et al., 1994; Krasnov et al., 2007).

The effect of the level of host specificity

In general, feeding performance on the principal host *versus* auxiliary hosts as well as among auxiliary hosts did not differ substantially between host-specific *P. chephrenis* and host-opportunistic *X. ramesis*. However, differences in the proportion of *P. chephrenis* that took a blood meal and the size of the blood meal between *Acomys* hosts and the remaining rodents were less

pronounced than those in *X. ramesis* between gerbilline and murine hosts (Fig. 1A *versus* Fig. 2A). On the one hand, this may be because a flea that is considered to be host-opportunistic can actually be host-opportunistic at the level of host species but host-specific at the level of host genus or family, which seems to be the case with *X. ramesis* (Krasnov et al., 1997). On the other hand, a host-specific flea may be able to extract resources from non-specific host species but not be able to translate these resources into offspring, which seems to be the case with *P. chephrenis* (Krasnov et al., 2002; Krasnov et al., 2003). In addition, a trend to compensate for a low quality resource by increasing the amount of extracted resource was found in host-opportunistic *X. ramesis* but not in host-specific *P. chephrenis*. This ability may be one of the factors determining the ability of *X. ramesis* to exploit multiple host species. Overall, these results suggest that the level of host specificity of a flea may play a role in the pattern of feeding performance on different host species.

In conclusion, this study suggests that the effect of phylogenetic position of an auxiliary host relative to the principal host on parasite performance in this host necessitates further investigation. These investigations should involve direct fitness-related measurements such as reproductive output and offspring quality of a parasite.

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