Parasitology

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Feeding performance on a novel host: no adaptation over generations and differential patterns in two flea species

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Research Article

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Abstract

To model the colonization of a novel host by fleas, *Synosternus cleopatrae* and *Xenopsylla ramesis*, we established experimental lines maintained for 15 generations on a principal or a novel host (either co-occurring with a flea or not). We compared the blood meal size and the energy expended for digestion by fleas from the 15th generation of each line on these hosts between hosts within a line and between lines within a host asking (a) whether fleas adapt to a novel host (increased blood consumption/decreased energy expended for digestion); (b) if yes, whether this adaptation leads to the loss of ability to exploit an original host, and (c) whether the success of adaptation to a novel host depends on its ecological co-occurrence with a flea. The blood consumption and digestion energetics of fleas fed on the principal host differed from those on other hosts. The effect of the principal host on feeding performance differed between fleas, with *S. cleopatrae* consuming less blood and expending more energy for digestion on the principal than on any other host, whereas the opposite was true for *X. ramesis*. No changes in feeding performance on a novel host over generations were found. We propose several explanations for the lack of adaptation to a novel host over time. We explain the poor performance of *S. cleopatrae* on its principal host *via* its immune response mounting pattern. We argue that the principal host of a parasite is not necessarily the host on which the parasite demonstrates the best performance.

Introduction

Although it is widely accepted that parasites and their hosts share an evolutionary history (Hoberg *et al.*, 1997; Paterson and Banks, 2001), our understanding of this relationship is far from being complete. Earlier studies of host–parasite coevolution were based on the underlying paradigm that the main evolutionary event during this shared history was contemporaneous speciation in both host and parasite lineages (=cospeciation), whereas other events, such as the colonization of a new host species by a parasite (=host switching), were rare (Hafner and Nadler, 1988; Barker, 1991; Hoberg *et al.*, 1997). Parasites cospeciating with their hosts would thus evolve as specialists adapted to a narrow range of closely related hosts, which seems to be the case for many parasitic species (e.g. Gregory *et al.*, 1991). However, host switching between both related and unrelated hosts has appeared to be a widespread event in various host–parasite associations (Paterson *et al.*, 1993; Beveridge and Chilton, 2001; Roy, 2001; Lu and Wu, 2005). It should be noted, however, that 'host-switching' in the most common use may actually encompass two different phenomena/processes, namely host-switches and host-shifts, with the former potentially leading to parasite speciation and the latter not (Rozsa *et al.*, 2015). As argued by Rozsa *et al.* (2015), the occurrence of these processes depends on whether a change of a host by a parasite occurs at either a leading or a rear edge of the expanding geographic range of a principal host of a given parasite.

'Ecological fitting' (Janzen, 1985), a key concept explaining host switching in parasites (e.g. Hoberg and Brooks, 2015), is thought to be its initial, and chief, mechanism (Agosta and Klemens, 2008; Agosta *et al.*, 2010). Regarding parasites, ecological fitting comes into play if the main requirement of a parasite is the resource itself rather than its 'representation' in nature (i.e. a particular host species) and if this resource is common among many host species. As a result, a parasite tracking the resource may: (a) invade new areas where the resource, but not its 'original representation' (=the host species to which the parasite is adapted), is present; (b) switch to a co-occurring (i.e. encountered in the past) but novel host species; and/or (c) switch to an invading (i.e. not encountered in the past) novel host (Brooks *et al.*, 2006; but see Rozsa *et al.*, 2015 for difference between host-switches and host-shifts). From this perspective, a novel host–parasite association can be established only if a parasite would be able to both use the resources represented by a new host and to extract these resources by overcoming

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its anti-parasitic defences (Combes, 2001; Araujo *et al.*, 2015). If a novel host is phylogenetically close to an original host, it likely represents a similar resource (Harvey and Pagel, 1991). However, the development of anti-parasitic defences may depend on the pattern of parasite pressure a host experiences. This is because the mounting and maintenance of immune responses is costly and does not confer a strong benefit if a host rarely encounters the parasite (Combes, 2001). Thus, the frequency and probability of parasite attacks may strongly affect the patterns of immune defence (Tella *et al.*, 2002). Consequently, whether or not a parasite species co-occurs with a novel host species within the same habitat may play a role in the probability of novel host individuals to successfully defend themselves from attacks by the parasite. On the one hand, an invasive host species that has not encountered a given parasite in the past may lack the necessary defences against this parasite. On the other hand, co-occurring hosts often harbour phylogenetically similar parasite assemblages (Krasnov *et al.*, 2010), so they are subject to similar parasitic challenges and may evolve similar defences. Thus, the probability of colonization may be lower if a novel host is presently co-occurring or has co-occurred in the past with the original host and a parasite than if a novel host is a recent invader.

Despite numerous phylogenetic, biogeographic and theoretical studies of host switching and, thus, colonizations of novel host species by parasites (e.g. Boeger *et al.*, 2003; Meinilä *et al.*, 2004; Araujo *et al.*, 2015), experimental studies are scarce (but see Giorgi *et al.*, 2004; Dick *et al.*, 2009; Arbiv *et al.*, 2012). Although the processes of adaptation to a new host species have been extensively studied in phytophagous arthropods (see reviews in Jaenike, 1990; Forister *et al.*, 2012), the results of these experiments may not be directly applied to parasites exploiting animal hosts. This is because, unlike plant hosts of phytophages, an animal host of a 'traditional' parasite responds to parasitism almost immediately by actively defending itself with an array of behavioural, physiological and immunological tools.

Ectoparasitic arthropods were earlier thought to be limited in their host colonization ability (e.g. Hafner and Nadler, 1988). Further studies demonstrated that this is not always the case (Krasnov and Shenbrot, 2002; Lu and Wu, 2005; Arbiv *et al.*, 2012). In this study, we investigated the colonization process of a novel host in two species of host-generalist fleas, *Synosternus cleopatrae* and *Xenopsylla ramesis*. Studying patterns of colonization of a novel host by ectoparasitic vectors of infectious diseases will be important for a better understanding of the spread of human and animal disease under the anthropogenic transformation of the environment and/or global climate changes in the framework of the One Health concept. We estimated the success of host colonization (i.e. adaptation to a novel host) by measuring their feeding performance (blood meal size and energy expended for blood digestion). This experiment models three possible scenarios that could occur in nature: (a) a principal host species of a parasite becomes extinct, so the parasite switches to another host species; (b) a parasite invades a new area where its principal host species does not occur; or (c) a new host invades an area inhabited by a parasite species. Furthermore, the adaptations to a new host may negatively affect a parasite's performance on an original host (Ebert, 1998), although trade-offs in parasite performance on alternative hosts have been reported in some (Dobson and Owen, 1977), but not other, systems (Jaenike and Dombeck, 1998).

We established experimental lines of the two flea species, maintained them for 15 generations on either their principal (=original) or a novel host species (either co-occurring in the same habitat with a flea or not) and compared the feeding performance of fleas from the 15th generation of each line when they exploited these hosts. We compared the flea feeding performances between host species within a line and between lines within a host species. This design was aimed at answering the following questions: (a) are fleas able to adapt to a novel host species over 15 generations?; (b) if yes, is an adaptation to a novel host accompanied by the loss of the ability to exploit an original host species?; and (c) does the success of adaptation to a novel host species depend on its identity in terms of ecological co-occurrence with a flea? Earlier studies of *X. ramesis* demonstrated that these fleas take relatively more blood and spend less energy for its digestion when they exploit the principal host (i.e. a host supporting the largest portion of a parasite's population) as compared to an auxiliary host (i.e. any other host) (Khokhlova *et al.*, 2012*a*). However, this was true only if an auxiliary host was closely related to the principal host (i.e. the same subfamily). In this study, all novel host species belonged to the same subfamily (Gerbillinae) as the principal hosts of both fleas. This selection allowed the avoidance of unpredictable flea responses to hosts phylogenetically distant from their principal host (Khokhlova *et al.*, 2012*a*, 2012*b*). Consequently, we expected that fleas maintained over generations on a novel host species would have a larger blood meal size and lower energy expenditure for digestion when feeding on this host than fleas maintained over generations on the principal (i.e. original) host that were forced to feed on the novel host only once. Additionally, if both of these flea groups were to feed on the principal host species and fleas reared on the novel host species were to take smaller blood meals and expend more energy for digestion, as compared to those reared on the principal host, then this would indicate the loss of these fleas' ability to exploit the principal host.

Materials and methods

Natural distribution of fleas and hosts

Synosternus cleopatrae and *X. ramesis* are common flea species in Israel's Negev Desert (Krasnov *et al.*, 1999). These species are allopatric, occur in different habitats and infest different host species. *Synosternus cleopatrae* is found solely in sandy habitats where it exploits a number of gerbilline species, being most abundant on *Gerbillus andersoni* (7.5–10.4 fleas per individual host; Hawlena *et al.*, 2005) and very often attaining 100% prevalence. It is only occasionally recorded on *Gerbillus henleyi* (abundance of less than one flea per individual host and prevalence <5%; Krasnov *et al.*, 1999). *Xenopsylla ramesis* inhabits mainly stony deserts and dry riverbeds. This species attains its highest abundance and prevalence on *Meriones crassus* (4.3–7.6 fleas per individual host and 60–80%, respectively; Krasnov *et al.*, 1998) while its infestation level in co-occurring *Gerbillus dasyurus* is much lower (abundance of 0.06–0.35 fleas per individual host and prevalence of 15%; Krasnov *et al.*, 1998). *Gerbillus andersoni* never inhabits stony habitats, whereas *G. dasyurus* never occurs in sands (Krasnov *et al.*, 1999). Thus, we considered *G. andersoni* and *M. crassus* as the principal hosts for *S. cleopatrae* and *X. ramesis*, respectively, whereas the host species co-occurring (=sympatric)/not co-occurring (=allopatric) with these fleas were *G. henleyi*/*G. dasyurus* and *G. dasyurus*/*G. andersoni*, respectively (Table 1).

Experimental animals

The rodents [*G. andersoni* (mean body mass 27.5 g), *G. dasyurus* (mean body mass 24.1 g), *G. henleyi* (mean body mass 10.7 g) and *M. crassus* (mean body mass 81.2 g)] and fleas (*S. cleopatrae* and *X. ramesis*) used in the experiments were randomly selected from our laboratory colonies, started from individuals collected in the field. Details on the colony maintenance have been published earlier (Krasnov *et al.*, 2001; Khokhlova *et al.*, 2012*a*, 2012*b*).

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Table 1. The design applied in the experiments

Flea species	Line	Maintenance host	Test hosts
S. cleopatrae	P	*Gerbillus andersoni*	*G. andersoni*, *G. henleyi*, *G. dasyurus*
	S	*Gerbillus henleyi*	*G. henleyi*, *G. andersoni*
	A	*Gerbillus dasyurus*	*G. dasyurus*, *G. andersoni*
X. ramesis	P	*Meriones crassus*	*M. crassus*, *G. dasyurus*, *G. andersoni*
	S	*Gerbillus dasyurus*	*G. dasyurus*, *M. crassus*
	A	*Gerbillus andersoni*	*G. andersoni*, *M. crassus*

P, line was maintained on the principal host of a flea species; S, line was maintained on a host species sympatric (i.e. co-occurring) with the flea; A, line was maintained on a host species allopatric (i.e. not co-occurring) to the flea.

Prior to the experiments, rodents were kept individually or in pairs in plastic cages (60 × 50 × 20 cm) at 25°C and a 12:12 light regime and were fed millet seed *ad libitum* and fresh alfalfa as a water source. Fleas were reared on their principal host species (*S. cleopatrae* on *G. andersoni* and *X. ramesis* on *M. crassus*). Rodent individuals used for flea rearing were transferred into another cage (60 × 50 × 40 cm) with a mesh floor (5 × 5 mm) with a mixture of sand and a flea larvae nutrient medium (94% dry bovine blood, 5% millet flour and 1% ground feces of the respective host species) underneath (3–5 mm). Cage substrate material was collected every 2 weeks, placed into plastic boxes and kept at 25°C and 90% relative humidity in an incubator (FOC225E, Velp Scientifica srl, Milano, Italy) until a new generation of fleas emerged. New rodents were then infested with these newly emerged fleas.

Experimental design

For each flea species, we established three lines that differed in host species identity on which they were maintained. We randomly selected newly emerged fleas from each colony and randomly assigned them to: (a) a line maintained on the principal host (line P: *G. andersoni* for *S. cleopatrae* and *M. crassus* for *X. ramesis*); (b) a line maintained on a host species sympatric (i.e. co-occurring) with the flea (line S: *G. henleyi* for *S. cleopatrae* and *G. dasyurus* for *X. ramesis*); and (c) a line maintained on a host species allopatric (i.e. not co-occurring) to the flea (line A: *G. dasyurus* for *S. cleopatrae* and *G. andersoni* for *X. ramesis*). These host species are hereafter referred to as maintenance hosts (Table 1). In each flea line, we measured blood meal size and energy expenditure for digestion when fleas were fed on either the maintenance host of this line or an alternative host (the principal host species for the S and A lines and the host species sympatric with or allopatric to the flea for the P line). These host species are hereafter referred to as test hosts (Table 1). Rodents used as maintenance and test hosts were 6- to 8-month-old males.

Establishing experimental lines started with 200 fleas of each species randomly taken from the respective colony and released into two plastic cages, each with two or three rodents of a given species. We repeated this procedure until the first generation of a new line emerged. Substrate material from each cage was collected every 2 weeks, as described above for flea colony maintenance, and placed in an individual plastic box and kept at 25°C and 90% relative humidity in an incubator. These flea species usually start oviposition 2 days after being released into a host cage, with the minimum duration of egg-to-imago development being 24 days and the peak of imago emergence at 30–35 days post-oviposition (Krasnov *et al.*, 2001; Khokhlova *et al.*, 2010; van der Mescht *et al.*, 2019). To guarantee that all emerged fleas belong to the same generation, we collected them from an incubated box on day 32. Then, we mixed newly emerged fleas of the same line from the two incubated boxes, randomly selected 200 newly emerged imagoes and placed them on the same host species as the previous generation. These procedures were repeated until the 15th generation of each line. Females of this generation were used for measuring feeding performance and digestion energetics.

Blood meal size and respirometry

To quantify the amount of blood consumed by a flea so that we could then measure the energy expended for digesting this blood, we first fed fleas on rodents placed individually in a wire mesh (5 mm × 5 mm) tube (15 cm in length and 5 cm in diameter for *M. crassus* and 10 cm in length and 2 cm in diameter for the other species). This design limited the rodent's movement and prevented self-grooming. Each tube with a rodent was placed in an individual white plastic pan. Twenty newly emerged females were weighed (±0.01 mg, 290 SCS Precisa Balance, Precisa Instruments AG, Switzerland) and then released onto the rodent. After allowing fleas to feed for 3 h, we collected them from the rodent and 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. We selected 10 satiated fleas and re-weighed them as described above. Based on flea body mass prior to feeding, we calculated the mean body mass of 10 unfed fleas and took the difference between the body mass of 10 fleas prior to and after feeding as the amount of blood consumed by 10 fleas. We then calculated the mean mass-specific amount of blood consumed as the difference between the body mass of 10 unfed and 10 fed fleas divided by the total body mass of 10 unfed fleas. Fleas that took a blood meal were at the early digestion stage (see Krasnov *et al.*, 2003; Khokhlova *et al.*, 2009 for details on digestion process), which is the most energetically costly stage (Sarfati *et al.*, 2005).

To estimate energy expenditure for digestion, 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) using a flow-through respirometry system. Incurrent air was scrubbed of H_2O vapour and CO_2 by drierite (700 mL) and ascarite (25 mL), respectively, columns and 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} controlled by a mass flow controller (model FC-260, Tylan, Rancho Dominquez, CA, USA). Dry and CO_2-free air constituted the baseline for all flow-through measurements. The carbon dioxide content (ppm) of air exiting the respirometer chamber was 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) with sampling every 2 s. Tygon tubing (3.3 mm internal diameter) was used to plumb the system. A stable temperature (25°C) for the air inside the respirometer tubing was achieved by placing the chamber and the preceding 6 m of incurrent tubing into an open-bath circulator [Thermo Haake V26, Thermo Electron Corp. (now Thermo Fisher Scientific), Waltham, MA, USA]. Carbon dioxide emission for fleas was recorded for 30 min with baseline measurements, each lasting 5 min, before and after each recording. Fleas were measured in groups of 10 females because the CO_2 emission of a single insect was only slightly above baseline levels. Fleas from each group were

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fed in the same feeding bout on the same rodent. Details of the protocol and the repeatability of measurements can be found elsewhere (Fielden *et al*., 2004; Sarfati *et al*., 2005). Measurement of blood meal size and CO_2 emission for each line of each flea species, either unfed or fed, on either a maintenance or a test host was replicated nine times.

The mass-specific energy expenditure by a flea for digesting 1 mg of host blood was calculated as follows. First, we calculated the difference in the volume of CO_2 emitted per hour per mg of body mass between a digesting flea and an unfed flea for each respirometry measurement carried out on the same day. Then, we divided this mass-specific difference in the volume of emitted CO_2 between digesting and unfed fleas by the mass-specific amount of consumed blood (see above) and considered this quotient as a mass-specific indicator of the energy cost of digesting 1 mg of blood. To convert the rate of CO_2 emission to energy expenditure, we used 24.5 J of energy produced per ml of CO_2 production (Schmidt-Nielsen, 1990) under the assumption of a respiratory quotient of 0.8 for hematophagous ectoparasites (previously determined for ticks; Lighton *et al*., 1993).

Data analysis

We analysed the effects of a maintenance host and a test host's identities on the mass-specific amount of blood consumed by a flea during one feeding bout and the mass-specific energy expenditure for digesting 1 mg of a host's blood separately for each flea species. The distribution of dependent variables did not significantly deviate from normality (Kolmogorov–Smirnov tests; $d = 0.117–0.132$, $P > 0.20$ for all), so no transformation was applied. We analysed the effect of a maintenance host and a test host's identities, as well as the interaction between these factors (=independent variables) on mass-specific blood meal size or mass-specific energy expended during 0.5 h digestion of 1 mg of blood (=dependent variables) using generalized linear models with the normal distribution and identity link function using the package 'stats' of the R 3.5.3 statistical environment (R Core Team, 2019). Initially, we constructed a model with both independent variables and their interaction for each flea species and each response variable. Then, we selected the best-fitted model based on the Akaike Information Criterion (AIC) using the 'dredge' function implemented in the R package 'MuMIn' (Barton, 2018).

Results

In both fleas, the best models of the relationships between either blood meal size or energy expenditure for digestion included only the identity of the test host, with the principal host being the reference level of effect of this explanatory variable (Table 2). In other words, independently of the identity of a host on which a given flea line was maintained, flea performance on the principal host, in terms of blood consumption and digestion energetics, differed from that on either a host sympatric with or allopatric to the flea.

Importantly, signs of the explanatory variables' coefficients in the best models (i.e. test hosts) were opposite (a) between response variables within flea species and (b) between flea species for the same response variable. In other words, (a) the direction of the principal host species' effect on response variables differed between blood meal size and energy expenditure for digestion and (b) in terms of either blood meal size or energy expenditure for digestion. *Synosternus cleopatrae* and *X. ramesis* responded differently to the principal host species as compared to the alternative host species. In particular, *S. cleopatrae* consumed relatively less blood when fed on the principal host than on either a sympatric or allopatric host, independently of which host species this flea was maintained on over generations, whereas the opposite was, in general, true for *X. ramesis* (Fig. 1), although the latter species maintained on the principal host consumed similar amounts of blood from it and from the co-occurring host (Fig. 1). In contrast, *S. cleopatrae* expended substantially more energy digesting the principal host species' blood than that of either the co-occurring or non-co-occurring host, whereas in *X. ramesis*, the energy expended for digesting the principal host's blood was either lower than or similar to, but never higher, than that expended for digesting the blood of either alternative host species.

Discussion

The results of this study directly supported a negative answer to our question about the ability of fleas to adapt to a novel host over 15 generations, so that the remaining questions appeared to be irrelevant. We did not find any indication that fleas maintained on novel hosts improved their feeding performance on these hosts in comparison with that on the principal host. In other words, fleas did not demonstrate any significant trend of adaptation to a novel host with regard to feeding performance. This does not, however, mean that fleas, in general, are unable to colonize a new host because previous phylogenetic studies provided evidence that they do (e.g. Krasnov and Shenbrot, 2002; Whiting *et al*., 2008; Zhu *et al*., 2015). There can be several, not necessarily mutually exclusive, reasons for the lack of adaptation to a novel host found in this study.

First, it is possible that the time span of 15 generations is too short for fleas with a higher efficiency to exploit a novel host to be selected and to reproduce to the level at which they constitute the majority of a given flea population. Furthermore, it is unknown whether a higher or lower performance of an individual flea on a given host has a genetic basis and is thus heritable, or is merely the result of phenotypic plasticity or else varies with parasite density (e.g. Tryjanowski *et al*., 2007; although this is highly unlikely in our experimental design). Although rapid adaptations to a novel host species have been demonstrated in some taxa, such as parasitoids (Jones *et al*., 2015) and phytophagous arthropods (e.g. Messina *et al*., 2009; Price *et al*., 2017), the time and number of generations required for this adaptation to be observed may be much longer and greater in other taxa, depending on generation time, selection pressure and/or the genetic mechanisms involved.

Second, in our earlier experiments, *X. ramesis* maintained on either *M. crassus* or *G. dasyurus* (Arbiv *et al*., 2012), but forced to feed on an alternative host (*G. dasyurus* or *M. crassus*, respectively), showed a reproductive decrease (in terms of egg production) after just four generations. This suggests that changes in feeding performance may not be a reliable measure of adaptation success to a novel host. Although flea feeding patterns may potentially serve as a proximate indicator of adaptation, its ultimate indicator is undoubtedly a reproductive success. Indeed, although flea feeding and reproductive performance in different host species are usually correlated, deviations from this correlation may also exist. For example, *X. ramesis* expended less energy when it digested blood taken from *M. crassus* than from *Acomys cahirinus* (Khokhlova *et al*., 2012*a*) and produced more eggs after feeding on the former than on the latter species (Khokhlova *et al*., 2012*b*). However, the energy expended for digestion after feeding on *A. cahirinus* did not differ from that after feeding on *Gerbillus pyramidum* (Khokhlova *et al*., 2012*a*), although egg production was significantly lower in the former than in the latter case (Khokhlova *et al*., 2012*b*). The reproductive performance of *S. cleopatrae* and *X. ramesis* from our experimental lines on different hosts remains to be studied.

Third, different flea species or higher taxa may have different evolutionary potentials for host switching. For example, among

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Table 2. Best-fit models of the effects of the maintenance and the test host species' (TH) identities on mass-specific blood meal size (BMS) and energy expenditure for 0.5 h of digestion of 1 mg of host blood (EE) in *Synosternus cleopatrae* and *Xenopsylla ramesis* maintained on a principal host (*Gerbillus andersoni* and *Meriones crassus*, respectively), a host sympatric with the flea (*Gerbillus henleyi* and *Gerbillus dasyurus*, respectively), and a host allopatric to the flea (*G. dasyurus* and *G. andersoni*, respectively)

Flea	Response	Equation	AIC	AIC weight	Likelihood ratio χ^2	*P*
S. cleopatrae	BMS	0.53 − 0.12 × TH	10.41	0.76	9.03	0.01
	EE	0.04 + 0.01 × TH	−263.09	0.70	6.95	0.03
X. ramesis	BMS	0.30 + 0.06 × TH	−69.37	0.84	10.65	0.01
	EE	0.06 − 0.01 × TH	−228.14	0.50	7.68	0.04

No best model contained the term of the maintenance host. The level of effect of the test host in all models was the principal host.

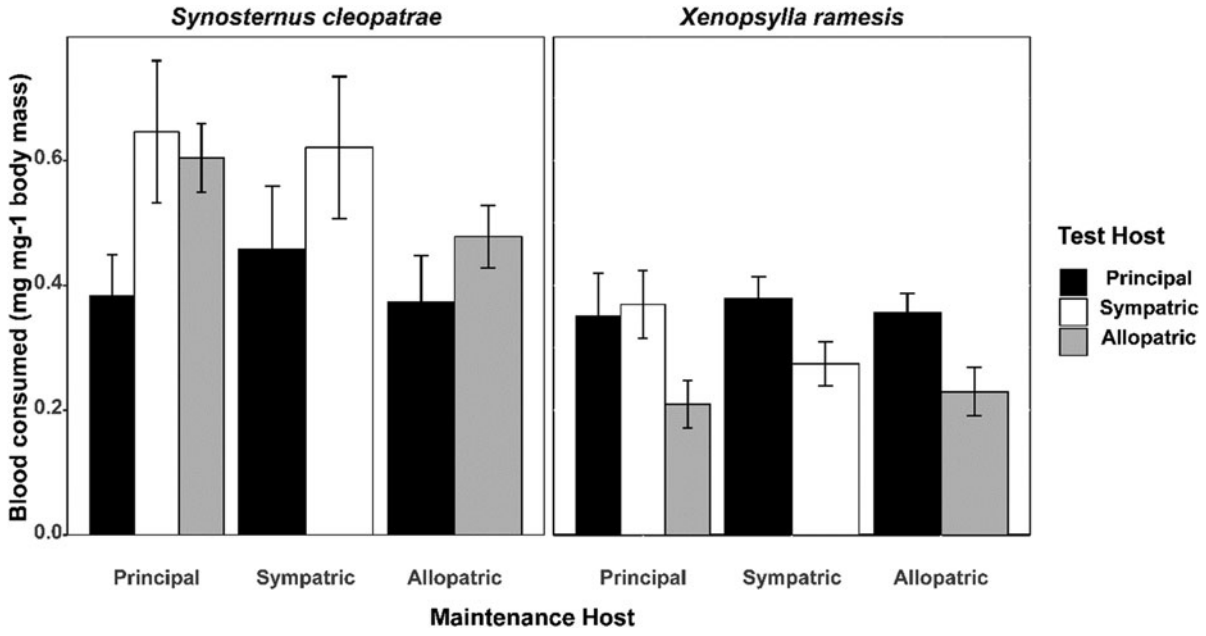

Fig. 1. Mean (±S.E.) mass-specific amount of blood taken from a host by *Synosternus cleopatrae* and *Xenopsylla ramesis*. A maintenance host is the host on which a given flea line was maintained over generations. A test host is the host on which fleas from a given line were fed and their blood meal size was measured. The maintenance host and the test host were represented by the principal host (*Gerbillus andersoni* for *S. cleopatrae* and *Meriones crassus* for *X. ramesis*), a host sympatric with the flea (*Gerbillus henleyi* for *S. cleopatrae* and *Gerbillus dasyurus X. ramesis*), and a host allopatric to the flea (*G. dasyurus* for *S. cleopatrae* and *G. andersoni* for *X. ramesis*).

numerous lineages of ceratophyllid fleas, only two (*Dasypsyllus gallinulae perpinnatus* + *Dasypsyllus stejnegeri* and *Ceratophyllus petrochelidoni* + *Ceratophyllus gallinae*) switched from rodents to birds (Whiting *et al.*, 2008), although it is unclear whether this switch occurred due to some inherent traits of these lineages (e.g. extreme host-opportunism) or due to historical incidents. Therefore, the lack of success in host colonization found in this study for these two flea species, both belonging to the same family (Pulicidae), does not mean that this is characteristic for the entire order. We, however, recognize that this explanation is highly speculative.

The most surprising result of this study was the differential pattern of feeding performance on the principal and an alternative host between the two fleas. Independently of the identity of the maintenance host, *X. ramesis* almost always took more blood and spent less energy for its digestion, whereas *S. cleopatrae* took less blood and expended more energy for digestion when fleas exploited the principal host as compared to any other host. The pattern demonstrated by *X. ramesis* is what has been reported earlier for this flea and for another species, *Parapulex chephrenis* (Sarfati *et al.*, 2005; Khokhlova *et al.*, 2012*a*). Obviously, different types of food (e.g. blood from different host species) entail different energy costs (Rechav and Fielden, 1997; Sarfati *et al.*, 2005; this study). In a hematophagous ectoparasite, these costs include the energy expenditure for blood extraction from a host, as well as the energetic cost of food processing that includes energy spent on enzyme production, transformation of food components, excretion of by-products and heat production during digestion (Clements, 1992; Lehane, 2005). The cost of food extraction by a parasite may differ among host species due to differences in their skin thickness, capillary depth and cutaneous immune response (Sokolov, 1983), whereas the cost of food processing by a parasite may differ among hosts due to differences in the blood's physical and chemical properties, such as viscosity, erythrocyte volume and count, platelet count, and the content of haemoglobin, glucose and lipids (Promislow, 1991). Consequently, different hosts present a given ectoparasite with resources of different values in terms of nutrition and the energy cost of processing. On the one hand, a lower energy expenditure for digestion would allow a parasite to allocate more energy to other activities such as reproduction, which would likely lead to increased fitness resulting from the selection of an appropriate host (see Khokhlova *et al.*, 2012*b* for *X. ramesis* and *M. crassus*), thus facilitating the adaptation of a parasite to a given host. On the other hand, differences in the energy cost of digesting a resource taken from one, rather than

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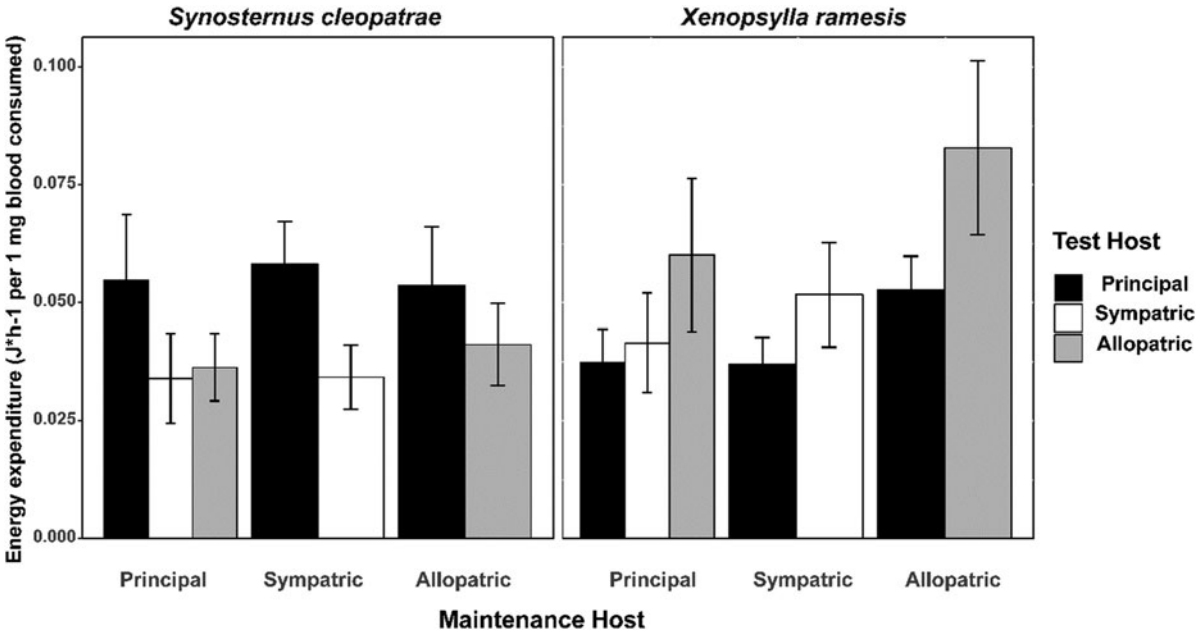

Fig. 2. Mean (±s.e.) mass-specific amount of energy expended for 0.5 h digestion of blood taken from a host by *Synosternus cleopatrae* and *Xenopsylla ramesis*. A maintenance host is the host on which a given flea line was maintained over generations. A test host is the host on which fleas from a given line were fed and their blood meal size was measured. The maintenance host and the test host were represented by the principal host (*Gerbillus andersoni* for *S. cleopatrae* and *Meriones crassus* for *X. ramesis*), a host sympatric with the flea (*Gerbillus henleyi* for *S. cleopatrae* and *Gerbillus dasyurus X. ramesis*), and a host allopatric to the flea (*G. dasyurus* for *S. cleopatrae* and *G. andersoni* for *X. ramesis*).

another, host species may reflect specific adaptations of a parasite to exploit a particular host species. This host may thus become a principal host for this parasite in terms of supporting the largest proportion of the parasite population (Dogiel *et al.*, 1961; Poulin, 2005).

However, the pattern demonstrated by *S. cleopatrae* directly contradicted the above scenario. Despite the fact that most individuals in this flea's natural populations are undoubtedly supported by *G. andersoni* (Krasnov *et al.*, 2005), the flea's feeding performance on this host appeared to be inferior to that on other hosts. The mechanism behind this is most likely associated with the immune response mounting pattern of *G. andersoni*. As mentioned above, immune response mounting and investment in immune defences depend on the pattern of parasite pressure (Combes, 2001; Tella *et al.*, 2002). The selection of immune resistance mechanisms is expensive and, thus, of little advantage if encounters with the parasite are rare (Poulin *et al.*, 1994). Consequently, if the frequency and/or probability of parasitic attacks are low, then a host can limit its energy allocation for immune responses by developing responses only after being attacked ('post-invasive'). If, however, the frequency and/or probability of parasitism are high, a continuous maintenance of a certain level of immune 'readiness' in the host is advantageous despite its high cost (Jokela *et al.*, 2000). *Gerbillus andersoni* is characterized by 100% prevalence of *S. cleopatrae*, whereas this is not the case for the other host species used in these studies (see Shenbrot *et al.*, 1994, 1997; Krasnov *et al.*, 1996). Khokhlova *et al.* (2004*a*) found that even non-parasitized *G. andersoni* showed immune responses against fleas, suggesting that the expected probability of flea attack plays an important role in determining its immune strategy. Therefore, the small blood meal size and high digestion cost of blood taken from *G. andersoni* by *S. cleopatrae* may be the result of the continuous immune 'readiness' of this host (see Rechav and Fielden, 1995), whereas other gerbillines (*M. crassus* and *G. dasyurus*) mounted immune responses to flea parasitism in a 'post-invasive' fashion (Khokhlova *et al.*, 2004*a*, 2004*b*). This explanation is also supported by *X. ramesis*'s smallest blood meal size and highest energy expenditure during blood digestion when *G. andersoni* was used as an allopatric host (Figs 1 and 2).

Blood meals taken by *S. cleopatrae* from *G. dasyurus* and *G. henleyi* were not only larger than those taken by this flea from *G. andersoni* but also were larger than blood meals taken by *X. ramesis* from any host species (Fig. 1). This may be a consequence of *S. cleopatrae*'s characteristic feeding pattern whereby the flea feeds on a host continuously with much of the blood passing through its gut unchanged (unpublished data). This behaviour is typical for sessile fleas (e.g. *Echidnophaga*; Vashchenok, 1967) but is also found in some flea species that feed on a host intermittently (e.g. *Leptopsylla segnis* and *Leptopsylla taschenbergi*; Kosminsky, 1965). Pumping the blood through the alimentary canal may be energetically costly, so fleas may compensate for this with larger blood meals and frequent feeding bouts (3–5 times more frequent than in other fleas that feed intermittently), as well as by starting a new bout even before completing digestion of the previously ingested blood (Kosminsky, 1965; Bryukhanova, 1966; Vashchenok, 1988). However, the immune 'readiness' of *G. andersoni* seems to preclude fleas from taking large blood meals from this particular host.

Nevertheless, field observations suggest that, despite the relatively 'poor' feeding performance of *S. cleopatrae* on *G. andersoni*, it successfully reproduces on this host, although its reproductive success there is somewhat lower than on other rodent hosts such as *G. pyramidum* (Messika *et al.*, 2017). However, *G. andersoni*'s substantially higher natural density than any other cohabitating rodent, including *G. pyramidum* (e.g. Abramsky and Pinshow, 1989), likely results in a larger proportion of a flea population being supported by the former host (see Krasnov *et al.*, 2002 for the relationship between host density and flea numbers). This suggests that a parasite's principal host from the ecological perspective (i.e. a host supporting the largest part of a parasite population) is not necessarily the best host from the physiological perspective (i.e. a host on which a parasite's performance is the highest).

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