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ORIGINAL PAPER

Experimental evidence of negative interspecific interactions among imago fleas: flea and host identities matter

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Abstract We investigated interspecific interactions between two flea species (*Parapulex chephrenis* and *Xenopsylla ramesis*) via evaluation of their feeding success (the size of a blood meal and time to death after a single blood meal) when they exploited rodent hosts [*Acomys cahirinus* (a characteristic host of the former) or *Meriones crassus* (a characteristic host of the latter)] in single-species or mixed-species groups. We predicted that the negative interactions between the two fleas will result in smaller blood meals and shorter survival time in mixed- versus single-species infestations. We also predicted that the negative effect of mixed-species infestation on feeding performance would be less pronounced when fleas exploited their characteristic host rather than a non-characteristic host. When exploiting a characteristic host, *P. chephrenis* took larger blood meals in single- than in mixed-species groups, whereas the blood meal size in *X. ramesis* did not differ between treatments. When exploiting a non-characteristic host, no effect of group composition was found in either flea species. Survival time after a single blood meal was not affected by co-infestation or host species in either flea. Our results suggest context-dependence of the negative effect of co-infestation on feeding performance in fleas with the manifestation of this effect varying in dependence of flea and host species identities.

Keywords Blood meal size · Fleas · Interspecific interactions · Rodents

Introduction

It is commonly accepted that interspecific interactions such as competition or facilitation may shape the structure of biological communities. Consequently, one of the most important tasks of community ecology is to understand what types of interactions prevail in a given community. There are a plethora of studies that aimed to reveal these interactions for a variety of plan and animal taxa, in various geographic regions and at a variety of scales (see, for example, Begon et al. 1996; Tilman and Kareiva 1998; Mittelbach 2012 for reviews). Species interactions in parasite communities have been repeatedly studied as well (e.g., Holmes and Price 1986; Bush et al. 1997; Combes 2001; Poulin 2007).

Depending on the presence or absence of interspecific interactions, isolationist and interactive parasite communities are distinguished (Holmes and Price 1986; Bush et al. 1997). A parasite community is considered as interactive if parasite species in this community exert selective pressures on each other (Holmes and Price 1986; Combes 2001). Studies of parasite communities produced contradictory results about what type of species interactions is predominant. Negative (competitive) interactions seemed to prevail in some communities (e.g., Patrick 1991 for intestinal helminths of a rodent, Rohde et al. 1995 for fish ectoparasites, Dawson et al. 2000 for copepods parasitic on fish, Dezfuli et al. 2001 for fish intestinal helminths), whereas positive (facilitating)

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interactions appeared to be characteristic for other communities (e.g., Shall and Bromwich 1994 for malarial parasites of a lizard, Krasnov et al. 2006a for fleas of small mammals, Krasnov et al. 2009 for gamasid mites of small mammals, Tello et al. 2008 for streblid flies of bats). However, in the majority of the studies of parasite interspecific interactions, the type of interaction was inferred from data on relative abundances and/or distribution of species rather than experimental investigations (e.g., Bush and Holmes 1986 for intestinal helminths of birds; Haukisalmi and Henttonen 1993 for helminths of a rodent; Forbes et al. 1999; Krasnov et al. 2006a, b for fleas of small mammals; Tello et al. 2008 for streblids of bats), whereas only a few studies present the results of manipulative experiments (e.g., Patrick 1991 with helminths of a rodent, Bush and Malenke 2008 with feather-feeding lice of a bird).

The main problem with interspecific interactions in parasitic species inferred from census and/or relative abundance data collected in the field is that they reflect not only the relationships between parasitic species but also the relationships between each of the parasites and a host. In other words, anti-parasitic defenses of a host may mediate or even mask true relationships among parasite species (e.g., Bush and Malenke 2008). This may result in contradictory conclusions about type of interspecific interactions in the communities of parasites of the same taxon. For example, both negative (Barnes et al. 1977; Day and Benton 1980; Lindsay and Galloway 1997) and positive (Krasnov et al. 2006a, b, 2010) interspecific interactions among flea species have been inferred from the observational data. Furthermore, the results of laboratory experiments with fleas repeatedly fed on a rodent host in mixed- and single-species groups suggested that one of the responses to co-infestation can be increased reproductive performance, although this response may vary among flea species (Khokhlova et al. 2015). However, when interspecific interactions among fleas were experimentally studied on their larvae (which are not parasitic and thus the mediating effect of host defenses on their interactions is obviously not relevant), clear competition among different species has been revealed (Krasnov et al. 2005). Consequently, experimental studies on interspecific interactions in parasites require experimental exclusion of the defense efforts of a host. Obviously, studies on helminths would require exclusion of mainly immunological defenses, whereas studies on ectoparasites would require exclusion of both behavioral and immunological defenses.

In this study, we investigated interspecific interactions between two flea species [*Parapulex chephrenis* and *Xenopsylla ramesis* (both Siphonaptera: Pulicidae)] in single-species or mixed-species groups and evaluated their feeding success as they exploited rodent hosts [*Acomys cahirinus* (Rodentia: Muridae: Deomyinae) or *Meriones crassus* (Rodentia: Muridae: Gerbillinae)]. Both rodents are commonly found in the southern part of the Negev desert of Israel. *M. crassus* is characterized by species-rich flea assemblages with *X. ramesis* being one of the most abundant and prevalent (Krasnov et al. 1998, 1999). In contrast, *A. cahirinus* is characteristically parasitized by *P. chephrenis*, which is rarely recorded on other hosts except for a congeneric species *Acomys russatus* (Krasnov et al. 1997, 1999). Nonetheless, *X. ramesis* and *P. chephrenis* have been occasionally found on *A. cahirinus* and *M. crassus*, respectively.

Fleas represent a convenient model for experimental studies. Imagoes of these insects are obligatory hematophagous parasites of mammals and birds, whereas larvae of the majority of species are not parasitic. Moreover, although fleas spend more time on their hosts than is required merely to obtain a blood meal, they spend much time off their hosts as well. This allows us to manipulate flea infestation on living hosts and to monitor changes in individual fleas over time. We used the mass-specific amount of blood that a flea consumed during a single feeding bout and the time that a flea survived after a single blood meal as estimates of feeding performance. We choose to use changes in flea feeding rather than reproductive performance as indicators of interspecific interactions because measuring flea feeding allows us to largely remove the effects of host anti-parasitic defenses from the experiments. First, fleas can be successfully fed on grooming-restricted hosts thus excluding host behavioral defenses (e.g., Khokhlova et al. 2009). Second, the majority of fleas require several blood meals for successful mating and egg production (see Krasnov 2008 for review). Repeated ectoparasite challenges cause a host to acquire anti-parasitic resistance that, in turn, suppresses ectoparasite feeding, reproduction and/or survival (e.g., Fielden et al. 1992). Thus, it is likely that a singular flea feeding event will cause only a negligible (if any) immunological response in an immune-naïve host.

We predicted that if negative interactions between the two flea species occur, then fleas would take less blood and survive for a shorter time after a single blood meal in mixed-species as compared to single-species infestations. In addition, we tested the response of fleas to mixed- versus single-species infestations when fleas fed on either their characteristic or a non-characteristic host. Earlier field observations suggested that the outcome of interspecific interactions in fleas may depend on host identity (Evans and Freeman 1950) due to differential suitability of a host to interacting parasites. In fact, even a host-opportunistic flea varies in its abundance or reproductive success among different host species with some host species providing better resources to a given parasite as compared to other host species (e.g., Krasnov et al. 2002; Khokhlova et al. 2012a). Consequently, competitive abilities of a parasite are expected to be better realized in a more suitable host, similar to the effect of habitat quality on the competitive outcome in free-living species (e.g., Steneck et al. 1991; Morris et al. 2000). We predicted that the negative effect of mixed-species infestations on feeding performance would be more pronounced in a non-characteristic host. In addition, we tested for the interrelationships between the two measures of feeding performance.

Material and methods

Rodents and fleas

We used rodents (*A. cahirinus* and *M. crassus*) and fleas (*P. chephrenis* and *X. ramesis*) from our laboratory colonies starting from specimens collected in the wild in 1999. Starting in 2004, we annually added 5 to 10 field-captured rodents of each species and 100 to 150 field-collected fleas of each species to the respective colonies. This was done to maintain genetic diversity of the colonies. Fleas were maintained on their natural host species. In particular, *X. ramesis* were maintained on *M. crassus* and *Gerbillus dasyurus* and *P. chephrenis* were maintained on *A. cahirinus* and *A. russatus*. Details of breeding and maintenance of flea and rodent colonies can be found elsewhere (e.g., Krasnov et al. 2002, 2003; Khokhlova et al. 2004, 2008, 2012a, b). In this study, we used 24–48-h-old fleas that have never fed prior to experiments and 6–8-month-old sexually naïve male rodents that have never experienced any contact with fleas prior to experiments. Fleas and rodents were randomly selected from the colonies. Each rodent individual was used in a single treatment.

Experimental procedures

Feeding performance and efficiency (estimated via resistance to starvation after a single blood meal; discussed earlier) was tested when fleas exploited either their characteristic or non-characteristic host in groups composed of either a target species alone or together with another species. Feeding performance and resistance to starvation of *P. chephrenis* was evaluated when it exploited *A. cahirinus* or *M. crassus* (either alone or together with *X. ramesis*). Feeding performance and resistance to starvation of *X. ramesis* was evaluated when it exploited *M. crassus* or *A. cahirinus* (either alone or together with *P. chephrenis*).

Each treatment (*P. chephrenis* or *X. ramesis* with *A. cahirinus* or *M. crassus* in single- or mixed-species infestations; two flea species × two host species × two types of infestation = eight treatments) was replicated 12–16 times. Experimental procedures were as follows. An individual rodent (either *A. cahirinus* or *M. crassus*) was placed in a wire mesh (5 mm × 5 mm) tube (15-cm length and 5-cm diameter) with caps on both ends to prevent the rodent from leaving the tube or turning around in the tube. Tubes with rodents were placed in individual white plastic baths. Fleas (*P. chephrenis* or *X. ramesis* or both) were then released into the hair of this rodent. To equalize flea density, single-species groups were composed of 50 (30 females and 20 males) *X. ramesis* or *P. chephrenis*, whereas mixed-species groups were composed of 25 (15 females and 10 males) *X. ramesis* and 25 (15 females and 10 males) *P. chephrenis*. Prior to releasing fleas to a rodent's hair, they were weighed (male and female groups of each species separately; ±0.01 mg, 290 SCS Precisa Balance, Precisa Instruments AG, Switzerland). After 6 h (time necessary for satiation of both flea species; see Khokhlova et al. 2012b), fleas were collected. We examined the fleas under a light microscope (×40 magnification) and randomly selected up to 10 female and up to 10 male fleas with blood in their midguts (that is, those having taken a blood meal). We weighed these fleas as described earlier (male and female groups of each species separately). The difference in mean flea's body mass before and after feeding was taken as the amount of blood consumed. Then, these fleas were placed individually into 0.5-ml Eppendorf tubes filled with 2 mm of clean sand and covered perforated lids, placed in an incubator (FOC225E, Velp Scientifica srl, Milano, Italy) and maintained at 25 °C and 90 % RH. The tubes were checked daily and the death of each flea was confirmed by examination under light microscopy.

Experimental protocol was approved by the Committee for the Ethical Care and Use of Animals in Experiments of the Ben-Gurion University of the Negev (authorization IL-52-07-2012).

Data analyses

Flea feeding performance was evaluated via the mass-specific amount of blood consumed (mean relative blood meal size per unit flea body mass) by a flea averaged within a group of fleas (females and males separately) that fed on the same rodent. This was calculated as the difference between total mass of fleas after feeding and total mass of fleas prior to feeding divided by total mass of fleas prior to feeding. Feeding efficiency was estimated as time to death after a single blood meal.

Obviously, whether in single- or mixed-species infestations, both female and male fleas of *P. chephrenis* and *X. ramesis* exploited the same individual rodent. Consequently, we applied linear mixed-effects models (LME, Zuur et al. 2009) with the individual number of a rodent as a random factor. We fitted the models using the lme function as implemented in "nlme" package (version 3.1-118; Pinheiro et al. 2014) in R (version 2.14; R Development Core Team 2013). Data entry for the former response variable (mean amount of blood consumed) was a group of male or female fleas fed simultaneously on a rodent, while data entry for the latter response variable (time to death after a single blood meal) was an individual flea. Explanatory variables were flea sex and co-infestation treatment (single-species versus mixed-species). To evaluate the overall fit of each model, we compared it with the model with an intercept and a random effect only using the likelihood ratio test. Reference levels for the fixed effects were female and mixed-species infestation, respectively.

To test for the relationship between flea feeding performance and efficiency, we applied LMEs with the mean blood meal size as a response variable and the mean time to death (for female or male fleas that fed on the same rodent simultaneously) after a single blood meal as an explanatory variable. Rodent individual number, flea sex, and co-infestation treatment were included in the models as random factors.

All analyses were carried out separately for *P. chephrenis* and *X. ramesis* when they fed on either characteristic (that is, *P. chephrenis* on *A. cahirinus* and *X. ramesis* on *M. crassus*) or non-characteristic (that is, *P. chephrenis* on *M. crassus* and *X. ramesis* on *A. cahirinus*) hosts. The reason for separate analyses with characteristic versus non-characteristic hosts is the strong effect of host identity on flea feeding variables (e.g., Krasnov et al. 2003; Khokhlova et al. 2012b) which makes the results for the same flea on different hosts incomparable in the context of this study. In addition, we did not compare feeding performance of the same flea on different hosts because this has been studied earlier (Khokhlova et al. 2012b). Prior to analyses, response variables were log+1 (or log)-transformed. Untransformed data are presented in figures.

Results

Mass-specific amounts of blood taken by *P. chephrenis* from *A. cahirinus* differed significantly between female and male fleas as well as between co-infestation treatments, whereas the effect of interaction between these two factors was non-significant (Table 1). In general, female fleas took larger blood meals per unit body mass than males (Fig. 1). Furthermore, fleas took larger blood meals in single-species than in mixed-species groups (Fig. 1). Similarly to *P. chephrenis*, female *X. ramesis* took larger mass-specific blood meals from *M. crassus* than males did (Table 1, Fig. 1). In contrast with *P. chephrenis*, *X. ramesis* consumed similar amounts of blood per unit body mass in single- and mixed-species infestations (Table 1, Fig. 1). When fleas exploited a non-characteristic host, neither factor affected the size of a blood meal in *P. chephrenis* (Table 2, Fig. 2), whereas patterns found in *X. ramesis* were the same as those revealed for conspecifics feeding on a characteristic host (compare Tables 1 and 2 and Figs. 1 and 2).

Time to death under starvation after a single blood meal taken from either a characteristic or a non-characteristic host was affected by flea sex, but not treatment or the interaction between the two factors in both *P. chephrenis* and *X. ramesis* (Tables 1 and 2). Female fleas survived significantly longer than male fleas (Figs. 3 and 4).

Responses in terms of survival after a single blood meal to the amount of blood of a characteristic host that fleas consumed from a characteristic host were similar in both species (Table 3). All else being equal (flea sex and co-infestation treatment), fleas that took more blood survived significantly longer when starved (Fig. 5). However, when fleas fed on non-characteristic hosts, a positive correlation was found between blood meal size and survival time for *X. ramesis* but not *P. chephrenis* (Fig. 6). Survival abilities of the latter feeding on blood of *M. crassus* did not depend on the size of a blood meal (Fig. 6).

Discussion

The results of this study only partly supported our predictions. In line with our predictions, we found evidence (in *P. chephrenis* but not in *X. ramesis*) of the negative effect of co-infestation on the mean size of a blood meal but not on the time of survival after a blood meal. Contrary to our prediction, the negative effect of co-infestation was pronounced only when *P. chephrenis* exploited a characteristic (*A. cahirinus*) but not a non-characteristic (*M. crassus*) host. This is the first experimental evidence of negative interspecific interactions between imago fleas. The occurrence of such interactions in fleas has been suggested previously (Day and Benton 1980; Lindsay and Galloway 1997) but has never been experimentally tested.

We also found the effects of flea sex on the mass-specific size of a blood meal (larger in females) and survival time under starvation (longer in females). These results support our earlier reports (see Krasnov 2008 for review), although the mass-specific amount of blood consumed by male and female fleas differed in some but not other experiments (Khokhlova et al. 2012b versus Sarfati et al. 2005). In the following section, we will not discuss the effect of flea sex on feeding performance because this effect was not the focus of the present study and has been discussed elsewhere (Krasnov 2008, Khokhlova et al. 2009).

Mechanisms of negative interspecific interactions

Negative interspecific interactions usually arise when the resources necessary for interacting species are limited (e.g., Tilman 1977). The main resource that fleas extract from a host is its blood which is definitely not limited for fleas. For example, during one meal, 50 *X. ramesis* fleas took in only about 0.17 % of the total blood volume of a small rodent host *G. dasyurus* (average body mass ca. 20–22 g) (Khokhlova et al. 2002). Both rodents used in this study are much larger (average body masses of male *A. cahirinus* and *M. crassus* are

Table 1 Summary of the linear mixed-effects models of the effects of fleas sex and treatment (T; single-species versus mixed-species infestations) on (a) mean mass-specific size of a blood meal taken by a flea (BMS) and (b) mean time to death under starvation after a single blood meal (TD) for *Parapulex chephrenis* and *Xenopsylla ramesis* fleas fed on their characteristic hosts (*Acomys cahirinus* or *Meriones crassus*, respectively) either alone or together with another flea species

Flea species	Response variable	Fixed effect	Coefficient estimate±SE	*t* value	AIC	LLR
P.chephrenis	BMS	Sex (male)	−0.04±0.01	−3.97**	−205.5	32.24***
		T (single)	0.03±0.01	2.42*		
		Sex×T	0.001±0.02	0.11^{ns}		
	TD	Sex (male)	−0.10±0.01	−8.51***	−1498.2	215.43***
		T (single)	0.02±0.01	1.75^{ns}		
		Sex×T	−0.02±0.01	-1.68^{ns}		
X. ramesis	BMS	Sex (male)	−0.09±0.01	−9.40***	−229.3	68.10***
		T (single)	0.03±0.01	1.75^{ns}		
		Sex×T	−0.01±0.01	-1.00^{ns}		
	TD	Sex (male)	−0.23±0.01	−19.59***	−1541.6	670.38***
		T (single)	−0.01±0.01	-0.68^{ns}		
		Sex×T	0.02±0.01	1.12^{ns}		

Individual rodent number was introduced as a random effect in each model. Reference levels for the fixed effects were female for sex and mixed-species infestation for treatment. AIC and LLR are related to the entire model. Coefficient estimates and *t* values are related to each fixed effect separately

AIC Akaike Information Criterion, *LLR* likelihood ratio statistics when comparing the best model with the model of intercept and a random effect only, *ns* non-significant

Significance levels: *–$p<0.05$; **–$p<0.01$; ***–$p<0.001$

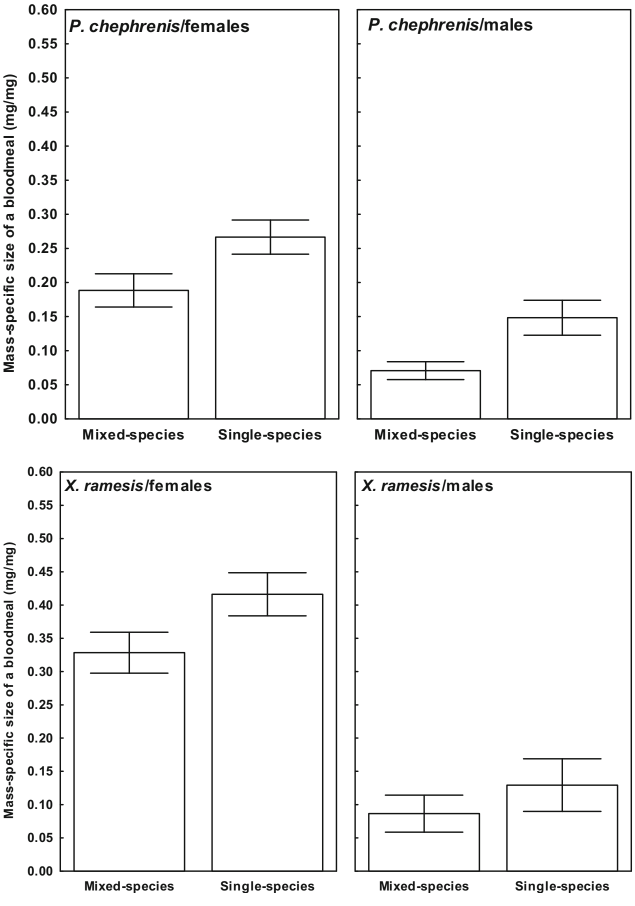

Fig. 1 Mean (±S.E.) mass-specific amount of blood taken by female and male *Parapulex chephrenis* and *Xenopsylla ramesis* when feeding during 6 h on a characteristic host (*Acomys cahirinus* and *Meriones crassus*, respectively) in single- or mixed-species infestations

40–45 and 80–100 g, respectively), so fleas take even smaller relative portions of total blood volume when they exploit these hosts. However, ectoparasites may compete for the pattern of resource (i.e., blood) acquisition. Ectoparasites belonging to different species or even higher taxa often demonstrate preferences to the same body parts of a host. This has not only been reported in purely descriptive and narrative studies (e.g., Linsdale and Davis 1956) but has also been recently supported by formal statistical analyses (Pilosof et al. 2012). The reasons for concentration of heterospecific ectoparasites in the same body areas of a host could be shared microclimatic preferences (e.g., Ma 1989), relative ease of blood extraction from certain body areas determined by skin thickness, hair density, or blood capillary depth (e.g., Klukowski 2004), as well as naturally selected preference to the "safest" (in terms of host's grooming or preening) body areas that are less accessible for hosts paws, teeth, or beak (see Krasnov 2008). One of the consequences of concentration of ectoparasites belonging to different species in the same body areas is a "local" increase in their density which may likely result in a decrease in the amount of blood taken during single feeding bout by an individual ectoparasite similar to the effects of a density on the size of a blood meal among conspecific ectoparasites (Tyre et al. 2003, Hawlena et al. 2007).

Possible consequences of negative interspecific interactions

Feeding performance of ectoparasites may further be translated into reproductive success as follows (a) the more blood a

Table 2 Summary of the linear mixed-effects models of the effects of fleas sex and treatment (T; see description in Table 1) on (a) mean mass-specific size of a blood meal taken by a flea (BMS) and (b) mean time to death under starvation after a single blood meal (TD) for *Parapulex chephrenis* and *Xenopsylla ramesis* fleas fed on their non-characteristic hosts (*Meriones crassus* or *Acomys cahirinus*, respectively) either alone or together with another flea species

Flea species	Response variable	Fixed effect	Coefficient estimate±SE	*t* value	AIC	LLR
P.chephrenis	BMS	Sex (male)	−0.004±0.01	−0.46^{ns}	−252.4	3.76^{ns}
		T (single)	−0.005±0.01	−0.45^{ns}		
		Sex×T	−0.01±0.01	0.75^{ns}		
	TD	Sex (male)	−0.09±0.01	−6.33***	−1033.6	107.75***
		T (single)	0.01±0.01	0.85^{ns}		
		Sex×T	−0.02±0.02	−0.87^{ns}		
X. ramesis	BMS	Sex (male)	−0.09±0.02	−3.69**	−117.1	19.73***
		T (single)	−0.01±0.02	−0.66^{ns}		
		Sex×T	0.02±0.03	0.50^{ns}		
	TD	Sex (male)	−0.18±0.02	−7.99***	−447.0	117.08***
		T (single)	−0.03±0.02	−1.21^{ns}		
		Sex×T	0.03±0.03	1.04^{ns}		

Individual number of a rodent was introduced as a random effect in each model. Reference levels for the fixed effects were female for sex and mixed-species infestation for treatment. See Table 1 for definitions of AIC and LLR as well. Asterisks denote significance level as in Table 1

flea takes, the more energy and nutrients it can transform into offspring and (b) the longer a flea lives after taking blood, the more offspring it may produce as a result of increased feeding and, consequently, reproductive opportunities (see Krasnov

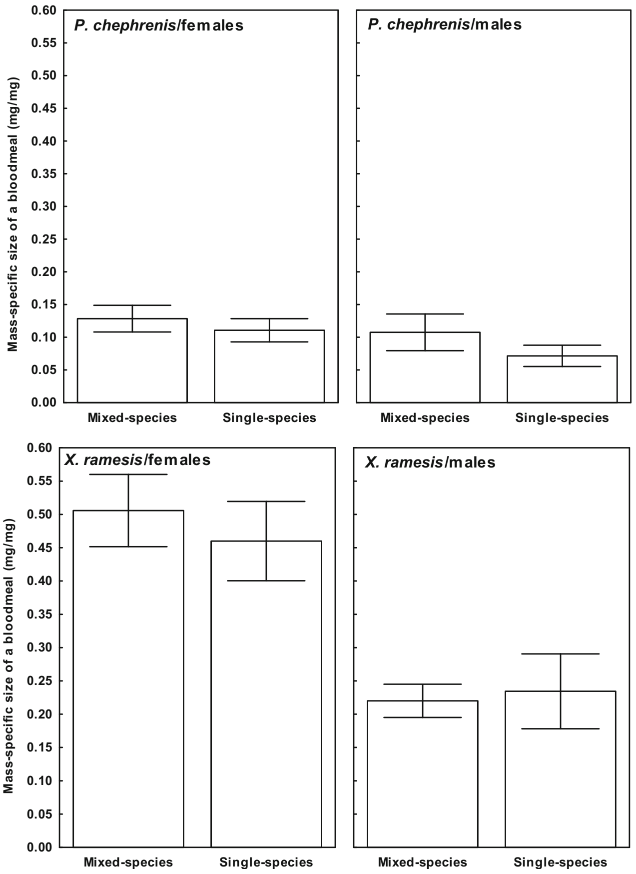

Fig. 2 Mean (±S.E.) mass-specific amount of blood taken by female and male *Parapulex chephrenis* and *Xenopsylla ramesis* when feeding during 6 h on a non-characteristic host (*Meriones crassus* and *Acomys cahirinus*, respectively) in single- or mixed-species infestations

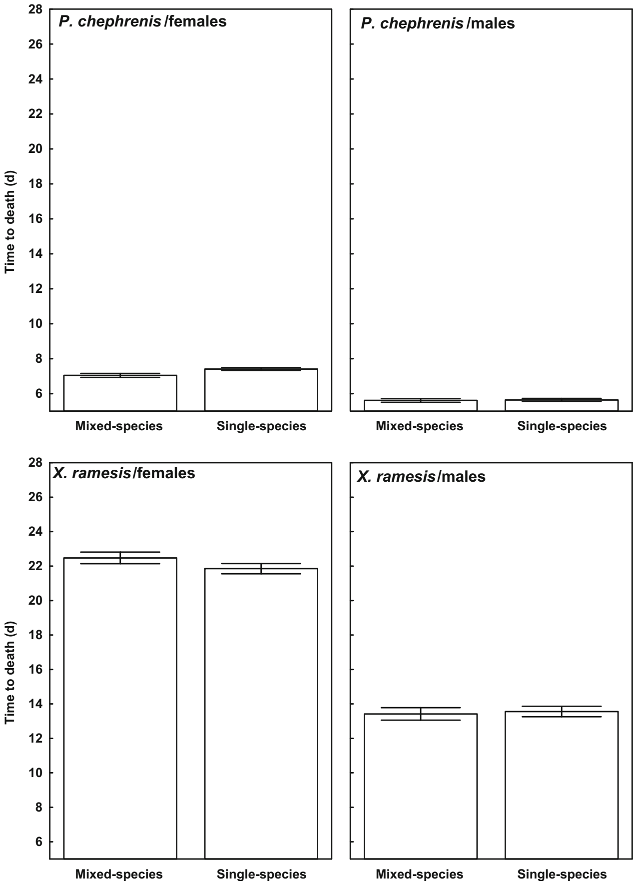

Fig. 3 Mean (±S.E.) time (days) to death of female and male *Parapulex chephrenis* and *Xenopsylla ramesis* after a single blood meal taken from a characteristic host (*Acomys cahirinus* and *Meriones crassus*, respectively) in single- or mixed-species infestations

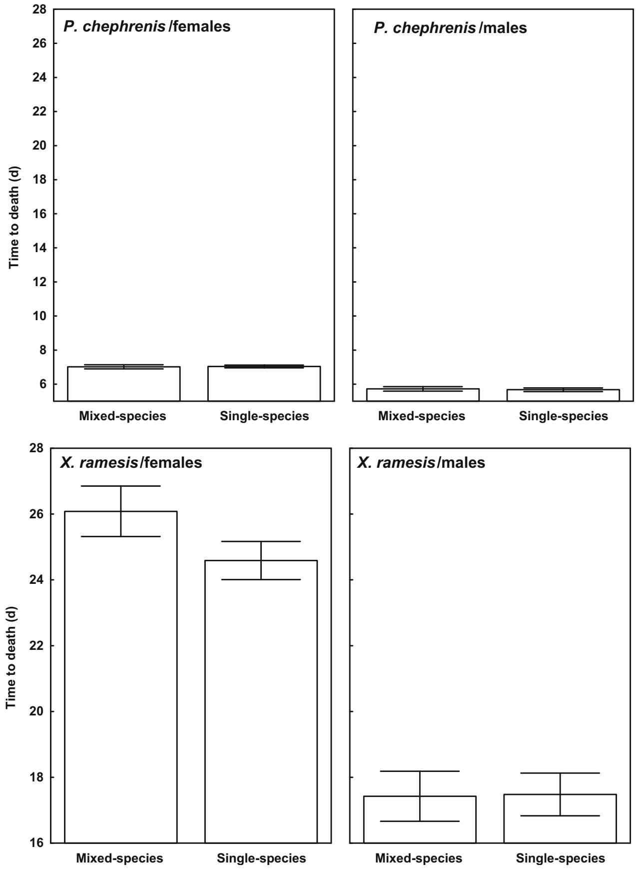

Fig. 4 Mean (±S.E.) time (days) to death of female and male *Parapulex chephrenis* and *Xenopsylla ramesis* after a single blood meal taken from a non-characteristic host (*Meriones crassus* and *Acomys cahirinus*, respectively) in single- or mixed-species infestations

2008 for the elaboration on these measures). In fact, the size of a blood meal was found to correlate positively with the number of eggs produced by various hematophagous arthropods (Shelton 1972; Leprince and Foil 1993). However, a decrease in the amount of blood taken from *A. cahirinus* by *P. chephrenis* when it co-fed with *X. ramesis* (this study) may not necessarily be followed by a decrease in the reproductive outcome of the former in terms, for example, of the number of eggs produced (Khokhlova et al. 2015). In contrast, *P. chephrenis* appeared to be somehow able to compensate for the negative effect of co-infestation on its feeding by producing larger eggs when it co-fed together with *X. ramesis* (Khokhlova et al. 2015).

Table 3 Summary of linear mixed-effects models of the effects of the mean mass-specific blood meal size of a flea on the mean time to death under starvation after a single blood meal for *Parapulex chephrenis* and *Xenopsylla ramesis* fed on either characteristic (*Acomys cahirinus* or *Meriones crassus*, respectively) or non-characteristic (*Meriones crassus* or *Acomys cahirinus*, respectively) hosts in single- or mixed-species infestations. Rodent individual number, flea sex and co-infestation treatment were introduced in the models as random factors

Flea species	Host species	Coefficient estimate±SE	*t* value	*F*
P.chephrenis	*A. cahirinus*	0.62±0.16	3.72**	13.84**
	M. crassus	0.38±0.92	0.40^{ns}	0.16^{ns}
X. ramesis	*M. crassus*	2.14±0.32	6.59***	43.40***
	A. cahirinus	1.30±0.23	5.65**	31.99**

Significance levels: **–$p<0.01$; ***–$p<0.001$

Linear mixed-effects models failed to reveal a direct effect of co-infestation on the resistance of individual *P. chephrenis* to starvation after a blood meal despite a positive correlation between blood meal size and survival time when the latter variable was averaged across fleas fed simultaneously on a host. This contradiction might arise due to differences in the dependent variables between the two analyses (a measurement of an individual flea versus averaged estimation across fleas). Another reason for this contradiction could be some, still unclear, context-dependent variation in the relationships between the two measures of feeding performance. For example, engorgement degree of *P. chephrenis* correlated with the survival time after a blood meal when fleas fed on *A. cahirinus* but not on *X. ramesis*.

Effects of flea and host identities

The most intriguing result of this study was the asymmetries in the response to co-infestation between the two flea species. In co-infestation treatments, presence of *X. ramesis* exerted negative effects on *P. chephrenis*, whereas presence of *P. chephrenis* has no effect whatsoever on *X. ramesis*. Moreover, negative effect of *X. ramesis* on *P. chephrenis* occurred only in *A. cahirinus* (characteristic host of *P. chephrenis*), whereas the lack of an effect of *P. chephrenis* on *X. ramesis* was found independently of whether feeding occurred on a characteristic host or not. These asymmetries are likely associated with differences between the two fleas in four ecological and/or behavioral traits. First, in the majority of hosts, *P. chephrenis* occur alone, whereas *X. ramesis* usually co-occur with a variety of other fleas (Krasnov et al. 1997, 1999). Second, *P. chephrenis* is a host specialist that mainly parasitizes congeneric *A. cahirinus* and *A. russatus* and rarely occur on other host species, whereas *X. ramesis* is a host opportunist that attacks hosts belonging to different species and genera (Hoogstraal and Traub 1965; Krasnov et al. 1997). Third, *P. chephrenis* and *X. ramesis* differ in the proportion of time spent in each of these two places. *P. chephrenis* is a “body” flea that spends most of its life on a host, whereas *X. ramesis* is a “nest” flea that stays on a host only to gain a blood meal, then leaves it and attacks it again for the next feeding bout (see Krasnov 2008 for definitions and explanations). Finally, *X. ramesis* is more mobile than *P. chephrenis*. It is able to move freely across a host’s body (although prefers specific body areas; discussed earlier), whereas *P. chephrenis* on a host usually do not move (Khokhlova, unpublished data).

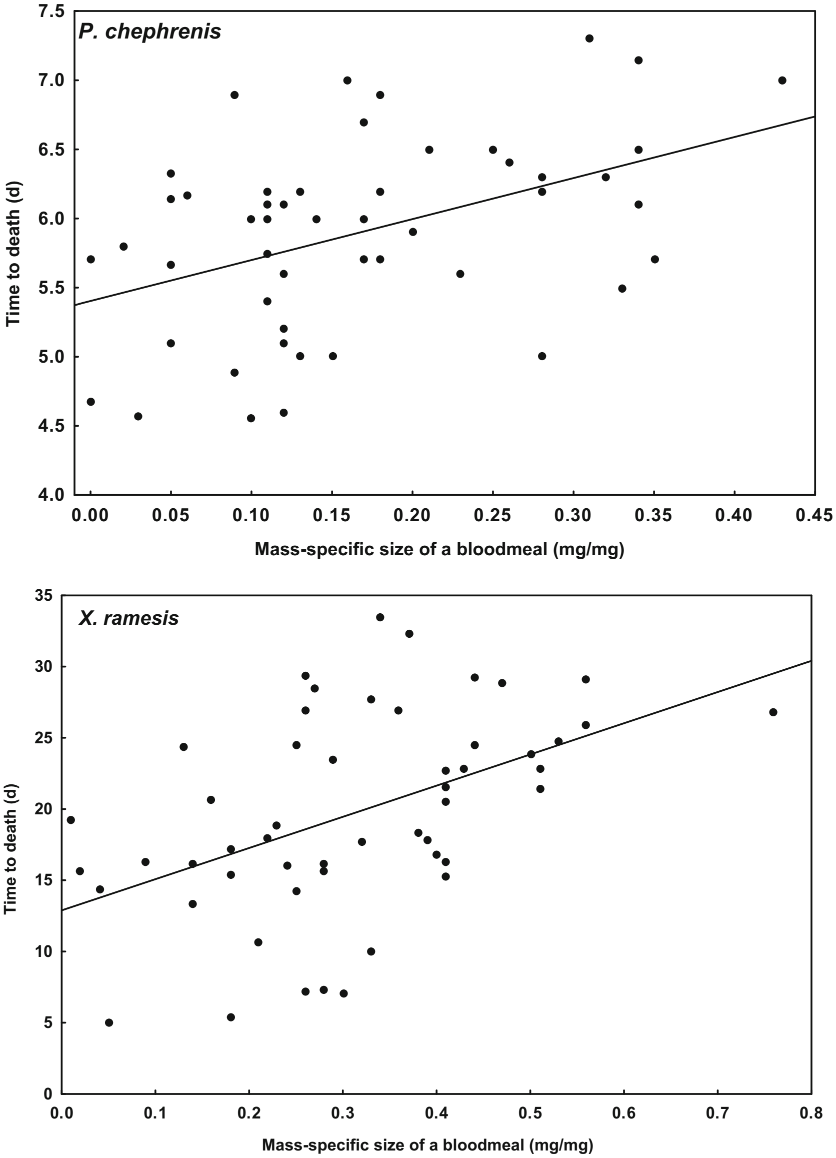

Fig. 5 Relationship between the mean mass-specific size of a blood meal and the mean time to death after a single blood meal taken by *P. chephrenis* or *X. ramesis* from a characteristic host (*A. cahirinus* and *M. crassus*, respectively)

As a result, some individuals of *X. ramesis* might avoid a contact with *P. chephrenis* by moving away from the areas of encounter, whereas *P. chephrenis* is unable to such avoidance.

Species that evolved in species-rich versus species-poor communities may differ in their responses to other species with members of species-rich communities developing some kind of ecological tolerance to other species. In other words, co-occurrence with other species is typical for *X. ramesis* but not for *P. chephrenis*. Consequently, *P. chephrenis* demonstrated a negative response to co-infestation with *X. ramesis* via decreased blood meal size, whereas feeding performance of *X. ramesis* did not depend on whether the other species was present or not. We failed to find any support for the ecological tolerance hypothesis in the literature and recognize that our explanation is, at present, speculative and warrants further investigation.

The occurrence of the negative effect of co-infestation on feeding performance of *P. chephrenis* in *A. cahirinus* but not *M. crassus* might be associated with its high degree of host-specificity. Blood of a non-characteristic host (*M. crassus*) presents a low-quality resource for this flea because of high energy expenditure for its digestion (Khokhlova et al. 2013). Nevertheless, *P. chephrenis* feeding on *M. crassus* invest in the size of the eggs at the expense of their number. Larger egg size promotes better egg survival, so the net result of feeding on this host is a moderate decrease in the number of imagoes of a new generation in comparison with conspecifics feeding on a characteristic host (Khokhlova et al. 2012a). Thus, the main strategy of *P. chephrenis* when only a non-characteristic

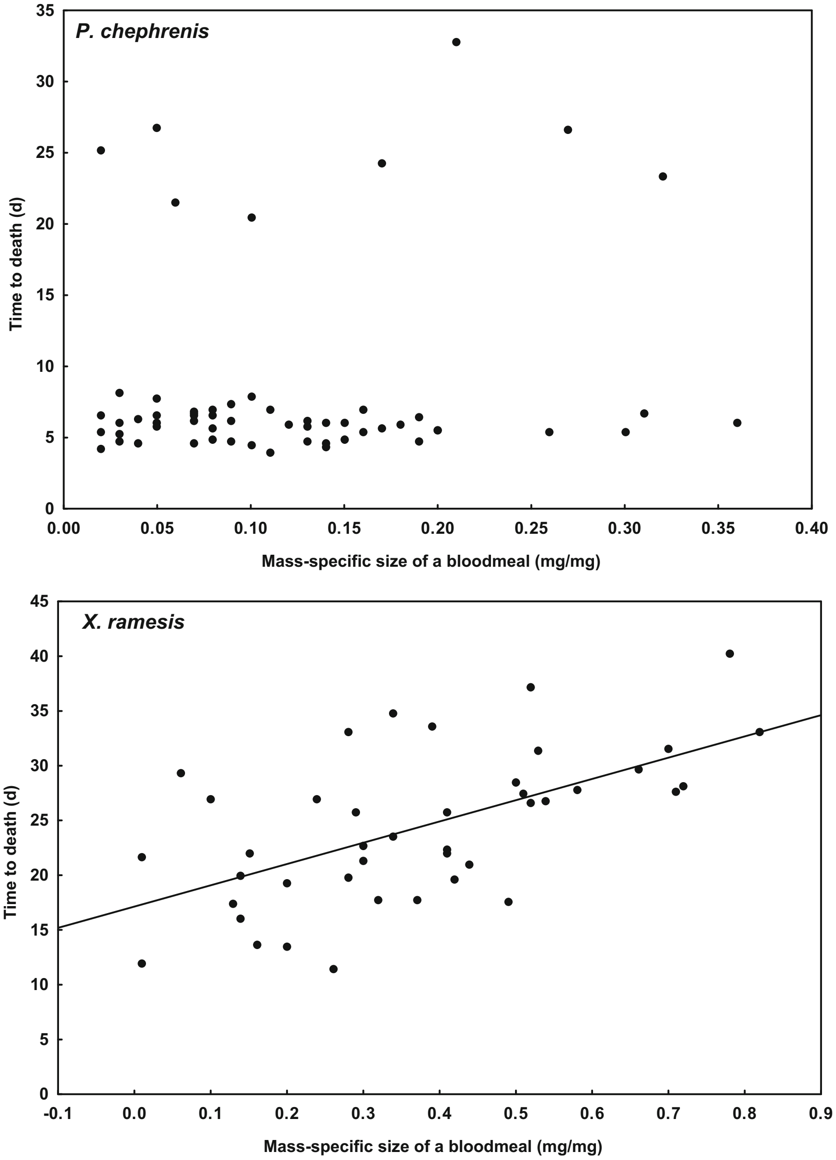

Fig. 6 Relationship between the mean mass-specific size of a blood meal and the mean time to death after a single blood meal taken by *P. chephrenis* or *X. ramesis* from a non-characteristic host (*M. crassus* and *A. cahirinus*, respectively)

host is available is to do "the best-of-the-bad job" and tolerate damage caused by low-quality resources. This strategy could be advantageous for a body flea because it rarely leaves its host individual so the probability of finding a more suitable host is likely low. Thus, the strong effect of host identity on feeding and/or reproductive effort of *P. chephrenis* may outweigh other effects such as co-infestation and flea sex (compare the effects of flea sex in Tables 1 and 2). On the contrary, *X. ramesis* encountering a non-characteristic host seems to rely mainly on the high probability that its next host will be more suitable, so it behaves similarly on both host species independently on whether it co-fed with other species or not, although it takes relatively more blood from a non-characteristic than a characteristic host (compare Figs. 1 and 2; see also Khokhlova et al. 2012b).

Interspecific interactions inferred from census data and experimental results

Results of our study suggest context-dependence of the negative effect of co-infestation on feeding performance in fleas with the manifestation of this effect varying among flea and host species. This variation may explain a contradiction between results of this study and patterns found when interspecific interactions among fleas were inferred from census data. The latter were found to be generally positive (e.g., Krasnov et al. 2006a, b). However, census data consider the entire assemblages of imago fleas on multiple hosts, whereas pairwise interactions between flea species can be either positive (*Xenopsylla hirtipes* and *Xenopsylla gerbilli* on *Rhombomys opimus*; Stepanova and Mitropolsky 1977) or negative (*P. chephrenis* and *X. ramesis* on *A. cahirinus*; this study) or neutral (*P. chephrenis* and *X. ramesis* on *M. crassus*; this study). Consequently, the net positive interactions revealed from census data can result from negative pairwise effects.

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