

Fitness responses to co-infestation in fleas exploiting rodent hosts

IRINA S. KHOKHLOVA¹, ELIZABETH M. DLUGOSZ² and BORIS R. KRASNOV^{2*}

¹Wylar Department of Dryland Agriculture, French Associates Institute for Agriculture and Biotechnology of Drylands, Jacob Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev, Sede-Boqer Campus, 8499000 Midreshet Ben-Gurion, Israel

²Mitrani Department of Desert Ecology, Swiss Institute for Dryland Environmental and Energy Research, Jacob Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev, Sede-Boqer Campus, 8499000 Midreshet Ben-Gurion, Israel

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SUMMARY

To understand mechanisms behind positive interspecific co-occurrences in flea infracommunities, we asked whether co-infestation results in an increase of flea fitness (quantity and/or quality of the offspring). We studied reproductive performance of *Xenopsylla ramesis* and *Parapulex chephrenis* when they exploited their characteristic host (*Meriones crassus* and *Acomys cahirinus*, respectively) either alone or together with another species. We used egg production, the number of new imagoes, pre-imaginal survival and egg size as fitness-related variables and predicted that fitness will be higher in fleas feeding in mixed- than in single-species groups. In both fleas, mean number of eggs produced per female flea did not depend on experimental treatment. No effect of single- vs mixed-species infestation on the mean number of new imagoes per female and the number of emerged imagoes per egg was found for *X. ramesis*, whereas both these numbers were higher in mixed- than in single-species groups for *P. chephrenis*. *X. ramesis* produced eggs of similar size independently of treatment, whereas eggs produced by *P. chephrenis* in mixed-species groups were significantly larger than eggs produced in single-species groups. We conclude that an increase in reproductive performance as a response to co-infestation may be one of the mechanisms behind aggregative structure of flea infracommunities. However, this response may vary among flea species.

Key words: Aggregation, co-infestation, fitness, infracommunity.

INTRODUCTION

Investigations of structure in the communities of ectoparasites infesting a host individual (hereafter known as infracommunities) in terrestrial ecosystems consistently demonstrated that these communities are non-randomly assembled (Krasnov *et al.* 2006a, b; Tello *et al.* 2008; Presley, 2011). In most cases, this non-randomness is reflected in the aggregative structure of ectoparasite infracommunities. In particular, analyses of ectoparasite co-occurrences using null models demonstrated that different ectoparasites species co-occurred on the same host individual significantly more often than expected by chance (Krasnov *et al.* 2006a; Presley, 2011). In other words, ectoparasites are characterized by positive co-occurrences on the same host. Comparison of infracommunity structure of various ectoparasites harboured by the same host showed that flea infracommunities demonstrated a higher degree of interspecific aggregation than lice, mites or ticks (Krasnov *et al.* 2010). Furthermore, flea communities consistently

demonstrated aggregative structure not only at the scale of infracommunities, but also at the component (xenocommunity; an assemblage of parasites of all species infesting a population of a host belonging to a particular species) (Krasnov *et al.* 2006b, 2011) and compound (an assemblage of parasites of all species infesting all co-occurring host species) community scales (Krasnov *et al.* 2005a). For example, the abundance of an individual flea species in a component community or a compound community was found to correlate positively with the abundance of all other co-occurring flea species (Faulkenberry and Robbins, 1980; Krasnov *et al.* 2005a; Brinkerhoff *et al.* 2006; see also Presley, 2007 for streblid bat flies).

Although positive co-occurrences of different ectoparasites (in particular, fleas) has often been reported from census data (see above), the mechanisms behind this interspecific aggregation remained unclear. It has been suggested (but never tested experimentally) that the mechanisms of positive co-occurrence of ectoparasites on an individual host include not only obvious shared preferences of different parasites but also apparent facilitation (*sensu* Levine, 1999) mediated via the host. Indeed, a host defends itself actively against a parasite using various behavioural and immunological tools. However, multiple challenges from a variety of parasite species may suppress host defence

* Corresponding author. Mitrani Department of Desert Ecology, Swiss Institute for Dryland Environmental and Energy Research, Jacob Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev, Sede-Boqer Campus, 84990 Midreshet Ben-Gurion, Israel. E-mail: krasnov@bgu.ac.il

systems (Bush and Holmes, 1986; Cox, 2001). Moreover, energetic and/or nutritional costs of the immune response are high (Demas and Nelson, 1998), so mounting different types of immune responses will likely be more costly than mounting one specific type of response (Taylor *et al.* 1998). As a result, an increase in the diversity of parasite attacks leads to a decrease in the effectiveness of energy allocation to immune defence (Jokela *et al.* 2000), so that the optimal strategy for a host subjected to multiple parasite challenges would be to tolerate damage and give up its defence (Jokela *et al.* 2000). From a parasite's perspective, this would be reflected in higher abundance of each parasite species that, in turn, would be caused by higher reproductive performances of parasites co-infesting a host. In other words, ectoparasites co-infestation is expected to result in an increase of their fitness in terms of either quantity or quality of the offspring or both. In this study, we tested this hypothesis using fleas *Xenopsylla ramesis* and *Parapulex chephrenis* parasitic on rodent hosts, *Meriones crassus* and *Acomys cahirinus*, in either single-species or mixed-species groups.

Both rodents are common species in the Negev desert of Israel. *Meriones crassus* is parasitized by several flea species among which the generalist *X. ramesis* is one of the most common (Krasnov *et al.* 1996, 1997). Host specialist *P. chephrenis* is a characteristic flea of *Acomys cahirinus* and a congeneric species *Acomys russatus*, whereas fleas of other species rarely attack it (Krasnov *et al.* 1997). Nevertheless, *X. ramesis* was sometimes recorded on *A. cahirinus* and *P. chephrenis* was sometimes recorded on *M. crassus* (G.I. Shenbrot and B.R. Krasnov, unpublished data).

In our experiments, we tested reproductive performance of fleas when they exploited their characteristic host either alone or together with another flea species. We used egg production, the number of imagoes of a new generation, pre-imaginal survival and size of eggs as fitness-related variables and predicted that the values of all these variables will be higher in fleas feeding on a host in mixed-species than in single-species groups.

MATERIALS AND METHODS

Fleas and rodents

We used fleas (*X. ramesis* and *P. chephrenis*) and rodents (*M. crassus* and *A. cahirinus*) from our laboratory colonies. These colonies started from field collected specimens in 1999. For the sake of maintaining genetic diversity of the laboratory populations, we annually (starting in 2004) added 100–150 fleas of each species and 5–10 rodents of each species captured in the wild to respective colonies. Fleas were maintained on their natural host species, namely *X. ramesis* on *M. crassus* and *Gerbillus dasyurus* and *P. chephrenis* on *A. cahirinus*

and *Acomys russatus*. Details of breeding and maintenance of flea and rodent colonies can be found elsewhere (e.g. Krasnov *et al.* 2001, 2002, 2003; Khokhlova *et al.* 2004, 2008, 2014; Sarfati *et al.* 2005). In this study we used newly emerged fleas 24–48 h old that have not fed prior to experiments and 6–8 month-old male rodents that have never been exposed to fleas prior to experiments.

Experimental design and procedures

The focus of our study was the effect of co-infestation on flea fitness. Consequently, reproductive performance of each flea species in single-species or mixed-species groups was tested when a flea exploited its characteristic host. In other words, reproductive performance of *X. ramesis* was evaluated when it exploited *M. crassus* either alone or together with *P. chephrenis*, whereas reproductive performance of *P. chephrenis* was evaluated when it exploited *A. cahirinus* either alone or together with *X. ramesis*. We did not measure reproductive variables in fleas fed on non-characteristic hosts (i.e. *X. ramesis* on *A. cahirinus* and *P. chephrenis* on *M. crassus*) intentionally because of the strong effect of host identity on reproductive performance of fleas with substantial decrease in, for example, egg production when fleas use non-characteristic host that is distantly related (but not too distantly; see Krasnov *et al.* 2007) to their principal host (e.g. Krasnov *et al.* 2003; Khokhlova *et al.* 2012), all else being equal.

Each treatment (single-species *vs* mixed-species infestations) for each host species was replicated 12–19 times. Experimental procedures were as follows. An individual rodent (either *M. crassus* or *A. cahirinus*) was placed in a plastic cage (60 × 50 × 40 cm³) with a floor of 3–5 mm of clean sand covered by a wire mesh (5 × 5 mm²). Then, we released a group of fleas into the cage for 3 days. To equalize ectoparasite pressure on a rodent, single-species groups were composed of 50 (30 females and 20 males) either *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*. This number of fleas is not higher than maximal number of fleas found simultaneously on an individual rodent (Krasnov *et al.* 1996, 1997). Under these conditions, fleas usually start to lay eggs no earlier than the 2nd day (Khokhlova *et al.* 2012). Three days of uninterrupted access to a host guaranteed that fleas were able to copulate and produce eggs. On the 4th day of the experiment, we collected fleas from both the rodent's body (over a white plastic pan using a toothbrush until no flea was recovered) and cage substrate and counted them. We placed female fleas of the same species recovered from the same individual rodent in a Petri dish and transferred it to an incubator (FOC225E, Velp

Table 1. Descriptive statistics for fitness-related variables in *Xenopsylla ramesis* and *Parapulex chephrenis* exploiting *Meriones crassus* and *Acomys cahirinus*, respectively, in single (S)- and mixed (M)-species infestations.

Flea species	Host species	Variable	Treatment	Mean	Range	SES
<i>X. ramesis</i>	<i>M. crassus</i>	EP	S	1.81	0.70–2.63	0.20
			M	1.69	0.55–3.00	
		NIP	S	1.49	0.47–2.43	0.08
			M	1.44	0.54–3.00	
<i>P. chephrenis</i>	<i>A. cahirinus</i>	NIE	S	0.81	0.63–0.92	–0.49
			M	0.86	0.59–1.00	
		EP	S	1.92	1.26–2.57	–0.14
			M	2.00	0.44–3.00	
		NIP	S	1.19	0.74–1.50	–0.72
			M	1.48	0.22–2.20	
NIE	S	0.63	0.49–0.77	–0.89		
	M	0.73	0.50–0.95			

Fitness-related variables were: egg production (EP; mean number of eggs produced per female), new imago production (NIP; mean number new imagoes produced per parent female) and mean number of new imagoes emerged per egg (NIE; mean number of new imagoes emerged per egg). Standardized effect size (SES) was calculated as mean differences divided by pooled standard deviation.

Table 2. Summary of general linear models of the effect of co-infestation (single-species *vs* mixed-species infestations) on egg production (EP; mean number of eggs produced per female), new imago production (NIP; mean number new imagoes produced per parent female) and mean number of new imagoes emerged per egg (NIE; mean number of new imagoes emerged per egg) in fleas *Xenopsylla ramesis* and *Parapulex chephrenis* exploiting their characteristic rodent host (*Meriones crassus* and *Acomys cahirinus*, respectively).

Flea species	Host species	Variable	Wald statistic	<i>p</i>	Estimation coefficient \pm SE
<i>X. ramesis</i>	<i>M. crassus</i>	EP	0.33	0.56	–0.03 \pm 0.06
		NIP	0.06	0.78	–0.02 \pm 0.08
		NIE	2.24	0.13	0.03 \pm 0.02
<i>P. chephrenis</i>	<i>A. cahirinus</i>	EP	0.15	0.70	0.02 \pm 0.05
		NIP	3.30	0.05	0.12 \pm 0.05
		NIE	5.77	0.02	0.08 \pm 0.06

Scientifica srl, Milano, Italy) at 25 °C air temperature and 90% relative humidity (RH) for 24 h. Then, we checked the dish and counted newly-laid eggs. We measured size of at least 10 eggs from each Petri dish (maximal length and maximal width) under light microscopy on a screen using a digital microscope camera Moticam 2000 with the Motic Images Plus 2.0ML program (Motic, Speed Fair Cp., Ltd., Causeway Bay, Hong Kong) up to nearest \pm 0.01 mm with 40x magnification and calibration using an object-micrometer.

After counting and measuring eggs, Petri dishes with eggs produced by the same group of females were filled with a 1 mm layer of sand and larval food medium [94% dry bovine blood, 5% millet flour and 1% grinded excrements of *M. crassus* (for *X. ramesis*) or *A. cahirinus* (for *P. chephrenis*)], and transferred into an incubator (see above) where they were maintained at 25 °C air temperature and 90% RH. Starting on the 18th day after oviposition (ca. a week less than minimal duration of pre-imaginal development; Krasnov *et al.* 2001), Petri dishes were checked daily until either all eggs developed

into new imago or for 60 consecutive days. We recorded the number of new adults that emerged from each group of eggs.

Fitness-related variables and data analyses

For each group of fleas of each species fed on each individual rodent hosts, we calculated two variables reflecting quantitative component of fitness (i.e. the variables describing the quantity of the offspring) and two variables reflecting qualitative component of fitness (i.e. the variables describing the quality of the offspring). Rationale for using these variables as indicators of flea fitness can be found elsewhere (Krasnov, 2008). The former variables were (a) egg production (mean number of eggs produced per female) and (b) new imago production (mean number of new imagoes produced per female). The latter variables were (a) mean (across eggs produced by the same group of females) number of new imagoes emerged per one egg and (b) egg volume. We considered mean number of new imagoes emerged per one egg as a proxy for pre-imaginal

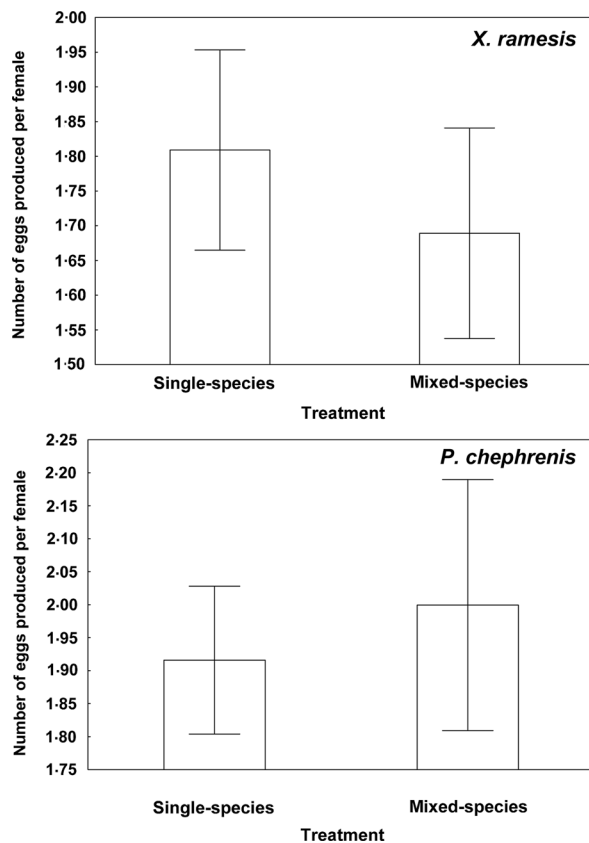


Fig. 1. Mean (\pm S.E.) number of eggs produced per female of *Xenopsylla ramesis* and *Parapulex chephrenis* after 3 days of uninterrupted feeding on a rodent host (*Meriones crassus* and *Acomys cahirinus*, respectively) in single-species or mixed-species infestations.

survival and, thus, an indicator of the offspring quality. Egg volume was calculated after Berrigan (1991) as $V = 1/6\pi * W^2 * L$, where V is egg volume, W is maximal egg width and L is maximal egg length.

Distribution of all four variables did not significantly deviate from normal (Shapiro–Wilk $W = 0.95–0.97$ for *X. ramesis* and Shapiro–Wilk $W = 0.96–0.97$ for *P. chephrenis*; $P > 0.29$ for all). We analysed the effect of treatment (single-species *vs* mixed-species infestations) on egg and new imago production as well as on the number of new imagoes emerged per egg using generalized linear models with normal distribution and log-link function. The effect of treatment on egg volume was analysed using linear mixed-effects models (Zuur *et al.* 2009) with the individual number of a flea group (i.e. fleas that exploited the same rodent individual) 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). All analyses were carried out separately for *X. ramesis* on *M. crassus* and *P. chephrenis* on *A. cahirinus*.

RESULTS

Descriptive statistics for fitness-related variables in the two fleas in single- and mixed-species

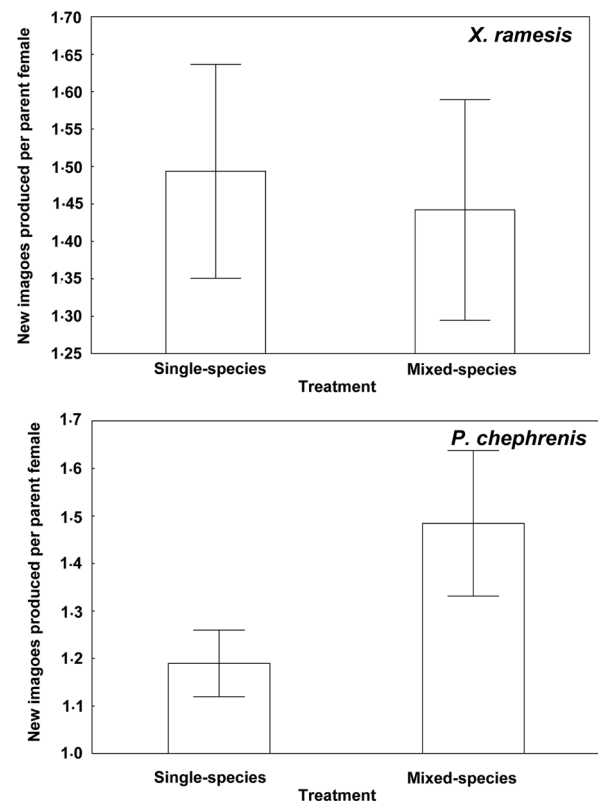


Fig. 2. Mean (\pm S.E.) number of new imagoes produced per female of *Xenopsylla ramesis* and *Parapulex chephrenis* after 3 days of uninterrupted feeding on a rodent host (*Meriones crassus* and *Acomys cahirinus*, respectively) in single-species or mixed-species infestations.

infestations is presented in Table 1. A summary of generalized linear models of the effect of single- *vs* mixed-species infestations of a host on egg and new imago production and mean number of new imagoes emerged per egg in the two flea species is presented in Table 2. In both *X. ramesis* and *P. chephrenis*, mean number of eggs produced per female flea did not depend on whether fleas fed on a host in single- or mixed species groups (Table 2, Fig. 1). No effect of single- *vs* mixed-species infestation on the mean number of new imagoes produced per female flea was found for *X. ramesis*, whereas the number of new imagoes per parent female of *P. chephrenis* was significantly higher in mixed- than in single-species groups (Table 2, Fig. 2). This difference was likely a result of higher survival of eggs from females in mixed-species groups as indicated by higher number of emerged imagoes per egg in these groups as compared with single-species groups, although this was true for *P. chephrenis* but not *X. ramesis* (Table 2, Fig. 3). The results of linear mixed-effects modelling indicated that *X. ramesis* in single- and mixed-species groups produced eggs of similar size (Table 3, Fig. 4). In contrast, eggs produced by *P. chephrenis* in mixed-species groups were significantly larger than eggs produced by conspecifics fed in single-species

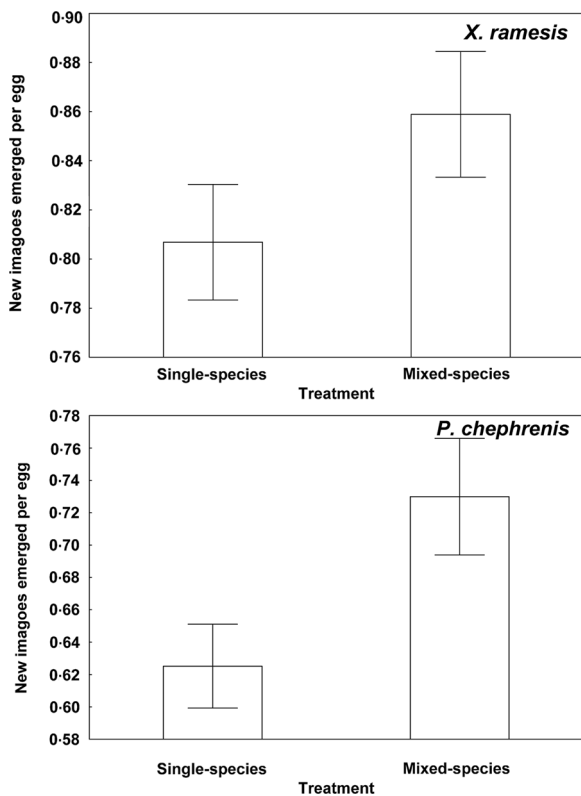


Fig. 3. Mean (\pm S.E.) number of new imagoes emerged per egg from eggs produced by *Xenopsylla ramesis* and *Parapulex chephrenis* after 3 days of uninterrupted feeding on a rodent host (*Meriones crassus* and *Acomys cahirinus*, respectively) in single-species or mixed-species infestations.

groups (Table 3, Fig. 4). The variation in the egg volume due to differences among rodent individuals (a random term in each model) was low (Table 3).

DISCUSSION

Our predictions appeared to be only partly true. The direct effects of single- vs mixed-species infestation were found (a) for the quality, but not quantity component of flea fitness and (b) for *P. chephrenis*, but not *X. ramesis*. Nevertheless, the quantity component of fitness (the number of imagoes of the new generation) of *P. chephrenis* was indirectly affected by co-infestation via its effect on survival ability of eggs.

From a mechanistic perspective, an increase in the quality of the offspring of *P. chephrenis* when fleas exploited their hosts simultaneously with *X. ramesis*, may be associated with a decrease in behavioural and/or immunological anti-parasitic defences of a host subjected to multiple parasite challenges (Jokela *et al.* 2000; but see Sánchez *et al.* 2014). In fact, host resistance is often defined as host-induced loss of fitness in a parasite and, thus, represents a characteristic of a host that is measured via the parasite (Combes, 2001). Accepting this idea, it is fair to state that the efficiency of the host's

anti-parasitic defences may be assessed via its effect on parasites (e.g. Khokhlova *et al.* 2008), although traditionally it is measured in terms of host-related variables such as, for example, leukocyte concentration (Heylen and Matthysen, 2008) or the level of inflammatory cytokines (Johnston *et al.* 2009). For example, multiple infestations by ticks likely led to a development of an acquired resistance in guinea pigs and resulted in decreased feeding and reproduction of ticks in subsequent infestations (Fielden *et al.* 1992). Similarly, increased reproductive performance of *P. chephrenis* in mixed-species groups likely indicates a decrease in anti-flea resistance of *A. cahirinus* under these conditions. A decrease of host defences under co-infestation by different parasites can also be indicated by general decrease of host's body conditions (Lochmiller and Deereberg, 2000). However, when a decrease in body condition and concomitant decrease in anti-parasitic defences under co-infestation are inferred from the field data on the records of different parasites collected from the same host individuals (e.g. Ranzani-Paiva and Silva-Souza, 2004; Risco *et al.* 2014; Serrano and Millán, 2014), it remains unclear whether this is because body condition (and, thus, anti-parasitic defences) deteriorates under multiple parasite attacks or because a host with originally low body condition (and, thus, low defences) represents a better source of resources for different parasites. The latter can be especially true because parasites belonging to the same taxon usually have similar trophic requirements. Nevertheless, the results of our study indicate that the former mechanism is not less likely than the latter.

Furthermore, we found *P. chephrenis* in mixed-species groups produced larger eggs that survived better as compared with those produced by fleas that exploited their hosts in single-species groups. Consequently, the net result of co-infestation was increased fitness of *P. chephrenis* under co-infestation with another flea species. Larger eggs are usually accompanied by better performance of new imagoes emerged from these eggs (Torres-Vila and Rodríguez-Molina, 2002; Pöykkö and Mänttari, 2012), although the size of new imagoes in holometabolous insects depends on other factors as well (e.g. larval food resources and density, air temperature, RH). Nevertheless, larger size and higher survival of eggs in *P. chephrenis* in mixed-species groups can be one of the mechanisms behind correlated abundances of co-infesting ectoparasites (Brinkerhoff *et al.* 2006; Presley, 2007). Another, not necessarily alternative explanation of the increase in egg quality (size and survival) in *P. chephrenis* in mixed-species groups can be a higher investment of females into offspring quality under unfavourable conditions such as some negative interactions with a co-occurring heterospecific fleas. Although the majority of studies on fleas indicate that interspecific

Table 3. Summary of linear mixed-effects models of egg volume in fleas *Xenopsylla ramesis* and *Parapulex chephrenis* dependent upon whether they exploited their host (*Meriones crassus* or *Acomys cahirinus*, respectively) in single-species or mixed-species groups.

Flea species	D.F.	F	Coefficient estimate \pm SE	t	p	PVRand
<i>X. ramesis</i>	33 870	1.97	-0.37 ± 0.27	-1.40	0.17	12.86
<i>P. chephrenis</i>	26 653	4.42	-0.27 ± 0.11	-2.12	0.04	4.70

The reference level for an independent variable was arbitrarily selected as the mixed-species treatment. PVRand—percentage of variation due to random term.

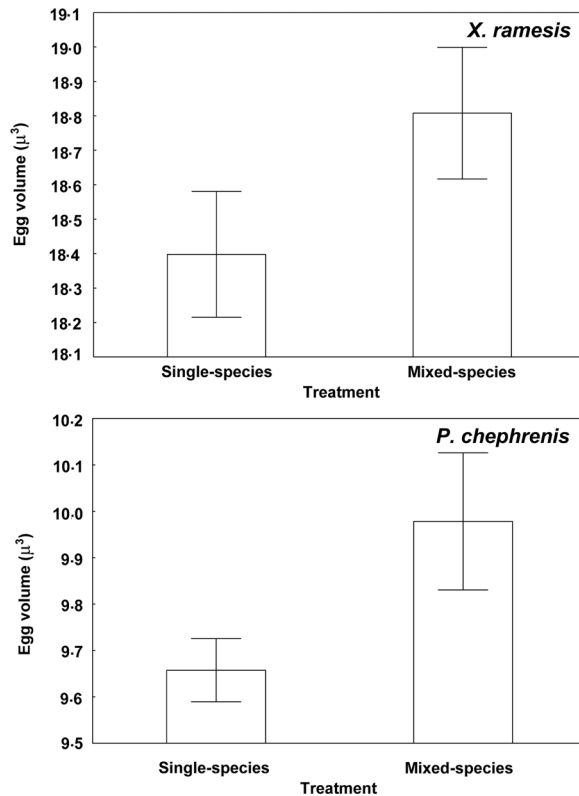


Fig. 4. Mean (\pm S.E.) volume of an egg produced by female of *Xenopsylla ramesis* and *Parapulex chephrenis* after 3 days of uninterrupted feeding on a rodent host (*Meriones crassus* and *Acomys cahirinus*, respectively) in single-species or mixed-species infestations.

interactions in their communities are either positive or neutral (Krasnov *et al.* 2005a, 2006a, b; Brinkerhoff *et al.* 2006; Pilosof *et al.* 2012), the possibility of the occurrence of some degree of inter-specific competition between flea imagoes (but not larvae; see Krasnov *et al.* 2005b) have also been suggested (e.g. Lindsay and Galloway, 1998). In our earlier experiments, we found that this flea responded to the unfavourable condition (feeding on a non-characteristic host) by increasing the egg size (Khokhlova *et al.* 2013, 2014). However, an increase in the egg size was accompanied by a decrease in egg number suggesting a well-known trade-off between quality and quantity of the offspring (Smith and Fretwell, 1974). In contrast, in this study *P. chephrenis* invested into the egg quality without sacrificing their quantity.

From an ecological perspective, the occurrence of a fitness-related response to co-infestation in *P. chephrenis* and the lack of such response in *X. ramesis* may be associated with the characteristic species composition of flea assemblages on their main hosts. In general, interactions in a host-flea network (a system that includes all co-habiting hosts and all co-habiting fleas in a given region or locality) have been shown to be asymmetric with host-specific fleas tending to interact with hosts with high flea richness whereas host-opportunistic fleas tend to interact with hosts with low parasite richness (Vazquez *et al.* 2005). However, *X. ramesis* and *P. chephrenis* do not both follow this rule. The absolute majority of *M. crassus* individuals harbour several flea species including host-generalist *X. ramesis*, whereas the absolute majority of *A. cahirinus* individuals harbour exclusively host-specialist *P. chephrenis* (Krasnov *et al.* 1997). In other words, co-infestation seems to be a usual condition for the *X. ramesis*, but definitely unusual for *P. chephrenis*. Consequently, *X. ramesis* may somehow be adapted to co-exploiting a host together with heterospecific fleas and thus its response to co-infestation is weak at best. In contrast, tolerance of *P. chephrenis* to co-occurring heterospecifics might be much lower, so it may mount a reproductive response. We recognize, however, that this explanation is highly speculative and warrants further investigation. Nevertheless, our study demonstrated that different flea species respond differently to co-infestation. In particular, this variation between flea species may explain why a positive correlation between abundance of a given flea species and abundance of co-occurring fleas of other species reported in earlier studies is, albeit general, but definitely not an universal rule (Krasnov *et al.* 2005a, b).

In conclusion, an increase in reproductive performance as a response to co-infestation may be one of the mechanisms behind aggregative structure of flea infra-communities. However, this response varies among flea species possibly dependent upon the level of diversity of flea assemblages on their principal hosts.

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REFERENCES

- Berrigan, D. (1991). The allometry of egg size and number in insects. *Oikos* **60**, 313–321.
- Brinkerhoff, R. J., Markeson, A. B., Knouft, J. A., Gage, K. L. and Monteneri, J. A. (2006). Abundance patterns of two *Oropsylla* (Ceratophyllidae: Siphonaptera) species on black-tailed prairie dog (*Cynomys ludovicianus*) hosts. *Journal of Vector Ecology* **31**, 355–363.
- Bush, A. O. and Holmes, J. C. (1986). Intestinal helminths of lesser scaup ducks: patterns of association. *Canadian Journal of Zoology* **64**, 132–141.
- Combes, C. (2001). *Parasitism. The Ecology and Evolution of Intimate Interactions*, University of Chicago Press, Chicago, USA.
- Cox, F. E. G. (2001). Concomitant infections, parasites and immune responses. *Parasitology* **122**, S23–S38.
- Demas, G. E. and Nelson, R. J. (1998). Photoperiod, ambient temperature, and food availability interact to affect reproductive and immune function in adult male deer mice (*Peromyscus maniculatus*). *Journal of Biological Rhythms* **13**, 253–262.
- Faulkenberry, G. D. and Robbins, R. G. (1980). Statistical measures of interspecific association between the fleas of the gray-tailed vole, *Microtus canicaudus* Miller. *Entomological News* **91**, 93–101.
- Fielden, L. J., Rechav, Y. and Bryson, N. R. (1992). Acquired immunity to larvae of *Amblyomma marmoratum* and *A. hebraeum* by tortoises, guinea-pigs and guinea-fowl. *Medical and Veterinary Entomology* **6**, 251–254.
- Heylen, D. J. A. and Matthysen, E. (2008). Effect of tick parasitism on the health status of a passerine bird. *Functional Ecology* **22**, 1099–1107.
- Johnston, N. A., Trammell, R. A., Ball-Kell, S., Verhulst, S. and Toth, L. A. (2009). Assessment of immune activation in mice before and after eradication of mite infestation. *Journal of the American Association for Laboratory Animal Science* **48**, 371–377.
- Jokela, J., Schmid-Hempel, P. and Rigby, M. C. (2000). Dr. Pangloss restrained by the Red Queen—steps towards a unified defence theory. *Oikos* **89**, 267–274.
- Khokhlova, I. S., Spinu, M., Krasnov, B. R. and Degen, A. A. (2004). Immune response to fleas in a wild desert rodent: effect of parasite species, parasite burden, sex of host and host parasitological experience. *Journal of Experimental Biology* **207**, 2725–2733.
- Khokhlova, I. S., Ghazaryan, L., Krasnov, B. R. and Degen, A. A. (2008). Effects of parasite specificity and previous infestation of hosts on the feeding and reproductive success of rodent-infesting fleas. *Functional Ecology* **22**, 530–536.
- Khokhlova, I. S., Fielden, L. J., Degen, A. A. and Krasnov, B. R. (2012). Ectoparasite fitness in auxiliary hosts: phylogenetic distance from a principal host matters. *Journal of Evolutionary Biology* **25**, 2005–2013.
- Khokhlova, I. S., Fielden, L. J., Williams, J. B., Degen, A. A. and Krasnov, B. R. (2013). Energy expenditure for egg production in arthropod ectoparasites: the effect of host species. *Parasitology* **140**, 1070–1077.
- Khokhlova, I. S., Pilosof, S., Fielden, L. J., Degen, A. A. and Krasnov, B. R. (2014). A trade-off between quantity and quality of offspring in haematophagous ectoparasites: the effect of the level of specialization. *Journal of Animal Ecology* **83**, 397–405.
- Krasnov, B. R. (2008). *Functional and Evolutionary Ecology of Fleas. A Model for Ecological Parasitology*, Cambridge University Press, Cambridge, UK.
- Krasnov, B. R., Shenbrot, G. I., Khokhlova, I. S., Degen, A. A. and Rogovin, K. V. (1996). On the biology of Sundevall's jird (*Meriones crassus* Sundevall) in Negev Highlands, Israel. *Mammalia* **60**, 375–391.
- Krasnov, B. R., Shenbrot, G. I., Medvedev, S. G., Vatschenok, V. S. and Khokhlova, I. S. (1997). Host-habitat relation as an important determinant of spatial distribution of flea assemblages (Siphonaptera) on rodents in the Negev Desert. *Parasitology* **114**, 159–173.
- Krasnov, B. R., Khokhlova, I. S., Fielden, L. J. and Burdelova, N. V. (2001). The effect of air temperature and humidity on the survival of pre-imaginal stages of two flea species (Siphonaptera: Pulicidae). *Journal of Medical Entomology* **38**, 629–637.
- Krasnov, B. R., Khokhlova, I. S., Oguzoglu, I. and Burdelova, N. V. (2002). Host discrimination by two desert fleas using an odour cue. *Animal Behaviour* **64**, 33–40.
- Krasnov, B. R., Sarfati, M., Arakelyan, M. S., Khokhlova, I. S., Burdelova, N. V. and Degen, A. A. (2003). Host-specificity and foraging efficiency in blood-sucking parasite: feeding patterns of a flea *Parapulex chephrenis* on two species of desert rodents. *Parasitology Research* **90**, 393–399.
- Krasnov, B. R., Mouillot, D., Shenbrot, G. I., Khokhlova, I. S. and Poulin, R. (2005a). Abundance patterns and coexistence processes in communities of fleas parasitic on small mammals. *Ecography* **28**, 453–464.
- Krasnov, B. R., Burdelova, N. V., Khokhlova, I. S., Shenbrot, G. I. and Degen, A. A. (2005b). Pre-imaginal interspecific competition in two flea species parasitic on the same rodent host. *Ecological Entomology* **30**, 146–155.
- Krasnov, B. R., Stanko, M. and Morand, S. (2006a). Are ectoparasite communities structured? Species co-occurrence, temporal variation and null models. *Journal of Animal Ecology* **75**, 1330–1339.
- Krasnov, B. R., Stanko, M., Khokhlova, I. S., Mosansky, L., Shenbrot, G. I., Hawlena, H. and Morand, S. (2006b). Aggregation and species coexistence in fleas parasitic on small mammals. *Ecography* **29**, 159–168.
- Krasnov, B. R., Korine, C., Burdelova, N. V., Khokhlova, I. S. and Pinshow, B. (2007). Between-host phylogenetic distance and feeding efficiency in haematophagous ectoparasites: rodent fleas and a bat host. *Parasitology Research* **101**, 365–371.
- Krasnov, B. R., Mathee, S., Lareschi, M., Korralo-Vinarskaya, N. P. and Vinarski, M. V. (2010). Co-occurrence of ectoparasites on rodent hosts; null model analyses of data from three continents. *Oikos* **119**, 120–128.
- Krasnov, B. R., Shenbrot, G. I. and Khokhlova, I. S. (2011). Aggregative structure is the rule in communities of fleas: null model analysis. *Ecography* **34**, 751–761.
- Levine, J. M. (1999). Indirect facilitation: evidence and predictions from a riparian community. *Ecology* **80**, 1762–1769.
- Lindsay, L. R. and Galloway, T. D. (1998). Reproductive status of four species of fleas (Insecta: Siphonaptera) on Richardson's ground squirrel (Rodentia: Sciuridae) in Manitoba, Canada. *Journal of Medical Entomology* **35**, 423–430.
- Lochmiller, R. L. and Deereberg, C. (2000). Trade-offs in evolutionary immunology: just what is the cost of immunity? *Oikos* **88**, 87–98.
- Pilosof, S., Lareschi, M. and Krasnov, B. R. (2012). Host body microcosm and ectoparasite infracommunities: arthropod ectoparasites are not spatially segregated. *Parasitology* **139**, 1739–1748.
- Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D. and R Core Team (2014). nlme: linear and nonlinear mixed effects models. R Package Version 3.1-118, <http://CRAN.R-project.org/package=nlme>
- Pöykkö, H. and Mänttari, S. (2012). Egg size and composition in an ageing capital breeder—consequences for offspring performance. *Ecological Entomology* **37**, 330–341.
- Presley, S. J. (2007). Streblid bat fly assemblage structure on Paraguayan *Noctilio leporinus* (Chiroptera: Noctilionidae): nestedness and species co-occurrence. *Journal of Tropical Ecology* **23**, 409–417.
- Presley, S. J. (2011). Interspecific aggregation of ectoparasites on bats: importance of hosts as habitats supersedes interspecific interactions. *Oikos* **120**, 832–841.
- R Core Team (2013). R: a Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria, URL <http://www.R-project.org/>
- Ranzani-Paiva, M. J. T. and Silva-Souza, A. T. (2004). Co-infestation of gills by different parasite groups in the mullet, *Mugil platamus* Günther, 1880 (Osteichthyes, Mugilidae): effects on relative condition factor. *Brazilian Journal of Biology* **64**, 677–682.
- Risco, D., Serrano, E., Fernández-Llario, P., Cuesta, J. M., Gonçalves, P., García-Jiménez, W. L., Martínez, R., Cerrato, R., Velarde, R., Gómez, L., Segalés, J. and Hermoso de Mendoza, J. (2014). Severity of bovine tuberculosis is associated with co-infection with common pathogens in wild boar. *PLoS ONE* **9**, e110123.
- Sánchez, S., Serrano, E., Gómez, M. S., Feliu, C. and Morand, S. (2014). Positive co-occurrence of flea infestation at a low biological cost in two rodent hosts in the Canary archipelago. *Parasitology* **141**, 511–521.
- Sarfati, M., Krasnov, B. R., Ghazaryan, L., Khokhlova, I. S., Fielden, L. J. and Degen, A. A. (2005). Energy costs of blood digestion in a host-specific haematophagous parasite. *Journal of Experimental Biology* **208**, 2489–2496.
- Serrano, E. and Millán, J. (2014). What is the price of neglecting parasite groups when assessing the cost of co-infection? *Epidemiology and Infection* **142**, 1533–1540.
- Smith, C. C. and Fretwell, S. D. (1974). Optimal balance between size and number of offspring. *American Naturalist* **108**, 499–506.
- Taylor, L. H., Mackinnon, M. J. and Read, A. F. (1998). Virulence of mixed-clone and single-clone infections of the rodent malaria *Plasmodium chabaudi*. *Evolution* **52**, 583–591.

Tello, J. S., Stevens, R. D. and Dick, C. W. (2008). Patterns of species co-occurrence and density compensation: a test for interspecific competition in bat ectoparasite infracommunities. *Oikos* **117**, 693–702.

Torres-Vila, L. M. and Rodríguez-Molina, M. C. (2002). Egg size variation and its relationship with larval performance in the Lepidoptera: the case of the European grapevine moth *Lobesia botrana*. *Oikos* **99**, 272–283.

Vazquez, D. P., Poulin, R., Krasnov, B. R. and Shenbrot, G. I. (2005). Species abundance patterns and the distribution of specialization in host-parasite interaction networks. *Journal of Animal Ecology* **74**, 946–955.

Zuur, A., Ieno, E. N., Walker, N., Saveliev, A. A. and Smith, G. M. (2009). *Mixed Effects Models and Extensions in Ecology with R*, Springer, New York, USA.