The effect of larval density on pre-imaginal development in two species of desert fleas

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SUMMARY

We studied the effect of density of larvae on pre-imaginal development in 2 flea species (*Xenopsylla conformis* and *Xenopsylla ramesis*) parasitic on 2 desert rodent species (*Dipodillus dasyurus*, adult body mass 20 g and *Meriones crassus*, 80 g). We predicted a decrease in duration of development with an increase in density of larvae. In general, in both flea species, duration of larva-to-pupa development decreased with an increasing larval density. In addition, this stage of development was longer in male fleas and in fleas from parents fed on *D. dasyurus*. The effect of larval density on larval development was manifested mainly when parent fleas fed on *D. dasyurus*. Duration of pupation decreased with increasing larval density only in offspring of fleas fed on *G. dasyurus*. In both fleas, pupation was longer in males. The effect of parent host on duration of pupation was found in *X. ramesis* only (longer if the host was *M. crassus*). Resistance of newly emerged fleas to starvation depended mainly on parent host species. Young *X. conformis* survived longer if their parents fed on *D. dasyurus*, whereas young *X. ramesis* survived longer if their parents fed on *M. crassus*. It was also found that (a) an individual flea that spent more time as a larva also spent more time as a pupa and (b) longer larval development resulted in a shorter time that a newly emerged flea was able to survive when starved.

Key words: density dependence, fleas, pre-imaginal development, rodents.

INTRODUCTION

Fleas (Insecta: Siphonaptera) are obligate haematophagous parasites most abundant and diverse in small and medium burrowing mammals (see Medvedev and Krasnov, 2006 and Krasnov, 2008 for reviews). They alternate between periods when they occur on the body of their host and when they occur in its burrow or nest. In the majority of flea species, pre-imaginal development is entirely off-host. With a few exceptions, flea larvae are not parasitic and feed on organic matter in the burrow and/or nest of the host.

The duration of development of pre-imaginal fleas, like that of all holometabolous insects, is highly dependent on extrinsic environmental factors such as ambient temperature, relative humidity and substrate structure (Margalit and Shulov, 1972; Silverman *et al*. 1981; Silverman and Rust, 1983, 1985; Metzger and Rust, 1997; Krasnov *et al*. 2001*a*,*b*; Krasnov *et al*. 2002*a*,*b*). In addition, it may be affected by host-related factors such as host species, body condition or reproductive activity (Tripet and Richner, 1999; Krasnov *et al*. 2004, 2005*a*). For example, Krasnov *et al*. (2004) found that the length of time that larvae of the rodent flea *Xenopsylla ramesis* took to hatch depended on the host species exploited by parent females. Development of eggs and larvae of *X. ramesis* took longer when parent females were fed on malnourished rodents (Krasnov *et al*. 2005*a*). Tripet and Richner (1999) reported that the development schedule of a hen flea *Ceratophyllus gallinae* was related to the timing and duration of the breeding period of its bird host.

Effects of extrinsic (that is, environment- and host-related) factors on flea pre-imaginal development have proven to be physiologically based (Silverman, 1981; Silverman and Rust, 1985; Metzger and Rust, 1997; Krasnov *et al*. 2005*a*). Effects of intrinsic (that is, flea-related) factors on their development have received less attention. Nevertheless, Tripet and Richner (2002) found that a high density of flea larvae resulted in a decreased survival in *C. gallinae*, although the duration of development was not affected, whereas Krasnov *et al*. (2008) reported a positive relationship between parent flea density and duration of pre-imaginal development in 2 *Xenopsylla* species.

Some studies suggested that pre-imaginal fleas may modify their developmental schedule (i.e. increase or decrease duration of development) in a way

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that maximizes their future survival. For example, Tripet and Richner (1999) found that some pre-imaginal *C. gallinae* traded off duration of development against survival. Bird hosts regularly clean their nests and search for and kill larval fleas. The intensity of cleaning behaviour is highest at the warmer centre of the nest and less so at the cooler periphery. As a result, larvae that cocoon at the nest periphery are better able to avoid host anti-parasite behaviour, but with the cost of delayed metamorphosis, i.e. longer pupal stage. In another study, Tripet and Richner (2002) reported that some newly emerged *C. gallinae* dispersed very early from the site of emergence, even prior to the nesting period of the bird hosts. Tripet and Richner (2002) argued that pre-imaginal development of these "early dispersers" took place after hosts departed from their nests, i.e. under shortage of food and independently of density. As a result, they had not enough energy reserves for a prolonged cocooned stage, so they had to emerge and initiate their host search as early as possible. They shortened their pupal stage and emerge early for the sake of finding a host.

These results suggest that an increased or decreased duration of development may both be advantageous for fleas, depending on ecological circumstances. For example, duration of pre-imaginal development may depend on density of flea larvae even when the larvae do not compete for food. Indeed, the amount of organic matter in a host burrow (where pre-imaginal development of fleas takes place) may be high (remnants of host food, its faeces etc.). However, even if fleas do not compete for food as larvae, it is likely that they compete for host blood as imagos, with this competition being often mediated by host behavioural or immunological anti-parasitic defences (e.g. Hawlena *et al*. 2007, 2008). As a result, fleas that emerge earlier may have an advantage over fleas that emerge later. Consequently, we hypothesized that flea larvae will respond to increased larval density by changes in their development schedule. We predicted a decrease in the duration of larval and pupal development with an increase in the density of larvae. Furthermore, this effect may be mediated by the host because of the aforementioned effect of host-related factors on pre-imaginal development of fleas (e.g. host identity; Krasnov *et al*. 2004).

We tested this prediction using 2 flea species, *Xenopsylla conformis* Wagner and *Xenopsylla ramesis* Rothschild, and 2 rodent host species, *Dipodillus dasyurus* Wagner (adult body mass about 20 g) and *Meriones crassus* Sundevall (adult body mass about 80 g), all common in the Negev desert. The 2 rodents co-exist in various non-sandy and non-rocky habitats, whereas the 2 flea species demonstrate paratopic distribution (they occupy different, albeit adjacent habitats with narrow zones of overlapping; Krasnov *et al*. 1998). Despite somewhat different environmental tolerance ranges within the habitats of the 2 fleas, both species showed highest survival and fastest development at similar air temperatures, relative humidities and in the same substrate (Krasnov *et al*. 2001*a*,*b*; 2002*a*,*b*). Both fleas attained higher fecundity when they exploit *M. crassus* than *D. dasyurus* (Krasnov *et al*. 2004).

MATERIALS AND METHODS

Fleas and rodents

We used rodents and fleas from our laboratory colonies established in 1997 and 1999, respectively. To guarantee genetic variability of rodent and flea colonies, approximately 20 wild-captured rodents and 200 wild-collected fleas were added to the colonies yearly. Details on the maintenance and breeding of rodents and fleas have been reported earlier (e.g. Krasnov *et al*. 2001*a*,*b*; 2002*a*,*b*; 2004, 2007; Khokhlova *et al*. 2009). In brief, rodents were maintained in plastic cages (60 by 50 by 40 cm or 20 cm) and offered millet seed and alfalfa (*Medicago* sp.) leaves *ad libitum*. To obtain fleas, an individual rodent host was placed in a cage that contained a steel nest box with a screen floor and a pan containing a mixture of sand and dried bovine blood (larvae nutrient medium) on the bottom. This rodent was infested with 10–15 (*M. crassus*) or 6–8 (*D. dasyurus*) newly emerged fleas. Every 2 weeks, we collected all substrate and bedding material from the cage and transferred it into an incubator (FOC225E, Velp Scientifica srl, Milano, Italy) where flea development and emergence took place at 25 °C and 75% relative humidity.

Larvae

We fed 250 newly emerged adult females and 100 newly emerged adult males of *X. conformis* or *X. ramesis* on adult male rodents (15–20 females and 5–10 males per individual *M. crassus* and 8–10 females and 3–5 males per individual *D. dasyurus*) for 2 h daily during 3 consecutive days. We used male rodents because host gender affects feeding and reproductive performance of fleas (Khokhlova *et al*. 2009). The numbers of fleas feeding simultaneously on *M. crassus* and *D. dasyurus* differed because of the size difference between the hosts. Consequently, the number of fleas per unit body surface of a host was approximately equal, so we assumed that parent fleas feeding on either host experienced a similar degree of competition. After feeding, fleas from each host were placed in plastic cups (200 cm^2), with a bottom covered by a thin layer of sand and small pieces of filter paper, transferred into an incubator and maintained at 25 °C and 95% RH. Each feeding of a flea was done on a different individual of the same host species. Details on this procedure can be found

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elsewhere (Khokhlova *et al*. 2007; Krasnov *et al*. 2007).

After the third feeding, randomly chosen gravid female fleas (200 fleas of each species) were placed individually into 20 ml glass vials containing small pieces of filter paper. Females oviposited on the pieces of filter paper and eggs were transferred into 20 ml glass vials that contained a thin layer of clean sand (8–10 eggs per vial). Vials were covered with a 5 × 5 cm nylon screen held by a rubber band. The eggs were monitored twice daily until larvae hatched (after 6–7 days; see Krasnov *et al*. 2001*b*). Experimental fleas and their eggs were maintained at 25 °C and 95% RH.

Experimental design and procedures

For each flea species, 1000 larvae of the same age (*ca*. 1 day old) were chosen randomly and transferred in groups of 5, 15 and 30 individuals into 50 ml glass vials (50 mm bottom diameter). The vials, covered by perforated plastic lids and containing a 3 mm layer of sand and a larval food medium (94% dry bovine blood, 5% millet flour, and 1% ground excrements of *M. crassus* or *D. dasyurus*), were placed in incubators at 25 °C and 95% RH. Larvae of the same species from different females feeding on different individuals of the same host species were randomly distributed among 3 treatments that differed in larval density. The daily amount of food (larvae medium) required for successful development of flea larvae was determined earlier as 0·07 ± 0·1 mg per individual larva (Krasnov *et al*. 2005*b*). The larvae were offered this amount in our experiments and food medium (0·07 mg per larva) was added daily to vials with larvae, so that the food amount per larva was equalized among and during treatments. Each treatment was replicated 10 times totalling 120 experiments (2 flea species × 2 host species × 3 densities × 10 replicates).

Vials with larvae were monitored twice daily until all larvae pupated and either spun cocoons or died. Cocoons were transferred into individual glass vials with 1 mm of clean sand and covered by a nylon screen (0·1 mm mesh) held by a rubber band. Vials with cocoons were checked twice a day. They were shaken slightly when checked, since vibration may stimulate flea emergence. Vials with cocoons were checked either until all adults emerged from their cocoons or were considered dead. Vials with newly-emerged adults were checked until all adults died. After death of each imago, we identified its gender by examination of its genitalia under light microscopy, so that gender of each larva that survived until emergence was known.

Data analyses

We estimated pupation and emergence success for each group of larvae as the proportion of cocooned larvae and the proportion of emerged adult fleas, respectively. To evaluate the effect of larval density on the duration of development and quality of newly emerged fleas we calculated (a) time to pupation of each larva; (b) time of pupation (from pupation to emergence) of each pupa and (c) the longevity of each newly emerged flea under starvation. This was done only for individuals that survived from the larval stage to emergence of the imago. We analysed the effect of host species of parent fleas, larval density and/or flea gender (independent variables) on pupation and emergence success, development time of larvae and pupae and time of death under starvation of newly emerged fleas (dependent variables) using 2-way (for pupation and emergence success) or 3-way ANOVAs (for remaining independent variables) separately for *X. conformis* and *X. ramesis*. In addition, we tested whether (a) duration of larval development affected duration of pupal development and time to death under starvation and (b) whether duration of pupal development affected time to death under starvation taking into account flea gender, parent host species and larval density. This was done separately for *X. conformis* and *X. ramesis* using Generalized Linear Models (GLM) with normal distribution and log-link function. Thus, 2 models were tested for each species. In one model, independent variables were flea gender, parent host species, larval density and duration of larval development, whereas the dependent variable was duration of pupal development. In another model, independent variables were those used in the previous model plus duration of pupal development, whereas the dependent variable was time of survival under starvation. We searched for the best model separately for *X. conformis* and *X. ramesis* using the Akaike's Information Criterion. We were interested in parameter estimates of a model. Significance and sign of these estimates for duration of a preceding time period (i.e. larval and/or pupal development) would indicate the occurrence of their negative or positive effect on duration of a subsequent time period (i.e. pupal development or time of survival under starvation).

Variables were log- or arcsin (pupation and emergence success) transformed prior to analysis. Untransformed data are presented in the figures. Tukey's HSD tests were used for multiple comparisons.

RESULTS

In total, 77·1% larval *X. conformis* and 83·1% larval *X. ramesis* survived until pupation, of which 87·8% and 97·2%, respectively, survived until emergence as new imagos. Survival of larval *X. conformis* did not depend on either host species, larval density or interaction between these two factors ($F_{1,54} = 0{\cdot}2$, $F_{2,54} = 1{\cdot}6$ and $F_{2,54} = 1{\cdot}0$, respectively; $P > 0{\cdot}20$ for all) (Fig. 1a). Survival of larval *X. ramesis* also did

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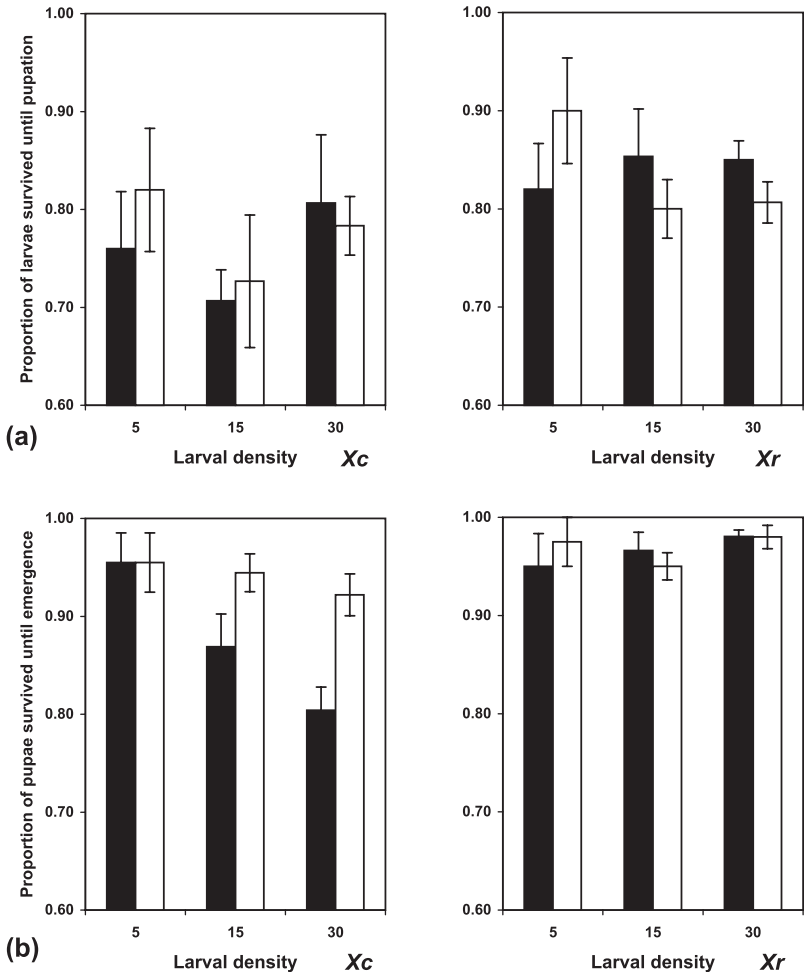

Fig. 1. Mean (± S.E.M.) proportion of (a) larvae that survived until pupation and (b) pupae that survived until emergence in pre-imaginal fleas *Xenopsylla conformis* (*Xc*) and *Xenopsylla ramesis* (*Xr*) from parents fed on *Meriones crassus* (black columns) or *Dipodillus dasyurus* (white columns) and maintained at different larval densities.

not depend on either host species or larval density ($F_{1,54}=0{\cdot}02$ and $F_{2,54}=2{\cdot}6$, respectively; $P>0{\cdot}08$ for both). However, interaction between these factors was significant ($F_{2,54}=2{\cdot}6$, $P<0{\cdot}04$) reflecting significantly higher larval survival at the lowest density but only when parent fleas fed on *D. dasyurus* (Tukey's HSD tests, $P<0{\cdot}05$; Fig. 1a). In contrast, pupal survival in *X. conformis* depended on both host species and flea density ($F_{1,54}=5{\cdot}5$ and $F_{2,54}=10{\cdot}0$, respectively; $P<0{\cdot}02$ for both), but was not affected by interaction between these factors ($F_{2,54}=1{\cdot}5$, $P>0{\cdot}20$). These effects were manifested in (a) significantly higher survival if a parent host was *D. dasyurus* than if a parent host was *M. crassus* (except for lowest larval density) and (b) significantly lower survival at higher density if a parent host was *M. crassus* (Tukey's HSD tests, $P<0{\cdot}05$; Fig. 1b). Neither factor or between-factor interaction affected pupal survival in *X. ramesis* ($F_{1,54}=0{\cdot}01$, $F_{2,54}=0{\cdot}8$ and $F_{2,54}=0{\cdot}9$, respectively; $P>0{\cdot}40$ for all) (Fig. 1b).

Summary of ANOVAs of duration of larval and pupal development of *X. conformis* and *X. ramesis* as affected by flea gender, host species on which parent fleas fed and larval density is presented in Table 1.

In *X. conformis*, duration of larval development depended on all 3 factors, being longer (a) in males, (b) in fleas born from parents fed on *D. dasyurus* and (c) at lower larval densities (Tukey's HSD tests, $P<0{\cdot}05$ for all; Fig. 2a). Duration of pupal development of this flea differed between males and females (being longer in males; Tukey's HSD tests, $P<0{\cdot}05$ for all), but did not generally depend on either parent host species or larval density, except for females born from parents fed on *D. dasyurus* (being longer at lower larval density; Tukey's HSD tests, $P<0{\cdot}05$; Fig. 2b). This explains the significance of two 2-way interactions both involving flea gender (Table 1).

Duration of larval development of *X. ramesis* depended on flea gender, parent host species and flea density (Table 1), being longer in males, in larvae produced by fleas fed on *D. dasyurus* and at lower density (Tukey's HSD tests, $P<0{\cdot}05$; Fig. 3a). However, the latter pattern occurred only in fleas born from parents fed on *D. dasyurus*, explaining the significance of host species × larval density interaction (Table 1, Fig. 3a). Pupal development of this flea was longer in males and in fleas born from parents fed on *M. crassus*. Male pupae produced

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Table 1. Summary of ANOVAs of the effect of flea gender, parent host species and larval density on duration of larval and pupal development in *Xenopsylla conformis* and *Xenopsylla ramesis*

Flea	Period of development	Factor	D.F.	*F*
X. conformis	Larval	Flea gender	1, 665	15·0***
		Parent host	1, 665	71·7***
		Larval density	2, 665	24·8***
	Pupal	Flea gender	1, 665	703·8***
		Parent host	1, 665	$2·7^{NS}$
		Larval density	2, 665	$0·2^{NS}$
		Flea gender × Parent host	2, 665	7·1**
		Flea gender × Larval density	2, 665	5·4**
X. ramesis	Larval	Flea gender	1, 796	4·9*
		Parent host	1, 796	42·3***
		Larval density	2, 796	3·7*
		Parent host × Larval density	2, 796	3·1*
	Pupal	Flea gender	1, 796	1710·2***
		Parent host	1, 796	40·5***
		Larval density	2, 796	3·1*
		Flea gender × Larval density	2, 665	6·2**

* $P<0·05$.
** $P<0·01$.
*** $P<0·001$.
NS, Non-significant.
Only significant interactions are shown.

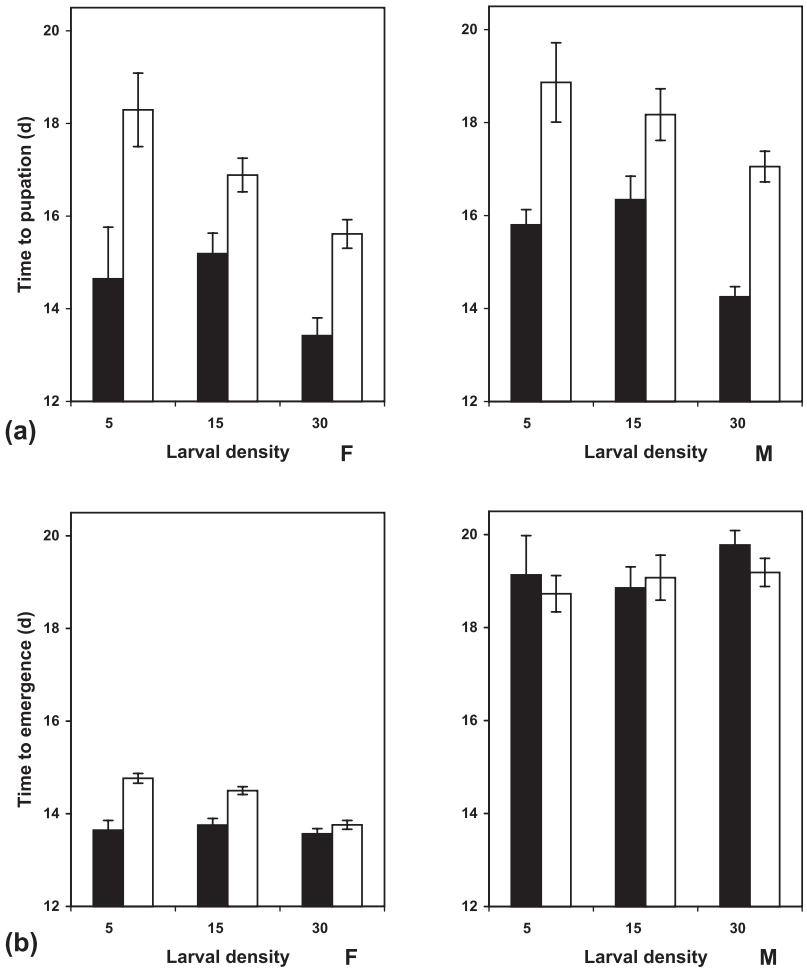

Fig. 2. Mean (± S.E.M.) duration of (a) larval and (b) pupal development in female (F) and male (M) *Xenopsylla conformis* from parents fed on *Meriones crassus* (black columns) or *Dipodillus dasyurus* (white columns) and maintained at different larval densities.

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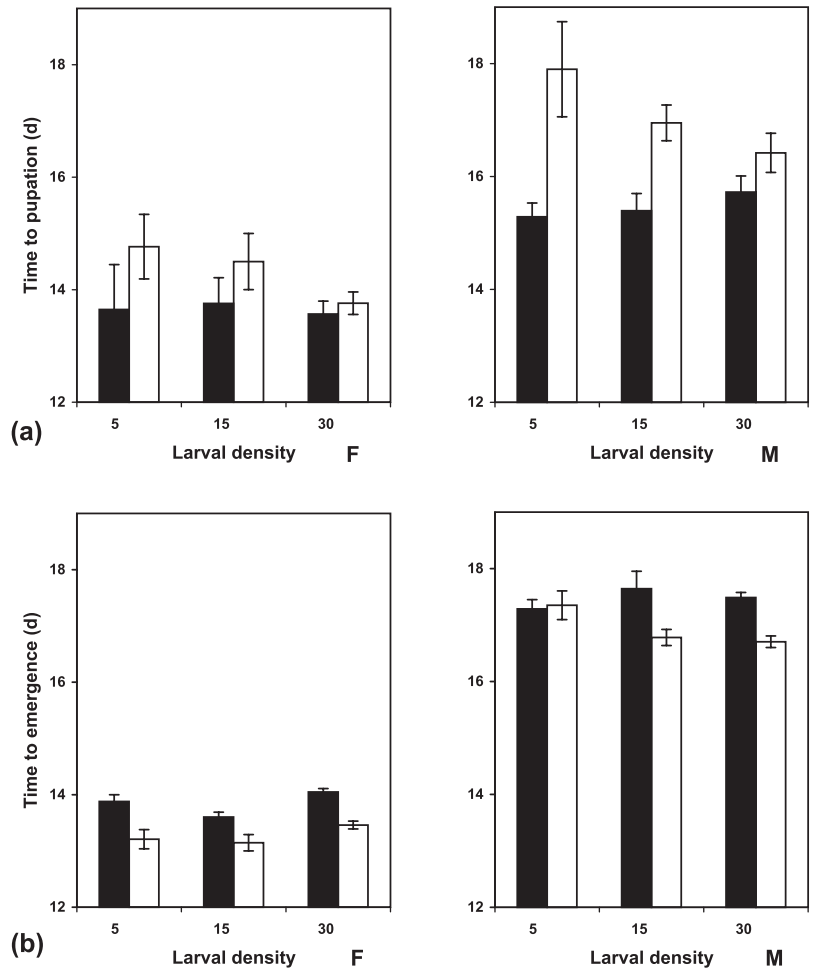

Fig. 3. Mean (±S.E.M.) duration of (a) larval and (b) pupal development in female (F) and male (M) *Xenopsylla ramesis* from parents fed on *Meriones crassus* (black columns) or *Dipodillus dasyurus* (white columns) and maintained at different larval densities.

by fleas fed on *D. dasyurus* developed significantly longer at lower larval density, but no effect of larval density on pupal development in female pupae or male pupae produced by fleas fed on *M. crassus* was found (Tukey's HSD tests, $P<0{\cdot}05$; Fig. 3b).

Results of ANOVAs of time to death under starvation in newly emerged fleas are presented in Table 2. In general, newly emerged female *X. conformis* survived longer than male conspecifics (Fig. 4a), although this was true mainly when their parents fed on *D. dasyurus* (Tukey's HSD tests, $P<0{\cdot}05$ if a parent host was *D. dasyurus* and $P>0{\cdot}05$ if a parent host was *M. crassus*). Both genders survived longer if their parents fed on *D. dasyurus* than on *M. crassus* (Tukey's HSD tests, $P<0{\cdot}05$; Fig. 4a). No effect of larval density on time to death of newly emerged *X. conformis* was found either in general or in pair-wise within-gender and within-parent host comparisons (Tukey's HSD tests, $P>0{\cdot}05$ for all). Time to death of newly emerged *X. ramesis* was affected by host species only (Table 2), being longer in fleas from parents fed on *M. crassus* (Fig. 4b).

Best models describing relationships between duration of larval development, duration of pupal development and survival time under starvation when all other factors were taken into account are presented in Table 3. Signs of coefficients in these models indicate positive correlation between duration of larval development and duration of pupal development and negative correlation between duration of larval development and time of survival under starvation. In other words, (a) an individual flea that spent a short time as a larva also spent a short time as a pupa and (b) longer larval development resulted in a shorter time that a newly emerged flea was able to survive when starved. In addition, models supported effects revealed in the ANOVAs (e.g. longer duration of development of male as compared to female pupae in *X. conformis* and longer time to death of *X. ramesis* produced by parents fed on *M. crassus* as compared to *D. dasyurus*).

DISCUSSION

In general, duration of flea development decreased with an increase in larval density. Consequently, our main prediction was supported. Moreover, manifestation and strength of the relationship between

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Table 2. Summary of ANOVAs of the effect of flea gender, parent host species and larval density on survival time under starvation of newly emerged *Xenopsylla conformis* and *Xenopsylla ramesis*

Flea	Factor	D.F.	*F*
X. conformis	Flea gender	1, 665	6·1*
	Parent host	1, 665	118·3***
	Larval density	2, 665	2·0^{NS}
	Flea gender × Parent host	1, 665	4·6*
	Flea gender × HS × Larval density	2, 665	7·1**
X. ramesis	Flea gender	1, 796	0·1^{NS}
	Parent host	1, 796	8·7***
	Larval density	2, 796	0·6^{NS}

* $P<0·05$.
** $P<0·01$.
*** $P<0·001$.
NS, Non-significant.
Only significant interactions are shown.

duration of development and larval density was mediated by host species and differed between flea species and developmental stages.

Shorter development with an increase of larval density

Despite an equal food amount per larva among treatments, larvae maintained at higher densities pupated earlier and their pupae tended to shorten the pupation period. This density-dependent response suggests that intraspecific larval competition occurs even when food does not represent a limiting factor. Intraspecific competition for food among insect larvae is well known (e.g. Nicholson, 1954; Klomp, 1964; Beaver, 1974; Averill and Prokopy, 1987; Burrak *et al*. 2009). For example, Nicholson (1954) described larval competition for food in the sheep blowfly *Lucilia cuprina* and used this study to introduce the concept of 'scramble competition' when all co-occurring individuals have equal access to the limited resource. Moreover, a change of the duration of development has been shown to be one of the consequences of competition (Klomp, 1964).

The ultimate reason behind the decreased duration of development of pre-imaginal fleas with an increase in density may be associated with prospective intraspecific competition among adult fleas. Increased competition may decrease feeding, and consequently, reproductive success of haematophagous arthropods (Kelly and Thompson, 2000; see Khokhlova *et al*. 2007 and Krasnov *et al*. 2007 for fleas). A higher density of flea larvae in a burrow of a host may result in a higher density of adult insects that will compete for blood from the same host individual, increasing thus the severity of competition. This is especially probable given that both *M. crassus* and *D. dasyurus* are solitary and usually a burrow is occupied by a single individual (Krasnov *et al*. 1996; Shenbrot *et al*. 1997; Gromov *et al*. 2000). As a result, under high pre-imaginal density, individuals that will emerge as imagoes earlier would more than likely have an advantage over individuals that emerge later as later-emerging fleas will more than likely feed on a host under a higher density of co-exploiters than earlier-emerging fleas. In other words, an earlier response of larval fleas to high densities may benefit their survival as adults. However, short duration of larval stage may negatively affect the viability of a pupa because an early pupated larva may not have enough time to accumulate energy reserves that will allow it to endure desiccation during the pupal stage or to break the wall of the cocoon and/or puparium (Silverman and Rust, 1985). Furthermore, experiments of Silverman and Rust (1985) suggested that flea emergence is triggered when energy reserves drop below a critical level. This was supported by the positive correlation between durations of larval and pupal stages in an individual flea in our study. In other words, longer development should benefit a flea larva. However, under high density, the larvae may face a trade-off between its current success as a larva and its future success as a new imago. Given this trade-off, natural selection may favour a flexible developmental schedule.

Proximate causes of density-dependent pre-imaginal development are likely to include some mechanisms that allow flea larvae to estimate the density level. For example, flea larvae were able to evaluate the amount of food available and respond by remaining in or leaving the patch (Shryok and Houseman, 2006). However, in our experiments the amount of food available per larva was equal and, thus, mechanisms other than direct food shortage may play a role. For example, the density may be indicated by the concentration of larval pheromones or larval faeces. Although the existence of larval pheromone has never been reported for fleas, it is well known for other insect orders (Hartman *et al*. 1978 for Diptera; Deneubourg *et al*. 1990 for Coleoptera; Jumean *et al*. 2005 for Lepidoptera). Larvae may also respond to the number of encounters with each other and/or frequency of tactile contacts. Another proximate cause could be variation in food selectivity among individual larvae. In many flea species, the most protein-rich part of the diet of larvae is faecal pellets that adult female fleas expel near the clutch (Cotton, 1970; Hinkle *et al*. 1991; Silverman and Appel, 1994; Larsen, 1995; Hsu *et al*. 2002). These pellets contain mainly blood of a host and, moreover, their protein content is higher than that of the blood upon which female fleas feed (Hinkle *et al*. 1991). In our experiments, the most protein-rich component of the larval medium was dry bovine blood which has been shown to be an adequate substitute of adult flea faeces as a food source for flea larvae (Moser *et al*.

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Table 3. The best models explaining variance in duration of pupal development and survival time under starvation as affected by flea gender, parent host species and duration of larval development in *Xenopsylla conformis* and *Xenopsylla ramesis* and parameter estimates for these models

(AIC - Akaike's Information Criterion, LR - likelihood ratio. Only significant coefficients are shown. Levels of effect of categorical variables are '*Dipodillus dasyurus*' for parent host species and 'female' for flea gender. All models are significant ($P<0{\cdot}0001$). See text for explanations.)

Flea	Independent variable	AIC	LR χ^2	Equation
X. conformis	Duration of pupal development	−2090·0	725·4	0·10+0·07*Larval development−0·06*Flea gender+0·01 *Parent host*Flea gender
	Time of survival	−802·6	196·1	0·23−0·15*Larval development+0·02*Flea gender+0·06 *Parent host
X. ramesis	Duration of pupal development	−2337·6	27·4	0·10+0·05*Larval development−0·01*Parent host
	Time of survival	−639·7	136·8	1·15−0·74*Larval development

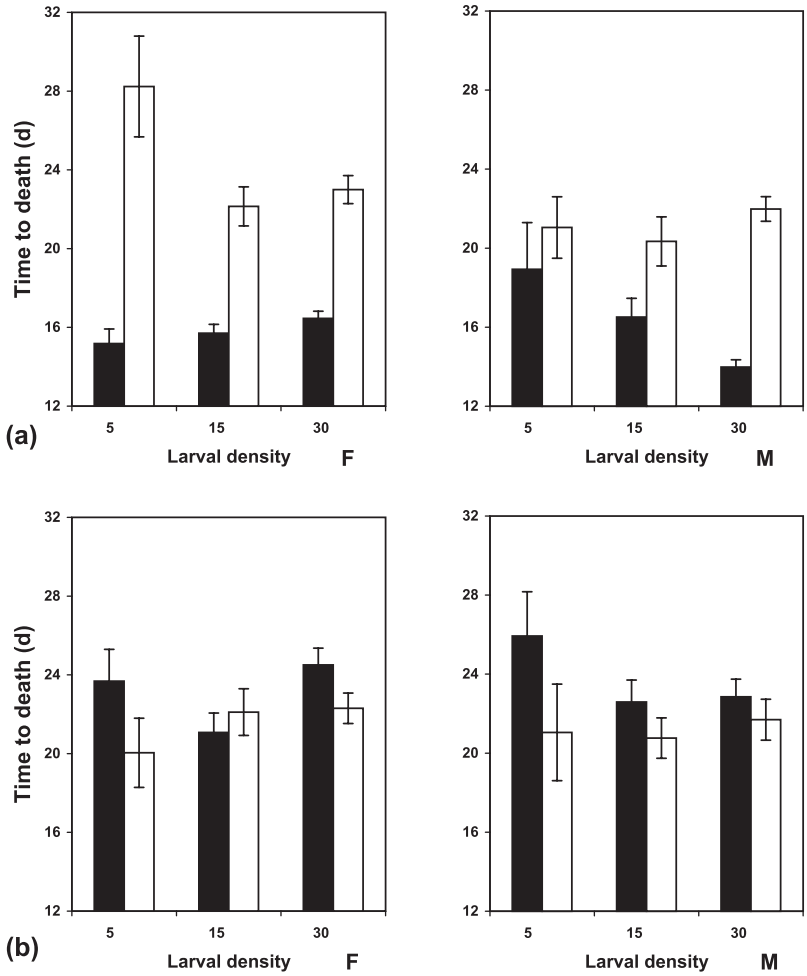

Fig. 4. Mean (±S.E.M.) survival time under starvation in newly emerged (a) *Xenopsylla conformis* and (b) *Xenopsylla ramesis* from parents fed on *Meriones crassus* (black columns) or *Dipodillus dasyurus* (white columns) and maintained at different larval densities.

1991). Although the amount of this component per larva was equal among treatments, its absolute amount per group of larvae was obviously greater in higher density treatments. If larvae vary in their food selectivity, then highly selective individuals will rapidly consume most of the dry blood, accumulate the necessary amount of energy reserves and pupate earlier. These explanations of the proximate causes of density dependence of development require further investigation.

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Effects of flea species, flea gender and host species

The effect of density on larval development was stronger in *X. conformis* than in *X. ramesis*, but the opposite was the case for pupal development. In other words, the between-flea difference in the magnitude of the response to density was stage specific. This may be associated with a different pattern of response to a variety of extrinsic factors between larvae and pupae of these fleas. Our earlier findings demonstrated that both larvae and pupae of the 2 species demonstrate species-specific sensitivity to the same factor. For example, larval *X. ramesis* are more sensitive to relative humidity than larval *X. conformis* (Krasnov *et al*. 2001*a*), whereas pupal *X. conformis* are more sensitive to air temperature than pupal *X. ramesis* (Krasnov *et al*. 2001*b*). However, the precise reason for differential stage-specificity in sensitivity to larval density between the 2 flea species is unknown.

In general, male fleas developed longer than female fleas and offspring of fleas on *D. dasyurus* developed longer than offspring of fleas on *M. crassus*. Gender difference in the duration of development in fleas has been reported for a number of flea species (Sharif, 1949; Hudson and Prince, 1958; Vaughan and Coombs, 1979; Amin *et al*. 1993; Metzger and Rust, 1997; Kern *et al*. 1999; Krasnov *et al*. 2001*b*). In the cat flea *Ctenocephalides felis*, it was suggested that the biological significance of a shorter development period by female fleas was to prevent inbreeding of fleas from the same cohort by increasing the probability that females will mate with males from other cohorts (Metzger and Rust, 1997). However, in our earlier studies (Krasnov *et al*. 2001*b*), as well as in this study, the time difference between male and female emergence seemed to be too small for the 'prevention-of-inbreeding' explanation to be feasible. Another reason for this pattern could be that pre-imaginal flea males are more sensitive to microclimatic factors than pre-imaginal flea females (Krasnov *et al*. 2001*b*).

Gender differences in the duration of development might be a reason for fluctuating sex ratio in populations of natural and laboratory reared fleas (Bossard *et al*. 2000). For example, in laboratory cultures of fleas it has been found that the peak of emergence of one gender alternates with that of the other gender, so that the snap-shots of young fleas from laboratory cultures demonstrated either strong male or strong female biases (Ma, 1993).

In our earlier study with the same flea and host species (Krasnov *et al*. 2004), we found that *D. dasyurus* as a host was inferior to *M. crassus* for both flea species in terms of lower egg production and/or slower egg development. This suggests that exploitation of *D. dasyurus* as opposed to *M. crassus* may result in the production of eggs of lower quality. Longer post-egg development may, therefore, be required to compensate for a lower quality of eggs. This compensation, in turn, may result in a higher amount of fat storage in newly emerged fleas that allows them to survive longer under starvation. However, this was found in *X. conformis* only, whereas young *X. ramesis* survived longer if their parents fed on *M. crassus*.

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