

THE EFFECT OF SUBSTRATE ON SURVIVAL AND DEVELOPMENT OF TWO SPECIES OF DESERT FLEAS (SIPHONAPTERA: PULICIDAE)

KRASNOV B.R.*, KHOKHLOVA I.S.** & FIELDEN L.F.*** & BURDELOVA N.V.*

Summary :

Fleas *Xenopsylla conformis mycerini* and *Xenopsylla ramesis* replace each other on the same rodent host (*Meriones crassus*) in two habitats that differ in substrate texture (sand and loess-like sediments, respectively). We hypothesized that the substrate is an important factor determining flea distribution and studied survival of larvae, pupae and newly emerged adults as well as the rate of pre-imaginal development of these flea species in sand and loess rearing medium (=substrate). Texture of rearing medium did not affect survival and development rate of eggs in either *X. c. mycerini* or *X. ramesis*. Larval survival and the rate of development were both affected by the factor of substrate. Survival of *X. c. mycerini* larvae was significantly higher in sand than in loess substrate, whereas survival of *X. ramesis* larvae did not differ in different substrates. Larvae of both species developed faster in sand substrate than in loess substrate. Maximal survival time of *X. c. mycerini* larvae that died before pupation did not depend on substrate, whereas *X. ramesis* larvae survived significantly longer in loess than in sand substrate. Most pupae of both species survived successfully on both substrates, but the duration of pupal stages in sand substrate was longer than that in loess substrate in both species. Newly emerged adults of both species survived similar time in both sand and loess substrate. Irrespective of substrate, adult *X. c. mycerini* survived for a shorter time than did adult *X. ramesis*. No between-sex within-species differences in survival time of newly emerged adults in sand versus loess substrate were found in *X. c. mycerini*. Survival time of males and females of *X. ramesis* differed in sand substrate but not in loess substrate.

KEY WORDS : *Xenopsylla conformis mycerini*, *Xenopsylla ramesis*, rearing medium, substrate texture, survival, development.

Résumé : EFFETS DU SUBSTRAT SUR LA SURVIE ET LE DÉVELOPPEMENT DE PUCES DE RÉGIONS DÉSERTIQUES (SIPHONAPTERA : PULICIDAE)

Deux puces, *Xenopsylla conformis mycerini* et *Xenopsylla ramesis*, se retrouvent l'une ou l'autre sur le même rongeur hôte (*Meriones crassus*) dans deux habitats qui diffèrent selon la texture du milieu (substrat : sable ou sédiment). Partant de l'hypothèse que la nature du substrat joue un rôle important dans la répartition de ces puces, nous avons étudié la survie de leurs larves, pupes et jeunes adultes, ainsi que le taux de développement pré-imaginal dans des milieux sablonneux ou sédimentaires (loess). La nature du substrat n'affecte pas la survie et le taux de développement des œufs, ni chez *X. c. mycerini*, ni chez *X. ramesis*. La survie des larves et leur développement sont tous les deux affectés par la qualité du substrat. La survie des larves de *X. c. mycerini* est plus élevée dans le sable, alors que celle des larves de *X. ramesis* n'est pas influencée par le substrat. Les larves de chacune des deux espèces se développent plus rapidement dans le sable. La durée maximale de survie des larves de *X. c. mycerini* qui meurent avant le stade de puppe ne dépend pas du substrat, tandis que les larves de *X. ramesis* survivent plus longtemps en milieu sédimentaire qu'en milieu sablonneux. La plupart des pupes des deux espèces survivent dans les deux substrats, mais la durée des stades de puppe est plus longue dans le sable. Les jeunes adultes des deux espèces survivent un temps égal dans les deux milieux. Indépendamment de la nature du substrat, les adultes de *X. c. mycerini* vivent moins longtemps que ceux de *X. ramesis*. On n'observe pas de différences inter-sexe et intra-espèce des temps de survie des jeunes adultes de *X. c. mycerini* entre les deux substrats. Les temps de survie des mâles et des femelles de *X. ramesis* sont différents en milieu sablonneux, mais pas en milieu sédimentaire.

MOTS CLÉS : *Xenopsylla conformis mycerini*, *Xenopsylla ramesis*, milieu, substrat, survie, développement.

INTRODUCTION

Fleas (Siphonaptera) alternate between periods of occurring on the host body and periods occurring in the nest or burrow of the host. Thus, flea distribution is determined not only by the distribution

of their hosts, but also by environmental factors. Furthermore, the flea life cycle also suggests strong environmental effects on their distribution because, in most cases, pre-imaginal flea development is entirely off-host and the survival of immature fleas is, thus, dependent on microclimatic conditions of host burrow or nest (e.g. Gerasimova, 1979; Zolotova & Afanasieva, 1971). These microclimatic parameters are variable among host habitats.

We studied the distribution of fleas among different rodent hosts occupying different habitats in the Negev desert, Israel (Krasnov *et al.*, 1997, 1998). It was found that the replacement of one species of flea, *Xenopsylla conformis mycerini* (Rothschild, 1904) with another *Xenopsylla ramesis* (Rothschild, 1904) occurred on the same rodent host *Meriones crassus* Sundevall, 1842 bet-

* Ramon Science Center, Jacob Blaustein Institute for Desert Research, Ben-Gurion University of the Negev, P.O.Box 194, Mizpe Ramon 80600, Israel.

** The Wyler Department of Dryland Agriculture, Jacob Blaustein Institute for Desert Research, Ben-Gurion University of the Negev, Beer-Sheva 84105, Israel.

*** Science Division, Truman State University, Kirksville, MO 63501, USA.

Correspondence: Boris R. Krasnov. Tel.: +972-8-6586337.

Fax: +972-8-6586369. E-mail: krasnov@bgumail.bgu.ac.il

ween two different habitats situated at the opposite sides of a steep precipitation gradient. Burrows of *M. crassus* in two habitats showed large differences in architecture (Shenbrot *et al.*, in press). Burrows in "mesic" habitat were much more ventilated than those in "xeric" habitats resulting in between-habitat differences in burrow microclimate. Air temperature was lower and relative humidity was higher in burrows in "mesic" habitats than those in burrows in "xeric" habitats. Attempting to explain the pattern of distribution of the two species, we studied the effect of air temperature and relative humidity on survival and rate of development of pre-imaginal fleas as well as on resistance to starvation of adult insects (Krasnov *et al.*, 2001a, b; 2002). We received contradictory results and concluded that paratopic habitat distribution of *X. c. mycerini* and *X. ramesis* cannot be explained by the differential response to different microclimatic conditions only. Other factors, such as between-habitat difference in soil texture, should also be involved.

The substrate in *X. c. mycerini* ("xeric") habitats is represented by sand, whereas that in *X. ramesis* ("mesic") habitats is represented by loess-like sediments (Krasnov *et al.*, 1997; Shenbrot *et al.*, in press). This difference presumably causes different soil moisture, which can influence both development time and survival of pre-imaginal fleas. Furthermore, a flea cocoon is covered by adhering particles of the nest substrate. Sand and loess particles differ in size and in physical properties. Consequently, we assumed that cocoon coverages built of sand or loess could differ in their abilities to absorb water, while active water vapour uptake has been shown to be important for cocooned fleas (Edney, 1947a; Rudolph & Knulle, 1982).

We hypothesized that the substrate is an important factor determining flea distribution. To test this hypothesis we studied survival and development of *X. c. mycerini* and *X. ramesis* in sand and loess rearing medium. We predicted that the texture of substrate would affect the survival of pre-imaginal and newly emerged adults of *X. c. mycerini* and *X. ramesis* as well as the rate of pre-imaginal development, all else being equal. We also predicted that survival and development of *X. c. mycerini* would be higher in sand rearing medium than in loess rearing medium whereas the opposite would be true for *X. ramesis*.

MATERIALS AND METHODS

FLEAS AND THEIR HOSTS

Flea eggs were obtained from a laboratory colony of fleas started from field-collected specimens on *M. crassus* using the rearing procedures following

by Silverman *et al.* (1981) and Metzger & Rust (1997). Details of flea rearing procedures are described elsewhere (Krasnov *et al.*, 2001a, b). The laboratory colony of *M. crassus* was established from animals captured in the field. Animals were bred and maintained at our laboratory for four years. Millet seeds and leaves of *Atriplex halimus* were fed *ad libitum*. Colonies of both fleas and rodents were maintained at 25°C and 75 % RH with a photoperiod of 12:12 (Light:Dark) h.

EXPERIMENTAL DESIGN

Flea eggs were collected daily on pieces of filter paper placed in a pan under the rodent's nest box. Female fleas oviposited on these pieces of filter paper. Eggs on filter paper were then transferred into 20 ml glass vials that contained a thin layer of clean sand or loess (from nine to 18 eggs per vial). Vials were covered with 5 x 5 cm nylon screen held by a rubber band. After eggs hatched, the larvae were transferred into another vial with clean sand or loess and dry bovine blood (larvae nutrient medium). There were eight to 12 larvae per vial. After pupation the procedure was repeated as described above. There were four to five cocoons per vial. After the imago emerged, each flea was transferred into a new individual vial with a thin layer of sand or loess. Sand and loess material were taken from the natural localities inhabited by *X. c. mycerini* and *X. ramesis*, respectively.

All eggs, larvae, cocoons and newly emerged fleas were maintained at 25°C air temperature and 75 % RH. Temperature was regulated in refrigerated incubators (FOC225E, Velp Scientifica srl, Milano, Italy). Humidity was regulated in 38 x 23 x 13 cm acrylic humidity chambers using saturated solution of potassium chloride as described by Winston & Bates (1960). Temperature and humidity were monitored using Fisherbrand Traceable Humidity/Temperature Pen with Memory (Fisher Scientific International, NJ, USA). Thus, there were two experimental treatments (sand substrate and loess substrate) for each of three pre-imaginal stages and newly emerged adults of each species replicated 10 to 40 times.

DATA RECORDING

Vials were checked twice a day. Egg vials were checked until all larvae hatched or for 30 consecutive days. An egg was considered dead if no larva hatched during this period. The vials of larvae were checked until all larvae cocooned or were dead. Finally, vials that contained cocoons were checked either until all adults emerged from their cocoons or for 40 consecutive days. We shook the cocoon vials slightly during checking to stimulate flea emergence, because vibration has been reported to stimulate flea emergence

from cocoons (Marshall, 1981). A pupa was considered dead if after 40 d no adult emerged. These cocoons were dissected and the stage at which the immature flea died (larva, pupa or pre-emerged imago) was identified. After death of each newly emerged imago, we identified its sex by examination of its genitalia under light microscopy. Identification of imago sex allowed us to identify whether each survivor to adulthood egg, larva or cocoon was male or female.

STATISTICAL PROCESSING

To adjust deviations from normality, we applied an arc-sine transformation on the proportion of eggs, larvae or pupae that survived. Log-transformation was applied on the duration of maximal survival time of a larva. Survival was analyzed using 2-way ANOVAs with species and substrate (=rearing medium) as independent variables and the proportion of pre-imaginal fleas that survived as a dependent variable. Construction of additional cocoons by larvae in dependence on substrate was analyzed using multi-way cross-tabulation tables and Pearson χ^2 tests for each 2-way table. The effect of the rearing medium on the maximal survival time of larvae and newly emerged adults was also tested using 2-way ANOVA with species and substrate as independent variables.

The duration of developmental time of each stage was log-transformed to adjust deviations from normality. Developmental time was analyzed using 2-way ANOVAs with species and substrate as independent variables. In addition, 2-way ANOVAs with substrate and sex as independent variables were applied for the analyses of sex differences in development time in dependence on substrate for each pre-imaginal stage in each flea species.

To test the effect of substrate on sex ratio of newly emerged adults we analyzed 2 x 2 contingency tables of emerged males and females using Yates corrected χ^2 tests. We (a) searched for deviation of sex ratio of newly emerged adults from 1:1 and (b) compared this sex ratio within-species between-substrate. Tukey's Honest Significant Difference (HSD) tests for unequal samples were applied for all multiple comparisons. Data are presented as mean \pm S.E.

RESULTS

SURVIVAL OF PRE-IMAGINAL FLEAS

Texture of rearing medium did not affect survival of eggs in either flea species ($F = 0.02$; $df = 1, 23$ and $F = 0.16$; $df = 1, 16$; respectively, $P > 0.05$ for both). In addition, no significant interaction between species and substrate factors in their influence

on egg survival was found as well ($F = 0.08$; $df = 1, 39$; $P > 0.05$). Survival of *X. c. mycerini* eggs averaged 76.5 ± 3.2 %, whereas that of *X. ramesis* eggs averaged 78.6 ± 3.7 % (Tukey's HSD tests, $P > 0.05$).

Larval survival was affected by substrate texture ($F = 12.11$; $df = 1, 136$; $P < 0.05$). Survival of *X. c. mycerini* larvae was significantly higher in sand substrate than in loess substrate, whereas survival of *X. ramesis* larvae did not differ significantly in different substrates (Tukey's HSD tests, $P < 0.05$ and $P > 0.2$, respectively) (Fig. 1). Maximal survival time of *X. c. mycerini* larvae that died before pupation did not depend on substrate texture and averaged 20.8 ± 1.5 days in sand and 21.6 ± 1.3 days in loess (Tukey's HSD test, $P > 0.5$). In contrast, *X. ramesis* larvae survived significantly longer in loess than in sand (25.9 ± 1.7 days versus 20.2 ± 1.3 days, respectively, Tukey's HSD test, $P < 0.05$). In addition, maximal survival time did not differ significantly among *X. c. mycerini* larvae in sand substrate, *X. c. mycerini* larvae in loess substrate and *X. ramesis* larvae in sand substrate (Tukey's HSD test, $P > 0.05$), whereas maximal survival time of *X. ramesis* larvae in loess substrate was significantly longer than in other 3 treatments (Tukey's HSD test, $P < 0.05$).

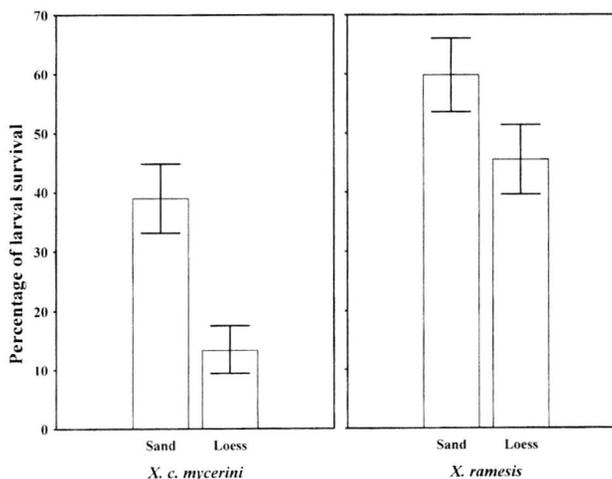


Fig. 1 – Mean (\pm S.E.) percentage of larval survival of *X. c. mycerini* and *X. ramesis* in different substrates.

Some larvae left their cocoons soon after construction and constructed new cocoons. In general, larvae of both fleas build these additional cocoons with similar frequency (in 49.5 % cases for *X. c. mycerini* and in 52.6 % cases for *X. ramesis*, Pearson's $\chi^2 = 1.82$; $df = 1$; $P > 0.05$). However, the frequency of the construction of additional cocoons in sand versus loess substrate did not differ significantly in *X. c. mycerini* (in 23.8 versus 25.7 % cases, respectively, Pearson's $\chi^2 = 0.03$; $df = 1$; $P > 0.05$). In contrast, *X. ramesis* constructed additional cocoons in sand significantly more often than they did

in loess (in 38.2 versus 14.4 % cases, respectively, Pearson's $\chi^2 = 8.03$; $df = 1$; $P > 0.05$). Nevertheless, the substrate had no effect on the amount of additional cocoons constructed by either species ($F = 2.62$; $df = 1$, 42 for *X. c. mycerini* and $F = 0.24$; $df = 1$, 48 for *X. ramesis*, $P > 0.05$ for both).

Most pupae of both species survived in both types of rearing medium. Only four of 263 experimental pupae died before emergence. All were *X. c. mycerini* (two in sand and two in loess) and died being in cocoons on a pre-pupal stage.

DEVELOPMENT OF PRE-IMAGINAL FLEAS

No general effect of substrate on the period of development from egg to larva was found in both flea species ($F = 0.1$, $df = 1$, 832, $P > 0.05$). Larvae of *X. c. mycerini* hatched from eggs slightly, albeit significantly, later than those of *X. ramesis*, all else being equal ($F = 6.2$, $df = 1$, 832, $P < 0.05$; Fig. 2). Female eggs of *X. c. mycerini* developed to hatching faster than male eggs did (5.40 ± 0.06 versus 5.62 ± 0.07 days, respectively, $F = 7.7$, $df = 1$, 105, $P < 0.05$), whereas no significant difference in time to hatching was found in *X. ramesis* (5.37 ± 0.06 days for females versus 5.33 ± 0.06 days for males, $F = 0.4$, $df = 1$, 151, $P > 0.05$). In addition, male eggs of *X. c. mycerini* developed faster in sand substrate than in loess substrate (5.58 ± 0.08 versus 6.00 ± 0.01 days, respectively, Tukey's HSD test, $P < 0.05$), whereas no effect of substrate was found for females of this species as well as for both sexes of *X. ramesis* (Tukey's HSD tests, $P > 0.05$).

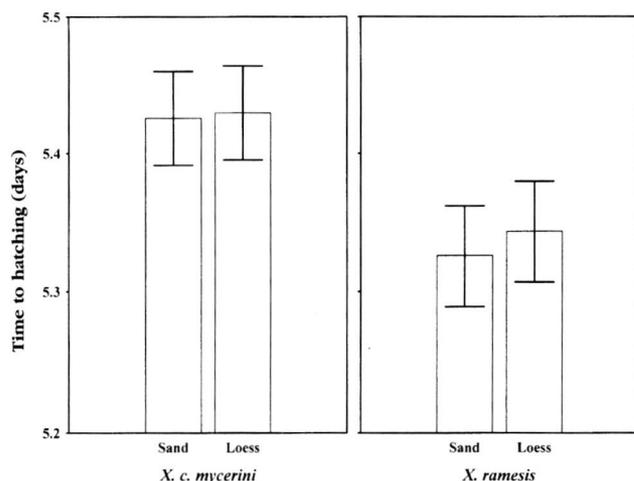


Fig. 2 – Mean (\pm S.E.) development time (days) of eggs of *X. c. mycerini* and *X. ramesis* in different substrates.

Duration of time from larva hatching to pupation was affected by species and substrate factors ($F = 17.87$; $df = 1$, 326 and $F = 18.16$; $df = 1$, 326; respectively, $P < 0.05$ for both). Larvae of both species developed

faster in sand substrate than in loess substrate (Fig. 3). In addition, larvae of *X. ramesis* attained pupation for shorter time period than those of *X. c. mycerini*, all else being equal (Fig. 3). The rate of development did not differ significantly between female and male larvae of occurring in the same substrate (*X. c. mycerini*: 18.52 ± 0.49 days for females and 18.20 ± 0.59 days for males; $F = 0.5$, $df = 1$, 323; *X. ramesis*: 17.18 ± 0.38 days for females and 17.24 ± 0.48 days for males; $F = 3.4$, $df = 1$, 455; $P > 0.05$ for both).

Substrate factor significantly affected the duration of pupal development ($F = 10.02$, $df = 1$, 258; $P < 0.05$), whereas the response to this factor did not differ between species ($F = 0.11$, $df = 1$, 258; $P > 0.05$). In addition, interaction between these two factors was non-significant ($F = 0.06$, $df = 1$, 258; $P > 0.05$). Duration of pupal stages in sand substrate was longer than that in loess substrate in both species (Fig. 4). In general, duration of female pupation was significantly shorter

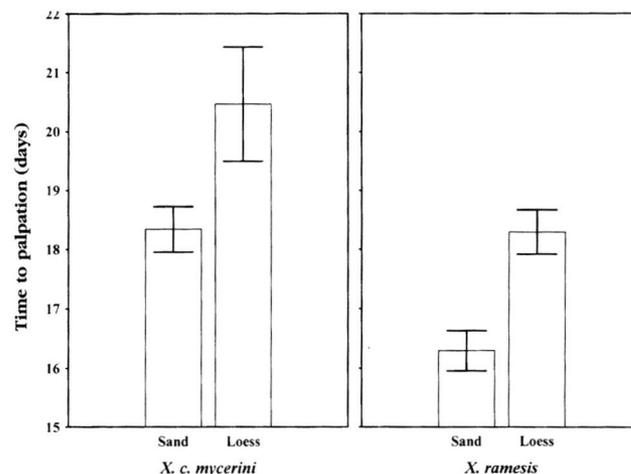


Fig. 3 – Mean (\pm S.E.) development time (days) of larvae of *X. c. mycerini* and *X. ramesis* in different substrates.

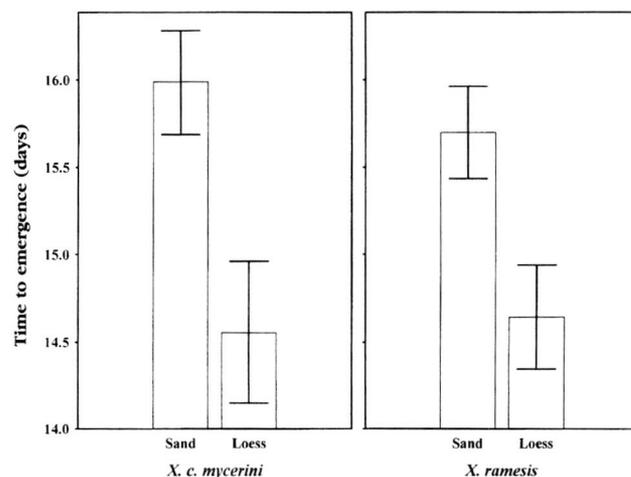


Fig. 4 – Mean (\pm S.E.) pupation time (days) of *X. c. mycerini* and *X. ramesis* in different substrates.

than that of male pupation in both species (*X. c. mycerini*: 14.77 ± 0.27 days for females and 17.45 ± 0.44 days for males; $F = 7.38$, $df = 1$, 105; *X. ramesis*: 13.75 ± 0.16 days for females and 17.54 ± 0.24 days for males; $F = 159.31$, $df = 1$, 149; $P < 0.05$ for both). Furthermore, pupation time of *X. c. mycerini* females did not depend on substrate texture (Tukey's HSD test, $P > 0.05$), whereas the opposite was true for males of this species (Tukey's HSD test, $P < 0.05$) with pupation time being shorter in loess substrate (Table D). In contrast, development rates of male and female pupae of *X. ramesis* were similar in the same substrate (Tukey's HSD tests, $P > 0.05$; Table I).

Species	Sex	Substrate	Duration of pupal development (days)
<i>X. c. mycerini</i>	Female	Sand	14.9 ± 0.3
		Loess	14.3 ± 0.4
	Male	Sand	17.7 ± 0.5
		Loess	15.5 ± 1.3
<i>X. ramesis</i>	Female	Sand	14.0 ± 0.2
		Loess	13.5 ± 0.2
	Male	Sand	17.4 ± 0.3
		Loess	17.4 ± 0.5

Table I – Mean (\pm S.E.) duration of development of male and female pupae of *X. c. mycerini* and *X. ramesis* in different substrates.

SURVIVAL TIME AND SEX RATIO OF NEWLY EMERGED ADULTS

In general, newly emerged adults survived for a similar time in both sand and loess substrate. This was true for both species ($F = 0.01$, $df = 1$, 256; $P > 0.05$). However, irrespective of substrate, adult *X. c. mycerini* survived for a shorter time than did adult *X. ramesis* ($F = 66.01$, $df = 1$, 256; $P < 0.05$; Fig. 5). No between-sex within-species differences in survival time of newly emerged adults in sand versus loess substrate were found in *X. c. mycerini* ($F = 0.02$ for sex factor, $F = 0.26$ for substrate factor, $F = 0.16$ for interaction of these factors; $df = 1$, 103; $P > 0.05$ for both). In contrast, males and females of *X. ramesis* showed difference in survival rate in sand substrate but not in loess substrate ($F = 6.43$, $df = 1$, 149; $P < 0.05$ for sex x substrate factor interaction) with females surviving less time than males (Tukey's HSD test, $P < 0.05$; Fig. 5).

Sex ratio of newly emerged adult *X. c. mycerini* did not differ significantly either from 1:1 (Yates corrected $\chi^2 = 2.07$ in sand and $\chi^2 = 1.93$ in loess, $df = 1$, $P > 0.05$) or between sand and loess substrate (Yates corrected $\chi^2 = 1.04$, $df = 1$, $P > 0.05$). Sex ratio of newly emerged *X. ramesis* in sand substrate did not deviate significantly from 1:1 (Yates corrected $\chi^2 = 0.05$, $df = 1$, $P > 0.05$), whereas significant female bias occurred in

loess substrate (71 % on newly emerged fleas were females, Yates corrected $\chi^2 = 5.87$, $df = 1$, $P < 0.05$).

DISCUSSION

SUBSTRATE TEXTURE EFFECT

The texture of substrate can determine its microclimatic conditions. For example, loess soils in all deserts absorb water best and their hydrothermic properties are similar to those of meadow soils (Korovin, 1961). This leads to increase of humidity that was shown to be very important in fleas (Sharif, 1949; Smith, 1951; Yinon *et al.* 1967; Krasnov *et al.*, 2001a). Our predictions appeared to be true for larval survival of *X. c. mycerini* only. Larval survival of this species was significantly higher in sand substrate than in loess substrate, whereas it did not differ significantly between substrates in *X. ramesis*. Unlike our predictions, there was no effect of the substrate on the survival and development of eggs and survival of pupae and of newly emerged adults. In addition, larvae of both species developed faster in sand substrate than in loess substrate, whereas the opposite was the case for pupal development.

Eggs, pupae and newly emerged adults proved to be the less sensitive stages to the variation in rearing medium. As mentioned above, the important difference between sand and loess lies in their ability to absorb water and, thus, to affect the relative humidity of the immature flea environment. However, flea eggs are highly resistant to low humidity (Krasnov *et al.*, 2001a) and, consequently, the variation in substrate texture had no evident effect on survival and rate of development.

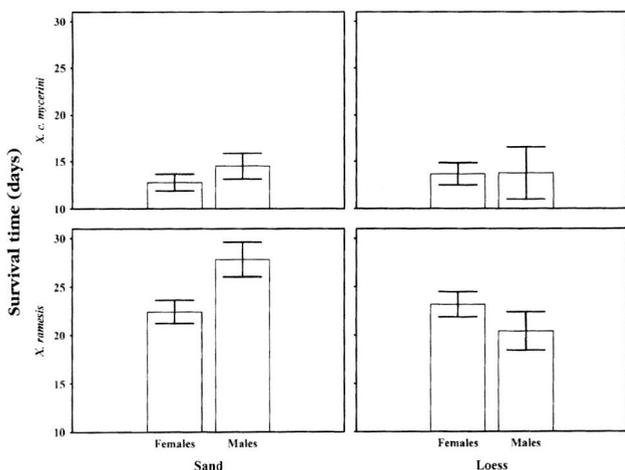


Fig. 5 – Mean (\pm S.E.) survival time of newly emerged male and female *X. c. mycerini* and *X. ramesis* in different substrates.

Flea pupae are enclosed within the protective microenvironment of a cocoon (Edney, 1947a) and, thus, are also resistant to low humidity, at least, in terms of survival (Krasnov *et al.*, 2001a). However, pupae are unable to absorb atmospheric water via their rectal sac at low humidity (Edney, 1947b). This can explain the lower pupation time of both fleas in sand than in loess rearing medium.

The absence of the substrate texture effect on imago fleas may be due to the relative tolerance of adult *X. c. mycerini* and *X. ramesis* to desiccation (Krasnov *et al.*, 2002), although earlier results demonstrated the decline of flea survival with the humidity decrease due to high water loss, presumably because much time is spent with the spiracles open (Silverman *et al.*, 1981; Cooke, 1999). This is especially true for newly emerged individuals (Krasnov *et al.*, 2002). These individuals have lower mass specific metabolic rates (Fielden *et al.*, 2001) and, consequently, lower oxygen requirements and water loss from the tracheal system (Bursell, 1974) than fed fleas.

The larval stage proved to be the most sensitive to the texture of the rearing medium. This sensitivity was manifested differently in the two fleas. In *X. c. mycerini*, survival of larvae was significantly higher in sand than in loess rearing medium, but maximal survival time of larvae that died before pupation did not depend on substrate texture. In *X. ramesis*, larvae survived similarly in different substrates, but maximal survival time in loess rearing medium was higher than that in sand rearing medium. Furthermore, survival of *X. c. mycerini* larvae in loess substrate was very low and attained only 13.4 %. These data support findings of Edney (1947b) that the longevity of larval *Xenopsylla brasiliensis* (Baker, 1904) and *Xenopsylla cheopis* (Rothschild, 1903) decreased drastically in the rearing medium with fine sand in comparison with that in the rearing medium with coarse sand. Nevertheless, in spite of sharp size differences of sand and loess particles, larvae of both species demonstrated an ability to use particles of both types to adhere to the cocoon for camouflage. However, higher frequency of the construction of additional cocoons by *X. ramesis* larvae in sand substrate in comparison with loess substrate suggests that this species is adapted to build cocoons using small substrate particles, whereas it leaves cocoons and constructs the new ones if only relatively large particles of substrate are available.

BETWEEN-SEX DIFFERENCES

In general, development of immature females in *X. c. mycerini* and *X. ramesis* was shorter than that of males on the egg and pupal stages. Previously, the shorter period of female development was reported only for pupal stages of *Ctenocephalides felis* (Bouché, 1835)

(see Hudson & Prince, 1958; Metzger & Rust, 1997). Metzger & Rust (1997) suggested that this prevents inbreeding of fleas from the same cohort. However, we mentioned earlier that the time difference between female and male development rate seems to be too small, albeit significant, for this explanation to be reasonable (Krasnov *et al.*, 2001b). Instead, we suggested that immature males are more sensitive to environmental factors than immature females, at least in *X. c. mycerini*. Indeed, male eggs and pupae of this species developed at a different rate in different rearing mediums, whereas no effect was found for females. Previously, we found that male and female eggs and larvae of *X. c. mycerini* responded differently to air temperature and relative humidity (Krasnov *et al.*, 2001b).

Another differential between-sex response to substrate texture was demonstrated by newly emerged adult *X. ramesis*. In this species, males and females survived differently in sand substrate but not in loess substrate with females being less tolerant than males. This suggests higher sensitivity of females to the substrate, unlike the pattern found in pre-imaginal *X. c. mycerini*, although adult female fleas have been shown to be less sensitive than males to fluctuations in air temperature and relative humidity (Krasnov *et al.*, 2002).

SUBSTRATE TEXTURE AND HABITAT DISTRIBUTION

Contrary to our expectations, the effect of substrate on both survival and development rate of pre-imaginal and adult fleas did not appear to be especially strong. Furthermore, results of this study demonstrated that it is difficult to explain habitat distribution of *X. c. mycerini* and *X. ramesis* by between-habitat difference in the substrate alone. Nevertheless, higher survival of *X. c. mycerini* larvae in sand rearing medium can partly explain the absence of this species from "mesic loess" habitats, whereas longer survival time of *X. ramesis* larvae in loess rearing medium can partly explain its absence from "xeric sandy" habitats. Both these patterns suggest that between-habitat substrate differences can presumably affect habitat distribution of fleas, all else being equal. Taking into account our previous results (Krasnov *et al.*, 2001a, 2001b, 2002), we propose that the factor of substrate texture interacts with other environmental factors (air temperature and humidity), and between-habitat distribution of the two fleas can be explained by an interaction of all three factors. Thus, the bottlenecks for the occurrence of *X. ramesis* in "xeric sand" habitats are (a) sensitivity of eggs, larvae and newly emerged imago to low relative humidity (Krasnov *et al.*, 2001a, 2002) and (b) fast mortality of larvae in sand substrate (this study). Bottlenecks for the occurrence of *X. c. mycerini* in "mesic loess" habitats are (a) sensitivity of pupae to low tempera-

ture (Krasnov *et al.*, 2001b) and (b) low survival of larvae in loess substrate (this study).

Alternatively, the two flea species can occupy different habitats due to the result of interspecific competition with adult fleas competing for blood of a host (Day & Benton, 1980) and/or larvae competing for space and food resources in the nest of a host (Marshall, 1981). Competitive outcome can also depend on microclimate conditions and/or larval food abundance because of difference in foraging efficiency between species. "Competition" and "environmental preferences" explanations of habitat distribution of *X. c. mycerini* and *X. ramesis* are not necessarily exclusive of each other. However, the "competition" hypothesis still requires experimental testing.

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