Bulletin of Entomological Research (2002) **92**, 375–384 DOI: 10.1079/BER2002184

Host location, survival and fecundity of the Oriental rat flea *Xenopsylla cheopis* (Siphonaptera: Pulicidae) in relation to black rat *Rattus rattus* (Rodentia: Muridae) host age and sex

S. Mears[1]*, F. Clark[1], M. Greenwood[2] and K.S. Larsen[3]

[1]Department of Biology, University of Leicester, University Road, Leicester, LE1 7RH, UK: [2]Department of Geography, University of Loughborough, UK: [3]KSL Consulting, Ramløsevej 25, Denmark

Abstract

Host choice and fecundity are two factors that may contribute to the variation in flea counts observed when assessing the potential risk of flea-borne transmission of pathogens from rodents to humans. Using the black rat, *Rattus rattus* Linnaeus, as host the effects of age and sex on host choice and fecundity of the Oriental rat flea, *Xenopsylla cheopis* Rothschild, were examined experimentally at 25°C and 80% rh. During the first two days of emergence from cocoons, female fleas dominated the sex ratio by 4:1 but from the third day onwards this switched to a male-dominated sex ratio of 4:1. The sex of the flea did not influence their host-seeking behaviour. Newly emerged fleas of both sexes were not influenced by the rat's presence and at seven days old both sexes demonstrated similar levels of attraction toward the rat host. The sex of the rat did not affect flea host-seeking behaviour. There was a 50–70% decline in the initial number of adult fleas during the first week after their release onto a rat host, and this decline was greatest on juvenile rats. Flea fecundity was also significantly lower on juvenile rat hosts but no differences due to the sex of the rat were observed. This experimental study supports the hypothesis that differences in flea count due to host sex, reported in field surveys, result from sexual differences in host behaviour and not from discriminatory host-seeking behaviour by *X. cheopis*. Differences in flea count due to host age may be affected by differences in *X. cheopis* fecundity, which may itself be mediated by host behaviour such as grooming.

Introduction

Monitoring the frequency of bubonic plague, *Yersinia pestis* Lehmann & Neumann (Enterobacteriales: Enterobacteriaceae), and murine typhus, *Rickettsia typhi* Wolbach & Todd (Rickettsiales: Rickettsiaceae), in rodent populations relies on calculating an accurate flea count (average number of a flea species caught per host and also commonly referred to as the flea index). The flea count is used to determine the potential risk of transmission of these pathogens from rodents to humans (Pollitzer, 1954; Traub *et al*., 1978). Both abiotic and biotic factors such as ambient temperature and host age and sex are known to influence the number of fleas per host at any one time (see for example Cole, 1945; Lang & Wills, 1991). In order to obtain an accurate flea count Hirst (1927) stated the need for hosts to be of the same species and weight.

Several authors conducting host-ectoparasite surveys have reported a relationship between the flea burden and host age or sex but have attributed these differences to the behaviour of the host rather than to flea behaviour leading

*E-mail: s.mears@cant.ac.uk

to greater aggregation on a 'preferred' host type (Mohr, 1961; Mohr & Stumpf, 1962; Haas, 1966; Cowx, 1967). Cowx (1967) for example in surveys of *Microtus agrestis* Linnaeus (Rodentia: Muridae), *Clethrionomys glareolus* Linnaeus (Rodentia: Muridae) and *Apodemus sylvaticus* Linnaeus (Rodentia: Muridae) found that adult males carried a greater population of fleas such as *Ctenophthalmus nobilis* Rothschild (Siphonaptera: Pulicidae) than adult females or juveniles of both sexes during the summer, but not during the winter months. These findings Cowx (1967) explained were the result of sexual differences in seasonal host behaviours such as a decline in mutual grooming for parasites among territorial males during the summer breeding season. Some experimental results, however, have pointed to the attractiveness of particular host types to fleas in order to explain the patterns in distribution. Shulov & Naor (1964) found that of the four species of rodent tested a male *Rattus norvegicus* var. *albino* Linnaeus (Rodentia: Muridae) was the most 'attractive' to *Xenopsylla cheopis* Rothschild (Siphonaptera: Pulicidae). Unfortunately further elaboration of this work concentrated on the effects of flea age and sex, rather than those of the host.

Others have noted the effects of host age and of different host species on the fecundity of various species of flea (Hirst, 1927; Buxton, 1948; Haas, 1965; Prasad, 1969). Hirst (1927) noted that counts of fleas, principally *X. cheopis* and *Xenopsylla astia* Rothschild (Siphonaptera: Pulicidae), on wild caught *Rattus rattus* Linnaeus (Rodentia: Muridae) increased with host weight (and therefore age) up to an optimum before declining. In the laboratory Buxton (1948) tested the effects of host age on fecundity of *X. cheopis* and found that *X. cheopis* reared on 2- to 10-day-old mice *Mus musculus* Linnaeus (Rodentia: Muridae) had a lower fecundity than those reared on adults. Hirst (1927) also mentioned differences in fecundity of *X. cheopis* when comparing those fed on human *Homo sapiens* Linnaeus (Primates: Hominidae) hosts with those fed on rodent hosts such as *R. rattus*. Haas (1965), however, was the only author to experimentally test host suitability in relation to host sex. With *Xenopsylla vexabilis* Jordon (Siphonaptera: Pulicidae) Haas (1965) found survival and feeding to be greatest on adult male, followed by adult female and then juvenile male *Rattus exulans* Peale (Rodentia: Muridae) hosts.

This paper examines experimentally at one temperature and relative humidity the host-seeking behaviour and fecundity of *X. cheopis* in relation to *R. rattus* host age and sex, and discusses the implications for flea count interpretation when assessing the risks of flea-borne disease transmission from rodent hosts to humans.

Materials and methods

All fleas were obtained from an established culture at the Danish Pest Infestation Laboratory using a Nilotic strain of *X. cheopis* originating from Tanzania, Africa (cf. Larsen, 1995). *Rattus rattus* used in the experiments originated from Tanzania and were maintained in a colony at the Danish Pest Infestation Laboratory. All the experimental work took place at the Danish Pest Infestation Laboratory, Skovbrynet 14, 2800 Lyngby, Denmark.

Host location

Twenty newly emerged *X. cheopis*, i.e. less than 24 h old, were obtained by checking cocoons daily for the presence of adults. These adults were separated from the cocoons (as described in Mears, 1996) and transferred to glass vials (diameter 20 mm, height 40 mm) and maintained in the dark at 19 ± 3°C and 80 ± 5% rh. A substrate of untreated deciduous wood shavings was added to minimize the activity of the unfed fleas and so reduce mortality (Hirst, 1927). To avoid the reduced activity of *X. cheopis* after carbon dioxide anaesthesia, as reported by Clark *et al*. (1993), determination of the sex of the fleas was deferred until after the experiment. Instead, advantage was taken of the protogyny demonstrated by fleas (Hirst, 1927; Webster, 1930; Edney, 1945). Fleas collected within the first two days of emergence from a batch of cocoons were tentatively assumed to be female; those collected from the third day onwards to be male. Final determinations are given in the results. The adult (sexually mature >12 months of age) and juvenile (sexually immature) *R. rattus* were transferred from the main colony at the Danish Pest Infestation Laboratory and maintained in individual cages one week prior to the experiments so as to acclimatize them to the new conditions and allow easy transfer to the experimental apparatus.

Experimental apparatus

The experimental design followed that of Shulov & Naor (1964). The apparatus (fig. 1) consisted of a transparent Perspex tube 50 cm long with a diameter of 5 cm. Half way along the length a 2.5 cm diameter hole was drilled to accommodate a bung (diameter 31 mm top, 25 mm bottom; height 30 mm) with a glass funnel inserted. A length of string to provide grip for the *X. cheopis* was stretched along the bottom of the tube by attaching its ends to an elastic band underneath. This was marked along its length to divide the tube into five equal sections. Muslin cloth was stretched over the ends of the tube and held in place by plastic O-rings to prevent the *X. cheopis* from escaping.

At the ends of the tube were placed large glass jars 20 cm high by 15 cm diameter. These were attached to the tube by means of a plastic tub pushed over the lid. A hole was cut to allow the tube to be pushed through the bottom of the tub and against the lid of the jar. Each lid had a 5 cm diameter hole cut in it, covered by a strong fine mesh, to allow ventilation of the rat odour into the apparatus. The apparatus was ventilated passively through the glass funnel in the centre of the tube so that any odour emanating from the jar passed over the *X. cheopis*. The use of an air pump, advocated by Shulov & Naor (1964), was found unnecessary and impracticable with 12 sets of apparatus in use simultaneously. The tube was fixed to a clamp stand and set horizontally using a spirit level. The jars were rested in a sponge trough so as to reduce vibrations created by movement of the rats. The whole apparatus was isolated within its own lightproof compartment. With each apparatus being held in its own dark compartment, counts of *X. cheopis* could be made in turn, without disturbance of the others. All experiments were conducted in a single climate-controlled room maintained at 25 ± 3°C and 80 ± 5% rh.

Experimental procedure

The test rat was encouraged into a jar, which along with a new empty jar at the opposite end, was connected to the apparatus. The end of attachment and particular apparatus

https://doi.org/10.1079/BER2002184 Published online by Cambridge University Press

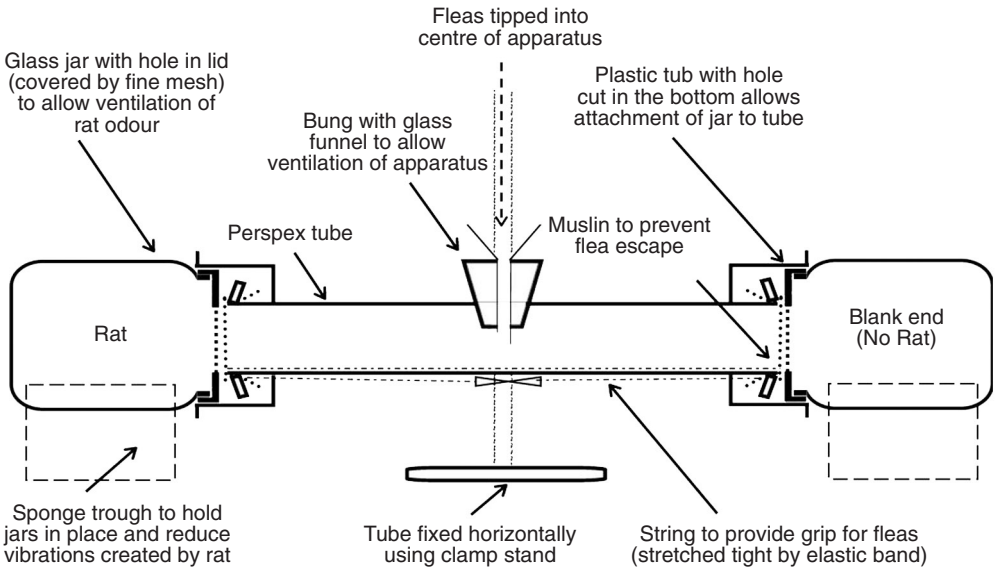

Fig. 1. Apparatus used to assess the host seeking behviour of *Xenopsylla cheopis* in the presence of *Rattus rattus*.

used by any particular rat was chosen at random. The rats, six males and six females of the same age, were left in the darkened compartments while the *X. cheopis* (12 vials, each with ~ 20 fleas) were collected from the incubator.

The *X. cheopis* were tipped into the centre of the tube via the glass funnel. The door of the compartment was closed and the process repeated with the next experimental apparatus. *Xenopsylla cheopis* were added to all sets of apparatus before the room was vacated, and left undisturbed for 1 h. The first compartment was then opened to allow illumination of the apparatus. This caused the *X. cheopis* to become motionless (see Clark *et al*., 1993) so that the number in each section of the tube could be counted. Very few *X. cheopis* were found to move during this process and rarely were any seen to cross from one section to another. *Xenopsylla cheopis* found on the dividing lines of the string were recorded as being in the section nearest the centre. Each experiment was checked in turn before the room was again vacated. The position of *X. cheopis* was recorded every hour with a final count being made at 6 h. The rats were returned to their cages and the *X. cheopis* collected into their original vials with a pooter. Finally, the *X. cheopis* were anaesthetised with carbon dioxide and the sex ratio determined.

To simplify analysis and interpretation of the count data a 'net taxis' of *X. cheopis* was calculated as described by Shulov & Naor (1964). This was done by subtracting the number of *X. cheopis* moving toward the blank end from those moving toward the rat end. A positive figure indicated the degree of flea taxis towards the rat and a negative figure the degree of flea taxis away from the rat. The net taxis provides a single value that gives the direction and degree of net movement in comparison to the opposite end. Statistical analysis of net taxis data was made using the Kruskall-Wallis one-way analysis of variance by ranks followed by multiple comparisons between treatments (Siegel & Castellan, 1988).

Flea fecundity

Eighteen male and 18 female sexually mature *R. rattus* (> 12 months of age), and 13 male and 5 female sexually immature juveniles, were weighed and transferred from stock cages, where they had been maintained singly since removal from the main colony, to the experimental cages. All experiments were conducted in a single climate-controlled room maintained at 25 ± 3°C and 80 ± 5% rh (cf. Larsen 1995).

Newly emerged *X. cheopis* were anaesthetized with carbon dioxide, sexed and placed into groups each of 10 males and 40 females using a dissecting microscope. There was a preponderance of female fleas available during the first days of emergence from a batch of cocoons. Therefore the sex ratio of fleas at the start of the experiment reflected the ratio of each sex available during this time. One group of fleas was introduced into the top of each nest box through the ventilation holes. Fresh food and water were provided *ad libitum* for the rat host but otherwise the cages were left undisturbed for one week.

On the seventh day after introduction of the *X. cheopis*, the nests were sieved (mesh size 2.5, 1.12, 0.5 mm) and the eggs and larvae separated using the methods described by Larsen (1995). The rat host (along with any fleas it carried) was retained in a large glass jar within the experimental cage. The *X. cheopis* from the nest material were counted and sexed. Only the fine debris from the nest material with the eggs and larvae was retained. The remaining nest material, along with the adult *X. cheopis*, was returned to the nest box prior to reintroduction of the rat host.

The fine debris from the nest material was weighed and shaken to evenly distribute it into a large glass Petri dish. This was divided into suitably sized portions, usually quarters. The top right-hand portion was aspirated into a pre-weighed vial using a motor driven pooter and weighed. Eggs and larvae were counted under a light microscope. The

https://doi.org/10.1079/BER2002184 Published online by Cambridge University Press

total numbers of eggs and larvae were estimated for the whole sample from the known weight of this material.

This procedure was repeated weekly for a total of four weeks. The numbers of *X. cheopis* remaining on each rat host were also counted at the end of the experiment so the total number of fleas surviving at the end of the experiment was known. As the size of the host can influence its grooming efficiency (see for example Lehane, 1991), all *R. rattus* hosts were killed at the end of the experiment, weighed and, after skinning, the surface area of skin was estimated using graph paper.

To account for differences in the survival of female *X. cheopis* on different *R. rattus* host animals the mean egg production per female (flea fecundity) was calculated. The weekly count of eggs and larvae from each experimental cage was divided by the number of female fleas, introduced initially or subsequently counted in the nest. It was assumed that *X. cheopis* counted in the nest were representative of the flea population as a whole. Fecundity data were analysed statistically using the Kruskal-Wallis one-way analysis of variance by ranks followed by multiple comparisons between treatments, i.e. host-types, as described in Siegel & Castellan (1988). Correlations involving rat weight, skin surface area and flea fecundity were analysed using the Spearman rank correlation coefficient for tied ranks (Siegel & Castellan, 1988). Variances between data sets were compared with the F-test (two-tailed) as described in Fowler & Cohen (1996).

Results

Host-choice

It was observed that during the first two days of emergence from cocoons female *X. cheopis* dominated the sex ratio by 4:1 but from the third day onwards this switched to a male dominated sex ratio of 4:1. Use was made of this observation, in order to avoid carbon dioxide anaesthesia affecting flea behaviour, and the sexing of fleas was deferred until after the host-choice experiment. Determination of the final sex ratio showed females to make up 80% (SD ± 20.2) of 'female' samples, i.e. those collected within the first two days of emergence of the first flea from its cocoon, and males to make up 78% (SD ± 16.3) of 'male' samples, i.e. those collected from the third day onwards after emergence of the first flea from its cocoon.

During initial control experiments (new empty jar at each end) at least 30% of the *X. cheopis* migrated from the centre, the point of introduction, within the first hour. More than 50% of the *X. cheopis* sample dispersed from the centre by the fifth hour and by the seventh hour they were evenly distributed across all sections (non-significant Kruskal-Wallis test = $6.18 < \chi^2_4 = 9.49$, $P = 0.05$). It was concluded that neither the apparatus nor the experimental procedure biased *X. cheopis* dispersal. A final count at 24 h found 15% of the *X. cheopis* remained in the centre with significantly more (KW = $17.5 > \chi^2_4 = 13.3$, $P = 0.01$) concentrated at the extreme ends of the tube.

In experiments with a *R. rattus* host at one end more than 50% of the *X. cheopis* had migrated from the central introduction point by the final count at 6 h, but in contrast to the control experiment a bias was demonstrated with more *X. cheopis* moving into one end of the tube than the other (table 1). The direction of this movement corresponded with the age of the *X. cheopis*. Newly emerged *X. cheopis* demonstrated a negative net taxis, i.e. more *X. cheopis* entered the blank end, in the presence of all rat types, whereas a positive net taxis was observed with 7-day-old *X. cheopis* with more entering the rat end. The null hypothesis, that host seeking behaviour is unaffected by *R. rattus* host age or sex irrespective of the age or sex of *X. cheopis*, was supported in all cases except one: the net taxis of 7-day-old male *X. cheopis* toward adult female *R. rattus* hosts was significantly different from the net taxis of newly emerged male *X. cheopis* toward juvenile female *R. rattus* hosts (KW = $71.0 > \chi^2_{15} = 30.6$, $P = 0.01$).

Discernible trends due to the sex of both *X. cheopis* and *R. rattus* host were also seen. There was a tendency amongst newly emerged *X. cheopis* for males to show a greater negative net taxis than females, regardless of *R. rattus* host type, and for 7-day-old unfed *X. cheopis* to exhibit a greater positive net taxis and therefore attraction toward female rather than male *R. rattus* hosts. No effects of *R. rattus* host age were obvious (table 1).

To investigate these trends further, sample sizes were increased and variances reduced by disregarding the age of the *R. rattus* host and combining the results (table 2). This was justified by the previous observations that these groups were not significantly different and that the variances between the combined groups were equal (non-significant: F-test result < critical value; $1.19 < 5.70_{7,6df}$; $2.19 < 5.12_{6,7df}$; $1.46 < 6.98_{6,5df}$; $1.15 < 5.99_{5,6df}$; $1.23 < 3.28_{14,12df}$; $1.17 < 3.61_{7,12df}$; $1.10 < 2.82_{10,19d}$; $2.19 < 6.33_{19,5df}$). A significant difference was found between the groups (KW = $66.4 > \chi^2_7 = 18.5$, $P = 0.01$). The only common factor in all cases was *X. cheopis* age; 7-day-old *X. cheopis* orientated toward the *R. rattus* host and newly emerged *X. cheopis* moved away

Table 1. Effects of *Xenopsylla cheopis* age and sex, and *Rattus rattus* host age and sex, on host-seeking behaviour of *X. cheopis* (net taxis*: mean ± SD).

Host	Newly emerged *X. cheopis*		7-day-old *X. cheopis*	
	Male	Female	Male	Female
Adult ♂	−5 ± 3	−2 ± 5	6 ± 5	3 ± 4
Adult ♀	−5 ± 5	−6 ± 5	7 ± 5	4 ± 5
Juvenile ♂	−4 ± 3	−3 ± 4	2 ± 6	6 ± 6
Juvenile ♀	−5 ± 4	−2 ± 5	7 ± 5	7 ± 4

*Net taxis = (no. fleas rat end) – (no. fleas blank end)
A negative (−) net taxis shows that the majority of *X. cheopis* entered the blank end away from the *R. rattus* host. A positive (+) net taxis shows that the majority of *X. cheopis* entered the rat end toward the *R. rattus* host.

https://doi.org/10.1079/BER2002184 Published online by Cambridge University Press

Table 2. Effects of *Xenopsylla cheopis* age and sex, and sex of *Rattus rattus* host (host ages pooled), on the host-seeking behaviour of *X. cheopis* (net taxis*: mean ± SD).

Host	Newly emerged *X. cheopis*		7-day-old *X. cheopis*	
	Male	Female	Male	Female
♂ *R. rattus*	−5 ± 3	−3 ± 4	4 ± 6	4 ± 5
♀ *R. rattus*	−5 ± 4	−4 ± 5	7 ± 5	5 ± 5

*Net taxis = (no. fleas rat end) – (no. fleas blank end)
A negative (−) net taxis shows that the majority of *X. cheopis* entered the blank end away from the *R. rattus* host. A positive (+) net taxis shows that the majority of *X. cheopis* entered the rat end toward the *R. rattus* host.

from the *R. rattus* host. The differences in net taxis due to the sex of *X. cheopis* or *R. rattus* host were not significant when *X. cheopis* age was not a factor. In essence, newly emerged *X. cheopis* were not attracted to the *R. rattus* hosts and 7-day-old *X. cheopis* were.

Flea fecundity

No trends were obvious in the ratio of eggs to larvae from week to week or among treatments, therefore, the numbers of eggs and larvae were combined to give weekly production for the *X. cheopis* population in each experimental cage. Throughout the experiment, weekly *X. cheopis* production of eggs and larvae was lower on the juvenile *R. rattus* hosts than on the adult *R. rattus* hosts (average no. of eggs and larvae per week: juvenile male hosts = 116; juvenile female hosts = 76; adult male hosts = 467; adult female hosts = 399, fig. 2). There was no significant difference in production due to *R. rattus* host sex, regardless of age but *X. cheopis* production on adult and juvenile *R. rattus* hosts was significantly different ($KW = 32.1 > \chi^2_3 = 11.3$, $P = 0.01$).

There was a reduction in the numbers of adult *X. cheopis* in the nests regardless of treatment. About 50–70% of the initial numbers of *X. cheopis* were found in the nest during the first week of the experiment. There was a steady decline in the number of adult *X. cheopis* thereafter. The rate of this decline was greater on juveniles than on adult *R. rattus* hosts (fig. 3). From the first week onwards a significant difference

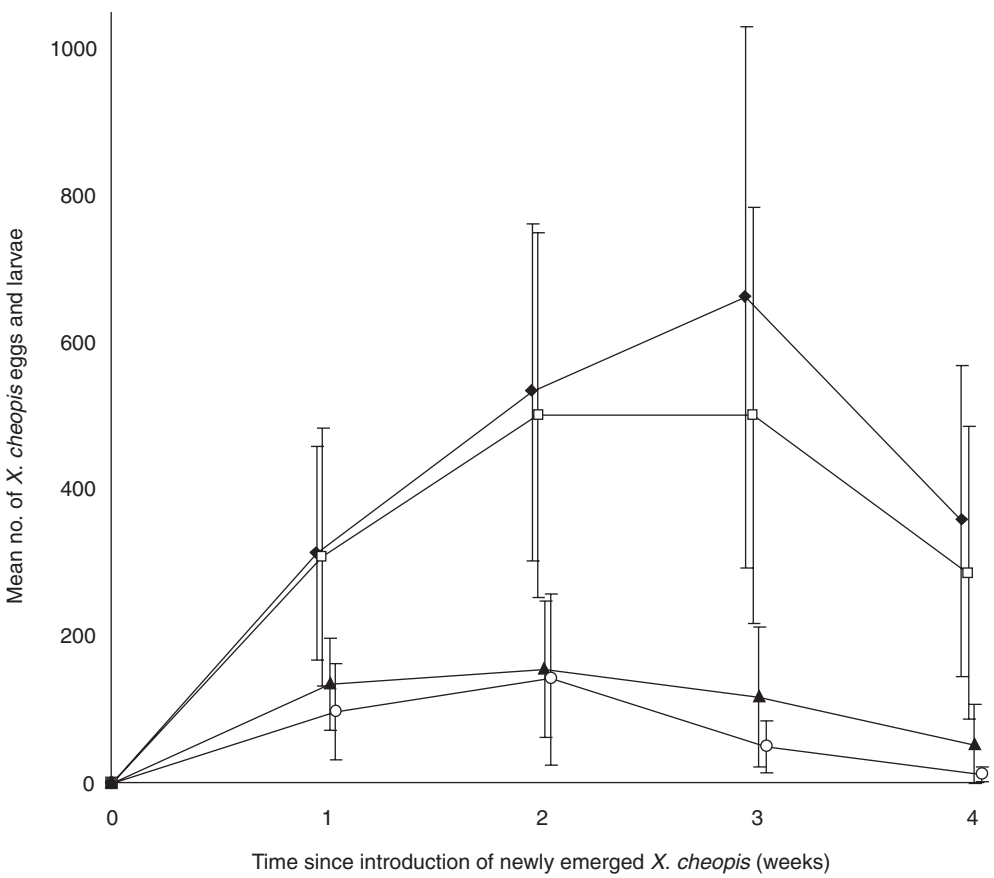

Fig. 2. Mean weekly production (± SD) of eggs and larvae of *Xenopsylla cheopis* on *Rattus rattus* hosts of different age and gender (◆, adult male; □, adult female; ▲, juvenile male; ○, juvenile female).

https://doi.org/10.1079/BER2002184 Published online by Cambridge University Press

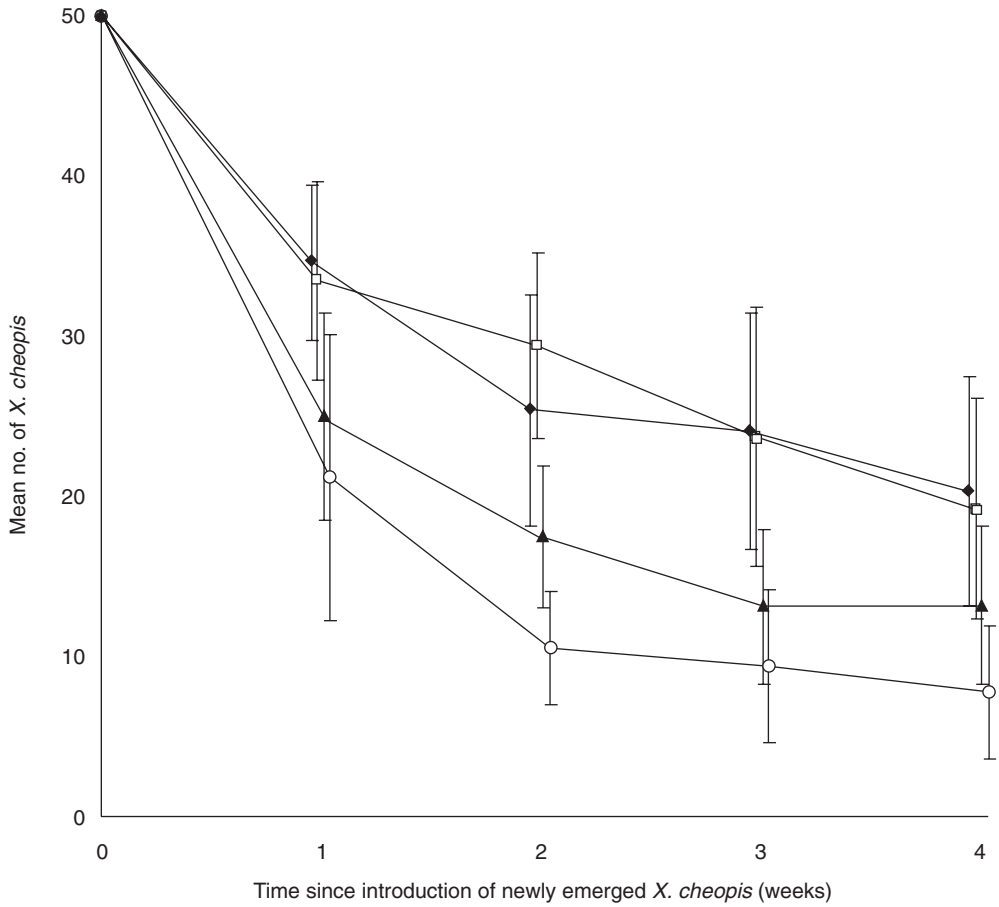

Fig. 3. Reduction of *Xenopsylla cheopis* populations (± SD) on *Rattus rattus* hosts of different age and gender (◆, adult male; □, adult female; ▲, juvenile male; ○, juvenile female).

was found in the *X. cheopis* nest population with more fleas associated with adult *R. rattus* nests (KW = 19.6, 28.5, 22.7, 16.9 > χ^2_3 = 11.3, P = 0.01, weeks 1–4 respectively).

Flea fecundity (numbers of eggs and larvae per female *X. cheopis*, counted in the nest, per week) on adult *R. rattus* hosts was significantly different from flea fecundity on juvenile *R. rattus* hosts in all weeks (KW = 19.8, 25.3, 27.9 and 27.2, respectively; > χ^2_3 = 11.3, P = 0.01). There were no significant differences in flea fecundity due to the sex of the *R. rattus* host (table 3).

At the end of the experiment all *R. rattus* hosts were examined for *X. cheopis* in order to obtain the true adult *X. cheopis* population, i.e. adult *X. cheopis* from the nest plus those from the body of the *R. rattus* host (for comparison with previous weeks, *X. cheopis* counted on the *R. rattus* host were not used in flea fecundity calculations). No significant differences were found in the proportion of the total *X. cheopis* population found in the fur of the different categories of *R. rattus* host (KW = 5.81 < χ^2_3 = 7.82, P = 0.05, table 4).

The ratio of male to female *X. cheopis* introduced at the start of the experiments was 1:4. At the end of the experiments, the sex ratio on the adult male *R. rattus* host was 1:5, on adult females 1:4, on juvenile males 1:6 and on juvenile females 1:2. There was no significant difference between the final percentage of introduced male *X. cheopis* remaining on the different host types, but there was a significant difference between the final percentage of introduced female *X. cheopis* found on adult male and on juvenile female *R. rattus* hosts (KW = 18.10 > χ^2_7 = 14.07, P = 0.05, table 4).

There was a significant difference in weight between adult and juvenile *R. rattus* hosts, (KW = 36.9 > χ^2_3 = 11.3, P = 0.01), but not between the sexes. There was a positive correlation between *R. rattus* host weight and skin surface area (r_s = 0.76, z = 5.76 > P = 0.00001, Spearman rank correlation coefficient corrected for tied ranks). Flea fecundity was also positively correlated with *R. rattus* host weight (r_s = 0.82, z = 6.07 > P = 0.00001). A weaker, but positive correlation, was also found between skin surface area and total flea fecundity (r_s = 0.60, z = 3.78 > P = 0.001).

Discussion

In obtaining *X. cheopis* of known age during the current study it was observed that female *X. cheopis* emerged from their cocoons earlier, i.e. had a shorter cocoon stage, than their male counterparts. A male to female sex ratio of 1:4 was recorded the first two days of *X. cheopis* emergence from cocoons and a 4:1 sex ratio from the third day onwards. The

https://doi.org/10.1079/BER2002184 Published online by Cambridge University Press

Table 3. Weekly production of eggs and larvae per female *Xenopsylla cheopis* surviving at the beginning of each week (calculated with the number of female *X. cheopis* introduced initially, or subsequently counted in the nest assuming the nest-flea : rat-flea ratio is similar on all host types).

Host	Mean weekly egg production per female *X. cheopis*				Total mean (± SD) egg production per female
	1	2	3	4	
Adult ♂	8	19	27	17	71.4 ± 26.8
Adult ♀	8	18	21	15	62.3 ± 25.1
Juvenile ♂	3	7	7	4	21.5 ± 11.1
Juvenile ♀	2	7	5	2	15.8 ± 8.6

Table 4. Mean number of male and female *Xenopsylla cheopis* in nests at the end of each week, and mean number (± SD) of male and female *X. cheopis* on the rat at the end of the experiment.

Host	*X. cheopis*	Mean no. of *X. cheopis* (± SD) counted week ending				
		1	2	3	4 (in nest)	4 (on rat)
Mature ♂	♂	6.5 ± 2.5	4.8 ± 2.9	4.4 ± 2.0	3.3 ± 2.4	0.9 ± 1.1
	♀	28.1 ± 4.5	20.6 ± 6.0	19.6 ± 6.4	17.1 ± 6.3	3.2 ± 4.9
	♂ : ♀ ratio	1:4	1:5	1:5	1:5	1:4
Mature ♀	♂	6.8 ± 2.3	6.2 ± 2.6	4.8 ± 2.2	3.6 ± 2.2	0.7 ± 1.2
	♀	26.7 ± 5.8	23.2 ± 5.3	19 ± 7.6	15.6 ± 5.9	4.1 ± 5.1
	♂ : ♀ ratio	1:4	1:4	1:4	1:4	1:6
Juvenile ♂	♂	3.9 ± 2.7	2.2 ± 1.5	2 ± 1.8	2 ± 1.5	0.1 ± 0.3
	♀	21.1 ± 4.8	15.2 ± 4.2	11.2 ± 4.8	11.2 ± 3.9	1.1 ± 1.4
	♂ : ♀ ratio	1:5	1:6	1:5	1:8	1:11
Juvenile ♀	♂	3.6 ± 2.7	2.6 ± 1.5	2.6 ± 1.5	2 ± 1.2	0.2 ± 0.4
	♀	17.6 ± 8.4	8 ± 4.3	6.8 ± 4.0	5.8 ± 3.8	0.6 ± 0.5
	♂ : ♀ ratio	1:5	1:3	1:3	1:3	1:3

demonstration of protogyny by *X. cheopis* during the current research agrees with previous workers such as Hirst (1927) and can help in the collection of sexed *X. cheopis* when the use of anaesthesia is undesirable.

In field studies, differential flea burdens due to the sex of the host are often attributed to host behaviour rather than flea preference (Mohr, 1961; Mohr & Stumpf, 1962; Mohr & Adams, 1963; Haas, 1966). Haas (1966) for example counted higher infestations of cat fleas *Ctenocephalides felis* Bouche (Siphonaptera: Pulicidae) on male mongooses *Herpestes auropunctatus* Hodgson (Carnivora: Viverridae) than on female mongooses in the field, but in laboratory experiments both sexes were equally suitable as hosts. Haas (1966) therefore concluded that the male mongoose's larger home range and size in comparison to the female resulted in greater exposure to, and retention of, *C. felis*. Similar experimental results were found in the current study where the sex of the *R. rattus* host did not significantly affect *X. cheopis* host-seeking behaviour.

Other studies have found flea infestations to correlate with host age and again quote behavioural differences of the host as an explanation (see for example Hirst, 1927; Stark & Miles, 1962; Cowx, 1967; Lundqvist & Brinck-Lindroth, 1990). Stark and Miles (1962), for example, collected greater numbers of *Hystrichopsylla linsdalei* Holland (Siphonaptera: Hystrichopsyllidae) and *Atyphloceras multidentatus* Fox (Siphonaptera: Hystrichopsyllidae) on 'young' wild caught meadow mice *Microtus californicus* Peale (Rodentia: Muridae) than on older meadow mice hosts. This, Stark & Miles (1962) suggested, was due to the younger meadow mice spending more time in the nest than the older meadow mice hosts and pointed out that *H. linsdalei* and *A. multidentatus* were 'nest fleas' and also spent more time in the nest than on the host. No previous studies have tested these assumptions directly however. The present experiments excluded sex or age related behavioural differences such as those due to differences in territory size or habitat preferences. The results of this study show that, with odour as the main stimulant, *X. cheopis* do not discriminate between *R. rattus* hosts of different age or sex. The host-seeking experiments therefore lend support to the theory that age and sex related differences in flea burden of wild caught hosts is a reflection of differences in host behaviour rather than flea host-seeking behaviour.

Host-seeking behaviour of *X. cheopis* was not influenced by the flea's sex. Newly emerged *X. cheopis* of both sexes were not influenced by the *R. rattus* hosts presence and at seven days old both *X. cheopis* sexes demonstrated similar levels of attraction toward the *R. rattus* host. These results do not support the findings of Shulov & Naor (1964) who found that at eight days of age more female than male *X. cheopis* were attracted to the *R. norvegicus* var. *albino* host. The male *X. cheopis* of Shulov & Naors (1964) experiments were found not to respond to, or were repelled by, the *R. norvegicus* var. *albino* hosts presence similar to that of newly emerged fleas in the current study. Hirst (1927) found male *X. cheopis* fed more often than female *X. cheopis* on *R. rattus* hosts and offered in explanation the males increased rate of dehydration as a result of their lower surface area to volume ratio. It may be expected that the male *X. cheopis* would begin host seeking earlier than females because they would become dehydrated sooner, but this was not tested in the

https://doi.org/10.1079/BER2002184 Published online by Cambridge University Press

present experiments. The *X. cheopis* in this study that demonstrated an attraction toward the *R. rattus* host had been starved for seven days and it is thought that both sexes would be stimulated to feed by this time.

In some flea species, such as *Ceratophyllus hirundinis* (Curtis), *Ceratophyllus farreni* Rothschild and *Ceratophyllus rusticus* Wagner (all Siphonaptera: Ceratophyllidae) for example, one sex (unusually the female in the aforementioned species) may be more active than the other (Marshall, 1981; Greenwood *et al*., 1991). This may have been expected to produce a difference in the current experiments with the more active sex responding to the *R. rattus* host to a greater degree. Direct experimental studies on the activity of *X. cheopis* found no difference in their activity levels attributable to sex however (Clark *et al*., 1993). This compares favourably with the results of the present study.

The post-emergence age of *X. cheopis* was important in the host-seeking experiments with the majority of newly emerged *X. cheopis*, in contrast to the 7-day-old *X. cheopis*, failing to respond positively toward the *R. rattus* host. Of the newly emerged *X. cheopis* that reacted to the presence of a rat in the test apparatus, a greater number (~20%) moved toward the blank end than toward the *R. rattus* host. A similar negative reaction with 1- to 3-day-old adult *X. cheopis* was reported in the experiments described by Shulov & Naor (1964) but no explanatory hypothesis was offered.

Others have also found a delay in the onset of host finding behaviours, or lower levels of response, in newly emerged adult fleas toward cues which otherwise trigger greater activity in older fleas (Hirst, 1927; Humphries, 1968; Osbrink & Rust, 1985; Dryden & Broce, 1993). Osbrink & Rust (1985), for example, noted that thermal and visual cues, which otherwise attracted 5- to 6-day-old starved *C. felis*, failed to elicit a response in newly emerged *C. felis*. They also noted that 4-day-old adult *C. felis* females placed on *Felis sylvestris catus* Linnaeus (Carnivora: Felidae) hosts would begin egg production after two days, whereas newly emerged adult females required 3–4 days on the host before egg production began. Osbrink & Rust (1985) therefore suggested that delayed host-seeking by *C. felis* would allow for maturation of the reproductive system off the host, thus lessening chances of mortality due to host grooming. *Xenopsylla cheopis* also emerge from their cocoons with immature sexual organs rendering fertilization, despite the copulations observed during this period, impossible (Rothschild *et al*., 1970). Male *X. cheopis* have been reported to require 18–24 h feeding on *R. norvegicus* var. *albino* before liberation of the sperm from the testis and female *X. cheopis* 34–54 h feeding before maturation of the oocytes (Rothschild *et al*., 1970). The delayed host-seeking response observed with the newly emerged *X. cheopis* during the present host-seeking experiments may therefore be an adaptation, similarly to that suggested for *C. felis* by Osbrink & Rust (1985), to allow for safe maturation of the sexual organs off the grooming host.

The reaction of *X. cheopis* to the *R. rattus* host varied greatly within all experimental treatments and although a significant movement toward the host may have been observed, this was not the case for all *X. cheopis* tested. The lack of an immediate response toward a host, and therefore a blood meal, is not uncommon (Hirst, 1927; Benton *et al*., 1959; Shulov & Naor, 1964; Benton & Lee, 1965). Shulov & Naor (1964), for example, also reported a lack of movement, in the presence of host stimuli, in 50% of *X. cheopis* tested. The lack of reaction of these fleas is unclear but would be worthy of further study. Generally, however, the variability in reaction to the stimulus of host odour during the present host-seeking experiments indicates that the locomotory movement of *X. cheopis* cannot be described in terms of a directional 'taxis'. Instead, the pattern of dispersal indicates a form of 'kinesis' in which the activity of *X. cheopis* is dependent upon the 'strength' of the stimulus, but the stimulus does not control the direction of the movement.

A number of authors have commented on the effects of host grooming on flea infestations (see Buxton, 1936; Cotton, 1970; Prasad, 1987; Wade & Georgi, 1988). Prasad (1987) noted that grooming efficiency in *M. musculus* var. *albino* increased with age leading to lower *X. cheopis* survival on adult hosts. In the present flea fecundity experiments, however, there was greater mortality of *X. cheopis* on juvenile than adult *R. rattus* hosts. This agrees with several authors (e.g. Mohr & Stumpf, 1962; Nilsson, 1981; Lehane, 1991) that have alternatively stated that grooming efficiency decreases with increase in host size because more areas become inaccessible to grooming on larger hosts. Further, it is suggested that the size of the host affects grooming which may interfere with egg production by reducing the number of blood meals taken. Prasad (1969) observed that *R. rattus* and *M. musculus* hosts often disturbed the *X. cheopis* on their bodies causing interruption in feeding activities in addition to *X. cheopis* mortality. Although the current flea fecundity experiments were not designed to test host grooming efficiency, the published observations and the present results may go some way to explaining the positive correlations between flea fecundity, rat weight and skin surface area found in this study.

Prasad (1987) observed higher *X. cheopis* mortality on adult and female *R. norvegicus* compared to young and male *R. norvegicus* hosts. The lack of a significant difference in flea fecundity due to the sex of the *R. rattus* host in this study suggests there is no difference in the grooming efficiency of male and female *R. rattus*. Female hosts of species that demonstrate considerable sexual size dimorphism may be expected to exhibit greater grooming efficiency than males due to their smaller size. The sizes of male and female *R. rattus*, as measured by their weight and skin surface area, were not found to be significantly different from each other in this study.

Several authors (Mohr & Adams, 1963; Bell & Clifford, 1964; Fraser & Waddell 1974) have noted differences in ectoparasite populations due to host sex and attributed these differences to host behaviour, such as the greater home range covered by males (see for example Haas, 1966; Cowx, 1967; Lodmell *et al*., 1970). Such factors were excluded in this experiment, therefore the lack of any difference in fecundity of *X. cheopis* due to the sex of the *R. rattus* host supports these assumptions. Other factors associated with host gender may have been expected to produce different *X. cheopis* fecundity rates, such as differences in the host's sex hormones (see for example Rothschild *et al*., 1970; Barnett, 1997).

It is noteworthy that *X. cheopis* mortality was the same in both sexes and on all host categories except juvenile females where the female fleas suffered greater mortality (table 4). It might be expected that female *X. cheopis* experienced greater mortality than males on all hosts, as female *X. cheopis* require frequent blood meals in order to mature their oocytes (Rothschild *et al*., 1970) which may have led them to spend

https://doi.org/10.1079/BER2002184 Published online by Cambridge University Press

more time on the host and increase their susceptibility to grooming. However, Hirst (1927) found male *X. cheopis* fed more often than females and he attributed this to their lower surface area to volume ratio, which increases their rate of dehydration in comparison to females. In the flea fecundity experiments, it would appear that both influences were equal and the vulnerability of both *X. cheopis* sexes was also the same. On juvenile female *R. rattus* hosts, female *X. cheopis* experienced greatest mortality. It would appear that female *X. cheopis* spent more time on these hosts than the *X. cheopis* females on other hosts. Why female fleas are more vulnerable to grooming on juvenile female rats as opposed to juvenile male rats is unknown.

This study supports the hypothesis that sexual differences in flea index result from sexual differences in host behaviour and not from discriminatory host-seeking behaviour by *X. cheopis*. Differences in flea index due to the age of the *R. rattus* host, however, can be affected by differences in *X. cheopis* fecundity, which may be mediated by host grooming behaviour. It is concluded that host age and sex are important factors to consider when comparing the variation in flea count of natural rodent populations.

Acknowledgements

The authors would like to thank all those whose support was offered during the experimental work at the Danish Pest Infestation Laboratory. Particular thanks go to Kristian Fordsman, Sarah Adams and Folmer Jensen for their technical support and also to Jørgen Jespersen for his help in other matters. Scott Mears was funded by the European Social Fund.

References

Barnett, A. (1997) Male hormones make for a lousy life. *New Scientist* March 15 (2073), 19.

Bell, J.F. & Clifford, C.M. (1964) Effects of limb disability on lousiness in mice. II. intersex grooming relationships. *Experimental Parasitology* **15**, 340–349.

Benton, A.H. & Lee, S.Y. (1965) Sensory reactions of siphonaptera in relation to host finding. *American Midland Naturalist* **74**, 119–125.

Benton, A.H., Cerwonka, R. & Hill, J. (1959) Observations on host perception in fleas. *Journal of Parasitology* **45**, 614.

Buxton, P.A. (1936) Quantitative studies on the biology of *Xenopsylla cheopis* (Siphonaptera). *Indian Journal of Medical Research* **26**, 505–530.

Buxton, P.A. (1948) Experiments with mice and fleas I. The baby mouse. *Parasitology* **39**, 119–124.

Clark, F., Greenwood, M. & Smith, S.J. (1993) Use of an insect activity monitor in behavioural studies of the flea, *Xenopsylla cheopis* (Rothschild). *Bulletin of the Society for Vector Ecology* **18**, 26–32.

Cole, L.C. (1945) The effect of temperature on the sex ratio of *Xenopsylla cheopis* recovered from live rats. *Public Health Report* **60**, 1337–1342.

Cotton, M.J. (1970) The life history of the hen flea, *Ceratophyllus gallinae* (Shrank) (Siphonaptera: Ceratophyllidae). *Entomologist* **103**, 45–48.

Cowx, N.C. (1967) Some aspects of the ecology and biology of some small mammal fleas from Yorkshire. *Journal of Biological Education* **1**, 75–78.

Dryden, M.W. & Broce, A.B. (1993) Development of a trap for collecting newly emerged *Ctenocephalides felis* (Siphonaptera: Pulicidae) in homes. *Journal of Medical Entomology* **30**, 901–906.

Edney, E.B. (1945) Laboratory studies on the bionomics of the rat fleas *Xenopsylla brasiliensis*, Baker, and *X. cheopis* Roths. I. Certain effects of light, temperature and humidity on development and adult longevity. *Bulletin of Entomological Research* **35**, 399–416.

Fowler, J. & Cohen, L. (1996) *Practical statistics for field biology*. John Wiley & Sons Ltd.

Fraser, D. & Waddell, M.S. (1974) The importance of social and self grooming for the control of ectoparasitic mites on normal and dystrotrophic laboratory mice. *Laboratory Practice* **23**, 58–59.

Greenwood, M.T., Clark, F. & Smith, J.S. (1991) Automatic recording of flea activity. *Medical and Veterinary Entomology* **5**, 93–100.

Haas, G.E. (1965) Comparative suitability of the four murine rodents of Hawaii as hosts for *Xenopsylla vexabilis* and *X. cheopis* (Siphonaptera). *Journal of Medical Entomology* **2**, 75–83.

Haas, G.E. (1966) Cat-flea–mongoose relationships in Hawaii. *Journal of Medical Entomology* **2**, 321–326.

Hirst, F.L. (1927) Researches on the parasitology of plague. *Ceylon Journal of Science, section D, Medical Science*, 155–455.

Humphries, D.A. (1968) The host-finding behaviour of the hen flea *Ceratophyllus gallinae* (Schrank) (Siphonaptera). *Parasitology* **58**, 403–414.

Lang, J.D. & Wills, W. (1991) Ecology of sylvatic plague in the San Jacinto Mountains of southern California. *Bulletin of the Society for Vector Ecology* **16**, 183–199.

Larsen, K. (1995) Laboratory rearing of the squirrel flea *Ceratophyllus sciurorum sciurorum* with notes on its biology. *Entomologia Experimentalis et Applicata* **76**, 241–245.

Lehane, M.J. (1991) *Biology of blood-sucking insects*. Harper Collins Academic (Pub).

Lodmell, D.L., Bell, J.F., Clifford, C.M., Moore, G.J. & Raymond, G. (1970) Effects of limb disability on lousiness in mice V. Hierarchy disturbance on mutual grooming and reproductive capacities. *Experimental Parasitology* **27**, 184–192.

Lundqvist, L. & Brinck-Lindroth, G. (1990) Patterns of co-existence: ectoparasites on small mammals in northern Fennoscandia. *Holarctic Ecology* **13**, 39–49.

Marshall, A.G. (1981) *The ecology of ectoparasitic insects*. 459 pp. London, Academic Press.

Mears, S. (1996) *The effects of rat host* (Rattus rattus) *age and gender upon the behaviour of the Oriental Rat Flea,* Xenopsylla cheopis. MSc thesis, University of Leicester.

Mohr, C.O. (1961) Relation of ectoparasite load to host size and standard range. *Journal of Parasitology* **47**, 978–984.

Mohr, C.O. & Adams, L. (1963) Relation of flea infestations to the spacing between cottontail rabbits. *Journal of Wildlife Management* **27**, 71–76.

Mohr, C.O. & Stumpf, W.A. (1962) Relation of ectoparasite load to host size and home area in small mammals and birds. pp. 174–183 in *Transactions of the 27th North American Wildlife and Natural Resources Conference* March 12–14, 1962, Denver, Wildlife Management Institute.

Nilsson, A. (1981) Spatial differentiation of ectoparasites on small mammals. *Holarctic Ecology* **4**, 184–190.

Osbrink, W.L.A. & Rust, M.K. (1985) Cat flea (Siphonaptera: Pulicidae): factors influencing host finding behaviour in the laboratory. *Annals of the Entomological Society of America* **78**, 29–34.

Pollitzer, R. (1954) *Plague*. WHO Monograph Series No. **22**, 698.

Prasad, R.S. (1969) Influence of host on fecundity of the Indian rat flea *X. cheopis*. *Journal of Medical Entomology* **6**, 443.

Prasad, R.S. (1987) Host dependency among haematophagous insects: a case study on flea–host association. *Proceedings of the Indian Academy of Science (Animal Sciences)* **96**, 349–360.

Rothschild, M., Ford, B. & Hughes, M. (1970) Maturation of the male rabbit flea (*Spilopsyllus cuniculi*) and the Oriental rat flea (*Xenopsylla cheopis*): some effects of mammalian hormones on development and impregnation. *Transactions of the Zoological Society of London*, **32**, 107–188.

Shulov, A. & Naor, D. (1964) Experiments on the olfactory responses and host specificity of the Oriental rat flea (*Xenopsylla cheopis*). *Parasitology* **54**, 225–231.

Siegel, S. & Castellan, N.J. (1988) *Nonparametric statistics for the behavioural sciences*. 2nd edn. McGraw-Hill Book Co.

Stark, H.E. & Miles, V.I. (1962) Ecological studies of wild rodent plague in the San Francisco Bay area of California. VI. The relative abundance of certain flea species and their host relationships on coexisting wild and domestic rodents. *American Journal of Tropical Medicine and Hygiene* **11**, 525–534.

Traub, R., Wisseman, A. & Farhang-Azad, C.L. (1978) The ecology of murine typhus – a critical review. *Tropical Disease Bulletin*, **75**, 237–317.

Wade, S.E. & Georgi, J.R. (1988) Survival and reproduction of artificially fed cat fleas, *Ctenocephalides felis* Bouche (Siphonaptera: Pulicidae). *Journal of Medical Entomology*, **25**, 186–190.

Webster, W.J. (1930) Observations on rat fleas and the transmission of plague. Part III. Flea bionomics. *Indian Journal of Medical Research* **18**, 391–405.

(Accepted 6 June 2002)