ABIOTIC FACTORS AFFECTING THE DEVELOPMENT OF FLEAS (SIPHONAPTERA) OF CALIFORNIA GROUND SQUIRRELS (RODENTIA: SCIURIDAE) IN SOUTHERN CALIFORNIA, USA

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Abstract - The California ground squirrel, *Spermophilus beecheyi* (Richardson), is a reservoir of bubonic plague throughout most of its range. Three species of fleas occur on these animals; *Oropsylla montana* (Baker), *Hoplopsyllus anomalus* (Baker), and the sticktight flea, *Echidnophaga gallinacea* (Westwood). Despite the importance of these fleas to plague transmission, there is only limited information regarding their biology, ecology, and how this might influence their control. Wild squirrels were deparasitized, tested for disease, and individually maintained in tub-style guinea pig cages. A novel nesting-box was designed to collect flea eggs without removing or handling the animal. Eggs were reared to adults on artificial media exposed to different temperature and relative humidity combinations similar to those reported from inside squirrel burrows. Survivorship from egg-to-adult varied for each flea species under different conditions. Results with *H. anomalus* and *E. gallinacea* suggest that these are more xeric-adapted species than *O. montana*. Average egg hatch of *H. anomalus* and *E. gallinacea* was substantially reduced at 31.5% RH and no larvae survived more than 48 hrs at less than 55% RH. In contrast, *O. montana* was highly susceptible to desiccation when reared at less than 75% RH, with none surviving at less than 65% RH. The developmental time of all species increased at lower temperatures and RH. Our initial results corroborate reported seasonal shifts in adult flea abundance on wild squirrels.
Key words - Plague, *Oropsylla montana*, *Hoplopsyllus anomalus*, *Echidnophaga gallinacea*

INTRODUCTION

Plague is a disease of rodents caused by the Gram-negative bacillus, *Yersinia pestis* ([Lehmann and Neumann] von Loghem), typically transmitted to humans and a variety of other mammals via the bite of infected fleas. The most important worldwide carriers of the disease are the roof rat, *Rattus rattus* L., and the Norway rat, *Rattus norvegicus* (Berkenhout), primarily due to their close associations with human dwellings (Pollitzer, 1954). Plague was first reported in North America from San Francisco in 1900 (Link, 1955). Since then, plague has been isolated from many species of rodents, lagomorphs, carnivores, and domestic pets in 15 states of the western USA (Barnes, 1982; Lang and Wills, 1991; Olsen, 1970).

In the United States, the primary reservoirs of plague are sylvatic rodents which pose a serious threat to public health in many of the parks and recreational areas of the west (Barnes, 1982; Lang and Wills, 1991). Confirmed cases of plague have averaged about 16 per year since 1974, with approximately an 18% morbidity rate despite effective antibiotics (Barnes, 1982). In 1994 and 1995, there were 21 confirmed cases of human plague reported in the United States, three of which were fatal (WHO, 1995). The principal public health concerns involve the possible introduction of plague into rural or urban commensal rat populations as well as the development of pneumonic forms of the disease in humans, both of which could lead to a plague epidemic (Barnes, 1982; Nelson *et al.*, 1986). State vector control authorities conduct extensive flea vector surveillance and control programs to reduce the risk of plague epidemics.

The California ground squirrel, *Spermophilus beecheyi* (Richardson), is the most important rodent species involved in plague epidemiology in California (Lang and Wills, 1991). There are three species of fleas associated with this rodent: *Oropsylla montana* (Baker), *Hoplopsyllus anomalus* (Baker), and the sticktight flea, *Echidnophaga gallinacea* (Westwood) (Ryckman, 1971b; Rutledge *et al.*, 1979;

Proceedings of the 3rd International Conference on Urban Pests.
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Lang and Wills, 1991). *O. montana* has been implicated as a competent vector transmitting plague to humans (Wheeler and Douglas, 1941; Hubbard, 1947). Although regarded as important sources of sylvatic plague among wild mammals, the plague risk to humans from the remaining two species is not understood (Stewart and Evans, 1941; Wheeler and Douglas, 1941).

There is little published information concerning the biology, ecology, and interaction of the three species of fleas associated with California ground squirrels. The limited information available deals primarily with the seasonal abundance of adult fleas on host animals, climatic factors which apparently affect adult population numbers, and the rates of adult flea transfer between hosts (Ryckman, 1971a,b,c; Stewart and Evans, 1941). Specific information concerning the growth, development and behavior of immatures is lacking. Such information could help explain and predict the fluctuations of adult populations which in turn could lead to more effective timing of treatment applications, thus improving current control procedures.

MATERIALS AND METHODS

Laboratory Animals. Fifteen squirrels were captured from wild populations on the UC Riverside campus because it was a low-risk area to collect squirrels and fleas because there was no history of sylvatic plague in Riverside, California (SOVE, 1996). The ground squirrels were live-trapped in cages and handled with heavy leather gloves. Captured squirrels were dusted with silica aerogel dust plus synergized pyrethrins (Drione®) to eliminate ectoparasites from the animals before being transferred to the University animal care facility. A second dusting approximately 10 days later prevented the re-establishment of anopluran lice from surviving nits in the hair.

The room in the vivarium had automatic air changes 15 times per hour and was maintained at 23 ± 2 ºC. Squirrels were housed in clear polycarbonate guinea pig cages (61 by 43 by 22 cm tall) with stainless steel wire lids. Each cage was provisioned with an ample supply of laboratory rodent chow, a water bottle, a two-piece nesting-box and an absorptive corn-cob bedding material. The nesting-boxes were constructed from 0.032 sheet aluminum (16.5 by 16.5 by 19 cm tall). The main upper section, available to the animal, was built with a hinged lid allowing access to the squirrel when necessary. The floor consisted of aluminum wire mesh (3.5 mm openings) riveted in place about 2 cm from the bottom. Entry and exit from the nesting-box was a single round opening (7.5 cm diameter) in one wall. The main section was designed to fit within a removable base pan which collected flea eggs as they dropped off the animals. Cages were sanitized and the bedding changed every week.

Fleas. California ground squirrels were used exclusively as hosts for all three flea species. After establishment of the 15 squirrels in the laboratory, additional wild squirrels were live-trapped and fleas were removed from the fur into a 38.75-liter, white plastic bucket using a fine-toothed flea comb. Collected fleas were aspirated from the bucket into vials and returned live to the laboratory where they were sexed and sorted according to species. Groups of 20 males and 20 females fleas of each species were placed on each of five individual squirrels to establish laboratory colonies.

Flea eggs and debris were brushed daily from the lower sections of the nesting-boxes through a series of sieves (ASTM Nos. 16, 35, and 60). The majority of eggs accumulated on the 60-mesh sieve screen from where they were collected. Eggs were then put into petri plates with artificial media (beef blood, dog chow, Centro-Nutrian 450B, and sand) and reared at 26.7 °C and 75% RH using the same technique developed for cat fleas (Metzger and Rust, 1996). Periodic additions of 20 males and 20 females adult fleas to each animal every 1-2 weeks to maintain adequate egg production.

Developmental studies. Three temperatures and six relative humidities (RH), encompassing most reported burrow conditions, were chosen for these experiments (Ryckman, 1971b; Stewart and Evans, 1941). Constant RH conditions of 35, 45, 55, 65, 75, and 85% were maintained in glass desiccators (11 liter) were prepared using saturated salt solutions (Winston and Bates, 1960). One desic-

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Table 1. Percent egg hatch of three flea species exposed to 18 different temperature and RH conditions.

Temperature	RH	*O. montana*	*H. anomalus*	*E. gallinacea*
26.7 °C	35%	—	35%	45%
	45%	6%	73%	68%
	55%	41%	94%	91%
	65%	88%	86%	98%
	75%	91%	78%	95%
	85%	89%	91%	99%
21.1 °C	35%	—	41%	59%
	45%	45%	84%	88%
	55%	82%	88%	93%
	65%	93%	88%	87%
	75%	90%	85%	83%
	85%	89%	88%	88%
15.5 °C	35%	3%	42%	49%
	45%	56%	72%	72%
	55%	88%	83%	93%
	65%	83%	80%	93%
	75%	92%	87%	94%
	85%	93%	84%	95%

Data based on 120 eggs.

Table 2. Percentage of adults of three flea species successfully developing and emerging from cocoons exposed to 18 different temperature and RH conditions..

Temperature	RH	*O. montana*	*H. anomalus*	*E. gallinacea*
26.7 °C	35%	—	—	—
	45%	—	—	—
	55%	—	13%	68%
	65%	—	73%	82%
	75%	78%	73%	89%
	85%	73%	81%	94%
21.1 °C	35%	—	—	—
	45%	—	—	—
	55%	—	73%	86%
	65%	12%	70%	81%
	75%	87%	80%	73%
	85%	86%	82%	83%
15.5 °C	35%	—	—	—
	45%	—	—	—
	55%	—	78%	87%
	65%	62%	77%	78%
	75%	84%	80%	90%
	85%	85%	70%	83%

Data based on 120 eggs.

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cator of each RH was placed in each of 3 rooms maintained at 12:12 (L:D) photoperiods and constant temperatures of 15.5, 21.1, and 26.7 °C. Developmental time and survivorship of individuals from egg-to-adult was determined using 96-well polystyrene, round-bottom ELISA plates (Cell Wells, disposable nonsterile assay plates [25855], Corning Glass, Corning, NY). ELISA plates were prepared for each flea species by placing one freshly harvested egg in each of the 60 wells with a fine camel's hair paint brush. ELISA plates loaded with eggs were then put inside one of the 18 desiccators and checked every 24 hrs for hatching. After hatching, each well was filled approximately 2/3 with larval rearing media. Plates were checked every 24 hrs to record cocoon formation and covered with clear tape during the cocoon stage to prevent emerging adult fleas from escaping (Metzger and Rust, 1996). Five ELISA plates were prepared for each temperature and humidity combination for each species of flea.

RESULTS AND DISCUSSION

S. beecheyi and its three flea species have been successfully colonized and maintained for over three years. We have developed new techniques for harvesting flea eggs from captive squirrels and for rearing immature fleas. The initial data analysis indicates that *H. anomalus* and *E. gallinacea* are more xeric-adapted species than *O. montana*. Egg hatch in these two species was only noticably affected at 35% RH, and no larvae survived more than 48 hrs at less than 55% RH. Development was completed at RH's as low as 55% (Tables 1 and 2). In contrast, eggs and larvae of *O. montana* were very susceptible to desiccation. Almost no eggs hatched at 35% RH and hatching was severely impaired at 45% (Table 1). Development from egg-to-adult generally required RH's of over 65%, although an appreciable number developed at 65% when maintained at 15.5 °C (Table 2).

Adult fleas of *S. beecheyi* are seasonally abundant on squirrels, but there is almost no information on other aspects of their biology and habits. Adult flea abundance data are mainly gathered by state and county plague surveillance crews who conduct annual adult flea surveys on wild rodents in the local mountain parks. In general, the presence of *O. montana* adults is associated with the high soil moisture and cool temperatures of southern California winter and spring, between the months of November and May (Stewart and Evans, 1941; Ryckman, 1971b; Lang and Wills, 1991). As the soil moisture decreases and the temperature increases, adults of *O. montana* are replaced by adults of *H. anomalus* and *E. gallinacea*.

The seasonality of these flea species can not be entirely explained by our data. The high humidity requirements of developing *O. montana* fleas may explain their prevalence during the winter and spring when soil moisture is high. Cool temperatures also seem to allow *O. montana* to develop under slightly drier conditions. However, no stages exhibited a diapause or quiescence during development which would explain their absence during the warm, dry months.

H. anomalus and *E. gallinacea* were able to tolerate much lower RH's during their development than *O. montana* which helps to explain their presence on animals during the hot, dry months. The absence of these two species during the winter and spring might be due to quiescent adults remaining inside cocoons. Individuals of both *H. anomalus* and *E. gallinacea* have been observed remaining within the cocoon for extended periods. In particular, several *H. anomalus* adults remained in the cocoon for over 1 year before emerging. Some individual *E. gallinacea* adults remained quescent inside cocoons at 15.5 °C for several months similarly to cat fleas, however, the quiescent, pre-emerged adults of *H. anomalus* did not appear to be affected by temperature or RH.

By rearing fleas in microwells of ELISA plates, we were able to monitor individual egg hatch, larval development, cocoon formation, and sex of emerging adults. Our initial results have begun to shed some light on some aspects of the biology of these fleas and may help explain seasonal shifts in adult flea abundance on wild rodents and lead to more effective timing and application of flea control techniques.

Proceedings of the 3rd International Conference on Urban Pests.
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ACKNOWLEDGEMENTS

We would like to thank Jody Hampton-Beesley and Elizabeth Cohen (University of California, Riverside) for their help with all aspects of our flea research. We also thank Wellmark International for partial support of the research.

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