

## NOTE

# Sex Differences in Susceptibility of House Crickets, *Acheta domesticus*, to Experimental Infection with *Serratia liquefaciens*

*Serratia liquefaciens* and *S. marcescens* are known to infect and cause mortality in a variety of insects including Orthopterans (P. A. D. Grimont and F. Grimont, in "Bergey's Manual of Systematic Bacteriology," (J. G. Holt and N. R. Krieg, Eds.), Vol. 1, pp. 477–484. Williams and Wilkins, Baltimore, MD, 1984; E. A. Steinhaus, *Hilgardia* 28, 351–377, 1959; G. E. Bucher and J. M. Stephens, *J. Insect Pathol.* 1, 356–373, 1959). Extracellular proteases and chitinase production are believed responsible for the observed toxicity (M. Kaska, *J. Invertebr. Pathol.* 27, 271, 1976; O. Lysenko, *J. Invertebr. Pathol.* 27, 385–386, 1976). In this note I report that last instar juvenile male and female house crickets, *Acheta domesticus* (Insecta: Orthoptera: Gryllidae) differ in their susceptibility to infection with *Serratia liquefaciens*.

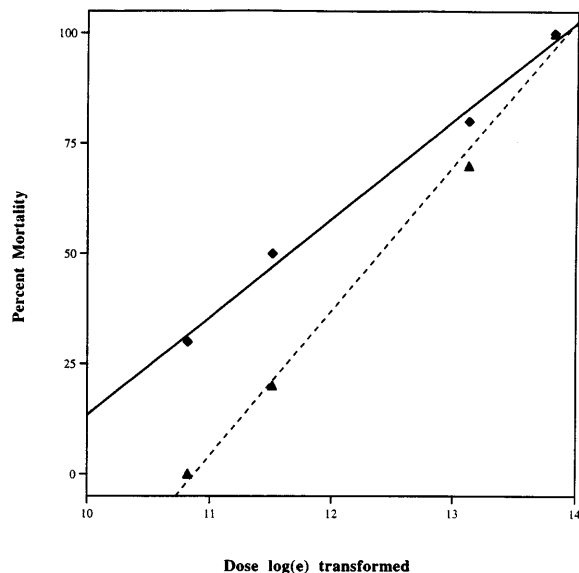
*Serratia liquefaciens* (American Type Culture Collection 27592) was cultured in nutrient broth (Difco 0003). One milliliter inoculant was introduced to 5 ml broth. The resulting mixture was incubated at 24°C for 24 h. Previous work (data not presented) has shown that after 24 h bacterial growth is exponential. Bacteria were quantified by cell counts in a hemocytometer. Penultimate instar juvenile crickets from laboratory stock (originally obtained from Bassett's Cricket Ranch, Visalia, CA) were cold anaesthetized and injected with 0.01 ml of bacteria/broth mixture. Injections were made with a Hamilton microsyringe directly into the hemocoel via the dorsal surface just posterior to the developing wing buds. The following dosages were used (numbers of bacteria per 0.01 ml):  $1 \times 10^6$ ,  $5 \times 10^5$ ,  $1 \times 10^5$ , and  $5 \times 10^4$ . Ten males and 10 females were infected with each dose, for a total of 40 crickets of each sex. Following injection, crickets were placed into individual containers and mortality was assessed after 24 h.

At the lower dosages, males appeared more susceptible than females (Fig. 1). Statistical analysis was performed using the CATMOD procedure (SAS, "SAS/STAT User's Guide, Version 6," Cary, North Carolina, 1989). CATMOD uses a maximum-likelihood approach to parameter estimation. A dummy variable, "sexid," was created to indicate sex: sexid, 0 for females and 1 for males. Both dose and sexid were significant predictors of survival. The best fit model to the data was

$20.166 - 1.6204*(\text{In dose}) - 1.4075*(\text{sexid})$ . [Dose: maximum likelihood estimate =  $-1.6204$ , standard error = 0.3263, Chi-square = 24.68,  $P < 0.0001$ ; Sexid, maximum likelihood estimate =  $-1.4075$ , standard error = 0.6897, Chi-square = 4.16,  $P < 0.0413$ ]. Solving for zero gave the LD<sub>50</sub> for males as  $1.066 \times 10^5$ ; for females, the LD<sub>50</sub> was  $2.539 \times 10^5$ . Thus the LD<sub>50</sub> data, although based on small samples, suggested a sex difference in susceptibility to infection.

Effect of sex and susceptibility was further tested by injecting a larger group of males and females from a later generation with  $1 \times 10^5$  bacteria in 0.01 ml broth as described above. Sterile broth was injected into control crickets. A table of random numbers was used to assign individuals to the treatment or the control group, with greater probability (70%) of entering the treatment group. Based on the LD<sub>50</sub> curves established above, approximately 47% of males and 18% of females were expected to be killed within 24 h. Crickets were injected on their 5th day after the penultimate molt. Results were as follows: 42.7% (58 of 136) of *S. liquefaciens*-injected males died, 7.1% (4 of 56) of control injected males died, 20.9% (14 of 67) of *S. liquefaciens*-injected females died, and 3.7% (1 of 27) of control-injected females died. The effect of the bacteria versus the control was clear (males: Likelihood Ratio Chi-square = 27.15,  $P < 0.001$ ; females, likelihood ratio Chi-square = 5.29,  $P < 0.025$ ). Comparing mortality in males and females injected with bacteria, males were more likely to die than were females (likelihood ratio Chi-square = 9.75,  $P < 0.002$ ).

The reason for differences between the sexes in susceptibility to infection is not clear. Although mortality among control males exceeded that among control females (7.1 vs 3.7%), this difference did not approach significance [likelihood ratio Chi-square = 0.41,  $P > 0.5$ ; Fisher's exact test,  $P = 1$ ], thus a general sex difference in mortality does not appear to account for the sex difference in susceptibility to bacterial infection. Male size slightly exceeded female size ( $X \pm \text{SD}$  mass at the penultimate molt in grams: males  $0.168 \pm 0.03$ , females  $0.159 \pm 0.03$ ,  $t$  test equal variances,  $t = 2.56$ ,  $df = 284$ ,  $P < 0.015$ ), thus, on a per gram basis, males received a slightly lower dose than females, yet had higher mortality.



**FIG. 1.** The relationship between dose and percentage of mortality by sex in *Acheta domesticus* injected with *Serratia liquefaciens*. Dose is  $\log(e)$  transformed. The solid line through diamonds represents males; the dashed line through triangles represents females.

It is likely that males are generally further from naturally selected optima for a variety of traits than are females (A. Zahavi, *J. Theor. Biol.* **53**, 205–214, 1975; R. Lande, *Evolution* **34**, 292–307, 1980). In vertebrates it is common for males to have higher mortality rates than females from a wide variety of factors, including disease (T. H. Clutton-Brock, S. D. Albon, and F. E. Guinness, *Nature* **313**, 131–133, 1985; D. E. L. Promislow, *Proc. R. Soc. Lond. B.* **247**, 203–210, 1992). This may be due to the negative effects of steroid sex hormones on the immune system (I. Folstad and A. J. Karter, *Am. Nat.* **139**, 603–622, 1992). Apparently, no such interaction has been described in invertebrates.

Nonetheless, results similar to those given here have been reported in tsetse flies, *Glossina* spp. (G. P. Kaaya and N. Darji, *Dev. Comp. Immunol.* **12**, 255–268, 1988; G. P. Kaaya, *Acta Trop.* **65**, 107–114, 1989; S. K. Mooloo, *Med. Vet. Entomol.* **7**, 369–372, 1993; Y. Nigam, I. Maudlin, S. Welburn, and N. A. Ratcliffe, *J. Invert. Pathol.* **69**, 279–281, 1997). Sex differences have also been reported for resistance to pesticides. Female larval *Locusta migratoria migratorioides* were less susceptible to the insecticide dieldrin than were males (F. A. Onyeocha and S. Fuzeau-Braesch, *Chronobiol. Int.* **8**, 103–109, 1991); adult male *Blattella germanica* were more susceptible to a variety of insecticides than were females (S. F. Abd-Elghafar, A. G. Appel, and T. P. Mack, *J. Econ. Entomol.* **83**, 2290–2294, 1990; G. Gecheva, *Wiad Parazytol.* **37**, 367–373, 1991; P. G. Koehler, C. A. Strong, R. S. Patterson, and S. M. Valles, *J. Econ. Entomol.* **86**, 785–792, 1993); male *Haematobia irritans* were more susceptible to the insecticide permethrin than females (P. T. McDonald and C. D. Schmidt, *J. Econ. Entomol.* **83**, 1715–1717, 1990). Despite these examples, the generality of sex-differences in susceptibility of invertebrates is unclear.

*Key Words:* *Acheta domesticus*; *Serratia liquefaciens*; susceptibility; LD50; sex; bioassay.

I am grateful to S. Adamo and O. Baca for their advice and comments. I also thank P. Albrecht-Gray for assistance with maintenance of the *Serratia* culture. This research was supported by an NSF Graduate Fellowship.

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Received January 6, 1997; accepted December 2, 1997

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