Sex-specific response of a mosquito to parasites and crowding

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Host–parasite interactions are significantly influenced by the sex of the host and the environment in which the host is found. Sex-specific responses to parasite infection, however, may change according to the host environment. I examine the combined effect of parasite infection and crowding on males and females of the mosquito *Aedes albopictus*. At a high larval density, infected males experienced a greater relative reduction in body size than did infected females, whereas the pattern was reversed at low density. This experiment demonstrates the importance of the environment on sex-specific responses to parasites and contributes to a growing body of work examining sources of variation in host–parasite interactions.

Keywords: *Aedes albopictus*; host–parasite interactions; density; sex-specific response

1. INTRODUCTION

Much current research on host–parasite evolutionary ecology examines the variation in the response of hosts to parasite infection. For example, the severity of parasite infection can be influenced by host food availability (Brown *et al.* 2000; Ferguson & Read 2002), rearing temperature (Blanford *et al.* 2003) and density (Wilson *et al.* 2002), as well as the sex of the host (Zuk & McKean 1996). Given that parasites have been implicated in a wide range of ecological and evolutionary phenomena, such as the evolution of sexual reproduction (Jaenike 1978; Hamilton 1980), the maintenance of sexually selected traits (Hamilton & Zuk 1982) and cyclical population dynamics (Scott & Dobson 1989), understanding how these sources of variation act and interact to influence host–parasite dynamics is of broad interest. The combined role of environmental variation and host sex on parasite infection severity is the focus of this study.

Sex-specific responses to larval competition have been documented in the mosquito *Aedes aegypti* (Bedhomme *et al.* 2003), and males and females of many invertebrate taxa have been shown to exhibit differential responses to parasitism (Sheridan *et al.* 2000). Sex-specific correlations between life-history traits and fitness have been proposed as one explanation for the differential responses to both intraspecific competition and parasites (Rolff 2002; Bedhomme *et al.* 2003). To explore whether sex-specific responses to parasite infection are consistent across multiple environments, I reared males and females of the mosquito *Aedes albopictus* (Diptera: Culicidae) with and without the protozoan parasite *Ascogregarina taiwanensis*

(Apicomplexa: Lecudinidae) and in crowded and uncrowded conditions. Parasite infection and increased density should have less of an effect on female body size than on male body size because a decrease in body size is more detrimental to female fitness than it is to male fitness (Christophers 1960; Benjamin & Bradshaw 1984).

2. MATERIAL AND METHODS

Aedes albopictus is a container-breeding mosquito with four larval instars and one pupal stage (Hawley 1988). *Ascogregarina taiwanensis* is a mildly virulent protozoan parasite specific to *Ae. albopictus*. Mosquito larvae become infected upon ingesting free-floating parasite oocysts (eggs). Parasites grow and develop in the larval mosquito gut. Adult mosquitoes release oocysts into breeding water during metamorphosis (Chen 1999). When larvae are food-limited, parasite infection increases larval development time and decreases size at maturity (Comiskey *et al.* 1999a), mainly via damage to gut epithelial cells and Malpighian tubules (Comiskey *et al.* 1999b). Infection by *A. taiwanensis* also increases the probability of transmission of the dog heartworm parasite, *Dirofilaria immitis* (Filarioidea: Onchocercidae) by *Ae. albopictus* (Comiskey *et al.* 1999b).

Mosquito larvae were reared in 150 ml plastic cups at high density (40 larvae per 100 ml of water) or at low density (17 larvae per 100 ml of water) and with or without *A. taiwanensis*. These densities fall within the range of larval densities found in nature (Willis & Nasci 1994). Each of the four treatments included six cups of larvae. A parasite oocyst solution was made using methods similar to Beier & Craig (1985); 75 infected adult *Ae. albopictus* were homogenized and diluted with 100 ml of tap water. Larvae were infected with an oocyst solution equivalent to ca. 0.3 ml of oocyst solution per larva. To verify the infectivity of the parasite dose, a sample of fourth-instar larvae was dissected.

Larvae were fed 0.2 mg of finely ground Nutriphase (PETsMART Inc., Arizona) rabbit chow every 3 days. This amount was *ad libitum* for the low-density larvae, but not for the high-density larvae. The experiment was conducted at $25 \pm 1^\circ\text{C}$ and $65 \pm 5\%$ relative humidity. Cups were haphazardly repositioned daily to minimize the effects caused by location.

The effect of density and parasites on the wing length of each sex was analysed using ANOVA. Density (high, low), parasite (present, absent) and sex (male, female) were included as fixed factors. Adult wing length is a reliable predictor of female fecundity (Armbruster & Hutchinson 2002) and this trait is one of the most commonly used indicators of mosquito fitness (e.g. Comiskey *et al.* 1999b; Koella & Offenberg 1999). ANCOVA were conducted to examine treatment effects on development time (number of days for all larvae per cup to metamorphose) and larval survival (number of surviving larvae per cup divided by the initial larval density). For both analyses, parasites and density were included as fixed factors and cup sex ratio was included as a covariate. Variances of treatment groups for each dependent variable were compared using the $F_{\max}$ -test (Hartley 1950) and were found to be homoscedastic. All statistical analyses were conducted using SPSS (v. 10.0.7; Norusis 2000).

3. RESULTS

Thirty-two larvae were dissected to confirm the infectivity of the parasite treatments. Parasite prevalence was 94% and mean parasite number per larva was 47.6 (s.d. = 50.7, range of 0–200). These values fall within the range of what is found in nature (Garcia *et al.* 1994). An ANOVA with cup nested within treatments revealed no significant cup effects on adult wing length ($F_{20,170} = 1.09$, $p = 0.362$). Consequently, each mosquito was treated as a unit of replication (Sokal & Rohlf 1995).

As commonly seen in mosquitoes, females metamorphosed with longer wings than did males (female mean $\pm$ s.d. = 2.9 ± 0.19 mm, $n = 105$, male mean $\pm$ s.d. = 2.3 ± 0.17 mm, $n = 95$). The three-way interaction among sex, density and parasites explained a significant amount of the variation in male and female wing length ($F_{1,191} = 3.912$, $p = 0.049$; figure 1). More specifically, for females at low density, parasitized individuals were 10.6% smaller than unparasitized individuals, but at high density parasitized females were only 1.4% smaller. The interaction

Figure 1. Response of *Aedes albopictus* wing length to parasite infection and density. Squares, females; circles, males; filled symbols, parasitized; open symbols, not parasitized; error bars = ± 1 s.e. Sample size (female, male): low density/not infected = 11,11; low density/infected = 21,25; high density/not infected = 39,29; high density/infected = 34,29. The dashed line separates the male and female data.

Figure 2. Effect of parasites and density on mosquito development time. Circles, low density; triangles, high density; filled symbols, parasitized; open symbols, not parasitized.

between parasites and density was not apparent in males: parasitized males were 5.5% smaller than unparasitized males in both density treatments. Other significant main effects and interactions in this analysis included: density ($F_{1,191} = 9.09$, $p = 0.003$), parasite ($F_{1,191} = 18.21$, $p < 0.001$), sex ($F_{1,191} = 349.3$, $p < 0.001$) and the density-by-parasite interaction ($F_{1,191} = 4.27$, $p = 0.040$).

Larval rearing cups with lower male-to-female ratios took longer to develop than did those that were more male-biased ($F_{1,24} = 4.49$, $p = 0.048$; figure 2). Cup development time was also significantly prolonged by increased density (high-density mean = 35.7 days, low-density mean = 26.3 days; $F_{1,24} = 8.77$, $p = 0.008$; figure 2), but

neither the effect of parasites ($F_{1,24} = 0.36$, $p = 0.553$) nor the parasite-by-density interaction ($F_{1,24} = 0.108$, $p = 0.747$) was statistically significant. Mean larval survival was 29%, with a range of 22–40%. Survival was not influenced by parasites ($F_{1,24} = 2.99$, $p = 0.100$), density ($F_{1,24} = 1.53$, $p = 0.231$), or the parasite-by-density interaction ($F_{1,24} = 3.82$, $p = 0.07$).

4. DISCUSSION

This study demonstrates that the host environment can alter the response of each sex to parasite infection. Infection decreased male mosquito wing length by a constant amount in both density treatments, whereas infection in females had a more severe effect at low density than at high density. This experiment builds on the recent evidence of environmentally dependent parasite virulence, and together these studies highlight the importance of the environment in determining the outcome of host-parasite interactions. For example, differences in the environment in which host-parasite experiments are conducted may be one reason why male invertebrates do not appear to consistently suffer more from parasite infection than do females (Sheridan *et al.* 2000) although theory predicts that they should (Rolff 2002).

A sex-specific response to competition was also reported in *Ae. aegypti* (Bedhomme *et al.* 2003). When exposed to competitors, male *Ae. aegypti* suffered a greater reduction in body size than did females. The authors reasoned that the differential response resulted from a cost of competition that was expressed through phenotypic modifications of traits least important for the fitness of each sex. In other words, in a stressful environment, attaining a larger body size is less important for male fitness than it is for female fitness. This hypothesis can be applied to the response of parasitized *Ae. albopictus* to high larval density. The difference in wing length between infected and uninfected males was greater than that in females. However, at low density, this pattern was reversed, suggesting that other factors may play a role in shaping sex-specific life-history responses when multiple stresses are involved. Further experiments are necessary to expose the underlying mechanisms of these responses.

High-density environments prolonged larval development time in both sexes but parasite infection did not affect this trait. This latter result is curious because previous studies have shown that infection by *A. taiwanensis* increases *Ae. albopictus* development time (Comiskey *et al.* 1999b; M. Tseng, unpublished data). It is possible that the effect of density overshadowed that of parasite infection. Mosquito development time has been shown to be very sensitive to rearing density (Agnew *et al.* 2002; Bedhomme *et al.* 2003).

In summary, this experiment demonstrates the importance of the role of the environment on sex-specific response to parasites, and it contributes to the growing body of work showing the importance of environmental variation on the effect of parasites on their hosts. Assessing host-parasite interactions across a variety of environments can greatly increase our understanding of the factors influencing variation in host-parasite interactions in nature.

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- Agnew, P., Hide, M., Sidobre, C. & Michalakakis, Y. 2002 A minimalist approach to the effects of density dependent competition on insect life-history traits. *Ecol. Entomol.* **27**, 396–402. (DOI 10.1046/j.1365-2311.2002.00430.x.)
- Armbruster, P. & Hutchinson, R. A. 2002 Pupal mass and wing length as indicators of fecundity in *Aedes albopictus* and *Aedes geniculatus* (Diptera: Culicidae). *J. Med. Entomol.* **39**, 699–704.
- Bedhomme, S., Agnew, P., Sidobre, C. & Michalakakis, Y. 2003 Sex-specific reaction norms to intraspecific larval competition in the mosquito *Aedes aegypti*. *J. Evol. Biol.* **16**, 721–730. (DOI 10.1046/j.1420-9101.2003.00576.x.)
- Beier, J. C. & Craig Jr, G. B. 1985 Gregarine parasites of mosquitoes. In *Integrated mosquito control methodologies*, vol. 2 (ed. M. Laird), pp. 167–184. London: Academic Press.
- Benjamin, S. N. & Bradshaw, W. E. 1984 Effects of flight activity and body size on male reproductive success in the pitcher-plant mosquito, *Wyeomyia smithii* (Diptera: Culicidae). *Ann. Entomol. Soc. Am.* **87**, 331–336.
- Blanford, S., Thomas, M. B., Pugh, C. & Pell, J. K. 2003 Temperature checks the Red Queen? Resistance and virulence in a fluctuating environment *Ecol. Lett.* **6**, 2–5. (DOI 10.1046/j.1461-0248.2003.00387.x.)
- Brown, M. J. F., Loosli, R. & Schmid-Hempel, P. 2000 Condition-dependent expression of virulence in a trypanosome infecting bumblebees. *Oikos* **91**, 421–427. (DOI 10.1034/j.1600-0706.2000.910302.x.)
- Chen, W.-J. 1999 The life cycle of *Ascogregarina taiwanensis* (Apicomplexa: Lecudinidae). *Parasitol. Today* **15**, 153–156.
- Christophers, S. R. 1960 *Aedes aegypti* (L.). *The yellow fever mosquito: its life history, bionomics and structure*. Cambridge University Press.
- Comiskey, N. M., Lowrie Jr, R. C. & Wesson, D. M. 1999a Role of habitat components on the dynamics of *Aedes albopictus* (Diptera: Culicidae) from New Orleans. *J. Med. Entomol.* **36**, 313–320.
- Comiskey, N. M., Lowrie Jr, R. C. & Wesson, D. M. 1999b Effect of nutrient levels and *Ascogregarina taiwanensis* (Apicomplexa: Lecudinidae) infections on the vector competence of *Aedes albopictus* (Diptera: Culicidae) for *Dirofilaria immitis* (Filarioidea: Onchocercidae). *J. Med. Entomol.* **36**, 55–61.
- Ferguson, H. M. & Read, A. F. 2002 Genetic and environmental determinants of malaria parasite virulence in mosquitoes. *Proc. R. Soc. Lond. B* **269**, 1217–1224. (DOI 10.1098/rspb.2002.2023.)
- Garcia, J. J., Fukuda, T. & Becnel, J. J. 1994 Seasonality, prevalence and pathogenicity of the gregarine *Ascogregarina taiwanensis* (Apicomplexa: Lecudinidae) in mosquitoes from Florida. *J. Am. Mosq. Control Assoc.* **10**, 413–418.
- Hamilton, W. D. 1980 Sex versus nonsex versus parasite. *Oikos* **35**, 282–290.
- Hamilton, W. D. & Zuk, M. 1982 Heritable true fitness and bright birds: a role for parasites? *Science* **218**, 384–387.
- Hartley, H. O. 1950 The maximum *F*-ratio as a short cut test for heterogeneity of variances. *Biometrika* **37**, 308–312.
- Hawley, J. A. 1988 The biology of *Aedes albopictus*. *J. Am. Mosq. Control Assoc.* **S1**, 1–40.
- Jaenike, J. 1978 A hypothesis to account for the maintenance of sex within populations. *Evol. Theor.* **3**, 191–194.
- Koella, J. C. & Offenberg, J. 1999 Food availability and parasite infection influence the correlated response of life history traits to selection for age at pupation in the mosquito *Aedes aegypti*. *J. Evol. Biol.* **12**, 760–769. (DOI 10.1046/j.1420-9101.1999.00071.x.)
- Norusis, M. J. 2000 *SPSS 10.0 guide to data analysis*. Upper Saddle River, NJ: Prentice-Hall.
- Rolff, J. 2002 Bateman's principle and immunity. *Proc. R. Soc. Lond. B* **269**, 867–872. (DOI 10.1098/rspb.2002.1959.)
- Scott, M. E. & Dobson, A. 1989 The role of parasites in regulating host abundance. *Parasitol. Today* **5**, 176–183.
- Sheridan, L. A. D., Poulin, R., Ward, D. F. & Zuk, M. 2000 Sex differences in parasite infections among arthropod hosts: is there a male bias? *Oikos* **88**, 327–334. (DOI 10.1034/j.1600-0706.2000.880211.x.)
- Sokal, R. R. & Rohlf, J. 1995 *Biometry*, 3rd edn. New York: Freeman.
- Willis, F. S. & Nasci, R. S. 1994 *Aedes albopictus* (Diptera: Culicidae) population density and structure in southwest Louisiana. *J. Med. Entomol.* **31**, 594–599.
- Wilson, K., Thomas, M. B., Blanford, S., Doggett, M., Simpson, S. J. & Moore, S. L. 2002 Coping with crowds: density dependent disease resistance in desert locusts. *Proc. Natl Acad. Sci. USA* **99**, 5471–5475. (DOI 10.1073/pnas.082461999.)
- Zuk, M. & McKean, K. A. 1996 Sex differences in parasite infections: patterns and processes. *Int. J. Parasitol.* **26**, 1009–1024.