

HOST SEX PREFERENCES AND TRANSMISSION SUCCESS BY THE WATER MITE *UNIONICOLA FOILI* (ACARI: UNIONICOLIDAE) PARASITIC ON THE MIDGE *CHIRONOMUS TENTANS* (DIPTERA: CHIRONOMIDAE)

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ABSTRACT: This study examined whether ecoparasitic larval *Unionicola foili* exhibited a sex bias when infecting laboratory populations of the host insect *Chironomus tentans* and whether an association with male or female midges increased the likelihood of larval mites returning to the aquatic habitat. When laboratory populations of *C. tentans* were exposed to larval *U. foili*, there was a higher prevalence of mites among female hosts at emergence (17 of 30 males vs. 25 of 30 females infected by mites). However, there was no significant difference in the distribution or abundance of larvae among infected male (mean = 2.3 larvae per host) and female (mean = 2.6 larvae per host) midges. Larval mites parasitizing both male and female chironomids were more likely to return to water than could be expected by chance. Mite larvae infesting female *C. tentans* were more likely to return to water when female hosts deposited egg masses in water, suggesting that oviposition plays an important role in cueing larvae parasitizing female midges to detach. The mechanism responsible for increasing the likelihood that mites parasitizing male hosts return to water remains unclear. Future studies will address the possibility of parasite-mediated changes in host behavior.

Sex differences in parasitism have been well documented in the parasitological literature. Males of many species of vertebrates are reportedly more heavily infected with parasites than females (Bundy, 1988; Poulin, 1996; Schalk and Forbes, 1997), and these biases often have been attributed to immunosuppression caused by hormones used for the development of male sexual traits (Alexander and Stimson, 1988; Zuk, 1990). An increase in the mean intensity of infection among male hosts has also been attributed to sexual selection because competition for mates can be stressful to males and may as a consequence increase their susceptibility to parasitic infections (Zuk, 1990). In addition, there is some evidence to suggest that ecological differences between males and females may influence sex-biased parasitism (see Zuk and McKean, 1996 for review). For example, male spadefoot toads are thought to be more heavily infected with the monogenean parasite *Pseudodiplorchis americanus* than female toads because they spend most of the breeding season immersed in water, where infective stages of the parasite are transmitted. Female toads visit ponds briefly to lay their eggs but do not return (Tinsley, 1989).

Sex differences in parasite infections have been addressed among invertebrate hosts also. Sheridan et al. (2000) performed a metaanalysis of published data for parasitic infections among a variety of arthropod species and found no differences in the prevalence or mean intensity of infections between the sexes. Similarity in the levels of parasitism among male and female arthropod hosts may be explained by the absence of the immunosuppressant effects of steroid sex hormones that generally make males of many species of vertebrates more prone to parasite infections than females (Alexander and Stimson, 1988; Sheridan et al., 2000).

Life history studies of a number of ectoparasitic water mites have reported a higher prevalence and abundance of mite larvae among female insect hosts (Davies, 1959; Mitchell, 1967; LeSage and Harrison, 1980). Lanciani (1988) reported a greater abundance of larval *Arrenurus novimarsallae* on female *Anopheles crucians* and showed that these mites prefer to parasitize female hosts in equal-exposure tests. Most studies reporting a higher mite abundance on female insects contend that

this pattern of distribution may increase the probability of their return to water given that female insects are more likely to return to water to engage in oviposition. Recently, McLachlan (1999) reported that larval *Unionicola ypsilophora* attached to male midges transfer to females during copulation and suggested that this behavior may increase the probability of larval mites returning to the aquatic environment.

With the exception of a study by Lanciani (1988), there is no additional experimental evidence to indicate that larval water mites prefer female hosts and there are no data to support the contention that a preference for females will increase the probability of mites returning to water. Interestingly, a number of additional studies examining the distribution and abundance of larval mites among insects have not reported a strong and consistent sex bias (Efford, 1963; Booth and Learner, 1978; Kouwets and Davids, 1984) or have argued that sex differences are dependent on the host's behavior (Smith, 1999) rather than active choice by the larvae. The relatively high prevalence and abundance of larval mites among male hosts reported by these and other studies raises an important question regarding the success with which larvae infesting male insects return to water.

Adults of the water mite *Unionicola foili* are common symbionts of the freshwater mussel *Utterbackia imbecillis*. Females deposit eggs in the gills of their molluscan hosts, with larvae emerging from the gills throughout spring and summer (Dimock, 1985). Larvae subsequently leave the mussels and establish a parasitic association with chironomids. Larval mites locate pupal chironomids, penetrate the cuticle of the host with their chelicerae, and firmly anchor themselves to the exoskeleton of the developing adult. When the insect molts, mite larvae are dragged through the pupal exuvium and begin to engorge on host hemolymph (Hevers, 1980). Larvae that are attached to a host insect after it becomes aerial must return to the aquatic environment to complete their life cycle. After a parasitic association with an insect, larvae reinvade a host mussel, undergo developmental transformation, and eventually become sexually mature adults. The present study addresses 2 questions related to the life cycle of *U. foili*. Do larval mites exhibit a sex bias when colonizing chironomid hosts? Are larvae infesting female midges more likely to return to the aquatic environment?

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TABLE I. Chi-square contingency table comparing the number of male and female *Chironomus tentans* infected with larval *Unionicola foili* at emergence.

Sex of host	Condition of host		Total	χ^2
	Infected	Uninfected		
Female	25	5	30	3.89*
Male	17	13	30	
Total	42	18	60	

* $P < 0.05$.

MATERIALS AND METHODS

Study animals

Unionicola foili was obtained from a population of *U. imbecillis* that was collected from Butler's Pond, a 4-ha farm pond located in Perry Co., Indiana (37°56'N, 86°43'W). Mussels were collected from June to July 2001, during which time mite larvae were emerging from their hosts. Larvae were obtained by teasing hosts' gills apart in petri dishes containing artificial pond water (APW) (Dietz and Alvarado, 1970). Larvae were washed several times in APW and were kept in this medium for no longer than 24 hr before use.

A laboratory-cultured population of *Chironomus tentans* was used as a host for larval *U. foili*. Laboratory cultures of *C. tentans* were established and maintained according to the procedures outlined by Batac-Catalan and White (1982). Chironomids were held in 38-L aquaria containing approximately 2 cm of artificial substratum (Batac-Catalan and White, 1982) and 10 L of APW. Each aquarium was supplied with an airstone and was covered with mosquito netting (1-mm mesh) to ensure that adult midges were confined when they emerged. Approximately 50 larval mites were pipetted into the aquarium weekly. When adult midges emerged, they were aspirated from the enclosure and were placed individually in 50-ml vials fitted with a small piece of mosquito netting. Each adult midge was then examined under a dissecting microscope for the presence of mite larvae. Chironomids carrying larval mites were sexed and the number of mites per host was recorded.

Host sex preferences

Two procedures were used to determine whether larval *U. foili* preferred male or female chironomids. First, 30 newly emerged male and female midges were removed from an aquarium, and the distribution of larval mites among these hosts was determined. Second, chironomids were collected from an aquarium over a 1-wk time interval, and the frequency distribution and abundance of larval *U. foili* among parasitized males ($n = 31$) and females ($n = 34$) were compared. A chi-square contingency test was used to compare the pattern of distribution of larval *U. foili* between male and female hosts. A comparison of mite loads between the 2 sexes was conducted using a Mann-Whitney U -test.

Host sex and the return of mites to water

To test the effects of host sex on the probability of mite larvae returning to water, midges (males: $n = 31$; females: $n = 34$) infected with larval *U. foili* were placed individually into 24 × 16 × 5 cm Tupperware® containers that were divided by the manufacturer into 2 equal parts (12 × 16 cm). Each container was fitted with mosquito netting (1-mm mesh), with a space of about 3 cm between the top edge of the container and the fitted piece of netting to allow the midges to move freely between the 2 compartments. Approximately 100 ml of APW was added to 1 of the 2 compartments in the container. Midges were held in these arenas until they were dead, at which time the following information was recorded: the date of death, the location (compartment with or without water) of death, whether females deposited an egg mass, the number of mite larvae remaining on a host insect, and the number of mites detaching from a host and their location (compartment with or without water) in the test arena. Larvae infesting individual midges were scored as returning to water if more than 50% were found in water after detachment from a host. A chi-square contingency test was used

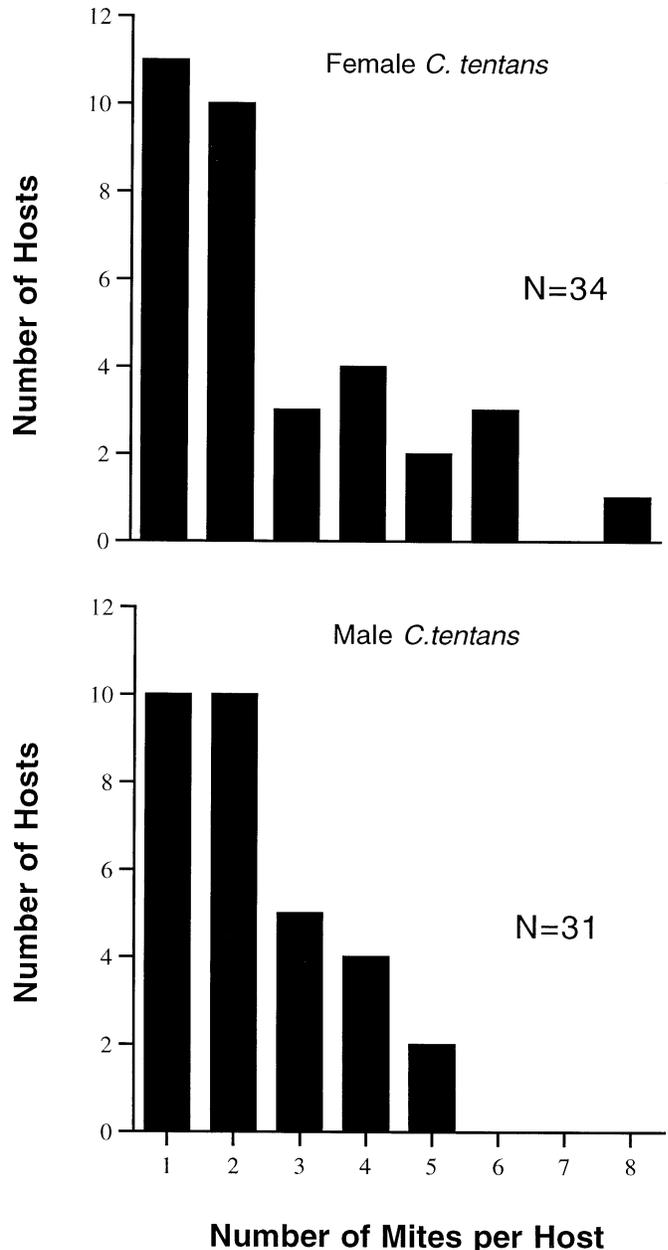


FIGURE 1. Frequency distribution of the number of larval *Unionicola foili* among infected male and female *Chironomus tentans*.

to compare the location of larval mites that had detached from male and female midges.

RESULTS

Host sex preferences

A chi-square contingency test revealed a significant difference in the number of infected and uninfected male and female *C. tentans* at emergence (Table I). The frequency distribution for the number of larval mites parasitizing male and female *C. tentans* is presented in Figure 1. The mean number of larvae among male midges was 2.3 larvae per host (SE = 0.23, range = 1–6), whereas larvae parasitizing females occurred at a mean

TABLE II. Chi-square contingency table comparing the distribution of dead male and female *Chironomus tentans* infected with larval *Unionicola foili* in partitioned Tupperware® containers.

Sex of host	Environment		Total	χ^2
	Water	No water		
Female	20	14	34	2.67*
Male	11	20	31	
Total	31	34	65	

* Not significant.

of 2.6 larvae per host (SE = 0.32, range = 1–8). There was no significant difference in the density of larvae per insect host when the data from males and females were compared ($U = 524.0$, $P > 0.05$).

Host sex and the return of mites to water

The distributions of dead male and female *C. tentans* in the test arena were not significantly different (Table II). A substantial number of mites did not detach from *C. tentans* after host death. Of 92 larval mites distributed among 34 female midges, 26 (28%) remained attached. Of 71 mites distributed among 31 male insects, 26 (37%) remained attached after host death. Among larval *U. foili* that detached from male and female host midges, 71% (32 of 45) and 66% (42 of 64), respectively, were found in water. There were significantly more instances in which 50% or more of the larvae that detached from a host were found in that portion of the test arena containing water (larvae parasitizing females: $\chi^2 = 5.8$, $P < 0.05$; larvae parasitizing males: $\chi^2 = 3.9$, $P < 0.05$). A chi-square contingency test indicated that the distribution of larvae that were previously parasitizing males and females was not significantly different (Table III).

An examination of the data for female *C. tentans* indicated that 29 of 66 females failed to oviposit before their death. Because female *C. tentans* return to water to oviposit (D. Edwards, pers. obs.) and because this behavior is presumed to play an important role in successfully returning larval water mites to the aquatic environment, the location of larvae in the test arena after parasitism of ovipositing and nonovipositing females was compared. Again, larvae infesting individual midges were scored as returning to water if more than 50% were found in water after detachment from a host. Seventy-eight percent (29 of 37) of larvae that detached from ovipositing females were found in water, whereas 52% of mites detaching from nonovipositing females were recovered from water. A significantly higher proportion of mite larvae returned to water after parasitism with ovipositing females (Table IV).

To further assess the relationship between oviposition and larval detachment, infected female *C. tentans* were placed individually in 50-ml vials containing approximately 15 ml of APW, and the water was examined daily for the presence of egg masses and larval mites. Each vial was monitored for the duration of an adult's life span (4.7 ± 0.17 SE days, range = 3–7, $n = 39$). There was no apparent relationship between the appearance of the first egg mass and the presence of larval mites in the container. Larval *U. foili* remained attached to female midges for an average of 2.6 days (SE = 0.21, $n = 39$),

TABLE III. Chi-square contingency table comparing the location of larval *Unionicola foili* in partitioned Tupperware® containers following parasitic associations with male and female *Chironomus tentans*. Values indicate whether larvae detaching from an individual host were found in water or out of water. Larvae were scored as returning to water if more than 50% were found in water following detachment from a host.

Sex of host	Environment		Total	χ^2
	Water	No water		
Female	24	10	34	0.004*
Male	21	10	31	
Total	45	20	65	

* Not significant.

whereas midges oviposited on average 1.1 days (SE = 0.54, $n = 39$) after eclosing. Only 16.5% of larvae (17 of 103) detached from female hosts during this oviposition episode. However, female *C. tentans* that produce eggs typically deposit 1 large egg mass followed by 1 or 2 smaller egg clusters (data not shown). Female midges in this study produced an average of 1.9 egg masses (SE = 0.06, $n = 39$). Among those females that oviposited twice, there was no significant difference ($U = 1063.5$, $P > 0.05$) between the average number of days a second egg mass was deposited (mean = 2.6 ± 0.12 SE) and the mean number of days larvae ($n = 48$) detached from female midges (mean = 2.7 ± 0.17 SE).

DISCUSSION

Female midges emerging from laboratory-reared cultures of *C. tentans* exposed to larval *U. foili* were more likely to be infected by mite larvae than were males. There was, however, no significant difference in the frequency distribution of mites among infected male and female chironomids. The latter observation is consistent with the pattern reported by Edwards and Dimock (1995) for larval *U. foili* and *U. formosa* parasitizing laboratory-reared populations of *C. tentans*.

Reports of sex differences in parasitism among water mite–insect associations are varied. Whereas data supporting a bias for female hosts are available (Davies, 1959; Mitchell, 1967; LeSage and Harrison, 1980), most studies examining the distribution and abundance of larval mites among male and female insects either have not found a difference (Booth and Learner, 1978; Kouwets and Davids, 1984; Rolff, 2000) or have reported

TABLE IV. Chi-square contingency table comparing the location of larval *Unionicola foili* in partitioned Tupperware® containers following parasitic associations with ovipositing and nonovipositing female *Chironomus tentans*. Values indicate whether larvae detaching from an individual host were found in water or out of water. Larvae were scored as returning to water if more than 50% were found in water following detachment from a host.

Condition of female	Environment		Total	χ^2
	Water	No water		
Ovipositing	18	4	22	4.03*
Nonovipositing	5	7	12	
Total	23	11	34	

* $P < 0.05$.

circumstances in which preferences for male or female hosts can vary depending on the species of host that is used (Reilly and McCarthy, 1991). Smith and McIver (1984) interpreted sex biases among females mosquitoes parasitized by *Arrenurus* larvae arising from temporal differences in the emergence of male and female hosts and as a consequence of temporal overlap with parasitic larval mites.

An explanation for the seemingly contradictory results (i.e., a higher prevalence of larvae among female hosts at emergence but no difference in the distribution and abundance of mites among infected males and females) in the pattern of distribution of larval *U. foili* among male and female *C. tentans* in the present study is unclear. It is possible that larvae prefer to colonize female insects but will parasitize males when females are difficult to find or unavailable. This pattern of response would be somewhat analogous to the searching behavior exhibited by phytophagous insects when gravid females accept less-preferred hosts as sites for oviposition as the duration of the search time increases (Singer, 1982). Jaenike (1990) interpreted this type of response pattern as evidence that the threshold stimulus necessary to elicit oviposition among insects decreases substantially with increased time spent searching. Unfortunately, it has proven to be extremely difficult to further address the possibility of sex preferences (e.g., by altering the availability of male and female hosts) or age-specific behavioral preferences among larval *U. foili*. The pupae of *C. tentans* almost invariably die when they are removed from culture aquaria and are confined to small vials (50 ml) containing larval mites. It is not clear whether declining oxygen tension or some other perturbation is responsible for the reduced survival of pupae in these isolated containers.

A substantial number of larvae remained attached to *C. tentans* at the time of host death. Most of these larvae appeared to be dead and were rarely found detached (4 of 52 larvae) from midges even when monitored for 2 to 3 days after the death of their hosts. This latter observation coupled with data indicating that male and female midges have an equal probability of dying in or out of water suggests that a large number of mite larvae parasitizing insects do not return to the aquatic habitat. There is very little additional information on the proportion of larvae that do not successfully complete their parasitic phase with insects, and the results appear to be highly variable. Collins (1975) reported that 89–94% of *Partnuniella thermalis* larvae parasitic on the brine fly *Ephedra thermophila* do not successfully detach from their hosts. Mitchell (1967) indicated that male *Leucorrhinia intacta* dragonflies lost more than 75% of their *Arrenurus reflexus* larval mites within 3 days of maturation.

Larval *U. foili* that detached from *C. tentans* had a high probability of returning to water regardless of whether they were associated with male or female insects. Mites infesting female midges were, however, more likely to return to water if their hosts had oviposited. The positive relationship between oviposition and the return of larval mites to water was further corroborated by the fact that larval *U. foili* detached from female midges at approximately the same time they were returning to water to deposit a second egg mass. Although these data suggest that detachment from a host is not a chance occurrence, the potential mechanism stimulating this response is unknown. Rolff and Martens (1997) found that detachment of mites from

host insects can be influenced by proximity to water. Larval *Arrenurus cuspidator* detached from *Coenagrion puella* dragonflies exposed at 10 mm above water but did not detach from hosts exposed at 25 mm. Whether these or other cues affect the detachment of *U. foili* from female *C. tentans* remains to be tested.

A parasitic association with male insects is thought to reduce the probability of larval water mites returning to water to complete their life cycle (Mitchell, 1967). McLachlan (1999) reported relatively high numbers of larval *U. ypsilophora* among male *Paratrichocladius rufiventris* but also noted that the average intensity of parasitism decreased in males and increased in females after mating. McLachlan (1999) concluded that larvae infesting males were transferred to females during copulation and that this behavior could be interpreted as an adaptation that increases the probability that larval *U. ypsilophora* returned to water. McLachlan's claim that larval *Unionicola* switch from male to female midges during host copulation is questionable given that he did not examine individual midges before and after mating but rather compared 2 separate populations (swarms vs. mated pairs) of midges. The results of the present study report a relatively high prevalence and abundance of mites among male midges but suggest that larval *U. foili* infesting male *C. tentans* return to water at a frequency greater than that expected by chance. There is, however, a possibility that some larval *U. foili* crawled to the compartment containing water even though they detached in an area of the arena without water. Indeed, Ullrich (1976) reported that *Sperchon setiger* larvae were capable of crawling at rates of 2 cm/min over dry land after parasitism on host insects. If the interpretation of the data reported from the present study is correct and reflects what is occurring in the natural habitat, future studies should address the mechanism(s) responsible for increasing the likelihood that mites parasitizing male midges return to water, including the possibility of parasite-mediated changes in host behavior.

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