Competitive interactions and persistence of two nematode species that parasitize *Drosophila recens*

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Abstract

*Drosophila recens* is parasitized in the wild by two nematodes, *Howardula aoronymphium*, a host generalist, and *Parasitylenchus nearcticus*, a host specialist known only from *D. recens*. In order to understand how these two parasite species coexist, we compared their ability to infect and grow in *D. recens*, their effects on host fecundity and survival, and whether one parasite species was competitively superior in double infections. The specialist nematode *P. nearcticus* had greater rates of infection and reproduction than the generalist *H. aoronymphium*, and completely sterilized females in single and mixed infections. The specialist was competitively superior in mixed infections, as generalist motherworms were significantly smaller than in single infections. These results suggest that *P. nearcticus* might competitively exclude *H. aoronymphium* if *D. recens* were the only host available. It is likely that *H. aoronymphium* persists in *D. recens* by transmission from other, more suitable host species.

Keywords

Allantonematidae, host specificity, interspecific competition, mycophagous *Drosophila*, parasite coexistence, virulence.

**INTRODUCTION**

Many host species typically harbour several species of parasites (Petney & Andrews 1998). However, because hosts are finite resources, parasites that share the same host species may adversely affect each other’s densities through interspecific competition, which can occur in two distinct ways (Dobson 1985). Exploitation competition does not require any direct interaction between parasite species. Instead, parasites affect each other by reducing host densities, possibly below thresholds necessary for parasite population growth. The most important properties that affect the nature of exploitation competition are a parasite’s virulence and transmission rate, and the statistical distribution of parasites among hosts. Interference competition results from coinfection of host individuals. In addition to competing directly for a common pool of resources within coinfected hosts, parasites can also interact in a directly antagonistic manner, for example, by releasing toxic compounds that inhibit growth or causing displacement from preferred attachment sites. Such interactions are often asymmetric (Poulin 1998). Interference between parasites can also be indirect, mediated for example by host defensive responses.

If a host species is considered a single limiting resource, then the competitive exclusion principle would suggest that only one parasite species could persist within a given host species. Given that individual host species are often infected by many different parasite species, this raises the question of how these parasites coexist.

One possible mechanism is parasite aggregation (Dobson 1985; Ives & May 1985; Roberts & Dobson 1995; Comins & Hassell 1996). By analogy with species that utilize patchy ephemeral resources (e.g. Wertheim *et al*. 2000), it has been suggested that if different parasite species exhibit independent aggregated distributions, then several species can be stably maintained within a single host species. As parasites become more aggregated, they have less effect on the host population, while being subject to greater within-host competition. If the distributions of the different parasite species are independent, this lessens the probability of co-occurrence, and the consequent interspecific competition within a single host individual. Many parasites exhibit highly aggregated distributions, so this mechanism is probably broadly relevant (Shaw & Dobson 1995).

Parasites may also coexist if there is both within-host resource partitioning and little or no virulence by the
parasite, so that host density is little affected by the different parasites. Within-host resource partitioning will reduce interference competition between parasites in the same host individual (Poulin 1998).

Coexistence of parasite species can also be maintained through tradeoffs between exploitation and interference competition (Hochberg & Holt 1990; Amarasekare 2000). A parasite that is a good colonizer, with high infection and reproductive rates under noncompetitive conditions, can coexist with a species that is superior in interference competition but which has a higher host threshold density. For example, one parasite species may release toxins that negatively affect the growth of a second species that has a higher pathogenicity. The better interference competitor might even require the presence of the other parasite species within a host for successful infection (see Schall & Brumwich 1994 for an example of facilitation).

Finally, different parasite species can coexist within a single host through source-sink dynamics involving more than one host species. Parasites infecting a single host species would consist of both core and satellite species (Poulin 1998). The core parasite species in a certain host would be maintained even if this were the only host species in a community, whereas satellite species would be present as a result of input from other host species.

**Drosophila recens** Wheeler and its parasitic nematodes

Mushroom-feeding Drosophila flies (Diptera: Drosophilidae) are often the most common insect visitors to decaying fleshy mushrooms on the forest floor. Associated with them are a number of parasitic nematodes that vary greatly in both host range and virulence. What contributes to the maintenance of this diverse host-parasite community? In this paper, we examine the factors involved in the coexistence of the two parasitic nematodes that infect *D. recens*. Specifically, we ask which parasite utilizes *D. recens* better in terms of infection rate and reproduction? Which parasite is more virulent and thus likely to have a more adverse effect on host population density? Finally, which parasite species is competitively superior in doubly infected hosts?

*Drosophila recens* (Diptera: Drosophilidae) is a mushroom-feeding fly found in the deciduous forests of northeastern North America. It is known to be parasitized by two species of nematode in nature, *Howardula aoronymphium* Welch and *Parasitylenchus nearcticus* Poinar, Jaenike and Dombeck (Tylenchida: Allantonematidae). In eastern North America, *H. aoronymphium* infects four species of Drosophila – *D. neotestacea* and *D. putrida* of the testacea species group, and *D. fulkii* Wheeler and *D. recens* of the quinaria group (Welch 1959; Jaenike 1992). *Parasitylenchus nearcticus* has been found only in *D. recens* (Poinar et al. 1997).

Both nematode species have direct life cycles, involving no intermediate hosts, and this greatly simplifies understanding their population dynamics. For both *Howardula* and *Parasitylenchus*, an inseminated female nematode infects the host by piercing the cuticle of a fly larva. When the adult fly emerges, the *Howardula* motherworm grows rapidly and then begins releasing juvenile nematodes into the haemocoel of the host. These juveniles are passed from the anus and ovipositor of the host as it visits mushrooms, and the nematodes subsequently mate inside these mushrooms and continue the cycle.

The most apparent fitness consequence of *Howardula* parasitism is reduced fertility of infected hosts. Females of the testacea group are completely sterilized by *H. aoronymphium*, whereas females of the quinaria group experience about a 50% reduction in fecundity (Jaenike 1992). Nematodes have also been shown to affect male mating success (Jaenike 1988) in *D. neotestacea*, as well as adult survival in *D. neotestacea* and *D. putrida* (Jaenike et al. 1995). The infection rate of *D. recens* varies from 0 to 20%, but only averages about 5% (Jaenike 1992), which is substantially less than the other host species of *H. aoronymphium* in northeastern North America (Jaenike 1992).

Nematodes of the genus *Parasitylenchus* have two obligate parasitic generations in their hosts. Inseminated motherworms infect fly larvae and then give birth to larval nematodes (F1), which then mate inside the same host. It is their larval offspring (F2) that are then passed to mushrooms. *Parasitylenchus nearcticus* completely sterilizes its female host (Poinar et al. 1997). The preliminary collections from which *P. nearcticus* was described in the Adirondack Mountains in New York reported a 5% infection rate in *D. recens*, 2% of collected flies were found to be infected with both *P. nearcticus* and *H. aoronymphium* (Poinar et al. 1997).

The following experiments were conducted to determine the suitability of *D. recens* for these two parasite species, their virulence to this host, and the nature of competitive interactions between them in coinfected individuals.

**MATERIALS AND METHODS**

**Stocks**

The strain of *D. recens* was established from multiple females collected in 1994 and 1995 from the Adirondack Mountains in New York. The *P. nearcticus* strain was descended from multiple nematodes collected along with the *D. recens*. These nematodes were maintained in the laboratory using *D. recens* as hosts. The *Howardula aoronymphium* strain was established from multiple nematodes collected from its four host species, which were collected in Rochester, New York in 1992. These nematodes were maintained in the laboratory using *D. neotestacea* as hosts.
Experimental infections

In order to collect fly eggs for infection, uninfected *D. reics* females were placed in Petri dishes with food plugs made from blended mushrooms, agar, and water. *Howardula aoronymphium* were obtained by grinding one to two-week-old-infected *D. neotestacea* flies in *Drosophila* Ringer’s solution (Roberts 1986). After determining the density of larval nematodes in this slurry, slurry volumes containing either 200, 400 or 800 larval nematodes were pipetted onto a 0.4-g piece of *Agaricus bisporus* mushroom. Larval *P. nearcticus* nematodes were obtained by grinding infected three-week-old *D. reics*.

Two experimental trials were conducted to assay parasite infection, reproduction, and effects on host fertility. In the first, the following treatments were set up: (1) no infection (n = 4 vials); (2) 200 larval *P. nearcticus* (n = 5); (3) 200 larval *H. aoronymphium* (n = 4); and (4) 200 larval *H. aoronymphium* and 200 larval *P. nearcticus* (n = 4). In the second trial, we set up 5 vials each at the following higher densities: (1) no infection; (2) 400 larval *P. nearcticus*; (3) 800 larval *P. nearcticus*; (4) 400 larval *H. aoronymphium*; (5) 800 larval *H. aoronymphium*; (6) 400 larval *P. nearcticus* and 400 larval *H. aoronymphium*; and (7) 800 larval *P. nearcticus* and 800 larval *H. aoronymphium*.

After the addition of the larval nematodes, 20 *D. reics* eggs were placed on the mushroom pieces, which were then placed in a vial with moistened cheesecloth on the bottom. Pieces of moistened mushroom were added to the vial to prevent starvation when necessary. Upon their emergence, adult flies were collected and transferred to fresh vials containing Instant *Drosophila* Medium (Carolina Biological Supply) plus a piece of fresh *A. bisporus* mushroom. Flies were frozen after 7 days. The following data were later recorded for each fly: number and size of *H. aoronymphium* motherworms, number of *P. nearcticus* F1 larvae, and number of mature eggs (stage 10 and later) in fly ovaries. Nematodes of the two species are readily distinguished. The much larger *H. aoronymphium* motherworms were traced with a camera lucida, and their longitudinal section area was then measured with a planimeter. *Parasitylenchus nearcticus* motherworms often disintegrate after a few days in their hosts and therefore cannot be counted or measured in week-old flies. *Howardula aoronymphium* motherworm size is a good predictor of parasite fecundity (Jaenike 1996), and the number of *P. nearcticus* F1 offspring within a host is a measure of motherworm reproduction, although one needs to correct for the number of motherworms per host.

A third experimental infection was conducted to assess the effect of parasites on the survival of adult flies. This experiment also provided data on infection rates. Six replicate vials of each of the following treatments were set up: (1) uninfected; (2) 800 larval *P. nearcticus*; (3) 800 larval *H. aoronymphium*; and (4) 800 larval *P. nearcticus* and 800 larval *H. aoronymphium*. After addition of the nematodes, 20 *D. reics* eggs per vial were added. Upon emergence of adult flies, males and females were put in separate vials to avoid any effects of mating on survival (e.g. Chapman et al. 1995). Every other day, flies were transferred to vials containing 8 mL of a mushroom/sucrose/agar medium. Vials were checked daily for dead flies, which were frozen and later dissected. Lifespan, infection status, number of *H. aoronymphium* motherworms, and number of *P. nearcticus* larvae were recorded for each fly in the experiment. Survival data were square root transformed to more closely approximate a normal distribution. Data were analysed using the statistical package JMP 3.1.5. (1989–95).

RESULTS

Host suitability for the two parasites

The rates of infection in the three experimental trials are presented in Table 1. The percent infection by *P. nearcticus* was much higher than that by *H. aoronymphium* in all three trials and at all densities of larval nematodes. In every case, at least 80% of *D. reics* were infected by *P. nearcticus*. Males and females of *D. reics* did not differ in susceptibility to infection (Table 2). The relative density of *H. aoronymphium*, defined as the total number of motherworms divided by the total number of flies (Margolis et al. 1982), was consistently low, in all cases less than or equal to one motherworm per host fly. Because *P. nearcticus* motherworms disintegrate after a few days in their hosts, it is not possible to census them in week-old flies. The number of F1 larvae is therefore only an indirect measure of relative density, since we are not able to measure the number of offspring per motherworm.

The reproductive rate of the different parasites can be compared indirectly, through their effects on host (female) fecundity, with greater reductions in host fecundity being correlated with greater parasite reproduction (Jaenike 1996b). Almost all females infected with *P. nearcticus* were completely sterile (average number of mature eggs = 0.28, n = 86 flies). For females infected only with *H. aoronymphium*, fecundity was reduced by about one quarter (average number of eggs for infected flies = 17.8 (n = 23 flies), and for uninfected flies = 24.6 (n = 95 flies)), a modestly significant difference (t = 2.07, d.f. = 116, P = 0.04) (Fig. 1). The greater reduction in host fertility suggests that *P. nearcticus* has a higher per capita rate of reproduction than does *H. aoronymphium* within *D. reics*. Because all *D. reics* females were rendered completely sterile by *P. nearcticus*, this strongly suggests that infection by even a single *P. nearcticus* motherworm is sufficient to bring about this sterility.
Mean longevity of female flies was substantially reduced by infection with either nematode species (Fig. 2; ANOVA: \( F = 5.72, \) d.f. = 3,78, \( P < 0.01 \)). There was no significant difference in the survival of male flies as a function of infection status [ANOVA: \( F = 0.80, \) d.f. = 3,86, NS]. Although both nematodes increased the mortality of infected flies, there was no difference in the mean longevity of female flies that were infected by \( H. \) aoronymphium only, \( P. \) nearcticus only, or coinfected with both nematodes simultaneously [ANOVA: \( F = 0.055, \) d.f. = 2,47, NS].

Interference competition between the two parasites

There was no effect of mixed infection on infection rate for either nematode species (Table 2). That is, the percent infection by either was not affected by the presence of the other.

There were however, significant effects of mixed infection on parasite reproduction. For \( H. \) aoronymphium, motherworm size was significantly less in flies that were also infected with \( P. \) nearcticus, for all larval densities tested [ANOVA: \( F = 17.78, \) d.f. = 1,81, \( P < 0.001 \)] (Fig. 3).
was no correlation between *H. aoronymphium* motherworm size and the number of *P. nearcticus* F1 offspring \( (r^2 < 0.001) \). Since *P. nearcticus* F1 number increases with infection density (and presumably motherworm number), this indicates that the reduction in *H. aoronymphium* motherworm size is independent of the number of *P. nearcticus* motherworms in the same individual fly. The size of *H. aoronymphium* motherworms was not affected by the number of conspecific motherworms per host \[ \text{ANOVA: } F \approx 0.24, \text{ d.f. } = 2,43, \text{ NS} \], although this was rarely more than two in our experiments. Other experiments have shown that reductions in motherworm size are evident at higher densities (Jaenike 1998).

*Parasitylenchus nearcticus* did not exhibit any negative effects of coinfection by *H. aoronymphium* (Fig. 4). For two of the experimental treatments (200 and 800 larval nematodes per treatment), the number of *P. nearcticus* F1 was greater in the presence of *H. aoronymphium* in the same host than in single infections. This difference was only significant at the highest nematode concentration \[ \text{ANOVA: effect of either single or mixed infection, } F = 7.62, \text{ d.f. } = 1.50, P = 0.02; \text{ nested effect of vial within treatment, } F = 41.17, \text{ d.f. } = 8,50, P = 0.003 \].

In mixed infections, the nematodes do not adversely affect each other through increased host mortality. There was no difference in host mortality in mixed compared with single infections (Fig. 2; see earlier section on mortality). In mixed infections, however, female hosts were completely sterile, probably due to the presence of *P. nearcticus* (Fig. 1).

**DISCUSSION**

The results presented above show that the host specialist *P. nearcticus* utilizes *D. recaen* more effectively than does...
the generalist *H. aoronymphium*. Prevalence of infection by *P. nearcticus* was consistently much higher than by *H. aoronymphium* using similar densities of infective nematodes. Female flies infected by *P. nearcticus* were almost always completely sterile, compared with only about a 25% reduction in female fertility caused by infection with *H. aoronymphium*. The greater reduction in host fertility caused by *P. nearcticus* suggests that a greater proportion of host resources is allocated to reproduction of this parasite. Although parasites did adversely affect host survival, there was no difference in the survival of flies as a function of the parasite species with which they were infected. This reduction in survival under benign laboratory conditions suggests that these parasites also reduce fly survival in the wild. It was previously shown that *H. aoronymphium* has a greater adverse effect on host survival in the field than in the laboratory (Jaenike *et al.* 1995). Because live flies transmit both nematodes, host mortality is detrimental to these parasites (e.g. Blower & Roughgarden 1987).

One important concern that needs to be addressed is whether maintaining nematodes for many generations in the laboratory on one fly species may have affected their ability to infect other hosts. Experimental laboratory infections have shown that both nematode species can successfully infect and reproduce in hosts other than the fly used for maintenance in the laboratory. For example, *H. aoronymphium* successfully parasitized *D. neotestacea* and *D. falleni* after several generations in *D. putrida* (Jaenike & Dombeck 1998). This study also revealed that *H. aoronymphium* harbours little if any adaptive genetic variation in ability to utilize different host species (Jaenike & Dombeck 1998). Also, *P. nearcticus* can successfully infect and reproduce in species other than *D. recens* (S. Perlman, unpublished data). We therefore think it unlikely that the *H. aoronymphium* used in the present experiments became maladapted to *D. recens* during laboratory culture, or that *P. nearcticus* has lost the ability to infect other hosts as a result of being brought into the laboratory.

The poor performance of *H. aoronymphium* in *D. recens* is not due to a generally low ability to utilize *Drosophila*. For instance, the decrease in fecundity of infected females was much less than that observed in the other *Drosophila* species infected by *H. aoronymphium* (Jaenike 1992). Of the four species of *Drosophila* infected by *H. aoronymphium* in northeastern North America, rates of infection in the wild and in the laboratory are lowest for *D. recens* (Jaenike 1992). Because these four species often occur on the same mushroom in the wild (Jaenike & James 1991), there are ample opportunities for interspecific transmission of *H. aoronymphium*. Thus, *D. recens* may be an incidental host for this parasite.

Besides having a better capacity to infect and reproduce within *D. recens*, *P. nearcticus* is also the better competitor in mixed infections. The most notable effect is on *H. aoronymphium* motherworm reproduction, as motherworms of this species were significantly smaller, and thus probably less fecund, in flies that also harboured *P. nearcticus*. The reproduction of *P. nearcticus*, as measured by offspring (F1) number, was not adversely affected by the presence of *H. aoronymphium*. Asymmetric competitive interactions are common in entomophagous nematodes (e.g. Koppenhöfer *et al.* 1995), and in many other parasites (Dobson 1985; Sousa 1993; Poulin 1998).

Inoculation of mushrooms with *H. aoronymphium* and *P. nearcticus* at the same time may have given us an underestimate of the competitive superiority of *P. nearcticus* within hosts, because this species is more likely to parasitize *D. recens* larvae prior to infection by *H. aoronymphium*. This is because *D. recens* adults come to mushrooms before *D. putrida* and *D. neotestacea*, two of the principal hosts of *H. aoronymphium*. These latter two flies are preferentially attracted to more rotten (older) mushrooms (Grimaldi 1985). Thus, the ratio of *P. nearcticus* to *H. aoronymphium* is likely to decrease with mushroom age. However, because motherworms develop much later than the window of time in which host penetration occurs, the effect of priority in this host-parasite system is not clear.

Infections by multiple parasite species can have a severe combined effect on host fitness (Petney & Andrews 1998), possibly because of the difficulty of resisting multiple parasites (Taylor *et al.* 1998). For *D. recens*, however, coinfections by *H. aoronymphium* plus *P. nearcticus* were no more virulent than infections by just a single species. Mortality of singly and doubly infected flies was similar. Both doubly infected female flies and those infected with only *P. nearcticus* were sterile.

**Coexistence of *H. aoronymphium* and *P. nearcticus***

How do infection rate, parasite reproductive rate, and virulence affect the population dynamics of these two parasite species? A macroparasite’s host threshold density (NT), or the minimum density of hosts required for positive parasite population growth (and parasite invasion of a host population), is inversely proportional to parasite transmission and reproductive rates (Anderson & May 1979; Jaenike 1998). Our experiments have shown that *P. nearcticus* has greater infection and reproductive rates (as inferred by their effects on host fertility) than *H. aoronymphium* in *D. recens*. In addition, NT will increase with the rate of parasite-induced host mortality. We found that both *H. aoronymphium* and *P. nearcticus* have similar adverse effects on the survival of *D. recens*. Considering the combined effects of infection rate, parasite reproductive rate, and parasite-induced host mortality, we conclude that the threshold population density of *D. recens* is lower for *P. nearcticus* than for *H. aoronymphium*. 

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Thus, in the absence of within–host interactions between the parasites, we would expect *P. nearcticus* to competitively exclude *H. aoronymphium* at the population level. The asymmetric competitive interactions within coinfected hosts – the greater adverse effect of *P. nearcticus* on *H. aoronymphium* than vice versa – further bolsters this conclusion. Thus, if *D. recens* were the only host species available, we predict that *P. nearcticus* would competitively exclude *H. aoronymphium*, both through within–host competitive interactions and through a greater reduction in host density, possibly below the threshold density for *H. aoronymphium*.

How does *H. aoronymphium* persist in *D. recens*, despite its poor performance and competitive inferiority? Here we evaluate the possible mechanisms for parasite species coexistence. First, coexistence due to independently aggregated distributions appears unlikely. Our field data shows no evidence of aggregation by *H. aoronymphium* in *D. recens*, with 21 out of 25 infected flies harbouring only a single motherworm. Both species of nematodes co-occur in the Adirondack Mountains. In a sample of 116 *D. recens* from the Adirondacks, 105 were uninfected, 5 were infected with *H. aoronymphium* only, 4 were infected with *P. nearcticus* only, and 2 were infected with both parasites (Poinar et al. 1997). In this sample of flies, the two parasites actually exhibit a positive association (*P = 0.04; Fisher’s exact test*).

Within-host resource partitioning is also unlikely as a mechanism for parasite coexistence in *D. recens*. Both *H. aoronymphium* and *P. nearcticus* are found in the haemocoel of the fly, and the present experiments revealed negative competitive interactions between them within coinfected hosts. Persistence of the two nematode species is not due to coexistence of a good colonizer and a good within-host competitor, because *P. nearcticus* is superior in both respects.

We suggest that *H. aoronymphium* persists in populations of *D. recens* as a result of transmission from other host species, especially *D. putrida* and *D. notestacea*, which are superior hosts for both infection and reproduction of *H. aoronymphium* (Jaenike 1992; Jaenike & Dombeck 1998). These *Drosophila* species share microhabitats and often breed in the same individual mushroom (Grimaldi & Jaenike 1984; Jaenike & James 1991). There is therefore great opportunity for horizontal transmission of *H. aoronymphium* between host species. Thus, the extent to which this generalist nematode encounters *D. recens* will depend on how frequently larvae of *D. recens* inhabit the same mushrooms as the other hosts of *H. aoronymphium*. Indeed, the poor performance of *H. aoronymphium* in *D. recens*, particularly its low infection rate, suggest that *D. recens* may represent a demographic sink for *H. aoronymphium*.

Host species that serve as demographic sinks for certain parasites can be profoundly affected by them. For example, endangered species can be sinks for parasites that are maintained in other hosts; parasitism may contribute to extinction of these threatened species, even when they fall below threshold density for their “own” parasites (McCallum & Dobson 1995). The role of source-sink dynamics on the coexistence of parasites and the broader community-level effects of such dynamics warrant further examination in natural populations.

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**BIOSKETCH**

Steve Perlman’s research focuses on the evolution and ecology of host range and virulence of nematode parasites of mushroom-feeding *Drosophila*.

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