

Ecology and Evolution of Host-Parasite Associations: Mycophagous *Drosophila* and Their Parasitic Nematodes

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ABSTRACT: Associations between mycophagous *Drosophila* and nematode parasites occur throughout the temperate and boreal regions of North America, Europe, and Asia. The nematode *Howardula aoronymphium* has substantial adverse effects on host survival and fertility on North American *Drosophila*. Long-term data show that rainy summers lead to a high prevalence of parasitism in the fall and the following spring, resulting in up to a 1-yr time lag between present rainfall and increased prevalence of *H. aoronymphium* parasitism. A biogeographic analysis of the relative abundance of different *Drosophila* species has shown that *H. aoronymphium* may facilitate the coexistence of different species of *Drosophila* that compete for larval food resources. The actual host range of parasites in nature is determined by the intrinsic suitability of potential hosts for parasite infection and reproduction and various ecological factors. For *H. aoronymphium* in eastern North America, intrinsically suitable hosts fall within a restricted clade within the genus *Drosophila*. However, the temperature sensitivity of *H. aoronymphium* prevents it from using several host species that occur outside the geographical range of the nematodes. Finally, the host range, virulence, and geographical range of *Drosophila*-parasitic nematodes appear to be highly dynamic over evolutionary timescales.

Keywords: biogeography, climate, density-dependence, host range, keystone effects, virulence.

Because of their ubiquity and negative impact on host fitness, parasites have become central figures in evolutionary biology in recent years, and they are now thought to be important in the maintenance of sexual reproduction, the evolution of recombination rates, the maintenance of genetic diversity, as agents affecting sexual selec-

tion, and even as factors bringing about speciation (see Telschow et al. 2002, in this issue). On the ecological side, it has long been recognized that parasites may regulate host populations at levels well below the carrying capacity set by resources (e.g., Anderson and May 1978).

Within food webs, parasites represent a terminal trophic level in natural communities. The occurrence of predator-mediated trophic cascades in both terrestrial and, especially, aquatic ecosystems (Pace et al. 1999; Chase 2000) raises the question of whether parasites may also be important in structuring natural ecological communities or whether they are merely a sort of trophic garnish, as their low biomass would suggest. Parasite impact depends on the structure of trophic connections involving the hosts of a given parasite, the host range, and two factors that influence the strength of trophic interactions (Paine 1980): virulence (i.e., the effect of parasite infection on host fitness; Combes 2001) as a function of parasite density per host and the statistical distribution of the number of parasites per host.

Some parasites clearly have major community-level effects. For instance, in Hawaii, native birds are more susceptible than exotic birds to an introduced species of malaria-causing *Plasmodium*, which is vectored by an introduced mosquito, *Culex quinquefasciatus*. The introduction of avian malaria to Hawaii has resulted in the native birds now being restricted largely to high elevations and xeric regions outside the mosquito's range (van Riper et al. 1986). Chestnut was formerly a dominant species in many deciduous forests of the eastern United States. The introduction of chestnut blight, *Endothia parasitica*, from eastern Asia resulted in their virtual extermination from these forests, resulting in a major shift in the tree species composition (Day and Monk 1974).

These cases show that parasites can act as major determinants of community structure. However, both of the examples cited above as well as several others involve introduced parasite species, raising the question of whether endemic parasites can have similar community-level ef-

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fects. It is possible that parasites that initially have such major effects evolve to become less virulent and the hosts more resistant, thus lessening the general importance of parasites to the structure of ecological communities.

Despite their potential importance, parasites are often neglected in field studies of natural populations. For instance, published food webs that include parasites are rare exceptions (e.g., Huxham et al. 1995; Polis and Strong 1996). Although a general theoretical basis for the potential importance of parasites in population biology has been developed (reviewed in Anderson and May 1991), we suspect that the principal reason for neglecting parasites is the difficulty in detecting and enumerating them in natural populations. The detection of animal parasites, in particular, typically requires dissection of the host, examination of blood or fecal samples, or close examination in the hand (for ectoparasites). The detection of certain types of parasites may be facilitated by the development of molecular methods (Singh 1997).

Our research is motivated by the goal of understanding host-parasite interactions from several perspectives: the effects of parasites on the fitness and behavior of infected individuals, the dynamics of interacting host and parasite populations, the effect of parasites on the structure of communities in which their hosts occur, the coevolution of hosts and their parasites, and the evolution of new host-parasite associations. To explore these questions, we have focused on a group of ecologically tractable and widespread species of *Drosophila*—those that use mushrooms as breeding sites—and their nematode parasites. In the discussion below, we briefly outline the natural history of these species and then review some of our findings on the host-parasite interactions from individuals to clades.

Natural History

Throughout the temperate and boreal forested regions of North America, Europe, and Asia, ectomycorrhizal fungi associated with trees reproduce via the production of sporophores—mushrooms. These substrates, as well as mushrooms produced by some saprophytic species, are used as breeding sites by a variety of species of *Drosophila*, in particular, by members of the *quinaria* and *testacea* species groups of the subgenus *Drosophila*. These *Drosophila* are highly polyphagous, using a wide variety of mushroom species (Jaenike 1978; Hackman and Meinander 1979; Shorrocks and Charlesworth 1980; Lacy 1984; Kimura and Toda 1989). The mushrooms serve not only as larval food resources but also as sites for adult feeding, courtship, and mating. Thus, all stages of the life cycles of these *Drosophila* can readily be observed in the vicinity of mushrooms. These mushroom-feeding *Drosophila* are also commonly infected with obligate parasitic nematodes of the genera

Howardula and *Parasitylenchus* (Tylenchida: Allantonematidae). Table 1 lists the known associations between *Drosophila* and allantonematid parasites. In addition to *Drosophila*, various other flies—including Sepsidae, Phoridae, and Sphaeroceridae, among others—use mushrooms as breeding sites (Buxton 1960; Hackman and Meinander 1979). In small-scale sampling of these flies, we have found that members of all of these fly families are parasitized by *Howardula* nematodes. Mitochondrial and nuclear ribosomal DNA (mtDNA and rDNA, respectively) sequence data clearly indicate that the nematodes parasitizing different families of flies are distinct species (S. J. Perlman and J. Jaenike, unpublished data). Thus, one compartment of the food web of these temperate forests can be summarized as trees → ectomycorrhizal fungi → Diptera → *Howardula* nematodes (fig. 1).

Nematodes of the genus *Howardula* have a simple, direct life cycle (Welch 1959; fig. 2). Following the deposition of juvenile nematodes on a mushroom, male and female worms mate. The inseminated female nematode then infects a fly larva by piercing the fly's cuticle with its harpoon-like stylet. The nematode (or motherworm) then grows within the hemocoel of the fly and begins producing offspring several days after the emergence of the adult fly. These juveniles are then passed from the fly via the ovipositor or anus when the fly visits a mushroom. Both male and female *Drosophila* are suitable hosts because there is little difference in infection rates between the sexes in nature (Jaenike 2002), and infected individuals of both sexes shed infective nematodes. The lack of intermediate hosts greatly simplifies understanding the population dynamics, evolution, and biogeography of these nematodes. The life cycle of *Parasitylenchus* is similar to that of *Howardula* except that there are two obligate parasitic generations in the fly host.

Fitness Effects

The ecological studies summarized in this and the following three sections have focused primarily on the nematode *Howardula aoronymphium* and its principal hosts in the eastern United States: *Drosophila falleni* and *Drosophila recens* of the *quinaria* group and *Drosophila neotestacea* and *Drosophila putrida* of the *testacea* group. The most obvious effect of *H. aoronymphium* infection is a reduction in female fertility (fig. 3). Wild-caught females of both *testacea* group species are almost invariably completely sterile because infected flies lack mature oocytes (stage 10 or later). The two *quinaria* group species are much less severely affected, with infected females carrying about half as many mature eggs on average as do uninfected females (Jaenike 1992).

Field experiments on the effects of *H. aoronymphium*

Table 1: Known associations between *Drosophila* species and nematode parasites of the family Allantonematidae

<i>Drosophila</i> species	Distribution	Nematode species	Reference
<i>testacea:</i>			
<i>Drosophila neotestacea</i>	North America	<i>Howardula aoronymphium</i>	Jaenike 1992
<i>Drosophila putrida</i>	North America	<i>H. aoronymphium</i>	Jaenike 1992
<i>Drosophila testacea</i>	Europe	<i>H. aoronymphium</i>	Gillis and Hardy 1997
<i>Drosophila orientacea</i>	Japan	<i>H. aoronymphium</i>	Kimura and Toda 1989
<i>quinaria:</i>			
<i>Drosophila falleni</i>	North America	<i>H. aoronymphium</i>	Jaenike 1992
		<i>Howardula</i> sp.	Jaenike 1996a
<i>Drosophila recens</i>	North America	<i>H. aoronymphium</i>	Jaenike 1992
		<i>Parasitylenchus nearcticus</i>	Poinar et al. 1997
<i>Drosophila munda</i>	North America	<i>Howardula neocosmis</i>	S. J. Perlman and J. Jaenike, unpublished data
<i>Drosophila suboccidentalis</i>	North America	<i>H. neocosmis</i>	Poinar et al. 1998
<i>Drosophila kuntzei</i>	Europe	<i>H. aoronymphium</i>	Gillis and Hardy 1997
<i>Drosophila phalerata</i>	Europe	<i>H. aoronymphium</i>	Gillis and Hardy 1997
<i>Drosophila transversa</i>	Europe	<i>H. aoronymphium</i>	Gillis and Hardy 1997
<i>Drosophila brachynephros</i>	Japan	<i>Howardula</i> sp.	Kimura and Toda 1989
<i>Drosophila curvispina</i>	Japan	<i>Howardula</i> sp.	Kimura and Toda 1989
<i>cardini:</i>			
<i>Drosophila acutilabella</i>	North America	<i>H. neocosmis</i>	Poinar et al. 1998
<i>immigrans:</i>			
<i>Drosophila immigrans</i>	Europe	<i>H. aoronymphium</i>	Gillis and Hardy 1997
<i>repleta:</i>			
<i>Drosophila nigrospiracula</i>	North America	<i>Howardula</i> sp.	Polak 1993
<i>histrio:</i>			
<i>Drosophila histrio</i>	Japan	<i>Howardula</i> sp.	Kimura and Toda 1989
<i>macroptera:</i>			
<i>Drosophila macroptera</i>	North America	<i>Howardula</i> sp.	S. J. Perlman and J. Jaenike, unpublished data
<i>obscura:</i>			
<i>Drosophila pseudoobscura</i>	North America	<i>Howardula</i> sp.	S. J. Perlman and J. Jaenike, unpublished data
<i>Drosophila obscura</i>	Europe	<i>Parasitylenchus diplogenus</i>	Welch 1959; Gillis and Hardy 1997
<i>Drosophila subobscura</i>	Europe	<i>P. diplogenus</i>	Welch 1959; Gillis and Hardy 1997
<i>Drosophila subsilvestris</i>	Europe	<i>P. diplogenus</i>	Welch 1959
<i>quadrivittata:</i>			
<i>Drosophila sexvittata</i>	Japan	<i>Howardula</i> sp.	Kimura and Toda 1989
<i>Drosophila trilineata</i>	Japan	<i>Howardula</i> sp.	Kimura and Toda 1989
<i>Drosophila trivittata</i>	Japan	<i>Howardula</i> sp.	Kimura and Toda 1989
<i>Drosophila confusa</i>	Europe	<i>Howardula</i> sp.	Welch 1959

Note: Except for the *repleta* and *obscura* group species, these flies are primarily mycophagous.

on the survival of adult *D. putrida* and *D. neotestacea* in the wild revealed that parasitized flies consistently experience a substantially greater mortality rate than do unparasitized flies (Jaenike et al. 1995). Although these nematodes do reduce host survival in the laboratory (S. J. Perlman and J. Jaenike, unpublished manuscript), the effects are much greater in the wild (Jaenike et al. 1995). The results on both host survival and fertility show that the effects of parasites depend on the environmental context: they are generally more severe under the more challenging conditions in nature than in the laboratory.

The effects of parasites on male mating success and sexual selection have received a great deal of attention since the pioneering work of Hamilton and Zuk (1982). Our

studies have not revealed a large effect of *H. aoronymphium* parasitism on male mating success in *D. neotestacea*. In one set of laboratory experiments employing wild-caught males, a weak negative effect of parasitism was found (Jaenike 1988). However, collections of copulating pairs directly off mushrooms in the wild revealed identical percentages of parasitized flies among those that were mating and in a simultaneously collected group of males that were not mating (James and Jaenike 1992). The latter finding suggests that *H. aoronymphium* parasitism does not reduce male mating success. However, because *H. aoronymphium* reduces the survival of adult flies in the wild, it is likely that parasitized flies are on average younger than those that are unparasitized. It is thus possible that the younger

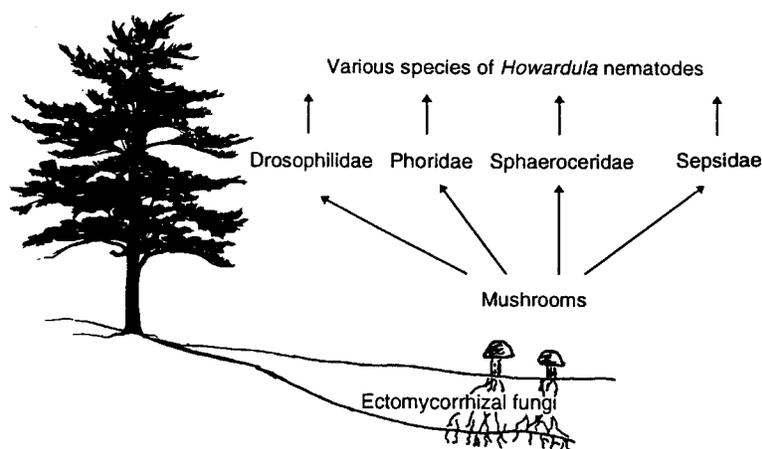


Figure 1: Trophic interactions involving mycophagous Diptera and parasitic *Howardula* nematodes. Other families of flies besides those shown also breed in mushrooms.

age of parasitized flies provides them with a mating advantage. Female flies may be able to use visual cues to distinguish parasitized from unparasitized males because these categories can be separated relatively well by human observers (Jaenike 1988).

Finally, we have found that both wild-caught and laboratory-reared males of *D. neotestacea* are considerably less fertile when they are parasitized by *H. aoronymphium* (Jaenike 1988; S. J. Perlman and J. Jaenike, unpublished manuscript). When mated to virgin, laboratory-reared females, wild-caught parasitized males sired only about one-third as many offspring per mating as did unparasitized males (laboratory-reared infected male sired even fewer offspring). Many females that mated with parasitized males failed to produce any offspring at all (Jaenike 1988; S. J. Perlman and J. Jaenike, unpublished manuscript). In most ecological and evolutionary models, offspring production is assumed to be limited by the female (Bateman 1948), so male fertility is an often overlooked component of fitness (but see Polak 1998). Our results with *D. neotestacea* indicate that male fertility can also be a limiting factor.

Taken together, these results of *H. aoronymphium* on its hosts show that these parasites have major adverse effects on the fitness of parasitized hosts; in some species (*D. putrida* and *D. neotestacea*), the fitness of infected females is reduced to zero because these flies are rendered completely sterile by *H. aoronymphium* infection. Whether these effects are important at the level of host populations depends on what fraction of the hosts are infected with nematodes. We turn to this question next.

Population Dynamics

Many macroparasites (like nematodes) of vertebrates exhibit highly aggregated distributions across hosts, with a small fraction of the host population harboring the bulk of the parasites (Shaw et al. 1998). As a result, a relatively small fraction of the hosts suffers significant parasite-caused reductions in fitness, and this makes it less likely that parasites can regulate populations of their hosts (Anderson and May 1978). The dispersion of *Howardula aoronymphium* across individuals within *Drosophila* populations does not follow this pattern. To a first approximation, these nematodes are randomly dispersed among hosts (fig. 4; Jaenike 1994, 2002). A slight degree of aggregation can be evident in some collections, but this is probably largely due to variation among individual mushrooms in the density of infective nematodes. Parasites are dispersed randomly among flies emerging from single mushrooms, and a combination of such random distributions with different means yields a slightly aggregated dispersion at the host population level (Jaenike 1994; see also Boulinier et al. 1996). Consequently, at low to intermediate prevalences of parasitism, as occurs in our study populations, most infected flies carry just one motherworm. We have therefore focused our analyses of infection on the prevalence of parasitism, that is, the fraction of flies in a host population that are infected at any one time.

From 1984 through 1997, monthly samples of the four host species of *H. aoronymphium* were obtained near Rochester, New York (Jaenike 2002). The flies were dissected in order to determine the prevalence of parasitism.

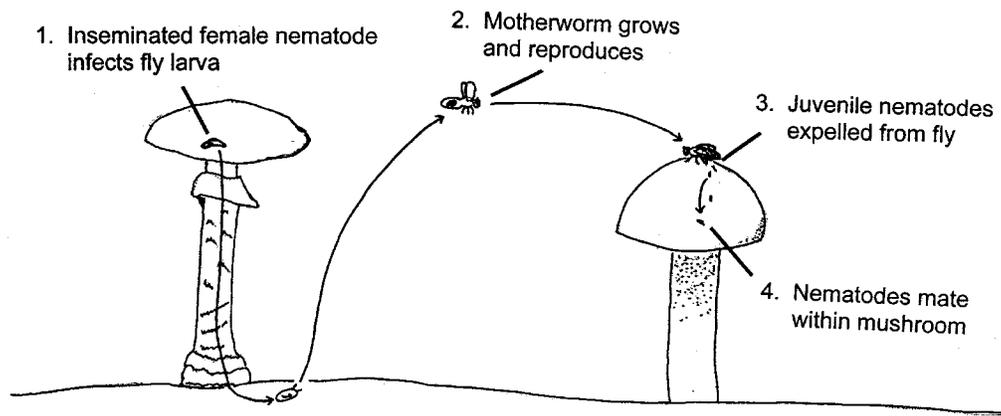


Figure 2: Life cycle of *Howardula* nematodes. Note the direct life cycle and lack of intermediate hosts. Nematodes of the genus *Parasitylenchus* have an additional generation of reproduction within infected adult flies.

These collections, first of all, revealed no long-term trends in the overall prevalence of parasitism in these populations. We did find substantial variation in prevalence among host species. Averaged across all samples, the mean prevalence of parasitism of the four host species was 23.0% of *Drosophila neotestacea*, 12.7% of *Drosophila putrida*, 11.4% of *Drosophila falleni*, and 4.8% of *Drosophila recens*. Thus, the two species that are most adversely affected by *H. aoronymphium*, at least in terms of female fertility, are also the most frequently infected. We should stress that a single motherworm suffices to all but guarantee complete sterility of a female of either of these host species. The greater rate of infection of these is likely due to two factors. First, controlled laboratory infections indicate that the two *testacea* group species are more susceptible to infection than are the *quinaria* group species (Jaenike and Dombeck 1998). Second, *D. putrida* and *D. neotestacea* tend to oviposit on older, more decayed fungi than do *D. falleni* and *D. recens*, and thus their larvae may be exposed to a higher density of infective nematodes (Grimaldi 1985; Montague and Jaenike 1985).

Seasonal changes in the average prevalence of parasitism across host species are also evident (Jaenike 2002). In general, the prevalence of parasitism is high in the spring (mean prevalence $\approx 16\%$ across host species), drops during midsummer (mean $\approx 10\%$), and then rises to a yearly maximum at the end of the season in late summer and early fall (mean $\approx 20\%$). We interpret these seasonal trends as follows: because these mycophagous *Drosophila* and their resident nematode parasites overwinter as adults (Jaenike 1992), there is a high correlation between the prevalence of parasitism in the spring and that in the previous fall in these species (Jaenike 2002). As a result of overwintering mortality, *Drosophila* populations tend to be at

low density in the spring. This low density probably results in a low rate of parasite transmission to the next generation of flies, in accordance with standard models of macro-parasite population dynamics (Anderson and May 1978), thus bringing about the low prevalence of infection typical of midsummer populations of flies. We believe that the relevant measure of *Drosophila* density for parasite transmission dynamics is the number of adult *Drosophila* per mushroom in the habitat. Field cage experiments confirm that high fly : mushroom ratios lead to increased parasitism in the next generation of flies (Jaenike and Anderson 1992). As the *Drosophila* population increases during the summer—if there has been sufficient rainfall to stimulate mushroom production—the fly : mushroom ratio will increase, resulting in greater parasite transmission and an increase in the prevalence of parasitism in late summer and early fall populations of *Drosophila*. Thus, our hypothesis is that seasonal changes in *Drosophila* density—or, more specifically, the fly : mushroom ratio—bring about changes in the rate of nematode transmission, which in turn cause seasonal changes in the prevalence of parasitism.

One interesting consequence of the above scenario is that the greater the increase in the *Drosophila* population density during a year, the greater the expected increase in the prevalence of parasitism in the fall. In fact, there is a strong correlation ($r = 0.66$, $P < .007$) between total May to August precipitation and the prevalence of parasitism in September of that year. This correlation probably results from the connections between rainfall and mushroom production, as documented by Worthen and McGuire (1990), and subsequent *Drosophila* population growth. As a result of the correlation between prevalence of parasitism in the fall of one year and in the spring of the next year, there is also a strong correlation between total May to August

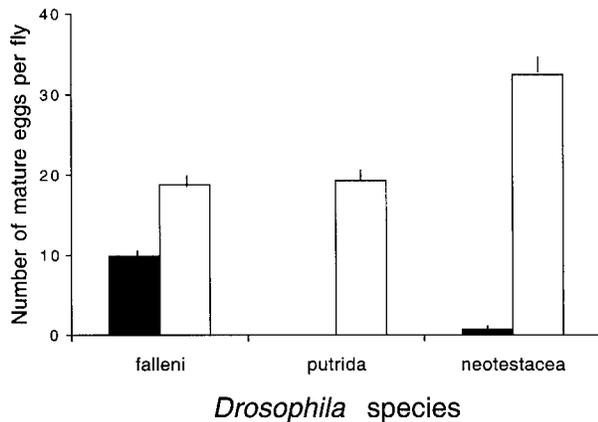


Figure 3: Effects of *Howardula aoronymphium* on fertility of wild-caught *Drosophila falleni*, *Drosophila putrida*, and *Drosophila neotestacea*. Mean \pm SE number of mature eggs per fly is plotted for parasitized (dark bars) and unparasitized (open bars) flies. Based on data from Jaenike 1992.

rainfall in one year and the mean prevalence of parasitism in the spring of the next year ($r = 0.77$, $P = .0013$; Jaenike 2002).

The time-delayed effect of precipitation on the prevalence of parasitism probably also affects *Drosophila* population growth rates. The prevalence of parasitism in the spring ranged from 4% to 50% for *D. neotestacea* and from 2% to 54% for *D. putrida* from 1984 through 1998. Because parasitized females of *D. putrida* and *D. neotestacea* are rendered sterile by *H. aoronymphium*, these data indicate that variation in the prevalence of parasitism can bring about at least a twofold difference in the potential rates of population growth from one year to the next. Thus, variation in precipitation can act with up to a 1-yr time delay on the population dynamics of *Drosophila* and their nematode parasites.

The finding that the rates of parasite transmission depend on patterns of precipitation suggests that long-term climatic change (e.g., changes associated with global warming) may bring about permanent changes in the dynamics of these host-parasite interactions. In the past century, both mean annual temperatures and precipitation throughout New York State have increased (U.S. Environmental Protection Agency 1997). If we can extrapolate from our current survey data, which encompass only 15 yr, then the predicted increases in temperature and precipitation may lead to higher rates of parasitism in spring populations of flies. Similarly, the incidence of various human diseases is correlated with climatic variation (e.g., Lobitz et al. 2000; Pascual et al. 2000; Curriero et al. 2001; Lindgren and Gustafson 2001). It is likely that global patterns of many diseases will be affected by climate change.

Our long-term survey of natural populations of *Drosophila* around Rochester, New York, has revealed that the prevalence of parasitism is significantly correlated between the four host species through time (fig. 5). That is, when parasitism is high in one *Drosophila* species, it tends to be high in all of them (Jaenike 2002). There are probably two causes acting in combination for this. First, the variation in rainfall discussed above probably affects the densities of all four host species similarly, as the mushrooms produced following abundant rainfall are used as breeding sites by all four species. Thus, population densities of the different host species probably vary in parallel through time. Second, the different species exhibited substantial interspecific aggregation across mushroom breeding sites; mushrooms yielding high numbers of one species tend to yield high numbers of the others (fig. 6; Worthen and McGuire 1988; Jaenike and James 1991). Other field studies also reveal that extensive sharing of fungal species among different species of mycophagous *Drosophila* is characteristic of all temperate region communities that have been examined (Shorrocks and Charlesworth 1980; Lacy 1984; Kimura and Toda 1989; Worthen et al. 1996). Because mushrooms are the sites of nematode infection, the sharing of mushrooms between species ensures a high level of interspecific transmission of these parasites. Consequently, if one species experiences a particularly high level of parasitism at some time, it will pass infective stages of these parasites to the other host species via the mush-

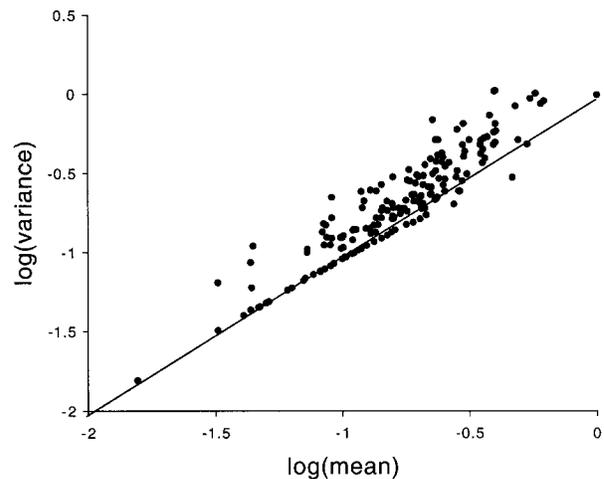


Figure 4: Statistical distribution of motherworms among flies. The mean and variance in the number of motherworms per fly is plotted, where each point represents a monthly collection of one *Drosophila* species (*Drosophila falleni*, *Drosophila putrida*, or *Drosophila neotestacea*) made from 1984 through 1998 in Monroe County, New York. Because the mean \approx variance in most cases, this indicates an approximately random dispersion of motherworms among flies. Redrawn from Jaenike 2002.

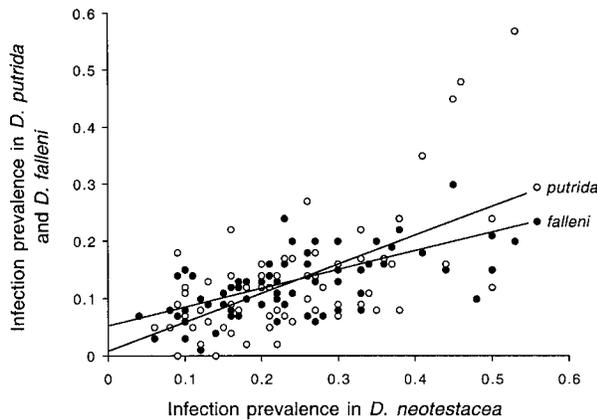


Figure 5: Correlation between *Drosophila* species in the prevalence of *Howardula* infection in collections made from 1984 through 1998 in Monroe County, New York. Each point represents the flies collected during the same month. Fitted lines are regressions of infection prevalence in either *Drosophila falleni* or *Drosophila putrida* as a function of prevalence in *Drosophila neotestacea*. The slopes of both regressions are significantly >0 ($P < .001$). Redrawn, with permission of the publisher, from Jaenike 2002.

rooms they share as breeding sites. In effect, this is a form of apparent competition (Holt 1977) in that one host species can exert an adverse effect on another via a shared parasite species. We discuss the community-level effects of such apparent competition in the next section. As a result of interspecific transmission and density-driven variation in parasite transmission, the prevalence of parasitism varies coordinately across the different host species. The use of the same individual mushrooms by different species of mycophagous Diptera opens up opportunities for interspecific transmission of nematodes. Over the short term, this results in a nematode lineage passing back and forth between its various host species, thus inhibiting the formation of host races (Jaenike and Dombeck 1998). Over evolutionary timescales, however, rare transmission events might initiate shifts to entirely new groups of host species. It will be interesting to determine the extent of host shifts that have occurred over evolutionary time between the nematodes that parasitize various families of mycophagous Diptera.

One question of considerable interest in host-parasite dynamics is whether parasites can regulate their host populations at densities below those set by resource availability. For simplicity, assume that the only fitness effect that *H. aoronymphium* has on its hosts is female sterility. Suppose that the average mushroom can support the complete development of K *Drosophila* larvae (half of which are females) and that the basic reproductive rate of the emergent female flies is R . Now consider the effect of

nematode parasites. Let the threshold population density of flies per mushroom be N_T ; that is, if the actual number of flies per mushroom is greater than N_T , then the rate of parasite population growth is positive. Thus, the parasite population density will increase until the actual number of flies per mushroom (N) is equal to the threshold density. Therefore, if $(1/2)N_T R < K$, then the *Drosophila* population density will be held at a level below which it becomes resource limited (Jaenike 1998). A field cage experiment on the dynamics of *H. aoronymphium* and *Drosophila* dynamics showed that the parasites exhibited rapid population growth even at *Drosophila* densities below those at which larval resources were limiting (Jaenike and Anderson 1992). Thus, at least in a simple homogeneous environment like a field cage, *H. aoronymphium* appears to be capable of regulating their *Drosophila* host populations. It remains to be seen whether the same would be true in a spatially complex environment.

A Possible Keystone Effect of *Howardula aoronymphium*

The concept of keystone species was introduced by Paine (1966), who showed that a predatory starfish, by preying selectively on competitively dominant mussels, opened up space for other, competitively inferior species in the intertidal zone. We have obtained evidence suggestive of a sim-

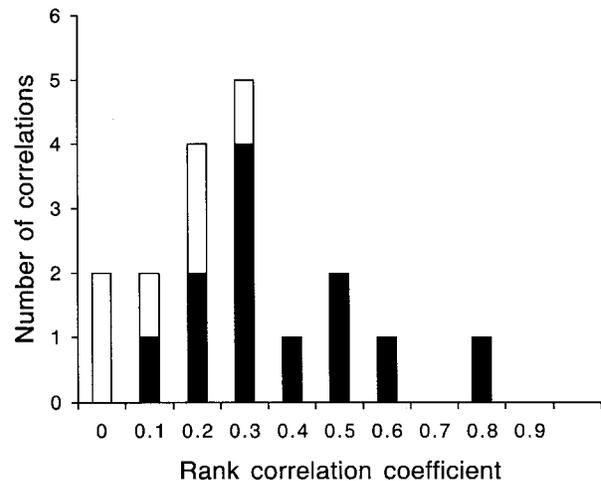


Figure 6: Correlations between *Drosophila* species (*Drosophila falleni*, *Drosophila neotestacea*, and *Drosophila putrida*) in the number of emergent flies per mushroom (data from Jaenike and James 1991). Data are based on six independent field collections of mushrooms. Positive correlations indicate that mushrooms yielding high numbers of one *Drosophila* species tended to yield high numbers of other species. Significant correlations shown in black; nonsignificant correlations shown in white.

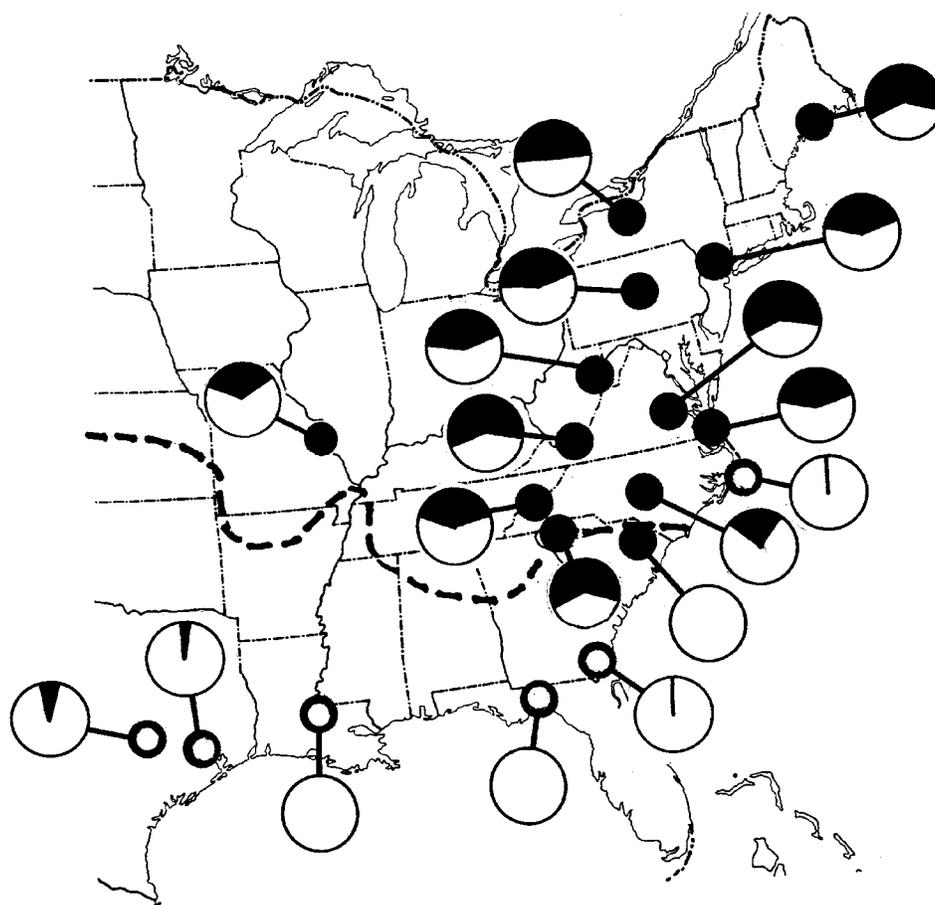


Figure 7: Apparent keystone effect of *Howardula aoronymphium*, as revealed by *Drosophila* community composition in relation to *Howardula* distribution. Small circles indicate presence (solid) or absence (open) of *H. aoronymphium* in the eastern United States. The dashed line is the 27°C July isotherm, south of which *H. aoronymphium* is absent. The pie diagrams indicate the relative abundance of mycophagous *Drosophila* belonging to the *quinaria* (black portion) and *testacea* (white portion) species groups. Reprinted, with permission of the publisher, from Jaenike 1995.

ilar, though less dramatic, effect involving *Howardula aoronymphium* as a keystone species.

As mentioned above, we have found that in eastern North America at least, mushrooms yielding high numbers of one species tend to yield high numbers of the others (Jaenike and James 1991). Similarly, Worthen et al. (1996) have shown that mycophagous Diptera exhibit nested patterns of resource utilization, with fly species being added as mushroom size increases. Consequently, the larvae of the different species frequently co-occur within mushrooms. Furthermore, experimental field studies in New York and Virginia involving manipulation of resource levels have clearly shown that *Drosophila* larvae often experience food limitation in nature (Grimaldi and Jaenike 1984; A. C. James, unpublished data). Resource limitation is not evident in all mushrooms, but it is clearly intense in some. The interspecific aggregation of larvae across

mushrooms indicates that competition for resources occurs both within and between species.

How might *H. aoronymphium* affect the dynamics of these competitive interactions? At the population level, *H. aoronymphium* has a much greater effect on the *testacea* group species (*Drosophila neotestacea* and *Drosophila putrida*) than on the *quinaria* group species (*Drosophila falleni* and *Drosophila recens*) because the prevalence of parasitism and the reduction in the fertility of female flies is considerably greater for the *testacea* group. Thus, all else being equal, one might expect that the presence of *H. aoronymphium* would tilt the competitive edge toward the *quinaria* group. Biogeographical patterns support this hypothesis. In virtually all areas in the eastern United States where *H. aoronymphium* is absent, the *testacea* group (specifically *D. putrida*) greatly outnumbers the *quinaria* group flies. In striking contrast, where *H. aoronymphium* is pre-

sent, the two species groups are consistently about equally abundant (fig. 7; Jaenike 1995). This pattern suggests that *H. aoronymphium* may reduce the competitive advantage of the *testacea* group species sufficiently to allow coexistence of the *quinaria* group species. Thus, a parasite that is deleterious to infected individuals of *D. falleni* may actually benefit *D. falleni* populations, in terms of both relative and absolute abundance, through its even more adverse effects on *D. falleni*'s competitors.

The presence or absence of *H. aoronymphium* is correlated with temperature. In laboratory studies, we have found that motherworm growth, reproduction, and transmission of *H. aoronymphium* are severely curtailed above 27°C (Jaenike 1995). Because the life cycle of these nematodes is completely tied to that of their *Drosophila* hosts, a population of *H. aoronymphium* may become extinct in just one generation at a temperature above 27°C. Because July is the hottest month of the year, July temperatures probably determine the southern limit of *H. aoronymphium*'s range. In our collections throughout the eastern United States, from Maine and Michigan down to Florida and Texas, we have found *H. aoronymphium* present in all localities (except on one offshore island) north of the 27°C July isotherm and absent in all areas south of the 27°C July isotherm. The offshore island (Cape Hatteras, N.C.) is particularly interesting because it is the only area north of the 27°C July isotherm lacking *H. aoronymphium*, and it is also the only area north of this isotherm in which the *testacea* group greatly outnumbers (by a ratio of 50 : 1) the *quinaria* group flies. Searches for other islands in the northern part of the country lacking *H. aoronymphium* have not been successful. Thus, while the biogeographical patterns are strongly suggestive of a keystone effect of *H. aoronymphium*, we cannot yet rule out temperature as the actual cause of the changes in the relative abundance of the *testacea* and *quinaria* groups.

If we assume that the keystone effect is real, how might these nematode parasites facilitate the coexistence of different host species? We suggest two categories of hypotheses, one based on population dynamics and the other on evolutionary changes. Our population dynamic hypothesis is based on the prediction that the prevalence of parasitism and, hence, the impact of parasites on a host population will increase as a function of host density. As mentioned above, the numbers of adult *Drosophila* emerging from individual mushrooms are significantly aggregated (Jaenike and James 1991). The intraspecific aggregation is due to two factors: the aggregated distribution of ovipositing females across mushrooms and the laying of multiple-egg clutches by individual females. We have also found that the level of intraspecific aggregation, as measured by the log (variance) : log (mean) ratio of emergent fly numbers, increases with the mean number of emergent flies per

mushroom. This is likely due to changes in the number of ovipositing females per mushroom, rather than changes in clutch size, as a function of *Drosophila* population density. Although the numbers of emergent flies per mushroom are correlated between the different *Drosophila* species, intraspecific aggregation is generally greater than interspecific aggregation, especially in comparisons between species from different species groups (e.g., *D. putrida* vs. *D. falleni*). As a consequence of these patterns, and because the prevalence of parasitism increases with the number of flies per mushroom (Jaenike and Anderson 1992), an increase in the density of one *Drosophila* species will bring about a greater increase in intraspecific aggregation than in interspecific aggregation. This assumes that parasitized (and, therefore, at least partially sterile) females are attracted to the same mushrooms as those that are unparasitized. Since nematodes are transmitted via mushrooms, an increase in the density of one species may result in increased parasite transmission to that species in comparison with other host species. Thus, a numerically dominant species may suffer greater rates of parasitism by *H. aoronymphium*. In support of this hypothesis, Gillis and Hardy (1997) found that in European collections of mycophagous *Drosophila*, there is a positive correlation between relative abundance of the different host species and the prevalence of *H. aoronymphium* parasitism. In this manner, these parasites might facilitate the coexistence of several competing host species. Although this finding supports the population dynamic hypothesis, it is also possible that abundant host species are infected with more than one nematode species, one of which may be a specialist on that host. In eastern North America, the taxonomic status and host ranges of *Howardula* nematodes have been well characterized. As a result, we have found that *D. falleni* is infected with the generalist *H. aoronymphium* and another as yet undescribed species of *Howardula* (Jaenike 1996a). Molecular markers can be used to determine whether such cryptic species of *Howardula* occur in other regions.

Another means by which *H. aoronymphium* could promote the coexistence of competing species is via evolutionary tracking of numerically dominant *Drosophila* hosts. Rapid parasite evolution might indirectly favor whatever species have been rare in a community. We explore this possibility in the next section.

Evolutionary Tracking of Hosts by *Howardula aoronymphium*?

Our evolutionary hypothesis for keystone effects of parasites assumes that parasites become specifically adapted to the host species that are currently most abundant. As a result, parasites would have the greatest deleterious ef-

fects on populations of the more abundant host species. For a parasite species to respond evolutionarily to changes in the relative abundance of hosts, the parasites must harbor genetic variation that differentially affects their ability to use the various host species, as manifested by variation in infection rates and/or virulence to these hosts.

We have conducted two experiments in a search for such genetic variation in *Howardula aoronymphium* (Jaenike and Dombeck 1998). In the first, we derived 35 isofemale strains of *H. aoronymphium* from sympatric populations of *Drosophila falleni*, *Drosophila putrida*, and *Drosophila neotestacea*, and then we tested the ability of these nematodes to infect and develop in these three host species. We found that nematodes obtained from any one host species were no better able to use that host species than were nematodes obtained from the other hosts. Both host-specific infection rates and motherworm size, which is positively correlated with parasite reproduction and negatively correlated with host reproduction (Jaenike 1996b), did not vary among the nematodes obtained from the different host species. Thus, our survey of isofemale strains revealed no evidence that the *H. aoronymphium* population comprises a set of host races.

In the second experiment, we established a large laboratory population of *H. aoronymphium* with flies harboring ~700 inseminated motherworms, approximately 99% of which were obtained from *D. falleni*, *D. putrida*, and *D. neotestacea* (1% were obtained from *Drosophila recens*). The offspring from this base population were used to establish replicate infected populations of either *D. putrida* or *D. falleni*. The nematodes were maintained solely on one host species for 25 generations. This maintenance regime was designed to select for ability to infect and reproduce in one particular host species. In assays of host specificity conducted after 11 and 25 generations of selection, we found no evidence of response to selection. For instance, the nematodes maintained on *D. putrida* were no better at using this host than were the nematodes maintained on *D. falleni*.

The results of the survey of isofemale strains and the selection experiment on *H. aoronymphium* indicate that populations of these parasites, at least in Upstate New York, harbor little genetic variation for differential adaptation, as measured by infection rate and motherworm size, to the different species within its normal host range. One possible explanation for these results is that general-purpose genotypes have been selected during the evolutionary history of *H. aoronymphium*, resulting in little adaptive polymorphism within populations (Jaenike and Dombeck 1998). Selection for host generalism might result from the correlated epidemiology of the different host species. Because of the interspecific aggregation of *Drosophila* species across individual mushrooms (discussed in "Pop-

ulation Dynamics"), the nematodes passed by one host species must frequently encounter as potential mates the nematodes transmitted by other host species, as well as the other hosts themselves. With widespread opportunities for random mating, and if nematodes randomly infect the larvae of host species encountered within mushrooms, then the conditions for the maintenance of an adaptive polymorphism are expected to be very stringent (Maynard Smith and Hoekstra 1980). While having opportunities for parasite transmission between host species may favor host generalism, it does not prevent the persistence of host specialists, as there are at least two host-specialist allantonematid nematodes that parasitize these *Drosophila* in the eastern United States (Jaenike 1996a; Poinar et al. 1997).

An alternative explanation is that the lack of genetic variation in New York populations of *H. aoronymphium* is due to the founder effect and also to the fact that this species recently colonized North America. In support of this idea, we have found no mtDNA or rDNA (ITS1) sequence variation in either North American or European populations of *H. aoronymphium* (S. J. Perlman and J. Jaenike, unpublished data). Furthermore, the North American and European sequences are identical, but they differ somewhat from Japanese sequences. These data suggest that the geographical ranges of these parasites may be highly dynamic over evolutionary time periods. The selective (general-purpose genotype) and historical (founder effect) hypotheses for the paucity of genetic variation in North American populations of *H. aoronymphium* could be distinguished by conducting similar surveys of adaptive polymorphism and DNA sequence variation in other populations or species of nematodes that parasitize multiple species of mycophagous *Drosophila*.

The apparent lack of host-specific adaptation within local populations of *H. aoronymphium* suggests that evolutionary change with respect to host utilization is likely to occur on a much longer timescale than competitive exclusion between *Drosophila* species. Thus, the apparent keystone effect of *H. aoronymphium* is probably not likely due to rapid evolutionary tracking of changes in relative abundance of the different host species.

Patterns of Virulence in the *Drosophila testacea* Group

Both the population dynamic and the evolutionary models of parasite keystone effects consider how the infection rate of a host species depends on its relative abundance. However, the net direct effect of a parasite on a given host species depends not only on the prevalence of infection but also on parasite virulence. Indeed, host-specific effects on female fertility appear to be a major factor in the potential keystone role of *Howardula aoronymphium* because

the competitively superior host species (*Drosophila putrida* and *Drosophila neotestacea*) are completely sterilized by these nematodes, while the competitively inferior species (*Drosophila recens* and *Drosophila falleni*) are much less affected. Over the ecological timescales that would be relevant for the population dynamic model, virulence is likely to be a constant for any given host-parasite pair. However, as we show below, virulence is likely to vary over longer time periods because of both host and parasite evolution.

In order to understand what determines sterility in the *H. aoronymphium*–*testacea* group association, we performed controlled infections of all four species of the *Drosophila testacea* group (*D. putrida* and *D. neotestacea* from North America, *D. testacea* from Europe, and *D. orientacea* from Japan), with *H. aoronymphium* from Europe, America, and Japan (S. J. Perlman and J. Jaenike, unpublished manuscript). All four species are infected by *H. aoronymphium* in the wild, yet only the two North American species are sterilized by their nematodes. The experimental infections yielded surprising results: sterility appears to be determined by the host species and not by the parasite. Hosts that are sterilized by their nematodes in the wild (i.e., *D. putrida* and *D. neotestacea*) are sterilized by all nematodes in the *H. aoronymphium* complex. Hosts that are not rendered sterile by their local nematode are not sterilized by any other nematodes in the complex (table 2). This suggests that there are two classes of host in the *testacea* group: species that are susceptible to parasite-induced sterility (*D. putrida* and *D. neotestacea*) and species that are resistant to it (*D. orientacea* and *D. testacea*). The high level of virulence evident in associations between *D. putrida* and *D. neotestacea* and North American *H. aoronymphium*—the basis for keystone effects described above—appears to be due to lack of host resistance rather than evolutionary tracking by parasites. It is unclear why *D. putrida* and *D. neotestacea* are so susceptible to parasite-induced sterility while *D. orientacea* and *D. testacea* are not. One possibility, which we are currently exploring, is that *H. aoronymphium* has only recently colonized North

America and that North American *testacea* group species have not yet evolved a tolerance to infection that would confer partial fertility. A recent colonization would be consistent with the apparent lack of genetic variation in North American *H. aoronymphium*, as mentioned above.

The general susceptibility to parasite virulence of the North American *D. putrida* and *D. neotestacea* is further highlighted in comparisons of parasite-induced mortality (table 2; S. J. Perlman and J. Jaenike, unpublished manuscript). Our *testacea* group–*H. aoronymphium* cross-infections reveal that all host species infected with local (sympatric) nematodes show similar reductions in adult life span of ~40%–45%. However, both *D. putrida* and *D. neotestacea* suffer ~80% reductions in life span when infected with allopatric nematodes. Because parasite-induced mortality is likely to be even greater in the wild (Jaenike et al. 1995), most infected hosts would probably die before transmitting any infective juvenile nematodes. We would then expect to see strong selection for reduced virulence (as manifested in parasite-induced mortality) in these nematodes. These experiments demonstrate significant geographic variation and thus evolutionary lability in virulence. These findings stand somewhat in contrast to those of Ebert (1994), whose studies of *Daphnia* and microsporidian parasites revealed that sympatric host-parasite combinations resulted in greater virulence than allopatric combinations. If the *Daphnia*-microsporidia associations are at evolutionary equilibrium, this would suggest that the correlations between virulence and parasite fitness differ between these microsporidia and *Drosophila*-parasitic nematodes.

The lack of parasite-induced sterility in European and Japanese species of the *testacea* group by *H. aoronymphium* suggests that these nematodes are less likely to play a keystone role in those regions. If virulence is evolutionarily variable, it is likely that the community-level keystone effects of these parasites can change over evolutionary time periods. If, some time in the future, *D. putrida* and *D. neotestacea* evolve resistance or tolerance to *H. aoronym-*

Table 2: Virulence of the *Howardula aoronymphium* complex to *Drosophila* species in the *testacea* group species, as determined in experimental laboratory infections

Host species	Fertility ^a			Longevity ^b	
	North America	Europe	Japan	North America	Japan
<i>Drosophila neotestacea</i> (North America)	Sterile (0%)	Sterile	Sterile	55%–60%	20%
<i>Drosophila putrida</i> (North America)	Sterile	Sterile	Sterile	55%–60%	20%
<i>Drosophila testacea</i> (Europe)	60%	No difference	No difference	55%–60%	55%–60%
<i>Drosophila orientacea</i> (Japan)	65%	Not infected	55%	55%–60%	55%–60%

Note: The most virulent interactions are indicated in bold.

^a Fertility of infected females in comparison with uninfected females. Fertility determined by the average number of mature eggs (stage 10 or later) per female.

^b Longevity of infected adults in comparison with uninfected adults.

phium infection and sterility, then these nematodes will play less of a keystone role.

Determinants of Host Range

The community-level effects of a parasite are determined not only by the strength of individual trophic connections but also by the number of host species actually used in nature. It is therefore of interest to identify the factors that determine a parasite's actual host range. To illustrate our approach to this question, we focus on *Howardula aoronymphium* in eastern North America. The four known host species of *H. aoronymphium* in this region—*Drosophila falleni*, *Drosophila recens*, *Drosophila neotestacea*, and *Drosophila putrida*—are connected geographically and ecologically to a larger community of other *Drosophila* species. Given the potential of the known hosts of *H. aoronymphium* to transmit nematodes to other host species, what determines which *Drosophila* species will fall within the realized host range of these nematodes?

To a first approximation, a potential host species will be included within the host range of a nematode if the hosts and parasites are sympatric, if the potential host is intrinsically suitable for infection and reproduction of the parasites, and if various ecological conditions are permissive for nematode transmission. The intrinsic suitability of a potential host species for a particular species of parasite may depend on host phylogeny, with some clades being, for example, biochemically or physiologically more suitable as hosts than others. In addition, intrinsic suitability might depend on derived characteristics of individual species, such as evolved resistance to infection. The role of intrinsic suitability as a determinant of host range will be discussed in the next section. Ecological suitability of a host species could be a function of the temperatures experienced by the flies and, thus, of the temperatures to which nematodes would be exposed, the suitability of various breeding sites for nematode transmission, population density (as parasite reproductive rate is likely to depend on host density), and the presence of other parasites competing for that host (Dobson 1985; Anderson and May 1991).

The actual host range of *H. aoronymphium* in eastern North America reflects the influence of several of these factors (fig. 8). First, the phylogeny of potential hosts constrains the host range of these nematodes. In experimental infections in the laboratory, we have found that *H. aoronymphium* is extremely limited in its ability to infect any flies outside the subgenus *Drosophila*, and within this subgenus, it does best on flies belonging to the *quinaria*, *testacea*, and *cardini* groups (S. J. Perlman and J. Jaenike, unpublished data). *Drosophila tripunctata* is broadly sympatric with these susceptible species and often emerges

from the same mushrooms, yet we have never found an infected fly in nature (J. Jaenike, unpublished data). Laboratory assays reveal that it is almost completely resistant to infection with *H. aoronymphium* (S. J. Perlman and J. Jaenike, unpublished data). Although the *tripunctata* group is closely related to the *quinaria*, *testacea*, and *cardini* groups (Throckmorton 1975), the exact phylogenetic relationships among them have not yet been determined. Therefore, it is not yet known whether resistance to parasitism in *D. tripunctata* is a derived state. Among the species that are susceptible to infection by *H. aoronymphium*, we have found considerable differences in probability of infection, as determined in controlled laboratory infections (Jaenike and Dombeck 1998). Thus, it is likely that intrinsic suitability is an evolutionarily labile trait, but not one that is so labile that there is no phylogenetic signal to host range.

Among the ecological filters restricting the host range of *H. aoronymphium*, the most obvious is temperature. Our laboratory assays indicate that two members of the *cardini* species group, *D. cardini* and *D. acutilabella*, are potentially excellent hosts for *H. aoronymphium*, yet they are never infected with this species in nature. *Drosophila acutilabella* occurs in Florida, as well as some Caribbean islands, while *D. cardini* is found from Florida to Texas. Both *D. falleni* and *D. putrida*, two of the actual hosts of *H. aoronymphium*, are sympatric with *D. cardini* and *D. acutilabella* in the south and thus could potentially transmit *H. aoronymphium* to them. However, this region of sympatry is well south of the southern limit of *H. aoronymphium*'s range in the eastern United States, which is limited by high temperature (see "A Possible Keystone Effect of *Howardula aoronymphium*"). Thus, the temperatures in regions inhabited by *D. cardini* and *D. acutilabella* prevent their use as hosts by *H. aoronymphium*.

We have also found a correlation between *Drosophila* breeding site and parasitism by *H. aoronymphium*. As discussed above, the normal host species of *H. aoronymphium* breed principally in mushrooms. However, all four of the host species—*D. falleni*, *D. recens*, *D. neotestacea*, and *D. putrida*—also breed occasionally in skunk cabbages. Interestingly, there are several species within the *quinaria* group, most notably *D. quinaria* and *D. palustris*, that often breed in skunk cabbages but which never use mushrooms (Grimaldi and Jaenike 1983). Although our laboratory assays indicate that both of these species are suitable hosts for *H. aoronymphium*, we have never found a wild-caught individual of either of these species infected with these nematodes (J. Jaenike, unpublished data). The occasional use of skunk cabbages by the normal hosts of *H. aoronymphium* suggests that infective nematodes may sometimes be present in skunk cabbages. *Drosophila limbata*, a European species that also breeds in decaying vegetation,

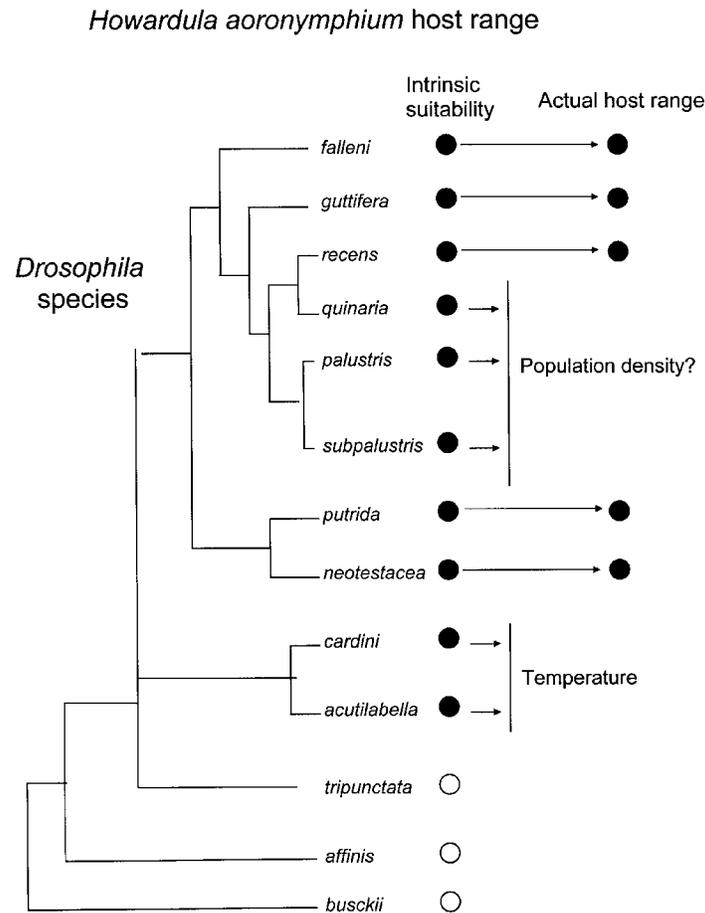


Figure 8: Phylogenetic and ecological determinants of *Howardula aoronymphium* host range in eastern North America. Intrinsic suitabilities were determined by controlled laboratory infections. Species outside the subgenus *Drosophila* are generally unsuitable as hosts (*open*), while all tested species within the *quinaria*, *testacea*, and *cardini* groups are intrinsically suitable (*solid*). Various ecological filters, such as temperature and host population density, restrict actual host range to a subset of intrinsically suitable species.

is also not parasitized by *H. aoronymphium* in nature (Klarenberg and Kersten 1994; Gillis and Hardy 1997), yet it too can readily be infected in the laboratory. Why, then, do decaying-vegetation breeders such as *D. quinaria* and *D. palustris* appear not to be parasitized in nature? One possibility is that they are, but at rates too low to be detected in samples of several hundred flies.

Alternatively, skunk cabbages may be unsuitable substrates for nematode transmission. That some breeding sites may be unsuitable is indicated by the finding that flies that develop in mushrooms containing high concentrations of α -amanitin, such as *Amanita virosa*, are not parasitized by *H. aoronymphium* because amanitin is toxic to these nematodes (Jaenike 1985). To test whether skunk cabbages are unsuitable, we introduced laboratory-reared, parasitized adults of *D. quinaria* into cages that enclosed skunk cabbages in the field. These flies were then allowed

to oviposit on the skunk cabbages, and the resulting emergent flies were collected and found to be parasitized with *H. aoronymphium*. Thus, skunk cabbage is a permissive substrate for nematode transmission to *D. quinaria* in the field (S. J. Perlman and J. Jaenike, unpublished data).

A final hypothesis to explain why *D. quinaria* and *D. palustris* are not infected concerns population density. Because skunk cabbages are only occasionally used as breeding sites by *D. recens*, *D. falleni*, *D. neotestacea*, and *D. putrida* (Grimaldi and Jaenike 1983), the populations of *H. aoronymphium* associated with skunk cabbages would be epidemiologically independent of those associated with mushrooms. For reasons not yet known, the densities of *D. quinaria* and *D. palustris* tend to be very low (Grimaldi and Jaenike 1983) and may be below the threshold density required for persistence of a skunk cabbage-associated population of *H. aoronymphium*. This hypothesis could be

tested by manipulating the densities of these potential host species within field enclosures. Furthermore, the dynamics of a parasite population are likely to be determined by the local density of *D. quinaria*, as mtDNA-based Φ statistics indicate that migration of flies between skunk cabbage patches is low (Shoemaker and Jaenike 1997). Consequently, if *H. aoronymphium* were to become extinct within a skunk cabbage patch, reestablishment would require migration of infected *D. quinaria* from other patches. Therefore, a high probability of local extinction of *H. aoronymphium* (caused by low host density) coupled with a low immigration rate of flies (as evidenced by significant Φ_{ST}) could result in global extinction of *D. quinaria*-associated populations of *H. aoronymphium*, as envisioned in models of metapopulation dynamics (Hanski 1994).

Finally, intrinsically suitable host species may be underused if they are also infected with other competing nematode species, as modeled by Dobson (1985). For example, *D. recens* is infected by both the generalist *H. aoronymphium* and a specialist, *Parasitylenchus nearcticus*, that is known only from this one host species. Motherworms of the generalist *H. aoronymphium* are significantly smaller in flies that are also infected with *P. nearcticus* than they are in flies that are not infected with *P. nearcticus*. Also, the specialist *P. nearcticus* uses *D. recens* more effectively than *H. aoronymphium*, as evidenced by greater rates of infection and reproduction in flies infected with only one nematode species. These results suggest that in the absence of other suitable host species, which serve as a source of *H. aoronymphium* to infect *D. recens*, *H. aoronymphium* might be competitively excluded by *P. nearcticus* (Perlman and Jaenike 2001).

Evolution of Host-Parasite Associations

If the biogeographical and ecological conditions are favorable, then the establishment of a new host-parasite association will depend largely on the intrinsic suitability of a potential host species for a given species of parasite. Given the capacity of hosts and parasites to evolve in response to their ongoing interactions, we expect intrinsic suitability to be an evolutionarily dynamic variable. One means by which to gauge the relative timescale over which these evolutionary changes occur is to examine how host and parasite phylogenetic relationships affect various aspects of host-parasite interactions. There have been few experimental studies of the phylogenetic determinants of host range. Previously, Reed and Hafner (1997) found that host suitability of lice on pocket gophers decreased with genetic distance, although their study considered only a small number of species. Tompkins and Clayton (1999) recently showed that barb size in the feathers of swiftlets was a better predictor of host suitability for louse parasites than

was phylogenetic position of the host species. A detailed description of our work on phylogenetic determinants of host range will be presented elsewhere, and here we highlight some of the major findings pertaining to infection of various *Drosophila* species by allantonematid nematodes.

We first consider how the phylogenetic position of different *Drosophila* species affects potential host range of a given nematode species. We assayed the ability of different nematode species to infect, grow, and reproduce in a host under controlled laboratory conditions. We also assayed virulence by measuring the reduction in fecundity of infected females. In all cases, our assays lead to high levels of infection of the natural hosts of the various nematode species. We infected a set of 24 species of *Drosophila* that either breed in mushrooms or are closely related to species that do. Host phylogeny is based on rDNA (28S) and mtDNA sequence data obtained by G. Spicer (unpublished data). The nematodes we have assayed are derived from North America, Europe, and Japan and represent six distinct species or populations. Our nematode phylogeny was based on mtDNA and rDNA (18S and ITS1) sequence.

Our principal findings can be summarized as follows. First, the phylogenetic position of a potential host species generally has some effect on its suitability to a given nematode species, but the effect of host phylogeny on nematode host range is quite imprecise in most cases. Nematodes are unable to infect distant relatives of their natural host, but among the species that are more closely related, many are potentially suitable hosts. Even a specialist nematode such as *Parasitylenchus nearcticus*, which is known from only one host species in the wild, can infect a large range of *Drosophila* in the laboratory. Thus, a geographical range expansion of *Drosophila recens*, the host of *P. nearcticus*, might lead to the incorporation of additional *Drosophila* species into the host range of these nematodes. Having a large potential host range, along with the frequent co-occurrence of different *Drosophila* species within mushrooms, provides the basis for both host range expansion as well as host switching. One evolutionary manifestation of these opportunities for transmission between *Drosophila* species is the lack of parallel cladogenesis in comparisons of *Howardula* and *Drosophila* phylogenies (S. J. Perlman and J. Jaenike, unpublished data).

Second, *Drosophila* species appear to vary in their general susceptibility to nematode parasitism. In our experimental infections, certain species appear to be inherently susceptible to infection by a variety of distantly related allantonematid nematode species, while others appear to be generally resistant to infection. Among the possible mechanisms that might affect general resistance are attractiveness of fly larvae to infective nematodes, larval irritability to nematodes attempting invasion, and resistance of larval cuticle to nematode penetration.

Table 3: Susceptibility of *Drosophila testacea* group species to *Howardula aoronymphium* infection

Host species	<i>Howardula aoronymphium</i> population		
	North America	Europe	Japan
<i>Drosophila neotestacea</i> (North America)	High	High	Moderate to high
<i>Drosophila putrida</i> (North America)	High	High	Low to moderate
<i>Drosophila testacea</i> (Europe)	High	Moderate	High
<i>Drosophila orientacea</i> (Japan)	Low to moderate	Not infected	High

Note: Susceptibility was determined in experimental laboratory infections. High is defined as >50% of flies infected; moderate is 10%–50% infected; low is <10% infected.

Furthermore, sister species of *Drosophila* can differ greatly in their susceptibility to infection by a given nematode species. For example, the European species *Drosophila testacea* is much more susceptible to *Howardula aoronymphium* from Europe (and North America) than is the Japanese species *Drosophila orientacea* (table 3). These sister species can produce fertile hybrid progeny in lab crosses (Grimaldi et al. 1992). These findings indicate that resistance (or susceptibility) to infection to a particular nematode species can evolve quite rapidly, and this is likely to blur the effect of host phylogeny on suitability for a given nematode.

Third, closely related nematodes can differ greatly in their ability to infect a particular host species. For example, *Drosophila putrida* is readily infected with *H. aoronymphium* from North America but not with Japanese *H. aoronymphium*. The Japanese *H. aoronymphium* infect *D. orientacea* at high frequencies in our experimental infections, but *H. aoronymphium* from Europe cannot infect these flies (table 3). These findings suggest that the potential host range of nematodes can evolve relatively quickly. As a consequence of this, the potential host ranges of different species of *Howardula* are not readily predictable from their phylogenetic relationships. Furthermore, evolutionary changes in the ability to use one host species may bring about correlated changes in ability to use related hosts, thus multiplying the potential for rapid host range evolution. For instance, both the European and the North American populations of *H. aoronymphium* can infect a broad range of the *Drosophila* species tested in our assays. In contrast, *H. aoronymphium* from Japan has a much smaller potential host range (S. J. Perlman and J. Jaenike, unpublished data).

Finally, as discussed earlier, parasite virulence can be highly variable within a closely related set of host and parasite species, depending on both the specific host and parasite involved in the association.

Conclusions

The nature of the associations between mycophagous *Drosophila* and their nematode parasites represents the inter-

play between certain predictable ecological constants and other variables that may change over evolutionary timescales. Among the ecological factors are the polyphagy and interspecific aggregation of these *Drosophila* across breeding sites, resulting in ample opportunities for transmission of parasites from one host species to another. These patterns of mushroom use by different species of *Drosophila* can also result in intense larval competition between these species. The existence of such interspecific competition sets the stage for a potential keystone role that could be played by nematode parasites. A second ecological constant is likely to be the causal connections between climate, mushroom production, *Drosophila* densities, and parasitism. The relation between *Drosophila* densities and rates of parasitism establishes the potential for these nematodes to play a role in the regulation of host populations.

However, whether parasites do regulate their hosts or serve as keystone species depends on their host range and virulence, among other things, both of which can be highly dynamic on an evolutionary timescale. Furthermore, it appears that these parasites can undergo vast and rapid range expansions, resulting in the sudden establishment of new host-parasite associations because several of the parasites we have studied have broad potential host ranges. These findings indicate that the ecological effects of these parasites are likely to vary considerably among communities. Thus, the capacity of these parasites to regulate host populations and the keystone community-level effects, as seen in eastern North America, may be but one point in a complex spectrum of interaction strengths.

It is well to bear in mind that the *Drosophila* and nematodes we have been considering represent an incredibly minute fraction of the species diversity and biomass of the ecological communities in which they reside. Yet we have no reason to think that the complexity of the interactions between these host and parasite species is in any way atypical.

Acknowledgments

This research was supported by grants from the National Science Foundation including, most recently, DEB-0074141.

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