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Inequalities in size and intensity-dependent growth in a mermithid nematode parasitic in beach hoppers

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Abstract

Inequality in body sizes is a common feature in populations of helminth parasites, with potential consequences for egg production and population genetics. Inequalities in body lengths and the effects of intraspecific competition on worm length were studied in a species of mermithid nematode parasitic in the crustacean *Talorchestia quoyana* (Amphipoda: Talitridae). The majority of the 753 worms recovered were relatively small, and an analysis using a Lorenz curve and Gini coefficient suggested that there were no marked inequalities in body lengths among the worms. Total worm length in the 356 infected amphipods (i.e. the sum of the lengths of all the worms in a host) increased steadily as a function of the number of worms per amphipod, whereas the length of the longest worm per amphipod peaked in amphipods harbouring intermediate numbers of worms. This last result was not significantly accounted for by the observed increase in host size with increasing intensity of infection, but resulted from a correlation between worm length and host size. As the number of worms per amphipod increased, the relative sizes of the second-, third-, and fourth-longest worms per host increased markedly. This means that relative inequalities in sizes become less pronounced, i.e. subordinate worms get closer in size to the longest worm, as the number of worms per host increases. The main consequence of this phenomenon is that worm sizes in the mermithid population are more homogeneous than they would be if intraspecific competition had stronger effects on worm growth.

Introduction

Variability in body sizes is a common feature among individual helminth parasites from the same population (Poulin, 1998). It can be extremely pronounced in some cases, reflecting the high degree of phenotypic plasticity displayed by many helminths. For instance, in the nematode *Raphidascaris acus*, a fish parasite, gravid female worms range in mass between 0.7 and 61.2 mg, an almost 90-fold difference in body size between the smallest and largest worm (Szalai & Dick, 1989). Such inequalities in size are not unusual (Dobson, 1986; Shostak & Dick, 1987). Given the aggregation of helminth parasites within their host population, various forms of intraspecific competition for space and other limiting resources have long been known to cause variability in helminth body sizes (Read, 1951; Bush & Lotz, 2000). The resulting size hierarchy typically consists of a few large worms and many small ones.

Inequalities in body size among helminth parasites of the same population can have important implications because parasite body size is almost universally related to fecundity (Poulin, 1998). In nematodes, for instance, larger female worms always produce more eggs per unit time or per lifetime than their smaller conspecifics (e.g. Mössinger & Wenk, 1986; Szalai & Dick, 1989; Sinniah & Subramaniam, 1991). This is also true across species, with larger-bodied nematode species being characterized by higher fecundity than their smaller-bodied sister taxa (Skorping *et al.*, 1991; Morand, 1996). Thus, a size

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hierarchy skewed toward small worm sizes means that the bulk of the eggs produced by a helminth population may come from a small subset of very large worms. This could reduce considerably the effective parasite population size and limit their genetic variability (Dobson, 1986).

This phenomenon has only been investigated in helminths parasitic in vertebrates (Dobson, 1986; Shostak & Dick, 1987; Szalai & Dick, 1989). In some host–parasite systems involving invertebrate hosts, the effects of intensity of infection on helminth growth are likely to be pronounced given the restricted space and limited resources available to growing worms inside these small hosts. For instance, mermithid nematodes maturing inside arthropod hosts can reach huge sizes within small hosts; because of space and nutrient constraints, inequalities in size may occur when two or more worms co-occur in the same host. Here, we investigated inequalities in body size among mermithid nematodes from a crustacean host, the beach hopper *Talorchestia quoyana* (Amphipoda: Talitridae). The mermithid is a new species, to be described elsewhere, that is relatively common in our study area (Poulin & Rate, 2001). Most infected hosts harbour a single worm, but several hosts harbour two or many more worms. Mermithids emerge from their hosts after reaching a relatively large size and mature in the external environment, where they lay eggs before dying (Poinar, 1983). The host is killed during worm emergence. Therefore, if one worm emerges from its arthropod host, other worms sharing the host with it will either die if they are too small, or also emerge and mature at a sub-optimal size if they are large enough. Competition for space and other resources among growing worms within the same hosts can thus result in greatly unequal sizes among adult worms.

Our objectives were to: (i) quantify the inequalities in body size among mermithid worms found in the amphipod *T. quoyana*; and (ii) determine how intensity of infection, i.e. the number of worms per host, influences the growth and size of mermithids. Our study thus combines a quantification of inequalities in size with an attempt to identify the intensity-dependent mechanisms responsible for them.

Materials and methods

Large numbers of the beach hopper amphipod *Talorchestia quoyana* were collected in November 2000 from Long Beach, north of Dunedin, South Island, New Zealand. The amphipods were maintained in moist sand overnight and were killed and preserved in ethanol the day following their capture. Amphipods were individually measured for body length (anterior end of the cephalon to posterior tip of the telson). Each amphipod was also dissected. The number of mermithid worms, if any, inside each amphipod was determined, and each worm was straightened without stretching and measured to the nearest mm.

A Lorenz curve and the Gini coefficient were used to evaluate the degree of inequality in body sizes among all the mermithid worms collected. These methods are described in detail in Weiner & Solbrig (1984) and Dobson (1986), and examples of their use are given in Shostak & Dick (1987) and Szalai & Dick (1989). In brief, a Lorenz curve is obtained by plotting the cumulative worm length against the cumulative number of worm individuals, when worms are ranked from smallest to longest in a sample. The more concave the curve, the greater the degree of inequality in sizes among worms. The Gini coefficient, or G, is an index of this inequality. When $G = 0$, every worm has the same length, but as G tends toward 1 inequalities in size become more pronounced, with increasingly fewer large worms accounting for most of the total worm length. The index was computed following Dobson (1986).

All statistical tests used are standard parametric tests. When comparisons are made among amphipods harbouring different numbers of worms, some infection classes had to be pooled because too few individuals occurred in them. This resulted in six groups of amphipods, those harbouring 1, 2, 3, 4–5, 6–9 and 10–14 worms. A single host was found containing 33 worms; data from this host are included in figures for illustrative purposes but not in the statistical analyses. The longest worm in each amphipod is referred to as the alpha-worm. For some analyses, the length of other worms is expressed as a percentage of the length of the alpha-worm, as a measure of their size relative to the alpha-worm.

Results

A total of 753 mermithid nematodes, ranging in length between 3 and 225 mm, were recovered from 356 infected amphipod hosts. The majority of worms measured between 20 and 80 mm, although several were longer than 100 mm (fig. 1). The Lorenz curve for these worms shows only a moderate degree of concavity

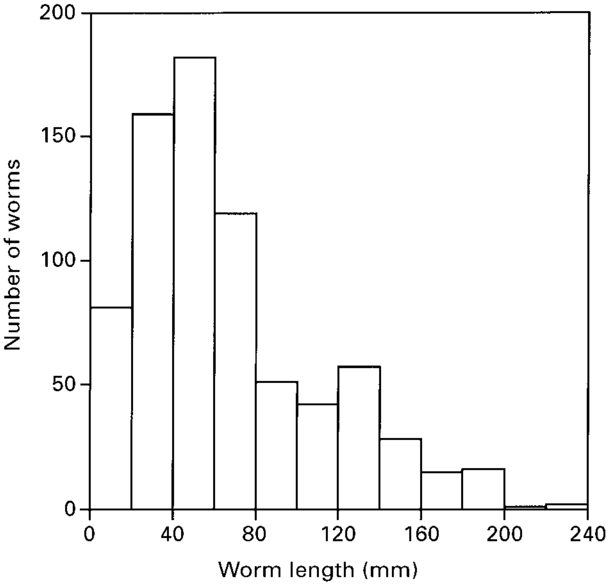

Fig. 1. Frequency (number of worms) distribution of body lengths among 753 mermithid nematodes recovered from 356 infected amphipods *Talorchestia quoyana*.

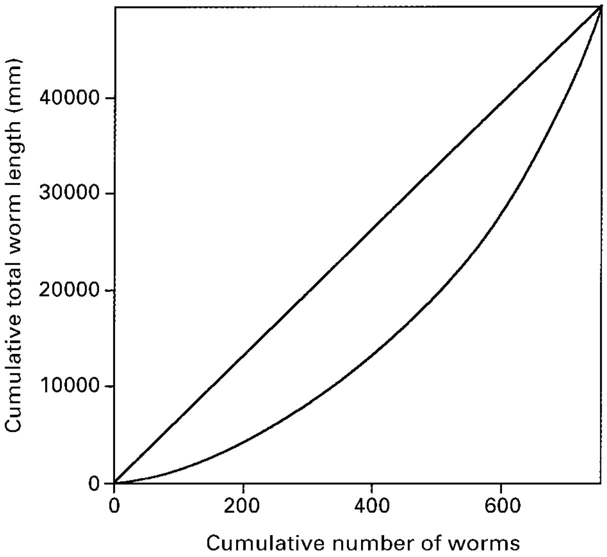

Fig. 2. Cumulative total worm length plotted against the cumulative number of individual worms, for 753 mermithid nematodes infecting the amphipod *Talorchestia quoyana*. The worms were ranked from smallest to longest prior to being cumulated.

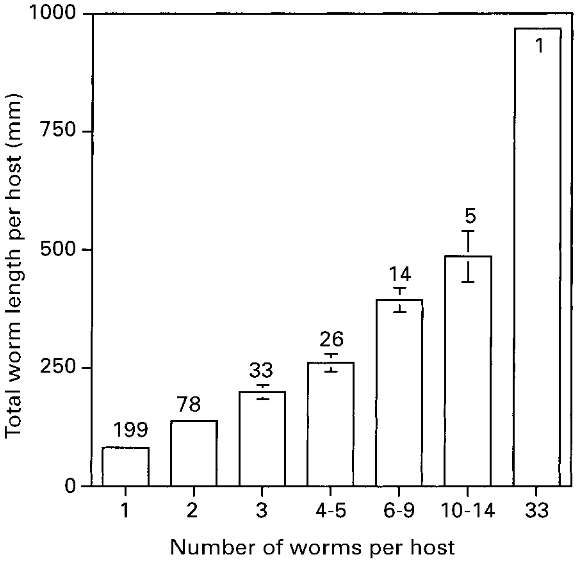

Fig. 3. Total worm length (mean±SE) per host as a function of the number of mermithid worms per amphipod host. Numbers above bars are sample sizes.

(fig. 2). Similarly, the Gini coefficient ($G = 0.431$) obtained from these data suggests that there is no marked inequality in body lengths among the 753 worms in the sample.

The total worm length in the 356 infected amphipods (i.e. the sum of the lengths of all the worms in an amphipod) increased significantly as a function of the

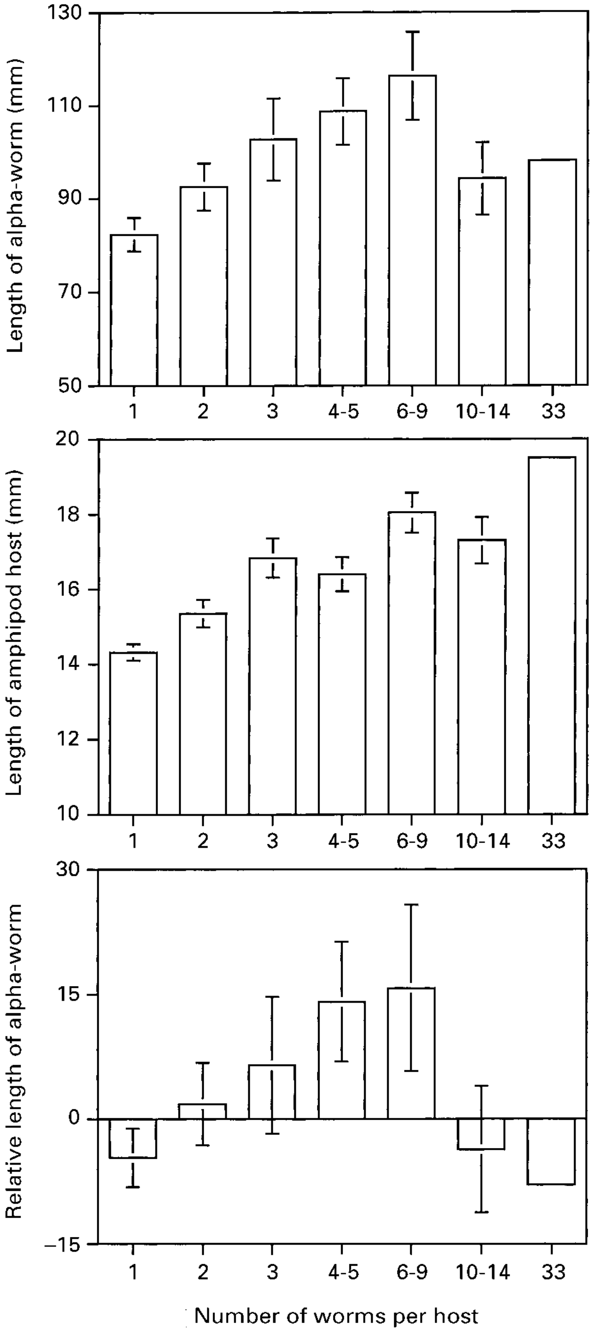

Fig. 4. Length of the longest worm or alpha-worm (mean±SE), host size, and relative length of the alpha-worm corrected for host size as a function of the number of mermithid worms per amphipod host. The relative lengths of the alpha-worms are the residuals of the regression of alpha-worm length versus host size; positive values indicate that worms are longer than expected based on host size, and negative values indicate that worms are shorter than expected. Sample sizes are as in fig. 3.

Table 1. Matrix of partial regression coefficients between the sizes of the longest (alpha) mermithid worms found in an amphipod and the lower ranked worms, and among the lower ranked worms themselves.

	Second longest (157)	Third longest (79)	Fourth longest (46)	Fifth longest (25)	Sixth longest (20)
Alpha-worm	0.467***	0.435***	0.231	0.204	0.008
Second longest worm		0.689***	0.402**	0.504*	0.458*
Third longest worm			0.667***	0.683***	0.718***
Fourth longest worm				0.890***	0.824***
Fifth longest worm					0.979***

*$P < 0.05$; **$P < 0.01$;*** $P < 0.001$.

The coefficients were obtained from multiple regressions in which host size was the other predictor variable; host size only had a significant influence ($r = 0.170$, $P = 0.0164$) in the alpha-worm versus second-longest worm regression (top left hand corner of the matrix). Actual sample sizes are the numbers of amphipods harbouring at least enough worms to be included in the regression, and are given in parentheses.

number of worms per amphipod (ANOVA: $F_{5,349} = 126.5$, $P = 0.0001$), reaching almost 0.5 m in amphipods harbouring 10–14 worms and almost 1 m in the amphipod with 33 worms (fig. 3).

The absolute size of the longest worm, or alpha-worm, found in an amphipod depended on how many other worms shared the host with it (ANOVA: $F_{5,349} = 3.213$, $P = 0.0075$); as a rule, alpha-worms in amphipods harbouring intermediate numbers of worms were longer than single alpha-worms or alpha-worms in heavy infections (fig. 4). However, the size of the amphipod host tended to increase with the number of worms harboured ($F_{5,349} = 8.947$, $P = 0.0001$; fig. 4), a factor which may confound the previous result. To control for host size, we used the residuals of a regression of length of the alpha-worm versus host size ($r = 0.244$, $P = 0.0001$) as measures of the relative size of the alpha-worm corrected for host size. Using these measures, we again found that alpha-worms tended to be longer in amphipods harbouring intermediate numbers of worms (fig. 4), but this result was no longer statistically significant ($F_{5,349} = 1.339$, $P = 0.2472$).

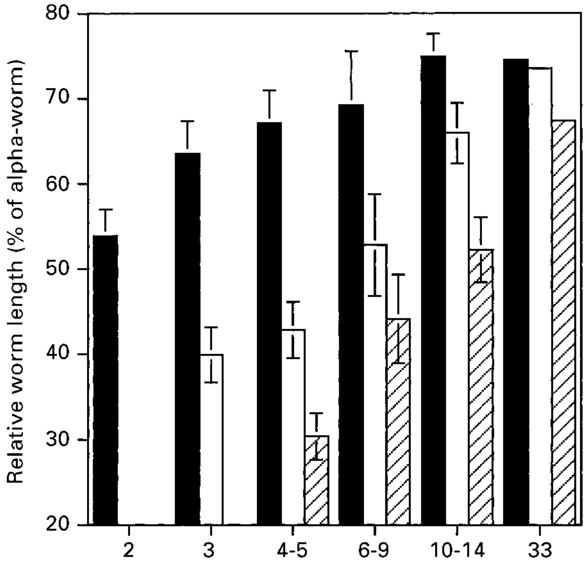

Fig. 5. Relative length (mean±SE) of the second-longest worm (black bars), third-longest worm (open bars), and fourth-longest worm (hatched bars) per host as a function of the number of mermithid worms per amphipod host. The relative length of the second-, third- and fourth-longest worms is expressed as a percentage of the length of the alpha-worm. Sample sizes are as in fig. 3.

When all amphipods with more than one worm are considered together irrespective of their exact worm load, there are almost always strong positive correlations between the size of the alpha-worm and that of lower ranked worms, as well as among the sizes of lower ranked worms. These relationships were assessed using a series of multiple regressions, in which both host size and the size of worms of a certain rank served as predictor variables, and the size of lower ranked worms was the dependent variable (table 1). Their results suggest that host size only affects the size of the second-longest worm (as it did the alpha-worm) but not that of smaller worms, and that the sizes of the various worms within the same hosts are not independent of one another. Thus, relative worm lengths must be used for comparisons among hosts with different worm loads. Using worm sizes expressed as a percentage of the sizes of alpha-worms, we found that as the number of worms per amphipod increases, the relative sizes of the second-longest ($F_{4,151} = 2.879$, $P = 0.0247$), third-longest ($F_{3,74} = 3.986$, $P = 0.0109$), and fourth-longest ($F_{2,42} = 6.314$, $P = 0.004$) worms increased markedly (fig. 5). The same trend was apparent for the fifth- and sixth-longest worms, but was not statistically significant; sample size limitations prevented analyses from being performed on worms of lower ranks. The above result indicates that relative inequalities in size become less pronounced, i.e. subordinate worms get closer in size to the alpha-worm, as the number of worms per host increases.

Discussion

Variability in body sizes is a normal feature of any natural helminth population. In the present study, we looked at a snapshot of the distribution of body lengths in a population of mermithid nematodes. The different worms included in our analyses were probably at different stages of development; subsequent growth might possibly have obscured the patterns observed here. However, because the emergence of one worm from the host precludes the further growth of other worms, we believe that the size variation seen inside the hosts is a good approximation of the size variation among adult worms living freely outside the hosts. Three mermithids emerged from amphipods held in moist sand during our study and survived outside the host until their capture. Data on these worms and their dead hosts were not included here, but the worms measured 95, 135 and 160 mm in length. Of all 356 alpha-worms recovered from amphipod hosts in this study, 157 (44%) were ≥95 mm, and thus potentially capable of emerging from the host, condemning other worms inside that host to either a small adult size or death. The size differences seen inside the host are maintained outside the host, or reduced if the smallest worms die. Therefore, since we only observed modest inequalities in worm size within the amphipod host, it is likely that free-living adult mermithids also display modest inequalities in body size.

This might suggest that, from a population genetics perspective, the effective population size of these mermithid parasites is not substantially reduced by intraspecific competition and its effects on body sizes. Earlier studies, however, have shown that inequalities in body size can underestimate inequalities in reproductive output (Shostak & Dick, 1987; Szalai & Dick, 1989). The main reason for this is that fecundity does not covary linearly with body size, with bigger worms producing disproportionately more eggs than small ones. Since we used immature worms, it was not possible to estimate reproductive output, and our measures of inequalities in body size may therefore not represent actual inequalities in egg production and in genetic contribution to the population.

The main cause of inequalities in body size is clearly competition for space and resources, i.e. intensity-dependent effects on growth. This mechanism, however, is itself not particularly strong. For instance, we found no evidence of a ceiling in total worm length inside a host (see fig. 3): the more worms in an amphipod, the longer their total length, approaching 1 m in a host harbouring 33 worms. Also, the absolute length of the longest worm in an amphipod (the alpha-worm) did not peak in hosts harbouring a single or only two or three worms, but in hosts with 4–9 worms. This counter-intuitive result can be explained by the tendency for amphipod size to increase with intensity of infection, such that amphipods harbouring many worms offer more room for worm growth than amphipods harbouring one or two worms. The size of the alpha-worm thus increases with intensity of infection, until the effects of competition become apparent when more than 10 worms share the same host. If competition were more intense, we would expect to see a decrease in the length of alpha-worms as intensity of infection increases from one worm per host to two per host, and a continued decrease as intensity increases further. In many mermithid-insect associations, the intensity of infection is often only one (Poinar, 1983), which suggests that the limited space inside the host does not allow multiple infections. From such observations, it is logical to conclude that higher intensities of infection should have a marked impact on the length of individual worms; our surprising results suggest that this is not necessarily the case.

In many systems, mermithids are acquired seasonally, over a short period of time, and then spend months developing inside their hosts (Poinar, 1983). In our study system, two sets of results also indicate that all worms inside one host are likely to have been acquired over a brief period of time. First, we found strong positive correlations between the size of the alpha-worm and that of lower ranked worms, as well as among the sizes of lower ranked worms. If the smaller worms were more recently acquired than the large worms, there would be no reasons to expect their sizes to be so tightly linked. Second, there is greater homogeneity in worm sizes among parasites from hosts harbouring several worms than among parasites from hosts with only few worms. In other words, relative inequalities in size become less marked, i.e. subordinate worms get closer in size to the alpha-worm, as the number of worms per host increases. The simplest explanation is that worms infect a host roughly simultaneously, and that host resources get partitioned among worms in a more equitable way than if one worm had a considerable head start on the others. The moderate size differences observed may result from genetic variability among worms in their ability to monopolize host resources, as well as from slight differences in the time at which they infected the host.

This last result may be the main reason why inequalities in body size were not more pronounced in the mermithid population. Sizes of worms in heavily-infected hosts are less unequal than in lightly-infected hosts. The relative sizes of the second-, third- and fourth-longest worms converge toward the size of the alpha-worm as the intensity of infection increases (see fig. 5). Apparently, the more conspecific worms share the same host, the more difficult it gets for a given worm to monopolize a disproportionate amount of host resources. This caused the value of the Gini coefficient, used as an index of inequalities in body size across all worms in the sample, to be lower than those found in earlier studies on helminth parasites of vertebrates (Shostak & Dick, 1987; Szalai & Dick, 1989). It may be that there are fundamental differences in the outcomes of intraspecific competition between systems involving mermithids in anthropods and those involving other helminths in vertebrates, and that inequalities in helminth body sizes vary among these systems as well.

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