CrossMark

ORIGINAL PAPER

Inequalities in body size among mermithid nematodes parasitizing earwigs

Fanny Maure[1] · Robert Poulin[1]

Abstract Variation among body sizes of adult parasitic worms determines the relative genetic contribution of individuals to the next generation as it affects the effective parasite population size. Here, we investigate inequalities in body size and how they are affected by intensity of infection in *Mermis nigrescens* (Mermithidae: Nematoda) parasitizing the European earwig *Forficula auricularia* in New Zealand. Among a population of pre-adult worms prior to their emergence from the host, we observed only modest inequalities in body length; however, among worms sharing the same individual host, inequalities in body sizes decreased with increasing intensity of infection. Thus, the more worms occurred in a host, the more the second-longest, third-longest and even fourth-longest worms approached the longest worm in body length. This pattern, also known from another mermithid species, suggests that worms sharing the same host may have infected it roughly simultaneously, when the host encountered a clump of eggs in the environment. Thus, the life history and mode of infection of the parasite may explain the modest inequalities in the sizes achieved by pre-adult worms, which are lower than those reported for endoparasitic helminths of vertebrates.

Keywords Body size · Competition · Gini coefficient · Mermithidae · *Mermis nigrescens* · *Forficula auricularia*

✉ Robert Poulin
  robert.poulin@otago.ac.nz

[1] Department of Zoology, University of Otago, PO Box 56, Dunedin 9054, New Zealand

Introduction

In most helminth parasites, size matters since larger worms produce more eggs than smaller ones (Poulin 1996). Among parasites from the same population, body size can vary dramatically from one individual to another (Poulin 2007), reflecting a high degree of phenotypic plasticity. Examples of such inequalities in body size among adult worms are common in the literature (Shostak and Dick 1987; Szalai and Dick 1989; Poulin and Latham 2002; Hanelt 2009), and this phenomenon may result in a reduction of the effective parasite population size and limit their genetic variability (Dobson 1986; Criscione and Blouin 2005). With size determining fecundity, genes in one generation may come mostly from a small proportion of individuals (the largest ones) in the previous generation. However, we still do not fully understand the consequences of this phenomenon, which has been mainly studied in helminth parasites in vertebrates.

In invertebrate hosts, growing parasites have to deal with restricted space and a limited amount of resources. When several worms are simultaneously developing in the same host, their growth may be even more affected by competition, and inequalities in body size may be more likely to occur. Assuming that juvenile size correlates with adult size (see Poulin and Latham 2003), these inequalities in body size among juvenile helminths can be transferred to the adult stage with the consequences on genetic variability described above.

Here, we investigated whether higher intensity of infection results in more pronounced inequalities in body size in *Mermis nigrescens* (Mermithidae: Nematoda) parasitizing the European earwig *Forficula auricularia*. Mermithid nematodes are parasites of insects, but have also been reported from other arthropods like spiders, scorpions and crustaceans. Once they complete their growth and just prior to their emergence from the host, they typically occupy all available space inside

their host and can reach impressive sizes. Unlike other nematodes that parasitize their hosts by penetrating through their cuticle, *M. nigrescens* infects host via passive infection: free-living gravid female worms deposit their eggs on vegetation to be eaten by herbivorous insects (Presswell et al. 2015). As eggs are commonly found in masses, arthropod hosts are likely to ingest more than one parasite at a time; thus, we may expect intraspecific competition within a shared earwig host to be quite common in this species. Moreover, the fact that an insect is infected does not prevent it from acquiring additional parasites later in life. When several worms share the same host, the emergence of one worm almost inevitably causes the death of the host; thus, if other worms have not yet completed their growth, they are forced to abandon the host at a suboptimal size. This may have severe consequences for the uniformity of body sizes among emerging pre-adult worms.

Our objectives were to (i) quantify inequalities in body sizes among mermithids inside their earwig hosts and draw comparisons with previously published estimates for other helminths and (ii) determine whether differences in body sizes among worms sharing the same host change as a function of increasing intensity of infection.

Material and methods

We collected a large number of adult earwigs (*F. auricularia*) between February and April 2015 from two nearby garden sites in Dunedin, New Zealand. Earwigs were maintained in a terrarium between 18 and 20 °C at 16:8 L:D until dissection. Earwigs were individually measured for body length (from the head to the base of the forceps) and sexed. The number of worms, if any, inside the abdomen of each earwig was recorded, and each worm was straightened without stretching and measured to the nearest millimetre. The longest worm per host is hereafter referred to as the alpha-worm.

We used a Lorenz curve and the Gini coefficient to quantify the degree of inequality in body lengths among all mermithid worms collected, to allow comparison with the few previously published studies that examined size inequalities among parasites. The methods are described in detail by Weiner and Solbrig (1984) and Dobson (1986). Briefly, a Lorenz curve is obtained by ranking individuals in a sample from the smallest to the largest and plotting the cumulative total worm length against the cumulative number of worms when they are added in order. The more concave the curve, the greater the degree of inequality in body lengths among worms. The Gini coefficient, G, is an index measuring this inequality. In essence, it quantifies the discrepancy between the concave Lorenz curve and the hypothetical straight line that would correspond to a situation in which all individuals have the same length. When $G = 0$, all worms have the same length,

but as G tends toward 1, inequalities in body lengths become more pronounced, with few large worms accounting for an increasing proportion of total worm length. The index was computed following Dobson (1986).

To determine how the number of parasites per host influences the growth and the size of worms, we categorized hosts based on their levels of infection by pooling different individual hosts according to the number of parasites they harboured. This was necessary because we obtained relatively few infected earwigs. We created the following infection classes: 1 worm only, 2–3 worms and 4–6 worms per host. We also found an individual harbouring 16 worms; although this individual is included in our figures for illustrative purposes, it was not included in our statistical analyses. To compare host length among the different classes, we used an ANOVA. To test the effect of infection level on total worm length, length of the alpha-worm and relative lengths of second- and third-longest worms, we conducted ANCOVAs using host length as a covariate in each model. Length data were log-transformed to meet the assumption of normality of the error terms.

Results

We found a total of 64 mermithid nematodes, ranging in length between 4 and 132 mm, from 24 infected earwig hosts. Most worms measured between 20 and 80 mm though several were considerably longer (Fig. 1). Both the Lorenz curve for these worms, which was only slightly concave (Fig. 2), and the Gini coefficient ($G = 0.308$) indicate that there is no substantial inequality in body sizes among worms in this population.
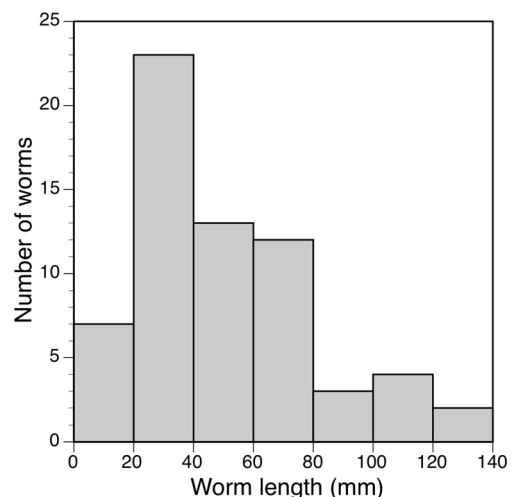
Fig. 1 Frequency distribution of body lengths among 64 mermithid nematodes, *Mermis nigrescens*, recovered from 24 infected earwigs
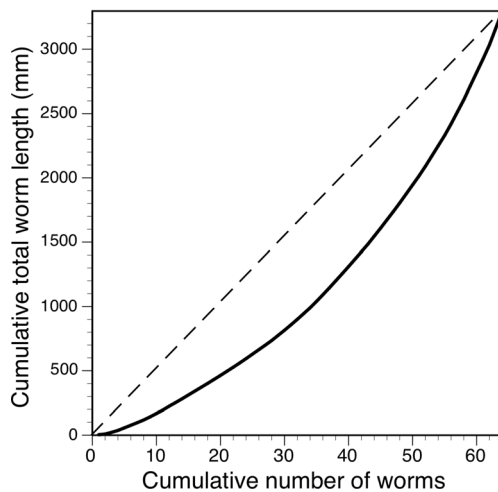
Fig. 2 Cumulative total worm length plotted against the cumulative number of individual worms, for 64 mermithid nematodes, *Mermis nigrescens*. The worms were ranked from shortest to longest prior to being cumulated. The *broken line* represents the hypothetical case in which all worms would have the same length

The number of mermithid nematodes per earwig ranged from 1 to 16 (mean intensity 2.6 ± 0.4) and was not correlated with the host's length (ANOVA: $F_{2,20} = 0.21$, $P = 0.81$; Fig. 3). However, the total worm length (i.e. cumulative length of all worms from the same host) increased significantly with the number of worms per host (ANCOVA: $F_{2,19} = 28$, $P < 0.0001$), reaching over 45 cm in the one earwig harbouring 16 different worms (Fig. 3).

This increase could not be explained by the growth of a single, dominant worm (i.e. the longest worm or "alpha-worm"), as the size of the alpha-worm tended to decrease as a function of the number of worms per host, though not significantly (ANCOVA: $F_{2,19} = 2.59$, $P = 0.10$; Fig. 3). Considering only earwigs harbouring more than one worm, we found positive correlations between the length of the alpha-worm and that of the second-longest worm (Pearson correlation coefficient, $r = 0.565$, $N = 12$, $P = 0.0556$) and between the lengths of the second-longest and third-longest worms ($r = 0.913$, $N = 7$, $P = 0.0041$). Additionally, when comparing relative sizes of worms infecting the same host, the inequality between the alpha-worm and the others was reduced as the level of infection increased. Indeed, the relative size of the second-longest (ANCOVA: $F_{1,8} = 5.93$, $P = 0.041$) and the third-longest (ANCOVA: $F_{1,3} = 14.57$, $P = 0.032$) worms was positively correlated with the number of worms per host (Fig. 4).

Discussion

Resource and space constraints often cause competition among growing helminth parasites. While in vertebrate hosts the resulting size hierarchy typically consists of a
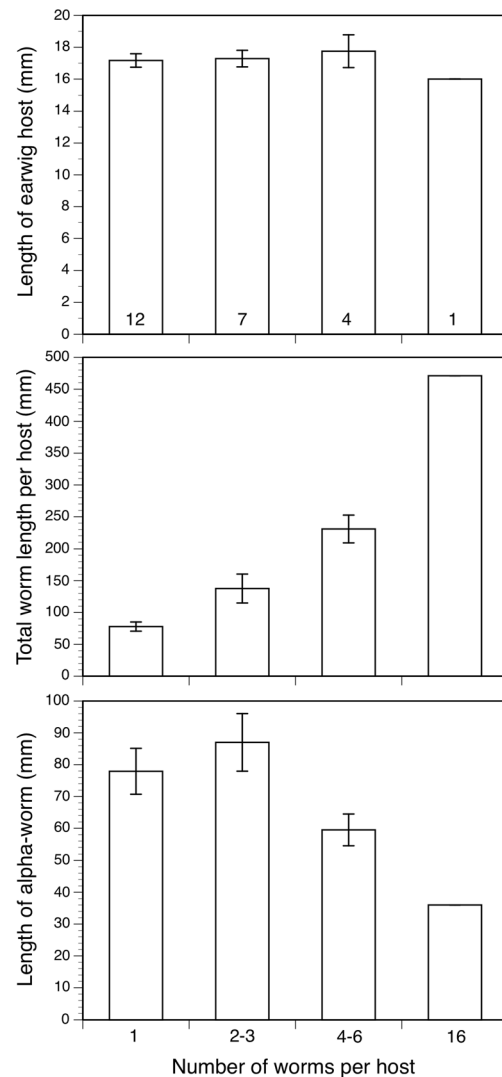
Fig. 3 Length of earwig hosts, total worm length per host and length of the alpha-worm (longest worm) per host as a function of the number of mermithid nematodes, *Mermis nigrescens*, per earwig. Values shown are means ± standard errors; sample sizes are shown on *bars* in the *top panel*

few large worms and many small ones, this phenomenon is still poorly documented in invertebrates (Poulin and Latham 2002; Hanelt 2009; Saldanha et al. 2009). Here, we investigated the effect of intraspecific competition on worm growth among mermithid nematodes parasitizing an insect host, the European earwig *F. auricularia*. We found that pre-adult worms prior to emergence from their host display only modest inequalities overall, but that among worms sharing the same host, inequalities in body sizes decrease with increasing intensity of infection. The worms we measured were probably at various stages of development. However, because the emergence of one worm from the host kills the host and prevents further growth by other worms, this snapshot of variation in worm sizes pre-emergence probably reflects that seen in the much harder to sample, free-living adult population.
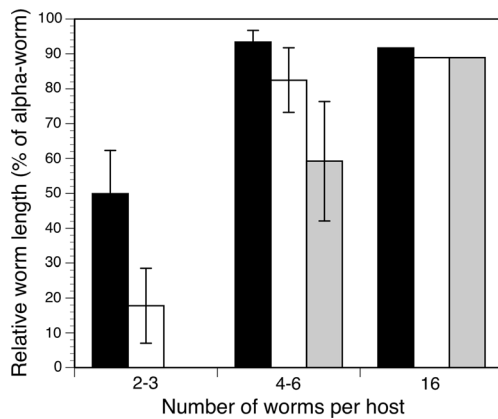
Fig. 4 Relative length (means ± standard errors) of the second-longest (*black bars*), third-longest (*open bars*) and fourth-longest (*grey bars*) worms per host as a function of the number of mermithid nematodes, *Mermis nigrescens*, per earwig. Relative lengths are expressed as a percentage of the length of the longest (alpha) worm in that host. See Fig. 3 for the sample sizes

The mermithid population studied here had a slightly skewed, unimodal body size distribution. Based on the Gini coefficient, it showed weaker inequalities in body sizes ($G = 0.308$ versus 0.431) than the only other mermithid population studied previously, that of *Thaumamermis zealandica* parasitic in semi-terrestrial sandhoppers (Poulin and Latham 2002). It also showed lower inequalities in either individual body sizes or actual reproductive output than what is seen in most populations of helminths parasitic in vertebrate hosts (Dobson 1986; Shostak and Dick 1987; Szalai and Dick 1989; Beck et al. 2015), with rare exceptions (Walker et al. 2010). Interestingly, the only published values of G for parasites that are distinctly lower than the ones observed here come from a study of hairworms (phylum Nematomorpha) parasitic in insects (Hanelt 2009). Mermithid nematodes and hairworms have very similar life cycles and patterns of host use: they are only parasitic as juveniles, grow to very large sizes inside an arthropod host before emerging as free-living adults and generally achieve only low prevalence and intensities of infection. These characteristics may limit intraspecific competition and lead to more uniform body sizes among the worm population than those seen in helminths endoparasitic in vertebrates, where both prevalence and intensities of infection are generally much higher.

We found that the total length of worms per host, i.e. the sum of the lengths of all worms found in the same host, increased with intensity of infection. A very similar pattern was observed in a different species of mermithid nematode (Poulin and Latham 2002). This is not a proportional relationship, however: for instance, total worm length in a host harbouring four worms is not four times greater than that in a host harbouring a single worm. In addition, we did not take into account the diameter of worms, and it is possible that a slight reduction in average worm diameter at higher

intensities of infection would limit parasite biomass and the overall burden on the host. Nevertheless, there appears to be no strong crowding effects resulting in much smaller worms at high intensities. This suggests a possible additive cost of infection to the host, with each extra worm extracting additional host resources.

The most interesting finding of our study is the increasing homogeneity of body sizes among worms sharing the same host as a function of increasing intensity of infection. The more worms in a host, the more the second-longest, third-longest and even fourth-longest worms approach the alpha-worm in body length. This parallels the findings of Poulin and Latham (2002) on the mermithid *T. zealandica* and therefore hints at an explanation that may apply generally to members of this nematode family. One obvious way in which multiple infections could be associated with similar lengths among worms would be if they occurred more or less simultaneously, i.e. the host acquires several parasites at the same time instead of acquiring them one at a time over a long period of its life. The latter scenario would allow the first worm to get a head start and achieve considerable size before subsequent worms enter the host; only the more or less simultaneous infection pattern provides a parsimonious scenario leading to roughly similar sizes. Given that female mermithids deposit their eggs in distinct clumps on vegetation (Baylis 1944), multiple infection at the same time through the ingestion of several eggs from the same clutch by a few unlucky earwigs appears plausible. Thus, the life history of the parasite may contribute to the limited inequalities in the sizes achieved by pre-adult worms. Testing this hypothesis would require the development of microsatellite markers to genotype worms sharing the same earwig hosts; if they tend to originate from the same egg clump laid by the same female, they should show greater genetic relatedness than that seen among worms chosen at random from the whole population.

Fecundity correlates strongly with body size in parasitic helminths in general (Poulin 1996) and in nematodes specifically (e.g. Mössinger and Wenk 1986; Szalai and Dick 1989; Sinniah and Subramaniam 1991). Inequalities in body sizes among adult worms can therefore greatly influence the effective population size, i.e. the proportion of individuals contributing genes to the next generation (Criscione and Blouin 2005; Poulin 2007). In this study, we found no strong inequalities in body sizes among individuals of *M. nigrescens* in their earwig hosts, as well as evidence suggesting that the timing of multiple infections of the same host may help to create more homogeneous worm sizes than would otherwise result from intra-host competition. This may apply to mermithid nematodes more broadly (see Poulin and Latham 2002) as a possible consequence of their particular life history strategy. As further data become available on other helminth species, it will become possible to assess how other life history strategies influence the homogeneity of adult worm sizes.

References

Baylis HA (1944) Observations on the nematode *Mermis nigrescens* and related species. Parasitology 36:122–132

Beck MA, Goater CP, Colwell DD (2015) Comparative recruitment, morphology and reproduction of a generalist trematode, *Dicrocoelium dendriticum*, in three species of host. Parasitology 142:1297–1305

Criscione CD, Blouin MS (2005) Effective sizes of macroparasite populations: a conceptual model. Trend Parasitol 21:212–217

Dobson AP (1986) Inequalities in the individual reproductive success of parasites. Parasitology 92:675–682

Hanelt B (2009) An anomaly against a current paradigm: extremely low rates of individual fecundity variability of the Gordian worm (Nematomorpha: Gordiida). Parasitology 136:211–218

Mössinger J, Wenk P (1986) Fecundity of *Litomosoides carinii* (Nematoda: Filarioidea) in vivo and in vitro. Z Parasitenkunde 72:121–131

Poulin R (1996) The evolution of life history strategies in parasitic animals. Adv Parasitol 37:107–134

Poulin R (2007) Evolutionary ecology of parasites, 2nd edn. Princeton University Press

Poulin R, Latham ADM (2002) Inequalities in size and intensity-dependent growth in a mermithid nematode parasitic in beach hoppers. J Helminthol 76:65–70

Poulin R, Latham ADM (2003) Effects of initial (larval) size and host body temperature on growth in trematodes. Can J Zool 81:574–581

Presswell B, Evans S, Poulin R, Jorge F (2015) Morphological and molecular characterization of *Mermis nigrescens* Dujardin, 1842 (Nematoda: Mermithidae) parasitizing the introduced European earwig (Dermaptera: Forficulidae) in New Zealand. J Helminthol 89:267–276

Saldanha I, Leung TLF, Poulin R (2009) Causes of intraspecific variation in body size among trematode metacercariae. J Helminthol 83:289–293

Shostak AW, Dick TA (1987) Individual variability in reproductive success of *Triaenophorus crassus* Forel (Cestoda: Pseudophyllidea), with comments on use of the Lorenz curve and Gini coefficient. Can J Zool 65:2878–2885

Sinniah B, Subramaniam K (1991) Factors influencing the egg production of *Ascaris lumbricoides*: relationship to weight, length and diameter of worms. J Helminthol 65:141–147

Szalai AJ, Dick TA (1989) Differences in numbers and inequalities in mass and fecundity during the egg-producing period for *Raphidascaris acus* (Nematoda: Anisakidae). Parasitology 98:489–495

Walker M, Hall A, Basáñez M-G (2010) Trickle or clumped infection process? An analysis of aggregation in the weights of the parasitic roundworm of humans, *Ascaris lumbricoides*. Int J Parasitol 40:1373–1380

Weiner J, Solbrig OT (1984) The meaning and measurement of size hierarchies in plant populations. Oecologia 61:334–336