

Benefits of fidelity: does host specialization impact nematode parasite life history and fecundity?

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SUMMARY

The range of hosts used by a parasite is influenced by macro-evolutionary processes (host switching, host–parasite co-evolution), as well as ‘encounter filters’ and ‘compatibility filters’ at the micro-evolutionary level driven by host/parasite ecology and physiology. Host specialization is hypothesized to result in trade-offs with aspects of parasite life history (e.g. reproductive output), but these have not been well studied. We used previously published data to create models examining general relationships among host specificity and important aspects of life history and reproduction for nematodes parasitizing animals. Our results indicate no general trade-off between host specificity and the average pre-patent period (time to first reproduction), female size, egg size, or fecundity of these nematodes. However, female size was positively related to egg size, fecundity, and pre-patent period. Host compatibility may thus not be the primary determinant of specificity in these parasitic nematodes if there are few apparent trade-offs with reproduction, but rather, the encounter opportunities for new host species at the micro-evolutionary level, and other processes at the macro-evolutionary level (i.e. phylogeny). Because host specificity is recognized as a key factor determining the spread of parasitic diseases understanding factors limiting host use are essential to predict future changes in parasite range and occurrence.

Key words: disease, fecundity, specificity, compatibility, trade-off, phylogenetic influences, micro-evolution, macro-evolution.

INTRODUCTION

The selective forces shaping the evolution of specialization have long been of interest to evolutionary ecologists, particularly with respect to symbiotic relationships (Futuyma and Moreno, 1988). Host use by parasites shows an incredible range, with some parasites (specialists) infecting only 1 or 2 related host species, while others (generalists) use many taxonomically diverse hosts. Understanding the forces driving and maintaining host specialization is a central issue in evolutionary parasitology and also critical for understanding parasitic infections, especially newly emerging diseases that are zoonotic in origin. Host specificity is recognized as a key factor determining the spread of parasitic diseases in the face of biological invasions, loss of natural habitats and climate change (Agosta *et al.* 2010), thus understanding what limits host range may prove valuable (Gemmill *et al.* 2000).

Why should parasites be either host generalists or specialists? This is naturally influenced by both macro- and micro-evolutionary processes. Host-switching and co-evolution of parasites with their hosts can have profound effects on host specificity

such that phylogeny (macro-evolution) could largely explain patterns of host use (for reviews, see Poulin, 2007 and Hoberg and Brooks, 2008). At the micro-evolutionary scale, host ecology and physiology have strong influences on the evolution of host specificity, with both factors acting as ‘filters’ (Euzet and Combes, 1980; Combes, 1991, 2001). While many hosts could potentially be used by a parasite, only a few are actually encountered, primarily owing to host ecology or behaviour. The ‘compatibility filter’ further narrows the range of potential hosts by eliminating those not providing the necessary resources for the parasite or those in which the parasite cannot survive and develop (Euzet and Combes, 1980; Combes, 1991, 2001). Parasite transmission modes also play an important role. Those with free-living and/or mobile infectious stages are likely to encounter a greater number of potential hosts relative to parasites that are directly transmitted, as are parasites entering hosts via ingestion compared with other routes such as penetration (Poulin, 2007).

Depending on interactions among various environmental and host factors, there are both advantages and disadvantages to either generalization or specialization. Parasites with a wide repertoire of host species might hedge their bets against extinction by reducing their dependence on any given resource base (Futuyma and Moreno, 1988; Bush and Kennedy, 1994). The evolution of host specialization may then

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be related to host availability and predictability, so as to minimize extinction rates (Ward, 1992; McCoy *et al.* 2001). This could confer a strong advantage to parasites with a wide host base to draw upon, but evidence suggests that across taxa, many parasite species are not extreme generalists (e.g. Poulin *et al.* 2006), implying countering disadvantages. As noted by Poulin (2005), in the study of ecological specialization, 'a common pattern is that jacks of all trades are often masters of none' (see Futuyma and Moreno, 1988; Thompson, 1994). This follows a central tenet in ecology, which presumes that adaptation to one environment reduces an organism's ability to exploit other environments (Gemmill *et al.* 2000). Simply put, the ability of parasites to exploit many host species is often assumed to result in a lower average efficiency of host exploitation, whereas specialists exploiting a single host species may be more efficient because they are specifically adapted to that host (Poulin, 2005). However, such negative correlations between habitat-specific fitness components with respect to parasitism have rarely been found (Gemmill *et al.* 2000).

If host-generalist parasite species have the advantage of lower odds of extinction, obvious possible fitness trade-offs could include reduced establishment, adult survivorship and/or fecundity in novel hosts (Gemmill *et al.* 2000), which are all critical life-history traits (Skorping *et al.* 1991; Anderson and May, 1992). Parasite life history and reproductive strategies show considerable variability, often associated with host or environmental factors, with no single end-point to parasite evolution for many aspects (reviewed by Poulin, 1995, 1996). This is demonstrated particularly well with the fecundity of parasitic nematodes. For instance, even within the family Ancylostomatidae, females of the species *Ancylostoma caninum* ('dog hookworm') produce approximately 2075 eggs/day (Sowemino and Asaolu, 2008) while those of *Ancylostoma duodenale* ('human hookworm') produce about 17 500 eggs/day (Morand, 1996a), but the reasons for this variation are poorly understood (Skorping *et al.* 1991). Host specialization could affect parasite life-history trade-offs in 2 possible ways: (i) specialization is a continuous variable life-history trait that correlates with other continuous traits (e.g. fecundity); or (ii) specialization is a mode of life such that this trait is categorical. In the second scenario, host specialization may affect a critical life-history trait such as body size, which in turn affects others (e.g. fecundity), as seen for larval nematode tissue migration and pre-patent period (Read and Skorping, 1995).

There is good evidence that the host immune response is a major determinant of parasite reproductive rate (Tinsley, 2004) and numerous studies have highlighted its role in controlling helminth populations (e.g. Stear *et al.* 1999; Meeusen and Balic, 2000; Sorci *et al.* 2003). Given that the host

immune system certainly represents a strong selective force on parasite success (Morand and Sorci, 1998), we may expect that parasites lacking the necessary adaptations would exhibit depressed fecundity and increased mortality (Trouve *et al.* 1998). If host-generalists need to develop counter-adaptations against the immune systems of many potential hosts, host-specialists could then allocate more resources to reproduction or other functions, resulting in a trade-off between the number of host species that can be successfully exploited and parasite success in those hosts (Poulin and Mouillot, 2004). Consequently, parasite species with low host specificity may have evolved in less advantageous conditions than those that are more host-specific (Combes, 1995), which could then affect the life-history strategies of each. Recent reports demonstrate the potential for correlated evolution between host immune defences and parasite life histories (e.g. Sorci *et al.* 2003).

Here we examine the importance of host specificity for parasitic nematodes of vertebrates with respect to trade-offs in parasite fecundity and other life-history traits. Previous work has demonstrated correlations among various nematode life-history traits, such as positive relationships between pre-patent period (time from initial establishment in host to first reproduction) and female fecundity, as well as between female size and fecundity (Skorping *et al.* 1991; Morand, 1996b). Given the wide range in fecundity exhibited by parasitic nematodes, this group is ideal to test hypotheses involving trade-offs between this trait and other aspects of life history. We predicted that females of relatively host-specific nematodes would have higher fecundity compared with generalist species, a larger size, a longer pre-patent period (via decreased mortality during establishment) and larger eggs. Well-adapted nematodes should have a longer pre-patent period if there is little pressure from the host's immune system, resulting in a longer period of growth and larger females, and ultimately, greater lifetime fecundity compared with those that mature quickly and begin reproduction earlier at a smaller size.

MATERIALS AND METHODS

Nematode life-history traits

We chose to examine only parasitic nematodes of vertebrates with single-host life cycles. Since several hosts can be involved in the life cycles of some parasites, this complicates determining host specificity and its potential causes (Desdevises *et al.* 2002). Restricting examinations to those with a simpler life cycle avoids such difficulties (Desdevises *et al.* 2002) and many parasitic nematodes have a direct life cycle with only 1 host (i.e. monoxenous). Presumably host defences are more pronounced in animals, especially

Table 1. Nematode parasites of animals included in the current study

(References given in footnotes. Values of host specificity (S_{TD}) are as calculated in the current study.)

Nematode species	Fecundity (eggs/female/day)	Female size (mm)	Egg surface area (μm^2) ^a	Prepatent period (days)	Host specificity (S_{TD})	Host access
<i>Trichuris trichiura</i>	5000 ¹	60 ¹	23 ²	49 ¹	2.3	Ingestion
<i>Trichuris muris</i>	6000 ¹	40 ¹	31 ³	14 ¹	2.2	Ingestion
<i>Trichinella spiralis</i>	800 ¹	3 ¹	15 ⁴	7 ¹	3.8	Ingestion
<i>Necator americanus</i>	15 000 ¹	10 ¹	38 ⁵	42 ¹	3.4	Penetration
<i>Ancylostoma duodenale</i>	17 500 ⁶	14 ¹	32 ⁵	39 ¹	3.3	Penetration
<i>Amidostomum anseris</i>	284 900 ¹	16.7 ¹	55 ⁷	50 ⁶	2.3	Ingestion
<i>Ostertagia ostertagi</i>	200 ¹	8.5 ¹	49 ⁸	21 ¹	2.6	Ingestion
<i>Trichostrongylus tenuis</i>	356 ¹	9 ¹	38.5 ⁹	7.5 ⁶	3	Ingestion
<i>Haemonchus contortus</i>	5000 ¹	24 ¹	46 ⁸	19 ¹⁰	2.7	Ingestion
<i>Nippostrongylus brasiliensis</i>	1250 ¹	7 ¹	31 ¹¹	6 ¹	1.7	Penetration
<i>Strongyloides stercoralis</i>	50 ¹	2.2 ¹	30 ¹⁰	18 ¹	4.2	Penetration
<i>Parascaris equorum</i>	270 000 ¹	150 ¹	95 ⁹	93.5 ¹	1	Ingestion
<i>Ascaris lumbricoides</i>	103 500 ¹	305 ¹	41 ¹²	65 ¹	3.7	Ingestion
<i>Trichostrongylus colubriformis</i>	351 ¹³	5.7 ¹³	46 ¹⁴	21 ¹⁰	3.2	Ingestion
<i>Ancylostoma caninum</i>	2075 ¹⁵	17 ¹⁶	38.8 ¹⁶	14 ¹⁰	2.9	Penetration
<i>Oesophagostomum bifurcum</i>	5055 ¹⁷	^b 14.6 ¹⁸	43 ¹⁹	60 ²⁰	1.9	Ingestion
<i>Oesophagostomum columbianum</i>	11 300 ²¹	18 ¹⁰	40 ¹⁰	40 ¹⁰	2.4	Ingestion
<i>Oesophagostomum venulosum</i>	11 098 ²²	19 ²³	50 ²³	29.5 ²⁴	2.6	Ingestion
<i>Heligmosomoides polygyrus</i>	^c 600 ¹	12 ¹	45 ²⁵	10 ⁶	2.2	Ingestion
<i>Nematodirus spathiger</i>	^d 78 ²⁶	19 ²⁷	100 ²⁸	22.5 ²⁹	3.1	Ingestion
<i>Chabertia ovina</i>	5000 ³⁰	16.5 ¹⁰	45 ¹⁰	60 ¹⁰	2.5	Ingestion
* <i>Teladorsagia circumcincta</i>	^d 267 ²⁶	^e 10.7 ³¹	^e 45 ³²	^e 18 ³²	2.6	Ingestion
* <i>Cooperia curticei</i>	^d 17 ²⁶	6.5 ³³	37.8 ²³	15 ³³	2.6	Ingestion
* <i>Marshallagia marshalli</i>	98 ³⁴	12.7 ³⁵	70 ³⁶	21 ³⁴	2.6	Ingestion

^a Based on surface area for an ellipse using egg length and width values as referenced.^b Only used value for human infection.^c Used value for original species name (*Nematospiroides dubia*).^d Used back-transformed data for 'normal' hay diet.^e Used values for original species name (*Ostertagia circumcincta*).

* Species additionally used in GLMM analysis.

¹Morand (1996b), ²Yoshikawa *et al.* (1989), ³Feliu *et al.* (2000), ⁴Berntzen (1965), ⁵Stoll (1946), ⁶Morand (1996a), ⁷Saif *et al.* (2008), ⁸Tetley (1950), ⁹Zajac and Conboy (2012), ¹⁰Foreyt (2001), ¹¹Haley (1961), ¹²Roberts and Janovy, Jr. (2009), ¹³Amarante *et al.* (2007), ¹⁴Thienpont *et al.* (1986), ¹⁵Sowemino and Asaolu (2008), ¹⁶Burrows (1962), ¹⁷Krepel and Polderman (1992), ¹⁸de Gruijter *et al.* (2006), ¹⁹Muller and Wakelin (2002), ²⁰Ziem (2006), ²¹Dobson (1964), ²²Coyne *et al.* (1991), ²³Andrews (1969), ²⁴Fowler (2010), ²⁵Ehrenford (1954), ²⁶Mu Peyo *et al.* (2011), ²⁷May (1921), ²⁸Tetley (1941), ²⁹Audebert *et al.* (2004), ³⁰Love and Hutchinson (2003), ³¹Lichtenfels and Pilitt (1991), ³²Threlkeld (1934), ³³Andrews (1939), ³⁴Morgan (2003), ³⁵Borji *et al.* (2011), ³⁶Popiolek *et al.* (2007).

selective pressure on endoparasites posed by immune systems. We thus excluded plant-parasitic nematodes given that their specificity may not involve the same trade-offs.

As a measure of fecundity, we chose to restrict our analysis to nematode species with published values of eggs/female/day. Because intra-uterine egg counts are far more common, this placed a constraint on the number of species available for inclusion in our study; however, egg counts represent point estimates of egg production in time and may not necessarily accurately reflect lifetime fecundity (Herreras *et al.* 2007a). With this restriction in mind, we were able to obtain our desired fecundity measure for 24 different nematode species (see Table 1 and references therein), 21 for which DNA sequence data could also be obtained to generate a phylogeny. In addition to average fecundity, we also acquired

average measures of female size, prepatent period and egg size (surface area) for each nematode species.

Host specificity

We compiled a record of host use for each nematode species by using published records in the host-parasite database of the Natural History Museum of London (<http://www.nhm.ac.uk/research-curation/research/projects/host-parasites/database/>).

Paratenic (i.e. transport) hosts were excluded such that only animal hosts within which adult nematodes resided were considered. We only included hosts identified to the species level such that subspecies were not considered separately, but grouped together as one. While such records may include errors due to misidentification and 'accidental hosts' not normally

Table 2. Nematode and outgroup taxa used to generate nematode phylogeny with GenBank Accession numbers and classification

Taxa (Classification and names)	GenBank accession numbers
Class Chromadorea	
Order Ascaridida	
Family Ascarididae	
<i>Ascaris lumbricoides</i>	U94366
<i>Parascaris equorum</i>	U94378
Order Rhabditida	
Family Amidostomatidae	AJ920353 ^a
<i>Amidostomum anseris</i> ^a	
Family Ancylostomatidae	
<i>Ancylostoma caninum</i>	AJ920347
<i>Ancylostoma duodenale</i>	EU344798
<i>Necator americanus</i>	AJ920348
Family Chabertiidae	
<i>Chabertia ovina</i>	AJ920341
<i>Oesophagostomum bifurcum</i> ^b	AJ920343 ^b
<i>Oesophagostomum columbianum</i> ^b	AJ920343 ^b
<i>Oesophagostomum venulosum</i> ^b	AJ920343 ^b
Family Haemonchidae	
<i>Haemonchus contortus</i>	EU086375
Family Heligmonellidae	
<i>Nippostrongylus brasiliensis</i>	AJ920356
Family Heligmosomatidae	
<i>Heligmosomoides polygyrus</i>	AJ920355
Family Molineidae	
<i>Nematodirus spathiger</i> ^c	AJ920360 ^c
Family Strongyloididae	
<i>Strongyloides stercoralis</i>	AF279916
Family Trichostrongylidae	
<i>Ostertagia ostertagi</i>	AJ920352
<i>Trichostrongylus colubriformis</i>	AJ920350
<i>Trichostrongylus tenuis</i> ^d	AJ920350 ^d
Class Enoplea	
Order Trichocephalida	
Family Trichinellidae	
<i>Trichinella spiralis</i>	U60231
Family Trichuridae	
<i>Trichuris muris</i>	AF036637
<i>Trichuris trichiura</i>	GQ352554
Class Priapulida	
Order	
Family Priapulidae	
<i>Priapulus caudatus</i> ^e	AF025927 ^e
Class Gordioida	
Order Chordodea	
Family Chrododidae	
<i>Chordodes morgani</i> ^e	AF036639 ^e

^a *Amidostomum anseris* 18S sequence data unavailable on GenBank, thus substituted with *A. cygni* as a representative taxon for the genus and Amidostomatidae family.

^b *Oesophagostomum* 18S sequence data unavailable on GenBank, thus substituted with *Petrovinema poculatum* as a representative taxon of an additional genus in the suborder Strongylida, not otherwise represented in our analyses, thus creating a 3-species polytomy.

^c *Nematodirus spathiger* 18S sequence data unavailable on GenBank, thus substituted with *N. battus* as a representative taxon for the genus and Molineidae family.

^d *Trichostrongylus tenuis* 18S sequence data unavailable on GenBank, thus substituted with *T. colubriformis* as a representative taxon of the genus, thus creating a 2-species polytomy.

^e Outgroups.

used by a parasite, only such published lists of known hosts allow comparisons to be made among large numbers of related parasite species (Poulin, 1992). The classification of each host species into its class, order, family and genus was done by using the Species 2000 & ITIS Catalogue of Life: Annual Checklist 2010, accessed through the Encyclopedia of Life website (<http://eol.org>). Given the re-naming of some host and nematode species (e.g. *Nematospiroides dubius* is now *Heligmosomoides polygyrus*), each was only counted once and their data combined.

As simply counting the number of host species used by a given parasite does not take into account the taxonomic or phylogenetic affinities of the various host species, we used the host specificity index (S_{TD}) developed by Poulin and Mouillot (2003). For a parasite using 2 distantly related host species (e.g. different orders), this greater taxonomic distinctness results in a higher value of S_{TD} compared with one using 2 hosts belonging to the same genus. As such, the value of S_{TD} for a given parasite is actually inversely proportional to specificity. This index is also preferable to counts of host species because it is independent of study effort, i.e. the number of published records of a parasite (Poulin and Mouillot, 2003).

Nematode phylogeny

Closely related species are likely to share features; hence, data obtained from organisms sharing a common evolutionary history are not necessarily independent from each other (Morand and Poulin, 2003; Poulin and Morand, 2004). In addition, host specificity is commonly believed to be the result of an adaptive process (Brooks and McLennan, 1991; Begon *et al.* 1996). Therefore, we took into account phylogenetic relationships between nematodes using the phylogenetically independent contrasts (PIC) method (Felsenstein, 1985) to control for confounding effects of phylogeny while investigating possible trade-offs between host specificity and other life-history traits. We computed these independent contrasts on continuous variables using the PDAP:PD TREE program (Midford *et al.* 2005) implemented in Mesquite version 2.5 for Mac OSX (Maddison and Maddison, 2007). We derived contrasts from a tree generated using an alignment consisting of previously published molecular data for the small subunit ribosomal DNA (18S) for 21 nematode species and 2 outgroups (Table 2), consisting of over 1900 sites. Sequences were aligned using MacClade 4.07 (Maddison and Maddison, 2005). Ambiguous regions or those containing gaps for most species were removed, resulting in a partition comprising 1184 sites. Modeltest 3.7 (Posada and Crandall, 1998; Posada and Buckley, 2004) determined the best nucleotide-substitution

model for the data. A transition model (TIM2) with gamma-distributed rate heterogeneity (G) was determined to provide the best fit to the data based on the Akaike Information Criterion corrected for small sample size (AIC_c). The dataset was analysed by methods of maximum likelihood (ML) and Bayesian inference (BI). ML and BI were performed using PhyML 3.0 (Guindon *et al.* 2010) and MrBayes v3.1.2 (Huelsenbeck and Ronquist, 2001), respectively, according to a more parameter-rich model (Generalized Time Reversible [GTR]). ML analyses were performed using 6 substitution rate categories, a gamma shape parameter set at 0.376 (estimated from Modeltest), tree improvement set at subtree pruning and regrafting (SPR), and 1000 bootstrap replicates. BI was performed using the covarion option according to a GTR + G nucleotide substitution model with no initial values assigned and with empirical nucleotide frequencies, 4 separate Markov chains were used to estimate posterior probabilities over 5×10^6 generations, sampling the Markov chains at intervals of 100 generations. The first 10 000 trees were discarded as 'burn-in' then a 50% majority-rule tree was constructed from the subsequent trees. Nodal support was estimated as the mean posterior probabilities (Huelsenbeck *et al.* 2001) using the *sumt* command. The trees were rooted on 2 outgroup taxa: a priapulid worm (*Priapululus caudatus*) and a nematomorph (*Chordodes morgani*) (see Blaxter *et al.* 1998).

Statistical analysis

All values were log-transformed prior to analysis, with the exception of S_{TD} which met the criteria of normality (Shapiro–Wilk test). For PIC, branch lengths used were those estimated by ML and their statistical adequacy verified according to Garland *et al.* (1992). Furthermore, we considered all unresolved nodes (polytomies) as 'soft polytomies' (Maddison, 1990; Purvis and Garland, 1993). Although polytomies were arbitrarily resolved by collapsing all unresolved branches (Felsenstein, 1985; Purvis and Garland, 1993), the number of degrees of freedom was reduced for hypothesis testing and set to between $N - 1$ and $p - 1$ (N , number of independent contrasts; p , number of nodes in the working phylogeny) in order to limit Type I error (Purvis and Garland, 1993). We computed contrasts following guidelines suggested by Garland *et al.* (1992). We also calculated phylogenetic inertia (Pagel's lambda) using the package Geiger (Harmon *et al.* 2008) in the program R (R Development Core Team, 2012).

In addition to the PIC analysis, we examined models predicting life-history traits for all 24 nematode species that were constructed using generalized linear mixed models (GLMM) fitted with a Gaussian distribution and nematode Order as a random

variable. We conducted these additional analyses to increase our sample size by removing the constraint of genetic data. The following fixed effects were examined: specificity (for fecundity, female size, pre-patent period and egg size), female size (for fecundity, pre-patent period and egg size), fecundity (for pre-patent period and egg size), pre-patent period (for fecundity, female size and egg size), and egg size (for fecundity, female size and pre-patent period). Models were fitted using the package lme4 (Bates and Maechler, 2009) in the program R. The GLMM method is likely the most robust for examining models with multiple predictors, including those involving parasitism (Fenton *et al.* 2010). Nematode Order was included as a random categorical variable given that the species used here belonged to 3 different Orders (see Table 2). The best model for each of these life-history traits was selected by virtue of the lowest AIC_c value. Based on this, ΔAIC_c values and weights (w_i) for each model were calculated. A model-averaging approach was used to obtain a candidate model for each life-history variable (see Burnham and Anderson, 2002; Anderson, 2008). We then calculated the relative importance weights [$w_+(i)$] and ranks for each predictor variable, as well as the weighted model average parameter estimates and 95% confidence intervals based on the unconditional variance obtained through model averaging (Anderson, 2008). After first conducting the GLMM procedure with host specificity as a continuous variable (i.e. life-history trait), we repeated the procedure using specificity as a random effect (i.e. as a mode of life). Given that the range of possible value of S_{TD} is 1–5 (Poulin and Mouillot, 2003), we chose a cut-off value of 3 to categorize host-generalists and specialists above and below this cut-off, respectively.

RESULTS

Values for the calculated host specificity measure (S_{TD}) and averages of nematode daily fecundity, female size, pre-patent period and egg size are shown in Table 1. The nematode phylogeny produced and used in the PIC analyses is shown in Fig. 1. Regressions of standardized contrasts using the PIC method did not find any significant relationships between host specificity and other nematode traits. Our calculations of Pagel's lambda for the various traits included in our analyses indicate a lack of phylogenetic signal (i.e. phylogenetic inertia), thus providing a strong rationale for re-analysing the data using GLMM or free of the biases introduced by the genetic data. The results of the GLMM procedure using host specialization as a continuous variable (fixed effect) are summarized in Tables 3 and 4. A combination of female size, egg size and pre-patent period resulted in the best model predicting fecundity; however, female size on its own was the only

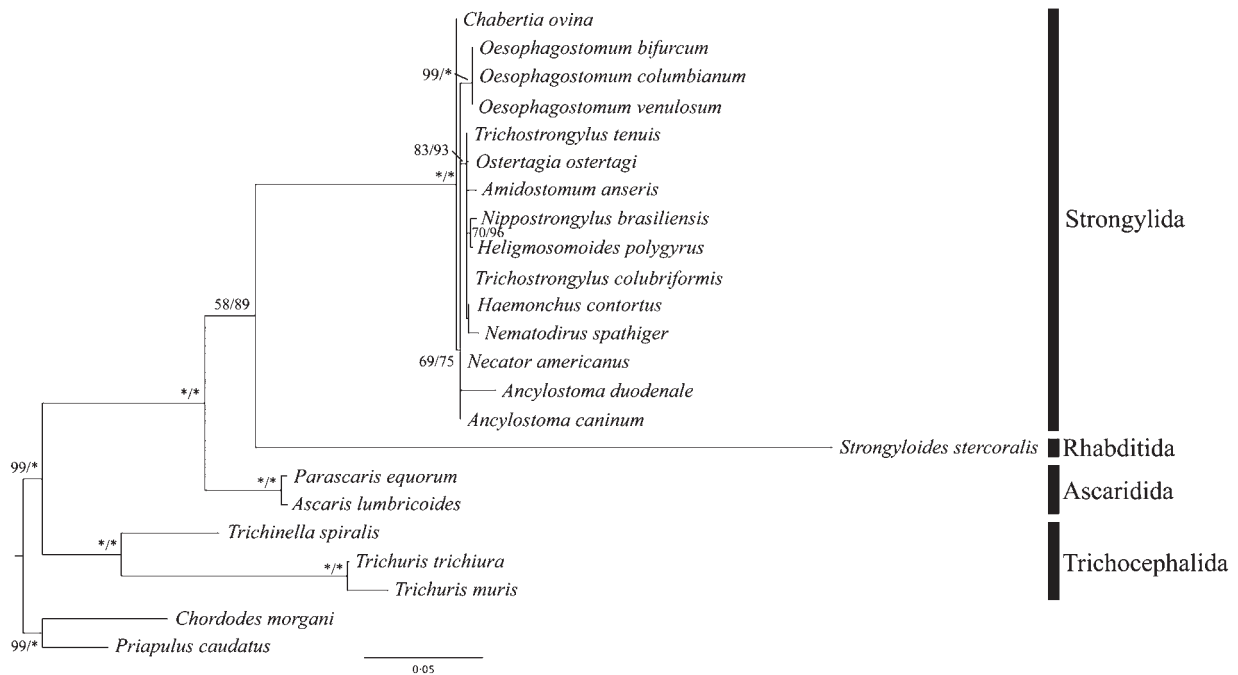


Fig. 1. Nematode phylogeny generated using GenBank sequences in Table 2.

Table 3. Akaike information criterion (AIC_c) values and model probabilities for models within 2 AIC_c of the ‘best’ model for each dependent variable

(The model with the highest probability is highlighted in bold.)

Fecundity			
Model	AIC _c	ΔAIC _c	Model probability
Female size + egg SA + pre-patent period	67.74	0	0.385
Female size + pre-patent period	69.31	1.57	0.176
Female size			
Pre-patent period	25.27	0	0.330
Egg SA + pre-patent period	25.89	0.62	0.241
Egg SA	25.96	0.69	0.234
Egg size (surface area)			
Female size	21.03	0	0.504
Pre-patent period			
Fecundity + egg SA	18.13	0	0.330
Female size	18.64	0.51	0.257
Fecundity	19.18	1.05	0.196

parameter with a confidence interval bounded away from ‘0’, indicating relatively little support for the other predictors. Female size was also the best predictor of egg size, explaining 21% of the variance in this trait but having a confidence interval bounded away from zero. The reverse was also seen, with egg size the best overall predictor of female size. Along with fecundity, female size accounted for a relatively

large proportion of the variation in pre-patent period as well. Overall, the random variable ‘nematode Order’ explained a large proportion of the variance for fecundity and female size (85.0% and 41.8%, respectively), and to a lesser degree that for egg size and pre-patent period (13.4% and 7.3%, respectively), as these traits varied among nematode groups (Fig. 2). T-tests revealed no statistically significant difference between life-history traits for specialist versus generalist nematodes (results not shown). These results were supported by the GLMM procedure, which revealed that there was no significant improvement in support for models resulting from using host specificity as a random effect.

DISCUSSION

Contrary to our predictions, high host specificity was not associated with an overall increase in advantageous life-history/reproductive measures for the parasitic nematodes examined here. Nematodes with high host specificity did not generally have greater fecundity, larger female size or increased pre-patency period (i.e. lower mortality during establishment) relative to species using a more diverse array of hosts. Since the nematodes included in this study do not appear to exhibit strong trade-offs in important life-history measures when using a wide range of hosts, this suggests that other forces are primarily maintaining/selecting host specificity. While our analyses intrinsically include a certain amount of variation inherent in the host and parasite measures obtained from the literature, we would expect that strong trade-offs with respect to host specificity

Table 4. Predictor variable relative importance weights [$w_+(i)$], ranks, weighted model average parameter estimates, and 95% confidence intervals

(Parameter estimates in bold indicate those bounded away from '0'.)

Fecundity				
Predictor variable	$w_+(i)$	Rank	Parameter estimate	Confidence interval
Host specificity	0.1469	4	-0.0390	-0.1303 to 0.0523
Female size	0.8208	1	1.0533	0.1636 to 1.9430
Egg surface area	0.6068	3	-0.6440	-1.4530 to 0.1650
Pre-patent period	0.8152	2	1.3865	-0.0230 to 2.7959
Female size				
Host specificity	0.1325	3	-0.0189	-0.0449 to 0.0070
Egg surface area	0.6276	1	0.3256	0.0290 to 0.6221
Pre-patent period	0.5479	2	0.2702	-0.0072 to 0.5477
Egg surface area				
Fecundity	0.1647	3	-0.0207	-0.0440 to 0.0027
Host specificity	0.0715	4	-0.0070	-0.0202 to 0.0062
Female size	0.7472	1	0.3735	0.0948 to 0.6522
Pre-patent period	0.1956	2	0.0567	-0.0521 to 0.1656
Pre-patent period				
Fecundity	0.6274	1	0.1107	0.0486 to 0.1728
Host specificity	0.0417	4	0.0011	-0.0057 to 0.0079
Female size	0.3917	3	0.1460	0.0035 to 0.2886
Egg surface area	0.4395	2	0.1489	-0.0043 to 0.3021

should still be detectable or appear as noticeable trends. Notably, our findings regarding other relationships among nematode life-history traits are in agreement with those previously reported in the literature (see below). Based on our results, macro-evolutionary processes (via phylogeny) are likely most important for host specificity, similar to recent findings for parasite diversity (Poulin *et al.* 2011). In addition to macro-evolutionary processes, the 'encounter filter' at the micro-evolutionary level (Euzet and Combes, 1980; Combes, 1991, 2001) may be the primary driver of host specificity for the nematodes examined here, rather than fitness trade-offs involving aspects of host physiology and immunity associated with the 'compatibility filter' which is also at the micro-evolutionary level. While we did find evidence of phylogenetic inertia, this is likely to be due to the high representation by species belonging to the order Rhabditida in our dataset and the poorly resolved tree generated from the molecular data.

Numerous studies have highlighted the role played by the host immune system in controlling helminth populations (e.g. Stear *et al.* 1999; Meeusen and Balic, 2000; Sorci *et al.* 2003) and there is good evidence that host immune response is a major determinant of parasite reproductive rate (Tinsley, 2004). Because the effectiveness of the host immune response is likely to vary across host species (e.g. John, 1994; Møller and Erritzoe, 1996), a similar covariation in parasite life history may be expected (Morand and Sorci, 1998). Even though an increase in pre-patency period confers a fitness advantage to

parasitic nematodes by increasing female size and reproductive lifespan (Skorping *et al.* 1991; Morand, 1996b), host specialization appears to have little impact on this measure here. Parasite fecundity is another fundamental element of parasite population growth, as well as influencing pathogenicity (Anderson and May, 1982), but again, there does not appear to be a strong trade-off with the range of hosts in this study. The variation in nematode life-history traits here may thus not be generally attributable to that in immunity factors across host taxa.

Outside of macro-evolutionary processes, if the 'encounter filter' (Euzet and Combes, 1980; Combes, 1991, 2001) plays the larger role in determining host specificity at the micro-evolutionary level, many parasitic nematodes may have limited opportunity to encounter and colonize new host species simply owing to their life history, or that of their hosts. As noted earlier, those entering hosts via ingestion rather than penetration are more likely to find themselves in new species (Poulin, 2007). We did not compare the host specificity for nematodes with ingestion versus penetration routes here as only 5 of our 24 species use the latter strategy. The 'mating hypothesis' may also explain the high host specificity of some parasite species if individuals achieve greater fitness by meeting on a single resource (the host) to mate in low-density populations (Rohde, 1979). Competitors or predators may also restrict the range of hosts used if parasites have been selected to seek 'enemy-free space' (Lawton, 1978; Bernays and Graham, 1988). Subsequent studies examining the degree of

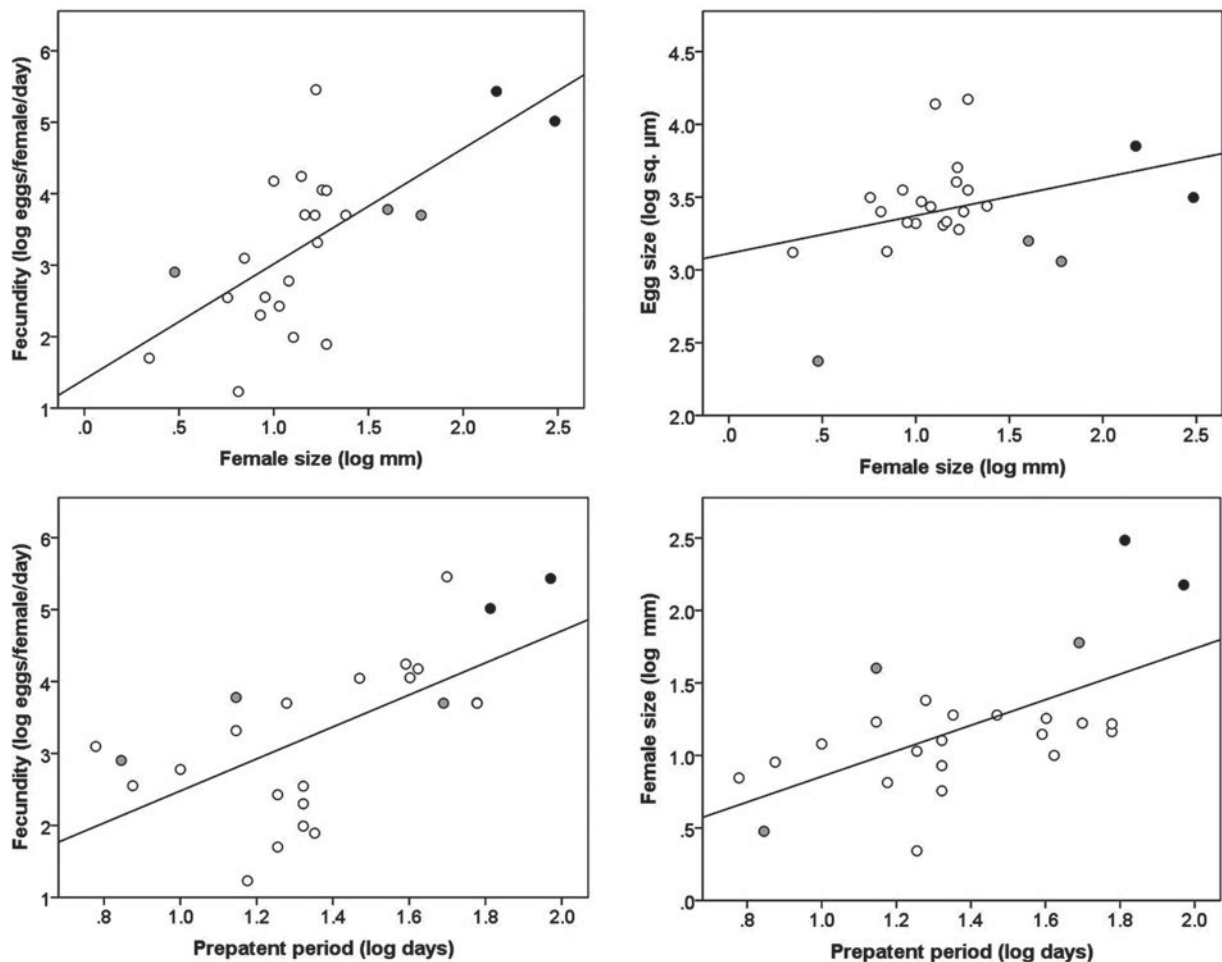


Fig. 2. Scatter-plots of nematode life-history traits based on models with support using a generalized linear mixed model (GLMM) analysis. Note that scatter-plot lines only indicate best fit. Black-filled circles denote species in the Order Ascaridida, open circles indicate species in the Order Rhabditida, and grey-filled circles indicate species in the Order Trichocephalida.

interspecific competition experienced by parasite nematodes in different host species (i.e. how many other nematode species use the same host) may aid in elucidating their variation in host specificity.

With respect to relationships among parasitic nematode life-history traits that do not involve host specificity, our findings largely agree with those of previous studies. We found positive relationships between female size and fecundity, between female size and pre-patent period, as well as between fecundity and pre-patent period. These correlations are in keeping with those reported by Skorping *et al.* (1991) and Morand (1996b) even though we considered a relatively restricted range of nematodes, i.e. only including single-host vertebrate nematodes for which daily fecundity values were available. We found that egg size was well predicted by female nematode size, contrary to the findings of Skorping *et al.* (1991). However, trade-offs with egg production and body size do occur for other parasites (Poulin, 1995, 2007; Poulin and Hamilton, 1997) and a trade-off between egg size and egg number has been

reported for some anisakid nematodes (Herrerias *et al.* 2007b).

While we did not find an overall advantage conferred by host specialization with respect to the life-history measures and species used here, this phenomenon has been reported for some outcomes in other host-parasite systems. Specifically, nematode abundance/infection intensity achieved in hosts seems to show a positive relationship with host specialization. For example, the specialist nematode *Parasitylenchus nearcticus* had greater rates of infection and reproduction than the generalist *Howardula aoronymphium* in its host *Drosophila recens* (Perlman and Jaenike, 2001). Similarly, *Strongyloides ratti* in mice exhibited lower establishment rates, earlier expulsion of established parasites and reduced *per capita* fecundity compared to infection in rats (Gemmill *et al.* 2000), but variation in the efficacy of thymus-dependent immunity between these two host species was insufficient to explain the majority of the observed differences. Poulin and Mouillot (2004) reported a relationship between host specialization

and the local abundance of helminth parasites of birds. Even though the patterns differed between nematodes and cestodes, they both showed evidence of a trade-off with host specialization. Low mean host abundance was generally seen in nematodes using host species that were distantly related, as well as in cestodes with one or a few host species very distantly related to the rest of their hosts (Poulin and Mouillot, 2004).

Although our analyses are based on a relatively small sample size (21 and 24 nematode species for the PIC and GLMM, respectively) with a taxonomic bias towards the order Rhabditida, a strong trade-off between range of host use and aspects of parasite life history should presumably still be detectable, or at least result in strong trends. However, further studies including a greater number of species (especially outside of the order Rhabditida) will be needed. A greater representation by more nematode groups would aid in the detection of general patterns. Measures of fecundity are particularly problematic since some nematode groups, such as the suborder Strongylida (Anderson, 1992), are characterized by continuous production of young during their lifetime (iteroparity), while others like the Oxyurida of some vertebrates (Adamson, 1989) show a single reproduction event followed by death (semelparity). In addition, it must be recognized that parasite fecundity values in the literature are often biased towards intensively studied parasites of medical and veterinary importance, which are generally maintained in the laboratory in convenient model hosts (Tinsley, 2004). This further narrows the diversity of nematodes for which measures have been obtained, and fecundity values may in fact be much larger in other animals if the models are not the 'ideal' hosts for the nematodes examined. Conversely, fecundity measures may be inflated by only examining these in very competent hosts. We also note that the nematode values used here are averages, which do not capture possible variation between host generalists and specialists and what it may reveal. Host records for parasites are also likely to be a source of error and under-representation. Recent genetic and experimental investigations have revealed that for many parasite species, host specificity is often either under- or overestimated when based on field surveys alone (e.g. Poulin and Keeney, 2008). Helminths that exploit more host species, and to a lesser degree those that exploit a broader taxonomic range of host species, tend to be discovered earlier than the more host-specific helminths such that more host-specific parasite species are found later than the generalist species (Poulin and Mouillot, 2005). It should also be noted that the presence of an adult worm inside a given host does not necessarily indicate that the parasite is able to successfully reproduce.

We did not find general trade-offs between range of host use and aspects of nematode parasite life history

and fecundity but the scope of constraints and processes operating at both the macro- and micro-evolutionary levels likely result in complex interactions among forces that together shape host specificity. As Begon *et al.* (1996) noted: '...we assume that the existence of a narrow and restricted range of hosts... represents some sort of evolutionarily optimal condition. We presume that natural enemies, chemical defenses or some other force would reduce the fitness of any parasites that extended the range. These are rash assumptions that beg to be tested.' Given the enormous influence that host specificity can have on parasite transmission and population dynamics, further studies investigating this aspect for both nematodes and other parasite taxa are needed and will play an important role in advancing our understanding regarding the evolution and consequences of specialization. For example, the success of many invasive species can be attributed to 'enemy release', including escape from their native parasites (Torchin *et al.* 2003; Torchin and Mitchell, 2004). However, non-indigenous species can also be highly competent hosts for native parasites, leading indirectly to increased infection in native hosts through what was recently termed parasite 'spillback' (Kelly *et al.* 2009). If encounter opportunity plays a larger role than host compatibility at the micro-evolutionary level, this could then result in greater parasite range spread than may be otherwise predicted. With emerging infectious diseases of wildlife an increasing concern (Daszak *et al.* 2000), factors influencing host specificity, which ultimately affect pathogen establishment and persistence, require further investigation.

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