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ORIGINAL PAPER

Disentangling phylogenetic constraints from selective forces in the evolution of trematode transmission stages

Anson V. Koehler · Brittni Brown · Robert Poulin · David W. Thieltges · Brian L. Fredensborg

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Abstract The transmission stages of parasites are key determinants of parasite fitness, but they also incur huge mortality. Yet the selective forces shaping the sizes of transmission stages remain poorly understood. We ran a comparative analysis of interspecific variation in the size of transmission stages among 404 species of parasitic trematodes. There are two transmission steps requiring infective stages in the life cycle of trematodes: transmission from the definitive to the first intermediate (snail) host is achieved by eggs and/or the miracidia hatched from those eggs, and transmission from the first to the second intermediate host is achieved by free-swimming cercariae. The sizes of these stages are under strong phylogenetic constraints. Our results show that taxonomy explains >50% of the unaccounted variance in linear mixed models, with most of the variance occurring at the superfamily level. The models also demonstrated that mollusc size is positively associated with egg volume, miracidial volume and cercarial body volume, but not with the relative size of the cercarial tail. In species where they encyst on substrates, cercariae have significantly larger bodies than in species penetrating chordates, although the relative size of the cercarial tail of species using chordates as second intermediate hosts was larger than in other trematode species. Habitat also matters, with larger cercarial tails seen in freshwater trematodes than in marine ones, and larger miracidial volumes in freshwater species than in marine ones. Finally, the latitude (proxy for local temperature) at which the trematode species were collected had no effect on the sizes of transmission stages.

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A. V. Koehler · R. Poulin (✉)
Department of Zoology, University of Otago, P.O. Box 56, Dunedin 9054, New Zealand
e-mail: robert.poulin@otago.ac.nz

B. Brown · B. L. Fredensborg
Department of Biology, The University of Texas–Pan American,
1201 W University Drive, Edinburg, TX 78539, USA

D. W. Thieltges
Marine Ecology Department, Royal Netherlands Institute for Sea Research (NIOZ),
P.O. Box 59, 1790 AB Den Burg, The Netherlands

We propose that resource availability within the snail host, the probability of contacting a host, and the density and viscosity of the water medium combine to select for different transmission stage sizes.

Keywords Body size · Cercariae · Latitude · Habitat type · Host type · Tail size

Introduction

Individual life history traits are shaped by physiological and phylogenetic constraints on the one hand, and by trade-offs with other traits on the other hand (Roff 1992; Stearns 1992). Phylogenetic constraints here refer to limitations on evolutionary change imposed not by phylogeny itself, but by genetic or developmental features shared by closely related species (Losos 2011). Within these constraints, selective pressures determine how various trade-offs are resolved, with factors affecting mortality rates at each life stage playing a key role (Partridge and Harvey 1988; Stearns 1992). In parasites with complex life cycles, there are several distinct life stages, incurring vastly different mortality rates. Transmission stages, in particular, experience extremely high rates of mortality, as they are generally very small, non-feeding, free-living stages with either no or very limited motility. In essence, these general properties and the huge mortality incurred also apply to the lecithotrophic larvae used as dispersal stages by many fish and marine invertebrates (Llodra 2002; Marshall and Keough 2003). The huge losses incurred by parasites during transmission from one host to the next are probably the reason for the high replication rates of many parasites (Jennings and Calow 1975; Poulin 1996). Production of transmission stages is subject to the same trade-off applying to offspring production (Messina and Fox 2001). For any amount of resources available, there must be a trade-off between the size of each individual and their total number. Indeed, in parasitic copepods, there is a negative relationship between the number and size of infective stages produced by adult females (Poulin 1995). Producing larger transmission stages, each better equipped to find a host, reduces the variance in their success at the expense of how many can be produced.

Selection on the size of transmission stages is likely to vary among parasite species depending on the characteristics of their life cycle, their hosts, or the external environment. Here, we use a comparative analysis to investigate interspecific patterns of variation in the size of transmission stages and their correlates among trematodes (flukes, phylum Platyhelminthes). The typical trematode life cycle (see Fig. 1) involves the production of transmission stages at two distinct periods in the life of an individual (Galaktionov and Dobrovolskij 2003). First, adult parasites inside their vertebrate definitive host produce eggs, usually released with the host's faeces. Among blood flukes (family Schistosomatidae), egg size varies among species and it shows a trade-off with fecundity (Loker 1983), suggesting that optimal egg size is under different selective pressures in different species. Depending on the trematode species, the egg either awaits accidental ingestion by a snail first intermediate host before hatching, or it hatches first into a flattened, ciliated transmission stage known as a miracidium, which then seeks and infects a snail (Galaktionov and Dobrovolskij 2003). Transmission to a new host in this part of the life cycle is therefore achieved by either the egg and/or the miracidium (Fig. 1). Second, after castrating its snail host and diverting host resources toward its asexual multiplication (Lafferty and Kuris 2009), the parasite releases free-swimming transmission stages known as cercariae, which generally proceed to locate and infect the second intermediate host. The latter can be an invertebrate or a vertebrate, depending on the trematode species; cercariae

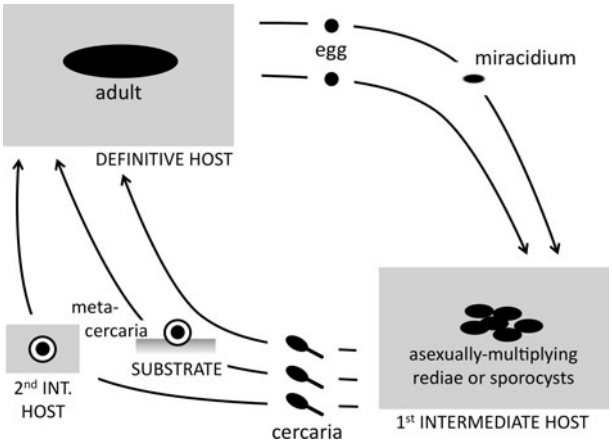

Fig. 1 Generalised trematode life cycle illustrating the two possible transmission routes from the definitive host to the first intermediate host (*top right*), and the three main return routes to the definitive host (*bottom left*). Parasite life stages are indicated in *lower case*, hosts in *upper case letters*. See text for further details

encyst within the second intermediate host to await ingestion by the definitive host and thereby complete their life cycle. There are some exceptions to this pattern, however (Fig. 1). In blood flukes, cercariae directly penetrate the vertebrate definitive host, and there is no second intermediate host; in some other trematode taxa, e.g. the marine Philophthalmidae and the terrestrial Fasciolidae, cercariae encyst on external surfaces, like vegetation or snail shells, awaiting ingestion by the definitive host. Cercarial body sizes vary among closely-related trematode species (Loker 1983), and may reflect the action of selective forces, just as specific cercarial swimming behaviours often appear well-suited to their target hosts (Combes et al. 1994; McCarthy et al. 2002). There also seems to be a negative interspecific correlation between cercarial body size and daily cercarial output from the snail host (Thieltges et al. 2008).

Interestingly, the cercaria consists of two parts: a body, which eventually grows into the adult worm, and a tail, which is jettisoned after the cercaria reaches its target host. Depending on the species, the tail has either a very simple morphology, or it is more complex, such as possessing branches (furca). It is used not only for swimming, but also for energy storage in the form of glycogen reserves near its base (Galaktionov and Dobrovolskij 2003). This sets up another potential trade-off, since for any finite amount of resources, the more is invested into the tail, the less is available for the cercarial body itself. Larger cercarial bodies generally end up as larger adult bodies (Poulin and Latham 2003), therefore the future fitness of the parasite is determined in part at the cercarial stage (larger adults produce more eggs than small ones). The relative investment into tail and body must therefore reflect the action of several factors, resulting in an optimum that must vary across trematode species.

Some aspects of a trematode's life cycle may influence the size of transmission stages favoured by selection. One of these may be the size of the snail first intermediate host. Snail size represents the size of the total pool of resources available for the production of cercariae. Using larger snails may relax any trade-off between total cercarial size and numbers produced, allowing larger cercarial sizes to evolve unconstrained by any reduction in output. While large hosts provide more space/nutrients for parasites, this often comes at the expense of low host abundance (White et al. 2007). We therefore expect a

large snail size to select for large eggs and/or miracidia to increase longevity and in turn the chance of encountering a snail host.

Another aspect of the life cycle that may be important is the identity of the second intermediate host. These range from relatively small and immobile invertebrates to large and mobile vertebrates. In parasitic copepods, species parasitising sessile invertebrates like ascidians or sponges produce few but very large eggs, hatching into relatively large infective stages, whereas species parasitising fish tend to produce numerous but very small eggs (Poulin 1995). Similarly, in marine trematodes, cercarial output is greater in species using fish second intermediate hosts than in those targeting invertebrates or encysting on substrates (Thieltges et al. 2008). We might expect a similar match between cercarial size and the nature of the target host among trematodes. In addition, since the swimming ability and the stored energy determining cercarial lifespan are determined by investments in the tail, we might also predict relatively larger tails in species targeting vertebrates than in those targeting invertebrates, as the former are generally more mobile targets. Taxa like blood flukes that directly infect the definitive host, and those that encyst on external surfaces, do not benefit from any additional growth period inside a second intermediate host before reaching adulthood, and we might therefore expect them to have relatively large cercarial bodies.

Environmental factors may also influence the evolution of trematode transmission stages. Ambient temperature is an obvious one, as it will determine the parasite's metabolism, and therefore its activity levels and the rate at which it will exhaust its finite energy supplies. The relative size of the cercarial tail might thus increase with decreasing latitude (a necessary proxy for temperature in large-scale comparative analyses); however, shorter lifespan at higher temperatures may easily be offset by higher activity and infectivity, and the selective pressures exerted on cercariae are complicated (Morley 2011). The sizes of all transmission stages may also change as a function of latitude, as seen with the eggs of parasitic copepods (Poulin 1995) and other invertebrates (Azevedo et al. 1996). Finally, except for a few terrestrial species in which cercariae encyst on vegetation, cercariae must swim in an aquatic environment to find their target host, and temperature affects important water properties such as density and viscosity. Since these properties combine to lower the sinking rates of cercariae, we might expect lower investments in cercarial tail versus body size in trematodes from high latitudes (i.e. cold dense water) compared to low latitudes (i.e. warm less dense water). Similarly, seawater has higher density and viscosity than freshwater, and we might expect lower investments in cercarial tail versus body size in marine trematodes than in freshwater ones.

In addition, turbulence associated with particular aquatic habitats should also select for certain characteristics in transmission stages. Larger size in eggs, miracidia and cercariae may be favoured as a means of reducing variance in survival in turbulent habitats with rapidly fluctuating conditions such as intertidal and estuarine habitats (corresponding roughly to 'marine' and 'brackish', respectively, in our dataset), compared to more stable habitats such as freshwater lakes. Also, we might expect greater investments in cercarial tail versus body size in turbulent habitats where cercarial swimming is challenging.

In this study, we use a large dataset on the sizes of trematode transmission stages to quantify patterns of interspecific variation in the characteristics of eggs, miracidia, and cercariae (body size and tail size) with respect to putative selective factors. We have two main goals. First, because of the inherited similarities among related species, we expect variability in transmission stages to be constrained by the shared features of phylogenetically related species. Therefore, we aim to quantify the phylogenetic heritability of egg, miracidial and cercarial properties, i.e. the proportion of their interspecific variance

explained by relationships among species (Housworth et al. 2004). Second, while accounting for this inherited similarity among species, we evaluate the effects of four factors (snail host size, second intermediate host taxon, latitude, and habitat) on the remaining variance in these traits. Our analysis provides the first large-scale test of the effects of selection on the evolution of trematode transmission stages.

Materials and methods

Data collection

Data on larval trematode morphometry were obtained mainly from species descriptions published in the following journals: *Journal of Parasitology*, *Systematic Parasitology*, *Parasitology Research*, *Transactions of the American Microscopical Society*, and *Biological Bulletin*; all issues available through the University of Texas library system were systematically surveyed for relevant articles. In addition, a search was conducted for additional sources on Web of Science using the keywords "tremat* and cercaria* and life cycle". To be included in our dataset, a species had to be fully named (i.e., descriptions of species named "*Cercariae* sp." were omitted), and data on the dimensions of its egg, miracidium and/or cercaria had to be provided. Data on all three of these stages were not always available, and therefore the number of species included in different analyses varies depending on the response variable involved. For each species included, we also collected data on the following variables:

Taxonomic affiliation: Each trematode species was placed in a family, superfamily and suborder based on the comprehensive systematic classification for trematodes (Gibson et al. 2002; Jones et al. 2005; Bray et al. 2008).

First intermediate host: The identity of the molluscan host was recorded for each trematode, and subsequently the mean shell length (mm), or mean diameter for species with a shell wider than their length, was obtained using a variety of published sources (online Appendix A). In molluscs, shell length correlates strongly with soft body mass (Benke et al. 1999), making it a good proxy of host body size. An average shell length was calculated for the few trematode species that utilise multiple molluscan hosts. In the few instances where annelids or tube snails (Vermetidae) serve as first intermediate hosts, host size was not included in the dataset.

Second intermediate host: The identity of the second intermediate host(s) of each trematode was recorded, sometimes requiring consultation of additional articles on life cycles. These were then classified into four groups: (i) molluscs, (ii) annelids and arthropods, which were combined due to the small number of trematodes using annelids as second intermediate hosts, (iii) chordates, and (iv) substratum, for those parasites that encyst on a variety of substrates including rocks, vegetation, and mollusc shells.

Habitat: For each trematode, the habitat in which the first intermediate host lives was recorded as either freshwater, brackish, marine or terrestrial. This also corresponds to the habitat in which eggs, miracidia and cercariae are released.

Latitude: The latitude (regardless of north or south) at which the trematode specimens measured were collected was recorded. Average latitude was used for the few cases where miracidium, egg, and cercarial data were collected from different locations.

The following morphological measurements were also obtained from the species descriptions: mean lengths and widths (mm) of trematode eggs, miracidia, and cercariae. Then, egg volume, miracidium body volume, and cercarial body volume were calculated as

the volume of an ellipsoid: $V = \frac{4}{3}\pi\left(\frac{W}{2}\right)^2\left(\frac{L}{2}\right)$ where W = width and L = length. After also recording the dimensions of the cercaria' tail, cercarial tail to body volume ratio (TBR) was calculated by dividing the tail volume, $V = \pi\left(\frac{W}{2}\right)^2 L$ by the cercarial body volume. If the cercaria possessed furcae, the volume of a cone, i.e. $V = 2\left(\frac{1}{3}\pi\left(\frac{FW}{2}\right)^2 FL\right)$ was used to calculate furcae volume, which was then added to the tail volume; here, *FW* represents the furca width and *FL* is the furca length. If descriptions lacked measurements (i.e. tail width) but included illustrations, the program ImageJ v1.44 was used to approximate the measurements from drawings.

Statistical analysis

All statistical analyses were performed using R v2.13.2 (R Development Core Team 2011). Continuous variables were either Box-Cox or $\log_e$ transformed to ensure assumptions of normality were met. The four response variables we considered (egg volume, miracidium body volume, cercarial body volume, and cercarial tail to body volume ratio or TBR) were each examined separately, using linear mixed models (LMMs) performed using the package 'lme4'. Each model included the following factors: mollusc size and latitude as continuous variables, second intermediate host (only for cercarial body volume and TBR) and habitat as categorical variables, and taxonomy (genus, family, superfamily) as a nested random factor. Phylogenetic influences must be taken into account in comparative analyses (Felsenstein 1985), and they are known to affect interspecific variation in parasite life history traits (Morand and Poulin 2003). However, the only available global phylogeny of trematodes (Olson et al. 2003) stops at the family level and does not include all recognized trematode families; given the wide range of trematode species in our dataset, applying methods such as phylogenetically independent contrasts would result in reduced statistical power. Therefore, we chose to use nested taxonomic levels as a random factor (see Clutton-Brock and Harvey 1977). The taxonomic scheme we used (detailed in Gibson et al. 2002; Jones et al. 2005; Bray et al. 2008) fully integrates the phylogenetic relationships uncovered by Olson et al. (2003), strengthening the inference of phylogenetic effects based on the detection of taxonomic ones. This allowed us to estimate how much of the variance in each response variable was due to taxonomic heritability, as an estimate of the phylogenetic signal in the data. We did this by calculating the proportion of the total variance accounted for by each of the taxonomic level.

Interactions were not included in the models as we had no a priori, biologically relevant reasons to include them. Model selection for the LMMs was performed in accordance with the 'top-down strategy' of Zuur et al. (2009) in which random factors are first assessed then fixed factors are removed from the full model based on AIC values. For this reason, not all final models included the full set of factors. Q–Q normal plots were used to assess normality, and residuals from the models were plotted against fitted values to assess heterogeneity. Predictor variables were checked for multicollinearity using the 'vif' function in the package 'car'.

Results

In total, 404 species of digenetic trematodes from 203 genera, 60 families, 20 superfamilies and 12 suborders are represented in the dataset (see online Appendix A; numbers used in

each analysis are given in Table 1). Cercarial volumes varied about 500-fold among trematode species, whereas egg volume ranged over 4 orders of magnitude, and miracidial volume over 3 orders of magnitude (Table 1). Cercariae ranged from having no tail (TBR = 0) to having a tail much larger than their body (Table 1). All four variables showed distribution of values highly skewed toward small sizes. There were some non-surprising positive correlations between the sizes of the three stages: egg volume versus miracidial volume ($r_s = 0.747$, $P < 0.0001$), egg volume versus cercarial body volume ($r_s = 0.307$, $P < 0.0001$), and miracidial volume versus cercarial body volume ($r_s = 0.196$, $P = 0.039$).

The results of LMMs investigating interspecific variation in each of the four response variables are shown in Tables 2, 3, 4, 5, 6, and the main findings are summarized below. We found no evidence of multicollinearity among the predictor variables (all variance inflations <5 and tolerances >0.2).

Taxonomic inheritance

From the LMMs, we calculated that taxonomy was responsible for between 53 and 91% of the unaccounted variance (variance not explained by the fixed effects), depending on which response variable is considered (Table 2). Superfamily was the most influential random effects factor, indicating that from 30 to 80% of the variance occurs at this taxonomic level. Except for one model, genus was dropped during the model selection process due to a lack of significant effect. Therefore, there was a strong overall phylogenetic signal in the data.

Main effects

First, the LMMs demonstrated that mollusc size was positively associated with egg volume, miracidial volume and cercarial body volume, but not with TBR (Tables 3, 5, 6). Note that because the Box-Cox transformation uses a negative exponent for the transformation of cercarial body volume and TBR, the estimates in the tables for both of these models should be interpreted with the opposite sign. Thus, trematode species using larger snail hosts had larger transmission stages, whether those infected the snail or were produced inside the snail.

Second, the LMMs indicate that in species where they encyst on substrates, cercariae have larger body volumes than those of species penetrating chordates (Table 3). The raw data suggest the opposite (Fig. 2), but they are uncorrected for what must be a strong effect of snail size. In contrast, the TBR of species using chordates as second intermediate hosts was significantly higher than that of species using either molluscs, arthropods (including annelids), or encysting on substrates (Fig. 3; Table 4). It must be pointed out that the vast

Table 1 Summary statistics of larval trematode measurements

Variable	No. species	Range	Mean	SD
Cercarial volume (mm^3)	379	1.02×10^{-5}–5.13	0.05	0.36
Cercarial tail to body volume ratio	376	0–177.9	1.72	10.4
Egg volume (mm^3)	254	1.03×10^{-7}– 0.035	0.0003	0.0025
Miracidium volume (mm^3)	126	3.9×10^{-7}–0.003	0.00016	0.0003

Table 2 Percentage of random variance attributable to each of the nested random effects (taxonomic levels) for four larval trematode measurements

Variable	Superfamily (%)	Family (%)	Genus (%)	Total (%)
Cercarial volume (mm^3)	43.3	22.4		65.7
Cercarial tail to body volume ratio	29.2	5.8	18.2	53.2
Egg volume (mm^3)	79.5	6.9		86.4
Miracidium volume (mm^3)	70.6	20.5		91.0

Table 3 Summary of LMM with Box-Cox-transformed cercarial body volume (mm^3) as the response variable, Log_e mollusc size, second intermediate host and Box-Cox-transformed latitude as fixed effects, and Genus nested within Family nested within Superfamily as the random effect

Random effects	Groups		Variance	SD
N = 340	Family: Superfamily		0.076	0.276
	Superfamily		0.147	0.383
	Residual		0.116	0.341
Fixed effects	Estimate	SE	*t* Value	95% CI
(Intercept)	2.6245	0.1388	18.90	2.3524 to 2.8967
Mollusc size	**−0.0635**	**0.0271**	**−2.34**	**−0.1167 to −0.0104**
2nd host: Arthropoda	0.1050	0.0842	1.25	−0.0601 to 0.2701
2nd host: Mollusca	−0.1443	0.0772	−1.87	−0.2955 to 0.0069
2nd host: Substratum	**−0.4323**	**0.1439**	**−3.00**	**−0.7144 to −0.1501**
Latitude	−0.0003	0.0002	−1.40	−0.0006 to 0.0001

NB the effect of Chordata as second intermediate hosts is included in the intercept

Fixed effect factors with significant parameter estimates (95% confidence interval bounded away from zero) are shown in bold. Because the Box-Cox transformation used a negative exponent for the transformation of the response variable, negative estimates should be interpreted as having a positive effect, and vice versa

majority (70%) of chordate second intermediate hosts among the trematode species in our dataset were freshwater fish or amphibians.

Third, the effect of the habitat type was manifested by larger TBR values in freshwater species than in marine ones (Table 4), though again the pattern is not clear from the raw data (Fig. 4), and also by larger miracidial volumes in freshwater species than in marine ones (Table 6; Fig. 5).

Finally, the latitude at which the trematode species studied were collected had no significant effect on any of the four response variables (Tables 3, 4, 5, 6).

Discussion

The phenotype of any organism is the product of phylogenetic inheritance and environmental factors that influence the fitness of the organism. Thus, phylogenetic inheritance provides the framework on which individual selective forces may act to favour phenotypes with the highest level of fitness (Stearns 1992). Among parasites, fitness is determined by the rate of successful transmission between hosts in the life cycle. Since the success rate of

Table 4 Summary of LMM with Box-Cox-transformed cercarial tail to body volume ratio as the response variable, Log_e mollusc size, second intermediate host and habitat as fixed effects, and Genus nested within Family nested within Superfamily as the random effect

Random effects	Groups		Variance	SD
N = 349	Genus: Family: Superfamily		0.012	0.110
	Family: Superfamily		0.004	0.062
	Superfamily		0.019	0.139
	Residual		0.031	0.176
Fixed effects	Estimate	SE	*t* Value	95% CI
(Intercept)	0.6142	0.0633	9.70	0.4902 to 0.7383
Mollusc size	0.0055	0.0150	0.37	−0.0238 to 0.0348
2nd host: Arthropoda	**0.1384**	**0.0461**	**3.01**	**0.0481 to 0.2287**
2nd host: Mollusca	**0.2497**	**0.0458**	**5.45**	**0.1599 to 0.3394**
2nd host: Substratum	**0.2281**	**0.0652**	**3.50**	**0.1004 to 0.3558**
Habitat: terrestrial	−0.0748	0.0835	−0.90	−0.2385 to 0.0889
Habitat: brackish	−0.0874	0.0636	−1.37	−0.212 to 0.0373
Habitat: freshwater	**−0.1138**	**0.0320**	**−3.56**	**−0.1765 to -0.0511**

NB the effects of Chordata as second intermediate hosts and marine as habitat are included in the intercept

Fixed effect factors with significant parameter estimates (95% confidence interval bounded away from zero) are shown in bold. Because the Box-Cox transformation used a negative exponent for the transformation of the response variable, negative estimates should be interpreted as having a positive effect, and vice versa

Table 5 Summary of LMM with Log_e egg volume (mm^3) as the response variable, Log_e mollusc size, habitat and Box-Cox-transformed latitude as fixed effects, and Genus nested within Family nested within Superfamily as the random effect

Random effects	Groups		Variance	SD
N = 237	Family: Superfamily		0.325	0.570
	Superfamily		3.744	1.935
	Residual		0.639	0.799
Fixed effects	Estimate	SE	*t* Value	95% CI
(Intercept)	−11.24000	0.54190	−20.74	−12.3021 to −10.1779
Mollusc size	**0.18270**	**0.07904**	**2.31**	**0.0278 to 0.3376**
Habitat: terrestrial	0.32210	0.40670	0.79	−0.475 to 1.1192
Habitat: brackish	−0.64100	0.36630	−1.75	−1.3589 to 0.0769
Habitat: freshwater	0.30440	0.16300	1.87	−0.0151 to 0.6239
Latitude	−0.00004	0.00056	−0.07	−0.0011 to 0.0011

NB the effect of Marine as habitat is included in the intercept

Fixed effect factors with significant parameter estimates (95% confidence interval bounded away from zero) are shown in bold

transmission from one host to the next is generally extremely low, the phenotype of transmission stages must be under an immense selection pressure that favours stages successfully transmitting to new hosts under a given set of environmental conditions.

Table 6 Summary of LMM with Log_e miracidium body volume (mm^3) as the response variable, Log_e mollusc size, habitat and Box-Cox-transformed latitude as fixed effects, and Genus nested within Family nested within Superfamily as the random effect

Random effects	Groups		Variance	SD
N = 111	Family: Superfamily		1.092	1.045
	Superfamily		3.763	1.940
	Residual		0.477	0.691
Fixed effects	Estimate	SE	*t* Value	95% CI
(Intercept)	−11.970	0.701	−17.09	−13.3434 to −10.5966
Mollusc size	**0.359**	**0.117**	**3.06**	**0.1288 to 0.5886**
Habitat: terrestrial	0.592	0.562	1.05	−0.509 to 1.6928
Habitat: freshwater	**0.694**	**0.260**	**2.67**	**0.1835 to 1.2043**
Latitude	0.001	0.001	1.09	−0.0006 to 0.0022

NB the effect of marine as habitat is included in the intercept

Fixed effect factors with significant parameter estimates (95% confidence interval bounded away from zero) are shown in bold

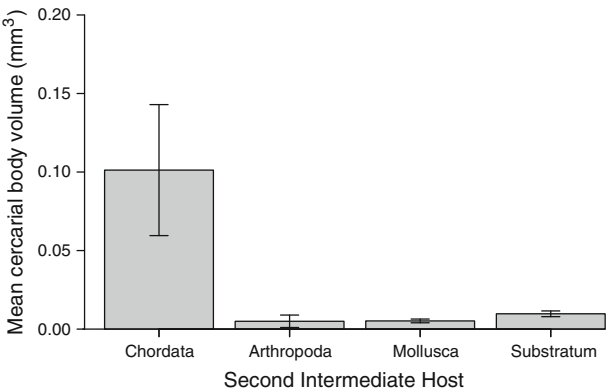

Fig. 2 Mean (±SE) cercarial body volume as a function of the type of second intermediate host. Numbers of trematode species in each category are 167, 78, 61 and 52, respectively

In this study, we used a large dataset including almost 400 species of trematodes to test the contribution of phylogeny and environmental factors to the morphology of free-living parasite transmission stages. Trematodes are ubiquitous and the most common metazoan parasites in aquatic ecosystems, and they therefore provide an excellent model system to test the contribution of phylogenetic inheritance and environmental factors to morphological evolution of dispersal stages.

Effect of phylogenetic inheritance

Our data demonstrated that taxonomy (a close proxy for phylogeny) accounts for a large proportion of the unexplained variance observed in the morphology of free-living stages (Table 2). The majority of the variance was explained at the level of superfamily with

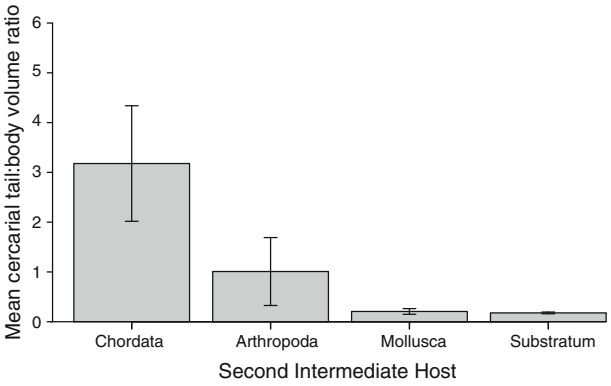

Fig. 3 Mean (±SE) cercarial tail to body volume ratio as a function of the type of second intermediate host. Numbers of trematode species in each category are 166, 78, 61 and 52, respectively

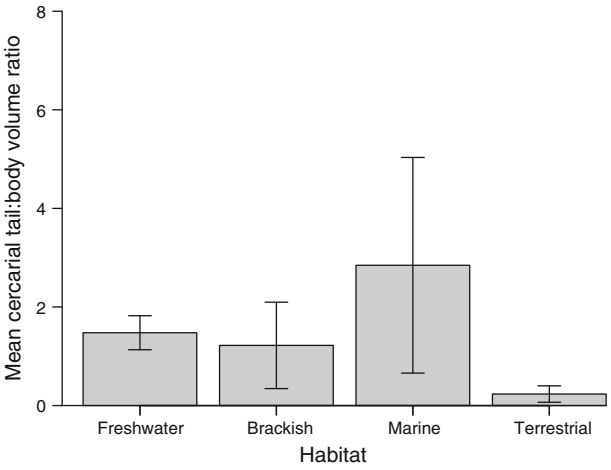

Fig. 4 Mean (±SE) cercarial tail to body volume ratio as a function of the type of habitat in which they are released. Numbers of trematode species in each category are 268, 12, 82 and 14, respectively

much less variance explained at the family and genus levels. Accordingly, much of the differences in life cycles, such as the general identity of host taxa, occur among superfamilies (Cribb et al. 2003).

The phylogenetic effect probably depends on the recency of common ancestry as well as adaptations to local environmental factors. A strong phylogenetic signal is commonly observed across taxa of free-living organisms (Felsenstein 1988, 2002; MacLeod and Forey 2002) and was therefore expected. Nevertheless, strong selection pressures (e.g. environmental gradients) may weaken the phylogenetic signal if those vary among related species (Straney and Patton 1980; Caumul and Polly 2005). Parasitic free-living stages are under strong selection pressure because they are at the mercy of local environmental conditions, and their success depends on finding and infecting a suitable host (Fingerut et al. 2003; Pietrock and Marcogliese 2003; Thieltges et al. 2008; Koprivnikar et al. 2010). At the same time, closely related trematode species share similar developmental pathways and patterns

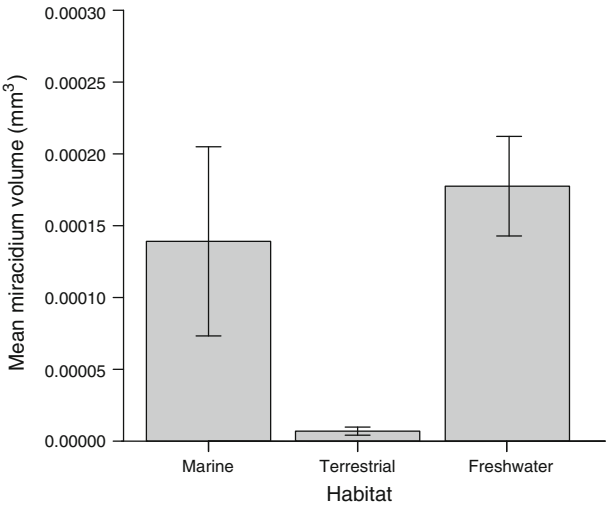

Fig. 5 Mean (±SE) miracidial volume as a function of the type of habitat in which they are released. Numbers of trematode species in each category are 15, 7 and 103, respectively

of genetic covariance that may limit the ways in which their traits can evolve (Galaktionov and Dobrovolskij 2003). It is therefore not surprising that both host and environmental factors explained a significant amount of the variation in the morphology of trematode free-living stages.

Effects of host factors

Snail host size was significantly and positively related to the size of eggs, miracidia, and cercariae. Cercariae are produced by asexual reproduction in the snail gonad, and previous studies found that the production of cercariae was positively related to snail size (Thieltges et al. 2008; Morley et al. 2010). Thus, larger snails have more gonad tissue and therefore provide more space for the production of cercariae. Our data indicate that larger snails also produce larger cercariae suggesting that the trade-off between size and number of cercariae is relaxed when sufficient space and/or nutrients are available. Cercarial size is positively related to the size and hence the fecundity of the adults (Poulin and Latham 2003), and is therefore indirectly related to the fitness of adult trematodes.

The advantage of a positive relationship between snail size and the size of egg and miracidium is less obvious but may be related to the likelihood of encountering a host. Earlier research has shown that egg sizes in trematodes are not influenced by the characteristics of the definitive host, such as body size or whether it is an endo- or ectothermic vertebrate, in which they are produced (Poulin 1997); it therefore seems likely that egg size is influenced by the probability of transmission to the snail host. Population density and the size (or mass) of species are often negatively related (White et al. 2007). The encounter rate between eggs/miracidia and the snail hosts will likely be lower for larger and less abundant snails. This could in turn select for larger and longer-lived transmission stages that remain infective until ingested by the snail (egg) or actively find and penetrate the snail (miracidia).

Apart from the effect of the snail host, the data also provided evidence that the next host in the life cycle had a strong effect on cercarial morphology. Thus, species that infect chordates have a larger TBR. Chordates are mobile and unpredictable in time and space. Such hosts should favour the evolution of cercariae that are capable of actively swimming towards habitats most frequented by the next host and that have a long lifespan to maximise the encounter rate with that host (McCarthy et al. 2002). Since cercariae are non-feeding stages, they completely rely on stored glycogen located in the tail. It was therefore expected that the tail volume is disproportionately large for cercariae infecting chordates.

In addition to a large TBR, chordate-infecting cercariae often also display a variety of morphological adaptations that are not easily quantified. For example, cercariae may have furca and other extensions of the tail to utilise the surface tension of the water to conserve energy and/or reduce the sinking rate (Galaktionov and Dobrovolskij 2003). Such modifications to the tail morphology allow cercariae to hang motionless at the surface and no doubt increase the transmission to the next host without considerably changing the volume of the tail. Our analysis therefore provides a conservative measure of the importance of the tail volume for trematodes infecting chordates.

Cercariae encysting in the open (no second intermediate host) had larger bodies than cercariae that penetrated a host. Cercariae that encyst outside a host experience no growth until reaching a definitive host. The size of the cercariae therefore determines the size, and hence the future fitness of the adult in the definitive host, presumably creating a strong selection pressure for a large cercarial body as previously discussed. The larger body volume is probably made possible by reducing the volume of the tail, and as a consequence cercariae that encyst in the open (e.g. fasciolids) do not swim for long before encysting (Roberts and Janovy 2010).

Effects of environmental factors

Our analysis demonstrated that habitat explained a significant amount of the variation in the morphology of free-living trematode stages. In freshwater habitats, the volume of miracidia and the TBR of cercariae were larger compared to marine species. This difference is probably related to the different properties of freshwater and saltwater. Saltwater has a higher density and viscosity compared to freshwater (by approximately 2.7%) which decreases the sinking rate of plankton, allowing them to float for longer and conserve energy (Vogel 1981). It remains to be tested whether the difference in density alone is sufficient to explain why cercariae in freshwater display a larger TBR than marine cercariae, and why miracidia are larger in freshwater habitats. Interestingly, diatoms that display no means of self-sustained locomotion are larger in marine ecosystems compared to freshwater ones, presumably due to a higher rate of turbulence in the former that keep them in suspension (Litchman et al. 2009). In the case of cercariae, we expected that turbulence would select for a larger investment in the tail to compensate for more demanding swimming conditions. However, as in the diatoms, turbulence may in fact help the cercariae stay suspended in the water column and allow a disproportionately larger investment towards increasing the body volume and therefore decreasing the TBR.

Another important factor determining the properties of water is temperature. Warm water is less dense than cold water. We therefore expected the morphology of cercariae and miracidia to experience different selection pressures under different temperature regimes. Surprisingly, our analysis showed no effect of latitude (a rough proxy for temperature) on any aspect of trematode free-living stage morphology. Many trematodes have a wide geographic range determined by the distribution of the snail first intermediate host. For

example, many trematodes use aquatic migratory birds as definitive hosts, which disperse them for hundreds or even thousands of kilometers during the migratory season (De Montaudouin et al. 2009). Such large-scale dispersal likely hinders the adaptation of trematodes to local environmental conditions, a supposition that is supported by molecular analyses indicating substantial gene flow over long distances along major avian flyways (Keeney et al. 2009, Louhi et al. 2010). Alternatively, latitude may be a better proxy of the duration of the transmission season than of actual temperature. With the temporal window of transmission decreasing from several months to a few weeks as one moves toward high latitudes, latitude might be more likely to impact the dynamics of cercarial release and transmission than cercarial morphometrics.

Conclusion

In conclusion, our study provides evidence that the morphology of trematode free-living transmission stages is significantly constrained by phylogenetic inheritance, but also substantially influenced by important host characteristics, such as host size and type of host, and by habitat (freshwater versus saltwater). In many ways, parasite free-living stages are under similar selection pressures to the lecithotrophic larvae of free-living organisms (Llodra 2002; Marshall and Keough 2003). A suitable substrate or host must be found within a short window of opportunity determined by the amount of stored energy reserves. The main difference is that suitable habitats for parasite transmission stages (i.e. hosts) often display a much lower predictability in time and space compared to substrate availability during the dispersal of free-living species. Comparative studies of free-living and parasitic larval stages are needed to examine the effect of predictability on the morphology of dispersal stages in organisms utilising the two life strategies.

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