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ORIGINAL PAPER

How large is the hand in the puppet? Ecological and evolutionary factors affecting body mass of 15 trematode parasitic castrators in their snail host

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Abstract Parasitic castration is an adaptive strategy where the parasite usurps its host's phenotype, most notably the host's reproductive effort. Though castrators are loosely known to be large relative to their hosts (compared to typical parasites), their mass has rarely been quantified and little is known about size variation, even if such variation exists. By cross-sectioning snails, we examined intra- and inter-specific variation in the parasite/host mass of 15 trematode species that castrate the California horn snail, *Cerithidea californica*. Trematode species occupied 14–39% (mean = 20.3%) of an infected snail's soft tissue mass. Intraspecific variation in castrator mass fluctuated with variables that covary with energy available for host reproduction. Specifically, trematode mass was 24% higher in summer than in winter, 15% greater in snails from intertidal flats than from tidal channels, and increased with host mass to the 1.37 power (a finding contrary to that previously documented for other types of parasites). Relative body mass differed across trematode species, varying interspecifically with: (1) taxonomic family, (2) host tissue use

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(larger species used more types of host-tissue), (3) position in the trematode interspecific competitive dominance hierarchy (the two most subordinate species were the largest, otherwise size tended to increase with dominance), and (4) type of host used by offspring (species whose offspring infect relatively predictably occurring benthic invertebrates were larger than those infecting transient vertebrates). Our findings suggest that ecological constraints and life history trade-offs between reproduction and survival influence the mass of these very large parasites.

Keywords Parasitism · Body mass · Virulence · Reproductive allocation · Consumer strategy · Life history

Introduction

> "All animals, if operated on when they are young, become bigger and better looking than their unmutilated fellows."
> Aristotle (350 BCE)

Castration forcibly frees reproductive energy for other uses. This explains the "post-operative" somatic development observed by Aristotle. A variety of parasites castrate their hosts. These "parasitic castrators" usurp their host's phenotype, most notably energy intended for reproduction (Kuris 1974; Baudoin 1975). In the simplest case, castrators may limit their energy consumption to host reproductive effort, unaffecting host energy allocation to growth and survival. However, a castrator's reproductive expectations may be quite different from those of the host. Therefore, these parasites may actively manipulate resource allocation of the hijacked host's phenotype in a way that reflects their life history and not that of the host. By the same reasoning, different castrators of the same host species may have different strategies of host use, each optimalized for its particular life history. Here, we quantify a correlate of parasite reproductive effort—relative body mass—for several species of parasitic castrator that use the same host species. We identify intra- and inter-specific variation in castrator relative mass and analyze factors associated with that variation to examine ecological and evolutionary issues underlying the energetics of parasitic castration.

A striking feature of parasitic castrators is that they grow large relative to their hosts, compared to typical parasites (Kuris 1974; Baudoin 1975). However, this is a generality largely based on casual visual inspection—there are few studies that have quantified the relative mass of castrators. Two previous studies quantified the mass of a single castrator species in a single host species. Trematode flatworm castrators comprised 20–28% of the infected host snail's tissue weight (Hurst 1927; Wilson and Denison 1980). Also, a few studies have shown that castrators grow along with their hosts, apparently tracking available resources. The size (length) of parasitic isopod crustaceans increased in proportion to size of their decapod crustacean hosts (reviewed in Kuris 1974; and, see Muñoz and George-Nascimento 1999; Kuris and Lafferty 2000).

Parasitic castrator mass might respond to variation in host resources beyond simply keeping pace with host size. For instance, for many iteroparous species, larger individuals allocate relatively more energy to reproduction than do smaller individuals (Pianka and Parker 1975; Kozlowski 1992). Therefore, a castrator may grow disproportionally large in larger hosts. If being in a larger host is advantageous, the castrator might manipulate host allocation to growth (Hall et al. 2007). Indeed, parasitic castrator trematodes sometimes cause gigantism of snail hosts (Rothschild 1936; reviewed by Sousa 1983; Sorensen and

Minchella 2001), suggesting that castrators may exercise different energy allocation strategies than do uninfected hosts. Reproductive investment may also differ between host sexes (Stearns 1992). Thus, if castrators are limited to pre-infection host allocation, they could have different masses in hosts of different sexes. Alternatively, if host sexes differ in reproductive effort, castrators may adaptively manipulate the host phenotype to an allocation pattern that better serves the parasite. This may be partly why many castrators of crustaceans feminize host morphology (e.g., see Reinhard 1956). Additionally, host reproductive effort can vary with seasonal change and with environmental quality. Thus, even without a reallocation strategy, parasitic castrator mass might intraspecifically vary in time or space (Hall et al. 2007).

It is less clear whether body mass should vary among parasitic castrator species. If castrators are constrained to simply use host reproductive allocation, we should see similar body masses across castrator species using the same host species. Alternatively, if castrators manipulate host energy allocation, interspecific variation in life-history trade-offs might alter optimal castrator body mass. Precise predictions of optimal energy allocation require knowledge of the fates of both the reproducing parasite and its young. For instance, mortality schedules may affect allocation to reproduction (Williams 1966; Pianka and Parker 1975; Stearns 1992). A shorter expected life span may select for castrators to increase current production (and thus increase relative mass) at the expense of maintenance. Supporting this, in a survey of the literature, Taskinen et al. (1997) reported that species of trematode castrators in snails with shorter life spans tended to infiltrate more tissues of the host. Theory also suggests that the mean and variance of expected survival of offspring affect reproductive allocation (Stearns 1992). Thus, for example, we predict that castrators with offspring that infect sporadically available hosts might be selected to have lower relative mass, reflecting a bet-hedging or variance discounting strategy. Hence, considering variation in mass among castrator species is one means to investigate the trade-offs involved in the evolution of parasitic castrators.

Because host species likely vary in many important aspects, a productive way to begin to investigate variation in body mass of parasitic castrators is to study several castrator species that parasitize the same host species. This can provide insight into the generality of intraspecific trends in castrator body mass, and allow interspecific comparisons. Trematode parasites that castrate snails provide perhaps the best systems for such inquiries because a single snail species at a single location can support a species-rich trematode assemblage (Kuris and Lafferty 1994; Poulin and Mouritsen 2003; Huspeni et al. 2005).

In this study, we quantified the parasite/host mass for 15 trematode species that castrate the California horn snail, *Cerithidea californica* (Haldeman 1840). Trematode castrator relative mass should directly reflect overall reproductive investment. This is because the main function of trematode tissue in a snail is the continual asexual reproduction of offspring, while the trematodes primarily use the host body for survival. Further supporting this reasoning are data from McCarthy et al. (2002). They reported on two confamilial trematode species that parasitize the same snail species. Our analysis of their data indicates that the trematode species that was twice as productive (volume of offspring) was also twice as large (volume of trematode tissue in snails) as was the other species (GLM, $F_{2,9} = 6.16$, $P = 0.021$).

We tested the following hypotheses and predictions. (1) If resource availability affects castrator relative mass, then relative mass would intraspecifically vary with resource quality as measured by host size, season and habitat type. (2) If castrator species adaptively manipulate infected host resource allocation, then relative mass would be the same in both host sexes, and (3) would differ between castrator species, doing so in ways predictable from life history theory. (4) If survivorship influences adaptive allocation, then relative

mass would be lower for competitively dominant trematode species than for subordinate species with shorter life expectancies. (5) If variance in offspring survivorship affects adaptive allocation, relative mass would be lower in trematode species whose offspring use more transient hosts compared to those infecting more predictably occurring hosts. (6) We also predicted that trematode relative mass would vary with host tissue use, and that species using the least commonly used tissue and a lesser number of host tissues would tend to have smaller relative masses. We found that parasitic castrator mass, although always large, varied intraspecifically in ways mirroring energy available for production. Additionally, trematodes interspecifically varied in relative mass such that they appear to differentially manipulate infected host resource allocation in response to species-specific trade-offs concerning survivorship and reproduction.

Materials and methods

Host–parasite system

At least 18 digenean trematode species, belonging to eight families and five orders (following Olson et al. 2003), parasitize the California horn snail, *Cerithidea californica* (Martin 1972; Lafferty et al. 2006). California horn snails live up to ~10 years (McCloy 1979; Sousa 1983) and individual trematode infections are also long-lived (Sousa 1983; Kuris 1990; Sousa 1990). Reproductive output of uninfected snails varies with habitat quality (Race 1981; Lafferty 1993; Armitage and Fong 2004) and season (McCloy 1979; Race 1981; Sousa 1983). Depending on trematode taxon, infection occurs either by a hatched free-swimming stage (miracidium) or by ingesting a trematode egg. Following infection, a trematode castrates the snail and grows via asexual production of clones (parthenitae). We equate the aggregate clonal mass to "body mass" because the clones cluster and function as a whole within the snail. Soon after infection, most parthenitae stop producing additional parthenitae (i.e., the infection grows very slowly, with the host), and switch to produce cercariae. These free-swimming offspring encyst in or on second intermediate hosts (e.g., annelids, mollusks, and fishes). Birds and mammals acquire adult parasites by preying on second intermediate hosts. One species does not use a second intermediate host, directly penetrating final host birds. Although the gonad is generally the most common site of infection, the species vary in tissue site use (Yoshino 1975; Kuris 1990; Sousa 1993). We classified the host tissue site based on our familiarity with this system (i.e., from direct observations of tens of thousands of dissected snails). Interference competition between trematodes within individual hosts is frequently strong and asymmetrical, resulting in the death of subordinate species (Kuris 1990; Sousa 1993; Lafferty et al. 1994). These trematodes do not cause gigantism in their snail host (Sousa 1983), nor appear to affect survivorship under most conditions (Sousa and Gleason 1989).

Field collection and laboratory work

We collected snails in 2005 from Carpinteria Salt Marsh (CSM), California, USA (34.40°N, 119.53°W) from two intertidal habitats (channels and mud flats). At CSM, flats have greater resource levels than do channels (J. Lorda, personal communication). Additionally, we sampled during two seasons, summer (when growth rates are high), and winter (when growth rates are low) (McCloy 1979; Race 1981; Sousa 1983). We haphazardly collected snails throughout the size range (~22–38 mm length) within which we commonly encounter developed trematode infections.

We brought snails to the lab and processed them within 24 h. After rinsing and air drying a snail, we carefully cracked the shell with a hammer and removed the tissue while viewing under a stereo-microscope. We then measured the total soft tissue weight (all weights are wet and to the nearest 0.0001 g). Then, we separated and measured the weight of the infected region(s) of the snail (digestive gland/gonad region, basal visceral mass, or mantle). We determined snail sex (by presence/absence of an ovipositor) and trematode species following Martin (1972) and Hechinger and Huspeni (unpublished manuscript).

To estimate trematode mass for each infected snail, we first determined the trematode proportion of the infected region of the snail using serial cross sections on AFA-fixed snails. We took six regularly-spaced cross-sections from each digestive gland/gonad and mantle region, and four cross-sections from each basal visceral mass. We determined the area (to the nearest 0.0001 mm^2) of trematode and snail tissue, using digital photographs of each section and ImageJ software (W. S. Rasband, 1997–2005, US NIH). We calculated the average proportion of trematode tissue (weighted by cross-section area) for each infected region. We obtained trematode mass by multiplying the average proportion trematode by the mass of the infected region and then calculated relative trematode mass for each snail by dividing trematode mass by the total snail soft tissue mass.

We sectioned 167 developed infections involving 15 trematode species (Table 1 and Table esm1). We excluded 13 immature heterophyid infections from analyses, since such infections are still rapidly growing. We also excluded mixed-species infections with overlapping host tissue occupancy (see esm) (the affect of such infections on total trematode mass is dealt with in another study (Hechinger et al. submitted)).

When calculating the trematode relative mass with our sectioning technique, we assumed equal density of trematode and surrounding snail tissue. To ensure that this assumption was reasonable, we compared the densities of infected and uninfected snail tissues. We estimated the densities by measuring wet mass and water displacement volume of 11 excised sections of each appropriate tissue type (trematode + digestive gland, digestive gland, basal visceral mass, and mantle). The various tissues did not significantly vary from an overall mean of 1.13 g ml^{-1} (one-way ANOVA, $F_{3,40} = 0.155$, $P = 0.93$), with 65% and 96% power to detect respective differences of 0.050 and 0.075 g ml^{-1} or greater.

We also estimated the relative mass of gonad tissue for a sample of uninfected snails, employing the same techniques that we used to quantify trematode mass. We did this for the testes of eight males and the ovaries of eleven females (all from a tidal channel in summer).

Data analysis

To examine how factors affected relative trematode mass, we primarily used general linear models (GLMs, see Neter et al. 1996; Quinn and Keough 2002) in JMP v. 7.0 (2007, SAS Institute, Inc.). We incorporated all categorical predictor variables as fixed effects. We first determined the effect on relative mass of species, and the potential covariates of season, habitat, host mass (combined host-parasite tissues), and host sex. To assess how relative mass was influenced by the four species-level predictor variables, we incorporated those into separate GLMs, nesting species within the variable of interest. We dropped snail sex from analyses because it did not affect relative trematode mass (see esm). To ensure that our findings concerning the relationship of relative mass to total mass were not the result of 'spurious self-correlations' (Kenney 1982; Jackson and Somers 1991), we performed a randomization test (Edgington 1995), using the

Table 1 Species of trematode used in this study with attributes of their biology and ecology

Family	Species	Species code[a]	Host-tissue use[b]	Dominance rank[c]	Offspring infect[d]	n[e]	CV[f] relative mass
Cyathocotylidae	Small cyathocotylid[f]	SMCY	g + dg + bvm	10.5	F	7	31.0
Schistosomatidae	*Austrobilharzia* sp.[g]	AUST	g + dg + bvm	1	B	1	–
Notocotylidae	*Catatropis johnstoni*	CATA	m	–	I	3	16.6
Echinostomatidae	*Acanthoparyphium spinulosum*	ACAN	g	5.5	I	6	13.3
	Himasthla rhigedana	HIMA	g	3	I	27	27.6
	Himasthla sp. B[g]	HIMB	g + bvm	4	I	27	27.1
Philophthalmidae	*Cloacitrema michiganensis*	CLOA	g + bvm	5.5	I	21	26.6
	Parorchis acanthus	PARO	g + bvm	2	I	19	27.1
Heterophyidae	*Euhaplorchis californiensis*	EUHA	g	8	F	22	20.1
	Phocitremoides ovale	PHOC	g	8	F	2	31.3
	Stictodora hancocki	STIC	g	8	F	4	19.7
Renicolidae	Large xiphidiocercaria[g]	LGXI	g + dg	10.5	I	9	36.1
	Renicola buchanani	RENB	m	–	F	10	30.4
Microphallidae	*Probolocoryphe uca*	PROB	g + dg + bvm	12.5	I	7	21.5
	Small microphallid[g]	SMMI	g	12.5	I	2	4.9

[a] Species codes will be used in subsequent Tables and Figures

[b] g = gonad, dg = digestive gland, bvm = basal visceral mass, m = mantle

[c] Lower-ranked species (dominants) kill higher-ranked species (subordinates). We did not rank the two mantle dwelling species because they do not linearly fit into the hierarchy and also are confounded by tissue-use

[d] "Offspring" refer to asexually produced cercariae, which leave the snail first intermediate host to infect either invertebrates (I), fishes (F), or birds (B)

[e] The number of individual infections of each species for which we quantified trematode mass ($n = 167$)

[f] CV = (standard deviation/mean) × 100 (based on data corrected for snail size, season, and habitat)

[g] We recognize these as species although they have not yet been described

Resampling Stats add-in 3.0 (2004, Resampling Stats, Inc.). To assess the relationship between absolute trematode mass and infected snail mass, we used non-linear regression with the Gauss–Newton method and parameter starting values derived from the equivalent model fit in log–log space (see esm). Ordinary least squares was justified because we expect our measurement error in trematode mass to be larger than that for the mass of the entire infected snail (Quinn and Keough 2002). We used an ordered-heterogeneity test (Gaines and Rice 1990; Rice and Gaines 1994) to assess statistically the trend of increasing relative mass with increased tissue-use. To generate the ordered-heterogeneity test statistic, r_sP_c, we used Spearman's rank correlation (r_s), and the complement of the P-value (P_c) from the partial F-test from the GLM testing the effect of tissue-use on relative mass. We ranked mantle-dwellers lowest, followed by other species in order of increasing numbers of tissues used. We performed a single-planned contrast to test whether mantle-dwelling species had the lowest relative mass. We assigned competitive

dominance ranks (Table 1) slightly modified from Kuris (1990) and Huspeni (2000). We only gave ranks to the 13 species that use the visceral mass because mantle-dwellers are confounded by tissue site use. The clearest way to categorize offspring environment predictability was by classifying their hosts as invertebrate (all sessile or localized benthos) or vertebrate (all mobile and transient fishes and birds). We examined the occurrence of statistical interactions between predictor variables. Interactions were non-significant ($P > 0.10$) in all cases that we examined (details in the esm).

We do not expect that phylogenetic constraint directly operates on trematode growth in snails to drive similarity in relative mass of related trematodes. These trematode infections grow modularly, by simply adding new, clonal parthenitae. Hence, trematode relative mass should be a trait that freely and rapidly evolves in response to factors affecting optimal allocation to survival and reproduction. Therefore, performing analyses on raw data is appropriate. However, even barring phylogenetic inertia, species may share numerous confounding traits due to common ancestry. Although our study included 15 trematode species, most robust phylogenetically explicit tests (Harvey and Pagel 1991) are precluded by the high covariation between trematode phylogeny, relative mass, and the predictor traits of interests (dominance rank and type of hosts infected by offspring). Nevertheless, instead of dismissing phylogenetic control, we examined two of our main interspecific comparisons in a phylogenetically corrected way. We constructed a phylogenic tree for our species (Figure esm1), using the phylogeny of trematode families provided by Olson et al. (2003), and information on generic or subfamilial relationships (Yamaguti 1971) to resolve within-family topography. We generated phylogenetically independent contrasts (Felsenstein 1985; Harvey and Pagel 1991) to examine how relative mass varies with rank in the competitive dominance hierarchy (treating rank as a continuous variable). To assess the influence on trematode relative mass of the type of host infected by offspring, we implemented Hansen's test for adaptation under optimizing selection (Hansen 1997), running the test for several values of evolutionary constraint (α). We also note the few independent comparisons in offspring host use that were available in the phylogeny. For all phylogenetically explicit comparisons, we used the software, COMPARE 4.6b (E. P. Martin, 2004, Indiana University).

Some species were poorly represented in our sampling. Although all the GLM-based analyses explicitly account for sample size, some of the comparative analyses are based solely on species' means. This was probably not an issue, since the coefficients of variation were relatively low for all sampled species (Table 1). Nevertheless, we performed parallel analyses excluding the five species with less than six sampled individuals. Because the results from those analyses were qualitatively (and effectively quantitatively) the same as the analyses using all available data, we present only the latter.

We ensured assumptions of approximate normality and variance homogeneity were met by inspecting plots of residuals versus predicted values, and by inspecting normal quantile plots with Lillifors 95% confidence limit curves. We analyzed untransformed data in all but one minor case where we used fourth root transformation to mitigate variance heterogeneity. All *P*-values are two-tailed, except for the test of the clearly directional and qualitatively substantiated hypothesis regarding the effect of tissue-use. Since we planned our testing before doing analyses, we focus on and present nominal *P*-values. We also show that all significant *P*-values remained so after controlling for multiple comparisons by holding the 'false-discovery rate' (Benjamini and Hochberg 1995) to 0.05 for our family of eight tests of the main effects on trematode mass and the single test of male versus female gonad size (Table esm7).

Table 2 Results of the general linear model demonstrating the effects of several variables on the relative mass of trematodes in infected snail hosts

Predictor	df	SS	*F*-ratio	R^2	*P*
Species	14	0.351	8.15	0.43	0.0000
Season	1	0.039	12.67	0.08	0.0005
Habitat	1	0.013	4.23	0.03	0.0414
Tissue mass	1	0.018	5.92	0.04	0.0162
Full model	17	0.665	12.69	0.59	0.0000
Residual	149	0.459			

Note: the intercept in this GLM was 0.153 ± 0.022 (±se)

Results

Single-species trematode infections comprised 6–49% of the tissue mass of parasitized snails ($n = 167$). Trematode species identity explained 43% of the variation in relative mass (Table 2). Mean relative mass ranged from 14 to 39% among the 15 trematode species (Fig. 1a), averaging 20.3 ± 3.5% (±95% cl) across species (holding to average values the effects of season, habitat, and host size). This compared with relative masses of 7.7 ± 1.9% (±95% cl) for uninfected female snail gonads and 14.6 ± 2.2% for male gonads (Fig. 1b), which further differed from each other ($F_{1,17} = 27.7$, $P < 0.0001$).

Effects on intraspecific variation in trematode relative mass

Relative trematode mass was higher under conditions of abundant resources. The relative mass of the average trematode infection was 23.9% higher in summer than in winter (22.3 ± 2.4% (±95% cl) in summer, vs. 18.0 ± 2.1% in winter; Table 2). Trematode relative mass was 15.0% greater on intertidal flats than in channels (21.5 ± 2.2% on flats, vs. 18.7 ± 2.4% in channels; Table 2).

Trematode relative mass increased with host size (Fig. 2a, Table 2) and this was not the result of spurious correlation (see esm for randomization test). Thus, absolute trematode mass scaled allometrically with infected snail mass (Fig. 2b). This allometric relationship was bolstered by our analysis in log-log space (esm). In contrast, there was no discernable relationship between relative gonad size and the size of uninfected snails (Fig. 2a; GLM on 4th root transformed data to mitigate variance heterogeneity, $F_{1,16} = 0.02$, $P = 0.89$), but we note the limited range of uninfected snail sizes caused by the rarity of uninfected large snails.

Effects on interspecific variation in trematode relative mass

Family-level differences explained a sizeable amount of the variation in relative trematode mass among species, after controlling for season, habitat, and host size ($P < 0.0001$, Table esm2). Species identity barely approached significance after accounting for family ($P = 0.053$, Table esm2).

The tissue site use of a trematode species was associated with variation in relative mass (Fig. 3, Table esm3). Additionally, relatively larger species used more tissues (Fig. 3). In particular, relatively larger species extended from the gonad to also use the basal visceral mass. Further, the species with the largest relative mass used the gonad, the digestive

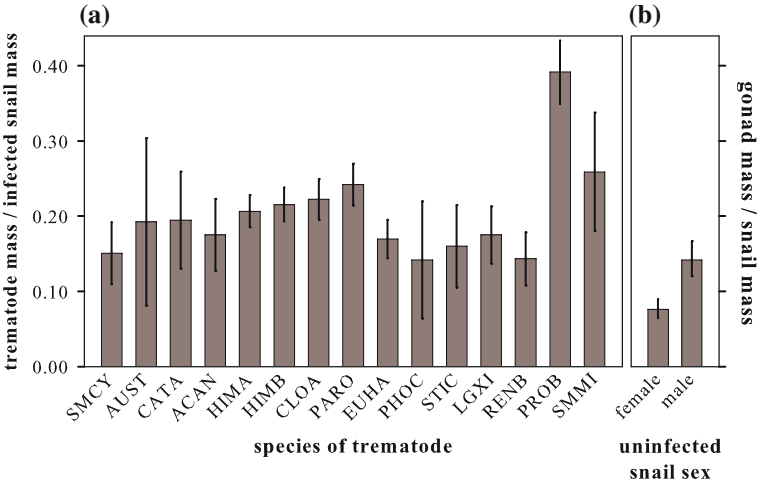

Fig. 1 (**a**) Relative mass of 15 species of trematodes in individual snail hosts and (**b**) relative mass of gonads of uninfected female and male *Cerithidea californica* snails. Values are estimated means (±95% confidence limits): for (**a**), from a general linear model holding to average values the effects of season, habitat, and host-tissue weight; and, for (**b**), from a general linear model holding snail weight constant (all uninfected snails came from a single habitat (channel) and season (summer)). Trematode species are arranged by taxon and species codes are in Table 1. Since the confidence limits are based upon the pooled variance, variation in their magnitude across species reflects only variation in sample size

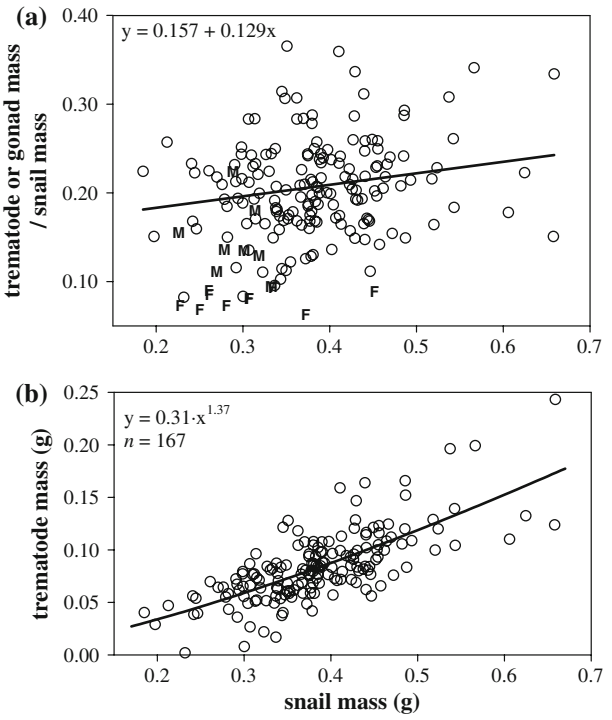

Fig. 2 (**a**) Relative trematode mass and (**b**) actual trematode mass increase with infected snail tissue mass. The data are leverage residuals (Sall 1990) obtained from general linear models controlling the effects of trematode taxon, habitat, and season. In (**a**), we also indicate the gonad relative mass of uninfected male (M) and female (F) snails. For (**b**), the 95% confidence interval for the exponent is 1.17–1.57, and that of the slope is 0.26–0.37

gland, and the basal visceral mass. Also, species occupying the mantle had 19% lower relative mass than did the species that use the visceral mass (Fig. 3; planned contrast, $t = 1.94$, df $= 149$, $P_{1\text{-tailed}} = 0.027$). Trematode species-specific variation still remained after accounting for tissues used ($F_{10,149} = 7.88$, $P < 0.0001$). Thus, diverse tissue use did not necessarily correspond with a larger relative mass. This occurred because some of the species that use more tissues (AUST, SMCY, and LGXI (species names and codes in Table 1)) were frequently less densely packed in host tissues than were species that only use host gonad (Figure esm2).

Although relative mass significantly varied among dominance ranks ($F_{7,138} = 7.2$, $P < 0.0001$, Table esm4), there was no consistently increasing relationship between a trematode species' relative mass and its rank in the competitive dominance hierarchy (Fig. 4). However, the two lowest ranking species had the highest relative masses. The general trend across the other species was that relative mass increased with increasing dominance. When using independent contrasts, there was no association between relative mass and dominance rank (Figure esm4, $R^2 < 0.001$, $P = 0.93$, $n = 12$), but we note the three apparently zero dominance rank contrast values may be due to a lack of resolution of the interactions between closely related species to whom are assigned the same dominance rank (for nodes i, j, and l; Figure esm1).

The relative mass of trematode species (excluding the two mantle-dwellers) differed between species whose offspring infect vertebrates and those whose offspring infect invertebrates ($F_{1,138} = 15.7$, $P = 0.0001$, Table esm5). The five vertebrate-users were, on average, 31% smaller than were the eight invertebrate-users ($16.5 \pm 0.03\%$ vs. $23.8 \pm 0.02\%$ ($\pm$95% cl)). Hansen's test for adaptation further supported this, effectively converging on our cross-species comparison throughout a large range of evolutionary constraints, including the maximum likelihood value for evolutionary constraint ($\alpha = 1.6$; Table esm6). Here, the optimum relative mass of vertebrate users was estimated to be 38% smaller than that for invertebrate users ($R^2 = 0.32$, $t_{12} = 2.43$, $P = 0.032$, Table esm6). Due to the general covariation between phylogenetic relatedness among trematodes and the hosts subsequently infected by their offspring, there were only two non-confounded independent character transitions to evaluate. However, the two groups using vertebrates had 26% and 37% lower relative mass than did the invertebrate-using species in their sister groups (Figure esm1, contrast codes b & c). We also note that the vertebrate-using

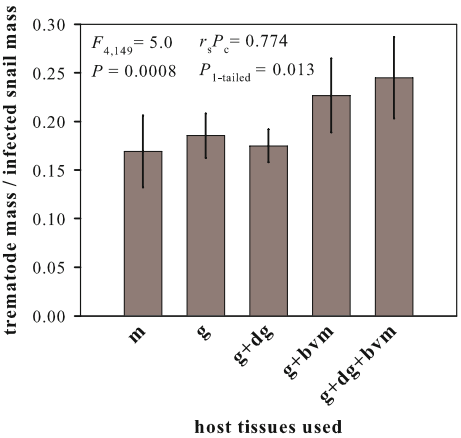

Fig. 3 Relative mass of trematodes in infected snails classified by host tissues used (m = mantle, g = gonad, dg = digestive gland, bvm = basal visceral mass). Values are means ($\pm$95% confidence limits, using the pooled variance), using a general linear model to hold constant the effects of season, habitat, and host-tissue weight (Table esm3). The *F*-statistic, and *P*-value are for the categorical effect of tissue-use on relative mass. The r_sP_c statistic and *P*-value are from an ordered-heterogeneity test assessing whether trematode mass increases with number of tissues used

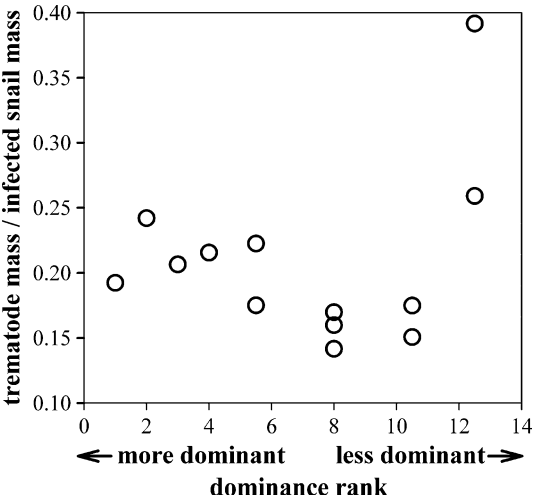

Fig. 4 The relative mass of trematode species, ordered by their rank in the dominance hierarchy (Table 1)

renicolid, RENB, was 18% smaller than its invertebrate-using congener, LGXI (Fig. 1). However, this association is confounded by RENB being a mantle dweller.

Discussion

Trematodes always took a large proportion of their host's mass and this proportion varied significantly among species. As outlined in the introduction, these interspecific differences in relative mass should directly reflect variation in the amount of energy allocated to reproduction. This idea is further supported by our evidence that much of the intraspecific variation in relative mass was associated with factors that covary with energy available to the host for reproduction and growth.

Intraspecific variation in relative castrator mass likely reflects energy availability

The intraspecific winter decline in trematode mass probably reflects diminished energy available for production. This is likely proximally driven by decreased availability of food (benthic diatoms) caused by shorter photoperiod and lower temperature. In the winter, the snails stop growing, stop reproducing, and become less active (McCloy 1979; Race 1981; Sousa 1983). We have observed that snail gonads regress in the winter and that trematode parthenitae become less densely packed in snail tissues and produce far fewer cercariae (unpublished data). Our findings quantify this qualitative observation of seasonal regression and show that the seasonal ebb in host reproductive effort is paralleled by decline in castrator mass.

Intraspecifically, relative trematode mass was higher in snails collected from flat than from channel habitats. Habitat quality for California horn snails generally varies at a small scale, due to factors such as food quality, sediment texture, or inundation time (Race 1981; Lafferty 1993; Armitage and Fong 2004). Indeed, recent sampling from our study areas indicates that benthic diatoms (horn snail food) are 3.4 times more abundant on the flats than in the channels (J. Lorda, personal communication). Mathematical modeling (Hall

et al. 2007) has also indicated that parasitic castrator volume can increase with an increase in food. Thus, as with seasonal variation, spatial variation in relative trematode mass is likely explained by energy availability.

Trematodes occupied a higher proportion of tissue in larger snails (Fig. 2). This contrasts interspecific patterns previously documented for other types of parasites. George-Nascimento et al. (2004) and Poulin and George-Nascimento (2007) respectively found that parasitic helminth biovolume in vertebrates either proportionally decreased or remained constant as host size increased. We expect that this difference reflects that parasitic castrators operate under different principles than do the macroparasites and trophically-transmitted larval parasites used in those studies. We suggest that the proportionally larger size achieved by parasitic castrators in larger hosts simply mirrors the increased reproductive effort that typifies many iteroparous species, where larger (and older) individuals shunt relatively more energy to reproduction than to growth (Pianka and Parker 1975; Kozlowski 1992). Like uninfected snails, parasitized horn snails grow more slowly when larger (Sousa 1983; Lafferty 1993), further supporting the idea that they allocate proportionally more to reproduction with increased size. The disproportionate increase in castrator mass with host size may be a general phenomenon, because castrators frequently are long-lived and iteroparous.

Thus, the combined evidence suggests that intraspecific variation in castrator mass largely reflects variation in the amount of energy available for production. Beyond this, variation in mass across castrator species likely reflects their differential manipulation of energy allocation of the castrated host's phenotype.

Does the documented interspecific variation in castrator relative mass represent adaptive responses to trade-offs between survivorship and reproduction?

Most of the variation in relative mass between trematode species was attributable to family differences (although some confamilial species still varied from each other in relative mass). This mirrors general trends across the animal kingdom, where most variation across species is bound up at the taxonomic familial and ordinal level (Harvey and Pagel 1991). However, what biological factors might drive the documented interspecific variation?

In some species, parthenitae were solely in the space normally occupied by the gonad (Table 1, Figure esm2). This fits in with the traditional view of a trematode parasitic castrator. By not affecting vital tissues, the parasite will minimally decrease host phenotype viability (Poulin 1998; Combes 2001; Esch et al. 2001). Hence, being restricted to the gonad area may constrain parasite mass and current production, but it also likely increases reproductive lifespan of the castrator.

Other trematode species used tissues beyond the gonad, but were less densely packed, not achieving masses much greater than species concentrating in the gonad space. This pattern is inconsistent with increasing production by escaping the limiting space of the gonad region. Why would these species incur the potential cost of reduced survivorship (by damaging vital tissue, see Yoshino 1976b) if not to achieve greater current production? One hypothesis is that the use of additional tissues by subordinates provides a partial refuge from competitive displacement by dominant species that concentrate in the gonad (DeCoursey and Vernberg 1974; Yoshino 1975; Kuris 1990). Supporting this idea, several of the species that used multiple tissue types are low in the dominance hierarchy (Table 1). Thus, increased survival provided by refuge from competition may offset the negative effects on host phenotype survival caused by using vital tissues.

Two of the trematode species localize in the mantle, completely segregated from the primary tissue sites used by other trematodes. This potentially minimizes competitive displacement by dominants (Yoshino 1975; Kuris 1990). However, these mantle dwellers damage vital tissues (Yoshino 1976a), likely decreasing survivorship. Offsetting this potential negative effect on survival, one of these species (RENB) had one of the lowest estimated relative masses of all the trematodes. In contrast, the other mantle species (CATA) had a high relative mass. Expecting that this species would therefore be associated with high snail mortality, we analyzed data from Sousa and Gleason (1989) and found that CATA-infected snails disproportionally died in their experiments (3 out of 7 CATA infections died, whereas only 15 of 811 other trematode infections died ($P = 0.0003$, $n = 818$, randomization test (Edgington 1995), stratified by treatment, 10,000 permutations).

Some trematode species appeared to achieve a higher relative mass by extending their tissue site use beyond the gonad. The two lowest members of the competitive dominance hierarchy had the highest relative masses of all the trematode species examined (Fig. 4). Because subordinates have a shorter life expectancy than do dominants, these two species may be manifesting a classic life history trade-off, increasing current production in the face of decreased survivorship (Williams 1966; Pianka and Parker 1975; Stearns 1992). However, survivorship did not appear to generally explain interspecific variation in trematode relative mass because there was no consistent relationship between dominance ranking and relative mass across all the examined species. Future work can more thoroughly examine this issue by determining survivorship probabilities based upon the amount of competitive losses actually occurring in the field (Lafferty et al. 1994).

Our findings support the hypothesis that castrators with offspring that infect sporadically available hosts are selected for lower relative mass, reflecting a bet-hedging or variance discounting strategy. Relative mass was smaller for castrators whose offspring infect vertebrates compared to those trematodes subsequently infecting invertebrates (in both our phylogenetically uncorrected and corrected analyses). This is consistent with the idea that infecting vertebrate hosts is a less predictable or lower return event relative to the likelihood or returns for infecting invertebrates. The vertebrate hosts in our system (birds and fishes) certainly move in space and time much more than do the invertebrate hosts (benthic crustaceans, mollusks, and polychaetes). Other factors important for host-parasite interactions differ between vertebrates and invertebrates (most notably, vertebrate adaptive immune systems). However, it is not clear whether such differences would affect the variance of cercarial or metacercarial survival. Our reasoning parallels that of McCarthy et al. (2002), explaining the variation in the total volume of cercariae produced by two trematode species. In their system, the trematode that infects vagile crabs produced approximately half the daily volume of cercariae than did the species infecting sessile and abundant barnacles.

On average, the mass of trematode tissue substantially exceeded the mass that snails devoted to their own gonad tissue, particularly when compared to females. This difference further supports our contention that castrators manipulate the energy budget of infected host phenotypes. Interestingly, most of the trematode species that only use the gonad space did not differ in relative mass from the relative mass of snail testes. Perhaps for these castrator species optimal allocation converges with that of uninfected snails. Also notable is that trematode mass did not differ between host sexes, despite intact female gonads being almost half the size of intact male gonads. Female horn snails have other reproductive structures (e.g., a large ovipositor and pallial gonoduct). These structures degenerate after infection, suggesting that trematodes masculinize female snails to obtain increased allocation to production. In any case, trematodes appeared to convert the physiological machinery of hosts of both sexes to the

same level of parasite tissue, substantiating adaptive control of energy allocation. Clearly, there is a need for studies that evaluate uninfected and parasitically castrated host allocation to survival, growth, and reproduction.

Multiple infections by the same trematode species probably occur undetected in our system. Such multi-clone infections are known to occur naturally in other larval trematode-snail systems (e.g. Minchella et al. 1995; Rauch et al. 2005; Keeney et al. 2007; Lagrue et al. 2007). Trematode species likely vary in the frequency of multi-clone infections, and, consequently, in the frequency of intraspecific competition. Intraspecific competition can affect adaptive variation in host use, as predicted by some models of virulence evolution (e.g., see Frank 1996). Because this could possibly explain interspecific differences in relative mass, study is warranted in this area.

We avoided the term virulence in our discussion of castrator trade-offs because virulence has many definitions, variously tied to effects on host or parasite fitness, survival, fecundity, and infectivity (Bull 1994; Read 1994; Frank 1996). For parasitic castrators, the effects on host fitness, fecundity, and survival do not correspond to each other. A castrated host has a reproductive value of zero. Thus, the parasite disastrously affects both host fitness and fecundity. In contrast, the castrator may not affect host survivorship and any effects of parasitism on host survival therefore matter only to the castrator (perhaps first recognized by Rothschild and Clay 1952). For this reason, the question of resource allocation seems best approached by applying basic life-history theory to parasite investment in either reproduction (of the castrator) or survival (of the host phenotype). This parallels theory on the evolution of virulence only where virulence corresponds only to the effects of decreased host survival on the parasite.

Conclusion

Zimmer (2000) likened parasitic castrators to “hands inside puppets.” This metaphor conveys the idea that apparently healthy, infected hosts are reproductively dead, serving only the spread of the parasite’s genes. Also like hands in puppets, parasitic castrators are large relative to their hosts. Although the hands of a puppeteer do not grow to fit larger puppets, our findings indicate that castrator size is affected by host size, as well as other factors related to available energy. Additionally, just as some puppeteers have larger hands than do others, trematode castrator species vary in relative mass. We propose that interspecific variation in relative mass is adaptively driven by castrator species differentially manipulating resource allocation of their infected hosts. This merits further comparative study including other castrator–host systems, each preferably with many castrator species, similar to the system we studied here.

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Methods-Mixed-species infections

We excluded mixed-species infections from this study except for five mantle dwellers (one CATA and four RENB) found in snails with a visceral mass dweller (three of which were mature). Because the trematodes in these mixed-species infections use different host tissues, we were able to separately calculate their relative masses. We included these measurements in our analysis to increase sample sizes, allowing better estimates of the masses of these species that are frequently found in mixed-species infections. Elsewhere, we present the effects of mixed-species infections on the aggregate mass of trematode tissue (Hechinger et al. submitted).

Methods & Results-Assessment of statistical interactions

We sought to examine all higher-order interactions (i.e., whether or not the predictor variables affected trematode mass differently depending on the value of the other predictor variables). Although we had a large overall sample size (167 infections), we did not have all possible combinations of the predictor variables for each trematode species. Thus, we could not include all three- and four-way interactions into GLMs containing trematode species as a factor. However, we determined whether interactions were important in two ways. We analyzed GLMs including all interactions up to fourth-order terms (between habitat, season, snail size, and snail sex), for all trematode infections pooled (i.e., ignoring taxon), and for the one superfamily for which we had the largest sample size (the Echinostomoidea, $n = 100$). To the extent that trematode taxon affects trematode mass, pooling trematodes would tend to contribute noise to the analysis when examining the effects of the other predictor variables on both trematode mass and on each other (i.e., their interactions). Given our large sample size, this would only be a problem for detection of very weak effects. We found no significant interactions in either the model with trematodes pooled (all $P \geq 0.21$, $n = 154$) or in the model limited to the Echinostomoidea (all $P > 0.37$, $n = 93$). Thus, we removed these non-significant interactions, simplifying subsequent analyses. However, trematode species could differ in how their relative mass varied across seasons, habitats, or with host size. While we could not examine such interactions for individual trematode species, we could assess two-way interactions between family, habitat, season, and host-size, for the four families that we most extensively sampled (namely, the Echinostomatidae, Philophthalmidae, Heterophyidae, and Renicolidae). This assessment of interactions should be robust because we found that most of the variation between species was due to family affiliation (see results). Here, also, all interactions were non-significant (all $P \geq 0.11$, $n = 147$) and we dropped them from subsequent analyses.

Methods & Results-lack of snail gender effect

We dropped snail sex from analyses, because it did not affect relative trematode mass in either the model pooling single trematode infections ($F_{1,138} = 0.74$, $P = 0.39$) nor in the model incorporating species identity ($F_{1,135} = 1.25$, $P = 0.27$). This allowed us to include 16 additional infections for which we had not recorded snail sex. There was no detectable bias, with these additional infections, regarding their influence on relative trematode mass for all pooled infections ($F_{1,191} = 0.43$, $P = 0.51$).

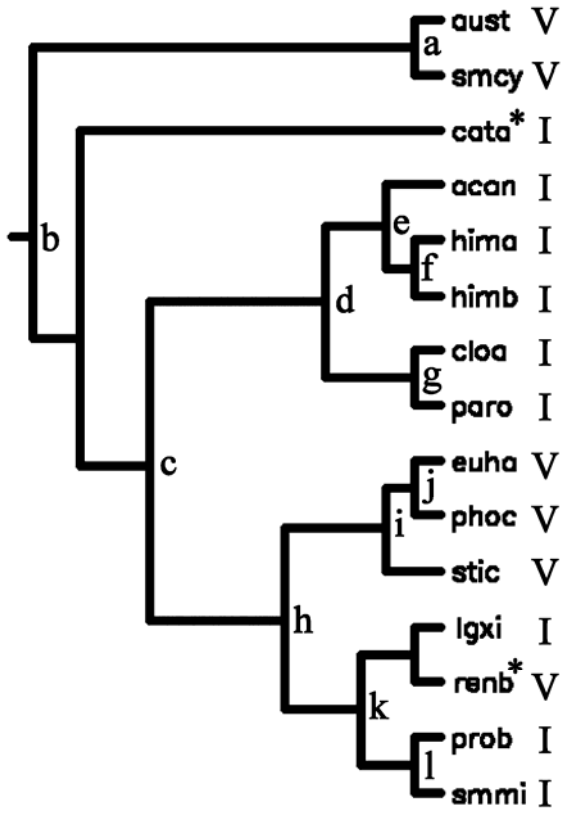

Figure esm1. The phylogenetic hypothesis used in comparative assessments (with equal branch lengths of one) to control for confounds due to common ancestry. Species codes are as in table 1. Symbols next to species codes indicate whether offspring (cercariae) infect vertebrates (V) or invertebrates (I). The letters below the nodes indicate the position of the independent contrasts used to assess the influence of dominance rank on castrator relative mass. We imposed vertebrate use at nodes a and i for Hansen's test examining adaptation of relative mass under the "selective regime" of offspring using vertebrates or invertebrates. The asterisks mark the two species that dwell in the mantle and were not used in comparative analyses (all other species reside in the visceral mass (full tissue-use indicated in table 1)).

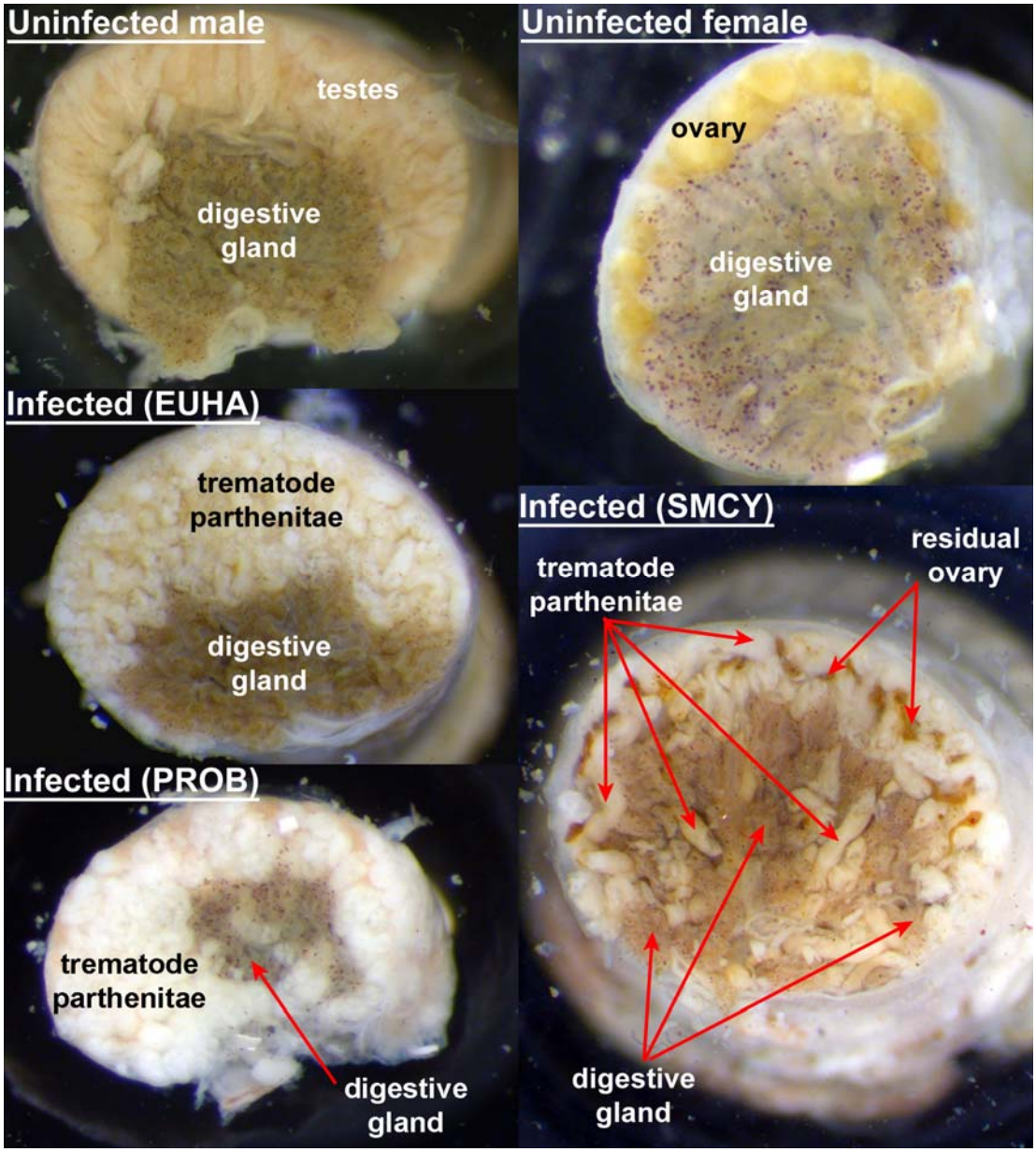

Figure esm2. Representative cross-sections of fixed gonadal-digestive gland regions of the visceral masses of uninfected and infected snails (*Cerithidea californica*). Red arrows indicate the major organs and trematode parthenitae. Note the difference in gonad size between uninfected males and females. EUHA (*Euhaplorchis californiensis*) represents one of the trematodes that take up the space in the visceral mass normally occupied by gonad. PROB (*Probolocoryphe uca*) and SMCY (Small cyathocotylid) represent species that also infiltrate the digestive gland. PROB achieves far greater mass by infiltrating additional tissues, whereas SMCY does not because its parthenitae are less densely packed. The residual (and non-functioning) ovary tissue is typical of SMCY infections.

Methods & Results-Trematode relative mass not spuriously self-correlated with infected snail mass

Measures of reproductive effort (Y/X) can spuriously self-correlate with total mass (X) (e.g., see Kenney 1982; Jackson and Somers 1991). Therefore, an appropriate test of the relationship between such variables should be based on a null distribution of the test statistic (e.g., slope) that corrects for any spurious self-correlation (Kenney 1982; Jackson and Somers 1991). The null distribution for the value of the slope may not be centered on 0, nor normally distributed.

Following (Jackson and Somers 1991), we performed a randomization test (Edgington 1995) by generating the null distribution for the slope of the relationship between trematode relative mass and infected snail mass. We did this by randomly permuting (10,000 iterations) values of absolute trematode mass and infected snail mass (first using a GLM to control for the effects of season, habitat, and trematode species), calculating relative mass, and scoring the slope of the relationship between relative mass and infected snail mass.

The results of this analysis supported our finding that trematode castrators were proportionally larger in larger snails. First, the observed relationship between trematode relative mass and infected snail weight, when we calculated relative mass on GLM-adjusted absolute masses, was statistically indistinguishable from the relationship observed when we incorporated relative mass directly into the GLM ($y = 0.20x + 0.14$, versus $y = 0.16x + 0.13$). Secondly, the null distribution of the slope was centered on -0.45 and was never more positive than -0.25 (thus, $P < 0.0000$) (Figure esm3). This validates that the trematodes were truly proportionally larger in larger snails.

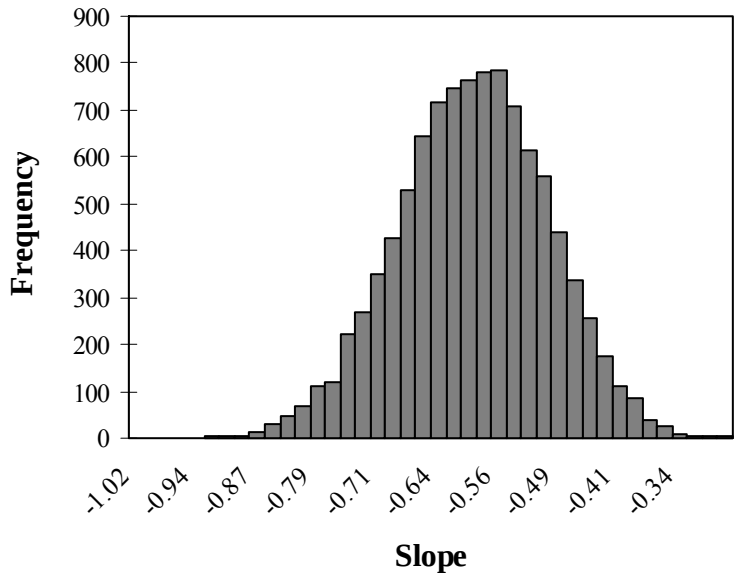

Figure esm3. Null distribution of the slope between relative trematode mass and infected snail weight, based on 10,000 randomizations.

Methods & Results-The scaling of absolute trematode mass with infected snail mass

We bolstered the non-linear regression in two general ways. First, we used a range of starting values (0, 1, 2) for the slope and the exponent, in addition to using the values obtained from the log-transformed variable analysis (described below). For all starting values, the parameter estimation converged within six iterations on the same slope and exponent (two iterations when starting with the values obtained by back-transforming from the log-log analysis). Second, we also performed a regression analysis on $\log_{10}$-transformed variables. The first analysis on log-transformed variables indicated an even larger scaling exponent than did the non-linear analysis. Here, trematode mass scaled with snail mass to the 1.5 ($P < 0.0000$, n = 167). However, when logged, the datum with the smallest relative mass value had undue influence on the slope (Cook's D > 1). We therefore performed the analysis without this outlier. The analysis on log-transformed masses then converged on the non-linear analysis. Here, trematode mass scaled with snail mass to the 1.34 (1.10-1.57 95% ci, $P < 0.0000$, n = 165) and with a coefficient of 0.29 (0.23-0.36 95% ci, $P < 0.0000$).

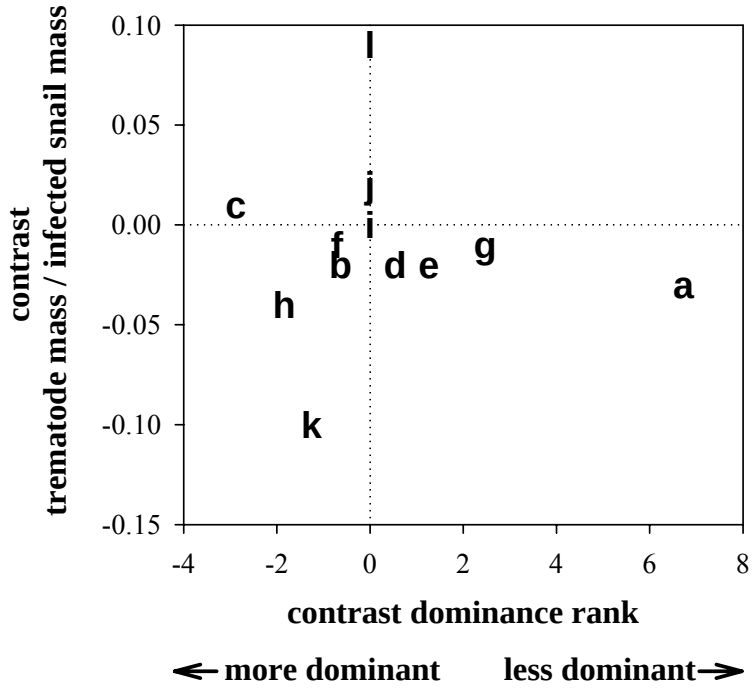

Figure esm4. Phylogenetically independent contrasts for trematode relative mass versus contrasts for rank in the competitive dominance hierarchy. Symbol letters match nodes in figure esm1.

Table esm1. Number of trematodes for which we quantified trematode mass, by habitat and season.

	Winter	Summer	Total
Channel	23	34	57
Flat	75	35	110
Total	98	69	167

Table esm2. Results of general linear model assessing the role of family on the relative mass of trematodes in infected snails.

predictor	df	SS	*F*-ratio	*P*
species [family][a]	7	0.044	2.05	0.0526
family	7	0.183	8.47	0.0000
season	1	0.039	12.67	0.0005
habitat	1	0.013	4.23	0.0414
tissue mass	1	0.018	5.92	0.0162
full model	17	0.665	12.69	<0.0001
residual	149	0.459		

[a] Species is nested within family.

Table esm3. Results of general linear model incorporating host-tissue site use of trematode species as an effect on their relative mass in infected snails.

predictor	df	SS	*F*-ratio	*P*
species [tiss-site][a]	10	0.243	7.88	0.0000
tissue-site	4	0.062	5.01	0.0008
season	1	0.039	12.67	0.0005
habitat	1	0.013	4.23	0.0414
tissue mass	1	0.018	5.92	0.0162
full model	17	0.665	12.69	0.0000
residual	149	0.459		

[a] Species are nested within their tissue-site use.

Table esm4. Results of general linear model assessing the role of dominance rank on the relative mass of trematodes in infected snails.

predictor	df	SS	*F*-ratio	*P*
species [dominance rank][a]	5	0.038	2.39	0.041
dominance rank	7	0.160	7.16	<0.0001
season	1	0.044	13.66	0.0003
habitat	1	0.012	3.61	0.0595
tissue mass	1	0.018	5.66	0.0188
full model	15	0.601	12.58	<0.0001
residual	138	0.440		

[a] Species is nested within dominance rank.

Table esm5. Results of general linear model assessing the role of the type of host infected by offspring (cercariae) on the relative mass of trematodes in infected snails.

predictor	df	SS	*F*-ratio	*P*
species [offspring host][a]	11	0.252	7.20	<0.0001
offspring host	1	0.050	15.68	0.0001
season	1	0.044	13.66	0.0003
habitat	1	0.012	3.61	0.0595
tissue mass	1	0.018	5.66	0.0188
full model	15	0.601	12.58	<0.0001
residual	138	0.440		

[a] Species is nested within type of host infected by offspring (vertebrate or invertebrate).

Table esm6. Results of Hansen's test for adaptation of relative mass for trematodes with offspring infecting vertebrates versus those using invertebrates.

evolutionary constraint (α)	invertebrate-user optimal relative mass (θ_2)±s.e.	vertebrate vs. invertebrate-user optimal relative mass (θ_1-θ_2)±s.e.	variation explained (R^2)	support (ln likelihood)	t[a]	P
0.1	0.25±0.083	-0.43±0.386	10.12	19.31	1.11	0.29
0.2	0.24±0.052	-0.25±0.193	13.3	20.07	1.30	0.22
0.3	0.24±0.039	-0.19±0.130	16.27	20.61	1.46	0.17
0.4	0.24±0.032	-0.16±0.098	18.97	21.00	1.63	0.13
0.5	0.23±0.028	-0.14±0.080	21.37	21.28	1.75	0.11
0.6	0.23±0.026	-0.12±0.068	23.46	21.49	1.76	0.10
0.7	0.23±0.024	-0.12±0.060	25.25	21.64	2.00	0.07
0.8	0.23±0.023	-0.11±0.054	26.76	21.74	2.04	0.06
0.9	0.23±0.022	-0.1±0.050	28.01	21.81	2.00	0.07
1	0.23±0.021	-0.1±0.047	29.05	21.86	2.13	0.05
1.6	**0.24±0.02**	**-0.09±0.037**	**32.2**	**21.94**	**2.43**	**0.03**
2	0.24±0.020	-0.08±0.035	32.83	21.94	2.29	0.04
3	0.24±0.020	-0.08±0.033	33.09	21.91	2.42	0.03
4	0.24±0.020	-0.08±0.032	33.04	21.90	2.50	0.03
5	0.24±0.020	-0.07±0.032	33.01	21.89	2.19	0.05
6	0.24±0.020	-0.07±0.032	32.99	21.89	2.19	0.05
7	0.24±0.020	-0.07±0.032	32.99	21.89	2.19	0.05
8	0.24±0.020	-0.07±0.032	32.98	21.89	2.19	0.05
9	0.24±0.020	-0.07±0.032	32.98	21.89	2.19	0.05
10	0.24±0.020	-0.07±0.032	32.98	21.89	2.19	0.05
100	0.24±0.020	-0.07±0.032	32.98	21.89	2.19	0.05

[a] t calculated with 12 d.f. (no. species – 1).

note: bold-font row indicates results for the value of evolutionary constraint (α) with maximum-likelihood support (using one digit in the grid search).

Table esm7. The family of tests for which we controlled the 'false discovery rate' (FDR).

effect[a]	observed *P*-value	FDR threshold[a]
taxon (family + species)	<0.0001	0.006
testes vs ovary	<0.0001	0.011
rank in dominance hierarchy	<0.0001	0.017
type of host used by offspring	0.0001	0.022
season	0.0005	0.028
host tissue use	0.0008	0.033
size	0.016	0.039
habitat	0.041	0.044
host sex	0.25	0.050

[a] This is the value below which a *P*-value must be to be deemed significant, holding the false discovery rate to 0.05.

Esm references

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