

EGG SIZE VARIABILITY IN TREMATODES: TEST OF THE BET-HEDGING HYPOTHESIS

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ABSTRACT: The hypothesis according to which egg size variability in hermaphroditic parasites results from bet-hedging was investigated in a comparative analysis using trematodes as a model. We hypothesized that the species reproducing mainly by self-fertilization should produce smaller eggs than those species that regularly practice cross-fertilization. Indeed, because self-fertilization is usually associated with inbreeding depression, selection should favor individuals spreading the risk of genetically disturbed development across more but smaller eggs, instead of producing fewer eggs, each possessing a large resource supply, of which many may fail to develop because of genetic deficiencies. On the basis of earlier theoretical and empirical studies, we assumed that the ratio length of testis–length of ovary positively correlates with the mating group size and, hence, with opportunities for cross-fertilization. In accordance with the bet-hedging hypothesis, we found, across trematode species, a positive relationship between this ratio and the mean egg volume produced by adults. This result was, however, observed only for the trematodes infecting birds and not for the species infecting fishes and mammals. In addition, once the influence of trematode phylogeny was taken into account, there was no significant trend, suggesting that phylogenetic legacies played a large role in generating the previous signal. Experimental tests of the bet-hedging hypothesis will be necessary to clarify the matter.

Understanding the life histories and reproductive strategies of parasites is of interest not only for parasitologists but also for anyone interested in epidemiology, control strategies, and evolutionary medicine (Thomas et al., 2002). Despite the increasing interest of scientists in this research area, parasites have undoubtedly received less attention than free-living species during the past decades (see Stearns, 1989, 1992; Roff, 1992). As a consequence, we are still struggling to understand what forces have shaped the life-history evolution of parasites (Combes, 1995; Poulin, 1996, 1998; Thomas et al., 2002).

The problem of the optimal partitioning of resources between egg number and egg size in parasites has been the subject of several studies and discussions. Conventional wisdom usually assumed that parasites typically produce huge amounts of tiny eggs to maximize their probability of transmission (e.g., Price, 1974). However, recent studies indicated that things are undoubtedly more complex (e.g., Poulin, 1995a, 1995b, 1995c, 1997). For instance, egg size can vary across parasite species independent of other traits (Skorping et al., 1991). Substantial variation may also exist among individuals in given populations (e.g., Yoshikawa et al., 1989). Thus, unexplained variation clearly exists among related parasites in egg number and egg size, suggesting that strategies other than the massive production of small eggs are likely to be adaptive in some cases (Poulin, 1998).

Recently, Wedekind et al. (1998) studied egg production in a hermaphroditic cestode (*Schistocephalus solidus*) in relationship with social situation. They found that *S. solidus* apparently adjusts its investment per egg depending on whether the offspring is the result of self- or cross-fertilization. Indeed, selfers produced a larger number of eggs, but these eggs were smaller than those resulting from outbreeding individuals. One possible explanation for this phenomenon is 'bet-hedging.' Indeed, an important cost of selfing is the higher frequency of genetic deficiencies among offspring due to inbreeding depression (D. Charlesworth and B. Charlesworth, 1987; Jarne and Charlesworth, 1993). Because these genetic deficiencies are likely to be expressed independently of egg size, individuals who are

forced to reproduce alone could react to this problem by spreading the risk of genetically disturbed development among more but smaller eggs, instead of producing fewer eggs, each equipped with a large resource supply, of which many fail to develop because of genetic deficiencies (Wedekind et al., 1998). The idea that bet-hedging strategies could be more common than is traditionally recognized in parasites has recently drawn increasing attention (see, for instance, Fenton and Hudson, 2002).

This article aims to test whether the bet-hedging hypothesis suggested by Wedekind et al. (1998) to explain egg size variation in *S. solidus* could be extended to account for variation in egg sizes across a larger number of parasite species. There are indeed several reasons to believe that this phenomenon could explain a significant part of the interspecific variability in egg sizes. Specifically, like *S. solidus*, most parasitic platyhelminths are simultaneous hermaphrodites. Several studies have also shown that cross-fertilization is preferred over self-fertilization whenever 2 conspecific helminths co-occur (Esch and Fernandez, 1993; Nollen, 1993; Rohde, 1994; Combes, 1995; Trouvé et al., 1999; Brown et al., 2001), suggesting that fitness benefits are gained by outbreeding individuals (Rohde, 1994; Wedekind et al., 1998; Lythgoe, 2000). However, because the mean infrapopulation size, i.e., mating group size, of parasite species is highly variable, it is reasonable to assume that opportunities for cross-fertilization also vary strongly between parasite species. Using trematode species as a model, we explored the idea that there should be a link between the social situation encountered by parasites during reproduction and the way they invest resources in their eggs.

MATERIALS AND METHODS

Data collection

Data were obtained from Yamaguti (1958). This source provides a random sample of trematode species worldwide. Measurements recorded for adult trematodes were mean body length, mean body width, mean testis length, and mean ovary length. The product of body length and body width was used as a measure of body size as in Poulin (1997). Because gonads do not have any particular shape, we kept their mean maximum length as a measure of their size in the analyses. We also recorded the mean length and width of eggs and computed their volume using the formula of an ellipsoid as in Trouvé et al. (1999). We obtained data for a total of 180 trematode species infecting fishes, birds, and mammals (definitive hosts). However, because data on all variables were

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not always available, sample sizes can vary in subsequent analyses. The data set is available upon request.

Estimating the opportunities for cross-fertilization

Assessing the opportunities for cross-fertilization would a priori require obtaining data on mean infrapopulation sizes in the definitive host for the different parasite species. Unfortunately, such data were available only for a small subset of species in our sample. In addition, we did not know what exactly are the links between infrapopulation size and mating group size in nature. For this reason, we used an indirect measurement, i.e., the ratio of testis length to ovary length. Indeed, theoretical (Fischer, 1981; Charnov, 1982, 1996; Lively, 1990) as well as empirical studies (e.g., Wedekind et al., 1998; Trouvé et al., 1999; Schärer and Wedekind, 2001) found a positive relationship between resource allocation to the male function and mating group size in hermaphroditic organisms as a consequence of local mate competition or sperm competition, or both. Thus, higher values of the ratio indicate greater opportunities for cross-fertilization. We also verified that, when controlling for worm surface area, the relationship between egg volume and ovary size was not significant (partial Kendall rank correlation, $r_k = -0.018$, $n = 104$, $P = 0.78$). Similarly, the relationships between testis size and both ovary size and egg volume were positive, suggesting no trade-off between these variables.

Statistical analysis

To assess the relationship between the ratio testis length–ovary length and egg volume, we first considered all available trematode species as independent points. Because conditions for the use of parametric statistics were violated, we used nonparametric statistical tests, i.e., Spearman rank order correlation coefficient and partial Kendall rank correlation (see Sokal and Rohlf, 1981; Siegel and Castellan, 1988). However, because 2 closely related species are more likely to resemble each other on account of common ancestry than are 2 unrelated species, it is perhaps inappropriate to use species as independent points in statistical analysis. To account for the potential influence of phylogeny on the nonindependence of species values, we used the phylogenetically independent contrasts method (Felsenstein, 1985; Harvey and Pagel, 1991). The phylogeny of digenean trematodes is still not fully resolved. Here, we constructed a working phylogeny using the proposed relationships among families and the higher taxa from Cribb et al. (2001), with additional information from the appendix in Brooks and McLennan (1993). Independent contrasts were computed among sister taxa in the trematode phylogeny, using the program CAIC, version 2.0 (Purvis and Rambaut, 1994). Contrasts in all continuous variables (worm surface area, egg volume, ovary length, and testis length) were derived from ln-transformed data. We included only the trematode species for which data were available on all 4 variables. Therefore, the number included is lower than that in the analyses across species described above. Analyses were first performed using all available trematode species and then using subsets of these species categorized according to the definitive host taxon that they use, i.e., fish, bird, or mammal. All regression or correlation analyses (or both) were forced through the origin (see Garland et al., 1992). Finally, knowing that independent contrast values can be positive or negative, we also performed sign tests to analyze the pattern of covariation among the variables under study.

RESULTS

The mean area of adult worms, the mean volume of eggs, and the mean value of the ratio testis length–ovary length were not significantly different among the 3 main groups of trematodes, i.e., infecting fishes, birds, and mammals (analyses of variance on ln-transformed data, all $P > 0.24$). In most cases, values of the ratio are greater than 1 (Fig. 1), possibly indicating that species invest on average more in the male function than in the female function if there are no other constraints acting on the size of the gonads. Overall, there were positive and significant relationships between worm area and (1) egg volume (Spearman rank order correlation coefficient $r_s = 0.53$, $n = 180$,

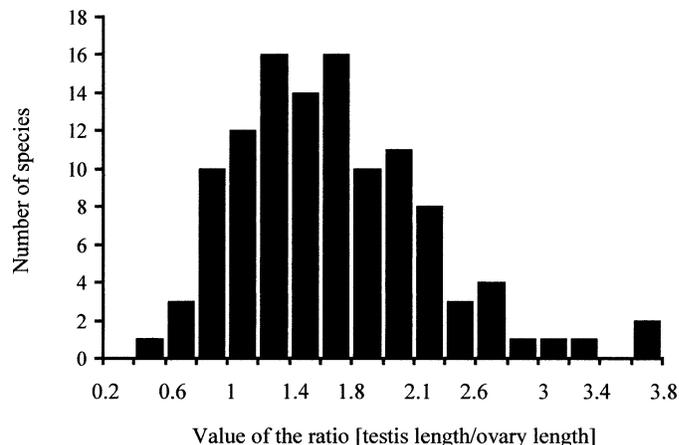


FIGURE 1. Frequency distribution for the ratio testis length–ovary length among different species of trematodes.

$P < 0.0001$; Fig. 2A), (2) testis length ($r_s = 0.86$, $n = 128$, $P < 0.000$; Fig. 2B), and (3) ovary length ($r_s = 0.87$, $n = 118$, $P < 0.0001$; Fig. 2C). The ratio length of testis–length of ovary was positively correlated with egg volume ($r_s = 0.48$, $n = 100$, $P < 0.0001$; Fig. 3), suggesting that, as predicted, species investing more in the male function also invest more resources in their eggs. When considering the 3 trematode groups separately, this relationship was significant only for trematodes infecting bird species, i.e., Spearman rank order correlation coefficient $r_s = 0.62$, $n = 56$, $P < 0.0001$ (trematodes of fish: $r_s = 0.06$, $n = 15$, $P = 0.84$; trematodes of mammals: $r_s = 0.26$, $n = 29$, $P = 0.16$). Considering all trematode species, the positive relationship between the ratio length of testis–length of ovary and egg volume was not due to worm area effects because it remains significant when controlling for this effect (partial Kendall rank correlation $r_k = 0.28$, $n = 100$, $P < 0.0001$). When controlling for worm area within each trematode categories, once again the tendency was significant for trematodes of birds only, i.e., partial Kendall rank correlation $r_k = 0.37$, $n = 56$, $P < 0.0001$ (trematodes of fish: $r_k = 0.09$, $n = 15$, $P = 0.32$; trematodes of mammals: $r_k = 0.15$, $n = 29$, $P = 0.26$).

Using independent contrasts, we found a nonsignificant relationship between egg volume and worm area ($n = 61$, $r = 0.08$, $P = 0.13$). However, there were significant relationships between worm area and (1) testis length ($n = 61$, $r = 0.59$, $P < 0.0001$) and (2) ovary length ($n = 61$, $r = 0.79$, $P < 0.0001$). Thereafter, we used the residuals of testis length (residuals 1) and ovary length (residuals 2). To assess the relationship between egg volume and sex allocation, we studied the relationship between egg volume and the ratio residuals 1–residuals 2. This relationship was not significant ($n = 61$, $r = -0.06$, $P = 0.49$). We also used testis length corrected for ovary length (residuals 3) as a measure of sex allocation. Residuals 3 were not significantly correlated to worm area ($n = 61$, $r = 0.13$, $P = 0.86$). The relationship between egg volume and residuals 3 was not significant ($n = 61$, $r = -0.16$, $P = 0.11$), suggesting that an increased resource allocation to the male function does not involve a greater resource allocation to eggs. None of the previous relationships was significant ($P > 0.05$) when examined within each category (fish, bird, or mammal hosts) of trematodes.

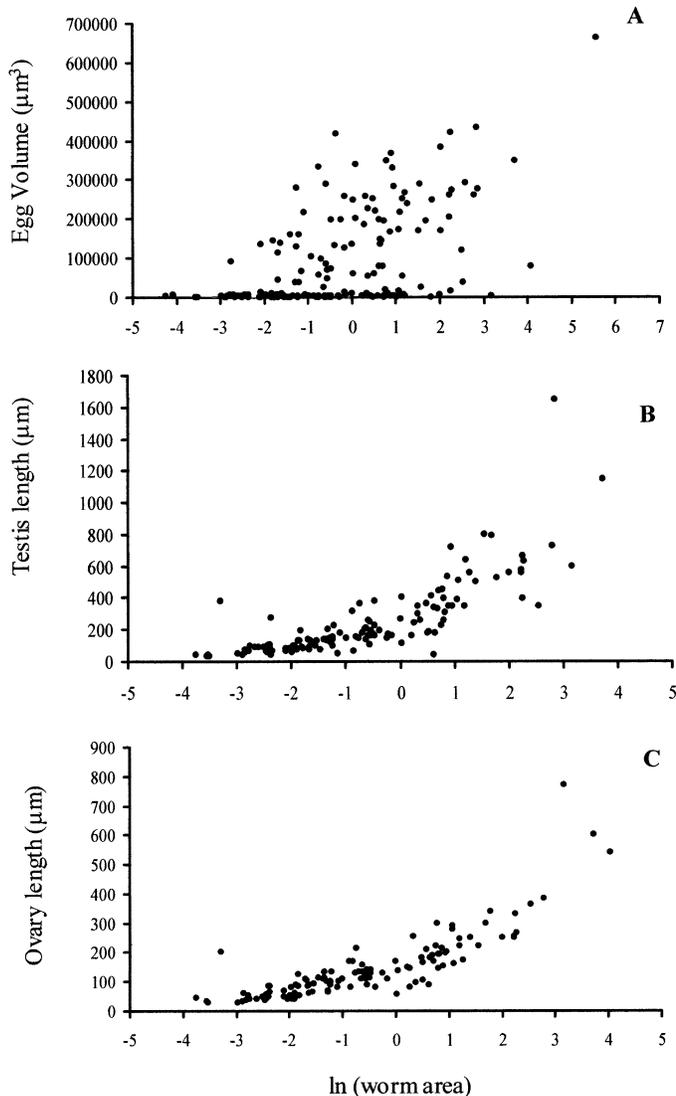


FIGURE 2. Relationships between worm area (\ln -transformed) and egg volume (A), testis length (B), and ovary length (C) among different species of trematodes.

Sign tests revealed that, as expected, there was a significant association between the contrast signs on testis length and ovary length ($\chi^2 = 11.3$, $df = 1$, $P = 0.0008$). However, sign tests were not significant when analyzing egg volume–testis length ($\chi^2 = 0.12$, $df = 1$, $P = 0.73$), egg volume–ovary length ($\chi^2 = 0.04$, $df = 1$, $P = 0.84$), egg volume–(residual 1–residual 2) ($\chi^2 = 0.40$, $df = 1$, $P = 0.70$), or egg volume–residual 3 ($\chi^2 = 2.81$, $df = 1$, $P = 0.09$), confirming the lack of association between resource allocation in male function and egg volume when using independent contrasts.

DISCUSSION

Variation in egg sizes is a central feature of the life history strategies of parasitic organisms, and yet it remains mostly unexplained (Poulin, 1998). Within trematode species, i.e., among conspecific worms, egg sizes vary considerably (Poulin and Hamilton, 2000). Some of this variation appears linked with

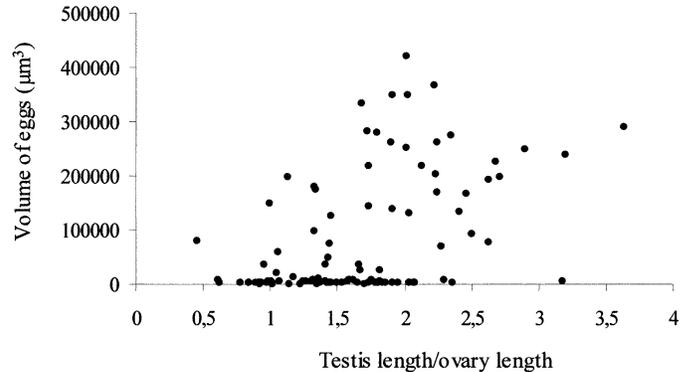


FIGURE 3. Relationship between egg volume and the ratio testis length–ovary length among different species of trematodes.

environmental unpredictability. For instance, trematode species in the tropics produce eggs of more variable size than those from higher latitudes; external conditions in tropical habitats tend to be more stable in time but more variable in space than those in temperate habitats, a factor that should promote the production of variable offspring (Poulin and Hamilton, 2000). Across trematode species, mean egg sizes are just as variable, ranging over 2 orders of magnitude (Poulin, 1997); some of this variation is the product of interspecific differences in adult worm body size, but the residual variation remains completely unexplained.

Assuming that the ratio length of testis–length of ovary is a reliable indicator of the opportunities for cross-fertilization, the bet-hedging hypothesis predicted that there should be a positive relationship between this ratio and egg size, independent of worm size. Indeed, the more a species has opportunities for cross-fertilization, i.e., high values of the ratio, the more resources it should invest in individual eggs because the risk of genetic deficiencies due to inbreeding depression is reduced. Our study produced conflicting results. Indeed, although across species values there was a positive relationship between the ratio and the mean egg volume, we must be cautious in our interpretation for 2 reasons. First, this tendency is observed only for 1 category of trematodes of the 3 considered, i.e., those infecting birds in contrast to those infecting fishes or mammals. Because there is a priori no reason to believe that trematodes infecting fishes or mammals experience unusual selective pressures compared with species infecting birds, we have at the moment no biological explanation for this lack of consistency in our results. However, given that sample sizes are relatively low for trematodes of fishes and mammals compared with trematodes of birds, we think that further analyses including more species would be necessary to confirm the present results.

A second reason to be cautious comes from the fact that the relationship was not observed when calculated from independent phylogenetic contrasts, even when considering only the species infecting birds. Thus, phylogenetic legacies obviously play an important role in generating the previous signal. Despite this, it remains possible that the adaptive value of inherited traits is maintained in derived groups if they still increase the fitness of their bearers. Finally, if we choose to view some of the tests performed as directed tests (see Rice and Gaines 1994a, 1994b), we would obtain marginal support for our hy-

pothesis from this analysis, i.e., a two-tailed *P*-value of 0.09 (as given with residuals 3) would be significant at $\alpha = 0.05$ in a directed test (one-tailed test). In conclusion, we feel that the bet-hedging hypothesis, although not fully supported here, remains an interesting notion to be considered when trying to explain egg size variability in hermaphroditic parasites. Further comparative analyses, but also experimental studies placing individual parasites in different social situations for mating, will be necessary to properly assess the relevance of this hypothesis.

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