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Phenotypic correlations reveal evidence for growth costs but not survival costs of reproduction in a freshwater snail

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ABSTRACT

Question: Do populations of freshwater snails experience trade-offs between reproduction and either growth or survival? Trade-offs among these traits is the most likely explanation for results from a related study.

Data studied: Growth rates, reproductive output, and survival in snails from 12 populations that were raised from eggs in the laboratory.

Search method: I examined correlations between reproductive output and growth and reproductive output and survival.

Conclusions: Trade-offs between reproductive output and growth were observed in laboratory conditions. However, trade-offs between reproduction and survival were not evident in laboratory conditions and, surprisingly, I found a positive relationship between reproductive output and maximum observed age. In the related study, selection for increased reproductive output may have been constrained by the negative relationship between reproduction and growth.

Keywords: cost of reproduction, genotype × environment interaction, life-history traits, phenotypic correlation, trade-offs.

INTRODUCTION

Trade-offs between life-history traits occur when changes that increase fitness through one trait decrease fitness through a correlated trait. Trade-offs can occur when limited resources mandate that two or more functions of an organism compete for the same pool of resources (physiological trade-offs) or when two or more life-history traits are linked genetically (evolutionary trade-offs) (Stearns, 1992). Trade-offs are central to life-history theory, because they are assumed to constrain variation in life-history traits (Roff, 2002). Without trade-offs, 'all organisms would be as fecund as the most fecund, as long-lived as the most long-lived and develop as rapidly as the most rapid developer' (Maynard Smith, 1991).

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In this study, I asked whether trade-offs among life-history traits occur in 12 populations of the freshwater snail *Helisoma anceps* (Menke 1830). In a related study of these same natural populations, I found no evidence that parasitic trematodes had selected on life-history traits of the snail (Krist, 2006); there was no relationship between prevalence of castrating trematodes and age at maturity or reproductive output in the offspring of snails collected from natural populations. This result was unexpected, because life-history theory predicts that consistent and high infection rates by castrating parasites should select for early maturity (Kozlowski and Uchmanski, 1987; Kozlowski and Weigert, 1987) and increased reproductive effort in uninfected individuals, under most conditions (Kozlowski and Uchmanski, 1987; Kozlowski and Weigert, 1987; Gandon *et al.*, 2002). One possible explanation for these results is that trade-offs among life-history traits constrained a response to selection. To determine whether trade-offs occur in these populations of *H. anceps*, I measured reproductive output, growth, and longevity in the same individuals that were part of the previous study.

Trade-offs between reproductive traits and other life-history traits are also referred to as costs of reproduction. These costs can be divided into a growth cost, the trade-off between reproduction and growth, a survival cost, the trade-off between reproduction and survival, and a fecundity cost, the trade-off between current and future reproduction. I directly examined only growth and survival costs of reproduction. However, growth costs are an indirect assessment of the fecundity cost, because allocation to current reproduction diverts resources away from growth and smaller size reduces expected future reproduction (Reznick, 1992).

Costs of reproduction can be physiological, resulting from the metabolic drain caused by reproduction, or ecological, resulting from risks such as nest guarding, which are associated with reproductive behaviours (Calow, 1979; Bell and Koufopanou, 1986; Reznick, 1992; Zera and Harshman, 2001). Costs of reproduction may also result from genetic correlations among life-history traits. Since ecological costs do not occur in the benign, enemy-free environment of the laboratory, I expected that any costs of reproduction would be due either to metabolic drain or pleiotropic interactions among life-history traits.

In this study, I found a negative relationship between growth and reproduction (a growth cost of reproduction) but I found no evidence for survival costs of reproduction. Because the animals were grown in benign laboratory conditions, I cannot preclude ecological survival costs that are likely to exist in natural conditions. These findings suggest that trade-offs between growth and reproductive output may have constrained a response to selection by parasites in an earlier study of the effects of variation in parasite prevalence on life-history traits (Krist, 2006).

MATERIALS AND METHODS

Study organism

Helisoma anceps (Planorbidae) is a freshwater pulmonate snail that occurs in lakes, ponds, and flowing waters throughout North America (Burch, 1982). In North Carolina, this animal has a lifespan of one year (Fernandez and Esch, 1991). The lifespan is similar in duration in north central Wisconsin; I observed that all adults senesced by mid-July of each year in the lakes that I studied. *Helisoma anceps* are hermaphroditic (Brown, 1991), hence isolated individuals begin laying fertilized eggs at sexual maturity.

Data collection

In late May 2002, I collected 75–100 adult *H. anceps* from 12 lakes in north-central Wisconsin by sampling haphazardly with a dip-net in the littoral zone. In the laboratory, I obtained eggs from each snail by placing them individually in 300-ml containers with de-chlorinated water for 7 days. The snails were then removed from the containers and the eggs were allowed to hatch. To maintain equal numbers of hatchlings per cup and to reduce density-dependent effects on growth, I reduced the density of juvenile snails in each cup twice in the first 8 weeks by choosing the survivors at random. I raised two offspring per parent to maturity, which resulted in 38–56 individuals per population (total = 575 individuals).

After 8 weeks, the juveniles were maintained individually in 300-ml, clear plastic cups. I fed the juvenile snails leaf lettuce that was supplemented with fish flakes (Tetramin® Tropical Flakes) after 9 April 2003. The snails were reared at 19°C with a 15½ : 8½ h light/dark cycle (corresponding to the daylength on 21 June 2002 in Eau Claire, WI). I changed the water in the rearing containers every 4 days.

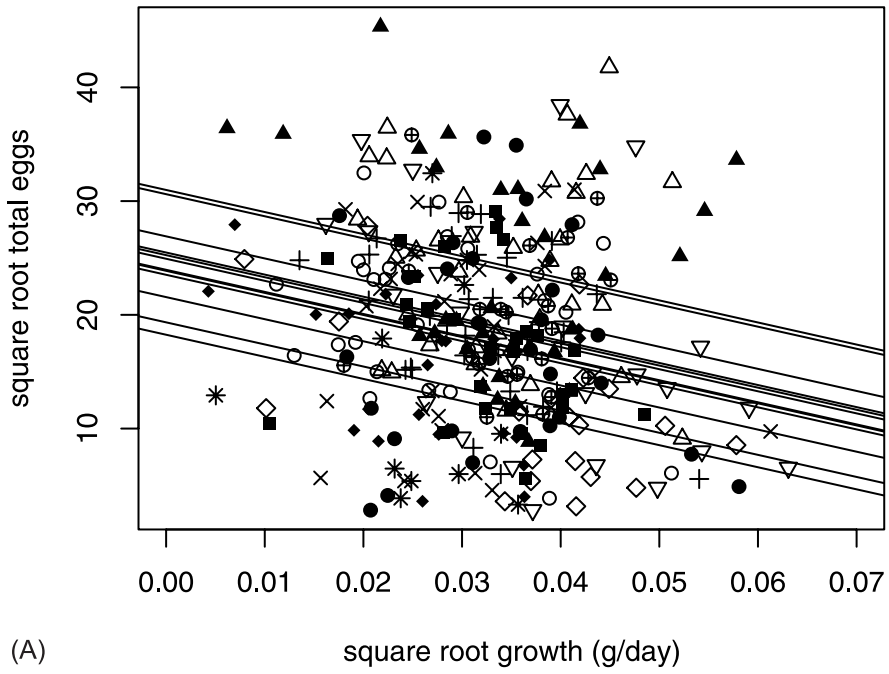
For each individual, I measured growth, reproductive output, and maximum observed age. To assess growth, I measured the mass of the snails at maturity (onset of egg laying) and again in March and May 2003. The first snail matured in early February 2003. In May 2003, the snails were nearly one year old (340–349 days old). Before measuring mass, I dried the snail by blotting the shell and foot onto paper towels. To measure reproductive output, I counted the number of eggs produced by each snail bi-weekly over 189 days (27 weeks), starting as soon as each snail began reproducing. Reproduction was measured over an extended period to ensure that the measure of reproductive output reflected a realistic period of time for an annual snail. I measured maximum observed age by monitoring the snails for mortality every 4 days. I calculated the maximum observed age of each snail as the number of days between their day of death and their 'birthday'. Because the snails hatched from eggs laid over a 7-day period, I estimated the 'birthday' as day 4 of that week.

Statistical analysis

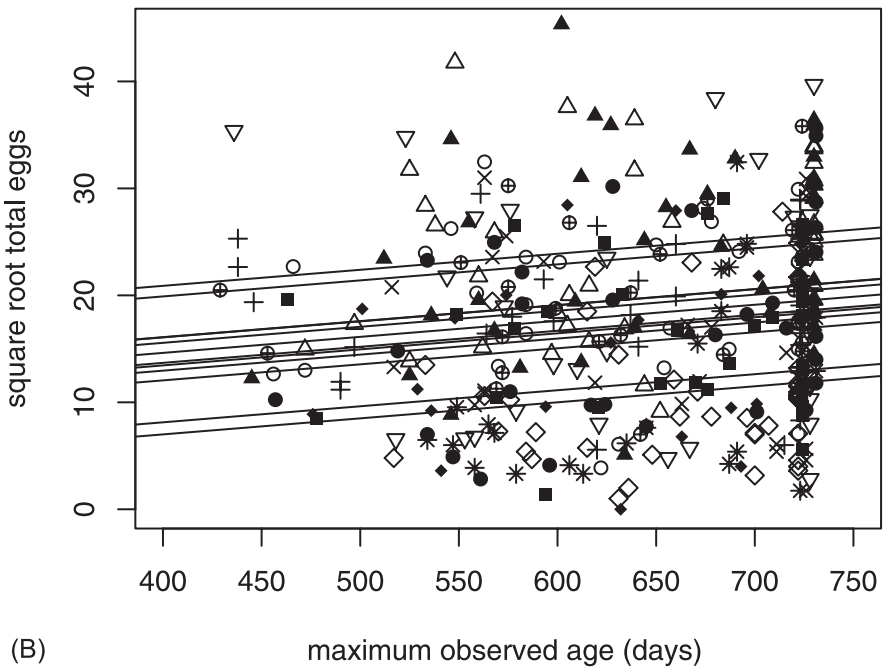
A square-root transformation was performed on the total egg counts to normalize the data. These transformed values of total eggs produced were used for all analyses of reproductive output. I did not correct the egg counts for body size because I found no relationship between size and fecundity in any of the 12 populations. The analysis included only animals that survived the 27-week period after maturity.

To determine whether there were growth costs of reproduction, I calculated a per day growth rate as [mass (g) at date of the last measurement – mass (g) at date of maturity)/ number of days]. I used a square-root transformation of the growth rate to increase homoscedasticity of variances. After determining that reproductive output varied with growth rate in a consistent manner across populations (i.e. the slopes were statistically homogeneous among populations), I used an analysis of covariance (ANCOVA) with growth and population (categorical) as predictors of reproductive output. Because I did not measure growth rate for snails that matured after May 2003, these animals were excluded from this analysis.

To determine whether there were survival costs of reproduction, I calculated maximum observed age as the age of the animal at the day of death. Again, after assessing that slopes


square root total eggs
10
20
30
40
0.00
0.01
0.02
0.03
0.04
0.05
0.06
0.07
square root growth (g/day)

(A)


square root total eggs
0
10
20
30
40
400
450
500
550
600
650
700
750
maximum observed age (days)

(B)

were statistically homogeneous among populations, I used ANCOVA, with survival and population as predictors of reproduction. To include the animals that survived the entire 24-month period of this study ($n = 123$), I assigned a date of death as the date of termination of the experiment. All statistics were conducted using the R statistical package (R Development Core Team, 2008).

RESULTS

There were significant negative effects of growth on reproduction (i.e. growth costs; Table 1A). A significant effect of population in the ANCOVA indicates that populations differed in overall reproductive rates (Table 1A, Fig. 1A).

Maximum observed age showed no negative effects on reproductive output (i.e. no survival costs) and, contrary to expectations, maximum observed age covaried positively with reproductive output (Table 1B, Fig. 1B). As in the analysis of growth costs, populations had different rates of reproduction (Table 1B, Fig. 1B). One explanation for the

Table 1. Analysis of covariance tables assessing (A) growth costs (reproductive output as a function of growth and source population) and (B) survival costs (reproductive output as a function of maximum observed age and source population)

Analysis	Source	d.f.	MS	*F*	*P*
(A) Growth costs	Growth	1	771.5	12.85	<0.001
	Population	11	350.7	5.84	<0.001
	Residuals	319	60.0		
(B) Survival costs	Maximum age	1	299.2	4.88	0.028
	Population	11	593.0	9.66	<0.001
	Residuals	388	61.4		

Note: After determining that slopes were homogeneous ($F_{\text{growth} \times \text{population}} = 0.71$, $P = 0.719$; $F_{\text{maximum age} \times \text{population}} = 1.17$, $P = 0.303$), the interaction term was omitted from the model (Dalgaard, 2002) and the above analysis of covariance tables was produced. For each analysis, population is a categorical factor. Growth was measured in grams per day from the date of maturity to May 2003. The values for reproductive output (total egg counts) and growth rate were square root transformed.

Fig. 1. The relationship between reproductive output (square root total eggs) and (A) growth (square root growth in grams per day) and (B) maximum observed age in days. Each symbol represents a different population. ○ = Axehandle, Δ = Bob, + = Boot, × = Bradley, ◇ = Chetek, ▽ = Goose, * = Howe, ■ = Lake 3, ⊕ = Lake 10, ▲ = Payne, ● = Snails, ◆ = South Shattuck). Since slopes were statistically homogeneous among populations, linear relationships for each population were drawn using the common slope [(A) –194.2, (B) 10.07)] and the intercept calculated from a linear regression model without interactions. Using the common slope, the intercept for Axehandle (reference population), and means for all animals, (A) on average a 25% increase in growth would decrease reproductive output by 67.9 eggs over 27 weeks (17% of mean egg production over all populations) and (B) on average a 25% increase in maximum age would increase reproductive output by 38.3 eggs over 27 weeks (9.7% of mean egg production over all populations).

positive relationship between maximum observed age and reproduction may be continued egg production throughout life. Two examples show continued reproductive output during the study period for many individuals in Howe and Snails lakes (Fig. 2).

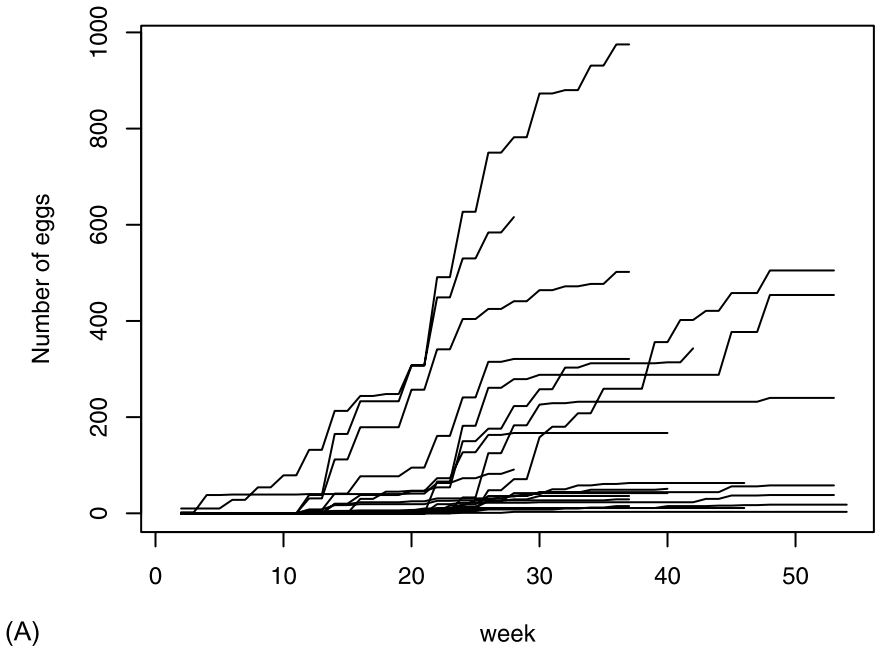
For many individuals, maximum observed age in the laboratory far surpassed the lifespan of one year that I observed in nature. When I terminated the experiment after 730 days, the surviving 123 snails were approximately 2 years old (individuals from Bob, Howe, and Snails Lakes were exactly 2 years old).

DISCUSSION

I found evidence for growth costs but not survival costs under laboratory conditions (Table 1). Growth costs may have resulted from either physiological drain or from genetic correlations between growth and fecundity. Metabolic drain is likely because I measured growth directly following sexual maturity when snails were simultaneously allocating resources to growth and reproduction. If the trade-off has a genetic basis, then antagonistic pleiotropy is the likely cause. When antagonistic pleiotropy occurs, genes that positively affect one component of fitness negatively affect another. To explain the trade-off between reproduction and growth in *H. anceps*, one or more genes would increase reproductive output or growth and decrease the corresponding function.

Regardless of the underlying nature, the trade-off between growth and reproduction observed in laboratory-reared *H. anceps* may have constrained a response to selection by parasites (Krist, 2006). Life-history theory predicts that consistently high infection rates by castrating parasites should select for individuals with increased reproductive effort (Kozlowski and Uchmanski, 1987; Kozlowski and Weigert, 1987; Gandon *et al.*, 2002). However, in a related study (Krist, 2006), I found no evidence for increased reproductive effort in populations of *H. anceps* with high rates of parasitism. If selection is acting on both growth and reproduction and this negative relationship between growth and reproduction also exists in nature, it could constrain a response to selection on reproductive output regardless of whether it has a genetic or only a phenotypic basis (Roff, 2002).

Although survival costs were also predicted, they were not found. In fact, reproductive output was positively related to maximum observed age. The absence of detectable trade-offs in this study does not preclude the presence of trade-offs in the natural environment. First, ecological costs, such as an increased risk of predation from greater time spent foraging, cannot be measured in laboratory conditions. Second, the novelty of laboratory conditions can cause genotype × environment interactions to mask potential costs of reproduction. Environmental factors can affect one life-history trait more than others, causing the phenotypic correlation to change in different environments (Stearns, 1992). Third, trade-offs may only be evident in suboptimal conditions (Rose and Bradley, 1998) and I raised the snails in benign laboratory conditions with ample food. When allocations are made with remaining resources, once high-priority needs have been filled, then many trade-offs will appear only when resources are limited (Stearns, 1992) or the environment is otherwise stressful (Bell and Koufopanou, 1986; Roff, 2002). For example, in a recent study of mammals and birds in zoos, Ricklefs and Cadena (2007) found no evidence for trade-offs between reproductive investment and longevity in this benign, resource-rich environment. In the current study, the lifespan of many individuals in the laboratory was as much as twice the duration of individuals in natural populations. This suggests the laboratory conditions in this study were benign and perhaps especially favourable to some individuals.



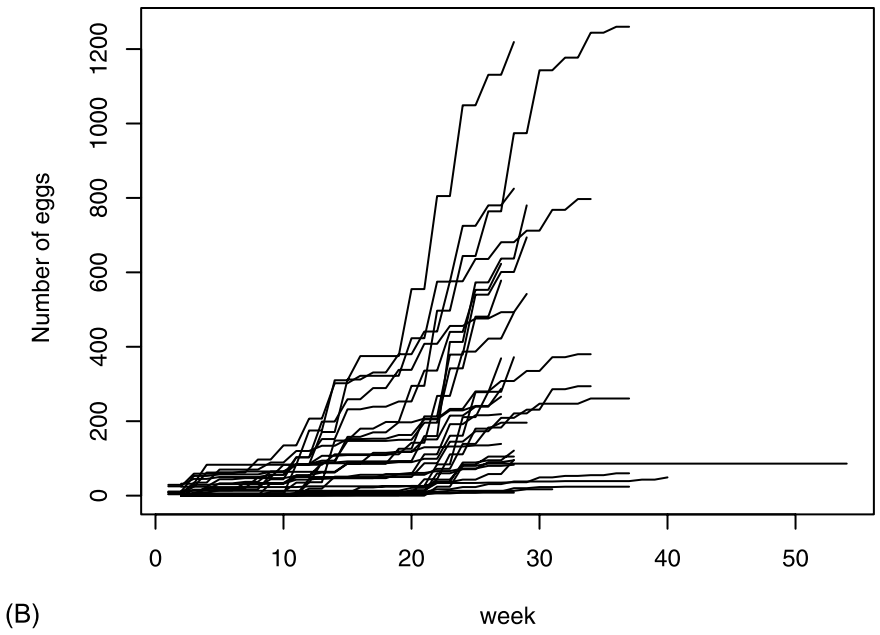


Fig. 2. Cumulative egg production for snails in (A) Howe and (B) Snails lakes. Reproductive output for each individual was measured for 27 weeks after maturity. Hence only individuals that survived for 27 weeks after maturity were included.

Not only were predicted survival costs of reproduction not found, but I found that reproduction and survival were positively related. This positive relationship occurs because for many individuals, egg production is fairly constant (Fig. 2). Hence for many snails, increased longevity leads to increased fecundity. In addition, positive relationships among life-history traits can occur, even when there is an underlying trade-off, if the variation in allocation of resources to reproduction is small and variation in acquisition of those resources is large (van Noordwijk and de Jong, 1986). Hence, variation in quality among individuals means that some individuals invest highly in both traits (see below). In this study, high levels of juvenile survivorship may have increased variation among individuals because both low- and high-quality individuals survived.

Genotype × environment interactions may also explain the positive relationship revealed in this study. The ability of 'super organisms' to reproduce at high rates and live long lives may be limited to conditions of abundant resources (Reznick *et al.*, 2000). This may result from a cost of resource acquisition that limits the advantage of 'super organisms' to certain environmental conditions (Reznick *et al.*, 2000) or because genes that enable high rates of nutrient acquisition can only be expressed in a resource-rich environment (Bell and Koufopanou, 1986). In the present study, snails were fed *ad libitum*; these high resource levels coupled with the possibility of high variation among individuals in resource acquisition may have contributed to the positive relationship between reproductive rates and survivorship.

Although only genetic correlations can lead to correlated responses to life-history selection, phenotypic correlations are often studied as proxies of genetic correlations because detecting genetic correlations often requires very large sample sizes (Pease and Bull, 1988). Using phenotypic correlations as a proxy is at best a conservative estimate of genetic correlations. At worst, phenotypic correlations are an inaccurate reflection of genetic correlations when groups vary in resource levels (Pease and Bull, 1988) or in their ability to acquire resources (van Noordwijk and de Jong, 1986). Despite these possible difficulties with phenotypic correlations, in a review of adult size and development time, phenotypic correlations were reliable indicators of both the sign and magnitude of genetic correlations (Roff, 2000). The sign of the phenotypic correlation was the same as the genetic correlation in 27 of 30 studies and the phenotypic correlation significantly predicted the genotypic correlation. Because the genetic correlations were consistently larger than the phenotypic correlations, this review suggests that the phenotypic correlation provides a minimum estimate of the genetic correlation. Additionally, phenotypic correlations are important because, when selection acts on both correlated traits, evolution will be tempered by phenotypic correlations between traits even in the absence of genetic correlations (Roff, 2002).

In summary, growth but not survival costs of reproduction were observed in this laboratory study of phenotypic correlations in freshwater snails. The trade-off between reproduction and growth may constrain selection on growth and reproduction in natural environments and may have constrained the response to selection by parasites in a related study (Krist, 2006). Genotype × environment interactions caused by laboratory conditions may explain the absence of a trade-off between reproduction and survival and the unexpected positive relationship between reproduction and survival. The positive relationship between reproductive output and maximum observed age is also consistent with continued egg production over the study period, suggesting that increased longevity leads to increased lifetime fecundity. However, the positive relationship between reproductive output and maximum observed age is probably weaker in most natural conditions because of the presence of ecological costs of reproduction.

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