Causes of Discordance between Allometries at and above Species Level: An Example with Aquatic Beetles

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ABSTRACT: Covariation among organismal traits is nearly universal, occurring both within and among species (static and evolutionary allometry, respectively). If conserved developmental processes produce similarity in static and evolutionary allometry, then when species differ in development, it should be expressed in discordance between allometries. Here, we investigate whether rapidly evolving developmental processes result in discordant static and evolutionary allometries attributable to trade-offs in resource acquisition, allocation, or growth across 30 species of aquatic beetles. The highly divergent sperm phenotypes of these beetles might be an important contributor to allometric evolution of testis and accessory gland mass through altered requirements for the production of sperm and seminal fluids. We documented extensive discordance between static and evolutionary allometries, indicating that allometric relationships are flexibly modified over short time periods but subject to constraint over longer time spans. Among species, sperm phenotype did not influence relative investment in accessory glands but was weakly associated with investment in testes. Furthermore, except when sperm were long and simple, sperm phenotype was not associated with species-specific modification of the allometry of testis/accessory gland mass and body size. Our results demonstrate the utility of allometric discordance to infer species differences in the provisioning and growth of concurrently developing traits.

Keywords: Dytiscidae, gonadosomatic index, Gyrinidae, paragonia, sperm conjugation.

Introduction

Covariance among traits at a single life stage within species (static allometry; Cock 1966) and covariance among traits among species (evolutionary allometry; Gould 1966) provide insight into developmental mechanisms, functional interactions between traits, and patterns of correlated evolution (Cheverud 1982). Conserved developmental pathways should produce similar static allometries among species (Gould 1966; Cheverud 1982; Riska 1986; Atchley and Hall 1991). However, sensitivity of these pathways to en-

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vironmental variations and adaptive evolution of covariance patterns among traits (Weber 1990; Wilkinson 1993; Emlen 1996; West-Eberhard 2003; Frankino et al. 2005; Cayetano et al. 2011) often result in divergent static allometries among closely related taxa (e.g., mammals [Cheverud 1982; Martin and Harvey 1985], birds [Badyaev and Hill 2000; Green et al. 2001], insects [Klingenberg and Zimmermann 1992; Simmons and Tomkins 1996; Hosken et al. 2005], and plants [Primack and Antonovics 1981; Mazer and Wheelwright 1993]). Nonetheless, even when static allometries are divergent, evolutionary allometry typically persists due to covariances diversifying along an axis, presumably as a consequence of constraint or selection (Lande 1979, 1985; Zeng 1988). We propose that because trait relationships evolve along this axis, evolutionary allometry can be considered a generalized model of trait covariance across species. Hence, when traits scale differently within and among species, this discordance between static allometry and evolutionary allometry can be used to infer the types of developmental modifications producing species-specific patterns of trait covariance.

Within species, scaling relationships often result from the use of shared resources and common factors regulating the growth of concurrently developing traits (Stern and Emlen 1999; Zera and Harshman 2001; Badyaev 2004, 2007). The characteristics of scaling relationships (e.g., slope, intercept, linearity) are determined by the relative initial size of the traits (e.g., precursor tissues) and the rate and duration of growth (Nijhout 2011). Due to common descent, the null expectation for related species is similar modes of trait production (i.e., similar patterns of resource acquisition, resource allocation, and growth regulation) and thus similar static allometries. In this scenario, evolutionary allometry will have a scaling coefficient and factor (i.e., slope and intercept, respectively) analogous to the static allometries, suggesting that a common mechanism (i.e., conserved developmental processes) underlies both static and evolutionary allometry (fig. 1A). In contrast, discordance between static and evolutionary allometry occurs when species-
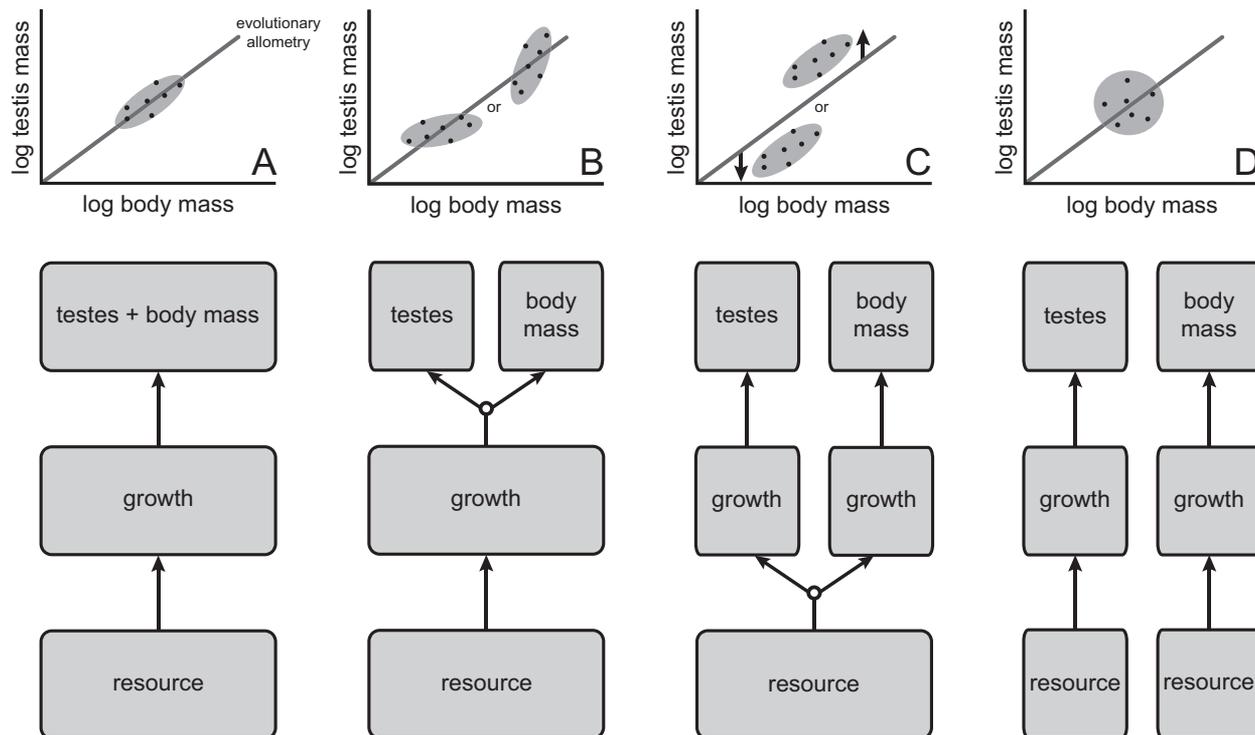
Figure 1: Types of discordance between static and evolutionary allometry resulting from trade-offs between provisioning and growth of two traits, using testis and body mass as an example. In the top panels, a shaded region represents static allometry, with individuals of a given species shown as filled circles. Evolutionary allometry may assume a variety of shapes. Here we focus on linear relationships due to their adequacy to describe trait relationships in many taxa (Gould 1966), including those in this study. *A*, When resource allocation and the timing and duration of growth of two traits are similar to the among-species mean, static allometry is expected to be concordant with evolutionary allometry. *B*, Species-specific change in the timing or duration of growth of the traits due to altered sensitivity to growth signals is predicted to result in changes in the allometric coefficient. *C*, Change to the initial resource allocation to a trait relative to body size is predicted to alter the scaling factor without corresponding changes in the allometric coefficient. *D*, Breakdown of an allometric relationship is predicted to occur when a developing trait becomes insensitive to signals of body size or is provisioned after determination of body size. Scenarios *B*, *C*, and *D* will result in discordance with evolutionary allometry.

specific modification of trade-offs between provisioning and growth of traits results in dissimilar static and evolutionary allometric scaling factors or coefficients. For example, discordance occurs when species differences in the rate and duration of growth alter the scaling coefficient (i.e., slope) between a trait and body size (fig. 1B), whereas changes in initial resource allocation to a trait relative to body size alter the scaling factor (i.e., intercept) without a corresponding change in the scaling coefficient (fig. 1C; Atchley and Hall 1991; Nijhout 2011; but see Emlen and Nijhout 2000). Discordance between static and evolutionary allometry can also occur when a developing trait does not covary with body size within species (i.e., an absence of allometry) despite covariation between the trait and the body size among species (fig. 1D). Within species, static allometry will be absent if a trait is insensitive to signals of body size or is provisioned after the determination of body size (among species, evolutionary allometry may persist). Thus, by using evolutionary allometry as a reference pattern of trait

covariance, discordance with static allometry can be used to test whether specific modifications of trait relationships are associated with ecological or selective contexts.

Because trait exaggeration associated with sexual selection is frequently achieved through growth-based trade-offs (e.g., Eberhard 1985; Emlen and Nijhout 2000) and results in diverse allometric relationships with body size (Bonduriansky and Day 2003; Badyaev 2004; Bonduriansky 2007), discordance between static and evolutionary allometry of reproductive traits and body size should be common. Testes and seminal fluid–producing accessory glands (e.g., paragonia in insects) are subject to indirect selection for male fertility and sperm competitiveness (e.g., Fairn et al. 2007; Simmons and Fitzpatrick 2012; Perry et al. 2013) and are of particular interest to the investigation of allometric evolution, because their ontogeny is often temporally separated from other aspects of organismal growth and these traits can vary widely in size relative to body mass without disrupting

whole-organism function. Diversification of sperm morphology in response to sexual selection (Snook 2005; Pitnick et al. 2009a; Pizzari and Parker 2009) might be an important contributing factor to the shaping of evolutionary allometry between testis/paragonial mass and body size. Larger or more complex sperm might be associated with increased investment in sperm production (Ramm and Schärer 2014) or reduced investment in nonsperm components of the ejaculate (i.e., seminal fluid); this hypothesis is supported by a significant correlation between sperm phenotype and testis mass in birds, mammals, fish, and insects (Gage 1994; Pitnick 1996; Stockley et al. 1997; Schulte-Hostedde and Millar 2004; Lüpold et al. 2009). However, elaboration of sperm phenotypes might not impact allometric scaling if it is achieved without substantial modification of individual sperm morphology, as is the case with sperm conjugation, where two or more spermatozoa join together for motility or transport through the female reproductive tract (Higginson and Pitnick 2011).

Here, we test for the presence and type of developmental modifications that result in discordance between static and evolutionary allometry using members of Hydradephaga, a monophyletic clade of aquatic beetles (Shull et al. 2001; Ribera et al. 2002) that vary dramatically in body size, sperm morphology, and sperm conjugation. We establish within- and among-species patterns of allometric scaling between testis/paragonial mass and body size for 30 species from the four major lineages of Hydradephaga (Dytiscidae, Gyrinidae, Haliplidae, and Noteridae) to (1) determine the prevalence of trade-offs between resource acquisition, allocation, and growth in species-specific modification of allometric relationships and (2) investigate the extent to which sperm phenotype is associated with the correlated evolution of testis/paragonial mass and body size. If developmental constraints on the production of testes or paragonia are strong, static and evolutionary allometries will show similar patterns of trait covariance, whereas changes in developmental trade-offs between reproductive traits and body size might produce discordance in one of three ways. First, if fertilization success conferred by testis or paragonial mass changes with body size or is dependent on sperm phenotype, then we would expect species-specific changes in the allometric scaling coefficient of testis or paragonial mass, achieved through modification of the timing or duration of growth of the traits. Second, if conjugation requires changes in testis structure or alters the need for accessory gland secretions, then conjugation is expected to change initial allocation to testes or paragonia, producing discordance in the scaling factors between static and evolutionary allometry. Third, as many species of aquatic beetles have extended adult life spans associated with delayed investment in reproductive organs (e.g., overwintering as adults prior to breeding; Larson et al. 2000; D. M. Higginson, personal observations), where reproductive organs may not reach their final size until months after adult eclosion (Heming 2003, p. 22), we expected a lack of covariation between reproductive traits and body mass to be the most common source of discordance between static and evolutionary allometry. Additionally, we assessed the contribution of evolutionary lability in body mass and testis/paragonial mass in driving interspecific variation in allometric scaling.

Methods

Testis/Paragonial Mass and Sperm Phenotype

For each species, males ($n = 12$) were euthanized and dissected in distilled water. Testes, paragonia, and soma were dried separately and weighed using an Orion Cahn C-35 ultra-microbalance. Mean testis, paragonia, and soma mass were calculated for each species (tables S1, S2 in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.j13m3 [Higginson et al. 2015]). Additional males ($n = 5$) were used to determine the sperm traits for each species. Seminal vesicles were ruptured to release mature sperm into phosphate-buffered saline on glass slides. Slides were then dried, fixed, and DNA-stained using Hoechst or 4′,6-diamidino-2-phenylindole (DAPI). Sperm length was measured from digitized images using dark field or epifluorescence microscopy and ImageJ (Rasband 2012). Presence and type of conjugation were recorded for each species (Higginson et al. 2012a; table S2 in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.j13m3 [Higginson et al. 2015]).

Phylogenetic Trees

Evolutionary relationships among species were inferred using 2,228 continuous or near-continuous base pairs of the mitochondrial genes COI, tRNA-Leu, and COII. Sequences were aligned by eye. The appropriate model of sequence evolution was selected using DT-ModSel (Minin et al. 2003). We used MrBayes (Ronquist and Huelsenbeck 2003) to generate phylogenetic trees based on four independent runs, with six Markov chain Monte Carlo (MCMC) chains each of 5×10^7 generations sampled every 4,000 generations, 0.1 heating, and uninformative priors (i.e., MrBayes defaults). The program AWTY (Nylander et al. 2008) was used to assess convergence, and the first 10^6 generations were discarded as burn-in. After the MCMC chains converge, they visit alternative phylogenetic trees in proportion to their probability, given the data, model, and priors. A majority consensus tree was constructed from the 40,000 post-burn-in trees (Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.j13m3 [Higginson et al. 2015]).

We explored alternative fossil time calibration scenarios using PATHd8 (Britton et al. 2007) before fixing the age of Hydradephaga at 219.8 Ma (Hunt et al. 2007) in addition

to eight other calibration points for generic and family divergence times (Dytiscoidea [Ponomarenko 1993; Beutel et al. 2013], dytiscid genera [Nilsson 2001], haliplid genera [Prokin and Ponomarenko 2013], Dytiscidae [Prokin and Ren 2010], timescale [IUGS 2013]).

Statistical Analyses

All continuous variables were $\log_{10}$ transformed. Intraspecific analyses also used $\log_{10}$-transformed data (Huxley 1932; Houle et al. 2011). We did not attempt to analyze the types of conjugation (Higginson et al. 2012*a*) separately, instead choosing to treat conjugation as a binary trait (present or absent) to maximize the degrees of freedom available for hypothesis testing.

Static and evolutionary allometry.   Estimation of static allometric coefficients and related analyses were conducted with JMP Pro 9.0.3 (SAS Institute, Cary, NC). Highly influential observations (Cook's $D > 1$), suspected to result from tissue-handling errors, were omitted from calculations of static allometric relationships. To reduce bias resulting from measurement error (Hansen and Bartoszek 2012), we calculated the evolutionary allometric coefficients, incorporating species standard error of mean trait values using the varFixed function in the R package nlme (Pinheiro et al. 2013) and the covariance structures resulting from alternative models of trait evolution using our time-calibrated phylogenetic tree with the package ape (Paradis et al. 2004). Model fit was evaluated using Akaike information criterion (AIC) and Bayesian information criterion (BIC) values.

Tests of discordance.   *T* ratios were used to test for differences between static and evolutionary allometric coefficients. When evolutionary and static allometric coefficients were equivalent, we tested for differences between the scaling factors using the *s* ratio method described by White and Gould (1965). Static allometry for a given species was considered equivalent to evolutionary allometry if there was no significant difference in coefficient relative to evolutionary allometry and if the difference in scaling factors was less than 0.1 (i.e., the *s* ratio).

Evolutionary rates.   We estimated relative rates of trait evolution, incorporating within-species trait covariance and measurement error (Adams 2013). This method allows direct comparison of the evolutionary rates of multiple traits, following a Brownian motion model. The measurement error of sperm length was unavailable for three species, *Celina hubbelli*, *Hydrovatus pustulatus*, and *Ilybius oblitus*, which were thus excluded from the analysis. To assess the appropriateness of this model for each of our traits, we first examined alternative models of trait evolution using the function fitContinuous in the R package geiger (Harmon et al. 2008). Due to the broad taxonomic sampling present in this study, we did not attempt to evaluate the suitability

of the κ model of evolution. Log testis and paragonial mass evolution were well described by Brownian motion (ΔAICc of Brownian motion vs. next-best-fit model = 1.32 and 1.88, respectively). However, log body mass was better described by a model with a linear trend in evolutionary rates over time (ΔAICc = 4.07). Nonetheless, the approach of Adams (2013) remains the best available method to compare relative rates of trait evolution while accounting for sources of error, but due caution should be exercised in the interpretation of the results.

Results

Across Hydradephaga, testes comprised a median of 3.34% of total body mass (range: 0.85%–8.06%), and 23 of 30 species had conjugated sperm. Sperm length (median = 0.60 mm, range: 0.10–6.84 mm) and body mass (median = 3.85; range: 0.26–65.02 mg) varied widely.

Evolutionary Allometry

Across species, testis mass scaled to body mass with an allometric coefficient greater than 1 ($\beta = 1.32$, $P < .0001$; test for difference from isometry: $t_{28} = 4.62$, $P = .0001$; fig. 2*A*). In no case did inclusion of a covariance structure based on phylogenetic relationships improve the fit of allometric models (ΔBIC = 3.33–4.37). The best-fit model of testis mass versus total body mass had a covariance structure that simulated a star phylogeny (Pagel's $\lambda = 0$; Pagel 1999) and was equivalent to generalized least squares regression. In contrast, paragonia mass scaled isometrically with body mass ($\beta = 1.13$, $P < .0001$; test for difference from isometry: $t_{28} = 1.09$, $P = .28$; fig. 2*B*), and the best-fit model included a covariance structure simulating Brownian evolution along the phylogenetic tree (Pagel's $\lambda = 1$ vs. 0 ΔBIC = 5.75; Pagel 1999; Freckleton et al. 2002). We additionally examined how combined testis/paragonial mass scaled with total body mass and found that it had an allometric coefficient greater than 1 ($\beta = 1.15$, $P < .0001$; test for difference from isometry: $t_{28} = 2.11$, $P = .04$; fig. 2*C*) and followed a Brownian motion model of evolution. To investigate whether allocation to one reproductive trait trades off with allocation to another concurrently developing trait, we regressed testis mass on paragonial mass and found that the traits scaled isometrically ($\beta = 0.91$, $P < .0001$; test for difference from isometry: $t_{28} = 0.81$, $P = .42$; fig. 2*D*), indicating no evidence of a trade-off. Models with or without a trait covariance structure based on species relatedness did not differ (ΔAIC < 0.51).

Static Allometry

To determine whether related species had similar static allometry, we tested alternative models of evolution of the
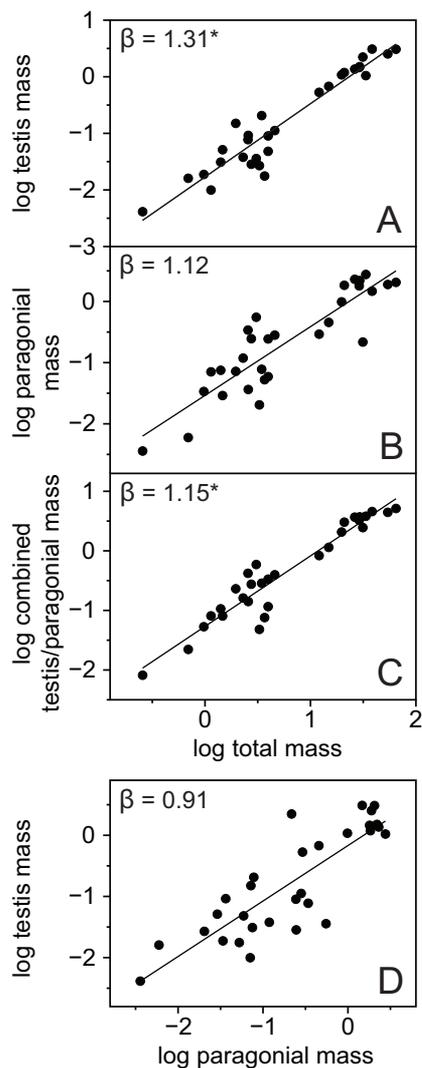
Figure 2: Evolutionary allometry of reproductive traits in Hydradephagan beetles. Each species is represented by a filled circle. *A*, Larger species have disproportionately larger testes than smaller species (i.e., positive allometry). *B*, Paragonial mass increases isometrically with body mass. *C*, Combined testis/paragonial mass scales to body mass with a coefficient slightly greater than 1, likely due to the contribution of testis mass. *D*, Species with large testes tend to have disproportionately small paragonia (i.e., negative allometry), but this trend is not statistically significant. An asterisk denotes a scaling coefficient significantly different from 1; $P < .05$.

standardized slopes of these relationships. The best-fit model for the evolution of the standardized slope of testis or paragonial mass versus total body mass assumed the data were drawn from a single normal distribution with no covariance among species, although the strength of support for this model was stronger for testes than for paragonia (e.g., Brownian motion vs. white noise ΔAIC = 13.81 and 3.05, respectively).

Similarly, a nonphylogenetic model fit as well or better than various evolutionary models of the standardized slope of combined testis/paragonial mass versus total body mass (ΔAIC = 0.47) or testes versus paragonia (ΔAIC = 3.38). Collectively, these results indicate that intraspecific allometries of the taxa included in this study are independent of phylogenetic relationships among species.

For the majority of species, the growth of reproductive traits and body size were decoupled, with no association between the two traits ($P > .05$; fig. 3; table 1). When allometric relationships did remain, they frequently deviated from evolutionary allometry through either altered allometric coefficients or scaling factors (table 1). The species-specific modification described above could have arisen through either changes in growth and provisioning of reproductive traits or body size. To investigate the underlying mechanisms driving changes in scaling, we estimated the rates of trait evolution and found that testis and paragonia mass evolved twice as fast as body mass (0.0030, 0.0029, and 0.0015, respectively; multiple evolutionary rate likelihood ratio = 8.27, $P = .016$). This suggests that changes in allometric scaling were primarily the result of changes in reproductive traits.

Sperm Phenotype and Allometry of Testis/Paragonial Mass

We examined the association between sperm length, the relative investment in testes or paragonia (e.g., testis mass/total body mass), and the presence of conjugation, incorporating measurement error and phylogenetic relationships. Phylogenetic generalized least squares regression using a Brownian motion model of evolution indicated that increased sperm length was marginally associated with increased relative investment in testes (main effect: $t_{23} = 2.00$, $P = .06$) and that single sperm were longer, with greater relative investment in testes than conjugated sperm ($t_{23} = 3.24$, $P = .004$; main effect of conjugation: $t_{23} = 0.29$, $P = .77$). There was no association between sperm length and relative investment in paragonia ($t_{25} = -0.56$, $P = .58$).

Discordance between static and evolutionary allometry was common (table 1), but in most cases, it was not associated with elaboration of the sperm phenotype. The presence of conjugation was independent of discordance between the scaling of testis mass, paragonial mass, and combined testis/paragonial mass to total body mass (Fisher's exact test, $P = 1.00$). Furthermore, conjugation did not affect the type of discordance (i.e., change in scaling coefficient or factor) between static and evolutionary allometry (Fisher's exact test, $P > .15$). Species with long, single sperm, however, were more likely to have discordance resulting from changes in the allometric coefficient of paragonial mass to total body mass relative to evolutionary

Species	T vs BM	P vs BM	T+P vs BM	T vs P	BM (mg)	sperm (mm)
Acilius mediatus	↑	0	<	0	38.66	0.177
Acilius semisulcatus	>	0	=	0	65.02	0.243
Graphoderus liberus	>	=	>	↑	31.77	0.200
Agabus ambiguus	0	=	↑	0	15.06	1.086
Ilybius oblitus	↓	<	↑	0	29.43	1.965
Laccophilus maculosus	↓	0	0	0	4.01	1.112
Rhantus binotatus	0	=	0	0	29.04	0.149
Coptotomus longulus	0	0	0	0	12.13	1.538
Nebrioporus rotundatus	0	<	0	0	3.98	0.681
Celina hubbelli	↑	0	↓	0	1.47	3.58*
Desmopachria convexa	0	↓	↑	0	0.26	1.517
Hydrovatus pustulatus	0	>	↓	<	0.70	4.49*
Heterosternuta pulcher	0	0	<	0	1.15	1.284
Neoporus dimidiatus	0	>	↑	0	1.97	2.099
Neoporus undulatus	0	0	↑	0	2.57	1.342
Hygrotus sayi	0	0	0	0	1.41	0.531
Hydroporus sp.	0	0	0	0	3.07	0.448
Haliplus immacucolis	0	0	0	0	0.98	0.135
Haliplus fasciatus	↓	0	0	0	3.29	0.314
Hydrocanthus iricolor	0	0	0	<	2.75	0.633
Dineutus ciliatus	<	↑	↑	0	54.38	0.102
Dineutus discolor	0	0	0	0	33.56	0.099
Dineutus emarginatus	↑	=	↓	0	19.82	0.146
Dineutus nigrior	0	=	↑	0	26.36	0.098
Dineutus horni	↑	0	↑	0	21.00	0.102
Gyretes iricolor	0	0	0	0	3.46	6.837
Gyrinus gibber	0	=	↓	0	4.61	0.580
Gyrinus latilimbus	0	<	0	0	2.31	0.447
Gyrinus fraternus	0	=	0	0	3.72	0.660
Gyrinus marginellus	0	0	0	0	2.60	0.621

Families: Dytiscidae, Haliplidae, Noteridae, Gyrinidae

250 mya 200 150 100 50 0

= equivalent to evolutionary allometry   0 no intraspecific relationship
↓↑ scaling factor less or greater than evolutionary allometry   < > slope less or greater than evolutionary allometry

single   pairs   aggregates   rouleaux   spermatodesms   spermatostyles

Figure 3: Time-calibrated tree, static allometric relationships, body mass, and sperm traits. Note the predominance of static allometry discordant with evolutionary relationships between traits. While this figure illustrates the multiple types of conjugation that were present (for description of types of conjugation, see Higginson and Pitnick 2011), conjugation was treated as a binary trait (present or absent) in our statistical analyses. T = testis mass, P = paragonial mass, BM = total body mass, T + P = combined testis/paragonial mass, sperm = sperm length.

Table 1: Summary of processes producing discordance between static and evolutionary allometry

Scaling relationship	Concordant	Discordant		
		Change in coefficient	Change in scaling	No relationship
Testis versus total mass	0	3	7	20
Paragonial versus total mass	7	5	2	16
Reproductive investment versus total mass	1	3	12	14
Testis versus paragonial mass	0	2	1	27

Note: Numbers indicate the number of species.

allometry, associated with the timing and duration of growth of the two traits (multinomial logistic regression, whole model: $X^2 = 23.03$, df $= 9$, $P = .006$; sperm length: $X^2 = 10.71$, df $= 3$, $P = .01$; conjugation: $X^2 = 5.89$, df $= 3$, $P = .12$; sperm length × conjugation: $X^2 = 14.33$, df $= 3$, $P = .003$). In all other scenarios, there was no association between sperm phenotype and allometric discordance ($P > .11$).

Discussion

Our study revealed strong evolutionary allometry between reproductive traits and body mass and extensive discordance between static and evolutionary allometries, indicating that different mechanisms determine patterns of trait covariation within and among species. Species-specific modification of static allometry was achieved through shifting the timing of trade-offs in resource acquisition, allocation, and growth between developing traits (Badyaev 2007), resulting, respectively, in a loss of covariance between reproductive traits and body mass (fig. 1*D*) or static allometries that differ in their scaling factors (fig. 1*C*) or coefficients (fig. 1*B*). The presence or type of discordance between static and evolutionary allometry was not associated with sperm phenotype, suggesting that there are multiple developmental modifications that can accommodate elaboration of sperm morphology.

Whereas among species, the ratio of paragonial mass and combined testis/paragonial mass to body mass conformed to expectations of traits evolving in a Brownian manner along the branches of a phylogeny, the ratio of testis mass to body mass and the static allometric coefficients of all traits contained no phylogenetic signal, indicating that clade-specific modification of scaling relationships is uncommon. A lack of phylogenetic signal can result from a number of evolutionary scenarios, including similar selection across species, strong constraint in the production of the traits, and divergent selection among species (Gittleman et al. 1996; Blomberg et al. 2003; Revell et al. 2008). As evolutionary allometry would be absent without a consistent relationship between testis and body mass, a lack of phylogenetic signal is likely to result from conservatism of scal-

ing relationships due to strong stabilizing selection or functional constraints. Conversely, we observed high evolutionary lability, resulting in an absence of phylogenetic signal among static allometries.

The contrasting patterns of diversification and constraint of static and evolutionary allometries in Hydradephaga suggest that species respond differently to contemporary and long-term selection. Both body size and testis/paragonial mass are important contributors to organismal fitness. When there are trade-offs in production of these traits, investment in reproductive traits relative to body size should maximize fitness and limit the observed trait covariances (Shoval et al. 2012; Sheftel et al. 2013). Contemporary trade-offs between testis/paragonial mass and body size might be limited due to the restricted range of trait covariances that can be produced with a given developmental pathway. Alternatively, trade-offs may be obscured by environmental contribution to phenotypes (e.g., resources might be abundant during the primary period determining body size but limited during testis maturation).

Physiological differences among taxa can strongly influence patterns of trait covariance. Whereas relative paragonial mass does not vary systematically with mating frequency (for opposing patterns, see, e.g., Baer and Boomsma 2004; Demary and Lewis 2007), among-species relative testis mass is a robust indicator of the intensity of postcopulatory sexual selection in a wide array of taxa (recently reviewed in Pizzari and Birkhead 2002; Calhim and Birkhead 2007; Vahed and Parker 2012). However, differences in the importance of gonadal contribution to circulating hormone titers may contribute to dissimilar allometric patterns of testis mass to body size among insects and vertebrates (i.e., scaling coefficients of >1 and <1, respectively; Harcourt et al. 1981; Short 1981; Gage 1994; Nijhout 1994; Pitnick 1996; Heming 2003, pp. 323–328; MacLeod and MacLeod 2009; Vahed et al. 2011; this study). In contrast to that of vertebrates, testis mass of insects does not relate to circulating hormone titers (Nijhout 1994; Heming 2003, pp. 323–328) and is thus expected to evolve freely in response to selection. In this study, the majority of species displayed no relationship between testis/paragonial mass and total body mass

(table 1), likely as a result of continued provisioning and growth of testes and paragonia after the cessation of larval growth, the primary determinant of total body mass. Here, reduction of within-species trade-offs between reproductive traits and body mass, enabled by conserved life-history traits, resulted in extensive discordance between within-species and among-species trait covariance.

Our analyses suggest that the evolution of static allometries, and thus discordance with evolutionary allometry, was driven by changes in reproductive traits. Specifically, we found that in Hydradephaga, testis and paragonial mass evolved two times faster than total body mass. While, to our knowledge, the relationship between sperm phenotype and paragonial mass is unknown for other taxa, increased investment in testes is associated with the evolution of longer sperm in butterflies (Gage 1994) and fruit flies (Pitnick 1996). In fruit flies, long sperm more effectively maintained preferred positions for fertilization by displacing rival sperm (Miller and Pitnick 2002; Pattarini et al. 2006; Lüpold et al. 2012). The selective advantage of long sperm, however, might be reduced by anchoring sperm conjugates at preferred positions within the female reproductive tract (Higginson et al. 2012b). In Hydradephaga, sperm length is not associated with paragonial mass and has only a marginal positive association with testis mass. Interestingly, there was an interaction between sperm length and conjugation; species with conjugation have typically shorter sperm and smaller testes than those without conjugation. Thus, because of a possible mitigating effect of conjugation, selection for increased sperm length is unlikely to explain the rapid evolution of testis and paragonial mass in Hydradephaga.

With the exception of changes in allometric coefficients being more common when sperm are long and single, elaboration of the sperm phenotype was not associated with the presence or type of discordance between static and evolutionary allometries of testis/paragonial mass and body mass. Taken together, these results show that, across Hydradephaga, evolution of sperm form requires minimal, if any, changes in reproductive investment in testes or paragonia. This might be the result of the complex nature of the trait subject to direct selection, ejaculate competitiveness, which provides multiple avenues of adaptation (Bjork et al. 2007). For example, evolution of sperm morphology may be accompanied by changes in sperm numbers (Parker et al. 2010; Immler et al. 2011) or accessory gland contributions to ejaculate composition (Wolfner 2002; Pitnick et al. 2009b), not altering investment in testis/paragonial mass.

As evolutionary allometry does not necessarily reflect functional relationships between traits, it has been suggested to lack biological information about its component species (e.g., Kozlowski and Weiner 1997; Schlichting and Pigliucci 1998; Voje and Hansen 2013). However, here we showed that discordance between static and evolutionary allometry can be used to infer species-specific modification of trade-offs in resource acquisition, allocation, and growth of concurrently developing traits. When applied to the evolution of allometric relationships between testis/paragonial mass and body size in Hydradephagan beetles, we found that the pattern and type of developmental modifications were not readily predicted by elaboration of sperm phenotype. Furthermore, the presence and type of developmental trade-offs are not necessarily associated with specific clades, indicating a high degree of lability of within-species mechanisms of trait production. Persistence of evolutionary allometry in this scenario could indicate strong selective or functional constraints on the ratio of testis/paragonial mass to body size among species.

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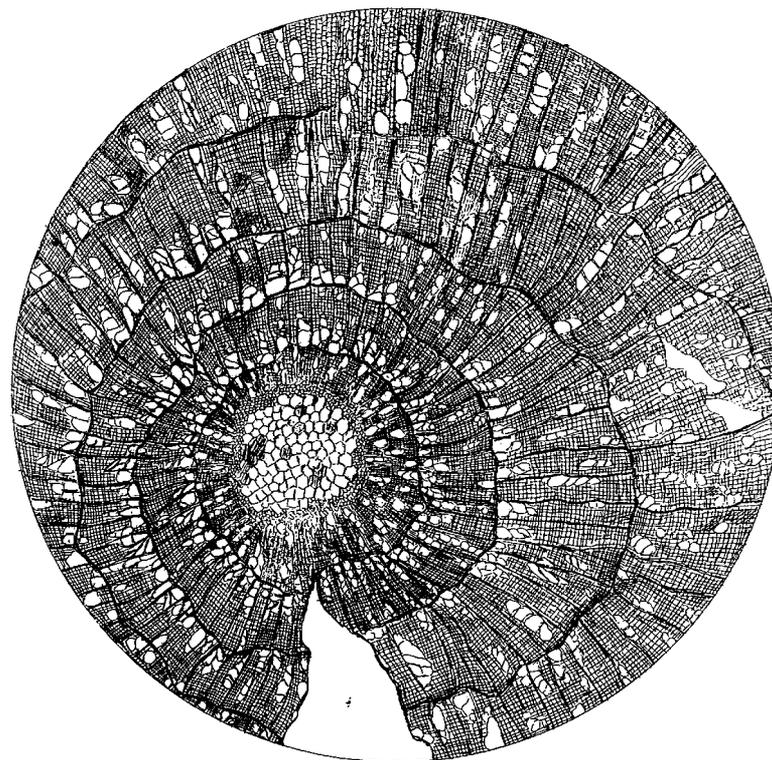
"The examination of any organic tissue, be it animal or vegetable, by means of the modern achromatic microscope, reveals such a world of beauty, and so much material for wonder, that the novice in such pastime is for a while very much puzzled what to observe, and what to leave unseen." Illustrated: "Section of oak wood cut transversely across the grain." From "Shavings Examined Microscopically" by A. M. Edwards (*The American Naturalist*, 1870, 3:561–568).