Experimental Evidence That Metamorphosis Alleviates Genomic Conflict

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ABSTRACT: Whenever genetically correlated traits experience antagonistic selection, an adaptive response in one trait can lead to a maladaptive response in the correlated trait. This is a form of genome-level conflict that can have important evolutionary consequences by impeding organisms from reaching their adaptive optima. Antagonistic selection should be pervasive in organisms with complex life histories because larval and adult life stages specialize in dramatically different environments. Since individuals express larval and adult morphologies from a single genome, genomic conflict across ontogenetic stages should also be prevalent. Using wood frogs as a study system, we measured natural selection on larval and postmetamorphic life stages and estimated genetic correlations among traits. Alternative life stages experienced a mix of both antagonistic and congruent viability selection. The integration between traits changed over the course of metamorphosis, reducing genetic correlations that cause conflict. Our results provide novel experimental evidence that metamorphosis can alleviate genomic conflict by partitioning life-history stages into modules that can more readily respond to selection. These results highlight the adaptive potential of metamorphosis as a means to avoid ecological specialization trade-offs. Moreover, they provide insights into the prevalence and evolutionary maintenance of complex life cycles.

Introduction

Antagonistic selection interacts with genomic architecture to influence adaptation. For instance, positive genetic correlations may promote adaptation when selection is congruent but impede adaptation when correlated traits experience antagonistic selection (Schluter 1996; Arnold et al. 2001; Ashman 2005; Bonduriansky and Chenoweth 2009; Abbott 2010; Berg and Maklakov 2012; Wright et al. 2018). Adaptation is impeded in this case because any response to selection in one trait would result in a correlated, maladaptive response in the other trait. A well-known example is the case of sexual conflict, in which genetic loci that are favored in one sex are disfavored in the opposite sex (i.e., sexually antagonistic selection or antagonistic pleiotropy; Chippindale et al. 2001; Chapman 2006; Delph and Ashman 2006; Rowe and Day 2006; Connallon et al. 2010; Delph et al. 2011). In the absence of a mechanism to reduce genetic correlations, one prediction is that sexual conflict could result, for example, in high-fitness individuals producing high-fitness progeny of the same sex but low-fitness progeny of the opposite sex. Empirical and theoretical studies of sexual conflict have outlined its important implications to the evolutionary dynamic of populations. For instance, sexual conflict has been shown to explain patterns of reduced fitness in populations (Berg and Maklakov 2012) and the maintenance of genetic variance in fitness-related traits (Hall et al. 2010). Moreover, sexual conflict is thought to play a role in within-species diversification (Bonduriansky 2011) and even speciation (Arnqvist et al. 2000; Martin and Hosken 2003).

Recent studies have highlighted the potential for extending studies of sexual conflict to cases of hermaphroditism, where a single individual expresses both sexes (Delph and Ashman 2006; Abbott 2010), as well as the case of alternative reproductive tactics within polymorphic species (Morris et al. 2013) and even different casts in social insects (Pennell et al. 2018). These generalizations pave the way for a quantitative genetics framework of genomic conflict that can be extended to any two alternative expressions of a genome. Here, we demonstrate this generality by investigating ontogenetic conflict experienced over the life stages of an organism with a complex life cycle.

We define genomic conflict broadly, sensu Rice (2013). This definition considers genomic conflict as any case of opposing selection between different genetic elements or loci within a single individual, between different genes in individuals of the same species, or between the expressions of a genetic locus in different backgrounds (e.g., sexes; Rice 2013). Although this definition is not without controversies (e.g., Gardner and Ubeda 2017), it allows for an immediate and

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intuitive recognition of how patterns of sexual conflict can be extended into a general framework.

Genomic conflict arises following two necessary conditions: one related to the genetic correlations between traits under linkage or pleiotropy or between the expression of a trait under different genetic backgrounds, and the other related to the direction of selection (Bonduriansky and Chenoweth 2009; Abbott 2010; Delph et al. 2011; Morris et al. 2013). Briefly, genomic conflict will occur whenever congruent selection acts on traits with negative genetic correlations or whenever antagonistic selection acts on traits with positive genetic correlations. Note that because positive genetic correlations do not result in genomic conflict when traits experience congruent selection, and antagonistic selection does not result in genomic conflict when traits are negatively correlated, the investigation of both conditions is necessary to demonstrate genomic conflict. Finally, as demonstrated in the sexual conflict literature, if the expression of the traits becomes decoupled such that genetic correlations are reduced (Chapman 2006; Bonduriansky and Chenoweth 2009; Connallon and Clark 2011; Delph et al. 2011; Cox et al. 2017; Wright et al. 2018), then genomic conflict is alleviated or resolved if genetic correlations become 0.

Genomic Conflict in Organisms with Complex Life Cycles

Complex life cycles require adaptation to different ecological challenges. For example, adaptation to aquatic and terrestrial environments experienced by larval and adult amphibians often depends on optimizing very different suites of phenotypic traits. Because larval and adult amphibians develop from a single genome, the expression of a certain gene could increase fitness in one life stage but decrease fitness in the other life stage. In the frog *Mantidactylus betsileanus*, for instance, 96% of all genes studied and 94% of the genes associated with morphological features are evenly expressed in both tadpole and frog stages (Wollenberg Valero et al. 2017). Genetic correlations between life stages can arise from the expression of these genes whether they affect the same trait in tadpoles and frogs (e.g., body length or body shape) or different traits in each life stage (e.g., tail length in tadpoles and leg length in frogs). Genetic correlations between ontogenetic stages are therefore expected, even when morphologies differ.

As amphibians transition through life stages and environments, the targets of natural selection change considerably, and thus antagonistic selection between larval and adult traits could be pervasive. However, metamorphosis might adaptively decouple phenotypes to resolve conflict between ontogenetic stages, a process analogous to the resolution of sexual conflict by sex-specific gene expression and sexual dimorphism (e.g., Chapman 2006; Bonduriansky and Chenoweth 2009; Connallon and Clark 2011; Cox et al. 2017; Wright et al. 2018). The dramatic changes in morphology and/or physiology that occur during metamorphosis are thought to decrease the genetic correlation between traits of larval and adult stages, allowing adaptation to proceed independently at each life stage (reviewed in Ebenman 1992; Moran 1994). Until now, no study has simultaneously measured the nature of both selection (antagonistic vs. congruent) and trait correlations (positive vs. negative) to fully test this hypothesis. Using wood frogs (*Rana sylvatica*) as a study system, we combined estimates of genetic correlations within and between life stages from a large-scale breeding design, including hundreds of tadpoles reared to metamorphosis, with the investigation of viability selection across both larval and juvenile ontogenetic stages. Through the investigation of these two necessary conditions under the quantitative genetics framework of genomic conflict, we provide the first test for the adaptive significance of metamorphosis as a means of alleviating ontogenetic conflict across life-history stages.

We report estimates of selection in tadpoles and juveniles that indicate a mix of both antagonistic and congruent selection are found across these two life stages. We then investigate the genetic architecture of the tadpole and juvenile traits under selection. We show that whereas traits that experience antagonistic selection after metamorphosis have high positive genetic correlations, traits that experience antagonistic selection across the metamorphosis boundary have comparatively lower genetic correlations.

It has been debated in the literature whether genetic decoupling is better reflected by estimates of genetic correlations that are 0 or simply those that are significantly <1 (e.g., Aguirre et al. 2014 and references within), as only perfect genetic correlations would truly constrain a response to selection. However, large (albeit imperfect) genetic correlations have been shown to impede adaptation for as many as 100 generations (Stewart and Rice 2018), and still weaker genetic correlations can cause deviations from the most direct evolutionary path toward the optimum (Via and Lande 1985). Moreover, in our interpretation, the adaptive decoupling hypothesis does not make predictions regarding the absolute magnitude of the genetic correlations but regarding the relative integration of traits within and between life stages. To make new steps toward clarifying these issues, we apply concepts of modularity studies, where the integration within subsets of traits is considered with respect to integration between subsets of traits. As applied to our case study, modularity indexes considered the genetic integration of traits within life stages with respect to the integration between life stages. This allowed us to test whether the life stages of wood frogs represent modules, in which the genetic integration within tadpole and frog stages is higher than the genetic integration across the metamorphosis boundary.

Finally, if metamorphosis is evolutionarily maintained as an adaptive means of decoupling genetic correlations (reviewed in Ebenman 1992; Moran 1994), opportunities for adaptive decoupling should be, by definition, limited to metamorphosis. Therefore, we predicted some degree of latent ontogenetic conflict following metamorphosis, as juvenile frogs continue to grow into mature adults. To further investigate the consequences of conflict persisting into adulthood, we measured viability selection on adult wood frogs.

Methods

Study System

Wood frogs (*Rana sylvatica*) are small to medium-sized anurans (35–60-mm snout-vent length in our study system) that inhabit forests throughout much of North America. Wood frogs in our study populations have a highly synchronized breeding period that follows the onset of above-freezing temperatures and spring rains occurring typically in mid-April. Adult wood frogs breed in vernal pools, where females lay egg masses that contain more than 600 eggs and that seem to be fertilized by a single male frog. Eggs hatch into larvae (tadpoles) after approximately 15–20 days and then, approximately 70–100 days later, metamorphose into juvenile frogs. Males mature within 1–2 years, while females take 2 years to reproduce (Berven 1982). Our study sites are located near Hanover, New Hampshire (43.7022°N, 72.2896°W), and Norwich, Vermont (43.7153°N, 72.3079°W).

Selection Estimates

Selection Experiments. We measured viability selection imposed by predators using uniquely marked individuals of each life stage that we released into seminatural field enclosures. We used larval diving beetles (Dytiscidae: *Dytiscus* sp.), a predator of wood frog tadpoles (Rubbo et al. 2006), and adult garter snakes (*Thamnophis sirtalis*), which prey on postmetamorphic wood frogs (Rittenhouse et al. 2009). Both predators are prevalent in our study sites.

To estimate selection on tadpoles, we used 200-L cattle tanks (approximately 131 cm × 78 cm × 30.5 cm) as mesocosms. We set up five mesocosms from June to August 2017, each containing 40 tadpoles collected from one of five different ponds (total $n = 200$ tadpoles), and a larva of a predacious diving beetle (minimum size: 3.5 cm). The predator was either collected in the experimental pond or introduced from a different pond, but no predator was used in more than one replicate. Mesocosms were set up by the margin of the pond where tadpoles were collected so that natural canopy cover provided shade, and the colder pond water cooled down the bottom of the tanks to prevent overheating. Tanks were filled with their respective pond water to a level of at least 20 cm deep, and a stick was added to the bottom as a perching site for predators. Pond water was filtered through a 2-mm grade mesh, allowing for only algae and other very small invertebrates to pass through and serve as food for the tadpoles. Tanks were fully covered with the same mesh and checked for survival rates after 7 days.

To estimate selection in juvenile frogs, we set up seminatural enclosures (18–20 m^2) near one of the ponds. We built the enclosures using mesh and fence poles so that the walls were at least 1 m in height. The ground of the enclosure was initially cleaned and inspected for other possible amphibians, snakes, or rodents using a garden rake and later recovered with leaf litter. One garter snake (minimum length of 30 cm) was used per enclosure, and no individual snake was reused across replicates. Two replicates were established in July–August 2015 and two in August 2017, each containing 40 juvenile frogs. In 2015, we used juvenile frogs (Gosner stage 46; Gosner 1960) originating from our breeding experiments in addition to juvenile frogs reared in captivity from wild caught tadpoles. In 2017, juvenile frogs (Gosner stage 45–46) were caught from six different populations as they left their ponds of origin. One replicate in each year had mortality rates of 97.5% and 100%. We removed these from the analyses given the likelihood that some unknown predator entered the enclosure, making the result incongruous with other replicates.

Finally, we used the same seminatural enclosures to estimate selection in adult frogs. Two replicates were established in April 2016 containing 50 and 46 frogs. Sex ratio was approximately equal between replicates (27 : 23 and 26 : 20, males : females), and combined survival was identical between sexes (18 males, 18 females). Two additional replicates were established in April 2017, but because of low rates of captures of females, only males were used in the experiment (50 males each). We did not introduce predators into the enclosures, but animals were exposed to predation from small mammals and birds of prey. In particular, American kestrels (*Falco sparverius*) are commonly seen and heard in the area, and evidence from kestrels feeding on frogs near the enclosures has been found at least once (D. Goedert and R. Calsbeek, personal observation).

Morphological measures for tadpoles, juvenile, and adult frogs were the same as for the breeding experiments. Tadpoles were individually marked using a polymer dye applied to their tail muscle in a unique color combination (https://www.nmt.us/visible-implant-elastomer). Juveniles and adults were individually marked via toe clipping.

Selection Estimates. We estimated viability selection using a multiple logistic regression between survival and traits. For tadpoles, we used the package lme4 (ver. 1.1-14; Bates et al. 2015) to fit a logit generalized mixed model, adding replicate as a random term because replicates presented variation in

survival rates (range: 43.2%–80%). We did not include a random effect term to the models for the estimates of selection on juvenile or adult traits because of the small number of groups, but we additionally present results of the models including replicate as a fixed effect. We applied a transformation to the logit coefficients (α) as suggested by Janzen and Stern (1998), following Lande and Arnold's (1983) demonstrations that the coefficient of relative fitness regressed on trait values represents the selection gradient. We estimated confidence intervals for the selection gradients (β') using nonparametric bootstrap ($n = 2{,}000$; Davison and Hinkley 1997; Canty and Ripley 2017). For the mixed effects model, we used parametric bootstrap ($n = 500$) with random effects estimates held fixed (bootMer function from lme4 package). We applied the same transformation as above to all bootstrap estimates before calculating the 0.025 and 0.975 quantiles. Because of sexual dimorphism and differences in the number of individuals of each sex used, we conducted selection analyses separately for adult males and females. However, we later pooled the data to test for differences in viability selection between males and females and refitted models including the interaction terms between traits of interest and sex.

All analyses were conducted in R (ver. 3.4.1; R Development Core Team 2017). Data and R codes have been deposited in the Dryad Digital Repository (https://doi.org/10.5061/dryad.jf653s3; Goedert and Calsbeek 2019).

Genetic Correlations

Fieldwork and Breeding. Adult wood frogs were collected in the spring of 2015 and 2016 using pitfall traps. Until enough animals were captured for breeding, animals were kept in a temperature-controlled room set to 4°C (Phillips 1998) in individual containers containing dechlorinated tap water and leaves of *Acer* and *Quercus* spp. We used animals from five different populations to meet the desired number of crosses (86 males and females): each male was crossed with two different females, and each female was crossed with two different males, with two replicates of approximately 10 offspring per family. This resulted in full siblings as well as paternal and maternal half-siblings in a pedigree with a total of 1,038 individuals (858 offspring of 93 full-sib families, with 59 dams and 56 sires).

Paternity was assured through in vitro fertilizations. Eggs were harvested from gravid females by gently pressing the female's abdomen in a motion to push the eggs out through the cloaca. In 2015, release of spermatozoid by males was stimulated by allowing males to amplex females not used in this study for 2 h. Males were then placed in individual clean closed containers and left to rest undisturbed for 30 min to 1 h. We captured males in a fast movement immediately on reopening the containers, startling the animals and triggering the release of urine containing spermatic fluid. The fluid was diluted in distilled water and immediately placed over the eggs for fertilization. Because this procedure resulted in low fertilization rates (55.2% of the replicates did not result in any hatchlings), in 2016 we stimulated production of spermatic fluid by injecting males with 10 IU per gram of body mass of human chorionic gonadotropin hormone (Chorulon; Waggener and Carroll 1998) 1 h before the harvesting.

Fertilized eggs were left unmolested for approximately 10 min in open petri dishes and then covered with distilled water and placed in an incubator at 10°C. At approximately Gosner stage 20, eggs were moved into 5.7-L plastic shoeboxes containing enough water so that eggs were always completely submerged and into a temperature-controlled room set to 14°C. Eggs were checked daily and water was refilled as necessary.

Tadpole Housing and Animal Care. Approximately 2 weeks after hatching, tadpoles were moved into the greenhouse, which provided a natural sunlight/photoperiod for the animals while allowing us to control temperature and humidity. Mean temperatures were 19.7°C in 2015 (measured as room temperature) and 20.5°C in 2016 (measured as water temperature).

Wood frog tadpoles preferentially associate with siblings (Waldman 1984); therefore, we kept tadpoles in family groups of up to 10 individuals for 2 weeks. Family groups were then split into groups of up to five individuals per container. Individuals were moved into isolated containers as they reached a body size of at least 1.5 cm or hind limb buds were visible to the naked eye (Gosner stage 30 or higher). We changed water on regular weekly intervals for large family containers and biweekly for individual containers. Food was a 2 : 1 mixture of rabbit chow and fish flakes (Tetra TetraMin Tropical Flakes; Relyea 2002), with occasional supplements of bloodworms and brine shrimp. Individuals were checked daily when additional water and food were added. Containers were rotated in the room weekly to biweekly.

As soon as forelimbs emerged, individuals were moved to a 5.7-L box with approximately 350 mL of water. Boxes were kept on a slight incline, allowing individuals to climb out to a dry area. Dry areas were covered with a damp paper towel and leaves, simulating a terrestrial environment while maintaining high levels of humidity. Once animals reached stages 43–44, water was drained from the box. Water was sprayed over the paper towel at least once a day, and paper towels were changed as necessary. Animals were given 10–20 fruit flies (*Drosophila melanogaster* or *Drosophila hydei*) daily after reaching Gosner stage 43.

Data Collection for Lab Animals. We measured snout-vent length of frogs and tadpoles (midbody length from the tip of the snout to the base of the tail). In addition to body size, we

measured traits related to body shape (e.g., head width and head length) and locomotion. Head width in frogs represented maximum head width, while for tadpoles it was measured as distance between the eyes. Head length was measured as the distance from the top of the eye to the tip of the snout for both tadpoles and juvenile frogs.

Locomotion-related traits change between aquatic and terrestrial stages. Locomotion-related traits of tadpoles were tail length (from the base to the tip of the tail) and maximum tail depth (representing the maximum height of the tail fin), as previous studies indicate relevance of both traits in predation contexts for wood frogs. For example, individuals reared in the presence of Aeshnidae predators have been shown to develop longer and deeper tails (Relyea 2002), while individuals reared in the presence of Dytiscidae predators have been shown to develop deeper tails (Michel 2012). Such plasticity is taken as adaptive since failed predation attempts can often result in clip off of tail tips (Relyea 2002), and larger fin area should lure predators away from vital body parts (Van Buskirk et al. 2003). Moreover, other studies provide direct evidence of viability selection for tail length and tail fin depth, although there is conflicting evidence on whether selection is positive or negative on these traits. For example, Calsbeek and Kuchta (2011) report positive selection for tail fin depth, Michel (2012) reports negative selection for the same trait, while Van Buskirk et al. (2003) show evidence for stabilizing selection. If tadpoles presented damaged tails, such that tail length or tail depth measurements were affected, we coded these variables as missing data.

Locomotion-related traits of frogs were leg length (taken as tibiofibula length) and foot length (taken as the maximum hindlimb forefoot length measured from the tarsus-metatarsus joint to the end of the distal phalange of the longest [fourth] toe). We did not measure foot length as distance from ankle to the end of the longest toe because frogs have a flexible tarsometatarsal joint, subdividing the foot into two units. The degree of freedom added by such flexible joints has been shown to be important in models of jumping kinematics (Kargo et al. 2002). Specifically, the consideration of these units as two degrees of freedom instead of one, even considering that the tarsometatarsal is a passive joint (i.e., not providing torque for jumping), improves prediction ability of jump distance models. This is so because modeling the tarsometatarsal extension in the final milliseconds of the jump results in an increase in the takeoff height and velocity of the jump as well as an increased horizontal distance covered by the center of mass of the frog during the metatarsophalangeal ground-contact phase of the jump (Kargo et al. 2002). Moreover, the added length of metatarsus and phalanges has been shown to be larger in strong jumpers (like *Rana* sp.) in comparison to weak jumpers (Zug 1972), indicating that this unit is important for locomotor performance and therefore could be under selection.

Tadpole traits were measured between Gosner stages 30–38 (mean = 35.1, median = 35), whereas frog traits were measured between stages 43–46 (mean = 44.6, median = 45). To avoid the use of anesthetics and excessive handling of tadpoles and frogs, individuals were photographed next to a ruler, and images were measured using NIH ImageJ (Schneider et al. 2012). Head width and snout size of tadpoles and juvenile frogs were measured from top view pictures, while body size and tail measurements of tadpoles were taken from side view pictures. Snout-vent length and frog exclusive morphological variables were measured using calipers, since the posture adopted by the frog can bias such measures if they are taken from the pictures (D. Goedert and R. Calsbeek, personal observation).

For all measures taken from pictures, we measured repeatability using the package irr (Gamer et al. 2012) in R (R Development Core Team 2017). Repeatability measures were above 0.76 and significant at the level of 0.1% (i.e., $P < .001$ for all traits; $n > 30$ for all traits). For all morphological traits, deviations from normality were assessed using quantile-quantile plots.

Animal Models. We fit multiresponse animal models using the MCMCglmm package (ver. 2.26; Hadfield 2010) in R (ver. 3.5.1; R Development Core Team 2017) All models were run for at least 4,000,000 iterations. Burn-in was 1% of total iterations, and sampling interval was 900–2,000. Run quality and convergence were assessed by visually inspecting the trace and density plots of the posterior distribution, ensuring that autocorrelation did not exceed 0.1 (Hadfield 2018), and using a Heidelberger and Welch's convergence diagnostic as implemented in the package coda (Plummer et al. 2006). Additionally, selected models were run a second time with different starting values for comparison of results.

All 10 variables were included as response variables. We included body size as a response variable, since body size could mediate genetic correlations measured between traits (Wilson 2008). We then ran the same models excluding body size as a response variable. For models with and without body size, we compared model fit with different random effects structures using a deviance information criterion (DIC; Spiegelhalter et al. 2002). The simplest model contained no additional random effects, and more complex models contained either dam or full-sib replicate. We considered a difference between DIC >10 to indicate strong support for the addition of the random effect. We used multivariate extension of the inverse gamma for the priors of the additive variance and for the priors of additional random effects. We also ran the models using a parameter extended prior for dam or replicate and using a proper prior with low variance and high nu for the additive variance in hopes that this would represent a flat prior for the genetic correlations (Hadfield 2018).

We estimated narrow sense heritability as the additive variance for each trait divided by the total phenotypic variance (Falconer and Mackay 1996). Because heritability values cannot be compared across taxa or studies (Houle 1992), we additionally present an index of evolvability (tables A1–A10, available online), calculated as the additive variance divided by the squared mean of each trait.

Genetic Correlations and Modularity Analyses. We used the mode of the posterior distribution of covariances for all of the analyses. We calculated the genetic correlation for all possible pairwise trait combinations as the additive covariance divided by the square root of the product of the additive variances. We used a *t*-test to compare the mean genetic correlation within life stages with the mean genetic correlation between life stages. The distribution of pairwise genetic correlations within and between life stages reasonably approximated normality, as estimated from quantile plot inspections.

In addition, we compared morphological integration within and across life stages using metrics of modularity: the RV Escoufier's coefficient (Klingenberg 2009) and the covariance ratio (CR; Adams 2016). Both of these metrics describe the degree of covariation between two sets of variables (i.e., between traits of two putative modules) with respect to the covariation within these sets of variables (i.e., between traits within each putative module), analogous to a multivariate squared correlation coefficient. The CR differs from RV by replacing the trait variances (which are sensitive to sample size) with 0, keeping only the pairwise covariances (Adams 2016). Therefore, if the two life stages function as modules, the covariance of pairwise traits between life stages should be low relative to the covariances of pairwise traits within life stages, and both RV and CR should be <1. An RV or CR of 1 would indicate that covariances between life stages are proportional to covariances within life stages, while a CR larger than 1 would indicate higher covariances for traits between life stages than within.

We calculated these indices for each saved iteration of the animal models, which provided us with a mean, mode, and credible interval. We then permuted traits within the **G** matrix to obtain all possible unique combinations of traits subdivided into two equal-sized modules (34 alternative combinations for the models not including body size and 125 for the models including body size). We recalculated RV and CR for these alternative modules and obtained the percentage of modules with equal or lower indexes values, analogous to a test statistic *P* value, by dividing the number of combinations resulting in equal or lower indexes than the candidate tadpole and frog module by the total number of combinations.

Results and Discussion

Selection Estimates: Tadpoles and Juveniles

Viability selection measured at each life stage revealed a mix of antagonistic and congruent selection on traits (fig. 1; tables A1, A2). Selection acted in opposite directions (i.e., antagonistic selection) between tail length of tadpoles ($\beta' = 0.212$; 95% confidence interval [CI]: 0.063–0.40) and head length of juvenile frogs ($\beta' = -1.287$; 95% CI: −2.18 to −0.627). By contrast, we detected positive directional selection (i.e., congruent selection) on tail length of tadpoles and foot length of juvenile frogs ($\beta' = 1.967$; 95% CI: 0.734–3.674). Consequently, selection was also antagonistic between head length and foot length of juveniles. No other tadpole trait had selection gradients that could be differentiated from 0 (table A1). For juvenile traits, probability of survival ($\alpha \pm$ SE) differed between replicates ($\alpha = -4.069 \pm 1.71$;

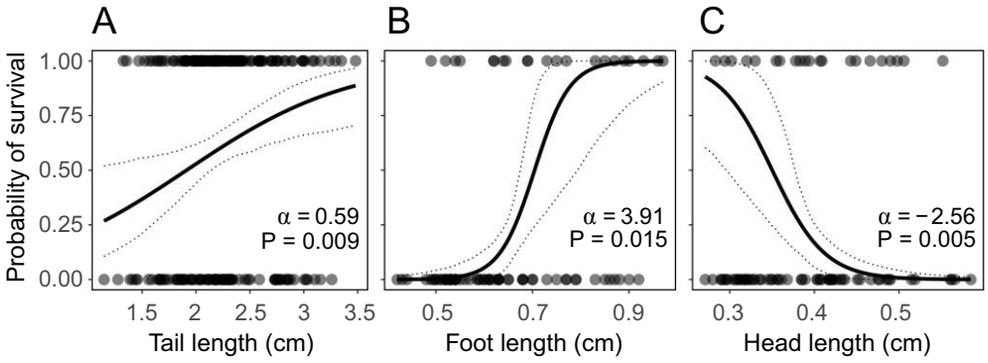

Figure 1: Probability of survival increases for tadpoles with longer tails (*A*) and for frogs with longer feet (*B*) but decreases for frogs with longer heads (*C*), indicating congruent selection between tadpole tail length and frog foot length but antagonistic selection between each of these two traits and frog head length. Alpha (α) values represent the coefficient estimates from the logistic regressions with their corresponding *P* values.

$P = .017$; table A2). The inclusion of replicate as a fixed effect rendered the probability of survival for head width statistically significant ($\alpha = 3.756 \pm 1.588$; $P = .018$; $\beta' = 1.686$; 95% CI: 0.34–3.446), suggesting that juveniles could at least occasionally experience additional antagonistic selection between head length and head width.

Genetic Correlations

For both models including or excluding body size as a response variable, model selection supported the choice of the simplest model for inferences (i.e., without the addition of dam or replicate as random effect terms; table A3). These results were robust to the use of different priors for the random effects (tables A3, A4). Moreover, adding maternal identity (representing the confounded effects of genetic and nongenetic maternal variance) as a random term in the model did not alter estimates of additive variance, which suggests that any such maternal effects would be small in magnitude relative to the genetic effects reported. However, this does not exclude the possibility that genetic and nongenetic maternal effects (or, for that matter, nongenetic paternal effects) are important, as it is possible that the number of families we measured was insufficient for estimating such effects. Therefore, heritability values should be interpreted with the caveat that nongenetic effects were not factored out of these estimates.

The posterior modes for heritability and evolvability were robust between models including or excluding body size as a variable in the animal models (tables A5, A6). The mode of the pairwise genetic correlations were also robust for the majority of trait combinations (table A7), with the exception of the correlation between tadpole tail length and frog foot length, which decreased from 0.297 in the model including body size to 0.093 in the model without body size. Yet both of these estimates had credible intervals including 0. Additionally, because some of the genetic correlations of low magnitude had 2.5% credible intervals that bordered on 0, these credible intervals were positive for some models and negative for others. Therefore, the interpretations regarding significance level of genetic correlations change depending on the model of choice. This is possibly due to the fact that our sample size limited the power to detect significance of weak genetic correlations. Consequently, we encourage the interpretation of our results on the basis of the differences in magnitude of the genetic correlations instead of absolute significance thresholds.

Heritability values for tadpole and juvenile traits ranged from 0.346 to 0.685 (mode of the posterior distribution) and had 2.5% credible intervals larger than 0 (table A5). These values are similar to some heritability values previously reported for wood frog morphological traits (Phillips 1998). Evolvability values ranged from 0.64 to 2.06 and also had 2.5% credible intervals larger than 0 (table A6). These results indicate that all traits evaluated present additive genetic variance that can respond to selection.

Genetic correlations measured within tadpole and within frog stages were stronger in magnitude than those measured between life stages (mean pairwise genetic correlation within tadpoles: 0.533; range: 0.391–0.769; within frogs: 0.542; range: 0.342–0.735; between stages: 0.201; range: 0.053–0.314; Welch two-sample t-test, $t_{32.6} = 11.22$, $P < .001$; fig. 2*A*; table A7). While all of the genetic correlations differed from 0 within life stages, the majority of genetic correlations between life stages exhibited credible intervals that overlapped 0 (fig. 2*B*–2*D*; table A7). Even if nonzero, however, genetic correlations within life stages were always significantly <1. These results suggest that none of the traits between or within life stages are rigidly constrained from evolving toward their optima. Yet relative to within–life stage genetic correlations, metamorphosis could still work as an adaptive decoupling mechanism if it significantly decreases the magnitude of genetic correlations between tadpole and juvenile frog traits in respect to genetic correlations within life stage.

In modularity analyses, both RV (mode: 0.02; mean: 0.109; 95% CI: 0.012–0.327) and CR values (mode: 0.425; mean: 0.530; 95% CI: 0.189–0.994) were significantly <1, indicating that genetic covariances between life stages are relatively lower than within life stages and therefore suggesting modularity across the metamorphosis boundary. Our initial candidate module separating tadpole and frog traits was within the lowest 5% of all possible modularity index values (0.8%; lowest of 126 combinations for both RV and CR), when accounting for all possible alternative combinations of traits into two modules of equal size. Removing body size did not affect RV values or the magnitude of CR. However, the range of the CR credible interval increased, causing it to overlap 1 (mode: 0.372; mean: 0.592; 95% CI: 0.224–1.128), even though permutations still indicated this to be within the lowest 5% of possible CR values (2.9%; lowest of 35 combinations). Thus, our results are consistent with the hypothesis that amphibian metamorphosis serves to decouple life-history stages. Moreover, although genetic covariances with body size account for important integration within life stages, this integration is also decoupled across life stages.

These results suggest that even though traits have similar degrees of integration within tadpole and frog life stages, metamorphosis results in significant reduction of genetic correlations across life stages. Therefore, wood frogs face stronger evolutionary constraints on morphological trait adaptation within each stage than across stages. Yet even if metamorphosis alleviates genomic conflict, it does not completely resolve it. Specifically, we found that selection was antagonistic between tail length of tadpoles and head length of juvenile

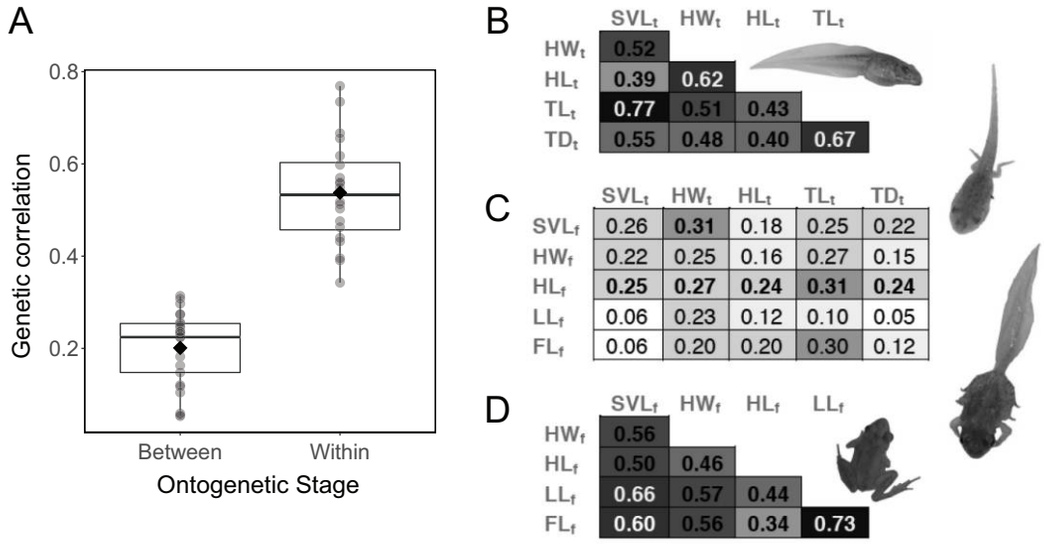

Figure 2: Mean values of genetic correlations were higher for pairs of traits within tadpole and frog life stages than were genetic correlations measured between ontogenetic stages (*A*). Boxplots show genetic correlation quartiles, while means are indicated by diamonds. **G** matrices show genetic correlations between pairs of tadpole traits (*B*), between tadpole and frog traits (*C*), and between pairs of frog traits (*D*). Shading corresponds to the magnitude of genetic correlations ranging from lower (lighter gray) to higher (darker gray). For full quantitative details, see table A7 (available online). SVL = snout-vent length; HW = head width; HL = head length; TL = tail length; TD = maximum tail depth; LL = leg length; FL = foot length; t = tadpole; f = frog.

frogs (fig. 1*A*, 1*C*), but these traits are not fully decoupled in their genetic correlation (fig. 2*B*–2*D*). Consequently, even if traits across the metamorphosis boundary exhibit reduced ontogenetic conflict in comparison to traits within the juvenile life stage, some degree of ontogenetic conflict remains.

We detected congruent positive directional selection on tail length of tadpoles and foot length of juvenile frogs (fig. 1*A*, 1*B*). These traits are positively genetically correlated with body size within their respective life stages (fig. 2*B*, 2*D*). For this reason, and because the foot develops during the tadpole stage, one might expect that these traits should share a positive genetic correlation. Yet tadpole and juvenile body size are decoupled by metamorphosis, and, consequently, so are tadpole tail length and juvenile foot length (fig. 2*C*). Decoupling traits that are subject to congruent selection could be an adaptive response to indirect antagonistic selection on correlated traits (e.g., frog head length). Interestingly, the magnitude of the genetic correlation between tadpole tail length and juvenile foot length is relatively high among the between–life stage genetic correlations (fig. 2*B*); in fact, it is higher than the magnitude of some genetic correlations with credible intervals not crossing 0, suggesting that a lack of power is unlikely the reason (table A7). Moreover, the removal of body size caused a threefold decrease in the genetic correlation between tadpole tail length and juvenile foot length. Therefore, it is possible that variation in body size caused by parental nongenetic effects—unaccounted for in this study—could influence the covariance between these two morphological traits.

Selection Estimates: Adults

In contrast to the positive selection acting on juvenile foot size, adult female frogs experienced negative selection for this same trait ($\beta' = -0.57$; 95% CI: -1.142 to -0.07; table A8). Adult female frogs also experienced positive selection for body size ($\beta' = 0.891$; 95% CI: 0.184–1.666), representing antagonistic selection with foot size. Selection for females did not differ between replicates (estimate $\pm$ SE $= 0.216 \pm 0.766$, $P > .7$; table A8), and body size (estimate $\pm$ SE $= 2.107 \pm 0.909$, $P = .020$) and foot size (estimate $\pm$ SE $= -1.382 \pm 0.696$, $P = .047$) were the only significant terms in the model including this fixed effect. Males, however, experienced selection on neither foot length ($\beta' = 0.067$; 95% CI: -0.35 to 0.459) nor body size ($\beta' = 0.128$; 95% CI: -0.361 to 0.586; table A9), and results were robust to the inclusion of replicate as a fixed effect in the model. The difference in selection between the sexes was statistically significant after pooling data sets (interaction of snout-vent length × sex: $P = .002$; interaction of foot length × sex: $P = .005$; table A10), irrespective of the inclusion of replicate as a fixed effect.

Thus, antagonistic selection appears to be pervasive across the developmental stages of wood frogs, including both ontogenetic and sexual components. Unfortunately, because wood frogs can take up to 2 years to reach sexual maturity (Berven 1982), we were unable to obtain genetic correlations between adults and the other life stages. Assuming that genetic correlations are perfect between juvenile and adult wood frogs, these results would indicate that although metamorphosis alleviates some of the ontogenetic conflict between tadpole and juvenile frogs, additional conflict remains.

Conclusions

Genomic conflict arises either when positively correlated traits experience antagonistic selection or when negatively correlated traits experience congruent selection. Using a quantitative genetics framework for studying genomic conflict, we have shown that metamorphosis works as an adaptive decoupling mechanism that allows life stages to more readily adapt to alternative phenotypic optima by partitioning ontogenetic stages into separate modules.

By extending our investigation of selection into the adult stage, we have further shown that a full understanding of genomic conflict is a highly complex task that will require comprehensive studies across multiple developmental stages. The differences in directional selection found between adult males and females also highlight the potential of a generalized quantitative genetics framework to unify the investigation between genomic conflict across life stages and between sexes. This connection sets the ground for a more complete understanding of how the genetic architecture of traits and the shape and form of selection can affect adaptation. The benefits of such a holistic approach could be expanded to, for example, the investigation of species with alternative reproductive tactics. For instance, intralocus tactical conflict could mediate dynamics of sexual conflict or vice versa, since the genetic correlations between sexes would likely differ depending on the reproductive tactic expressed (e.g., Abbott and Svensson 2010).

Additional investigations of ontogenetic conflict in other species as well as a comprehensive study of ontogenetic conflict in species with direct development will further enrich our understanding of the prevalence of ontogenetic conflict and the mechanisms by which it can be resolved. Just as sexual conflict is known to have important consequences for the evolutionary dynamics of species, other forms of genomic conflict (such as tactical or ontogenetic conflict) may have similar consequences for standing genetic variation or species-level diversification. We encourage the investigation of these potentially significant evolutionary consequences for the full range of contexts in which genomic conflicts may arise.

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Wood frog juvenile in the final stage of metamorphosis. Photo credit: Debora Goedert.