Environmental Stress Increases the Magnitude of Nonadditive Genetic Variation in Offspring Fitness in the Frog *Crinia georgiana*

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abstract: When organisms encounter heterogeneous environments, selection may favor the ability of individuals to tailor their phenotypes to suit the prevailing conditions. Understanding the genetic basis of plastic responses is therefore vital for predicting whether susceptible populations can adapt and persist under new selection pressures. Here, we investigated whether there is potential for adaptive plasticity in development time in the quacking frog *Crinia georgiana*, a species experiencing a drying climate. Using a North Carolina II breeding design, we exposed 90 family groups to two water depth treatments (baseline and low water) late in larval development. We then estimated the contribution of additive and nonadditive sources of genetic variation to early offspring fitness under both environments. Our results revealed a marked decline in larval fitness under the stressful (low water) rearing environment but also that additive genetic variation was negligible for all traits. However, in most cases, we found significant sire-by-dam interactions, indicating the importance of nonadditive genetic variation for offspring fitness. Moreover, sire-by-dam interactions were modified by the treatment, indicating that patterns of nonadditive genetic variance depend on environmental context. For all traits, we found higher levels of nonadditive genetic variation (relative to total phenotypic variation) when larvae were reared under stressful conditions, suggesting that the fitness costs associated with incompatible parental crosses (e.g., homozygous deleterious recessive alleles) will only be expressed when water availability is low. Taken together, our results highlight the need to consider patterns of nonadditive genetic variation under contrasting selective regimes when considering the resilience of species to environmental change.

Keywords: genetic compatibility, genotype-by-environment interaction, reaction norm, metamorphic duration, climate change.

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Introduction

Almost all organisms face the possibility of unstable environments, which in recent times is increasing due to human activities (e.g., climate change, invasive species, habitat fragmentation). Whether populations can persist under rapidly changing conditions depends on their ability to employ one or a combination of up to three response mechanisms: evasion, phenotypic plasticity, and genetic adaptation (Holt 1990; Davis et al. 2005). In principle, evasion can allow populations to move to favorable locations (Parmesan 2006; Thomas 2010), but successful range shifts require new habitats to be accessible, which is increasingly impeded by habitat fragmentation (Fahrig 2003; Pecl et al. 2017). Moreover, the rapid rate of environmental change experienced by many populations may require organisms to travel distances that exceed their capabilities (Schloss et al. 2012; Hetem et al. 2014). Therefore, as evasion will not always be an option for persistence as environments change, phenotypic plasticity and genetic adaptation will play key roles in determining the survival of species that do not shift their distributions (Moritz and Agudo 2013).

A considerable body of research has focused on the ability of populations to respond to environmental changes via phenotypic plasticity—the ability of a given genotype to adjust its phenotype according to its environment. Phenotypic plasticity has been widely documented in natural populations (West-Eberhard 2003; Hollander et al. 2015) and is particularly prevalent in organisms such as amphibians that inhabit highly heterogeneous environments (Urban et al. 2014). Meta-analysis has revealed that 71% of amphibian traits show plasticity in response to climatic variation (Urban et al. 2014). However, phenotypic plasticity is not necessarily adaptive (Visser et al. 2006; Ghalambor et al. 2007, 2015; Urban et al. 2014). Furthermore, not all traits are plastic, and there are often inherent costs that limit plasticity (DeWitt et al. 1998; Relyea 2002). Thus, while plastic responses are important

for buffering the effects of changes in environmental conditions in the short term, most organisms require a microevolutionary response to persist under continued directional change in their environments (Gienapp et al. 2008).

Plastic responses can themselves evolve and contribute to environmental adaptation (Hoffmann and Sgrò 2011). If there is genetic variation in phenotypic plasticity (i.e., genotype-by-environment interaction [GEI]), and if plastic responses increase fitness, selection can target the degree of plasticity (Crispo et al. 2010; Tedeschi et al. 2015). However, while plastic responses to environmental change are documented for many groups, the genetic basis of such responses is poorly understood. This is largely due to the difficulty in reliably distinguishing phenotypic and genetic responses to changed environments (Chown et al. 2010; Merilä and Hendry 2014), which is analogous to the difficulty in partitioning the causal components of variance when the trait itself is a variance rather than a mean. Teplitsky et al. (2008), for example, showed that phenotypic shifts in mean body size observed in birds, originally attributed to genetic adaptation, were in fact a consequence of phenotypic plasticity.

Quantitative genetics experiments offer an empirical framework for investigating the potential for genetic responses to environmental change (Lynch and Walsh 1998). Early quantitative genetics studies revealed that patterns of additive genetic variance underlying trait expression can vary with environmental conditions due to genotype-by-environment interactions (Hoffmann and Parsons 1991; Hoffmann and Merilä 1999), indicating that heritabilities may not be constant as abiotic variables change (Visser 2008), with a trend toward heritabilities being lower in unfavorable environments (Charmantier and Garant 2005). Whether the lower heritabilities are due to less additive genetic variance, relatively greater nonadditive variance, and/or greater environmental variance remains unclear.

Amphibians are ideal models for investigating patterns of genetic variation under unstable environments, as their generally large clutch sizes, external fertilization, and—for the most part—lack of parental care means they are especially suited to quantitative genetic analyses (Laurila et al. 2002; Merilä et al. 2004; Laugen et al. 2005; Eads et al. 2012). Moreover, amphibians have experienced substantial species losses and population declines over recent decades (Alroy 2015; Catenazzi 2015). While disease has been identified as the primary driver for many such declines (Skerratt et al. 2007), environmental stresses associated with a drying climate are exerting additional selection pressures on susceptible populations and have likely been a second major driver of the extinction process (Kiesecker et al. 2001; Wake 2012). However, the severity of this threat to amphibians remains unclear, largely due to disagreements among researchers about the resilience of threatened populations. We therefore require far better understanding of the potential for microevolutionary responses if we are to predict the resilience of amphibians to environmental uncertainty (Urban et al. 2014).

A central reason for the vulnerability of many amphibians is their strong dependence on freshwater for reproduction (Carey and Alexander 2003; Walls et al. 2013), a resource that has declined in quality and availability in many regions (Milly et al. 2005). Most amphibians inhabit highly variable environments and have evolved mechanisms for dealing with low water depth, such as by accelerating development to metamorphosis (Newman 1992; Gomez-Mestre et al. 2013). Such plasticity potentially allows metamorphs to escape drying pools. However, rapid development may come at a cost of smaller body size at metamorphosis (Doughty and Roberts 2003; Mueller et al. 2012), which in turn is likely to impede survival in the terrestrial environment (Semlitsch et al. 1988; Berven 1990; Cabrera-Guzmán et al. 2013).

In this study, we apply an experimental quantitative genetic framework to determine whether there is the potential for adaptive plasticity in developmental rate in the quacking frog *Crinia georgiana*, a polyandrous species that occurs in southwestern Australia (Roberts et al. 1999). *Crinia georgiana* is a highly suitable model for investigating the impact of a drying climate on early development, as the larvae show accelerated rates of maturation relative to related amphibian species with similarly sized eggs (Mueller et al. 2012). Low water depth is a frequent challenge for *C. georgiana* larvae as eggs are deposited in shallow (~1–2 cm deep) temporary pools that frequently dry out between bouts of rain (Byrne and Roberts 2000; Doughty 2002; Doughty and Roberts 2003). Furthermore, *C. georgiana* inhabits a region that has experienced a substantial decline in winter rainfall over the past 40 years (19% reduction since the 1970s; Smith 2004; IOCI 2012; Andrich and Imberger 2013; CSIRO and BoM 2016), and this region is expected to become warmer and drier in the coming decades (Gallant et al. 2007; Bates et al. 2008; Smith and Power 2014; CSIRO and BoM 2015). It is therefore likely that populations will face strong selection pressure on larval traits that provide resilience to drying.

We used a cross-classified (North Carolina II) breeding design to determine whether *C. georgiana* exhibits underlying genetic variation in its response to changes in water depth, focusing on a range of putative fitness traits, including embryonic and juvenile survival, time to metamorphosis, body size, morphology, and jumping performance. Importantly, this design enabled us to determine whether there is a genetic basis to plasticity in the expression of these traits (i.e., GEI)—and thus the potential for selection to target such plastic responses. Furthermore, as our breeding design involved a series of factorial crosses between parental genotypes, we were able to determine whether variation in water depth modifies patterns of nonadditive genetic variance in offspring fitness, for example, attributable to variation in parental compatibility, which constitutes an important source of variation in em-

bryonic survival in this species (Dziminski et al. 2008). Taken together, our analyses were designed to offer insights into the fitness implications associated with changes in breeding environments and the capacity of amphibian populations to respond to such changes.

Material and Methods

Ethics Statement

All animal work was conducted in accordance with the University of Western Australia's (UWA) Animal Ethics Committee (permit no. RA/3/100/1395). Fieldwork was conducted under permit SF010360 issued by the Western Australian Department of Biodiversity, Conservation and Attractions.

Study Species

Crinia georgiana is a small (19–47 mm snout-to-vent length) species of myobatrachid frog widely distributed throughout the southwest of Western Australia. Breeding occurs between late autumn and the middle of spring (Main 1965), when males aggregate in shallow, temporary water and call to attract females. Females entering the chorus are amplexed by 1–9 males (Buzatto et al. 2015) and release eggs, which are fertilized externally. Multiple mating by females (polyandry) is common in this species, with approximately 50% of all matings involving more than one male (Roberts et al. 1999), and results in multiple paternity of egg clutches (Roberts et al. 1999; Buzatto et al. 2017). The environment in which embryos and larvae develop is generally unstable, as eggs are deposited within shallow (1–2 cm) temporary pools or seepages that can dry and flood several times within the breeding season (Seymour et al. 2000; Doughty and Roberts 2003). Consequently, both the embryos and free-swimming larvae are at high risk of desiccation.

Animal Collection

Adult *C. georgiana* were collected by hand from a large population near Kangaroo Gully, approximately 40 km southeast of Perth, Western Australia (lat. 32°06′35″S, long. 116°08′54″E). In total, 30 gravid females and 30 adult males were collected from within a breeding chorus over 5 nights between August 9 and 22, 2015. Frogs were transported to the University of Western Australia in Perth on the night of collection.

Breeding Design

We performed controlled laboratory crosses according to a North Carolina II (NCII) block breeding design (Lynch and Walsh 1998). In each block, eggs from three females were crossed with the sperm from three males (fig. 1). We established 10 such blocks, thus yielding 90 families. Each family included at least 20 eggs, resulting in a total sample size of 2,067 eggs across the 10 blocks. The NCII design generates full siblings, paternal half-siblings, and maternal half-siblings, making it possible to partition sources of phenotypic variation into additive genetic (i.e., sire) effects, maternal (genetic and environmental) effects, and nonadditive effects (Comstock and Robinson 1948; Lynch and Walsh 1998).

In Vitro Fertilizations

All procedures outlined below were performed on the same night that animals were collected. Male frogs were euthanized via ventral immersion in <0.03% benzocaine solution, followed by double pithing. Their testes were then removed, weighed, and crushed within an Eppendorf tube in 0.3–1.1 mL (adjusted according to the weight of the testes) of chilled standard amphibian ringer (SAR; 113 mM NaCl, 2 mM KCl, 1.35 mM $CaCl_2$, and 1.2 mM $NaHCO_3$). Testes macerates were immediately placed on ice and sperm concentrations were measured using an improved Neubauer hemocytometer (Hirschmann Laborgeräte, Eberstadt, Germany).

Eggs were gently squeezed from each female onto a clean surface. They were then moistened with SAR and divided equally among three plastic weigh pans and placed on ice until fertilization. Following Dziminski et al. (2008), a calculated volume of sperm suspension was pipetted onto one edge of the pan, followed by a volume of stream water (collected from the breeding site) at 16°C. When mixed, the two solutions produced a sperm concentration of 0.2×10^6

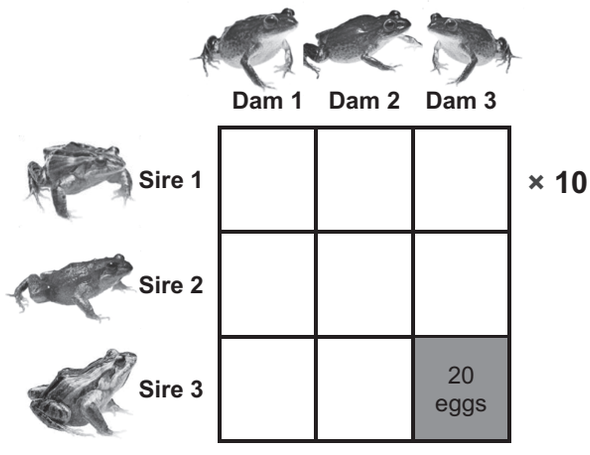

Figure 1: Experimental North Carolina II block-breeding design (Lynch and Walsh 1998). In each block, the eggs of three females were fertilized with sperm from three males in all nine combinations. Ten such blocks were created, thus yielding 90 *Crinia georgiana* families.

sperm/mL, which leads to asymptotic rates of fertilization (Dziminski et al. 2008, 2009*a*). Each pan was manually agitated for 20 s to mix the diluted sperm suspension among the eggs to promote fertilization. After 15 min, pans were backlit and submerged eggs were photographed with a stage micrometer for calibration. These images were used to measure the diameter (later converted to volume) of 50 eggs from each female, using ImageJ software (Abràmoff et al. 2004). All remaining eggs from each female were frozen at −20°C for analysis of yolk corticosteroids. Both ovum volume and yolk corticosterone levels can have significant effects on offspring fitness in amphibians (Dziminski and Roberts 2006; Love and Williams 2008), and thus these factors were included as covariates in all of our analyses (see below).

Eggs were transferred to round plastic containers (base diameter = 9 cm, height = 6.5 cm; 5 eggs per container) and covered with stream water (collected from the breeding site) to a depth of 2 cm. Containers with eggs were maintained in a temperature-controlled room at 16°C with a 11L : 13D photoperiod to match ambient (winter) conditions. Fluorescent lights (Grolux, Sylvania, Padstow, Australia) provided UV light for 3 h each day. Two hours after combining eggs and sperm, fertilization success was scored in each container by visualizing the eggs under a microscope at ×32 magnification. Fertilized embryos were at the 2- or 4-cell stage (Gosner stage 3 or 4) at this point in time.

Tadpole Rearing and Measurements

Containers were checked for hatchlings every 12 h, and time to hatching was recorded to the nearest minute. Once all five tadpoles in a dish had hatched, the water was replaced with fresh stream water to a depth of 2 cm. Embryonic survival was recorded for each dish as the proportion of fertilized eggs that hatched. A macro picture of each tadpole (dorsal view) was taken (Canon PowerShot G16, along with a microruler for calibration) for later analysis of tadpole morphology (fig. 2*A*). Five morphological variables were measured to the nearest 0.01 mm using ImageJ (ver. 1.50b) software: total length, tail length, body length, body width, and tail muscle width. Each of these traits affects swimming performance (Van Buskirk and McCollum 2000; e.g., Teplitsky et al. 2005; Wilson et al. 2005; Johnson et al. 2015), which is important for predator escape (Watkins 1996).

Feeding was initiated once tadpoles developed mouthparts (Gosner stage 21, ~3 days after hatching). Tadpoles were fed a ground and sieved 3 : 1 mixture of rabbit pellets (Lucerne) and TetraMin tropical fish food (TetraWerke, Melle, Germany), with 25 mg of this mixture added to each container every 3 days, resulting in approximately 5 mg of food per tadpole. This feeding regime ensured a size at metamorphosis consistent with sizes that occur in the wild (Doughty and Roberts 2003; Dziminski and Roberts 2006). Containers were frequently cleaned with a sponge to remove debris, and stream water was changed daily. Once tadpoles reached Gosner stage 34 (hind limb buds developing with early differentiation of toes, ~28 days after fertilization), manipulation of water depth began. The decision to initiate treatment at this stage was based on Doughty and Roberts (2003), who reduced water depth at a range of larval stages and found the strongest response when the treatment was ini-

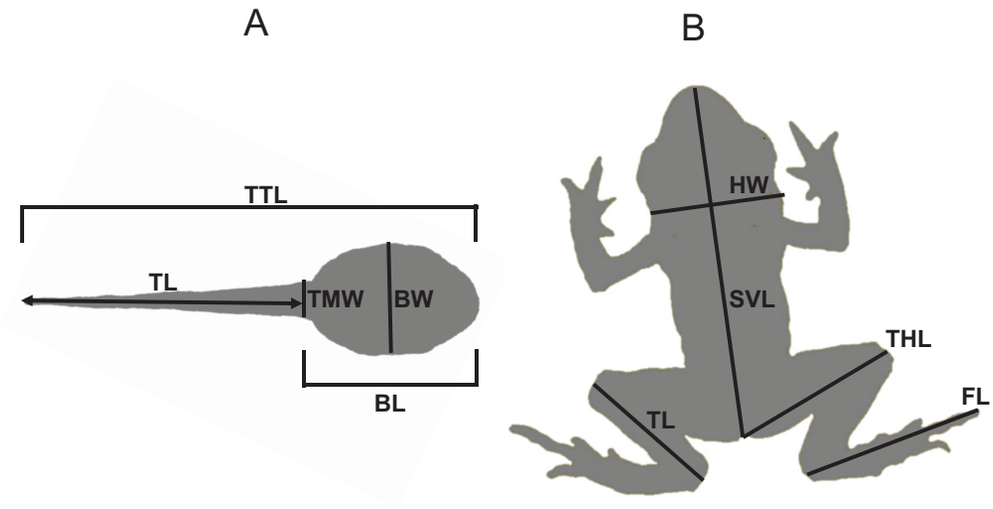

Figure 2: Morphological measurements taken for *Crinia georgiana* hatchlings (*A*) and metamorphs (*B*). Tadpole labels are as follows: TTL = total tadpole length, TL = tail length, BL = body length, BW = body width, TMW = tail muscle width. Metamorph labels are as follows: SVL = snout-vent length, HW = head width, THL = thigh length, TL = tibia length, FL = foot length.

tiated at stage 34. Tadpoles were subjected to one of two water depth treatments: a baseline treatment, where tadpoles continued to experience a constant water depth of 2 cm, or a low water depth treatment, where the water depth was reduced to 0.5 cm. This water depth was chosen because it was low enough to simulate pool drying while still allowing tadpoles to swim and feed. Thus, any effects of treatment were likely attributable to water depth and not food intake.

The developmental stage of the tadpoles in each container was determined every 12 h using a binocular microscope. Tadpole survival was recorded as the proportion of fertilized eggs that survived to Gosner stage 42 (emergence of at least one forelimb), which marks the end of the larval period and the beginning of metamorphosis, and the stage at which individuals switch from gill breathing to lung breathing (Anstis 2013). The length of the larval period was calculated as the time between hatching (approximately Gosner stage 28, achieved at ~14 days after fertilization) and Gosner stage 42 (achieved ~49 days after fertilization). Individuals at stage 42 were placed in new containers with perforated lids, and containers were placed onto a sloping shelf to provide wet and dry areas that allowed metamorphs to leave the water. Food was not provided from this point onward, as late developmental stages do not feed (Williamson and Bull 1989).

Time to metamorphosis was interpreted as the time between fertilization and the completion of metamorphosis (i.e. complete reabsorption of the tail; Gosner stage 46), and survival was calculated as the proportion of fertilized eggs that metamorphosed. We also calculated the metamorphic duration—the time (in days) it took tadpoles to progress from Gosner stage 42 to Gosner stage 46—as this is a vulnerable stage in amphibian life history, when major internal reorganization occurs (Downie et al. 2004).

On the day metamorphosis was achieved, jumping performance was assessed for each metamorph. Jumping trials were conducted in a temperature-controlled room at 16°C. Prior to each trial, metamorphs were placed in a Petri dish containing 3-mm-deep stream water for 15 min to ensure that they were fully hydrated but did not swim. Metamorphs were then positioned into the middle of an A3-sized paper. A syringe was used to apply a small amount of food coloring (Queen blue color) onto the hind limbs of each metamorph (Whitehead et al. 1989). Metamorphs were then induced to jump five times by lightly tapping the urostyle with a pen (Zug 1978). Distances between ink marks on the paper were measured to the nearest millimeter, and the average jumping distance was calculated for each individual.

Following the measurement of jump performance, metamorphs were euthanized in <0.03% benzocaine solution and preserved in 10% neutral buffered formalin. Wet mass was later recorded to the nearest 0.001 g after blotting on tissue. Preserved metamorphs were photographed in dorsal view (while submerged in water to minimize refraction) using a digital imaging camera (Leica DFC320) attached to a light microscope (Leica MZ7.5) at ×6.3 magnification. ImageJ was used to measure the following five morphological traits to the nearest 0.01 mm: snout-vent length (SVL), head width (HW), thigh length (THL), tibia length (TL), and foot length (FL; fig. 2*B*).

Yolk Corticosterone Analysis

Maternal steroid hormones deposited in the egg, such as the glucocorticoid corticosterone, can influence offspring fitness (Love and Williams 2008) and development rate (Kulkarni and Buchholz 2012). We therefore measured corticosterone concentrations in spare eggs from each female for inclusion as a covariate in our analyses. For this purpose, egg samples (yolk + jelly) were weighed and homogenized in 300 μL of double-distilled (DD) water using an Eppendorf micropestle (Eppendorf, Hauppauge, NY). The micropestle was rinsed with 100 μL of DD water. Corticosterone was extracted by adding 4 mL of pure diethyl ether, and the samples were then vortexed for 10 min. Samples were kept frozen at −20°C overnight, and the organic phase was transferred into a 12 × 75-mm glass tube and dried under airflow. The dry samples were reconstituted in 300 μL of phosphate-buffered saline, vortexed for 5 min, and centrifuged at 2,000 g for 5 min. Duplicates of 100 μL of egg extract were assayed using the ImunoChem Cortiscosterone l 125 kit (MP Biomedicals, Orangeburg, NY). All samples were prepared in a single assay. The limit of detection was 2.9 ng/mL, and the intra-assay coefficients of variation for quality-control samples containing 72.1 and 485.5 ng/mL were 8.9% and 7.1%, respectively. Corticosterone concentrations were then calculated as nanograms corticosterone/milligrams fresh egg sample from each female.

Statistical Analysis

All analyses were performed using R, version 3.3.1 (R Development Core Team 2016). We used linear mixed effects models, with restricted maximum-likelihood methods (REML), to partition sources of phenotypic variation in each trait among genetic and environmental effects and to reveal potential genotype-by-environment interactions underlying their expression. The REML models were performed with the lme4 package in R (Bates et al. 2015). Models of traits that were measured before the water depth treatment was initiated (fertilization success, time to hatching, tadpole morphology) included only the random effects of sire, dam, block, and the sire-by-dam interaction. For all other traits, treatment was added as a fixed effect, and the models also included the random effects sire by treatment, dam by treatment, and sire by dam by treatment. The significance of the fixed treatment ef-

fect was evaluated using a Wald χ^2 test on the full model. The significance levels of the random effects were obtained from likelihood ratio tests, in which each random effect is excluded in turn and the fit of the reduced model was compared with the full model (Shaw 1987). No adjustments for multiple comparisons were performed. Tadpole morphological traits (total length, tail length, body length, body width, and tail muscle width) and metamorph morphological traits (head width and lengths of the snout-vent, tibia, thigh, and foot) were all highly correlated with one another. In order to simplify the analysis, we conducted a principal component analysis (PCA) for both tadpole and metamorph morphological traits and performed the linear mixed effects models on the first principal component (see table 1 for PC factor loadings, eigenvalues, and variance explained by each PC). Analysis was restricted to the first principal component because the eigenvalues of the subsequent PCs were below 1 and because including other PCs did not significantly alter any results.

In the light of significant three-way sire-by-dam-by-treatment interactions for most traits (see "Results"), we further explored the causal basis for such interactions. Briefly, genotype-by-environment interactions may arise either due to a change in the magnitude of variance between environments (variance GEI; i.e., where there is substantially more nonadditive genetic variance in one environment than another) or a change in the ordering of rank family means, where genotypic values cross each other in different environments (ecological crossover; Fry et al. 1996; Conner and Hartl 2004). In order to distinguish between these factors, we tested the correlations between trait scores for specific combinations of males and females between the two environmental treatments. The prediction from such an analysis is that the correlation in offspring fitness for any given sire-dam combination should be increasingly weaker as ecological crossover becomes more important. Conversely, a significantly positive correlation between the ordering of fitness scores between environments would indicate no significant change in the rank order of fitness between treatments (consistent with variance GEI). To test these alternative scenarios, we used a randomization approach to extract mean family trait scores for the three independent sire-by-dam families (i.e., each involving a different sire-dam combination to avoid pseudoreplication) in each block for each water depth treatment. For each draw of the data, this process generated a sample comprising

Table 1: Principal component (PC) analysis on five tadpole and five metamorph morphological traits

Morphology, PC	Eigenvalue	Variance (%)	Cumulative variance (%)
Tadpole:			
1	3.3942	67.9	67.9
2	.8447	16.9	84.8
3	.6110	12.2	97.0
4	.1501	3.0	100
Metamorph:			
1	3.9490	79.0	79.0
2	.4199	8.4	87.4
3	.3258	6.5	93.9
4	.1795	3.6	97.5
5	.1258	2.5	100

	Factor loadings				
Morphology, variable	PC1	PC2	PC3	PC4	PC5
Tadpole:					
Total length	.523	−.172	.262	−.179	−.772
Body length	.510	.134	.016	.829	.188
Tail length	.507	−.178	.328	−.485	.607
Body width	.250	.959	.132	−.001	0
Tail muscle width	.384	.023	−.898	−.215	0
Metamorph:					
Snout-vent length	.455	.400	.088	−.790	−.037
Thigh length	.460	−.120	−.555	.110	.674
Tibia length	.464	−.210	−.429	.148	−.731
Foot length	.418	−.672	.602	−.037	.098
Head width	.437	.575	.371	.584	.013

Note: Eigenvalues (*top*) and factor loadings (*bottom*) are presented for each principal component.

$n = 30$ independent crosses, from which we calculated the Spearman's rank correlation in mean fitness scores between treatments. We repeated this procedure 1,000 times using the software package PopTools (Hood 2011) and calculated the mean and 95% confidence limits for the distribution of correlation coefficients generated by all independent correlations (Evans et al. 2007). To further explore how patterns of nonadditive genetic variation change between treatments, we contrasted the ratios of nonadditive genetic variation ($V_{\text{sire}\times\text{dam}}$) to total phenotypic variation (V_P) between water depth treatments. These latter comparisons enabled us to determine whether the level of expressed nonadditive variation (e.g., attributable to dominance and/or epistatic variance and thus the expression of deleterious recessive alleles) is greater under stressful environments.

Fertilization rates and survival data were binomial variables, and thus a generalized linear mixed effects model (GLMM) with a logit-link function was used for the analysis of these traits. The significance of the treatment effect was evaluated using Wald's Z test, and the significance of the random effects was evaluated using log-likelihood ratio tests.

In order to control for some environmental aspects of maternal effects, ovum size and corticosterone concentrations in the yolk were used as covariates in all of our analyses. Because jumping distance was strongly positively correlated with metamorph morphological traits (head width and snout-vent, thigh, tibia, and foot lengths), we used the first principal component of these traits as a covariate in the analysis. Jumping distance was also correlated with metamorph wet weight. However, since morphological traits and wet weight were highly correlated ($P < .001$, $R^2 > 0.76$), we restricted the covariates to metamorph morphology only. In order to ensure that data complied with assumptions of normality, quantile-quantile plots of residuals were inspected, and where necessary, data were treated with the following transformations: metamorph wet mass and tadpole tail muscle width were subject to $\log_{10}$ transformations, metamorph tibia length data were squared, and length of larval period and time between Gosner stages 42 and 46 data were transformed using the Box-Cox method (Box and Cox 1964). We checked for overdispersion in our GLMM models using the overdisp_fun function proposed by Bolker et al. (2009). Only two traits—metamorph wet mass and time between Gosner stages 42 and 46—were overdispersed; we added observation level as an extra random factor to account for overdispersion when analyzing these traits (Harrison 2014).

We used untransformed variables to estimate causal components of genetic variation (Garcia-Gonzalez et al. 2012), and data were centered around sample means for each treatment separately to allow comparison of variances across treatments. Additive genetic variance (V_A) was estimated as four times the sire variance component. For nonbinomial data, total phenotypic variance (V_P) was calculated by summing the variance components of all random effects in the model. For the binomial fertilization success and survival data, V_P was calculated by summing the variance components of all random effects in the GLMM model and adding this value to an estimate of residual variance, calculated according to Nakagawa and Schielzeth (2010; residual variance $= \omega \times [\pi 2/3]$). Narrow-sense heritability estimates (h^2) were calculated as $h^2 = V_A/V_P$ for each trait within each treatment group (note that for traits that were measured before treatment was initiated, h^2 was estimated across the whole sample). We also present CV_A, the coefficient of additive genetic variation, and its square I_A to provide estimates of evolvability (sensu Houle 1992). Unlike heritability, these measures are standardized by the trait mean and are therefore independent of other sources of variance, making the comparison of evolvability among traits and taxa possible (Houle 1992; Hansen et al. 2011; Garcia-Gonzalez et al. 2012). We calculated CV_A as $CV_A = (V_A)^{1/2}/\bar{X}$ ($\bar{X}$ = phenotypic mean) and I_A as $I_A = V_A/\bar{X}^2$ (Garcia-Gonzalez et al. 2012). Data are deposited in the Dryad Digital Repository: https://doi.org/10.5061/dryad.98j4qv2 (Rudin-Bitterli et al. 2018).

Results

Treatment Effects

Crinia georgiana tadpoles responded to the low water depth treatment by accelerating their development, significantly reducing the length of their larval period by an average of 3.5 days (9% difference) and significantly reducing metamorphic duration by an average of 3.5 days (20% difference) compared to tadpoles reared at higher water depths (fig. 3*A*, 3*B*; table 2). However, survival was reduced slightly (6% difference; $P = .01$) in tadpoles reared in the low water environment (fig. 3*C*), and tadpoles that accelerated their development were smaller at metamorphosis and had a poorer jumping performance, even when corrected for their smaller body size (fig. 3*D*–3*I*; table 2).

Sources of Phenotypic Variation: Maternal, Additive, and Nonadditive Genetic Effects

As expected, ovum volume explained significant variance in many offspring traits measured in our study (tables 2, 3), including the length of larval period, metamorphic duration (time between Gosner stages 42 and 46), size at metamorphosis, and tadpole and metamorph morphology (e.g., snout-to-vent, tibia, and thigh lengths tended to be greater in tadpoles from larger eggs). Conversely, ovum volume did not explain variance in fertilization success, time to hatching, survival, or jumping performance. Concentrations of maternal steroid hormones in the egg yolk ranged between 4.14 and 250.86 ng

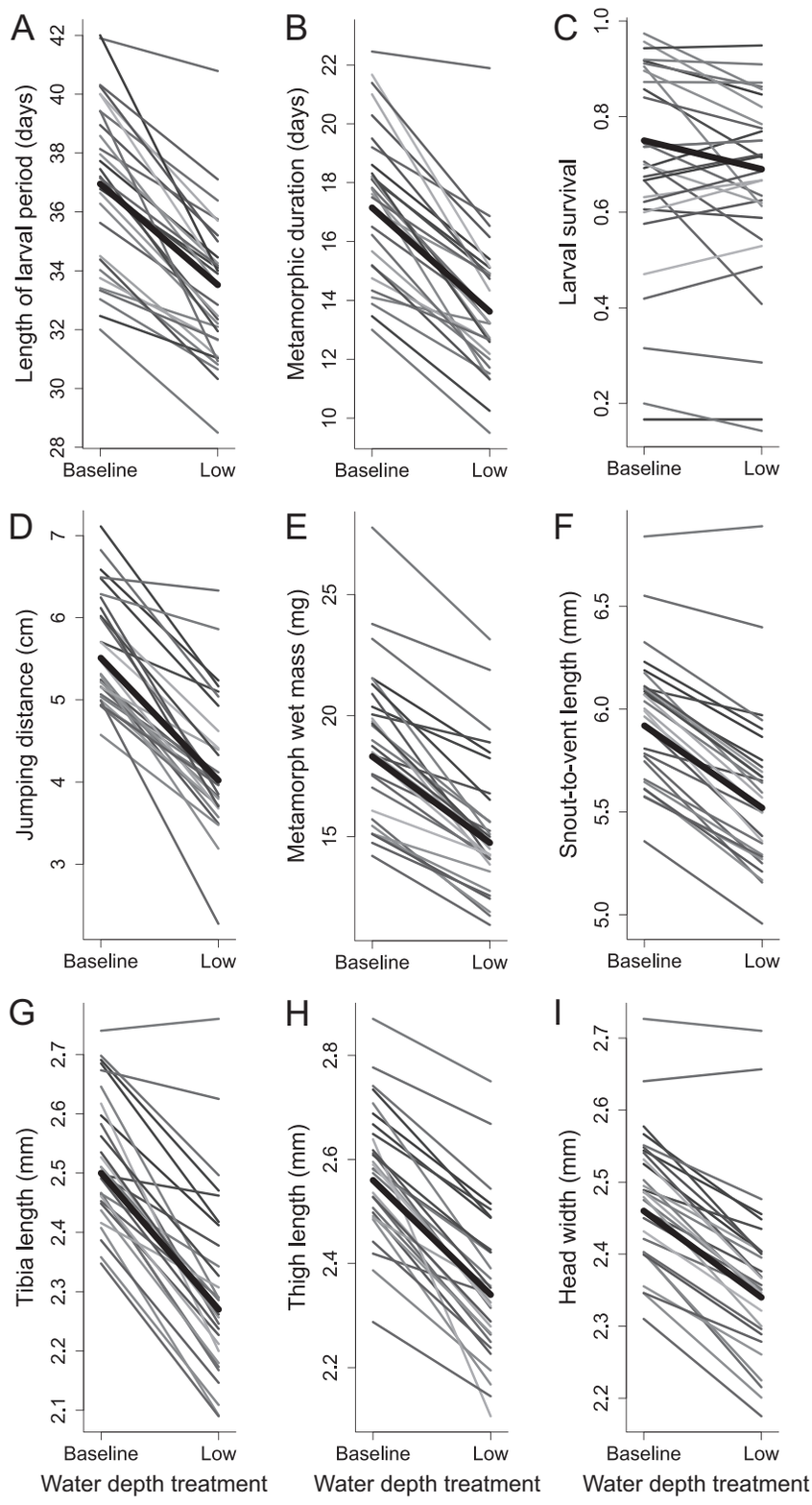

Figure 3: Reaction norms for various *Crinia georgiana* fitness traits. *A*, Length of larval period. *B*, Metamorphic duration. *C*, Larval survival (proportion of fertilized eggs reaching Gosner stage 42). *D*, Metamorph jumping distance (average of five jumps). *E*, Wet weight at metamorphosis. *F–I*, Metamorph morphology. Each line represents the mean score for each sire family ($n = 30$ sires). Please note that any crossings of reaction norms between sires are not significant. The thick line represents mean scores within each treatment across all sires.

corticosterone/mg egg sample for all females (mean = 34.53 ng corticosterone/mg egg sample) but had no significant influence on any offspring trait measured in our study. Despite including ovum size and yolk corticosterone levels as covariates, dam effects were still highly significant for most traits, with the exception of metamorph wet mass, morphology, and jumping performance (tables 2, 3).

Sire-by-dam interactions were significant for all traits except jumping performance (tables 2, 3), suggesting that nonadditive (i.e., epistatic and/or dominance) genetic variance is important in determining the expression of these traits. Nonadditive effects explained up to 32% of the phenotypic variance (table 4). There were no significant sire effects on any of the traits measured ($P < .05$). Accordingly, narrow-sense heritability estimate (h^2) values were low for most traits, with the exception of embryonic and larval survival, where values ranged between 0.22 and 0.36 (table 4). Some traits appeared to show higher heritability under the low water depth treatment (table 4), but these differences were not significant, as no sire-by-treatment interactions were significant for any of the traits. Hence heritability values in table 4 are simply shown for completeness.

Genotype-by-Environment Interactions

Our analysis revealed significant three-way sire-by-dam-by-treatment interactions for most traits, including the metamorphic duration and the wet mass and morphology of metamorphs (table 2). The slopes of the reaction norms for the trait metamorphic duration, for example, differed between each sire-by-dam combination (=full-sib family), as illustrated in figure 4. While most families accelerated development at low water depths, there were some families where developmental rate was unaffected or where offspring took longer to metamorphose under the low water depth treatment relative to the baseline (positive slopes). Despite some variation in rank order changes of sire-by-dam families across the two water depth treatments (appendix, available online), we found limited evidence for ecological crossover in traits revealing significant three-way interactions. The independent correlations from the randomization approach (see "Material and Methods") were positive and significant (length of larval period: r mean = 0.64, 95% confidence limits [CL] = 0.44/0.82; metamorphic duration: r mean = 0.58, 95% CL = 0.33/0.79; metamorph wet mass: r mean = 0.69, 95% CL = 0.47/0.86; metamorph morphology: r mean = 0.75, 95% CL = 0.56/0.90; proportion of fertilized eggs surviving to metamorphosis: r mean = 0.81, 95% CL = 0.65/0.924; see appendix for a visualization of the distributions of correlation coefficients). When we compared the ratio of nonadditive genetic variation to total phenotypic variation (i.e., $V_{\text{sire}\times\text{dam}}/V_{\text{P}}$) between water depth treatments, we found consistently higher levels of nonadditive genetic variation in the low water (stressful) environments (see fig. 5).

Finally, we found no evidence of sire-by-treatment or dam-by-treatment interactions for any of the offspring traits investigated in this study (tables 2, 4).

Discussion

Our findings emphasize clear fitness consequences associated with variation in water depth for the larval stage of *Crinia georgiana* but also demonstrate that there is nonadditive genetic variance underlying traits that are responsive to water depth. Moreover, our analyses reveal that the magnitude of nonadditive genetic variation contributing toward the fitness of offspring depends on the environment in which they emerge. Specifically, all traits that revealed evidence for three-way genotype-by-environment interaction (i.e., where the level of nonadditive genetic variation differed between treatments) exhibited a higher magnitude of nonadditive genetic variation in the stressful (low water) rearing environment. These results have important evolutionary implications by providing genetic insights into how climatic variables drive life-history traits in amphibians. We discuss these key findings in turn below.

Treatment Effects

Crinia georgiana tadpoles facing low water depths were able to accelerate their development and metamorphose significantly earlier than tadpoles in the baseline treatment. While this plastic response would allow metamorphs to escape drying pools earlier, our analysis shows that allocating energy toward rapid development comes at a cost, as implied by earlier studies on this species (Doughty and Roberts 2003; Mueller et al. 2012). Specifically, faster developers exhibited slightly reduced larval survival (although survival to metamorphosis was unaffected by the treatment), a reduction in body size, and poorer jumping performance compared to their slower-developing counterparts. Importantly, work on other amphibian species has shown that metamorph size and jumping performance are strong predictors of future fitness in the terrestrial environment. For example, reduced jumping distance may lead to increased vulnerability to terrestrial predators (Marsh 1994), while smaller metamorphs can experience increased risks of desiccation (Newman and Dunham 1994) and may be less adept at catching and consuming prey (Cabrera-Guzmán et al. 2013). Furthermore, larger size at metamorphosis may convey physiological advantages, particularly with regard to juvenile aerobic performance (Pough and Kamel 1984; Taigen and Pough 1985). Size at metamorphosis is also linked to reproductive success, with smaller metamorphs taking longer to mature (Smith 1987; Semlitsch et al. 1988), maturing at a smaller body size (Semlitsch

Table 2: Mixed effects model results of *Crinia georgiana* traits measured after water depth treatments were initiated

Trait, variances	*N*	Mean ± SD	χ^2	*P*
Length of larval period (days)	1,373	35.25 ± 4.82		
Treatment			102.04	**<.001**
Ovum volume			8.44	**.004**
Ovum volume × treatment			2.10	.15
Yolk corticosterone			.25	.61
Sire			0	1
Dam			7.10	**.008**
Sire × dam			12.55	**<.001**
Sire × treatment			0	1
Dam × treatment			3.34	.07
Sire × dam × treatment			38.15	**<.001**
Block			.09	.76
Metamorphic duration (days)	1,074	15.35 ± 4.25		
Treatment			112.48	**<.001**
Ovum volume			12.12	**<.001**
Ovum volume × treatment			1.02	.31
Yolk corticosterone			2.46	.12
Sire			2.36	.12
Dam			6.24	**.01**
Sire × dam			5.09	**.02**
Sire × treatment			.09	.76
Dam × treatment			1.59	.21
Sire × dam × treatment			6.20	**.01**
Block			0	1
Metamorph wet mass (mg)	1,059	16.48 ± 4.51		
Treatment			204.43	**<.001**
Ovum volume			38.68	**<.001**
Ovum volume × treatment			1.40	.24
Yolk corticosterone			1.11	.29
Sire			0	1
Dam			2.63	.11
Sire × dam			8.70	**.003**
Sire × treatment			0	1
Dam × treatment			0	1
Sire × dam × treatment			5.79	**.02**
Block			.33	.56
Metamorph morphology (residuals of the first principal component of all five morphological traits measured)	1,058	…		
Treatment			354.54	**<.001**
Ovum volume			35.84	**<.001**
Ovum volume × treatment			5.25	**.02**
Yolk corticosterone			.98	.32
Sire			0	1
Dam			.95	.33
Sire × dam			13.76	**<.001**
Sire × treatment			0	1
Dam × treatment			0	1
Sire × dam × treatment			5.04	**.05**
Block			1.96	.16
Metamorph jumping performance (cm)	908	4.73 ± 1.47		
Treatment			53.23	**<.001**
Ovum volume			.08	.78
Yolk corticosterone			.05	.82
PCA of metamorph morphology			208.16	**<.001**

Table 2 (*Continued*)

Trait, variances	*N*	Mean ± SD	χ^2	*P*
Sire			.34	.56
Dam			2.52	.11
Sire × dam			.15	.70
Sire × treatment			.09	.76
Dam × treatment			.06	.81
Sire × dam × treatment			1.34	.25
Block			.11	.74
Proportion of fertilized eggs reaching Gosner stage 42	1,373	.72		
Treatment			6.60	**.01**
Ovum volume			1.77	.18
Yolk corticosterone			.03	.86
Sire			3.47	.06
Dam			11.48	**<.001**
Sire × dam			27.47	**<.001**
Sire × treatment			1.64	.20
Dam × treatment			0	1
Sire × dam × treatment			0	1
Block			.37	.54
Proportion of fertilized eggs completing metamorphosis	1,059	.55		
Treatment			.58	.45
Ovum volume			3.02	.08
Yolk corticosterone			.01	.91
Sire			3.03	.08
Dam			10.43	**.001**
Sire × dam			15.00	**<.001**
Sire × treatment			0	1
Dam × treatment			0	1
Sire × dam × treatment			6.58	**.01**
Block			.18	.67

Note: Sample sizes (*N*), trait means, and standard deviations are presented for each trait, and χ^2 values (and associated *P* values) are presented for each model. Significant results are highlighted in boldface. Ovum volume and yolk corticosterone concentrations were added as covariates to each model.

et al. 1988; Berven 1990; Altwegg and Reyer 2003), and having reduced fecundity and lower mating success (Howard 1980; Berven 1981).

Nonadditive Genetic Variance

We found significant and strong sire-by-dam interactions in all but one offspring trait, implying the existence of nonadditive genetic variation due to dominance or epistatic effects (Lynch and Walsh 1998). Our results therefore suggest that the interaction between male and female haplotypes plays an important role in determining offspring fitness in *C. georgiana*, in line with earlier studies on this species (Dziminski et al. 2008) and other amphibians (Travis et al. 1987; Laurila et al. 2002; Merilä et al. 2004; Eads et al. 2012). However, in our study we also found that the magnitude of nonadditive effects was modified by the environment (water depth) in which offspring developed, as evidenced by the significant sire-by-dam-by-treatment interactions (see also Nystrand et al. 2011; Eads et al. 2012; Lymbery and Evans 2013). Specifically, our analyses revealed consistently higher levels of nonadditive genetic variation under stressful (low water) rearing conditions (fig. 5). One interpretation of this finding is that individuals harboring deleterious recessive alleles in the homozygous state will suffer greater fitness costs when they encounter new (stressful) environments. Our supplementary analyses revealing significant positive correlations between treatment groups for the different components of offspring fitness support this interpretation. Specifically, this finding is consistent with the idea that some individuals carry more deleterious alleles than others, irrespective of context (i.e., their rank order for fitness does not change between environments) but that the phenotypic effects of such alleles are stronger under certain (stressful) conditions. A number of studies, for example, have suggested that inbreeding depression is amplified in stressful (or novel) environments (for a review, see Armbruster and Reed 2005), and thus the sire-by-dam-by treatment effects observed here could be a manifestation of context-dependent inbreeding effects.

Table 3: Mixed effects model results of *Crinia georgiana* traits measured before treatment was initiated

Trait, variances	*N*	Mean ± SD	χ^2	*P*
Proportion of eggs fertilized	2,067	.93		
Ovum volume			1.08	.30
Yolk corticosterone			1.61	.20
Sire			.05	.82
Dam			12.36	**<.001**
Sire × dam			5.01	.03
Block			1.30	.26
Proportion of fertilized eggs hatching	1,913	.80		
Ovum volume			.17	.68
Yolk corticosterone			.01	.91
Sire			.91	.34
Dam			11.75	**<.001**
Sire × dam			67.21	**<.001**
Block			.16	.69
Time to hatching (days)	1,536	14.06 ± 1.35		
Ovum volume			.003	.96
Yolk corticosterone			.30	.58
Sire			.68	.41
Dam			7.84	.005
Sire × dam			64.12	**<.001**
Block			3.20	.07
Tadpole morphology (residuals of the first principal component of all five measured traits)	1,536	…		
Ovum volume			12.29	**<.001**
Yolk corticosterone			.01	.91
Sire			0	1
Dam			31.77	**<.001**
Sire × dam			71.48	**<.001**
Block			8.34	**.004**

Note: Sample sizes (*N*), trait means, and standard deviations are presented for each trait, and χ^2 and associated *P* values are presented for each model. Significant results are highlighted in boldface. Ovum volume and yolk corticosterone concentrations were added as covariates to each model.

Accordingly, relatively common environments (in our case, the baseline water depth treatment) may act as evolutionary capacitors (see Masel 2013), in that they allow populations to accumulate deleterious (or potentially advantageous) alleles that have limited phenotypic effects. According to this scenario, as environmental conditions change, or when individuals move to new environments, the deleterious and/or beneficial effects of those alleles will be realized, rendering them visible to natural selection (Kim 2007; Trotter et al. 2014). Therefore, phenotypic plasticity in *C. georgiana* tadpoles in response to changes in water depth may not be adaptive, since the complex sire-by-dam-by-environment interactions may produce phenotypes that differ from the local phenotypic optimum. According to this view, nonadaptive plasticity may facilitate evolutionary responses to new environments by increasing the strength of directional selection (Ghalambor et al. 2015), although this subject is still highly debated (Mallard et al. 2018; Van Gestel and Weissing 2018). The accumulating evidence for sire-by-dam-by-environment interactions reported in crickets (Nystrand et al. 2011), sea urchins (Lymbery and Evans 2013), and frogs (Eads et al. 2012) suggests that such effects may be more common than currently appreciated. Collectively, these studies highlight the importance of estimating levels of genetic variation across multiple contexts in order to better assess the potential for evolutionary responses to environmental change.

Maternal Effects

Consistent with previous reports in anurans (Kaplan 1998; Pakkasmaa et al. 2003; Räsänen et al. 2003; Merilä et al. 2004; Dziminski et al. 2008; Eads et al. 2012), maternal effects were strong determinants of most offspring fitness traits we measured. Ovum size, in particular, was an important source of phenotypic variance in a range of traits considered in our analysis, and we found a significant interaction between ovum volume and treatment in the trait metamorph morphology (table 2). In accordance with dynamic energy budget (DEB) theory, larger reserves of maternally derived yolk within an individual ovum will enable offspring to partition

Table 4: Patterns of genetic variation for each of the *Crinia georgiana* fitness traits measured

Variable, traits	Treat	N	Mean (SD)	V_{sire} (SE)	V_{dam} (SE)	$V_{sire\times dam}$ (SE)	V_{block} (SE)	V_{res} (SE)	V_P	V_A	h^2	CV_A	I_A
Survival:													
Proportion of eggs fertilized	NA	2,067	.93	.06 (.01)	1.65 (.03)	.53 (.02)	.81 (.02)	1.33 (.03)	4.38	.24	.05	.53	.28
Proportion of fertilized eggs hatching	NA	1,913	.80	.54 (.02)	3.43 (.04)	2.50 (.04)	.52 (.02)	2.68 (.02)	9.67	2.16	.22	1.84	3.38
Proportion of fertilized eggs reaching Gosner stage 42	Baseline	1,373	.75	.62 (.02)	2.14 (.04)	1.77 (.04)	1.06 (.03)	2.86 (.02)	8.45	2.48	.29	2.10	4.41
	Low	1,373	.69	.70 (.02)	1.17 (.03)	.73 (.02)	.41 (.02)	4.68 (.04)	7.69	2.80	.36	2.43	5.89
Proportion of fertilized eggs completing metamorphosis	Baseline	1,059	.54	.14 (.01)	1.12 (.03)	.96 (.03)	.28 (.02)	4.88 (.05)	7.38	.56	.08	1.39	1.92
	Low	1,059	.56	.81 (.03)	1.96 (.04)	1.56 (.04)	<.00 (~0)	4.47 (.04)	8.80	3.24	.37	3.21	10.33
Development rate:[a]													
Time to hatching (days)	NA	1,536	14.06 (1.35)	.21 (.20)	6.29 (1.11)	.94 (.06)	.03 (.01)	4.43 (.71)	11.9	.84	.07	.03	.0009
Length of larval period (days)	Baseline	695	36.95 (4.41)	.50 (.84)	4.04 (2.41)	2.60 (.93)	1.84 (1.63)	5.57 (2.83)	14.55	2	.14	.04	.002
	Low	678	33.52 (4.61)	<.00 (~0)	4.34 (2.53)	5.38 (.81)	.68 (1.00)	6.38 (3.07)	16.78	~0	~0	0	0
Metamorphic duration (days)	Baseline	528	17.15 (3.91)	.14 (.44)	1.04 (.03)	.56 (.002)	.43 (.02)	1.75 (.14)	3.92	.56	.14	.04	.002
	Low	546	13.62 (3.82)	.42 (.84)	.96 (.12)	.55 (.07)	.04 (.03)	1.61 (.16)	3.58	1.68	.47	.10	.009
Tadpole morphology:[a]													
Total length (cm)	NA	1,536	1.21 (.15)	<.00 (~0)	3.43 (.59)	.92 (.12)	6.14 (.78)	4.86 (.69)	15.35	~0	~0	0	0
Body length (cm)	NA	1,536	.37 (.04)	<.00 (~0)	1.80 (.42)	.27 (.09)	3.89 (.62)	2.96 (.54)	8.92	~0	~0	0	0
Tail length (cm)	NA	1,536	.84 (.12)	<.00 (~0)	4.59 (.67)	1.39 (.37)	7.21 (.85)	7.07 (.71)	20.26	~0	~0	~0	~0
Body width (cm)	NA	1,536	.22 (.04)	<.00 (~0)	2.93 (.29)	<.00 (~0)	2.40 (.24)	30.41 (1.74)	35.74	~0	~0	0	0
Tail muscle width (cm)	NA	1,536	.09 (.01)	.18 (.13)	2.49 (.50)	.43 (.21)	6.91 (.83)	12.46 (1.16)	22.47	.72	.03	2.22	4.94
Metamorph fitness:[a]													
Metamorph wet mass (mg)	Baseline	518	18.31 (4.45)	<.00 (~0)	7.90 (4.22)	10.18 (2.54)	<.00 (~0)	43.64 (19.60)	61.72	~0	~0	~0	~0
	Low	541	14.74 (3.82)	<.00 (~0)	2.16 (2.80)	17.58 (2.89)	3.07 (1.34)	38.87 (10.66)	61.68	~0	~0	0	0
Jumping distance (cm)	Baseline	431	5.51 (1.31)	2.38 (1.35)	10.12 (4.84)	16.61 (6.21)	<.00 (~0)	139.52 (37.35)	168.63	9.52	.06	.05	.003
	Low	477	4.02 (1.22)	<.00 (~0)	4.42 (3.04)	23.74 (7.05)	<.00 (~0)	110.37 (33.22)	138.53	~0	~0	.07	.005
Metamorph morphology:[a]													
Residuals of the first PC of all five morphological traits	Baseline	517	…	.02 (.01)	.14 (.09)	.07 (.02)	.07 (.05)	.95 (.34)	1.25	.08	…	…	…
	Low	541	…	.02 (.01)	<.00 (~0)	.07 (.02)	<.00 (~0)	.79 (.29)	.88	.08	…	…	…

Note: Data are presented for each water depth treatment separately where possible. Traits that were measured before treatment was initiated are indicated NA (not applicable). Sample size and trait mean (±SD) are presented for each trait. Variance components for sires (V_{sire}), dams (V_{dam}), their interaction ($V_{sire\times dam}$), block (V_{block}), and residual variance (V_{res}) were obtained from the mixed effects models. For most traits, total phenotypic variance (V_P) was calculated by summing the variance components of all random effects in the model. For fertilization success and survival data, V_P was calculated by summing the variance components of all random effects in the generalized linear mixed effects model and adding this value to an estimate of residual variance. See "Material and Methods" for further information. Heritability values are reported for completeness.

[a] Data were centered around sample means for each treatment separately before calculating variances, and all values are $\times 10^3$.

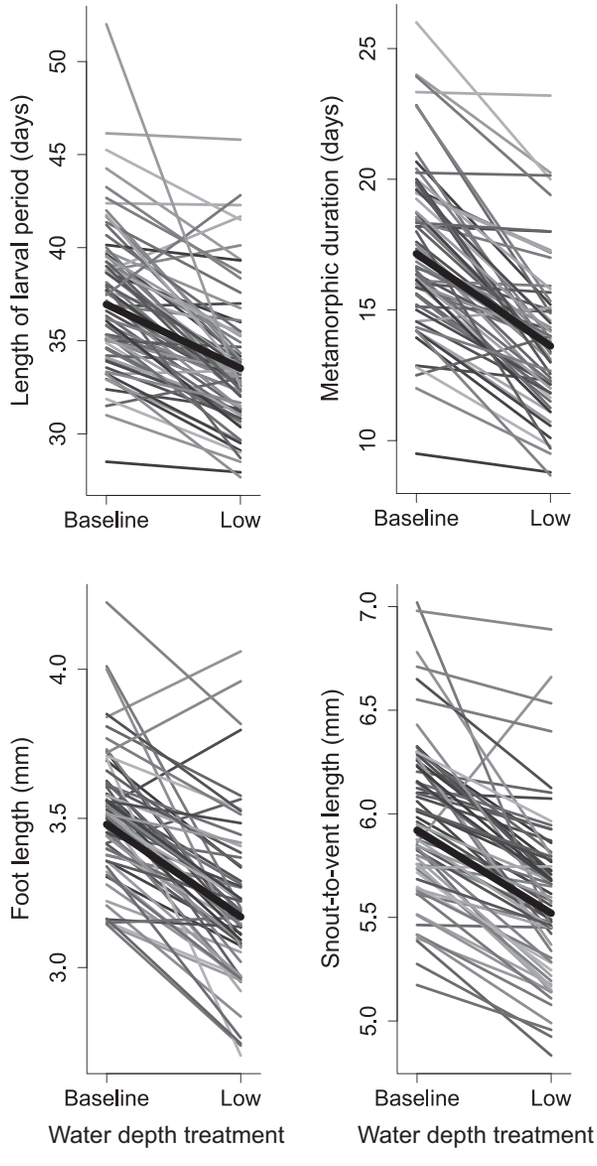

Figure 4: Reaction norms for selected *Crinia georgiana* fitness traits, illustrating mean trait values for each sire-by-dam combination across the two water depth environments. The thick black line represents mean scores within each treatment across all families.

more energy into maturation and growth (Mueller et al. 2012), which in *C. georgiana* leads to higher survival, larger size at metamorphosis, and shorter development time (Dziminski and Roberts 2006; Dziminski et al. 2009*b*). Therefore, at low water depths, maternal fitness is increased when fewer but larger ova are produced (Dziminski and Roberts 2006), as offspring can develop plastically when hydroperiods are short. As ovum size is independent of female size in this species (Dziminski and Roberts 2006), this trait has the potential to be selected for (and evolve) separately, suggesting that maternal provisioning could play a key role in *C. georgiana*'s adaptation to climate change (Doughty 2002; Pakkasmaa et al. 2003).

Significant dam effects remained for most traits after accounting for variation in ovum size, which suggests that other nongenetic or genetic maternal effects contribute to offspring phenotypes. Aside from the amount of yolk available to the embryo, yolk composition can also influence offspring quality. In birds and reptiles, for example, differences in the maternal allocation of antioxidants, antibodies, and hormone concentrations in the yolk can affect various offspring traits (Schwabl 1996; Royle et al. 2001; Saino et al. 2003). Maternally derived steroid hormones, such as the glucocorticoid corticosterone, have been linked to offspring phenotypes and quality (Sinervo and DeNardo 1996; McCormick 1998; Seckl 2001; Meylan and Clobert 2005; Saino et al. 2005; Love and Williams 2008), and in amphibians, corticosterone concentrations in the yolk can influence development rate (Wada 2008; Kulkarni and Buchholz 2012). In the present study, corticosterone concentrations differed substantially between clutches from different females but had no significant effect on any offspring trait, suggesting that other factors were a significant source of phenotypic variation in developing *C. georgiana*. Hence our results are in alignment with the increasing awareness that ovum size is only a crude proxy for maternal allocation of compounds to the offspring (Giron and Casas 2003; Lock et al. 2007; Geister et al. 2008). Future work examining the role of specific egg components that affect embryonic and larval development would greatly benefit

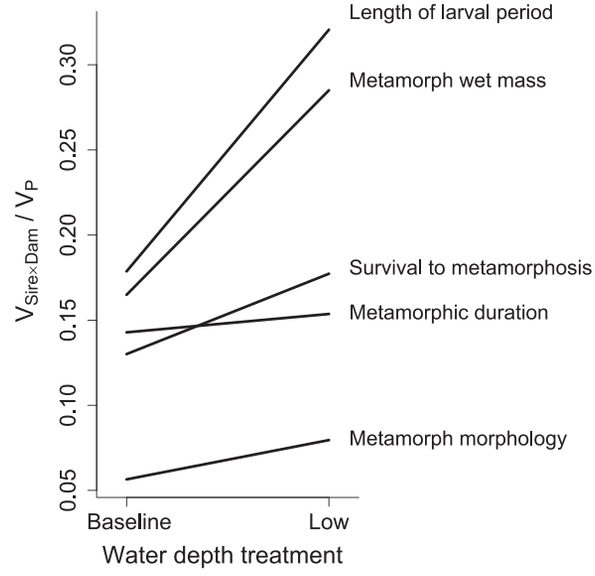

Figure 5: Relative magnitude of nonadditive genetic variation (ratio of variance components for nonadditive genetic variation to total phenotypic variation; $V_{\text{sire}\times\text{dam}}/V_{\text{P}}$) in baseline and low water depth treatments.

our understanding of the mechanisms underlying nongenetic maternal effects. There is tentative evidence, for example, that differential allocation of free amino acids to eggs may influence offspring fitness in some insect species (Geister et al. 2008; Newcombe et al. 2015).

Conclusions

While our results suggest that *Crinia georgiana* embryos can respond plastically to drying conditions by accelerating their development, we found no evidence for additive genetic variation underlying the expression of this response, pointing to limited potential for this population to respond genetically to drying conditions. Overall, we show that larval fitness is reduced under low water (stressful) rearing environments, but our results also highlight how environmental factors can alter patterns of nonadditive genetic variation and thus potentially change the way in which deleterious alleles affect individual fitness. Collectively our findings suggest that the consequences of deleterious alleles may become apparent only under certain environmental conditions, which may have important implications for a population's resilience to changing environments. While complex patterns of nonadditive variation—and sensitivity to environmental conditions thereof—have been found in laboratory studies of invertebrates (e.g., *Drosophila*), this study is one of only a handful to show evidence for similar complexity in a wild vertebrate population.

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A gravid female *Crinia georgiana*. Photo: Corné van der Linden.