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Evolution under unpredictable environmental conditions: quantitative genetics of larval life-history traits in a myobatrachid frog *Crinia georgiana*

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The southwestern Australian frog *Crinia georgiana* (Anura; Myobatrachidae) inhabits ephemeral pools in which the tadpoles often face desiccation. Under these conditions selection on tadpoles can be severe and can directly affect fitness during the aquatic as well as the terrestrial developmental stages. A quantitative genetic study using a half-sib breeding design was conducted to understand the genetic effects on larval life-history traits. We found no significant additive genetic variance in any of larval traits. Except for hatching period, heritability estimates based on females were high in egg size, larval period, snout-vent length, and weight at metamorphosis, suggesting non-additive genetic effects. These results indicate that any response to selection during hatching and larval periods should be predominately governed by non-additive genetic effects in *C. georgiana*.

Keywords: additive genetic variation; larval period; maternal effect; multiple mating

Introduction

Natural selection favors those individuals whose traits allow them to deal with unpredictable environmental conditions (Darwin 1871). In facing unpredictable conditions, two strategies may be employed: (1) individuals develop traits that enable them to avoid unfavorable conditions until favorable circumstances return (e.g., hibernation; Holenweg and Reyer 2000) or (2) individuals develop traits that allow them to directly cope with the unfavorable conditions (e.g., accelerated larval development to counteract pond drying; Abrams et al. 1996).

Dealing with unpredictable environmental conditions is especially important during early development because it can not only have a direct impact on fitness at the current life-history stage, but can also effect future development, survival, and fecundity (Semlitsch et al. 1988; Berven 1990). For example, organisms that inhabit temporary pools are likely to have evolved in physiological, ontogenic, and behavioral responses to the threat of desiccation such as the ability to accelerate development to metamorphosis (Leips et al. 2000). However, several potential costs associated with accelerating development during the larval life-history stage have been detected. In frogs, accelerating larval development may lead to a smaller size at metamorphosis which may influence the chances of surviving to maturity during the terrestrial life-history stages (Smith 1987) and affect the maturation phenotype such as size and age at maturity (Altwegg and Reyer 2003). The maturation phenotype can in turn directly influence reproductive success and fecundity (Halliday and Tejedo 1995).

Although the survival benefits of accelerated development are clear in the case of drying ponds, the genetic basis underlying such responses to selection pressures are still poorly understood. Quantitative genetic techniques are now commonly employed to reveal the amount of genetic variability in traits that are expressed during development and the genetic relationships between these traits (Roff 1996, 1997; Collins et al. 1998; Jang and Greenfield 2000; Dziminski et al. 2008). Considerable phenotypic variability in amphibian larval traits has been detected, even in frog species that reside in permanent water bodies (Travis et al. 1987), and subsequent quantitative genetic studies have detected considerable additive genetic variation in these traits (Berven 1987; Newman 1988; Sommer and Pearman 2003). Additive genetic variation refers to the amount of variation that is attributable to allelic differences between individuals (Falconer and Mackay 1996). Heritability, which is defined as additive genetic variance divided by phenotypic variance, determines the extent to which genetic differences contribute to differences in a phenotypic trait (Falconer and Mackay 1996).

In southwestern Australia, *Crinia georgiana* (Anura; Myobatrachidae) inhabits temporary pools that are extremely variable in terms of size and duration (Doughty and Roberts 2003). Pair formation in this species is characterized by male acoustic signaling and female phonotaxis (Gerhardt et al. 2000; Jang et al. 2011; Yoo and Jang 2012). However, polyandrous matings often occur when intruding males

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join mating pairs (Byrne and Roberts 1999; Roberts et al. 1999). *C. georgiana* breeds during the winter months (June to September) and deposits eggs in small temporary pools where the larvae often face desiccation (Doughty 2002; Doughty and Roberts 2003). There is a high variability in egg size both within and between clutches, independent of female phenotype in *C. georgiana,* which may have evolved in response to the unpredictability in pond drying (Dziminski and Roberts, 2006). A quantitative genetic study found significant nonadditive genetic effects on larval fitness traits, but no additive genetic effects were found on these traits (Dziminski et al. 2008). Eggs are usually laid in discrete clumps (Seymour and Roberts 1995), and tadpoles can survive to metamorphosis without feeding (Byrne and Roberts 2000). Thus, the natural history of *C. georgiana* suggests strong selection for rapid development. In this study, we investigated the genetic basis of embryonic and larval life-history traits in the myobatrachid frog, *C. georgiana* using quantitative genetic techniques. Findings of this study are discussed within the context of adaptation to highly variable and unpredictable environmental conditions.

Materials and methods

Population studied and experimental pairing

From 23 June to 3 July 2000 adult frogs were collected from two localities in southwestern Australia, Kangaroo Gully and Boulder Rock, which were 3 km apart. The study areas were described thoroughly by Byrne and Roberts (1999, 2000), Roberts et al. (1999), Doughty (2002), Doughty and Roberts (2003), Smith and Roberts (2003a, 2003b, 2003c), and Smith et al. (2003). At both sites frogs breed in the numerous temporary shallow pools (up to 30 cm deep) that form around the edge of granite outcrops which are in turn surrounded by eucalypt forests (Roberts et al. 1999).

We used a half-sib breeding protocol (Becker 1984; Falconer and Mackay 1996) to estimate genetic parameters. A male (sire) was mated with three randomly selected female frogs (dam). The male was placed with a female in a 500-ml plastic container with about 50 ml of filtered and deionized water. Males were then washed down with water and similarly paired with the second and then the third female. The remating time for males ranged from one hour to one day. No male was kept in the lab for more than three days, and female frogs were only mated once. All pairs produced fertilized eggs within one hour.

Eggs were housed singly in 500-ml plastic containers with 250 ml of filtered and deionized water until metamorphosis. The containers were placed into eight shelves in a randomized order. The rearing room was kept under a 12:12 h light:dark photoperiod, and the temperature was maintained with an 18:15°C cycle between the light and dark photoperiod. The diet for tadpoles proportionally consisted of 3.5 parts of "Prepact" rabbit and guinea pig pellets, 1.5 parts of "Tetramin Tropical Fish Food", and a tablet of "Bob Martin" vitamin and mineral supplement (Smith and Roberts 2003c). These diet components were ground and sieved through a 0.2-mm mesh. The tadpoles were fed 10 mg of food twice every week until metamorphosis. Water was changed weekly.

Genetic parameter estimation

For the half-sib breeding experiment, 27 males and 81 females were used for mating, and five eggs were randomly chosen from each mating. Twenty three tadpoles died during the course of the experiment: one tadpole from each of 19 full-sib families and two tadpoles from each of two full-sib families. Thus, the breeding experiment consisted of 81 full-sib families and 381 offspring (F_1).

Traits measured for estimation of genetic parameters were egg size, hatching period, larval period, snout-vent length (SVL) at metamorphosis, and weight at metamorphosis. Egg size was measured with a Leica MZ6 dissection microscope ($\pm$ 0.01 mm) within 1.5 hours of fertilization. Each egg was placed in a Petri dish with 70 ml of water. The egg was rotated and measured three times and the largest diameter was recorded. Hatching period was the number of days from egg fertilization to hatching. Larval period was the number of days from hatching to metamorphosis, which was defined as complete tail resorption (Gosner 1960). The metamorphs were gently blotted dry and weighed to the nearest milligram. Metamorphs were then placed under a plastic sheet with 0.01-mm grids printed on it, and their SVLs were measured under a Leica MZ6 dissection microscope.

Because all traits were not severely deviated from the normal distribution (skewness $\leq$ 0.96), untransformed values of traits were used for statistical analyses (see Table 1). A nested analysis of variance (ANOVA)

Table 1. Descriptive statistics of the embryonic and larval traits that were measured from *Crinia georgiana*. $N = 381$.

	Mean	SD	Min	Max
Egg size (mm)	2.168	0.1502	1.9	3.2
Hatching period (day)	13.33	1.709	3	21
Larval period (day)	54.28	8.339	40	88
SVL at met. (mm)	6.845	0.6100	4.8	9.0
Weight at met. (g)	0.0310	0.0077	0.0112	0.0607

met., metamorphosis.

for unequal sample sizes was used (Sokal and Rohlf 1995) to estimate genetic parameters. The nested ANOVA partitions the phenotypic variance between sires, between dams within sires, and within dams. The degree of resemblance between sibs is the between-sire variance and estimates one-fourth of the heritability. We used WOMBAT (Meyer 2007), which employs restricted maximum likelihood (REML), to estimate heritability values. REML is widely used for analyses of continuous traits and uses a likelihood function calculated from a transformed set of data (Meyer 2007). Two linear mixed effects models were fitted for the larval life-history data: (1) with dam as a random effect and (2) without dam (Wilson et al. 2009). Comparison between these two models provides estimates of additive and non-additive genetic effects on larval life-history traits. We also estimated additive genetic covariance among larval life-history traits. Because WOMBAT does not provide capacity to determine significance of estimates, we judge significance of heritability estimates when an estimate was larger than twice the standard error of the estimate. To assess the significance of the inclusion of the dam random effect, we compared the final log-likelihoods between models (with and without the dam random effect) with the chi-square test statistics with one degree of freedom (Wilson et al. 2009).

Results

Body size of adults

Adults of *C. georgiana* captured in the field for this study showed no sexual size dimorphism. Both SVL ($SVL_{Sire} = 35.63 \pm 5.070$ mm, $N = 27$; $SVL_{Dam} = 35.22 \pm 2.966$ mm, $N = 81$; $t = 0.396$, $P = 0.695$, two-tailed t test with unequal variance) and weight ($Weight_{Sire} = 4.07 \pm 1.730$ g, $N = 27$; $Weight_{Dam} = 4.00 \pm 1.029$ g, $N = 81$; $t = 0.214$, $P = 0.832$, two-tailed t test with unequal variance) did not differ between the sexes. The ranges of adult SVL in both sexes were also similar to those reported in a previous field survey by Smith and Roberts (2003c), which showed no sexual size dimorphism in this species.

Phenotypic correlations

Phenotypic variation in all measured traits was high (Table 1). Analyses of phenotypic correlations between parent and offspring traits revealed that the overall body size of dams, measured by SVL and weight, was significantly correlated with all offspring traits, except for SVL at metamorphosis (Table 2a). The strength of these phenotypic correlations was ordered, from strongest to weakest, as egg size, hatching period, larval period, and weight at metamorphosis which also reflected a positive association with time from hatching. Sire weight was correlated with larval period only.

Both hatching period and larval period were significantly and negatively correlated with egg size (Table 2b). In other words, individuals from eggs with larger yolks tended to hatch sooner and reached metamorphosis quicker. Significant and positive phenotypic correlations were also detected between hatching period and egg size and between SVL and weight at metamorphosis. Weight at metamorphosis was negatively correlated with both hatching period and larval period. This suggests that larger metamorphs had shorter hatching and larval periods.

Table 2. Pearson's product–moment correlation coefficients between (a) parent and offspring traits and among (b) offspring traits ($N = 381$).

	SVL_{Dam}		$Weight_{Dam}$		SVL_{Sire}		$Weight_{Sire}$	
(a)								
Egg size	**0.451**	<0.001	**0.467**	<0.001	−0.002	0.967	−0.017	0.747
Hatching period	**−0.230**	<0.001	**−0.140**	0.006	0.001	0.980	0.032	0.528
Larval period	**−0.211**	<0.001	**−0.179**	<0.001	0.099	0.053	**0.113**	0.027
SVL at met.	0.079	0.123	0.075	0.143	−0.006	0.911	−0.024	0.639
Weight at met.	**0.123**	0.016	**0.106**	0.038	0.019	0.705	0.001	0.979
	Egg size		Hatching period		Larval period		SVL at met	
(b)								
Hatching period	**−0.221**	<0.001						
Larval period	**−0.193**	<0.001	**0.430**	<0.001				
SVL at met.	0.031	0.547	−0.054	0.295	−0.003	0.958		
Weight at met.	0.066	0.196	**−0.124**	0.015	**−0.149**	0.004	**0.851**	< 0.001

Note: Correlation coefficients are presented with significance probabilities. Values in boldface are P values < 0.05.

Estimation of genetic parameters

Results of the nested ANOVA showed that sire significantly affected all traits measured (Table 3). Dam also had a significant effect for all traits except for larval period. The heritability estimates with both sire and dam included as random effects were not significant for all traits (Table 4). However, the heritability estimates with only sire included as a random effect were significant for egg size, hatching period, and both SVL and weight at metamorphosis, suggesting a strong nonadditive genetic effect. In other words, with the exception of larval period, the inclusion of the dam random effect reduced the additive genetic variation estimates, indicating similarity among maternal siblings. We also found no significant additive genetic covariance among larval life-history traits in *C. georgiana* (Table 5).

Discussion

Our quantitative genetic study of *C. georgiana* using a half-sib breeding design revealed no significant additive genetic variances in any of the larval fitness traits, once maternal effects were accounted for (Tables 4 and 5). Our results are consistent with another quantitative genetic study of *C. georgiana* that utilized a cross-classified breeding design to simulate stressful conditions (Dziminski et al. 2008). The feeding regime used by Dziminski et al. (2008) was 2 mg of food per tadpole every 3 days, whereas we fed 5 mg of food per tadpole every 3.5 days. Tadpoles in the more "stressful" conditions (e.g., less food; Dziminski et al. 2008) reached metamorphosis 14.52 days sooner and were 30% lighter at metamorphosis than in this study. Hence, when compared, these two studies suggest that tadpoles of *C. georgiana* can respond to varying environmental conditions, despite a lack of additive genetic variation in larval traits. However, we do stress that these two studies were conducted under different experimental conditions and consequently, any comparisons are speculative. Nonetheless, if we are to learn more about the response of frogs to environmental variability, continued study of additive genetic variation in species like *C. georgiana* under a range of stressful conditions, including limited water availability or presence of predators, is warranted because quantitative genetic measures are only valid in the environmental condition in which they are measured (Hoffmann and Parsons 1991; Charmantier and Garant 2005).

Interestingly, unlike *C. georgiana*, significant heritability estimates were detected in larval fitness traits in other amphibian species. For example, larval period has significant additive genetic variance in *Rana sylvatica* (Berven 1987), *Rana temporaria* (Uller et al. 2002; Sommer and Pearman 2003), *Hyla crucifer* (Travis et al. 1987), and *Scaphiopus couchii* (Newman 1988). Of these, the last two species typically experience unpredictable environmental conditions often facing desiccation.

Several traits had significant nonadditive genetic variance (Table 4) which suggests considerable similarity among maternal siblings. In addition, the significant phenotypic correlations between egg size and both dam size and weight at metamorphosis could be indicative of a maternal influence (Laugen et al. 2002). Studies that incorporate designs that are better suited to examine maternal effects on egg size and other life-history variables such as size and weight at

Table 3. Results of nested ANOVA of offspring traits.

Source	Trait	*df*	MS	*F*	*P*
Sire	Egg size	26	0.12	22.38	>0.001
	Hatching period	26	6.24	2.60	>0.001
	Larval period	26	114.79	1.71	0.019
	SVL at met.	26	0.76	2.59	>0.001
	Weight at met.	26	0.01	2.21	0.001
Dam	Egg size	54	0.07	12.61	>0.001
	Hatching period	54	4.18	1.74	0.002
	Larval period	54	63.17	0.94	0.591
	SVL at met.	54	0.61	2.06	>0.001
	Weight at met.	54	0.01	2.09	>0.001
Error	Egg size	300	0.01		
	Hatching period	300	2.40		
	Larval period	300	67.00		
	SVL at met.	300	0.30		
	Weight at met.	300	0.01		

Note: In a half-sib breeding design, each sire ($N=27$) was mated with three dams ($N=81$). Five eggs were randomly selected for rearing in each full-sib female.

Table 4. Heritability estimates of larval life-history traits in *C. georgiana*. We used two linear mixed effects models to estimate heritabilities based on sire and dam ($N=381$).

	Heritability $\pm$ SE		
Trait	Sire random effect only	Sire and dam random effect	Significance of dam effect
Egg size	**0.985 $\pm$ 0.221**	0.478 $\pm$ 0.413	$P<0.10$
Hatching period	**0.349 $\pm$ 0.110**	0.181 $\pm$ 0.177	ns
Larval period	0.103 $\pm$ 0.074	0.103 $\pm$ 0.121	ns
SVL at met.	**0.407 $\pm$ 0.117**	0.099 $\pm$ 0.177	$P<0.10$
Weight at met.	**0.374 $\pm$ 0.116**	0.013 $\pm$ 0.163	$P<0.10$

Note: Heritability values that are greater than twice of SE (e.g., assumed to be significant at $P<0.05$) are emphasized in boldface.

Table 5. Additive genetic covariances between larval life-history traits ($COV_A \pm SE$) in *C. georgiana* ($N = 381$).

	Egg size	Hatching period	Larval period	SVL at met
Hatching period	-0.197 ± 0.200			
Larval period	-0.125 ± 0.165	0.079 ± 0.115		
SVL at met.	-0.026 ± 0.187	-0.065 ± 0.126	-0.040 ± 0.105	
Weight at met.	0.019 ± 0.187	-0.069 ± 0.127	-0.044 ± 0.106	0.097 ± 0.170

metamorphosis (Semlitsch and Schmiedehausen, 1994; Laugen et al. 2002; Pakkasmaa et al. 2003) are needed to test this possibility.

We detected a negative phenotypic correlation between larval period and weight at metamorphosis. From an evolutionary perspective, this is of particular interest because the metamorphic phenotype not only reflects the larval environment, but can also influence survival and fitness during the terrestrial life-history stages in amphibians. Previous studies have shown that age and size at metamorphosis can influence survival and both age and size at maturity in frogs (Altwegg and Reyer 2003). Although no additive genetic variation in larval period was detected, small metamorphic size coupled with the considerable scope for postmetamorphic growth suggest that *C. georgiana* may be a particularly appropriate species to examine the effects of the metamorphic phenotype on the adult phenotype.

An important aspect of the mating behavior of *C. georgiana* with regard to larval development is the occurrence of simultaneous multiple-male matings (Byrne and Roberts 1999, 2000; Roberts et al. 1999). Polyandry can be thought of as a bet hedging strategy. Most of the fertilization success is shared between the focal ventral and dorsal males (Byrne and Roberts 1999, 2000; Roberts et al. 1999). By mating with multiple males, females could increase their overall fitness in unpredictable environmental conditions by increasing the degree of genetic variability in their offspring (Perreault et al. 1997). Furthermore, there is evidence that interactions between male and female haplotypes play an important role in the successful combinations of sperm and eggs at fertilization (Dziminski et al. 2008). However, a study by Byrne and Roberts (2000) failed to detect any genetic benefits to multiple paternity in the form of larval performance in clutches from single and multiple (two males) fathers in *C. georgiana*.

Here we show that information on the heritability of embryonic and larval life-history traits is critical in understanding how frog populations might respond to selection in unpredictable conditions and to understand the adaptive significance and maintenance of phenotypic variation in larval life-history traits. Such studies also help to better explain the existence of evolutionary mechanisms such as simultaneous multiple-male matings. Finally, the results reported here broaden the taxonomic breadth of studies that are concerned with the genetics of life-history traits in frogs which are currently restricted to only a few taxa.

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References

Abrams PA, Leimar O, Nylin S, Wiklund C. 1996. The effect of flexible growth rates on optimal sizes and development times in a seasonal environment. Am Nat. 147:381–395.

Altwegg R, Reyer H-U. 2003. Patterns of natural selection on size at metamorphosis in water frogs. Evolution 57:872–882.

Becker WA. 1984. Manual of quantitative genetics. 4th ed. Pullman (WA): Academic Enterprises.

Berven KA. 1987. The heritable basis of variation in larval developmental patterns within populations of the wood frog (*Rana sylvatica*). Evolution 41:1088–1097.

Berven KA. 1990. Factors affecting population fluctuations in larval and adult stages of the wood frog (*Rana sylvatica*). Ecology 71:1599–1608.

Byrne PG, Roberts JD. 1999. Simultaneous mating with multiple males reduces fertilization success in the myobatrachid frog *Crinia georgiana*. Proc R Soc Lond B. 266:717–721.

Byrne PG, Roberts JD. 2000. Does multiple paternity improve fitness of the frog *Crinia georgiana*? Evolution 54:968–973.

Charmantier A, Garant D. 2005. Environmental quality and evolutionary potential: lessons from wild populations. Proc R Soc Lond. B 272:1415–1425.

Collins RD, Jang Y, Reinhold K, Greenfield MD. 1998. Quantitative genetics of ultrasonic advertisement signalling in the lesser waxmoth, *Achroia grisella* (Lepidoptera: Pyralidae). Heredity 83:644–651.

Darwin C. 1871. The descent of man, and selection in relation to sex. London: John Murray.

Doughty P. 2002. Coevolution of developmental plasticity and large egg size in *Crinia georgiana* tadpoles. Copeia 2002:928–937.

Doughty P, Roberts JD. 2003. Plasticity in age and size at metamorphosis of *Crinia georgiana* tadpoles: responses to variation and dete. Aust J Zool. 51:271–278.

Dziminski MA, Roberts JD. 2006. Fitness consequences of variable maternal provisioning in quacking frogs (*Crinia georgiana*). J Evol Biol. 19:144–156.

Dziminski MA, Roberts JD, Simmons LW. 2008. Fitness consequences of parental compatibility in the frog *Crinia georgiana*. Evolution 62:879–886.

Falconer DS, Mackay TFC. 1996. Introduction to quantitative genetics. Essex (UK): Longman.

Gerhardt HC, Roberts JD, Bee MA, Schwartz JJ. 2000. Call matching in the quacking frog (*Crinia georgiana*). Behav Ecol Sociobiol. 48:243–251.

Gosner KL. 1960. A simplified table for staging anuran embryos and larvae with notes and identification. Herpetologica 16:183–190.

Halliday T, Tejedo M. 1995. Intrasexual selection and alternative mating behaviour. In: Heatwole H, Sullivan BK, editors. Amphibian biology. Chipping Norton (NSW): Surrey Beatty & Sons. p. 419–468.

Hoffmann AA, Parsons PA. 1991. Evolutionary genetics and environmental stress. New York (NY): Oxford University Press.

Holenweg AK, Reyer HU. 2000. Hibernation behavior of *Rana lessonae* and *R. esculenta* in their natural habitat. Oecologia 123:41–47.

Jang Y, Greenfield MD. 2000. Quantitative genetics of female choice in an ultrasonic pyralid moth, *Achroia grisella*: variation and evolvability of preference along multiple dimensions of the male advertisement signal. Heredity 84:73–80.

Jang Y, Hahm EH, Lee H-J, Park S, Won Y-J, Choe JC. 2011. Geographic variation in advertisement calls in a tree frog species: gene flow and selection hypotheses. PLoS ONE 6:e23297.

Laugen AT, Laurila A, Merila J. 2002. Maternal and genetic contributions to geographical variation in *Rana temporaria* larval life-history traits. Biol J Linn Soc. 76:61–70.

Leips J, McManus MG, Travis J. 2000. Response of treefrog larvae to drying ponds: comparing temporary and permanent pond breeders. Ecology 81:2997–3008.

Meyer K. 2007. WOMBAT-A tool for mixed model analyses in quantitative genetics by restricted maximum likelihood (REML). J Zhejiang Univ Sci B. 8:815–821.

Newman RA. 1988. Genetic variation for larval Anuran (*Scaphiopus couchii*) development time in an uncertain environment. Evolution 42:763–773.

Pakkasmaa S, Merila J, O'Hara RB. 2003. Genetic and maternal effect influences on viability of common frog tadpoles under different environmental conditions. Heredity 91:117–124.

Perreault S, Lemon RE, Kuhnlein U. 1997. Patterns and correlates of extrapair paternity in American redstarts (*Setophaga ruticilla*). Behav Ecol. 8:612–621.

Roberts JD, Standish RJ, Byrne PG, Doughty P. 1999. Synchronous polyandry and multiple paternity in the frog *Crinia georgiana* (Anura: Myobatrachidae). Anim Behav. 57:721–726.

Roff DA. 1996. The evolution of genetic correlations: an analysis of patterns. Evolution 50:1392–1403.

Roff DA. 1997. Evolutionary quantitative genetics. New York (NY): Chapman & Hall.

Semlitsch RD, Schmiedehausen S. 1994. Parental contributions to variation in hatchling size and its relationship to growth and metamorphosis in tadpoles of *Rana lessonae* and *Rana esculenta*. Copeia 1994:406–412.

Semlitsch RD, Scott DE, Pechmann JHK. 1988. Time and size at metamorphosis related to adult fitness in *Ambystoma talpoideum*. Ecology 69:184–192.

Seymour RS, Roberts JD. 1995. Oxygen uptake by the aquatic eggs of the Australian frog *Crinia georgiana*. Physiol Zool. 68:206–222.

Smith DC. 1987. Adult recruitment in chorus frogs: effects of size and date at metamorphosis. Ecology 68:344–350.

Smith MJ, Roberts JD. 2003a. An experimental examination of female preference patterns for components of the male advertisement call in the quacking frog, *Crinia georgiana*. Behav Ecol Sociobiol. 55:144–150.

Smith MJ, Roberts JD. 2003b. Call structure may affect male mating success in the quacking frog *Crinia georgiana* (Anura: Myobatrachidae). Behav Ecol Sociobiol. 53: 221–226.

Smith MJ, Roberts JD. 2003c. No sexual size dimorphism in the frog *Crinia georgiana* (Anura Myobatrachidae): an examination of pre- and postmaturational growth. J Herpetol. 37:132–137.

Smith MJ, Withers PC, Roberts JD. 2003. Reproductive energetics and behavior of an Australian myobatrachid frog *Crinia georgiana*. Copeia 2003:248–254.

Sokal RR, Rohlf FJ. 1995. Biometry. 3rd ed. New York (NY): Freeman.

Sommer S, Pearman PB. 2003. Quantitative genetic analysis of larval life history traits in two alpine populations of *Rana temporaria*. Genetica (Dordrecht) 118:1–10.

Travis J, Emerson SB, Blouin M. 1987. A quantitative-genetic analysis of larval life-history traits in *Hyla crucifer*. Evolution 41:145–156.

Uller T, Olsson M, Stahlberg F. 2002. Variation in heritability of tadpole growth: an experimental analysis. Heredity 88:480–484.

Wilson AJ, Reale D, Clements MN, Morrissey MM, Postma E, Walling CA, Kruuk LEB, Nussey DH. 2009. An ecologist's guide to the animal model. J Anim Ecol. 79:13–26.

Yoo E, Jang Y. 2012. Abiotic effects on calling phenology of three frog species in Korea. Anim Cell Syst. 16:260–267.