

Characterising patterns of genetic variation in desiccation tolerance in embryos of the terrestrial-breeding frog, *Pseudophryne guentheri*

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Amphibians have experienced global declines for various reasons (Alford & Richards 1999; Lawler *et al.* 2010), and southwest Australian amphibians are especially susceptible to environmental changes. This region has experienced a substantial decline in rainfall since the 1970s (Smith 2004) and, even in optimistic scenarios, future projections indicate this region will become drier and warmer (Bates *et al.* 2008). Wet conditions are essential to the successful development of terrestrial frog embryos as the outer capsule of their eggs is almost completely permeable to water (Bradford & Seymour 1988; Mitchell 2002). A drying climate could produce small, under-developed, or deformed tadpoles, which may be competitively disadvantaged, have decreased swimming performance, and be at a higher risk of predation (Andrewartha *et al.* 2008). Under human-induced climate change, populations risk extinction unless they can respond adaptively to new selection pressures (Bates *et al.* 2008; Gienapp *et al.* 2008). Additive genetic variance, governed purely by parental alleles, determines the heritability of a trait, and effectively reflects the ‘evolvability’ of a population (Falconer & Mackay 1996; Lynch & Walsh 1998). The expression of additive genetic variance may differ widely depending on the environmental conditions under which it has been estimated, due to genotype-environment interactions (Lynch & Walsh 1998). Finding a low level of additive genetic variation in tolerance traits would imply that there are limited possibilities for adapting to climate change (Hoffmann *et al.* 1995; Merilä 2009).

The aim of this study was to provide estimates of phenotypic variance in traits of *Pseudophryne guentheri* (Myobatrachidae) hatchlings, developed under a range of optimal and stressful environments, to gain a better understanding of the genetic and environmental mechanisms of variation. A cross-classified breeding experiment was used to generate full-siblings, paternal half-siblings, and maternal half-siblings under controlled environmental conditions, to disentangle sources of variance in offspring traits and enable controlled tests of genotype by environment effects (Lynch & Walsh 1998). Embryos were subjected to one of three water-stress treatments, and variances in survival, time taken to hatch after inundation, and hatchling morphology were attributed to the effects of the male, female, treatment, or interactions between these factors. Significant similarity between paternal half-siblings was used as an indication of the presence of heritable variation, because this covariance corresponds to pure additive genetic variance. Interactions between genetic factors and environmental treatment can show evidence of differential adaptation to stress, and indicate that heritable variation estimates are dependent on environmental conditions (Lynch & Walsh 1998).

Lower soil water availability during embryo development had significant negative effects on all hatchling traits examined, including survival rate, and therefore may exert strong selective pressure simply via reduced embryonic survival (Räsänen & Kruuk 2007). Maternal influences

were significant across traits, consistent with previous studies that have recognised maternal effects as an important source of phenotypic variation in amphibian hatchling traits (Räsänen *et al.* 2003; Merilä *et al.* 2004). Maternal provisioning, through variation in yolk content or quality, or egg maturity, is an important source of early amphibian fitness and can also increase adult fitness, and will often play a large role in facilitating population persistence under increasing environmental stress (Räsänen & Kruuk 2007). Only tadpole shape was found to have a heritable component of variation, though the potential for selection on shape was not changed under stressful conditions. Therefore, no evidence was found for water-stress tolerance in shape variation among genotypes.

Genotype-by-environment interactions indicated that variance component estimates for the time it took tadpoles to hatch after being inundated were dependent on the environmental conditions under which they were measured. Non-additive genetic effects (e.g. genetic compatibilities) had a significant effect under benign conditions; however, under more stressful conditions, the main effects of the male and female were seen instead. This result may indicate that there are greater adaptive benefits of maternal provisioning under more stressful conditions, while genetic interactions may become more significant under benign conditions (Räsänen & Kruuk 2007). No heritable variation was detected for survival of the embryos; thus, it is clear that a drying climate will likely have negative consequences on *P. guentheri* recruitment. As minimal heritable variation was observed overall, along with large environmental variances, it is expected that there will be limited adaptation opportunities in this population, especially under a rapid rate of environmental change (Sgrò *et al.* 2010).

This study highlights the importance to further research conservation methods which concern increasing genetic diversity, such as genetic translocation. Genetic translocation involves introducing genes from one population to another, and has been suggested by NCCARF's 'National Adaptation Research Plan for Terrestrial Biodiversity' as a potential tool to increase the resilience of isolated populations to environmental change. In translocations, it will be critical to know whether the genetic variation within a source population is sufficient to sustain the required selection response during rapid environmental change. Local adaptation to water-stress is necessary to even begin to consider genetic translocation as a viable conservation tool, and this was not found in this study. However, levels of genetic variation, and therefore the potential for adaptive evolution, may not be uniform among populations throughout a species' ranges (Etterson 2004). As heritable variation may differ between populations and can also depend on environmental conditions (Hoffmann *et al.* 1995), it is necessary to study this species across populations and environmental conditions, and through generations, to reveal consistent patterns produced by specific conditions, and therefore potential influences on evolutionary rates. Comparing across populations, including those near the edge of this species' range, would be useful to further investigate the potential of local adaptation.

This study may be considered a critical starting point upon which to build an assessment of the potential of genetic translocation to increase resilience in frog populations that will experience progressively drier years under climate change. The results could potentially inform the management of other amphibians, such as the closely related Corroboree frog (*P. corroboree*) from alpine Australia, a highly endangered species that is also likely to experience systematic drying of its habitat under climate change (NSW National Parks & Wildlife Service 2001).

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