Informing compensatory habitat creation with experimental trials: a 3-year study on a threatened amphibian

JOSE W. VALDEZ, KAYA KLOP-TOKER, MICHELLE P. STOCKWELL, LOREN FARDELL
SIMON CLULOW, JOHN CLULOW and MICHAEL J. MAHONY

SUPPLEMENTARY MATERIAL Laboratory methods

To determine whether breeding events occurred amongst released *Litoria aurea* individuals at the trial site, we genotyped adults and tadpoles from our breeding population. We sampled 37 adults, obtaining biopsies from the webbing between the second and third digits of the right foot. It was difficult to determine the parentage of captive-bred tadpoles, as multiple breeding events occurred between several males and females, sometimes simultaneously, in a community breeding tub. Therefore, we sampled 10 tadpoles for every female present (n=100) in the breeding facility. Twelve days after egg masses were laid, tissue samples were removed from their tail tips (c. 2 mm of the tip) using sterile scissors. Tail tips were then stored in 80% ethanol, and tadpoles were placed in a holding tank for monitoring and recovery.

For captive-bred individuals we used a Puregene DNA isolation kit (Gentra Systems, Minneapolis, USA), following the manufacturer's protocol for DNA purification of solid tissue. For unknown *L. aurea* tadpoles or juveniles found at the trial site, a modified glass fibre extraction protocol (Ivanova et al., 2006) was used. Analyses were conducted at the Australian Genome Research Facility in Adelaide, Australia. We amplified samples using eight previously identified microsatellite primers (DeBoo et al., 2012), and microsatellite profiles were scored using *Genemapper 3.7* (Applied Biosystems, Foster City, USA). Based on previous genotyping research on *L. aurea*, we assumed a genotyping error rate of 1.27% per allele.

For unknown offspring found at the trial site, we determined ancestry by determining relatedness with potential parental clutch (captive-bred released individuals) and grandparents (captive breeding adults). Individuals that are more closely related have a higher pairwise relatedness (r) than individuals that are more distantly related. The relative difference in pairwise relatedness between (a) offspring descended from our released individuals and (b) offspring from wild, unsampled parents would be higher or lower if we sampled both ancestry situations. If we sampled offspring exclusively from either set of descendants (a) or (b) we could not infer probable ancestry.

Pairwise relatedness estimates were calculated with *GeneAlEx 6.5* (Peakall & Smouse, 2006; Peakall & Smouse, 2012), using the mean of the Lynch & Ritland (1999) methods. Individuals that failed to amplify at more than four loci were excluded from the analysis. A total of 37 captive breeding adults, 84 released offspring and nine unknown offspring found at the trial site were used in our analysis. There was an approximately 50:50 split between positive and negative pairwise relatedness scores between released offspring and captive breeding adults, which was assumed to be normal for this population. Deviations from this trend when comparing unknown offspring to the captive breeding adults and released individuals were considered to be evidence that unknown offspring may not have descended from released individuals, and probably descended from the local wild population.

Results

Juveniles that were too young to have been part of the release programme and did not possess visible implant elastomer tags were found in the unfenced portion of the trial plot in the second year. Relatedness estimates identified two unknown juveniles as likely descendants of our released individuals. High and low levels of relatedness between the unknown offspring indicate several clutches are present. Furthermore, we found high pairwise relatedness between several individuals but relatively low pairwise relatedness between others, which is indicative of half siblings, and one individual successfully breeding with several in the wild.