Science for Saving Species

Research findings factsheet

Project 4.1.6.1

Threatened Species Recovery Hub

National Environmental Science Programme

Testing assisted gene flow for terrestrial-breeding frogs in a drying climate

In brief

Assisted gene flow (AGF) is an emerging strategy for helping threatened populations to adapt to climate change by introducing genetic material from other populations that have desirable traits.

We tested the ability of AGF to improve the capacity of crawling frogs to survive drier future climate conditions, through cross-breeding frogs from different populations in wetter and drier parts of the species range in the laboratory, and assessing how their offspring tolerated development on dry soils.

The crosses revealed very varied results; one cross showed increased tolerance to drier soils, while other crosses showed neutral results, or negative results where offspring developed abnormally.

We recommend more testing to tease out the causes of positive and negative outcomes in crosses before applying it to manage wild populations.

This research is relevant to other terrestrial-breeding frog species, such as Critically Endangered northern and southern corroboree frogs.

Background

Amphibians are very vulnerable to climate change. Around a third of amphibian species worldwide have declined or become extinct since 1970. These global declines have been caused by a range of factors such as habitat fragmentation, disease, increased UV-B radiation and introduced species, the effects of which are compounded by climate change; but climate change also directly impacts amphibian populations by causing their physiological tolerances to be breached, and by reducing the ability of populations to disperse.

Crawling frogs (Pseudophryne guentheri) *are vulnerable to climate change. Image: Nicki Mitchell*

For many species of amphibian, shifting their distribution to more favourable climates is not possible due to habitat fragmentation. Their capacity to adapt will depend on their ability to evolve to suit their new environments, which in turn will depend on the level of genetic variation within a population on which natural selection can act. If there is heritable variation in traits that promote tolerance to changes in their microclimates, short-lived species like amphibians can evolve rapidly.

Projections of the impacts of climate change on ecological communities and species have most commonly been based on studies of the effects in single locations. However, individuals and populations vary, sometimes very widely, within species.

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Background (continued)

It may not be valid, therefore, to assume that populations will respond in the same way to climate change. In particular, looking at the environmental sensitivities of populations at the edge of the range will be vital to understanding how species' distributions might change as the climate changes, as it is at range edges where colonisations and local extinctions take place.

For many amphibian species, climate change will be experienced as increasing temperatures, decreasing rainfall and more frequent and severe droughts. This will likely have a negative effect on both aquatic- and terrestrial-breeding frogs, as breeding success and dispersal are tightly associated with the presence of water.

Frogs in the Australian *Pseudophryne* genus lay their eggs in shallow burrows in the soil. Moist soils keep the embryos hydrated and rainfall eventually floods the burrows, which initiates hatching and allows tadpoles to feed and to complete metamorphosis. The effects of climate change are apparent across the continent-wide distribution of this genus but are particularly severe in the south-west of Australia.

Over the past 40 years, this bioregion has experienced substantial declines in the autumn and winter rainfall that promotes successful frog breeding, and it is projected that temperature and evaporation will continue to increase while the availability of rainfall and soil water will further decrease.

This research looked at variation in drying tolerance in crawling frogs *Pseudophryne guentheri*, one of 17 species of terrestrial-breeding amphibians found only in Australia's south-west. Crawling frogs are an ideal model for studying amphibian responses to climate change, due to their broad distribution across a rainfall gradient, and because of evidence of a high degree of variation in embryos and adults to withstand dry conditions.

The species now consists of numerous isolated populations due to extensive clearing of habitat in the Western Australian wheatbelt, and its genetic diversity has been found to have declined due to the effects of this fragmentation, and possibly also due to increasingly frequent droughts. If the populations remain isolated, we would expect to see further declines in genetic diversity, which would make the populations even less able to adapt to changing environments or to evolve resistance to introduced diseases.

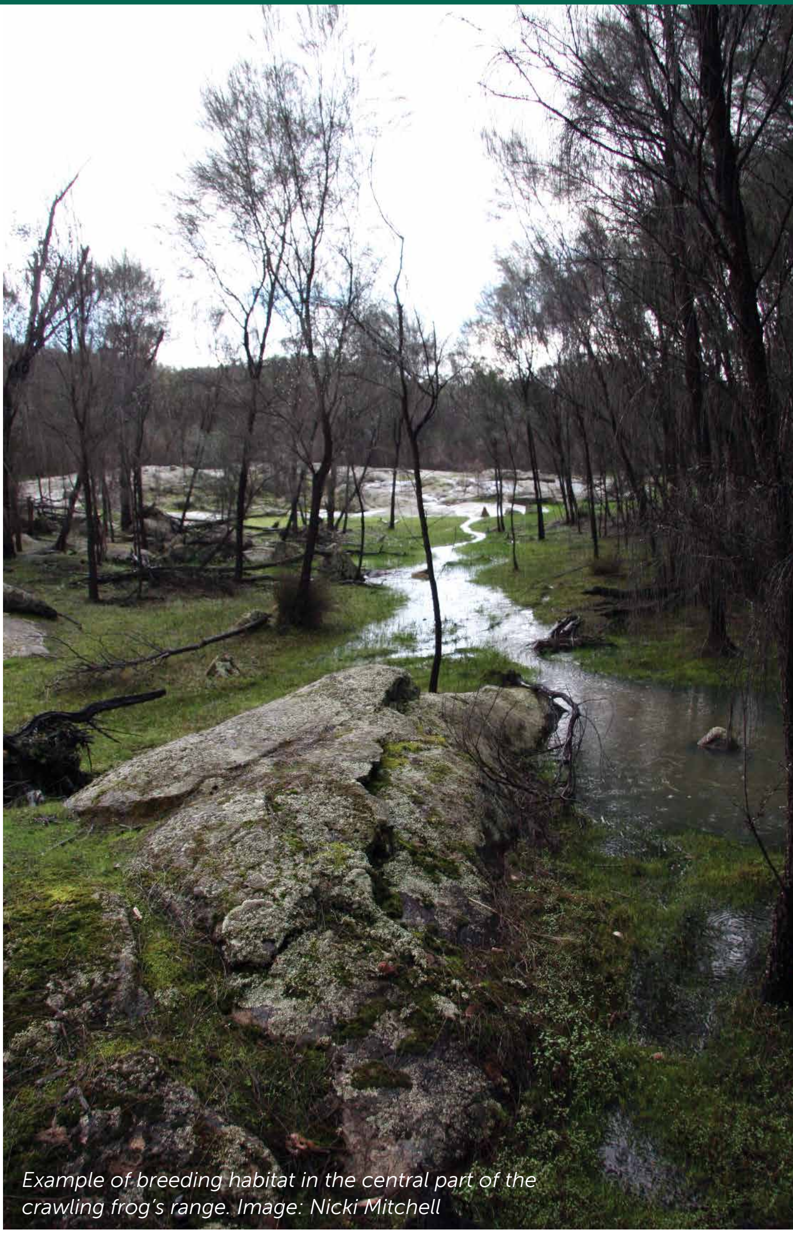
Assisted gene flow (AGF; also known as targeted gene flow, TGF) is an emerging strategy for bolstering the adaptive potential of isolated populations threatened by climate change. It can take advantage of naturally occurring geographic variation in environmental tolerance to increase the genetic variation of recipient populations in a desired direction. Testing of AGF is so far lacking for amphibians.

Main aim of the research

The aim of this research was to test whether assisted gene flow (AGF) could increase the capacity of crawling frogs to tolerate future climate conditions, through cross-breeding different populations.

Male crawling frogs construct shallow breeding burrows where embryos develop. Image: Nicki Mitchell

Example of breeding habitat in the drier parts of the crawling frog's range. Image: Nicki Mitchell



Example of breeding habitat in the central part of the crawling frog's range. Image: Nicki Mitchell

What we did

We collected adult crawling frogs from populations in areas with rainfall varying from 300 to 1250 mm per year and assessed how quickly they were able to dehydrate and rehydrate (see Figure 1). We also measured how their embryos (unhatched eggs) responded to development on soils with different moisture content.

Next, we investigated variation in male reproductive traits from crawling frog populations from different areas.

Because rainfall cues breeding activity in frogs, including in crawling frogs, the timing and duration of breeding seasons differ among geographically separated populations. This can promote local differences in sperm traits that have the potential to influence the outcomes of AGF efforts.

We found dramatic differences in sperm morphology and swimming performance across the populations that we sampled.

Finally, we tested the outcomes of AGF for crawling frogs in a laboratory environment.

We reciprocally crossed four populations, two from the drier northern edges of its range, and two from the moister higher rainfall southern centre of its range; reared embryos on wet and dry soils; and quantified a range of fitness traits upon hatching related to their tolerance to development on drier soils.

BELOW: *Tabitha conducting dessication tolerance experiments in the laboratory. Image: Emily Hoffmann*

Key findings

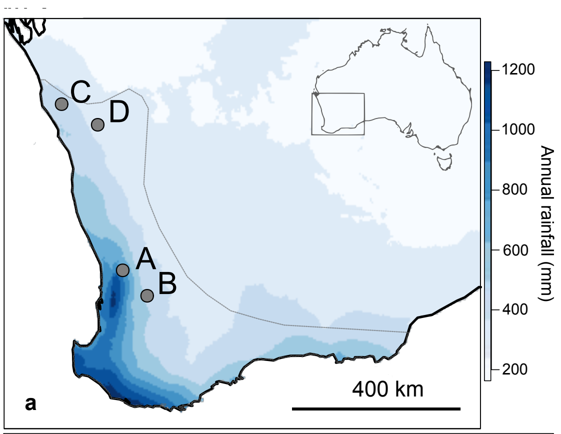
Our findings strongly suggest that different crawling frog populations will respond differently to the regional reduction in rainfall projected across the species' range.

We found significant variation between individuals on almost all traits associated with tolerance for dryness, both in adult males and in their first-generation offspring.

Adult males from different areas showed differences in how they lost and gained water. Populations from drier sites showed a greater tolerance to desiccating conditions, losing and gaining water more slowly than frogs from moister sites. The first-generation offspring from drier sites also showed greater tolerance to drier conditions in the laboratory experiments.

The results of cross-breeding experiments, which we conducted one year later, provide the first insights into factors that will influence AGF outcomes in amphibians.

The laboratory crossings of isolated frog populations resulted in diverse outcomes for traits linked to desiccation tolerance. Figure 2 shows the populations crossed and whether outcomes were positive (blue arrow), neutral (grey arrow) or negative (orange arrow). Arrow direction shows where sperm originated from, and the differences in the sperm images reflect that sperm morphology was different in northern and southern populations.



Population	A	B	C	D
		Distance (km)		
A	–	90	456	380
B	0.196	–	540	458
C	0.310	0.278	–	109
D	0.330	0.304	0.126	–

b (F_{ST})

Figure 1. a) locations of breeding sites in southwestern Australia from which adult crawling frogs were collected for crossing experiments, overlaid onto the annual rainfall gradient; b) matrix of pairwise geographic and genetic distances between populations where higher values and darker shading indicate greater separation; c) a gravid female; d) an adult male

Extinction risk may be greater than currently recognised for the crawling frog. Image: Nicki Mitchell

Crawling frog tadpoles. Image: Marion Anstis

Key findings (continued)

All crosses between populations from the northern drier range-edge (C or D) and a populations several hundred kilometres south (A or B) had negative outcomes. Populations from the northern and southern distribution edges of this species are separated by a maximum distance of around 460 km and showed a high level of genetic divergence. Embryonic survival was very low when eggs from a southern population were fertilised with sperm from a northern population, suggesting that these populations are in effect reproductively isolated.

Crosses between the two southern populations had a neutral and a positive outcome depending on which way they were crossed (see Figure 2). When sperm from A were crossed with eggs from B the outcome was neutral, but when sperm from B were mixed with eggs from A the offspring showed increased tolerance to drying compared to within-population (i.e., AA or BB) crosses. Crosses between two northern populations (sperm from D with eggs from C), were neutral. We did not test sperm from C with eggs from D due to not encountering females when we visited site D.

These results show that the outcomes of AGF in the crawling frog depend on the geographic and genetic distances between the source and recipient population, and also, more surprisingly, on the direction of the cross, with sperm from B males and eggs from A females producing the most favourable cross. Specifically, this version of the AB cross featured higher mass for tadpoles, better swimming performance, faster time to hatching and fewer hatchling malformations compared to AA and BB offspring.

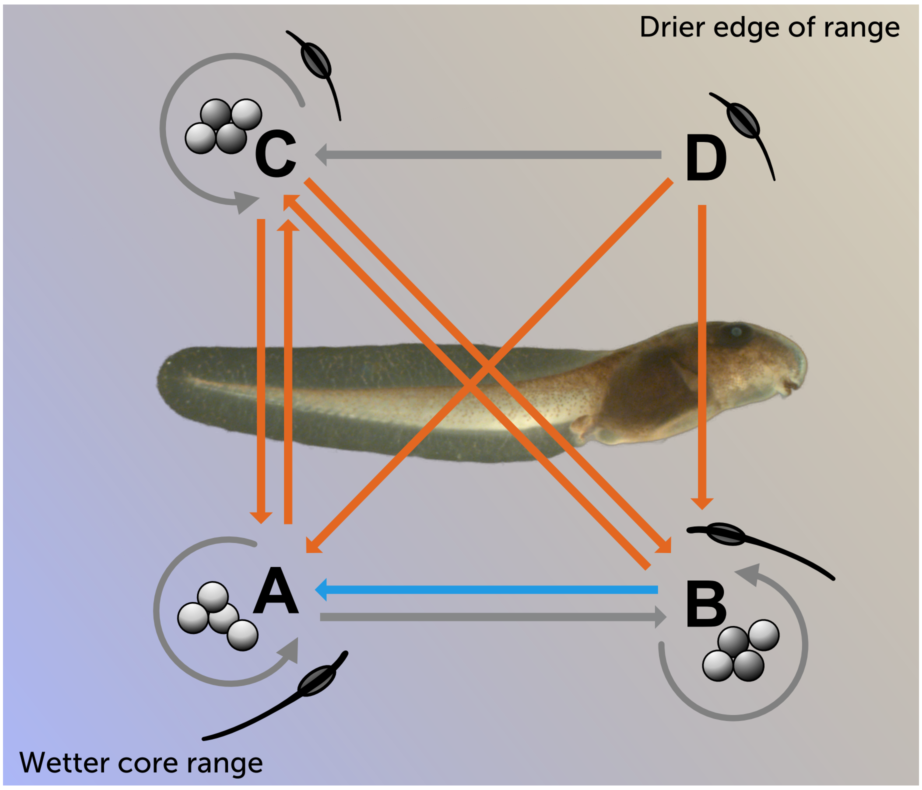


Figure 2. Summary of the effects of male (population) origin on overall offspring fitness in crawling frog hatchlings originating from crosses with females from populations A, B or C. Orange = negative effects, blue = positive effects and grey = neutral effects

These traits are likely to provide fitness benefits in a drying climate, for example, faster hatching times can allow larvae to be washed into stable pools of standing water where they can feed and seek refuge. Furthermore, faster development rates allow embryos to hatch sooner if flooded, reducing risks such as predation or fungal infection of eggs. Finally, traits such as malformations and body size affect swimming performance at hatching, which in turn influences the ability to avoid predators and forage efficiently.

Outside the laboratory setting, where there is potential for sperm competition, sperm from "foreign" males may be less competitive for fertilisations relative to sperm from local males. Hence, to achieve the positive effects of AB crosses in AGF in wild populations, it may be best to artificially fertilise egg clutches taken from wild females with sperm from populations with the target traits for greater tolerance to drying conditions, rather than directly introduce males to these populations. Fortunately, the sperm of crawling species can be stored for several weeks and can remain viable, so this is a practical solution. Eggs stripped from females, however, only have a few hours of viability before fertilisation success declines.

Finally, these results support the view that the risks of cross-breeding (e.g., low embryonic survival and high rates of malformation) increase with genetic, geographic and environmental distance.

Collectively, our findings highlight the need for laboratory trials as an important step before AGF is used to support the adaption of increase the genetic diversity of threatened wild frog populations.

Implications and recommendations

All parts of the crawling frog's range in south-west Western Australia are drying, but this species has clearly adapted to different rainfall regimes, including its seasonality. Limited gene flow between isolated populations means that much of the variation in physiological traits that exists at the species level is not available to populations threatened by climate change.

Range-edge populations are generally thought to harbour the greatest amount of a species' adaptive variation and could be important for targeted conservation to reduce extinction risk as the climate dries. Given the finding that some populations of the crawling frog are more tolerant to desiccation stress than others, this research shows that AGF has the potential to be strategically employed to enhance the resilience of populations in drying regions.

We recommend multi-generational studies to separate out the factors responsible for the effects of tolerance for drier environments in some crosses. It is difficult to disentangle whether the increased tolerance for dryness in AB offspring was the result of introduction of genes that promote tolerance for dryness, or due to favourable interactions between the nuclear genome (transferred via eggs and sperm) and the mitochondrial genome (transferred via eggs only).

Finding out will matter a great deal for determining whether AGF will be a useful tool in frog conservation. We therefore recommend further empirical tests of AGF in suitable model species and, when practical, the monitoring of long-term consequences.

There is a growing urgency to explore AGF in threatened Australian frog species to target adaptive traits. The *Pseudophryne* genus includes some of Australia's most imperilled and charismatic frog species, such as the Critically Endangered northern and southern corroboree frogs (*P. pengileyii* and *P. corroboree*), which are being considered for a form of AGF to improve resistance to the chytrid fungus.

While crawling frogs are not listed as threatened, our work showed that they are subject to population isolation, decline and inbreeding, suggesting that the species may be at greater risk of extinction than is currently recognised.

A female crawling frog. Image: Nicki Mitchell

Cited material

Rudin-Bitterli, T.S., Evans, J.P., Mitchell, N.J. 2021. Fitness consequences of targeted gene flow to counter impacts of drying climates on terrestrial-breeding frogs.*Communications Biology*. In press.

Rudin-Bitterli, T. S., Evans, J. P., & Mitchell, N. J. 2020. Geographic variation in adult and embryonic desiccation tolerance in a terrestrial-breeding frog. *Evolution*, 74(6), 1186-1199. https://doi.org/10.1111/evo.13973

Rudin-Bitterli, T.S., Mitchell, N.J., Evans, J.P. 2020. Extensive geographical variation in testes size and ejaculate traits in a terrestrial-breeding frog. *Biology Letters*. 16:20200411. http://dx.doi.org/10.1098/rsbl.2020.0411

Cummins, D., Kennington, W.J., Rudin-Bitterli, T.S., Mitchell, N.J. 2019. A genome-wide search for local adaptation in a terrestrial-breeding frog reveals vulnerability to climate change. *Global Change Biology*, 00:1–13. DOI: 10.1111/gcb.14703

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