Targeted gene flow for conservation:

northern quolls and the invasive cane toad

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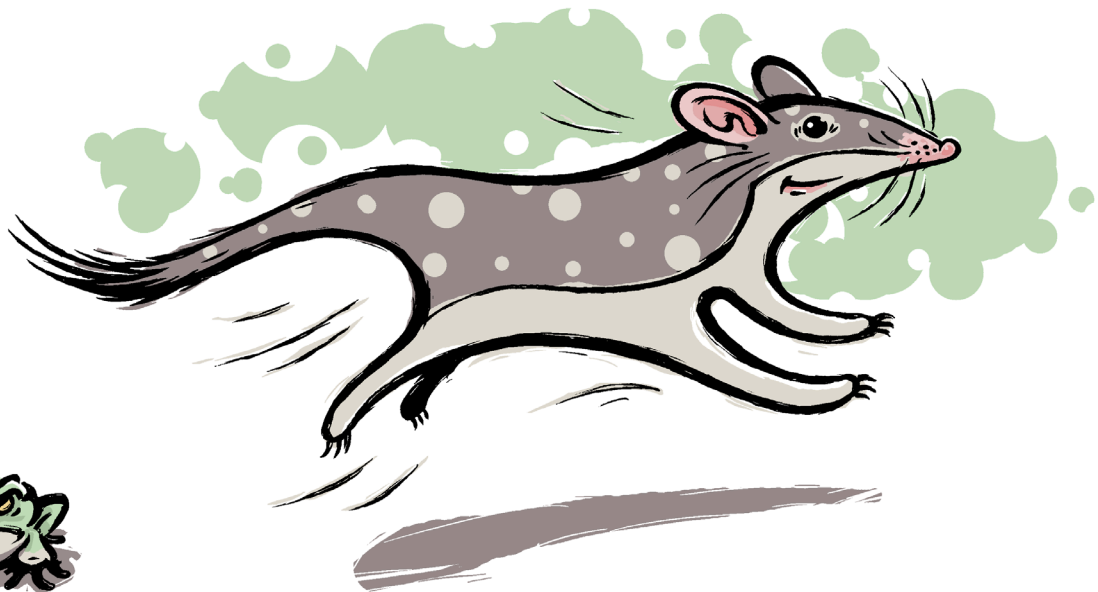
Abstract

GLOBAL biodiversity is declining at an unprecedented rate. Within declining populations, however, there are some individuals who are able to survive the threat. Unfortunately in many cases these adaptive traits are not common enough to prevent extinction, particularly when threats are rapid and severe. But by understanding how species respond to certain threats conservationists may be able to boost adaptive potential in threatened populations. Targeted gene flow is a novel conservation tool that involves moving individuals with relevant traits to areas where they could be beneficial for conservation. Although the implications are wide reaching, this idea is yet to be attempted on a wild population.

In this thesis, I set out to test the feasibility of targeted gene flow as a conservation tool, using the endangered northern quoll (*Dasyurus hallucatus*) as a model species. Northern quolls have experienced dramatic declines since the introduction of the invasive cane toad (*Rhinella marina*) because the quolls unsuspectingly attack the toxic toads. There are, however, a small number of remnant quoll populations that have survived the toad invasion, seemingly because they do not attack toads. It is this potential "toad-smart" behaviour I hoped to harness using targeted gene flow. If it was possible to breed toad-smarts into still threatened areas of the northern quoll's range, managers could boost adaptive potential and population survival.

The first step was to understand how some individuals could survive alongside toads. In the preliminary chapters of this thesis, I examine toad-exposed northern quolls to see how they react to cane toads. I found that quolls from areas invaded by cane toads were indeed toad-smart – they didn't attack toads. Using a common garden experiment, I then demonstrated this toad-smart behaviour had a heritable basis, meaning I could potentially breed the trait into threatened populations.

The next step was to explore how best to implement targeted gene flow for quolls, including investigating any potential negative impacts. I used population

modelling to explore the optimal timing and number of individuals introduced to maximise population survival whilst maintaining species-level genetic diversity. I then set up an experimental field trail, releasing both toad-smart and toad-naïve northern quolls onto a toad-infested island. Despite unforeseen circumstances that resulted in a dramatic reduction in population size, I was able to demonstrate no negative implications of targeted gene flow from the first stage of the experiment.

This thesis shares the process of exploring a new conservation strategy, from initial conception to field trials. I provide evidence that targeted gene flow could reverse declines of northern quoll populations – demonstrating a genetic basis for toad-smart behaviour, showing little evidence of outbreeding depression, and presenting the ideal management approach for implementing the tool in threatened populations. The resulting strategy is not limited to northern quolls, but instead has widespread applications for other threatened populations. Even the most endangered populations often have some individuals who are resistant to a threat. If conservationists can understand and harness these adaptive traits, targeted gene flow could prove an invaluable tool for conserving threatened species.

Declaration page

This is to certify that:

    (i)   the thesis comprises only my original work towards the Doctor of Philosophy except where indicated in the Preface,

    (ii)   due acknowledgement has been made in the text to all other material used; and

    (iii)   the thesis is fewer than 100,000 words in length, exclusive of tables, maps, bibliographies and appendices.

Signed: Date: 18th November 2018

Preface

I have structured this thesis into seven chapters, comprising of an introductory chapter and six data chapters. The introductory chapter sets up the central question of the thesis, which the following chapters address and answer. Chapters 2-6 are peer-reviewed manuscripts that have been or (in the case of Chapter 6) are in the process of being published, details of which are listed below. As a result, each chapter is a standalone work, with enough detail so they can be viewed independently to the rest of the thesis. I am the primary author and principle contributor on all published chapters. They are all co-authored by my supervisor, Ben Phillips, who contributed to the conception, experimental design and manuscript preparation for the studies. Jonathon Webb also contributed to the experimental design and manuscript preparation of Chapter 5. The final chapter, Chapter 7, reports on the first stage of a field trail that was always meant to run beyond the timeline of my PhD. The intention of Chapter 7 is to present preliminary data from this field trial, as well as bring together the thesis narrative and present concluding remarks.

CHAPTER CONTRIBUTIONS:

Chapter 2

Kelly, E and Phillips, BL (2016) Targeted gene flow for conservation. Published by *Conservation Biology* on 1st April 2016.

Kelly E Conceived and developed ideas and wrote manuscript.

Phillips BL Conceived and developed ideas and contributed manuscript preparation.

Chapter 3

Kelly, E and Phillips, BL (2017) Get smart: native mammal develops toad-smart behaviour in response to a toxic invader. Published by *Behavioural Ecology* on 1st May 2017.

Kelly E Conceived and developed ideas, designed and conducted experiments, preformed analysis and wrote manuscript.

Phillips BL Conceived and developed ideas, assisted in experiment design, analysis and manuscript preparation.

Chapter 4

Kelly, E and Phillips, BL (2018) Targeted gene flow and rapid adaptation in an endangered marsupial. Published by *Conservation Biology* on 13th June 2018.

Kelly E Conceived and developed ideas, designed and conducted experiments, preformed analysis and wrote manuscript.

Phillips BL Conceived and developed ideas, assisted in experiment design, analysis and manuscript preparation.

Chapter 5

Kelly, E, Phillips BL and Webb JK (2018) Taste overshadows less salient cues to elicit food aversion in endangered marsupial. Published by *Applied Animal Behaviour Science* on 25th September 2018.

Kelly E Conceived and developed ideas, designed and conducted experiments, preformed analysis and wrote manuscript.

Phillips BL Assisted in experiment design, analysis and manuscript preparation.

Webb JK Assisted in development of ideas and manuscript preparation.

Chapter 6

Kelly, E and Phillips, BL (2019) How many and when? Optimising targeted gene flow for a step change in the environment. Published by *Ecology Letters* on 7th January 2019.

Kelly E Conceived and developed ideas, wrote and executed model and wrote manuscript.

Phillips BL Conceived and developed ideas, assisted in model writing and contributed to manuscript preparation.

by

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Chapter 1
Introduction

Changing practices in a changing world

ANTHROPOGENIC environmental change is causing unprecedented declines in global biodiversity (Barnosky *et al.* 2011; Ceballos *et al.* 2015). Habitat destruction has led to dramatic declines in available habitat and increases in fragmentation (Haddad *et al.* 2015). Global human colonisation has facilitated the spread of invasive species and disease (Altizer *et al.* 2003; Clavero & García-Berthou 2005). Anthropogenic climate change is already beginning to effect the planet, with further change predicted to come (Travis 2003). All this change is leading to mounting numbers of threatened species, hanging on in increasingly isolated populations. Adaptation to these changes is often not fast enough to allow threatened species to persist: many populations are, or will soon be, dangerously maladapted to their environment (Hoffmann & Sgrò 2011; Sih *et al.* 2011).

In this landscape of rapid environmental change, conservationists race to develop new methods to protect threatened species (Johnson *et al.* 2017). Previously, biodiversity conservation focused on maintaining local genetic diversity – or "evolutionary significant units" – and managing populations in isolation (Moritz 1999). The aim of this guiding principle was to preserve as much species diversity as possible, maintain local adaptations, and conserve significant populations that are genetically unique (Petit *et al.* 1998). In increasingly fragmented landscapes, however, this approach is becoming less feasible (Ralls *et al.* 2017). Small isolated populations suffer losses of genetic diversity, meaning they are at risk of lower fitness from inbreeding depression

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as well as reduced adaptive potential to future changes to their habitat (Bijlsma *et al.* 2000; Edmands 2007).

Thus, conservation focus has turned to increasing genetic variation within populations, and rescuing isolated populations from the impacts of inbreeding depression (Frankham *et al.* 2017; Ralls *et al.* 2017). In many cases this can be achieved relatively easily, by introducing individuals from neighbouring populations or subspecies (Frankham 2015; Whiteley *et al.* 2015). This strategy, termed genetic rescue, was famously successful in helping the critically endangered Florida panther bounce back after the population had reached less than 30 individuals (Hedrick & Fredrickson 2010). Conservationists introduced eight female panthers from the Texan subspecies to the Florida population. The introduced panthers bred with the locals, producing fitter hybrids by masking deleterious alleles. This boosted genetic diversity and ultimately led to population recovery (Frankham 2015).

Since the trial on the Florida panther in 1995, calls to use genetic rescue to conserve isolated populations of threatened species have risen (Hedrick *et al.* 2011; Weeks *et al.* 2017). In response, there have been concerns raised around crossbreeding individuals from different gene-pools, primarily due to cultural values and fears of outbreeding depression (Love Stowell *et al.* 2017; Ralls *et al.* 2017). Recent work, however, suggests the risk of outbreeding depression – where crossing genetically distinct individuals produces hybrids who are less fit than purebred individuals – has been overstated (Frankham *et al.* 2011). Generally, the fitness costs from allelic incompatibilities is transient and outweighed by the benefits that genetic rescue provides. Loss of local adaptation is also usually a minor and manageable issue, particularly as small isolated populations are often maladapted to their local environments already, due to genetic drift (Frankham *et al.* 2017; Ralls *et al.* 2017).

As the current environment becomes increasingly mismatched with the historical environment, conservationists begin to consider tactics for helping species adapt. Instead of arbitrarily increasing genetic diversity through genetic rescue, an idea formed to introduce specific individuals who are adapted to the future environment of the site. This concept – assisted gene flow – is being explored to combat the impacts of climate change (Aitken & Whitlock 2013). It involves the translocation of warm-adapted individuals within a species' range to areas that are anticipating warming to reduce maladaptation in threatened populations (Aitken & Whitlock 2013). Assisted gene flow is being promoted to rescue plant populations threatened by climate change who show adaption along climatic gradients (Vitt *et al.* 2010; Aitken & Bemmels 2016), and also is currently being considered as a strategy to buffer the Great Barrier Reef from warming (Dixon *et al.* 2015; Van Oppen *et al.* 2014).

Climate change, however, is not the only process impacting threatened populations. There are many threatening processes leading to declines in biodiversity, but at the same time there are often also individuals within threatened populations that can survive these threats (Stockwell *et al.*

2003). It is important to recognise how standing genetic variation within a threatened species may influence how that species responds (Bolnick *et al.* 2003, 2011). Generally, even among widespread declines, not all individuals respond identically to a threat. Potentially, you could apply the same concept of artificially increasing adaptive potential – as is envisaged in assisted gene flow for climate change – to threatened populations more generally: through targeted translocations of pre-adapted individuals.

A more targeted approach?

This new concept involves purposefully translocating individuals who carry a favourable trait to areas of a species' range where that trait is useful (Kelly & Phillips 2016; Frankham *et al.* 2017). The idea, targeted gene flow, is a generalisation of assisted gene flow. It is not only applicable to combating climate change, but to any threatening process where trait variation can be brought to bear (Stockwell *et al.* 2003; Sih *et al.* 2011). This can include promoting an adaptive trait in a population that is threatened by more immediate impacts, such as the arrival of a disease or invasive species. Alternatively, targeted gene flow could also be used to promote traits for lowered dispersal in populations that conservationists wish to contain (i.e. genetic backburn, where targeted gene flow aims to reduce the dispersal ability of an invasive species; Phillips *et al.* 2016). These wide-ranging applications are only just beginning to be explored, and in this thesis I will be focusing on one potential application: promoting adaptive traits in threatened populations. By selecting individuals that are adapted to a certain threat and translocating them to areas that are about to meet that threat, it may be possible to bolster declining populations and increase their adaptive potential (Ralls *et al.* 2017).

The aim of promoting adaptive traits through targeted gene flow is to not only decrease a threatened population's probability of extinction, but to also help maintain species-level genetic diversity (Reed & Frankham 2003). By introducing pre-adapted genes to threatened populations, we would expect these genes to integrate with the local genome. Once the population came under selection (after the threat arrived), hybrids carrying both adaptive genes and the local genome would survive, maintaining both the (introduced) adaptive genes, as well as (local) pre-existing genetic variation and local adaptations. This conservation of species-level genetic diversity, and therefore adaptive potential, is extremely important in a world experiencing rapid environmental change (Jump *et al.* 2009; Schierenbeck 2017). Threatening processes do not occur in isolation, so species need to be able to adapt to unpredictable future threats – all else being equal, having a higher genetic diversity increases the chance that adaptation will occur (Whiteley *et al.* 2015).

Although there have been some examples of the use of genetic rescue and assisted gene flow (Hedrick & Fredrickson 2010; Vitt *et al.* 2010; Aitken &

Bemmels 2016; Weeks *et al.* 2017), the idea of targeted gene flow has yet to be explored in a field setting. New theories need to be thoroughly tested prior to implementation. This is particularly important for conservation strategies, that are to be used in complicated environmental settings (Kujala *et al.* 2013; Game *et al.* 2014). Predicting outcomes in complex ecosystems is extremely difficult – and translocations are inherently risky and often unpredictable (Fischer & Lindenmayer 2000; Miller *et al.* 2014). When translocating animals, in particular, we need to be sure we will achieve anticipated outcomes, as well as avoid any negative consequences to our actions (Weeks *et al.* 2011; IUCN/SSC 2013). As a result, thorough testing of targeted gene flow is required before we can be sure of its usefulness as a tool for conservation.

A model system: northern quolls and cane toads

To test the idea of targeted gene flow, I needed a case study. This required a threatened species that had variation in a trait that allowed them to survive a threat. I needed to be able to measure that trait, as well as examine its fitness benefits and heritability, in a relatively short time frame. The invasion of cane toads (*Rhinella marina*) across northern Australia presented the ideal situation for achieving this. The cane toad was introduced to Far North Queensland in 1935 and continues to spread across northern Australia (Shine 2010; Tingley *et al.* 2017). Cane toads have now invaded the Northern Territory and into Western Australia, and will likely colonise the rest of the Kimberly within the next decade (Kearney *et al.* 2008). Conservationists and land managers are continually looking for ways to stop the toad invasion and remove them from their current range – but, as is common for invasive species, this is proving extremely difficult (Saunders *et al.* 2010). As a consequence, there are now northern ecosystems that have been: long exposed to toads; recently invaded; and predicted to be invaded in the coming years.

The arrival of the cane toad has dramatically altered northern Australian ecosystems, having a particularly strong impact on native predators (Shine 2010; Llewelyn *et al.* 2014). Cane toads are poisonous, excreting a powerful cardiac toxin from glands on their backs. As Australia has no native toxic anurans, our predators unknowingly attack the toads and have uniformly low resistance to the poison (Ujvari *et al.* 2013). This has led to drastic declines in native predatory species in areas that the toads have invaded. Species impacted by toxic toads include freshwater crocodiles, elapid snakes, varanid and scincid lizards, and dasyurids – all of which experience population crashes following toad arrival (e.g. Phillips 2004; Woinarski *et al.* 2008; Letnic *et al.* 2008; Jolly *et al.* 2016). No species has yet gone extinct due to the cane toad, however, and there is mounting evidence that impacted species are beginning to adapt.

Adaptations range from morphological (snakes developing smaller jaws so they cannot ingest lethal sized toads; Phillips & Shine 2004) to behavioural (raptors who only consume non-lethal parts of the toads; Beckmann & Shine 2011). Some species are simply beginning to ignore toads as a prey item all together (Webb *et al.* 2008; Somaweera *et al.* 2011; Llewelyn *et al.* 2014; Ward-Fear *et al.* 2017).

These instances of rapid adaptation to an invasive species are encouraging, but not entirely unsurprising (Mooney & Cleland 2001). The selective pressure that toads place on populations is potentially extremely strong – any individual who attacks a toad is immediately removed from the gene pool. If there is genetic variation in traits that mediate individual survival (e.g. a trait that stops them attacking toads), these surviving individuals will pass on these adaptive genes to following generations. It is this combination of genetic variation and selection that leads to adaptation, however this must occur prior to the population going extinct (thus, there is a race between adaptation and extinction; Gomulkiewicz & Holt 1995). Unfortunately, adaptation does not occur rapidly enough in many threatened populations of native predators, likely because adaptive traits are not in high enough proportions in declining populations. As a result, after toad arrival most predator populations experience rapid declines, range retractions and loss of genetic diversity (Woinarski *et al.* 2008; Llewelyn *et al.* 2014). Left alone, native predators may be able to persist in small, isolated populations following the toad invasion, but this leaves them increasingly vulnerable to future threats (Jump *et al.* 2009).

Potentially, we could promote traits that help threatened species survive alongside cane toads through targeted gene flow, bolstering threatened populations and conserving genetic diversity. The predictable trajectory of the cane toad invasion means we could promote adaptive potential in populations prior to cane toad arrival (Urban *et al.* 2007; Kearney *et al.* 2008), if we could identify heritable traits that help predatory species survive cane toads. For this, we would need to return to Queensland, where toads were first introduced, and find populations which have adapted to the presence of toads (Woinarski *et al.* 2008). If we could identify the trait that helped these small remnant populations survive, we could potentially use it to promote adaptation in soon to be invaded populations, or help species return to areas where they have gone locally extinct. To test this idea, we needed a model species.

The northern quoll (*Dasyurus hallucatus*; Figure 1.1) is one of Australia's native predators threatened by the arrival of the cane toad (Woinarski *et al.* 2008). Northern quolls are marsupial mesopredators and members of the Dasyuridae family (Schmitt *et al.* 1989; Oakwood 1997). They are the smallest of the four quolls species found in Australia, weighing up to 1300g (Oakwood 1997). Northern quolls are short-lived, breeding annually with offspring becoming fully mature by the following breeding season (Oakwood 2000). Females can breed for multiple seasons, but males often experience a "die-off" (characteristic of smaller dasyurids), where they lose condition following the breeding season

Figure 1.1. A male northern quoll (photo: Ella Kelly)

and generally do not survive to the next (Dickman & Braithwaite 1992; Oakwood *et al.* 2001). Northern quolls are opportunistic feeders, consuming a range of insects, amphibians, small mammals and, sometimes, fruit and plant matter (Dunlop *et al.* 2017). Being nocturnal, they generally spend the day in dens (tree hollows, rock crevices, logs or other available burrows) and forage overnight. They have been found in a wide variety of habitats, including rocky escarpments, savanna woodlands, monsoon rainforests and close to human settlements (Braithwaite & Griffiths 1994; Oakwood 2000). In areas where their range has reduced, however, they will often become restricted to higher quality habitat with topographic complexity and rocky refugia (Woinarski *et al.* 2008).

Prior to European settlement, northern quolls were distributed over much of northern Australia (Figure 1.2). Their range has since contracted due to a combination of habitat destruction, inappropriate fire regimes, and the impacts of mammalian predators (dingoes and feral cats; Hill & Ward 2010). As with other native predators, they have experienced further declines since the arrival of cane toads, which they attack and are immediately poisoned by (Cremona *et al.* 2017). As a consequence, northern quolls are now listed as nationally endangered and critically endangered in the Northern Territory (EPBC 1999). Eventually, cane toads are predicted to invade the entire range of the northern quoll (Kearney *et al.* 2008), and with no current way to stop the invasion, conservationists have turned to investigating ways to help the northern quolls survive alongside toads (Cremona *et al.* 2017; Tingley *et al.* 2017; Indigo *et al.* 2018a).

One technique being trialled to protect northern quolls from toad arrival is conditioned taste aversion, where an individual learns to avoid a food-type based on a single bad experience (O'Donnell *et al.* 2010). This concept is being used to teach northern quolls to avoid cane toads by feeding an individual quoll a non-lethal portion of toad accompanied by a chemical that induces nausea (Garcia *et al.* 1985). The quoll then associates the cane toad with this bad experience and avoids attacking them thereafter. This technique has been successful in training northern quolls in captivity to avoid cane toads (O'Donnell *et al.* 2010; Indigo *et al.* 2018a), and these trained individuals survive in the short term after being released into toad-infested environments (Cremona *et al.* 2017; Jolly *et al.* 2017). But the practical implications and long-term effects of this method are still being assessed, and new evidence suggests that deploying the technique on a landscape scale is both challenging, and may not have the desired outcomes (Indigo *et al.* 2018b; Indigo *et al. unpublished manuscript*).

There are also populations of northern quolls that appear to have naturally developed this aversion to attacking cane toads – making them "toad-smart".
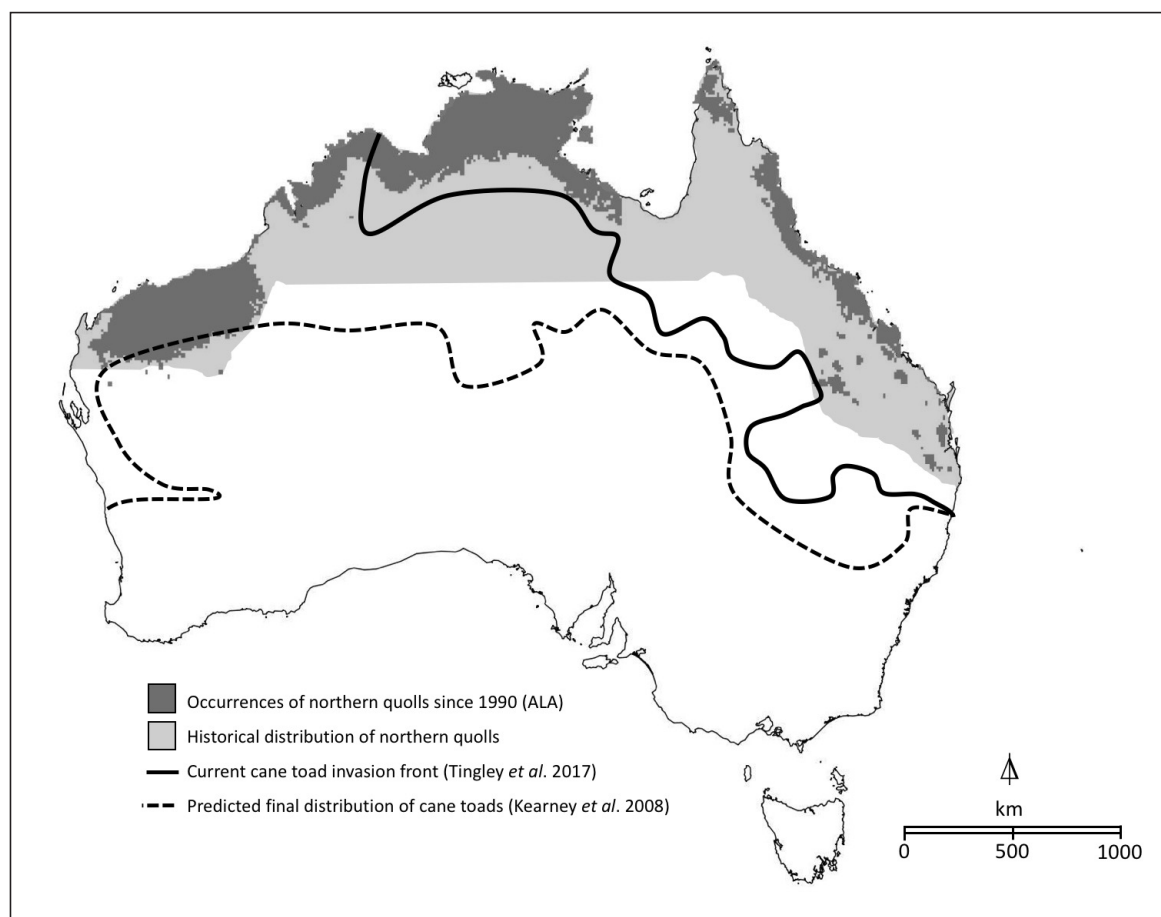
Figure 1.2. Map of Australia showing the distribution of northern quolls (light grey: historical range; dark grey: range based on occurrences since 1990) and cane toads (solid line: current range of cane toad; dotted line: approximate final extent of cane toad invasion (as predicted by Kearney et al. 2008). Distribution data from the Atlas of Living Australia (website at http://www.ala.org.au. Accessed 9 April 2018) for quolls and from Tingley et al. (2017) for toads. Figure adapted from Kelly and Phillips 2018. Note, toads have spread across the Northern Territory since 1990, so the vast majority of quoll populations indicated in the NT are likely extinct.

These remnant populations of northern quolls in Queensland have survived alongside cane toads for the past ~80 years since the initial invasion (Woinarski *et al.* 2008; Figure 1.2). Because northern quolls have no immunity to the cane toad toxin (Ujvari *et al.* 2013), these quolls must be surviving because they do not attack toads in the first place – they are toad-smart. The populations in Queensland that live alongside toads are still restricted and small. The large reduction in population size they have experienced likely also means a loss in genetic diversity, making them vulnerable to future threats or environmental changes. Despite this, these isolated Queensland populations may hold the key for northern quoll survival. If this toad-smart behaviour is heritable, it could be possible to use targeted gene flow to introduce this trait into northern quoll populations that are still under threat from cane toads.

Here, I have used the northern quoll as a model to test targeted gene flow as a conservation strategy. This unique system was an ideal model to test the theory for number of reasons. First, northern quolls are relatively fast breeders, making the experiments viable in a shorter time frame (Oakwood 2000). Second, the threat – cane toads – causes a step change in the environment, where it shifts from one state to another. I could categorically class the presence of the threat and measure its effects immediately, instead of waiting to examine a more gradual change (e.g. climate change). Finally, cane toads have presented a novel system in which to work on these ideas. Because of their predicable movement across the northern quoll's range, I am able to compare long-exposed groups to those who have never seen a toad before: ready-made experimental treatments. All this made for an ideal model species: by using a fast-moving system such as this I am able to test the broader applicability of the idea for slower moving systems (such as climate change).

Thesis objectives

In this thesis, I examine targeted gene flow as a conservation tool. In **Chapter 2**, I discuss the idea of targeted gene flow broadly, and the differences between this and other similar conservation tools. I discuss the diverse potential applications for targeted gene flow, as well as the implications and possible risks.

I then place these ideas in a real-world context, examining the use of targeted gene flow to reverse declines in northern quoll populations. Throughout the rest of the thesis I assess whether targeted gene flow could be a viable strategy for the species. There are a number of baseline criteria that need to be met for targeted gene flow to be an appropriate tool, and I address these questions in Chapters 2 and 3. Broadly, for targeted gene flow to be a viable strategy requires three minimum necessary conditions:

1. variation in the trait that allows some individuals to survive a threat;
2. the trait must be heritable; and

3. individuals carrying the trait must be able to be interbreed successfully with those from threatened populations.

Although we believed that long toad-exposed populations of northern quolls do ignore cane toads, this behaviour had never been empirically verified. Therefore, in **Chapter 3**, I examine wild-caught northern quolls from toad-exposed areas in Queensland and compare their response to toads with northern quolls from toad-free areas. This allows me to see any variation in the toad-smart trait in wild populations.

In **Chapter 4**, I then examine toad-smart behaviour in more detail – testing if the trait is heritable. To do this, I use captive bred quolls from both toad-smart and toad-naïve parents, in a common garden experimental design. This allows me to remove the environmental or learning factors associated with toad-smarts and instead just examine underlying genetic factors. In addition, by crossbreeding populations, I am able measure whether crosses are viable.

In **Chapter 5**, a chapter somewhat tangential to the central thrust of the thesis, I examine which cues the quolls are using to identify and avoid unpalatable prey. By doing so, I take a step back from examining toad-smart behaviour, and instead examine the process underlying learning responses in the quolls. Therefore, instead of using naturally toad-smart quolls, I instead use conditioned taste aversion to elicit the aversion. These results give me a better idea of the process behind toad-smart behaviour, helping me tease apart the importance of olfactory, visual and taste cues in the northern quoll's foraging behaviour.

Once I am able to show that northern quolls are indeed appropriate candidates for targeted gene flow, I set out to test the practicality of this strategy for the species. First, in **Chapter 6**, I use population viability modelling to address questions around implementing targeted gene flow. I start out by developing a generic individual based population model to investigate the broader uses of targeted gene flow for conserving species threatened by a step change in their environment. I use the model to demonstrate how these management levers can influence the outcome of targeted gene flow, both by lowering extinction probability but also maintaining local genetic diversity. I then customise this model to investigate the northern quoll example, looking at when targeted gene flow should be implemented in relation to toad arrival, and how many toad-smart quolls need to be introduced for the strategy to be effective.

Finally, I test targeted gene flow on a wild population of northern quolls. I aimed to examine the selection of toad-smart traits over multiple generations in the presence of toads. To do this, I set up an experiment that will run beyond the timeline of my thesis, and release both toad-smart and toad-naïve northern quolls onto an offshore, toad infested island. By knowing the initial population composition and using genetic analysis, I can track the genes from the two populations, and indirectly measure the selection of toad-smart traits in the presence of toads. By tracking the survival of subsequent generations of

hybrids in the wild, I am also able further measure the impact of outbreeding depression, as well as identify other unforeseen negative impacts of the strategy. In **Chapter 7**, I present the results of the first stage of this experiment – the first generation born on the island – to examine if targeted gene flow can help a northern quoll population survive alongside cane toads.

Overall, this thesis aims to address the applicability of targeted gene flow by using a logistically-attractive case study – toad-smart northern quolls. In doing so I hope not only to present targeted gene flow as a viable strategy for this endangered species, but to also demonstrate the potential broader implications. In a world experiencing rapid environmental change, novel conservation strategies such as targeted gene flow may help us improve adaptive potential of threatened populations and conserve biodiversity.

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Chapter 2
Targeted gene flow for conservation

Abstract

ANTHROPOGENIC threats often impose strong selection on affected populations, causing rapid evolutionary responses. Unfortunately, these adaptive responses are rarely harnessed for conservation. We suggest that conservation managers should pay close attention to adaptive processes and geographic variation, with an eye to using them for conservation goals. Translocating pre-adapted individuals into recipient populations is currently considered a potentially important management tool in the face of climate change. Here we point out that targeted gene flow, which involves moving favourable traits to areas where they would have a conservation benefit, could have much broader application in conservation. Across a species' range there may be long-standing geographic variation in traits, or variation may have rapidly developed in response to a threatening process. Targeted gene flow could be used to promote natural resistance to threats to increase species resilience. We suggest that targeted gene flow is a currently underappreciated strategy in conservation that has applications ranging from the management of invasive species and their impacts to controlling the impact and virulence of pathogens.

Introduction

Recently, assisted gene flow has emerged as a strategy to increase the adaptive potential of populations affected by climate change (Sgro *et al.* 2011; Aitken & Whitlock 2013; Shoo *et al.* 2013). By translocating individuals adapted to relatively warmer environments to populations adapted to colder environments, conservationists can increase the proportion of genes that match the future climate of the warming site (Weeks *et al.* 2011; Aitken & Whitlock 2013; Dixon *et al.* 2015). These pre-adapted genes act to both increase the recipient population's initial fitness in a warmer world, but also to increase the adaptive capacity of that population. As temperatures increase, warm-adapted genes are favoured by selection and so further increase in frequency within the population. Assisted gene flow utilizes the naturally occurring geographic variation across a species' range and enhances the natural rate of gene flow to artificially manipulate a recipient population's evolutionary resilience (Sgro *et al.* 2011).

Climate change, however, is not the only threatening process affecting biodiversity and causing rapid evolution (Stockwell *et al.* 2003), nor is it the only threatening process for which geographic variation exists in relevant traits. If we generalize the idea behind assisted gene flow, we can see that its aim is to exploit geographic variation in traits- moving trait variants to places where they will have a conservation benefit. While assisted gene flow increases the presumed rate of natural gene flow, there is no need to restrict ourselves to merely amplifying natural flow. A more aggressive approach can be taken by moving variants to wherever they will achieve a conservation goal. Such targeted gene flow could potentially be applied to a wide range of circumstances ranging from habitat loss and degradation, to exotic species introductions, to disease.

Targeted gene flow is only of use when it is possible to predict the trajectory of environmental change. This can be done (at least approximately) for climate change and for many other threats. Invasive species and diseases, for example, often spread in a roughly predictable way (Crowl *et al.* 2008; Kearney *et al.* 2008). Many threatening processes also worsen over time, such as accelerating climate change (IPCC 2013) and pathogens evolving to become more virulent (Hawley *et al.* 2013; Phillips & Puschendorf 2013). In all these cases, where there is geographic variation at relevant traits and we can predict a likely environmental trajectory, targeted gene flow can be implemented.

Geographic variation is universal, and in the majority of cases geographic variation reflects local adaptation (Kawecki & Ebert 2004). Across a species' range there may be long-standing geographic variation in traits (e.g., local adaptation to environmental gradients; Leimu & Fischer 2008; Hereford 2009), but the variation may also rapidly develop in response

to a threatening process. Threatening processes typically impose selection on populations even as those populations decline (e.g., Olsen *et al.* 2004). Selection strengths are expected to be highest in declining populations (Reiss 2013), and there is abundant evidence that these selection pressures often lead to rapid evolutionary shifts (Thompson 1998; Hendry & Kinnison 1999; Stockwell *et al.* 2003). Rarely, however, are threatening processes homogenous across a species' range: some populations are affected sooner, or more strongly, than others. Additionally, populations vary in their evolutionary starting positions. By chance some populations have higher initial fitness than others to the threatening process in question, and some populations go extinct. Together, geographic variation in the strength of a threat and in populations' response to that threat can lead to rapidly emergent geographic variation in adaptation (Sorte *et al.* 2011; Schiffers *et al.* 2013). Targeted gene flow can exploit this emergent geographic variation in traits by identifying well-adapted or partially adapted populations and translocating individuals from these populations to currently maladapted recipient populations.

The idea of targeted gene flow is similar to that of genetic rescue, in which populations with low genetic diversity, (consequently affected by low mean absolute fitness) have their genetic diversity bolstered by the introduction of individuals from elsewhere (Figure 2.1; Tallmon *et al.* 2004; Hedrick *et al.* 2011; Whiteley *et al.* 2015). Genetic rescue has been credited with the persistence of a number of isolated populations; recent meta-analysis shows beneficial effects of evolutionary rescue to gene flow in 91.1% of cases (Frankham *et al.* 2017). The critical difference between genetic rescue and targeted gene flow as we envision it is that with targeted gene flow we are deliberately trying to bolster genetic variation along a particular axis of selection. While genetic rescue attempts to simply increase genetic variation, targeted gene flow attempts to increase genetic variation in a direction that is relevant to the problem at hand. This biased increase in genetic variation may provide the same benefits as genetic rescue – a general increase in adaptive potential or an increase in absolute fitness in inbred recipient populations – but these are side-effects of the aim of matching trait variants to particular problems (Table 2.1).

Targeted gene flow is also similar to assisted gene flow, but the latter implies assistance of what would, given time, be the natural flow of genes. Targeted gene flow encompasses assisted gene flow but also encompasses more aggressive strategies, including the movement of particular variants to areas outside the current range. We present some examples of where targeted gene flow could be a viable conservation strategy. We acknowledge some of these ideas are speculative. Rather than developing specific actions, we seek to stimulate thought and discussion by demonstrating the breadth of potential uses for targeted gene flow.

Table 2.1. Comparison between genetic rescue, assisted gene flow and targeted gene flow.

	Targeted gene flow	Assisted gene flow	Genetic rescue
Circumstances	Populations with higher proportions of favourable genes due to rapid adaption or existing genetic	Geographic variation in genetic traits Populations with higher proportions of favourable genes due to existing genetic variation	Low genetic diversity Small isolated populations (possibly with inbreeding)
	Low/no natural gene flow and landscape connectivity	Low natural gene flow between populations	
Mechanism	Translocation within the species' current, former, or future range (including across landscape barriers)	Translocation to enhance the rate of natural gene flow amongst populations	
	Individuals are selected for translocation based on genetic makeup: Pre-adapted genes are moved to populations were they are needed/suited		Any individual can be translocated
Outcome	Increased genetic variation in a particular direction (e.g. increased adaptive capacity to respond to a threat)		Increased genetic variation in any direction
	Increased fitness of individuals leading to overall population growth		

Potential uses for targeted gene flow

Reducing vulnerability to pathogens and parasites

Host-parasite interactions exhibit a variety of coevolutionary outcomes, often with rapid evolution in both the host and the parasite (Kaltz & Shykoff 1998; Altizer *et al.* 2003). After a pathogen or parasite has been present in a landscape for some time, we would expect local co-adaptation in the genes of both host and parasite (Kaltz & Shykoff 1998) and the adaptive equilibrium to differ spatially, creating a complex geographic mosaic of coevolutionary outcomes (Gomulkiewicz *et al.* 2000). By exploiting these mosaics, targeted gene flow could be used to reduce the negative effects of pathogens and parasites – especially when there are less virulent strains evolving or there are areas where the host is becoming immune to the threat (Best & Kerr 2000; Vander Wal *et al.* 2014). This concept is already being applied to white pine blister rust. Managers are moving genetically resistant strains of white pines to control sudden outbreaks of the fungus (Kinloch 2003). Because the spread of a disease may be roughly predictable, this method can target areas that are soon to be affected by the pathogen or parasite and increase the population's evolutionary resilience once the disease hits.

Conservationists could, in principle, use remnant populations to increase overall host resilience. An example of this is currently playing out in southern Australia. An endemic predator, the Tasmanian devil (*Sarcophilus harrisii*), has declined rapidly over the past 12-15 years due to the spread of a contagious facial cancer (Hollings *et al.* 2014). There remains, however, a disease-free population of Tasmanian devils, located at the northwestern tip of Tasmania (Jones *et al.* 2007). It appears this remnant population is disease free not because it has not been exposed but because it has genetic resistance to the disease (Cheng *et al.* 2012; Hamede *et al.* 2012; Lane *et al.* 2012). Researchers are examining the genetic makeup of devils to identify variation in genes that could correlate to disease resistance in wild populations (Morris *et al.* 2015). If there are variants that drive disease immunity, these populations might provide genetic resources that can be used to secure other populations of devils (currently quarantined on offshore islands or in captive breeding programs) that are not immune to the disease (Jones *et al.* 2007). Alternatively, this genetically pre-adapted population could be used to reintroduce individuals to areas in which the species has been extirpated. Disease-free Tasmanian devils are being bred in captive breeding programs (Jones *et al.* 2007), and work is progressing on the development of a vaccine (Pinfold *et al.* 2014). If there are naturally occurring resistant alleles, a vaccine would not need to be developed (which, even if effective, would be logistically difficult to administer). Instead, resistant alleles could be introduced into the captive population through targeted gene flow. The captive population would then have the genetic diversity needed for successful reintroduction into the wild.

In the case of host-parasite systems, targeted gene flow could involve the movement of genes from hosts, pathogens, or both. A more complex and controversial possibility is the use of targeted gene flow to counteract the effects of the amphibian disease chytridiomycosis (caused by the fungal pathogen *Batrachochytrium dendrobatidis*), which has devastated amphibian populations across the globe (Fisher *et al.* 2009). Genetic analyses show substantial genetic variation within the pathogen, and a number of hypervirulent strains have emerged (Farrer *et al.* 2011; Rosenblum *et al.* 2013). The large degree of variation seen in hosts, pathogen, and the environment gives conservationists a complex coevolutionary mosaic with which to work. Some populations of amphibians persist despite the disease (Newell *et al.* 2013; Gervasi *et al.* 2014). Persistence may be in ecological refugia but may also be related to host-parasite coevolution. Certainly, after initial invasion by the fungus, we would expect to see rapid coevolutionary shifts toward lower pathogenicity because hosts are under strong selection for resistance and the fungus itself may be more transmissible at lower virulence (Brown *et al.* 2012). This rapid shift toward lower pathogenicity is exactly what occurred following the introduction of myxoma virus in Australian and British rabbit populations (Best and Kerr 2000; Kerr *et al.* 2013). In the chytrid case, rapid shifts in fungus virulence are hinted at (Farrer *et al.* 2011; Phillips and Puschendorf 2013), and there appears to be a genetic basis to disease resistance in hosts (Savage and Zamudio 2011). Thus, there may be local host-parasite systems that have evolved in this case that are stable; they do not cause the extinction of host populations. If true, targeted gene flow could help spread these stable systems across the landscape, thus decreasing extinction probability in populations that previously had not evolved resistance.

Although there has been a recent push toward applying evolutionary dynamics to wildlife disease management (Joseph *et al.* 2013; Vander Wal *et al.* 2014), the concept of using targeted gene flow to combat pathogens is a new one and not without risks. The first step for managers would be to develop a thorough understanding of the system they hope to influence. For instance, a manager would need to determine the genetic variation that relates to immunity in Tasmanian devils or understand the underlying factors that influence lower virulence of chytrid fungus (Vander Wal *et al.* 2014). Due to the complexity of host-parasite systems and the stochasticity of evolutionary dynamics, managers need to think carefully before individuals or pathogens are translocated across a landscape.

Reducing the impact of invasive species

Evidence for contemporary evolution of native species in response to invasive species is abundant (Strauss *et al.* 2006; Carroll 2007). The impact of invasive species might be diminished through the use of targeted gene flow to capitalize on these naturally occurring adaptive responses. This approach may be

particularly appropriate when invasive species are still spreading across the target species' range. Managers could translocate individuals from populations that have evolved in the presence of the invasive species to areas where the invader does not occur. This would bolster the genetic composition of populations that have yet to experience the threat and increase their evolutionary resilience before the invader arrives.

The cane toad (*Rhinella marina*) invasion in northern Australia (Shine 2010) provides an example of such a possibility. Populations of native predators have dramatically declined since the introduction of the toxic cane toads in 1935 (Shine 2010). However, there is evidence that some remnant populations are persisting in toad-colonized areas (e.g. snakes: Phillips & Shine 2006; crocodiles: Letnic *et al.* 2008; quolls: Woinarski *et al.* 2008; goannas: Shine 2010). In at least some of these cases there is strong evidence that this persistence is enabled by rapid evolution, including morphological, physiological, and behavioural changes (Phillips & Shine 2006; Llewelyn *et al.* 2014). While these evolutionary shifts are naturally occurring in response to toads, they only occur fast enough in a small number of populations. In most cases when toads arrive, populations of large predators are extirpated (Shine 2010).

Cane toads are continuing to spread across Australia and are likely to cross the Kimberley region in the next 7-10 years (Kearney *et al.* 2008). Therefore, toads have not yet covered the entire geographic range of susceptible species such as goannas and northern quolls. By translocating individuals of these species from long-exposed populations with a high frequency of toad-smart genes, we could improve the adaptive capacity of toad-naïve populations before cane toads invade the area. Once toads arrive toad-smart traits would be under intense selection and should introgress rapidly into the recipient population.

Controlling the spread of invasive species

Native species can mount rapid evolutionary change in response to an invader, but invaders evolve rapidly as well. Invaders can evolve in response to interactions with native species or novel environments or due to selection pressure at the invasion front (Mooney & Cleland 2001; Prentis *et al.* 2008). Selection pressure on the invasion front, in particular, favors the evolution of highly dispersive phenotypes (Prentis *et al.* 2008; Phillips *et al.* 2010). Increases in dispersal likely come at a cost to competitive ability. Invasive phenotypes are incredibly successful on an invasion front where competition with conspecifics is low but are less successful when placed in competition with conspecifics (Burton *et al.* 2010; Shine *et al.* 2011). These shifts in dispersal are increasingly observed in invasions, with examples ranging from cane toads, to birds, to damselflies (Phillips & Suarez 2012).

This increased dispersal rate causes invasions to accelerate (Perkins 2012) and makes them harder to stop (Travis *et al.* 2010). Targeted gene flow could

be used to halt the spread of invasive species that have evolved increased dispersal capacity, especially in conjunction with a natural dispersal barrier. If individuals from long-established populations are translocated to this hypothetical barrier, we can set up a "genetic backburn" (Figure 2.1). In this case, the less dispersive phenotypes cannot breach the barrier but instead spread back toward the oncoming invasion front. When the two invasion fronts meet, the less-dispersive phenotypes are fitter than the invasion-front phenotypes, so less-dispersive genes replace the highly dispersive invasion front genes. The end result is that dispersal rates are rapidly reduced and the landscape barrier to spread remains effective. Although the idea of a genetic backburn is new, the idea of controlling invasive species by swamping them with particular variants is not without precedent (Klassen & Curtis 2005). The screwworm (*Cochliomyia hominivorax*), for example, was eradicated from parts of North America when sterile males were released to mate with wild type females and produce unfertilized eggs (Marsula & Wissel 1994; Williams *et al.* 2013). In the genetic backburn scenario, instead of swamping the population with infertile males, we swamp it with less dispersive genes, rendering effective an otherwise permeable landscape barrier.

Cane toads will never be eradicated from Australia, but managers now hold hope of halting the invasion before it enters the Pilbara region (Florance *et al.* 2011; Tingley *et al.* 2013), and, in doing, keeping toads out of 268,000 km^2 of their potential Australian range. Biophysical and simulation modelling, as well as detailed field data, suggest the toad invasion is about to encounter a potential landscape barrier (Florance *et al.* 2011). Ensuring the barrier's effectiveness will require restricting water access across a 100-km-wide strip of coastal habitat (Tingley *et al.* 2013). The barrier needs to be this wide because cane toads at the invasion front have evolved to disperse faster than their counterparts in eastern Queensland (Phillips *et al.* 2006; Phillips *et al.* 2010). The invasion front now moves more that 50 km in a season, whereas their long-established conspecifics typically travel less than one-fifth this distance (Phillips *et al.* 2007). If the toads were dispersing only at one-fifth the rate of individuals on the invasion front, the barrier would be substantially more effective. Given the potential conservation potential, it seems sensible to consider genetic backburn as a mechanism to reduce toad dispersal ability and increase the barrier's effectiveness.

The cane toad is not the only invasive species to develop increased dispersal ability at the invasion front (Phillips & Suarez 2012). When initial eradication has failed and management has switched to a containment strategy (Sharov *et al.* 1998; Sharov 2004), genetic backburn could be used to improve the effectiveness of a natural barrier that otherwise would not slow or halt an invasion. Even if barriers to spread do not halt an invasion indefinitely, the delay may nonetheless be both economically and ecologically beneficial (Sharov 2004), and may also allow time for additional methods of control or eradication to be developed.

(a) Invasion front populated with highly dispersive phenotype

(b) Translocation of individuals with normal phenotype

(c) Translocated individuals spread

(d) Populations merge

(e) Normal phenotype outcompetes highly dispersive

Normal phenotype ⟶ Highly dispersive phenotype
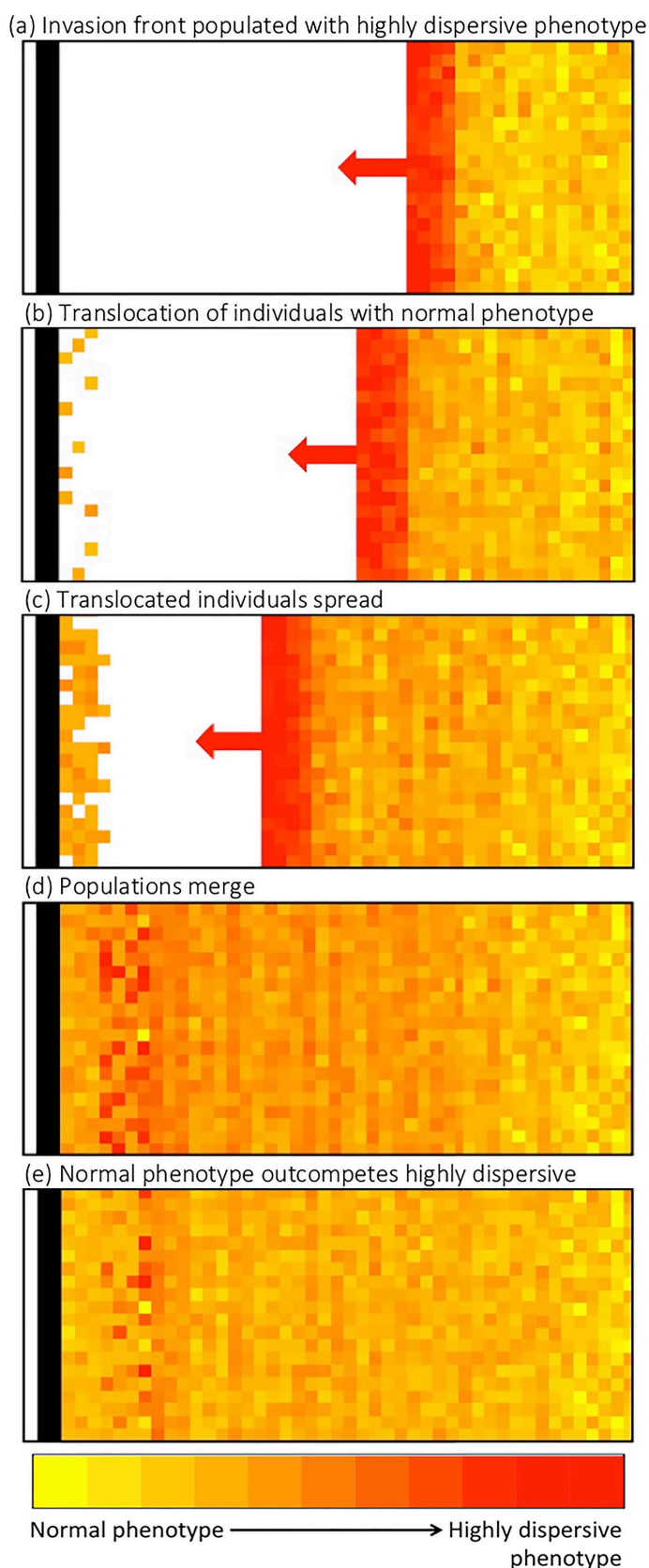
Figure 2.1 Graphical representation of genetic backburn **(a)** Spread toward a barrier (black) of a population consisting of a highly dispersive phenotype and a normal phenotype, **(b)** targeted gene flow implemented (individuals with normal phenotype translocated ahead of the invasion front), **(c)** spread of both phenotypes, **(d)** merging of the phenotypes, and **(e)** normal phenotype outcompetes highly dispersive phenotype when invasion is halted by barrier.

Implementation and potential risks

As with any conservation action, there are risks and costs associated with targeted gene flow. We certainly do not advocate an ad hoc, unplanned, and wholesale movement of plants, animals, and their pathogens. Evolutionary outcomes are difficult to predict, particularly when there is a high degree of stochasticity; thus, targeted gene flow will always require careful thought with weighting of risks, costs, and benefits. Rather than provide fully developed case studies, we point to some clear examples where the movement of a number of individuals within the species current, former, or future range may have profound conservation benefits because of the genes these individuals carry. In pointing out these possibilities, we hope to generate discussion and thought about the broader possibilities available to conservation managers when they look to exploit geographic trait variation. Targeted gene flow is a strategy with much broader application than as a response to climate change, and this breadth of application is currently underappreciated.

The success or failure of a targeted gene flow will, however, often be difficult to predict. For example, the risk of failure for translocations of threatened species is high and is affected by the existence of threatening processes, maladaptation to the new environment, small translocation sizes, and genetic problems (Chauvenet *et al.* 2013; IUCN/SSC 2013). These issues are further exacerbated by environmental and demographic stochasticity, all of which conspire to make the outcome of translocations difficult to predict (Lindenmayer & Burgman 2005). Targeted gene flow has many of the same problems as translocation but may also be subject to outbreeding depression and reduction in local adaption. Outbreeding depression, where hybrids of local and translocated parents have lower fitness, could arise from local adaptation, chromosomal incompatibilities, or the breakdown of co-adapted gene complexes, all of which are difficult to predict in advance (Edmands 2007; Frankham *et al.* 2011). Although outbreeding is an obvious problem, a number of recent reviews suggest the impact of outbreeding depression has been overemphasized in translocation literature (Edmands 2007; Frankham *et al.* 2011; Weeks *et al.* 2011; Aitken & Whitlock 2013). Simulations show that the effect of outbreeding depression is often weak and temporary, and can be rapidly outweighed by the benfit of incorporating the new genetic variance (Aitken & Whitlock 2013). Consequently, unreasonable fear of outbreeding depression may be slowing practical implemention of genetic management in threatened populations (Frankham 2010).

Because conservationists have traditionally avoided genetic translocation approaches, there are few tools to assist decision makers with genetic translocation decisions. How does one best identify source and recipient populations? What is the optimal time to execute a genetic translocation? How many individuals should be moved so as to avoid the worst excesses of stochasticity? If individuals are translocated too early or too few individuals

are translocated, selection may not be strong enough to capture the variants needed. If individuals are translocated too late or too many are translocated, the recipient population may be extirpated or swamped by the introduced genome. The scientific community needs to develop these decision tools. In the absence of these tools, however, the first two questions to be answered are whether there is useful geographic variation in the desired traits and whether that variation is heritable. Next, we need to know whether there is reproductive and genetic compatibility between source and recipient populations, sufficient such that genes can introgress into the recipient population (IUCN/SSC 2013). These questions could be answered by examining the genetic variation of a population in relation to the specific trait (Morris *et al.* 2015) or by comparing phenotypes of the source and recipient populations. Captive breeding programs are also a possible avenue for cross-breeding populations to compare phenotypic and genetic variation and determining the impact of outbreeding (Rollinson *et al.* 2014).

If the conditions appear favourable for a targeted gene flow action, a raft of nuanced decisions need then to be made such as the choice of source and recipient population and the size and timing of a translocations effort. These are important considerations that will likely have a big impact on the success of a targeted gene flow intervention (Tallmon *et al.* 2004; Grueber *et al.* 2013). Experiences of what influences the success and failure of translocations are a useful guide when planning for management involving targeted gene flow. For instance, there are decision trees for genetic depression and the likelihood of success of translocations that could also be applied to targeted gene flow (Weeks *et al.* 2011; Chauvenet *et al.* 2013; IUCN/SSC 2013). We also suggest the use of trait-explicit population modelling, which can assist in determining the impact of different management decisions on population survival. Of course, it is extremely important for managers to maintain *in situ* monitoring of populations following translocation to assess the success of management and inform future decisions.

Conclusion

Species almost never go extinct all at once; typically there is widespread range contraction first. That is, the majority of populations are extirpated, but a few remnant populations persist (IUCN Species Survival Commission 2001; Fisher 2011). Ecologists almost always assume these remaining populations are occur in places that act as refugia (Ashcroft 2010; Hampe *et al.* 2013). While this may often be true, it is also possible that particular populations (including those in ecological refugia) are persistent because of adaptation. Thus, rather than simply assuming persistent populations are there purely because of attributes of their environment, we should carefully consider the possibility that these populations persist because of genetic variation in relevant traits. Where

adaptation is indicated, these persistent populations can be exploited for both targeted gene flow and reintroduction efforts.

More broadly, geographic variation and local adaptation is ubiquitous and this is as true for problem species (invasive species and pathogens) as it is for threatened species. By paying careful attention to the geographic variation exhibited by problem species, we can also use targeted gene flow to actually mitigate a threat rather than just ameliorate its impact. Examples of this include introducing less pathogenic forms of a parasite or less dispersive forms of an invasive species.

We suggest that targeted gene flow is a currently underappreciated strategy in conservation that has applications well beyond its current remit as a strategy against climate-change impacts. Targeted gene flow may provide novel solutions to a number of conservation problems across a wide range of species and threatening processes. Targeted gene flow will be of particular importance when mitigation of the threat has failed, where the threat will play out predictably, and where the target species plays an important role in its ecological community. Although targeted gene flow is not without its uncertainties, it is, nonetheless, well worth considering.

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Chapter 3
Get smart: native mammal develops toad-smart behaviour in response to a toxic invader

Abstract

ALTHOUGH invasive species can cause major declines in native populations, some individuals in a native population are better equipped to deal with the threat than others. Existing trait variation – especially in highly flexible behavioural traits – may thus buffer populations, and allow natural selection to proceed. Cane toads (*Rhinella marina)* have caused dramatic declines in native Australian predators, which unwittingly attack the poisonous toads. The northern quoll (*Dasyurus hallucatus)* is one such predator, with declines and local extinction of quoll populations typically occurring rapidly after toads arrive. Despite this, some quoll populations persist in areas where toads have been present for 70+ years. Here we compare northern quolls from toad-infested and toad-free areas to test whether this persistence is enabled by behavioural traits. We demonstrate

that northern quolls from long-term toad-infested areas have indeed become "toad-smart", spending significantly less time investigating a toad compared to a control prey item, and limiting this investigation time to investigatory rather than attacking behaviour. By contrast quolls from toad-naïve populations vary in their response to toads, with many exhibiting attack behaviour. These results demonstrate that behavioural variation exists within naïve populations and the few persisting northern quoll populations in toad-infested areas have naturally developed toad-smart behaviour. Population modelling suggests this behaviour likely persists across generations. Although the mechanism is unknown, the observed shift in toad-smart behaviour may be due to rapid adaption, and if so could become a vital tool for conserving this endangered species.

Introduction

Trait variation is universal and plays an important role in how populations respond to a threatening process (Bolnick *et al.* 2003; 2011). Individuals possessing certain traits may be better equipped to deal with particular threats. Thus, the impact of a threat is not equal amongst individuals in a population, resulting in natural selection favoring certain traits (Darwin 1859; Wilson 1998; Bolnick *et al.* 2011). A response to natural selection will cause traits to shift, but of course traits can shift rapidly through plastic and stochastic processes also (Stockwell *et al.* 2003; Bolnick *et al.* 2011). Behavioural traits are particularly flexible, with adaptive shifts occurring both within and across generations (Dall *et al.* 2004; Wong & Candolin 2015; Caro 2016). Following the introduction of a novel threat, then, existing and *de novo* variation in behavioural traits can act to buffer a species from extinction (Buchholz 2007; Ghalambor *et al.* 2007).

Due to long-term evolutionary and biogeographical isolation, Australia's native fauna are considered particularly vulnerable to invasive species. One of Australia's most infamous invaders is the cane toad (*Rhinella marina*). Introduced to north-east Australia in 1935, it has since spread eastwards across the continent (Urban *et al.* 2007; Kearney *et al.* 2008; Shine 2010). These toxic anurans are novel to Australian predators, who unwittingly attack them and die, causing local extinctions in native predator populations across northern Australia (Shine 2010). Despite the widespread decline, however, some predator species exhibit rapid adaptation to the presence of toads, and now persist alongside them (frogs and fish, Nelson *et al.* 2011; monitor lizards, Llewelyn *et al.* 2014). These adaptations include changes to morphological traits, such as decreasing jaw size in snakes so that they cannot consume lethal-sized cane toads (Phillips & Shine 2004). Perhaps most importantly, there have also been changes in behaviour, with many predators – such as goannas (Jolly *et al.* 2016; Ward-Fear *et al.* 2017), snakes (Phillips & Shine 2006), frogs (Greenlees *et al.* 2010), dasyurids (Webb *et al.* 2008; Kämper *et al.* 2013) and freshwater crocodiles (Somaweera *et al.* 2011) – either learning or evolving to avoid toads

as prey. Some raptors have even learnt to selectively consume only the parts of the toad with low concentrations of toxin (Beckmann & Shine 2011). The toad invasion is an ideal system to observe the effect of invasive species on behaviour of natives because it is often possible to find predator populations ranging from long-exposed (70+ years) through to completely toad naïve.

One native predator at risk of extinction due to the cane toad is the northern quoll (*Dasyurus hallucatus*). These cat-like marsupials are generalist mesopredators, consuming a varied diet of reptiles, amphibians, insects and mammals (Oakwood 1997). They used to occur over much of northern Australia, but are now classified as endangered under Federal legislation, chiefly due to the impact of cane toads (Woinarski *et al.* 2008; Woinarski *et al.* 2014). Cane toads have already fatally poisoned countless quolls, causing rapid declines of quoll populations in Queensland and parts of the Northern Territory. Toads are expected to eventually colonize the quolls' entire range (Kearney *et al.* 2008; Woinarski *et al.* 2008). Although detailed data on pre- and post-toad quoll densities are only available for the recent invasions, it is clear that the vast majority of northern quoll populations that have come into contact with toads since 1935 have gone locally extinct (Burnett 1997; Woinarski *et al.* 2008). However, a small number of remnant populations have persisted in Queensland over the past 70+ years since toad arrival (Woinarski *et al.* 2008). Northern quolls in these populations have not developed an increased resistance to the toxin (Ujvari *et al.* 2013), suggesting that behavioural mechanisms are likely at play.

It seem likely then that quolls from these remnant populations are "toad-smart" (Woinarski *et al.* 2008) – they avoid poisoning themselves on toads – a trait that, if it could be induced in toad-naïve populations, could be very valuable to conservationists (Kelly & Phillips 2016). As yet, researchers have been unable to determine the mechanism whereby quolls acquire this behaviour. Previous work shows quolls can be trained to be toad-smart through conditioned taste aversion (Garcia *et al.* 1974; O'Donnell *et al.* 2010), but such training occurs under controlled circumstances: evidence from radiotelemetry and mark-recapture studies of toad-naïve populations suggest that very few quolls survive to learn in field conditions (Woinarski *et al.* 2008; O'Donnell *et al.* 2010; Cremona 2015; Jolly *et al.* 2017). It is this high mortality rate that is argued to have caused the rapid population declines, but the link between toad mortality and population decline has not been firmly established. We make this link explicit here using population viability analysis to determine what proportion of a population would need to be toad-smart for the population to persist. We then go on to ask whether there is variation for toad-smart behaviour in toad-naïve quolls, and whether these behavioural traits appear to have shifted between toad-naïve and toad-exposed quoll populations. We predict that toad-exposed quolls will have less interest in toad prey compared to their naïve counterparts, and generally exhibit less attacking behaviour when interacting with toads.

Methods

Population viability analysis

To predict the level of toad-smarts required for a northern quoll population to survive the cane toad invasion, we developed an individual-based simulation model (Shettleworth 1984; Grimm & Railsback 2005). Using this, we executed a population viability analysis to estimate how the probability of individuals attacking and dying from toads (from 0-1 in 0.01 increments) influences population viability. The model captures all relevant aspects of quoll life-history and was parameterized using published data from wild and captive-bred northern quolls. Model details are reported in Appendix I.

Toad-response experiment

To measure responses to toads in wild northern quoll populations, we collected northern quolls from toad-infested and toad-free areas of northern Australia and brought them into captivity at the Territory Wildlife Park, NT. The toad-exposed group (n = 18) was collected from two toad-infested areas in Far North Queensland, Mareeba (Mareeba Wetlands and Mareeba Crocodile Farm) and Cooktown (South Endeavor). Both areas had similar densities of cane toads, and predators of northern quolls were present at both sites. The toad-naïve group (n = 40) was collected from Astell Island, NT, a predator and toad free island set up in 2003 as an insurance population of northern quolls from Kakadu National Park (where quolls are now almost locally extinct) prior to the cane toad invasion (for more details see Rankmore *et al.* 2008). These collection sites were selected because they fit with our scientific objectives, as well as being logistically possible. We were unable to collect even sample sizes from each location due to practical (collection and housing) constraints, as well as limitations on permits.

To measure toad-smarts, each individual was presented with a dead adult cane toad or a dead adult mouse (the "prey" treatment) in a wire cage, so that they could see and smell the prey item but not access it. The mouse was selected as a control item as it was a prey the quolls were familiar with. Both prey items were presented dead to control for any prey behavioural differences. The experiment was run over six nights with prey alternating so each prey type was presented three times, with the starting prey item being randomly allocated. Experiments began at sunset and ran for two hours, after which the prey item was removed and the quolls were fed their regular diet (a rotating combination of chicken necks, live insects, fish, and vet-recommended cat biscuits). The response was filmed (GoPro HERO) and analyzed for overall time the quoll spent investigating the prey item in each two-hour trial. "Inspecting" behaviour was defined as the quoll being engaged with the cage containing the prey item, exhibiting either sniffing, pawing or biting behaviour. Videos were scored by the same observer who was blind to the quoll's origin (toad-infested or free), but was not blind to prey treatment as it was visible in the video. We

used a linear mixed effects model to analyze the time spent inspecting the prey item. As fixed effects, the model included the effect of sex, trial night (1-3 for each prey type), toad-exposure group, and prey treatment. We also included the interaction between prey treatment and toad-exposure category, this being the primary effect of interest. Individual was included as a random effect. P-values were obtained by likelihood ratio tests of the full model with the effect in question against the model without the effect in question. This analysis was performed using R (R Core Team 2016) with the *lme4* software package (Bates 2015). The two toad-exposed populations were pooled after we added collection location to the model and found no significant effect of where the quolls originated from ($\chi^2(1) = 0.16$, p = 0.69; Table 3.1).

To determine the type of behaviour the quolls exhibited when first encountering the prey item, the first minute of each interaction (where the quoll did approach the prey item; time spent investigating > 0 seconds; toad-exposed (*n* = 18) and toad-naïve (*n* = 37)) on the first night was analyzed for the presence of either investigatory behaviour: "sniffing" or attacking behaviour: "pawing" or "biting" the cage containing the prey item. For the purpose of analysis, we pooled the pawing and biting responses to create a binary response of either "investigating" or "attacking". We performed a logistic regression on these data using GLM function with a binomial distribution, including effects for toad exposure category (toad-exposed or toad-naïve) and prey type (toad treatment or mouse control) and the interaction between them (R Core Team 2016). We calculated relative risk to generate an intuitive metric for effect sizes.

Results

Population viability analysis

The results from the population viability analysis indicates that northern quolls would experience a population collapse when the probability of attacking (and being killed by) a toad for each individual was greater than 0.68 (Figure 3.1).

Toad-response experiment

The interaction between toad exposure category and treatment showed northern quolls from toad-infested areas of northern Australia (toad-exposed) spent significantly less time investigating the toad (mean inspection time(±SE) = 33±17 seconds) compared to the mouse control (212±17s) and their naïve counterparts (225±10s, $\chi^2(1) = 10.21$, p < 0.001; Figure 3.2; Table 3.1). The time spent investigating the prey declined over the trial period for all treatments and populations (-74 seconds/day, $\chi^2(2) = 53.60$, p < 0.001). Male quolls spent significantly more time investigating than females (90.5 seconds more, on average, $\chi^2(2) = 17.44$, p < 0.001).
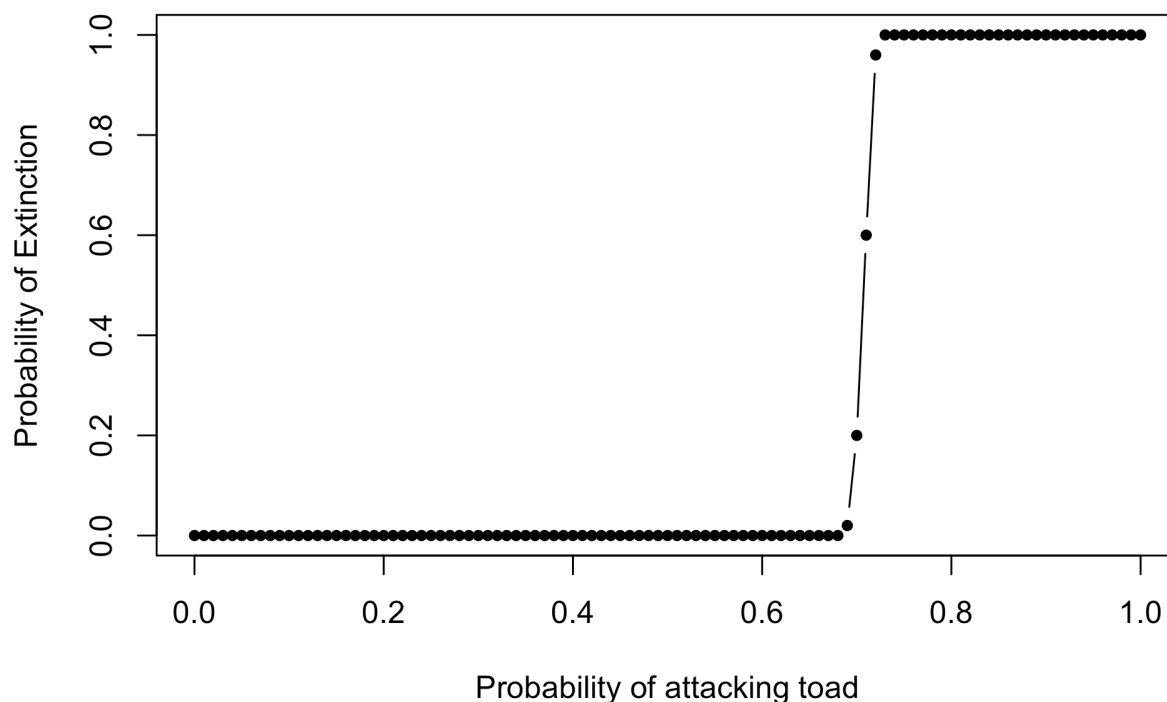
Figure 3.1. Estimated probability of extinction for northern quoll populations from of an individual based simulation model (runs=100) for a range of probabilities (between 0-1) of each individual attacking a toad.

Table 3.1. Linear mixed-model analysis of time spent investigating prey items for northern quolls from toad-infested and toad-free populations.

Fixed Effects	Estimate	SE	P
Intercept	294.55	33.58	
Prey type (toad)	-59.37	20.73	< 0.001
Toad-exposed group	31.80	39.72	0.01
Day	-74.58	10.71	< 0.001
Sex (male)	90.52	32.46	< 0.001
Prey type*Exposure group	-122.80	38.07	< 0.001

Random Effects	Variance	Std.Dev	
Quoll	10591	102.9	
Residual	25795	160.61	

As well as spending substantially less time investigating toads overall, toad-exposed quolls were also much less likely to exhibit attacking behaviour (biting or pawing) during the first minute interacting with the toad (Figure 3.3). Toad-naïve quolls were 8.22 times more likely (Relative Risk, 95% CI [1.73, 47.1])

to attack (paw+bite) the offered toad than their toad-exposed conspecifics, and the model showed the interaction between exposure category and prey type to be statistically significant ($\chi^2(1) = 10.44$; p = 0.001).
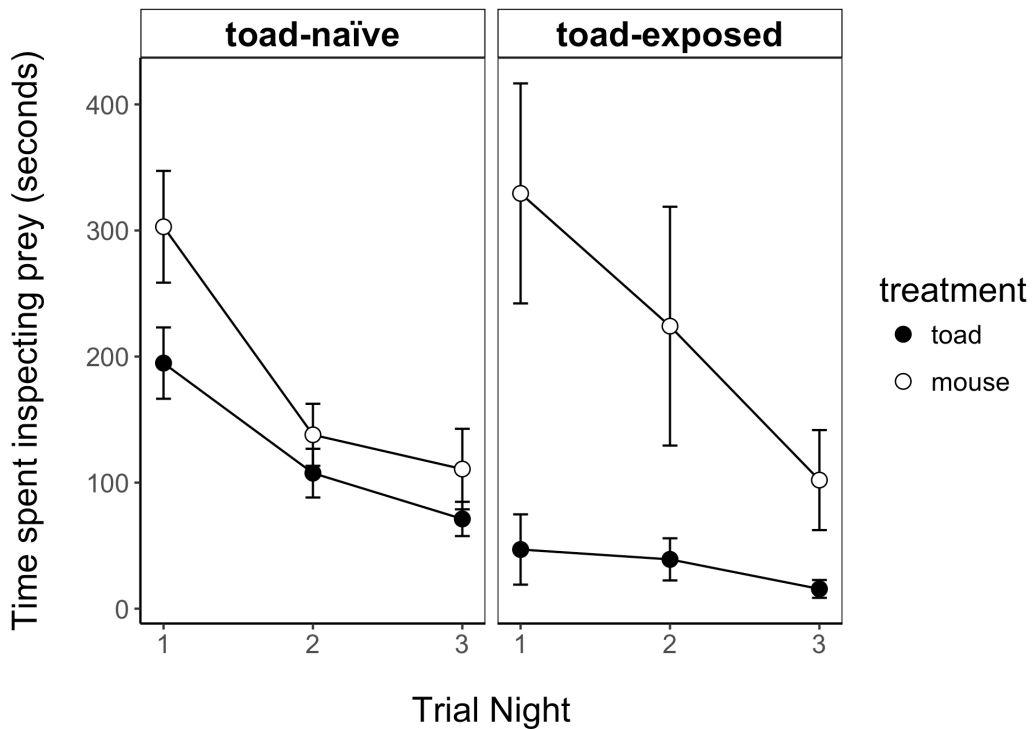
Figure 3.2. Mean±SE time spent investigating two treatment prey types (dead adult toad or control dead adult mouse) in a two-hour period (in seconds). Results shown over three nights for each treatment for two populations: toad-naïve (NT) and toad-exposed (QLD).
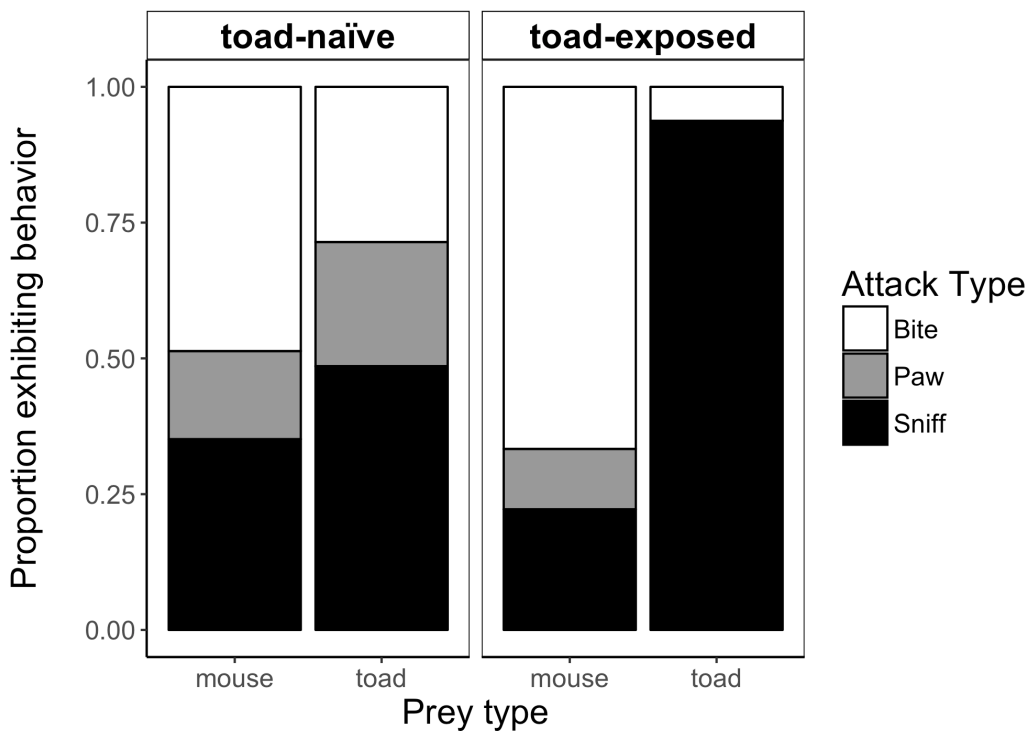
Figure 3.3. Proportion of individuals exhibiting the three different attack types (bite, paw, sniff) in the first minute of interaction with prey items (mouse and toad) for each population, toad-exposed (QLD, n = 18) and toad-naïve (NT, n = 37).

Discussion

The results of the population viability analysis suggest that, if less than 68% of quolls in a population attack a toad, then the population should persist. By contrast, 51.4% of quolls from toad-free areas exhibiting attack behaviour *within the first minute* of encountering an unmoving (dead) toad. Given the contrived circumstances, we consider this the absolute lower bound for the true percentage of toad-naïve quolls that would attack toads. Certainly, toad-induced mortality of these toad-naïve animals appears substantially higher in the field (e.g., 85.7% of toad-naïve female quolls attacked cane toads within 3 days of release; Jolly *et al.* 2017). Also, the behaviour of toad-naïve quolls was generally no different when they were interacting with a dead adult toad compared to a dead adult mouse, suggesting they treat cane toads as they would any potential prey item. Taken together, these results indicate that the initial proportion of wary individuals that avoid toads in a toad-naïve population will often not be high enough to allow that population to persist.

Quolls collected from toad-exposed areas spent substantially less time investigating toads compared to a control prey item, and only one of the toad-exposed quolls displayed attacking behaviour towards toads. This confirms also that quolls from populations long-exposed to toads are typically toad-smart, and show little variation in this trait due to the strong selection acting upon toad-smart behaviour. By contrast, toad-naïve quolls were more than eight times more likely to exhibit these attack behaviours. Relative to quolls from toad-naïve populations, quolls from toad-exposed populations were both disinterested in toads, and disinclined to attack them. Certainly, the rates of toad attack in toad-exposed quolls appears sufficiently low that toads do not threaten the population.

Being generalist predatory mammals, quolls are likely capable learners (Shettleworth 1984). Our results support this by showing a rapidly declining interest in the prey item over the course of the experiment as the quolls learned they could not access the prey item. It has also been shown that conditioned taste aversion (Garcia *et al.* 1974; Pearce 2013) can be used to elicit toad avoidance in quolls (O'Donnell *et al.* 2010; Cremona 2015). Our toad-exposed quolls were collected as adults from toad-infested areas. Because of this they clearly grew up with toads and must have avoided them in the wild (otherwise they would not have survived). It remains possible, then, that all quolls in these populations learn toad avoidance *de novo* and that we simply sampled animals that had already learnt to avoid toads (and did not sample the dead individuals from this population that failed to learn). This strict possibility seems unlikely simply because toad-induced mortality rates in naïve animals in the field appear too high for populations to persist (above the 68% threshold determined by our population model; O'Donnell *et al.* 2010; Jolly *et al.* 2017). More likely is that individuals from toad-exposed populations either innately avoid toads, learn via cultural transmission from their parents not to eat toads, or have an innately

increased propensity for learning relative to their toad-naïve conspecifics. Together, the results from the population model and our knowledge of quoll behaviour suggests that toad-smarts are likely inherited – yet further work, likely involving a common garden experiment, would be required to confirm this. A genetic basis to prey choice has been shown in a broad array of taxa (Ayres & Arnold 1983; Lindström *et al.* 1999), including possible innate cane toad avoidance in some Australian reptiles (with no maternal care; Phillips & Shine 2006; Llewelyn *et al.* 2011). A genetic basis would also be consistent with the obviously very strong natural selection acting upon toad-smart behaviour (e.g. Phillips *et al.* 2010). This said, we cannot rule out the possibility of pure cultural transmission either (i.e., that mothers teach their offspring to avoid toads, or the offspring learn through observation/imitation e.g., Oakwood 2000; Thornton & Raihani 2008; Thornton & Raihani 2010).

Our study is the first to demonstrate natural individual variation in toad-smart behaviour in toad-naïve populations of northern quolls and suggests that some quolls are innately better equipped to survive the cane toad invasion. This variation is magnified when we compare toad-smart behaviour across naïve and toad-exposed populations. Variation in quoll behaviour protects some individuals from toad-induced mortality, at once buffering the population from the impact and allowing natural selection to occur. This is supported by results from northern quoll toad aversion training, which shows variation amongst individuals in their ability to learn via conditioned taste aversion (O'Donnell *et al.* 2010; Jolly *et al.* 2017). While our results suggest toad-smarts can be passed between generations – either genetically or through cultural transmission – additional work would be needed to confirm this. And the answer matters: if we know how toad-smarts are passed between individuals we can develop specific management actions to increase the proportion of individuals with toad-smart behaviour in threatened populations (Kelly & Phillips 2016).

More generally, behaviour is becoming increasingly recognized as an important tool for buffering the impacts of human-induced environmental change. Many are urging more focus on individual level traits and how behaviour can be used practically to improve conservation benefits (Berger-Tal *et al.* 2015; Wong & Candolin 2015; Caro 2016). Our study supports these calls.

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Chapter 4
Targeted gene flow and rapid adaption in an endangered marsupial

Abstract

TARGETED gene flow is an emerging conservation strategy. It involves translocating individuals with favorable genes to areas where they will have a conservation benefit. The applications for targeted gene flow are wide-ranging but include preadapting native species to the arrival of invasive species. The endangered carnivorous marsupial, the northern quoll (*Dasyurus hallucatus*), has declined rapidly since the introduction of the cane toad (*Rhinella marina*), which fatally poisons quolls that attack them. There are, however, a few remaining toad-invaded quoll populations in which the quolls survive because they know not to eat cane toads. It is this toad-smart behaviour we hope to promote through targeted gene flow. For targeted gene flow to be feasible, however, toad-smarts must have a genetic basis. To assess this, we used a common garden experiment, comparing offspring from toad-exposed and toad-naïve parents raised in identical environments, to determine if toad-smart behaviour was heritable. Offspring from toad-exposed populations were substantially less likely to eat toads than those with toad-naïve parents. Hybrid offspring showed

similar responses to quolls with two toad-exposed parents, indicating the trait may be dominant. Together, these results suggest a heritable trait and rapid adaptive response in a small number of toad-exposed populations. Although questions remain about outbreeding depression, our results are encouraging for targeted gene flow. It should be possible to introduce toad-smart behaviour into soon to be affected quoll populations.

Introduction

Anthropogenic environmental change often requires species to rapidly adapt or risk extinction (Hoffmann & Sgrò 2011; Sih *et al.* 2011). The rate at which a population can adapt depends critically on how much genetic variation exists for relevant traits. Most adaptive responses to threatening processes will be fueled by existing trait variation within a population ("standing variation"; Barrett & Schluter 2008) rather than by mutation. Recently conservationists have begun to consider the use of species-wide standing variation in the management of threatened populations (Aitken & Whitlock 2013; Kelly & Phillips 2016). One such strategy, targeted gene flow, involves translocating individuals carrying favorable traits to appropriate areas of a species' range (Kelly & Phillips 2016). Done effectively, this strategy could provide relevant genetic variation and so speed the adaptive response, promoting evolutionary rescue of recipient populations faced with environmental change. However, targeted gene flow is yet to be trialed in a wild population (Kelly & Phillips 2016). To execute targeted gene flow, relevant traits need to be heritable. If the traits are not heritable, of course, targeted gene flow cannot be used.

Invasive species are a potent agent of environmental change. They have permanently altered many ecosystems across the globe and led to local species declines and extinctions (Clavero & García-Berthou 2005; Crowl *et al.* 2008). Because invasive species are difficult to eradicate, adaptation may be the only way for natives to persist (Mooney & Cleland 2001). Although rapid evolution may be a common response to an invasive species, it may not occur quickly enough to allow population survival (Strauss *et al.* 2006; Carroll 2007). If, however, targeted gene flow could be used to introduce appropriate heritable trait variation into a population, it should be possible to artificially increase the speed of adaptation (Weeks *et al.* 2011; Kelly & Phillips 2016).

The relentless invasion of the toxic cane toad (*Rhinella marina*) throughout northern Australia has led to widespread declines of native fauna (Tingley *et al.* 2017). Unfamiliar with toxic anurans, Australian predators attack cane toads and are killed by the toxin they secrete (Shine 2010). As a result, predators such as snakes, goannas, freshwater crocodiles, and northern quolls have declined rapidly, and often go locally extinct immediately following cane toad arrival (Shine 2010). Northern quolls (a medium-sized marsupial predator; *Dasyurus hallucatus*), for example, have declined by >75% since the arrival of toads; thus,

this species is listed as nationally endangered under the Australian Environment Protection and Biodiversity Conservation Act (EPBC 1999; Fig 1). Despite widespread local extinctions, however, no species has yet gone completely extinct since the arrival of toads in 1935. A small number of populations of native predators appear to have adapted to toads and are recovering (Woinarski *et al.* 2008; Llewelyn *et al.* 2014). Adaptations to toads include morphological changes (e.g. decrease in jaw sizes of snakes; Phillips & Shine 2006) as well as behavioural changes (e.g. selectively consuming only nonpoisonous areas of toads (Beckmann & Shine 2011) or simply not attacking them at all (e.g.,Webb *et al.* 2008; Greenlees *et al.* 2010; Ward-Fear *et al.* 2017).

Recent work has shown that northern quolls can behaviourally avoid cane toads in the wild (Kelly & Phillips 2017). This behaviour can also be taught to naïve quolls (through conditioned taste aversion; O'Donnell *et al.* 2010; Cremona *et al.* 2017; Indigo *et al.* 2017), but whether the trait also has a genetic basis remains unresolved (Kelly & Phillips 2017). Despite almost universal declines in quoll numbers immediately following toad arrival, toad-smart behaviour allows a small number of populations of northern quolls to persist in areas of northeastern Australia that have been invaded by toads for over 70 years (Woinarski *et al.* 2008). Behavioural experiments show that quolls from these toad-infested areas avoid attacking cane toads entirely (Kelly & Phillips 2017). Population modelling suggests that this toad-smart behaviour must be transmitted across generations (Kelly & Phillips 2017), but we are still unsure if this is happening genetically, through cultural transmission (i.e. offspring learning from their mother; Thornton & Raihani 2008; Thornton & Raihani 2010), or a combination of the two. Despite the existence of toad-smart behaviour, however, the majority (~95%) of naïve quoll populations go extinct once cane toads arrive (EPBC 1999), suggesting the behaviour is, in most quoll populations, either too rare or not effectively transmitted. If toad-smart behaviour has a genetic basis, targeted gene flow could be used to introduce this rare trait into soon to be affected populations, increasing the chance of evolutionary rescue (Figure 4.1).

To execute targeted gene flow in quolls, we would need to cross quolls from across their geographic range to incorporate toad-smart behaviour into the genome of threatened populations. In the past, four subspecies of northern quolls were recognized but recent work suggests northern quolls have only weak phylogeographic structure across their range (Firestone 2000; Cardoso *et al.* 2009; Hohnen *et al.* 2016). Such weak structure is consistent with simple isolation by distance, possibly coupled with more recent drift caused by declines and fragmentation since European arrival (How *et al.* 2009). The climatic environment – a wet-dry monsoonal climate – is fairly consistent across the quoll's northern range. Mitochondrial DNA shows genetic distances of 0.035-0.046 between populations of quolls from the central part of their range (Northern Territory) and those 1500 km away in their far eastern extent (Queensland; Figure 4.1; Firestone 2000). Generally, crosses with <5% sequence divergence (indicating they have not been isolated for long) are unlikely to suffer

ill effects from outbreeding depression – particularly when they occupy similar environments and have a history of gene flow (Edmands 1999; Frankham 2015, 2016). Therefore, outbreeding depression seems unlikely to be a barrier in this instance. Once toads arrive, of course, toad-smart traits would be under intense selection, and if these traits are at high enough frequency (such that population extinction is avoided) they should introgress rapidly into the recipient population.

We examined whether toad-smart behaviour has a genetic basis. A genetic basis to prey choice has been demonstrated in a broad array of taxa (Ayres & Arnold 1983; Lindström *et al.* 1999), including in Australian reptiles responding to cane toads (Phillips & Shine 2006; Llewelyn *et al.* 2011). The large difference in toad-smart behaviour between naïve quoll populations and the few remaining toad-exposed populations suggest rapid evolution (Kelly & Phillips 2017), and a genetic basis to toad smarts in quolls. But we cannot rule out the possible role of cultural transmission, especially as adult quolls can be trained to avoid toads (O'Donnell *et al.* 2010; Cremona *et al.* 2017), and quoll offspring do spend time with their mothers learning to hunt (Oakwood 2000). Common garden experiments have long been used to uncouple genetic and environmental influences on adaptive traits (de Villemereuil *et al.* 2016). We used this method to determine the mechanism of transfer of toad-smart behaviour between quoll parents and offspring. We examined a range of traits that may be associated with toad-smart behaviour, from foraging (interest and acquisition behaviour) to the final decision of whether to consume a toad. Although foraging behaivour is important, the choice of whether to attack and consume the toad or not will be the crucial behaviour for investigating toad-smarts. By breeding quolls from toad-exposed and toad-free areas in a captive, toad-free environment we eliminate any environmental or cultural effects to focus solely on the quolls' innate responses to cane toads. We also determined the feasibility of crossbreeding populations of northern quolls, and assess any obvious adverse effects of outbreeding depression in the F1 hybrids.

Methods

To determine whether there are genetically based differences in toad-smart behaviour, we used a common garden experiment to measure the innate response to cane toads of northern quolls from toad-exposed and toad-naïve origins. Initially, northern quolls were collected from toad-exposed and toad-free areas of their range (Figure 4.1) and brought into captivity to breed at the Territory Wildlife Park, Northern Territory (NT), Australia. The toad-exposed group (*n* = 18) was collected from two toad-infested areas in Far North Queensland (QLD) Australia, Mareeba (Mareeba Wetlands and Mareeba Crocodile Farm), and Cooktown (South Endeavour), both of which have had high densities of cane toads for >70 years. The toad-naïve group (*n* = 18) was collected from predator- and toad-free Astell Island, NT, which was set up as an insurance
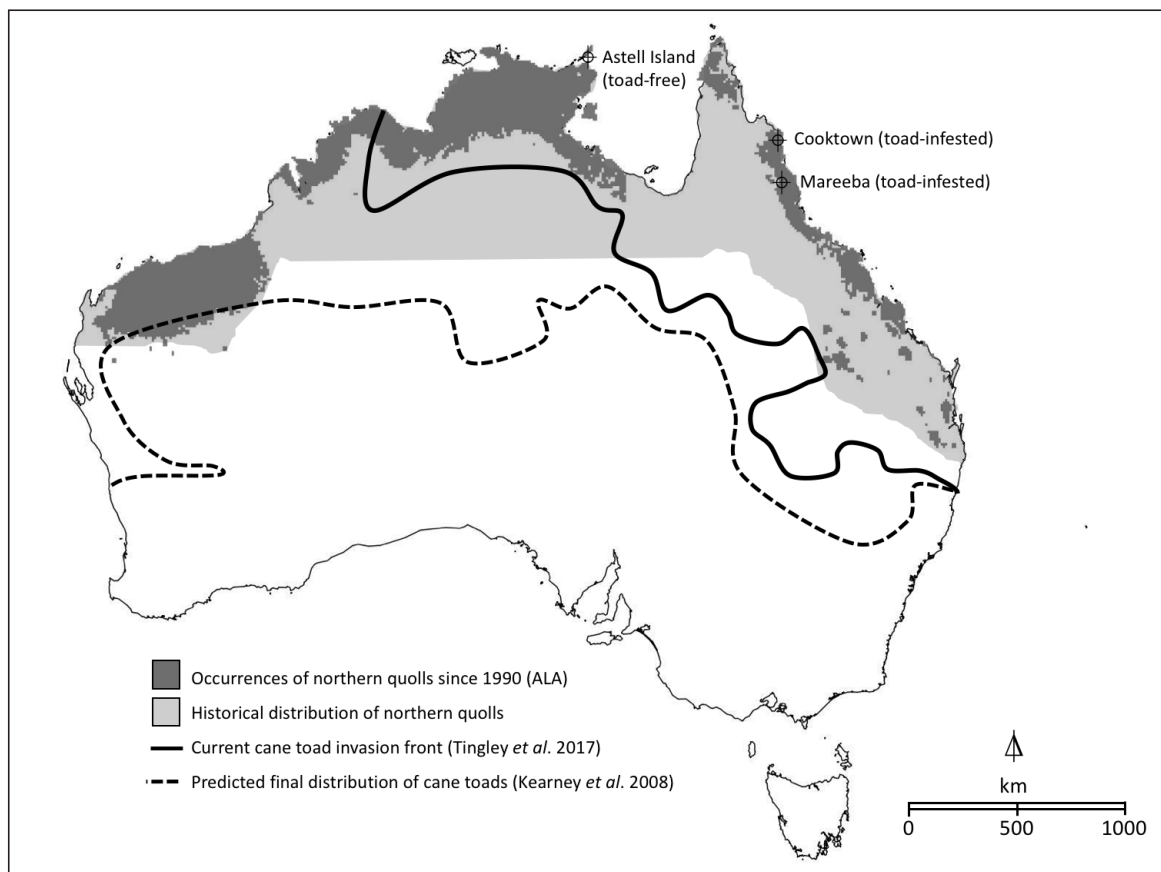
Figure 4.1. Map of Australia showing the distribution of northern quolls and cane toads (solid line, current range of cane toad; dotted line, approximate final extent of cane toad invasion (as predicted by Kearney et al. 2008). Locations on map (Astell Island, Cooktown and Mareeba) show where northern quolls were collected for this study. Distribution data are from the Atlas of Living Australia (website at http://www.ala.org.au. Accessed 9 April 2018) for quolls and from (Tingley et al. 2017) for toads.

population of northern quolls from Kakadu National Park, NT, in 2003 (for more details see Rankmore *et al.* 2008). All collections and experiments were undertaken with approval from The University of Melbourne Animal Ethics Committee (ID number: 1413369.2), and with all relevant permits from State, Territory, and Indigenous authorities.

Once in captivity, we bred the quolls to produce three lines of captive bred offspring: purebred toad-naïve offspring (NT x NT; $n = 42$; eight litters in 2016); purebred toad-exposed offspring (QLD x QLD, $n = 52$; four litters in 2015, four in 2016); and hybrid offspring (NT x QLD; $n = 13$; two litters in 2016). An analysis of variance (ANOVA) was used to compare litter sizes across origin groups. The breeding occurred over two breeding seasons (which equates to 2 years, as quolls reach sexual maturity at age one; Oakwood 2000) as quolls reach sexual maturity at age one; Oakwood 2000. The 2015 breeding season produced 24 toad-exposed offspring, and the remainder were born in the 2016 season (sample sizes in Table 4.1). Unfortunately, the 2016 breeding season had some logistical difficulties outside the realm of the experiment, leading to the uneven litter numbers and small sample size of hybrids. We therefore had two generations of toad-exposed quolls (2015 F1 and 2016 F2), and only one

generation of toad-naïve quolls (2016 F1). We produced two litters of hybrid quolls (2016 F1) – one with a captive born QLD mother and wild caught NT father and the other with a wild-caught NT mother and captive-born QLD father. All offspring were raised in similar conditions and were not exposed to cane toads until the beginning of the experiments, including the time they spent housed with their mother (offspring are weaned at ~5 months). We examined reproductive output (litter size and offspring survival) to measure any impacts of outbreeding depression on the body condition and fitness of captive F1 hybrids.

Table 4.1. Sample sizes of experiment 1 (northern quoll foraging and acquisition behaviour) and experiment 2 (northern quoll consumption of a cane toad) in the 2015 and 2016 litters for three groups of tested quoll litters (number of litters in parentheses).

Litter	Experiment 1		Experiment 2	
	2015	*2016*	*2015*	*2016*
Toad-naïve	-	41(8)	-	41(8)
Hybrid	-	13(2)*	-	13(2)*
Toad-exposed	24(4)	27(4)*	-	21(4)*

Definitions; *toad-naïve*, litters whose parents were never exposed to cane toads; *toad-exposed*, both parents exposed to cane toads; *hybrid*, parents from both origins. *At least one captive-born parent.

Once the offspring were weaned and living in individual cages, we measured their response to cane toads. In the first experiment, we scored each quoll's foraging behaviour in response to a dead cane toad in a cage, as well as a control dead mouse. In both 2015 and 2016, each individual quoll was presented with a dead adult cane toad or a dead adult mouse (the prey treatment) in a wire cage, so that they could see and smell the prey item but not access it. These prey items were dead to control for any behavioural differences in the prey. A prey item was presented to the quoll for two hours from sunset for two consecutive nights (with order of prey type randomly assigned). If the quoll did not approach the cage during the first night for either prey item, we repeated the experiment until they did (up to three nights).

We measured foraging behaviour by scoring interest in the prey item (the time each individual spent investigating the prey item) and acquisition behaviour (the type of behaviour they exhibited within the first minute of interaction). Acquisition behaviour was categorized as attack (bite or paw) or investigate (sniff). In 2016 this experiment was conducted prior to the toad leg test described below. For analysis, we collapsed bite and paw behaviours into a single behaviour attack, and then modelled the probability that an animal would exhibit an attack behaviour. We performed a generalized linear mixed-effects model with a binomial distribution, including fixed effects for toad-

exposure category (toad-exposed, toad-naïve or hybrid origin) and prey type (toad treatment or mouse control) and the interaction between them. Litter was included as a random effect. The p values were obtained by likelihood ratio tests. Time spent investigating each prey item was analyzed using a mixed-effects model as above but with a normal error distribution and log transformation of time spent investigating (data in Figure 4.2 are presented untransformed).

In the second experiment, we determined whether a quoll would consume a cane toad by presenting each individual with a toad leg (which does not contain enough poison to harm the quoll). The leg was left in the quoll's enclosure overnight instead of their regular food. In the morning, we recorded whether the quoll had eaten the toad leg. This experiment was conducted only in 2016 due to logistical considerations, so the sample size was slightly reduced (toad-naïve = 42; toad-exposed = 21; hybrid = 13). This experiment was conducted in conjunction with conditioned taste aversion training (O'Donnell *et al.* 2010; Jolly *et al.* 2017) for a separate project, so some toad legs were laced with the odorless and tasteless nausea-inducing chemical thiabendazole. This factor was considered in the analysis but there was no significant effect of thiabendazole on the probability of consuming the toad leg ($\chi^2(1) = 3.13$, p = 0.08). Generations in captivity (F1 or F2) could not be factored into the analysis because it could not be uncoupled from origin (i.e. all purebred toad-exposed quolls were F2 offspring; Table 4.1). These data on consumption were analyzed using generalized linear mixed-effects model with a binomial distribution, with toad-exposure category (toad-exposed, toad-naïve, or hybrid origin) and presence of thiabendazole as fixed factors and litter as a random effect. The p values were obtained by likelihood ratio tests. All analysis was performed using R (R Core Team 2016) with the *lme4* software package (Bates 2015).

Results

We successfully breed 18 litters of captive-born offspring over 2 years. For husbandry reasons (stressed female northern quolls will kill their babies) we were not able to monitor survival rates from day 0, but all individuals survived from their first check-up (which occurred between approximately 110 and 160 days). Litter size varied between 3-9 offspring (mean litter size [SE]: purebred toad-exposed, 6.6 [1.8]; hybrid: 6.5 [1.5] (These 2 litters had 6 and 7 offspring, respectively.); purebred toad-naïve: 5.3[2.3]). There was no significant difference in litter sizes across the three populations origins (ANOVA: $F2,15) = 0.96$, $p = 0.14$).

Quolls spent significantly more time investigating the mouse relative to the toad ($\chi^2(1) = 46.55$, $p < 0.001$) (Figure 4.2). Although the model also estimated a sizeable interaction between quoll origin and prey type (indicating greater discrimination between mouse and toad in animals from toad-exposed origin), this effect fell short of significance ($\chi^2(2) = 5.00$, p = 0.082) (Figure 4.2 & Appendix II).
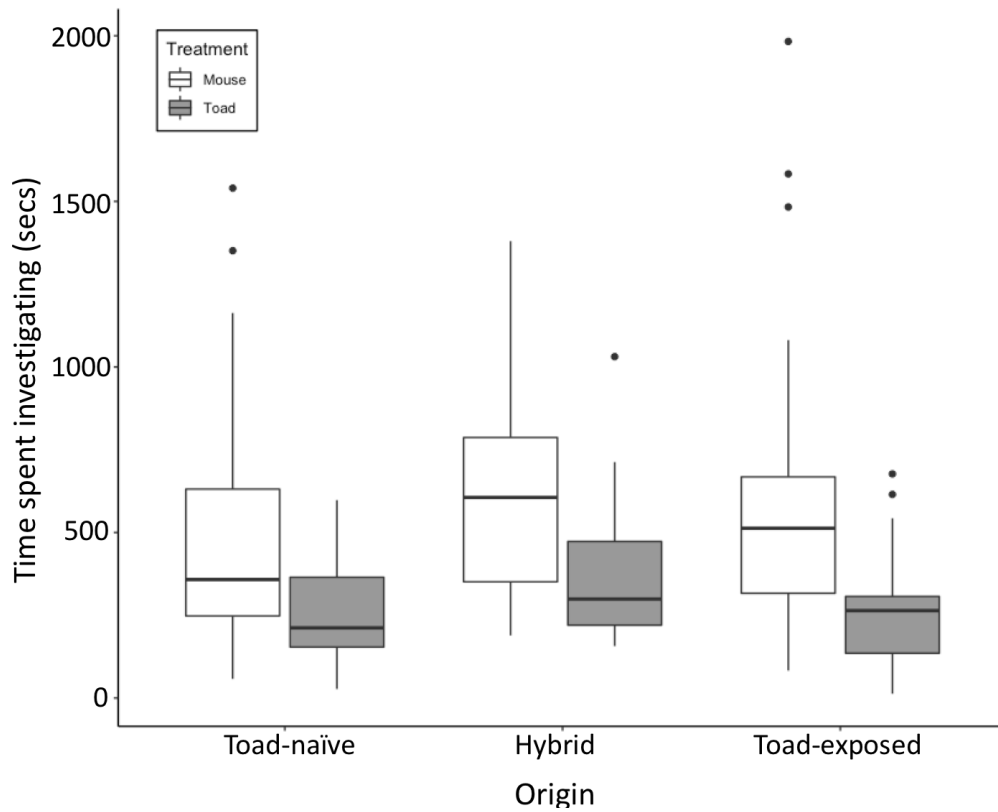
Figure 4.2. Time captive-bred northern quolls spent investigating a dead toad (grey) and dead mouse (white) for offspring of toad-naïve, hybrid and toad-exposed origins (box, 25th, 50th and 75th percentile; whiskers, 1.5 * inter-quartile range from the box; points, outliers).
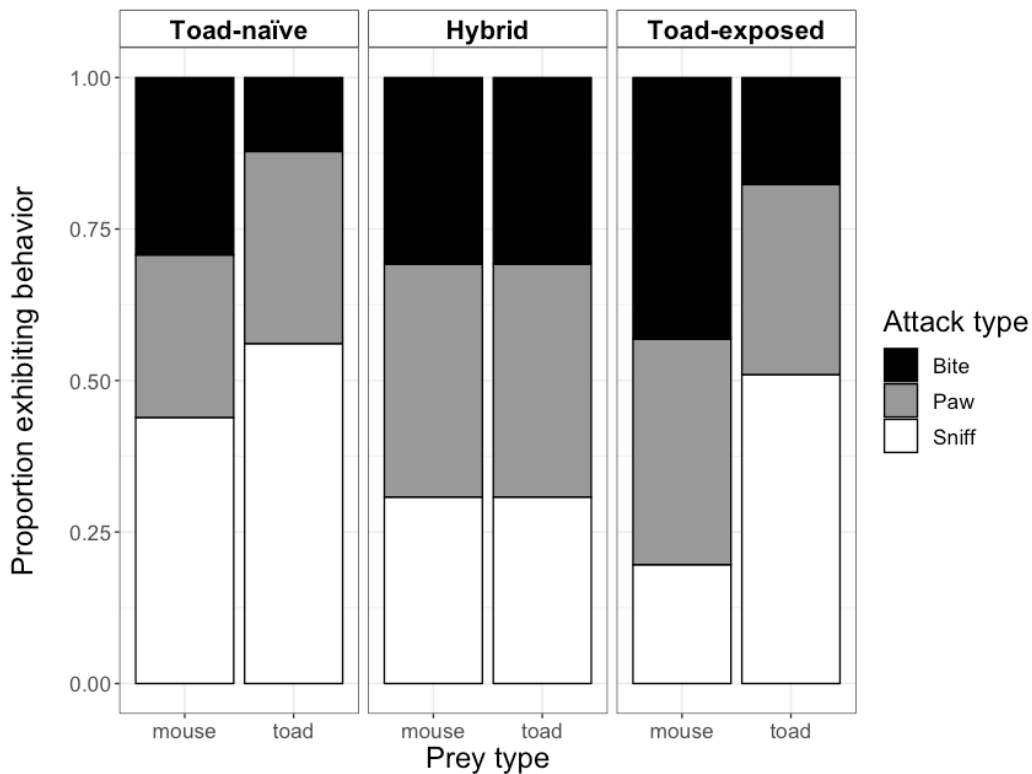
Figure 4.3. Proportion of captive-bred northern quolls exhibiting the 3 different attack types (bite, paw, sniff) in the first minute of interaction with prey items (mouse and cane toad) for each origin population (toad-naïve parents, n = 41; hybrid parents, n = 13; toad-exposed parents, n = 50).
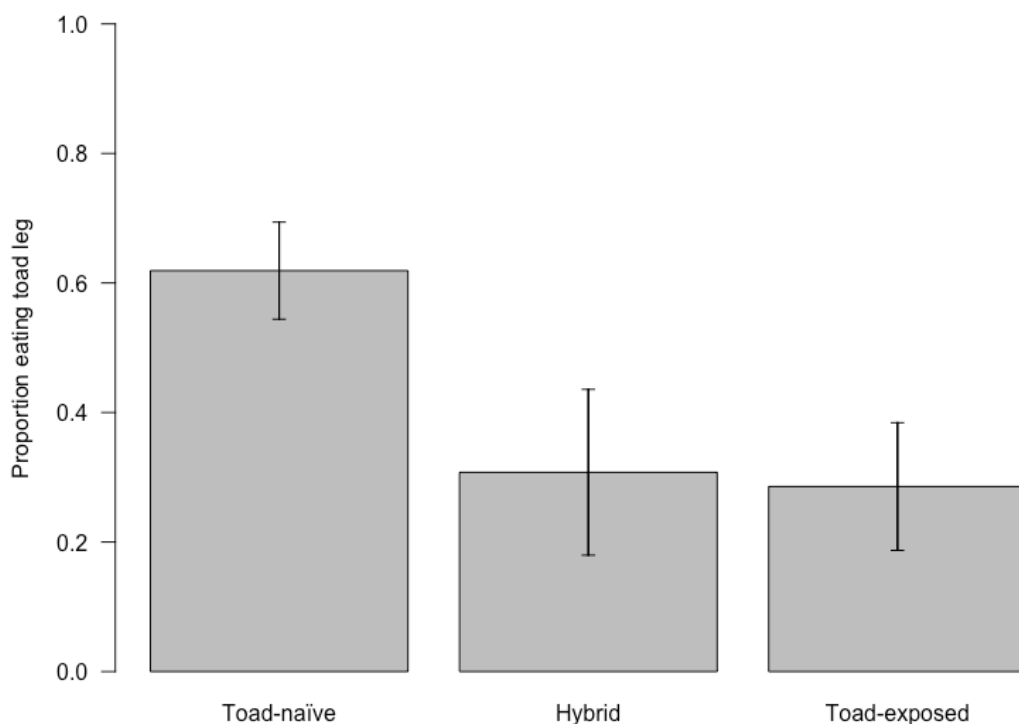
Figure 4.4. The proportion of captive-bred northern quolls with toad-naïve, hybrid, and toad-exposed parents eating a cane toad leg presented to them overnight (±SE).

Acquisition behaviour results suggest quolls from a toad-exposed origin may show a greater discrimination between the prey types compared with animals from a toad-naïve population and hybrids. This interaction between toad-naïve and toad-exposed populations was, however, not significant ($\chi^2(2)$ = 3.51, p = 0.17) (Figure 4.3 & Appendix II). There was no overall difference in attack behaviour between the populations ($\chi^2(2)$ = 4.40, p = 0.11), but again, there was a significant difference between prey items, with quolls more likely to attack mice than toads ($\chi^2(2)$ = 9.05, p = 0.002).

The results from the final experiment showed northern quolls with toad-exposed parents were significantly less likely to consume a toad leg than those with toad-naïve parents ($\chi^2(2)$ = 9.13, p = 0.010;) (Figure 4.4 & Appendix II) and that hybrid origin animals also had a low tendency to consume toads, similar to the response of animals with a toad-exposed origin.

Discussion

Our results show a clear difference in the choice of whether to eat a toad or not in quolls from different origins. Despite being raised in identical toad-free environments, quolls born to parents from toad-infested areas were significantly less likely to eat a cane toad than those from toad-naïve lineages. We controlled the environmental effects that may have influenced this behaviour – quolls had no prior exposure to (so chance to learn to avoid) cane toads. Therefore, we

can conclude that this is an innate, genetically based trait that has likely been under strong selection in populations of northern quolls surviving in toad-infested areas.

The differences between the populations was less clear, however, when we examined behaviours associated with foraging and acquisition behaviour. Although in both cases our data hint that quolls with parents from toad-exposed populations showed greater discrimination between mouse and toad prey, the results were nonsignificant in both cases. Together, our results suggest that although the final decision to eat a toad is strongly innate, foraging behaviour is more plastic.

That foraging and acquisition behaviour was plastic is not surprising; many predators have been observed to rapidly shift foraging and acquisition behaviours, (particulary when housed in captivity; Bremner-Harrison *et al.* 2004; Watters & Powell 2012; Reading *et al.* 2013). Quolls are no exception. Earlier work shows, for example, that individual quolls can be trained not to attack toads (Cremona *et al.* 2017) and that captive-born offspring (F1 and F2) tend to be bolder in prey acquisition trials (Kelly & Phillips 2017). In wild-caught quolls (many of whom were parents of the animals we tested here) we previously observed large differences in toad-smart foraging and acquisition behaviour between toad-naïve and toad-exposed populations (Kelly & Phillips 2017). These differences are either substantially weaker, or non-existent in the captive born generation tested here. Because we removed the chance for learning in response to toads, our results indicate that although the decision to eat a toad appears strongly innate, environmental learning is likely involved in the more plastic behaviours, such as foraging. Thus, it seems likely that – in the presence of toads – an innate tendency not to eat toads eventually translates into toad-smart foraging and acquisition behaviour. That is, animals with a tendency to not consume toads eventually learn not to expend effort acquiring them.

Our breeding experiment also demonstrated that it is possible to cross breed quolls from distant populations. Litter sizes and survival of population crosses were indistinguishable from those within populations. Although our sample sizes are small – particularly for the hybrid group – it is interesting to note that hybrid litter sizes were large, and almost identical to those in the toad-exposed group. It remains possible that incompatibilities will be expressed in F2 or greater generations, however, so further hybrid litters and deeper generations are needed to exclude outbreeding depression entirely. The hybrid quolls, however, also had interesting behavioural responses. Regarding acquisition, hybrids showed the highest proportion of attacking behaviour when interacting with the dead prey items inside a cage. This attacking behaviour was also identical across the two prey types offered, suggesting that hybrid quolls were overall more aggressive in their interactions with prey, and showed much lower discrimination between a mouse and a toad. These results were not statistically significant but are intriguing nonetheless; hybrids may exhibit acquisition

behaviours more extreme than either parental phenotype, a result that suggests overdominance for this trait (Parsons & Bodmer 1961).

Despite this potentially increased aggression, the results of the consumption experiment indicated hybrids tended not to eat toads. This aspect of toad-smart behaviour in the hybrids, being much closer in value to the toad-exposed population, suggests a standard dominance effect (Mendel 1866; Veitia *et al.* 2017). If the trait were due only to additive genetic effects, we would expect hybrids to have trait values intermediate between the two parental populations (although our experiment may not have the power to dectect this; Hill *et al.* 2008). Instead, there is a hint that whatever alleles influence the decision to eat a toad, these alleles are dominant – having one toad-smart parent made the offspring toad-smart. This makes sense because such alleles would rapidly come to high frequency following hard selection (Hazel 1943), such as undoubtedly occurred in the toad-invaded part of the quolls' range. All such interpretations are, of course, speculative. Unequivocal demonstration of dominance and outbreeding effects can only be achieved with a substantially larger sample size and more complex pedigree than were available to us (Lynch & Walsh 1998).

We have, however, been successful in answering the question – does toad-smart behaviour have a genetic basis? Despite the variability in foraging behaviour, our data strongly imply that important aspects of toad-smart behaviour do have a genetic basis, with observed phenotypic differences occurring in an adaptive direction. A genetic basis to prey choice has been demonstrated in a broad array of taxa (Ayres & Arnold 1983; Lindström *et al.* 1999), including in Australia reptiles responding to cane toads (Phillips & Shine 2006; Llewelyn *et al.* 2011). However, it remains possible that maternal effects may be influencing our results (Mousseau & Fox 1998), but in our case this seems unlikely due to hybrid behaviour being similar whether the mother was toad-exposed or toad-naïve. We examined quolls only from a few local populations and are assuming the behavioural differences we observed are broadly relevant to toad-naïve and -exposed populations. Our results also hint that the genetic effects are not simply additive; instead, patterns were consistent with dominance and overdominance. Plasticity in behaviour (particularly foraging) likely also plays a role in fine-tuning innate tendencies. Previous studies suggest that toad-smart behaviour exists at low levels in toad-naïve populations (Kelly & Phillips 2017), and our study clearly indicates that even animals from toad-naïve populations are able to discriminate toads from other prey types. Therefore, it seems likely that this pre-existing trait variation is rapidly selected for once cane toads arrive, leading to either extinction or evolutionary rescue occurring over a short period of time. Certainly, evolution can occur over contemporary timescales (Schoener 2011; Colautti & Lau 2015), particularly in response to sudden anthropogenic change such as the arrival of invasive species (Stockwell *et al.* 2003). Such rapid evolution has already been demonstrated in other taxa responding to cane toads (Phillips & Shine 2004, 2006), and we can now add northern quolls to the list of species exhibiting rapid adaptation to toads.

Although this adaptive response can allow northern quolls to live alongside cane toads, the widespread extinction of quoll populations following toad arrival suggest that extinction usually occurs before adaptation is complete. That toad-smarts have a genetic basis means we could use targeted gene flow to shift the balance in favor of adaptation rather than extinction. By introducing toad-adapted individuals into target populations we could potentially improve the resilience of northern quoll populations prior to cane toad arrival, or facilitate population recovery or reintroduction efforts. This study represents the first step toward this goal, but more needs to be investigated before targeted gene flow for quolls can become a reality. Targeted gene flow aims to increase population viability whilst still maintaining local genetic diversity, so investigating this trade-off will need to be done prior to any actions. Population models could help managers predict how best to integrate toad-smart traits without replacing the local genome, by examining how adjusting timing and number of individuals introduced to ensure toad-smarts are in high enough proportion to maintain the population but the local genome is not overwhelmed. This could also help us assess the risks associated with reduced hybrid fitness as well as to investigate the practicalities of implementing the management strategy. As well as population models, targeted gene flow needs to be tested in the field, to determine if individuals with toad-smart genes do indeed have higher fitness and improve population persistence. Our work demonstrates that it is possible to crossbreed individuals from different areas of the quolls' range to produce hybrids, and that these hybrids do not have any obvious fitness deficits. However, monitoring of more litters and additional generations is still required as our sample size was small and many aspects of outbreeding depression may not become obvious until the F2 or F3 generation (Fenster & Galloway 2000; Aitken & Whitlock 2013; Frankham 2016). At this point, however, the potential benefits of targeted gene flow in this situation appear to outweigh the risks.

Cane toads have already caused local extinctions of many northern quoll populations, and the toads' invasion appears unstoppable. The aim now should be to ensure northern quolls persist in areas after cane toads arrive, that quolls maintain their functional role in the ecosystem, and that we preserve as much of the species standing genetic variation as possible. Here, we have shown targeted gene flow is a potential strategy to this end. We know we quolls can be trained to not attack cane toads via conditioned taste aversion, and the tactic is currently being deployed ahead of the invasion front (O'Donnell *et al.* 2010; Cremona *et al.* 2017; Jolly *et al.* 2017). Training is, however, neither universally effective nor is there compelling evidence that there is cultural transmission of this acquired aversion to offspring. Effectiveness of this training strategy could be improved by introducing individuals carrying toad-smart genes. Although much of the practical complexities require further thought, by combining targeted gene flow with existing conservation strategies this endangered species may well be its best chance of survival.

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Chapter 5

Taste overshadows less salient cues to elicit food aversion in endangered marsupial

Abstract

CONDITIONED taste aversion is an emerging conservation tool that can be used to limit inter-species conflict, for example decreasing predation on endangered species, or limiting the consumption of invasive toxic prey. Typically, managers wish to elicit an aversion that will be associated with visual or odour cues so that the target species maintains an "arm's length" relationship, and does not have to attack or taste the prey. Combining multiple cues in conditioned taste aversion can cause cue overshadowing, which reduces the effectiveness of the training. Here, we examine the northern quoll (*Dasyurus hallucatus*), a carnivorous marsupial threatened because they attack the toxic invasive cane toad (*Rhinella marina*). Conditioned taste aversion has been suggested as a way to reduce quoll's predation on toads, but for training to be effective it must elicit an aversion to stimuli before the quolls attack the

toad. Using baits containing distinct novel meat, odour and visual cues, we test whether quolls will generalise their aversion to visual or odour cues. We found that quolls associate their aversion with the compound stimulus of meat taste and meat odour, and that this overshadows the less salient artificial odour and visual cues. Quolls ate 87% of taste control substituted baits, compared to 38% and 42% of the odour and visual control substituted baits respectively, which represented a significant interaction between baits substituted with the control cue and bait type ($\chi^2(4) = 14.70$, $p = 0.005$). We show that although quolls do not generalise their aversions to novel artificial stimuli, they can distinguish subtle visual and odour cues in meat that still elicit an aversion without them needing to attack and taste prey.

Introduction

Conditioned taste aversion is increasingly used as a conservation tool to reduce inter-species conflict (Gustavson *et al.* 1983; Conover 1995; Indigo *et al.* 2017). A highly conserved learning response mechanism present in all vertebrates, conditioned taste aversion occurs when an animal associates a certain food type with illness caused by ingestion of a toxic substance (Garcia *et al.* 1974, 1985; Bernstein 1999). This allows foragers to learn rapidly and avoid poisoning when tasting novel food in their environment. Since this phenomenon was first described, conservationists have trialled the use of conditioned taste aversion as a non-lethal alternative to pest control (Gustavson & Nicolaus 1987; Conover 1990), or to reduce the impact of invasive species (Cremona *et al.* 2017; Ward-Fear *et al.* 2017).

Harnessing conditioned taste aversion for a conservation benefit requires knowledge about the cues that animals use to distinguish edible from toxic prey after their initial encounter with the toxic substance. Often, animals associate taste with illness, but they may also generalise their aversion to odour or visual cues (Pearce 2013). From a conservation perspective, conditioned taste aversion is likely be more effective in situations where target species can use visual or odour cues to recognise unpalatable prey from a distance, without the need to attack or taste the prey (Cowan *et al.* 2000; Baker *et al.* 2007). For example, to reduce crow predation on green-coloured eggs, researchers placed toxic green eggs in territories of American crows. After this intervention, the crows that had consumed toxic green eggs subsequently avoided palatable green eggs, but they continued to consume palatable white eggs (Nicolaus *et al.* 1983). Although some species may generalise their aversion to odour or visual cues, this response is not universal. In some circumstances, associating stimuli (such as taste with novel odours) may also reduce the individual effectiveness of the cues through a process called overshadowing. That is, when two cues are presented together they gain less associative strength each than if they were presented alone alongside the negative experience (Pearce 2013).

Whether predators generalise their aversion of taste to odour and visual cues is particularly relevant in conservation programs where the aim is to protect eggs from predators (Nicolaus *et al.* 1983), reduce predation on livestock or endangered species (Gustavson *et al.* 1974), or reduce native predation on introduced toxic prey species (O'Donnell *et al.* 2010). In Australia, the invasive cane toad *Rhinella marina* has spread across much of northern Australia (Kearney *et al.* 2008; Tingley *et al.* 2017), and has caused population declines of native predators such as goannas, snakes, crocodiles and quolls (Letnic *et al.* 2008; Doody J. S. *et al.* 2009; Woinarski *et al.* 2011). These predators are naïve and susceptible to the toad's toxins, and individuals that consume them are fatally poisoned (Shine 2010). Conditioned taste aversion was suggested as a tool for mitigating the impact of toads on these predators (Webb *et al.* 2008), and recent trials have been promising (Cremona *et al.* 2017; Indigo *et al.* 2017; Ward-Fear *et al.* 2017).

Toad aversion training involves offering the predator a non-lethal cane toad (with toxin squeezed out) or a small toad (or toad meat) laced with a nausea-including chemical. This approach has produced an aversion to attacking live cane toads in goannas (*Varanus panoptes* ; Ward-Fear et al. 2017), blue-tongue lizards (*Tiliqua scincoides intermedia*; Price-Rees, Webb, and Shine 2013) and northern quolls (Dasyurus hallucatus; O'Donnell *et al.* 2010). Northern quolls, an endangered mesopredator that used to inhabit much of northern Australia, can develop an aversion to cane toads after being fed toad meat (either small metamorphs; (O'Donnell *et al.* 2010) or minced toad legs; (Indigo *et al.* 2017)) combined with the nausea inducing chemical thiabendazole. This process elicits an aversion to dead adult toads and live metamorphs in captive quolls (O'Donnell *et al.* 2010; Indigo *et al.* 2017), and can increase a quoll's survival following reintroduction to toad-infested environments (Cremona *et al.* 2017; Jolly *et al.* 2017). The deployment of toad sausages ahead of the toad invasion front may also increase the survival rates of wild quolls, with tests of this possibility currently underway (Indigo *et al.* 2017). Landholders and conservation managers are currently trialling broad-scale aerial deployment of toad sausages in the Kimberley, Western Australia, in an attempt to reduce the impact of toads on these soon-to-be-invaded populations.

Many practical challenges remain for developing an effective toad-aversion bait. Although past trials have shown overall positive responses, there has been variation in the techniques used, and their effectiveness across individuals. For instance, male quolls appear much harder to train than females (O'Donnell *et al.* 2010). The mass deployment of baits creates additional problems, such as the need to include preservatives to stop baits degrading, and the question of uptake by non-target species (Indigo *et al.* 2017). Because quolls use visual, olfactory and auditory cues to detect and attack prey, and will attack prey swiftly without hesitation (Kelly & Phillips 2017), any toad training must elicit an aversion to the sight and smell of live adult toads. We cannot, however, use live

adult toads as the conditioning stimulus (the quolls would die from attacking them), but nonetheless require a stimulus that creates a strong aversion to live cane toads in wild quolls.

Here, we examine the process of conditioned taste aversion in northern quolls. We use novel baits combining distinct taste, odour and visual cues to examine which cues quolls associate with illness. We investigate whether quolls generalise their aversion to visual or odour cues, a question that is crucial for conserving northern quolls, and is relevant to the broader theory of associative learning. We predict that being nocturnal carnivores, northern quolls may generalise their aversion practically to the smell and taste of the meat.

Methods

Experimental setup

The experiment was conducted at a northern quoll captive breeding facility at the Territory Wildlife Park, Northern Territory, Australia in May 2016 (immediately prior to the northern quoll breeding season). We used two groups of northern quolls in the experiment, the first were wild caught quolls from the offshore predator- and toad-free Astell Island, Northern Territory, Australia that were brought into captivity in February 2016 (n(males) = 10, n(females) = 15). The second group were first generation captive born northern quolls with parents from toad-infested Mareeba, Queensland, Australia (n(males) = 4, n(females) = 7). The disparity in sample sizes and origins were due to logistical and ethical difficulties associated with working on an endangered short-lived mammal. To control for this, we included origin as a factor in the analysis.

The northern quolls used in the experiment were housed individually in 2x4m enclosures at the Territory Wildlife Park (adult northern quolls are aggressive and will attack conspecifics when housed together). Quolls were checked each morning at 8am and fed at 4pm each evening (except for nights of the experiment, where regular food was withheld). Being nocturnal species, all experiments were conducted overnight, beginning at dusk. The University of Melbourne Animal Ethics Committee gave its permission (ID number: 1413369.2) to carry out the experiment. After the experiment, all individuals remained at the Territory Wildlife Park as part of an ongoing captive breeding program.

Experimental design

Each individual was allocated a treatment and control bait from two completely novel bait types (with novel taste, smells and shapes). The baits were 1) pork mince laced with orange essence in a star shape and 2) kangaroo mince laced with vanilla essence in a heart shape. These meat, odour and visual cues were

all novel to the quolls. They were trained to avoid the treatment bait using conditioned taste aversion. After training, each individual was given choices to eat their treatment, control and one control substituted bait – where the control substituted bait was their treatment bait with one cue (taste, smell, look) swapped for the control equivalent. The experimental design is summarised in Figure 5.1.

Experimental procedure

The experiment began with a training phase, where each individual was randomly allocated to either group 1 or 2, with a control and treatment bait from either the pork or kangaroo bait types (Figure 5.1). To begin with, we gave each quoll their allocated control bait (5g; not laced with thiabendazole) for three repeated nights or until the bait was eaten. Then, we presented each quoll with the treatment bait (Conditioned Stimulus) which was laced with chemical thiabendazole (Unconditioned Stimulus; Sigma Aldridge, Sydney, Australia) at a dose rate of 400 mg kg^{-1} quoll mass in a 5g bait – a dose rate that has been shown to elicit illness in northern quolls previously (O'Donnell *et al.* 2010). We repeated the dosed treatment bait until the bait was eaten or for up to three nights. Baits were given instead of the quoll's regular food and if a quoll did not consume either the control or the treatment bait within the three nights it was removed from the experiment ($n = 2$ removed; sample sizes above represent final numbers of quolls who remained in the experiment).

After training we moved onto the testing phase, where each individual was presented with a three-way choice: treatment bait (without thiabendazole), control bait and a "control substituted bait". The control substituted bait was the treatment bait, with one of the three cues (taste, odour, visual) substituted with the control equivalent. This choice experiment was repeated over three nights with the control substituted changing so that each cue was substituted with its control equivalent (in randomised order). For example, if an individual was trained with the pork treatment bait (pork mince laced with orange essence in a star shape), their taste control substitute bait would be *kangaroo mince* laced with orange essence in a star shape (Figure 5.1). The morning after each choice experiment we recorded which baits had been consumed overnight.

Data analysis

We performed a logistic regression in *R* to determine the effect of bait type and substituted cue on the likelihood of a quoll eating a bait (R Core Team 2013). Sex, timing (the order the substituted baits were given in over the three nights) and origin were also included as factors in the model. P-values were obtained by likelihood ratio tests and are presented as chi squared and p-values (with significance determined as <0.05). Results are presented as percentage of individuals who consumed a bait type.
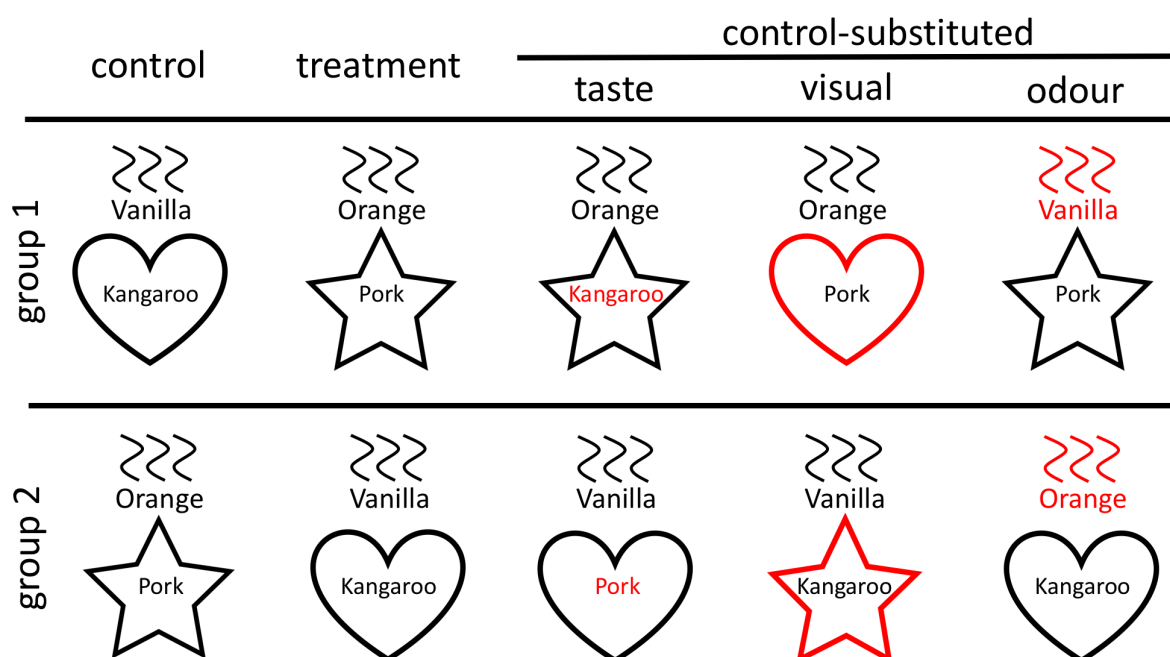
Figure 5.1. Control, treatment and control substituted baits given for each group.

Results

Our results demonstrated that northern quolls were associating meat type with the unconditioned stimulus, while the novel odour and visual cues alone did not elicit an aversion (Figure 5.2). Bait type had a significant effect on the likelihood of consumption ($\chi^2(6) = 111.12$, $p < 0.001$; Table 5.1), with 92% of the control baits being eaten, versus only 36% of the treatment baits. There was a significant interaction between bait type and substituted cue ($\chi^2(4) = 14.70$, $p = 0.005$), with 87% of the taste control substituted baits being consumed compare to 38% and 42% of the odour and visual control substituted baits respectively.

Table 5.1. Binary logistic regression analysis of likelihood on consuming treatment, control and control substituted baits for northern quolls.

Coefficients	Estimate	SE	P
Intercept	2.00	0.75	
Bait type (treatment)	-3.45	0.76	< 0.001
Control substituted (taste)	0.20	0.99	< 0.001
Bait type * control substituted	2.49	1.20	< 0.001
Sex (male)	1.80	0.33	< 0.001
Origin (Queensland)	-1.16	0.34	< 0.001
Order of control substitution	0.23	1.90	0.22
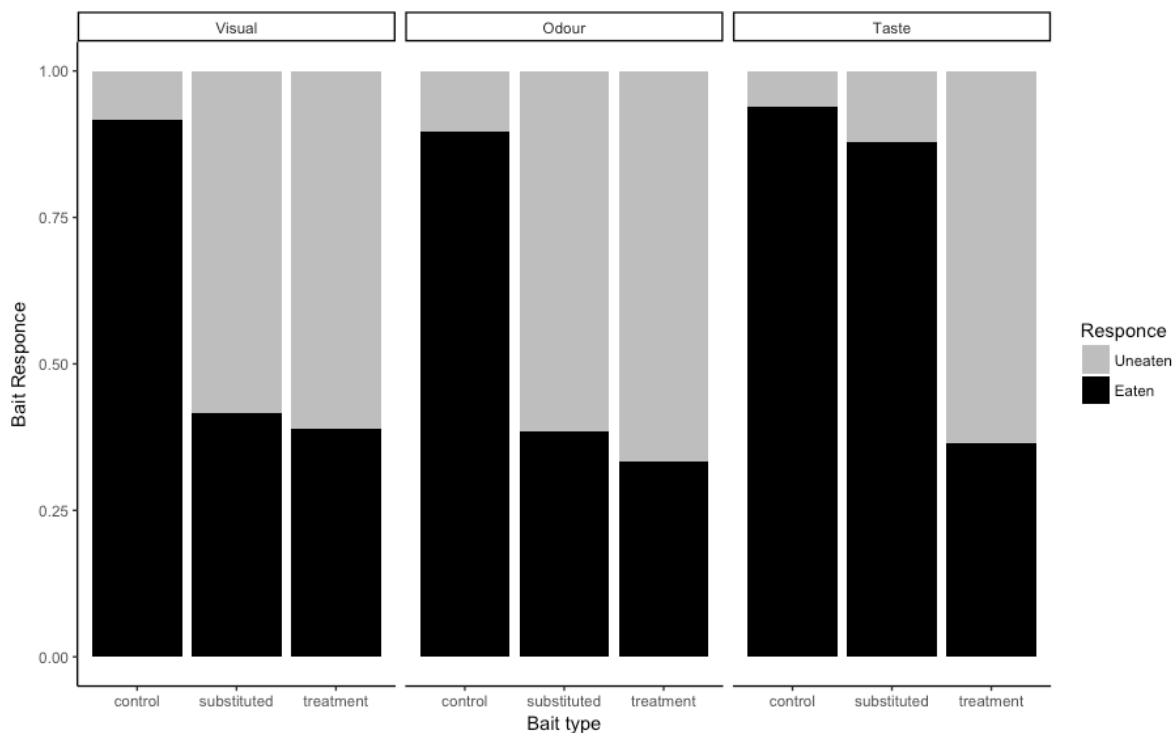
Figure 5.2. Response of northern quolls (n = 36) to baits following conditioned taste aversion (eaten = black, uneaten = grey). Three bait types were treatment (bait they associated with negative stimulus), control (bait they were not trained to avoid with conditioned taste aversion) and substituted (control substituted bait which was the treatment bait with one cue (look, smell, taste) substituted with the control cue). Panels indicate which cue was substituted.

Males were significantly more likely to consume baits than females ($\chi^2(1) = 37.80$, $p < 0.001$), and wild caught Astell Island quolls were significantly more likely than captive born Queensland quolls to eat the baits ($\chi^2(1) = 12.56$, $p < 0.001$). Order that the control substituted baits were presented in had no effect on the likelihood of consumption ($\chi^2(1) = 1.50$, $p = 0.22$).

Discussion

Our results demonstrate that northern quolls use novel taste cues to identify and avoid food that previously made them ill. Novel artificial odour and visual cues, however, did not produce an aversion when not coupled with the treatment meat. Due to the nature of the experiment, we could not completely uncouple the taste of the meat with its odour and visual properties – kangaroo and pork mince both have their own smell, colour and texture that could not be removed from the experiment. Thus, our results show that northern quolls develop an aversion to the compound stimulus of meat and meat characteristics, but do not generalise their aversion to the novel odour or visual cues that were paired with the meat. We found that some groups were more likely to consume treatment baits than others. Males, for instance were more likely to eat their treatment

baits – a result that has been found in other conditioned taste aversion studies on quolls (O'Donnell *et al.* 2010).

Additionally, wild-caught quolls were also more likely to consume treatment baits, perhaps reflecting behavioural changes that occur in captive born animals (McDougall *et al.* 2006). Perhaps quolls raised in captivity are likely more confident of consistent food supply, and therefore more likely to reject food. Previous work showed that captive born quolls were bolder than their wild-caught parents, spending more time investigating prey items (Kelly & Phillips 2018). An alternative explanation here is natural selection on the conditioned taste aversion apparatus. Quolls from Queensland have been under strong selection from toads (80+ years since cane toad arrival), and conditioned taste aversion response may be one of the traits under selection. By contrast, animals from the island have not been exposed to toads, and may also have experienced no other toxic prey for the last 13 generations. These 13 generations have been sufficient time for their innate response to predators to be lost (Jolly *et al.* 2018), so it is likely that other traits have also shifted as dramatically. Further work would be required to discriminate these possibilities, however.

We did not find any evidence of stimulus generalisation: quolls did not associate novel odour or visual cues with illness. Instead, the associative strength of the meat characteristics overshadowed the less salient artificial odour and visual cues. Quolls were able to discriminate between these different stimuli, and associate the aversion with the more natural meat cues – potentially because it was something more likely to be associated with their negative experience of feeling ill (Nachman *et al.* 1977). These results support the theory that compound cues overshadow less salient cues in conditioned taste aversion, something that has also been demonstrated in rats in previous laboratory experiments (Mikulka *et al.* 1982; Mondragón & Hall 2002).

Our result is interesting, because previous studies have found that some species generalise their aversions to visual stimuli. For example, crows that ingested painted green eggs containing an emetic subsequently avoided non-toxic green painted eggs thereafter (Nicolaus *et al.* 1983). Additionally, wild badgers that consumed baits paired with ziram (a fungicide that induces nausea) and clove oil subsequently avoided untreated baits containing clove oil (Baker *et al.* 2007). This stimulus generalisation can also occur when predators encounter the natural cues associated with live prey. For example, planigales that consumed toxic cane toads and became ill subsequently developed an aversion to the smell of all amphibians, irrespective of their colour (Webb *et al.* 2008). Engendering stimulus generalisation in predators is important if want to modify their behaviour such that they sniff and reject prey from a distance. However, we did not find evidence that quolls generalise their aversion to novel odour or visual cues. Potentially, this could be a result of the intensity of the illness, or the time delay between the consumption of food and onset of the illness (Revusky 1968). In quolls, thiabendazole does not induce vomiting, but

the planigales in previous experiments experienced sickness near-death (Webb *et al.* 2008).

However, quolls presented with the compound cue of meat and meat odour by and large completely ignored (did not touch or taste) the baits – the outcome we were after. Unfortunately, we were unable to uncouple the meat cues (taste, odour, look and texture) to determine what cue elicited this aversion in northern quolls. Perhaps it is the combination of these associated meat cues, but the most likely stimuli is meat odour, considering the quolls rejected the treatment baits without tasting the meat. This demonstrates that quolls can distinguish between the novel odour and the meat odour, and generalise their aversion to natural meat stimuli. Although not tested, presumably northern quolls have a well refined sense of smell (marsupial olfactory bulbs are generally quite large; Delbridge *et al.* 2010), which allowed them to distinguish between the meat and artificial cues.

The knowledge of how conditioned taste aversion occurs in this endangered species will help us to conserve them. Toad aversion training is fast becoming the management strategy of choice for protecting populations of quolls prior to toad invasion, but there still remains problems with its deployment (Indigo *et al.* 2017). For instance, bait longevity has emerged as a problem for mass deployment of toad aversion baits, because the climate quolls inhabit is tropical and hot, leading to rapid decomposition of the baits. We have demonstrated that quolls do not generalise aversions to novel odour or visual cues, suggesting that the inclusion of preservatives in toad baits is unlikely to endanger the associative strength of the toad stimuli.

We have demonstrated that northern quolls associate natural (meat) stimuli with an aversion, and that this overshadows less salient artificial cues. Presumably, this would translate to avoidance of a live prey item, as has been demonstrated in previous toad-aversion training studies. In our study, however, we see a much higher aversion to our meat baits compared to studies in which aversion to live prey is measured following training with a meat bait (O'Donnell *et al.* 2010; Jolly *et al.* 2017; Indigo *et al.* 2018). There is clearly dissociation occurring in some individuals between the meat bait and the live prey stimuli.

More broadly, our results fit with the current learning theory that more salient compound cues overshadow that of artificial odour and visual cues (Pearce 2013), but contrast with studies showing that some species generalise their aversion to odour or visual cues (Gustavson *et al.* 1974; Clarke *et al.* 1979). This inconsistency in results across studies is likely due to differences in stimulus strength (i.e. degree of illness), as well as underlying differences in target species and their foraging behaviour (crows are much more visual species than quolls, for example). Future research on this topic is clearly necessary to fine tune conditioned taste aversion as a tool for wildlife conservation.

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Chapter 6
How many and when? Optimising targeted gene flow for a step change in the environment

Abstract

TARGETED gene flow is an emerging conservation strategy that involves introducing individuals with particular traits to places where these traits are of benefit. One obvious application is to adapt a recipient population to a known threat, but questions remain as to how best to achieve this. Here, we vary timing and size of the introduction to maximise our objective – survival of the recipient population's genome. We explore a generic population model as well as a specific example – the northern quoll, an Australian marsupial predator threatened by the toxic cane toad. We reveal a trade-off between preserving the recipient genome and reducing population extinction risk, but key management levers can often optimise this so that nearly 100% of the recipient population's genome is preserved. Any action was better than none but the size of the benefit was sensitive to outbreeding depression, recombination rate, and the timing and size of the introduction.

Introduction

Rapid environmental change is causing declines in biodiversity across the globe (Barnosky *et al.* 2011), and as threats become harder to mitigate, threatened species must adapt to survive (Hoffmann & Sgrò 2011). Existing genetic variation in relevant traits coupled with strong selection imposed by a threatening process may allow some populations to rapidly adapt; staving off extinction through evolutionary rescue (Bolnick *et al.* 2011; Sih *et al.* 2011). Unfortunately, for many threatening processes, beneficial traits are either locally absent or at low frequencies, and this makes extinction more likely than evolutionary rescue (Gomulkiewicz & Holt 1995; Frankham 2015). Targeted gene flow has emerged as a conservation strategy for helping promote these favourable traits in threatened populations (Kelly & Phillips 2016). This strategy involves translocating individuals with key traits to areas where the traits would have a conservation benefit.

Although targeted gene flow is being explored with regard to climate change ("assisted gene flow", e.g., Aitken & Bemmels 2016), it has yet to be robustly assessed: climate change is a relatively slow change, making assessment difficult. Targeted gene flow, however, could provide benefits for populations facing more rapidly-acting threats, such as disease and invasive species. Here it can enhance the capacity of these populations to adapt to predictable, imminent changes in their environment, and so skew outcomes towards evolutionary rescue rather than extinction (Kelly & Phillips 2016). Targeted gene flow is similar to the idea of genetic rescue, where populations of low genetic diversity are bolstered by the introduction of individuals from elsewhere, however targeted gene flow aims to increase the proportion of specific traits instead of just increasing genetic variance in a non-directed way (Tallmon *et al.* 2004; Whiteley *et al.* 2015; Frankham *et al.* 2017). By increasing the chance of evolutionary rescue, we also promote the persistence of the local genome: the aim being to manipulate populations so that they are not only locally adapted, but also carry genes that allow them to survive the current threat (Kelly & Phillips 2016; Ralls *et al.* 2017).

Here, we address questions on the implementation and risks of targeted gene flow using a population viability analysis that incorporates micro-evolutionary process. Conservation managers considering any adaptive relocation must decide on the optimal timing for their translocation, as well as the composition of the introduced cohort (McDonald-Madden *et al.* 2011). Targeted gene flow is no different, with timing and number of introductees likely to have large effects on the benefits for conservation. This presents managers with a "management space" within which we seek the optimum solution. Evolutionary processes clearly have strong influences on population persistence, but are rarely included in models of population viability (Pierson *et al.* 2015). For those models that do incorporate rapid evolution, few look at the potential impact of outbreeding depression (Frankham *et al.* 2011), and none have yet assessed the potential use of targeted gene flow (Frankham *et al.* 2017). For any conservation action, it

is wise to assess the action prior to implementation – particularly to determine the best way to implement the strategy, and to assess possible negative effects (Coulson *et al.* 2001). We addressed these key aspects of conservation decision making (timing and number of introductees) in our model. We also examined how key uncertainties (i.e. population dynamics, outbreeding depression, recombination rate) changes outcomes.

We seek a strategy that, within our management space (timing and size of introduction), maximises expected return for conservation. To identify this strategy requires a clear statement of our management objective (Regan *et al.* 2005). In our case we would like to keep our recipient population extant, but we would like to do so without replacing the local genome. Replacing the local genome with a genome from elsewhere is equivalent to a reintroduction, but one of the main potential benefits of targeted gene flow is the possibility that we preserve local genetic diversity. A sensible objective, then, is analogous to a gambler's expected return: the probability of winning, multiplied by the payout. In our case the payout is the proportion of the recipient population's genome still extant, (calculated as mean proportion of recipient genome within each individual; $\bar{r}_i$), and our probability of winning it, $1 - x$, where x is the extinction probability. Thus, our objective is to maximise the expected return:

$$E(Y) = \bar{r}_i(1 - x)$$

The problem here is a general one: how does varying key management levers (timing and number of introductees) influence the expected return of targeted gene flow following a stepwise change in the environment? A step change, where the environment goes from one state to another, is the simplest possible example to begin to explore these ideas. This could be applicable to any population dealing with a rapid change in their environment (i.e. arrival of a disease or invasive predator), where the population has some proportion of individuals who are resistant to the threat. While modelling evolutionary response to a step change is possible (e.g. Gomulkiewicz & Holt (1995)), modelling population response following targeted gene flow is complicated by the non-normal trait distribution, and linkage disequilibrium that results from the introduction. These issues render an analytical model intractable, so we require an individual-based population model to examine this idea. We execute this here, exploring the idea of targeted gene flow with a generic population model and a generic adaptive trait, and then grounding the ideas in reality with a particular case study – the endangered carnivorous marsupial, the northern quoll (*Dasyurus hallucatus*) and the arrival of toxic cane toads.

Northern quolls are a potential candidate for targeted gene flow: they are threatened by the invasion of the toxic cane toad (*Rhinella marina*) because quolls unknowingly eat the toxic toads and are fatally poisoned (Tingley *et al.* 2017). The great majority of quoll populations (more than 95%) go extinct after the large step-change in their environment caused by the arrival of cane toads (EPBC 1999). Cane toads continue to spread westward, and will eventually

inhabit the quolls' entire range (Tingley *et al.* 2017). Evidence suggests a small number of scattered populations have adapted to the threat (Kelly & Phillips 2017, 2018) by evolving to avoid toads as prey. Recent work shows the behaviour has a heritable basis (Kelly & Phillips 2018). It is this "toad-smart" behavioural trait that we can make use of with targeted gene flow.

Here, we optimise targeted gene flow following a step change in the environment for both a generic individual-based population model and one whose demographic sub-model has been rendered specific to northern quolls. We use a population viability analysis, to explore our management space (timing of the introduction and size of the introduced cohort) to maximise the expected return for conservation.

Methods

We developed two discrete-time individual-based population models. The first was a generic model, with generalised population dynamics. We then used northern quolls as an example to apply the model to a specific scenario. The models differ only in their population dynamics: the mechanics of sexual reproduction and trait inheritance remained consistent across both. In both models we introduced a stepwise change in the environment, an arrival of a threat that imposed hard selection – in the quoll model this represented the arrival of toads. The step change led to selection-driven mortality imposed only in the first year juvenile stage. Animals surviving this event were considered to have survived the threat thereafter. For both models we explored assumptions using a sensitivity analysis of key parameters.

Population dynamics

The two models – the generic population model and the northern quoll specific model – differed only in their population dynamics. The general population model was developed using the Beverton–Holt model of density dependence (Beverton & Holt 2012). For the quoll model, we gave individuals age- and sex-specific annual survival and fecundity rates, and imposed density dependence on juvenile survival.

GENERIC POPULATION MODEL

Individuals in the generic population have a maximum rate of reproduction, R_{max}, modified by density dependence, described using the Beverton–Holt model (Beverton & Holt 2012), which yields their expected reproductive output, $E(W)$:

$$E(W) = \frac{R_{max}}{1 + (\frac{R_{max} - 1}{N^*}N)}$$

In this formulation, N is the number of individuals in the population. $N*$ represents the carrying capacity that would be achieved if all individuals achieved a fecundity of R_{max}. We set $R_{max}=3$ and $N*=500$, but also ran a sensitivity analysis on R_{max} and $N*$ to determine the impact of population dynamics on our results (see Scenarios). All individuals are treated as sexual hermaphrodites and all have the chance to breed with a randomly selected mate. The population is considered an annual species, with individuals only living for one breeding season (survival = 0 for all individuals over one year of age).

NORTHERN QUOLL POPULATION MODEL

The northern quoll population model is more complex, grounded in demographic data collected by previous studies. It incorporates survival rates that differ by sex and age. Within time intervals, reproduction (including pre-weaning survival of babies) is followed by survival of juveniles and adults. Baseline survival probabilities and fecundity were inferred for this model using the average of values drawn from the literature, and density dependence (driven by density of adult females) acted on the probability that offspring survived to weaning (full details in Appendix 3; Table S6.1).

Sexual reproduction

IN both the generic and northern quoll-specific population models the animal's genotype consists of a number of diploid, biallelic loci. A number of loci, n_p, contribute to the organism's phenotype; n_c are involved in incompatibility; and another subset n_n were neutral and used to track the recipient genome (see below). Each offspring from each pair inherits a genotype determined by the fusion of gametes from the sire and dam. In all cases, a gamete is the result of random recombination of the parent's genotype into haploid form. All loci are randomly placed on a chromosome (represented as a line from 0 to 1) at the beginning of a simulation (so that, across simulations we integrate over all possible linkage arrangements). For each gamete, random cuts are made to produce a recombined haploid genome. Genome-wide pairwise recombination rate was calculated as the average proportion of pairwise crossover events between loci. We manipulated recombination rate by adjusting the expected number of cuts made (actual number drawn from a poisson distribution). In the primary models, we set the expected number of cuts on the chromosome to 50 to simulate an approximately 0.5 recombination rate (based on 30 loci). We also explored a lower recombination rates (0.25, set by 1.33 cuts) in the sensitivity analysis (Appendix 3).

Evolutionary dynamics

EACH individual expresses a continuous trait, A, determining whether or not the individual survive the threat (generic, or toads for the northern quoll

model), or not. Thus, *A* experiences threshold selection. The trait is determined by the animal's genotype, and also by environmental variation according to the underlying mechanisms of a simple quantitative genetic model in which the total phenotypic variance is the sum of genetic and environmental contributions: $V_T = V_G + V_E$. Within all our simulations, the environmental variation imposed on the trait remains constant ($V_E = 1$).

THE GENOTYPE AND EXPECTED TRAIT VALUE

A specified number of loci, n_p, contribute to the organism's phenotype. Each phenotype-influencing locus has an equal additive effect on the individual's expected trait value, *E(A)*. Two alleles are possible at each locus, with alleles having an additive effect size of either 0, or *e*, where e represents an increment towards being fitter after the environmental step-change (more toad-smart in the quoll model). The effect size, e is calculated as a function of the environmental variance (V_E), and the heritability (h^2), and is chosen such that the stated heritability is achieved at initialisation given the stated environmental variance and number of loci (Falconer & Mackay 1996). Under a simple quantitative genetic model (with no dominance or epistasis),

$$h^2 = \frac{V_G}{V_G + V_E}$$

or equivalently,

$$V_G = \frac{h^2 V_E}{1 - h^2}$$

Under a binomial distribution, the expected genetic variance is,

$$V_G = 2e^2 n_p f_0 (1 - f_0),$$

where f_0 is the initial frequency of favourable alleles (i.e., those with effect sizes of *e*). Thus, at initialisation, our effect size can be calculated as:

$$e = \sqrt{\frac{h^2 V_E}{2 n_p f_0 (1 - h^2)(1 - f_0)}}$$

An individual's genotypic value (its expected phenotype) is then:

$$E(A_i) = e \sum_{j=1}^{n_p} \sum_{k=1}^{2} a_{ijk}$$

where a_{jk} references the allelic value (either 0 or 1) at allele *k* of locus *j*. *i* indexes the individual.

In the results presented here, the heritability of the trait in the recipient population (h^2) was set to 0.2, based on the estimate of behavioural trait heritability given by Roff (2012). We explored the impact of h^2 in the sensitivity analysis (Figure 6.3 & S6.3). We assume that the population initially has low mean fitness with regard to the threat, and that alleles conferring threat-adapted behaviour are rare in the population. We set the initial frequency of threat-adapted alleles in our recipient population, $f_0 = 0.05$.

THE PHENOTYPE

AN individual's realised phenotypic value, A_i incorporates environmental variation on the expected trait value, and is determined stochastically, as a draw from the normal distribution.

$$A_i \sim N(E(A_i), V_E)$$

SELECTION

THE model implements threshold selection in which all individuals with trait values for $A_i \leq A^*$, will be killed prior to breeding, while all individuals with $A_i > A^*$ will survive. The selection threshold, A^*, is defined as the $(1 - \bar{w}_0)$th quantile of the initial expected phenotype distribution, where $\bar{w}_0$ is the initial post step-change fitness at a population level. $\bar{w}_0$ was determined by running the simulations with different values of $\bar{w}_0$ to get the model to generate an observed population extinction rate (with no management intervention) of 0.95 after the stepwise change. Selection threshold is determined as a function of the environmental variance and the heritability at initialisation. Therefore values of $\bar{w}_0$ varied based on these parameters and are presented in Table S6.2 & S6.3. The expected phenotype distribution is,

$$N(2en_p f_0, V_T)$$

Where V_T is the total phenotypic variation, $V_G + V_E$.

As is likely the case in reality (small population sizes and short time spans), selection acts on this standing variation in the population; we do not consider mutation important (Elena *et al.* 2007).

LOCI INVOLVED WITH INCOMPATIBILITY

TO allow us to incorporate outbreeding depression, each individual also carries loci involved with incompatibility. These loci carry fixed differences between recipient (=0) and introduced (=1) populations.

These loci were used to implement outbreeding depression using the model of two-locus incompatibilities developed by (Turelli & Orr 2000). This model encapsulates the idea that lowered hybrid fitness can be explained by between-locus Dobzhansky-Muller incompatibilities. The Turelli and Orr model includes three types of incompatibilities: those between heterozygous loci (H_0), those

between a heterozygous and a homozygous (or hemizygous) locus (H_1), and those between homozygous loci (H_2). Using this model, we determine the "hybrid breakdown score" $E(S)$ of each individual based on the composition of alleles in their set of loci involved with incompatibility (proportion of loci that are homozygous from population 1 (p_1), the proportion that are homozygous from population 2 (p_2), and the proportion that are heterozygous for material from the two populations (p_H)). Following Turelli and Orr, the hybrid breakdown score is given as,

$$E(S) = n_c[p_1 p_2 h^2 + (p_1 + p_2)p_H h_1 + p_H^2 h_0]$$

where n_c is the number of loci that are contributing to incompatibilities. We then used a simple negative exponential function to link hybrid breakdown score to fitness,

$$s_h = e^{-\alpha E(S)}$$

where α is a constant value, and s_h is the probability of survival from outbreeding depression. When outbreeding depression is activated in the model, all baseline survival probabilities are multiplied by individual s_h values, and survival is then determined by a draw from a Bernoulli distribution with the resultant survival probability.

We used simple dosage ratios for the different classes of incompatibilities: (H_1) = 0.5, (H_2) = 1 and (H_0) = 0.25, to generate the hybrid breakdown score. We then varied the value of α in the fitness function to manipulate the strength of outbreeding depression.

NEUTRAL LOCI

TO track the proportion of the recipient population's genome remaining, we also initialised n_n neutral loci, also carrying fixed differences between recipient (=0) and introduced (=1) populations. We measured changes in the recipient genome in two different ways — the first ($\bar{r}_i$) was calculated as the proportion of recipient population alleles within each individual, averaged over all individuals in the population. This was used, along with probability of extinction (x) to calculate the expected return of our management objective ($E(Y)$: the proportion of the local genome surviving) for each scenario. Therefore, maximum expected return equated to the population with the highest proportion of recipient genome likely to survive the threat (calculated by $E(Y) = \bar{r}_i(1 - x)$).

$\bar{r}_i$, however, underestimates the true proportion of the recipient genome still extant in the population, because the original genome will be scattered across individuals within the population. Therefore, we also calculated $\bar{r}_p$, the proportion of loci which retained recipient alleles across the population. This gives us the true estimate of recipient genome retention, because it counts alleles even when they are at low frequency. While $\bar{r}_p$ is the true proportion of

the recipient genome still extant, it will likely decline over time due to loss of rare alleles through drift. Because of this, we ran our optimisation using the more conservative metric, $\bar{r}_i$, and make a comparison of the two metrics using the generic population model (Figure S6.2).

Scenarios

ALL our scenarios begin with an initially poorly adapted population of individuals — our "recipient population" (where N = carrying capacity – $N = N^*$ for generic model , and $N = K$ for the quoll model). The recipient population is initiated with initial fitness of $\bar{w}_0$ where post step-change probability of extinction with no management is 95% (Table S6.2 & S6.3). The number of loci was set to 30 (n_c = 10 relating to incompatibility, n_p = 10 relating to the trait and n_n = 10 neutral loci). Carrying capacity of breeding individuals (N^* or K in generic and quoll models respectively) was set to 500. The recipient population was allowed to grow for 30 years before the step change in the environment, which happens at the beginning of a generation. For the quoll model, this sudden change reflects the reality of toad arrival (Phillips 2007).

In each scenario we recorded whether the population went extinct or not over 50 years following the step change. For populations that survived we also calculated the proportion of the recipient population's genome remaining. Using these measures, we calculated the expected return — the proportion of recipient genome likely to survive the step change.

TARGETED GENE FLOW

To simulate targeted gene flow we introduced a number of threat-adapted individuals to our recipient populations prior to reproduction in a specified year. Our "source population" was a threat-adapted population, initialized with f_0 = 0.9, age = 1, and (in the quoll model) sex randomly allocated. We explored a management space, varying the timing of introduction and the number of introductees: introduction times ranged from ten years prior to ten years post the step change (in 1 year increments); and the proportion of threat-adapted individuals introduced ranged from 0 – 0.3 in 0.02 increments. This proportion was converted to number of individuals introduced by multiplying by the carrying capacity of the population and rounding down to the nearest whole number. For our standard population size (carrying capacity = 500) this meant we introduced a range of 0-150 threat-adapted individuals. We ran 100 simulations for each scenario to estimate extinction probability, x, and the proportion of the recipient genome remaining, $\bar{r}_i$ or $\bar{r}_p$ (averaged over the 100 simulations).

SENSITIVITY ANALYSIS

THE above scenario was repeated across a broad sample of parameter space

to produce a global sensitivity analysis around key parameters (Table S6.2 & S6.3).

For the generic model we systematically sample the 3-dimensional parameter space in a fully factorial design to determine the impact of varying carrying capacity (N^*), growth rate (R_{max}) and heritability (h^2) on model output (Table S6.2; 27 parameter sets). For each parameter set, we explore the full management space and record the maximum expected return ($E(Y)_{max}$) as well as where in the management space this maximum occurred.

For the northern quoll model we ran the global sensitivity analysis on just carrying capacity (K) and trait heritability (h^2; Table S6.3). We sampled this 2-dimensional parameter space (9 parameter sets), and again for each parameter set record the maximum expected return ($E(Y)_{max}$), and the management strategy that delivered the maximum expected return.

For both models, we also examined the impact of outbreeding depression (leading to lowered fitness of hybrids) and lower recombination rate. For the scenarios with increased outbreeding depression we ran the simulations described above ("Scenarios") but added in varying recombination rates and strengths of outbreeding depression. We set outbreeding depression to have a low and high impact on hybrid individuals by reducing the fitness of F1 hybrids by 10% and 50% of baseline fitness. This was achieved by changing the value of α that converts hybrid breakdown score into fitness (10% reduction in F1 hybrids α = 0.04; 50% reduction in F1 hybrids α = 0.28). For recombination rate, we lowered pairwise recombination rate from 0.5 to 0.25 by reducing the number of cuts on the chromosome. In the generic model, we also explored the combined effect of recombination rate and outbreeding depression (10% and 50% reduction in hybrid fitness combined with 0.25 recombination rate; Table S6.2 & S6.3).

Model simulations were implemented in *R* (R Core Team 2016) and run using *Spartan*, a High Performance Computing system operated by Research Platform Services at The University of Melbourne (Lafayette *et al.* 2016). All code is available at https://github.com/elkelly/TGFquolls.

Results

We found that the success of targeted gene flow was strongly influenced by the timing of the introduction, and the proportion of individuals introduced (Figure 6.1 & 6.2). The results varied between the two population models (generic and quoll), however a general pattern did emerge. Our management objective (Y) was optimised when a larger proportion of individuals (> 0.1 × carrying capacity) were introduced in the years immediately preceding the step change (Figure 6.1 & 6.2).
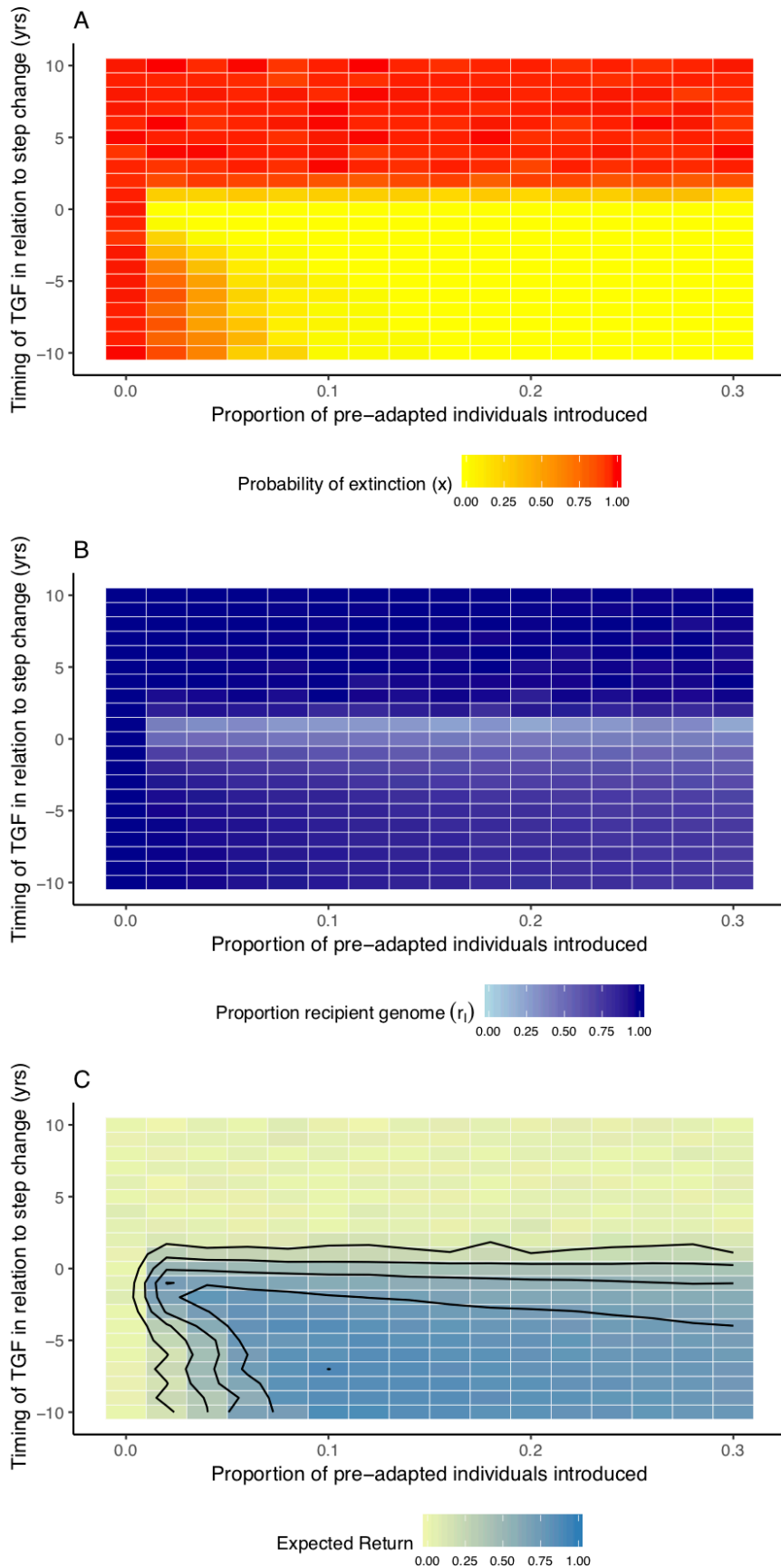
Figure 6.1. Generic population model results across our management space: varying the timing of targeted gene flow (years) and the proportion of pre-adapted individuals introduced. **A:** The probability of extinction of a generic population (*x*; red = high chance of extinction) for varying implementations of targeted gene flow. **B:** The proportion of recipient population genome ($\bar{r}_i$; dark blue is recipient genome) in eventual population after varying implementations of targeted gene flow. **C:** Expected return of the recipient genome (i.e. the proportion of the recipient genome surviving, calculated by $E(Y) = \bar{r}_i(1 - x)$). Management scenario that produced the maximum expected return is represented by a black point ($E(Y)_{max} = 0.89$).
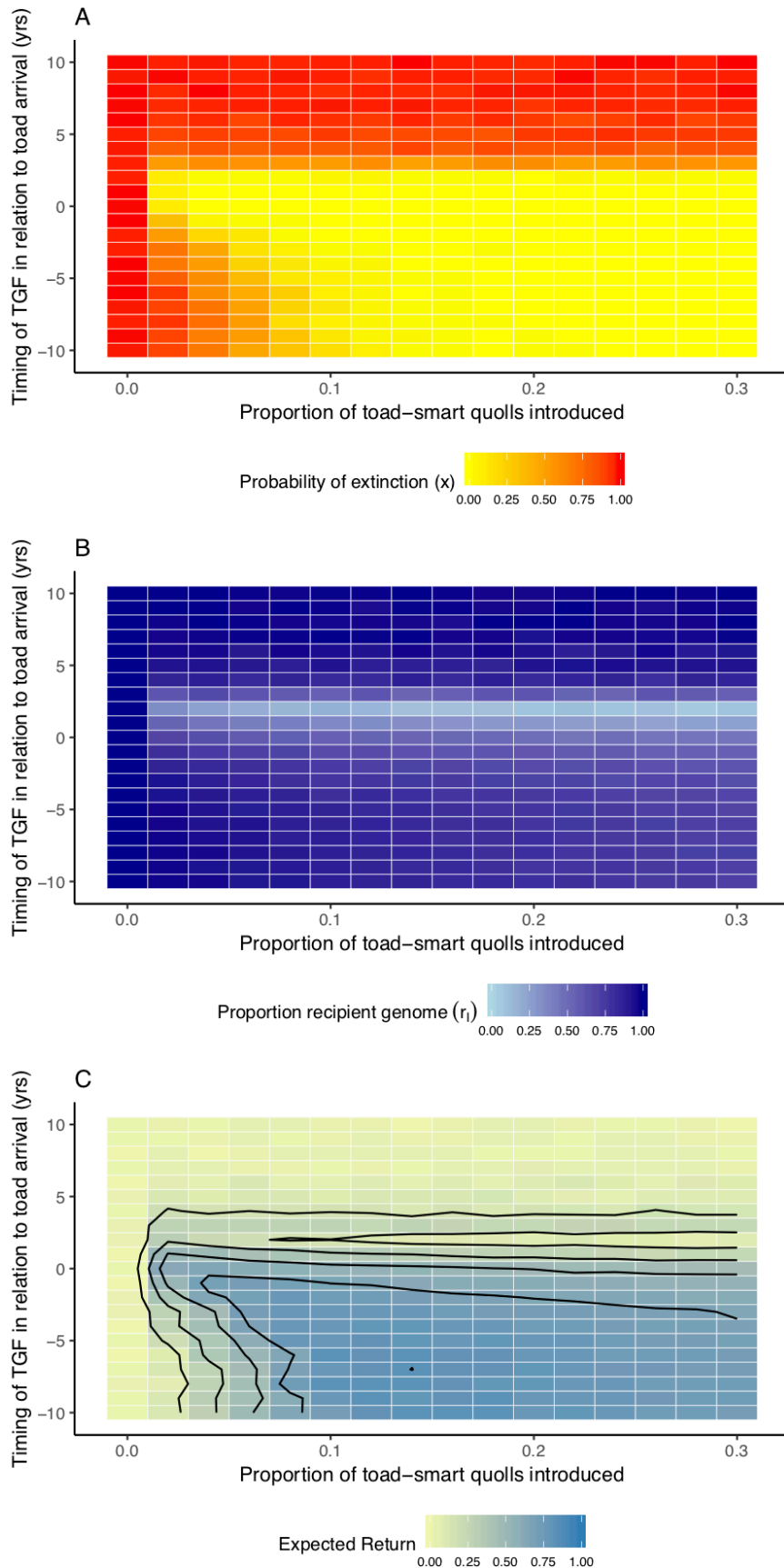
Figure 6.2. Northern quoll population model results across our management space: varying the timing of targeted gene flow (years) and the proportion of toad-smart individuals introduced. **A:** The probability of extinction of a northern quoll population (x; red = high chance of extinction) for varying implementations of targeted gene flow. **B:** The proportion of recipient population genome ($\bar{r}_i$; dark blue is recipient genome) in eventual population after varying implementations of targeted gene flow. **C:** Expected return of the recipient genome (i.e. the proportion of the recipient genome surviving, calculated by $E(Y) = \bar{r}_i(1-x)$ using probability of extinction (x) and proportion of recipient genome ($\bar{r}_i$)). Management scenario that produced the maximum expected return is represented by a black point ($E(Y)_{max} = 0.83$).
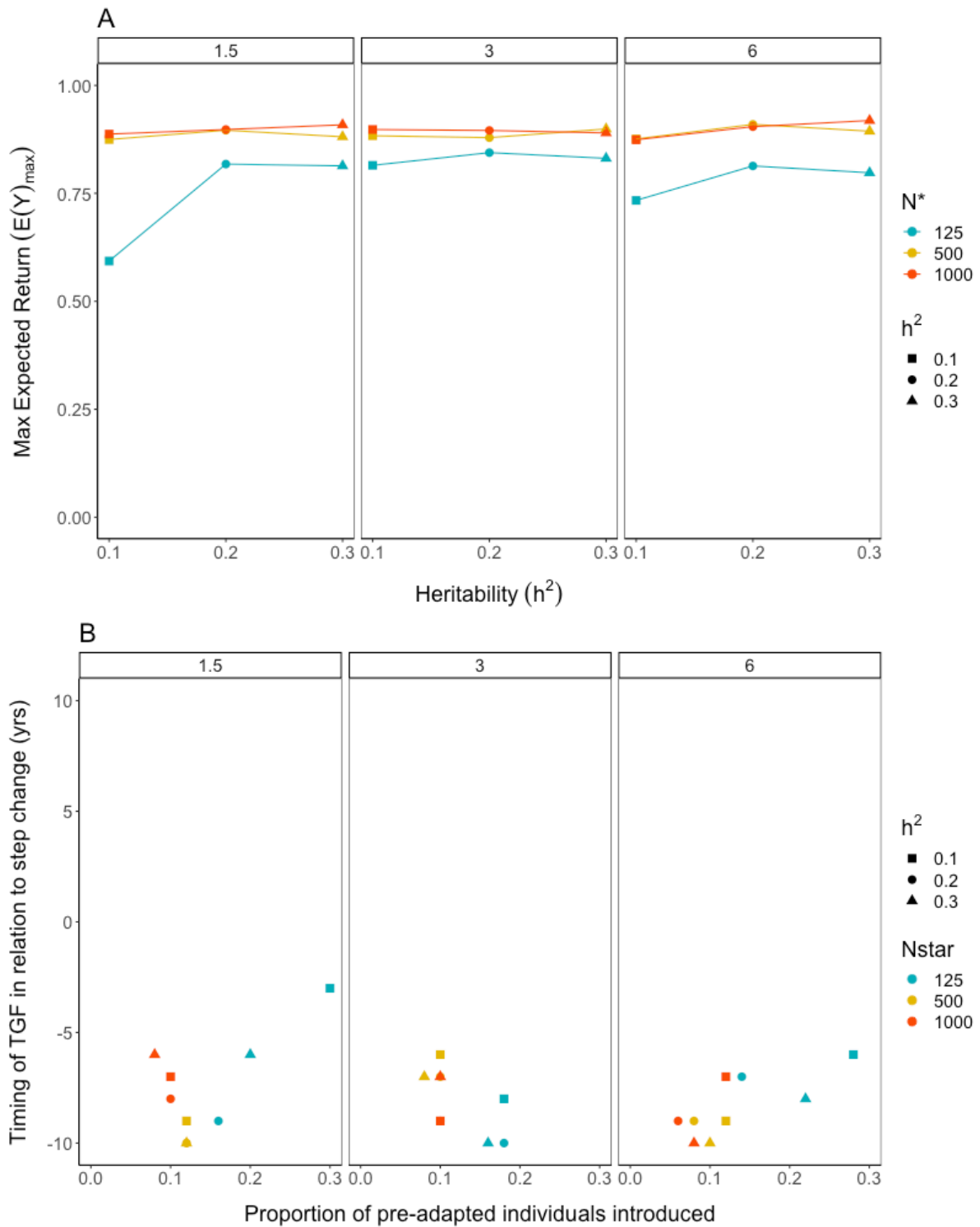
Figure 6.3. Generic population model: global sensitivity analysis exploring three dimensional parameter space: population size (N*: represented by point colours), growth rate (R_{max}: represented in panels) and heritability (h^2: represented as point shapes). Showing **A:** Maximum expected return ($E(Y)_{max}$) from a scenario, and **B:** the location in the management space (the timing of targeted gene flow and the proportion of pre-adapted individuals introduced) that produced maximum expected return.
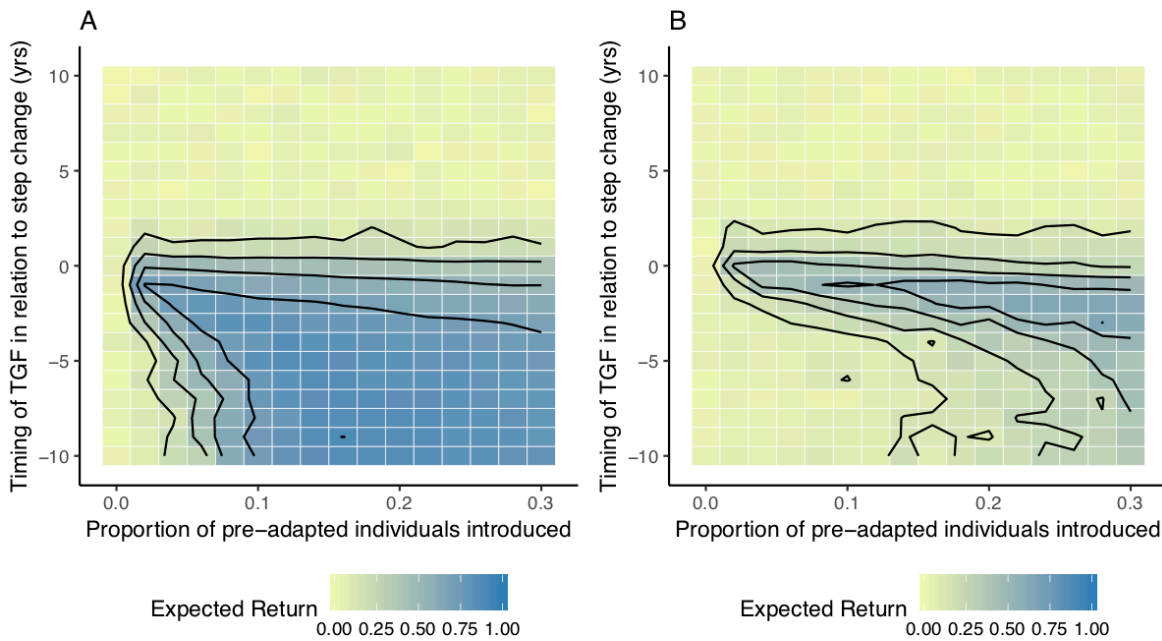
Figure 6.4. Generic population model results across our management space considering **A:** 10% and **B:** 50% reduction of fitness for F1 hybrids. Expected return of the recipient genome (calculated by $E(Y) = \bar{r}_i(1 - x)$ using probability of extinction (x) and proportion of recipient genome ($\bar{r}_i$)).

Although this pattern remained relatively consistent, adjusting population parameters did alter the effectiveness of targeted gene flow, and the optimal management strategy (Figure 6.3 & Figure S6.3 – S6.5). From the global sensitivity analysis conducted on the genetic population model, we were able to capture these trends (Figure 6.3). We found that as population size (N^*) increased, the effectiveness of targeted gene flow increased. For scenarios with a higher population size ($N^* = 500$ or 1000) our maximum expected return was higher, and we required a small proportion of threat-adapted introducees to gain this benefit. Changing the growth rate of a population (R_{max}) had little effect on the outcome of targeted gene flow (Figure 6.3). Heritability (h^2) also impacted the success of the management action, with higher heritability leading to an increase in population survival – particularly in small populations, driving an increase in expected return (Figure 6.3). These broad patterns were also seen in the sensitivity analysis for the northern quoll population model (Figure S6.3), suggesting they are consistent trends robust to population dynamics.

Generally, outbreeding depression reduced the success of targeted gene flow in both models. 10% reduction in fitness produced relatively similar results to no outbreeding depression, however a 50% reduction in fitness increased the probability of extinction and decreased the proportion of recipient population genes, reducing the proportion of scenarios with a high value of Y (Figure 6.4 & Figure S6.1). Outbreeding depression also drove optimal timing towards later action: with weak outbreeding depression the introduction needs to happen closer to the step change to effect high returns; with extremely high outbreeding depression, the optimal timing occurs after the step change (Figure

S6.1). Lowering recombination rate led to a lower retention of the recipient genome, and thus lower overall expected returns for both the generic and quoll population model (Figure S6.4). The combined affect of high outbreeding depression and low recombination rate caused extremely low expected returns (though still better than no action; Figure S6.5).

Discussion

Our model demonstrates that our management objective – retention of the recipient population and genome, Y – is sensitive to the timing and size of the introduction. There is also an apparent trade-off between maintaining the local recipient genome, and population survival. Generally, a larger number of introductees in the years immediately preceding and following the stepwise threat produce the lowest probability of extinction, but this also produces lower retention of the recipient genome. The trade-off can be optimised, however, with the highest expected return when we introduce a higher proportion of pre-adapted individuals prior to the arrival of the threat. This strategy produces hybrids in the years prior to the step change so that when selection begins, individuals who carry the recipient genome as well as resistant genes survive. These more optimal strategies retained almost 100% of the recipient population alleles spread across the genome ($\bar{r}_p$). Our results fit with previous assessments of assisted colonisation (that do not consider evolution) that show the timing and number of introductees to be primary considerations for conservation managers undertaking such endeavours (McDonald-Madden *et al.* 2011).

Despite difference in population dynamics, the optimal strategy for targeted gene flow was similar for both the generic population model and specific northern quoll model. This indicates that the underlying trade-off between timing and size of the introduction is likely a general one. The specific population dynamics still play an important role in determining the best course of action, however. For instance, implementing targeted gene flow after the step change exhibited low returns in the generic model. Here, individuals only breed for one year, so the population cannot sustain itself under selection for as long as in the quoll model, which had individuals breeding over multiple years. This suggests that timing of an introduction will perhaps be particularly sensitive in short lived species.

The importance of population dynamics was further demonstrated when we considered broader parameters in our global sensitivity analyses. For instance, carrying capacity had a major bearing on the effectiveness of targeted gene flow, with larger populations generating a substantially higher return than small populations. This result matches theoretical expectations in that larger populations are less affected by stochastic processes. As such, natural selection is more effective in these populations, and they are less prone to stochastic extinction processes (Lande 1993; Charlesworth 2009). By contrast to carrying

capacity, growth rate (explored only in the generic model), had only a minor impact on expected return. This result may reflect model assumption in that we chose initial fitness levels guaranteed to generate 95% extinction probability for each growth rate, and relative fitness in our model was predicated on survival rather than reproductive rate. While the effect of growth rate may be larger than we unearth here, in reality managers focussing on a particular species may get to choose among sites varying in carrying capacity, but will have substantially less choice with regard to growth rate. Finally, heritability of the threat-adapted trait also affected returns, with less heritable traits causing an increased chance of extinction (particularly in small populations). This is unsurprising given heritability determines the response to selection, but there are still some management strategies that are effective even under low heritability. Overall, targeted gene flow decreased extinction probability, and this is consistent across all scenarios.

There is, however, an oft-cited risk when hybridising populations: outbreeding depression can reduce population fitness (Edmands 2007; Frankham *et al.* 2011). Reduced fitness in hybrids could arise from breakdown of local adaptation, or from genetic incompatibilities, both of which are difficult to predict (Frankham *et al.* 2011). Our model incorporated the possibility of genetic incompatibilities and showed outbreeding depression generally reduced the success of targeted gene flow. These results are unsurprising: we have introduced both a barrier to introgression and a mechanism for reducing fitness. Importantly, however, even with high hybrid dysfunction it was typically still beneficial to act. Outbreeding depression did not increase extinction probability above the baseline "do nothing" level of 95% (scenario where 0 individuals are introduced). Although every situation has its own peculiarities, recent reviews suggest that the risk of outbreeding depression is overstated in the literature: in most realistic cases outbreeding should cause only minor and transitory effects (Frankham *et al.* 2011; Aitken & Whitlock 2013), and work on hybrid zones show that beneficial alleles rapidly introgress despite strong selection against hybrids (e.g., Barton & Bengtsson 1986). Of course, crossing small inbred populations sometimes also leads to hybrid vigour, by masking deleterious alleles (Frankham 2015), an effect which should decrease the extinction probability (Weeks *et al.* 2017), though potentially at some cost to the recipient genome. In our particular case study, it is likely that northern quolls will experience only minor, if any, effects of outbreeding depression (due to low genetic divergence across their range; Firestone 2000). Generally, the likely fitness benefits gained from carrying favourable alleles that help individuals survive a current and overwhelming threat will likely outweigh any small impact of outbreeding depression (How *et al.* 2009; Weeks *et al.* 2011, 2016; Aitken & Whitlock 2013), and recombination ensures that maladaptive genetic combinations are rapidly lost.

Recombination, however, is the key variable affecting linkage disequilibrium, and, by affecting the independence of loci can potentially lower the effectiveness

of targeted gene flow. When recombination rate was set to 0.25, there were lower expected returns overall because, although populations survived, a lower proportion of the recipient genome was retained. Lower recombination causes selection to capture larger chunks of the introduced genome: the introduced population's neutral alleles are carried along with the threat-adapted (strongly favoured) alleles (Aquadro 1997). Therefore in a scenario where recombination is low, the introduction of pre-adapted individuals would need to occur a significant time prior to the step change, to allow time for linkage disequilibrium to decay. When outbreeding depression is also a factor, however, selection against hybrids renders early introductions ineffective. Thus when low recombination rate and strong outbreeding depression occur together, we see low returns. Unfortunately, both of these factors are complex and difficult to predict in advance (Jensen-Seaman *et al.* 2004; Frankham *et al.* 2011). Again, however, even with outbreeding and low recombination, our returns for a targeted gene flow action are still greater than a do nothing approach.

We have, of course, been unable to capture the full potential complexity in our model. We used timing, population sizes and number of introductees that would be feasible for terrestrial fauna, but using this parameter space we were still able to capture the variation in the global sensitivity analysis. The model lacks complexity around genetic architecture: in reality, genes influence traits to varying degrees (i.e., there is a distribution of effect sizes, *e*), loci are non-randomly linked, and there are interactions within and between loci (Gomulkiewicz & Holt 1995). Although we have incorporated recombination, the picture is, of course, far more complex in reality. For example, dominance in the target trait (which is hinted at in northern quolls; Kelly & Phillips 2018) would result in a faster adaptive shift if dominance effects are in the direction of selection, and depending upon the distribution of dominance effects, may also generate heterosis. But in the absence of detail on the distribution of fitness effects and genetic architecture of the key trait, we have chosen the simplest model possible. Our model was simplified also to not include spatial effects, giving all individuals (including introductees) equal chances of survival and finding a mate.

Here we consider a threat that constitutes a step change in the environment. This is broadly relevant to such threatening processes as invasive species and disease that move into a population and alter it from one state to another. There are, however, other threatening processes, such as climate change, that cause gradual change (McDonald-Madden *et al.* 2011; Aitken & Whitlock 2013). Our management objective – maximising the expected proportion of recipient genome, $\bar{r}_i(1 - x)$ – can of course be applied to a gradual change also, and this is a challenging future problem. For now, however, our results with regard to a step change suggest that timing is important. Introducing beneficial traits early and generating hybrids prior to the step change is our optimal action (particularly with low recombination rates), but the timing for this becomes tighter as outbreeding depression increases, generating selection against hybrids.

Ultimately, at high levels of outbreeding depression, the introduction is best made after the step change. Unfortunately, we will rarely know in advance the strength of outbreeding depression, nor the genetic architecture of a polygenic trait. Such uncertainty is common in conservation management (Kujala *et al.* 2013), and modelling, along with decision-theoretic approaches can provide useful framework for optimising actions in particular cases (Regan *et al.* 2005; Polasky *et al.* 2011).

Overall our model suggests that targeted gene flow could provide substantial benefits to populations at risk from step change to their environment. No scenario we explored caused more damage than not acting at all. Despite the variation of different population dynamics, our model suggests it is generally possible to use targeted gene flow to reduce population extinction risk while still maintaining much, if not all, the local genetic diversity of the population. We have also identified a useful objective to optimise: the surviving proportion of the recipient population's genome. This objective directly addresses the trade-off between reducing extinction probability and retaining the recipient genome, and it proved sensitive to management levers. It would be straightforward, in many circumstances, to anchor this optimisation process with a cost model that adds the economic and logistical constraints particular to a given system (e.g., Southwell *et al.* 2017). While each case will have its own particularities, it appears that targeted gene flow can be a valuable tool in an era of rapid environmental change.

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Chapter 7
Trialling targeted gene flow in the endangered northern quoll

Abstract

TARGETED gene flow is a novel conservation strategy that involves translocating individuals with favourable genes to areas where they will have a conservation benefit. Although it may have widespread applications, targeted gene flow is yet to be tested in a wild environment. Here, we used the northern quoll as a model to test this conservation strategy. Northern quolls are endangered by the spread of the invasive cane toad, which they unknowingly attack and are fatally poisoned by. There are, however, a small number of quolls that are "toad-smart" – they possess a heritable trait that means they don't attack toads. It is this trait we hoped to promote through targeted gene flow. We aimed to test the use of targeted gene flow for this species by releasing 54 toad-smart and toad-naïve northern quolls onto a small offshore, toad-infested island in 2017. We trained our released animals to avoid toads, and hoped to monitor selection of toad-smart genes in the following generations in the wild. Here we present the results from the first year of monitoring. Genetic data suggests some selection toward toad-smarts, with the toad-smart proportion of the genome increasing from 29.2% in the release population to 39.3-43.2% in the first island generation. Our results also demonstrate the viability of both F1, and F2 backcross hybrids between toad-smart and toad-naïve populations. Unfortunately, we trapped

only six island-born individuals during a two-week trapping effort, so our sample population sizes are much smaller than anticipated (best population size estimate 16 individuals; 95% CI: 12-40). The small population size was likely due to a combination of toad-mortality, as well as stochastic processes including fire, a cyclone, predation and ineffective breeding. Additional monitoring of the population in 2019 will conclusively determine the population's fate. While our data demonstrate successful hybridisation between populations and hint at the value of toad-smart genes, logistical issues (that would not have occurred in an established population) hampered the experiment. Although questions remain, we recommend employing targeted gene flow in established threatened quoll populations – particularly given the unstoppable force of the toad invasion. As well as achieving an outcome for an endangered species, we also hope to have illustrated the broader applications of targeted gene flow for conservation.

Introduction

In a climate of rapid environmental change and widespread biodiversity decline, conservationists are racing to devise effective strategies to conserve threatened species (Johnson *et al.* 2017). Conservationists face numerous interacting challenges, including habitat loss, climate change, invasive species and disease, and to address these many recognise the need to expand the toolkit (Mawdsley *et al.* 2009). Many of these threats are becoming increasing difficult to mitigate – so focus has also turned to helping species adapt to their changing world. This has led to the development of strategies that use the species-wide standing variation to promote adaptation in threatened populations (Aitken & Whitlock 2013; Whiteley *et al.* 2015). Yet as with any new concept, these new tools require rigorous testing to determine if they will be effective in complex environmental settings (Kujala *et al.* 2013; Game *et al.* 2014).

Targeted gene flow is one such emerging conservation strategy aimed at helping species adapt to threatening processes (Kelly & Phillips 2016). The idea is a generalisation of the concept of assisted gene flow, which aims to help populations adapt to climate change by introducing warm-adapted individuals to populations that will experience temperature rises (Sgro *et al.* 2011; Aitken & Whitlock 2013; Shoo *et al.* 2013). Climate change is, however, not the only threatening process causing species declines, and we could potentially use this same approach to help species experiencing a wide range of threats – particularly if these threats are difficult to remove (Kelly & Phillips 2016). One application of targeted gene flow involves introducing individuals with adaptive genes into areas of the species range that are under threat (Aitken & Whitlock 2013; Kelly & Phillips 2016; Frankham *et al.* 2017). In theory, this idea could be applicable to any declining population that has variation in a trait that results in a fitness advantage with regard to a given threat. For instance, introducing disease-resistant individuals into a population threatened

by disease, or individuals with traits that allow them to survive in the presence of an invasive species.

Despite the broad applications of this strategy, it is yet to be tested in a wild setting (Kelly & Phillips 2018b). Here, we have selected a candidate species to test the idea. The endangered northern quoll (*Dasyurus hallucatus*) is a marsupial mesopredator threatened by the arrival of the invasive toxic cane toad (*Bufo marinus*; Shine 2010). Northern quolls unsuspectingly attack the poisonous toads and are fatally poisoned, causing dramatic crashes in populations following toad arrival (Woinarski *et al.* 2008). Toads are currently moving across northern Australia, where the quoll lives, and will eventually invade the entirety of the northern quoll's range (Kearney *et al.* 2008; Tingley *et al.* 2017). There are, however, a small number of northern quoll populations surviving alongside cane toads, because the quolls who live there are "toad-smart" – they know not to attack toads (Kelly & Phillips 2017). Unfortunately, this trait is at very low levels in toad-naïve populations, so following toad arrival population size and genetic diversity dramatically decrease – with the vast majority of populations going locally extinct (EPBC 1999).

Targeted gene flow could be a tool for conserving populations of northern quolls that are yet to be invaded by cane toads (Kelly & Phillips 2018b). If the toad-smart behaviour could be introduced ahead of the toad front, the recipient population would increase its adaptive potential and so be more likely survive the invasion. For the strategy to work, the toad-smart trait must be heritable. Several years of experimental work on captive populations of toad-smart and toad-naïve quolls demonstrates that there is indeed a genetic basis to toad-smarts (Kelly & Phillips 2018b), so we could breed this behaviour into naïve populations. Doing so, however, also requires successful hybridisation between individuals from toad-smart and toad-naïve populations. When crossing populations there is a risk of outbreeding depression, where hybrids are less fit than purebred individuals due to allelic incompatibilities and loss of local adaptation (Frankham *et al.* 2011). This risk increases with populations with fixed chomosomal diffeences or with more distantly related the populations that have local adatative differences(Frankham *et al.* 2011, 2017). Due to the low divergence – between 3-7% divergence at mtDNA loci (Firestone 2000; Cardoso *et al.* 2009; Hohnen *et al.* 2016) – between populations of northern quolls we believe the risk is low for this species. Previous captive breeding experiments have produced healthy F1 hybrid offspring (Kelly & Phillips 2018b). But outbreeding depression often does not manifest until the F2 generation (Frankham *et al.* 2011), so questions remain as to the reproductive fitness of these F1 hybrids, and the fitness of the F2 generation.

We aimed to test the effectiveness of targeted gene flow for northern quolls. The aim of the strategy is to not only reduce extinction probability of the population, but to also maintain local provenance (so that the local genome is not entirely replaced by the adapted genome, as occurs in a reintroduction). Recent modelling work has shown that these two objectives – probability

Table 7.1. Sample sizes of wild caught northern quolls collected in 2015 & 2016; sample sizes of successful litters and captive born northern quolls for the 2015-2016 & 2016-2017 breeding seasons; and sample size of northern quolls released onto Indian Island in 2017. Sample sizes presented for male and female northern quolls from both toad-smart (QLD) and toad-naïve (NT) areas of their range.

	Generation	Year	QLD (toad-smart)		Hybrid		NT (toad-naïve)		total
			female	male	female	male	female	male	
Number of wild caught quolls	parental	2015	5	6	-	-	-	11	
	parental	2016	7	6	-	-	40	28	
# of litters	-	2015–2016	4		-	-	-	-	4
	-	2016–2017	4		-	2	8	-	14
Number of captive born quolls	parental	2015–2016	10	15	-	-	-	-	25
	F1	2016–2017	9	18	7	6	24	18	82
Number released onto Indian Island	F1	2017	-	10	7	6	18	13	54
Caught on Indian Island	F2	2018	-	-	4	1	-	-	6

of survival and local genome retention – can be maximised using standard conservation levers (Kelly & Phillips 2018a). By adjusting the timing of the introduction, and the number of introduced toad-smart quolls into a naïve population, managers could create a population of hybrids prior to the arrival of cane toads. These hybrids would carry both toad-smart genes as well as the local genome, and would be selected for once toads arrived. Here, we used an offshore toad-infested island to empirically test this idea. By releasing both toad-naïve and toad-smart hybrids, we hoped to track selection over multiple generations and detect any presence of outbreeding depression in subsequent hybrid cohorts. In this chapter, we present the data from the first year of monitoring.

Methods

Initial capture and captive breeding

In preparation for the experiment, we collected our parental population of northern quolls from toad-smart and toad-naïve populations across northern Australian and brought them into captivity at the Territory Wildlife Park, Northern Territory (NT). In 2015, we began the project by collecting 11 toad-smart northern quolls from Mareeba, Queensland (QLD). The following year, we collected a further 13 toad-smart individuals from Cooktown, QLD to augment our toad-smart stock. Additionally, in 2016 we also trapped 68 toad-naïve northern quolls from the offshore toad-free Astell Island, NT (which was set up in 2009 as a refuge population prior to cane toads arriving in Kakadu National Park; Rankmore *et al.* 2008). Twenty-nine of these toad-naïve NT quolls were later released back into Kakadu National Park as part of another project (Jolly *et al.* 2017), and the rest were used for breeding in 2016. These groups were shown to differ in their response to cane toads, with quolls from Queensland being far less interested in or likely to attack toads (Kelly & Phillips 2017). Henceforth, we will refer to our two source populations as QLD (toad-smart) and NT (toad-naïve).

Northern quolls will breed from May-September, with the young being separated from their mother the following year (approximately January/ February), and then becoming sexually mature by the breeding season (Oakwood 2000). In the wild, male quolls will often die following the breeding season but females can live for two (or sometimes three) breeding seasons (Oakwood *et al.* 2001). In our study, however, all litters were produced by first time breeders because both sexes generally only breed once in captivity. There was no multiple paternity or multiple litters for either sex.

In 2015-2016 breeding period, we bred just wild caught toad-smart QLD northern quolls, to produce four litters of offspring (Table 7.1). The 2016-2017 breeding season we began the crossing experiment using our parental generation
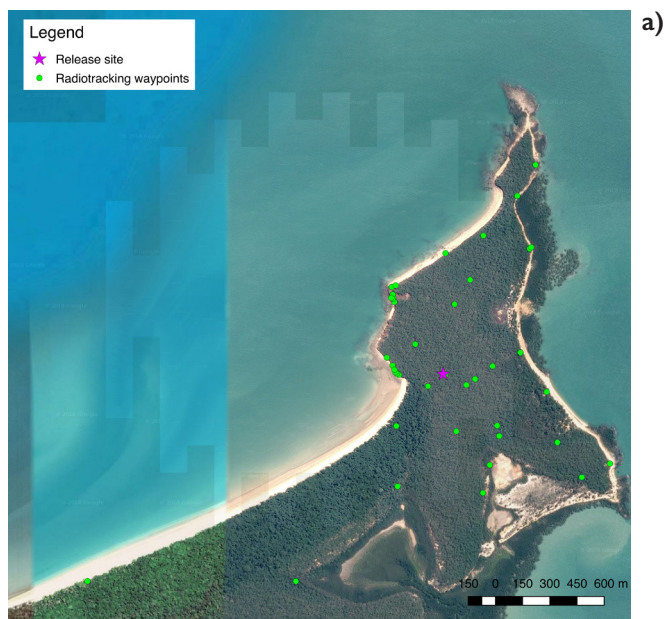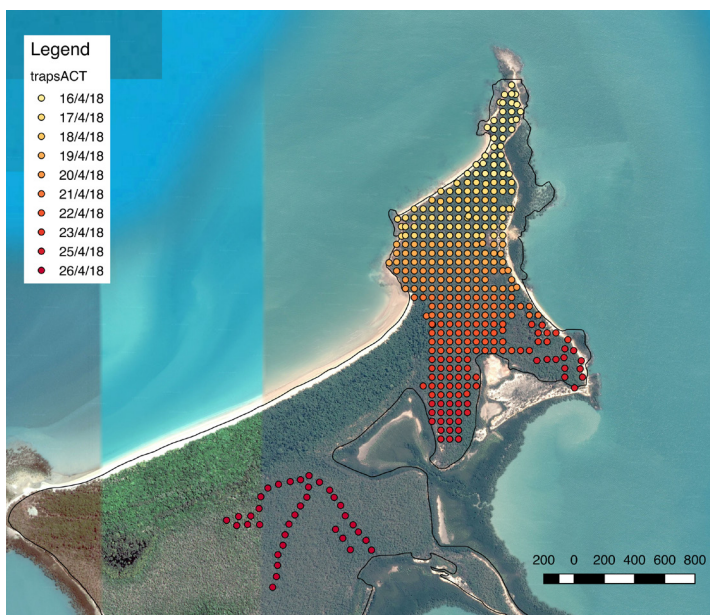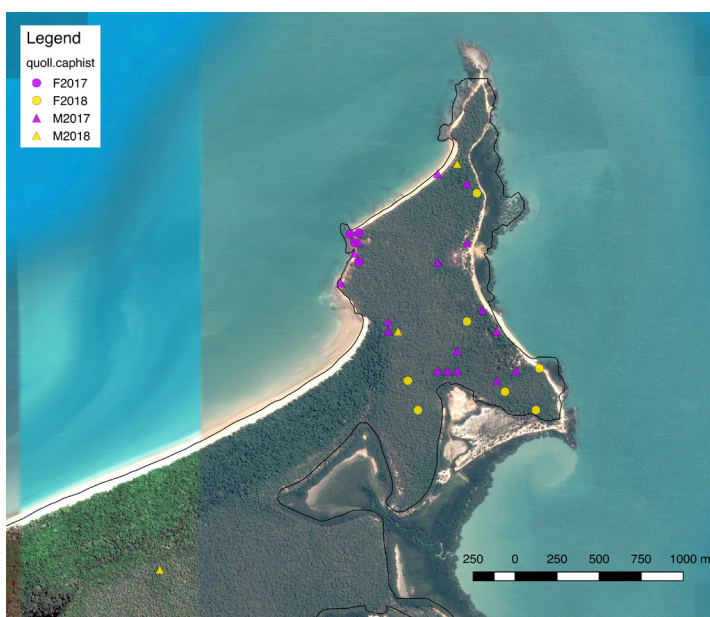
Figure 7.1. Maps of northern part of Indian Island showing **a)** release site (purple star) and radio tracking waypoints for 29 radio tracked northern quolls for first four days of release (green dots); **b)** 2018 trapping grid colour coded by date of deployment (traps were set for four days and placed 70m apart; **c)** locations of trapped northern quolls in 2018 colour coded by cohort with circles representing females and triangles representing males.

of wild caught and captive born parental quolls, who were either purebred QLD or purebred NT. The 2016-2017 breeding season involved pairs of QLD x QLD, QLD x NT and NT x NT parents, which produced three lines of offspring: purebred toad-smart, hybrid and purebred toad-naïve. These offspring born in 2017 are classed as our "F1" generation, and designated one of three groups: F1 QLD, F1 HYBRID or F1 NT. The litter sizes listed in Table 7.1 show the number of successful litters and the numbers of surviving offspring. Some pairings in captivity did not produce offspring due to logistical difficulties, hence the uneven number of litters for each line, and the overall small samples sizes considering our initial numbers of wild caught individuals. The two F1 Hybrid litters represented crosses in both directions: being produced by one QLD dam and one NT dam. The captive born offspring were raised in completely toad-free environments with their mother, until they reached maturity and were separated into individual enclosures.

Island selection and release

For the release location we selected Indian Island, an offshore island located ~40km southwest of Darwin along the coast of the Northern Territory (-12.632, 130.508). Indian Island met all our criteria for the release site: there was an established cane toad population on the island, it was good northern quoll habitat, and it was free of mammalian predators of the northern quoll (cats and dingos) as well as small endangered fauna that the quolls might impact. Indian Island has no permanent human population but is visited and managed by Kenbi Traditional Owners, who agreed to the quoll release and became important partners in the project.

We selected 54 captive-born F1 northern quolls for release onto Indian Island. This included 13 F1 Hybrids, 31 F1 NT (toad-naïve) and 10 F1 QLD males (toad-smart; Table 7.1). We did not release any F1 QLD females onto the island so as to avoid the release of QLD mitochondria (thus helping us to maximise local NT provenance in the released population). All quolls were vet checked, microchipped and ear-clipped for DNA before release.

In the week prior to release, each F1 quoll was trained using condition taste aversion not to eat cane toads – a method that has shown to be successful in training captive quolls previous studies (O'Donnell *et al.* 2010; Indigo *et al.* 2018). This involved offering each quoll a non-lethal toad leg laced with 400 mg kg^{-1} quoll mass thiabendazole overnight instead of regular food. The following morning, we recorded whether the quoll ate the thiabendazole laced toad meat, if they did not, they were offered another leg. We repeated this for three nights or until they ate the toad leg. Those who didn't consume the toad leg were still considered trained (or naturally toad-smart). There was a significant difference in toad leg consumption depending on origin (those with at least one toad-smart parent were less likely to consume the toad leg; results presented in Kelly and Phillips 2018b).

We released the trained F1 northern quolls onto the northern part of Indian Island in two batches – the first batch of 35 quolls on the 12th May 2018, and the second of 19 quolls on the 24th May 2018 (Figure 7.1a). A subset of the first batch ($n = 29$) were radio-telemetered and tracked over four nights. Transmitters were equipped with mortality sensors, and on the last day of tracking we used a helicopter to search for lost animals. Experience from earlier releases of northern quolls to toad-infested areas suggested that toad mortality would likely occur in the first night or two post-release, so we expected to measure mortality from toads in this time (Jolly et al. 2017; Cremona et al. 2017). We monitored the quolls' location and if the mortality sensor was activated, we determined the cause of death with a field post-mortem. The population was then left to breed for the 2017-2018 breeding period. Assuming random mating (and for simplicity, a single locus), we calculated the expected proportions of purebred NT and hybrid offspring in the 2018 F2 cohort (including QLD and NT backcrosses). We used this to calculate the expected proportion of QLD and NT genome in the 2018 F2 cohort.

Monitoring island population

In May 2018, we returned to Indian Island to monitor the population using live cage trapping. We placed a rolling 70m x 70m trapping grid starting at the far northern end of the island and moving traps south after four nights of trapping (see Figure 7.1b). This covered the area immediately surrounding the release site and the majority of the space that quolls were recording to use during the radio tracking (Figure 7.1a). Three additional lines ($n = 12$ traps each) were put out in the second, more southern patch of woodland to explore for more dispersed offspring. Traps in the second woodland block were only deployed for three nights. Traps were baited with chicken necks or fish: the traditional peanut-butter and oat mix was eschewed in an effort to avoid traps filling with *Melomys burtoni*, which are extremely abundant on the island.

Known quolls from the released 2017 F1 cohort were scanned, weighed and pouch checked prior to release. New quolls (2018 F2 cohort) who were caught were microchipped for identification, weighed and pouch checked before being released. We took ear clippings placed in 100% ethanol for DNA analysis. Recaptures were scanned and released.

To estimate population size on the island in 2018, we used our trapping data to perform a mark-recapture analysis using a closed-population model, executed using a Bayesian approach. Our observations consist of a capture history for each captured individual over the number of trapping nights. We denote N_{tot} as the total estimated number of quolls on the island, made up of the estimated number of individuals in the 2017 F1 cohort and 2018 F2 cohort N_c (N_{2017} and N_{2018}). We assumed each cohort had a different detection probability, so estimated N_{2017} and N_{2018} separately. To estimate N_c for each cohort, we used a closed population mark-recapture analysis in which each

individual, i, was either observed, or not, at a given time, t, according to a Bernoulli distribution:

$$O_{itc} \sim \text{Bernoulli}(d_{tc})$$

Where d_{tc} denotes detection probability for that cohort, i, at time, t, which we assume declines over time according to:

$$logit\,(d_{tc}) \;=\; \mu_{dc} \;+\; \beta t$$

Where μ_{dc} is the expected detection probability for a cohort at $t = 0$, β is the change in log odds of detection over time.

We used the "data augmentation" method (Tanner & Wong 1987) in combination with this detection probability to estimate N_c. Under this approach, the data are padded by adding an arbitrary number of zero-only encounter histories of potential unobserved individuals. The augmented dataset is modelled as a zero-inflated model (Royle *et al.* 2007) and changes the problem from estimating a count, to estimating a proportion. This was executed by adding a latent binary indicator variable, R_c, to classify each row in the augmented data matrix as a 'real' individual or not, where $R_c \sim Bernoulli(\Omega_c)$. The parameter Ω_c is estimated from the data and cohort size was estimated by $N_c = \sum_{i\,(c)} R_{ic}$.

The model was fitted using Bayesian Markov Chain Monte Carlo (MCMC) methods within the package *rjags* (Plummer *et al.* 2018) using the *R* statistical program (R Core Team 2013). We used minimally informative priors (available in Table 7.3) except for Ω_c, which were bounded by priors based on the maximum number of individuals known to be possible on the island for each cohort. Parameter estimates were based on 100,000 iterations with a thinning interval of 5 following a 10,000 sample burn-in. Three MCMC chains were run, and model convergence assessed by eye, and using the Gelman-Rubin diagnostic (Gelman & Rubin 1992).

Genetic analysis

Diversity Arrays Technology Pty Ltd (Canberra, Australia) obtained single nucleotide polymorphisms (SNPs) by performing DArTseq™ complexity reduction and sequencing of the genome using Next Generation Sequencing platforms. Diversity Arrays Technology preformed DNA extraction from the tissue samples and optimised the DArTseq method for northern quolls using six samples from our dataset. Analysis of the genomic data including summary statistics, relatedness and parentage analysis was done using the *R* statistical program (R Core Team 2013) including packages *dartR* (Gruber *et al.* 2018), *ASRemlR* (Butler *et al.* 2007), *rrBLUP* (Endelman 2011) and *sequoia* (Huisman 2017). The program *STRUCTURE 2.3.2* was used to compute the proportion of the genome of an individual originating from each inferred population using a

quantitative clustering method (Pritchard *et al.* 2000).

Results

Release and radio-tracking

Over the four nights of radio-tracking following release, only three of the 29 tracked animals succumbed to toads (two F1 NT males; one F1 Hybrid male) and one other F1 NT male was killed and consumed by a rufous owl. The remaining 25 radio-tracked animals survived the first four days, and many of the females had already apparently settled in the rocky cliffs on the northern tip of the island (Figure 7.1a). Other observations from this effort suggested quolls were using the beaches and woodlands, but largely avoiding the dense rainforest patches (which informed our trapping regime for 2018). In August 2017, a large fire burned the entire woodland block in which quolls were released, and on 17th March 2018, Tropical Cyclone Marcus passed over the island. The fire started on the eastern side of the island and burned for almost three weeks; the cyclone brought winds in excess of 200km/hr, and extreme rainfall.

Monitoring island population

In total, we caught 12 quolls in 2018 – fewer animals than we had anticipated,

Table 7.2. Northern quolls captured on Indian Island in May 2018, including ID, sex, cohort and (if known) population.

Cohort	ID	Sex	Population
2017 F1	941000018496110	male	NT
2017 F1	941000018496025	female	Hybrid
2017 F1	941000018496031	male	QLD
2017 F1	941000018495953	female	NT
2017 F1	941000017807570	male	Hybrid
2017 F1	941000018495966	male	QLD
2018 F2	A52	female	unknown
2018 F2	CF2	female	unknown
2018 F2	DBC	male	unknown
2018 F2	DC1	female	unknown
2018 F2	14F	male	unknown
2018 F2	DF1	female	unknown

Detection probability (μ_d)
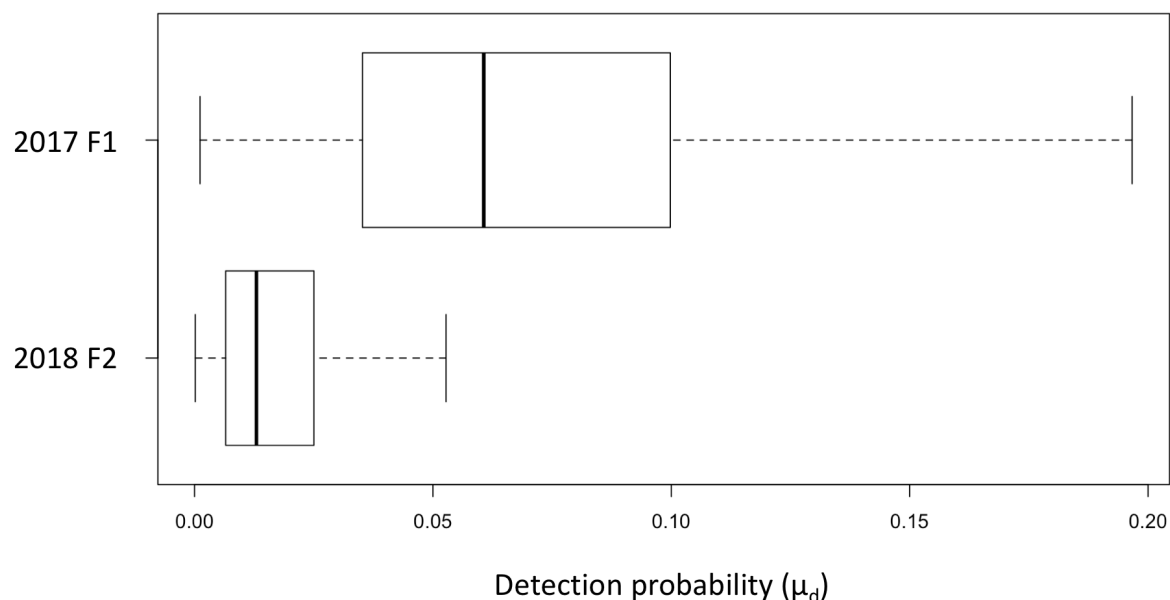
Figure 7.2. Boxplot showing density of the two posteriors – detection probability (µd) for 2017 F1 and 2018 F2 cohorts of northern quolls trapped on Indian Island in 2018.

Table 7.3. Mark-recapture model for Indian Island northern quoll population. Table presents model parameters and their priors including prior distributions, standard deviation, estimated posterior means and their 95% credible intervals. N denotes normal probability distribution N(mean, SD) and U denotes uniform distribution U(min, max).

Name for parameter	Parameter	Prior Distributions	Posterior mean	95% CI
Intercept for detection (2017 F1 cohort)	μ_{d2017}	N(0, 1e-6)	0.0606	(0.011, 0.229)
Intercept for detection (2018 F2 cohort)	μ_{d2018}	N(0, 1e-6)	0.0130	(0.002, 0.07)
Intercept for Omega (2017 F1 cohort)	Ω_{2017}	U(0, 54)	6	(6,7)
Intercept for Omega (2018 F2 cohort)	Ω_{2017}	U(0, 200)	10	(6, 34)
Slope of time effect on detection	β	N(0, 1e-6)	0.0146	(0.002, 0.028)

and these animals included both young of the year (2018 F2 cohort, $n = 6$) and older animals who we had released (2017 F1 cohort, $n = 6$; Table 7.2). Animals were captured across the entire woodland block, and also deep into the second woodland block (Figure 7.1c). The one quoll caught in the second woodland block was a recaptured 2018 F2 male, last caught three days previously in the target block (1.93km away). Interestingly, old females (2017 F1 cohort) were only found in rocky cliff area adjacent to the release site – the location to which they had previously been radiotracked less than four days post release. Younger females (2018 F2 cohort), by contrast, were captured across the trapping grid.

There was a strong difference in the detectability between the two cohorts, with almost all of the 2017 F1 cohort being recaptured multiple times, but only two of six 2018 F2 cohort being recaptured. The mark-recapture analysis showed this clear difference in detection probabilities between the two cohorts (Figure 7.2) and yielded a total population size estimate of 16 individuals (N_{tot} = 16 individuals; 95% CI: 12-40; Table 7.3). This is the sum of the estimated number of 2017 F1 animals (N_{2017} = 6 individuals; 95% CI: 6-7) and the 2018 F2 animals (N_{2018} = 10 individuals; 95% CI: 6-34).

Genetic analysis

SUMMARY STATISTICS

Genomic DaRT sequencing produced 81,010 binary SNPs with 23.18% missing data. Loci with a call rate lower than 0.95 were removed from the set. SNP datasets generated by DArT include fragments with more than one SNP. These multiple SNP loci within a fragment (secondaries) are linked, so to minimise linkage disequilibrium we randomly selected one locus from each fragment.

Table 7.4. The number of individuals (n) heterozygosity (He) and number of pairwise fixed alleles for each group of northern quolls. NT (parental wild caught quolls and their purebred F1 offspring), QLD (parental wild caught quolls and their purebred F1 offspring), F1 Hybrid (F1 hybrid quolls born in captivity with known heritage) and F2 Island (F2 quolls born on Indian Island).

Population	n	He	Pairwise fixed differences			
			NT	QLD	HYBRID	ISLAND
NT	71	0.11	-	111	0	1
QLD	53	0.06	111	-	0	1
F1 HYBRID	13	0.15	0	0	-	0
F2 ISLAND	6	0.10	1	1	0	-
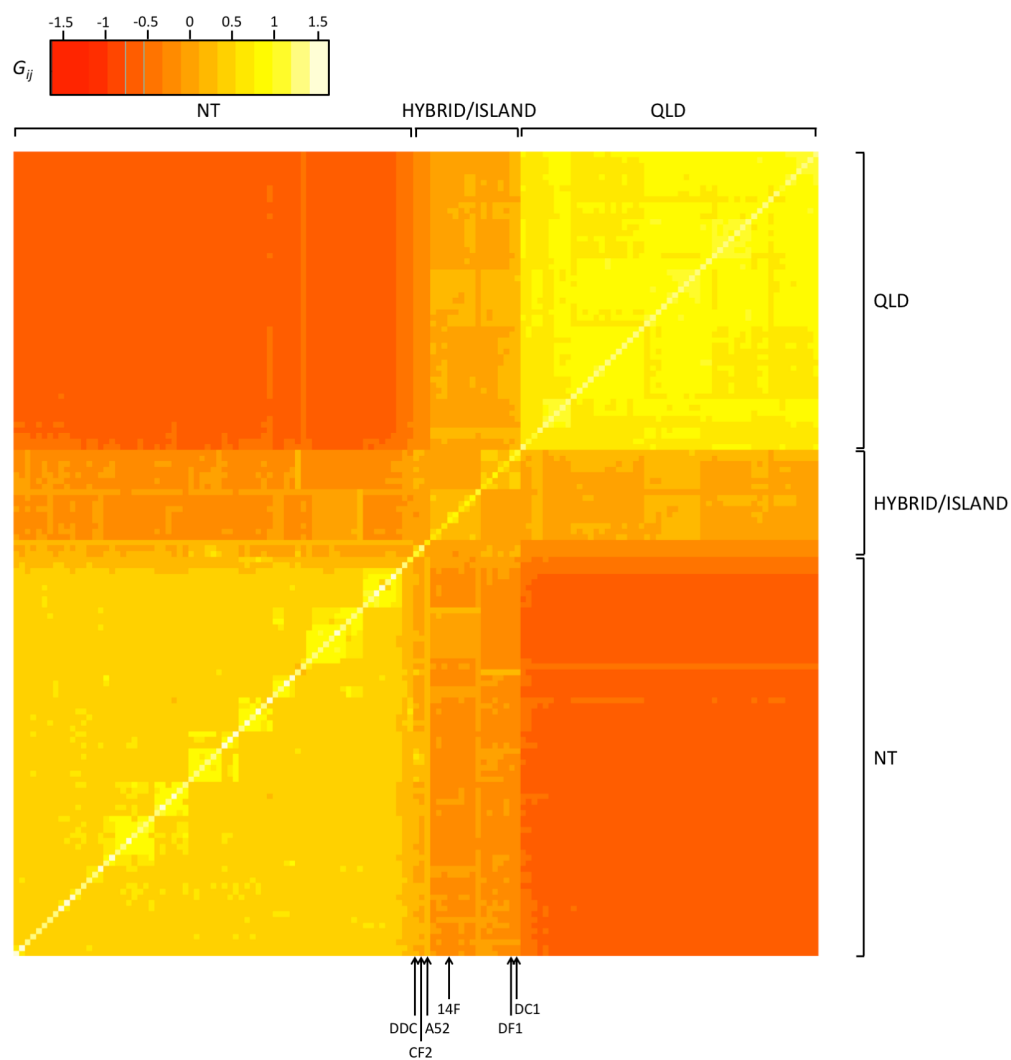
Figure 7.3. Heatmap of the genomic relationship matrix. Shows the individual × individual values of Gij calculated with 14,041 SNPS using the GBLUP method in the Package ASRemlR. Each square denotes pairwise relatedness between two individuals. Gij = 0 (orange) indicates average degree of relatedness between two individuals, Gij >0 (yellow) indicated higher relatedness between the two individuals compared to average, Gij <0 (red) lower relatedness between the two individuals compared to average. Population structure showed on y-axis and six unknown F2 Island quolls identified in x-axis.

This left us with a dataset of 14,041 binary SNPs with 2.53% missing data. There were two known source populations in the dataset (QLD and NT), but we also divided the individuals into four natural groups: NT (parental wild caught quolls and their purebred F1 offspring), QLD (parental wild caught quolls and their purebred F1 offspring), F1 Hybrid (F1 hybrid quolls born in captivity with known heritage) and F2 Island (2018 F2 quolls born on Indian Island). The heterozygosity for each group and pairwise fixed allelic differences between each group are shown in Table 7.4.

GENOMIC RELATEDNESS MATRIX

We used these 14,041 binary SNPs to calculate the pairwise relatedness between individuals, producing a genomic relationship matrix (Figure 7.3). We used the GBLUP method employed in the R packages *ASRemlR* and *rrBLUP* (Butler *et al.* 2007; Endelman 2011) to derive the genomic relationship matrix (computing the value G_{ij}). This measure, G_{ij} represents proportion of the genome that is identical by descent between individuals i and j (VanRaden 2008). $G_{ij} = 0$ indicates i and j have the average relatedness of two individuals within the population. Measure of $G_{ij} > 0$ indicate that i and j are more related than the average individual, while $G_{ij} < 0$ indicate they are less related than the average individual. These results show clear population structure between the QLD and NT population, with F1 Hybrid and F2 Island quolls showing relationships with both populations (as expected). There is a high amount of relatedness within both QLD and NT populations, with litters emerging as clearly closely related.
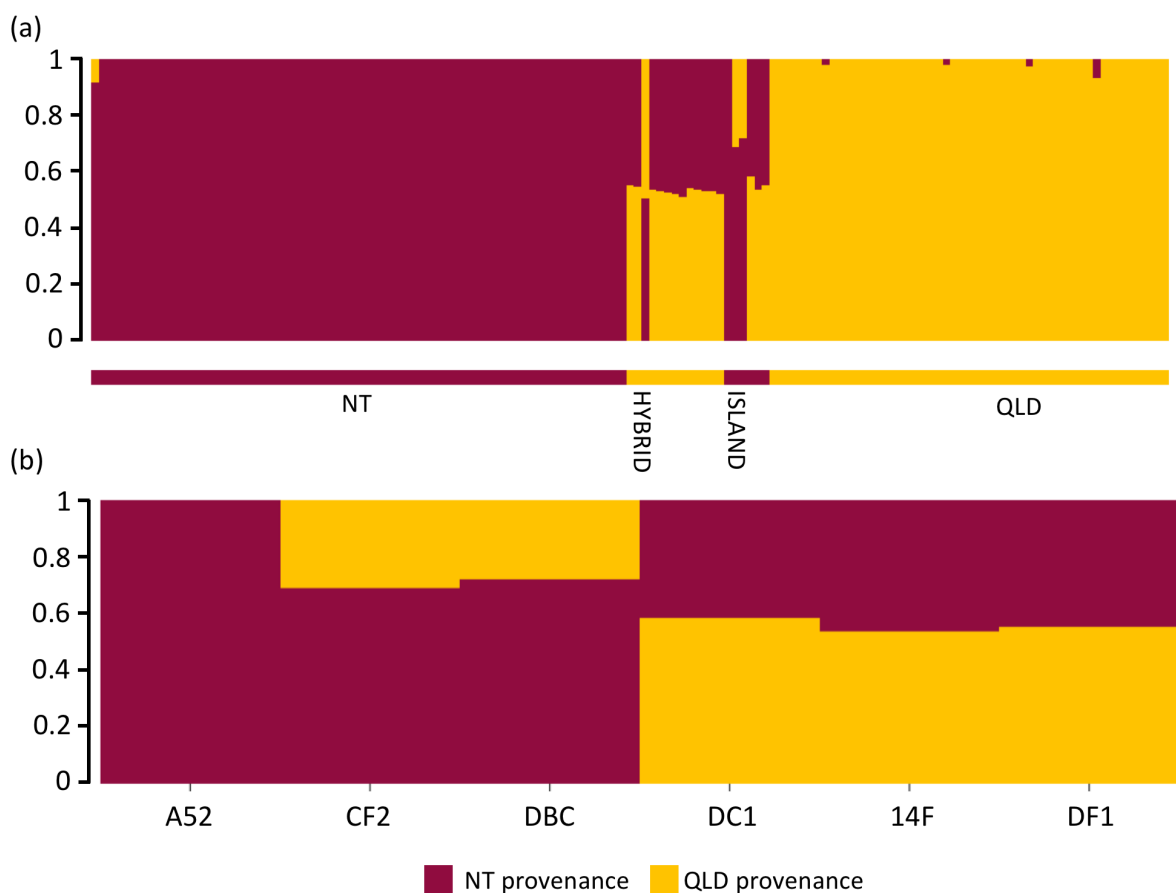
Figure 7.4. Population structure analysis estimated using 14,041 SNPs with call rate above 0.95 with two clusters (K = 2), representing the two source populations (QLD in yellow; NT in maroon). Each individual is represented by a vertical line that is divided by K coloured segments representing the estimated fraction belonging to each cluster. (a) All individuals. The bar and labels at the bottom represent the four groups (NT (n = 71) & QLD (n = 53): purebred parental and F1 NT and QLD quolls; HYBRID (n = 13): F1 Hybrid quolls born in captivity to known parents; ISLAND (n = 6): F2 Island quolls born on Indian Island. (b) Only F2 Island quolls born on Indian Island (n = 6) with individual ID labels.

POPULATION STRUCTURE

We ran a population structure analysis on the 14,041 SNPs. Figures 7.4 and 7.5 show the results for population structure provided by *STRUCTURE* and PCoA. Figure 7.4 shows the clusters provided by *STRUCTURE*, assuming two source populations (K = 2). For K = 2, the two source populations, NT and QLD, were clearly distinguished. There were low levels of admixture between these two populations, but the hybrids showed the expected level (~50% of each source population group). The PCoA mirrored these results, with the purebred QLD and NT groups forming two clusters with high levels of admixture for F1 Hybrid and F2 Island groups (Figure 7.5). The two F1 Hybrid litters were also clustered due to sibling relationships. The F2 Island group showed varying levels of admixture with five out of six individuals having suggested genetics from both populations (Figure 7.4b), and at least two individuals (CF2, DBC, and possibly A52) appearing to be F2 backcrosses (against NT background). These results from the *STRUCTURE* analysis suggest that 39.3% of the 2018 F2 Island cohort genome is of QLD origin.

HYBRID INDEX

We also calculated a hybrid index for each quoll in the F2 Island group using the 111 SNPs from the filtered 14,041 SNP dataset that had fixed alleles between
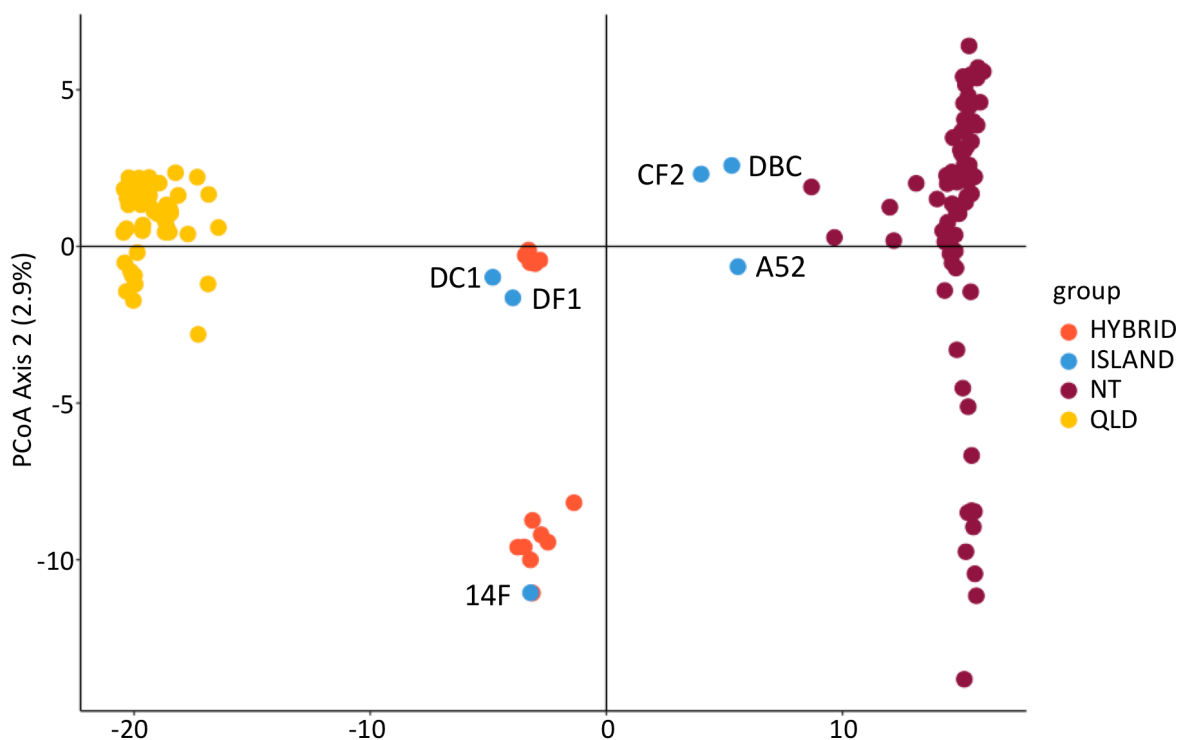
Figure 7.5. First and second principal components. Each point represents one quoll and individuals are coloured according to their group. NT (parental wild caught quolls and their purebred F1 offspring), QLD (parental wild caught quolls and their purebred F1 offspring), HYBRID (F1 Hybrid quolls born in captivity with known heritage) and ISLAND (F2 Island quolls born on Indian Island). Six F2 Island quolls labelled with ID number.
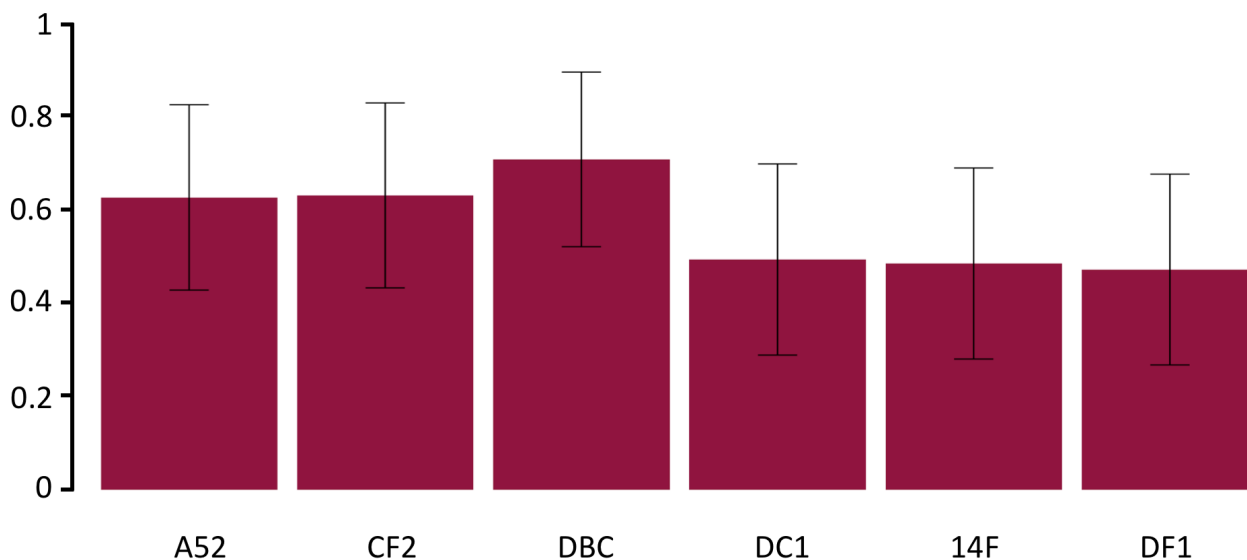
Figure 7.6. Hybrid index calculated by the proportion of NT fixed alleles present in each individual of the 2018 F2 Island cohort. n = 111 fixed alleles between QLD and NT populations.

the QLD and NT populations (Figure 7.6). Our hybrid index is the proportion of fixed loci with alleles private to purebred NT population and not present in the QLD population. With this index we found that F2 Island individuals were made up of 43.2% QLD genome, and 56.8% of the NT genes. These results mirrored the results of the PCoA and *STRUCTURE* analysis. Five out of six F2 Island individuals had a hybrid index very similar to that calculated by *STRUCTURE*, except for A52, who was classified as entirely NT by *STRUCTURE*, but who had a hybrid index of 0.63.

We used an exact binomial test to compare the observed frequency of NT fixed alleles with the probabilities we would expect if the individual was a QLD F2 backcross (hybrid index = 0.25), F2 Hybrid (0.5), NT F2 backcross (0.75) or F2 NT (1). The results of the binomial test indicated that the proportion of NT genome was not significantly different to 0.75 for DBC (p = 0.27), and not significantly different to 0.5 for DF1 (p = 0.70), 14F (p = 0.70) and DC1 (p = 1; Table 7.5). This indicates that DF1, 14F and DC1 are F2 Hybrids, and DBC is an NT F2 Backcross. A52 and CF2 could not conclusively be assigned a group, however both individuals were clearly not F2 NT (p<0.001) or QLD F2 Backcross (p<0.001). Both were slightly more likely to be F2 hybrids than F2 backcrosses (Likelihood ratio, hybrid/backcross: A52 = 0.964; CF2 = 0.321).

PARENTAGE ANALYSIS

Finally, we attempted to identify the parents of the six unknown F2 Island quolls using the R package *sequoia* (Huisman 2017). To do this, the 14,041 SNPs with a call rate above 95% and secondaries deleted was further subsetted according to recommendations for parentage analysis. We selected loci that had high minor allele frequencies (>0.4) in either the purebred QLD or purebred NT groups, which produced a dataset of 781 SNPs. We ran this dataset through

Table 7.5. Results of exact binomial test comparing the number of NT fixed alleles observed in six 2018 F2 Island quolls. Calculated using 111 fixated SNPs for each individual with a hypothesised probability of success, based on the four expected NT genome proportions: (0.25 = QLD F2 backcross; 0.5 = F2 hybrid; 0.75 = NT F2 backcross; 1 = F2 NT). The null hypothesis that the real proportion of NT fixed alleles is equal to the expected proportion.

Individual	Observed proportion	Expected proportion			
		0.25	*0.5*	*0.75*	*1*
A52	0.631	<0.001	0.008	0.006	<0.001
CF2	0.622	<0.001	0.013	0.003	<0.001
DBC	0.703	<0.001	<0.001	**0.273**	<0.001
14F	0.477	<0.001	**0.704**	<0.001	<0.001
DC1	0.505	<0.001	**1.000**	<0.001	<0.001
DF1	0.523	<0.001	**0.704**	<0.001	<0.001

sequoia, which assigned at least one parent to five out of the six F2 Island quolls (Table 7.6). Some individuals were assigned multiple potential parents, which were ranked based on likelihood ratios indicating the degree of fit of the match of parent-offspring pair (LLR: the ratio between the likelihood of the assigned parent being the parent, versus the most likely alternative type). As we had all possible parents genotyped, this meant the program had some problems assigning parents – likely due to high relatedness between the individuals. To test the effectiveness of the technique, we used the program to assess our captive parent-offspring from the 2015-2016 and 2016-2017 breeding seasons. *Sequoia* correctly identified a total of 50 parent-offspring pairs (33 dams and 17 sires) out of our known 147 parent-offspring pairs. Although there were no false assignments, *sequoia* was unable to identify 66% of known pairings using the 781 SNP dataset.

Despite the uncertainty in assigning parents, we can use these results to assign litters. The results indicate that DF1 and DC1 are the result of a full-sibling cross within hybrid litter "B60071/B60007", one of the two F1 Hybrid litters. This was confirmed by a sibling analysis, that suggested they were a full-sibling pair (LLR: 9.04). The results suggested that BDC had the same sire as DF1 and DC1, a F1 Hybrid male from litter "B60071/B60007" – making these three individuals half-siblings. BDC was assigned a F1 NT dam, supporting the results from the hybrid index that this individual is a NT-backcross. 14F is also likely the result of a full-sibling F1 Hybrid cross from within the second F1 Hybrid litter ("B60011/B60075"). Finally, results suggest that A52 has a NT dam and sire from either two F1 NT litters, both are different litters to that of

Table 7.6. Parentage estimates using package R sequoia for unknown F2 Island individuals. Shows potential parental (Dam and Site) IDs, origins, and litter ID. LLR is the log10 likelihood ratio, indicating the degree of fit of the match of parent-offspring pair (this is the ratio between the likelihood of the assigned parent being the parent, versus the most likely alternative type). Matches are ranked according to highest likelihood ratio.

Offspring	Dam ID	Dam Origin	Dam litter ID	Dam LLR	Sire ID	Sire Origin	Sire litter ID	Sire LLR
A52	941000018496033	NT FI	B60090/B60094	142.27				
A52					941000018496007	NT FI	B60090/B60094	133.46
A52	941000018496012	NT FI	B60044/B60043	94.49				
A52	941000018496011	NT FI	B60044/B60043	88.63				
A52					941000018496009	NT FI	B60044/B60043	48.76
DBC					941000018496026	Hybrid FI	B60071/B60007	27.98
DBC	941000018495950	NT FI	B60065/B60076	1.44				
DCI	941000018496025	Hybrid FI	B60071/B60007	7.37				
DCI					941000018496026	Hybrid FI	B60071/B60007	-19.34
14F	941000017807572	Hybrid FI	B60011/B60075	12.18				
14F	941000017807573	Hybrid FI	B60011/B60075	12.05				
14F					941000017807571	Hybrid FI	B60011/B60075	8.31
14F					941000017807570	Hybrid FI	B60011/B60075	3.31
DFI					941000018496026	Hybrid FI	B60071/B60007	12.55
DFI					941000018496026	Hybrid FI	B60071/B60007	4.46

Table 7.7. Summary of results to determine origin of six unknown 2018 F2 Island northern quolls. Results indicating origin form STRUCTURE, PCoA, hybrid index and parentage analysis. − represents an unknown/unclear result, with suggested origins in brackets.

Quoll	STRUCTURE	PCoA	Hybrid Index	Parentage
A52	F2 NT	- (F2 NT Backcross)	F2 NT Backcross or F2 Hybrid	NT F2 or F2 NT Backcross
CF2	- (F2 NT Backcross)	- (F2 NT Backcross)	F2 NT Backcross or F2 Hybrid	-
DBC	- (F2 NT Backcross)	- (F2 NT Backcross)	F2 NT Backcross	F2 NT Backcross
I4F	F2 Hybrid	F2 Hybrid	F2 Hybrid	F2 Hybrid
DCI	F2 Hybrid	F2 Hybrid	F2 Hybrid	F2 Hybrid
DFI	F2 Hybrid	F2 Hybrid	F2 Hybrid	F2 Hybrid

Table 7.8. Expected and observed proportion of QLD and NT provenance in 2018 F2 Island cohort and expected and observed frequencies of the genome and crosses in 2018 F2 Island cohort. Expected proportions and frequencies calculated using random mating and assuming a single locus. Observed frequencies based on both STRUCTURE analysis and hybrid index. Chi-squared test preformed on observed frequencies.

	QLD	NT	Hybrid			χ^2 (p)
			QLD b/cross	Hybrid	NT b/cross	
Expected proportion of provenance	0.292	0.707				
Observed proportion (STRUCTURE)	0.393	0.607				
Observed proportion (Hybrid index)	0.423	0.577				
Expected frequencies (genome)	0	0.324	0.095	0.303	0.276	
Expected frequencies (crosses)	0	2	0	4		
Observed frequencies (STRUCTURE)	0	1	0	5		0.67 (0.4)
Observed frequencies (Hybrid index)	0	0	0	6		2.88 (0.09)

the NT dam of BDC. None of the parentage analysis produced results for CF2. In total, these parentage results suggest our six F2 Island individuals came from five dams (of the 25 females that were introduced). Potentially microsatellites could be used to improve the certainty of parentage analysis, as the technique would provide substantially more power. For the current study, however, we were primarily interested in acquiring a large number of markers across the genome to measure introgression between population genomes, as well as the potential alignment to a future genome.

CONCLUSIONS FROM DNA ANALYSIS

Although there is some uncertainty from the individual tests, we can draw conclusions based on the consistencies between tests to help identify the origins of the six unknown F2 Island quolls. A summary of the results is shown in Table 7.7. We then used the results from the both hybrid index and *STRUCTURE* analysis to produce our observed proportions of QLD and NT provenance, and the frequencies of different crosses compared to our expected proportions/ frequencies based on random mating. Due to the small sample size, we did not include backcrosses in the frequencies, instead just comparing pure NT and hybrids (which encompassed F2 QLD backcross, F2 Hybrids and F2 NT backcross). Using the observed frequencies, we performed chi-squared tests and Fisher's exact tests on the data. Sample sizes are very small, and this badly violated the assumption that each cell of the contingency table should be >5. Neither test produced a significant difference (Table 7.8).

The genetic results indicated our six sampled individuals came from five litters (4 x 1 per litter, 1 x 2 per litter). Using this, we were able to estimate the number of litters born on the island (k) based on the observed distribution of litter sizes and the number of offspring we observed in 2018 ($n = 6$). We assumed each of our observed offspring has an equal (and independent) chance of being from a particular litter, and so the probability of arriving in a particular litter is $1/k$. If there is no limit on litter size, we expect the probability of observable litter sizes (x) should to be distributed according to:

$$P(X = x) = \binom{n}{x} \frac{1}{k^x}\left(1 - \frac{1}{k}\right)^{(n-x)} \bigg/ 1 - (1 - \frac{1}{k})^n$$

Where denotes the the number of individuals arriving in a particular litter and is the binomial coefficient $\binom{n}{0}$. We used our distribution of litter sizes to get a maximum likelihood estimate on k. We then used bootstrapping to get the 95% CI on number of litters (k). Our best estimate of number of litters (k) was 14 (95% CI: 5-25). The upper and lower estimates match what we would expect (we observed five litters and only released 25 females). The best estimate of 14 (meaning, based on mean litter sizes, we would expect approximately 81 offspring) indicates there was some F1 mortality (or failed breeding), but the majority of mortality occurred post-weaning.

Discussion

Due to unforeseeable circumstances, our results are not as clear as we could have hoped. We are, however, still able to draw some conclusions from the field trial. First, we have demonstrated the reproductive viability of the F1 Hybrids and fitness of F2 Hybrids and backcrosses. This suggests that outbreeding depression caused by genetic incompatibilities is unlikely to be an issue in this system. Before this field trail, we had no evidence to show F1 Hybrids were sexually viable, but the genetics analysis has demonstrated that F1 Hybrids (both males and females) were able to breed and produce healthy offspring on the island. Our results suggest three out of six 2018 F2 Island individuals were a result of hybrid/hybrid crosses (from full sibling pairings). One other 2018 F2 Island quoll was an NT backcross, with a F1 NT dam and F1 Hybrid sire – indicating that these backcrosses also produce healthy offspring. Although the remaining two individuals could not be conclusively assigned a group, the hybrid index indicated they were also the result of either a NT backcross or hybrid/hybrid pairing. Thus, we have no evidence of outbreeding depression in either the F1 or F2 generations. Outbreeding depression in northern quolls could have provided a barrier to the implementation of targeted gene flow, but these results demonstrate that the toad-smart and toad-naïve populations are genetically compatible.

Second, but more speculatively, we have tentative evidence for selection of the toad-smart genome. The genetic results suggest a much higher proportion of Queensland genes (from the toad-smart population) in the 2018 F2 Island cohort (39.3% from *STRUCTURE*; 43.2% from hybrid index) compared to the expected proportion based on random mating of the released population (29.2%; Table 7.8). Our expected frequency of NT and Hybrid crosses differed from what we observed, but only slightly. If these observed frequencies did indeed represent the true frequencies, and were not due to random sampling error, this would represent a departure from a null expectation, with fewer pure NT offspring than expected. With the current data, however, we cannot conclusively say that the Queensland genome is under selection.

Our conclusions are limited due to the extremely low sample size of the 2018 F2 cohort. Although the initial release of northern quolls on Indian Island was successful, the following year the population was far smaller than expected. Our best estimate for population size one year post release was 16 individuals (including 6 F1 individuals), far smaller than the original 54 released. We released 25 females onto the island, so based on mean litter sizes in captivity (mean = 5.8; Kelly and Phillips 2018b), the expected number of offspring in the 2018 cohort would be approximately 145. We estimate the current population size of 2018 individuals to be between 4 and 24% of this number. This dramatic decrease has several potential causes, which are difficult to disentangle: toads, fire, weather, predation, failed breeding and dispersal. Here, we discuss the likelihood of these factors influencing mortality.

First, the impact of toads. We trained all release animals to avoid toads so that they would survive to reproduce, and natural selection would begin to take effect on the first generation born on the island (F2); ideally a larger population than the release cohort. We know the original released generation were effectively trained to avoid toads (because they largely survived the first four radio-tracked days). We expected that natural selection from toads would kill a large number of the 2018 F2 cohort. If we assume that inheritance of toad-smarts in the wild follows what we measured in captivity (Kelly and Phillips 2018b), then only 29.7% of heterozygous and pure QLD types should be killed by toads, compared with 61.9% killed of pure NT animals. If we assume random mating and, for simplicity, a single locus, then 6% of the offspring would be pure toad-smart type; 47% would be pure toad-naive; and 47% would be heterozygous. This equates to an expectation that 55% of the 2018 cohort should have survived toads, as they inherited toad-smart gene(s). Clearly this calculation involves a number of uncertainties in trait expression, so it still remains possible that toads alone have driven the reduction in population size we have observed (an approximately 93% reduction). But toad-mortality under these assumptions seems unlikely to be the sole cause.

Our assumption that all released animals that survived their initial encounter with toads were toad-smart thereafter has, however, recently come into question. While results from captive and short-term field trials suggested that conditioned taste aversion training is effective at reducing toad mortality in quolls (O'Donnell *et al.* 2010; Cremona *et al.* 2017; Jolly *et al.* 2017), recent results from a large-scale field trial suggest that taste aversion is transitory, and declines over the course of several months (~120 days; Indigo et al *unpublished manuscript*). If this is the case for our released cohort, those individuals who were trained through conditioned taste aversion prior to release would have lost the lesson by September, which is towards the end of the breeding season. Females with pouch young may have therefore been poisoned by toads before they could raise their offspring to maturity, greatly reducing the population size, and this could be the sole cause for our small population size in 2018.

A failure of conditioned taste aversion training would have consequences for the current experiment, because it would cause the full impact of toads to occur a generation earlier than we planned. We purposely trained the release population to avoid toads so that we could establish a mixed population of toad-smart and toad-naïve quolls that would survive to breed. The offspring born on the island would then be subject to toad selection, theoretically with only those who carry toad-smart genes surviving. This would have resulted in a skew towards QLD genes, as we saw from the genetic data. However, this pattern could have also occurred if training had decayed and the release population was reduced through toad-mortality. We do know that two purebred NT quolls did survive, because we caught them in 2018 (neither of which were selected as potential dams of our 2018 F2 Island quolls, meaning there were potentially four F2 NT quolls which survived to breed). However, this is only a very small

proportion of those released, and these individuals are likely part of the small proportion of naïve quolls that are toad-smart without training. Although it seems likely, we cannot be sure if training did decay and lead to reduction in population size we observed, and other potential causes remain.

One other possibility is that there was increased mortality on both adult and juvenile quolls due to fire, weather, and predation. A bushfire, the first on the island in over two decades, occurred in August at the end of the dry season and burnt for three weeks. The fire ranged from hot (leaf litter burned, mid-story survived) through to severe (mature canopy killed), and burnt the majority of the previously abundant hollow logs. This caused a major reduction in shelter for the quolls and, importantly, the fire occurred during a time when female quolls were denning their young (Oakwood 2000). The lack of shelter for the females and their young offspring at this crucial time would likely have increased mortality both during the fire and left them vulnerable to predation in the months afterwards. Another massive stochastic disturbance – a category 2 cyclone – also impacted the island in March 2018. The storm surge may well have flooded the rocky cliffs where females prefer to den, and the wind would certainly have stripped foliage from the canopy, creating opportunities for predators. Although Indian Island is free of mammalian predators – there are no dingos or feral cats – there is still the chance of predation by birds. During the 2017 radio tracking one male was killed by a rufous owl. Owls (and to a lesser extent, diurnal raptors) could be a continuous source of mortality for quolls on the island, particularly of dispersing juveniles. This, coupled with the lack of shelter caused by the fire and cyclone, may have caused surprisingly high mortality on the Island.

The lack of F2 offspring could be a result of failed breeding attempts in 2017. This seems unlikely given many of the females were in oestrus when they were released, and a mating was observed on the night of release. If the stress of release caused a failure of the first oestrus, the females will have gone into a second oestrus. The genetic data also provides evidence that breeding was successful in 2017. Our results suggest the six 2018 F2 Island individuals came from five litters (4 x 1 per litter; 1 x 2 per litter) and using this we were able to estimate that there were likely 14 (95% CI: 5-25) litters born on the island. This suggests breeding was relatively successful in 2017, and mortality of the F2 generation occurred after weaning. If this is the case, decay in toad aversion training is the less likely explanation, with toad-mortality in F2 offspring and predation (facilitated by fire and cyclone) driving the small population numbers.

A final possibility is that there are, in fact, many juveniles surviving, but they have dispersed very widely across the island such that we simply did not encounter them. This is a possibility, particularly given this cohort's very low detection probability, and that we encountered 2018 cohort animals across the entire first woodland block (Figure 7.1c). Our trapping in the second woodland block, however, generated no additional animals to those already observed in the first block. Additionally, in a monitoring site in the south of Indian Island (approximately 8km south of the release point and separated by a

mangrove mudflat) no sign of quolls (tracks or in traps) has yet been recorded. Thus, while there has undoubtedly been some dispersal out of the study area, it would not appear to be a major cause of the small population we observed.

Conclusions

So where does this leave the population on Indian Island? No matter the cause, the population is now even smaller than the original released cohort – making it highly vulnerable to genetic drift and stochastic demographic processes. Alleles in small populations reach fixation at a faster rate, and those under selection (such as the toad-smart genes) will have a higher chance of fixation (Kimura & Ohta 1969). Unfortunately, even with this high chance of selection of toad-smart genes, the small population is still at risk of stochastic failure, such as predation or failed breeding. Even if toad-smart genes reach fixation in the population, the risk of extinction is high until numbers recover. Additional monitoring of the population on Indian Island over the next few years will ultimately reveal its fate. Either the population will recover through successful breeding seasons, or else go extinct.

Despite the uncertainty in our results, we have been able to draw conclusions from this first stage of the experiment. We can now effectively rule out one important possible negative outcome – severe outbreeding depression – as we find no evidence for it in either F1 or F2 generations. Encouragingly, we also found a high proportion of QLD genome in our F2 population, which – despite the small sample size – suggests that this introduced variation in toad-smart genes has been an important factor in the survival of our population. If the population survives, we hope to track this selection over multiple generations. This would not only boost our sample size, but also provide more information on the selection dynamics of the toad-smart and toad-naïve genomes that would not have been apparent from a year-long study. This experiment was always meant to surpass the timeline of this thesis, requiring several years' monitoring to gain adequate conclusions on selection.

In the meantime, a clear distinction must be made between the failings of the experiment and the failings of targeted gene flow. The issues that may have contributed to the small population size are consequences of conducting an experiment on a small closed population. Our aim to establish a population on a toad-infested island was risky – previous northern quoll reintroductions into areas with toads and predators have all failed (Cremona *et al.* 2017; Jolly *et al.* 2017). Our small population was also astonishingly unlucky to experience two major rare stochastic events – fire and cyclone – in the year of establishment. This left our population particularly vulnerable to predation. These problems do not speak to the issue of targeted gene flow. They do, however, highlight an important risk of the stratergy - the risk of population failure. There are ways to minimise this risk. If we were to deploy targeted gene flow in a mainland population, previous modelling work suggests this can be best achieved though

introducing toad-smart quolls into an established population prior to cane toad arrival (Kelly and Phillips 2018a). Introducing toad-smart quolls into an established toad-free population avoids entirely the issues encountered by the current experiment (possible toad aversion training failure and small population size at risk from stochastic processes), allowing the generation of toad-smart hybrids prior to toad arrival. We would then expect toad smart traits to be selected once toads arrive.

Overall this thesis has met its objectives – demonstrating that northern quolls are a suitable candidate for this targeted gene flow and developing an ideal strategy for implementing targeted gene flow – but as always, questions remain. Firstly, there are still uncertainties regarding the underlying mechanism of toad-smart behaviour. Although we are clear there is a heritable toad-smart trait that means an individual will not attack a toad, the cognitive process behind this shift in diet remains a mystery. In addition, we have only scratched the surface of the genetic component to this work. There is much more to uncover regarding the underlying genetic aspects of toad-smart behaviour, as well as the impact that toads have had on genetic diversity of northern quolls. The preliminary analyses of genetic data here show very low levels of genetic diversity in toad-exposed populations, suggesting an historical bottleneck that was likely driven by toad arrival (Gattepaille *et al.* 2013). Future work could take advantage of the significant recent progress in genome sequencing and analysis techniques, meaning we could identify areas of the genome that are potentially under selection from toads (Goddard & Hayes 2009; Narum *et al.* 2013). In doing so, we could detect regions of the genome that may contribute to the complex toad-smart trait.

Although questions remain, the undeniable threat of cane toads and limited time frame means conservationists may need to take a risk to ensure the continued survival of northern quolls. Given the evidence presented in this thesis, we recommend employing targeted gene flow on an established mainland population prior to the arrival of toads. Despite knowledge gaps, we have demonstrated that targeted gene flow is a feasible strategy for northern quolls. As well as achieving this outcome for an endangered species, we also hope to have illustrated the broader applications of targeted gene flow for conservation. In the face of rapid environmental change, many species are locked in a desperate race between adaptation and extinction. Targeted gene flow could prove an important tool for giving the advantage to adaptation and conserving threatened species.

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Appendix I
Supplementary material for Chapter 3

Methods

FOR the population viability analysis we created an individual-based population model in the R statistical environment (R Core Team 2016). Individuals were simulated in discrete time (years). Within years, reproduction is followed by aging, stochastic mortality, male die offs and selection (if toads are present). Each simulation was run for 100 years, with toads arriving at year 30. When toads were present, survival of each individual was determined by drawing from a binomial distribution where the probability of attacking a toad was altered for each scenario to capture the entire possible range of responses (0-1 in 0.01 increments). Each scenario was run 100 times and the population viability was calculated from the number of surviving simulations.

Populations were initialized through the specification of individual traits, with sex drawn randomly for each individual from a binomial distribution, and age set to one for all initial individuals. The population size was initially set at 2000 individuals (2K where K = 1000 = carrying capacity of females; see below). To mimic natural quoll breeding systems, males were able to mate with more than one female per mating season. To maintain the population within reasonable bounds, the expected number of offspring that survived to adulthood was determined by the density of females. We applied a density dependence function to female reproductive output, which determined the number of offspring a female had that survived to reproduce. As female population size increased, the number of offspring surviving through to the next generation decreased. When the population

reached the specified carrying capacity (*K*) the proportion of females breeding is 0.3 (i.e. the proportion of females required to breed to keep population levels constant). The equation for the function (bounded between 0-1) is:

$$P(N) = e^{N\frac{\log(0.3)}{K}}$$

Where *P(N)* is the proportion of females breeding, *N* is the female population size and *K* is the carrying capacity of females. Because we applied the function to act on the number of females in the population, the *K* we specify in the function is actually not the true *K* of the population because it excludes males and juveniles.

Population parameters for the model were drawn from data collected from captive and wild populations of northern quolls (Begg 1981; Schmitt *et al.* 1989; Dickman & Braithwaite 1992; Braithwaite & Griffiths 1994; Oakwood 2000; Rankmore *et al.* 2008). Reproductive output (number of offspring per female) was drawn randomly from a binominal distribution fitted to data collected on litter sizes from captive bred quolls at the Territory Wildlife Park in 2015 and 2016 (bounded at 8, max teats for a northern quoll; Oakwood 2000; Kelly et al. unpublished data). Sex and age specific survival was determined by comparing model outcomes to the population sizes of mark-recaptured populations of northern quolls on Astell and Pobassoo Islands to determine the most likely values of demographic parameters (Rankmore *et al.* 2008; Table S1). We began with priors from the literature (Schmitt *et al.* 1989; Dickman & Braithwaite 1992; Braithwaite & Griffiths 1994; Oakwood 1997; Hill & Ward 2010), and then we used the model with these priors to predict population trajectories on Astell and Pobassoo. We then used Approximate Bayesian computation (ABC) to generate posteriors for each of these parameters (Table S3.1). For each run of the model, it draws upon these posteriors to estimate age and sex-specific survival probability, which is then drawn randomly for each individual from a binominal distribution.

Tables

Table S3.1. Raw data priors, sources and estimates from ABC computation for age and sex specific survival for simulated northern quoll population.

Demographic parameter	Proportion surviving	Reference	Estimated survival
Male survival	0.00	(Dickman & Braithwaite 1992)	0.044
	0.00	(Oakwood 2000)	
	0.13	(Begg 1981)	
	0.05	(Schmitt et al. 1989)	
Female survival (first year)	0.27	(Oakwood 2000)	0.123
	0.08	(Braithwaite & Griffiths 1994)	
	0.21	(Begg 1981)	
	0.38	(Schmitt et al. 1989)	
Female survival (second year)	0.00	(Oakwood 2000)	0.030
	0.00	(Braithwaite & Griffiths 1994)	
	0.06	(Begg 1981)	

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Appendix II
Supplementary material for Chapter 4

Tables

Table S4.1. Linear mixed model fit by maximum likelihood of the effect of origin (pure toad-exposed, hybrid or pure toad-naïve) and prey type on the time captive born northern quolls spent investigating a cane toad or mouse in a cage. P values determined by log likelihood tests and significance indicated by *.

Fixed Effects	Estimate	SE	P
Intercept	6.44	0.25	
Origin	-	-	0.24
(toad-naïve)	-0.43	0.28	
(toad-exposed)	-0.41	0.28	
Prey type (toad)	-0.70	0.10	<0.001*
Origin (toad-exposed) * Prey type (toad)	-0.47	0.28	0.06

Random Effects	Variance	Std.Dev
Litter	0.08	0.29
Individual	0.06	0.24
Residual	0.49	0.70

Table S4.2. Generalized linear mixed model fit by maximum likelihood (family = binomial) of the effect of origin (pure toad-exposed, hybrid or pure toad-naïve) and prey type on the likelihood of a captive born northern quolls attacking a cane toad or mouse in a cage. P values determined by log likelihood tests and significance indicated by *.

Fixed Effects	Estimate	SE	P
Intercept	-1.28	0.48	
Origin	-	-	0.11
(toad-naïve)	0.84	0.51	
(toad-exposed)	0.21	0.50	
Prey type (toad)	0.87	0.29	<0.01*
Origin (toad-exposed) * Prey type (toad)	1.46	0.97	0.17

Random Effects	Variance	Std.Dev	
Litter	0.03	0.16	
Individual	2.89×10^{-9}	5.38×10^{-5}	

Table S4.3. Generalized linear mixed model fit by maximum likelihood (family = binomial) of the effect of origin (pure toad-exposed, hybrid or pure toad-naïve) on the likelihood of a captive born northern quolls eating a toad leg. P values determined by log likelihood tests and significance indicated by *.

Fixed Effects	Estimate	SE	P
Intercept	1.07	1.46	
Origin	-	-	0.01*
(toad-naïve)	1.52	0.94	
(toad-exposed)	-1.51	1.42	
Thiabendazole present	-1.90	1.21	0.08

Random Effects	Variance	Std.Dev	
Litter	0.54	0.73	
Individual	5.99×10^{-7}	0.8×10^{-3}	

Appendix III
Supplementary material for Chapter 6

Methods

Northern quoll population dynamics

FEMALE FECUNDITY AND SURVIVAL OF BABIES

FECUNDITY of females was considered density dependent. We considered that all females have the capacity to produce 8 babies, but that the survival of these babies to weaning is density dependent: declining from a base survival rate with an increasing density of adult females in the population. Male density was ignored because almost all adult northern quoll males die before young quolls are weaned (Dickman & Braithwaite 1992). Thus, the expected number of weaned offspring for each female is $8s_b$, where s_b is the probability that each baby survives to weaning. This survival probability is dependent on the density of adult females in the population, n such that:

$$s_b = s_0 e^{n\log 0.3/K}$$

s_0 is the base survival rate in the absence of density effects. Here K is the density of females at which survival probability of babies equals $0.3s_0$. The constant, 0.3, is chosen as approximately the value of s_b at which the population stops growing when $s_0 = 1$. While this process gives us an expected number of weaned offspring, for each female, the realised number of weaned offspring is stochastic; determined, for each female, as a draw from a binomial distribution Binom(s_b, 8). Mate choice is random and males can mate with multiple females,

litter) is not allowed.

SURVIVAL OF JUVENILES AND ADULTS

We used data from previous mark-recapture studies to estimate yearly survival probabilities of male and female quolls (Begg 1981; Schmitt et al. 1989; Braithwaite & Griffiths 1994; Oakwood 2000). We used raw published data that indicated age and sex of the mentioned quolls, and that followed the individuals for at least one year, as this meant we could estimate annual survival (Table S6.1). We took the unweighted means of these data to get the survival probabilities that were used in our model. Unfortunately, there was no way of accounting for survey effort or detection probability, as these details were not reported. However, our estimates were extremely close to those from Cremona et al. (2017), which used their own mark-recapture study and estimated annual survival incorporating recapture probability. Therefore, we believe our parameter estimates are the best that can be determined, given the available data.

Juvenile survival probability (s_o) was set to 0.38 based on estimates from Oakwood (2000) of the survival of juveniles between when they were first denned to when they first became trappable. Male quolls mature within a year and, with high probability, die before their second year. In our model, this was captured with two parameters: $s_{m1} = 0.042$, $s_{m2} = 0$. Female quolls also mature within a year, but have a greater chance to survive through to a second reproductive season. In our model, this was captured with three parameters: $s_{f1} = 0.234$, $s_{f2} = 0.03$, and $s_{f3} = 0$. In all cases, the realised survival of an individual was treated as a draw from a Bernoulli distribution with the specified survival probability.

Tables

Table S6.1. Raw survival data from previous mark-recapture studies used to estimate annual sex-based survival rates

| Study	Sex	Age	n	surv	die	survival
Oakwood 2000	female	1	11	3	8	0.2727273
Begg 1980	female	1	53	11	42	0.2075472
Braithwaite and Griffiths 1994	female	1	150	12	138	0.0800000
Schmitt 1989	female	1	16	6	10	0.3750000
Oakwood 2000	female	2	11	0	10	0.0000000
Begg 1980	female	2	33	2	31	0.0606061
Oakwood 2000	male	1	26	0	26	0.0000000
Begg 1980	male	1	32	4	28	0.1250000
Braithwaite and Griffiths 1994	male	1	57	0	57	0.0000000
Schmitt 1989	male	1	46	2	44	0.0434783

Table S6.2. Generic population model: model scenario parameters including global sensitivity analysis, where $\bar{w}_0$ is the initial post step-change fitness at a population level.

Population size	Growth rate	Heritability	Outbreeding depression	Recombination rate	$\bar{w}_0$
125	1.5	0.1	none	0.5	0.02
125	1.5	0.2	none	0.5	0.05
125	1.5	0.3	none	0.5	0.02
125	3	0.1	none	0.5	0.05
125	3	0.2	none	0.5	0.02
125	3	0.3	none	0.5	0.005
125	6	0.1	none	0.5	0.005
125	6	0.2	none	0.5	0.005
125	6	0.3	none	0.5	0.0005
500	1.5	0.1	none	0.5	0.1
500	1.5	0.2	none	0.5	0.035
500	1.5	0.3	none	0.5	0.005
500	3	0.1	none	0.5	0.03
500	3	0.2	none	0.5	0.005
500	3	0.3	none	0.5	0.003
500	6	0.1	none	0.5	0.01
500	6	0.2	none	0.5	0.005
500	6	0.3	none	0.5	0.001
1000	1.5	0.1	none	0.5	0.08
1000	1.5	0.2	none	0.5	0.015
1000	1.5	0.3	none	0.5	0.005
1000	3	0.1	none	0.5	0.02
1000	3	0.2	none	0.5	0.002
1000	3	0.3	none	0.5	0.0005
1000	6	0.1	none	0.5	0.005
1000	6	0.2	none	0.5	0.002
1000	6	0.3	none	0.5	0.03
500	3	0.2	50%	0.5	0.005
500	3	0.2	10%	0.5	0.005
500	3	0.2	none	0.25	0.005
500	3	0.2	50%	0.25	0.005
500	3	0.2	10%	0.25	0.005

**Table S6.3.** Northern quoll population model: model scenario parameters including sensitivity analysis

Population size	heritability	Outbreeding depression	Recombination rate	$\bar{w}_0$
500	0.2	none	0.5	0.05
1000	0.1	none	0.5	0.15
1000	0.3	none	0.5	0.03
125	0.1	none	0.5	0.3
125	0.3	none	0.5	0.08
500	0.2	50% reduction	0.5	0.05
500	0.2	10% reduction	0.5	0.05
500	0.2	none	0.25	0.05

Figures
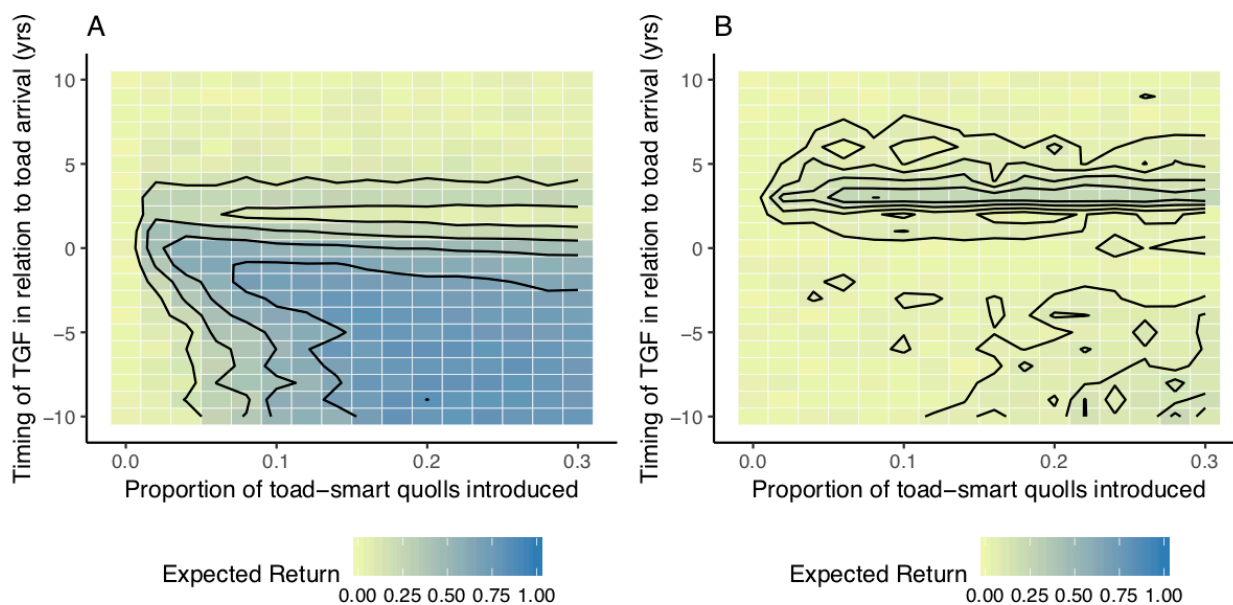
Figure S6.1. Northern quoll population model: The impact of outbreeding depression on targeted gene flow outcomes in relation to the timing of targeted gene flow (years) and the number of toad-smart individuals introduced. Expected return of the recipient genome (calculated by E using probability of extinction () and proportion of recipient genome ()) for targeted gene flow with A: 10% and B: 50% reduction of fitness for F1 hybrids.
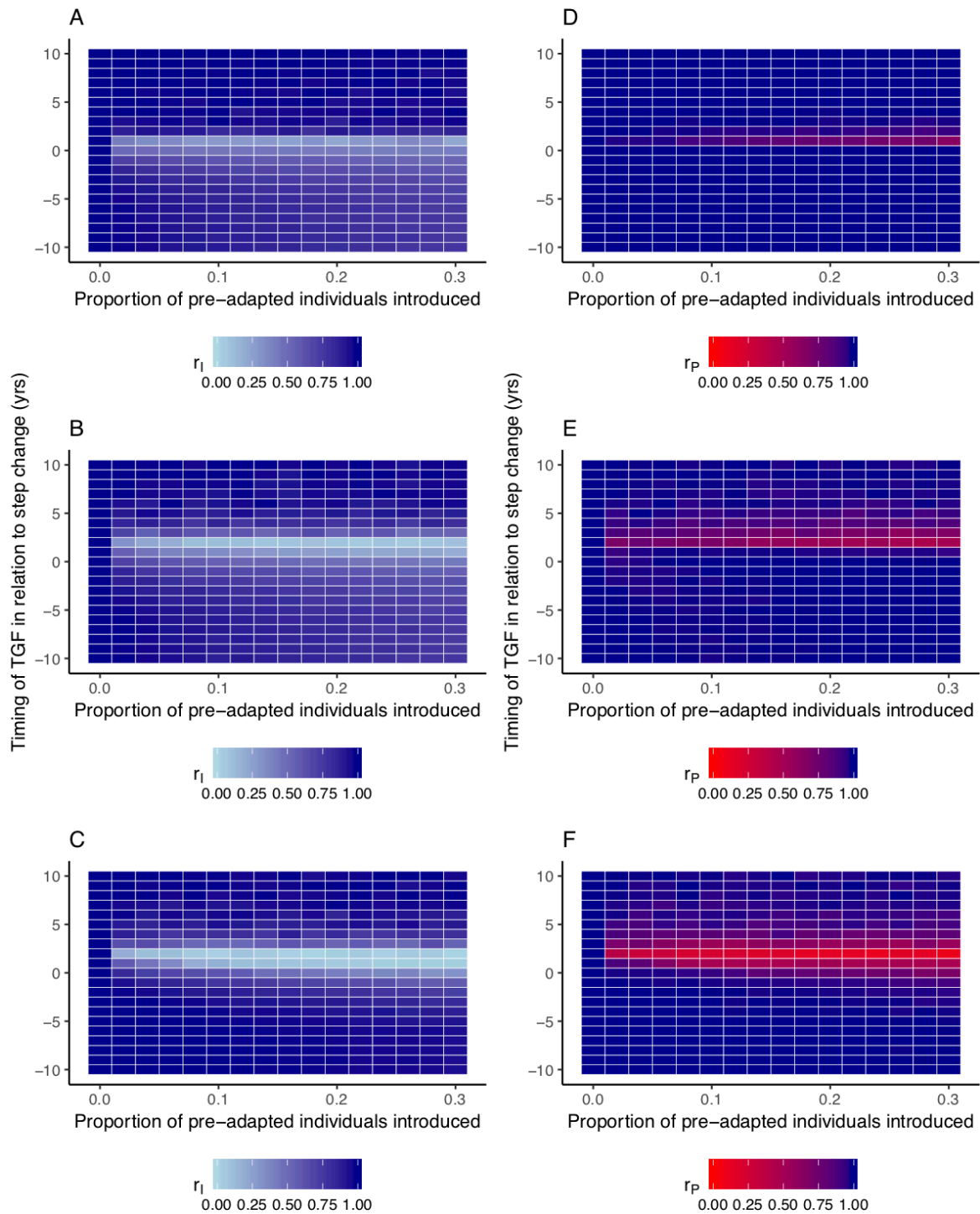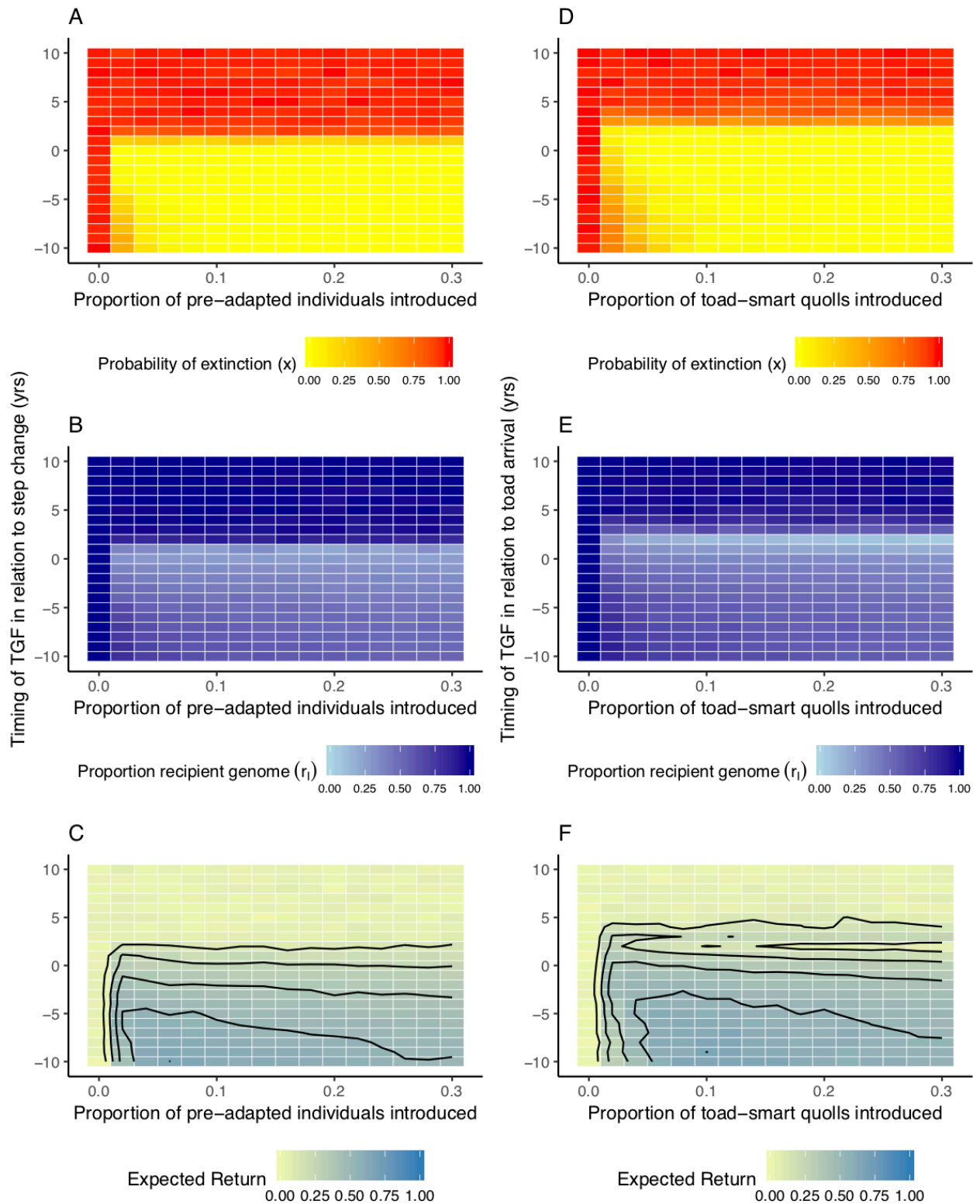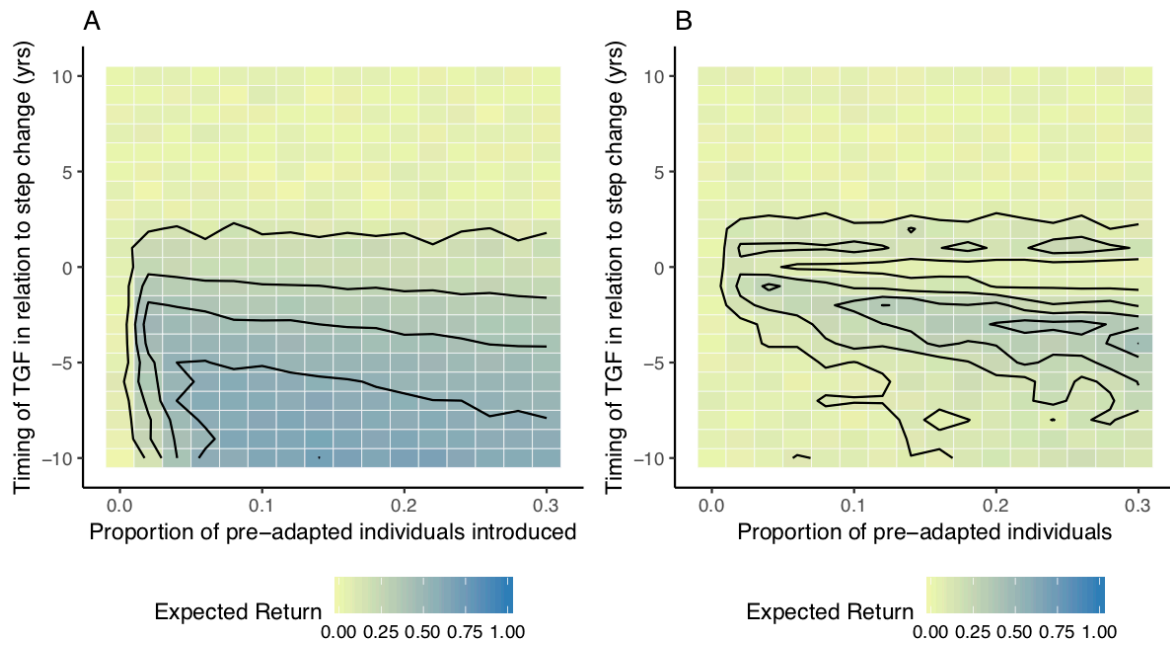
Figure S6.2. Generic population model: Comparison of two methods for measuring the proportion of recipient genome remaining, and . A-C: calculated as the proportion of recipient alleles remaining in each individual averaged over individuals. D-F: proportion of loci with recipient alleles remaining anywhere within the whole population. A & D: Model without outbreeding depression. B & E: 10% reduction in F1 hybrid fitness. C & F: 50% reduction in F1 hybrid fitness.
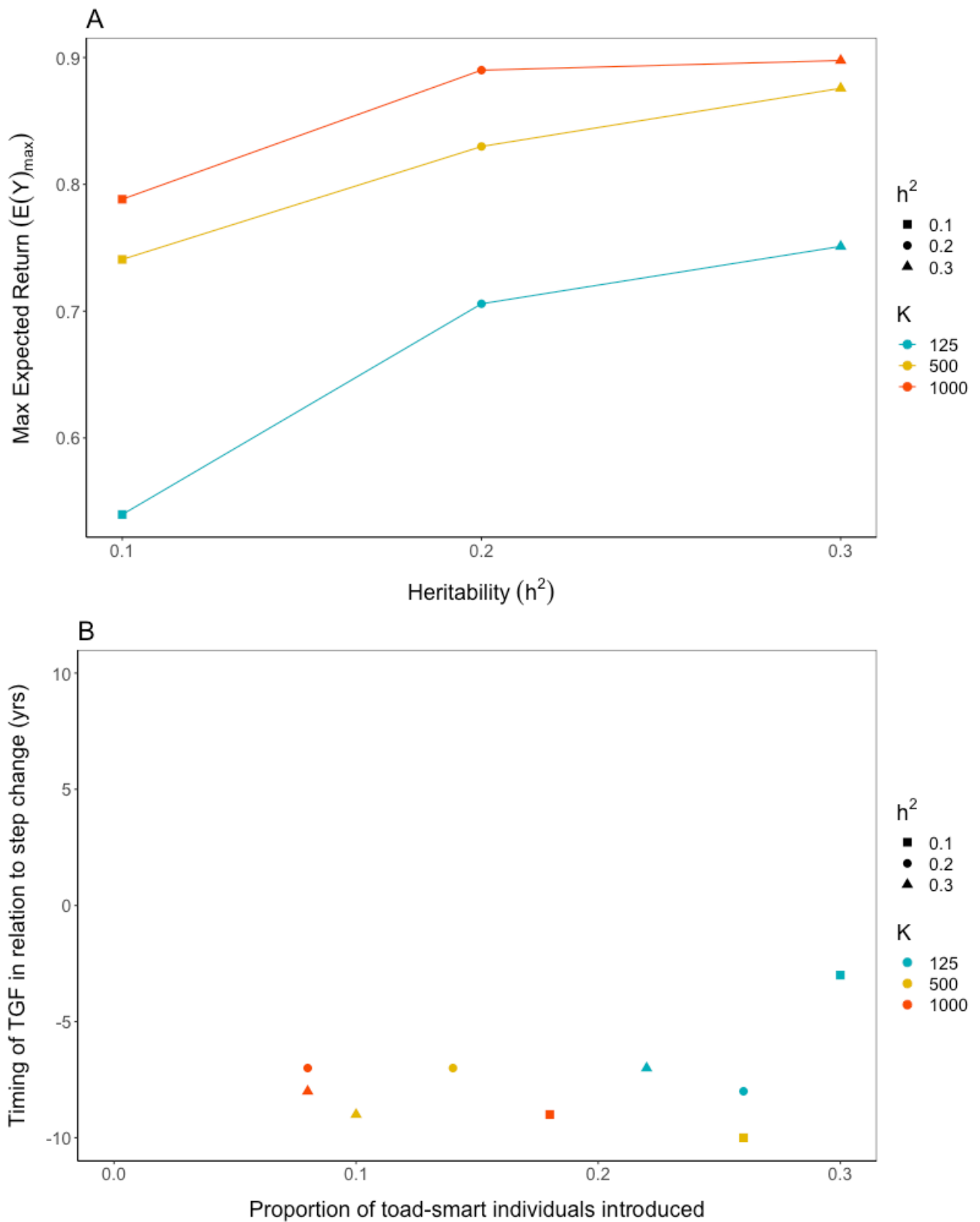
Figure S6.3. Northern quoll population model: global sensitivity analysis exploring two dimensional parameter space: population size (: represented by point colours) and heritability (: represented as point shapes). Showing A: Maximum expected return from a scenario, and B: the location in the management space (the timing of targeted gene flow and the proportion of toad-smart individuals introduced) that produced maximum expected return.

Figure S6.4. Sensitivity analysis of recombination rate for A-C: generic population model and D-F: northern quoll population model. Recombination rate set to 0.25 (simulated by setting the number of cuts to 1.33), A & D: The probability of extinction (; red = high chance of extinction) for varying implementations of targeted gene flow. B & E: The proportion of recipient population genome (; dark blue is recipient genome) in eventual population after varying implementations of targeted gene flow. C & F: The expected return of the recipient genome (i.e. the proportion of the recipient genome surviving, calculated by using probability of extinction () and proportion of recipient genome ())

Figure S6.5. Generic population model: The impact of outbreeding depression and lower recombination rate (0.25) on targeted gene flow outcomes in relation to the timing of targeted gene flow (years) and the proportion of pre-adapted individuals introduced. Expected return of the recipient genome (calculated by E using probability of extinction () and proportion of recipient genome ()) for targeted gene flow with A: 10% and B: 50% reduction of fitness for F1 hybrids.

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