Genetic Rescue and the conservation of small populations.

George West

Thesis submitted for the degree of Doctor of Philosophy

University of East Anglia

School of Biological Sciences

April 2025

Thesis abstract

Genetic rescue is a key tool in the conservation of endangered species by alleviating inbreeding depression and enhancing adaptive potential. However, there is reluctance from conservation practitioners to attempt genetic rescue due to perceived risks. To overcome such concerns, we must expand our understanding of how gene flow from different sources affects inbred populations, allowing genetic rescue to be achieved safely. This thesis utilises the model species *Tribolium castaneum* to test how different rescuer characteristics affect the success of genetic rescue. In chapter two, I rescued inbred populations with rescuers of either sex from an outbred population; both were equally successful at achieving genetic rescue. I then attempted rescue of inbred populations using a rescuer originating from populations maintained under either sexual selection or no sexual selection. Results show that only the rescuers from a sexual selection background improved population fitness. In chapter three, I tested for differences in the success of genetic rescue using rescuers from either outbred or inbred source populations. As predicted a rescuer from an outbred population increased the fitness of recipient populations more compared to inbred rescuers. Whole genome analysis found that outbred rescuers reduced inbreeding within recipient populations more than inbred rescuers. Outbred rescuers also introduced more mutational load than did inbred rescuers, though importantly this was as masked load and did not appear to impact the fitness of the populations even after nine generations. Finally in chapter four, I used inbred populations previously adapted to higher temperatures to discover if genetic rescue would disrupt the local adaptation. Non-adapted rescuers still improved population fitness, however a rescuer from another adapted population produced greater fitness benefits. I then synthesise these findings in context of the wider literature to understand how they further our understanding of genetic rescue and how it may be implemented in conservation.

Table of contents

Table of figures

Table of tables

Acknowledgements

I would like to thank everyone who has helped me for the past 4+ years both with this work and in life. I am a very different person than the one who came to Norwich in 2020 because of the people I have met.

I would like to thank my supervisors for their guidance and patience. My final supervisory team: Dave who took on my project and put a lot of time and effort into making sure this thesis was more precise and concise. Becky whose work led to this project in the first place and helped me with anything *Tribolium* related. Will who helped tremendously with the genomic analysis and made sure to put my wellbeing first. I also thank my former supervisors for their assistance in the first years of my PhD and the planning of my experiments and thesis – Lewis, Matt and Jen.

I also need to thank all of my colleagues and friends for their support. Everyone who worked in the Tribolium lab and was vital in my training and for their assistance with the experiments – Mike, Ben, Ram, Tash and Ginny. Those that have been a part of the lab group and helped in all possible ways – Chuen, Alessandro, Claire, Calista, Sarah, Nel and Claudia. Members of the ARIES DTP (Aries Fairies) and Floor 01 who I relied on for their help and let me get involved in their own projects – Becky, Lili, Sarah, Karolina, Emily and Sam. Also, the undergraduate students whom I supervised and assisted me with my projects.

Finally, I would like to thank my family for their support. My mom and dad who had to deal with my constant phone calls and helped me through my struggles with anxiety. My brother for helping to distract me from the stress of the PhD. My dog Sadie for always being excited to see me when I visited home.

Chapter One
General Introduction

1.1 Inbreeding depression

Anthropogenic effects on the natural world, such as climate change and habitat destruction, are driving species to extinction (Ceballos et al., 2017; Pimm et al., 2014). A principal issue is the fragmentation of habitats and populations (Haddad et al., 2015), resulting in isolated populations that no longer experience gene flow (Ralls et al., 2018). Small and isolated populations suffer as the result of genetic effects that can further drive them towards extinction (Frankham et al., 2014). Restricted mate choice in the now isolated population can both remove the beneficial effects of sexual selection and also lead to inbreeding (Frankham et al., 2014). As a result, such a population may suffer from inbreeding depression, the loss of fitness from inbreeding (Ebel and Phillips, 2016). Inbreeding depression has been shown to occur across a wide range of organisms and is a threat to the persistence of isolated populations (Hedrick and Garcia-Dorado, 2016).

Inbreeding depression occurs partly because of recessive deleterious alleles in a population, known as the population's mutation load (Hedrick and Garcia-Dorado, 2016). In heterozygous state these recessive alleles are not expressed (thus termed hidden load). Inbreeding results in these alleles being converted to a homozygous state in offspring, thus becoming expressed load (Robinson et al., 2023; van Oosterhout, 2020; Hedrick and Garcia-Dorado, 2016). In small populations genetic drift and inbreeding result in reduced genetic variation which negatively effects the ability of the population to adapt to environmental changes i.e., it's adaptive potential (Ralls et al., 2020; Ørsted et al., 2019). Such adaptive potential is key to a populations ability to persist in the face of future challenges (Ralls et al., 2020). Additionally, inbreeding and the loss of genetic variation leads to the loss of heterozygote advantage, where heterozygosity provides additional (dominance or overdominance) benefits (Sellis et al., 2011). All the genetic effects outlined above result in a loss of fitness and adaptability and thus lead to increased extinction risk of small, isolated populations (Frankham et al., 2017; Chan et al., 2019). Furthermore, these effects can interact with environmental risks further increasing extinction likelihood, this interacting effect is known as an extinction vortex (Soule and Gilpin, 1986). For example, inbreeding reduces a populations fitness and adaptability making them more vulnerable to environmental changes. If these changes occur population size is reduced leading to a further increase in inbreeding. This is a simple example, as the extinction vortex can be influenced by several factors, such as disease, invasive species or

loss of habitat (Figure 1.1), that can all drive a population towards extinction (Fagan and Holmes, 2006; Godwin et al., 2020).

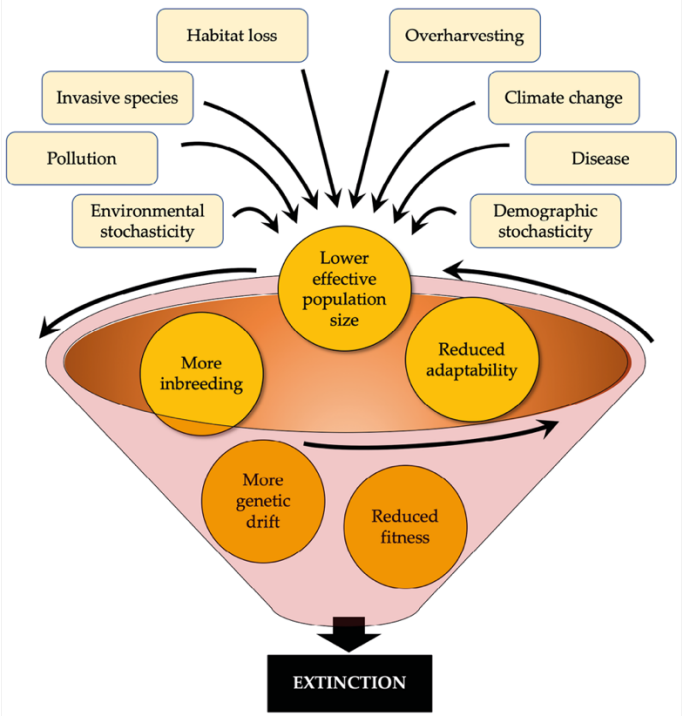

Figure 1.1: **Extinction vortex diagram**. Reproduced from Wilson, J. W., & Primack, R. B. (2019). *Conservation Biology in Sub-Saharan Africa* (Figure 8.10, p. 284). Open Book Publishers. Licensed under CC BY 4.0. Available at: https://commons.wikimedia.org/wiki/File:The_extinction_vortex_describes_a_process_whereby_the_factors_that_affect_small_populations_can_drive_its_size_progressively_downward_towards_extinction.png

The extinction vortex causes a problem for the conservation management of isolated populations, because multiple threats to population persistence need to be addressed and it can be difficult to determine which is most detrimental. Mitigating other threats such as habitat loss or disease can improve population survival but will not address reduced genetic diversity and inbreeding (Quinn et al., 2019). Inbreeding depression and reduced adaptive potential means that the population is vulnerable to any future threats such as a new disease or climate change, which could restart the extinction vortex. Genetic variation may recover eventually if the population persists, but this would occur on an evolutionary time scale through mutation and recombination (Love Stowell et al., 2017). Threats to the population are likely to occur during this time so it is important to reduce inbreeding depression and improve the populations probability of surviving. Reducing inbreeding will improve fitness and provide standing genetic variation for the population to be able to adapt to future changes, this can be done utilising translocations of individuals into the recipient population to attempt genetic rescue.

1.2 Genetic rescue

Genetic rescue is the increase in the fitness of a population as a result of newly introduced genetic variation (Bell et al., 2019). Genetic rescue is attempted by augmenting gene flow either by the movement of organisms or their gametes from one population to another. The hope being that variation introduced will increase the fitness in the recipient population via reduction of mutation load and increase adaptive potential (Hoelzel et al., 2019; Mable, 2019; Willi et al., 2006). For endangered species that still have multiple populations and/or subpopulations from which to source the genetic variation, genetic rescue can be a key management technique to alleviate genetic load and reduce extinction risk (Bell et al., 2019; Frankham, 2015, 2016; Whiteley et al., 2015).

Genetic rescue has been underutilised in conservation due to a fear of negative effects as a result of mixing genetic variation from different, divergent, populations (Frankham et al., 2017; Bell et al., 2019; Whiteley et al., 2015). A key worry is outbreeding depression, the loss of fitness when outbreeding occurs (Edmands, 2007). This can be due to fixed chromosomal differences, disruption of local adaptation, reproductive isolation or introducing mutation load (Kyriazis et al., 2021; Frankham et al., 2011). Another major issue is that if the introduced variation proves to enable greater fitness genetic swamping and homogenization of the population could occur (Kolodny et al., 2019; Bell et al., 2019; Willi et al., 2006). This is counter to the general conservation policy of trying to conserve genetic uniqueness (Moritz, 1994.; Fraser and Bernatchez, 2001). In recent years several publications have argued that genetic rescue needs to be utilised much more if we are to conserve currently inbred species (Bell et al., 2019; Ralls et al., 2018). However, there are still questions that need to be answered for its use to become widespread such as what makes a good rescuer or a good source population. There is already evidence across a wide range of taxa for the success of genetic rescue either in experiments or in conservation action (Bell et al., 2019; Pérez-Pereira et al., 2025; Nichols et al., 2024). Despite this there is still a reluctance to implement genetic rescue (Fitzpatrick et al., 2023), therefore more research is needed to answer the outstanding questions.

1.3 Implementing genetic rescue

There are several guidelines that have been developed over the years to advise on genetic rescue procedures (Figure 1.2) (Hedrick and Fredrickson, 2010; Frankham et al., 2017; Ralls et al., 2018). Guidelines start with identifying a population that needs rescue as it is suffering from inbreeding depression, the population must also have sources for genetic variation to be introduced from. An important consideration is that the population should be isolated by

human activity in the last 500 years (Ralls et al., 2018). This is because isolation from geneflow is a mechanism of speciation and conservation is trying to mitigate the impact of humans on the natural world. Restoring or augmenting geneflow should only be undertaken if it was disrupted because of anthropogenic activities, to enable processes such as speciation to occur naturally.

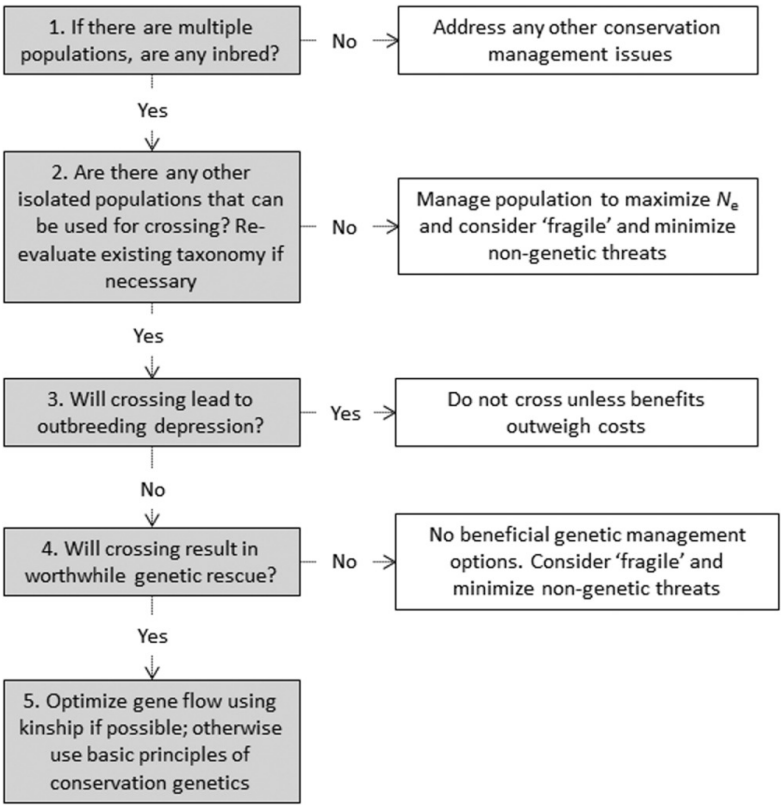

Figure 1.2: A decision tree for genetic management of isolated populations. Figure reproduced from (Ralls et al., 2018), *Conservation Letters*, DOI: 10.1111/conl.12412, under CC BY 4.0 license.

Once a candidate for rescue is identified a source of genetic variation must be selected (Frankham et al., 2017; Hedrick and Fredrickson, 2010). It is recommended that a closely related population living in a similar habitat is used, to avoid the risk of outbreeding depression (Frankham et al., 2017; Bell et al., 2019; Whiteley et al., 2015). Genetic variation from a population in a different habitat could disrupt locally adapted gene complexes resulting in outbreeding depression. Therefore, it is suggested in guidelines to experimentally test the outcome of crosses between populations to look for signs of outbreeding depression (Frankham et al., 2017). Collecting this data can be difficult depending on the species in question, there may be few or no captive populations/organisms to cross, the generational time may be too long to collect data in a relevant timeframe, or the species may be difficulty to breed in captivity (Hedrick and Fredrickson, 2010). As a result, when planning a genetic

rescue, risk of outbreeding depression may have to be weighed against the risks of waiting for experimental data to be available.

The Florida panther (*Puma concolor couguar*) is one of the best-known cases of genetic rescue and a key example of how rescue can be implemented (Pimm et al., 2006). A small population of this species containing only ~25 individuals existed in the 1990's (McBride et al., 2008). This population had low genetic diversity and was suffering from inbreeding depression, evidenced through a range of physiological defects (Roelke et al., 1993). This included kinked tails, cowlicks, seminal defects, cryptorchidism and cardiac defects. In 1995 eight female pumas (Puma concolor couguar) from Texas were translocated into the population and interbred. Studies showed that subsequently the Florida panther population's heterozygosity levels doubled, survival and reproductive success improved and, consequently, the population tripled in size (Johnson et al., 2010). Furthermore, over time the range of the Florida panther expanded, and they colonised habitat that had been thought to be unsuited to the species (Pimm et al., 2006). This is, therefore, seen as an exemplar of a successful genetic rescue, but at the time the rescue was controversial due to the fears of genetic swamping or outbreeding depression due to the difference in habitat between Texas and Florida. Despite this the translocation went ahead, but according to the subsequently developed guidelines it should not have been attempted. In addition to habitat differences and biogeographical differences the Florida population was considered a separate subspecies (*Puma concolor coryi*) (Kitchener et al., 2017), which explains the resistance to the rescue attempt. But in 2005, 10 years after the genetic rescue, the Florida panther was reclassified to be a population of the North American cougar *(Puma concolor couguar)* rather than a distinct subspecies. Modern guidelines recommend reviewing the taxonomic classifications of populations when considering rescue for this reason (Ralls et al., 2018). Additionally, despite differences in habitat and a large geographical distance it was believed that there had been gene flow between the populations in the 19th century within the 500-year separation suggested in guidelines (Seal et al., 1994; Ralls et al., 2018). Improvements in knowledge and tools allow us to make more confident decisions on the outcomes of genetic rescue.

The natural genetic rescue of the Isle Royale wolves (*Canis lupus*) provides the second key example of the issues important in genetic rescue. Wolves colonised Isle Royale in Lake Superior around 1950 establishing a small population that suffered from inbreeding depression, the population peaked at 50 individuals but dropped following a disease outbreak (Robinson et al., 2019; Hedrick et al., 2014). It is thought that they received occasional gene flow from the mainland by the formation of ice bridges during winter (Hedrick et al., 2014). However, in the past 50 years climate change has resulted in bridges becoming rarer, slowly

reducing gene flow to the island. Then in 1997 a male wolf migrated into the population across an ice bridge becoming dominant in the population. This resulted in 34 offspring and, nearly 60% of individuals had ancestry from this male within a decade (Hedrick et al., 2014). As of 2011 his was the only variant of the Y chromosome in the population showing how much the male and his offspring dominated the gene pool (Adams et al., 2011). This resulted in the wolf population increasing. However, in the early 2000's the population declined dramatically, with only two individuals remaining in 2017 (Hedrick et al., 2019). This is blamed on the success of the immigrant male which lead to a highly related population in a restricted habitat. The increased inbreeding due to the high relatedness of the population increased homozygosity, exposing hidden mutation load introduced by the male and resulting in severe inbreeding depression (Robinson et al., 2019). This is the key evidence of genetic rescue potentially leading to population extinction. Crossing populations not only risks outbreeding depression but can introduced additional mutation load.

The two examples of genetic rescue outlined above give us examples of what should be considered when planning a rescue. These two studies also allow us to compare between a planned and natural rescue, so that we may learn from the negative results seen on Isle Royale. The environment was key in both rescues, the Florida panther population could expand their range whereas the wolves were restricted to an island preventing dispersal and promoting inbreeding. The number of rescuers is important, the single wolf resulted in a highly related population, multiple panthers meanwhile meant that rescuer offspring would have different ancestry reducing inbreeding in subsequent generations. The fact the panther was a planned rescue also meant the rescuers were eventually removed, whilst in the wolves the male bred with his own offspring worsening the inbreeding process. This highlights the importance of monitoring so that intervention can occur to prevent negative effects of cross-breeding between populations. In the panthers a source population was selected to try and reduce outbreeding depression, whilst the wolves experienced a natural immigration from the closest population yet had a negative outcome. In a planned rescue of the wolves the mainland population that the male came from would be the main candidate as a source population, as they are closely related to the island population. This shows that the above considerations are vital, not just the selection of a source population.

A key consideration when implementing a genetic rescue is the potential for stressful environmental conditions affecting the recipient population. Inbreeding depression is exacerbated under stressful conditions putting populations at greater risk of extinction (Armbruster and Reed, 2005). Genetic rescue could become important to offset the environmental effects, reducing inbreeding depression can allow the population to persist.

Additionally, an aim of genetic rescue is to restore adaptive potential by increasing genetic diversity, this should allow a population to adapt and overcome the stress in the future. As such genetic rescue may allow continued persistence in a stressful environment by improving fitness and increasing adaptive potential.

1.4 Optimising genetic rescue

When optimising genetic rescue, selecting which individuals to use as rescuers is important. For example, there are different pros and cons of using male or female rescuers. It has been shown that a given sex can be more vulnerable to inbreeding depression depending on the species in question (Ebel and Phillips, 2016). Factors that may cause these differences include sexual selection, heterogametic sex differences or differences in the cost of inbreeding (Vega-Trejo et al., 2022a). Moreover, how effective an individual of a specific sex may be in terms of introgression into a new population may depend on species specific patterns of mating and sexual selection. Conservation efforts and experimental studies have found that adding male immigrants increased the fitness benefits of rescue more than adding female immigrants, though this will depend on the biology and social structure of the species in question (Zajitschek et al., 2009; Trinkel et al., 2008; Madsen et al., 2020).

In some attempts at genetic rescue, such as in the Florida panther (Pimm et al., 2006) female rescuers were preferred over males. This was because females were judged to be less likely to cause conflict, or to disperse from the area (Pimm et al., 2006; Seal et al., 1994), though again patterns of sex biased dispersal differs across species. However, using female rescuers does raise another potential risk linked to mitochondrial incompatibility (Havird et al., 2016). In animals, mitochondrial genomes are nearly always inherited only through the maternal line, female rescuers introducing new mitochondrial genomes may have incompatibilities with the recipient population nuclear genome affecting fitness of offspring (Havird et al., 2016). The mitochondrial and nuclear genome co-evolve, a different mitochondrial genome may not share these co-adaptations resulting in reduced offspring fitness (Havird et al., 2016). Incompatibilities with the nuclear genome can even contribute to a reproductive barrier in some cases (Ma et al., 2016). There are examples of mitochondrial incompatibilities reducing fitness in yeasts (*Saccharomyces bayanus, Saccharomyces cerevisiae* Lee et al., 2008) and in *Drosophila melanogaster* (Meiklejohn et al., 2013). On the other hand, it has been argued that if there are deleterious alleles present in the mitochondrial genome, only a female rescuer would be able to reduce this genetic load (Gemmell and Allendorf, 2001; Gemmell et al., 2004). Like the nuclear genome, deleterious mutations can occur in the mitochondrial genome but, unlike the nuclear genome, there is no introgression during the crossing of populations as

it only comes from the female. Only a female rescuer introducing a new mitochondrial genome into the population could remove this mutation load (Gemmell and Allendorf, 2001).

The selection of a population to use as the donor is, perhaps, the most critical aspect of genetic rescue (Hoffmann et al., 2020). The donor population will determine what new genetic variation will be introduced into the recipient population and thus affect the possibility of outbreeding depression and genetic swamping (Robinson et al., 2023). Therefore, the genetic divergence between populations must be accounted for, a more divergent population could be more likely to result in outbreeding depression, but a closely related population may not (re)introduce much genetic diversity. As mentioned above, it is recommended by the guidelines to utilise a population of the same species where geneflow has only recently been disrupted (Ralls et al., 2018). This should avoid reproductive barriers between the populations preventing rescue. However, there have been suggestions that other subspecies should be considered for improving genetic diversity through hybridisation (Chan et al., 2019). This would introduce more genetic diversity as the divergence between subspecies will be greater than populations within a species and could even introduce alleles novel to the subspecies. However, this is a controversial stance to take as sub-species are usually formed via natural speciation events and would not have geneflow that had been interrupted within the past 500 years by human activity (Ralls et al., 2018). There could be serious genetic incompatibilities between subspecies that could endanger the population if introduced. Hybridisation should not be dismissed out of hand, as we know from the Florida Panther taxonomic designations are changeable and what we call a subspecies today may be considered the same species in the future (Kitchener et al., 2017; Clavero et al., 2024).

If there are multiple populations that meet the criteria for genetic divergence, the evidence supports the use of a large, outbred population as a source of genetic rescuers, (Ralls et al., 2020; Frankham, 2015, 2016). Outbred populations will introduce more genetic diversity, thus reducing homozygosity, restoring any heterozygote advantage and increasing adaptive potential (Ralls et al., 2020; Willi et al., 2006). However, as large, outbred populations will also harbour considerable hidden genetic load the risk of outbreeding depression is increased (Robinson et al., 2023). When introduced into a small, inbred population, deleterious alleles from such outbred rescuers may spread through the population and lead to detrimental effects. This appears to be what caused the near extinction of the population following a natural genetic rescue in the Isle Royale wolves (Robinson et al., 2019). Therefore, it may be more beneficial to introduce rescuers from another isolated, inbred population (Robinson et al., 2018, 2019). Such populations will likely have already undergone the purging of (some) deleterious alleles. Purging occurs when deleterious alleles are in a homozygous state,

through inbreeding, and are removed from the population via natural selection (Dussex et al., 2023). However, these populations may not help introgress enough positive genetic variation. Simulations that attempt to understand this trade off, advise selecting rescuers from moderate sized populations with a lower frequency of highly deleterious alleles as using high genetic diversity rescuers results in increased extinction risk for small populations (Kyriazis et al., 2021). This study suggests the focus should be on reducing deleterious variation introduced to the population, rather than maximising genetic variation overall.

Avoiding the introduction of deleterious variation by utilising inbred populations as the source of rescuers could pose problems. While purging will have removed some deleterious variation, genetic drift acts more strongly in small populations. As a result, mildly deleterious alleles can become fixed in the population as purging is less effective at removing deleterious alleles with weaker effects. This accumulation of fixed genetic load can risk future survival of a population (Grossen et al., 2020). Using these populations as a source for rescue could introduce this accumulated mutation load into the recipient population. Fitness may increase in the environment that purging took place in but if the environment changes the population may struggle to adapt due to loss of adaptive potential (Ralls et al., 2020). This could also pose an issue if the recipient population is in a different habitat, the non-purged deleterious alleles could be more detrimental in the new environment. For all these reasons maximising the amount of genetic diversity being introduced is more favourable than utilising purged, inbred populations.

Guidelines for genetic rescue also recommend using a source population found in a similar habitat or environment to the recipient population (Hedrick and Fredrickson, 2010). Populations adapt to local conditions and new genetic diversity can break up locally adapted gene complexes, which could disrupt to expression of traits that are beneficial in that environment (Lenormand, 2002; Kawecki and Ebert, 2004). There may also be the introduction of adaptations to a different environment (the source populations habitat) which may be deleterious in the recipient populations habitat. If either issue occurs, fitness in that habitat will be reduced, endangering the population. It is important to conserve populations across differencing habitats as they could be sources of genetic adaptation, especially with the changing climate. Currently beneficial adaptations may become deleterious in future conditions, changing which source populations are preferred. In fact, there have been attempts at targeted geneflow, trying to introduce a specific adaptation from one population to another (Kelly and Phillips, 2019b; Rudin-Bitterli et al., 2021). Differences between population habitats are important to consider in genetic rescue. An assessment weighing the

benefits and risks of rescue if there is no source population in a similar habitat available should be made before crossing populations from two different habitats (Seal et al., 1994).

Another population feature that may be considered is sexual selection, which can vary across populations (Kasumovic et al., 2008). Levels of inbreeding, genetic variation and adaptation are all affected by sexual selection and may influence the selection of a source population or rescuer (Parrett et al., 2022; Parrett and Knell, 2018; Vega-Trejo et al., 2017). Sexual selection should improve not only population fitness but also reduce genetic load, producing good rescue candidates that should introduce fewer deleterious alleles into the recipient population (Cally et al., 2019; Whitlock and Agrawal, 2009). Additionally, sexual selection should make rescuers preferred and more competitive at reproduction, increasing the introgression of their genome into the population and improving the efficacy of rescue. There are potential issues with this however, the ability to identify differences in sexual selection between populations may be difficult without large-scale monitoring. Sexual selection could promote assortative mating, this could risk genetic swamping as rescuers and their offspring are more likely to mate with each other than the recipient population (van Doorn et al., 2009). Sexual selection could enhance genetic rescue by producing better rescuers and improving their chances to reproduce in the recipient population, but little research has been done on this question.

1.5 Measuring genetic rescue

There are different ways to measure the success of any genetic rescue attempts, Population measures look at how the whole population responds to genetic rescue using factors such as population productivity (number of offspring produced), growth rate or persistence probability. Population growth rate is a popular measure as it shows that a population's increased fitness, as a result of the genetic rescue, is allowing it to recover to a larger, healthier size (Madsen et al., 2004). However, population growth depends upon the environment being able to support a larger population meaning that positive effects of rescue could be missed if there is no room for growth (Hedrick et al., 2011). Population size is another indicator that has been utilised in studies, though like population growth can be limited by habitat, to see the increase in individuals following rescue (Madsen et al., 2020; Hostetler et al., 2013). Additionally, populations can experience extension of their range showcasing the effects of genetic rescue, this was seen in the Florida panther where individuals were detected outside their expected range (Pimm et al., 2006; Johnson et al., 2010). A population's persistence probability can be a valuable measurement for how effective a rescue is, as it will ensure short-term survival so that further conservation action can be taken. Persistence can be calculated

using matrix or individual based modelling providing demographic predictions following a rescue attempt (van de Kerk et al., 2019). Many other metrics that can be used to measure the fitness of a population that has undergone a genetic rescue attempt such as population density or reproductive success (Madsen et al., 1999; Fitzpatrick et al., 2020; Quinn et al., 2019).

Genetic analysis can be a powerful tool enabling genetic rescue. First it can help us identify when populations are in need of genetic rescue, e.g. confirming that inbreeding depression is occurring in a population and is likely responsible for its poor fitness (Kardos et al., 2016). It can also estimate a population's adaptive potential (Ørsted et al., 2019). Genetic information can then tell us how a genetic rescue attempt has affected a population's genetics. Measures such as allelic richness, migrant ancestry and heterozygosity are used to determine if introgression has occurred (Miller et al., 2020b; Madsen et al., 2020; Hedrick et al., 2019). Some measures linked to population fitness can provide evidence of reduced inbreeding like the inbreeding coefficient, mutation load or runs of homozygosity (Charlesworth and Willis, 2009; Ceballos et al., 2018). Our ability to utilise this data is increasing as sequencing becomes more affordable and accessible. Studies can now examine the whole genome improving our estimates of genetic diversity and the effects of geneflow. Using tools that can identify deleterious alleles we can examine the genetic load to reveal how geneflow is affecting expression of these alleles (Kumar et al., 2009). Increased genetic diversity and therefore heterozygosity will prevent recessive deleterious alleles from being expressed improving population fitness (Smeds and Ellegren, 2022; Pérez-Pereira et al., 2025). Genomic data has been used to support the implementation of genetic rescue in some species (Sundell et al., 2023; Al Hikmani et al., 2024; Chege et al., 2024). These powerful tools can allow us to improve our understanding and confidence in the effects of genetic rescue.

Monitoring is a key part of conservation and is especially important following translocations to understand what the effect has been on the population. There are many metrics and measures that will all provide information about the ongoing processes and a combination of these may be the best technique (Robinson et al., 2020). A key criticism of genetic rescue studies is the lack of long-term data following the rescue, often only three generations (Bell et al., 2019; Clarke et al., 2024). This means there is limited information on how long the benefits of genetic rescue last. Genetic diversity could become reduced if inbreeding resumes, potentially exposing mutation load as was observed on Isle Royale (Hedrick et al., 2019). On the other hand, introduced deleterious variation could instead be purged reducing mutation load but maintaining beneficial variation that was introduced. In recent years more studies are

attempting to address this issue but the effort and cost to collect data consistently over multiple generations is a barrier (Nichols et al., 2024; Pérez-Pereira et al., 2025).

1.6 Model species *Tribolium castaneum*

Tribolium castaneum, the red flour beetle, is a small darkling beetle thought to originally have fed on rotting wood, it is now a global agricultural pest found infesting grain stores (Dawson, 1977). This representative of the species rich order, *Coleoptera*, has been utilised as a model species for over a century, including in ecology and evolution (Pointer et al., 2021; Park, 1932; Duval et al., 1939). Its relatively quick generation times, roughly one month, make it suitable for multi-generation studies. *T. castaneum* is also highly fecund, which makes maintaining and crossing populations easier. Due to their co-habitation with humans, they are simple to maintain requiring only flour and yeast to live and feed on, making it an ideal system to carry out population level studies. Another benefit is that sex is apparent at the pupal stage allowing individuals to be isolated before sexual maturity, this ensures individuals are virgins when initiating populations.

T. castaneum has already been utilised to test both genetic and evolutionary rescue with conflicting results (Hufbauer et al., 2015; Lewis et al., 2024). A test of both evolutionary and genetic rescue on populations adapted to higher temperatures (38°C compared to 30°C standard) found no evidence of genetic rescue. Populations were kept at 100 or 10,000 individuals at the higher temperature and received no migration or 10% of the population was replaced with migrants from an outbred, non-adapted population every generation (10 generations total). Migration reduced the fitness of these large populations, though testing at the individual level found improved fitness (Lewis et al., 2024). Another study found that genetic rescue did improve population fitness, using populations of either 50 or 150 individuals. Migrants were randomly selected and replaced an equal number of residents, one in the smaller populations and three in the larger. The rescue was a single event, and populations were followed for four generations after. The genetic rescue treatment reduced extinction and increased fitness, showing that a genetic rescue effect can be achieved in this species (Hufbauer et al., 2015) The difference between the outcomes of these studies could be due to the different population sizes, frequency/size of rescue or adaptation to a higher temperature preventing rescue. Importantly there are also a range of genomic resources available for *T. castaneum*, including a sequenced genome and gene annotation enabling genomic analysis (Brown et al., 2003; Yates et al., 2022). The genome consists of 9 autosomes and the XY sex chromosomes. There are continued updates improving the quality of these

resources using long-read sequencing (Volarić et al., 2024; USDA ARS Ag100Pest Initiative, 2022).

1.7 Thesis aims

In this thesis, I utilise the *T. castaneum* system to understand how genetic rescue can affect the fitness of inbred populations. In the second chapter I examine if the sex of a rescuer will affect the outcome of genetic rescue. I then test if different levels of sexual selection in the source populations could impact the success of a rescue attempt, by measuring the productivity (number of adult offspring produced) over many generations to capture any drop in productivity. In Chapter three, I use both population fitness assays and genomic methods to assess the relative efficacy of genetic rescue in being either an inbred or outbred rescuer. In Chapter four, I quantify the risk of genetic rescue disrupting local adaptation by rescuing *T. castaneum* lines adapted to a higher temperature. I then discuss the overall results of the thesis and its implications for genetic rescue and conservation.

Chapter Two

Sexual selection matters in genetic rescue, but productivity benefits fade over time; a multi-generation experiment to inform conservation.

2.1 Abstract

Globally, many species are threatened by population decline because of anthropogenic changes leading to population fragmentation, genetic isolation and inbreeding depression. Genetic rescue, the controlled introduction of genetic variation, is a method used to relieve such effects in small populations. However, without understanding how the characteristics of rescuers impact rescue attempts interventions run the risk of being sub-optimal, or even counterproductive. We use the red flour beetle (*Tribolium castaneum*) to test the impact of rescuer sex, and sexual selection background, on population productivity. We record the impact of genetic rescue on population productivity in 24 and 36 replicated populations for ten generations following intervention. We find little or no impact of rescuer sex on the efficacy of rescue but show that a background of elevated sexual selection makes individuals more effective rescuers. In both experiments, rescue effects diminish 6–10 generations after the rescue. Our results confirm that the efficacy of genetic rescue can be influenced by characteristics of the rescuers and that the level of sexual selection in the rescuing population is an important factor. We show that any increase in fitness associated with rescue may last for a limited number of generations, suggesting implications for conservation policy and practice.

2.2 Introduction

Populations worldwide increasingly face extinction after becoming fragmented by human activity (Ceballos and Ehrlich, 2023). Fragmentation reduces population size and increases risk of genetic isolation, leading to increased impact of genetic drift and loss of genetic variation. Consequentially, many small populations suffer inbreeding depression (reduction in fitness when recessive, deleterious alleles appear in homozygous form, and/or the loss of heterozygote advantage) and reduced adaptive potential (Charlesworth and Willis, 2009; Crnokrak and Roff, 1998). Individuals within such populations are also more prone to environmental stress, which can exacerbate inbreeding depression (Frankham, 2005; Armbruster and Reed, 2005; Fox and Reed, 2011; Reed et al., 2002; Richardson et al., 2004).

The interaction between these factors can lead to population or species extinction (Soule and Gilpin, 1986; Blomqvist et al., 2010; Palomares et al., 2012).

Genetic rescue, increasing population fitness through the introduction of novel alleles beyond the demographic effects of immigration, is one way to relieve inbreeding depression (Ingvarsson, 2001; Hedrick et al., 2011). This requires the introduction of rescuers (conspecific individuals from a different population), allowing reproduction with the inbred population. The aim is to introduce new genetic diversity, reducing homozygosity and the expression of deleterious alleles in offspring. Introducing genetic variation also increases adaptive potential, providing standing variation for selection to act on (Hoelzel et al., 2019; Mable, 2019) and increasing the potential for evolutionary rescue (Bell and Gonzalez, 2009; Schiffers et al., 2013; Lindsey et al., 2013).

Genetic rescue has been studied in wild, captive, and laboratory populations across many taxa (reviewed in (Frankham, 2015, 2016; White et al., 2023)) and has seen many successful implementations (Miller et al., 2020b; Davis et al., 2021; Robinson et al., 2017; Pregler et al., 2022; Pavlova et al., 2023). Reviews and meta-analyses support its utility as a conservation tool (Waller, 2015; Whiteley et al., 2015; Robinson et al., 2020; Frankham, 2016, 2015). Theoretical studies have modelled the outcome of genetic rescue in specific situations to assess the risks and benefits to wild populations (Ash et al., 2023; Robinson et al., 2020). This allows for the exploration of the potential impact of different variables, such as inbreeding in the rescuing population (Kyriazis et al., 2021) There is also a growing body of experimental research testing how factors, such as the sex or degree of inbreeding in rescuers, and level of environmental stress, impact genetic rescue attempts (Zajitschek et al., 2009; Jørgensen et al., 2022; Heber et al., 2012; Hufbauer et al., 2015; Lewis et al., 2024). However, failures and negative effects have also been observed. For example, the Isle Royal wolf (*Canis lupus*) population collapsed following (naturally occurring) genetic rescue (Hedrick et al., 2014). In the Hihi (*Notiomystis cincta*) genetic rescue resulted in increased inbreeding 10 years later due to high ancestry from one rescuer (Nichols et al., 2024); and in the Macquarie perch (*Macquaria australasica*) little or no mixing occurred between the rescuers and inbred population leading to a failed rescue attempt (Pavlova et al., 2024).

Despite the publication of guidelines as to when and where to attempt genetic rescue (Hedrick and Fredrickson, 2010; Frankham, 2015), there is still considerable reluctance by conservation stakeholders to attempt rescue in wild populations (Fitzpatrick et al., 2023). This is, to some degree, understandable due to potential risks such as outbreeding depression (Bell et al., 2019). This loss of fitness due to the crossing of two genetically divergent populations

(Edmands, 2007) is associated with the breakdown of locally adapted gene complexes (Lenormand, 2002). An additional risk is genetic swamping, the rescuing population replacing unique genetic variation in the rescued population (Rhymer and Simberloff, 1996). Despite evidence to suggest such risks may be overstated, and that mixing divergent populations can provide considerable benefits (Kronenberger et al., 2017, 2018; Fitzpatrick et al., 2016), these risks highlight the importance of understanding what characterises the most effective rescuer(s) (Whiteley et al., 2015). Genetic structure of the rescuing population is an essential consideration (Kyriazis et al., 2021; Robinson et al., 2018; Ralls et al., 2020), as well as the number (van de Kerk et al., 2019; Kelly and Phillips, 2019a) and the sex of rescuers (Zajitschek et al., 2009; Havird et al., 2016). These factors affect how much genetic diversity and load is introduced, how quickly it can introgress, and potentially how long the rescue effect will last.

A central criticism of many genetic rescue studies is the fact that the longevity of rescue effects is not captured, due to the number of generations observed (Bell et al., 2019; Clarke et al., 2024). Laboratory studies on species with short generation times greatly facilitate our ability to monitor outcomes over multiple generations. Consequently, by using these species we can better test if and how quickly genetic rescue occurs, how long it lasts and whether there are any negative effects in the long term. In wild studies, where it is often extremely difficult and/or expensive to follow the rescue long-term, populations are often only monitored over a few consecutive generations (Hasselgren et al., 2018; Lotsander et al., 2021) or sporadically over generations (Miller et al., 2020b).

Sex of rescuing individuals may be a key factor in the efficacy of genetic rescue as females are typically more limited in the number of offspring they can produce than males (Bateman, 1948). In many systems (i.e. promiscuous, polygynous, socially monogamous with extra-pair paternity), this means a male rescuer should speed the impact of genetic rescue. A male should sire more offspring carrying rescuing alleles and higher heterozygosity (Bateman, 1948) than a female, meaning that this additive variation is quicker to spread in the population. This effect has been shown in both guppies (*Poecilia reticulata*) (Zajitschek et al., 2009) and African lions (*Panthera leo*) (Miller et al., 2020b; Trinkel et al., 2008). In addition, purging of genetic load is more effective in males due to differences in gamete investment between the sexes (Whitlock and Agrawal, 2009; Grieshop et al., 2021). In a rescue scenario, an individual with less genetic load should be favoured under sexual selection and have greater reproductive success.

Despite putative advantages of male rescue, female rescuers can be advantageous in other systems or scenarios. In the Florida panther (*Puma concolor couguar*), females were used for

rescue (Pimm et al., 2006) as they were less likely to disperse or cause social conflict (Seal et al., 1994). Genetic load can also accumulate in mitochondrial DNA (mtDNA), which is commonly inherited through females (Gemmell et al., 2004). Thus, only female rescuers can introduce mtDNA variants to a population to reduce mtDNA genetic load (Gemmell and Allendorf, 2001). However, there is a risk of mitochondrial mismatch reducing offspring fitness (Havird et al., 2016). Female rescuers may also introduce maternal effects, the mother's phenotype influencing that of the offspring (Wolf and Wade, 2009), which may affect rescue efficiency.

Furthermore, another key consideration related to both rescuer sex and the genetic structure of rescuing populations is the background of sexual selection the rescuing population has experienced. Sexual selection can vary across populations (Kasumovic et al., 2008) affecting patterns of genetic variation (Parrett et al., 2022), facilitating adaptation (Parrett and Knell, 2018) and reducing inbreeding (Vega-Trejo et al., 2017). Stronger sexual selection has been shown to improve population fitness (Cally et al., 2019) and can also reduce genetic load in a population (Whitlock and Agrawal, 2009). Individuals from high sexual selection populations should also be more competitive in securing mates, thus gaining greater reproductive success, and increasing the speed at which genetic diversity introgresses during rescue if preferences for sexually selected traits are shared across the populations. An increase in population fitness due to sexual selection has been observed in *Tribolium castaneum;* experimental populations experiencing elevated sexual selection were shown to be less likely to go extinct under stressful conditions than those that evolved under monogamy (Godwin et al., 2020; Lumley et al., 2015). Although beneficial, sexual selection may also promote assortative mating (van Doorn et al., 2009), and potentially reduce subsequent interbreeding between rescuers and rescued, thus hindering rescue attempts. However, to our knowledge, no studies have tested if the effect of sexual selection background increases or decreases the efficacy of genetic rescue.

Here, we use the *Tribolium castaneum* model (Pointer et al., 2021) to experimentally address key omissions in the understanding of genetic rescue of inbred populations. *T. castaneum* has been utilised previously to study genetic rescue with one finding evidence of rescue (Hufbauer et al., 2015) and the other not observing a rescue effect (Lewis et al., 2024). First, we test if the sex of a rescuer has an impact on genetic rescue. We predict that a male rescuer will result in a greater fitness increase in inbred populations due to the ability of males to produce more offspring than females, allowing for faster introgression. Second, we test if rescuers evolved under different levels of sexual selection differentially impact the outcome of genetic rescue. We predict that a rescuer from a strong sexual selection background will be more effective,

due to lowered genetic load. Importantly, we utilise the short generation time of *T. castaneum* to follow the effects of genetic rescue over 10 generations, allowing observation of both the speed and longevity of rescue effects. Additionally, we replicate our experimental populations under nutrient stress. We predict that stress will exaggerate the effects of inbreeding depression so that the magnitude of the rescue effect will be greater under stress than under benign conditions.

2.3 Methods

2.3.1 Ethics

No ethics approval was required for this study as experiments were conducted on an unregulated invertebrate species.

2.3.2 Husbandry

T. castaneum were kept in a controlled environment at 30°C and 60% humidity with a 12:12 light-dark cycle. Populations were kept on standard fodder consisting of 90% organic white flour, 10% brewer's yeast and a layer of oats for traction unless otherwise stated. During the husbandry cycle, 2mm and 850µm sieves were used to remove pupae and adults from fodder. The following cycle was started by a set number of adults (line dependent, see below) being placed into containers with fresh standard fodder. The oviposition phase: populations were given seven days to mate and lay eggs before adults were removed by sieving to prevent overlapping generations. The fodder containing eggs was returned to the container. The development phase: eggs were kept in the containers for 35 days to allow the eggs to develop into mature adults. Around day 21 of the development phase, pupae were collected to obtain known-sex virgin individuals which were then used to start the next generation. The pupae were kept as virgins in single-sex groups of 20 for 10 days to allow them to complete development. Once mature, the cycle began again with those beetles going into fresh fodder to form a population of males and females.

2.3.3 *Tribolium castaneum* lines

Krakow Super Strain (KSS): KSS was created by mixing fourteen laboratory strains to maximise genetic diversity in a single strain (Laskowski et al., 2015). This was used as the outbred treatment in the genetic rescue experiments.

Inbred Lines: Founded from KSS and inbred through three single-pair bottlenecks in the first, fifth and seventh generations. Between bottlenecks, the lines were maintained at a maximum population size of 100 randomly selected adults. Of the initial 30 lines, 24 survived the inbreeding treatment and 12 lines were maintained and used for experiments.

Sexual Selection Lines: Polyandrous and monogamous lines were created from the Georgia 1 stock (Haliscak and Beeman, 1983; Lumley et al., 2015). Each polyandrous line (n=3) was maintained each generation in twelve groups each consisting of five males and one female. Following oviposition, the eggs from all groups in a line are mixed to form one population from which the next generation's groups will be sourced. For each monogamous line (n=3) twenty separate mating pairs are bred. Following oviposition, the eggs from all pairs are mixed and the next pairs are sourced from this population to maintain that line. The number of groups and pairs in each regime results in a theoretical Ne = 40 in each treatment (Godwin et al., 2020). These regimes had been maintained for 150 generations when rescuers were taken. The polyandrous lines are hereafter referred to as sexual selection lines, and monogamous as no sexual selection.

2.3.4 Genetic rescue protocol

Replicate experimental inbred populations were created from the inbred lines to serve as populations to be rescued. Pupae were sexed and placed into plastic dishes with lids, containing 10ml standard fodder in single-sex groups. 10±2 days after eclosion, ten males and ten females from a given line were placed in a 125ml tub with 70ml of standard fodder creating populations each containing twenty adult beetles at a 1:1 sex ratio for the oviposition phase. On day 20±1 of the development phase, pupae were again taken from the populations using the method outlined above to create the next non-overlapping generation.

Populations were maintained using twenty reproducing adults per generation, not allowing population growth. This allowed us to maintain a roughly constant population density during offspring development across generations, avoiding the confounding influence of negative density-dependence on offspring production (Duval et al., 1939; King and Dawson, 1972; Janus, 1989).

Each experimental population was randomly assigned an ID number, to avoid bias when handling. After being established at the experimental size, the populations were maintained in experimental conditions for one generation to avoid transgenerational density effects affecting the genetic rescue results (Đukić et al., 2021). The rescue treatments were applied in

the second generation under experimental conditions. In each population, a single beetle was replaced with a rescuer thus maintaining the 1:1 sex ratio and population size, avoiding any increase in productivity due to a demographic rescue. Rescuers taken from their source populations as pupae were age-matched as closely as possible to individuals in experimental populations. On day 37 of the development phase experimental populations were frozen at -6°C and mature offspring were counted as a measure of productivity (our metric for population fitness). If a population was removed from the experiment because of slow development (pupae were not available to establish the next generation), that population was analysed as part of all generations prior but excluded henceforth.

2.3.5 The sex of the rescuer in genetic rescue

Due to logistic issues with ventilation, four out of the 12 experimental inbred populations failed to produce offspring in generation 0. From each of the remaining eight inbred lines, three replicate populations were created and assigned to one of three treatments; No Rescue control (ten inbred line males, ten inbred line females); Male Rescue (nine inbred line males, one KSS male, ten inbred line females); and Female Rescue (ten inbred line males, nine inbred line females, one KSS female; Figure 2.1). Populations were maintained for ten, non-overlapping generations.

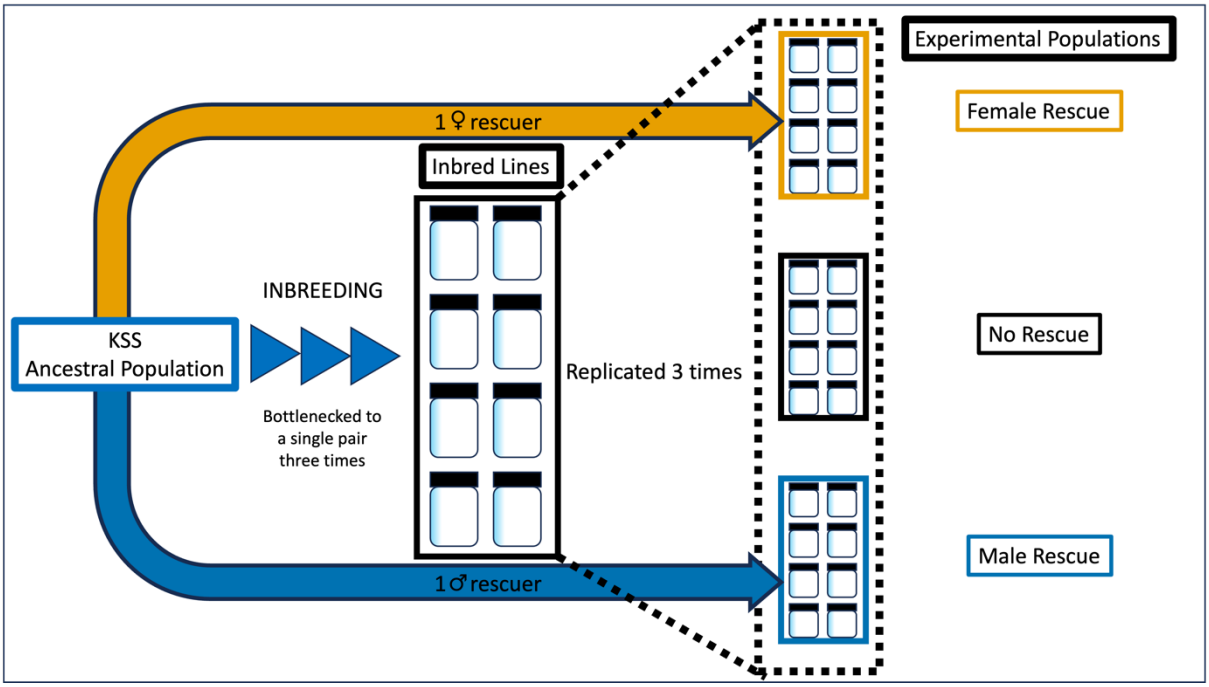

Figure 2.1: Experimental set-up of the creation and attempted genetic rescue of small, inbred *T. castaneum* populations (N_e = 20) by a single male or female rescuer from the outbred ancestral population. Three experimental populations were created from each of 8 inbred lines resulting in 24 experimental populations, every line represented once in a treatment.

2.3.6 Sexual selection and genetic rescue

We investigated the impact of a rescuer's sexual selection history on the effectiveness of genetic rescue. From 12 inbred lines, three replicate populations were created and assigned to one of three treatments; No Rescue Control (ten inbred line males, ten inbred line females); Sexual Selection Rescue (nine inbred line males, one polyandrous male and, ten inbred line females); No Sexual Selection Rescue (nine inbred line males, one monogamous male, ten inbred line females; Figure 2.2). A single polyandrous and single monandrous line were used as the source for rescuers. Populations were maintained for nine generations.

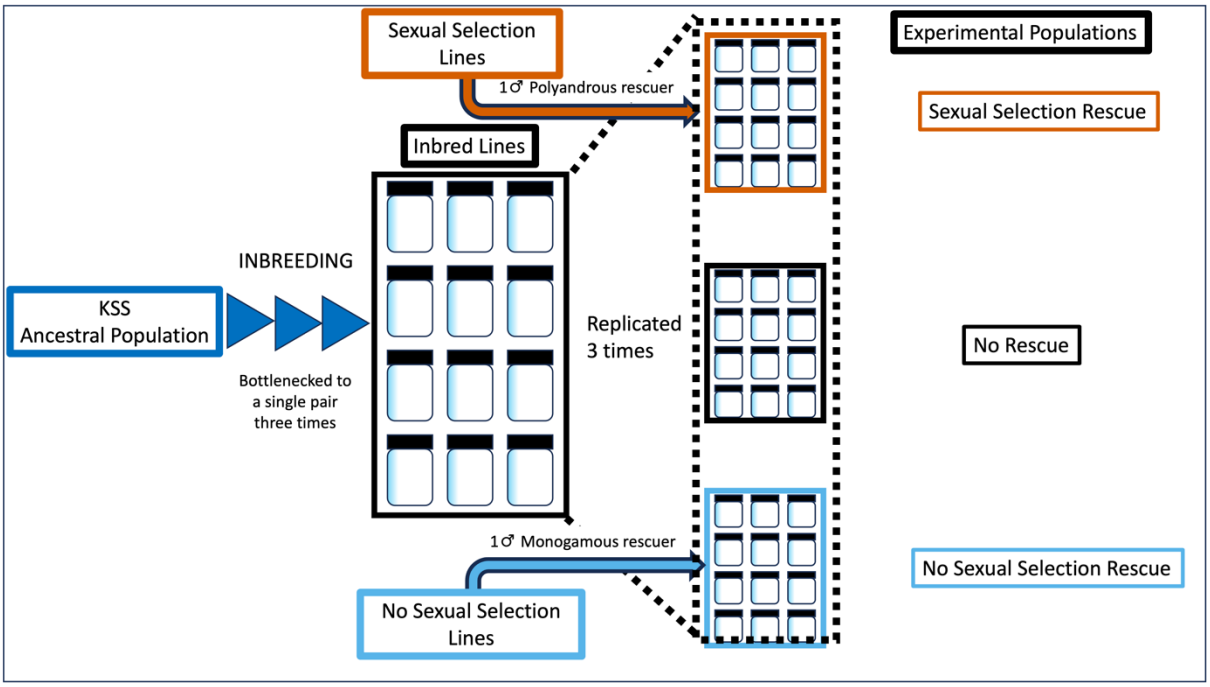

Figure 2.2: Experimental procedure for the creation and attempted genetic rescue of small, inbred *T. castaneum* populations (N_e = 20) by a single male rescuer from either a sexual selection or no sexual selection line. Three experimental populations were created from each of 12 inbred lines resulting in 36 experimental populations, every line represented once in a treatment.

2.3.7 Stressful conditions

To test if genetic rescue makes populations more resilient to environmental change and/or stressful conditions, duplicate rescue populations were established from each rescued line at generation five in the 'sex' experiment, and generation six in the 'sexual selection' experiment. This was done at these generations to allow time for the rescuer genome to

introgress into the recipient population before the environmental change. These populations were maintained as in the main experiments (until generation ten and nine respectively), but with a reduction in the yeast content of the fodder, which is the main source of protein for the experimental populations. This reduction generates nutrient stress in *T. castaneum* (Godwin et al., 2020). In the 'sex' experiment fodder contained 0% yeast and 1% yeast in the 'sexual selection' experiment (because of low survival with zero yeast).

2.3.8 Statistical analyses

Statistical analyses were carried out in R V4.4.1 (R Core Team, 2024) utilising R studio version 2024.04.2+764 (Posit team, 2024). Tidyverse (Wickham et al., 2019), stats (R Core Team, 2024), Rmisc (Hope, 2022) and googlesheets4 (Bryan, 2023) were used for data management and exploration. Plots were created using ggplot2 (Wickham, 2016). The distribution of data was checked using the shapiro.test function (R Core Team, 2024). Generalised Linear Mixed Models (GLMMs) were fitted to test for differences in productivity between the experimental treatments using glmmTMB (Brooks et al., 2017) Model fit was checked using DHARMa (Hartig, 2022). Model parameters were checked for collinearity using variance inflation factor (Vif) scores with the check_collinearity function from performance (Lüdecke et al., 2021). There were no issues with overdispersion or collinearity (VIF: <3 for all variables) in any models. R^2 was determined using the r.squaredGLMM function in MuMIn (Bartoń, 2024). Post-hoc pairwise Tukey tests were carried out using multcomp (Hothorn et al., 2008). Ggeffects (Lüdecke D, 2018) package was used for model predictions.

Within each experiment, we fitted GLMMs with the same model structure, using a negative binomial distribution to model productivity counts, which provided better model fit than a Poisson distribution. Productivity was the response variable, with treatment, generation and generation^2 as fixed effects. Inbred line of origin and experimental population ID were included as random effects, with ID nested within inbred line. Interaction terms (treatment x generation, treatment x generation^2) were initially included but removed from the model if not significant. The generation^2 factor was not significant in the models for populations under stressful conditions and was therefore removed. When a quadratic effect of generation was detected in a model, we plotted the model prediction to show the non-monotonic effect of generation on productivity and to identify the generation at which the slope changed. Then, to test if there was both a significant increase and, importantly, a significant decrease in productivity two separate GLMMs (with the same factors as previously) were run on the data split into generations 1-5 and 5-10 (either side of the peak). These GLMMs were fitted with treatment and generation as fixed effects, ID nested within inbred line as a random effect. GLMMs were

also fitted on generations 2 and 3 individually (Table S1.3 + S1.4) in the 'sex' experiment, these single-generation models used a Poisson distribution, productivity as a response variable and treatment as a fixed effect. Random effects were the same as above. This was to test at which point the rescue treatments resulted in a significant difference from the control, to see if there were differences in the speed of male or female rescue.

2.4 Results

2.4.1 The sex of the rescuer in genetic rescue

Twenty-four populations were initiated, but in generation two one population in the control inbred populations failed to pupate in time for the next generation. Generations 0 and 1 for this population were included in the data set.

Male and female rescuer treatments both resulted in significantly higher productivity than the control (see Table 2.1, Figure 2.3). Generation^2 also had a significant negative effect. Interactions between rescuer sex treatment x generation (and generation^2) were not significant. In post-hoc tests there was no significant difference across all generations between the male and female rescue treatments (Estimate = 0.015, SE = 0.040, z = 0.374, P = 0.926, 95% CI = -0.079, -0.109).

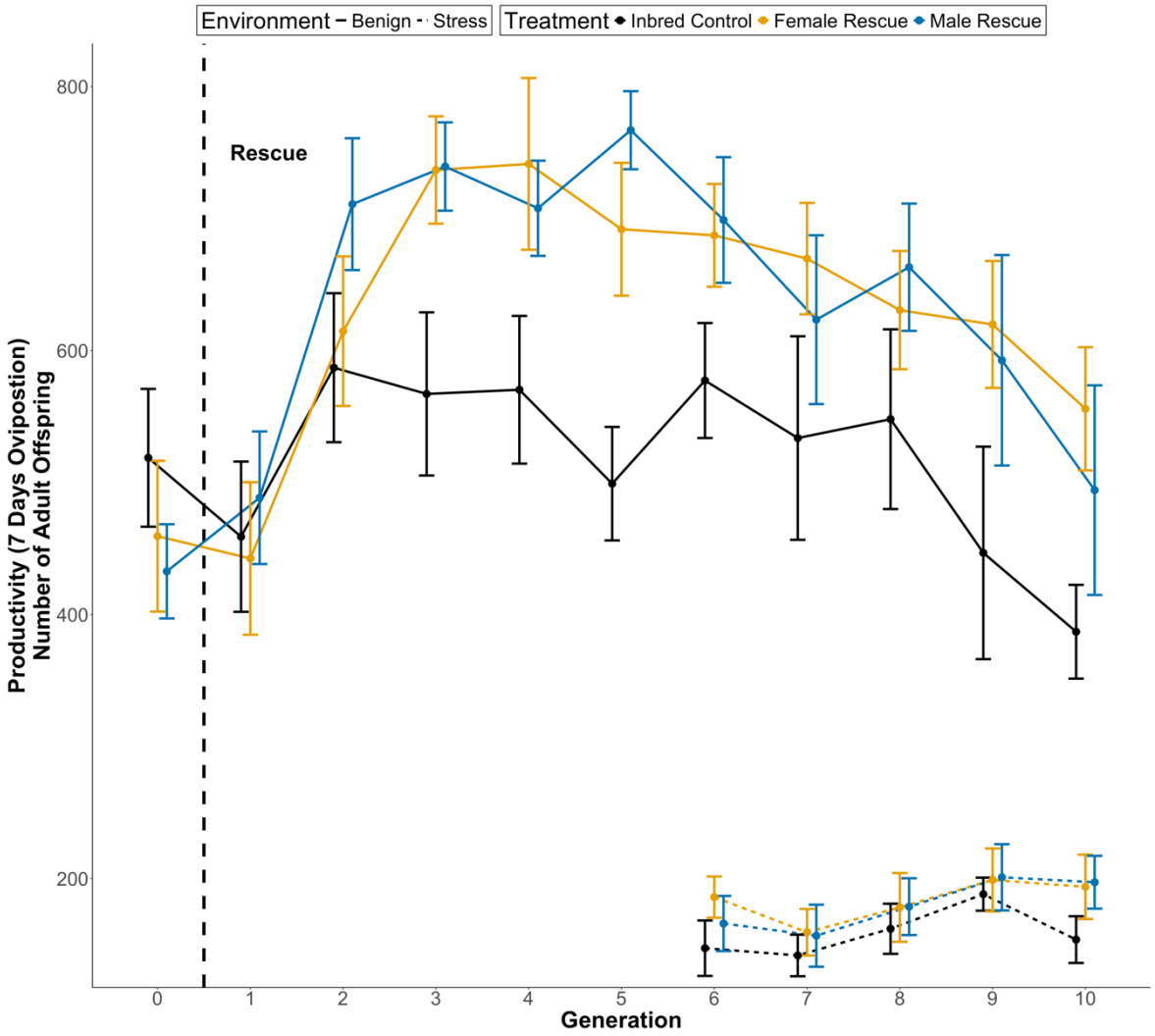

Figure 2.3: The effect of introducing a male or female rescuer on the mean productivity of small, inbred populations of *T. castaneum* (N_e = 20, n = 24/23) over 10 generations after an introduction event. A single male or female rescuer was used to replace one individual of the same sex (dashed vertical line) within the populations of 10 females and 10 males. Populations were kept in either benign (solid line) or stressful (dashed line - starting only at generation 6) environmental conditions (fodder with or without yeast respectively). Under benign conditions, there was a significant increase in productivity for both male (Blue), and female (Orange) rescue treatments compared to the control treatment (Black). There was also a quadratic interaction with generation (See Table 2.1). Standard errors are shown.

Table 2.1: Factors impacting the productivity of small, inbred populations of *T. castaneum* (N_e = 20, n = 24) receiving a single male or female genetic rescuer, or no rescue, tested using a GLMM.[1]

Predictor	Estimate	SE	*z*	*P*	95% CI
Intercept	5.944	0.075	79.570	<2e-16	5.798 6.091
Treatment (baseline = Control)					
Female Rescue	**0.220**	**0.042**	**5.220**	**<0.001***	**0.138** **0.303**
Male Rescue	**0.235**	**0.042**	**5.590**	**<0.001***	**0.153** **0.318**
Generation	**0.160**	**0.027**	**6.030**	**<0.001***	**0.108** **0.212**
Generation^2	**-0.015**	**0.002**	**-6.580**	**<0.001***	**-0.020** **-0.011**
Random	231 Observations		Variance		
ID:Inbred line	24 Populations*		7.056e-10		
Inbred line	8 Lines		8.295e-03		

Post-hoc tests showed that by generation two the productivity of the male rescue lines was significantly higher than the control lines (see Figure 2.3 & Table S1.3; Estimate = 0.189, SE = 0.092, z = 2.060, P = 0.040, 95% CI = 0.009, 0.370), but the productivity of the female rescue lines was not (see Figure 2.3 & Table S1.3; Estimate = 0.035, SE = 0.092, z = 0.370, P = 0.708, 95% CI = -0.146, 0.216). However, the productivity of male and female rescued lines in that generation (2) was not significantly different (see Figure 3; Estimate = 0.155, SE = 0.088, z = 1.760, P = 0.183, 95% CI = -0.051, 0.361). There was no significant difference between the male and female rescued lines in any other single generation (see Figure 2.3).

Plotting the model prediction shows that productivity increased until Generation 5 then began to decline as expected by the negative estimate (see Table 2.1 & Figure S1.3). When modelled separately post-hoc, over generations 1-5 productivity increased significantly (see Table S1.1; Estimate = 0.070, SE = 0.016, z = 4.450, P < 0.001, 95% CI = 0.039, 0.101), then over generations 5-10 productivity decreased significantly (see Table S1.2; Estimate = -0.058, SE = 0.014, z = -4.26, P < 0.001, 95% CI = -0.085, -0.031).

[1] Productivity was measured over 10 generations following the rescue event. Predictors in bold are significant (P < 0.05). Marginal R^2 = 0.247, Conditional R^2 = 0.330. *One population was lost in Generation 2, so there are 23 populations from Generation 2 onwards.

Under stress conditions (0% yeast in fodder) productivity greatly decreased (Figure 2.3), and there were no significant differences between the treatments. There was a significant linear effect of generation on productivity (see Table 2.2).

Table 2.2: Factors impacting the productivity of small, inbred *T. castaneum* populations (N_e = 20, n = 23) under nutrient stress that had either a male or female rescuer from an outbred population introduced five generations prior, tested using a GLMM.[2]

Predictor	Estimate	SE	*z*	*P*	95% CI
Intercept	4.716	0.136	34.570	<2e-16	4.449 4.984
Treatment (baseline = Control)					
Female Rescue	0.221	0.117	1.890	0.059	-0.008 0.451
Male Rescue	0.113	0.118	1.040	0.300	-0.109 0.354
Generation	**0.038**	**0.012**	**3.190**	**0.001***	**0.015 0.062**
Random	78 Observations		Variance		
ID:Inbred line	23 Populations		0.040		
Inbred line	8 Lines		0.021		

2.4.2 Sexual selection and genetic rescue

Thirty-six populations were initiated, but in both generations two and five one population in the control inbred populations failed to pupate in time for the next generation. These populations were included in the analyses.

When introducing a rescuer from a sexual selection population, productivity interacted with generation^2; i.e., there was an increase in productivity followed by a later decline . There was no evidence of an interaction between 'no sexual selection' rescue and generation^2 (Figure 2.4, Table 2.3). There was no significant effect when the interaction was removed.

[2] Predictors in bold are significant ($P < 0.05$).

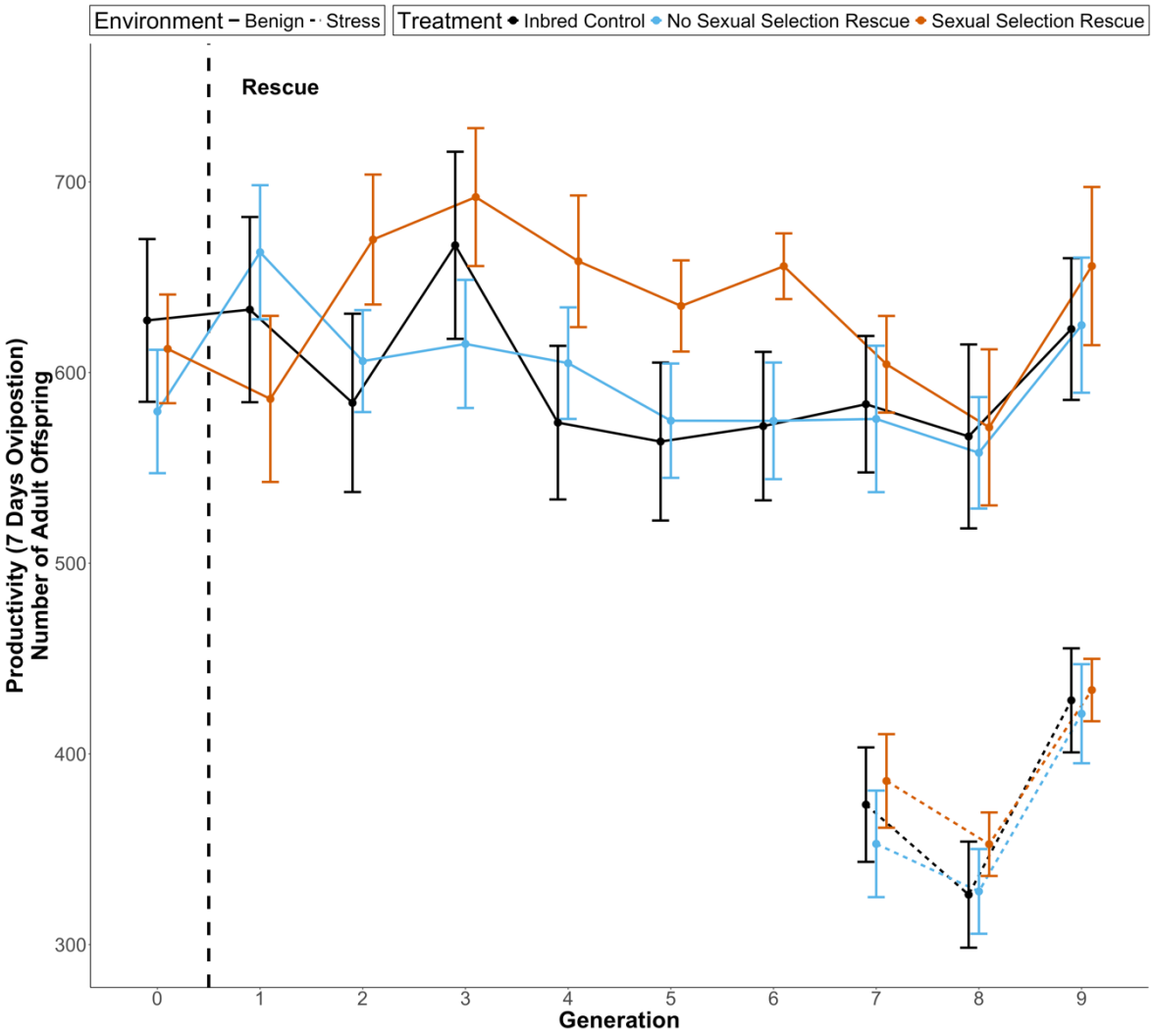

Figure 2.4: The effect of introducing a single male genetic rescuer from a sexual selection background or no sexual selection background on the productivity of small, inbred *T. castaneum* populations (N_e = 20, n = 36/34) over nine generations. Populations were in either a benign (solid line) or stressful (dashed line) environment. The rescue was a single event (dashed vertical line) where the rescuer replaced a male in the inbred population. Compared to the control (black) there was a significant increase in productivity in the sexual selection rescue treatment (orange), which had a quadratic interaction with generation, but no significant effect of the no sexual selection treatment (blue) (See Table 2.3). Standard errors are shown.

Table 2.3: Factors impacting the productivity of small, inbred populations (N_e = 20, n = 36) of *T. castaneum* that received a single rescuer from either a sexual selection or no sexual selection background line population, tested using a GLMM.[3]

Predictor	Estimate	SE	*z*	*P*	95% CI
Intercept	6.482	0.068	94.800	<2e-16	6.348 6.616
Treatment (baseline = Control)					
No sexual selection	0.063	0.090	0.700	0.486	-0.114 0.240
Sexual selection	-0.082	0.090	-0.920	0.360	-0.259 0.094
Generation	-0.047	0.026	-1.760	0.078	-0.100 0.005
Generation^2	0.004	0.003	1.560	0.120	-0.001 0.009
Treatment*Gen (Control)					
No sexual selection*Gen	-0.019	0.037	-0.500	0.614	-0.091 0.054
Sexual selection*Gen	**0.082**	**0.037**	**2.240**	**0.025***	**0.010 0.154**
Treatment*Gen^2 (Control)					
No sexual selection*Gen^2	0.001	0.004	0.370	0.710	-0.006 0.009
Sexual selection*Gen^2	**-0.008**	**0.004**	**-2.260**	**0.024***	**-0.015 -0.001**
Random	311 Observations		Variance		
ID:Inbred line	36* Populations		0.013		
Inbred line	12		0.006		

Under stress conditions, there were no significant differences between the treatments' productivity, but productivity did increase over generations (Figure 2.4, Table 2.4).

[3] Predictors in bold are significant ($P < 0.05$). Marginal $R^2 = 0.077$, Conditional $R^2 = 0.512$.
*One population was lost in Generation 2 and one in Generation 5.

Table 2.4: Factors impacting the productivity of small, inbred populations (N_e = 20, n = 34) of *T. castaneum* under nutrient stress that had been rescued by either a sexual selection or no sexual selection background male rescuer seven generations prior, tested using a GLMM.[4]

Predictor	Estimate	SE	*z*	*P*	95% CI
Intercept	5.281	0.180	29.385	<2e-16	4.929 5.633
Treatment (baseline = Control)					
No sexual selection	-0.023	0.066	-0.352	0.725	-0.152 0.106
Sexual selection	0.048	0.065	0.741	0.459	-0.080 0.177
Generation	**0.079**	**0.021**	**3.731**	**<0.001***	**0.038 0.120**
Random	102 Observations		Variance		
ID:Inbred line	34 Populations		0.013		
Inbred line	12		0.011		

2.5 Discussion

We tested how the sex and sexual selection evolutionary history of a rescuing individual affects the duration of genetic rescue using small, inbred populations of *T. castaneum*. Our results show that a male or a female rescuer was equally effective; both improved productivity compared to the control, though there was some evidence that a male rescuer led to faster rescue. In the second experiment, the introduction of a male from an elevated sexual selection background resulted in a significant increase in productivity, whilst a male from a monogamous background did not. Importantly, in both experiments we observed temporal effects; in the successful rescue treatments productivity increases were observed in the initial generations after the introduction of rescuers, before declining in later generations. When these experiments were replicated under severe nutrient stress conditions we saw no significant effect of rescue on productivity.

Male rescuers have been suggested to enable faster/greater genetic rescue than females due to their higher reproductive potential, as generating more offspring will spread introduced genetic diversity faster (Zajitschek et al., 2009). In our results, females are as effective at rescuing the inbred populations as males. We did find some evidence that males may enable faster rescue of productivity; with male rescue lines showing a significantly earlier increase in productivity compared to control lines (by generation 2) than female (by generation 3) rescue

[4] Predictors in bold are significant ($P < 0.05$).

lines (See Figure 2.3 & Table S1.3). This did not translate into a significant difference between the productivity of male and female rescue lines in generation 2. This result contrasts with previous studies: in wild lions, males were more effective rescuers despite potential issues of social disruption and infanticide (Trinkel et al., 2008) in guppies, faster population growth was observed following male rescue (Zajitschek et al., 2009). It is difficult to compare the results of a wild, large mammal genetic rescue to our laboratory insect species. The study on guppies measured population growth rather than productivity, which may also result in different outcomes. Another aspect which may explain these differences is the extreme disparity between the mating systems of target species, coupled with our experimental approach. We used smaller populations (N_e = 20) than in other studies of genetic rescue in *T. castaneum* (Durkee et al., 2023; Hufbauer et al., 2015; Lewis et al., 2024), which may have limited the advantage that male rescuers had over female rescuers. As female *T. castaneum* can mate with 4-6 males in an hour (Pai and Yan, 2003), the 10 females available to a male in our populations over seven days is far less than his mating potential, and thus the impact of genetic rescue. More experimentation is needed, factoring in population size and testing species with different variation in reproductive success between sexes.

T. castaneum is a promiscuous and highly fecund species (Pointer et al., 2021) and our results are applicable to species with similar life history strategies and mating systems. Females in this system may act as equivalent rescuers to males as there is evidence of inbreeding avoidance in the female reproductive behaviour (Attia and Tregenza, 2004; Michalczyk et al., 2011) meaning negative impacts of inbreeding (Hedrick and Garcia-Dorado, 2016; Vega-Trejo et al., 2022b) may be minimised. However, *T. castaneum* females do not exhibit care for offspring (Kölliker, 2012), eliminating a potential advantage provided by a female rescuer (Mattey et al., 2018; Pooley et al., 2014).

We predicted that rescuers drawn from populations with elevated sexual selection would be more fit (with less genetic load) and more competitive, resulting in a more effective genetic rescue. Our results support this, rescuers with a high sexual selection background improved productivity in the inbred populations whereas rescuers from a no sexual selection background did not. The lines from which our sexually selected rescuers were sourced have previously been shown to resist extinction in the face of inbreeding, relative to lines with no history of sexual selection (Lumley et al., 2015; Godwin et al., 2020) suggesting that these lines have a higher fitness due to sexual selection. Using males from these lines as rescuers may have increased productivity for several reasons, including increased mating competitiveness and increased fitness in offspring with lower genetic load. Furthermore, lower introduced genetic load should result in less re-emergent inbreeding depression in later generations in

these small populations. However, the rescue may fail if populations have divergent traits or differences in trait preference. The rescuer, and their offspring, may be selected against due to differences in sexual selection, inhibiting introgression and thus reducing any fitness benefits. Further work is needed to unravel these possibilities.

The effects of inbreeding depression on endangered populations are often exacerbated by exposure to environmental stress (Armbruster and Reed, 2005; Richardson et al., 2004). However, when testing rescue treatments under stressful (nutrient) conditions we found no significant differences between treatments in either sex or sexual selection experiments. This was unexpected as stress should magnify inbreeding depression and disproportionally affect the productivity of populations that had not been rescued. This lack of effect may be due to the harshness of the nutrient stress treatment we used, as this has been shown to greatly reduce female fecundity and slow offspring development (Demont et al., 2014). Nutrient stress could also increase cannibalism, which occurs in *T. castaneum* when food is scarce (King and Dawson, 1972). This may have had more impact on rescued populations due to increased competition for resources when initial productivity (eggs laid) is higher. However, stressful conditions do not always exaggerate inbreeding depression (Armbruster and Reed, 2005; Sandner et al., 2022). Our finding that stress repressed genetic rescue points to the importance of improving environmental conditions for species before attempting to recover population numbers (Bell et al., 2019; Root, 1998; Ferreras et al., 2001).

A regular criticism of genetic rescue studies is that they fail to monitor populations over sufficient timescales (Clarke et al., 2024). Our study continued monitoring rescue outcomes over multiple (9-10) generations. We see genetic rescue effects begin in the second generation after rescue. Rescue effects are not seen in the generation immediately following rescue, likely because, even in a promiscuous population, it will take more than one generation for the variation from a single rescuer to introgress widely into the population and influence overall productivity. In both experiments, the treatments that result in rescue have peak productivity around generation 5-6. This suggests the beneficial introgression of the rescuer's genetic diversity into the population takes several generations, as seen in previous studies (Hufbauer et al., 2015).

Importantly, we saw productivity benefits of rescue began to decline by the sixth generation in both experiments. Many genetic rescue studies are relatively short-term projects relative to the generation time of the species involved (Frankham, 2015). Owing to the short generation times of *T. castaneum* (Pointer et al., 2021), we are the first to show that rescue effects may not be long-lasting. This has important implications for studies in wild systems, reinforcing

suggestions that monitoring must continue in the long-term, but also that single rescue introductions are potentially not sufficient to rescue populations. We suggest our findings are associated with the resumption of inbreeding effects in later generations due to small population size (N = 20). In other systems, increases in population size resulting from genetic rescue may allow for the introduced genetic diversity to be maintained. If population growth had been allowed, the decline in productivity seen in our experiment may not have occurred as it may have been a product of the continued restricted small size of the populations, leading to the re-emergence of inbreeding depression. However, it must be noted that in some cases endangered populations where genetic rescue may be attempted may also be restricted to small sizes because of factors such as habitat restrictions etc (Robinson et al., 2020). This does not reduce the relevance to conservation contexts, as similar effects have been seen in wild systems (Hedrick et al., 2014, 2019; Robinson et al., 2019). The genetic rescue of the Florida Panther resulted in benefits for five generations after rescue (Onorato et al., 2024), our results suggest that in the coming generations these benefits may start to decline.

In conclusion, we find that both male and female rescuers can be effective genetic rescuers. This is likely linked to the dynamics of promiscuous mating systems such as that seen in *T. castaneum* but serves to highlight the importance of such species-dependent traits when planning conservation interventions. Importantly, and for the first time, we show sexual selection background affects the efficacy of genetic rescue. Given these results, we suggest that, where feasible, using a rescuer from a high sexual selection background when attempting genetic rescue could be beneficial in conservation programs. Overall, our results add important evidence to our understanding of the effectiveness of genetic rescue and support the argument that it should be considered an important tool to conserve endangered populations.

Chapter Three

Inbred versus outbred rescuers in genetic rescue; An experimental test of productivity and genetic load in *Tribolium castaneum*.

3.1 Abstract

Anthropogenic change is causing many natural populations to become small and isolated, often leading to inbreeding depression and increased risk of extinction. Genetic rescue aims to reduce inbreeding depression by reintroducing genetic variation. However, this can also lead to the introduction of new deleterious alleles (mutation load). Recent theoretical advances recommend using individuals from a closely related, outbred population to rescue an inbred population. However, such outbred populations also harbour deleterious alleles in a heterozygous state which could be introduced into inbred populations. Deleterious alleles may become expressed in later generations due to inbreeding, lowering population fitness. Consequently, other divergent but inbred populations, which should have purged more of their deleterious alleles, may be a better source of rescuers. We used *Tribolium castaneum* to experimentally test how productivity differed in inbred populations rescued with outbred versus different inbred rescuers over nine generations. Furthermore whole-genome re-sequencing of individuals from the stock and inbred populations from the 4th generation after rescue (for each treatment) enabled us to measure mutation load. Our results show that outbred rescuers improved productivity the most, but rescuers from inbred populations still improved population productivity compared to not receiving a rescuer. The outbred rescuers significantly reduced the population's inbreeding coefficient and total length of ROH compared to receiving no rescue. Finally, populations that received outbred rescuers had significantly less heterozygous deleterious mutations (hidden load) compared to populations with rescuers from inbred populations or no rescue which did not differ from each other. Importantly, the higher hidden mutation load in outbred rescued populations compared to inbred rescued populations may have negative consequences in the long run if populations remain small and suffer further inbreeding. Given this pattern regarding mutation load, the best rescuers to use may, therefore, depend on a populations ability to increase in size following the rescue attempt.

3.2 Introduction

Anthropogenic effects worldwide have resulted in small, genetically isolated populations of many species (Ceballos and Ehrlich, 2023). These populations often suffer inbreeding depression; increased homozygosity leading to recessive deleterious alleles being expressed and the loss of any heterozygote advantage, both reducing individual and population fitness (Charlesworth and Willis, 2009; Crnokrak and Roff, 1998). Deleterious alleles within a population are known as mutation load. Most such mutations are recessive, as dominant deleterious alleles are immediately exposed to selection and therefore more likely to be purged (Charlesworth and Willis, 2009). When expressed in homozygote form these deleterious alleles are termed realised load (or expressed load) while those in heterozygous form are included in what is called hidden load (Bertorelle et al., 2022; van Oosterhout, 2020). Loss of genetic diversity also reduces adaptive potential, increasing vulnerability to further environmental change and exacerbating extinction risk (Fox and Reed, 2011; Blomqvist et al., 2010; Soule and Gilpin, 1986; Armbruster and Reed, 2005).

Genetic rescue can potentially reduce inbreeding depression, increasing individual and population fitness (beyond demographic effects) by introducing novel alleles (Ingvarsson, 2001; Hedrick et al., 2011). This can be achieved by translocating rescuers, from another closely related (normally conspecific) population, to reproduce within the inbred population. In doing so the rescuer (re)introduces genetic diversity , thus reducing homozygosity (and therefore realised load) and restoring any heterozygote advantage. The increase in genetic diversity in the population will also increase adaptive potential providing resilience for future challenges (Hoelzel et al., 2019; Mable, 2019).

There have been various genetic rescue attempts across taxa in wild, captive and laboratory settings (Frankham, 2015, 2016; White et al., 2023; Clarke et al., 2024). Notable examples of successful rescue attempts include the Florida panther (*Puma concolor couguar*), Adder (*Vipera berus*) and Brook trout (*Salvelinas fontinalis*) (Robinson et al., 2017; Pimm et al., 2006; Madsen et al., 2020). A key criticism of many of these studies is the lack of long-term monitoring of the populations following rescue to see if fitness benefits are maintained (Bell et al., 2019; Clarke et al., 2024). Experimental testing can allow us to observe long-term trends more easily using short generation time systems. They also allow greater control and monitoring of populations and the ability to test practices that could not be applied to a wild population (Lewis et al., 2024; Pérez-Pereira et al., 2025; West et al., 2025). Such studies are providing a small but growing body of evidence to support and inform the use of genetic rescue

in conservation (Ralls et al., 2018; Whiteley et al., 2015). However, there are risks associated with genetic rescue that have discouraged attempts to achieve it (Fitzpatrick et al., 2023).

Outbreeding depression, the loss of fitness from crossing populations, poses a potential risk when attempting genetic rescue (Edmands, 2007). Fitness can be lost due to the disruption of locally adaptive gene complexes, the swamping of unique variation and the introduction of deleterious alleles (Lenormand, 2002; Rhymer and Simberloff, 1996). These process make genetic rescue a risk and there are examples of genetic rescue attempts having little effect or even resulting in population declines (Hedrick et al., 2019; Nichols et al., 2024; Pavlova et al., 2024). However, it has been argued that these risks are overstated (Frankham et al., 2011). To reduce the risk of outbreeding depression, and maximise the success of genetic rescue, guidelines for practitioners have been published (Hedrick and Fredrickson, 2010; Frankham, 2016), including how to select a source population for rescuers (Kronenberger et al., 2018; Fitzpatrick et al., 2015; Powell, 2023; Ralls et al., 2020). However, a reluctance to facilitate genetic rescue remains despite such advice (Frankham, 2016; Hedrick and Fredrickson, 2010; Fitzpatrick et al., 2023).

Most evidence supports the use of individuals from large, outbred populations for genetic rescue, which will introduce greater genetic diversity than inbred populations (Ralls et al., 2020). A meta-analysis on outcrossing inbred populations found using outbred immigrants resulted in greater fitness increases than those using inbred immigrants (Frankham, 2015). However, such outbred populations will harbour hidden mutation load that later could be converted to realised load in populations that suffer further inbreeding if they remain small (e.g. due to other constraints (Dussex et al., 2023; Mathur and DeWoody, 2021; van Oosterhout, 2020). This could potentially lead to population crashes in the future, as observed in wolves (*Canis lupus*) on Isle Royale. A single male wolf migrated into the population resulting in a brief improvement in population fitness, but inbreeding in later generations resulted in a drastic population crash as a result of recessive, deleterious alleles becoming expressed (Hedrick et al., 2014).

Inbreeding increases homozygosity converting hidden load to realised load, thus exposing it to selection, which can remove it from the population (purging) (Hedrick and Garcia-Dorado, 2016). Various small, inbred populations have persisted for long-periods and undergone a certain amount of purging (Robinson et al., 2016, 2018). Rescuers from such inbred populations may be preferable because of the reduction in mutation load. Simulations have shown that rescuers from small/moderate-sized populations will have a lower risk of introducing deleterious genetic variation (Kyriazis et al., 2021).However, these purged

populations may have lost beneficial variants and fixed deleterious variants through genetic drift. However, to our knowledge, only one other study has directly tested the difference between outbred and inbred rescuers finding that outbred rescuers performed better than inbred ones (Pérez-Pereira et al., 2025).

Here, we use captive populations of *Tribolium castaneum* to experimentally test the impact and duration of attempted genetic rescue using inbred or outbred rescued populations monitored over ten generations. This species has been utilised previously as an amenable model in which to study genetic rescue (West et al., 2025; Lewis et al., 2024; Hufbauer et al., 2015; Pointer et al., 2021). We also sequenced the genomes of individuals prior to the experiments, and four generations after the genetic rescue, to determine how the genetic diversity, homozygosity and mutation load have changed. We predict that 1) both treatments should lead to increased productivity, but outbred rescuers should improve productivity more than inbred rescuers. 2) outbred rescuers should lead to a greater reduction in genetic homozygosity than inbred rescuers. However, 3) outbred rescuers will lead to higher hidden mutation load within the rescued populations than inbred rescuers. Because our populations were kept at a restricted size (n=20 adults) for the 9 generations after rescue, we expect the inbreeding to accumulate over these generations. Consequently, we expect the differences in introgressed mutation load to lead to 4) faster declines in productivity in later generations (due hidden load being converted into realised load) when outbred rescuers were used than when inbred rescuers were used. The study therefore provides a comprehensive and rigorous test of the impact different levels of genetic diversity in source populations have on genetic rescue.

3.3 Methods

3.3.1 Husbandry

Tribolium castaneum populations were maintained at 30°C and 60% humidity in a controlled environment with a 12:12 light-dark cycle. Populations were provided with a standard fodder mixture of 90% organic white flour, 10% brewer's yeast and a layer of oats for traction. For each line of beetles, a generation is initiated by virgin adults (see lines below for population size) which have seven days to mate and oviposit on fresh fodder. After those seven days adults were removed using 2mm and 850µm sieves and leaving only eggs and fodder. After ~21 days the eggs have progressed to the pupal stage and can be sexed. The fodder is sieved, and pupae are divided into same-sex groups. After 10 days these same-sex groups will be mature virgin adults, and the required number will be placed into new fodder to start the next generation. A generational cycle last for ~38 days.

2.3.2 *Tribolium castaneum* Lines

Krakow Super Strain (KSS) – A combination of 14 laboratory strains to maximise genetic diversity in a single strain (Laskowski et al., 2015) - was the ancestral population and a source for outbred genetic rescuers (hereafter outbred stock).

Inbred stocks -12 inbred stocks separately descended from KSS were created by three single-pair bottlenecks in the first, fifth, and seventh generations. These populations were then maintained at 100 randomly selected adults between and following bottlenecks, used to create the experimental populations, and were the source of genetic rescuers. The productivity of the inbred stocks was determined from previous experiments (West et al., 2025) by taking their average productivity when they received no rescue (See Table S2.1). Inbred rescuers were sourced from the three highest and three lowest productivity stocks.

3.3.3 Rescued populations

80 pupae (40 male, 40 female) were taken from each inbred line and kept in single sex groups of 20 on 10ml of standard fodder for 10±2 days in plastic dishes with lids. After this time, when pupae had eclosed into mature adults, 10 males and 10 females were placed into 125ml tubs containing 70ml of standard fodder. Four experimental populations were created from each inbred line, producing a total of 48 (4 sets of 12) experimental populations containing 20 adult beetles with a 1:1 sex ratio, kept under standard rearing conditions. Maintaining the populations at 20 individuals preventing any negative density effects on offspring production (Duval et al., 1939; King and Dawson, 1972; Janus, 1989). To avoid bias during handling, populations were randomly assigned ID numbers so treatments were not known. Before the start of the experiment, populations were maintained under experimental conditions for one generation to control for transgenerational density effects (Generation 0). Following this, a single male from each population was replaced with a ‘rescuer’ when the 10 males and 10 females went into the fresh fodder. This replacement, rather than just adding an additional male beetle, was to avoid the effects of demographic rescue (increased population size causing increased population fitness). Experimental populations were maintained for nine non-overlapping generations post rescue.

3.3.4 Rescue treatments

Four experimental treatments were created, defined by rescued populations receiving a 'rescuer' male from a different background (Figure 3.1). No Rescue was the control treatment. Inbred Rescue was a treatment that received rescue from another inbred stock. This was split into High Rescue (rescuer from the highest productivity lines) or Low Rescue (rescuer from the lowest productivity lines) treatments (Figure 3.1). The Outbred Rescue treatment received rescue from the outbred stock population.

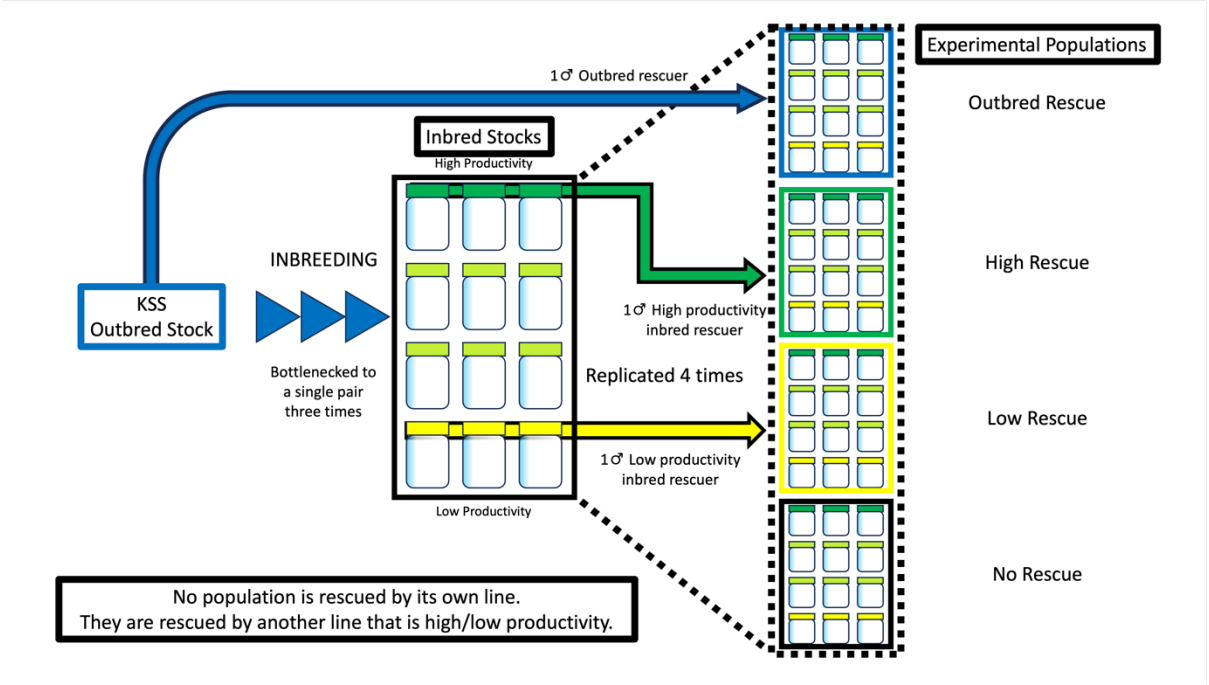

Figure 3.1: Experimental design to test genetic rescue of inbred *T. castaneum* populations (Population size = 20). Inbred rescuers were sourced from inbred populations (either high or low productivity). Twelve inbred lines were replicated once in each treatment, resulting in a total of 48 populations. Outbred rescuers were sourced from the ancestral KSS population. Rescue was carried out by replacing a single male beetle with a male rescuer.

3.3.5 Productivity

At the end of a generational cycle (~38 days) all mature adult beetles not used for starting the next generation were frozen at -6°C and counted to measure population productivity. The number of mature adults from the sexed pupae (including those that founded the population) were also included in productivity.

3.3.6 Stressful conditions

To test if the effects of genetic rescue were amplified under stressful conditions new parallel populations were established by taking double the number of pupae at generation six of the main experiment. These new populations were maintained as in the main experiment but with the yeast content of the fodder reduced to 1%, a diet that leads to nutrient stress in these beetles due to reducing the main source of protein (Godwin et al., 2020).

3.3.7 Genomic sequencing

168 adult females were sampled at 35 days old from the 4th generation following genetic rescue. Two individuals were sampled from each control population (2 x 12 = 24) and three individuals per experimental population, from all other populations (Outbred Rescue, High Recue and Low Rescue) (3 x 12 = 36 samples per treatment = 108 in total). From the inbred stocks, two individuals were sampled from six populations that did not provide rescuers (12 samples), three samples were taken from the six populations that were the source of rescuers (18 samples). Finally, six samples were taken from the outbred KSS stock to supplement 24 individuals (12 males, 12 females) from the outbred stock that had been sequenced previously (Pointer, 2025). DNA extraction was conducted using the Qiagen DNeasy blood and tissue kit (insect tissue protocol). Each whole individual was ground in liquid nitrogen prior to extraction. A 1x bead cleanup was used to clean and concentrate the extractions using the AMPure XP SPRI protocol (Beckman Coulter). This resulted in average DNA concentrations of 23.56ng/μL (range 8.72 - 53.2) (See Table S2.2). Library preparation and sequencing was carried out at the Earlham Institute (Norwich, UK) using a low-input transposase-enabled (LITE) pipeline (Supplementary methods). Samples were sequenced on a single flow cell on the Illumina Novaseq X Plus platform. The 24 additional KSS samples from a previous study had been sequenced using the Illumina Novaseq 6000 platform.

3.3.8 Read mapping and variant calling

FastQC (0.11.9, Andrews, 2010) was used to quality check raw reads and trimmomatic (0.39, Bolger et al., 2014) was used to remove adapter sequences, with the adapters.fa file from bbmap2 (Bushnell, 2014) as reference. Reads were mapped to the *T. castaneum* reference genome (GCA_000002335.3, Tcas 5.2, Yates et al., 2022) using BWA-MEM (v0.7.17, Li, 2013). SAMtools (v1.18, Li et al., 2009) fixmate was used to ensure correct read pairing and resulting BAM files were sorted using SAMtools sort. PCR duplicates were removed using Picard

MarkDuplicates (v2.26.2,Picard Team, 2019) and remaining mappings were filtered for complete read pairs and those with a mapping quality (MAPQ) >25 using SAMtools view.

Joint genotyping was conducted using BCFtools mpileup (v1.18.0, Danecek et al., 2021). BCFtools call was then used to call variant sites under the multi-allelic model (-vm). Following this, BCFtools filter was used to remove SNPs or Indels within 3bp of other Indels, with a variant quality score <30, at a locus with sequencing depth <637 and >5733 (+/- 3x average sequencing depth), and variants that were represented by data at that locus in less than 50% of individuals (-g 3 -G 3 -e 'DP < 637 || DP > 5733|| F_MISSING > 0.5 || QUAL < 30'). Single nucleotide polymorphisms SNPs) were extracted using BCFtools view. At this stage, they were further filtered to remove sites with minor allele count <3. This file is referred to hereafter as the SNP vcf.

3.3.9 Population structure

The SNP vcf was linkage pruned using PLINK v.1.9 (Purcell et al., 2007) with the following flags: --double id, -- allow-extra-chr, --set-missing-var-ids @:#, --indep-pairwise 50 10 0.1. A principal component analysis (PCA) was performed using PLINK v.1.9 to check the population structure matched the experimental design and there was no batch effect of sequencing (See Figure S2.1).

3.3.10 Inbreeding

Heterozygosity was calculated as the genomic inbreeding coefficient (F) on a per-individual basis using vcftools --het command on the SNP vcf. Plink v.1.9 was used to detect ROH from the SNP vcf. The minimum length for a ROH was 200kb containing 30 SNPs and the minimum gap allowed was 100kb. SNP density was set at 1 per 50kb. The window size was set as 30 SNPs, 2 heterozygous and 5 missing genotypes were allowed per window, the threshold was set at 0.05. The flags for extra chromosomes and double ID were included. These settings were based on studies in insects with similar genome sizes (Gmel et al., 2023; Izutsu et al., 2012; Pérez-Pereira et al., 2025).

3.3.11 Mutation load

A SIFT database was created using the SIFT4G_Create_Genomic_DB for *T. castaneum* using the Tcas 5.2 reference genome (.fa and .fa.fai files), genetic code table 1 Standard, mito-genetic code table 5 Invertebrate Mitochondrial, gene annotation and protein files (.gtf and

.pep.all.fa files). The protein database used was uniref90.fasta. The SIFT 4G annotator was used to assign a SIFT score for each SNP, labelling it as deleterious or tolerated on the SNP vcf. SIFT predicts the impact of an amino acid substitution on protein function using sequence homology and physical properties (Kumar et al., 2009). The annotated SNP vcf was filtered to only the SNPs annotated as being deleterious by SIFT (SIFT score < 0.05). Any SNPs with low confidence annotations were removed. This resulted in a total of 9004 SNPs. bcftools stats was used to generate per sample statistics on the number and zygosity of SNPs annotated as deleterious.

3.3.12 Statistical analysis

R V4.4.1 (R Core Team, 2024) was used to conduct analysis of productivity data in R studio version 2024.04.2+764 (Posit team, 2024). For data management, the packages tidyverse (Wickham et al., 2019), stats (R Core Team, 2024), rmisc (Hope, 2022) and googlesheets4 (Bryan, 2023) were used. ggplot2 (Wickham, 2016) and ggforce (Pedersen, 2024) were used for visualisation. The Shapiro.test function was used to check the dispersal of the data. The glmmTMB (Brooks et al., 2017) package was used to fit generalised linear mixed models (GLMMs) to test for differences in productivity between treatments. A negative binomial distribution was fitted as productivity is count data and Poisson was a poor fit. The response variable was productivity, fixed variables were treatment interacting with generation and generation^2. The treatment generation^2 interaction was dropped as it was non-significant. Random factors were population ID nested within Inbred line. This model was repeated splitting the inbred rescuer treatment into those rescuers from high and low productivity. The DHARMa package (Hartig, 2022) was used to check model fit and parameters were checked for collinearity using the check_collinearity function from the performance package (Lüdecke et al., 2021). No overdispersion or collinearity issues were found. The emmeans package (Lenth R, 2024) was used to conduct post-hoc pairwise Tukey tests on the above models to identify differences between pairs of treatments. The predict function from stats (R Core Team, 2024) was used to extract model predictions for plotting the total length of ROH and total number of deleterious SNPs to account for differences in individual sample coverage.

3.3.13 Genomic statistics

To test for differences between treatments for the different genomic measures we fit the following statistical models (glmmTMB unless otherwise stated). The nlme package (Pinheiro et al., 2025) was used to fit a linear mixed model, using nlme::lme, to account for heteroscedasticity amongst treatments for inbreeding coefficient. The response variable was

F, fixed variable was treatment, and population was included as a random effect. A linear mixed effects model for total length of ROH was fit with a gaussian distribution, the response variable was total length of ROH, the fixed variables were treatment and average individual genome coverage, with populations as a random factor. Values were extracted from the model to plot Figure 3.4. To test for differences between the count of deleterious SNPs between treatments a GLMM was fitted. A negative binomial distribution as used to account for count data, the response variable was number of deleterious SNPs, fixed variables were treatment and average individual genome coverage, with a random factor of population. Values were extracted from the model to plot Figure 3.5a. To model the proportion of homozygous deleterious SNPs, a GLLM was fit with a Beta distribution and logit link function, the response variable was the proportion of homozygous deleterious SNPs, the fixed variable was treatment and individual cover, with population as a random factor.

3.4 Results

3.4.1 Population productivity

Population productivity was significantly affected by treatment. All separate treatments showed significantly higher productivity (in interaction with generation) than the No Rescue (Figure 3.2a, Table 3.1). In post hoc pairwise testing all treatments were significantly different from each other (Table 3.2).

Table 3.1: A GLMM of factors impacting the productivity of small, inbred, *T. castaneum* populations (Population size = 20, Populations = 48) receiving a rescue by either Outbred Rescue, Inbred Rescuer or No Rescue. Productivity was measured over nine generations following the rescue event. Predictors in bold are significant ($P < 0.05$). Marginal $R^2 = 0.136$, Conditional $R^2 = 0.578$.

Predictor	Estimate	SE	*z*	*P*
(Intercept)	6.358	0.049	127.96	<2e-16
Treatment (No Rescue)				
Inbred Rescue	-0.016	0.042	-0.38	0.702
Outbred Rescue	0.086	0.047	1.81	0.071
Generation	**0.051**	**0.011**	**4.46**	**< 0.001**
Generation^2	**-0.006**	**0.001**	**-5.50**	**< 0.001**
Treatment*Generation (No Rescue*Generation)				
Inbred Rescue* Generation	**0.017**	**0.006**	**2.85**	**0.004**
Outbred Rescue*Generation	**0.016**	**0.007**	**2.33**	**0.020**
Random	432 observations		Variance	
ID:Inbred stocks	48 populations		0.005	
Inbred stocks	12 stocks		0.011	

Table 3.2 - **A pairwise comparison of the productivity of small, inbred, *T. castaneum* populations (Population size = 20, Population = 48) receiving either Outbred Rescue, Inbred Rescue or No Rescue.** Productivity was measured over nine generations following the rescue event. Predictors in bold are significant ($P < 0.05$).

Pair	Estimate	SE	*z*	*P*
No Rescue – Inbred Rescue	**-0.069**	**0.029**	**-2.346**	**0.050**
No Rescue – Outbred Rescue	**-0.163**	**0.034**	**-4.853**	**< 0.001**
Inbred Rescue – Outbred Rescue	**-0.094**	**0.029**	**-3.262**	**0.003**

To further explore the marginal effect of the Inbred Rescue treatment we split it into the fitness categories of the rescuer populations (See Table S2.3). We found that High Rescue had significantly higher productivity than Low Rescue or No Rescue but not from Outbred Rescue (Table 3.3). Low Rescue did not have significantly different productivity from No Rescue but was significantly lower than High Rescue and Outbred Rescue. When the populations were replicated onto nutrient stress they had lower productivity and there were no significant differences between the treatments (See Table S2.4). As there were productivity differences between High Rescue and Low Rescue they remained split for the rest of the analysis.

Table 3.3 - **A pairwise comparison of the productivity of small, inbred, *T. castaneum* populations (Population size = 20, Population = 48) receiving either Outbred Rescue, High Rescue, Low Rescue or No Rescue.** Productivity was measured over nine generations following the rescue event. Predictors in bold are significant ($P < 0.05$).

Pair	Estimate	SE	*z*	*P*
No Rescue – Low Rescue	-0.027	0.031	-0.886	0.822
No Rescue – High Rescue	**-0.110**	**0.031**	**-3.568**	**0.002**
No Rescue – Outbred Rescue	**-0.163**	**0.031**	**-5.313**	**< 0.001**
Low Rescue – High Rescue	**-0.083**	**0.031**	**-2.701**	**0.035**
Low Rescue – Outbred Rescue	**-0.136**	**0.031**	**-4.448**	**0.001**
High rescue – Outbred Rescue	-0.053	0.030	-1.746	0.300

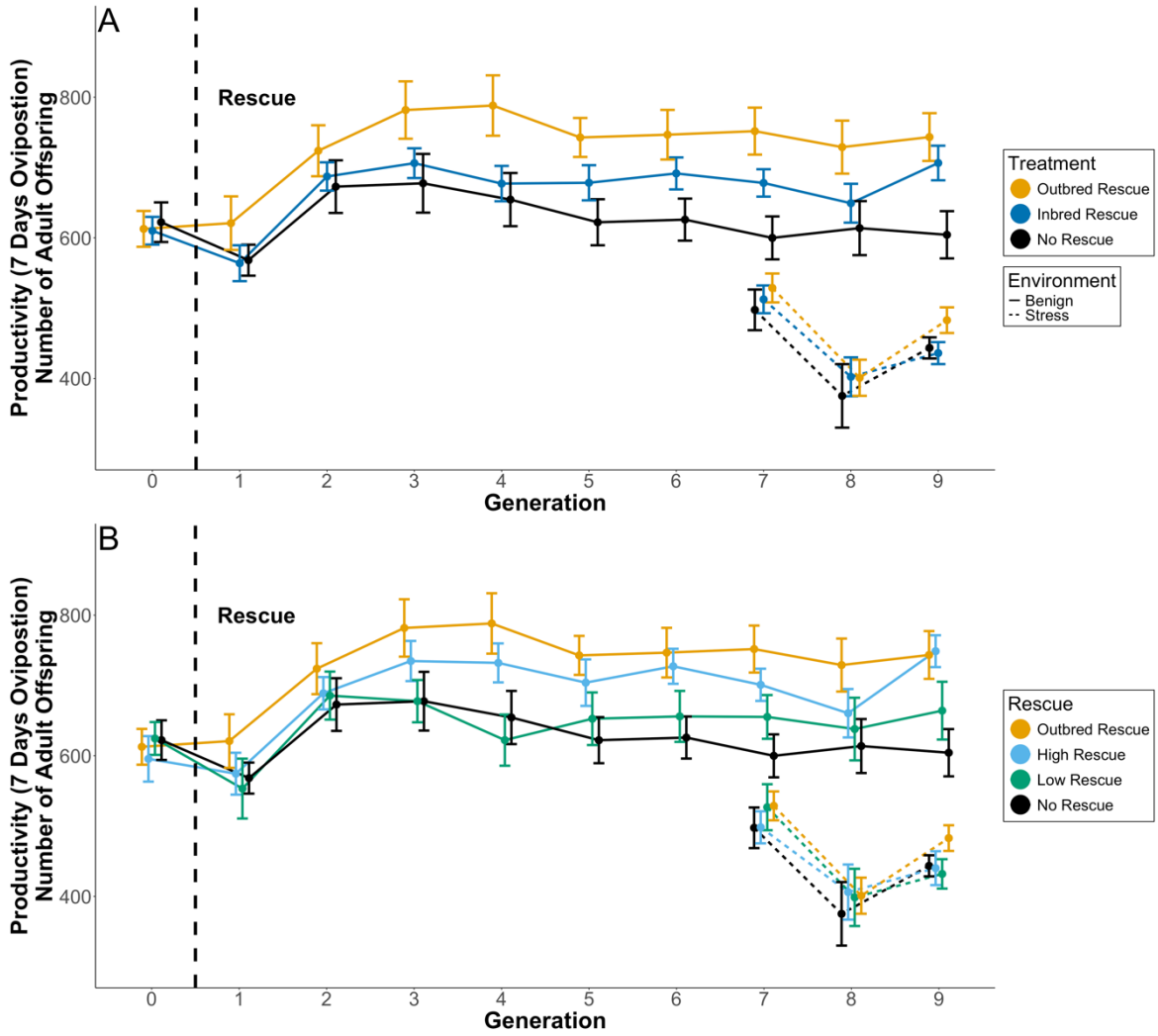

Figure 3.2: The effect of introducing a male rescuer from either an outbred population or inbred populations on the mean productivity of small, inbred populations of *T. castaneum* (Population size = 20, Number of populations = 48) over nine generations after an introduction event. A single male was used to replace one individual of the same sex (dashed vertical line) in populations of 10 females and 10 males. Populations were kept in either benign (solid line) or stressful (dashed line - starting only at generation 7) environmental conditions (fodder with reduced yeast for stress). Standard errors are shown, points are jittered for clarity. **A:** Rescue treatment of Outbred Rescue and Inbred Rescue. Under benign conditions, there was a significant interaction between productivity and generation for Outbred Rescue (Orange) and Inbred Rescue (Green) treatments compared to the No Rescue (Black). **B:** Inbred Rescue treatment split into High Rescue and Low rescue based on productivity of source populations (See Methods). Under benign conditions, there was a significant interaction between productivity and generation for Outbred Rescue (Orange), High Rescue (Blue) and Low Rescue (Green) treatments compared to the No Rescue treatment (Black).

3.4.2 Genomic sequencing

We obtained whole genome SNP data from 192 individuals. Two samples (one from a High Rescue, one from an Outbred Rescue population) had low coverage and were removed from the analysis. The average coverage for the remaining 190 individuals was 10.1x and we identified 4,277,024 SNPs. In downstream analysis it was necessary to filter out 12 male samples due to hemizygosity of the sex chromosomes.

The PCA (See Figure S2.1) shows 13 clusters with the ancestral outbred population as the central cluster, surrounded by 12 cluster reflecting the genetic variation in the 12 initial inbred populations (populations to be rescued) as expected under the experimental design. There were no batch effects of the different runs on the outbred stock samples.

3.4.3 Inbreeding

As expected, the outbred stock had the lowest median inbreeding coefficient and was increased significantly in the inbred stocks (estimate = 0.459, SE = 0.056, t ratio = 8.256, $p < 0.001$). In relation to the rescue treatments, we found that Outbred Rescue significantly reduced population inbreeding coefficient compared to Low Rescue and No Rescue (Figure 3.3). The difference between High rescue and Outbred Rescue was marginally insignificant. There were no significant differences between No Rescue, Low Rescue or High Rescue (See Table S2.5).

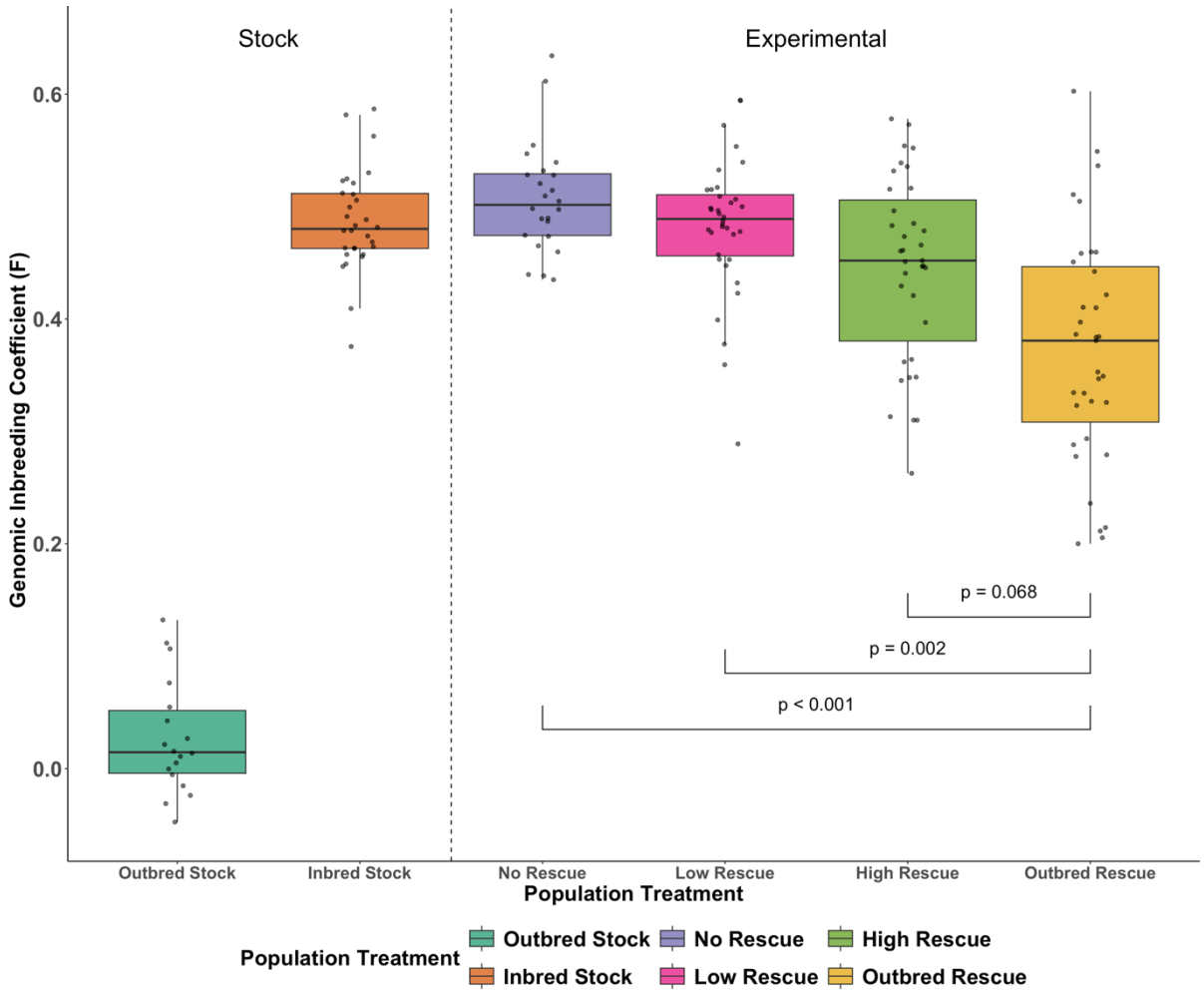

Figure 3.3: Genomic inbreeding coefficient (F) for individual T. *castaneum* samples from experimental populations (Population size = 20) in a genetic rescue experiment (Outbred Rescue, High Rescue. Low Rescue and No Rescue), inbred stock populations and the outbred stock population. Each point represents an individual. The populations left of the dashed line are the ancestral, outbred stock that was the source of outbred rescuers (Dark Green), and the inbred stock populations that were the source of the experimental populations and inbred rescuers (Orange). The experimental populations right of the dashed line are the No Rescue (Blue), Low Rescue (Pink), High Rescue (Light Green) and Outbred Rescue (Yellow). Each point represents an adult individual (35 days old) sampled four generations following genetic rescue of the population by replacing a single male with a rescuer male. The boxplots show the median and interquartile ranges. Significance was calculated using a linear model and post hoc Tukey tests.

The total length of ROH was shortest in the outbred stock and total length was increased in the inbred stocks (Figure 3.4). Regarding the experimental treatments, the Outbred Rescue treatment had significantly shorter total ROH than all the other experimental treatments. The other treatments were not significantly different from each other (See Table S2.6).

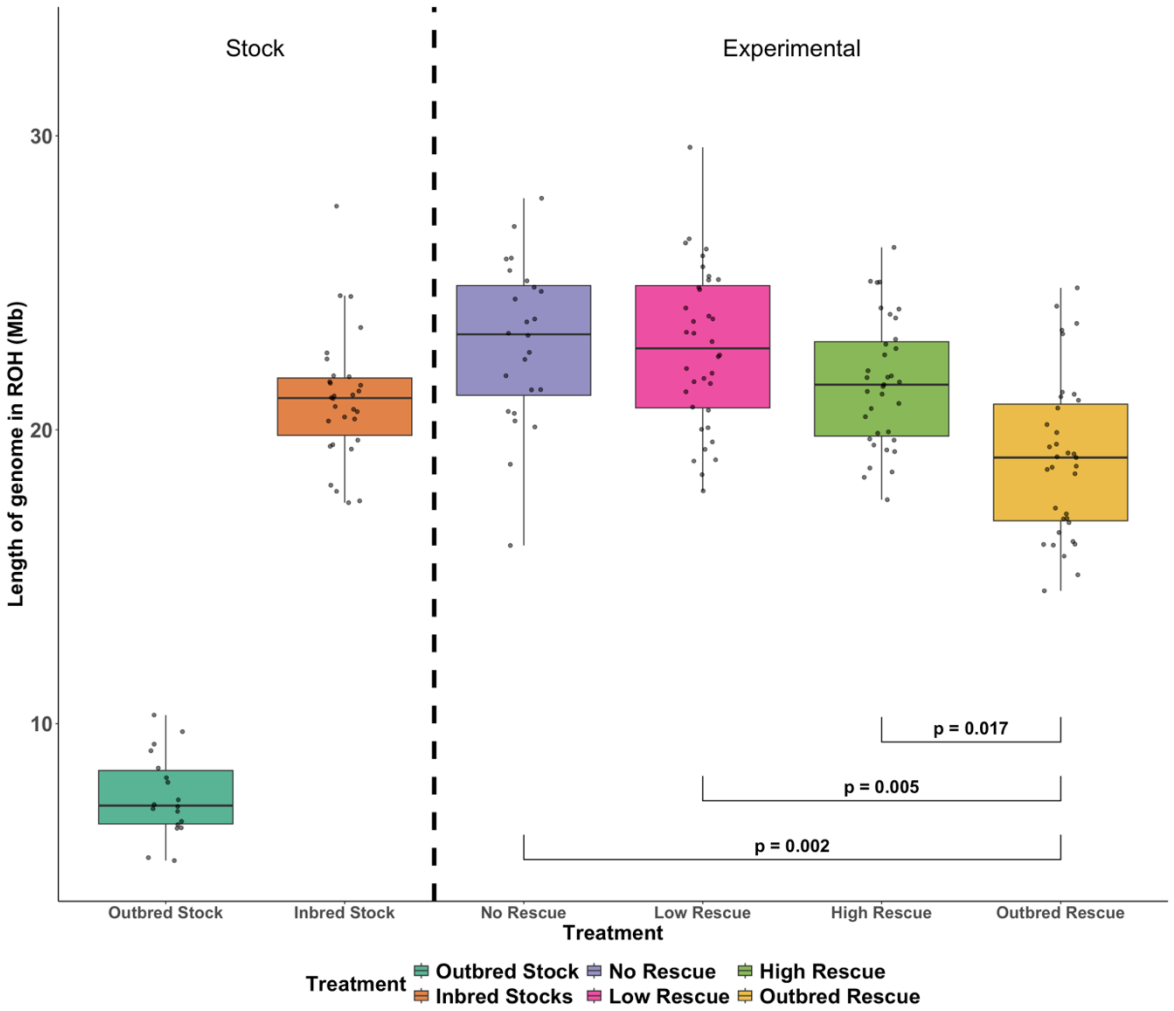

Figure 3.4: The total length of ROH (>200Kb) in individual genomes in populations of *T. castaneum* involved in a genetic rescue experiment. Values shown were extracted from a model with the formulae: response variable total length of ROH, fixed variables treatment and average individual genome coverage, with population as a random factor. Each point represents an individual. Whole genome sequence data was generated for individuals taken from the four experimental populations, the inbred stock populations (Orange) and the ancestral outbred population (Dark Green). In the experiment small, inbred populations were rescued by Outbred Rescue (Yellow), High Rescue (Light Green), Low Rescue (Pink) or received No Rescue (Blue). Calculated using PLINK. The boxplots show the median and interquartile ranges of total length. Significance was calculated using a linear model and post hoc Tukey tests.

3.4.4 Mutation load

The outbred stock had the highest count of deleterious SNPs, and the count was reduced in the inbred stock populations (Figure 3.5a). The Outbred Rescue treatment had significantly more deleterious SNPs than the other experimental populations, which did not differ from each other (See Table S2.7). The proportion of deleterious SNPs that were homozygous was

lowest in the Outbred stocks and higher in the inbred stocks (Figure 3.5b). The Outbred Rescue treatment had significantly less deleterious SNPs in a homozygous state compared to the other experimental treatments, which were not significantly different from each other (See Table S2.8).

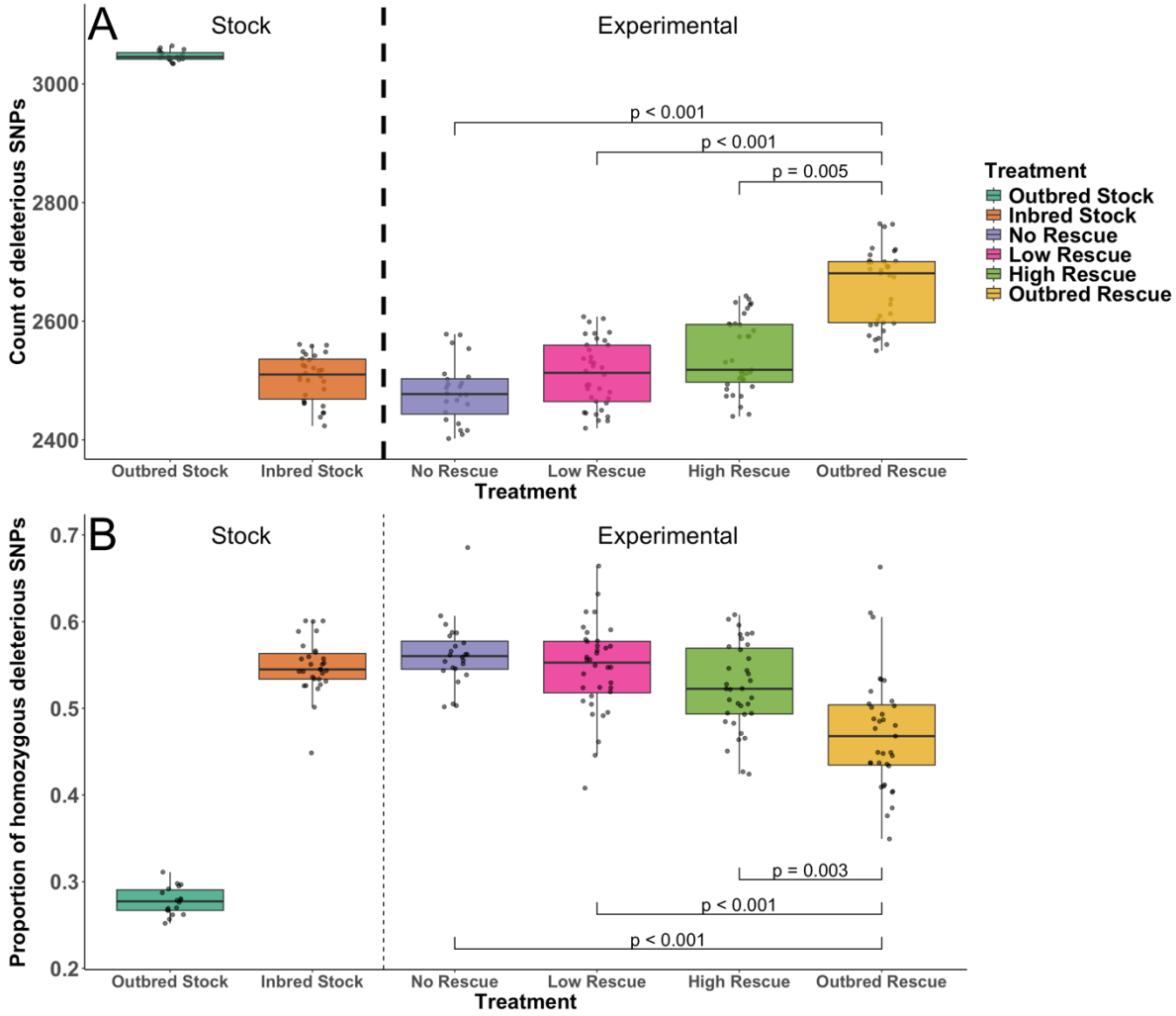

Figure 3.5: The change in the amount and expression of deleterious SNPs in small, inbred populations of *T. castaneum* that received a genetic rescuer from different source populations. Each point represents an individual. Whole genome sequence data was generated for individuals from the four experimental populations, the inbred stock populations (Orange) and the ancestral outbred population (Dark Green). Stock populations are left of the vertical dashed line, experimental populations to the right. Experimental treatments were Outbred Rescue (Yellow), High Rescue (Light Green), Low Rescue (Pink) and No Rescue (Blue). A single male rescuer replaced a single male in the population (Population size = 10 males and 10 females each generation). **A:** The count of deleterious SNPs in individuals of each treatment. Values shown were extracted from a model with the formula**:** a negative binomial distribution, response variable number of deleterious SNPs, fixed variables treatment and average individual genome coverage, with a random factor of population. **B:** The

proportion homozygous deleterious SNPs in individuals of each genetic rescue treatment. Significance was calculated using a linear model and post-hoc Tukey tests.

3.5 Discussion

Our results show that using a rescuer from an outbred population increased population productivity of an inbred population. A rescuer from another (genetically divergent) inbred population also produced an increase in productivity, but this increase was less than for a population rescued by an outbred rescuer. When sorting the inbred rescuer source populations into high and low productivity, we find that High Rescue had a greater rescue effect than Low Rescue as expected. Under nutrient stress, there was no difference between any of the rescue treatments as all populations have low productivity. The genomic data from the 4th generation after rescue reveals that only the Outbred Rescue treatment resulted in statistically significant differences from the other rescue treatments. Outbred Rescue had reduced inbreeding coefficient and shorter length of the genome in ROH, showing that more genetic diversity was introduced than in the Inbred Rescue treatments, which did not differ statistically from No Rescue. When examining deleterious SNPs, the Outbred Rescue did introduce more mutation load than the Inbred Rescue but (in the 4th generation) significantly more of that load was heterozygous and therefore unexpressed in the populations.

Established guidance and recent studies suggest that rescuers sourced from outbred populations should provide greater fitness benefits in genetic rescue by introducing more genetic diversity (Frankham, 2015; Ralls et al., 2020). In our experiment, Outbred Rescue did produce the highest overall population fitness benefit with consistently higher productivity than Inbred Rescue or No Rescue (Figure 3.2a). This fitness benefit was probably due to the Outbred Rescue reducing levels of inbreeding in the inbred recipient population more than the other treatments as evidenced by lower F and lower total length of ROH. Thus, our results confirm that decreased inbreeding due to the genetic diversity introduced is the key benefit of using a rescuer from an outbred population over one from an inbred population. A recent study in *Drosophila melanogaster* found analogous results with rescuers from a larger donor population proving more effective than rescuers from smaller inbred populations (Pérez-Pereira et al., 2025).

The Inbred Rescue also significantly increased the productivity of the inbred recipient populations but not as much as the Outbred Rescue (Figure 3.2a). This fits with our expectations that new genetic diversity improves fitness, but a less genetically diverse rescuer was not as effective as an outbred rescuer(Ralls et al., 2020). Furthermore, the productivity of

inbred rescued populations stayed lower than outbred rescued populations in all generations despite our expectation that outbred rescuers would introduce mutation load, affecting fitness in later populations. Our genomic measurements of inbreeding (F and length of ROH) were only slightly reduced by the inbred rescue treatments compared with the No Rescue treatment, likely a result of a small amount of genetic variation being introduced. When looking at the zygosity of the mutation load there was also a slight change when comparing the Inbred Rescues to the No Rescue. This was surprising as there was a clear fitness benefit of inbred rescue, especially High Rescue, but that was not reflected in the genomic data. This shows that inbred rescuers can be effective to some degree, but they will not improve genetic health of a population as much as an outbred rescuer would. However, the key benefit of an inbred rescuer is avoiding the introduction of mutation load, and only the Outbred Rescue treatment had a significant increase in load. This is key to preventing fitness loss if continued inbreeding occurs in the population.

We also tested whether the productivity of an inbred population affected its ability to rescue. Despite going through the same inbreeding procedure there will be variations in fitness between inbred populations due to founder effects and the stochastic nature of genetic drift (Charlesworth and Charlesworth, 1987). Our results show that High Rescue was consistently nearly as effective as the Outbred Rescue treatment at increasing productivity (Figure 3.2b), while the Low Rescue treatment was only slightly more productive than the No Rescue treatment. Thus, the productivity of the source populations appeared to be a good indication of the productivity increase that would occur in rescued populations. The F statistic, total length of ROH and, the proportion of homozygous deleterious SNPs followed the pattern we expected with reductions from No Rescue to Low Rescue and reduced further in High Rescue (Figure 3.3, 3.4, 3.5b). Although these were not significant differences, they do follow the pattern expected with increasing amounts of genetic diversity being introduced. There were also only small differences in the number of deleterious SNPs between No Rescue, Low Rescue or High Rescue populations which is a key benefit of inbred rescuers (Kyriazis et al., 2021; Robinson et al., 2023). The genomic analyses indicates that the inbred rescues may be less successful than outbred rescued populations because they don't result in such high levels of heterozygosity and therefore fewer deleterious alleles are 'hidden' (Ralls et al., 2020). However, we see a large difference in productivity between High Rescue and Low Rescue, despite small differences in genomic measures. This difference could be due to cumulative effects of moderately or weakly deleterious alleles that can be difficult to detect, resulting in the low productivity of the inbred stocks used for Low Rescue (Hewett et al., 2024). The fixation of weakly deleterious alleles in the low productivity inbred stocks could prevent effective rescue, as the Low Rescue will be introducing these deleterious alleles that we could not

detect. The combined effect of a large amount of weakly deleterious alleles could potentially explain the productivity difference between High and Low Rescue and why we did not detect a significant difference in our analysis of deleterious SNPs. These results suggest that if conservation practitioners are considering inbred source populations for genetic rescue, those populations should be assessed in detail to select the population that will be the most effective at rescue.

Genetic swamping, where the unique genetics of a population is replaced, is another risk factor associated with genetic rescue (Roberts et al., 2010). Our PCA results (See Figure S2.1) show the rescued population individuals still clearly cluster with the pre-rescue inbred population they derive from, indicating that genetic swamping has not occurred. Our experiment involved just a single rescue event, showing that (limited) genetic introgression/rescue can work without leading to genetic swamping. Other attempts at rescue utilising a single rescue event similarly avoided genetic swamping (Onorato et al., 2024; Fitzpatrick et al., 2020). While these cases show that a small amount of gene flow can provide benefits, it may not be optimal for a conservation scenario. It is recommended that gene flow occurs consistently to maintain fitness benefits (Al Hikmani et al., 2024; Pazhenkova et al., 2025) for multiple reasons; 1) It is less likely a single rescuer would successful survive or reproduce in the wild (Griffith et al., 1989), 2) A single rescuer may not introduce enough genetic variation (Frankham et al., 2010), 3) multiple rescuers are unlikely to carry the same recessive deleterious alleles helping prevent unmasking of mutation load (Charlesworth and Willis, 2009; Hedrick et al., 2014). Multiple rescuers have been utilised in previous attempts in conservation scenarios; as many as 20 rescuers in some species (Madsen et al., 2004; Nichols et al., 2024). This increases the chance of introgression but will also increase the risks of outbreeding depression and genetic swamping. Furthermore, if gene flow is too low, inbreeding will resume in the population and any introduced recessive deleterious alleles will become expressed leading to lowered population fitness again. Clearly it is important to find a balance between reducing inbreeding and preventing genetic swamping and outbreeding to improve fitness.

In these experiments population size was limited each generation but the ability of the recipient inbred population to expand following rescue could be key to deciding between outbred or inbred source populations. If population growth is expected (i.e. because habitat is not limited) an outbred rescuer should be favoured as the future likelihood of inbreeding will decrease as the population size increases. Genetic rescue can enable population expansion, as was seen in the Florida panther (Pimm et al., 2006). In a situation where population growth is limited, for example by habitat constraints like for the Isle Royale wolves, the chance of

inbreeding resuming is much greater (Hedrick et al., 2019, 2014). This makes it more likely that introduced mutation load will be expressed in future generations. in this situation utilising an inbred population that has experienced purging may be preferred. However, in our experiment the population size was limited to 20 adults every generation mimicking a constrained population, yet Outbred Rescue was the most successful treatment. If the experiment had continued past generation nine the masked load may have been expressed, and fitness could have declined (Kyriazis et al., 2021). Alternatively, due to such a small population size purging may have been acting upon the introduced genetic diversity. This emphasises the need for long-term, well monitored studies of genetic rescue to understand the changes in mutation load and its expression. Risk of inbreeding resuming must be assessed before deciding between an inbred or outbred rescuer, as this could avoid outbreeding depression.

We also tested the effects of nutrient stress on the different rescue treatment populations. We would expect the rescued populations to be more able to cope with the stress than the No Rescue populations (Fox and Reed, 2011). Under nutrient stress we see no differences between the treatments. We also found this in previous experiments (West et al., 2025) suggesting that this stress may be too harsh, or the populations are unable to adjust to a change in diet. Future experiments could use different stressful conditions, such as heat stress, to examine if genetic rescue makes populations more resilient to environment change, which is expected as genetic rescue should increase adaptive potential (Sexton et al., 2011; Hoffmann et al., 2020).

A limitation of this study is that genomic data was only collected from the 4th generation following the rescue attempt, exploring genomic changes across each generation after rescue could be very informative. It would be possible to track the changes in genetic diversity, increasing initially and then declining if inbreeding resumes in later generations. The expression of mutation load could also be followed through generations, seeing the input from a rescuer and the hiding of the load through increase heterozygosity followed by conversion to expressed load in later generations. These predictions currently rely on simulations of population genetics due to the cost of WGS (Kyriazis et al., 2021), but if it could be done in a population it would be extremely insightful. Eventually, the effects of genetic rescue should be lost from a population that continues to inbreed. Comparisons across generations would allow in depth examination of introduced genetic diversity and mutation load changes, ideally following more generations than we did here, as we did not see a decline in productivity.

We find that attempting genetic rescue using an outbred source population is the most effective way to improve population fitness, supporting current recommendations (Ralls et al.,

2020). However, an inbred source population can provide effective rescuers, leading to increased fitness compared to No Rescue. How much of an increase in fitness an inbred rescuer provides depends on the productivity/fitness of the source inbred population in question. While inbred populations can be used in genetic rescue, it is important to know the relative fitness of the population for rescue to be a success. We found that genomic analysis gave insight into how rescue attempts improved fitness, with a clear signature of reduced inbreeding and less realised genetic load when rescued by an outbred rescuer. However, outbred rescuers did increase the number of deleterious SNPs in the rescued population (mutation load). We recommend that where possible outbred source populations should be utilised for genetic rescue, but with precautions taken to prevent future inbreeding., i.e. because of a population being unable to expand.

Chapter Four

Does genetic rescue disrupt local adaptation? An experimental test using thermally adapted *Tribolium castaneum* lines

4.1 Abstract

As a result of anthropogenic effects, many species now find themselves in small, genetically isolated populations prone to inbreeding depression and, therefore, at increased risk of extinction. Genetic rescue, the reduction in inbreeding via introducing genetic variation from one population to another, can alleviate inbreeding depression. However, a major worry in conservation -that has restricted the use of this technique- is that such augmented gene flow risks disrupting local adaptation, which may be crucial for the population's persistence. Using the red flour beetle (*Tribolium castaneum*), we assess if genetic rescue attempts disrupt the adaptation of populations that previously evolved to be adapted to reproduction at higher temperatures. We initiated genetic rescue by introducing rescuers drawn from populations adapted to either 30°C or 38°C, into inbred populations adapted to 38°C. We recorded subsequent population productivity over three generations at 38°C as a measure of population fitness. We found that a rescuer adapted to 38°C improved productivity the most, although using a rescuer adapted to 30°C still significantly increased productivity of the recipient inbred populations. In conclusion, using rescuers from a population with adaptations to similar selection pressures may be the best option, if it is possible to identify such factors/populations. However, this may rarely be the case for wild populations and using non-locally adapted individuals can still benefit overall population fitness, even in the face of considerable local adaptation.

4.2 Introduction

Climate change and habitat destruction are causing species to become fragmented into small and isolated populations, disrupting gene flow and leading to inbreeding, inbreeding depression and, consequently, an increased risk of extinction (Haddad et al., 2015). Fitness is reduced partly because of the accumulation of deleterious alleles within the population known as genetic load. Individual and population level fitness is reduced in such populations as inbred individuals are more likely to be homozygous for any recessive, deleterious alleles present in the population (Crnokrak and Roff, 1998; Charlesworth and Willis, 2009). Inbred

individuals may also lose the benefits of heterozygote advantage due to the increased homozygosity throughout the genome (Hedrick and Garcia-Dorado, 2016). Inbreeding depression can interact with environmental factors to drive populations into an extinction vortex (Soule and Gilpin, 1986; Blomqvist et al., 2010; Palomares et al., 2012). Therefore, mitigating the effects of inbreeding is a crucial component of conservation.

Genetic rescue refers to the increase in fitness observed when novel genetic variation is introduced by a rescuer (a conspecific from another population) to an inbred population (Ingvarsson, 2001; Hedrick et al., 2011). This process increases genomic heterozygosity within the target population, thereby reducing the genetic load expressed in individuals and improving both individual and population fitness. Genetic rescue has been extensively studied, reviewed, and successfully implemented in several endangered populations (Clarke et al., 2024; Frankham, 2016, 2015; White et al., 2023), including the Florida panther (*Puma concolor couguar*) (Pimm et al., 2006; Onorato et al., 2024). Furthermore, studies conducted in experimental systems have deepened our understanding of this process (Hufbauer et al., 2015; Fitzpatrick et al., 2019; Zajitschek et al., 2009; Bijlsma et al., 2010). However, risks potentially associated with attempting genetic rescue have led to a reluctance among conservation managers to utilise this method (Frankham et al., 2011; Edmands, 2007).

Outbreeding depression is one of the potential risks associated with implementing genetic rescue as a conservation measure (Tallmon et al., 2004; Bell et al., 2019). One reason that this may occur is because populations may be adapted to local conditions, and the input of novel genetic variation from other populations could disrupt the adaptive gene complexes that provide local adaptation. Rescuers may introduce non-locally adapted alleles, the expression of which could prevent adaptations from functioning, and this could exacerbate, not alleviate, fitness issues in the vulnerable recipient population (Kawecki and Ebert, 2004; Lenormand, 2002). To date, there are few such examples of outbreeding depression (Hedrick et al., 2019; Turček and Hickey, 1951; Loope et al., 2024), and it has been argued that the risk of such a detrimental impact is overstated if genetic rescue guidelines are followed (Frankham et al., 2011; Ralls et al., 2020; Powell, 2023)

Climate change poses a significant challenge for local adaptation and endangered populations (Hoegh-Guldberg et al., 2018). Species can survive such environmental shifts if they adapt to new conditions, however the rate of change is unprecedented, and genetically depauperate populations possess a limited capacity to adapt (Bellard et al., 2012). Introducing genetic variation into isolated populations (as is the case with genetic rescue

attempts) could be essential to enable them to adapt to the rapidly evolving climate by improving standing genetic variation (Bell and Gonzalez, 2009; Bell et al., 2019).

Populations which are already adapted to specific local conditions (e.g. high temperatures) could serve as important sources for genetic rescue, if those adaptations are suited to future climatic conditions. Alternatively, they could be used for targeted geneflow: a process to introduce specific adaptations to improve population fitness, which has been attempted a few times with mixed results (Kelly and Phillips, 2019b; Rudin-Bitterli et al., 2021). If such adapted populations are affected by inbreeding depression, it would be crucial to restore fitness while avoiding outbreeding depression and the loss of local adaptation so they can be utilised in the future.

Tribolium castaneum, a beetle in the Tenebrionidae family, has long been a model species (Pointer et al., 2021) utilised to study population genetics, genetic rescue, and thermal tolerance (Hufbauer et al., 2015; Wade and Goodnight, 1991; Sokal and Sonleitner, 1968; Sales et al., 2021). In the present study we used *T. castaneum* populations previously selected, over 150 generations, to reproduce successfully at elevated temperatures; specifically at 38°C compared to the original population optimum of 30°C (Vasudeva et al., 2019; Skourti et al., 2022). These thermally adapted populations are less fit than the 30°C beetles which produce more offspring at both 30°C and 38°C. (Lewis, 2020). However, when developing from an egg at 38°C the thermally adapted beetles produce more eggs and a greater proportion will hatch than a 30°C reared at 38°C (Lewis, 2020). The key adaptation in these lines is the ability to develop at 38°C and maintain the ability to reproduce. Genetic rescue has been attempted with these adapted lines before, but no evidence of rescue was observed (Lewis et al., 2024).

We replicated these adapted lines and inbred them to further reduce genetic diversity potentially increasing inbreeding depression and the likelihood of genetic rescue succeeding. We utilise them as recipient populations in an experiment to assess the impact of genetic rescue on local adaptation for thermal tolerance. We predicted that introducing a rescuer from another thermally adapted population will enhance population fitness by reducing inbreeding depression (i.e. genetic rescue) without disrupting the local adaptation to high temperatures. In contrast, using a rescuer from a non-thermally adapted line reduces the recipient population's fitness by disrupting local adaptation, which will prevent reduction of inbreeding depression. If the non-adapted rescuer produces many offspring (as it did not develop at 38°C) it could lead to the extinction of the population. All populations in the

experiment were monitored by measuring the number of adult offspring produced for three generations following the rescue attempt.

4.3 Methods

4.3.1 Husbandry

T. castaneum in experimental populations were maintained on standard fodder (90% white organic flour, 10% brewer's yeast and a layer of oats for traction) in a controlled environment of 30°C (unless otherwise stated) and 60% humidity with a 12:12 light-dark cycle. Populations were maintained following a standard cycle of virgin adults having seven days of mating and oviposition followed by the removal of adult beetles from the fodder, using 2 mm and 850 µm sieves, so that only eggs remain. Each generation is initiated with a number of adults (line dependent, see below) that are given 7 days to mate and lay eggs before being removed. The eggs are left for 35 days to develop into mature adults.

4.3.2 *Tribolium castaneum* lines

Krakow super strain (KSS) combined fourteen laboratory strains to maximise genetic diversity in one population maintained at 600 individuals (Laskowski et al., 2015). This line is highly productive at 30°C but has reduced fitness at 38°C. This was used as the non-adapted rescuer population.

Thermal lines: Ten populations (100 adults per population) founded from KSS that had been experimentally evolved for ~150 generations at an environment temperature of 38°C (Dickinson, 2018), thus imposing selection for development and reproduction at this temperature, considerably above the thermal optimum for *T. castaneum* (Howe, 1962). All other conditions were as described above except for a shorter development period of 27 days. These were used as the thermally adapted rescuer populations.

4.3.3 Inbred lines

Ten inbred populations were created from the ten thermal lines described above. Adult beetles from each thermal line were isolated for two weeks to ensure any fertilized eggs were laid. Pairs from the same line were formed from these isolated adults resulting in a single pair bottleneck for each thermal line. Their full sibling offspring were paired again for a second bottleneck. After these two single pair bottlenecks, populations of 10 male and 10 female

beetles were created. These beetles were full sibling offspring of full sibling pairs. Six inbred experimental populations were created from each of the ten inbred thermal populations, creating 60 experimental populations. However, one inbred population only produced enough offspring for four experimental populations, resulting in 58 experimental populations split over two temporal blocks – 30 in set one and 28 in set two. The two blocks were maintained one day apart for ease of handling but otherwise treated the same. Each population received a random ID number to blind the experiment and avoid bias. These experimental populations initiated every generation at a size of ten males and ten females sourced from the offspring of the previous generation to reduce the effects of density dependence (Duval et al., 1939; King and Dawson, 1972; Janus, 1989). All these experimental inbred recipient populations were kept at 38°C in A.B. Newlife 75 Mk4 forced air egg incubators (A.B. Incubators, Suffolk, UK); all other conditions were kept as described in the husbandry section.

4.3.4 Genetic rescue protocol

Populations were maintained at a populations size of 10 males and 10 females in 125 ml tubs containing 70 ml of standard fodder. After seven days of oviposition eggs were left to develop for ~21 days to the pupal stage when pupae (10 Male, 10 Female) were taken to establish the next generation. The remaining individuals in the populations were left for another ten days to complete development and then were frozen before manual counting. Pupae taken at day ~21 were kept in plastic dishes containing 10ml standard fodder in single-sex groups until they matured into adults after 10±2 days, and the next generational cycle began with virgin adults, avoiding overlapping generations.

A single male from each inbred recipient population (10 males and 10 females) was removed and replaced with a single male rescuer to avoid demographic rescue effects (increased population fitness due to increased population size) (Bell et al., 2019; Ingvarsson, 2001). Three treatments were undertaken, with each of the 10 initial inbred thermal population represented by two experimental populations in every treatment, except for one inbred thermal population, which only produced four experimental populations resulting in one fewer control and one fewer thermally adapted rescue populations (See figure 4.1). The three treatments were as follows: 1) controls – nineteen populations received no rescue (the male was not removed). 2) locally adapted rescue – 19 populations received a 38°C-adapted rescuer (a male from a different initial thermally adapted population than the one from which the inbred line was derived). 3) non-locally adapted rescue – 20 populations received a non-thermally adapted rescuer (a KSS male, see above).

Population fitness was measured using productivity, i.e. the number of mature adult offspring the population produced each generation. Populations were maintained for three non-overlapping generations following rescue (each generation initiated with 10 females and 10 males). Fifty-eight populations were initiated, but two populations were lost after generation two from human error in maintenance. Generation three therefore consisted of 19 control populations 18 thermally adapted rescue populations and 19 non-thermally adapted rescue populations. Data for those populations lost in generation three was still included in the analysis for generations one and two.

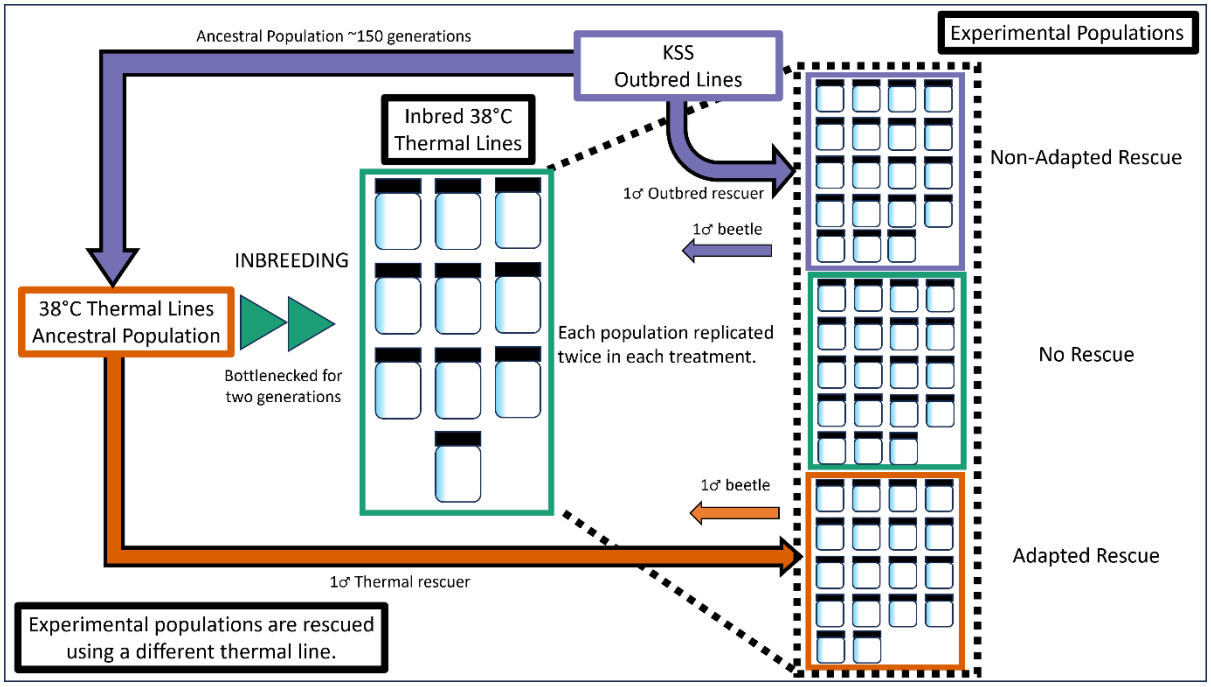

Figure 4.1: Experimental design for the attempted genetic rescue of inbred thermally adapted *T. castaneum* populations by a single thermally adapted or non-thermally adapted rescuer. Inbred, thermally adapted lines were created by inbreeding lines thermally adapted to 38°C over ca 150 generation (Dickinson, 2018) with two generations of full sibling matings, before being kept for three generation at n = 20 (10 females and 10 males) during the experiment. The ten inbred thermal lines were replicated to be represented in each experimental treatment twice. The final sample size was 56 experimental populations (see main text).

4.3.5 Statistical analysis

R V.4.4.1 (R Core Team, 2024) was used with R Studio version 2024.04.2+764 (Posit team, 2024). Data management and exploration were performed with Tidyverse (Wickham et al., 2019), stats (R Core Team, 2024), Rmisc (Hope, 2022) and googlesheets4 (Bryan, 2023). Ggplot2 (Wickham, 2016) was used to visualise results. Data distribution was checked using the shapiro.test function (R Core Team, 2024). The glmmTMB package (Brooks et al., 2017)

was used to fit generalised linear mixed models (GLMMs). DHARMa (Hartig, 2022) was used to check model fit and the check_collinearity function from the performance package (Lüdecke et al., 2021) to test variance inflation factor scores (VIF). No overdispersion or collinearity (VIF<3 for all variables) was found. R^2 was determined using the r.squaredGLMM function in MuMIn (Bartoń, 2024).

A GLMM was run using the productivity data for all generations as the response variable to test if there were differences between the treatments. Fixed variables included treatment, generation and, generation^2 as well as interactions between these variables, the random factors of ID nested within thermal line were included. There was no significant interaction with generation^2 so the interaction was dropped from the model. A negative binomial distribution was used as productivity is count data and the fit was better than Poisson distribution. The Control (no rescue) treatment was set as the baseline factor for comparison. The baseline was changed to non-adapted to compare between the two rescue treatments post-hoc. Generation 0 was not included as it was before treatment (rescue) was applied.

GLMMs, constructed as described above, but excluding the generation variable, were then run on each generation individually to test if there were significant differences between the treatments in each generation. The baseline was changed to non-adapted rescuers, to compare between the two rescue treatments post-hoc.

4.4 Results

Only the thermally adapted rescuer treatment resulted in significant interaction with generation compared with the no rescue control treatment (Table 4.1, Figure 4.2). In a post hoc test, the two rescue treatments interactions with generation were not significantly different (Estimate = 0.038, SE = 0.037, z = 1.01, P = 0.312, 95% CI = -0.035, 0.111).

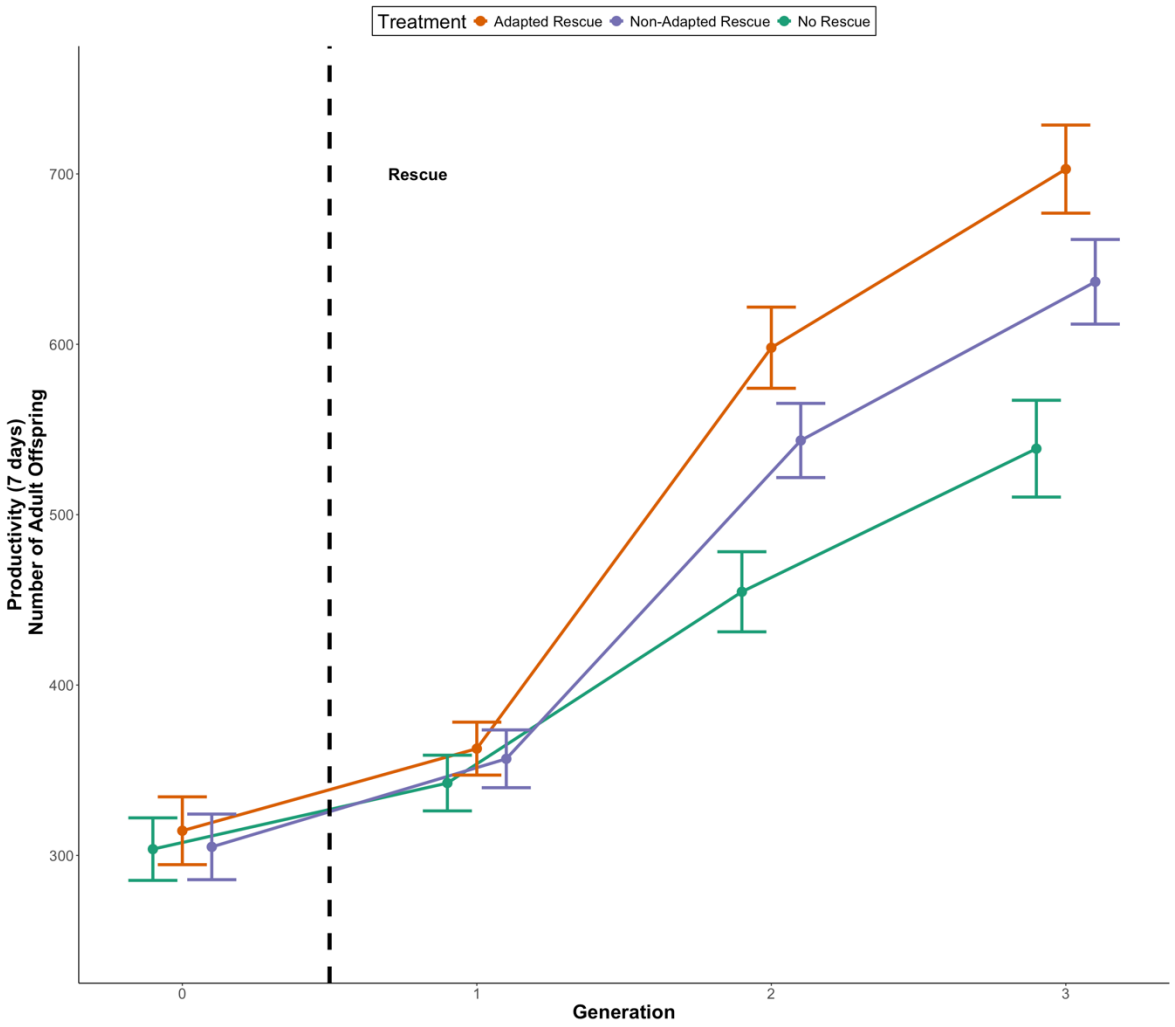

Figure 4.2: The effect of introducing a 1) thermally adapted (orange) or 2) non-adapted (blue) rescuer, compared to control populations (green) on the mean productivity of small inbred thermally adapted populations of *T. castaneum* (Population size = 20, Number of experimental populations = 58 (56 in generation 3)) over three generations after a single introduction event (dotted vertical line) while maintained at 38°C. A single adapted or non-adapted male rescuer replaced one male in the inbred population containing ten males and ten females. There was a significant difference in productivity between both rescue treatments and control treatments (Table 4.1), but no significant difference between the two rescue treatments (Estimate = 0.038, SE = 0.037, z = 1.01, P = 0.312, 95% CI = -0.035, 0.111). Plot is jittered to aid in visualisation, standard errors are shown.

Table 4.1: Summary of a GLMM fitted to model the productivity of small, inbred, thermally adapted *T. castaneum* populations (Population size = 20, Number of populations = 58) after receiving a rescue by a thermally adapted, or non-adapted, male rescuer or no rescue over three generations. Predictors in bold are significant ($P < 0.05$). Marginal R^2 = 0.637, Conditional R^2 = 0.740. ^Two populations were lost in Generation 3.

Predictor	Estimate	SE	*z*	*P*
Intercept	5.192	0.118	44.01	< 2e-16
Treatment (No Rescue)				
Thermally Adapted Rescue	0.022	0.092	0.23	0.815
Non-adapted Rescue	0.029	0.092	0.31	0.755
Generation	**0.724**	**0.110**	**6.56**	**< 0.001**
Generation²	**-0.122**	**0.026**	**-4.71**	**< 0.001**
Treatment (No Rescue) * Generation				
Treatment (Thermally Adapted Rescue) * Generation	**0.093**	**0.039**	**2.38**	**0.017**
Treatment (Non-adapted Rescue) * Generation	0.055	0.039	1.41	0.160
Random	172 observations		Variance	
ID:Thermal line	58^		0.003	
Thermal line	10		0.007	

Independent tests of productivity differences in each separate generation were then undertaken (Table 4.2). In generation one, no significant differences existed between any of the treatments (Non-Adapted – Adapted: Estimate = 0.015, SE = 0.057, $z = 0.26$, $P = 0.794$). In both generation two and three, productivity in the rescue treatments both differed significantly from the no rescue control (Table 4.2), they were not significantly different from each other in generation 2 (Estimate = 0.095, SE = 0.057, $z = 1.67$, $P = 0.095$) but they were in generation 3 (Estimate = 0.097, SE = 0.045, $z = 2.17$, $P = 0.030$).

Table 4.2: Composite table of three GLMM results for each generation of the productivity of small, inbred, thermally adapted *T. castaneum* populations (Population size = 20, Experimental populations = 58 or 56) after receiving a rescue by a thermally adapted, or non-adapted, male rescuer or no rescue.

	Gen 1 Estimate	SE	*z*	*P*
Intercept (No Rescue)	5.810	0.055	105.84	<2e-16
Thermally Adapted Rescue	0.061	0.058	1.05	0.291
Non- Adapted Rescue	0.056	0.057	0.81	0.420
	Gen 1 observation		Gen 1 variance	
Random	58			
ID:Thermal	58		<0.001	
Thermal	10		<0.001	
	Gen 2 Estimate	SE	*z*	*P*
Intercept (No Rescue)	6.100	0.057	107.29	< 2e-16
Thermally Adapted Rescue	**0.284**	**0.058**	**4.90**	**< 0.001**
Non- Adapted Rescue	**0.188**	**0.057**	**3.28**	**0.001**
	Gen 2 observation		Gen 2 variance	
Random	58			
ID:Thermal	58		0.030	
Thermal	10		0.005	
	Gen 3 Estimate	SE	*z*	*P*
Intercept (No Rescue)	6.263	0.051	122.93	< 2e-16
Thermally Adapted Rescue	**0.277**	**0.045**	**6.20**	**< 0.001**
Non- Adapted Rescue	**0.180**	**0.044**	**4.07**	**< 0.001**
	Gen 3 observation		Gen 3 variance	
Random	56			
ID:Thermal	56		0.017	
Thermal	10		0.016	

4.5 Discussion

We tested the effect of introducing a thermally adapted or a non-adapted genetic rescuer on the fitness of inbred, thermally adapted populations of *T. castaneum*. Results show that populations receiving thermally adapted rescue had increasing productivity over the generations compared to the no-rescue populations. When looking at each generation individually, both rescue treatments improved productivity compared to the no-rescue control in the second and third generations. In the third generation, thermally adapted rescue had improved productivity over both the no-control and non-adapted rescue treatments.

Introducing new genetic diversity into an inbred population via augmented gene flow (the introduction of a rescuer individual from another populations) should improve population fitness, i.e. genetic rescue, as has been shown previously (Madsen et al., 2020; Johnson et al., 2010). The results of our study support this, we show that the productivity of the inbred populations improved when rescued (with a single introduced male) by either rescue treatment. Importantly, in our present study we also found that non-adapted rescuers improved productivity compared with no rescue, despite the risk of disrupting adaptations. There was only a statistically significant difference between the productivity of the two rescue treatments in the final generation, despite our hypothesis of reduced fitness from non-adapted rescue (Bachmann et al., 2020). The non-adapted rescue population was a highly outbred ancestral population, the type recommended for use in genetic rescue (Ralls et al., 2020). However, our experimental populations have been genetically isolated for 150 generations, considerably more than the 'less than 20 generations' of divergence recommended for genetic rescue if the populations are in different environmental conditions (Frankham et al., 2011). Guidelines suggest geneflow within the last 500 years, 150 generations reflects, for example, ca 675 years in the Florida panther (Hostetler et al., 2013). Our results show that despite the length of isolation and different adaptations genetic rescue improved fitness, adding to the growing evidence that the risk of outbreeding depression in genetic rescue may be exaggerated (Powell, 2023; Fitzpatrick and Reid, 2019; Fitzpatrick et al., 2015).

The rescue by a thermally adapted rescuer had the highest productivity, showing more effective rescue than a non-thermally adapted male. This matches with recommendations that genetic rescue should utilise source populations from similar environmental conditions as it reduces the risk of disrupting local adaptation (Lenormand, 2002). The ten thermal lines all originated from the same, very genetically diverse, KSS stock populations but were

separated prior to experimental evolution for adaptation to 38°C (Vasudeva et al., 2019). Consequently, while being very closely related, the different lines may all contain different subsets of genetic variation, thus providing the substrate for genetic rescue between the inbred thermal populations. The different lines mimic populations that have diverged from a larger outbred population into similar environmental conditions, higher temperatures in this case, under natural conditions. The selection process for thermal adaptation did result in bottlenecking of the lines, they should be much less genetically diverse than the outbred stock (Dickinson, 2018). The higher productivity from the attempted rescue with these lines is likely due to the similar adaptations to 38°C.

In our experiment, the productivity difference between the rescue treatments may be caused by some disruption of local adaptation. The key adaptation for these thermal lines is the capacity to remain fertile while developing at 38°C which the non-adapted individuals may disrupt (Sales et al., 2018; Vasudeva et al., 2019; Lewis, 2020). If the increase in fitness were solely attributable to the introduction of increased genetic variation reducing inbreeding, one would expect that the outbred (but not thermally adapted) rescue would yield greater productivity than the thermally adapted rescue (Ralls et al., 2020). However, we see that the thermally adapted rescue was more productive than the non-adapted (outbred) rescue suggesting some disruption of local adaptation. This fits with the suggestion that it is important to rescue from adaptively similar populations (Edmands, 2007). Translocations between populations have been recommended to enable adaptation to thermal changes in some species, though they have yet to occur (Miller et al., 2020a). Such conservation translocations from adapted populations could be important in the future if they improve population resilience to a changing climate (Fitzpatrick and Reid, 2019).

Our study using experimentally evolved lines *T. castaneum* provides evidence of how genetic rescue of an inbred population with an environmental adaptation is affected by the adapted characteristics of the rescuer population. Rescuing with another similarly locally adapted population yielded the greatest fitness benefit, supporting current recommendations for genetic rescue. The increase in fitness conferred by non-adapted rescuers adds to the evidence that the risks of outbreeding depression may be overstated, or at least that benefits of alleviating inbreeding depression can surpass any negative impacts of disrupting local adaptation. This was seen in the Florida panther rescue, where individuals from an arid environment were used to rescue a tropical population and was very successful at restoring population fitness. Future work should focus on the genomic and functional basis of adaptation to study in detail how rescue may disrupt these adaptations. Additionally, experimental tests on the efficacy of targeted gene flow could improve confidence in its use.

Clearly, where possible, similarly adapted, closely related, populations should be utilised as sources of genetic rescue for the best results. Nevertheless, genetic rescue of inbred populations may be beneficial for improving fitness and survival even if there are adaptive differences between the populations.

Chapter 5

General Discussion

5.1 Overview

The overall aim of this thesis was to improve our understanding of the traits that can affect the outcome of genetic rescue attempts. I used the model species *Tribolium castaneum* to create inbred populations that I could then attempt to rescue. My first objective was to see if I could successfully rescue the inbred populations, measured through increased productivity, i.e. the number of offspring the population produces. The second objective was to test if utilising individual rescuers with different traits, or rescuers from source populations evolved under different conditions, would alter the effectiveness of genetic rescue. This allowed me to examine if different rescuers or source populations achieved genetic rescue and how the productivity of the rescued populations was affected by those differences.

In my first data chapter (chapter two) I tested if genetic rescue could be achieved using either a male or female rescuer from an outbred source population. I found both treatments improved the productivity of the inbred populations compared with the no rescue control populations, and there were no significant differences between the two sexes ability to rescue. Male rescuers improved the productivity of rescued populations earlier than the female rescuers, likely as a result of differences in reproductive output. I followed the populations for ten generations post-rescue attempt to capture the effects of rescue beyond the initial fitness increase. I then tested if a background of sexual selection would produce a better rescuer. Sexual selection results in improved fitness and can lower genetic load in populations, so individuals from such populations should be good candidates as rescuers. Using male rescuers from either a population maintained under sexual selection or a population with no sexual selection, I attempted to rescue inbred populations. The results show that only the rescuer from a sexually selected background resulted in a successful rescue. These populations were monitored for nine generations post rescue to attempt to observe the full effects of a genetic rescue on the recipient populations.

In chapter three I attempted to rescue inbred populations using male rescuers sourced from either an outbred population or inbred populations. Outbred populations are genetically diverse and introducing this diversity should have the greatest fitness effects on the recipient population. However, this could introduce hidden mutation load which may reduce population fitness in later generations, inbred populations should have purged their mutation

load which may make them better rescuers in the long term. Productivity was measured for nine generations after the rescue treatments were applied to capture the full effects of rescue on fitness. I also took samples from the fourth-generation post rescue attempt to test how the inbreeding coefficient, runs of homozygosity and mutation load in the recipient populations had been affected by the rescue treatments. I found that the outbred rescue was the most effective at improving productivity and genetic measures. Outbred Rescue lowered the populations inbreeding coefficient, reduced the proportion of the genome in runs of homozygosity and reduced the number of deleterious alleles in homozygous state. Introduced genetic diversity had increased heterozygosity lowering inbreeding and increasing the adaptive potential of recipient populations. Additionally, mutation load had been converted to heterozygous, improving population fitness by preventing expression of deleterious alleles.

Inbred rescuers still rescued the populations, but they had lower productivity than the Outbred Rescue and did not have a significant effect on the genomic measures compared to No Rescue. Outbred rescuers did introduce more mutation load than the inbred rescuers, but this load was mostly hidden in a heterozygous state. The fitness of the population inbred rescuers were sourced from effected the success of genetic rescue. Rescuers from more productive inbred populations improved the productivity of the recipient populations more than those from a low productivity source population. The level of fitness of a source population was key to the outcome of rescue, there was a correlation between fitness and level of inbreeding.

In the fourth chapter, I tested whether genetic rescue disrupted local adaptation, a key concern in conservation. Using populations that had been adapted to reproduce at 38°C, rather than 30°C, I created small, inbred populations that I could attempt to rescue. The inbred populations were rescued using either an outbred population not adapted to higher temperature or another temperature adapted population. The outbred population should have disrupted the adaptation reducing fitness, while the temperature adapted population should have improved fitness and not disrupted the adaptation. I found that the non-adapted outbred rescuer still improved population fitness but the rescue from another thermally adapted population improved fitness more. This supports recommendations of using source populations from similar habitats to the recipient populations.

Overall, I find that genetic rescue can be achieved in these small, inbred populations and selection of the source population is important to the outcome. I will now discuss my findings and future directions for research.

5.2 Findings

In all my experiments I observed improvements in the productivity of rescued inbred populations compared with the controls for at least one rescue treatment. Rescue attempts using a single rescuer in a single event managed to consistently achieve a genetic rescue effect despite different source and recipient populations being utilised. Combined with previous studies testing genetic rescue (Lewis et al., 2024; Hufbauer et al., 2015), and the availability of genomic resources *T. castaneum* makes for a suitable system to test genetic rescue. There are a number of available stock populations that can be utilised in experiments, and populations are easily manipulated to create unique traits such as the thermally adapted, sexually selected and inbred populations used in my thesis. The availability of the reference genome and annotations provided the resources to combine experiments with genomic data to further our understanding of genetic rescue.

The results of my data chapters also show that introducing genetic diversity to improve population fitness was a reproducible process, occurring in multiple experiments using different source populations for rescuers. In addition to my own work and studies in *T. castaneum*, other model species have been utilised to test genetic rescue (Pérez-Pereira et al., 2025; Bijlsma et al., 2010; Zajitschek et al., 2009). Outside of model laboratory species, experimental tests on populations have also resulted in genetic rescue (Robinson et al., 2017; Lindsay et al., 2020; Fitzpatrick et al., 2019) as well as the results of planned conservation or natural occurring genetic rescues (Miller et al., 2020b; Hasselgren et al., 2018). The wide range of studies on different organisms, in different conditions and with different objectives all reporting successful fitness increases from rescue attempts shows how reproducible the process is. The success of genetic rescue attempts across taxa adds to the growing evidence that genetic rescue is a viable and achievable strategy for the conservation of inbred populations.

Importantly, my experiments were conducted under benign conditions, unlike wild populations which may be suffering from environmental stress. Inbreeding depression can be exacerbated under environmental stress reducing population fitness, genetic rescue alleviating the inbreeding depression could allow the population to better cope with the environmental stress (Armbruster and Reed, 2005; Fox and Reed, 2011). Alternatively, the stress, if harsh enough, could negate the fitness benefits of reducing inbreeding depression, preventing population recovery despite reduced inbreeding. I specifically set out to test the interacting effect of environmental stress and genetic rescue in my nutrient stress experiments, I found that a highly stressful condition repressed the benefits of genetic rescue.

In all experiments with a stress treatment the productivity differences between treatments disappeared when replicated onto the nutrient stress (See chapters 2 & 3). The nutrient stress was reducing the amount of yeast, the primary protein source, that the beetles had access to. This slows development and can encourage behaviours such as cannibalism which will reduce the number of individuals surviving to maturity (Sonleitner and Gutherie, 1991). As a result, this stress may have been too severe for reduced inbreeding depression to counteract which is why there were no differences between treatments under nutrient stress.

However, another study on *T. castaneum* found genetic rescue effects when utilising nutrient stress with reduced yeast and a novel carbohydrate source (Hufbauer et al., 2015). Unlike my study the whole experiment was on stressful media with populations having a generation to adjust before rescue occurred. The sudden switch to stressful conditions in my experiment could explain why there were no differences between treatments as there was no selection for nutrient stress on the populations until that point. Perhaps if more generations had been monitored under stressful conditions we may have seen differences in how well the treatments adapted with the pressure in place. Replicating the rescued populations onto stress happened multiple generations after the rescue attempt and the benefits of rescue may have been reduced by multiple generations of inbreeding. If the rescue had been under stress conditions or stress was applied sooner the populations may have had the genetic diversity to cope with the stress. Other measures of fitness such as the number of eggs laid, may have been a better metric under nutrient stress as this would be less affected by slower development or cannibalism if counted before hatching began. Genetic rescue alone may improve fitness and contribute to population persistence, but it is key that other threats such as habitat degradation or disease are addressed so that populations can recover. Factors like environmental stress may supress the benefits of genetic rescue preventing an increase in population numbers.

Some treatments did not provide significant fitness benefits within experiments. Rescuers from a no sexual selection population (chapter two) and rescuers from a low productivity inbred population (chapter three) did not significantly change the productivity of recipient populations from the no rescue treatments in those experiments. These two source populations are expected to be low fitness populations due to their population history. In the no sexual selection populations mate choice is eliminated via isolation of single pairs, the lack of sexual selection allows unfit individuals to contribute to the next generation meaning low fitness traits are maintained and preventing the removal of mutation load. They are also maintained at a small population size of 40 individuals so are unlikely to be highly genetically diverse. The low productivity inbred populations had been through three bottlenecks of a

single pair and are expected to be highly inbred and suffering from inbreeding depression. These were the inbred populations that were showing the lowest fitness, measured by productivity, likely suffering the most from inbreeding depression. Both these source populations therefore should have low genetic diversity and low individual fitness. Other studies have found little to no effect of genetic rescue attempts on inbred populations. Genetic rescue can fail for numerous reasons such as failure of the translocated individuals to reproduce or benefits being short lived leading to inbreeding in later generations (Pavlova et al., 2024; Nichols et al., 2024). Comparing these studies to mine we did not see even short-term benefits in these treatments, so the resumption of inbreeding was not the issue. Perhaps, failure of the rescuers to reproduce could explain the lack of rescue effect but this is unlikely given how promiscuous my study species is. My experiments were lab based with replication making it even more unlikely that either of these two effects occurred in all populations preventing rescue, this one of the advantages of model species over wild populations. It is likely my treatments failed to rescue the inbred populations as they are introducing low amounts of genetic diversity and may carry weak or mildly deleterious alleles as a result of genetic drift (Ralls et al., 2020).

It is important to have fitness knowledge of the source populations, the sexually selected population has the same effective population size as no sexual selection but improved the productivity of the recipient populations (Lumley et al., 2015). The high productivity inbred lines underwent the same inbreeding procedure as the low productivity but improved recipient population productivity nearly as well as an outbred rescue. Despite being the same population size and having similar demographic histories to their contrasting treatment in the experiments there were clear differences in how effective they were as rescuers. The additional fitness information allowed me to separate these source populations and find that genetic rescue is affected by these differences. While simulations and experiments have been carried out looking at the differences between outbred and inbred rescue (Kyriazis et al., 2021; Pérez-Pereira et al., 2025), to my knowledge there is no research on the differences between inbred populations as sources of rescue. If carrying out a genetic rescue attempt as much information as possible must be collected on potential source populations to maximise success and the difference in fitness between inbred populations could be important.

A key criticism of genetic rescue experiments is the lack of long-term monitoring, though this is increasingly being addressed (Nichols et al., 2024; Pérez-Pereira et al., 2025). In my first three experiments the populations were monitored for nine or ten generations after the rescue attempts to try and capture the long-term effects of rescue. In these experiments there was a clear change in productivity over the generations, with an initial increase in productivity

following the rescue then a plateau, and in some cases a significant decline (chapter two). The increase in productivity significantly above control treatments occurred in generation two or three depending on the treatment and experiment, showing that the rescuers were not having a significant impact immediately rather it was their offspring that improved population productivity. The peak in productivity seems to range from generation three to generation five, again depending on the experiment and treatment, suggesting that by four or five generations the maximum benefits of rescue are achieved. This is important as many studies on genetic rescue only observe for a few generations following the rescue and in our experiments this would only capture the increase and potentially the peak in fitness (Clarke et al., 2024).

Monitoring the populations for nine or ten generations meant that the decline from the peak productivity was observed. This varied in each experiment with some treatments declining from the peak (chapter two) and some seeming to slightly decline then plateau (chapter three). The consistent negative effect of generation on productivity in the models shows that at this small population size it is likely inbreeding was occurring and affecting productivity. Limiting the population size in my experiments mimics a population in a restricted habitat that prevents population growth. A restricted population will lead to inbreeding resuming which can reduce the benefits that the genetic rescue may have introduced, potentially repeating the population crash of the Isle Royale wolves (Hedrick et al., 2014). The rescue treatments, when effective, increased productivity but did not seem to prevent this negative generational effect. The finding that generation was affecting the population fitness shows that genetic rescue can improve fitness, but without a holistic approach addressing other threats, such as restricted habitat, these benefits may be short lived. My findings support the criticisms of short-term genetic rescue studies. I found that generation is a significant factor in my experiments having a negative effect on the productivity of inbred populations. Short term studies may only be capturing the initial increase and peak in fitness improvement, missing the decline in later generations. Even after the peak in rescue effects only in the sexual selection experiment did the rescued populations productivity return to the same levels as the no rescue. The few long-term studies on genetic rescue do find beneficial effects many generations after the rescue attempt, more research is needed to fully understand the duration of genetic rescue and how long monitoring should be in place (Pérez-Pereira et al., 2025; Madsen et al., 2020).

The no rescue control treatments were highly important in my experiments to identify when differences in productivity occurred. Notably the control treatments often increased in productivity in a similar pattern to the rescue treatments. A potential explanation is the reduction in density as population size was smaller in the experimental setup which can affect egg-laying (Sonleitner and Gutherie, 1991). *T. castaneum* females secrete chemicals into the

fodder to discourage other females from laying eggs, in less dense fodder there are less secretions encouraging egg laying (El-Desouky et al., 2018). However, these increases in productivity occurred in generations after the treatments had been applied. Potentially, this was caused by transgenerational effects, stress has been shown to have transgenerational effects on offspring production in *T.castaneum* (Gilad and Scharf, 2019). I tried to account for transgenerational effects by having populations in the experimental setup for a generation before treatments were applied, so that they were not directly moved from a dense population. Another explanation could be fluctuations in the controlled environment, in chapter two the sexual selection rescue had an increase in productivity for all treatments, including nutrient stress, in generation nine. This mostly likely is due to some change in their environment (temperature, humidity, fodder) affecting all populations and treatments equally. The no rescue treatments show that despite these increases and decreases in productivity that were part of the experimental setup genetic rescue improved productivity, following the same patterns but at a higher fitness.

The experiment that tested outbred and inbred source populations utilised genomic information alongside the productivity data, allowing for a direct comparison of these two measures of genetic rescue success. Importantly I found that the genomic information very closely matched the expectations for each treatment, with Outbred Rescue having the biggest effect on genomic measures. The Inbred Rescue treatments were not significantly different from No Rescue, but the slight changes were in the expected direction. The Low Rescue populations were more similar to No Rescue in genomic measures than the High Rescue populations were. The High Rescue interestingly had nearly as high productivity as Outbred Rescue, but this wasn't reflected in the genomic data. This shows that in a conservation situation where data can be difficult to collect genomic information can give good estimates of the productivity benefits the population is likely to accrue. However, the difference in genomic measures and productivity for the High Rescue populations shows that genomic data may not give the whole picture. It is unlikely we would predict the productivity seen for High Rescue based on the genomic data, where it was not significantly different from No Rescue. Genomic data generally correlates with observed fitness data (Pérez-Pereira et al., 2025; Nichols et al., 2024; Onorato et al., 2024), but my experiment shows that there may be some exceptions. This could be due to that fact the differences in fitness between similar inbred populations have not yet been studied in regards to genetic rescue. Genomic data is powerful and can tell us much about the genetics of a population, through measures such as inbreeding coefficient and mutation load, allowing us to infer the population fitness. However, as much data as possible must be collected about a population to be confident in the outcome of a genetic rescue as shown by the High Rescue treatment in chapter three.

A criticism of my studies is that these experiments used a model species in a laboratory setting and do not reflect the conditions found in a wild population. The results cannot be directly mapped on to a conservation scenario because of this, but suggestions for conservation action can be made. As stated above the rescues were performed in benign controlled conditions, an extremely unlikely scenario for species of conservation concern. In a wild population there will be multiple interacting factors that affect a populations fitness and that cannot be replicated in a laboratory (Soule and Gilpin, 1986). Additionally, our species is highly productive, so may only be representative of species with similar life history strategies. It could be difficult to apply these results where twenty individuals can produce hundreds of offspring to a species where individuals produce one or two offspring a generation. The stochasticity of a wild population will make genetic rescue more difficult to achieve, than in a laboratory setting. There are advantages to my approach also, my controlled experiments show that genetic rescue occurred consistently, and we can improve the chance of success by accounting for traits of rescuers. The lack of many interacting factors affecting populations allows the focus to be on the traits being tested. The ability to have replicated populations to increase sample size improves the power of the findings compared to studies done on examples in a single wild population (Nichols et al., 2024; Pavlova et al., 2024). Being able to control the populations allows mimicking of some natural populations. Only twenty individuals contributed to each generation in the experiments despite the production of many offspring, artificially restricting the population size and testing the effects this has without risk to a wild population.

5.3 Future research

The findings of this thesis have opened future avenues to explore how genetic rescue affects inbred populations. The experimental setup I used was consistent across experiments but these variables (i.e. population size, number of rescuers, non-overlapping generations) could be changed to examine how genetic rescue is affected. Increasing the population size may give males a greater advantage over female rescuers due to differences in reproductive output. Females are limited by the number of eggs they can lay while males are limited by the number of mates available. In a larger population a female will still produce the same number of eggs, whereas a male will be the father of more eggs than it would in a small population. A larger population size may also help to prevent inbreeding resuming after rescue, this could remove the effect of generation I found. A larger population means more mate choice so individuals are less likely to mate with a close relative, this will reduce inbreeding in the population and as generations pass the resumption of inbreeding will be slowed. Reducing inbreeding should

also reduce any loss of genetic diversity introduced by the rescuer and prevent the expression of mutation load. Single rescuer and rescue event may result in a slower rescue effect as the rescuer and their offspring are a smaller proportion of the population. A larger population size might result in a slower but longer lasting rescue. In addition to these experiments utilising a population size of 20 individuals, previous studies of genetic rescue in *T. castaneum* have used differing population sizes of 50, 150 and 10,000. The test of genetic rescue at sizes of 50 (one rescuer) and 150 (three rescuers) under nutrient stress found improved fitness and lower extinction risk from the rescue treatment (Hufbauer et al., 2015). The other study attempted to rescue thermally adapted populations by introducing 1000 rescuers into populations of 9000 thermally adapted individuals (Lewis et al., 2024). Fitness was measured by reproductive output of pairs and survival of individuals in a heatwave, they found no differences between the treatment receiving migration and the control. These two studies had different measurements of fitness one looking at the population level and the other at pairs or individuals, but the difference in population size may also explain the contrasting results.

Using multiple rescuers or multiple rescue attempts may improve fitness more than a single rescuer/attempt because it would introduce more genetic diversity than a single rescuer/attempt. Different individuals are unlikely to carry the same alleles if sourced from an outbred population. Introducing more genetic diversity with more rescuers should improve fitness more than a single rescuer. On the other hand, more rescuers may also lead to the introduction of more mutation load, diverse populations carry more deleterious alleles so multiple rescuers will introduce more mutation load than a single rescuer. However, the introduction of multiple genomes from multiple rescuers may prevent any deleterious alleles from being expressed in later generations. If individuals are descended from a single rescuer they are more likely to carry the same alleles and risk their offspring being homozygous for a deleterious recessive gene. As multiple rescuers are unlikely to carry the same alleles there is a reduced risk of offspring becoming homozygous for a deleterious variant. Another risk of using multiple rescuers is more introduced genetic diversity could lead to the genetic swamping of the original population, which could be even more of a risk if there is assortative mating. Using different numbers of rescuers could allow for testing of both expression of mutation load following rescue and the risk of genomic swamping. Simulations carried out on the Florida Panther population found that too many rescuers could have a negative impact due to population size fluctuations. It was calculated that an ideal management would be the introduction of five rescuers every 20 years (van de Kerk et al., 2019). A similar study on bobcats found that a single female every four years would be enough to stabilise heterozygosity (Miller-Butterworth et al., 2021). These two studies show that the number of rescuers and how often rescue is required can differ greatly between scenarios. Further

research testing different numbers of rescuers, different numbers of rescue events and the time between the events could provide crucial information for the planning of genetic rescue attempts.

Stress is a key area that could be investigated as we know that stress affects inbreeding depression and therefore may affect genetic rescue (Armbruster and Reed, 2005). In my experiments nutrient stress was applied five to six generations after rescue was attempted by replicating populations onto stressful fodder. This removed the differences in productivity between rescue treatments and the controls that were seen under benign conditions. Testing different levels of, for example, nutrient stress could find a level where the benefits of genetic rescue are maintained even under the stress or exaggerate the differences in fitness between the treatments. In *T. castaneum* temperature is another commonly used stress (Pointer et al., 2021), which could be applied. The changing climate makes temperature a stress that many populations could face, testing the interaction between inbreeding depression and temperature could provide vital information for conservation efforts. Testing of genetic rescue under stressful conditions has been done, even in *T .castaneum* (Hufbauer et al., 2015), but the stress itself is not the variable being tested. Understanding the interactions between stress, inbreeding depression and genetic rescue is important to our knowledge of genetic rescue.

A key criticism of genetic rescue studies is how long populations are monitored following rescue, and current studies tend to only follow populations for a few generations following the rescue attempt (Clarke et al., 2024). As discussed above this could miss affects in later generations such as fitness declines following the resumption of inbreeding. It is key that more studies look at the duration of genetic rescue, knowing how long the benefits of genetic rescue will last allows for planning of further conservation action to maintain fitness. A recent study followed populations of *Drosophila melanogaster* for 33 generations after a rescue attempt, finding that a higher proportion of populations survived when rescued compared to non-rescue populations over all generations (Pérez-Pereira et al., 2025). More studies like this are needed to improve our understanding of the duration of genetic rescue, finding differences 33 generations after rescue shows that the nine or ten generations in my experiments could have been too few.

My study was limited to collecting genomic data at a single generation after the rescue, sampling in multiple generations would allow tracking changes in genetic diversity and genetic load. Longitudinal genomic data would allow us to monitor how many generations introduced genetic diversity remains in the recipient population. It could also allow observation of

mutation load being converted between a hidden and expressed state. Genomic monitoring over multiple generations is especially important if the recipient population begins to inbreed again. The duration of genetic rescue is an important avenue of research to enable its use in the planning of conservation actions.

References

Åkesson, M., Liberg, O., Sand, H., et al. (2016) Genetic rescue in a severely inbred wolf population. *Molecular ecology*, 25 (19): 4745–4756. doi:10.1111/mec.13797.

Al Hikmani, H., van Oosterhout, C., Birley, T., et al. (2024) Can genetic rescue help save Arabia's last big cat? *Evolutionary Applications*, 17 (5). doi:10.1111/eva.13701.

Andrews, S. (2010) *FastQC: A Quality Control tool for High Throughput Sequence Data*.

Armbruster, P. and Reed, D.H. (2005) Inbreeding depression in benign and stressful environments. *Heredity*, 95 (3): 235–242. doi:10.1038/sj.hdy.6800721.

Ash, E., Cushman, S., Kaszta, Ż., et al. (2023) Female-biased introductions produce higher predicted population size and genetic diversity in simulations of a small, isolated tiger (Panthera tigris) population. *Scientific Reports*, 13 (1): 11199. doi:10.1038/s41598-023-36849-z.

Attia, F.A. and Tregenza, T. (2004) Divergence revealed by population crosses in the red flour beetle Tribolium castaneum. *Evolutionary Ecology Research*. Available at: https://eprints.whiterose.ac.uk/.

Bachmann, J.C., Jansen Van Rensburg, A., Cortazar-Chinarro, M., et al. (2020) Gene Flow Limits Adaptation along Steep Environmental Gradients. *The American Naturalist*, 195. doi:10.5061/dryad.41ns1rn96.

Bartoń, K. (2024) *MuMIn: Multi-Model Inference*.

Bateman, A.J. (1948) Intra-sexual selection in Drosophila. *Heredity*, 2 (3): 349–368. doi:10.1038/hdy.1948.21.

Bell, D., Robinson, Z., Funk, W.C., et al. (2019) The Exciting Potential and Remaining Uncertainties of Genetic Rescue. *Trends in Ecology and Evolution*, 34 (12): 1070–1079. doi:10.1016/j.tree.2019.06.006.

Bell, G. and Gonzalez, A. (2009) Evolutionary rescue can prevent extinction following environmental change. *Ecology Letters*, 12 (9): 942–948. doi:10.1111/j.1461-0248.2009.01350.x.

Bellard, C., Bertelsmeier, C., Leadley, P., et al. (2012) Impacts of climate change on the future of biodiversity. *Ecology Letters*. 15 (4) pp. 365–377. doi:10.1111/j.1461-0248.2011.01736.x.

Bertorelle, G., Raffini, F., Bosse, M., et al. (2022) Genetic load: genomic estimates and applications in non-model animals. *Nature Reviews Genetics*. 23 (8) pp. 492–503. doi:10.1038/s41576-022-00448-x.

Bijlsma, R., Westerhof, M.D.D., Roekx, L.P., et al. (2010) Dynamics of genetic rescue in inbred Drosophila melanogaster populations. *Conservation Genetics*, 11 (2): 449–462. doi:10.1007/s10592-010-0058-z.

Blomqvist, D., Pauliny, A., Larsson, M., et al. (2010) Trapped in the extinction vortex? Strong genetic effects in a declining vertebrate population. *BMC Evol Biol*, 10 (33). Available at: http://www.biomedcentral.com/1471-2148/10/33.

Bolger, A.M., Lohse, M. and Usadel, B. (2014) Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics*, 30 (15): 2114–2120. doi:10.1093/bioinformatics/btu170.

Brooks, M.E., Kristensen, K., Van Benthem, K.J., et al. (2017) *glmmTMB Balances Speed and Flexibility Among Packages for Zero-inflated Generalized Linear Mixed Modeling*.

Brown, S.J., Denell, R.E. and Beeman, R.W. (2003) Beetling around the genome. *Genetical Research*. 82 (3) pp. 155–161. doi:10.1017/S0016672303006451.

Bryan, J. (2023) *googlesheets4: Access Google Sheets using the Sheets API V4*.
Bushnell, B. (2014) *BBMap: A Fast, Accurate, Splice-Aware Aligner*.
Cally, J.G., Stuart-Fox, D. and Holman, L. (2019) Meta-analytic evidence that sexual selection improves population fitness. *Nature Communications*, 10 (1). doi:10.1038/s41467-019-10074-7.
Ceballos, F.C., Joshi, P.K., Clark, D.W., et al. (2018) Runs of homozygosity: Windows into population history and trait architecture. *Nature Reviews Genetics*. 19 (4) pp. 220–234. doi:10.1038/nrg.2017.109.
Ceballos, G. and Ehrlich, P.R. (2023) Mutilation of the tree of life via mass extinction of animal genera. *Proceedings of the National Academy of Sciences of the United States of America*, 120 (39). doi:10.1073/pnas.2306987120.
Ceballos, G., Ehrlich, P.R. and Dirzo, R. (2017) Biological annihilation via the ongoing sixth mass extinction signaled by vertebrate population losses and declines. *Proceedings of the National Academy of Sciences of the United States of America*, 114 (30): E6089–E6096. doi:10.1073/pnas.1704949114.
Chan, W.Y., Hoffmann, A.A. and van Oppen, M.J.H. (2019) Hybridization as a conservation management tool. *Conservation Letters*. 12 (5). doi:10.1111/conl.12652.
Charlesworth, D. and Charlesworth, B. (1987) Inbreeding Depression and its Evolutionary Consequences. *Annual Review of Ecology and Systematics*, 18: 237–268. Available at: http://www.jstor.org/stable/2097132.
Charlesworth, D. and Willis, J.H. (2009) The genetics of inbreeding depression. *Nature Reviews Genetics*. 10 (11). doi:10.1038/nrg2664.
Chege, M., Sewalt, B., Lesilau, F., et al. (2024) Genetic diversity of lion populations in Kenya: Evaluating past management practices and recommendations for future conservation actions. *Evolutionary Applications*, 17 (3). doi:10.1111/eva.13676.
Clarke, J.G., Smith, A.C. and Cullingham, C.I. (2024) Genetic rescue often leads to higher fitness as a result of increased heterozygosity across animal taxa. *Molecular Ecology*. doi:10.1111/mec.17532.
Clavero, M., Naves, J., Lucena-Perez, M., et al. (2024) Taxonomic inflation as a conservation trap for inbred populations. *Evolutionary Applications*, 17 (5). doi:10.1111/eva.13677.
Crnokrak, P. and Roff, D.A. (1998) Inbreeding depression in the wild. *Heredity*.
Danecek, P., Bonfield, J.K., Liddle, J., et al. (2021) Twelve years of SAMtools and BCFtools. *GigaScience*, 10 (2). doi:10.1093/gigascience/giab008.
Davis, M.M., Smyser, T.J., Johnson, S.A., et al. (2021) Reproductive success of captive-reared Allegheny Woodrats (Neotoma magister) released into genetically depauperate populations. *Conservation Genetics*, 22 (6): 903–912. doi:10.1007/s10592-021-01372-z.
Dawson, P.S. (1977) LIFE HISTORY STRATEGY AND EVOLUTIONARY HISTORY OF TRIBOLIUM FLOUR BEETLES. *Evolution*, 31 (1): 226–229. doi:10.1111/j.1558-5646.1977.tb01001.x.
Demont, M., Grazer, V.M., Michalczyk, Ł., et al. (2014) Experimental Removal of Sexual Selection Reveals Adaptations to Polyandry in Both Sexes. *Evolutionary Biology*, 41 (1): 62–70. doi:10.1007/s11692-013-9246-3.
Dickinson, M. (2018) *The impacts of heat-wave conditions on reproduction in a model insect, Tribolium castaneum*. University of East Anglia.
van Doorn, G.S., Edelaar, P. and Weissing, F.J. (2009) On the Origin of Species by Natural and Sexual Selection. *Science*, 326 (5960): 1704–1707. doi:10.1126/science.1178883.

Đukić, N., Radonjić, A., Popović, B., et al. (2021) Development and progeny performance of Tribolium castaneum (Herbst) in brewer's yeast and wheat (patent) flour at different population densities. *Journal of Stored Products Research*, 94. doi:10.1016/j.jspr.2021.101886.
Durkee, L.F., Olazcuaga, L., Szymanski, R., et al. (2023) Genetic mixing facilitates adaptation to a novel environmental constraint. *Ecological Entomology*. doi:10.1111/een.13242.
Dussex, N., Morales, H.E., Grossen, C., et al. (2023) Purging and accumulation of genetic load in conservation. *Trends in Ecology and Evolution*. 38 (10) pp. 961–969. doi:10.1016/j.tree.2023.05.008.
Duval, C., Park, T., Miller, E.V., et al. (1939) Studies in Population Physiology. IX. The Effect of Imago Population Density on the Duration of the Larval and Pupal Stages of Tribolium. *Ecology*, 20 (3): 365–373.
Ebel, E.R. and Phillips, P.C. (2016) Intrinsic differences between males and females determine sex-specific consequences of inbreeding. *BMC Evolutionary Biology*, 16 (1). doi:10.1186/s12862-016-0604-5.
Edmands, S. (2007) Between a rock and a hard place: Evaluating the relative risks of inbreeding and outbreeding for conservation and management. *Molecular Ecology*. 16 (3) pp. 463–475. doi:10.1111/j.1365-294X.2006.03148.x.
El-Desouky, T.A., Elbadawy, S.S., Hussain, H.B.H., et al. (2018) Impact of Insect Densities Tribolium Castaneum on the Benzoquinone Secretions and Aflatoxins Levels in Wheat Flour During Storage Periods. *The Open Biotechnology Journal*, 12 (1): 104–111. doi:10.2174/1874070701812010104.
Fagan, W.F. and Holmes, E.E. (2006) Quantifying the extinction vortex. *Ecology Letters*, 9 (1): 51–60. doi:10.1111/j.1461-0248.2005.00845.x.
Ferreras, P., Gaona, P., Palomares, F., et al. (2001) Restore habitat or reduce mortality? Implications from a population viability analysis of the Iberian lynx. *Animal Conservation*, 4 (3): 265–274. doi:10.1017/S1367943001001317.
Fitzpatrick, S.W., Bradburd, G.S., Kremer, C.T., et al. (2019) Genetic rescue without genomic swamping in wild populations. *bioRxiv*. doi:10.1101/701706.
Fitzpatrick, S.W., Bradburd, G.S., Kremer, C.T., et al. (2020) Genomic and Fitness Consequences of Genetic Rescue in Wild Populations. *Current Biology*, 30 (3): 517-522.e5. doi:10.1016/j.cub.2019.11.062.
Fitzpatrick, S.W., Gerberich, J.C., Angeloni, L.M., et al. (2016) Gene flow from an adaptively divergent source causes rescue through genetic and demographic factors in two wild populations of Trinidadian guppies. *Evolutionary Applications*, 9 (7): 879–891. doi:10.1111/eva.12356.
Fitzpatrick, S.W., Gerberich, J.C., Kronenberger, J.A., et al. (2015) Locally adapted traits maintained in the face of high gene flow. *Ecology Letters*, 18 (1): 37–47. doi:10.1111/ele.12388.
Fitzpatrick, S.W., Mittan-Moreau, C., Miller, M., et al. (2023) Genetic rescue remains underused for aiding recovery of federally listed vertebrates in the United States Zamudio, K. (ed.). *Journal of Heredity*. doi:10.1093/jhered/esad002.
Fitzpatrick, S.W. and Reid, B.N. (2019) Does gene flow aggravate or alleviate maladaptation to environmental stress in small populations? *Evolutionary Applications*, 12 (7): 1402–1416. doi:10.1111/eva.12768.
Fox, C.W. and Reed, D.H. (2011) Inbreeding depression increases with environmental stress: An experimental study and meta-analysis. *Evolution*, 65 (1): 246–258. doi:10.1111/j.1558-5646.2010.01108.x.
Frankham, R. (2005) "Stress and adaptation in conservation genetics." In *Journal of Evolutionary Biology*. July 2005. pp. 750–755. doi:10.1111/j.1420-9101.2005.00885.x.

Frankham, R. (2015) Genetic rescue of small inbred populations: meta-analysis reveals large and consistent benefits of gene flow. *Molecular Ecology*, 24 (11): 2610–2618. doi:10.1111/mec.13139.
Frankham, R. (2016) Genetic rescue benefits persist to at least the F3 generation, based on a meta-analysis. *Biological Conservation*, 195: 33–36. doi:10.1016/j.biocon.2015.12.038.
Frankham, R., Ballou, J.D. and Briscoe, D.A. (2010) *Introduction to Conservation Genetics*. 2nd ed. Cambridge: Cambridge University Press. doi:DOI: 10.1017/CBO9780511809002.
Frankham, R., Ballou, J.D., Eldridge, M.D.B., et al. (2011) Predicting the Probability of Outbreeding Depression. *Conservation Biology*, 25 (3): 465–475. doi:10.1111/j.1523-1739.2011.01662.x.
Frankham, R., Ballou, J.D., Ralls, K., et al. (2017) Genetic Management of Fragmented Animal and Plant Populations. *Livro*.
Frankham, R., Bradshaw, C.J.A. and Brook, B.W. (2014) Genetics in conservation management: Revised recommendations for the 50/500 rules, Red List criteria and population viability analyses. *Biological Conservation*. 170 pp. 56–63. doi:10.1016/j.biocon.2013.12.036.
Fraser, D.J. and Bernatchez, L. (2001) Adaptive evolutionary conservation: towards a unified concept for defining conservation units. *Molecular Ecology*, 10 (12): 2741–2752. doi:10.1046/j.0962-1083.2001.01411.x.
Gemmell, N.J. and Allendorf, F.W. (2001) Mitochondrial mutations may decrease population viability. *Trends in Ecology & Evolution*, 16 (3): 115–117.
Gemmell, N.J., Metcalf, V.J. and Allendorf, F.W. (2004) Mother's curse: The effect of mtDNA on individual fitness and population viability. *Trends in Ecology and Evolution*, 19 (5): 238–244. doi:10.1016/j.tree.2004.02.002.
Gilad, T. and Scharf, I. (2019) Separation between maternal and paternal effects on offspring following exposure of adult red flour beetles to two stressors. *Ecological Entomology*, 44 (4): 494–501. doi:10.1111/een.12726.
Gmel, A.I., Guichard, M., Dainat, B., et al. (2023) Identification of runs of homozygosity in Western honey bees (Apis mellifera) using whole-genome sequencing data. *Ecology and Evolution*, 13 (1). doi:10.1002/ece3.9723.
Godwin, J.L., Lumley, A.J., Michalczyk, Ł., et al. (2020) Mating patterns influence vulnerability to the extinction vortex. *Global Change Biology*, 26 (8): 4226–4239. doi:10.1111/gcb.15186.
Grieshop, K., Maurizio, P.L., Arnqvist, G., et al. (2021) Selection in males purges the mutation load on female fitness. *Evolution Letters*. 5 (4) pp. 328–343. doi:10.1002/evl3.239.
Griffith, B., Scott, J.M., Carpenter, J.W., et al. (1989) Translocation as a Species Conservation Tool: Status and Strategy. *Science*, 245 (4917): 477–480. doi:10.1126/science.245.4917.477.
Grossen, C., Guillaume, F., Keller, L.F., et al. (2020) Purging of highly deleterious mutations through severe bottlenecks in Alpine ibex. *Nature Communications*, 11 (1). doi:10.1038/s41467-020-14803-1.
Haddad, N.M., Brudvig, L.A., Clobert, J., et al. (2015) Habitat fragmentation and its lasting impact on Earth's ecosystems. *Science Advances*, 1 (2). doi:10.1126/sciadv.1500052.
Haliscak, J.P. and Beeman, R.W. (1983) Status of Malathion Resistance in Five Genera of Beetles Infesting Farm-Stored Corn, Wheat, and Oats in the United States1. *Journal of Economic Entomology*, 76 (4): 717–722. doi:10.1093/jee/76.4.717.

Hartig, F. (2022) *DHARMa: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models*.
Hasselgren, M., Angerbjörn, A., Eide, N.E., et al. (2018) Genetic rescue in an inbred Arctic fox (Vulpes lagopus) population. *Proceedings of the Royal Society B: Biological Sciences*, 285 (1875). doi:10.1098/rspb.2017.2814.
Havird, J.C., Fitzpatrick, S.W., Kronenberger, J., et al. (2016) Sex, Mitochondria, and Genetic Rescue. *Trends in Ecology and Evolution*. 31 (2) pp. 96–99. doi:10.1016/j.tree.2015.11.012.
Heber, S., Briskie, J. V. and Apiolaza, L.A. (2012) A test of the "genetic rescue" technique using bottlenecked donor populations of Drosophila melanogaster. *PLoS ONE*, 7 (8). doi:10.1371/journal.pone.0043113.
Heber, S., Varsani, A., Kuhn, S., et al. (2013) The genetic rescue of two bottlenecked south island robin populations using translocations of inbred donors. *Proceedings of the Royal Society B: Biological Sciences*, 280 (1752). doi:10.1098/rspb.2012.2228.
Hedrick, P.W., Adams, J.R. and Vucetich, J.A. (2011) Reevaluating and Broadening the Definition of Genetic Rescue. *Conservation Biology*. 25 (6) pp. 1069–1070. doi:10.1111/j.1523-1739.2011.01751.x.
Hedrick, P.W. and Fredrickson, R. (2010) Genetic rescue guidelines with examples from Mexican wolves and Florida panthers. *Conservation Genetics*, 11 (2): 615–626. doi:10.1007/s10592-009-9999-5.
Hedrick, P.W. and Garcia-Dorado, A. (2016) Understanding Inbreeding Depression, Purging, and Genetic Rescue. *Trends in Ecology and Evolution*. 31 (12) pp. 940–952. doi:10.1016/j.tree.2016.09.005.
Hedrick, P.W., Peterson, R.O., Vucetich, L.M., et al. (2014) Genetic rescue in Isle Royale wolves: genetic analysis and the collapse of the population. *Conservation Genetics*, 15 (5): 1111–1121. doi:10.1007/s10592-014-0604-1.
Hedrick, P.W., Robinson, J.A., Peterson, R.O., et al. (2019) Genetics and extinction and the example of Isle Royale wolves. *Animal Conservation*, 22 (3): 302–309. doi:10.1111/acv.12479.
Hewett, A.M., Johnston, S.E., Morris, A., et al. (2024) Genetic architecture of inbreeding depression may explain its persistence in a population of wild red deer. *Molecular Ecology*. doi:10.1111/mec.17335.
Hoegh-Guldberg, O., Jacob, D., Taylor, M., et al. (2018) *2018: Impacts of 1.5°C Global Warming on Natural and Human Systems. In: Global Warming of 1.5°C. An IPCC Special Report on the impacts of global warming of 1.5°C above pre-industrial levels and related global greenhouse gas emission pathways, in the context of strengthening the global response to the threat of climate change, sustainable development, and efforts to eradicate poverty*.
Hoelzel, A.R., Bruford, M.W. and Fleischer, R.C. (2019) Conservation of adaptive potential and functional diversity. *Conservation Genetics*. 20 (1). doi:10.1007/s10592-019-01151-x.
Hoffmann, A.A., Miller, A.D. and Weeks, A.R. (2020) Genetic mixing for population management: from genetic rescue to provenancing. *Evolutionary Applications*, p. eva.13154. doi:10.1111/eva.13154.
Hope, R. (2022) *Rmisc: Ryan Miscellaneous*.
Hostetler, J.A., Onorato, D.P., Jansen, D., et al. (2013) A cat's tale: The impact of genetic restoration on Florida panther population dynamics and persistence. *Journal of Animal Ecology*, 82 (3): 608–620. doi:10.1111/1365-2656.12033.
Hothorn, T., Bretz, F. and Westfall, P. (2008) Simultaneous Inference in General Parametric Models. *Biometrical Journal*, 50 (3): 346–363.

Howe, R.W. (1962) The effects of temperature and humidity on the oviposition rate of Tribolium castaneum (Hbst.) (Coleoptera, Tenebrionidae). *Bulletin of Entomological Research*, 53 (2): 301–310. doi:DOI: 10.1017/S0007485300048148.
Hufbauer, R.A., Szűcs, M., Kasyon, E., et al. (2015) Three types of rescue can avert extinction in a changing environment. *Proceedings of the National Academy of Sciences*, 112 (33): 10557. doi:10.1073/pnas.1504732112.
Ingvarsson, P.K. (2001) Restoration of genetic variation lost - the genetic rescue hypothesis. *TRENDS in Ecology & Evolution*, 16 (2).
Izutsu, M., Zhou, J., Sugiyama, Y., et al. (2012) Genome features of "dark-fly", a drosophila line reared long-term in a dark environment. *PLoS ONE*, 7 (3). doi:10.1371/journal.pone.0033288.
Janus, M.C. (1989) Phenotypic diversity of Tribolium confusum pupae in heterogeneous environments. *Entomologia Experimentalis et Applicata*, 50 (3). doi:10.1111/j.1570-7458.1989.tb01203.x.
Johnson, W.E., Onorato, D.P., Roelke, M.E., et al. (2010) Genetic restoration of the Florida panther. *Science*, 329 (5999). doi:10.1126/science.1192891.
Jørgensen, D.B., Ørsted, M. and Kristensen, T.N. (2022) Sustained positive consequences of genetic rescue of fitness and behavioural traits in inbred populations of Drosophila melanogaster. *Journal of Evolutionary Biology*. doi:10.1111/jeb.14015.
Kardos, M., Taylor, H.R., Ellegren, H., et al. (2016) Genomics advances the study of inbreeding depression in the wild. *Evolutionary Applications*. 9 (10) pp. 1205–1218. doi:10.1111/eva.12414.
Kasumovic, M.M., Bruce, M.J., Andrade, M.C.B., et al. (2008) Spatial and temporal demographic variation drives within-season fluctuations in sexual selection. *Evolution*, 62 (9): 2316–2325. doi:10.1111/j.1558-5646.2008.00446.x.
Kawecki, T.J. and Ebert, D. (2004) Conceptual issues in local adaptation. *Ecology Letters*. 7 (12) pp. 1225–1241. doi:10.1111/j.1461-0248.2004.00684.x.
Kelly, E. and Phillips, B. (2019a) How many and when? Optimising targeted gene flow for a step change in the environment. *Ecology Letters*. 22 (3) pp. 447–457. doi:10.1111/ele.13201.
Kelly, E. and Phillips, B.L. (2019b) Targeted gene flow and rapid adaptation in an endangered marsupial. *Conservation Biology*, 33 (1): 112–121. doi:10.1111/cobi.13149.
van de Kerk, M., Onorato, D.P., Hostetler, J.A., et al. (2019) Dynamics, Persistence, and Genetic Management of the Endangered Florida Panther Population. *Wildlife Monographs*, 203 (1): 3–35. doi:10.1002/wmon.1041.
King, C.E. and Dawson, P.S. (1972) "Population Biology and the Tribolium Model." In *Evolutionary Biology*. doi:10.1007/978-1-4757-0256-9_5.
Kitchener, A., Breitenmoser, C., Eizirik, E., et al. (2017) A revised taxonomy of the Felidae. The final report of the Cat Classification Task Force of the IUCN/SSC Cat Specialist Group. *Cat News Special Issue*, p. 80 pp.
Kölliker, M. (2012) *The Evolution of Parental Care*. Royle, N.J. and Smiseth, P.T. (eds.). Oxford University Press. doi:10.1093/acprof:oso/9780199692576.001.0001.
Kolodny, O., McLaren, M.R., Greenbaum, G., et al. (2019) Reconsidering the management paradigm of fragmented populations. *bioRxiv*. doi:10.1101/649129.
Kronenberger, J.A., Funk, W.C., Smith, J.W., et al. (2017) Testing the demographic effects of divergent immigrants on small populations of Trinidadian guppies. *Animal Conservation*, 20 (1): 3–11. doi:10.1111/acv.12286.

Kronenberger, J.A., Gerberich, J.C., Fitzpatrick, S.W., et al. (2018) An experimental test of alternative population augmentation scenarios. *Conservation Biology*, 32 (4): 838–848. doi:10.1111/cobi.13076.
Kumar, P., Henikoff, S. and Ng, P.C. (2009) Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. *Nature Protocols*, 4 (7): 1073–1081. doi:10.1038/nprot.2009.86.
Kyriazis, C.C., Wayne, R.K. and Lohmueller, K.E. (2021) Strongly deleterious mutations are a primary determinant of extinction risk due to inbreeding depression. *Evolution Letters*, 5 (1): 33–47. doi:10.1002/evl3.209.
Laskowski, R., Radwan, J., Kuduk, K., et al. (2015) Population growth rate and genetic variability of small and large populations of Red flour beetle (Tribolium castaneum) following multigenerational exposure to copper. *Ecotoxicology*, 24 (5): 1162–1170. doi:10.1007/s10646-015-1463-3.
Lee, H.Y., Chou, J.Y., Cheong, L., et al. (2008) Incompatibility of Nuclear and Mitochondrial Genomes Causes Hybrid Sterility between Two Yeast Species. *Cell*, 135 (6): 1065–1073. doi:10.1016/j.cell.2008.10.047.
Lenormand, T. (2002) Gene flow and the limits to natural selection. *Trends in Ecology & Evolution*, 17 (4): 183–189. doi:https://doi.org/10.1016/S0169-5347(02)02497-7.
Lenth R (2024) *emmeans: Estimated Marginal Means, aka Least-Squares Means*.
Lewis, R., Pointer, M.D., Friend, L., et al. (2024) Tests of evolutionary and genetic rescue using flour beetles, Tribolium castaneum, experimentally evolved to thermal conditions. *Ecology and Evolution*, 14 (5). doi:10.1002/ece3.11313.
Lewis, R.C. (2020) *Thermal adaptation in a model pest insect*. University of East Anglia. Available at: https://ueaeprints.uea.ac.uk/id/eprint/79830 (Accessed: 10 February 2025).
Li, H. (2013) *Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM*. Available at: http://arxiv.org/abs/1303.3997.
Li, H., Handsaker, B., Wysoker, A., et al. (2009) The Sequence Alignment/Map format and SAMtools. *Bioinformatics*, 25 (16): 2078–2079. doi:10.1093/bioinformatics/btp352.
Lindsay, W.R., Madsen, T., Wapstra, E., et al. (2020) Long term effects of outbreeding: experimental founding of island population eliminates malformations and improves hatching success in sand lizards. *Biological Conservation*, 249. doi:10.1016/j.biocon.2020.108710.
Lindsey, H.A., Gallie, J., Taylor, S., et al. (2013) Evolutionary rescue from extinction is contingent on a lower rate of environmental change. *Nature*, 494 (7438): 463–467. doi:10.1038/nature11879.
Loope, K.J., DeSha, J.N., Aresco, M.J., et al. (2024) Common-garden experiment reveals outbreeding depression and region-of-origin effects on reproductive success in a frequently translocated tortoise. *Animal Conservation*. doi:10.1111/acv.12977.
Lotsander, A., Hasselgren, M., Larm, M., et al. (2021) Low persistence of genetic rescue across generations in the arctic fox (Vulpes lagopus). *Journal of Heredity*, 112 (3): 276–285. doi:10.1093/jhered/esab011.
Love Stowell, S.M., Pinzone, C.A. and Martin, A.P. (2017) Overcoming barriers to active interventions for genetic diversity. *Biodiversity and Conservation*. 26 (8) pp. 1753–1765. doi:10.1007/s10531-017-1330-z.
Lüdecke D (2018) *ggeffects: Tidy Data Frames of Marginal Effects from Regression Models*.
Lüdecke, D., Ben-Shachar, M., Patil, I., et al. (2021) performance: An R Package for Assessment, Comparison and Testing of Statistical Models. *Journal of Open Source Software*, 6 (60): 3139. doi:10.21105/joss.03139.

Lumley, A.J., Michalczyk, Ł., Kitson, J.J.N., et al. (2015) Sexual selection protects against extinction. *Nature*, 522 (7557): 470–473. doi:10.1038/nature14419.
Ma, H., Marti Gutierrez, N., Morey, R., et al. (2016) Incompatibility between Nuclear and Mitochondrial Genomes Contributes to an Interspecies Reproductive Barrier. *Cell Metabolism*, 24 (2): 283–294. doi:10.1016/j.cmet.2016.06.012.
Mable, B.K. (2019) Conservation of adaptive potential and functional diversity: integrating old and new approaches. *Conservation Genetics*. 20 (1). doi:10.1007/s10592-018-1129-9.
Madsen, T., Loman, J., Anderberg, L., et al. (2020) Genetic rescue restores long-term viability of an isolated population of adders (Vipera berus). *Current Biology*, 30 (21): R1297–R1299. doi:https://doi.org/10.1016/j.cub.2020.08.059.
Madsen, T., Shine, R., Olsson, M., et al. (1999) Restoration of an inbred adder population. *Nature*, 402 (6757): 34–35. doi:10.1038/46941.
Madsen, T., Ujvari, B. and Olsson, M. (2004) Novel genes continue to enhance population growth in adders (Vipera berus). *Biological Conservation*, 120 (1): 145–147. doi:10.1016/j.biocon.2004.01.022.
Mathur, S. and DeWoody, J.A. (2021) Genetic load has potential in large populations but is realized in small inbred populations. *Evolutionary Applications*, 14 (6): 1540–1557. doi:10.1111/eva.13216.
Mattey, S.N., Richardson, J., Ratz, T., et al. (2018) Effects of Offspring and Parental Inbreeding on Parent-Offspring Communication. *The American Naturalist*, 191 (6). doi:10.5061/dryad.fn909.
McBride, R.T., McBride, R.T., McBride, R.M., et al. (2008) Counting pumas by categorizing physical evidence. *Southeastern Naturalist*, 7 (3). doi:10.1656/1528-7092-7.3.381.
Meiklejohn, C.D., Holmbeck, M.A., Siddiq, M.A., et al. (2013) An Incompatibility between a Mitochondrial tRNA and Its Nuclear-Encoded tRNA Synthetase Compromises Development and Fitness in Drosophila. *PLoS Genetics*, 9 (1). doi:10.1371/journal.pgen.1003238.
Michalczyk, L., Millard, A.L., Martin, O.Y., et al. (2011) Experimental evolution exposes female and male responses to sexual selection and conflict in tribolium castaneum. *Evolution*, 65 (3): 713–724. doi:10.1111/j.1558-5646.2010.01174.x.
Miller, A.D., Coleman, M.A., Clark, J., et al. (2020a) Local thermal adaptation and limited gene flow constrain future climate responses of a marine ecosystem engineer. *Evolutionary Applications*, 13 (5): 918–934. doi:10.1111/eva.12909.
Miller, S.M., Druce, D.J., Dalton, D.L., et al. (2020b) Genetic rescue of an isolated African lion population. *Conservation Genetics*, 21 (1): 41–53. doi:10.1007/s10592-019-01231-y.
Miller-Butterworth, C.M., Diefenbach, D.R., Edson, J.E., et al. (2021) Demographic changes and loss of genetic diversity in two insular populations of bobcats (Lynx rufus). *Global Ecology and Conservation*, 26. doi:10.1016/j.gecco.2021.e01457.
Moritz, C. (n.d.) *Defining "Evolutionarily Significant Units" for conservation*.
Mussmann, S.M., Douglas, M.R., Anthonysamy, W.J.B., et al. (2017) Genetic rescue, the greater prairie chicken and the problem of conservation reliance in the Anthropocene. *Royal Society Open Science*, 4 (2). doi:10.1098/rsos.160736.
Nichols, S., Ewen, J.G., Gottelli, D., et al. (2024) Genetic rescue attempt in a small, inbred population of a wild endangered passerine. *Biological Conservation*. doi:10.1016/j.biocon.2023.110430.
Onorato, D.P., Cunningham, M.W., Lotz, M., et al. (2024) Multi-generational benefits of genetic rescue. *Scientific Reports*, 14 (1). doi:10.1038/s41598-024-67033-6.

van Oosterhout, C. (2020) Mutation load is the spectre of species conservation. *Nature Ecology and Evolution*. 4 (8) pp. 1004–1006. doi:10.1038/s41559-020-1204-8.
Ørsted, M., Hoffmann, A.A., Sverrisdóttir, E., et al. (2019) Genomic variation predicts adaptive evolutionary responses better than population bottleneck history. *PLoS Genetics*, 15 (6). doi:10.1371/journal.pgen.1008205.
Pai, A. and Yan, G. (2003) Rapid female multiple mating in red flour beetles (Tribolium castaneum). *Canadian Journal of Zoology*, 81 (5): 888–896. doi:10.1139/z03-070.
Palomares, F., Godoy, J.A., López-Bao, J.V., et al. (2012) Possible Extinction Vortex for a Population of Iberian Lynx on the Verge of Extirpation. *Conservation Biology*, 26 (4): 689–697. doi:10.1111/j.1523-1739.2012.01870.x.
Park, T. (1932) Studies in Population Physiology: The Relation of Numbers to Initial Population Growth in the Flour Beetle Tribolium Confusum Duval. *Ecology*, 13 (2). doi:10.2307/1931067.
Parrett, J.M., Chmielewski, S., Aydogdu, E., et al. (2022) Genomic evidence that a sexually selected trait captures genome-wide variation and facilitates the purging of genetic load. *Nature Ecology and Evolution*, 6 (9): 1330–1342. doi:10.1038/s41559-022-01816-w.
Parrett, J.M. and Knell, R.J. (2018) The effect of sexual selection on adaptation and extinction under increasing temperatures. *Proceedings of the Royal Society B: Biological Sciences*, 285 (1877). doi:10.1098/rspb.2018.0303.
Pavlova, A., Petrovic, S., Harrisson, K.A., et al. (2023) Benefits of genetic rescue of a critically endangered subspecies from another subspecies outweigh risks: Results of captive breeding trials. *Biological Conservation*, 284: 110203. doi:10.1016/j.biocon.2023.110203.
Pavlova, A., Schneller, N.M., Lintermans, M., et al. (2024) Planning and implementing genetic rescue of an endangered freshwater fish population in a regulated river, where low flow reduces breeding opportunities and may trigger inbreeding depression. *Evolutionary Applications*, 17 (4). doi:10.1111/eva.13679.
Pazhenkova, E., Bartol, M., Boljte, B., et al. (2025) Genetic Rescue of the Dinaric Lynx Population: Insights for Conservation From Genetic Monitoring and Individual-Based Modelling. *Evolutionary Applications* , 18 (1). doi:10.1111/eva.70045.
Pedersen, T. (2024) *ggforce: Accelerating "ggplot2."*
Pérez-Pereira, N., Kleinman-Ruiz, D., García-Dorado, A., et al. (2025) A Test of the Long-Term Efficiency of Genetic Rescue With Drosophila melanogaster. *Molecular Ecology*. doi:10.1111/mec.17690.
Picard Team (2019) *Picard: Remove Duplicates*.
Pimm, S.L., Dollar, L. and Bass, O.L. (2006) The genetic rescue of the Florida panther. *Animal Conservation*, 9 (2): 115–122. doi:10.1111/j.1469-1795.2005.00010.x.
Pimm, S.L., Jenkins, C.N., Abell, R., et al. (2014) The biodiversity of species and their rates of extinction, distribution, and protection Background Rates of Species Extinction. *Science*, 344 (6187). Available at: http://science.sciencemag.org/.
Pinheiro, J., Bates, D. and R Core Team (2025) *nlme: Linear and Nonlinear Mixed Effects Models*.
Pointer, M.D. (2025) *Beetling about: using experimental evolution to understand dispersal behaviour in the pest/model insect Tribolium castaneum*. University of East Anglia.
Pointer, M.D., Gage, M.J.G. and Spurgin, L.G. (2021) Tribolium beetles as a model system in evolution and ecology. *Heredity*. doi:10.1038/s41437-021-00420-1.
Pooley, E.L., Kennedy, M.W. and Nager, R.G. (2014) Maternal inbreeding reduces parental care in the zebra finch, Taeniopygia guttata. *Animal Behaviour*, 97: 153–163. doi:10.1016/j.anbehav.2014.09.012.

Posit team (2024) *RStudio: Integrated Development for R*.
Powell, D.M. (2023) Losing the forest for the tree? On the wisdom of subpopulation management. *Zoo Biology*. 42 (5) pp. 591–604. doi:10.1002/zoo.21776.
Pregler, K.C., Obedzinski, M., Gilbert-Horvath, E.A., et al. (2022) Assisted gene flow from outcrossing shows the potential for genetic rescue in an endangered salmon population. *Conservation Letters*. doi:10.1111/conl.12934.
Purcell, S., Neale, B., Todd-Brown, K., et al. (2007) PLINK: A tool set for whole-genome association and population-based linkage analyses. *American Journal of Human Genetics*, 81 (3): 559–575. doi:10.1086/519795.
Quinn, C.B., Alden, P.B. and Sacks, B.N. (2019) Noninvasive Sampling Reveals Short-Term Genetic Rescue in an Insular Red Fox Population. *Journal of Heredity*, 110 (5): 559–576. doi:10.1093/jhered/esz024.
R Core Team (2024) *R: A Language and Environment for Statistical Computing*.
Ralls, K., Ballou, J.D., Dudash, M.R., et al. (2018) Call for a Paradigm Shift in the Genetic Management of Fragmented Populations. *Conservation Letters*, 11 (2). doi:10.1111/conl.12412.
Ralls, K., Sunnucks, P., Lacy, R.C., et al. (2020) Genetic rescue: A critique of the evidence supports maximizing genetic diversity rather than minimizing the introduction of putatively harmful genetic variation. *Biological Conservation*. 251. doi:10.1016/j.biocon.2020.108784.
Reed, D.H., Briscoe, D.A. and Frankham, R. (2002) *Inbreeding and extinction: The effect of environmental stress and lineage*.
Rhymer, J.M. and Simberloff, D. (1996) *EXTINCTION BY HYBRIDIZATION AND INTROGRESSION*.
Richardson, D.S., Komdeur, J. and Burke, T. (2004) Inbreeding in the Seychelles warbler: Environment-dependent maternal effects. *Evolution*, 58 (9): 2037–2048. doi:10.1111/j.0014-3820.2004.tb00488.x.
Roberts, D.G., Gray, C.A., West, R.J., et al. (2010) Marine genetic swamping: Hybrids replace an obligately estuarine fish. *Molecular Ecology*, 19 (3): 508–520. doi:10.1111/j.1365-294X.2009.04501.x.
Robinson, J., Kyriazis, C.C., Yuan, S.C., et al. (2023) Deleterious Variation in Natural Populations and Implications for Conservation Genetics. *Annual Review of Animal Biosciences*, Vol. 11:93-114. doi:10.1146/annurev-animal-080522.
Robinson, J.A., Brown, C., Kim, B.Y., et al. (2018) Purging of Strongly Deleterious Mutations Explains Long-Term Persistence and Absence of Inbreeding Depression in Island Foxes. *Current Biology*, 28 (21): 3487-3494.e4. doi:10.1016/j.cub.2018.08.066.
Robinson, J.A., Ortega-Del Vecchyo, D., Fan, Z., et al. (2016) Genomic Flatlining in the Endangered Island Fox. *Current Biology*, 26 (9): 1183–1189. doi:10.1016/j.cub.2016.02.062.
Robinson, J.A., Räikkönen, J., Vucetich, L.M., et al. (2019) *Genomic signatures of extensive inbreeding in Isle Royale wolves, a population on the threshold of extinction*. Available at: http://advances.sciencemag.org/.
Robinson, Z., Bell, D., Dhendup, T., et al. (2020) Evaluating the outcomes of genetic rescue attempts. *Conservation Biology*. doi:10.1111/cobi.13596.
Robinson, Z.L., Coombs, J.A., Hudy, M., et al. (2017) Experimental test of genetic rescue in isolated populations of brook trout. *Molecular Ecology*, 26 (17): 4418–4433. doi:10.1111/mec.14225.
Roelke, M.E., Martenson, J.S. and O'brien, S.J. (1993) The consequences of demographic reduction and genetic depletion in the endangered Florida panther. *Current Biology*, 3 (6).

Root, K. V. (1998) Evaluating the effects of habitat quality, connectivity, and catastrophes on a threatened species. *Ecological Applications*, 8 (3): 854–865. doi:10.1890/1051-0761(1998)008[0854:ETEOHQ]2.0.CO;2.
Rudin-Bitterli, T.S., Evans, J.P. and Mitchell, N.J. (2021) Fitness consequences of targeted gene flow to counter impacts of drying climates on terrestrial-breeding frogs. *Communications Biology*, 4 (1): 1195. doi:10.1038/s42003-021-02695-w.
Sales, K., Vasudeva, R., Dickinson, M.E., et al. (2018) Experimental heatwaves compromise sperm function and cause transgenerational damage in a model insect. *Nature Communications*, 9 (1). doi:10.1038/s41467-018-07273-z.
Sales, K., Vasudeva, R. and Gage, M.J.G. (2021) Fertility and mortality impacts of thermal stress from experimental heatwaves on different life stages and their recovery in a model insect. *Royal Society Open Science*, 8 (3). doi:10.1098/rsos.201717.
Sandner, T.M., Dotzert, A., Gerken, F., et al. (2022) Inbreeding depression changes with stress response over time in flooded Mimulus guttatus. *Perspectives in Plant Ecology, Evolution and Systematics*, 57. doi:10.1016/j.ppees.2022.125697.
Schiffers, K., Bourne, E.C., Lavergne, S., et al. (2013) Limited evolutionary rescue of locally adapted populations facing climate change. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 368 (1610). doi:10.1098/rstb.2012.0083.
Seal, U.S., Lacy, R.C. and Eds (1994) *A Plan for Genetic Restoration and Management of the Florida Panther (Felis concolor coryi) (Report to the Florida Game and Fresh Water Fish Commission, Conservation Breeding Specialist Group, Apple Valley, MN, 1994)*.
Sellis, D., Callahan, B.J., Petrov, D.A., et al. (2011) Heterozygote advantage as a natural consequence of adaptation in diploids. *Proceedings of the National Academy of Sciences of the United States of America*, 108 (51): 20666–20671. doi:10.1073/pnas.1114573108.
Sexton, J.P., Strauss, S.Y. and Rice, K.J. (2011) Gene flow increases fitness at the warm edge of a species' range. *Proceedings of the National Academy of Sciences of the United States of America*, 108 (28): 11704–11709. doi:10.1073/pnas.1100404108.
Skourti, A., Kavallieratos, N.G. and Papanikolaou, N.E. (2022) Demographic responses of Tribolium castaneum (Coleoptera: Tenebrionidae) to different temperatures in soft wheat flour. *Journal of Thermal Biology*, 103. doi:10.1016/j.jtherbio.2021.103162.
Smeds, L. and Ellegren, H. (2022) From high masked to high realized genetic load in inbred Scandinavian wolves. *Molecular Ecology*. doi:10.1111/mec.16802.
Sokal, R.R. and Sonleitner, F.J. (1968) The Ecology of Selection in Hybrid Populations of Tribolium castaneum. *Ecological Monographs*, 38 (4): 345–379.
Sonleitner, F.J. and Gutherie, J. (1991) Factors affecting oviposition rate in the flour beetle Tribolium castaneum and the origin of the population regulation mechanism. *Researches on Population Ecology*, 33 (1). doi:10.1007/BF02514569.
Soule, M.E. (ed.) and Gilpin, M.E. (1986) *Conservation biology: the science of scarcity and diversity*. Sunderland, MA (USA) Sinauer Associates.
Sundell, T., Kammonen, J.I., Mustanoja, E., et al. (2023) Genomic evidence uncovers inbreeding and supports translocations in rescuing the genetic diversity of a landlocked seal population. *Conservation Genetics*. doi:10.1007/s10592-022-01497-9.
Tallmon, D.A., Luikart, G. and Waples, R.S. (2004) The alluring simplicity and complex reality of genetic rescue. *Trends in Ecology and Evolution*. 19 (9) pp. 489–496. doi:10.1016/j.tree.2004.07.003.

Trinkel, M., Ferguson, N., Reid, A., et al. (2008) Translocating lions into an inbred lion population in the Hluhluwe-iMfolozi Park, South Africa. *Animal Conservation*, 11 (2): 138–143. doi:10.1111/j.1469-1795.2008.00163.x.
Turček, F.J. and Hickey, J.J. (1951) Effect of Introductions on Two Game Populations in Czechoslovakia. *The Journal of Wildlife Management*, 15 (1): 113–114. Available at: https://www.jstor.org/stable/3796784.
USDA ARS Ag100Pest Initiative (2022) Tribolium castaneum genome assembly icTriCast1.1. *https://www.ncbi.nlm.nih.gov/datasets/genome/GCF_031307605.1/*.
Vasudeva, R., Sutter, A., Sales, K., et al. (2019) Adaptive thermal plasticity enhances sperm and egg performance in a model insect. *eLife*. doi:10.7554/eLife.49452.001.
Vega-Trejo, R., de Boer, R.A., Fitzpatrick, J.L., et al. (2022a) Sex-specific inbreeding depression: A meta-analysis. *Ecology Letters*, 25 (4): 1009–1026. doi:10.1111/ele.13961.
Vega-Trejo, R., de Boer, R.A., Fitzpatrick, J.L., et al. (2022b) Sex-specific inbreeding depression: A meta-analysis. *Ecology Letters*, 25 (4): 1009–1026. doi:10.1111/ele.13961.
Vega-Trejo, R., Head, M.L., Keogh, J.S., et al. (2017) Experimental evidence for sexual selection against inbred males. *Journal of Animal Ecology*, 86 (2): 394–404. doi:10.1111/1365-2656.12615.
Volarić, M., Despot-Slade, E., Veseljak, D., et al. (2024) Long-read genome assembly of the insect model organism *Tribolium castaneum* reveals spread of satellite DNA in gene-rich regions by recurrent burst events. *Genome Research*, 34 (11): 1878–1894. doi:10.1101/gr.279225.124.
Wade, M.J. and Goodnight, C.J. (1991) *Wright's Shifting Balance Theory: An Experimental Study*.
Waller, D.M. (2015) Genetic rescue: a safe or risky bet? *Molecular Ecology*, 24 (11): 2595–2597. doi:10.1111/mec.13220.
West, G., Pointer, M., Nash, W., et al. (2025) Sexual selection matters in genetic rescue, but productivity benefits fade over time: a multi-generation experiment to inform conservation. *Proceedings of the Royal Society B: Biological Sciences*, 292 (2039). doi:10.1098/rspb.2024.2374.
White, S.L., Rash, J.M. and Kazyak, D.C. (2023) Is now the time? Review of genetic rescue as a conservation tool for brook trout. *Ecology and Evolution*, 13 (5). doi:10.1002/ece3.10142.
Whiteley, A.R., Fitzpatrick, S.W., Funk, W.C., et al. (2015) Genetic rescue to the rescue. *Trends in Ecology and Evolution*. 30 (1) pp. 42–49. doi:10.1016/j.tree.2014.10.009.
Whitlock, M.C. and Agrawal, A.F. (2009) Purging the genome with sexual selection: Reducing mutation load through selection on males. *Evolution*. 63 (3) pp. 569–582. doi:10.1111/j.1558-5646.2008.00558.x.
Wickham, H. (2016) *ggplot2: Elegant Graphics for Data Analysis*.
Wickham, H., Averick, M., Bryan, J., et al. (2019) Welcome to the Tidyverse. *Journal of Open Source Software*, 4 (43): 1686. doi:10.21105/joss.01686.
Willi, Y., Van Buskirk, J. and Hoffmann, A.A. (2006) Limits to the adaptive potential of small populations. *Annual Review of Ecology, Evolution, and Systematics*. 37 pp. 433–458. doi:10.1146/annurev.ecolsys.37.091305.110145.
Wolf, J.B. and Wade, M.J. (2009) What are maternal effects (and what are they not)? *Philosophical Transactions of the Royal Society B: Biological Sciences*, 364 (1520): 1107–1115. doi:10.1098/rstb.2008.0238.

Yates, A.D., Allen, J., Amode, R.M., et al. (2022) Ensembl Genomes 2022: An expanding genome resource for non-vertebrates. *Nucleic Acids Research*, 50 (D1): D996–D1003. doi:10.1093/nar/gkab1007.
Zajitschek, S.R., Zajitschek, F. and Brooks, R.C. (2009) Demographic costs of inbreeding revealed by sex-specific genetic rescue effects. *BMC Evolutionary Biology*, 9 (1). doi:10.1186/1471-2148-9-289.

Appendix 1 – Supplementary material for Chapter Two

Supplementary Tables

Table S1.1: Factors impacting the productivity of small, inbred populations (N_e = 20, n = 24) of *T. castaneum* rescued by either a male or female rescuer in the first five generations following rescue. Tested using a GLMM. Predictors in bold are significant ($P < 0.05$).

Predictor	Estimate	SE	*z*	*P*	95% CI
Intercept	6.054	0.073	82.800	<2e-16	5.911 6.197
Treatment (Control)					
Female Rescue	**0.191**	**0.054**	**3.550**	**<0.001**	**0.086** **0.296**
Male Rescue	**0.255**	**0.054**	**4.760**	**<0.001**	**0.150** **0.360**
Generation	**0.070**	**0.016**	**4.450**	**<0.001**	**0.039** **0.101**
Random	116 Observations		Variance		
ID:Inbred line	24 Populations		<0.001		
Inbred line	8 Lines		<0.001		

Table S1.2: Factors impacting the productivity of small, inbred populations (N_e = 20, n = 24) of *T. castaneum* rescued by either a male or female rescuer in generations five to ten following rescue. Tested using a GLMM. Predictors in bold are significant ($P < 0.05$).

Predictor	Estimate	SE	*z*	*P*	95% CI
Intercept	6.638	0.113	58.890	<2e-16	6.417 6.858
Treatment (Control)					
Female Rescue	**0.259**	**0.057**	**4.530**	**<0.001**	**0.147** **0.371**
Male Rescue	**0.250**	**0.057**	**4.370**	**<0.001**	**0.138** **0.363**
Generation	**-0.058**	**0.014**	**-4.260**	**<0.001**	**-0.085** **-0.031**
Random	116 Observations		Variance		
ID:Inbred line	23 Populations		<0.001		
Inbred line	8 Lines		<0.001		

Table S1.3: Factors impacting the productivity of small, inbred populations (N_e = 20, n = 24) of *T. castaneum* that had been rescued by either a male or female rescuer in the second generation following rescue. Tested using a GLMM. Predictors in bold are significant ($P < 0.05$).

Predictor	Estimate	SE	*z*	*P*	95% CI
Intercept	6.360	0.083	76.550	<2e-16	6.198 6.523
Treatment (Control)					
Female Rescue	0.035	0.092	0.370	0.708	-0.146 0.216
Male Rescue	**0.189**	**0.092**	**2.060**	**0.040**	**0.009** **0.370**
Random	23 Observations		Variance		
ID:Inbred line	23 Populations		0.171		
Inbred line	8 Lines		0.135		

Table S1.4: Factors impacting the productivity of small, inbred populations (N_e = 20, n = 24) of *T. castaneum* rescued by either a male or female rescuer in the third generation following rescue. Tested using a GLMM. Predictors in bold are significant ($P < 0.05$).

Predictor	Estimate	SE	*z*	*P*	95% CI
Intercept	6.290	0.073	86.42	<2e-16	6.147 6.432
Treatment (Control)					
Female Rescue	**0.302**	**0.077**	**3.900**	**<0.001**	**0.150** **0.454**
Male Rescue	**0.309**	**0.077**	**3.990**	**<0.001**	**0.157** **0.460**
Random	23 Observations		Variance		
ID:Inbred line	23 Populations		0.171		
Inbred line	8 Lines		0.135		

Supplementary Figures

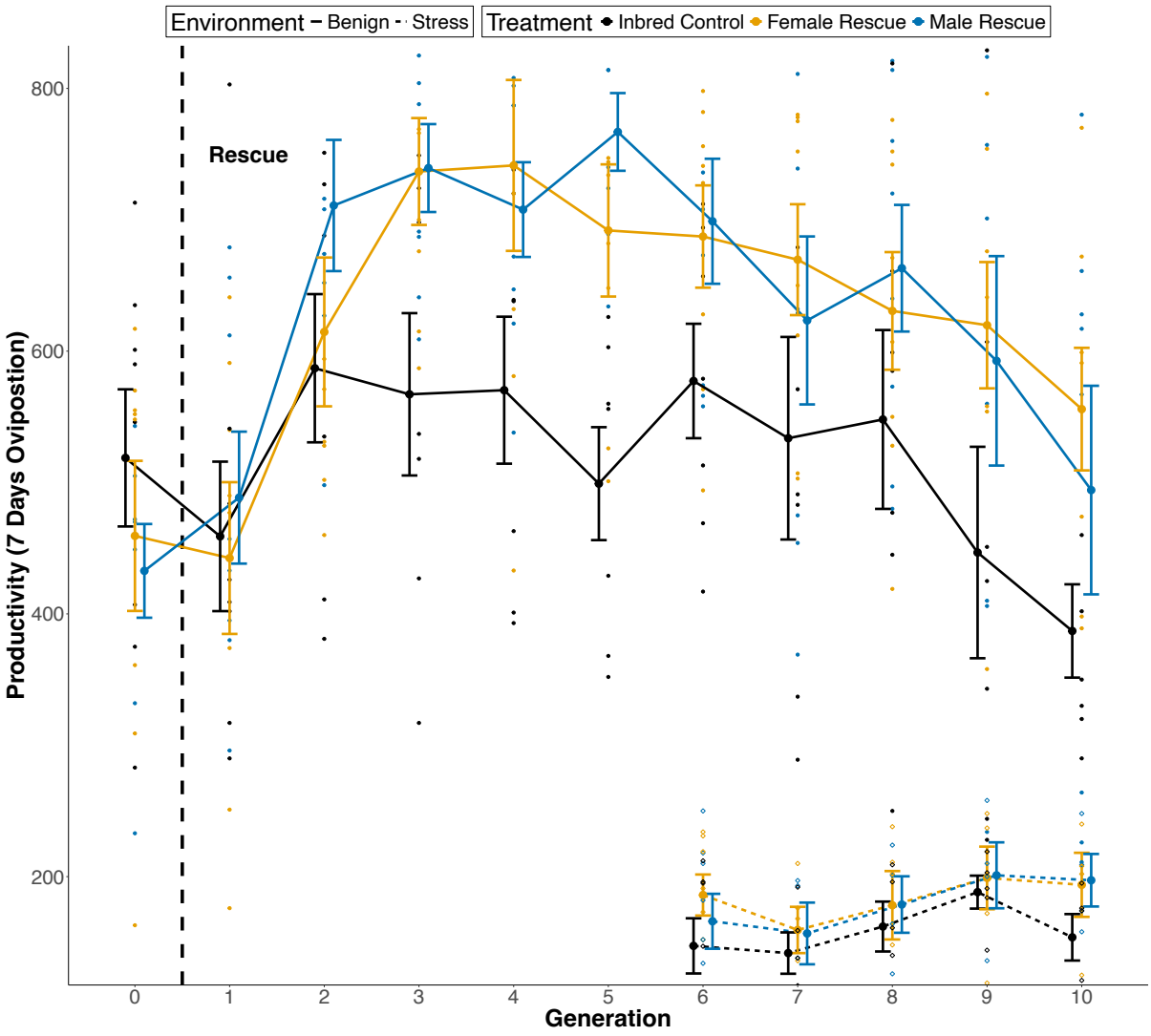

Figure S1.1: The effect of introducing a male or female rescuer on the mean productivity of small, inbred populations of *T. castaneum* (N_e = 20, n = 24/23) over 10 generations after an introduction event with raw data shown. A single male or female rescuer was used to replace one individual of the same sex (dashed vertical line) within the populations of 10 females and 10 males. Populations were kept in either benign (solid line) or stressful (dashed line – starting only at generation 6) environmental conditions (fodder with or without yeast respectively). Under benign conditions, there was a significant increase in productivity for both male (Blue), and female (Orange) rescue treatments compared to the control treatment (Black). There was also a quadratic effect of generation (See Table 1). Standard errors are shown.

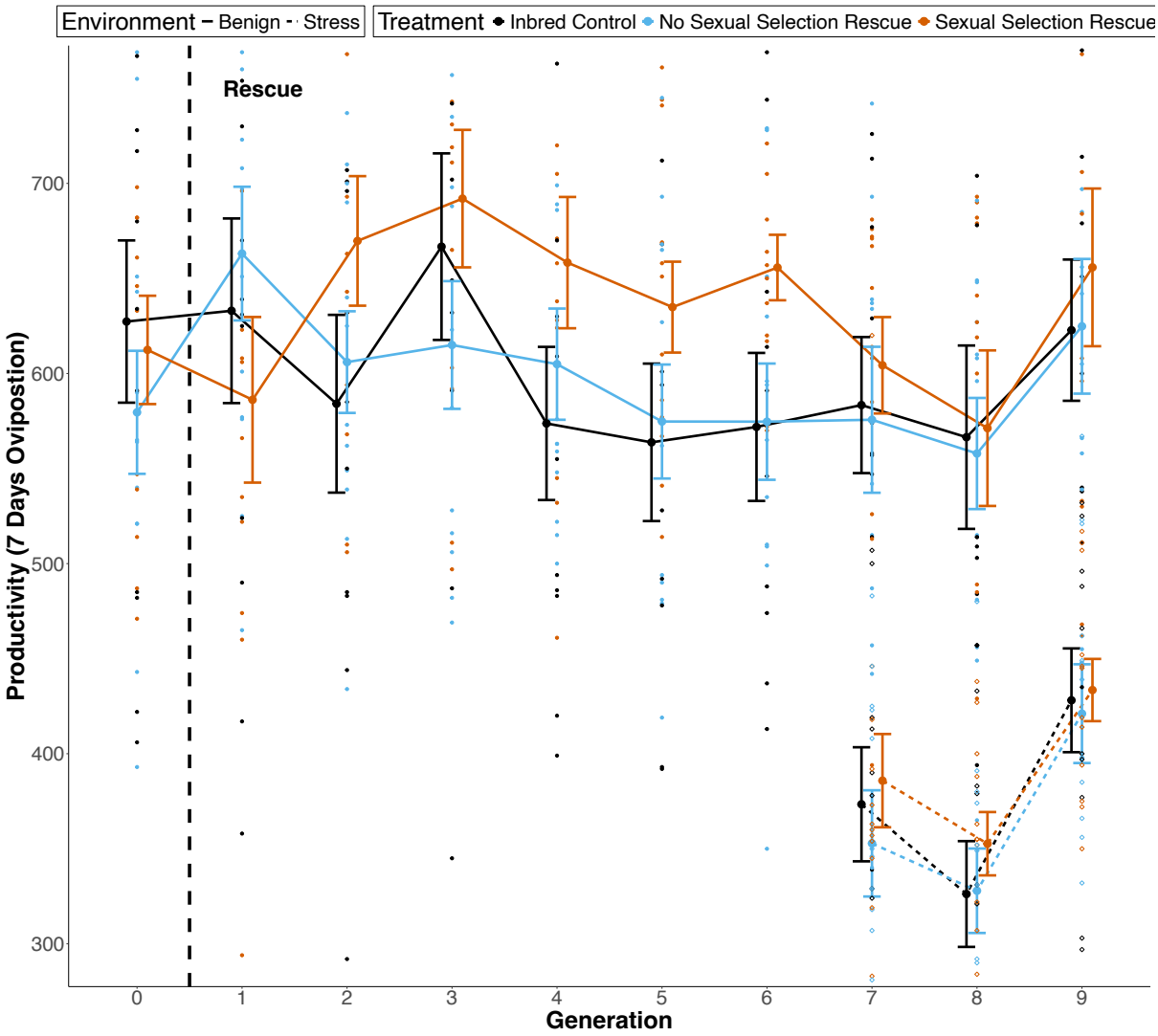

Figure S1.2: The effect of introducing a single male genetic rescuer from a sexual selection background or no sexual selection background on the productivity of small, inbred *T. castaneum* populations (N_e = 20, n = 36/34) over nine generations with raw data shown. Populations were in either a benign (solid line) or stressful (dashed line) environment. The rescue was a single event (dashed vertical line) where the rescuer replaced a male in the inbred population. Compared to the control (black) there was a significant increase in productivity in the sexual selection rescue treatment (orange), which had a quadratic interaction with generation, but no significant effect of the no sexual selection treatment (blue) (See Table 3). Standard errors are shown.

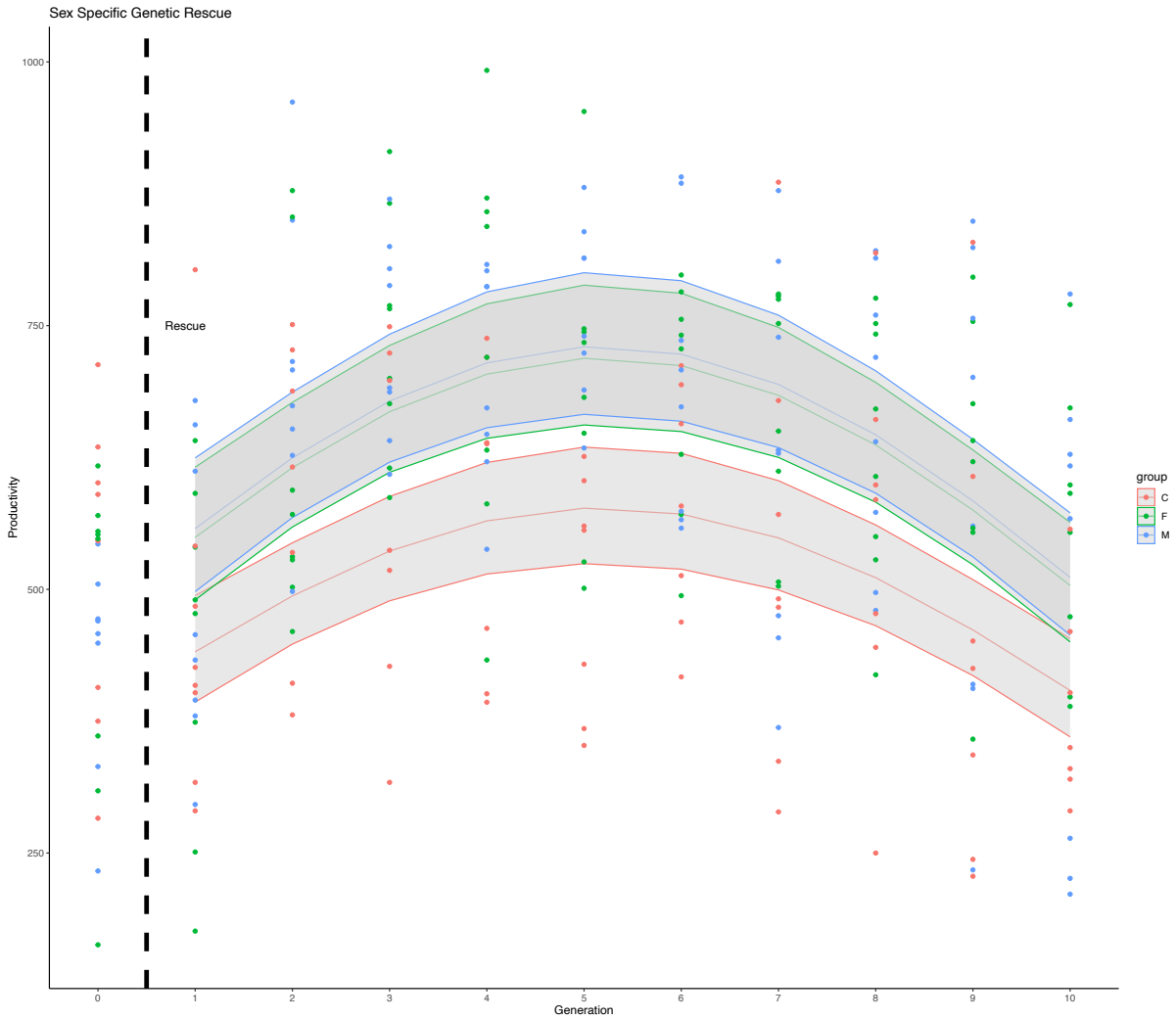

Figure S1.3: A model prediction of the effect of introducing a male or female rescuer on the mean productivity of small, inbred populations of *T. castaneum* (N_e = 20, n = 24/23) over 10 generations after an introduction event with raw data shown. A single male or female rescuer was used to replace one individual of the same sex (dashed vertical line) within the populations of 10 females and 10 males. Under benign conditions, there was a significant increase in productivity for both male (Blue), and female (Green) rescue treatments compared to the control treatment (Red). The grey ribbon shows 95% confidence intervals.

Published version of Chapter 2

West, G., Pointer, M., Nash, W., et al. (2025) Sexual selection matters in genetic rescue, but productivity benefits fade over time: a multi-generation experiment to inform conservation. *Proceedings of the Royal Society B: Biological Sciences*, 292 (2039). Doi:10.1098/rspb.2024.2374.

PROCEEDINGS B

royalsocietypublishing.org/journal/rspb

Research

Cite this article: West G, Pointer M, Nash W, Lewis R, Gage MJG, Richardson DS. 2025 Sexual selection matters in genetic rescue, but productivity benefits fade over time: a multi-generation experiment to inform conservation. *Proc. R. Soc. B* **292**: 20242374.
https://doi.org/10.1098/rspb.2024.2374

Received: 4 October 2024
Accepted: 17 December 2024

Subject Category:
Global change and conservation

Subject Areas:
evolution, genetics, genomics

Keywords:
genetic rescue, sexual selection, inbreeding depression, small populations, genetic variation, tribolium

Authors for correspondence:
George West
e-mail: gwest16@hotmail.com
David S. Richardson
e-mail: david.richardson@uea.ac.uk

Electronic supplementary material is available online at https://doi.org/10.6084/m9.figshare.c.7634288.

THE ROYAL SOCIETY PUBLISHING

Sexual selection matters in genetic rescue, but productivity benefits fade over time: a multi-generation experiment to inform conservation

George West[1], Michael Pointer[1], Will Nash[2,3], Rebecca Lewis[1], Matt J. G. Gage[1] and David S. Richardson[1]

[1]University of East Anglia School of Biological Sciences, Norwich, UK
[2]Natural History Museum, London, England, UK
[3]Earlham Institute, Norwich, England, UK

GW, 0000-0002-0489-1109; MP, 0000-0002-7926-330X; WN, 0000-0002-6790-1167; RL, 0000-0003-4739-0280; MJGG, 0000-0003-3318-6879; DSR, 0000-0001-7226-9074

Globally, many species are threatened by population decline because of anthropogenic changes leading to population fragmentation, genetic isolation and inbreeding depression. Genetic rescue, the controlled introduction of genetic variation, is a method used to relieve such effects in small populations. However, without understanding how the characteristics of rescuers impact rescue attempts interventions run the risk of being sub-optimal, or even counterproductive. We use the red flour beetle (*Tribolium castaneum*) to test the impact of rescuer sex, and sexual selection background, on population productivity. We record the impact of genetic rescue on population productivity in 24 and 36 replicated populations for ten generations following intervention. We find little or no impact of rescuer sex on the efficacy of rescue but show that a background of elevated sexual selection makes individuals more effective rescuers. In both experiments, rescue effects diminish 6–10 generations after the rescue. Our results confirm that the efficacy of genetic rescue can be influenced by characteristics of the rescuers and that the level of sexual selection in the rescuing population is an important factor. We show that any increase in fitness associated with rescue may last for a limited number of generations, suggesting implications for conservation policy and practice.

1. Introduction

Populations worldwide increasingly face extinction after becoming fragmented by human activity [1]. Fragmentation reduces population size and increases risk of genetic isolation, leading to increased impact of genetic drift and loss of genetic variation. Consequentially, many small populations suffer inbreeding depression (reduction in fitness when recessive, deleterious alleles appear in homozygous form and/or the loss of heterozygote advantage) and reduced adaptive potential [2,3]. Individuals within such populations are also more prone to environmental stress, which can exacerbate inbreeding depression [4–8]. The interaction between these factors can lead to population or species extinction [9–11].

Genetic rescue, increasing population fitness through the introduction of novel alleles beyond the demographic effects of immigration, is one way to relieve inbreeding depression [12,13]. This requires the introduction of rescuers (conspecific individuals from a different population), allowing reproduction with the inbred population. The aim is to introduce new genetic

diversity, reducing homozygosity and the expression of deleterious alleles in offspring. Introducing genetic variation also increases adaptive potential, providing standing variation for selection to act on [14,15] and increasing the potential for evolutionary rescue [16–18].

Genetic rescue has been studied in wild, captive and laboratory populations across many taxa (reviewed in [19–21]) and has seen many successful implementations [22–27]. Reviews and meta-analyses support its utility as a conservation tool [19,20,28–30]. Theoretical studies have modelled the outcome of genetic rescue in specific situations to assess the risks and benefits to wild populations [30,31]. This allows for the exploration of the potential impact of different variables, such as inbreeding in the rescuing population [32]. There is also a growing body of experimental research testing how factors, such as the sex or degree of inbreeding in rescuers, and level of environmental stress, impact genetic rescue attempts [33–38]. However, failures and negative effects have also been observed. For example, the Isle Royal wolf (*Canis lupus*) population collapsed following (naturally occurring) genetic rescue [39], in the hihi (*Notiomystis cincta*) genetic rescue resulted in increased inbreeding 10 years later [40]; and in the Macquarie perch (*Macquaria australasica*) little or no mixing occurred between the rescuers and inbred population [41].

Despite the publication of guidelines as to when and where to attempt genetic rescue [19,42], there is still considerable reluctance by conservation stakeholders to attempt rescue in wild populations [43]. This is, to some degree, understandable due to potential risks such as outbreeding depression [44]. This loss of fitness due to the crossing of two genetically divergent populations [45] is associated with the breakdown of locally adapted gene complexes [46]. An additional risk is genetic swamping, the rescuing population replacing unique genetic variation in the rescued population [47]. Despite evidence to suggest such risks may be overstated, and that mixing divergent populations can provide considerable benefits [48–50], these risks highlight the importance of understanding what characterizes the most effective rescuer(s) [29]. Genetic structure of the rescuing population is an essential consideration [32,51–53], as well as the number [54,55] and the sex of rescuers [34,56]. These factors affect how much genetic diversity and load is introduced, how quickly it can introgress, and how long the rescue effect will last.

A central criticism of many genetic rescue studies is the fact that the longevity of rescue effects is not captured, due to the number of generations observed [44,57]. Laboratory studies on species with short generation times greatly facilitate our ability to monitor outcomes over multiple generations. Consequently, we can better test if and how quickly genetic rescue occurs, how long it lasts and whether there are any negative effects in the long term. In wild studies, where it is often extremely difficult and/or expensive to follow the rescue long-term, populations are often only monitored over a few consecutive generations [58,59] or sporadically over generations [22].

Sex of rescuing individuals may be a key factor in the efficacy of genetic rescue as females are typically more limited in the number of offspring they can produce than males [60]. In many systems (i.e. promiscuous, polygynous, socially monogamous with extra-pair paternity), this means a male rescuer should speed the impact of genetic rescue. A male should sire more offspring carrying rescuing alleles and higher heterozygosity [60] than a female, meaning that this additive variation is quicker to spread in the population. This effect has been shown in both guppies (*Poecilia reticulata*) [34] and African lions (*Panthera leo*) [22,61]. In addition, purging of genetic load is more effective in males due to differences in gamete investment between the sexes [62,63]. In a rescue scenario, an individual with less genetic load should be favoured under sexual selection and have greater reproductive success.

Despite putative advantages of male rescue, female rescuers can be advantageous in other systems or scenarios. In the Florida panther (*Puma concolor couguar*), females were used for rescue [64] as they were less likely to disperse or cause social conflict [65]. Genetic load can also accumulate in mitochondrial DNA (mtDNA), which is commonly inherited through females [66]. Thus, only female rescuers can introduce mtDNA variants to a population to reduce mtDNA genetic load [67]. However, there is a risk of mitochondrial mismatch reducing offspring fitness [56]. Female rescuers may also introduce maternal effects, the mother's phenotype influencing that of the offspring [68], which may affect rescue efficiency.

Another key consideration related to both rescuer sex and the genetic structure of rescuing populations is the background of sexual selection the rescuing population has experienced. Sexual selection can vary across populations [69] affecting patterns of genetic variation [70], facilitating adaptation [71] and reducing inbreeding [72]. Stronger sexual selection has been shown to improve population fitness [73] and can also reduce genetic load in a population [62]. Individuals from high sexual selection populations should also be more competitive in securing mates, thus gaining greater reproductive success, and increasing the speed at which genetic diversity introgresses during rescue if preferences for sexually selected traits are shared across the populations. An increase in population fitness due to sexual selection has been observed in *Tribolium castaneum;* experimental populations experiencing elevated sexual selection were shown to be less likely to go extinct under stressful conditions than those that evolved under monogamy [74,75]. Although beneficial, sexual selection may also promote assortative mating [76], and potentially reduce subsequent interbreeding between rescuers and rescued, thus hindering rescue attempts. To our knowledge, no studies have tested if the effect of sexual selection background increases or decreases the efficacy of genetic rescue.

Here, we use the *T. castaneum* model [77] to experimentally address key omissions in the understanding of genetic rescue of inbred populations. *T. castaneum* has been utilized previously to study genetic rescue with one finding evidence of rescue [37] and the other not observing a rescue effect [33]. First, we test if the sex of a rescuer has an impact on genetic rescue. We predict that a male rescuer will result in a greater fitness increase in inbred populations due to the ability of males to produce more offspring than females, allowing for faster introgression. Second, we test if rescuers evolved under different levels of sexual selection differentially impact the outcome of genetic rescue. We predict that a rescuer from a strong sexual selection background will be more effective, due to lowered genetic load. Importantly, we utilize the short generation time of *T. castaneum* to follow the effects of genetic rescue over 10 generations, allowing observation of both the speed and longevity of rescue

effects. Additionally, we replicate our experimental populations under nutrient stress. We predict that stress will exaggerate the effects of inbreeding depression so that the magnitude of the rescue effect will be greater under stress than under benign conditions.

2. Methods

(a) Husbandry

T. castaneum were kept in a controlled environment at 30°C and 60% humidity with a 12:12 light-dark cycle. Populations were kept on standard fodder consisting of 90% organic white flour, 10% brewer's yeast and a layer of oats for traction unless otherwise stated. During the husbandry cycle, 2 mm and 850 µm sieves were used to remove pupae and adults from fodder. The following cycle was started by a set number of adults (line dependent, see below) being placed into containers with fresh standard fodder. The oviposition phase: populations were given 7 days to mate and lay eggs before adults were removed by sieving to prevent overlapping generations. The fodder containing eggs was returned to the container. The development phase: eggs were kept in the containers for 35 days to allow the eggs to develop into mature adults. Around day 21 of the development phase, pupae were collected to obtain known-sex virgin individuals which were then used to start the next generation. The pupae were kept as virgins in single-sex groups of 20 for 10 days to allow them to complete development. Once mature, the cycle began again with those beetles going into fresh fodder to form a population of males and females.

(b) *Tribolium castaneum* lines

Krakow super strain (KSS) was created by mixing 14 laboratory strains to maximize genetic diversity in a single strain [78]. This was used as the outbred treatment in the genetic rescue experiments.

Inbred lines were founded from KSS and inbred through three single-pair bottlenecks in the first, fifth and seventh generations. Between bottlenecks, the lines were maintained at a maximum population size of 100 randomly selected adults. Of the initial 30 lines, 24 survived the inbreeding treatment and 12 lines were maintained and used for experiments.

Sexual selection lines: polyandrous and monogamous lines were created from the Georgia 1 stock [75,79]. Each polyandrous line ($n = 3$) was maintained each generation in 12 groups each consisting of 5 males and one female. Following oviposition, the eggs from all groups in a line are mixed to form one population from which the next generation's groups will be sourced. For each monogamous line ($n = 3$), 20 separate mating pairs are bred. Following oviposition, the eggs from all pairs are mixed and the next pairs are sourced from this population to maintain that line. The number of groups and pairs in each regime results in a theoretical $N_e = 40$ in each treatment [74]. These regimes had been maintained for 150 generations when rescuers were taken. The polyandrous lines are hereafter referred to as sexual selection lines, and monogamous as no sexual selection.

(c) Genetic rescue protocol

Replicate experimental inbred populations were created from the inbred lines to serve as populations to be rescued. Pupae were sexed and placed into plastic dishes with lids, containing 10 ml standard fodder in single-sex groups. 10 ± 2 days after eclosion, 10 males and 10 females from a given line were placed in a 125 ml tub with 70 ml of standard fodder creating populations each containing twenty adult beetles at a 1:1 sex ratio for the oviposition phase. On day 20 ± 1 of the development phase, pupae were again taken from the populations using the method outlined above to create the next non-overlapping generation.

Populations were maintained using 20 reproducing adults per generation, not allowing population growth. This allowed us to maintain a roughly constant population density during offspring development across generations, avoiding the confounding influence of negative density dependence on offspring production [80–82].

Each experimental population was randomly assigned an ID number, to avoid bias when handling. After being established at the experimental size, the populations were maintained in experimental conditions for one generation to avoid transgenerational density effects affecting the genetic rescue results [83]. The rescue treatments were applied in the second generation under experimental conditions. In each population, a single beetle was replaced with a rescuer thus maintaining the 1:1 sex ratio and population size, avoiding any increase in productivity due to a demographic rescue. Rescuers taken from their source populations as pupae were age-matched as closely as possible to individuals in experimental populations. On day 37 of the development phase experimental populations were frozen at −6°C and mature offspring were counted as a measure of productivity (our metric for population fitness). If a population was removed from the experiment because of slow development (pupae were not available to establish the next generation), that population was analysed as part of all generations prior but excluded henceforth.

(d) The sex of the rescuer in genetic rescue

Due to logistic issues with ventilation, 4 out of the 12 experimental inbred populations failed to produce offspring in generation 0. From each of the remaining eight inbred lines, three replicate populations were created and assigned to one of three treatments; no rescue control (ten inbred line males, ten inbred line females); male rescue (nine inbred line males, one KSS male, ten inbred line females); and female rescue (ten inbred line males, nine inbred line females, one KSS female; figure 1). Populations were maintained for ten, non-overlapping generations.

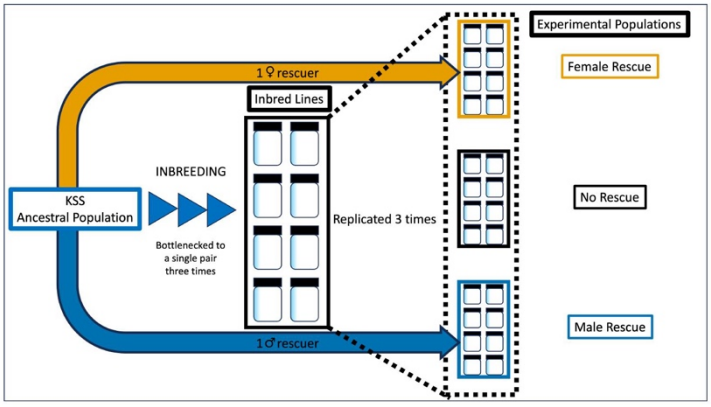

Figure 1. Experimental set-up of the creation and attempted genetic rescue of small, inbred *T. castaneum* populations ($N_e = 20$) by a single male or female rescuer from the outbred ancestral population. Three experimental populations were created from each of 8 inbred lines resulting in 24 experimental populations, every line represented once in a treatment.

(e) Sexual selection and genetic rescue

We investigated the impact of a rescuer's sexual selection history on the effectiveness of genetic rescue. From 12 inbred lines, three replicate populations were created and assigned to one of three treatments; no rescue control (ten inbred line males, ten inbred line females); sexual selection rescue (nine inbred line males, one polyandrous male and ten inbred line females); no sexual selection rescue (nine inbred line males, one monogamous male, ten inbred line females; figure 2). A single polyandrous and single monandrous line were used as the source for rescuers. Populations were maintained for nine generations.

(f) Stressful conditions

To test if genetic rescue makes populations more resilient to environmental change and/or stressful conditions, duplicate rescue populations were established from each rescued line at generation five in the 'sex' experiment, and generation six in the 'sexual selection' experiment. This was done at these generations to allow time for the rescuer genome to introgress into the recipient population before the environmental change. These populations were maintained as in the main experiments (until generation ten and nine, respectively), but with a reduction in the yeast content of the fodder, which is the main source of protein for the experimental populations. This reduction generates nutrient stress in *T. castaneum* [74]. In the 'sex' experiment, fodder contained 0% yeast and 1% yeast in the 'sexual selection' experiment (because of low survival with zero yeast).

(g) Statistical analyses

Statistical analyses were carried out in R v. 4.4.1 [84] utilizing R studio version 2024.04.2 + 764 [85]. Tidyverse [86], stats [84], Rmisc [87] and googlesheets4 [88] were used for data management and exploration. Plots were created using ggplot2 [89]. The distribution of data was checked using the shapiro.test function [84]. Generalized linear mixed models (GLMMs) were fitted to test for differences in productivity between the experimental treatments using glmmTMB [90]. Model fit was checked using DHARMa [91]. Model parameters were checked for collinearity using variance inflation factor (Vif) scores with the check_collinearity function from performance [92]. There were no issues with overdispersion or collinearity (VIF: < 3 for all variables) in any models. R^2 was determined using the r.squaredGLMM function in MuMIn [93]. Post-hoc pairwise Tukey tests were carried out using multcomp [94]. Ggeffects [95] package was used for model predictions.

Within each experiment, we fitted GLMMs with the same model structure, using a negative binomial distribution to model productivity counts, which provided better model fit than a Poisson distribution. Productivity was the response variable, with treatment, generation and generation^2 as fixed effects. Inbred line of origin and experimental population ID were included as random effects, with ID nested within inbred line. Interaction terms (treatment × generation, treatment × generation^2) were initially included but removed from the model if not significant. The generation^2 factor was not significant in the models for populations under stressful conditions and was therefore removed. When a quadratic effect of generation was detected in a model, we plotted the model prediction to show the non-monotonic effect of generation on productivity and to identify the generation at which the slope changed. Then, to test if there was both a significant increase and, importantly, a significant decrease in productivity two separate GLMMs (with the same factors as previously) were run on the data split into generations 1–5 and 5–10 (either side of the peak). These GLMMs were fitted with treatment and generation as fixed effects, ID nested within inbred line as a random effect. GLMMs were also fitted on generations 2 and 3 individually (electronic supplementary material, tables S3 and S4) in the 'sex' experiment, these single-generation models used a Poisson distribution, productivity as a response variable and treatment as a fixed effect. Random effects were the same as above. This was to test at which point the

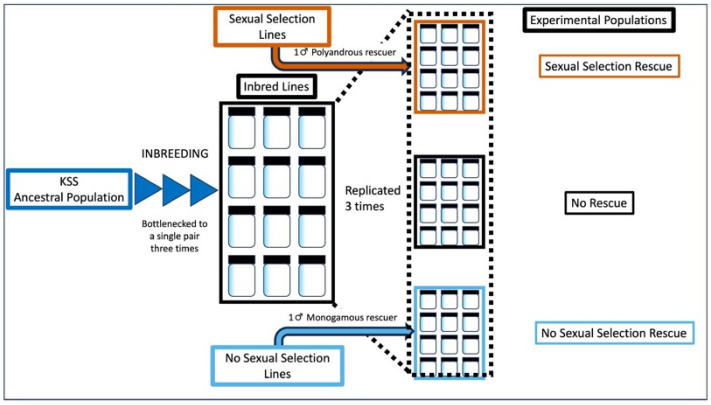

Figure 2. Experimental procedure for the creation and attempted genetic rescue of small, inbred *T. castaneum* populations ($N_e = 20$) by a single male rescuer from either a sexual selection or no sexual selection line. Three experimental populations were created from each of 12 inbred lines resulting in 36 experimental populations, every line represented once in a treatment.

rescue treatments resulted in a significant difference from the control, to see if there were differences in the speed of male or female rescue.

3. Results

(a) The sex of the rescuer in genetic rescue

Twenty-four populations were initiated, but in generation 2 one population in the control inbred populations failed to pupate in time for the next generation. Generations 0 and 1 for this population were included in the data set.

Male and female rescuer treatments both resulted in significantly higher productivity than the control (see table 1; figure 3). Generation^2 also had a significant negative effect. Interactions between rescuer sex treatment × generation (and generation^2) were not significant. In post-hoc tests, there was no significant difference across all generations between the male and female rescue treatments (estimate = 0.015, s.e. = 0.040, $z = 0.374$, $p = 0.926$, 95% CI = −0.079, −0.109).

Post-hoc tests showed that by generation 2 the productivity of the male rescue lines was significantly higher than the control lines (see figure 3; electronic supplementary material, table S3; estimate = 0.189, s.e. = 0.092, $z = 2.060$, $p = 0.040$, 95% CI = 0.009, 0.370), but the productivity of the female rescue lines was not (see figure 3; electronic supplementary material, table S3; estimate = 0.035, s.e. = 0.092, $z = 0.370$, $p = 0.708$, 95% CI = −0.146, 0.216). However, the productivity of male and female rescued lines in that generation 2 was not significantly different (see figure 3; estimate = 0.155, s.e. = 0.088, $z = 1.760$, $p = 0.183$, 95% CI = −0.051, 0.361). There was no significant difference between the male and female rescued lines in any other single generation (see figure 3).

Plotting the model prediction shows that productivity increased until generation 5 then began to decline as expected by the negative estimate (see table 1; electronic supplementary material, figure S3). When modelled separately post-hoc, over generations 1–5 productivity increased significantly (see electronic supplementary material, table S1; estimate = 0.070, s.e. = 0.016, $z = 4.450$, $p < 0.001$, 95% CI = 0.039, 0.101), then over generations 5–10 productivity decreased significantly (see electronic supplementary material, table S2; estimate = −0.058, s.e. = 0.014, $z = -4.26$, $p < 0.001$, 95% CI = −0.085, −0.031).

Under stress conditions (0% yeast in fodder) productivity greatly decreased (figure 3), and there were no significant differences between the treatments. There was a significant linear effect of generation on productivity (see table 2).

(b) Sexual selection and genetic rescue

Thirty-six populations were initiated, but in both generations two and five one population in the control inbred populations failed to pupate in time for the next generation. These populations were included in the analyses.

When introducing a rescuer from a sexual selection population productivity interacted with generation^2 (i.e. there was an increase in productivity followed by a later decline). There was no evidence of an interaction between 'no sexual selection' rescue and generation^2 (figure 4; table 3). There was no significant effect when the interaction was removed.

Under stress conditions, there were no significant differences between the treatments' productivity, but productivity did increase over generations (figure 4; table 4).

4. Discussion

We tested how the sex and sexual selection evolutionary history of a rescuing individual affects the duration of genetic rescue using small, inbred populations of *T. castaneum*. Our results show that a male or a female rescuer was equally effective; both

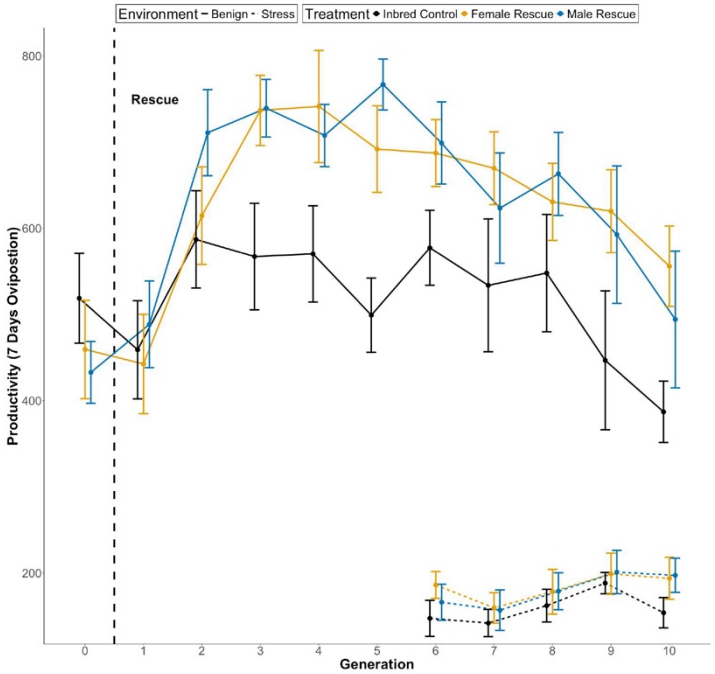

Figure 3. The effect of introducing a male or female rescuer on the mean productivity of small, inbred populations of *T. castaneum* ($N_e = 20$, $n = 24/23$) over 10 generations after an introduction event. A single male or female rescuer was used to replace one individual of the same sex (dashed vertical line) within the populations of 10 females and 10 males. Populations were kept in either benign (solid line) or stressful (dashed line—starting only at generation 6) environmental conditions (fodder with or without yeast, respectively). Under benign conditions, there was a significant increase in productivity for both male (blue), and female (orange) rescue treatments compared to the control treatment (Black). There was also a quadratic interaction with generation (see table 1). Standard errors are shown.

Table 1. Factors impacting the productivity of small, inbred populations of *T. castaneum* ($N_e = 20$, $n = 24$) receiving a single male or female genetic rescuer, or no rescue, tested using a GLMM. Productivity was measured over 10 generations following the rescue event. Predictors in bold are significant ($p < 0.05$). Marginal $R^2 = 0.247$, conditional $R^2 = 0.330$. *One population was lost in generation 2, so there are 23 populations from generation 2 onwards.

predictor	estimate	s.e.	*z*	*p*	95% CI
intercept	5.944	0.075	79.570	<2e−16	5.798, 6.091
treatment (baseline = control)					
female rescue	**0.220**	**0.042**	**5.220**	**<0.001***	**0.138, 0.303**
male rescue	**0.235**	**0.042**	**5.590**	**<0.001***	**0.153, 0.318**
generation	**0.160**	**0.027**	**6.030**	**<0.001***	**0.108, 0.212**
generation^2	**−0.015**	**0.002**	**−6.580**	**<0.001***	**−0.020, −0.011**
random	231 observations		variance		
ID:inbred line	24 populations*		7.056e−10		
inbred line	8 Lines		8.295e−03		

improved productivity compared to the control, though there was some evidence that a male rescuer led to faster rescue. In the second experiment, the introduction of a male from an elevated sexual selection background resulted in a significant increase in productivity, while a male from a monogamous background did not. Importantly, in both experiments we observed temporal effects; in the successful rescue treatments, productivity increases were observed in the initial generations after the introduction of rescuers, before declining in later generations. When these experiments were replicated under severe nutrient stress conditions we saw no significant effect of rescue on productivity.

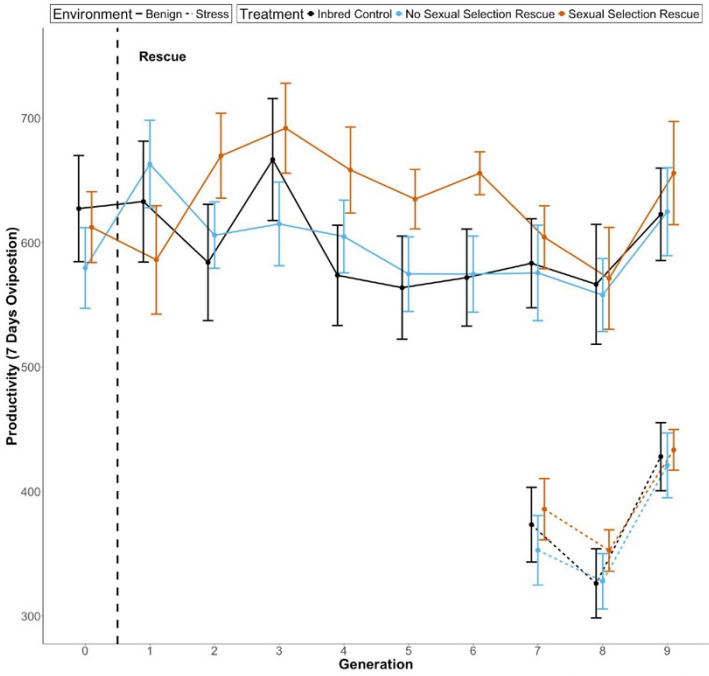

Figure 4. The effect of introducing a single male genetic rescuer from a sexual selection background or no sexual selection background on the productivity of small, inbred *T. castaneum* populations ($N_e = 20$, $n = 36/34$) over nine generations. Populations were in either a benign (solid line) or stressful (dashed line) environment. The rescue was a single event (dashed vertical line) where the rescuer replaced a male in the inbred population. Compared to the control (black) there was a significant increase in productivity in the sexual selection rescue treatment (orange), which had a quadratic interaction with generation but no significant effect of the no sexual selection treatment (blue) (see table 3). Standard errors are shown.

Table 2. Factors impacting the productivity of small, inbred *T. castaneum* populations ($N_e = 20$, $n = 23$) under nutrient stress that had either a male or female rescuer from an outbred population introduced five generations prior, tested using a GLMM. Predictors in bold are significant ($p < 0.05$).

predictor	estimate	s.e.	*z*	*p*	95% CI
intercept	4.716	0.136	34.570	<2e−16	4.449, 4.984
treatment (baseline = control)					
female rescue	0.221	0.117	1.890	0.059	−0.008, 0.451
male rescue	0.113	0.118	1.040	0.300	−0.109, 0.354
generation	**0.038**	**0.012**	**3.190**	**0.001***	**0.015, 0.062**
random	78 observations		variance		
ID:inbred line	23 populations		0.040		
inbred line	8 lines		0.021		

Male rescuers have been suggested to enable faster/greater genetic rescue than females due to their higher reproductive potential, as generating more offspring will spread introduced genetic diversity faster [34]. In our results, females are as effective at rescuing the inbred populations as males. We did find some evidence that males may enable faster rescue of productivity; with male rescue lines showing a significantly earlier increase in productivity compared to control lines (by generation 2) than female (by generation 3) rescue lines (see figure 3 and electronic supplementary material, table S3). This did not translate into a significant difference between the productivity of male and female rescue lines in generation 2. This result contrasts with previous studies: in wild lions, males were more effective rescuers despite potential issues of social disruption and infanticide [61] in guppies, faster population growth was observed following male rescue [34]. One aspect that may explain these differences is the extreme disparity between the mating systems of target species, coupled with our experimental approach. We used smaller populations ($N_e = 20$) than in other studies of genetic rescue in *T. castaneum* [33,37,96], which may

Table 3. Factors impacting the productivity of small, inbred populations ($N_e = 20$, $n = 36$) of *T. castaneum* that received a single rescuer from either a sexual selection or no sexual selection background line population, tested using a GLMM. Predictors in bold are significant ($p < 0.05$). Marginal $R^2 = 0.077$, conditional $R^2 = 0.512$. *One population was lost in generation 2 and one in generation 5.

predictor	estimate	s.e.	z	p	95% CI
intercept	6.482	0.068	94.800	<2e−16	6.348, 6.616
treatment (baseline = control)					
no sexual selection	0.063	0.090	0.700	0.486	−0.114, 0.240
sexual selection	−0.082	0.090	−0.920	0.360	−0.259, 0.094
generation	−0.047	0.026	−1.760	0.078	−0.100, 0.005
generation^2	0.004	0.003	1.560	0.120	−0.001, 0.009
treatment × generation (Control)					
no sexual selection × generation	−0.019	0.037	−0.500	0.614	−0.091, 0.054
sexual selection × generation	**0.082**	**0.037**	**2.240**	**0.025***	**0.010, 0.154**
treatment × generation^2 (control)					
no sexual selection × generation^2	0.001	0.004	0.370	0.710	−0.006, 0.009
sexual selection × generation^2	**−0.008**	**0.004**	**−2.260**	**0.024***	**−0.015, −0.001**
random	311 observations		variance		
ID:inbred line	36* populations		0.013		
inbred line	12		0.006		

Table 4. Factors impacting the productivity of small, inbred populations ($N_e = 20$, $n = 34$) of *T. castaneum* under nutrient stress that had been rescued by either a sexual selection or no sexual selection background male rescuer seven generations prior, tested using a GLMM. Predictors in bold are significant ($p < 0.05$).

predictor	estimate	s.e.	z	p	95% CI
intercept	5.281	0.180	29.385	<2e−16	4.929, 5.633
treatment (baseline = Control)					
no sexual selection rescue	−0.023	0.066	−0.352	0.725	−0.152, 0.106
sexual selection rescue	0.048	0.065	0.741	0.459	−0.080, 0.177
generation	**0.079**	**0.021**	**3.731**	**<0.001***	**0.038, 0.120**
random	102 observations		variance		
ID:inbred line	34 populations		0.013		
inbred line	12		0.011		

have limited the advantage that male rescuers had over female rescuers. As female *T. castaneum* can mate with 4–6 males in an hour [97], the 10 females available to a male in our populations over 7 days is far less than his mating potential, and thus the impact of genetic rescue. More experimentation is needed, factoring in population size and testing species with different variations in reproductive success between sexes.

T. castaneum is a promiscuous and highly fecund species [77] and our results are applicable to species with similar life history strategies and mating systems. Females in this system may act as equivalent rescuers to males as there is evidence of inbreeding avoidance in the female reproductive behaviour [98,99] meaning negative impacts of inbreeding [100,101] may be minimized. However, *T. castaneum* females do not exhibit care for offspring [102], eliminating a potential advantage provided by a female rescuer [103,104].

We predicted that rescuers drawn from populations with elevated sexual selection would be more fit (with less genetic load) and more competitive, resulting in a more effective genetic rescue. Our results support this, rescuers with a high sexual selection background improved productivity in the inbred populations, whereas rescuers from a no sexual selection background did not. The lines from which our sexually selected rescuers were sourced have previously been shown to resist extinction in the face of inbreeding, relative to lines with no history of sexual selection [74,75] suggesting that these lines have a higher fitness due to sexual selection. Using males from these lines as rescuers may have increased productivity for several reasons, including increased mating competitiveness and increased fitness in offspring with lower genetic load. Furthermore, lower introduced genetic load should result in less re-emergent inbreeding depression in later generations in these small

populations. However, the rescue may fail if populations have divergent traits or differences in trait preference. The rescuer, and their offspring, may be selected against due to differences in sexual selection, inhibiting introgression and thus reducing any fitness benefits. Further work is needed to unravel these possibilities.

The effects of inbreeding depression on endangered populations are often exacerbated by exposure to environmental stress [5,8]. However, when testing rescue treatments under stressful (nutrient) conditions we found no significant differences between treatments in either sex or sexual selection experiments. This was unexpected as stress should magnify inbreeding depression and disproportionally affect the productivity of populations that had not been rescued. This lack of effect may be due to the harshness of the nutrient stress treatment we used, as this has been shown to greatly reduce female fecundity and slow offspring development [105]. Nutrient stress could also increase cannibalism, which occurs in *T. castaneum* when food is scarce [81]. This may have had more impact on rescued populations due to increased competition for resources when initial productivity (eggs laid) is higher. However, stressful conditions do not always exaggerate inbreeding depression [5,106]. Our finding that stress-repressed genetic rescue points to the importance of improving environmental conditions for species before attempting to recover population numbers [44,107,108].

A regular criticism of genetic rescue studies is that they fail to monitor populations over sufficient timescales [57]. Our study continued monitoring rescue outcomes over multiple (9–10) generations. We see genetic rescue effects begin in the second generation after rescue. Rescue effects are not seen in the generation immediately following rescue, likely because, even in a promiscuous population, it will take more than one generation for the variation from a single rescuer to introgress widely into the population and influence overall productivity. In both experiments, the treatments that result in rescue have peak productivity around generations 5 and 6. This suggests the beneficial introgression of the rescuer's genetic diversity into the population takes several generations, as seen in previous studies [37].

Importantly, we saw productivity benefits of rescue began to decline by the sixth generation in both experiments. Many genetic rescue studies are relatively short-term projects relative to the generation time of the species involved [19]. Owing to the short generation times of *T. castaneum* [77], we are the first to show that rescue effects may be short-lasting. This has important implications for studies in wild systems, reinforcing suggestions that monitoring must continue in the long-term, but also that single rescue introductions are potentially not sufficient to rescue populations. We suggest our findings are associated with the resumption of inbreeding effects in later generations due to small population size (n = 20). In other systems, increases in population size resulting from genetic rescue may allow for the introduced genetic diversity to be maintained. If population growth had been allowed, the decline in productivity seen in our experiment may not have occurred as it may have been a product of the continued restricted small size of the populations, leading to the re-emergence of inbreeding depression. However, it must be noted that in some cases endangered populations where genetic rescue may be attempted may also be restricted to small sizes because of factors such as habitat restrictions, etc. [30]. This does not reduce the relevance to conservation contexts, as similar effects have been seen in wild systems [39,109,110]. The genetic rescue of the Florida Panther resulted in benefits for five generations after rescue [111], our results suggest that in the coming generations, these benefits may start to decline.

In conclusion, we find that both male and female rescuers can be effective genetic rescuers. This is likely linked to the dynamics of promiscuous mating systems such as that seen in *T. castaneum* but serves to highlight the importance of such species-dependent traits when planning conservation interventions. Importantly, and for the first time, we show sexual selection background affects the efficacy of genetic rescue. Given these results, we suggest that, where feasible, using a rescuer from a high sexual selection background when attempting genetic rescue could be beneficial in conservation programs. Overall, our results add important evidence to our understanding of the effectiveness of genetic rescue and support the argument that it should be considered an important tool to conserve endangered populations.

Ethics. No ethics approval was required for this study as experiments were conducted on an unregulated invertebrate species.

Data accessibility. All data and scripts are available at Dryad [112].

Supplementary material is available online [113].

Declaration of AI use. We have not used AI-assisted technologies in creating this article.

Authors' contributions. G.W.: conceptualization, data curation, formal analysis, investigation, methodology, project administration, visualization, writing—original draft, writing—review and editing; M.P.: investigation, methodology, resources, writing—review and editing; W.N.: supervision, writing—review and editing; R.L.: conceptualization, investigation, methodology, supervision, writing—review and editing; M.J.G.G.: conceptualization, funding acquisition, supervision; D.S.R.: conceptualization, formal analysis, funding acquisition, methodology, project administration, resources, supervision, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare we have no competing interests.

Funding. This work was supported by the Natural Environment Research Council, including an ARIES DTP PhD [NE/S007334/1] to George West, and a Research Grant (Understanding heatwave damage through reproduction in insect systems) [NE/T007885/1] to Matt Gage. The authors also acknowledge support from the Biotechnology and Biological Sciences Research Council (BBSRC), part of UK Research and Innovation, Core Capability Grant BB/CCG2220/1 at the Earlham Institute (EI) and its constituent work packages (BBS/E/T/000PR9818 and BBS/E/T/000PR9819), and the Core Capability Grant BB/CCG1720/1 and the National Capability BBS/E/T/000PR9816 (NC1—Supporting EI's ISPs and the UK Community with Genomics and Single Cell Analysis), BBS/E/T/000PR9811 (NC4—Enabling and Advancing Life Scientists in data-driven research through Advanced Genomics and Computational Training),and BBS/E/T/000PR9814 (NC 3—Development and deployment of versatile digital platforms for 'omics-based data sharing and analysis). Also support from BBSRC Core Capability Grant BB/CCG1720/1 and the work delivered via the Scientific Computing group, and the physical HPC infrastructure and data centre delivered via the NBI Computing infrastructure for Science (CiS) group.

Acknowledgements. We thank the members of the UEA Tribolium Lab for assistance with line maintenance and data collection. We also thank the editors and two anonymous reviewers for their feedback.

References

1. Ceballos G, Ehrlich PR. 2023 Mutilation of the tree of life via mass extinction of animal genera. *Proc. Natl Acad. Sci. USA* **120**, e2306987120. (doi:10.1073/pnas.2306987120)
2. Charlesworth D, Willis JH. 2009 The genetics of inbreeding depression. *Nat. Rev. Genet.* **10**, 783–796. (doi:10.1038/nrg2664)
3. Crnokrak P, Roff DA. 1999 Inbreeding depression in the wild. *Heredity* **83**, 260–270. (doi:10.1038/sj.hdy.6885530)
4. Frankham R. 2005 Stress and adaptation in conservation genetics. *J. Evol. Biol.* **18**, 750–755. (doi:10.1111/j.1420-9101.2005.00885.x)
5. Armbruster P, Reed DH. 2005 Inbreeding depression in benign and stressful environments. *Heredity* **95**, 235–242. (doi:10.1038/sj.hdy.6800721)
6. Fox CW, Reed DH. 2011 Inbreeding depression increases with environmental stress: an experimental study and *META*-analysis. *Evol. Int. J. Org. Evol.* **65**, 246–258. (doi:10.1111/j.1558-5646.2010.01108.x)
7. Reed DH, Briscoe DA, Frankham R. 2002 Inbreeding and extinction: the effect of environmental stress and lineage. *Conserv. Genet.* **3**, 301–307. (doi:10.1023/A:1019948130263)
8. Richardson DS, Komdeur J, Burke T. 2004 Inbreeding in the seychelles warbler: environment-dependent maternal effects. *Evol. Int. J. Org. Evol.* **58**, 2037–2048. (doi:10.1111/j.0014-3820.2004.tb00488.x)
9. Usher MB, Soule ME. 1987 Conservation biology: the science of scarcity and diversity. *J. Ecol.* **75**, 1212. (doi:10.2307/2260338)
10. Blomqvist D, Pauliny A, Larsson M, Flodin LA. 2010 Trapped in the extinction vortex? Strong genetic effects in a declining vertebrate population. *BMC Evol. Biol.* **10**, 33. (doi:10.1186/1471-2148-10-33)
11. Palomares F, Godoy JA, López-Bao JV, Rodríguez A, Roques S, Casas-Marce M, Revilla E, Delibes M. 2012 Possible extinction vortex for a population of Iberian lynx on the verge of extirpation. *Conserv. Biol.* **26**, 689–697. (doi:10.1111/j.1523-1739.2012.01870.x)
12. Ingvarsson PK. 2001 Restoration of genetic variation lost—the genetic rescue hypothesis. *Trends Ecol. Evol.* **16**, 62–63. (doi:10.1016/s0169-5347(00)02065-6)
13. Hedrick PW, Adams JR, Vucetich JA. 2011 Reevaluating and broadening the definition of genetic rescue. *Conserv. Biol.* **25**, 1069–1070. (doi:10.1111/j.1523-1739.2011.01751.x)
14. Hoelzel AR, Bruford MW, Fleischer RC. 2019 Conservation of adaptive potential and functional diversity. *Conserv. Genet.* **20**, 1–5. (doi:10.1007/s10592-019-01151-x)
15. Mable BK. 2019 Conservation of adaptive potential and functional diversity: integrating old and new approaches. *Conserv. Genet.* **20**, 89–100. (doi:10.1007/s10592-018-1129-9)
16. Bell G, Gonzalez A. 2009 Evolutionary rescue can prevent extinction following environmental change. *Ecol. Lett.* **12**, 942–948. (doi:10.1111/j.1461-0248.2009.01350.x)
17. Schiffers K, Bourne EC, Lavergne S, Thuiller W, Travis JMJ. 2013 Limited evolutionary rescue of locally adapted populations facing climate change. *Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci.* **368**, 20120083. (doi:10.1098/rstb.2012.0083)
18. Lindsey HA, Gallie J, Taylor S, Kerr B. 2013 Evolutionary rescue from extinction is contingent on a lower rate of environmental change. *Nature* **494**, 463–467. (doi:10.1038/nature11879)
19. Frankham R. 2015 Genetic rescue of small inbred populations: meta-analysis reveals large and consistent benefits of gene flow. *Mol. Ecol.* **24**, 2610–2618. (doi:10.1111/mec.13139)
20. Frankham R. 2018 Corrigendum to Genetic rescue benefits persist to at least the F3 generation, based on a meta-analysis. *Biol. Conserv.* **219**, 174. (doi:10.1016/j.biocon.2018.01.019)
21. White SL, Rash JM, Kazyak DC. 2023 Is now the time? Review of genetic rescue as a conservation tool for brook trout. *Ecol. Evol.* **13**, e10142. (doi:10.1002/ece3.10142)
22. Miller SM, Druce DJ, Dalton DL, Harper CK, Kotze A, Packer C, Slotow R, Bloomer P. 2020 Genetic rescue of an isolated African lion population. *Conserv. Genet.* **21**, 41–53. (doi:10.1007/s10592-019-01231-y)
23. Robinson ZL, Coombs JA, Hudy M, Nislow KH, Letcher BH, Whiteley AR. 2017 Experimental test of genetic rescue in isolated populations of brook trout. *Mol. Ecol.* **26**, 4418–4433. (doi:10.1111/mec.14225)
24. Pregler KC, Obedzinski M, Gilbert-Horvath EA, White B, Carlson SM, Garza JC. 2023 Assisted gene flow from outcrossing shows the potential for genetic rescue in an endangered salmon population. *Conserv. Lett.* **16**. (doi:10.1111/conl.12934)
25. Davis MM, Smyser TJ, Johnson SA, Duchamp J, Larkin JL, Swihart RK, Doyle JM. 2021 Reproductive success of captive-reared Allegheny Woodrats (*Neotoma magister*) released into genetically depauperate populations. *Conserv. Genet.* **22**, 903–912. (doi:10.1007/s10592-021-01372-z)
26. Pavlova A *et al*. 2023 Benefits of genetic rescue of a critically endangered subspecies from another subspecies outweigh risks: Results of captive breeding trials. *Biol. Conserv.* **284**, 110203. (doi:10.1016/j.biocon.2023.110203)
27. Madsen T, Loman J, Anderberg L, Anderberg H, Georges A, Ujvari B. 2020 Genetic rescue restores long-term viability of an isolated population of adders (*Vipera berus*). *Curr. Biol.* **30**, R1297–R1299. (doi:10.1016/j.cub.2020.08.059)
28. Waller DM. 2015 Genetic rescue: a safe or risky bet? *Mol. Ecol.* **24**, 2595–2597. (doi:10.1111/mec.13220)
29. Whiteley AR, Fitzpatrick SW, Funk WC, Tallmon DA. 2015 Genetic rescue to the rescue. *Trends Ecol. Evol.* **30**, 42–49. (doi:10.1016/j.tree.2014.10.009)
30. Robinson ZL, Bell DA, Dhendup T, Luikart G, Whiteley AR, Kardos M. 2021 Evaluating the outcomes of genetic rescue attempts. *Conserv. Biol.* **35**, 666–677. (doi:10.1111/cobi.13596)
31. Ash E, Cushman S, Kaszta Ż, Landguth E, Redford T, Macdonald DW. 2023 Female-biased introductions produce higher predicted population size and genetic diversity in simulations of a small, isolated tiger (*Panthera tigris*) population. *Sci. Rep.* **13**, 11199. (doi:10.1038/s41598-023-36849-z)
32. Kyriazis CC, Wayne RK, Lohmueller KE. 2021 Strongly deleterious mutations are a primary determinant of extinction risk due to inbreeding depression. *Evol. Lett.* **5**, 33–47. (doi:10.1002/evl3.209)
33. Lewis R, Pointer MD, Friend L, Gage MJG, Spurgin LG. 2024 Tests of evolutionary and genetic rescue using flour beetles, *Tribolium castaneum* , experimentally evolved to thermal conditions. *Ecol. Evol.* **14**, e11313. (doi:10.1002/ece3.11313)
34. Zajitschek SRK, Zajitschek F, Brooks RC. 2009 Demographic costs of inbreeding revealed by sex-specific genetic rescue effects. *BMC Evol. Biol.* **9**, 289. (doi:10.1186/1471-2148-9-289)
35. Jørgensen DB, Ørsted M, Kristensen TN. 2022 Sustained positive consequences of genetic rescue of fitness and behavioural traits in inbred populations of *Drosophila melanogaster*. *J. Evol. Biol.* **35**, 868–878. (doi:10.1111/jeb.14015)
36. Heber S, Briskie JV, Apiolaza LA. 2012 A test of the 'genetic rescue' technique using bottlenecked donor populations of *Drosophila melanogaster*. *PloS One* **7**, e43113. (doi:10.1371/journal.pone.0043113)
37. Hufbauer RA, Szűcs M, Kasyon E, Youngberg C, Koontz MJ, Richards C, Tuff T, Melbourne BA. 2015 Three types of rescue can avert extinction in a changing environment. *Proc. Natl Acad. Sci. USA* **112**, 10557–10562. (doi:10.1073/pnas.1504732112)

38. Bijlsma R, Westerhof MDD, Roekx LP, Pen I. 2010 Dynamics of genetic rescue in inbred *Drosophila melanogaster* populations. *Conserv. Genet.* **11**, 449–462. (doi:10.1007/s10592-010-0058-z)
39. Hedrick PW, Peterson RO, Vucetich LM, Adams JR, Vucetich JA. 2014 Genetic rescue in Isle Royale wolves: genetic analysis and the collapse of the population. *Conserv. Genet.* **15**, 1111–1121. (doi:10.1007/s10592-014-0604-1)
40. Nichols S, Ewen JG, Gottelli D, Grueber CE, Santure AW, Trask A, Brekke P. 2024 Genetic rescue attempt in a small, inbred population of a wild endangered passerine. *Biol. Conserv.* **290**, 110430. (doi:10.1016/j.biocon.2023.110430)
41. Pavlova A, Schneller NM, Lintermans M, Beitzel M, Robledo-Ruiz DA, Sunnucks P. 2024 Planning and implementing genetic rescue of an endangered freshwater fish population in a regulated river, where low flow reduces breeding opportunities and may trigger inbreeding depression. *Evol. Appl.* **17**, e13679. (doi:10.1111/eva.13679)
42. Hedrick PW, Fredrickson R. 2010 Genetic rescue guidelines with examples from Mexican wolves and Florida panthers. *Conserv. Genet.* **11**, 615–626. (doi:10.1007/s10592-009-9999-5)
43. Fitzpatrick SW, Mittan-Moreau C, Miller M, Judson JM. 2023 Genetic rescue remains underused for aiding recovery of federally listed vertebrates in the United States. *J. Hered.* **114**, 354–366. (doi:10.1093/jhered/esad002)
44. Bell DA, Robinson ZL, Funk WC, Fitzpatrick SW, Allendorf FW, Tallmon DA, Whiteley AR. 2019 The exciting potential and remaining uncertainties of genetic rescue. *Trends Ecol. Evol.* **34**, 1070–1079. (doi:10.1016/j.tree.2019.06.006)
45. Edmands S. 2007 Between a rock and a hard place: evaluating the relative risks of inbreeding and outbreeding for conservation and management. *Mol. Ecol.* **16**, 463–475. (doi:10.1111/j.1365-294x.2006.03148.x)
46. Lenormand T. 2002 Gene flow and the limits to natural selection. *Trends Ecol. Evol.* **17**, 183–189. (doi:10.1016/s0169-5347(02)02497-7)
47. Rhymer JM, Simberloff D. 1996 Extinction by hybridization and introgression. *Annu. Rev. Ecol. Syst.* **27**, 83–109. (doi:10.1146/annurev.ecolsys.27.1.83)
48. Kronenberger JA, Funk WC, Smith JW, Fitzpatrick SW, Angeloni LM, Broder ED, Ruell EW. 2017 Testing the demographic effects of divergent immigrants on small populations of Trinidadian guppies. *Anim. Conserv.* **20**, 3–11. (doi:10.1111/acv.12286)
49. Kronenberger JA, Gerberich JC, Fitzpatrick SW, Broder ED, Angeloni LM, Funk WC. 2018 An experimental test of alternative population augmentation scenarios. *Conserv. Biol.* **32**, 838–848. (doi:10.1111/cobi.13076)
50. Fitzpatrick SW *et al*. 2016 Gene flow from an adaptively divergent source causes rescue through genetic and demographic factors in two wild populations of Trinidadian guppies. *Evol. Appl.* **9**, 879–891. (doi:10.1111/eva.12356)
51. Robinson JA, Brown C, Kim BY, Lohmueller KE, Wayne RK. 2018 Purging of strongly deleterious mutations explains long-term persistence and absence of inbreeding depression in island foxes. *Curr. Biol.* **28**, 3487–3494.(doi:10.1016/j.cub.2018.08.066)
52. Ralls K, Sunnucks P, Lacy RC, Frankham R. 2020 Genetic rescue: A critique of the evidence supports maximizing genetic diversity rather than minimizing the introduction of putatively harmful genetic variation. *Biol. Conserv.* **251**, 108784. (doi:10.1016/j.biocon.2020.108784)
53. Heber S, Varsani A, Kuhn S, Girg A, Kempenaers B, Briskie J. 2013 The genetic rescue of two bottlenecked South Island robin populations using translocations of inbred donors. *Proc. Biol. Sci.* **280**, 20122228. (doi:10.1098/rspb.2012.2228)
54. van de Kerk M, Onorato DP, Hostetler JA, Bolker BM, Oli MK. 2019 Dynamics, persistence, and genetic management of the endangered florida panther population. *Wildl. Monogr.* **203**, 3–35. (doi:10.1002/wmon.1041)
55. Kelly E, Phillips B. 2019 How many and when? Optimising targeted gene flow for a step change in the environment. *Ecol. Lett.* **22**, 447–457. (doi:10.1111/ele.13201)
56. Havird JC, Fitzpatrick SW, Kronenberger J, Funk WC, Angeloni LM, Sloan DB. 2016 Sex, mitochondria, and genetic rescue. *Trends Ecol. Evol.* **31**, 96–99. (doi:10.1016/j.tree.2015.11.012)
57. Clarke JG, Smith AC, Cullingham CI. 2024 Genetic rescue often leads to higher fitness as a result of increased heterozygosity across animal taxa. *Mol. Ecol.* **33**, e17532. (doi:10.1111/mec.17532)
58. Hasselgren M, Angerbjörn A, Eide NE, Erlandsson R, Flagstad Ø, Landa A, Wallén J, Norén K. 2018 Genetic rescue in an inbred Arctic fox (*Vulpes lagopus*) population. *Proc. R. Soc. B* **285**, 20172814. (doi:10.1098/rspb.2017.2814)
59. Lotsander A, Hasselgren M, Larm M, Wallén J, Angerbjörn A, Norén K. 2021 Low persistence of genetic rescue across generations in the arctic fox (*Vulpes lagopus*)). *J. Hered.* **112**, 276–285. (doi:10.1093/jhered/esab011)
60. Bateman AJ. 1948 Intra-sexual selection in *Drosophila*. *Heredity* **2**, 349–368. (doi:10.1038/hdy.1948.21)
61. Trinkel M *et al*. 2008 Translocating lions into an inbred lion population in the Hluhluwe-iMfolozi Park, South Africa. *Anim. Conserv.* **11**, 138–143. (doi:10.1111/j.1469-1795.2008.00163.x)
62. Whitlock MC, Agrawal AF. 2009 Purging the genome with sexual selection: reducing mutation load through selection on males. *Evol. Int. J. Org. Evol.* **63**, 569–582. (doi:10.1111/j.1558-5646.2008.00558.x)
63. Grieshop K, Maurizio PL, Arnqvist G, Berger D. 2021 Selection in males purges the mutation load on female fitness. *Evol. Lett.* **5**, 328–343. (doi:10.1002/evl3.239)
64. Pimm SL, Dollar L, Bass OL. 2006 The genetic rescue of the Florida panther. *Anim. Conserv.* **9**, 115–122. (doi:10.1111/j.1469-1795.2005.00010.x)
65. Seal US, Lacy RC. 1994 *A plan for genetic restoration and management of the Florida panther (Felis concolor coryi)*. Report to the Florida Game and Fresh Water Fish Commission, Conservation Breeding Specialist Group. See https://myfwc.com/media/3130/planforgeneticrestoration1994.pdf.
66. Gemmell NJ, Metcalf VJ, Allendorf FW. 2004 Mother's curse: the effect of mtDNA on individual fitness and population viability. *Trends Ecol. Evol.* **19**, 238–244. (doi:10.1016/j.tree.2004.02.002)
67. Gemmell NJ, Allendorf FW. 2001 Mitochondrial mutations may decrease population viability. *Trends Ecol. Evol.* **16**, 115–117. (doi:10.1016/s0169-5347(00)02087-5)
68. Wolf JB, Wade MJ. 2009 What are maternal effects (and what are they not)? *Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci.* **364**, 1107–1115. (doi:10.1098/rstb.2008.0238)
69. Kasumovic MM, Bruce MJ, Andrade MCB, Herberstein ME. 2008 Spatial and temporal demographic variation drives within-season fluctuations in sexual selection. *Evol. Int. J. Org. Evol.* **62**, 2316–2325. (doi:10.1111/j.1558-5646.2008.00446.x)
70. Parrett JM, Chmielewski S, Aydogdu E, Łukasiewicz A, Rombauts S, Szubert-Kruszyńska A, Babik W, Konczal M, Radwan J. 2022 Genomic evidence that a sexually selected trait captures genome-wide variation and facilitates the purging of genetic load. *Nat. Ecol. Evol.* **6**, 1330–1342. (doi:10.1038/s41559-022-01816-w)
71. Parrett JM, Knell RJ. 2018 The effect of sexual selection on adaptation and extinction under increasing temperatures. *Proc. R. Soc. B* **285**, 20180303. (doi:10.1098/rspb.2018.0303)
72. Vega-Trejo R, Head ML, Keogh JS, Jennions MD. 2017 Experimental evidence for sexual selection against inbred males. *J. Anim. Ecol.* **86**, 394–404. (doi:10.1111/1365-2656.12615)
73. Cally JG, Stuart-Fox D, Holman L. 2019 Meta-analytic evidence that sexual selection improves population fitness. *Nat. Commun.* **10**, 1–10. (doi:10.1038/s41467-019-10074-7)
74. Godwin JL, Lumley AJ, Michalczyk Ł, Martin OY, Gage MJG. 2020 Mating patterns influence vulnerability to the extinction vortex. *Glob. Chang. Biol.* **26**, 4226–4239. (doi:10.1111/gcb.15186)

75. Lumley AJ *et al*. 2015 Sexual selection protects against extinction. *Nature* **522**, 470–473. (doi:10.1038/nature14419)
76. van Doorn GS, Edelaar P, Weissing FJ. 2009 On the origin of species by natural and sexual selection. *Science* **326**, 1704–1707. (doi:10.1126/science.1181661)
77. Pointer MD, Gage MJG, Spurgin LG. 2021 *Tribolium* beetles as a model system in evolution and ecology. *Heredity* **126**, 869–883. (doi:10.1038/s41437-021-00420-1)
78. Laskowski R, Radwan J, Kuduk K, Mendrok M, Kramarz P. 2015 Population growth rate and genetic variability of small and large populations of Red flour beetle (*Tribolium castaneum*) following multigenerational exposure to copper. *Ecotoxicology* **24**, 1162–1170. (doi:10.1007/s10646-015-1463-3)
79. Haliscak JP, Beeman RW. 1983 Status of malathion resistance in five genera of beetles infesting farm-stored corn, wheat, and oats in the United States. *J. Econ. Entomol.* **76**, 717–722. (doi:10.1093/jee/76.4.717)
80. Park T, Miller EV, Lutherman CZ. 1939 Studies in population physiology. IX. The effect of imago population density on the duration of the larval and pupal stages of *Tribolium confusum* Duval. *Ecology* **20**, 365–373. (doi:10.2307/1930389)
81. King CE, Dawson PS. 1972 Population biology and the *Tribolium* model. In *Evolutionary biology*, pp. 133–227. Springer US. (doi:10.1007/978-1-4757-0256-9_5)
82. Janus MC. 1989 Phenotypic diversity of *Tribolium confusum* pupae in heterogeneous environments. *Entomol. Exp. Et Appl.* **50**, 281–286. (doi:10.1111/j.1570-7458.1989.tb01203.x)
83. Đukić N, Radonjić A, Popović B, Andrić G. 2021 Development and progeny performance of *Tribolium castaneum* (Herbst) in brewer's yeast and wheat (patent) flour at different population densities. *J. Stored Prod. Res.* **94**, 101886. (doi:10.1016/j.jspr.2021.101886)
84. R Core Team. 2024 R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.
85. Posit team. 2024 RStudio: integrated development for R. See http://www.posit.co/.
86. Wickham H *et al*. 2019 Welcome to the Tidyverse. *J. Open Source Softw.* **4**, 1686. (doi:10.21105/joss.01686)
87. Hope R. 2022 Rmisc: Ryan miscellaneous. See https://CRAN.R-project.org/package=Rmisc.
88. Bryan J. 2019 googlesheets4. (doi:10.32614/cran.package.googlesheets4)
89. Wickham H. 2016 ggplot2: elegant graphics for data analysis (doi:10.1007/978-3-319-24277-4_9)
90. Brooks M, Kristensen K, Benthem K, Magnusson A, Berg C, Nielsen A, Skaug H, Mächler M, Bolker B. 2017 glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *R. J.* **9**, 378. (doi:10.32614/RJ-2017-066)
91. Hartig F. 2016 DHARMa: residual diagnostics for hierarchical (multi-level / mixed) regression models. See https://cran.r-project.org/web/packages/DHARMa/vignettes/DHARMa.html.
92. Lüdecke D, Ben-Shachar M, Patil I, Waggoner P, Makowski D. 2021 performance: an R package for assessment, comparison and testing of statistical models. *J. Open Source Softw.* **6**, 3139. (doi:10.21105/joss.03139)
93. Bartoń K. 2010 MuMIn: multi-model inference. See https://cran.r-project.org/web/packages/MuMIn/index.html.
94. Hothorn T, Bretz F, Westfall P. 2008 Simultaneous inference in general parametric models. *Biom. J.* **50**, 346–363. (doi:10.1002/bimj.200810425)
95. Lüdecke D. 2018 ggeffects: tidy data frames of marginal effects from regression models. *J. Open Source Softw.* **3**, 772. (doi:10.21105/joss.00772)
96. Durkee LF, Olazcuaga L, Szymanski R, Melbourne BA, Hufbauer RA. 2023 Genetic mixing facilitates adaptation to a novel environmental constraint. *Ecol. Entomol.* **48**, 517–522. (doi:10.1111/een.13242)
97. Pai A, Yan G. 2003 Rapid female multiple mating in red flour beetles (*Tribolium castaneum*). *Can. J. Zool.* **81**, 888–896. (doi:10.1139/z03-070)
98. Attia FA, Tregenza T. 2004 Divergence revealed by population crosses in the red flour beetle *Tribolium castaneum*. *Evol. Ecol. Res.* **6**, 927–935.
99. Michalczyk Ł, Millard AL, Martin OY, Lumley AJ, Emerson BC, Gage MJG. 2011 Experimental evolution exposes female and male responses to sexual selection and conflict in *Tribolium castaneum*. *Evol. Int. J. Org. Evol.* **65**, 713–724. (doi:10.1111/j.1558-5646.2010.01174.x)
100. Hedrick PW, Garcia-Dorado A. 2016 Understanding inbreeding depression, purging, and genetic rescue. *Trends Ecol. Evol.* **31**, 940–952. (doi:10.1016/j.tree.2016.09.005)
101. Vega-Trejo R, de Boer RA, Fitzpatrick JL, Kotrschal A. 2022 Sex-specific inbreeding depression: a meta-analysis. *Ecol. Lett.* **25**, 1009–1026. (doi:10.1111/ele.13961)
102. Kölliker M. 2012 *The evolution of parental care*. Oxford, UK: Oxford University Press. (doi:10.1093/acprof:oso/9780199692576.001.0001)
103. Mattey SN, Richardson J, Ratz T, Smiseth PT. 2018 Effects of offspring and parental inbreeding on parent-offspring communication. *Am. Nat.* **191**, 716–725. (doi:10.1086/697236)
104. Pooley EL, Kennedy MW, Nager RG. 2014 Maternal inbreeding reduces parental care in the zebra finch, *Taeniopygia guttata*. *Anim. Behav.* **97**, 153–163. (doi:10.1016/j.anbehav.2014.09.012)
105. Demont M, Grazer VM, Michalczyk Ł, Millard AL, Sbilordo SH, Emerson BC, Gage MJG, Martin OY. 2014 Experimental removal of sexual selection reveals adaptations to polyandry in both sexes. *Evol. Biol.* **41**, 62–70. (doi:10.1007/s11692-013-9246-3)
106. Sandner TM, Dotzert A, Gerken F, Matthies D. 2022 Inbreeding depression changes with stress response over time in flooded *Mimulus guttatus*. *Perspect. Plant Ecol. Evol. Syst.* **57**, 125697. (doi:10.1016/j.ppees.2022.125697)
107. Root KV. 1998 Evaluating the effects of habitat quality, connectivity, and catastrophes on a threatened species. *Ecol. Appl.* **8**, 854–865. (doi:10.1890/1051-0761(1998)008[0854:eteohq]2.0.co;2)
108. Ferreras P, Gaona P, Palomares F, Delibes M. 2001 Restore habitat or reduce mortality? Implications from a population viability analysis of the Iberian *lynx*. *Anim. Conserv.* **4**, 265–274. (doi:10.1017/s1367943001001317)
109. Hedrick PW, Robinson JA, Peterson RO, Vucetich JA. 2019 Genetics and extinction and the example of Isle Royale wolves. *Anim. Conserv.* **22**, 302–309. (doi:10.1111/acv.12479)
110. Robinson JA, Räikkönen J, Vucetich LM, Vucetich JA, Peterson RO, Lohmueller KE, Wayne RK. 2019 Genomic signatures of extensive inbreeding in Isle Royale wolves, a population on the threshold of extinction. *Sci. Adv.* **5**, eaau0757. (doi:10.1126/sciadv.aau0757)
111. Onorato DP *et al*. 2024 Multi-generational benefits of genetic rescue. *Sci. Rep.* **14**, 17519. (doi:10.1038/s41598-024-67033-6)
112. West G, Pointer M, Nash W *et al*. 2024 Data for: Sexual selection matters in genetic rescue, but productivity benefits fade over time: A multi-generation experiment to inform conservation. Dryad Digital Repository (doi:10.5061/dryad.g1jwstr1f)
113. West G, Pointer M, Nash W, Lewis R, Gage MJG, Richardson DS. 2025 Supplementary material from: Sexual selection matters in genetic rescue, but productivity benefits fade over time; a multi-generation experiment to inform conservation. Figshare. (doi:10.6084/m9.figshare.c.7634288)

Appendix 2 – Supplementary material for Chapter Three

Supplementary Methods

The sample preparation and sequencing for this project was delivered by the Technical Genomics Team at the Earlham Institute, Norwich, UK with a bespoke protocol using the Illumina Tagment DNA TDE1 enzyme and buffer kit (Illumina 20034197/20034198).

A total of 1ng of DNA was combined with 0.9µl of Tagment DNA buffer, 0.1µl Tagment enzyme TDE1 and 2µl nuclease free water in a reaction volume of 5µl and incubated for 10 minutes at 55˚C. Following the initial incubation, 5µl of custom barcoded P5 and P7 compatible primers (2µM), 4µl 5x Kapa Robust 2G reaction buffer B, 0.4µl 10mM dNTPs, 0.1µl Kapa Robust 2G enzyme (Sigma Aldrich: KK5005) and 5.5µl water were mixed, giving a total PCR volume of 20µl. The DNA was enriched with 14 cycles of PCR (72˚C for 3 minutes, 98ºC for 3 minutes, 14 cycles of: 95ºC for 10 seconds, 62ºC for 30 seconds, 72ºC for 3 minutes, final hold at 4 ˚C). Post PCR, the DNA was cleaned up with 1.25x volume of KAPA Pure Beads from Roche (07983298001) utilising the Tecan Fluent 780 liquid handling platform and final libraries were eluted in EB. The size distribution of each library was determined using the Perkin Elmer GX Touch DNA High Sensitivity assay (DNA High Sensitivity Reagent Kit CLS760672), and a smear analysis on a 450-650bp size range was performed, to equimolar pool the libraries. The pool of libraries were then subjected to size selection on a Blue Pippin 1.5% agarose cassette (R2 marker) from SAGE Science (BDF1510) recovering library molecules between 450-650bp. The final pool was quantified by q-PCR and sequenced on two lanes of a 300 cycle Illumina NovaSeq X Series 10B Reagent Kit (Illumina 20085594). For this run the library was diluted down to 0.75nM using EB (10mM Tris pH8.0) in a volume of 40ul before spiking in 1% Illumina phiX Control v3. This was denatured by adding 10ul 0.2N NaOH and incubating at room temperature for 5 mins, after which it was neutralised by adding 150ul of Illumina's preload buffer, 160ul of this was loaded onto the sequencing cartridge. The flow cell, sequencing cartridge, and buffer cartridge were then loaded onto the NovaSeq X Plus for sequencing. The NovaSeq X Plus had control software version 1.1.0.18335 and was set up to sequence 150bp PE reads. The data was demultiplexed and converted to fastq using bcl2fastq2.

Supplementary Tables

Table S2.1: **The average productivity of No Rescue treatments descended from each of the 12 inbred stock populations from two previous experiments.** This productivity measure was used to select the high and low productivity inbred lines. N varies due to different numbers of generations and replicates in the experiments for each population.

Inbred Stock	N	Productivity	SD	SE	CI
Iso 12	9	784.222	69.819	23.273	53.668
Iso 23	4	656.500	66.736	33.368	106.192
Iso 14	15	651.800	147.753	38.150	81.823
Iso 15	15	643.533	137.925	35.612	76.381
Iso 1	7	621.857	124.596	47.093	115.232
Iso 8	15	604.800	89.973	23.231	49.825
Iso 5	9	572.111	88.632	29.544	68.128
Iso 16	15	553.733	103.138	26.630	57.116
Iso 3	9	539.444	105.156	35.052	80.830
Iso 21	11	478.727	127.042	38.305	85.348
Iso 20	15	440.000	78.258	20.206	43.338
Iso 13	15	419.267	82.213	21.227	45.528

Table S2.2: The DNA concentrations for each individual sample following DNA extraction and bead clean up. Samples were quantified using Qubit fluorometer.

Sample ID	ID	Population	Stock	Treatment	Clean Up (ng/µL)
R1	01_C_01	Rescue	Iso 21	Control	21
R2	01_C_02	Rescue	Iso 21	Control	24.8
R3	02_H_01	Rescue	Iso 1	High	24.4
R4	02_H_02	Rescue	Iso 1	High	23
R5	02_H_03	Rescue	Iso 1	High	22
R6	03_O_01	Rescue	Iso 21	Outbred	26.8
R7	03_O_02	Rescue	Iso 21	Outbred	26.4
R8	03_O_03	Rescue	Iso 21	Outbred	22.2
R9	04_O_01	Rescue	Iso 12	Outbred	28.8
R10	04_O_02	Rescue	Iso 12	Outbred	31
R11	04_O_03	Rescue	Iso 12	Outbred	30.8
R12	05_O_01	Rescue	Iso 1	Outbred	29.6
R13	05_O_02	Rescue	Iso 1	Outbred	25.2
R14	05_O_03	Rescue	Iso 1	Outbred	19
R15	06_O_01	Rescue	Iso 8	Outbred	18.5
R16	06_O_02	Rescue	Iso 8	Outbred	24
R17	06_O_03	Rescue	Iso 8	Outbred	22.4
R18	07_L_01	Rescue	Iso 15	Low	15.1
R19	07_L_02	Rescue	Iso 15	Low	33.8

R20	07_L_03	Rescue	Iso 15	Low	21
R21	08_H_01	Rescue	Iso 13	High	24.6
R22	08_H_02	Rescue	Iso 13	High	17.3
R23	08_H_03	Rescue	Iso 13	High	27
R24	09_H_01	Rescue	Iso 5	High	17.1
R25	09_H_02	Rescue	Iso 5	High	27.8
R26	09_H_03	Rescue	Iso 5	High	23.6
R27	10_O_01	Rescue	Iso 3	Outbred	10.5
R28	10_O_02	Rescue	Iso 3	Outbred	21.6
R29	10_O_03	Rescue	Iso 3	Outbred	24.6
R30	11_H_01	Rescue	Iso 23	High	27.2
R31	11_H_02	Rescue	Iso 23	High	39
R32	11_H_03	Rescue	Iso 23	High	36.8
R33	12_L_01	Rescue	Iso 1	Low	16.4
R34	12_L_02	Rescue	Iso 1	Low	8.86
R35	12_L_03	Rescue	Iso 1	Low	12.7
R36	13_O_01	Rescue	Iso 5	Outbred	18.6
R37	13_O_02	Rescue	Iso 5	Outbred	16.5
R38	13_O_03	Rescue	Iso 5	Outbred	8.72
R39	14_O_01	Rescue	Iso 14	Outbred	35.4
R40	14_O_02	Rescue	Iso 14	Outbred	20.6
R41	14_O_03	Rescue	Iso 14	Outbred	13.7
R42	15_C_01	Rescue	Iso 13	Control	24
R43	15_C_02	Rescue	Iso 13	Control	26
R44	16_C_01	Rescue	Iso 1	Control	32.6
R45	16_C_02	Rescue	Iso 1	Control	16.4
R46	17_L_01	Rescue	Iso 21	Low	29.6
R47	17_L_02	Rescue	Iso 21	Low	30.8
R48	17_L_03	Rescue	Iso 21	Low	27
R49	18_C_01	Rescue	Iso 3	Control	32.4
R50	18_C_02	Rescue	Iso 3	Control	26.2
R51	19_L_01	Rescue	Iso 3	Low	38.8
R52	19_L_02	Rescue	Iso 3	Low	21.8
R53	19_L_03	Rescue	Iso 3	Low	17.8
R54	20_L_01	Rescue	Iso 20	Low	33
R55	20_L_02	Rescue	Iso 20	Low	38.2
R56	20_L_03	Rescue	Iso 20	Low	26.4
R57	21_H_01	Rescue	Iso 20	High	53.2
R58	21_H_02	Rescue	Iso 20	High	32
R59	21_H_03	Rescue	Iso 20	High	17.4
R60	22_L_01	Rescue	Iso 13	Low	36.6
R61	22_L_02	Rescue	Iso 13	Low	23.4
R62	22_L_03	Rescue	Iso 13	Low	27.8
R63	23_C_01	Rescue	Iso 5	Control	24.2
R64	23_C_02	Rescue	Iso 5	Control	24.6
R65	24_C_01	Rescue	Iso 20	Control	28.6
R66	24_C_02	Rescue	Iso 20	Control	20.2

R67	25_H_01	Rescue	Iso 12	High	34.2
R68	25_H_02	Rescue	Iso 12	High	26
R69	25_H_03	Rescue	Iso 12	High	26.8
R70	26_C_01	Rescue	Iso 16	Control	26.6
R71	26_C_02	Rescue	Iso 16	Control	24.8
R72	27_C_01	Rescue	Iso 15	Control	22.6
R73	27_C_02	Rescue	Iso 15	Control	32.6
R74	28_L_01	Rescue	Iso 23	Low	14.4
R75	28_L_02	Rescue	Iso 23	Low	32.8
R76	28_L_03	Rescue	Iso 23	Low	24.8
R77	29_H_01	Rescue	Iso 8	High	29.2
R78	29_H_02	Rescue	Iso 8	High	22.4
R79	29_H_03	Rescue	Iso 8	High	33
R80	30_L_01	Rescue	Iso 8	Low	37.4
R81	30_L_02	Rescue	Iso 8	Low	27
R82	30_L_03	Rescue	Iso 8	Low	31.6
R83	31_H_01	Rescue	Iso 15	High	20.4
R84	31_H_02	Rescue	Iso 15	High	20.2
R85	31_H_03	Rescue	Iso 15	High	23.6
R86	32_O_01	Rescue	Iso 16	Outbred	22.8
R87	32_O_02	Rescue	Iso 16	Outbred	36.4
R88	32_O_03	Rescue	Iso 16	Outbred	20.4
R89	33_L_01	Rescue	Iso 12	Low	18.2
R90	33_L_02	Rescue	Iso 12	Low	18.5
R91	33_L_03	Rescue	Iso 12	Low	12.7
R92	34_C_01	Rescue	Iso 8	Control	15.7
R93	34_C_02	Rescue	Iso 8	Control	26.2
R94	35_H_01	Rescue	Iso 21	High	19.3
R95	35_H_02	Rescue	Iso 21	High	21.4
R96	35_H_03	Rescue	Iso 21	High	19.6
R97	36_O_01	Rescue	Iso 23	Outbred	22.8
R98	36_O_02	Rescue	Iso 23	Outbred	25.8
R99	36_O_03	Rescue	Iso 23	Outbred	21.8
R100	37_O_01	Rescue	Iso 13	Outbred	23.4
R101	37_O_02	Rescue	Iso 13	Outbred	23
R102	37_O_03	Rescue	Iso 13	Outbred	21.4
R103	38_L_01	Rescue	Iso 5	Low	14.9
R104	38_L_02	Rescue	Iso 5	Low	20.6
R105	38_L_03	Rescue	Iso 5	Low	19.2
R106	39_C_01	Rescue	Iso 23	Control	24.4
R107	39_C_02	Rescue	Iso 23	Control	19.2
R108	40_L_01	Rescue	Iso 14	Low	16.8
R109	40_L_02	Rescue	Iso 14	Low	17.3
R110	40_L_03	Rescue	Iso 14	Low	14.2
R111	41_H_01	Rescue	Iso 14	High	15.5
R112	41_H_02	Rescue	Iso 14	High	15.7
R113	41_H_03	Rescue	Iso 14	High	14.9
R114	42_L_01	Rescue	Iso 16	Low	25.4

R115	42_L_02	Rescue	Iso 16	Low	21
R116	42_L_03	Rescue	Iso 16	Low	31.8
R117	43_H_01	Rescue	Iso 3	High	25.8
R118	43_H_02	Rescue	Iso 3	High	19.3
R119	43_H_03	Rescue	Iso 3	High	23.2
R120	44_O_01	Rescue	Iso 20	Outbred	23.4
R121	44_O_02	Rescue	Iso 20	Outbred	23.2
R122	44_O_03	Rescue	Iso 20	Outbred	22.8
R123	45_H_01	Rescue	Iso 16	High	23.2
R124	45_H_02	Rescue	Iso 16	High	26
R125	45_H_03	Rescue	Iso 16	High	23.8
R126	46_C_01	Rescue	Iso 12	Control	17.1
R127	46_C_02	Rescue	Iso 12	Control	18.6
R128	47_O_01	Rescue	Iso 15	Outbred	30.4
R129	47_O_02	Rescue	Iso 15	Outbred	16.2
R130	47_O_03	Rescue	Iso 15	Outbred	15.4
R131	48_C_01	Rescue	Iso 14	Control	14.4
R132	48_C_02	Rescue	Iso 14	Control	16.8
I1	01_ISO_01	Isoline	Iso 1	Isoline	13.5
I2	01_ISO_02	Isoline	Iso 1	Isoline	13.8
I3	03_ISO_01	Isoline	Iso 3	Isoline	17.7
I4	03_ISO_02	Isoline	Iso 3	Isoline	16.2
I5	05_ISO_01	Isoline	Iso 5	Isoline	12.6
I6	05_ISO_02	Isoline	Iso 5	Isoline	20.6
I7	08_ISO_01	Isoline	Iso 8	Isoline	32.6
I8	08_ISO_02	Isoline	Iso 8	Isoline	26
I9	12_ISO_01	Isoline	Iso 12	Isoline	15.3
I10	12_ISO_02	Isoline	Iso 12	Isoline	14.9
I11	12_ISO_03	Isoline	Iso 12	Isoline	17.6
I12	13_ISO_01	Isoline	Iso 13	Isoline	22.2
I13	13_ISO_02	Isoline	Iso 13	Isoline	29.4
I14	13_ISO_03	Isoline	Iso 13	Isoline	17.2
I15	14_ISO_01	Isoline	Iso 14	Isoline	17.8
I16	14_ISO_02	Isoline	Iso 14	Isoline	17.5
I17	14_ISO_03	Isoline	Iso 14	Isoline	19.4
I18	15_ISO_01	Isoline	Iso 15	Isoline	23.8
I19	15_ISO_02	Isoline	Iso 15	Isoline	29
I20	16_ISO_01	Isoline	Iso 16	Isoline	30
I21	16_ISO_02	Isoline	Iso 16	Isoline	23.2
I22	20_ISO_01	Isoline	Iso 20	Isoline	21
I23	20_ISO_02	Isoline	Iso 20	Isoline	18
I24	20_ISO_03	Isoline	Iso 20	Isoline	21.4
I25	21_ISO_01	Isoline	Iso 21	Isoline	22.4
I26	21_ISO_02	Isoline	Iso 21	Isoline	25.6
I27	21_ISO_03	Isoline	Iso 21	Isoline	19.7
I28	23_ISO_01	Isoline	Iso 23	Isoline	33
I29	23_ISO_02	Isoline	Iso 23	Isoline	23.8
I30	23_ISO_03	Isoline	Iso 23	Isoline	21.2
K1	KSS-1	KSS	KSS	KSS	47.4
K2	KSS-2	KSS	KSS	KSS	36.6
K3	KSS-3	KSS	KSS	KSS	42.8

K4	KSS-4	KSS	KSS	KSS	21
K5	KSS-5	KSS	KSS	KSS	28.6
K6	KSS-6	KSS	KSS	KSS	11.6

Table S2.3: A GLMM of factors impacting the productivity of small, inbred, *T. castaneum* populations (Population size = 20, Populations = 48) receiving either an Outbred Rescue, High Rescue, Low Rescue or No Rescue. Productivity was measured over nine generations following the rescue event. Predictors in bold are significant ($P < 0.05$).

Predictor	Estimate	SE	*z*	*P*
Intercept	6.358	0.049	129.88	<2e-16
Treatment (No Rescue)				
High Rescue	0.012	0.046	0.27	0.788
Low Rescue	-0.045	0.046	-0.96	0.337
Outbred Rescue	0.085	0.043	1.88	0.060
Generation	**0.051**	**0.011**	**4.46**	**< 0.001**
Generation^2	**-0.006**	**0.001**	**-5.50**	**< 0.001**
Treatment*Generation (No Rescue*Generation)				
High Rescue*Generation	**0.020**	**0.007**	**2.89**	**0.004**
Low Rescue*Generation	**0.014**	**0.007**	**2.07**	**0.039**
Outbred Rescue*Generation	**0.016**	**0.007**	**2.34**	**0.020**
Random	432 Observations		Variance	
ID:Inbred stocks	48 Populations		0.004	
Inbred stocks	12 stocks		0.012	

Table S2.4: A GLMM of factors impacting the productivity of small, inbred, *T. castaneum* populations (Population size = 20, Populations = 48) receiving a rescue by either an outbred male rescuer, inbred male rescuer or No Rescue under nutrient stress. Productivity was measured over nine generations following the rescue event. Predictors in bold are significant ($P < 0.05$).

Predictor	Estimate	SE	*z*	*P*
Intercept	18.915	0.550	34.42	<2e-16
Treatment (No Rescue)				
High Rescue	0.016	0.051	0.32	0.750
Low Rescue	0.026	0.051	0.51	0.611
Outbred Rescue	0.085	0.051	1.67	0.096
Generation	**-3.181**	**0.138**	**-23.06**	**< 0.001**
Generation^2	**0.195**	**0.009**	**22.57**	**< 0.001**
Random	144 observations		Variance	
ID:Inbred stocks	48 populations		0.015	
Inbred stocks	12 stocks		0.031	

Table S2.5: **A pairwise comparison of inbreeding coefficient of small, inbred, *T. castaneum* populations (Population size = 20, Population = 48) receiving either Outbred Rescue, High Rescue, Low Rescue or No Rescue.** From the 4th generation after rescue. Predictors in bold are significant ($P < 0.05$).

Pair	Estimate	SE	*t*	*P*
No Rescue – Low Rescue	0.025	0.023	1.062	0.894
No Rescue – High Rescue	0.060	0.023	2.586	0.118
No Rescue – Outbred Rescue	**0.136**	**0.027**	**5.072**	**< 0.001**
Low Rescue – High Rescue	0.035	0.023	1.503	0.664
Low Rescue – Outbred Rescue	**0.111**	**0.027**	**4.118**	**0.002**
High rescue – Outbred Rescue	0.076	0.027	2.830	0.068

Table S2.6: A pairwise comparison of the total length of genome in ROH (> 200kbs) of small, inbred, *T. castaneum* populations (Population size = 20, Population = 48) receiving either Outbred Rescue, High Rescue, Low Rescue or No Rescue. From the 4th generation after rescue. Predictors in bold are significant ($P < 0.05$).

Pair	Estimate	SE	*t*	*P*
No Rescue – Low Rescue	398	1090	0.367	0.999
No Rescue – High Rescue	944	1090	0.867	0.953
No Rescue – Outbred Rescue	**3884**	**1090**	**3.565**	**0.009**
Low Rescue – High Rescue	546	1040	0.523	0.995
Low Rescue – Outbred Rescue	**3486**	**1040**	**3.345**	**0.018**
High rescue – Outbred Rescue	2940	1050	2.803	0.073

Table S2.7: A pairwise comparison of the number of deleterious SNPs of small, inbred, *T. castaneum* populations (Population size = 20, Population = 48) receiving either Outbred Rescue, High Rescue, Low Rescue or No Rescue. From the 4th generation after rescue. Predictors in bold are significant ($P < 0.05$).

Pair	Estimate	SE	*z*	*P*
No Rescue – Low Rescue	-0.011	0.013	-0.883	0.951
No Rescue – High Rescue	-0.024	0.013	-1.886	0.411
No Rescue – Outbred Rescue	**-0.069**	**0.013**	**-5.311**	**< 0.001**
Low Rescue – High Rescue	-0.013	0.013	-1.041	0.904
Low Rescue – Outbred Rescue	**-0.057**	**0.012**	**-4.600**	**< 0.001**
High rescue – Outbred Rescue	**-0.044**	**0.013**	**-3.536**	**0.005**

Table S2.8: A pairwise comparison of the proportion of homozygous deleterious SNPs of small, inbred, *T. castaneum* populations (Population size = 20, Population = 48) receiving either Outbred Rescue, High Rescue, Low Rescue or No Rescue. From the 4th generation after rescue. Predictors in bold are significant ($P < 0.05$).

Pair	Estimate	SE	*z*	*P*
No Rescue – Low Rescue	0.067	0.063	1.057	0.898
No Rescue – High Rescue	0.143	0.064	2.253	0.214
No Rescue – Outbred Rescue	**0.382**	**0.064**	**5.862**	**< 0.001**
Low Rescue – High Rescue	0.076	0.061	1.244	0.815
Low Rescue – Outbred Rescue	**0.306**	**0.061**	**5.005**	**< 0.001**
High rescue – Outbred Rescue	**0.229**	**0.061**	**3.738**	**0.003**

Supplementary Figures

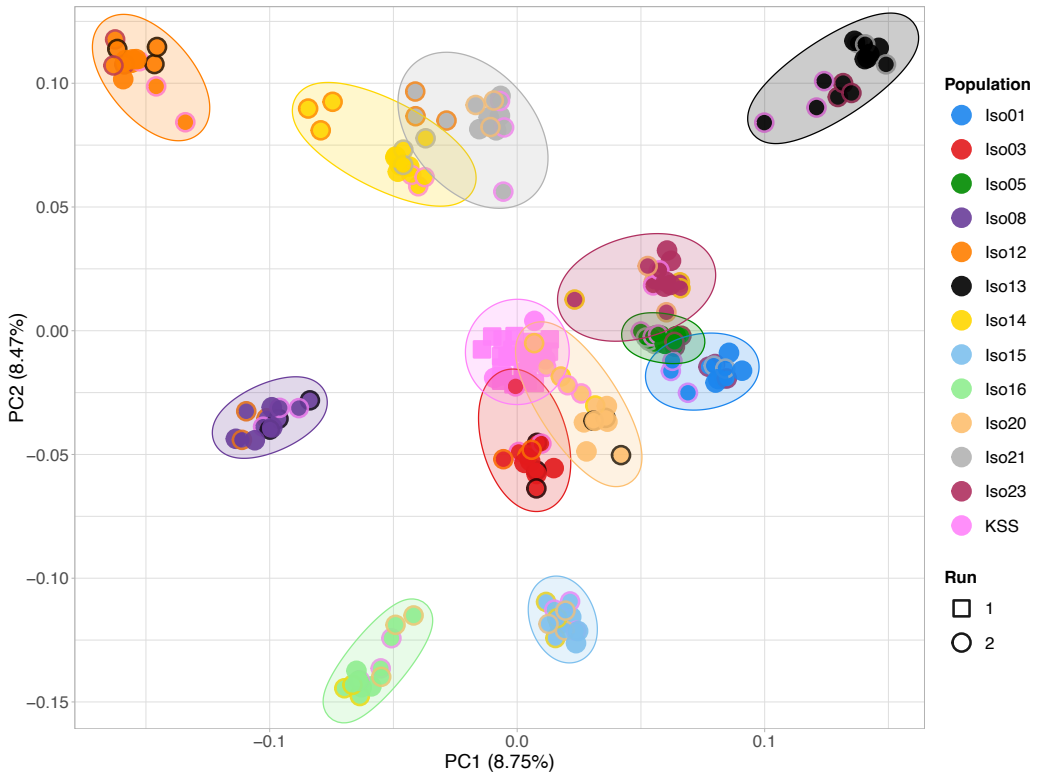

Figure S2.1: A PCA plot based on genome-wide SNPs within populations of *T. castaneum* in the genetic rescue experiment, stock inbred lines, and the stock outbred line. Each point represents an individual beetle. The inner colour of the circle and the ellipses represent the line it originated from; the outer colour of a circle is the line that rescued it (Solid colour means the line was not rescued or was a stock population) and the shape represents the run it was sequenced on. The central pink cluster represents the ancestral, outbred, stock population KSS, the surrounding clusters are the 12 inbred lines used to create the inbred populations that were rescued. Rescuers were sourced from the outbred or inbred stock populations.