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Effects of local thermal adaptation in genetic rescue of Red flour Beetles

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Abstract:

The effects of human-induced climate change have greatly affected the ability of wild animal populations across the globe to maintain their population size and genetic diversity. These populations have been fragmented and diminished due to the loss of land and environmental changes such as rising temperatures. Species which are highly susceptible to these environmental pressures often dwindle in number in number and genetic diversity leading to inbreeding. This report aims to investigate some of the potential mechanics of genetic rescue in inbred populations and the rate of adoption of the thermal adaptation. *Tribolium castaneum*, which is a widespread food store pest, is the chosen species for this study. Inbred populations which underwent 3 genetic bottlenecks were kept at 38°C for 2 generations. During this, a genetic rescue was carried out using beetles from a population adapted to 38°C as donor supply. This resulted in an increase of offspring successfully making it to adulthood in the second generation. Further experimentation into how the rescue and control populations would perform during a simulated heatwave at 42°C showed no increased survival fitness in the rescued populations. An additional investigation was conducted focusing on why the beetle populations were not producing as many offspring using egg counts on pairs from the second generation. Both rescue treatments had significantly higher egg counts than the control population and it was found that the rescue with the thermal adaptation yielded higher rates of eggs surviving to adulthood. The result of this is promising for the potential efficacy of genetic rescue on inbred populations, especially in those under thermal pressure.

Introduction:

One of the most important global issues in the field of conservation is the unprecedented environmental change as a result of human activity. Since the industrial revolution, humans have both directly and indirectly impacted Earth's marine and terrestrial ecosystem on a scale previously unrecorded. Modern farming techniques and new land required to accommodate the infrastructure needed for the increasing human population have left only 3% of the world's land ecosystems intact (Plumptre et al., 2021). Furthermore, modern industry and personal greenhouse emissions are at an all-time high despite the growing awareness of climate change (Vuuren and Riahi, 2008). The resulting temperature fluctuations not only affect land ecosystems, but, since the ocean is the earth's largest carbon sink it absorbs a vast amount of excess greenhouse gasses and the resulting atmospheric heat. This causes a process called ocean acidification in which CO_2 dissolves in seawater which eventually causes higher concentrations of hydrogen ions to be present lowering overall pH (Guinotte and Fabry, 2008). This accompanied by the excess atmospheric heat being absorbed has caused the loss of 14% of the world’s total coral reefs in only the past 15 years (Souter et al., 2021). As a result of these issues, an ever-increasing amount of research is directed towards understanding the extent of the impact caused to ecosystems globally due to climate change. It is necessary to understand the mechanisms behind how a changing climate affects organisms in order to manage our resources sustainably and conserve the planet’s dwindling wildlife.

The aforementioned loss of wild habitats has catastrophic consequences on wildlife populations, this is especially true in migratory species. This is seen in the WWF’s Living Planet Report 2022 which reported that on average a staggering 69% of wildlife populations have plummeted since 1970 (Almond, Grooten, Juffe Bignoli, 2022). Desertification is another cause of dwindling wildlife populations; it is caused in part by natural climate patterns and other natural systems however it is increasingly being linked to human intervention (Sagdic, 2022). It is estimated that 75% of the world’s drylands have been either partially or significantly impacted by desertification (Abdi et al., 2013). It is also theorised that desertification can itself affect climate change due to it causing reduced levels of surface moisture which focuses less of the sun's energy on evaporation causing more of it to be absorbed by the ground and consequently the lower atmosphere (European Committee of the Regions, 2011). One of the main impacts of dwindling population sizes is the increased risk of genetic bottlenecks. Once a population has substantially reduced in size, the genetic diversity of the population is greatly limited as only a small proportion of the original population’s genetics will be passed on to the next generation (Frankham et al., 1999). The result of this is a diminished ability to adapt to rapidly changing environmental conditions such as climate change which can sometimes cause an ecological negative feedback loop causing populations and even species to go extinct (Frankham et al., 1999). Climate change isn’t the only cause of genetic bottlenecks, these can also occur as a side effect of factors such as natural disasters, being outcompeted by another species and overhunting (Nei, 2005).

Because genetic bottlenecks lead to diminished population counts, inbreeding depression becomes a risk as overall relatedness increases over many generations (Hedrick and García-Dorado, 2016). This lack of gene flow is commonly found in populations that have been contained in smaller national parks and aren’t able to migrate, some parks such as Banff National Park allowing wildlife crossings to create sufficient gene flow in the park’s grizzly and black bear populations (Sawaya, Kalinowski and Clevenger, 2014). Furthermore, allelic fixation due to genetic drift can further lower a population’s genetic diversity and overall fitness, increasing the risk of extinction (Whitlock, 2000). Genetic rescue may offer a solution to these genetic diversity problems associated with dwindling population numbers. As long as there is a separate population of the species from which individuals can be introduced to the diminished populations, genetic rescue can be considered as a potentially applicable conservational measure. The potential benefits of genetic rescues include a higher genetic diversity of a population which in turn will lead to a higher overall fitness (Whiteley et al., 2015). However, despite this being a compelling tool for sustaining wild populations and ecosystems, it has not been widely applied in the conservation of threatened populations (Whiteley et al., 2015). An example of a genetic rescue was in 1995 when conservation managers introduced 8 female pumas from Texas to a dwindled population of panthers in the southern Florida swampland (Johnson et al., 2010). The experiment was considered a success as genetic diversity and population increased as well as a decline in the indicators for inbreeding depression. (Johnson et al., 2010)

However, there are also risks associated with genetic rescue and it may not benefit different populations in differing species equally. One of these risks is outbreeding depression, where the overall reproductive fitness of a population is decreased as a result of the rescuer being too genetically distant from the population (Tallmon, Luikart, and Waples, 2004). This could be caused by the donor population being adapted to a different set of environmental factors which are too far from the recipient population's environment (Derry et al., 2019). Interestingly, inbreeding and outbreeding depression can happen simultaneously, meaning that it can be more difficult to understand the mechanisms affecting population fitness (Ralls, Ballou and Frankham, 2001). Another potential risk is the spread of maladapted alleles as a result of genetic rescue further lowering the fitness of a population, for example, this might occur due to hybridization between divergent source populations (White, Rash and Kazyak, 2023). This means it's very important to carefully consider the use of genetic rescue before deploying it as a solution. Furthermore, it is imperative to collect data after the rescue to monitor its efficacy and effects. Factors such as genetic heterozygosity, population size, measures of fitness and survival, and inbreeding rates should be considered for observation.

One way to investigate and understand the potential effects of genetic rescue without performing one is by carrying out experiments on model species. This carries the benefit of the ability to test which parameters might be successful in a genetic rescue without putting endangered wild populations at risk of further degradation. This brings us to *Tribolium castaneum*, also known as the red flour beetle, which is the main focus species for this study. The red flour beetle is most commonly known for being one of the most common secondary pests in plant-based food stores around the globe (Pires et al., 2017). This is because they are very active, can sometimes be attracted to light, and have the ability to fly, which are all traits that are associated with a species having the ability to disperse quickly. moreover, females can lay up to 300 to 400 eggs, per generation 5 or 6 times a year eggs during their entire life span making it easy for them to quickly establish large populations (Calderwood, 1961). The factors that make them problematic pests throughout the world also make them ideal for use in population-based research as they can quickly establish populations and multiple generations. Moreover, both the males and females of the species exhibit polygamous behaviour leading to greater reproductive success within a polygamous population when compared to monogamous populations (Pai and Bernasconi, 2008). The beetles were used as a model in order to understand what effects can potentially be expected when genetic rescue is applied to wild populations of different species.

The goal of this study is to investigate the rate of adoption and efficacy of high-temperature survival and reproductive adaptations introduced via genetic rescue on an inbred population facing thermal stress. Further interest is taken into how genetic rescue impacts the early stages of development in inbred populations under thermal stress. The results from this study will help broaden the understanding of how genetic rescue can be used in helping dwindling natural populations recover genetic diversity using genetic rescue. This is especially relevant as migratory genetic rescue is currently an underutilised tool in the realm of conserving diminished wild populations.

Materials and Methods:

The experiments use stock from the Krakow Super Strain (KSS) of *T. castaneum*, from which 12 inbred isolated lines were created. The 'iso-line' populations were created by subjecting the lines to 3 extreme 1:1 genetic bottlenecks using individual pairs. For the genetic rescue, stock from the thermal selection lines was used which were created in 2010 from the KSS stock. These thermal lines are kept at 38°C resulting in the beetles being adapted to reproducing at a temperature which would typically drastically reduce fecundity. Furthermore, a stock of outbred beetles from the KSS was used for the non-adapted rescue populations. The use of inbred populations as the main stock of the study was in order to incentivise breeding with the outbred rescue beetles. Each of the 12 iso-lines was made into 3 new populations resulting in a total of 36 populations with each population consisting of 10 males and 10 females. Each of the 12 lines was randomly subjected to 3 separate treatments, a 'no rescuer' treatment in which each of the 10 males in the populations are inbred as a control population, a rescue with 1/10 of the male beetles in each population having an adaptation to a higher temperature, and lastly a rescue with 1/10 of the males being outbred but non-adapted to high temperature. All of the females used in the experiments were from the inbred stock. The study used two primary types of pots to contain the beetle populations, a 125ml tub with 75ml of fodder which would contain the mixed male and female populations for breeding and smaller 2oz plastic dishes with 10ml of fodder for pupae growth after sexing. The fodder consisted of 90% organic white flour, 10% brewer's yeast, and oats on the surface for traction. The beetles are kept in an incubator at 38°C and 60% relative humidity for the duration of the experiments except for the heatwave experiment.

During the experiment, a range of equipment was utilised, one of the primary ones being multi-layered sieves which were used in order to separate the beetles in their various stages of development from the fodder. The top layer sieve had an aperture of 2mm, the following layer had an aperture of 850 µm followed by a collection tray at the bottom. Further equipment used in the study consisted of stereoscopic microscopes for sexing and egg counting, 99.9% isopropyl alcohol, brushes for sieve cleaning and incubators used to simulate temperatures. No ethical approval is required for this study as *T. castaneum* is considered a common house pest.

Experiment 1 (Population Size):

The goal of the primary experiment was to identify the effectiveness of a genetic rescue on reproductive success in an inbred population facing thermal pressure. Furthermore, the generational rate of adoption of the rescue gene was of great interest as un-adapted adult *T. castaneum* are still able to reproduce at 38°C, however, the offspring developing at this temperature sometimes display lower fertility meaning that the effects of the thermal pressure would be delayed by at least 1 generation(Sales, Vasudeva and Gage, 2021). The data collected for this experiment was in the form of beetle population counts, these were acquired after being frozen and subsequently counted. After the initial assembly of the 36 populations (generation 0) mentioned earlier, these pots were placed into the incubator at 38°C for 7 days and allowed to mate. The adults were frozen and discarded according to the correct procedure, the eggs were then allowed to develop for 16 days. Following this period, 15 male and 15 female pupae were collected from each population and placed into separate plastic dishes which would make up the next generation (generation 1), the remaining pupae were allowed to grow for 17 days and subsequently frozen for counting. The plastic dishes containing the 15 male and 15 female pupae were given 10 days to develop into adults at which point 10 of each were combined into plastic tubs. 5 Extra male and female pupae were collected in case any of them didn't make it to adulthood. These populations of 20 were given a further 7 days to mate thus creating generation 2 at which point the entire cycle restarted.

All of the analysis for this experiment was carried out using R version 4.3.3 running on Posit cloud (Ubuntu 20.04). The data tidying and graph generation were accomplished using the help of the 'tidyverse' package (Wickham et al., 2019). A general linear model (GLM) was fitted onto the data from both generations 1 and 2 of the experiment. The size of the beetle populations which had made it to adulthood was used as the response variable in both cases. Furthermore, two boxplots showing population size plotted against treatment were created as a visual illustration, a consistent colour code was used which consisted of grey for control, green for the rescue with the adaptation and red for the rescue without the adaptation.

Experiment 2 (Heatwave Survival):

In order to further investigate the rate at which the rescue gene was being adapted, it was decided that the populations in each generation would be subject to a heatwave procedure. The aim of this was to understand how well the populations fared under extreme thermal pressure (42°C) with the newly introduced rescue gene. The data collected during this experiment consisted of counting the number of surviving beetles in each population after the heatwave. This was chosen due to *T. castaneum* being unable to reproduce at this temperature leaving survival rate to be the next best choice. The study system was set up by utilising the adults of generation 1 after their 7-day mating period. If not all 20 beetles in each population survived to this stage, then it was noted and then all of the populations were placed into an incubator at (42°C) for 5 days. Afterwards, the number of surviving beetles in each population was recorded and the beetles were then frozen and discarded. After the first generation, over half of the populations were unable to successfully reproduce, including all of the control populations, leaving Generation 2 with greatly diminished numbers. This meant that some of the beetles which were used for egg counts were re-used in the heatwave, otherwise, despite the lack of control populations, the protocol remained the same.

For the analysis of this experiment, a further two GLMs were fitted onto the data, one for each generation. Due to high mortality rates before this assay, there were varying sample sizes, so the survival percentage was calculated, instead of using raw survival counts. This percentage survival rate was used as the response variable in the models, furthermore, any populations containing less than 10 beetles prior to this heatwave assay were excluded from the model. Due to the lack of a control group in generation 2, the original isolines were used to provide a control for this experiment. Once again two boxplots were created, one for representing each generation, this time showing survival percentages plotted against treatment using the same consistent colour code.

Experiment 3 (Egg Counts):

As a final delve into understanding the patterns that were observed in experiment 1, the focus was narrowed to the developmental stage of the beetle’s life cycle. This stage is especially crucial to study because it is during its developmental phase when *T. castaneum* develops lowered fertility when kept at high temperatures (Sales, Vasudeva and Gage, 2021). The experiment devised for this looked at egg counting and successful development rate as the metric to judge this study. The procedure for the experiment was comprised of taking 5 pairs of 1 male and 1 female beetle from each population from generation 2. All of the males are marked on the back of their thorax with ink to distinguish them from the females, and then the 5 pairs of males and females a put into a small vial together containing 2/3 teaspoon fodder. They are left in the vial for 2 days in order to mate after which the male is taken out and returned to its original population and the female is transferred to a new vial in order to lay eggs. After 1 day in the new vial, each female is transferred back to their original population and the number of eggs laid is counted under a microscope. Since no control populations had survived in the 2nd generation, inbred pairs were selected from iso line stocks that didn't take part in the main experiment meaning they can only be considered baselines and not true controls.

Another 2 GLMs were fitted onto the data, the linear models were fitted with total eggs laid per population and beetles that made it to adulthood as their response variables respectively. Moreover, 2 boxplots were created showcasing the aforementioned response variables plotted against treatment, using the constant colour code.

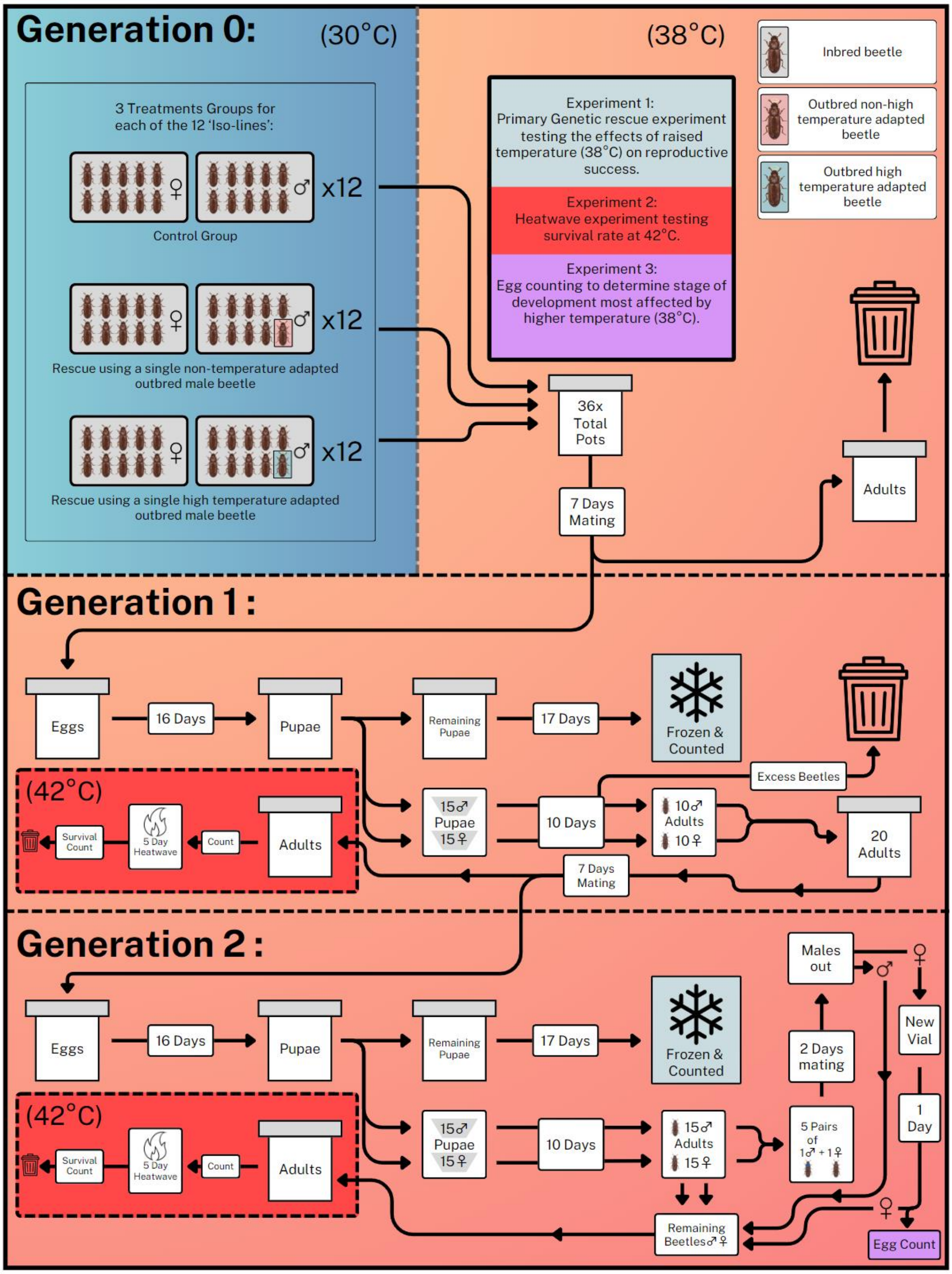

Figure 1: Complete experiment plan for experiments 1-3. Diagram showing the flow of experimental populations, generations, and temperatures throughout the experiments. Procedure temperatures are categorised by 30°C (blue), 38°C (orange), and 42°C (red). The data collection locations are also categorised by colour with Grey/Light blue showing experiment 1, red showing experiment 2 and purple showing experiment 3.

Results:

The first generation of experiment 1 showed no greater reproductive success in the rescue treatments over the control treatment group (Figure 2, Table 1).

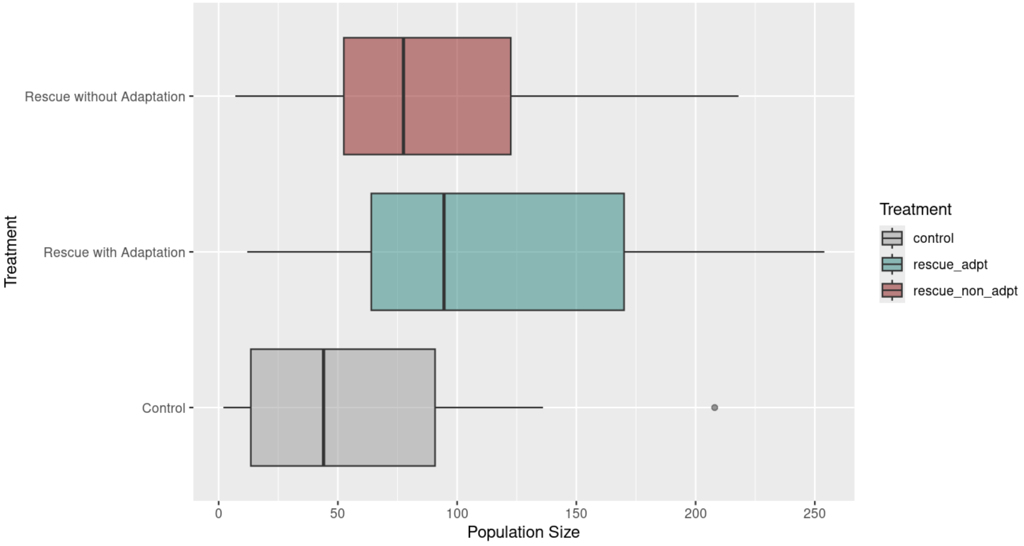

Figure: 2: The number of *T. castaneum* offspring successfully reaching adulthood per population at 38°C and 60% RH in the first generation after genetic rescue. After 3 generations of genetic bottlenecks, 36 populations of beetles were rescued either with a high-temperature adapted outbred beetle (green), a non-adapted outbred beetle (red) or no rescue (grey) and left to mate and produce offspring at 38°C. The boxplot shows the medians, interquartile ranges, minimums, maximums and outliers (dots). The table shows a slight tendency for the rescue treatments to have higher population sizes however they were not statistically significant.

Table 1: A GLM modelling the number of *T. castaneum* offspring successfully reaching adulthood per population at 38°C and 60% RH in the first generation after genetic rescue. Beetle count in the populations of generation 1 was used as the response variable. The model was fitted with the three treatment groups as factors including a control group. There were no statistically relevant correlations found in the model.

Coefficients	Estimate	Standard Error	T value	Pr(>|t|)
(Intercept)	63.83	19.97	3.197	0.00306
Rescue with adaptation	55.67	28.24	1.971	0.05712
Rescue without adaptation	28.67	28.24	1.015	0.31741

The second generation of experiment 1 showed that the thermally adapted rescue treatment had higher reproductive success over the control treatment group (Figure 2.1, Table 1.1, P <0.05). Meanwhile, the non-adapted rescue group showed no such trend (Figure 2.1, Table 1.1).

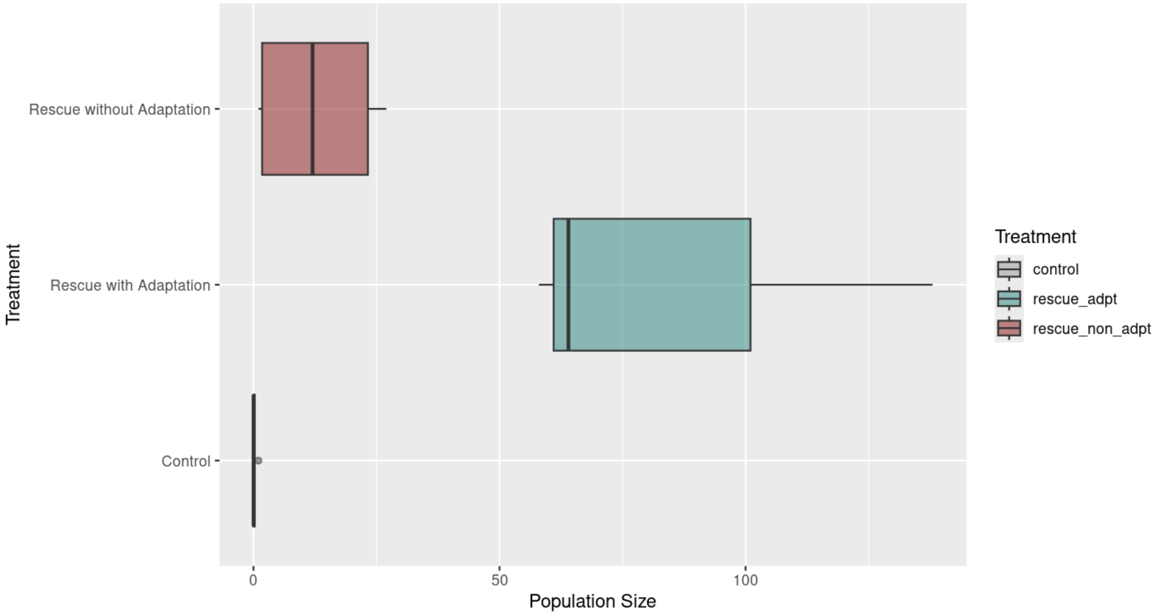

Figure 2.1: The number of *T. castaneum* offspring successfully reaching adulthood per population at 38°C and 60% RH in the second generation after genetic rescue. Populations of beetles were rescued either with a high-temperature adapted outbred beetle (green), a non-adapted outbred beetle (red) or no rescue (grey) and left to mate and produce offspring at 38°C. The data shows the number of Generation 1's offspring successfully reaching adulthood.

Table 1.1: A GLM modelling the number of *T. castaneum* offspring successfully reaching adulthood per population at 38°C and 60% RH in the first generation after genetic rescue. Beetle count in the populations of generation 1 was used as the response variable. The model was fitted with the three treatment groups as factors including a control group. Rescue with adaptation had higher reproductive success than control with a P< 0.05 and an estimate of 86.42.

Coefficients	**Estimate**	**Standard Error**	**T value**	**Pr(>|t|)**
(Intercept)	0.25	11.88	0.021	0.98372
Rescue with adaptation	86.42	18.14	4.764	0.00142
Rescue without adaptation	12.75	16.80	0.759	0.46955

The data from the first-generation heatwave shows no increased survival rates in the rescue treatment groups over the control groups (Figure 3, Table 2).

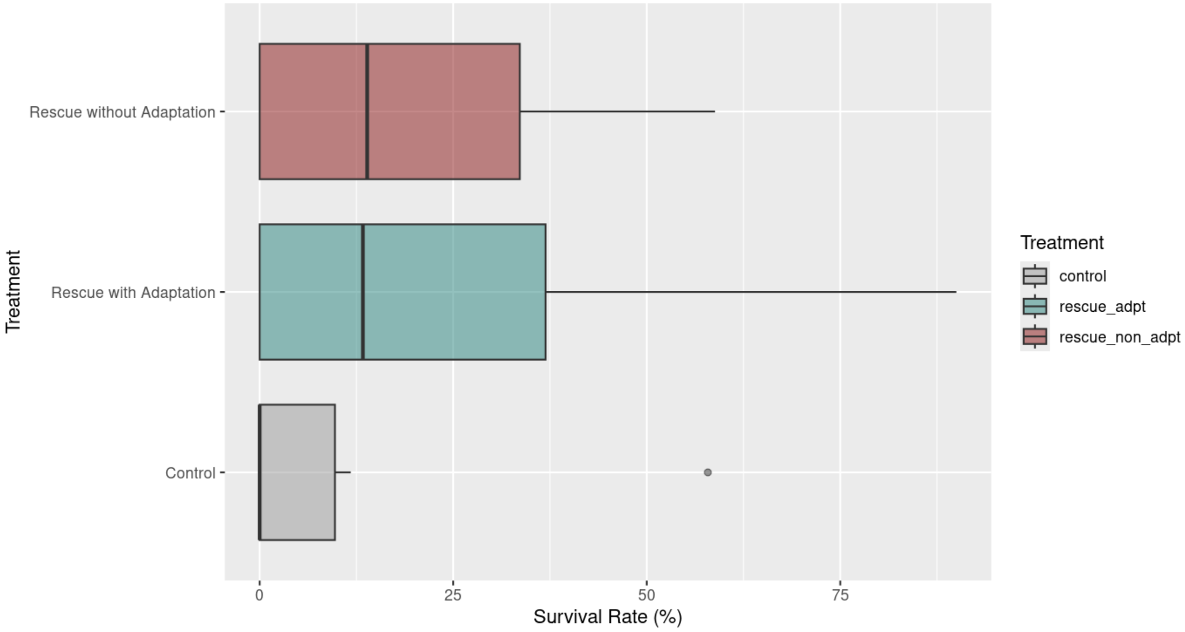

Figure 3: The percentage of *T. castaneum* surviving 5 days in a 42°C heatwave in the first generation after genetic rescue. The beetles used for the heatwave were from generation 1 and subjected to the same conditions as Figure 2. There was no substantial difference between beetle population survival rates which were subjected to rescue with or without adaptation, or in relation to the control.

Table 2: A GLM modelling the percentage of *T. castaneum* surviving 5 days in a 42°C heatwave in the first generation after genetic rescue. The survival rate in the first-generation populations as a percentage was fitted as the response variable. The model was fitted with the three treatment groups as factors including a control group. There were no statistically relevant correlations found in the model.

Coefficients	Estimate	Standard Error	T value	Pr(>|t|)
(Intercept)	8.633	7.428	1.162	0.255
Rescue with adaptation	14.706	10.263	1.433	0.163
Rescue without adaptation	11.175	10.505	1.064	0.297

The data from the second-generation heatwave shows non increased survival rate in the thermally adapted rescue treatment versus the non-adapted rescue treatment (Figure 3.1, Table 2.1).

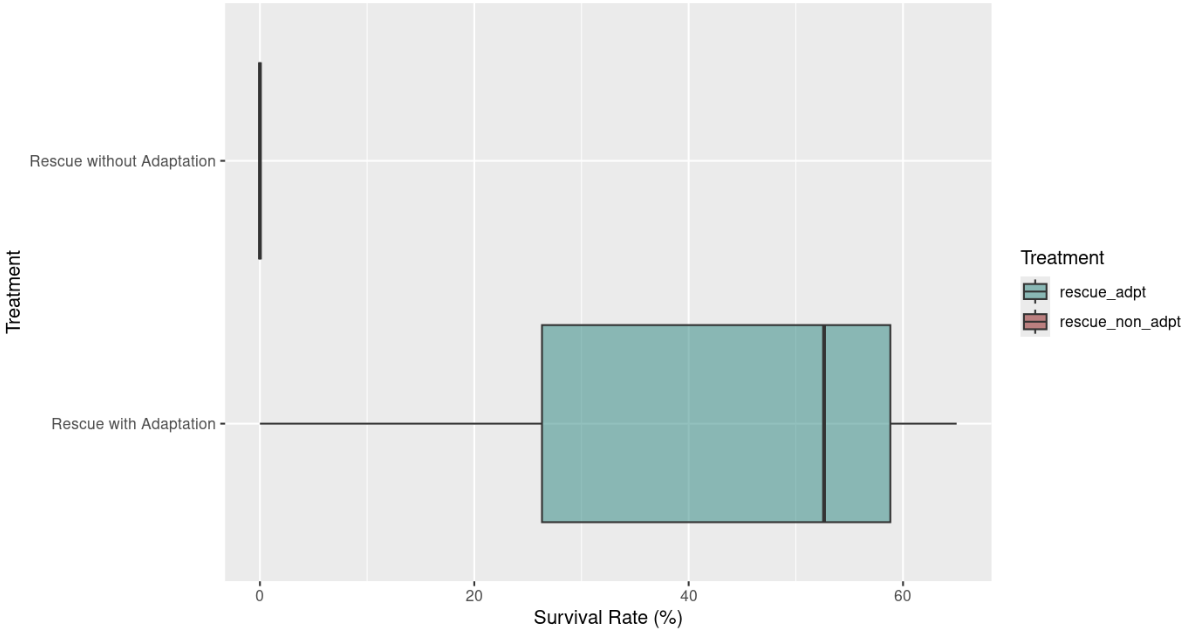

Figure 3.1: The percentage of *T. castaneum* surviving 5 days in a 42°C heatwave in the second generation after genetic rescue. The beetles used for the heatwave were from generation 2 and subjected to the same conditions as Figure 2.1. As there were no surviving control populations in Generation 2, the second heatwave experiment had to be run without a control. However, it should be noted that the populations undergoing the rescue with adaption treatment outperformed the same treatment in the previous generation and the rescue without adaption treatment.

Table 2.1: A GLM modelling the percentage of *T. castaneum* surviving 5 days in a 42°C heatwave in the second generation after genetic rescue. The survival rate in the second-generation populations as a percentage was fitted as the response variable. The model was fitted with the two treatment groups as factors not including a control group due to limitations. There were no statistically relevant relationships found in the model.

Coefficients	**Estimate**	**Standard Error**	**T value**	**Pr(>|t|)**
(Intercept)	-6.355e-15	1.993e+01	0.000	1.000
Rescue with adaptation	3.921e+01	2.573e+01	1.524	0.225

There was an increased egg count in both rescue treatments over the baseline treatment (Figure 4, Table 3, P<0.05).

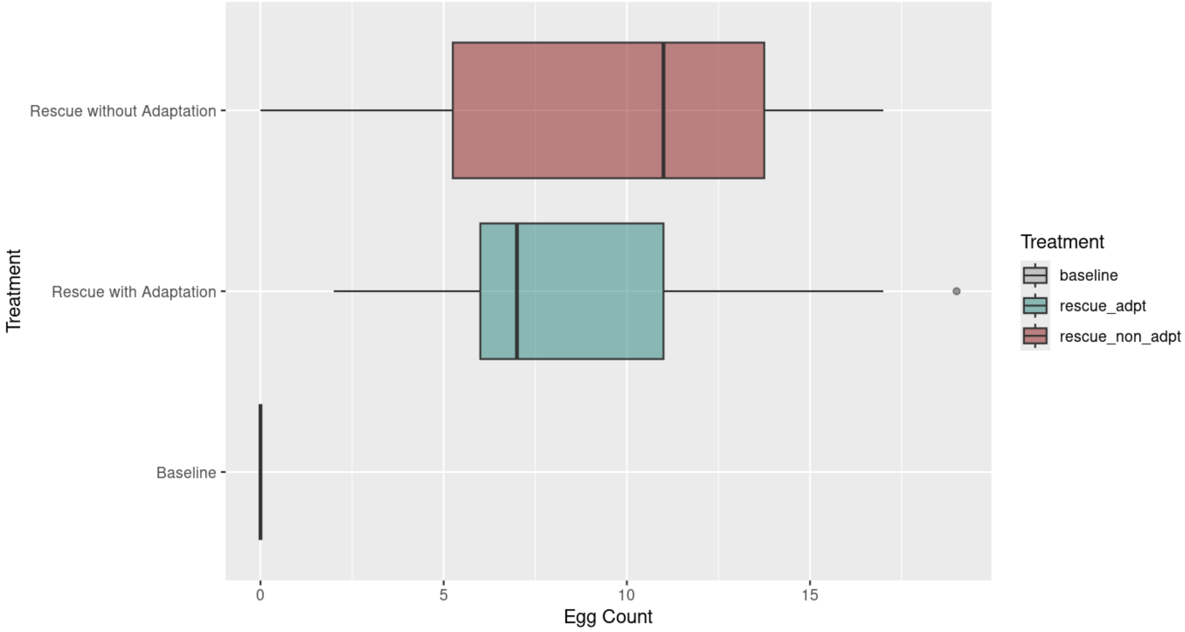

Figure 4: The number of eggs laid by *T. castaneum* pairs at 38°C and 60% RH in the second generation after genetic rescue. No controls survived in the second generation so inbred beetles from the original stock were used as baseline. None of the baseline pairs laid eggs at 38°C. Both rescue treatments performed similarly. Both rescue treatments outperformed the baseline pairs in egg quantity.

Table 3: A GLM modelling the number of eggs laid by *T. castaneum* pairs at 38°C and 60% RH in the second generation after genetic rescue. Number of eggs laid was used as the response variable. The model was fitted with the three treatment groups as factors including a baseline group. Both rescue treatments have higher egg counts than the baseline pairs with both p-values being < 0.05.

Coefficients	Estimate	Standard Error	T value	Pr(>|t|)
(Intercept)	-2.741e-15	9.825e-01	0.000	1
Rescue with adaptation	8.600e+00	1.435e+00	5.993	5.30e-07
Rescue without adaptation	9.600e+00	1.614e+00	5.946	6.15e-07

There was a greatly increased rate of eggs reaching adulthood in the thermally adapted treatment group over baseline (Figure 4.1, Table 3.1, P<0.05). The non-adapted rescue treatment showed no increase in eggs reaching adulthood over baseline (Figure 4.1, Table 3.1).

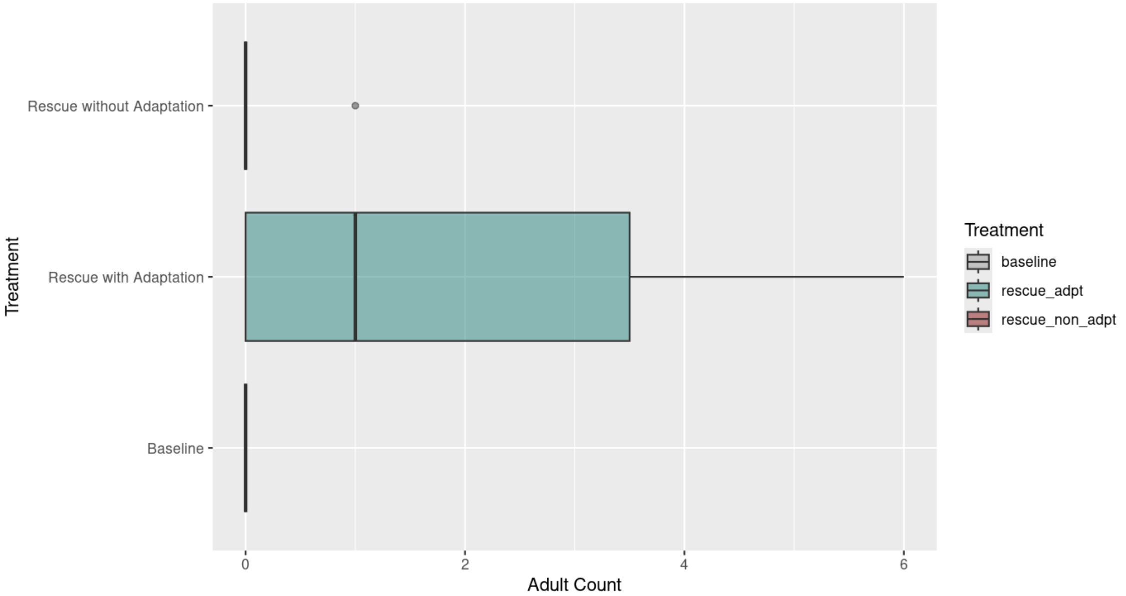

Figure 4.1: The number of eggs reaching adulthood, laid by *T. castaneum* pairs at 38°C and 60% RH in the second generation after genetic rescue. No controls survived in the second generation so inbred beetles from the original stock were used as baseline. As no baseline pairs laid eggs, there were no adults. The rescue with adaptation treatment pairs performed significantly better than the rest of the treatments.

Table 3.1: A GLM modelling the number of eggs reaching adulthood, laid by *T. castaneum* pairs at 38°C and 60% RH in the second generation after genetic rescue. Number of eggs laid was used as the response variable. Number of eggs laid was used as the response variable. The model was fitted with the three treatment groups as factors including a baseline group. The rescue with adaptation treatment pairs statistically had a higher egg survival rate to adulthood than the baseline pairs in egg quantity with the p-value being < 0.05.

Coefficients	Estimate	Standard Error	T value	Pr(>|t|)
(Intercept)	5.482e-16	3.382e-01	0.000	1.000000
Rescue with adaptation	1.933e+00	4.940e-01	3.914	0.000354
Rescue without adaptation	1.000e-01	5.557e-01	0.180	0.858126

Discussion:

The goal of this study was to investigate how genetic rescue using outbred high-temperature-adapted beetles on an inbred population facing thermal pressure would impact beetle reproductive success. This ties together with the motivations of this study to investigate how populations undergoing genetic rescue might react to conditions similar to those created by climate change and elevated temperatures. The results suggest that there was a relationship between the genetic rescue treatment with adapted beetles and higher overall reproductive success at 38°C than 'rescue without adaptation' and no genetic rescue groups in generation 2 (Figure 2.1, Table 1.1). During the first generation, I expected there to be no significant differences between the rescue groups and the control group due to the 'generational lag' effect seen in this study. This happens because *T. castaneum* developing at high temperatures often has lower fertility, however, the initial generation 0 developed at 30°C meaning the counts of the first generation would not represent a true reproductive cycle at high temperature (Sales, Vasudeva and Gage, 2021). This was the case as there was no difference in reproductive success at 38°C found between the rescue treatments and control group in the first generation (Figure 2, Table 1). Looking at the second generation, there is a significant correlation between higher reproductive success at 38°C in the 'rescue with adaptation' treatment group. This is similar to another study that found genetic rescue effective in *T. castaneum*, however, this study focused on differing population sizes as treatment (Hufbauer et al., 2015). It should be noted that around 2/3 of the total populations from each treatment in Generation 2 had failed to make it to adulthood. The control group had 4/12 populations remaining, rescue without adaptation had 4/12 populations and the rescue with adaptation treatment group only had 3/12 of the initial populations remaining by the second generation. So it's important to note whilst more beetles in the rescue with adaptation treatment made it to adulthood in the second generation, a smaller amount of the total populations survived.

The second experiment was aimed at investigating how the genetic rescue beetle populations would respond to conditions mimicking the heatwaves caused by climate change. Taking a look at the first generation's heatwave data and Figure 3, it initially appears to depict that the rescue treatments did better than the control group however the difference was not statistically significant. Figure 2.1 which is the linear model for the second generation also shows no significant correlation between any of the treatments. It's important to note that there were no control populations which survived to adulthood in generation meaning that there was no control for the second heatwave experiment. This could be possibly skewing the results. A study looking at inbred *T. castaneum* populations with differing levels of genetic bottlenecks saw much lower overall extinction rates than the rates observed in this investigation, a possible reason for this is the 38°C temperature used was too intense for the level of bottlenecking in this investigation (Olazcuaga et al., 2023). One of the possible reasons why there were no increased survival rates in the rescue groups is that they only displayed reproductive fitness benefits and not survival fitness. Furthermore, it's possible that the adaptation that helps the thermal line beetles maintain their populations at 38°C doesn’t have an effect at 42°C due to being highly specialised for the prior temperature. A genetic rescue study on inbred Drosophila populations showed that deleterious alleles which pose temperature dependant lowered fitness can be passed on to recipient generations using genetic rescue, however, the study also found these risks can be mitigated using sufficient immigrants and certain demographic measures (Bijlsma et al., 2010).

The egg count results indicate that the genetic rescue treatments successfully increased the egg count laid by pairs of the second generation at 38°C over baseline egg counts. This is further backed up by Table 3 which is a linear model showing that both rescue treatments showed statistically significant increases in egg counts over the baseline group. The egg count data is especially valuable as it creates insight into the possible reason why the control treatment populations failed to survive in generation 2, showing how a baseline group of inbred beetles failed to reproduce at the egg-laying stage of reproduction. However, it isn’t a perfect comparison as the egg counts had no control group as none survived, meaning that the baseline beetles used for the egg counts were not from the same populations and should only be used as an approximation. The total number of eggs which made it to adulthood was also significantly higher in the beetle population undergoing the rescue with adaptation treatment as seen in Figure 4.1. The linear model in Table 3.1 shows that this greater survival rate to adulthood is statistically significant.

A study performing a genetic rescue on an inbred Drosophila population found similar results to this report as it concluded that the genetic rescue successfully increased overall fitness in the rescue population however the rescue did not have a thermal adaptation treatment which is where this report found a correlation (Jørgensen et al., 2022). Another study using genomic approaches mentions how genetic rescue can be an effective solution to inbreeding depression however due to the risk it should not be considered the solution for high inbreeding depression (Hedrick and García-Dorado, 2016). Focusing on the heatwave results, a study on *T. castaneum* found that exposure to a 5-day heatwave at 42°C reduces reproductive output in males, which is a somewhat similar result to what this report has shown as *T. castaneum* could not reproduce at that temperature, moreover, the mortality rate increased highly during the heatwave (Sales et al., 2018; Mahroof et al., 2003). One potential future experiment to improve low *T. castaneum* heatwave survival rates is beetle preconditioning, which in a study investigating heat preconditioning in *T. castaneum* larvae found that the preconditioning was able to increase survival rates during exposure to heat (Soderstrom, Brandl and Mackey, 1992). Finally, reviewing the egg count experiments, the results of this report showing an increase in egg counts as a result of genetic rescue with outbred donor populations over baseline is potentially contradicted by this study which concluded that egg laying as a trait does not present inbreeding depression (López-Fanjul, 1977).

Although the standard of practice was generally high in this report, there were a few limitations causing a few approximations to be made. One of the effects faced was the high rate of population extinction experienced in the second generation of beetles, this caused the second heatwave experiment and egg counts to lack a control group. A possible solution to this could be changing the number of genetic bottlenecks the beetles are subjected to prior to the experiment, or if that isn't feasible then increasing the overall population count in each treatment could help mitigate the effect. For future studies, it would be valuable to understand how differing population sizes affect how well the thermal adaptation is adopted. This would not only build on this report but also a previously mentioned study which used population size as treatment and could give further insight into the interaction between population sizes and thermal adaptation treatments (Hufbauer et al., 2015). Furthermore, it would also be beneficial to investigate if any of the maladapted alleles were possibly transferred from the donor population using genetic testing. If so, could increasing the rescue donor count mitigate the risk of this transfer? A study found that using a highly inbred donor population for genetic rescue restored South Island robin populations and increased genetic diversity, further studies building upon this one could add inbred rescue for the control, rescue with adaptation and rescue without adaptation populations (Heber et al., 2013).

Conclusion:

Climate change and other human activity have fragmented wild populations and created environmental pressures such as rising temperatures forcing populations to adapt at a rapid rate. Genetic rescue is an underutilised technique in the conservation of diminished wild populations. This study has investigated the efficacy of genetic rescue as a measure to increase fitness in inbred *T. castaneum* populations under thermal pressure. More specifically genetic rescue with the donor population possessing an adaptation to the higher thermal pressure. The study found that the genetic rescue with the thermal adaption was successful at increasing reproductive success over multiple generations and egg survival rate. The heatwave experiments showed no effect of a statistically significant increase in survival as a result of the genetic rescue treatments over control. On the other hand, both of the rescue treatments were effective at increasing egg count in the inbred populations, 2 generations after rescue. This data shows promise for further investigation into the applicability of genetic rescue in the conservation of wild populations. However, it is important to consider the risks such as the introduction of maladaptive alleles and lowered overall fitness due to differing environmental adaptations of the donor population. With proper precautions such as a substantial migrant count, these possible risks can be lowered. However, this isn’t always feasible as endangered wild populations may not have the necessary population counts for a substantial migrant donation. This leaves genetic rescue as a seemingly favourable technique for managing fragmented and diminished wild populations being impacted by human activity.

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