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Author for correspondence:

Tim S. Jessop

e-mail: tjessop@unimelb.edu.au

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Conservation biology

Assortative mating among animals of captive and wild origin following experimental conservation releases

Brendan Slade¹, Marissa L. Parrott², Aleisha Paproth^{1,2}, Michael J. L. Magrath², Graeme R. Gillespie¹ and Tim S. Jessop¹

¹Department of Zoology, University of Melbourne, Parkville, Victoria 3010, Australia

²Wildlife Conservation and Science, Zoos Victoria, Parkville, Victoria 3056, Australia

Captive breeding is a high profile management tool used for conserving threatened species. However, the inevitable consequence of generations in captivity is broad scale and often-rapid phenotypic divergence between captive and wild individuals, through environmental differences and genetic processes. Although poorly understood, mate choice preference is one of the changes that may occur in captivity that could have important implications for the re-introduction success of captive-bred animals. We bred wild-caught house mice for three generations to examine mating patterns and reproductive outcomes when these animals were simultaneously released into multiple outdoor enclosures with wild conspecifics. At release, there were significant differences in phenotypic (e.g. body mass) and genetic measures (e.g. G_{st} and F) between captive-bred and wild adult mice. Furthermore, 83% of offspring produced post-release were of same source parentage, inferring pronounced assortative mating. Our findings suggest that captive breeding may affect mating preferences, with potentially adverse implications for the success of threatened species reintroduction programmes.

1. Introduction

To prevent imminent extinction, individuals of threatened species may be removed from the wild to establish captive breeding programmes, usually with the aim of providing individuals to supplement or re-establish wild populations after the key threats have been mitigated [1–3]. Such conservation approaches are employed, or proposed increasingly, as part of recovery plans for threatened species worldwide [1–3]. However, conservation breeding programmes are expensive and reintroductions from captivity usually have low or variable success [1–3]. Yet, as these approaches are often the final management option remaining to prevent extinction, identifying and resolving factors that limit their success remain vital to improving these breeding programmes [3].

Most animals maintained or bred in captivity are exposed to vastly different conditions from their natural environments, which can promote rapid phenotypic change [4,5]. An increasing number of studies show that captive breeding can result in rapid selection or plastic responses in phenotypic or life-history traits that can reduce an individual's fitness on release and compromise the chances of successful reintroduction [4–7].

Change in mate preference is one such modification that may occur in captivity, but has received very little attention [8]. Established theory and an increasing number of empirical studies attest to how ecological divergence between populations can rapidly alter mate choice and cause non-random, or assortative mating, initial steps needed for subsequent reproductive isolation and ecological speciation [9,10]. If changes in mate preferences and choosiness arise in captivity, then released animals may have limited value if matings with

wild resident individuals are avoided [8]. For example, avoidance of captive–wild matings would compromise the effectiveness of ‘genetic rescue’ attempts, where the aim is to incorporate new genetic diversity into genetically depauperate wild populations [6].

Here, we experimentally investigated the mating outcomes of releasing captive-bred animals into a wild population using house mice, *Mus musculus*, as a model species. We reared mice from wild founders in captivity for three generations then introduced captive adults into outdoor semi-natural enclosures alongside wild mice. We then determined reproductive outcomes over a period of 20 weeks and assessed the extent of assortative mating between captive and wild mice post-release.

2. Material and methods

(a) Captive husbandry

A captive breeding colony was established using trapped wild founder mice (11 male and 9 females) from an open grassland at Werribee Open Plains Zoo, Victoria (electronic supplementary material, method S1). Captive founders were housed in individual rodent laboratory cages (400 × 120 × 250 mm) provisioned with shelter, nesting substrate and *ad libitum* food and water. Breeding of founder, F₁ and F₂ adults employed outbreeding protocols. Founder pairs comprised individuals that were captured from spatially separated trap locations (over an area of more than 10 ha) to minimize relatedness among mice. F₁ and F₂ pairs were drawn from genetically unrelated individuals using pedigree mapping. All founders were represented equally in the third-generation release group.

(b) Reintroduction protocol

Third-generation adult captive-bred mice ($n = 54$) and adult wild mice ($n = 54$) were released into nine semi-natural outdoor circular enclosures (3.14 m²) in the area from which founders were captured. To minimize the possibility of inbreeding, we stocked each enclosure with unrelated captive and wild mice. Enclosures were supplied with four nesting-boxes, scattered tree branches for cover, and supplemental food and water. All enclosures were covered with shade cloth to exclude predators. Each enclosure was stocked with 12 mice (captive: 3M:3F; wild: 3M:3F). The period of post-release monitoring lasted 20 weeks (May–September) and enabled mice to freely reproduce. At the end of the experiment, all mice were trapped then euthanased.

(c) Phenotypic and reproductive measures

Immediately pre-release, the body mass of all captive-bred and wild-caught individuals was recorded. Each week post-release we captured and weighed adult mice and checked nest-boxes for offspring. All offspring found were collected and euthanased, and tissue was stored for parentage analysis.

(d) Genetic measures

Ear tissue samples for genetic analyses were taken from all adult and juvenile mice during the post-release study. Mouse genomic DNA was extracted using the Qiagen DNeasy tissue kit (Qiagen, USA). In total, 297 individuals were screened for eight mouse-specific microsatellite loci (D4Mit1, D6Mit138, D10Mit14, D11Mit4, D13Mit1, D14Mit132, D16Mit1 and D18Mit17) by the Australian Genome Research Facility. Genotyping was carried out using GENE PROFILER 4.05 software (Scanalytics, Fairfax, VA).

Genetic diversity in captive and wild mice was estimated by allelic diversity, observed heterozygosity (H_o) and the inbreeding

coefficient (F) using programs GENALEX v. 6.501 [11] or COANCESTRY [12]. Simulation procedures were used to test for significant group differences in genetic distance (G_{ST}) and in the inbreeding coefficient (electronic supplementary material, method S2). The program CERVUS [13] was used to assign parentage of offspring to captive and wild-born adults. Because parent–pup pairs could not be identified in enclosures, the probability of exclusion was estimated with no parent known. Log-likelihood ratio probabilities of parentage for each candidate parent were calculated, taking into account genotyping errors. Confidence intervals (CIs) of 80% (relaxed) and 95% (strict) were calculated using a default error rate of 1% per locus. We accepted parentages without mismatches only (resulting in exclusion of 14 individuals).

(e) Statistical analyses

Generalized linear models (GLMs) incorporating random effects (e.g. mouse identity and enclosure identity) were used to test for differences between captive and wild adults with respect to their body mass (i.e. a phenotypic trait related to mate choice) and timing of reproduction (i.e. birth of offspring). From parentage–offspring assignments, we tested for frequency differences in assortative mating (i.e. in litters from the four possible parentage combinations) and multiple paternity between captive and wild mice, using one-way χ^2 contingency tests. Differences between captive and wild mice reproductive success (i.e. number of offspring produced per parent) were statistically evaluated using GLM.

3. Results

At release, there were significant phenotypic and genetic differences between captive and wild mice. Captive mice were consistently heavier in body mass than wild mice throughout the post-release monitoring period (Wald $\chi^2 = 654.72$, $p = 0.001$; figure 1a). There was no significant difference between parental source and the timing of litter production throughout the monitoring period (Wald $\chi^2 = 0.01$, $p = 0.973$), indicating similar breeding schedules between captive and wild mice.

Third-generation captive-bred mice were subtly, but significantly genetically different from wild mice (pairwise $G_{ST} = 0.10$, $p = 0.001$), with captive-bred mice having lower allelic diversity, higher observed heterozygosity and a lower inbreeding coefficient ($p < 0.001$) compared with wild mice (figure 1b,c).

Offspring were sired from pairings between 40 of the original 108 adult mice released. During the 20-week post-release period, 59 litters of 189 offspring were produced. Eighty-three per cent of these 59 litters resulted from the same source parental pairings ($\chi^2 = 35.71$, $p < 0.001$; figure 1d), inferring that mating was strongly assortative between captive and wild mice. Captive-bred females produced more litters during the 20-week trial period (figure 1d), but the mean *per capita* reproductive success (i.e. offspring produced) between captive (3.98 ± 0.60) and wild (4.02 ± 0.72) parents was not significantly different (Wald $\chi^2 = 0.14$, $p = 0.96$). Multiple paternity was evident in both captive (33%) and wild (16%) litters, but frequencies did not significantly differ ($\chi^2 = 1.81$; $p = 0.179$), suggesting a similar mating system in captive and wild mice.

4. Discussion

Understanding phenotypic and genetic consequences of captive breeding is extremely important to better reintroduction success [1–7]. Our findings reveal a potential concern for release

Figure 1. Comparison of (a) phenotypic and (b,c) genetic differences between F_3 captive and wild sourced mice and (d) the percentage of litters ($n = 59$) produced in the post-release period in relation to sources of the two parents.

programmes that appears to have received very little previous consideration. After just three generations in captivity, we found that captive–captive and wild–wild pairs accounted for 83% of all offspring produced, inferring strong assortative mating between animals in relation to their origin.

Multiple causes for the observed assortative mating are possible [14]. First, captive conditions (e.g. diet) could cause plastic or even trans-generational responses in traits important to mouse mate preferences, including body size, behaviour and odour [15,16]. Thus, captive-bred individuals, exhibiting plasticity in mate choice criteria, may be more likely to mate assortatively with other captive-born animals [10]. Second, the necessity to manage pairings in captivity to prevent inbreeding and retain genetic diversity (as achieved here) could influence genetic quality or compatibility attributes that affect patterns of mate choice (e.g. compatible genes hypothesis) [17]. Third, any other phenotypic difference (e.g. variation in daily activity schedules or microhabitat use) arising between captive and wild parents that reduced mating opportunities post-release could further limit frequency of mixed-origin mating [18].

Other potential explanations appear unlikely. Selection in captivity (i.e. an evolutionary response), which is a common concern for most multi-generational breeding programmes [4], would have been negligible in this case, because all

captive founders were represented in the third-generation release group. Elevated rates of fertilization failure or embryonic/juvenile mortality following matings between captive-bred and wild individuals also seem unlikely given the small number of generations and lack of opportunity for selection in captivity [6]. Even the fastest instances of genetic incompatibility require 10s (but typically 100–1000s) of generations before reproductive isolation evolves to reinforce population divergence [10,19]. However, we do acknowledge that our data would represent only the subset of matings that resulted in the production of offspring that survived until they were collected.

Importantly, pronounced assortative mating between captive and wild animals in reintroduction programmes could be problematic, particularly where there is an urgent need to improve the fitness of wild individuals by the integration of new genes to bolster genetic diversity [20]. A low frequency of matings between captive-bred and wild animals also means that any deleterious genetic changes acquired in captivity could be expressed in their offspring, limiting their fitness [4]. Moreover, in species with parental care, there are significant direct fitness benefits to captive-bred individuals from pairing with wild-reared individuals, such as greater familiarity with the environment and transference of skill, that improve foraging and predator avoidance [21].

5. Conclusion

This study highlights that assortative pairing and mating could be a largely unrecognized issue for reintroduction programmes worldwide. Given the limited literature on the mating patterns between individuals from captive-bred (or indeed other translocated wild populations) and existing wild populations, we call for greater reporting of the social pairing and mating outcomes following reintroductions. This information would alert conservation managers to

potentially serious conflicts with the goals of reintroduction, and enable changes to the management of breeding and or reintroduction programmes that could be implemented to address the problem [3].

Ethics statement. Research procedures were conducted under Zoos Victoria Animal Ethics Committee permit ZV07006.

Data accessibility. Data are held in the Dryad repository (<http://data.dryad.org>): doi:10.5061/dryad.15mk5.

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