Conservation Cupid: Incorporating mate choice into the Vancouver Island marmot (*Marmota vancouverensis*) conservation breeding program

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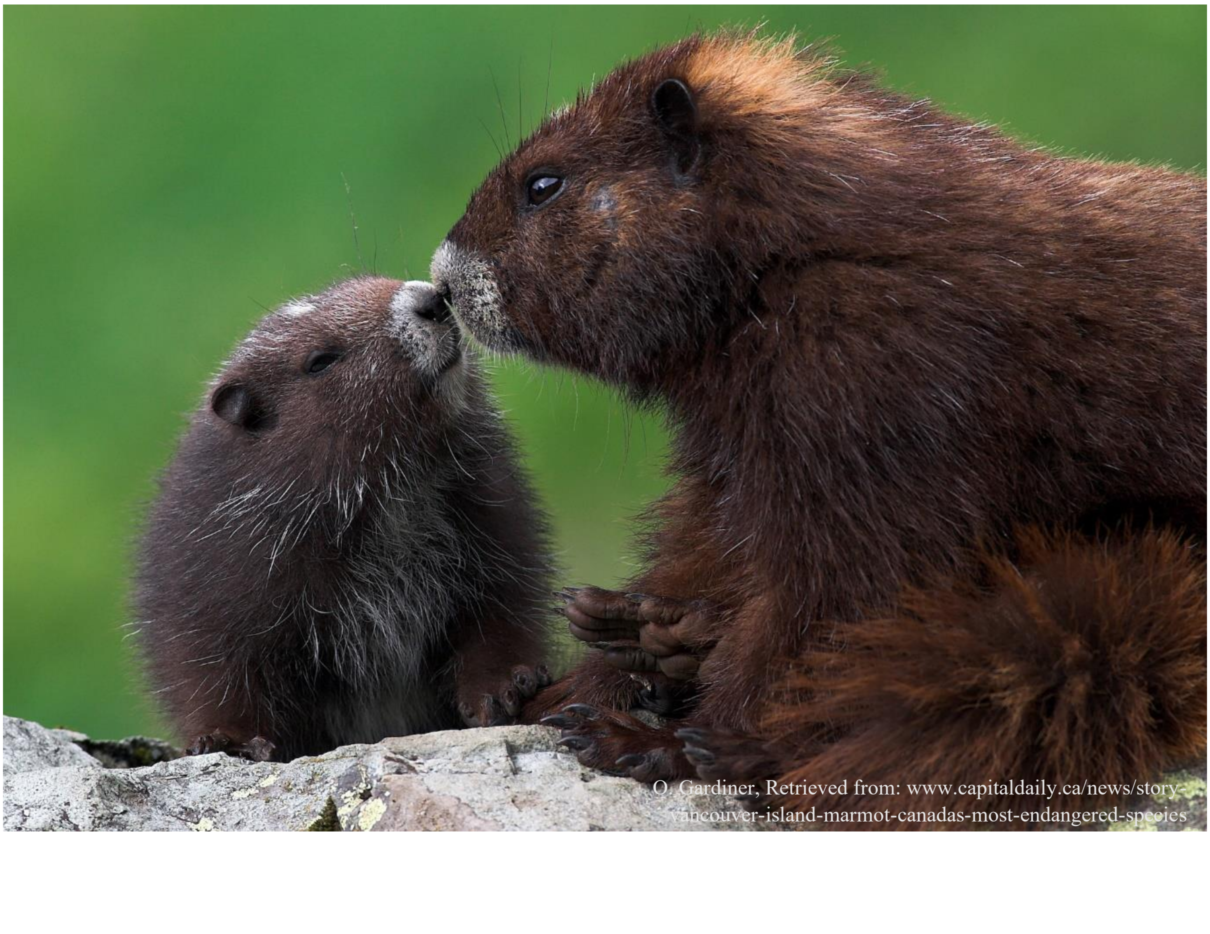

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Contents

1. Introduction

Conservation breeding is an important tool to combat species declines considering current, rapid biodiversity loss (Bowkett, 2009; Seddon et al., 2007; Taylor et al., 2017). There have been several notable examples of conservation breeding and release/reintroduction programs preventing species extinctions, for example the Golden Lion Tamarin (*Leontopithecus rosalia*; Kierulff et al., 2012), and the Mauritius Kestrel (*Falco punctatus*; Jones et al., 1995). Despite these important examples, not all conservation breeding programs are successful (Leus et al., 2011). To increase the success of conservation breeding programs, many researchers are advocating for careful consideration of evolutionary processes, particularly mate-choice, in management practices (Martin-Wintle et al., 2019; Schulte-Hostedde et al., 2015).

It has long been recognized that mate-choice is an important driver of evolution (Darwin, 1872), and in natural systems most animals have a choice of mate because of competition, preference, or breeding style. Typically, females are regarded as the “choosy” sex (Bateman, 1948) and there are extensive studies on female mate-choice (see: Clutton-Brock & McAuliffe, 2009), however depending on the mating strategy and life history of the species male preference can also affect breeding (Johnstone et al., 1996; Pryke & Griffith, 2007). In contrast, most conservation breeding programs pair individuals for breeding based on genetic distance to safeguard genetic diversity (Lacy, 2013). This is often done through a pedigree and assigning a mean kinship (MK) value to assess relatedness (Asa et al., 2011). While preserving genetic diversity is important, especially for critically small populations, failure to consider evolutionary processes like sexual selection can reduce breeding success or reduce the genetic quality of offspring, which is counterproductive to the goal of maintaining genetic diversity (Asa et al., 2011; Pitcher & Neff, 2007; Schulte-Hostedde et al., 2015; Wedekind, 2002). Moreover, failure of some individuals to breed can create genetic scarcity if their genes are not being represented in the population (Asa et al., 2011).

There is a growing body of literature demonstrating incorporating natural mate-choice behaviours increases breeding success (Martin-Wintle et al., 2015; Parrott et al., 2019) and increases the genetic quality of offspring (Hartnett et al., 2018; Pitcher & Neff, 2007). For example, allowing giant pandas (*Ailuropoda melanoleuca*) to pair with a chosen partner increased copulation and birthing rates (Martin-Wintle et al., 2015). Moreover, offspring from preferred pairings have demonstrated higher fitness (Drickamer et al., 2000). Ultimately allowing for mate-choice can help improve conservation breeding programs both by producing more offspring, and offspring that are more likely to survive, which will be crucial in preventing species extinctions (Martin-Wintle et al., 2019).

There are several ways in which mate-choice can be incorporated in conservation breeding programs. The most straightforward of which is simply combining all animals in the program into one enclosure and allowing them free mate-choice. This strategy has been commonly employed for birds in aviaries (Pryke & Griffith, 2007), but has also been successful for some mammal species (Gallie & Hayes, 2020). This strategy is not always favoured however because of the possibility of competition, and consequently injury or death, and it is challenging to track the genetics or maintain a studbook accurately. Therefore, many programs prefer to assess preference first, and create pairs accordingly. Preference can be determined through assessing behaviour through a partition (Martin-Wintle et al., 2015; Parrott et al., 2019) or by scent. Scent has been identified as an important component for mate-choice, especially for various species of rodents including golden hamsters (*Mesocricetus auratus;* William Huck et al., 1985), Harvest mice (*Micromys minutus*; Roberts & Gosling, 2004) and house mice (*Mus musculus;* (Drickamer et al., 2000). Experiments using scent to assess female preference have been used in breeding programs to increase breeding success (Parrott et al., 2019).

2. Background

The Vancouver Island marmot (*Marmota vancouverensis;* henceforth marmot) is a species that is currently maintained in a conservation breeding program. These marmots are a social, burrowing rodent endemic to Vancouver Island, British Columbia, Canada (Nagorsen et al., 1996). They live in colonies, often with more than one family group in sub-alpine meadows between 1000 and 1400m (Bryant & Janz, 1996). Marmots are obligate hibernators usually immerging in hibernacula in September- - October and emerging in the spring around March – April (Bryant & McAdie, 2003).

First listed as endangered by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) in 1978, marmot populations continued to decline throughout the 1990's with as few as 70 individuals counted in the wild in 1998 (MRF, 2020). Therefore, it was determined that the target population of 400-600 individuals would not be accomplished without a conservation breeding program (McAdie, 2004). In 1997, several marmots were brought into care at the Toronto Zoo (TZ), followed by the Calgary Zoo (CZ). The Mountain View Conservation and Breeding Society (MVF) bred marmots until 2014, and the Tony Barrett Mount Washington Marmot Recovery Centre (TBMWMRC) have also housed and bred marmots. 587 captive bred marmots have been released since 2003, and the current population is estimated to be 204 individuals at 27 colonies (Gourlay et al., 2022).

There is limited information on the reproductive behaviour of these marmots because reproduction, birth, and early rearing of pups is done in burrows pre-emergence. Marmots produce 1 litter a year, but do not always breed each year (Bryant, 2005). Litters usually consist of three to four pups, however litter sizes ranging from one to seven have been observed (Bryant, 2005). Litter size and age at first reproduction is similar for captive and wild marmots (Bryant, 2005), however not all breeding pairs breed successfully in human care (Casimir et al., 2007), and there are lower probabilities of breeding for captive marmots in certain age classes compared to wild (Bryant, 2005). This is a challenge in the marmot conservation breeding program, as the wild population is still dependent on supplementation from captive bred individuals.

3. Goal

To date, there has been no evaluation of mate-choice strategies to improve the breeding success of marmots in human care. Given that marmots are highly social animals in the wild, I hypothesize that allowing for mate-choice will increase the breeding success of marmots. My goal is to provide the first, preliminary experiment to incorporate mate-choice in the conservation breeding program of marmots.

3.1 Objectives

Specifically, my objectives are:

1. To determine if female marmots show preference to "high-quality" males (i.e., genetically advantageous as selected for by the studbook) based on scent
2. To evaluate what factors may be contributing to female mate-choice based on scent

4. Methods

4.1 Study Site

Currently, marmots are housed at TZ, CZ and the TBMWMRC. I will conduct the mate-choice experiment using the population in care at TZ. Given that previous research found no significant difference in breeding between facilities (Bryant, 2005), the population in care at CZ will be used as an experimental control. At TZ, marmots are usually held in breeding pairs with one male and one female. Occasionally, same-sex sibling pairs are held together. Marmots are housed in indoor enclosures only, or enclosures with indoor and outdoor access. Enclosures are between 12.7 and 17.7 m^2 and each enclosure has two nest boxes measuring 1.2 x 0.8 x 0.6m (Casimir et al., 2007). There are 8 breeding pairs of marmots at TZ and 15 at CZ.

4.2 Mate-choice experimental design

I will evaluate mate preference using a T-maze experimental set up. The T-maze will be a 0.5m strait connected to two 1m long wings with a 0.5 by 0.5m box at each end to provide the novel scents (Figure 1). The T-maze will be made from wire to allow me to assess time and behaviour. The maze without scents will be placed in the female enclosure at least 1 week before the experiment to allow the female to acclimatize to the structure. Similar methods have been used to assess behaviour in response to olfactory cues for the Black footed ferret (*Mustela nigripes*; Potratz et al., 2022).

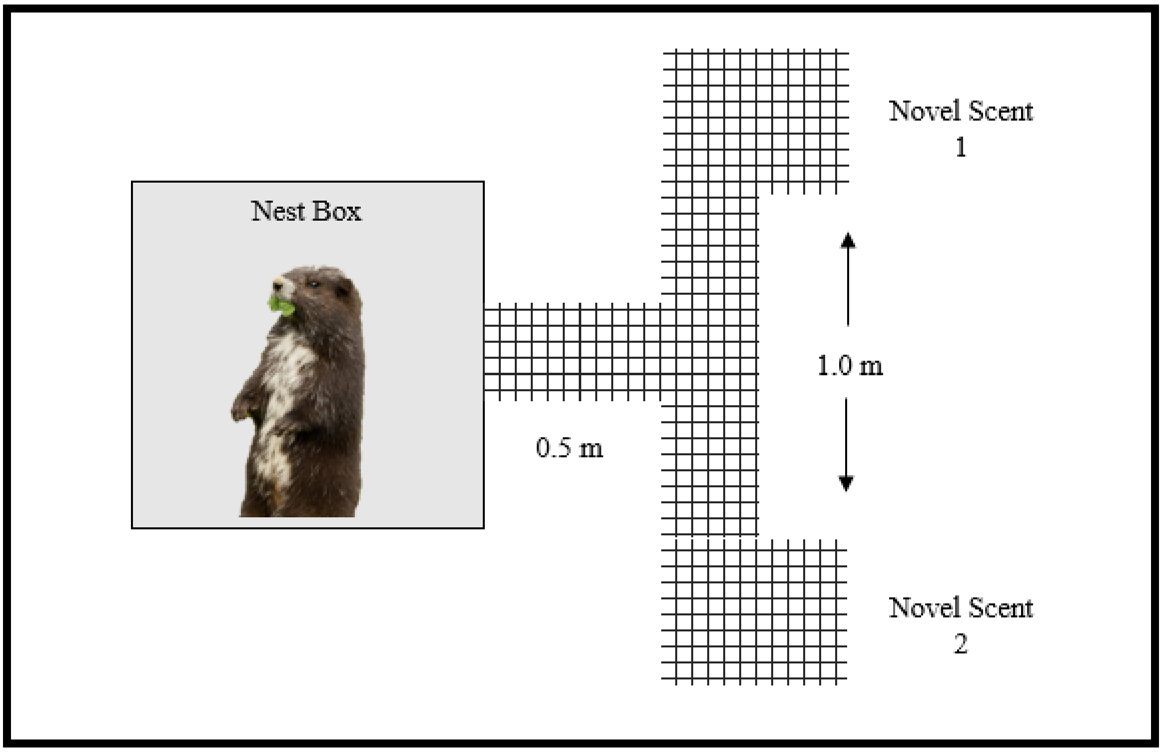

Figure 1. Experimental design for T-maze that would be fit in each enclosure

With the female contained in the nest box, I will fit the T-maze over the nest box opening with the scents contained in the boxes at each end. All trials will take place in the active season prior to immergence to hibernation. Trials will last 30 minutes, and each female will be assessed 3 times for a total of 24 trials. Each female will have two trials containing the scents of high-quality male and low-quality male using soiled shavings, and then another trial using the scent of the high-quality male and another scent as a control (Table 1). Soiled shaving as scents will be used so there will be no direct contact between marmots prior to introduction to eliminate possible negative behavioural responses. Trials will be randomized throughout the day and the order in which the scent pairings are presented. To not influence behaviour, trials will be recorded, and the video footage will be analyzed remotely. This will also allow trials to be conducted simultaneously.

Table 1. Scent combinations for each proposed trial. Each female will undergo all trial pairings

Trial	Scent 1	Scent 2
1	High-quality male	Low-quality male
2	Low-quality male	High-quality male
3	High-quality male	Other Scent

4.3 Statistical Analysis

Depending on the distribution of the data and whether the assumption of normality is violated, either an unpaired student's t-test (parametric) or a Wilcoxon rank-sum test (nonparametric) will be used to determine if there was a significant difference in the time females spent with the high and low-quality male's scent.

To assess other factors that may influence female choice, I will use a general linear mixed model (glmm) with a binomal family. All statistical analysis will be done in R (R core team 2020) using the package *glmm* (Knudson et al., 2022). Preference shown for the high-quality male will be converted to a binary response for this analysis (i.e., higher proportion of time spent with high-quality male scent is 1, higher proportion of time spent with low-quality male is 0). I will include a random effect of female ID to account for multiple trials from the same female, and female age, age at first reproduction, production of young in the previous year will be evaluated as fixed effects as these were previously determined to affect reproduction in marmots (Casimir et al., 2007).

5. Project Schedule

Task	**Year 1**				**Year 2**				**Result/Indicator of Success**
	Q1	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**	
Write formal project proposal									Proposal document
Get data collection permission from TZ									Signed data collection agreement
Apply for animal care permits									Animal care permit obtained
Construct T-maze									T-maze constructed
Conduct scent trial experiments									Data collected for all trials
Analyse data from scent trials									Working *glmm* models to represent data
Write paper using trial results									Peer reviewed paper
Write protocol to be distributed to other institutions									Protocol developed

6. Expected Outputs

I expect two major outputs from this study. First, enough data to publish an article in a peer reviewed journal detailing the outcomes of the experiment. Although many zoos have a mandate for being research facilities, there is a lack of scientific literature published from zoos in the field of conservation (Pyott & Schulte-Hostedde, 2020). This study could help bridge that gap.

Second, this experiment will provide the basis of a protocol that could be distributed to other zoological institutions to employ with their conservation breeding programs. Clearly outlining the approach, and any pitfalls and successes will key for other institutions utilizing the approach.

7. Expected Outcomes

As a result of this study, I expect we will have a better understanding of marmot breeding behaviour. We will know whether scent is an important factor for female mate choice in the species and we will know which factors contribute to this decision. This information has the potential to influence management practices in the future to maximize breeding success.

8. Future Research Directions

This experiment is intended to be a preliminary evaluation of female mate-choice using scent in the program. The results of this study do not directly address breeding success in the program, nor do they address the genetic quality of the offspring produced. Nonetheless, it is an important first step in being able to answer these questions.

If female marmots do show preference to high quality males based on the scent trials, this technique could be used in future to allow female marmots to choose a preferred mate based on suggestions from the pedigree. Then, a study could be conducted to see whether there is increased breeding success with a preferred partner vs. a forced partner, as is consistent with the literature. Secondarily, studies could be conducted on the fitness of offspring from preferred partner pairs too see whether these offspring are more likely to survive post-release, which is imperative for the success of the conservation program.

9. Resources Required

Table 2. Summary of budget items for the project

Item	Quantity	Cost	Purpose
Chicken wire	2	2 *180.2 = $360 CAD	Construct T-mazes (qty. 4)
Seagate One Touch 4TB USB 3.0 Portable External hard Drive	1	$139.99 CAD	Video storage
Adobe Express Subscription	3	$13.46 CAD	Three-month subscription to analyze video

			footage
Salary for researcher	1	$17,500 CAD	*Based on average graduate student salary for CAD
	Total:	**$18,013.45 CAD**	

10. Risks/Assumptions

This study was designed to minimize risk to the individual marmots, and to the overall success of the program. Using scent eliminates the possibility of injury from competition and I am not proposing altering any breeding pairs for the purposes of this study, which could influence breeding success. The biggest risk would be the possibility of stress to the female marmots by introducing a novel stimulus to their environment (i.e., the T-maze). However, precautions would be taken to introduce the T-maze to allow for acclimatization, moreover they are provided with similar structures, like plastic tubing, as enrichment so I suspect the physiological repercussions will be minimal.

The biggest assumption in this study is that the olfactory response is important in mate-choice for this species. However, there is an existing body of literature to suggest this may be the case for similar species, and this study is designed to test this assumption with minimal disruption to how the program operates.

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