Disease and unsuccessful reintroduction of Vancouver Island marmots (*Marmota vancouverensis*)

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ABSTRACT

In 1996 we conducted an experimental reintroduction of Vancouver Island marmots (*Marmota vancouverensis*). Six animals were captured from 3 colonies in recently logged habitats and released in a natural sub-alpine meadow. Two males dispersed from the site shortly after release. One male was found dead 5 km from the release site after being killed by a predator; the other was not seen again. The remaining 4 animals bonded to the release site, excavated burrows, foraged, showed typical weight gains and entered hibernation normally. These animals died during the winter of 1996-97. Death may have been caused by a bacterial infection. Our experience underscores the fragility of reintroductions based on small numbers of animals, and illustrates the potential of disease threats to small reintroduced populations.

INTRODUCTION

Reintroduction is becoming an increasingly important tool for wildlife managers (Griffith et al. 1989). Although some reintroductions have achieved notable success, many have failed and causes of failure are often unknown (Wolf et al. 1996). In particular, the role of disease in limiting reintroduction success has received little attention but may be of great importance (Griffith et al. 1993, Cunningham 1996).

The Vancouver Island marmot (*Marmota vancouverensis*: Swarth 1911) is endemic to Vancouver Island, British Columbia (Nagorsen 1987). It is listed as an endangered species by the Committee on the Status of Endangered Wildlife in Canada, and by the Province of British Columbia (Munro et al. 1985). The current population is mostly confined to a small (150 km²) geographic area on southern Vancouver Island (Bryant and Janz 1996), and is estimated to contain fewer than 150 individuals (Bryant et al. in prep.). This represents a decline in numbers of approximately 60% within the past decade, and a substantially reduced geographic range in recent decades (Bryant and Janz 1996). Recovery of this species from endangered status depends on

increasing numbers and distribution, and this in turn requires development of suitable reintroduction methods (Janz et al. 1994).

In this paper we report on initial attempts to restore *M. vancouverensis* to a formerly occupied natural sub-alpine meadow on Vancouver Island. Our objectives were to 1) describe the theoretical and practical rationale behind transplant methods for this species, 2) describe results to date, and 3) provide a case-study for others contemplating reintroduction as a management tool for endangered species.

METHODS

Guiding principles

Detailed reintroduction planning began in the early 1990s, after results from population surveys confirmed the critically low distribution and abundance of Vancouver Island marmots (Bryant and Janz 1996). At the outset it was recognized that lack of previous experience with *M. vancouverensis* transplants, limited financial resources, incomplete knowledge of habitat requirements, and small size of potential "donor" colonies would severely restrict the scale of reintroduction efforts, at least initially (Bryant 1995).

The project was designed to incorporate features associated with successful reintroductions elsewhere (Griffith et al. 1989). A number of "guiding principles" were adopted given this experience and knowledge of *M. vancouverensis* ecology.

1. Wild-captured animals. Wild-captured animals generally provide superior reintroduction results (e.g., Griffith et al. 1989, Wolf et al. 1996). In our case wild-captured marmots were the only option. No releasable Vancouver Island marmots have yet been produced in captivity.
2. Animals from sub-optimal donor habitats. Marmots living in clearcuts show reduced survival rates and these habitats may act as population "sinks" (Bryant 1996).
3. "Stepping-stone" approach. Initial reintroductions were planned to occur close to extant colonies. This was deemed prudent in case animals immediately dispersed from the release site, as it would maximize the probability that dispersers encountered other marmots.
4. Complete social units. A matrilineal social group (adult female with offspring) was selected in order to minimize the probability of dispersal and maximize the probability of successful communal hibernation (Arnold 1990).
5. Appropriate age-sex structure. An adult female with yearling offspring was selected because female *M. vancouverensis* breed only every 2nd year on average in the wild (Bryant 1996). This minimized the possibility of translocating a pregnant female, yet made use of animals that had already successfully hibernated.
6. Maximum genetic variation. An adult male and presumed 2 year-old male immigrant from different colonies were included in the transplant group to maximize genetic variation in the small founder colony. This also prevented social disruption of the donor colony through removal of the resident adult male.
7. Appropriate timing. Late June was selected for translocation because this allowed sufficient time for early-spring identification of donor colonies and implantation of radio transmitters. This timing also permitted confirmation that pregnant females would not be moved, yet

allowed over two months for habituation to the new site before onset of winter hibernation (Bryant 1996).

8. Monitoring protocols. It was clear in advance that reintroduction of small numbers of an endangered mammal was inherently risky. The Recovery Team could not afford a "try and try again" philosophy. Accordingly the experiment was performed in combination with observation and radio-telemetry monitoring protocols.
9. "Measures of reintroduction success". Several authors have commented on the absence of evaluation targets in other reintroductions (e.g., Ramousse et al. 1992). Our *a priori* measures of success were basic: a) evidence of site-bonding or bonding to nearby sites in the first year, b) high rate of overwintering survival within the first 2 years, and c) successful reproduction within 3 years of release.

Release-site selection

Habitat evaluation efforts at historic and extant marmot colonies began in 1993 and biophysical habitat data were available (Demarchi et at. 1996). Criteria for selecting potential release sites included historic occupancy by marmots, high relative abundance of plant species eaten by *M. vancouverensis* (Martel and Milko 1986), deep colluvial soils and talus slopes associated with marmot hibernation, and boulders associated with marmot thermoregulation and "lookout" sites (Bryant 1997). Of 8 potential release sites examined all met the minimum criteria but only one site was rated as "high potential". Based on this assessment the Recovery Team selected a primary release site on the south face of Mount McQuillan (1120 meters elevation). This site is within 5 km of where the first scientific collection of *M. vancouverensis* was made in 1910 (Swarth 1912).

Pre-transplant capture and implantation of transmitters

Monitoring activities began in late April 1996 to select candidate donor groups. Choices were limited but by late May a family group on Mount Franklin was selected. Capture, ear-tagging and surgical implantation of radio-transmitters in the adult female and yearling offspring animals began on 4 June and was completed by 10 June following established protocols (see Bryant 1996 for details of trapping, chemical immobilization and ear-tagging techniques).

We used transmitters from Telonics® (Mesa, AZ; model IMP 300) or Custom Telemetry® (Watkinsville, GA: see Van Vuren 1989). Both transmitters had similar overall dimensions (~90 x 25 mm) and weight (35-40 grams). Transmitters were encased in beeswax and disinfected in povidone-iodine (Betadine®, Purdue Frederick Co., Norfolk, CT) solution for 24 hours prior to surgeries. Implantations were performed in the field by a veterinarian (see Acknowledgments). Procedures followed Van Vuren (1989) with several refinements.

After sedation with a ketamine/midazolam combination to facilitate handling (see Bryant 1996) marmots were anaesthetized using 2.0-3.0% isoflurane gas (Aerrane®, Anaquest, Missisauga, ON) administered with bottled oxygen and Isotec® vaporizer (Ohmeda, Madison WI) mated to a small animal mask. Oxygen flow rates were 2 to 3 liters/minute. After induction, anesthesia was maintained at a reduced isoflurane concentration (1.5-2.0%). The use of isoflurane greatly shortened recovery time (to 15-30 minutes) and allowed more precise control of anesthetic depth than injectable agents alone. Transmitters were introduced into the peritoneal cavity through a midline incision. Other deviations from Van Vuren's procedure included incision through the

linea alba to minimize muscle trauma and blood loss, and use of methyl-methacrylate glue (Vetbond®, 3M, St. Paul, MN) in addition to subcuticular sutures to seal and reinforce the incision site. All animals were released within 1.5 hours of capture into their pre-capture habitat, and were allowed to recover from the effects of surgery for at least 8 days before transplant.

Release site preparation and transplant

Site preparations were minimal. A plywood shelter box (dimensions = 2.5 x 1.2 x 1.2 m) was erected on the site to provide temporary housing for marmots. The box was painted white to reflect sunlight and contained an interior partition to provide separate housing for the adult male and female. Access to each compartment was available through a hinged door. On the meadow itself, 0.3-0.5 meter deep "starter" burrows were dug using hand tools in the hope that marmots would use them to construct proper burrows.

While trapping was in progress on any given morning, previously captured animals were kept in plastic dog travel kennels that were kept shaded from direct sunlight. Trapping sessions typically ended before noon, at which time a helicopter was called to the site by radio. Capture of marmots targeted for movement was more difficult than anticipated. One of the 4 available yearlings from Mt. Franklin was never captured, and we were unable to capture a 2nd suitable adult female, although trapping efforts continued through early July.

RESULTS

Transplant

Six marmots were moved to the Mount McQuillan release site between 18 and 22 June. The transplanted group included one adult female, 2 female and 1 male yearling offspring (from Mt. Franklin), and 2 adult males (from Sherk Lake and Butler Peak). The female and yearlings were equipped with radio transmitters but logistic reasons precluded implantation of the adult and 2 year-old males.

The actual transplant was free of complications. Marmots did not whistle or appear agitated in the helicopter. The release plan called for marmots to be confined in shelter boxes overnight with the intention of allowing them to calm down before release on the following morning. This was altered by irregular trapping success and by marmots themselves. Marmots chewed through aluminum ventilation grates and two yearlings released themselves though an unsecured door. Staggered arrival of later marmots at the release site compounded the situation: only two yearlings and two adults were actually confined to the shelter box overnight. The adult male from Butler Peak was released directly into an existing burrow. The male yearling from Mount Franklin was transplanted 3 days after all other animals and was also released directly.

Post-release behavior

Marmots explored their new meadow thoroughly within hours of release. In a few days they excavated old marmot burrows, enlarged starter burrows and lined the shelter box with vegetation. The adult male from Butler Peak disappeared shortly after release, and without radio-telemetry it was impossible to determine his eventual fate.

The yearling male from Mount Franklin exhibited unusually large daily movements during the next 2 weeks, and was observed in locations >2 km from the release meadow. Release crews noted aggressive behavior directed towards this individual by the Sherk Lake male and by the yearling siblings from Mount Franklin. He disappeared 3 weeks after release. Hopes of successful dispersal were raised when field crews re-located the transmitter signal on 26 September in a recently logged habitat approximately 5 km from the release site. Unfortunately just the transmitter and traces of fur were found on 1 October. The evidence indicated predation, probably by cougar (*Felis concolor*) or wolf (*Canis lupus*).

The remaining animals (adult female, adult male and 2 yearling females) stayed within the general area of the release meadow and exhibited normal foraging behavior and activity rhythms (Heard 1977) for at least 3 weeks after release. Daily monitoring was suspended in mid-August, but weekly checks were made through onset of hibernation in late September. All 3 telemetered animals were confirmed to be in the same hibernaculum at 1140 meters elevation. Radio-transmitters were periodically checked during the winter of 1996-97 and monitoring efforts increased in May (*M. vancouverensis* normally emerge from hibernation between 21 April and 15 May: Bryant 1997).

Unsuccessful hibernation

No signs of emergence were observed during early spring monitoring efforts. On 19 June the hibernaculum was excavated. This burrow measured over 4 meters long and the hibernaculum chamber was approximately 1 meter below ground level. No signs of flooding or burrow collapse were observed, and the single entrance showed normal evidence of being plugged with soil from the inside. Two dead telemetered yearlings and the untelemetered male from Sherk Lake were retrieved. Telemetry indicated that the adult female was located in the same burrow, but this animal could not be retrieved as she was apparently positioned behind a large boulder. The 3 retrieved animals showed severe signs of decomposition but appeared to be in very fat body condition compared to animals of similar age captured in early spring after normal hibernation (i.e., they were judged to be “curiously heavy for spring 2 year-olds”; AB field notes). Unfortunately the carcasses were not weighed at this time or during subsequent necropsy.

Necropsy

Carcasses were extremely autolysed but necropsies verified “high to good” body fat content. Implanted transmitters in the 2 telemetered yearlings were present within folds of mesentery of the two yearlings and there was no associated inflammation. Detectable gross abnormalities were confined to the lungs and heart. The hearts were globose with engorged vessels or ecchymotic haemorrhage of the epicardium. The lungs were mottled with a lobular pattern of atelectasis. Despite autolysis and freezing artifact in tissue sections, histology demonstrated some inflammatory changes in the myocardium, lung, and renal tubules. Unfortunately, the state of the tissues made it impossible to reach a definitive histological diagnosis.

A heavy growth of *Yersinia frederiksenii* and *Carnobacterium divergens* (formerly *Lactobacillus divergens*) was cultured from multiple samples of lung, liver and kidney from all recovered marmots. No viruses were isolated from the tissues.

The three carcasses were frozen and maintained for further study. One year later, samples were resubmitted for bacterial culture to a different laboratory, to further identify the bacterial strains involved (see Acknowledgments). This time, *Yersinia enterocolitica* was isolated in low numbers from the bone marrow of one animal and the bladder of a second marmot.

The failure of any of the four marmots hibernating together to survive the winter, in combination with the similarity of gross post-mortem lesions and consistent culture of a *Yersinia* from multiple organs led to a presumptive diagnosis of yersiniosis as the cause of mortality.

DISCUSSION

The loss of transplanted marmots was heartbreaking for those involved in the planning and execution of their movement. More significantly, failure of the first reintroduction attempt for *M. vancouverensis* underscores the vulnerability of this species, which is characterized by metapopulation structure and individual colonies that normally contain fewer than 10 individuals (Bryant and Janz 1996). On theoretical grounds, it is unsurprising that small re-introduced populations are especially vulnerable to disastrous events (Griffith et al. 1993). On practical grounds, to our knowledge this is one of the few cases in which mortality of marmots appears to be associated with disease (Barash 1989).

Y. frederiksenii is closely related to *Y enterocolitica*, but has not been reported in the literature as a cause of disease in animals or people. *Y. enterocolitica* is a commonly encountered bacterial infection in deer and other mammals and birds (Zwart 1993, Blake et al. 1991, Henderson 1983, Martyny and Botzler l976). *Y. enterocolitica* is found in a wide variety of hosts and there are apparently large species and genus-specific differences in susceptibility (Fox and Lipman 1991). It is considered to be an opportunistic pathogen with low virulence and minimal clinical signs, and has been isolated from soil, water and feces (A. Borczyk pers. com., National Yersinia Reference Service, Etobikoke, ON). The route of infection is ingestion (Obwolo 1976). Increased virulence in *Y. enterocolitica* has been associated with stressors including age, unfavorable climatic conditions, poor nutrition or high population density (Zwart 1993, Blake et al. 1991).

Carnobacterium divergens has been isolated from a variety of meats stored at low temperatures (Collins et al. 1987, Brooks et al. 1992). *Y. fredericksenii* also survives at low temperatures (Botzler 1987). It is postulated that the low temperatures in the burrows “selected” for the growth of these two cold tolerant organisms after the death of the marmots.

At this time, it is uncertain as to what agent actually killed these marmots. Although *Y. fredericksenii* or *enterocolitica* would seem the most likely suspect, it may also simply have been an opportunistic post-mortem contaminant.

It remains unclear what factors precipitated the disease outbreak. It is possible that animals were suffering from increased stress resulting from the reintroduction. The fact that the remaining uncaptured yearling at Mount Franklin survived through 1997 may lend credence to this hypothesis. However, transplanted animals had over two months in which to habituate to their new environment. This fact combined with good body fat condition makes us doubt that transplant-related stress was a significant factor.

A second hypothesis is that a bacteria or virus was present on the release site but not in the transplanted animals. Indeed, it is possible that disease caused local extinction of marmots at this site originally. We note that other *Yersinia* spp. persist in the kinds of damp and cool conditions probably associated with marmot burrows (Little et al. 1992).

A third possibility is that one or more of the transplanted animals carried disease to the release site. Later population counts at donor colonies support this hypothesis. In particular, the Butler Peak clearcut colony showed higher than average mortality in 1996-97 and several animals also disappeared from the Sherk Lake donor colony.

The final hypothesis is that certain bacteria or viruses are a normal but low-level mortality factor in *M. vancouverensis* and that the physiological demands of hibernation allow the agent to be manifested. Perhaps the transplanted marmots were just unlucky. Arctic ground squirrels (*Spermophilus parryii*) show poor wound healing, reductions in white cell counts and platelet numbers during hibernation (J.E. Blake pers. com., University of Alaska, Fairbanks AK). Study of marmot immune function during hibernation may show similar changes in susceptibility to infection. Without further information about the natural distribution or vectors of disease-causing agents on Vancouver Island, or within extant or historic marmot colonies, it is impossible to distinguish among these possibilities.

The first reintroduction of *M. vancouverensis* is perhaps best described as “a successful operation except that the patient died.” Despite achieving only the first of the pre-established measures of success, we consider the 1996 reintroduction to have been highly useful in teaching us the mechanics of transplanting Vancouver Island marmots. We learned that marmots can be moved safely and that most will bond to the release-site and enter hibernation normally. Movement of complete family groups is justified. Ultimately, the loss of animals from dispersal, predators and disease illustrates the need to base reintroductions upon larger numbers of animals, and gives us strong encouragement to pursue efforts to breed *M. vancouverensis* in captivity.

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