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Unexpected genetic composition of a reintroduced carnivore population

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ABSTRACT

In an attempt to maximize genetic diversity, species reintroductions often target numerous source groups and occur over multiple years. However, the serial introduction of individuals from disparate locations can create unique patterns of genetic structure, with potential implications for demography and population connectivity. To investigate the genetic structure and connectedness of contemporary populations of serially reintroduced American martens (*Martes americana*) in Wisconsin, we sampled the source populations of Colorado, Minnesota, and Ontario, and the two reintroduction sites within the state. In a relatively small area (~7000 km^2), we detected six distinct genetic clusters, partitioned according to the original source groups. Source groups exhibited differing degrees of success, with Minnesota and Ontario signatures persisting and Colorado disappearing from the landscape. Two of the genetic clusters had unknown sources not attributable to the reintroduced populations, potentially arising from an early reintroduction attempt or the persistence of a cryptic native population of martens. Limited admixture between clusters was detected, yet we found evidence for the occasional dispersal of martens from each site to the other. Our results suggest that reintroductions can create strong and novel patterns of genetic structure − unseen for this species elsewhere or other vertebrates in the region − across small spatial scales.

1. Introduction

As a result of global declines in abundance and distribution (Estes et al., 2011), carnivores have frequently been targeted for reintroductions in an attempt to restore ecosystem functioning (Breitenmoser et al., 2001; Ripple et al., 2014). Source populations for reintroductions are often selected to maximize resilience of the recipient population against demographic and genetic stochasticity, while balancing logistical constraints and considerations of viability for the source populations (Hedrick and Fredrickson, 2010; Miller et al., 1999). Consequently, individuals selected for reintroductions are often pulled from geographically disparate locations and genetically distinct populations, and sampled over a number of years (Olson et al., 2013). This spatial and temporal variation, and serial shuffling of individuals, can alter the genetic composition of the recipient population and create a novel patchwork of genetic structure on the landscape (Groombridge et al., 2012). Yet, in many cases, little information exists on the genetic structure of the translocated individuals or the previously existing populations (Vernesi et al., 2003).

Goals for the genetic composition of reintroduced populations typically include maintaining a genetically viable population (Forbes and Boyd, 1997) by avoiding inbreeding depression (Frankham et al., 2011) and maximizing genetic diversity (Hedrick and Miller, 1992). However, the genetic consequences of drawing from divergent stocks are difficult to predict. In some cases (e.g., translocated panthers from Texas rescuing the insular and inbred population of Florida panthers), introduced individuals contribute genetic variation to local populations while maintaining genetic uniqueness (Hedrick and Fredrickson, 2010). In extreme cases, the reintroduced population remains genetically indistinguishable from the source population. Specifically, this can occur in first-time reintroductions in areas where natural populations were driven to extinction and replaced by a single stock of individuals (Wisely et al., 2003; Whittaker et al., 2004), or when augmentations genetically swamp small recipient populations (Tallmon et al., 2004). In other instances, established carnivore populations diverge measurably from their founding groups. For example, strong differentiation from

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the original source was found in reintroduced brown bears (*Ursus arctos*) to northern Italy (De Barba et al., 2010), and grey wolves (*Canis lupus*) to Yellowstone National Park, USA (vonHoldt et al., 2008). Multiple translocations of American martens (*Martes americana*) from genetically panmictic source populations (Kyle and Strobeck, 2003) to northern Michigan produced strongly structured subpopulations, even at fine-scales (Williams and Scribner, 2010). Thus, even carefully planned reintroductions have been observed to create novel genetic structures and unexpected patterns of relatedness across the landscape.

Many translocations involve the release of animals over multiple years from several sources, and thus take the form of augmentations (Vinkey et al., 2006). Consequently, animals from divergent genetic stocks are released onto a landscape with a backdrop of preexisting patterns of genetic diversity. The resultant genetic structure can be further altered depending on connectivity and the mixing of historic and released populations (Vandewoestijne and Baguette, 2004). For example, the connectivity of habitat patches has been shown to impact dispersal following augmentation (Dzialak et al., 2005) and is an important driver of population persistence (Schadt et al., 2002). Despite the importance of connectivity (Steury and Murray, 2004), few studies account for dispersal across populations in a translocation framework. Following the release of potentially diverse individuals, the genetic contributions of reintroduced groups also depend on the extent of admixture between or among subpopulations (Hendricks et al., 2016). In some translocations, admixing creates hybrids in the overlapping ranges of expanding populations (Vernesi et al., 2003); in others, minimal mixing is detected, isolation by distance may not occur, and signatures of the sources are well preserved (Mowry et al., 2015). Reintroductions, then, produce questions about what patterns of dispersal and admixture can be detected (Fredrickson and Hedrick, 2006), which genotypes and individuals are persisting and contributing to population establishment (Cullingham and Moehrenschlager, 2013), and how translocation efforts alter patterns of genetic diversity on the landscape.

American martens were previously abundant throughout their southern range in the continental United States (Dawson and Cook, 2012), but have experienced numerous local extirpations (Laliberte and Ripple, 2004) due to overharvesting and habitat loss (Proulx et al., 2005; Krohn, 2012). As managers increasingly turn to reintroductions and augmentations to resurrect community composition and ecosystem functioning, American martens have become the most widely translocated carnivore in North America (Powell et al., 2012). Reintroductions in the northern Great Lakes region have drawn from multiple sources, including Minnesota, Ontario, and even a different species of martens in Colorado, and have taken place over a span of six decades. Attempts to re-establish three populations of martens in Wisconsin began with the reintroduction of a mixed stock of ten individuals to the Apostle Islands National Lakeshore, an event considered to be a failure (Kohn and Eckstein, 1987). Subsequent, and more concerted, efforts in the Chequamegon and Nicolet National Forests in northern Wisconsin drew from different sources and had distinct reintroduction histories (Fig. 1: Williams et al., 2007). Recent research on the demographic status of the Chequamegon National Forest (Manlick et al., 2017a) suggests the marten population is limited by competition (Manlick et al., 2017b) and has gained minimal reproductive benefit from subsequent augmentations, although the contemporary genetic structure of these groups has not yet been quantified.

Herein, we assessed the contribution of individual reintroduction events by exploring current genetic structure in the populations of American martens in Wisconsin, and explored patterns of dispersal, as informed by genetic structuring, in the region. In ascertaining the contributions of the original reintroduction events (Woodford et al., 2013), we predicted that genetic signatures from each of the founding populations from Colorado, Minnesota, and Ontario would be present in their respective reintroduction sites. Considering the proximity of the Nicolet National Forest to established marten populations in northern Michigan, we also predicted that we would find Michigan marten genetic signatures and evidence of admixture in eastern Wisconsin. However, we further hypothesized that there would be limited admixture between the other source groups due to high amounts of genetic differentiation, and limited admixture or movement between the Wisconsin populations due to their geographic isolation.

2. Methods

2.1. Biological sampling

We obtained biological samples of martens in Wisconsin via non-invasive sampling (Manlick et al., 2017a). Field sampling occurred in the Great Divide district of the Chequamegon National Forest (hereafter, Chequamegon) for 8 weeks each January–March of 2012–2014 and in the Eagle River-Florence district of the Nicolet National Forest (hereafter, Nicolet) from 2015 to 2016. We randomly placed non-invasive hair traps (modified from Pauli et al., 2008) along accessible winter roads and snowmobile trails at least 1 km apart using ArcGIS 10.1 (ESRI, Inc. 2011. Redlands, CA) and set traps in suitable marten habitat within a 250-meter radius of the original point. We attached traps to trees or snags with bait at the center and wire brushes on either end to obtain hair samples. Traps were checked every 6–8 days (200 traps in the Chequamegon, 175 in the Nicolet) and hair samples were collected from brushes with sterile forceps and stored frozen until analysis. All sampling methods were approved by the Institutional Animal Care and Use Committee at the University of Wisconsin-Madison (Project A005239-A01).

To document the genetic characteristics of the sources from Colorado and Ontario and the neighboring population in Michigan, we collected tissue samples from local agencies and fur trappers. We obtained tissue samples from martens trapped in Garfield, Grand, Summit, and Gunnison counties in Colorado, from within the Temagami fur management district neighboring Algonquin Provincial Park in Ontario, and from Iron, Gogebic, Marquette, Ontonagon, Houghton, and Baraga counties in Michigan's Upper Peninsula. Samples were obtained with the help of local natural resources agencies and the assistance of fur trappers. We cut a small section of the parietal muscle from marten skulls and stored tissue samples in falcon tubes at − 20 °C. We also used the genotypes of 68 previously analyzed marten samples from Saint Louis and Cook counties in Minnesota that were obtained directly from skin biopsies of the most recent augmentation to the Chequamegon population (Woodford et al., 2013; Manlick et al., 2017a).

2.2. Laboratory methods

We genotyped hair and tissue samples through initial DNA extraction in a clean room facility at the University of Wisconsin-Madison using QIAmp DNA micro kits and DNEasy blood and tissue kits (Qiagen, Valencia, CA), respectively. All possible marten samples (n = 279) were screened first with a microsatellite Gg3 (to differentiate martens from fishers [*Pekania pennanti*] and weasels [*Mustela* spp.]) and questionable samples (n = 19) were also sequenced with cyt b (Pauli et al., 2015), visualized in FinchTV (Geospiza, Inc., USA), and aligned and blasted in MEGA 7 (Kumar et al., 2016) against martens, fishers, and weasels. Confirmed marten samples were then genotyped using 9 additional microsatellite markers (Williams et al., 2009): Gg7, Ma1, Ma2, Ma5, Ma11, Ma14, Tt4, Mer041, and Mvis072. Polymerase chain reaction (PCR) conditions for all microsatellites included 2.5 μL of template, 0.16 μM labeled forward primer, 0.16 μM reverse primer, 0.4 μg/μl BSA, 0.2 μM $MgCl_2$, 200 μM dNTPs, 200 μM 10 × PCR buffer, and 0.5 units of Taq DNA polymerase. DNA was amplified at 94 °C for 2 min, followed by 42 cycles of [94 °C for 45 s, marker specific annealing temperature for 60 s, 1 min at 72 °C], followed by 72 °C for 10 min (Williams et al., 2009). We genotyped PCR product according to HEX, FAM, or TAM fluorescently labeled microsatellite markers on ABI 3730xl DNA analyzers (Applied Biosystems) at the UW-Madison