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Factors influencing red wolf–coyote hybridization in eastern North Carolina, USA

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ABSTRACT

Understanding the mechanisms that govern interspecific hybridization is vital to mitigating its impacts on endangered species. Research suggests that behavioral mechanisms such as mate choice and social disruption can regulate the rate at which hybridizing species interbreed. We investigated hybridization events between endangered red wolves (*Canis rufus*) and coyotes (*Canis latrans*) in eastern North Carolina to evaluate potential factors that may promote hybridization between these species. Specifically, we examined spatial location, breeding experience, breeder origin (captive vs wild), breeder ancestry (pure vs hybrid), and past history of the animal. There were over four times (126 vs 30) as many red wolf litters as hybrid litters over a 13 year time period. Over half of the hybridization events followed the disruption of a stable breeding pair of red wolves due to mortality of one or both breeders. Of these 69% were due to anthropogenic causes, primarily gunshot mortality prior to the red wolf breeding season. Both male and female red wolves interbred with coyotes, although a majority (90%) of the events we observed involved females. Wolves that produced hybrid litters tended to be young, first-time breeders with slightly higher levels of coyote ancestry. Only 16% of the hybrid litters were produced in the inner core of the red wolf recovery area. Our results suggest that disruption of stable breeding pairs of red wolves facilitates hybridization, jeopardizing future recovery of the red wolf. They also indicate the importance of behavioral forces, especially social stability, in regulating hybridization.

1. Introduction

Human activity has the potential to disrupt dynamics between hybridizing species, which can cause hybridization and introgression to emerge as conservation threats (Rhymer and Simberloff, 1996; Allendorf et al., 2001). As with any conservation problem, developing solutions requires recognizing the mechanisms that influence the process. For hybridization, that requires understanding the mechanisms that cause previously reproductively isolated species to interbreed. Species introductions (Rhymer and Simberloff, 1996; Stigall, 2010), habitat destruction and ecological homogenization (Seehausen et al., 2008; Crispo et al., 2011), and the spread of domesticated species (Likre et al., 2010; Champagnon et al., 2012) have been implicated as processes that may facilitate these shifts.

Still, these forces primarily influence whether hybridizing species come into contact, not necessarily whether individuals will interbreed. There is increasing recognition that behavioral processes such as mate choice (Pfennig, 2007; Reyer, 2008; Svedin et al., 2008; Gilman and Behm, 2011; Robbins et al., 2014), interspecific competition (Wolf et al., 2001; Krosby and Rohwer, 2010; Sacks et al., 2011), and Allee effects (Lode et al., 2005) can influence the rate of hybridization. The potential for behavioral forces to moderate hybridization and introgression may be a critical factor that would influence conservation schemes.

Understanding the mechanisms that govern interspecific mating is vital to recovery of species threatened by hybridization, especially the critically endangered red wolf (*Canis rufus*) in eastern North Carolina. Historically red wolves were distributed across eastern North America, but overharvest, habitat destruction, and hybridization with coyotes (*Canis latrans*) led to extinction in the wild by 1980 (Paradiso and Nowak, 1972; Nowak, 2002; USFWS, 1990). Beginning in 1987, captive red wolves were reintroduced into eastern North Carolina and today a population of about 80–100 individuals occupies the 600000 hectare Albemarle Peninsula (Phillips and Parker, 1988; Phillips et al., 2003; Bartel and Rabon, 2013).

At the same time, coyotes expanded their range into North Carolina (Hill et al., 1987) and in 1993 the first hybridization event between a reintroduced red wolf and a coyote was detected (Phillips et al., 2003). A subsequent population viability analysis

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suggested that hybridization was the greatest threat to red wolf recovery (Kelly et al., 1999). This led to the development of an aggressive adaptive management plan by the US Fish and Wildlife Service (USFWS) and the Red Wolf Recovery Implementation Team (RWRIT, Stoskopf et al., 2005) to limit hybridization and introgression. The genetic composition of the population is managed by an active monitoring program combined with genetic testing to remove hybrid individuals from the landscape (Stoskopf et al., 2005; USFWS, 2007; Bartel and Rabon, 2013).

Such aggressive practices have been implemented based on the hypothesis that a small red wolf population would be genetically swamped by coyotes without human intervention (Kelly et al., 1999). This is predicated on the assumption that when sympatric, red wolves and coyotes will breed indiscriminately. However, this assumption has not been tested empirically. Fredrickson and Hedrick (2006) modeled red wolf viability and found that positive assortative mating and aggressive interactions between the species were the most important factors in maintaining population viability. They developed hypothetical values for those parameters because empirical estimates did not exist. USFWS field biologists have observed red wolves displacing and occasionally killing coyotes and hybrids (USFWS, 2007). Otherwise, there is little understanding of how mate choice and social structure influence interactions between these species. Given the importance of social dynamics on the ecology of mammalian carnivores, there is potential for behavioral processes such as mate choice, social structure, and competition to limit hybridization (Rutledge et al., 2010; Sacks et al., 2011; Shurtliff, 2011). Conversely, disrupting these social systems may in turn influence reproductive patterns (Brainerd et al., 2008; Borg et al., in press) and hybridization rates (Rutledge et al., 2010).

We examined breeding records and individual histories of red wolves involved in hybridization events from 2001 to 2013 to elucidate factors that facilitate interbreeding between these species. Specifically, we asked the following questions: (1) Do age, prior breeding experience, and origin of the wolf influence the likelihood of hybridization, (2) are individuals with mixed red wolf/coyote ancestry more likely to hybridize, (3) are hybridization events evenly distributed across the recovery area, and (4) are hybrid litters produced under particular scenarios or breeder histories? If breeding between these species is indiscriminate, we would expect young dispersing red wolves to be the most likely individuals to encounter and breed with a coyote. Breeding opportunities within wolf packs are often restricted to a dominant breeding pair, which forces individuals in search of mates to disperse outside the pack (Mech and Boitani, 2003; Sparkman et al., 2012). In this system dispersing would increase the likelihood of an individual encountering a coyote considering that wolf packs are known to exclude coyotes within their range (USFWS, 2007). This is similar to observations of eastern wolf (*Canis lycaon*) packs in southern Canada (Benson and Patterson, 2013). We predicted that hybridization would increase from east to west, since the western portion of the study area has the fewest wolves, least stringent management, and closest proximity to the mainland coyote population. Also, we hypothesized that individuals with mixed red wolf/coyote ancestry would be involved in more hybridization events. By examining the characteristics and history of red wolves responsible for hybridization events with coyotes, we can better understand the mechanisms that govern hybridization, aiding recovery of this species.

2. Materials and methods

2.1. Genetic monitoring

Every spring USFWS biologists track female red wolves to locate active dens that contain pups. Blood samples are collected from pups and genetic testing is conducted to assess their ancestry and place them within the red wolf pedigree (Adams, 2006; Bohling et al., 2013). Since the implementation of the adaptive management plan in 2000 monitoring of red wolf dens and genetic testing of captured canids, including pups, became standard, which improved detectability of hybridization events (Stoskopf et al., 2005; USFWS, 2007). As a result, we only considered hybridization events that have occurred since 2000 for this study. USFWS biologists possessed permits for capturing and handling red wolves that have been jointly issued by the USFWS, Association of Zoos and Aquariums Reintroduction Scientific Advisory Group, and IUCN Species Survival Commission Reintroduction Specialist Group.

Parentage for red wolf and hybrid litters was determined following the methods of Adams (2006) and Bohling et al. (2013). To summarize, each pup was genotyped at 17 microsatellite loci and assigned to red wolf parents using genetic and field data, allowing for $\leqslant 1$ mismatch for a parent pair. Based on this pedigree we were able to estimate an individual's ancestry by averaging the amount of red wolf ancestry possessed by the parents as traced through the pedigree. In the case of hybrid litters, typically only the red wolf parent was identified, although in several situations the non-red wolf parent was later captured and determined to be a parent using genetic analysis. Several hybrid litters were detected when hybrid offspring were captured as juveniles and later assigned to a red wolf parent. Three hybrid litters fit this scenario; thus, the exact size of those litters could not be determined.

2.2. Location

The adaptive management plan divided the peninsula into three zones with different management goals (Stoskopf et al., 2005) (Fig. 1). Zone 1, the easternmost portion of the peninsula, serves as the core red wolf population and coyotes and hybrids captured in this area are euthanized. In Zone 2, directly west of Zone 1, hybrid individuals are euthanized but coyotes are sterilized under the hypothesis that sterile individuals would serve as territorial placeholders that discourage undetected coyotes from dispersing into the peninsula (Bartel and Rabon, 2013). Zone 3 is the furthest west section and falls at the junction of the peninsula and the mainland. Management practices in Zone 3 vary, but many sections of this area are managed similarly to Zone 2. This entire region has been designated as the Red Wolf Experimental Population Area (RWEPA).

We classified each hybrid and red wolf litter to a Zone based upon where it was detected (Zone 1, 2, or 3) and used a χ^2-squared test of independence to evaluate the distribution of each type of litter across all three zones. As noted, some hybrids were discovered as adults. In these situations, once the red wolf parent was identified via genetic testing we assigned the location of these litters according to the home range of that red wolf during the prior breeding season.

2.3. Breeder experience

To examine the impact of breeder experience on hybridization we compared both the age and prior breeding experience of red wolves that produced hybrid and red wolf litters. For breeding experience we classified each litter according to whether it was produced by a first-time breeder or an experienced breeder. This was only performed for females since the sample size of male red wolves was low (see Section 3.1). We defined first-time breeder as any individual producing its first known litter of pups, regardless of whether it was a hybrid or red wolf litter. An individual was considered an experienced breeder once it had produced a second litter. We compared the proportion of total red wolf and hybrid litters that were born to experienced breeders using a χ^2