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Original Article

Diameter Thresholds for Distinguishing Between Red Wolf and Other Canid Scat

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ABSTRACT Differentiation between scats of sympatric canid species is important for determining species-specific presence and movements, but distinction in the field is difficult. We calculated upper and lower thresholds of scat diameters to distinguish between scats of red wolves (*Canis rufus*) and scats of coyotes (*C. latrans*) and coyote–wolf hybrids in the field, in North Carolina, USA, from February 2009 to March 2010. We used DNA genotyping to identify scats collected in the field and took diameter measurements of those scats. Based on normal-distribution probability functions of scat diameters, scats $\geq$29 mm in diameter were $\geq$95% certain to be of red wolf origin. Conversely, scats $\leq$14 mm in diameter were 95% certain to be of coyote or hybrid origin. Scats $>$14 mm and $<$29 mm in diameter could not be identified by diameter alone. We suggest these upper and lower thresholds of scat diameters be used in concert with other methods (e.g., DNA genotyping) to monitor for red wolf, coyote, and hybrid activity to help conserve a lone, free-ranging population of wild red wolves. © 2011 The Wildlife Society.

KEY WORDS *Canis latrans*, *Canis rufus*, coyote, DNA genotyping, hybrid, red wolf, scat.

Since 1987, the U.S. Fish and Wildlife Service has managed the only free-ranging population of red wolves (*Canis rufus*) in the 6,650-km^2 Red Wolf Recovery Experimental Population Area (RWREPA) on the Albemarle Peninsula in North Carolina, USA. A major threat to this endangered species in the wild is hybridization with coyotes (*C. latrans*; Phillips et al. 2003). Biologists routinely monitor location and movement of packs of red wolves within the recovery area, as well as co-occurring coyotes, to attempt to reduce hybridization between the 2 canids.

Current monitoring techniques include tracking animals fitted with Global Positioning System and very high frequency collars and identification of scats using fecal DNA genotyping methods (Adams and Waits 2007, Chadwick et al. 2010). Although fecal DNA genotyping is a generally reliable method, it has some drawbacks (e.g., high cost [approx. US$60/sample], taking several months to conduct genetic testing to determine species of origin of scats, and a requirement of high-quality DNA, typically from fresh scats; Adams et al. 2003). Direct identification of scats in the field would aid in monitoring presence and movement of red wolves across the RWREPA, but criteria to distinguish scats of red wolves from scats of coyotes and coyote–wolf hybrids are not available. Herein, we describe guidelines for distinguishing scats of coyotes and hybrids from red wolves based on scat morphology.

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STUDY AREA

The RWREPA was comprised of $>$6,650 km^2 of federal, state, and private lands in 5 counties (Beaufort, Dare, Hyde, Tyrrell, and Washington) on the Albemarle Peninsula in North Carolina. Federal lands included Alligator River National Wildlife Refuge, Pocosin Lakes National Wildlife Refuge, and the United States Navy and Air Force Dare County bombing range. State lands included numerous state-owned hunting and fishing areas, while private lands were primarily pine plantations and agricultural fields. Types of land cover and approximate percentage of area were agricultural fields (30%); commercial pine (*Pinus* spp.) plantations (15%); pocosin (15%; *Pinus serotina* and *Persea palustris*); nonriverine swamp forests (10%; *Nyssa* spp., *Liquidambar styraciflua*, *Acer rubrum*, and *Chamaecyparis thyoides*); saltwater marsh or open water (10%); and other types of land cover (10%). Climate was characterized by 4 full seasons of nearly equal length with annual precipitation averaging 127 cm. Temperatures averaged 5° C in winter and 27° C in summer. Elevation was from sea level to 50 m (Beck et al. 2009). Potential prey included white-tailed deer (*Odocoileus virginianus*), rabbits (*Sylvilagus floridanus*, *S. palustris*), raccoons (*Procyon lotor*), feral hogs (*Sus scrofa*), nutria (*Myocastor coypus*), muskrats (*Ondatra zibethicus*),

small rodents (*Sigmodon hispidus*, *Mus musculus*, *Oryzomys palustris*, and *Reithrodontomys humulis*), and ground-dwelling birds (*Colinus virginianus* and *Meleagris gallopavo*; Phillips et al. 2003). Co-occurring carnivores included gray foxes (*Urocyon cinereoargenteus*), red foxes (*Vulpes vulpes*), red wolves, coyotes, coyote–red wolf hybrids, feral dogs, bobcats (*Lynx rufus*), and American black bears (*Ursus americanus*).

METHODS

During February 2009–March 2010, we collected scats of canids by systematically traveling game trails and unpaved roads within the RWREPA at least once per month (Fig. 1). We measured maximum diameter of scats at the widest point to the nearest 1 mm using calipers. Following measurements, fecal matter was removed from each scat and stored in a buffer solution for DNA genotyping (Adams et al. 2003). We attempted to identify all scats using fecal DNA genotyping. We identified fecal matter to species following the methods of Adams and Waits (2007) and Adams et al. (2003), in which 2 polymerase chain reaction (PCR) tests were performed and the same 9 microsatellite loci were amplified for both tests (Ostrander et al. 1993, Holmes et al. 1995, Mellersh et al. 1997). Scats that failed to amplify at $\geq$5 loci were removed from further analysis. Genotypes of scats that amplified at $\geq$5 loci for the 2 PCRs combined were compared to genotypes of known red wolves and coyotes within the RWREPA (Adams and Waits 2007). Scats with genotypes not matching those of known individuals, we analyzed in Program Structure 2.3.3 (Pritchard et al. 2000). Scats with genotypes not matching those of known individuals but having $\geq$85% probability of being red wolf or coyote based on Program Structure 2.3.3, we labeled accordingly; otherwise, we labeled the scats as hybrid (Pritchard et al. 2000). Once fecal DNA genotyping was complete, all comparative analyses involved 2 groupings: 1) scats of red wolves, and 2) scats of coyotes and hybrids combined.

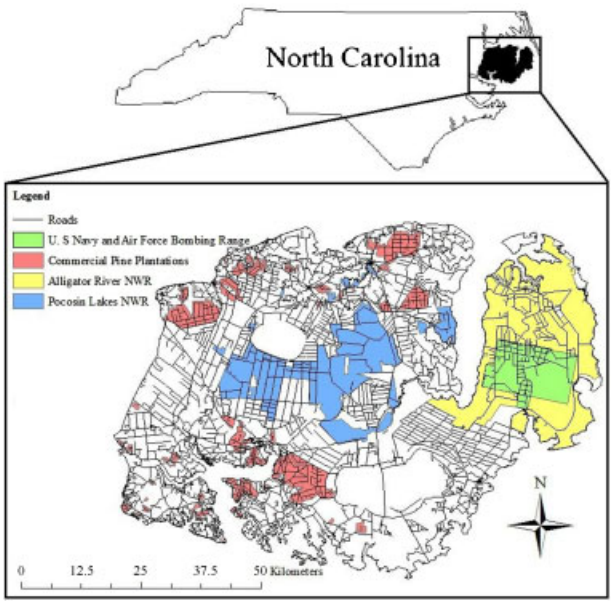

Figure 1. Landownership in the Red Wolf Recovery Experimental Population Area in northeastern North Carolina, USA (2009–2010).

Because items in scats could potentially influence scat diameters, we determined composition of scats. We individually washed scats and dried them for 48 hr, and we identified food items using reference keys. We used percent frequency of occurrence to determine contribution of prey items to scats (Ciucci et al. 1996). Scats containing >1 prey item, we listed as containing only the prey item representing the majority of the scat. In all cases, prey items representing the majority of the scat accounted for the majority of the mass. An Anderson–Darling test for normality demonstrated that diameters of scats grouped by prey item were not normally distributed ($P < 0.05$); furthermore, sample sizes were unequal. Thus, we used a Kruskal–Wallis test to assess the influence of prey items in scats on diameters of scats of red wolves and scats of coyotes and hybrids.

An Anderson–Darling test for normality demonstrated that diameters of scats grouped by species of origin were not normally distributed ($P < 0.05$), furthermore sample sizes were unequal. Thus, we used a Mann–Whitney *U*-test to determine whether diameters of scats of red wolves and scats of coyotes and hybrids differed. We constructed normal-distribution probability functions to estimate an upper threshold in diameter of scats of coyotes and hybrids, above which one could be 95% certain scats greater than or equal to this diameter were not of coyote or hybrid origin. Similarly, we used normal-distribution probability functions to estimate a lower threshold in diameter of scats of red wolves, below which one could be 95% certain scats less than or equal to this diameter were not of red wolf origin. All normal-distribution probability functions were based on mean and standard deviation of scats of interest (i.e., diam of scats of coyotes and hybrids for upper threshold and diam of scats of red wolves for lower threshold).

RESULTS

Of 1,377 scats collected, we identified 254 (18.4%) as red wolf, 57 (4.1%) as coyote, and 54 (3.9%) as hybrid using fecal DNA genotyping. We were unable to identify the remaining scats using fecal DNA genotyping due to low quality of DNA of some scats, which is a result of weathering of DNA. Diameters of scats of the 2 groups overlapped considerably (Fig. 2). Mean (±1 SD) maximum diameter of scats of coyotes and hybrids was 19 ± 6 mm (range = 10–35 mm). Mean (±1 SD) maximum diameter of scats of red wolves was 24 ± 6 mm (range = 10–43 mm). Median diameters of scats of red wolves (24 mm) and scats of coyotes and hybrids (19 mm) were different ($P < 0.01$).

Analyses of scats of red wolves revealed 7 prey groups (Table 1). The dominant prey item in scats had no effect on median diameters of red wolf scats ($P = 0.28$) or median diameters of coyote and hybrid scats ($P = 0.32$).

Normal-distribution probability functions resulted in upper and lower 95% certainty thresholds of 29 mm and 14 mm, respectively. Scats within the RWREPA $\geq$29 mm in diameter are 95% certain not to be of coyote

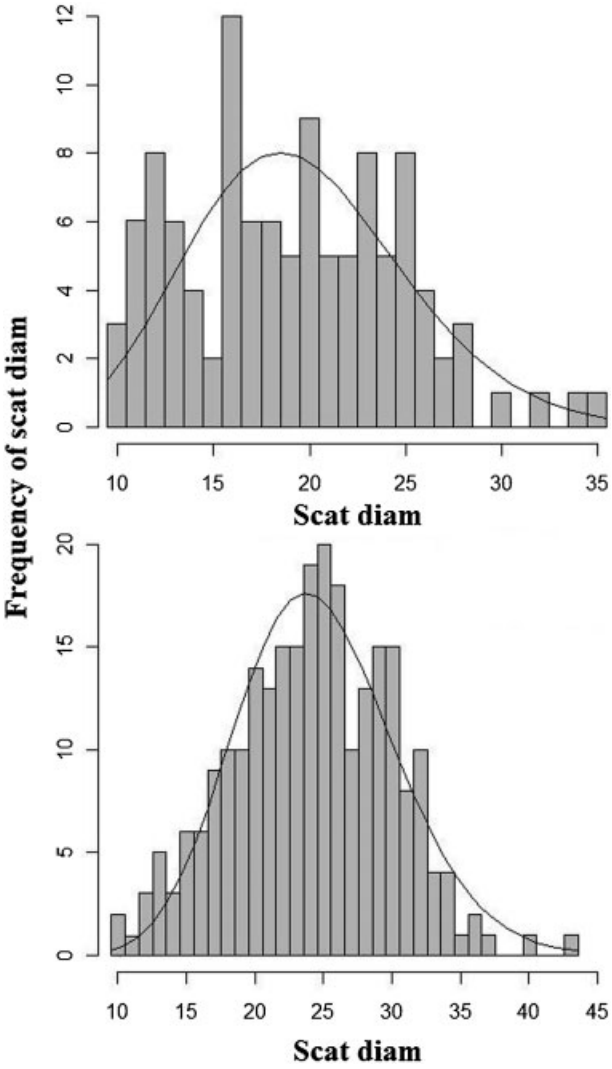

Figure 2. Diameters (mm) of coyote and hybrid scats (top; $n = 111$) and red wolf scats (bottom; $n = 254$) in the Red Wolf Recovery Experimental Population Area in northeastern North Carolina, USA (2009–2010).

or hybrid origin. Conversely, scats within the RWREPA ≤14 mm in diameter are 95% certain not to be of red wolf origin. Scats with diameters >14 mm and <29 mm could not be assigned based on diameter alone.

Four percent of scats of coyotes and hybrids were equal to or exceeded the separation point of 29 mm established using normal-distribution probability functions. The largest diameter for scat of a coyote or hybrid was 35 mm. Conversely, 24% of scats of red wolves in our study were equal to or exceeded this same separation point. Five percent of scats of red wolves were equal to or less than the separation point of 14 mm established using normal-distribution probability functions. The smallest diameter for scat of a red wolf, at 10 mm, was equal to the smallest diameter for scat of a coyote or hybrid. Conversely, 24% of scats of coyotes and hybrids were ≤14 mm.

DISCUSSION

Scat diameters and ranges from our study were similar to those of Weaver and Fritts (1979), who reported mean diameters of 21 mm and 27 mm (range = 7–34 and 13–47 mm) for coyotes and gray wolves (*C. lupus*), respectively. Also, diameters of scats and ranges were similar to those of Reed (2004), who reported mean diameters of 23 mm and 26 mm (range = 17–28 and 16–36 mm) for coyotes and Mexican gray wolves (*C. l. baileyi*), respectively. Our results agree with Weaver and Fritts (1979) that the dominant prey item has no effect on median diameters of scats of large canids. Diameters and ranges from these studies have been accepted and used to study and compare diets and movements of both Mexican and gray wolves with those of coyotes where they co-occur (Arjo et al. 2002, Carrera et al. 2008). Thus, we suggest diameters and ranges from our study are acceptable standards for distinction between coyote and red wolf scats where they co-occur.

Domestic dogs are present in the RWREPA, but in low numbers, and they experience low survival (C. Lucash, United States Fish and Wildlife Service, personal communication). Thus, canid scats ≥29 mm in diameter are likely red wolf. We suggest 29 mm as an upper threshold for distinguishing scats of red wolves from scats of coyotes, hybrids, and smaller canids (e.g., red foxes and gray foxes) within the RWREPA. We suggest DNA genotyping need not be used to identify scats of red wolves when the diameter is ≤14 mm or ≥29 mm.

Table 1. Diameters of scats of red wolves and scats of coyotes and hybrids grouped by primary prey found in scats collected within the Red Wolf Recovery Experimental Population Area, North Carolina, from 2009 to 2010. *N* = number of scats with corresponding prey as primary prey item. *M* = median diameter of scats with corresponding primary prey item.

	Red wolf		Coyote and hybrid	
Prey item	***N***	**M (mm)**	***N***	**M (mm)**
White-tailed deer (*Odocoileus virginianus*)	97	25	36	20
Large rodent[a]	13	25	2	24
Small rodent[b]	32	23	22	20
Rabbit[c]	84	23	49	16
Feral and domestic hog	11	23	2	26
Raccoon (*Procyon lotor*)	12	28		n/a
Insect[d]	5	22		n/a

[a] Nutria (*Myocastor coypus*) and muskrat (*Ondatra zibethicus*).
[b] Primarily hispid cotton rat (*Sigmodon hispidus*) and house mouse (*Mus musculus*).
[c] Marsh rabbit (*Sylvilagus palustris*) and eastern cottontail (*S. floridanus*).
[d] Primarily grasshoppers family Acrididae.

Use of these thresholds alone is likely to lead to considerable loss of information due to exclusion of scats of red wolves <29 mm in diameter. In this study, 76% of red wolf scats collected could not be distinguished from coyote and hybrid scats based on diameter. Similarly, 76% of coyote and hybrid scats collected could not be distinguished from red wolf scats based on diameter. Scats of canids with diameters of 15–28 mm will not be identifiable based on diameter alone, so other techniques such as DNA genotyping will be required (Adams et al. 2003, Adams and Waits 2007). Co-occurrence of scats ≥29 mm in diameter and scats <29 mm in diameter could represent the pairing of a red wolf with a coyote or hybrid, different-sized scats from the same red wolf or pack of red wolves, or a transient coyote or hybrid.

Though the above thresholds only appear to allow for identification of approximately 25% of red wolf scats and coyote and hybrid scats in the RWREPA, this cost-effective monitoring alternative translates into a savings of US$1,500 for every 100 scats sampled at present cost for analyses (US$60/sample). Although diameters of scats can be influenced by environmental factors, we feel that the simplicity of this method coupled with financial savings facilitates its use. Fecal DNA genotyping is precise, but requires fresh scats, with high-quality DNA, costly equipment, training to use the equipment, and an advanced understanding of genetics (Adams et al. 2003). Use of scat diameters to identify scats would be most beneficial to studies with low budgets and interested in monitoring the distribution of a species at the population level, while fecal DNA genotyping would be most beneficial to studies wanting to monitor and distinguish individuals within a population.

Though the above thresholds are only immediately applicable to biologists in and around the RWREPA, it is important to realize that the methodology is applicable to other species: for example, distinguishing scats of endangered Canada lynx (*Lynx canadensis*) from those of bobcats, or scats of grizzly bears (*U. arctos*) from those of American black bears. This method could allow biologists to rapidly and cost-effectively monitor the distribution and location of a number of rare and endangered species. However, data sets used to develop diameter thresholds of scats for distinguishing among co-occurring species should be as large as is feasibly possible to develop robust thresholds. Failing to do so could result in thresholds that are poor at discriminating scats of co-occurring species and could lead to misinterpreting the location or distribution of the species of interest. For example, misidentification of a coyote scat in the RWREPA as a red wolf scat could result in the managers' assuming the existence of a red wolf territory, when, in reality, the territory is occupied by a coyote. This individual then has the potential to mate with a red wolf, resulting in a hybrid offspring, which is the primary threat to the existence of the red wolf (Adams et al. 2003).

MANAGEMENT IMPLICATIONS

Biologists routinely monitor location and movement of packs of red wolves within the recovery area, as well as co-occurring coyotes, to attempt to reduce hybridization between the 2 canids. Effective restoration and management of the only free-ranging population of red wolves requires biologists to have access to, and knowledge of, fast and efficient field-identification techniques. Rapid identification of scats of red wolves from scats of coyotes, coyote–wolf hybrids, and smaller canids, based on diameters of scats provides a cost-effective alternative to DNA genotyping for monitoring movements of red wolves and co-occurring canids. However, DNA genotyping is an important method for distinguishing between red wolf scats and coyote and hybrid scats and will likely be required to identify approximately 75% of canid scats collected in the RWREPA. Use of such field-identification techniques, whether based on diameters of scats or other metrics of identification (e.g., mass of scats or size of tracks), is easily adapted to other situations of management concern and would be useful elsewhere to rapidly and cost-effectively monitor the distribution and location of a number of rare and endangered species.

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