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Investigation of captive red wolf ejaculate characteristics in relation to age and inbreeding

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ABSTRACT

An evaluation of a large database of red wolf fresh ejaculate characteristics (n = 427 ejaculates from 64 wolves) was undertaken to increase knowledge of seminal characteristics in the red wolf and evaluate possible relationships between inbreeding, age, and seminal quality. Phase microscopy analysis of electroejaculates collected over 14 natural breeding seasons was compared with animal ages and inbreeding coefficients. Ejaculate volume increased and sperm concentration and total count decreased as wolves aged ($P < 0.01$, 0.001, and 0.05, respectively), and the proportion of sperm cell morphological abnormalities was greater in animals with higher coefficients of inbreeding ($P < 0.001$), particularly for older animals ($P < 0.001$). Moreover, the mean coefficient of inbreeding of animals that had failed to reproduce given at least one opportunity during their lifetimes was significantly greater than that of wolves with proven fertility, and wolves of proven fertility exhibited higher sperm concentrations and total counts than nonproven wolves. Thus, as the captive red wolf population becomes more inbred, the maximum age of reproduction is likely to decrease; an important finding to consider when projecting population dynamics and determining pairing recommendations.

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1. Introduction

The red wolf once ranged throughout the south-eastern United States and possibly as far north as Maine [1,2]. However, numerous factors including private and government-based persecution, habitat loss, and hybridization with the coyote (*Canis latrans*) combined to cause a severe and rapid decline in the red wolf population during the 19th and 20th centuries. Drainage of marshlands and clearing of forests for agriculture and oil exploration reduced and fragmented the available habitat for red wolves and their primary prey species [2–4]. At the same time, urbanization created a niche suitable for the coyote [2]. The coyote was able to significantly expand its range eastward [2,5,6], whereas fragmentation of the red wolf population into ecologically isolated patches compromised their ability to disperse and locate appropriate mates, leading to the occurrence of interspecific breeding in areas in which red wolves and coyotes cohabitated [3,7,8]. Owing to the morphological similarity between the red wolf and coyote and the fact that coyotes were not historically known in the region [2,9], coyotes and hybrids were often misidentified as red wolves [2], and the decline of red wolves went virtually unnoticed until the species was facing extinction. Finally, in the late 1960s, the rarity of the red wolf was recognized, and it was listed as endangered [10,11].

Once it became clear that red wolves were a minority within their range relative to coyotes, and that the pressures of habitat loss, hybridization, and local antiwolf sentiment were not solvable in the near term, efforts at

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preserving the species in the wild were abandoned in favour of planned extirpation with the long-term goal of reestablishing the species in protected portions of its historical range [12]. Of over 400 animals evaluated, 43 met the morphological standards to be considered nonhybrids. Of those, ultimately only 14 became founders for the captive breeding program [12–16]. Because the founder group for the extant red wolf population is small, and because the zoo-based population has been skewed toward older animals [17], there is a need to understand the implications of both inbreeding and age on reproductive success in this species to make optimal breeding decisions for the future of the species. Currently, the captive red wolf population is managed through a zoo-based Species Survival Plan (SSP) in combination with the Red Wolf Recovery Plan, administered by the United States Fish and Wildlife Service.

Little information exists on the specific effects of aging and inbreeding on ejaculate characteristics and/or sperm quality in wild canids. There have been two previous reports of seminal traits in the red wolf (*Canis rufus*) [18,19]. However, these studies considered relatively small numbers of animals sampled over one and two breeding seasons, respectively. The semen parameters and sperm characteristics for the red wolf reported in these studies, while comparable to other canids, tended to be on the extreme ends of the canid spectrum and exhibited a high range of variability both within and among wolves. The significance of these findings in regard to red wolf fertility has not been established.

An evaluation of a large database of red wolf fresh ejaculate characteristics compiled over a 14 year span and including multiple samples from individual wolves, was undertaken. The objective of this study was to improve and build upon current knowledge of seminal characteristics in the red wolf and evaluate possible relationships between fertility, age, inbreeding, and seminal quality.

2. Materials and methods

2.1. Animals

Adult, male red wolves (*C. rufus*; n = 64) were maintained at 10 facilities in various geographical locations across the United States. All housing facilities adhered to husbandry protocols set by the Red Wolf SSP. Wolves ranged in age from 1 to 14 years. All wolves were housed singly, in conspecific pairs, or in family units. Animals were exposed to natural photoperiod and housed in pens that contained natural substrate, foliage, and sheltered dens with bedding material. Wolves were fed a commercially available dry dog food daily and provided water *ad libitum*. Animals that sired at least one litter during their lifetimes were considered to be of proven fertility, whereas males that did not produce a litter given at least one opportunity to breed during their lifetime (i.e. housed with a female conspecific for breeding purposes for the duration of one or more natural breeding seasons) were considered nonproven. Eight of the 64 study animals were of indeterminate fertility (i.e. unpaired) and as such were excluded from fertility-based analyses.

2.2. Semen collection and evaluation

Semen collection was performed during 14 natural breeding seasons; from mid December until early April 1990 to 2004. One to 10 collections were performed per animal per year, for a total of 427 ejaculates from 64 animals. Wherever possible, care was taken to ensure that collections were not aligned with mating events. Consistency in collection procedures and evaluation parameters was assured in that all technicians were trained by the same individual and used standardized operating procedures and a specific data collection form designed for this study.

Wolves were fasted 1 day before collection. On the day of collection, animals were anesthetized using Telazol (teletamine hycrochloride and zolazepam; 6.5 mg kg^{-1}) administered by hand syringe. Before semen collection, the penis was cleaned and the bladder drained of urine *via* catheterization with a five Fr, 55.8-cm long polypropylene catheter (Sherwood Medical, St. Louis, MO, USA). Electroejaculation was performed using a PT Electronics model 302 ejaculator (no. 4 probe: 1.6-cm diameter; Boring, OR, USA). Using previously described methods [18,20,21], ejaculation was achieved through a set of three to five stimulation series, each consisting of multiple on-off stimuli in increasing voltages ranging from 3 to 8 volts. A rest period of 5 to 7 minutes was allowed between each series. Semen was collected into plastic containers.

For each ejaculate, fresh semen from all series was pooled and the total volume, pH, concentration, and percent motile cells were determined using previously described methods for this species [18,20,21]. Specifically, concentration was measured and percent motile cells estimated using a hemocytometer [18,20,21]. The forward progressive status of motile cells was rated on a scale from 0 to 5 (0 = no motility, 1 = side-to-side flipping without forward progression, 2 = slow meandering progression, 3 = moderate meandering progression, 4 = moderate linear progression, 5 = rapid linear progression). To assess morphology and evaluate acrosome integrity, aliquots of 5 μL of each ejaculate were smeared on separate, clean glass slides, and allowed to dry before fixation in methyl alcohol for 60 seconds. Beginning in 1993, fixed slides were stained with Spermac (FertiPro, Belgium; supplied by Meditech first Canada, Inc.) and examined using phase microscopy as described by Goodrowe et al. [18]. A total of 300 spermatozoa from each ejaculate were evaluated, and the percentages of normal spermatozoa, each abnormality type, and spermatozoa with intact, partial, and missing acrosomes were determined. Intact acrosomes were determined by a uniform blue color in the distal portion of the sperm head, whereas the postacrosomal region was stained pink, as described by Goodrowe et al. [22]. Partial acrosomes were identified as those in which the blue stain in the distal portion of the sperm head was disrupted or irregular in appearance. Missing acrosomes were identified by blue color in the equatorial region only, or by even pink color in the acrosomal region. Morphological abnormalities were categorized as those involving the head, midpiece, or flagellum. Neither urine-contaminated nor aspermic samples were included for analysis.

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