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Evaluation of a Formula that Categorizes Female Gray Wolf Breeding Status by Nipple Size

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Abstract - The proportion by age class of wild *Canis lupus* (Gray Wolf) females that reproduce in any given year remains unclear; thus, we evaluated the applicability to our long-term (1972–2013) data set of the Mech et al. (1993) formula that categorizes female Gray Wolf breeding status by nipple size and time of year. We used the formula to classify Gray Wolves from 68 capture events into 4 categories (yearling, adult non-breeder, former breeder, current breeder). To address issues with small sample size and variance, we created an ambiguity index to allow some Gray Wolves to be classed into 2 categories. We classified 20 nipple measurements ambiguously: 16 current or former breeder, 3 former or adult non-breeder, and 1 yearling or adult non-breeder. The formula unambiguously classified 48 (71%) of the nipple measurements; based on supplemental field evidence, at least 5 (10%) of these were incorrect. When used in conjunction with an ambiguity index we developed and with corrections made for classifications involving very large nipples, and supplemented with available field evidence, the Mech et al. (1993) formula provided reasonably reliable classification of breeding status in wild female Gray Wolves.

Introduction

Some important areas regarding reproduction in wild female *Canis lupus* L. (Gray Wolf, herefter Wolf) are not yet fully understood. Although some female Wolves breed at 1-y old (Medjo and Mech 1976, Seal et al. 1979, Sidorovich and Rotenko 2014), some wild Wolves do not breed until they are 4–5 years old (Mech and Seal 1987, Mech et al. 1998). The proportion of females in a population that breed in any given year is unknown, but this metric is important because it is fundamental to understanding Wolf population dynamics, is useful for modeling Wolf populations (Miller and Lacey 2005), and it is required for calculation of generation time in Wolf genetic research.

We were interested in whether an equation from an earlier study (Mech et al. 1993) designed to categorize female Wolf breeding status based on nipple sizes of captive Wolves, could be applied to our long-term data set and enable us to determine the proportion of female breeders in any year. Mech et al. (1993) recorded nipple measurements of 29 captive Wolves with known breeding histories every 2 weeks and categorized them into 4 breeding classes (yearling, adult non-breeder, former breeder, and current breeder). The mean and standard deviation of nipple sizes were calculated for each category every 2 weeks with sample sizes ranging

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from 4 to 17 for each category each period. The researchers then developed the following classification formula based on nipple size and time of year:

$$t_s = (Y_1 - Y_2)/(s_2 \sqrt{[n_2+1]/n_2}),$$

Where, Y_1 = length + width nipple measurement from Wolf, Y_2 = sample mean from appropriate period, s_2 = sample deviation from period, n_2 = sample size, t_s = probability of nipple measurement under H_o: same population, and $n_2 - 1$ = degrees of freedom. We applied this formula to wild Wolves captured in the Superior National Forest in Minnesota, to determine the formula's reliability and applicability. Mech (2006) conducted a preliminary study of Wolf breeding status for various ages, but that investigation involved only 23 Wolves distributed over ages 2–12 y and did not directly apply the Mech et al. (1993) classifying formula (hereafter, the formula) as we did in this study.

Methods

Our study area covered 2060-km^2 in the Superior National Forest, MN, (48°N, 92°W; see Heinselman 1993 and Nelson and Mech 1981). As part of a long-term study of Wolves and *Odocoileus virginianus* Zimmermann (White-tailed Deer; Mech 2009), we captured wild Wolves with modified foot-hold traps (either Newhouse 14 or EZ Grip 7, Rancher's Supply, Alpine TX) from 1972 to 2013 (Mech 2009) and followed guidelines of the American Society of Mammalogists (Gannon and Sikes 2007) during capture and processing (see Barber-Meyer and Mech 2014 for details). We anesthetised all adult trapped Wolves (all non-pups) with a standard dose of 250 mg ketamine (Ketaset1, ketamine hydrochloride; Fort Dodge Animal Health, Fort Dodge, IA; 1988–1991) or 286mg telazol (R) (tiletamine hydrochloride and zolazepam hydrochloride; Pfizer, New York, NY, and Fort Dodge Animal Health; 1992–2011) and 37 mg xylazine (Anased(R); Llyod Laboratories, Shenandoah, IA) given intramuscularly via a pole syringe (approximate length 4 feet [1.2 m]). An additional 100–200 mg of ketamine was handinjected intramuscularly if required. We recorded standard morphological measurements, collected specimens such as blood (0–28 cubic centimeter [cc]) and sometimes scat and hair, and applied ear tags (jumbo rototag, Nasco, Fort Atkinson, WI; or sheep/goat tag, Destron Fearing, Farmington, MN) and a VHF radiocollar (Telonics, Inc., Mesa, AZ). Beginning in 2000, we used tooth wear to estimate Wolf age by comparing our samples with the chart in Gipson et al. (2000). Prior to 2000, unless the Wolf was of known age (i.e., captured as a pup), we assigned a known-minimum age of 1 year and updated the age if a Wolf was recaptured. We administered antibiotics and an antagonist to the anesthetic. We handled Wolves for ~1 h in total.

We excluded yearlings and pups from our analyses because it is not common for Wolves <2 y old to breed. We classified Wolves with inconspicuous nipples as adult non-breeders. We entered the observed nipple size for all measured Wolves into the formula and added values corresponding to the appropriate period. We calculated *t*-values for each of the 4 potential breeder categories and then used these values in Arc version 1.06 (Cook and Weisberg 1999) to generate a 2-tailed probability (as

detailed in Mech et al. 1993) that the observed nipple size belonged to the same statistical population as each Mech et al. (1993) category. We classified the Wolf in the category with the highest probability among all categories. We encountered some problems when the second highest probability was very close to that of the assigned category. Therefore, we created an ambiguity index for each Wolf by summing all the resulting probabilities and determining what fraction of the total was contributed by each category (contribution fractions summed to 1). If the difference between the contribution fraction from the selected category and the next highest probability category was less than an arbitrarily chosen value of 0.20, we categorized the Wolf as potentially in either category (e.g., current or former breeder, former or adult non-breeder, and yearling or adult non-breeder).

Results

We classified 86 individual Wolves 2 y and older into breeding categories based on their nipple sizes. Sixty-eight capture events included Wolves with conspicuous nipples that we measured and classified according to the formula. Of these 68, we considered 20 (29%) to be ambiguous classifications (1 was adult non-breeder or yearling, 3 were former breeders or adult non-breeders, and 16 were current or former breeders). For example, one ambiguous classification was a 3-y-old, Wolf (#7057) with a nipple size of 1.0 cm measured on 25 August 2008. For this period, the Mech et al. (1993) mean nipple size for adult non-breeders was 0.85 cm (SD = 0.16, n = 17) and mean nipple size for former breeders was 1.45 cm (SD = 0.33, n = 6). When we applied the formula and ambiguity index, we classified Wolf #7057 ambiguously as an adult non-breeder (unscaled probability of belonging to that category = 0.375) or former breeder (unscaled probability of belonging to that category = 0.262) because the difference between these top 2 categories in scaled contributing probabilities was 0.145 (less than the 0.20 cutoff). We excluded known yearlings from our analysis; thus, we concluded Wolf #7057 was an adult non-breeder. Three Wolves classified as former or adult non-breeders. In 1 case, we had information from a previous capture to conclude that the Wolf was actually a former breeder. Of the 16 animals that were classified as current or former breeders using the formula and ambiguity index, there were 2 cases in which current breeders were erroneously classed ambiguously because they had large nipples, 1 case where pups were observed (and the female was estimated at 4-y old), and 1 case where we concluded that the 3-y old female was likely a current breeder because radio telemetry and visual observations indicated that she was alone with her mate the previous winter, she showed restricted movements based on her 3 locations during the beginning of the denning period (suggesting she denned) before her radio-signal ceased, and because her pack totaled at least 5 Wolves the following November (suggesting successful reproduction).

Of the 68 classifications based on conspicuous nipple measurements, 48 (71%) were unambiguous. Of the unambiguous classifications, at least 5 (10%) were likely erroneous. Three of these were due to sample-size and rank-order issues. Specifically, at 5-y, nipple size of Wolf #979 was 2.3 cm on 28 August 28 2005.

For this period, the Mech et al. (1993) mean nipple size for current breeders was 1.52 cm (SD = 0.34, n = 14) and mean nipple size for former breeders was 1.45 cm (SD = 0.53, n = 4). A larger sample and smaller SD of current breeders (relatively narrower confidence interval) for this date resulted in Wolf #979 being categorized erroneously as a former breeder (relatively wider confidence interval). Similarly, 2 other Wolves that were current breeders with larger nipple sizes were erroneously classed as former breeders.

The remaining 2 incorrect unambiguous classifications included Wolves with relatively smaller nipples that were classed as former breeders but were lactating. For categorization, we considered lactating Wolves to be current breeders, but both of these Wolves could have been pseudopregnant (Jochle 1977, Seal et al. 1979; but see also Mech and Seal 1987).

We did not detect any other inconsistencies in the rest of the classifications as compared to available supplemental field evidence (e.g., observed pups, female lactating, female localizing during denning and rendezvous seasons, female the only known adult female in the pack, etc.).

Discussion

In general, we determined that in wild Wolves, the formula reliably separated yearlings and adult non-breeders from current breeders. It did not perform well at separating current and former breeders, and also, to a lesser extent, former from adult non-breeders. These problems were due to several factors: small samples in the captive wolves used for comparison, the formula's inability to account for the order of nipple-sizes among categories (i.e., nipple sizes should generally be yearling < adult non-breeder < former breeder < current breeder; thus, Wolves with very large nipples should be placed in the current breeder category), inadequate information on nipple regression, and individual variation in Wolves.

To increase the applicability of the formula, additional information is needed on how quickly a former breeder's nipple measurements return to the size-range of an adult non-breeder. Four Wolves (ages 6, 7, 9, and 9) had inconspicuous nipples (L.D. Mech and S.M. Barber-Meyer, unpubl. data). This finding raises the questions: (1) were they incorrectly aged? (2) had they never bred? or (3) had they bred and their nipples regressed? Future research may help to answer these questions. We know that at least 1 of our Wolves had very small (0.6 cm) nipples at 7 y, but bred at least when she was 2-, 3-, and 5-y old (L.D. Mech and S.M. Barber-Meyer, unpubl. data). Results of studies of the mammary glands of domestic dogs would likely help us understand nipple size in wolves; however, such studies have not been conducted (Evans and de Lahunta 2013:399). Also, it would be useful to gather additional data from Wolves from other regions where body size differs from the Wolves we evaluated for this study to determine if the formula applies equally.

Some uncertainty in classifying Wolves will remain regardless of refinements to the formula and increases in the sample of known-breeding status wolf nipple sizes simply due to inherent variation among individual Wolves. As a supplement

to the classifications generated by the formula, researchers may wish to use available field evidence to class some females as current breeders if they are known to be the only adult female in their pack and have been observed with pups or had localizing movements during denning and rendezvous season, etc. Additionally, we recommend that during necropsies, researchers collect placental-scar information whenever possible along with nipple sizes for comparisons.

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Literature Cited

Barber-Meyer, S.M., and L.D. Mech. 2014. How hot is too hot? Live-trapped Gray Wolf rectal temperatures and 1-year survival. Wildlife Society Bulletin 38(4):767–772.

Cook, R.D., and S. Weisberg. 1999. Applied Regression Including Computing and Graphics. John Wiley, New York, NY. 368 pp.

Evans, H.E., and A. De Lahunta. 2013. Miller's Anatomy of the Dog. Elsevier Health Sciences, Waltham, MA. 872 pp.

Gannon, W.L., R.S. Sikes, and Animal Care and Use Committee of the American Society of Mammalogists. 2007. Guidelines of the American Society of Mammalogists for the use of wild mammals in research. Journal of Mammalogy 88:809–823.

Gipson, P.S., W.B. Ballard, R.M. Nowak, and L.D. Mech. 2000. Accuracy and precision of estimating age of Gray Wolves by tooth wear. Journal of Wildlife Management 64:752–758.

Heinselman, M. 1993. The Boundary Waters Wilderness Ecosystem. University of Minnesota Press, Minneapolis, MN. 334 pp.

Jochle, W. 1997. Review: Prolactin in canine and feline reproduction. Reproduction in Domestic Animals 32:183–193.

Mech, L.D. 2006. Age-related body mass and reproductive measurements of Gray Wolves in Minnesota. Journal of Mammalogy 87:80–84

Mech. L.D. 2009. Long-term research on wolves in the Superior National Forest. Pp. 15–34, *In* A.P. Wydeven, E.J. Heske, and T.R. Van Deelen (Eds.). Recovery of Gray Wolves in the Great Lakes Region of the United States: An Endangered Species Success Story. Springer, New York, NY. 350 pp.

Mech, L.D., and U.S. Seal. 1987. Premature reproductive activity in wild wolves. Journal of Mammalogy 68:871–873.

Mech, L.D., T.J. Meier, and U.S. Seal. 1993. Wolf nipple measurements as indices of age and breeding status. American Midland Naturalist 129:266–271.

Mech, L.D., L.G. Adams, T.J. Meier, J.W. Burch, and B.W. Dale. 1998. The Wolves of Denali. University of Minnesota Press, Minneapolis, MN. 227 pp.

Medjo, D., and L.D. Mech. 1976. Reproductive activity in nine- and ten-month-old wolves. Journal of Mammalogy 57(2):406–408.

Miller, P.S., and R.C. Lacy. 2005. VORTEX: A stochastic simulation of the extinction process. Version 9.50 User's Manual. Conservation Breeding Specialist Group (SSC/IUCN), Apple Valley, MN.

Nelson, M.E., and L.D. Mech. 1981. Deer social organization and wolf depredation in northeastern Minnesota. Wildlife Monographs 77.

Nelson, M.E., and L.D. Mech. 1986. Mortality of White-tailed Deer in northeastern Minnesota. Journal of Wildlife Management 50:691–698.

Seal, U.S., E.D. Plotka, J.M. Packard, and L.D. Mech. 1979. Endocrine correlates of reproduction in the wolf. Biology of Reproduction 21:1057–1066.

Sidorovich, V., and I. Rotenko. 2014. Abnormal features of the wolf population in Belarus: Pack multi-breeding, breeding of yearling females, and hybridization with stray dogs. Is this an influence of the rich prey-supply or low density of the wolf population that is affected by human persecution? Pp. 37–38, *In* European Large Carnivores: Problems of Small-sized Populations, Study on Reproduction, and Challenges of Reintroduction Programs. Abstracts and program of the International Scientific Conference in the Krasny Bor Terrain (Dobroplyosy Village, Verkhnedvinsk district, Belarus, 15–22 September 2014). NiktagraphicsPlus Publisher, Minsk, Belarus.