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Wolf Nipple Measurements as Indices of Age and Breeding Status

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ABSTRACT.—We measured nipple sizes of 29 captive wolves (*Canis lupus*), of known breeding histories, throughout the year and tested distinctions among various known breeding statuses of 20 wild wolves examined in northeastern Minnesota from May through September. For ca. 8 mo of the year only breeders and nonbreeders can be classified. Distinctions between current and former breeders were not reliable.

INTRODUCTION

In field studies involving live trapping of mammals it is valuable to be able to determine the age and breeding history or status of any animal handled. However, with wild wolves (*Canis lupus*) only two age classes can readily be recognized: individuals less than 6 mo of age and those older (Van Ballenberghe and Mech, 1975). Van Ballenberghe *et al.* (1975), Mech (1977) and Peterson *et al.* (1984) tried to draw inferences about the breeding status of individual wolves from the size and appearance of their nipples. However, no such information about female wolves of known age and breeding status has been available. Therefore, we present here seasonal measurements of the posterior-most inguinal nipples of captive wolves known to be yearlings, adult nonbreeders, former breeders, and current breeders. We then test the accuracy of using these measurements to assess the age and breeding history of wild wolves. Inguinal nipples were used because if any nipples are being suckled it is the posterior-most inguinals.

METHODS

The wolves were housed in enclosures N of the Twin Cities, Minnesota, and fed Purina Dog Chow supplemented with road-killed deer (*Odocoileus virginianus*) and Purvinal, a vitamin supplement (Seal *et al.*, 1979). Every other week from April 1977 through December 1983 the wolves were anesthetized, and the length and width of one posterior inguinal nipple from each wolf was measured with calipers. The animals were observed daily, and records were kept of their breeding status (Seal *et al.*, 1979; Packard *et al.*, 1983, 1985). A total of 29 wolves were measured over several years during which many changed in breeding status. Thus we have data from 10–24 nonbreeding yearlings (age based on known histories in captivity), 14–26 nonbreeding adults, 5–13 former breeders, and 5–13 current breeders per 2-wk period. Current breeders were individuals producing and nursing pups during

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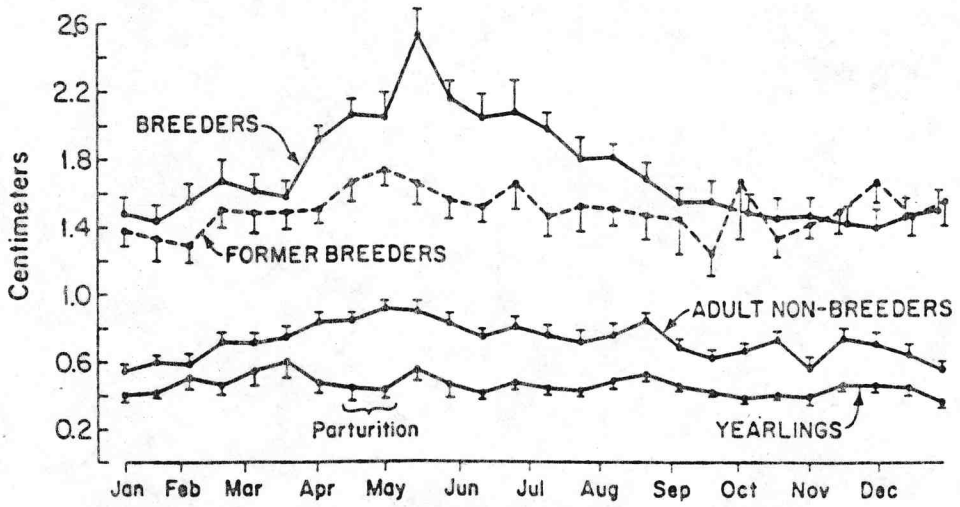

FIG. 1.—Seasonal changes (mean and SE) in length plus width of one posterior-most inguinal nipple on captive wolves of known age and breeding history

the year their measurements were tabulated, and former breeders were those that had produced one litter but not during the year they were measured. Statistical analyses included one-way and two-way analyses of variance (Sokal and Rohlf, 1981).

RESULTS

Nipple sizes of wolves in each category began increasing about February each year, peaked in April and May, and except for those of the former breeders, returned to their base sizes by September (Fig. 1). These changes are apparently mediated by the hormonal effects of pregnancy in breeding animals and pseudopregnancy in nonbreeders (Seal *et al.*, 1979). Pseudopregnancy is known to cause extensive mammary development in dogs (Forsyth and Hayden, 1977), and lactation has been observed in nonbreeding wolves housed with pups (Klinghammer, *vide* Allen, 1979). The dramatic increase in nipple sizes of breeding wolves after parturition is probably due to the mechanical stimulation of nursing.

The average nipple sizes of animals classified in the four reproductive categories were significantly different during the year, although their ranges overlapped (Table 1). This overlap precludes simple placement of an animal into a unique category based on an individual measurement. Grouping the animals as either nonbreeders or breeders (former or current) also yielded significant differences and with less overlap of the ranges (Table

TABLE 1.—Average nipple sizes for each breeding category of captive female wolves

Category	n	Mean	SE	SD	Range
Yearlings	331	0.43	0.010	0.18	0.2–1.1
Adult nonbreeder	419	0.73	0.012	0.25	0.3–1.3
Former breeder	188	1.44	0.026	0.36	0.7–2.2
Current breeder	330	1.69	0.028	0.51	0.8–3.2

$F = 956$ (df 3, 1264), $P < 0.0001$

TABLE 2.—Average nipple sizes for the combined breeders and former breeders compared with the combined yearlings and adult nonbreeders

Category	n	Mean	SE	SD	Range
Nonbreeders	750	0.60	0.009	0.26	0.2–1.3
Breeders	518	1.60	0.018	0.41	0.7–3.2

$F = 2274$ (df 1, 1266), $P \ll 0.0001$

Note: Comparisons of these combined groups for each biweekly set of data, by 2-way ANOVA, yielded significant differences for each of the 26 biweekly samples. The range of means for the nonbreeders was 0.44 to 0.72, and for the breeders the range was 1.34 to 2.12

2). The distribution of the values from the four reproductive categories into size ranges (Table 3) indicates that if 1.1 cm is taken as a boundary, then 2.4% of these nonbreeders would have been misclassified as breeders. Conversely, 13.2% of breeders would have been misclassified as nonbreeders. The distribution of such classification errors in an unknown population would depend upon the proportion of animals in each category and their probability of capture for measurement.

Analysis of the data pooled from the entire year neglects the possible contribution of changes during the year (Fig. 1), which might be expected to occur as a result of pregnancy and nursing. The ANOVAs comparing the four groups at each period were significant for all 26 periods. This reflects in part the distinct separation between breeders and nonbreeders (Fig. 1). The least significant difference (LSD) ($P < 0.01$) for comparing the means between groups at each period (Table 4) can be used to identify the significant between-mean differences.

Mean nipple size of yearling wolves did not increase significantly during the year (Table 4). Measurements from 21 yearlings ranged from 0.2 to 1.2 cm. However, 11 animals in this group had no values greater than 0.6 cm, and two animals accounted for 44 of the 54 measurements >0.6 in these yearlings. Nine of the other 11 values, distributed over eight yearlings, were 0.7 cm, and two were 0.8 cm. Thus all of the 29 values 0.9–1.1 cm were observed in two animals. The high values in these two animals were concentrated in March through June.

Mean nipple size of older wolves that had never bred increased gradually from March through May during the reproductive season and then declined. The least significant difference (LSD) ($P < 0.01$) for comparing the means within these adult nonbreeders between periods is 0.23. They were significantly larger on the average than in the yearlings during the reproductive season. Most of these animals ovulated and maintained an active corpus luteum for 60–90 days as measured by serum progesterone (Seal *et al.*, 1979).

TABLE 3.—Proportion of nipple measurements of each reproductive group in each of four nipple size classes

Size class	Yearlings (%)	Adult nonbreeder (%)	Former breeders (%)	Current breeders (%)
0.2–0.6	87.4	39.6	0	0
0.7–1.1	12.6	56.0	17.8	10.6
1.2–2.0	0	4.4	75.2	66.8
2.1+	0	0	7.0	22.6

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	Former breeders (%)	Current breeders (%)
	0	0
	17.8	10.6
	75.2	66.8
	7.0	22.6

TABLE 4.—Mean nipple sizes measured at 2-wk intervals from January through December of each year of captive female wolves classified as yearling, adult nonbreeder, former breeders and breeding wolves during each year

	Yearling			Adult nonbreeders			Former breeders			Current breeders			
Date	$\bar{x}$	SD	n	$\bar{x}$	SD	n	$\bar{x}$	SD	n	$\bar{x}$	SD	n	$LSD_{0.01}$
1	0.37	0.07	7	0.54	0.19	18	1.32	0.32	9	1.45	0.32	12	0.24
2	0.36	0.07	8	0.58	0.21	19	1.25	0.44	10	1.42	0.33	12	0.32
3	0.45	0.22	8	0.57	0.28	17	1.25	0.34	10	1.45	0.37	12	0.32
4	0.41	0.20	11	0.73	0.24	18	1.45	0.40	10	1.55	0.45	11	0.36
5	0.48	0.30	9	0.74	0.26	17	1.39	0.42	10	1.58	0.37	10	0.35
6	0.52	0.33	8	0.75	0.24	19	1.44	0.37	10	1.51	0.39	18	0.35
7	0.44	0.24	14	0.86	0.20	18	1.46	0.28	10	1.84	0.39	20	0.35
8	0.46	0.29	9	0.85	0.26	18	1.60	0.35	8	2.13	0.38	17	0.32
9	0.42	0.20	12	0.90	0.24	18	1.71	0.28	8	2.02	0.57	12	0.38
10	0.48	0.24	14	0.91	0.30	17	1.53	0.39	8	2.41	0.51	16	0.39
11	0.42	0.22	14	0.84	0.27	16	1.52	0.34	8	2.10	0.39	14	0.32
12	0.39	0.13	17	0.76	0.22	16	1.50	0.26	8	2.13	0.51	10	0.29
13	0.46	0.15	12	0.83	0.23	15	1.63	0.43	7	2.08	0.62	11	0.41
14	0.45	0.17	13	0.79	0.23	14	1.48	0.31	6	1.94	0.35	10	0.31
15	0.42	0.13	15	0.73	0.28	15	1.43	0.45	6	1.75	0.44	11	0.36
16	0.47	0.14	13	0.76	0.30	14	1.57	0.36	7	1.80	0.31	12	0.29
17	0.54	0.14	12	0.85	0.16	17	1.45	0.33	6	1.73	0.44	13	0.33
18	0.45	0.14	15	0.71	0.19	17	1.45	0.53	4	1.52	0.34	14	0.26
19	0.41	0.11	15	0.64	0.20	16	1.20	0.36	6	1.49	0.42	13	0.30
20	0.39	0.14	15	0.66	0.18	16	1.44	0.71	5	1.40	0.42	13	0.38
21	0.39	0.15	16	0.73	0.20	13	1.30	0.29	5	1.38	0.41	10	0.29
22	0.35	0.13	14	0.62	0.30	13	1.36	0.15	5	1.36	0.38	11	0.31
23	0.48	0.17	15	0.75	0.27	17	1.38	0.28	5	1.44	0.34	12	0.28
24	0.42	0.18	16	0.74	0.22	16	1.60	0.26	5	1.37	0.38	13	0.30
25	0.41	0.20	15	0.66	0.24	14	1.38	0.31	6	1.40	0.36	12	0.31
26	0.35	0.12	14	0.53	0.21	13	1.45	0.36	6	1.38	0.50	11	0.31
F	0.84			3.39			0.85			7.32			
P <	NS			0.0001			NS			0.00001			
$LSD_{0.01}$				0.22			0.44						

Note: Values are given as a mean and standard deviation to allow calculation of the probability of a single measurement collected on a specific date being a member of each of the respective groupings. This would provide a direct estimate of the risk of error. A formula that can be used is given in the text

Changes in mean nipple sizes of the former breeders were not significant during the year (Table 4). Their mean nipple sizes were similar to those of the breeders except during the time of pregnancy and nursing.

Mean nipple size in the breeders varied significantly during the year with larger mean sizes during intervals 8–14 (16–28 wk) relative to the remainder of the year and relative to the former breeders (Table 4). The LSD ($P < 0.01$) is 0.44 for between-mean comparisons at each period for the breeders. This separation in nipple sizes is the first clear external morphological difference we have observed between pregnant and pseudopregnant wolves. Endocrine differences have not been found (Seal *et al.*, 1979).

TABLE 5.—Accuracy of age class and breeding history estimation by inguinal nipple length plus width applied to wild wolves of known age class and history from northeastern Minnesota examined between 21 May and 22 September, except for one adult nonbreeder examined on 10 January

n	Known breeding history	No. estimated in each class			
		Yearling	Adult nonbreeder	Former breeder	Current breeder
5	Yearling	5			
5	Adult nonbreeder	1	4		
6	Current breeder			3	3
4[a]	Former breeder		1	3	

[a] Two individuals were 2 yr old and apparently had lost their pups a few weeks before measurements were made, based on the animals' movements

DISCUSSION

These analyses indicate that for ca. 8 mo of the year the only significant distinction in mean nipple size between reproductive groups is that between nonbreeders and breeders. Pooling of these data by period blurs the distinction between weeks within group, but the differences between groups are significant at every interval (Table 5).

Classification of individual measurements can be evaluated by use of the single sample comparison with the respective sample means for the appropriate period (Sokal and Rohlf, 1981:230). The formula is:

$$t_s = \frac{Y_1 - \bar{Y}_2}{s_2 \sqrt{\frac{n_2 + 1}{n_2}}}$$

where Y_1 is the nipple measurement from the wolf, $\bar{Y}_2$ is the sample mean from the appropriate period, s_2 is the sample standard deviation, n_2 is the sample size, and there are $n_2 - 1$ degrees of freedom. The t_s value obtained may be evaluated as the probability of obtaining such a value under the hypothesis that both samples are from the same statistical population (Sokal and Rohlf, 1981).

Classification, with a probability estimate based upon the t-value, into reproductive and age group of individually collected nipple measurements from females captured from wild populations can be done by comparison with the standard data of the appropriate period (Table 4). During ca. 8 mo of the year the primary distinction will be between breeder and nonbreeder, with further refinement possible if the demographic structure of the population is known.

Wild wolves of known breeding histories from northeastern Minnesota whose nipples were measured between 21 May and 22 September were generally classified correctly as never having bred or having bred (Table 5). However, distinction between current and former breeders was not reliable, and one of 10 current or former breeders (nipple length plus width equaled 0.9 cm on 14 August) was misclassified as an adult nonbreeder. Nevertheless, the measurements are useful in allowing confirmation of nonbreeders and of most current breeders as breeders.

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