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Preliminary Results of Parentage Analysis Using Microsatellite Markers from an Exploited Wolf Population in Central Alaska

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PRELIMINARY RESULTS OF PARENTAGE ANALYSIS USING MICROSATELLITE MARKERS FROM AN EXPLOITED WOLF POPULATION IN CENTRAL ALASKA

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Abstract: Parentage and familial relationships within a highly exploited wolf population in central Alaska were examined to investigate how social disruption affects wolf reproductive performance. Skin tissue or blood samples were collected from 123 live–captured wolves. DNA extraction, genotyping, and parentage analyses were performed by Wildlife Genetics International, Nelson, B.C. Twenty-two locus genotypes were obtained from all samples. Parentage analysis was performed for both parents by plotting 2-parent mismatch distributions for all potential offspring. Parent–offspring relationships were identified for 66 offspring from 10 male and 15 female parents. Multiple litters were identified genetically in 2 packs; in both cases a single male, the primary male, sired the multiple litters. In 1 pack 3 different females produced surviving offspring. In all cases, females that produced surviving offspring in both single litter and multiple litter packs were not daughters of the primary male. Production of multiple litters within our study area resulted when a primary male was replaced and secondary females sired by the previous primary male were retained within the pack.

Introduction

High pregnancy rates and multiple litters in single packs have been found in heavily hunted and trapped wolf populations (Rausch 1967; Ballard et al. 1987). Woolpy (1968) suggested exploitation caused a breakdown of socially induced breeding restrictions, allowing pregnancy in several females within a single pack. However, multiple litters also occur in unexploited wolf populations (Mech et al. 1998), and Haber (1996) suggested inbreeding was a common characteristic of unexploited populations. If so, then multiple litters could result from primary males breeding their subordinate female daughters. Alternatively, multiple litters could result from matings between subordinate females and males from different packs. Meir et al. (1995) found genetic variation within the packs of Denali National Park that indicated genetic exchange between packs. That could result either from adoption of nonpack members or by interpack breeding. However, to date no one has identified the genetic relationships among multiple breeding females, their offspring, and potential fathers of those offspring within multiple litter wolf packs.

We studied familial relationships among wolves in a highly exploited wolf population in central Alaska between 1995 and 2001. This report provides preliminary results of genetic-based parentage analysis among wolves that were live captured during our study.

Study Area

Our study area (11,500 km^2, 64° 10′ N 147° 45′ W) within Alaska's Game Management Unit 20A (GMU 20A) was the site of previous studies on moose, caribou, and wolves. (Gasaway et al. 1983; Boertje et al. 1996; Valkenburg et al. 2004). Elevations range from 300 m to 4000 m sloping upward north to south from poorly drained "flats" of boreal spruce/birch forest (*Picea* spp., *Betula* spp.) through a foothill zone of alpine shrubs (*Salix* spp., *Alnus* spp., *Betula* spp., *Populus* spp.) and tundra sedges (*Carex* spp., *Eriophorum* spp.) to the crest of the Alaska Range. The terrain above 2000 m is mostly rock covered and supports little vegetation. It has areas of permanent snow or glacial ice. The study area is roadless except for seasonal mining trails and trails to homestead sites along the western boundary. Hunting for wolves in GMU 20A was allowed from 10 August to 30 April, and trapping was allowed from 1 November to 30 April. Denali National Park lies adjacent to the study area, and wolves are protected within the park.

Methods

From March 1995 through March 2000 we live-captured wolves by darting the animals from helicopters with 3cc Palmer Cap-Chur® (Palmer Cap-Chur Equipment, Douglasville, Georgia) darts loaded with 500–560 mg of Telazol® (tiletamine HCl and zolazepam HCl, Fort Dodge Lab, Fort Dodge, Iowa), and propelled by low velocity (brown) charges. We attached numbered ear tags to all live-captured wolves and fitted mortality-sensing radio collars to most (Telonics, Inc, Mesa, Arizona USA). From each captured wolf we recorded weight, gender, and various body measurements. We collected whole blood and punched an approximately 3-mm diameter disk of skin, cartilage and hair from the ear to apply ear tags. Ear punch samples were air dried in paper envelopes, frozen and stored in plastic cryotubes. Samples were shipped to Wildlife Genetics International (WGI, Nelson, British Columbia) for DNA extraction, genotyping and preliminary parentage analysis. WGI developed 22 microsatellite markers for this project using the following criteria for acceptable markers:

1) Marker had to be mapped to chromosome, and no chromosome could contribute more than 1 marker to ensure markers were not linked.

2) The microsatellite repeat had to contain at least 17 uninterrupted tandem repeats to ensure variability.

3) Repetitive sequences on either side of the core repeat sequence had to be minimal to reduce the chance for compound variation.

4) Total length of amplified sequences had to be < 250 base pairs, because shorter lengths of DNA are more likely to amplify from poor quality samples.

5) Primer sequences had to produce strong, legible results.

WGI used the exclusion method for parentage analysis, in which hypothesized parent–offspring sets that did not have matching alleles at all examined loci were excluded (Jones

and Arden 2003). Our data set was suitable for that analysis because the sample came from an intensively studied population in which putative relationships were already identified. I used cementum or known ages of sample wolves to eliminate nonsensical parent–offspring relationships that remained after the exclusion analysis.

I use the terms primary and secondary rather than the more traditional terms "alpha" and "subordinate" to differentiate social status among reproductive-aged females (i.e., ≥22 months) within a single pack. Primary females exhibited high pack fidelity, were associated with the primary male more often than other pack members during winter and early spring, and were the oldest females within a pack. Primary females did not disperse or exhibit extraterritorial movements alone. Secondary females were younger than the primary, often exhibited predisperal movements outside of their territory, and most eventually dispersed from multiple female packs.

Pups (≤11 months of age) were identified by incomplete eruption of canine teeth and by the prominent swelling at the distal end of the radius that indicated incomplete ossification of the metaphysis. I identified yearling females (12–23 months of age) from known ages if they had been initially captured as pups, by tooth cementum age from the 1st upper premolar (Ballard et al. 1995) if a postmortem sample was available, or by using a combination of nipple size (Mech et al. 1993) and tooth wear similar to that described by Gipson et al. (2000). Live-captured animals lacking pup characteristics were considered yearlings if they had slight or no wear on incisors and a combined width + length nipple measurement of less than 8 mm. The 8-mm value was assigned because it was below the 90% confidence interval (8.3–10.6) of the mean nipple size of cementum aged and known aged 29- to 36-month-old wolves (n = 9) in the sample.

Results

WGI scored 22 locus genotypes for 123 wolves live captured in GMU 20A between 1995 and 1999. Heterozygosity in the 22 loci genotypes averaged 0.76 with an average of 5.6 alleles per locus (Table 1). Parentage was identified for 66 offspring based on complete 22 loci matches with candidate mother–father pairs (Table 2).

Ten primary males and 10 primary females were identified in 11 packs based on genotypes with supporting evidence from behavior of wolves observed during radiotracking, tenure within the pack, and relative ages. Five productive secondary females were identified in 3 of those packs. Multiple litters in a single pack in the same year were confirmed in 2 packs (Pack #7 and Pack #8). In each case the multiple litters were sired by a single male, the primary male.

None of the 5 secondary females identified as mothers in multiple litter packs were daughters of the primary male; therefore, we found no evidence of inbreeding. However, in all 5 cases the secondary females were the daughters of the primary female. We identified 1 secondary female (#190) that was not the daughter of the primary female (139). That secondary was confirmed pregnant by ultrasound in both 1996 and 1997 (McNay et al. 2006), but our only genetic sample from a pup (331) in that pack during those years came from an offspring of

the primary female (139). Therefore, we could not document production of pups by unrelated females within the same pack during the same year.

Parentage analysis indicated female 462 produced surviving pups in 2 different packs. First, as a secondary female in the Jumbo pack (pack #7) she produced pup 187 in 1995. During the same year, the Jumbo pack primary female (199) also produced pups (185, 186). Female 462 then dispersed and became the primary female in the Boulder Creek pack (pack # 46). Those pups were sired by male 150. Male 150 had sired pups in the adjacent Mystic Creek pack (pack #5) in 1995, but after the primary female was trapped, he dispersed and formed a pair bond with 462 to form the Boulder Creek pack. Therefore, those 2 wolves produced pups in 2 different packs, but only after dispersal from their original pack. We found no evidence of males producing offspring simultaneously in more than 1 pack, and found no evidence that pups in any pack were sired by males other than the primary male of that pack.

Discussion

We used exclusion for molecular parentage analysis among radiomarked wolf packs. The exclusion method uses genetic incompatibilities (i.e., mismatches of alleles) to reject parent–offspring hypothesis. Perfect exclusion can be difficult to attain if genetic variability within the sample is too low, if too few loci are genotyped, or if the pool of candidate parents contains siblings. Human error in genotyping, naturally occurring mutations, and null alleles also may introduce uncertainty into the exclusion parentage analysis (Jones and Arden 2003).

Our review of early studies of wolf genetic variability suggested that parentage analysis would be difficult in wolves because of relatively low heterozygosity and few alleles per locus. Heterozygosity is the sum of the frequencies of heterozygous genotypes at a given locus and is the most commonly used measure of genetic diversity. The heterozygosity over a number of loci is the mean of heterozygosities of individual loci (Chambers 1983). Among 3 different populations in southern Canada heterozygosity (H_o) ranged from 0.58 to 0.63 with 4.4–4.5 alleles per locus in 10 loci genotypes (Forbes and Boyd 1997). However, the 22 new markers developed specifically for this study by WGI revealed a substantially higher level of genetic diversity (H_o=0.76,) than reported by Forbes and Boyd (1997).

That high level of heterozygosity and the large number of loci analyzed allowed clear parentage discrimination in our sample. In 65 cases a candidate offspring's genotype matched at all loci (i.e., 0 mismatches) with only a single putative mother–father pair. In 1 case a 22 loci match was found for a single mother, but 2 males were candidate fathers. Investigation of the field data for the candidate males showed that 1 was a known-aged wolf born the year prior to the putative offspring, thereby excluding that male as a parent and identifying it as an older sibling.

Although a single mismatch is technically sufficient to exclude a parent–offspring hypothesis, errors in genotyping, or a mutation, could result in a single mismatch score from a true parent–offspring relationship. Our sample contained single mismatches (i.e., matches at 21 of 22 markers) for 10 sets of candidate offspring–parents. We used age data and field observation data to confirm that in 8 of those cases the parents of the candidate offspring had already been identified with perfect 22 loci matches, and the 21 loci match reflected a candi-

date pairing between the offspring's father and a full sister. In the other 2 cases data on relative age clearly identified the relationships as siblings or as an offspring being identified as a potential parent to its known parent. Therefore, the single mismatches were totally explained and did not represent parent–offspring relationships, further supporting our assumption that the 66 perfect matches represented true offspring–parent relationships.

Conclusions and Management Implications

Previous work on wolves indicated low heterozygosity, but our results indicate that parentage analysis is possible with 22 locus genotypes. We found no evidence of multiple paternity within packs, but multiple dams were identified in 2 packs. Previous studies using ultrasound for pregnancy diagnoses indicated that multiple litters were common in this population (McNay et al. 2006). Our genetic data revealed that primary males breed nondaughter secondary females that are daughters of the current primary female, and those females produce surviving pups. Pack social structure therefore contributes to multiple littering. A change in the alpha male within an established pack immediately changes the status of secondary females from daughter to nondaughters, making them eligible for breeding. Turnover among primary males may occur through natural mortality or by exploitation by humans. Low exploitation rates by hunting and trapping therefore may contribute to multiple litters if alpha males are removed and other pack members remain. This change in social structure could conceivably increase reproductive output of a wolf population sufficient to offset population declines from human exploitation.

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Table 1 Characteristics of 22 DNA microsatellite markers chosen for parentage analysis of wolves in GMU 20A, Alaska.

Marker	Chromosome	Repeat Sequence	Number of Alleles	Heterozygosity
C01.251	01	$(CA)_{17}$	6	0.88
C02.030	02	$(GT)_4(CT)_{13}$	8	0.60
REN233H01	03	$(CA)_{19}$	5	0.77
REN144A06	04	$(CA)_{19}$	6	0.81
REN69B24	07	$(CA)_{18}$	4	0.73
REN68B08	10	$(CA)_{22}$	6	0.89
REN105L03	11	$(CA)_{26}$	5	0.62
AHT121	13	$(CA)_{28}$	8	0.89
CPH9	29	$(GT)_{18}$	5	0.77
REN145P07	09	$(CA)_{21}$	6	0.73
REN262I12	12	$(CA)_{24}$	5	0.77
REN66E15	15	$(CA)_{19}$	6	0.92
REN85N14	16	$(CA)_{20}$	4	0.73
REN112G06	17	$(CA)_{22}$	6	0.85
REN183B03	18	$(CA)_{19}$	6	0.77
REN297N05	19	$(CA)_{21}$	5	0.73
REN316E23	20	$(CA)_{20}$	6	0.69
REN199O08	21	$(CA)_{18}$	5	0.77
REN210D03	23	$(CA)_{19}$	4	0.42
REN106I06	24	$(CA)_{21}$	7	0.88
REN94H15	25	$(CA)_{19}$	6	0.56
REN181L14	27	$(CA)_{20}$	5	0.85

Table 2 Parentage determined by genotype among sampled wolf packs in GMU 20A for offspring born between 1994 and 1999.

Pack of Capture for Offspring	Male Parent	Female Parent	Offspring
2	02-294	02-139	295,325
45	02-294	02-139	331
4	04-148	04-149	195,196,298,299,312,313,314,315, 316,317,318,319,365,367
5	05-150	05-152	192,193
46	46-150	46-362	350,351,352,357,358,363
33	33-153	33-265	270
7	07-156	07-199	155,157,158,184,185,186,302,303
50	07-156	07-199	353
7	07-156	46-362	187
38	38-284	38-285	286,327,339
8	08-159	08-160	183,343,344,345,348
8	08-159	08-161	181,347,349
8	08-159	08-162	329,346,
8	08-159	08-179	180,182
14	14-175	14-272	297
17	17-169	17-170	307,309,311
17	17-169	17-200	166,167,168,308,310,330,332
31	31-322	31-338	173,320,323,369
Totals	10 Males in 11 packs	15 Females	66 Offspring