Using Microsatellites to Identify Mountain Goat Kids Orphaned During Capture and Translocation Operations

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Abstract: We used molecular markers to test the hypothesis that orphaned mountain goat (*Oreamnos americanus*) kids were driving recent observations of kid mortality in translocated groups in Oregon. To address this hypothesis we collected genetic samples (N = 55) during 3 years of mountain goat captures (2007-2009) in the Elkhorn Mountains of Oregon. Using genotypes from these samples at 12 microsatellite loci, we conducted parentage analyses and estimated relatedness within each year's translocation group to identify kids without a mother. Based on the results of our parentage analyses, 6 of 15 kids were assigned to potential mothers with a level of confidence < 80%, levels at which the validity of the assignment is questionable. Using the more liberal assignments based on maximum likelihood estimates of relatedness, 3 of 15 kids could not be assigned to a mother within their capture group. Therefore, at least 3 kids (20% of 15 total) but as many as 6 kids (40%) were orphaned as a result of translocation operations. In addition, at least 4 but as many as 6 candidate mothers that were not assigned to a kid were lactating at the time of capture. Thus, orphaning of mountain goat kids may have occurred as a result of mothers being transported without their offspring as well as through offspring being transported without their mothers. Our results indicate that biologists conducting translocations of mountain goats should anticipate some orphaning as a result of capture operations.

KEY WORDS *Oreamnos americanus*, orphans, parentage analysis, reintroduction, relatedness, mountain goat, supplementation, translocation.

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Translocations are a commonly used tool in the conservation and management of wildlife species (Griffith et al. 1989, Fischer and Lindenmayer 2000). Many large fauna in North America have benefitted from reintroduction campaigns with success stories including white-tailed deer (*Odocoileus virginianus*; DeYoung et al. 2003), Rocky Mountain elk (*Cervus elaphus*; Hicks et al. 2007), and bighorn sheep (*Ovis canadensis*; Krausman 2000). Although reintroduction programs have re-

established populations of many species to areas throughout their historic ranges, the success of individual reintroductions often is not assured (Risenhoover et al. 1988, Fischer and Lindenmayer 2000).

Of the factors affecting the success of reintroductions, the size of translocated groups has been shown to have a dramatic impact on population persistence (Forsyth and Duncan 2001). Large group sizes hasten the growth of populations away from small population sizes, effectively buffering them from the negative effects of demographic and environmental stochasticity (Lande 1988), Allee effects (Deredec and Courchamp 2007), and inbreeding and genetic drift (Lacy 1987, Keller and Waller 2002). All of these processes affect small populations more than large populations (Pimm 1991) and could potentially act to create an extinction vortex (Gilpin and Soulé 1986). Thus, improvements in the efficiency of translocations, either in terms of increasing the group size associated with translocations or by improving survival of translocated individuals, can improve the success of supplementations or reintroductions (Rhodes and Latch 2010).

Oregon Department of Fish and Wildlife (ODFW) has been conducting mountain goat (*Oreamnos americanus*) translocations since 1950, when the first successful reintroduction of mountain goats to Oregon took place in the Wallowa Mountains (Coggins et al. 1996). Since that reintroduction Oregon's mountain goats have increased in number to over 800 animals (Myatt 2010), due in large part to a translocation campaign intended to restore goats to their historic range across the state (ODFW 2003). However, during recent capture efforts, ODFW biologists noticed poor kid survival after translocation (Myatt et al. 2010). One hypothesis proposed to explain high kid mortality after translocation was that translocated groups could include orphaned kids. Due to the logistical challenges associated with staging capture operations in the alpine environment that mountain goats inhabit (e.g., poor access), goat captures in Oregon occur during times of year when kids are dependent on their mothers (Rideout and Hoffmann 1975). Thus, orphaning could occur if dependent kids are captured and translocated without their mothers in the group or vice versa.

Identifying the prevalence of orphans resulting from mountain goat capture operations is important because it is likely that orphaned kids exhibit poor survival in translocated groups. While understanding that there is an ethical obligation to improve kid survival after translocation, from a biological perspective orphaning of goat kids could also effectively reduce translocation group size: a critical component in the long-term success of reintroductions and supplementations. In addition, recent simulations have predicted that juveniles in reintroductions may be even more valuable than adults in terms of population persistence when genetics and demography were considered simultaneously (Robert et al. 2004). Alternatively, the negative aspects of orphaning mountain goat kids may not be restricted to the reintroduced population. Although productivity is expected to be reduced in the source population due to the export of captured individuals, further reduced productivity could occur if capture operations orphan kids at the site of capture by translocating their mothers.

In this study we used a suite of microsatellites, a type of molecular marker that is hyper-variable and biparentally-inherited, to address the hypothesis that mountain goat capture operations resulted in the orphaning of goat kids. We used parentage analysis and estimates of relatedness derived from our suite of

molecular makers to assign kids in each capture group to candidate mothers. Thus, kids that we could not assign to a mother likely would be orphans. The specific objectives of this study were to 1) identify the proportion of goat kids captured without their mother as a result of translocation operations, and 2) identify the proportion of lactating mothers captured and translocated without an associated kid during capture operations.

STUDY AREA

Mountain goats were captured at Goodrich Lake in the Elkhorn Mountains of northeastern Oregon. The Elkhorns, a part of the larger Blue Mountains, are located immediately west of Baker City, and rise approximately 5,000 ft from adjacent Baker Valley (Johnson 2004). A population of mountain goats was re-established in the Elkhorns through reintroduction efforts in 1983-1986 that involved 21 goats translocated from Idaho, the Olympic Peninsula of Washington, and Misty Fjord in Alaska (Coggins et al. 1996). The Elkhorn population has increased steadily since reintroduction: 301 goats were counted during the 2010 herd inventory conducted by ODFW (Myatt 2010). As such, the Elkhorn population has been valuable as a source for mountain goat translocations in Oregon since 2000.

METHODS

Field Methods

Goats were captured using a drop-net once each July from 2007-2009. The net was baited with salt and dropped on a group only when we noted no other goats in visible range of the net. Groups were observed for a considerable amount of time in order to ensure that nanny-kid groups were not accidentally separated when the net was dropped. Once captured, we blindfolded and hobbled goats prior to recording their sex, age, and lactation status if applicable. We tagged all individuals with uniquely numbered ear-tags and some received radio-collars as part of a larger study of goat movement behavior and survival. Tissue samples were collected from each goat for genetic analysis using a 6.3-mm (i.e., 0.25-inch) ear punch, and sampling equipment was sterilized between each use to avoid cross-contamination. Each sample was stored in a 2 mL screw-top vial filled to 1 mL with desiccant beads. The vials were shipped immediately to the genetics lab at Purdue University where full desiccation of the sample was ensured before samples were stored at -80° C until DNA extraction.

Laboratory Methods

Genomic DNA was extracted using a modified ammonium acetate protocol (see Fike et al. 2009). Quality of the extracted DNA was assessed visually using gel electrophoresis prior to DNA quantification on a NanoDrop 8000 spectrophotometer (Thermo Scientific, Waltham, MA, USA). Genomic DNA was stored at -80° C after an aliquot of working stock was diluted to 20 ng/μL.

From each sample we used polymerase chain reaction (PCR) to amplify 13 microsatellite loci (Table 1). These loci were chosen from a suite of those used in previous mountain goat studies based on their level of polymorphism and ease of amplification in our lab. The PCR amplification of genomic DNA was carried out in 10-μL reactions which consisted of 20 ng DNA template, 0.25 μM of each primer, 0.2 mM of each dNTP, 1.25 mM $MgCl_2$, 1× reaction buffer (10 mM Tris-HCL, 50 mM KCL, 0.05 mg/μL BSA), and 1 unit of *Taq* DNA polymerase. We used the following thermocycler profile for all loci: 94 °C for 2 min; 30 cycles of 94 °C for 30 sec, locus-specific annealing temperature (Table 1) for 15 sec, and 72 °C for 15 sec; then 72 °C for 10 min and a final extension at 60 °C for 45 min. PCR amplification products were

Table 1. Microsatellite loci used in this study were genotyped in 55 mountain goats captured in the Elkhorn Mountains of northeastern Oregon during 2007-2009. Listed for each locus are number of alleles (A), annealing temperature (T_A), observed (H_O) and expected (H_E) heterozygosity, and a test of Hardy-Weinburg proportions (F_{IS}) with its associated p-value.

Marker[a]	Primer Sequence (5' → 3')	Allele size range (bp)	A	T_A (°C)	H_O	H_E	F_{IS}	P
LS15[4]	F: gtagaaacccctaaagattc	98-116	5	60	0.661	0.638	-0.036	1.000
	R: ctgagtgttaattttctatcct							
OarCP26[2]	F: gcctaacagaattcagatgatgttgc	129-148	3	64	0.500	0.434	-0.169	1.000
	R: gtcaccatactgacggctggttcc							
BM121[1]	F: tggcattgtgaaaagaagtaaa	162-176	6	60	0.893	0.774	-0.215	0.013
	R: actagcactatctggcaagca							
INRA3[7]	F: ctggaggtgtgtgagccccattta	201-228	5	64	0.625	0.651	0.133	0.530
	R: ctaagagtcgaaggtgtgactagg							
BM203[1]	F: gggtgtgacattttgttccc	244-277	3	64	0.278	0.522	0.673	<0.004
	R: ctgctcgccactagtccttc							
RT9[8]	F: tgaagtttaatttccactct	129-137	4	55	0.786	0.679	-0.222	0.247
	R: cagtcactttcatcccacat							
BM4028[1]	F: acggaagcagcatctcttac	148-150	2	64	0.109	0.167	-0.040	1.000
	R: atggaaacatggtctcctgc							
INRA11[6]	F: cgagtttctttcctcgtggtaggc	193-207	5	64	0.429	0.629	0.191	0.375
	R: gctcggcacatcttccttagcaac							
BM1818[1]	F: agctgggaatataaccaaagg	243-248	3	60	0.093	0.09	-0.020	1.000
	R: agtgctttcaaggtccatgc							
BMS599[5, b]	F: agtaggagctgtcttctgtggc	166-172	6	64				
	R: gtcactgggacttctctgagc							
MCM527[3]	F: gtccattgcctcaaatcaattc	165-169	2	64	0.536	0.502	-0.198	0.450
	R: aaaccacttgactactccccaa							
BMC5221[1]	F: agcaaggagaacaggcattc	185-215	7	64	0.875	0.754	-0.144	0.721
	R: cttctttggcagcacagtttc							
BM1225[1]	F: tttctcaacagaggtgtccac	278-290	5	64	0.696	0.665	-0.034	0.604
	R: acccctatcaccatgctctg							

[a]Originally described in [1]Bichop et al. (1994), [2]Ede et al. (1995), [3]Hulme et al (1994), [4]Maddox et al. (2000), [5]Stone et al. (1995), [6]Vaiman et al. (1992), [7]Vaiman et al. (1994), and [8]Wilson et al. (1997)

[b] Locus BMS599 was difficult to score and was removed from all analyses

electrophoresed at the Purdue Genomics Core Facility on an ABI 3730xl automated sequencer (Applied Biosystems, Carlsbad, CA, USA). The electrophoretic data were imported into GeneMapper version 3.7 (Applied Biosystems) where fragments were sized based on internal ROX size standards (DeWoody et al. 2004). We used the following methods to ensure the quality of our microsatellite dataset: (i) allelic standards were included for each locus in each submission to the core facility, (ii), an experienced researcher independently scored each locus/sample combination to assess genotyping error rates, and (iii) all ambiguous or low-quality genotypes (signal strength <100 in GeneMapper) were re-amplified to confirm the genotype.

Data Analysis

We used program CREATE version 1.33 (Coombs et al. 2008) to facilitate data conversion for all analyses. We tested for locus-specific deviations from Hardy-Weinberg equilibrium (HWE) using Fisher's exact tests and for pairwise deviations among loci from linkage equilibrium in GENEPOP version 4.0 (100,000 steps in the Markov chain; 100 batches with 1000 iterations; Raymond and Rousset 1995). We used only genotypes from adult goats (i.e., > 1 year old) in these tests to avoid violating assumptions associated with sampling across generations. Deviations from equilibrium expectations were assessed for significance after correction for multiple tests using Bonferroni's method (Rice 1989). Using the full dataset, we calculated the number of alleles, observed heterozygosities, and expected heterozygosity for each locus in program GENALEX version 6.3 (Peakall and Smouse 2006). Because there is some uncertainty involved in the assignment of offspring to parents when the sample of candidate parents is incomplete or when using a finite number of molecular markers (Glaubitz et al. 2003, Jones and Ardren 2003), we used 2 methods to identify mountain goat orphans from our genetic data.

First, we conducted parentage analysis using program CERVUS version 3.0 (Kalinowski et al. 2007) to identify likely mother-offspring dyads within each capture group. Separate analyses were conducted for each group (i.e., goats captured in 2007, 2008, and 2009), but genotype frequencies were simulated using the full dataset. Within each capture group we identified offspring as goat kids (i.e., goats < 1 year old) and candidate mothers as nannies ≥ 2 years old. Within CERVUS, we simulated 10,000 offspring genotypes with the proportion of loci mistyped set to 1.0%. The sampling rate was set to 80% based on the observations of field personnel.

CERVUS uses simulated genotypes to create a critical likelihood value (critical ΔLOD; where a LOD score is the logarithm of the likelihood ratio) beyond which there is some level of confidence in a mother-offspring dyad. Briefly, LOD scores are calculated for the most-likely candidate mother and the second most-likely mother for each offspring. Then, the difference between the ratios is compared to the critical ΔLOD in order to assign confidence in the pairing (Marshall et al. 1998). In this study, assignments were made at a relaxed level of 80% confidence and a strict level of 95% confidence which are standard for the program. Loci exhibiting a heterozygote deficit were excluded from this analysis. Although there are many causes of heterozygote deficiency, null alleles are particularly problematic in parentage analysis (Dakin and Avise 2004).

The second method we used to identify goat orphans was via maximum likelihood estimates of relatedness calculated in program ML-Relate (Kalinowski et al. 2006). Offspring and candidate mothers were defined as above within each capture group. ML-Relate produces a matrix containing the most likely of 4 relationship categories for each pair of individuals (i.e., unrelated, half-sib, full-sib, or parent-offspring). Thus, to identify putative goat orphans, we recorded all offspring lacking a parent-offspring assignment to any candidate mother in their capture group. Then, for those putative orphans, we specifically tested any assigned relationship to a candidate mother (i.e., at the half-sib or full-sib level) to determine if that relationship was statistically more likely than a parent-offspring relationship (using the specific hypothesis test option with 100,000 simulated genotypes in ML-Relate). Putative orphans with statistical support for

each of such assignments were considered true orphans. Because ML-Relate is capable of adjusting its simulation to accommodate null alleles where they have been identified *a priori* (Kalinowski et al. 2006), loci exhibiting heterozygote deficits were retained in this analysis, but were flagged as potentially harboring null alleles.

Although mountain goat kids usually remain close to their mother for the first year of life (Rideout and Hoffmann 1975), it is possible for capture operations to orphan kids at the site of capture by translocating a mother without her kid as opposed to translocating a kid without its mother. We identified potential instances of orphaning at the site of capture by noting lactating candidate mothers that remained unassigned to a kid from our analyses.

RESULTS

We successfully extracted genomic DNA from all mountain goat samples (N = 55). Cohort size was 19 in 2007 including 5 kids and 10 candidate mothers (i.e., nannies > 2 years old), 19 in 2008 including 6 kids and 8 candidate mothers, and 17 in 2009 including 4 kids and 5 candidate mothers. One microsatellite locus, BMS599, was difficult to score and was excluded from all analyses. Our genotyping error rate for the remaining 12 loci was < 0.5 % overall and our missing data rate was < 4.0 % for each locus (i.e., 2 missing genotypes) and was < 1.0 % overall. Locus BM203 exhibited a significant deficit of heterozygotes ($P < 0.004$, Table 1). Thus, we excluded this locus from analyses in program CERVUS, but retained it for our analyses using program ML-Relate after we flagged the locus as potentially harboring null alleles. No pair of loci deviated from linkage equilibrium after corrections for multiple tests (i.e., all $P > 0.0003$).

Parentage analysis allowed us to assign 3 of 5 offspring to mothers in the 2007 group, 3 of 6 offspring to mothers in the 2008 group, and 3 of 4 offspring to mothers in the 2009 group with confidence exceeding the relaxed assignment threshold of 80% (Table 2), which is a standard threshold for the program and commonly used in parentage studies (e.g., Richardson et al. 2001). Thus, our analysis revealed the potential for 2 orphans in the 2007 group, 3 orphans in the 2008 group, and 1 orphan in the 2009 capture group. These numbers should be interpreted with caution as the likelihood scores used to calculate confidence in CERVUS are dependent on the relative strength of evidence for other candidate mothers. For example, assignments with < 80% confidence can indicate 1) that the assignment was truly unlikely (i.e., that the offspring was an orphan), or 2) that the second-strongest candidate mother was also likely to be the true mother. Thus, these results likely represent the maximum number of orphans in each group.

All mother-offspring dyads identified in program CERVUS were nominally identical to those we identified using the maximum likelihood estimates of relatedness in program ML-Relate (Table 2). However, this analysis revealed 5 putative orphans rather than the 6 identified using parentage analysis (Table 2). Further simulations indicated that 3 putative orphans had statistical support for status as true orphans (Table 3). Therefore, a minimum of 3 mountain goat kids were captured as orphans during 3 years of capture activities.

Based on our parentage analyses, additional potential orphaning occurred at the site of capture in 2008 (N = 4) and 2009 (N = 2) when candidate mothers were identified as lactating but remained unassigned to offspring in the capture group (Table 2). Based on our more liberal estimates of relatedness, potential orphaning at the site of capture similarly occurred in 2008 (N = 2) and 2009 (N = 2). No

Table 2. Assignments of offspring to candidate mothers using parentage analysis (CERVUS) and estimates of relatedness (ML-Relate) derived from genetic data. Samples were obtained from 3 years (2007-2009) of capture operations in Oregon's campaign to restore mountain goats to their historic range in the state.

	CERVUS						ML-Relate	
Year Offspring	Candidate mother	Lactation status[a]	Loci compared (mismatching)		Pair ΔLOD[b]	Pair confidence	Candidate mother	Relation[c]
2007								
RMG10	RMG1	NL	11	(0)	6.07	> 95%	RMG1	PO
RMG11	RMG3	L	11	(0)	3.73	80-95%	RMG3	PO
RMG12	RMG6	NL	11	(0)	4.52	80-95%	RMG6	PO
RMG14	RMG17	NL	11	(0)	0.40	< 80%	RMG17	FS
RMG15	RMG16	unk	11	(0)	0.99	< 80%	RMG16	PO
	RMG4	NL				Unassigned		
	RMG5	NL				Unassigned		
	RMG13	NL				Unassigned		
	RMG19	unk				Unassigned		
	RMG20	unk				Unassigned		
2008								
RMG21	RMG36	L	10	(0)	1.76	< 80%	RMG36	PO
RMG27	RMG24	L	11	(0)	2.28	< 80%	RMG24	PO
RMG28	RMG25	L	11	(0)	2.95	80-95%	RMG25	FS
RMG29	RMG26	L	11	(1)	0.20	< 80%	RMG26	HS
RMG30	RMG36	L	11	(0)	4.51	80-95%	RMG36	PO
RMG31	RMG32	L	11	(0)	5.28	> 95%	RMG32	PO
	RMG22	NL				Unassigned		
	RMG23	NL				Unassigned		
	RMG33	L				Unassigned		
2009								
RMG40	RMG2778	L	11	(0)	4.88	80-95%	RMG2778	PO
RMG41	RMG2279	L	10	(0)	3.33	80-95%	RMG2279	FS
RMG42	RMG2284	L	11	(0)	3.16	80-95%	RMG2284	PO
RMG43	RMG2279	L	10	(0)	-0.11	< 80%	RMG2279	HS
	RMG2282	L				Unassigned		
	RMG2288	NL				Unassigned		

[a]NL= not lactating, L = lactating, or unk = no data

[b]Critical ΔLOD (logarithm of likelihood ratio) was 5.02 for strict (95%) and 2.57 for relaxed (80%) confidence

[c] Most likely relationship: U = unrelated, HS = half-sib, FS = full-sib, and PO = parent-offspring

Table 3. P-values from simulations conducted in ML-Relate to test if the most-likely relationship (i.e., half-sib or full sib) of putative orphans and candidate mothers was significantly more likely than that of a parent-offspring relationship. P-values < 0.05 indicate support for the half-sib or full-sib designation and that a parent-offspring relationship was not likely. Putative orphans with statistical support for each such relationship were considered true orphans.

Putative Orphan	Candidate Mother			
2007	RMG3	RMG6	RMG13	RMG17
	Half-sib	Half-sib	Full-sib	Half-sib
RMG14	<0.001	<0.001	<0.001	0.030
2008	RMG25	RMG33	RMG36	
	Full-sib	Full-sib	Full-sib	
RMG28	0.061	<0.001	<0.001	
	RMG26			
	Half-sib			
RMG29	<0.001			
2009	RMG2279			
	Full-sib			
RMG41	0.057			
	RMG2279	RMG2288		
	Half-sib	Half-sib		
RMG43	0.029	<0.001		

potential orphaning at the site of capture was apparent in the 2007 group (using parentage analyses or estimates of relatedness), although 2 candidate mothers were assigned to offspring even though they were not lactating at their time of capture (Table 2).

DISCUSSION

Mountain goat kids were captured without their mothers at surprisingly high rates: at least 3 kids (20% of 15 captured overall), and potentially as many as 6 kids (40%), were orphans in their translocated groups across 3 years of captures in Oregon. Biologists often strive to achieve the highest, logistically-feasible number of animals in their translocation efforts to maximize the positive effects of supplementation on target populations (Van Houtan et al. 2009) and/or to bolster the ability of introduced populations to resist stochastic demographic processes and avoid potential Allee effects (Deredec and Courchamp 2007, Armstrong and Seddon 2008). Large translocation groups also are a viable strategy to combat reductions in genetic diversity often associated with reintroduction programs (DeYoung et al. 2003, Mock et al. 2004, Hicks et al. 2007, Rhodes and Latch 2010). Thus, it is possible that orphaned goat kids are reducing the effectiveness of expensive capture operations by reducing the size of translocation groups. However, in a concomitant, independent assessment of kid

mortality after translocation using radio-collared kids from this study, ODFW biologists identified near complete kid mortality after translocation possibly as a result of kids being separated from their mothers during their release (Myatt et al. 2010). Therefore, while orphaning of mountain goat kids during translocation operations likely reduced the probability of kid survival and, thus, the effectiveness of translocation efforts, other factors such as release method may supersede the impacts of orphaning on kid survival in some instances.

In addition to goat kids being captured without their mothers, another concern associated with capture operations is translocating mothers without their offspring. Mountain goat kids typically are weaned in September (Rideout and Hoffmann 1975) and capture-related orphaning during earlier phases of development undoubtedly increases the risk of kid mortality. The potential for orphaning at the site of capture – identified as lactating candidate mothers that were unassigned to offspring – was evident in 6 of 23 possible assignments (i.e., 26%) from our parentage analysis and 4 of 23 possible assignments (17%) from our more liberal estimates of relatedness. While the prevalence of lactating females that were not assigned to an offspring could track the true rate of orphaning at the site of capture, it is possible that some portion of these candidate mothers experienced kid mortality prior to capture operations, had not yet stopped lactating, and that the true rate of orphaning at the site of capture was slightly lower.

Lactation status failed to predict offspring assignment twice in the 2007 capture group, where candidate mothers identified as not lactating in the field were assigned to offspring. These assignments (supported in both cases by both our analytical approaches) may be indicative of imperfect assessment of lactation status in the field, but could also evidence mothers weaning offspring earlier than has been previously reported (i.e., July as opposed to September; Rideout and Hoffman 1975). Further research will be necessary to address this issue, but these cases may serve to highlight the imperfection of physiological cues that prevent biologists from identifying orphaned goat kids in the field.

Estimates of parentage and relatedness derived from genetic data have found a variety of uses in the field of wildlife management (DeYoung and Honeycutt 2005). Herein we applied parentage analysis and estimates of relatedness to a novel problem in mountain goat management in which we identified offspring without mothers and lactating females without offspring in translocated groups using microsatellite markers. If orphaning reduces kid survival as we would expect, our study could indicate that these translocated groups of mountain goats are effectively smaller than they seem. Biologists should consider the potential effects of orphaning, and the potential for kid mortality independent of orphaning, on translocation group sizes when designing future mountain goat reintroductions and supplementations.

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