Supplemental Data

Cougar captures

Cougar capture and handling procedures followed Quigley (2000) and were approved by the Hornocker Wildlife Institute/WCS Animal Care and Use Committee and Yellowstone National Park.

Microsatellite data

DNA was extracted from the blood or tissue samples using a QIAGEN DNA extraction kit. We used the following 11 microsatellite markers originally developed for domestic cats and lynx (Carmichael 2000; Menotti-Raymond et al. 1999) to genotype all sampled individuals: *Fca30*, *Fca35*, *Fca57*, *Fca77*, *Fca90*, *Fca96*, *Fca132*, *Fca176*, *Fca391*, *Fca559*, *Lc109*. Except for *Lc109*, all loci had been chromosome mapped for the domestic cat; distances among loci on the same chromosome were all >100cM (Menotti-Raymond et al. 1999).

Polymerase chain reaction (PCR) components consisted of up to 200 ng genomic DNA, 10 mM Tris–HCl (pH8.3), 2.0 mM MgCl2, 20 μM of each dNTP, 0.2 μM of each primer (10nM for labeled primer) and 0.8 U Taq DNA polymerase and water for a total volume of 10 μl. PCR conditions were: initial incubation at 3 minutes for *Fca* primers and 5 minutes for *Lc109*, followed by 10 cycles of denaturation at 94 ^{0}C for 15 sec, annealing at 55^{0}C for 15 sec, and extension at 72^0 C for 30 sec. All PCR testing included samples containing only purified water and DNA from an initially analyzed individual as negative and positive controls, respectively.

Each amplified locus was resolved and visualized on a polyacrylamide gel using the LICOR DNA analyzer (Lincoln, Nebraska, USA) and allele sizes for all samples were scored visually relative to the positive control. Results were verified through independent scoring by a

second independent observer. Individuals for which fewer than eight loci were scored were removed from the data set.

Descriptive statistics

We used GenePop 3.1(Raymond & Rousset 1995) to determine expected and observed heterozygosity, number of alleles, and test for linkage disequilibrium (LD) and deviations from Hardy-Weinberg equilibrium (HWE), expressed as F_{IS} (Weir & Cockerham 1984), using the method of Guo and Thompson (1992). After Bonferroni correction, none of the loci deviated significantly from HWE (Table S1) and no pair of loci showed LD (Results not shown).

Table S1**.** Descriptive statistics for microsatellite data collect from 69 cougars within the greater Yellowstone ecosystem for 11 loci. H_{exp}/H_{obs} = expected/ observed heterozygosity.

Locus	Alleles	H_{exp}	H_{obs}	F_{IS}
Fca30	6	0.24	0.26	-0.086
Fca35	3	0.53	0.55	-0.037*
Fca57	6	0.63	0.55	0.127
Fca77	2	0.07	0.07	-0.030
Fca90	5	0.67	0.70	-0.041
Fca96	5	0.73	0.77	-0.048*
Fca132	4	0.64	0.59	0.066
Fca176	6	0.66	0.72	-0.094
Fca391	5	0.51	0.55	-0.081
Fca559	5	0.62	0.65	-0.053
Lc109	5	0.53	0.51	0.049
Average	**4.7**	**0.53**	**0.54**	**-0.021**

* = significantly different from Hardy-Weinberg-proportion before Bonferroni correction, but not after

Relatedness among first-order relatives

We created the distribution of *R*-values from 53 mother-kitten pairs and 32 full-sibs, each representing first-order relatives with a theoretically expected $R = 0.5$ (Queller & Goodnight 1989). Overall, relatedness averaged 0.36 (Fig.S1); means for mother-kitten pairs during Phase I and Phase II and for full siblings were equivalent (0.39, 0.33, and 0.35, respectively). The lower than expected *R* have also been reported in previous studies of wild populations (e.g. van Horn 2004) and at least in our case were not due to wrong scoring, since all pairs shared at least one allele for each locus.

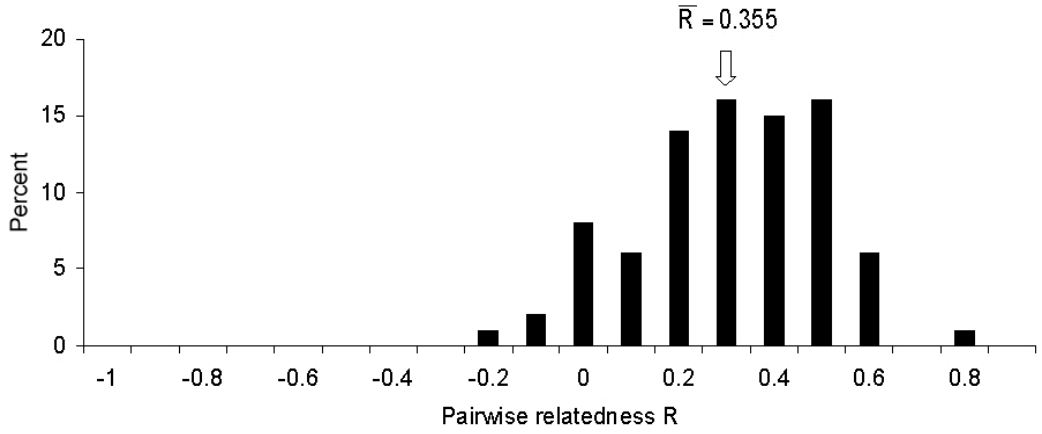

Fig. S1: Distribution of pair-wise relatedness among cougar mother-kitten and full sib pairs in the northern Yellowstone ecosystem 1988 - 2004.

We suspect that the deflation of values may be caused by kittens carrying rare alleles from their fathers, which they are unlikely to share with their mother and often their siblings and which weigh more heavily than common alleles in the calculation (Queller & Goodnight 1989). Defining rare alleles as those occurring at frequency <0.1, we found that kittens had on average 0.13 rare alleles per locus (n=53) compared to 0.1 for mothers (n=18). Although this difference

was not statistically different (Student t-test, $p = 0.145$), it may have been sufficient to reduce the probability of mother and kittens sharing rare alleles, resulting in a slight reduction in average relatedness.

References

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