

MOLECULAR GENETIC APPLICATIONS FOR BEAR RESEARCH

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Abstract: Recent advances in molecular genetic techniques have generated a new and exciting range of possible applications of genetic methods to bear (*Ursus* spp.) research, conservation, and management. These advances have led to an explosion in genetic research on bear populations and an increased interest in genetic technology among researchers working in other scientific disciplines. This paper provides an overview of genetic techniques and markers, recent publications, and future directions. Five major applications of genetic analyses are addressed: forensics, population structure and gene flow, genetic diversity, maternity and paternity analysis, and non-invasive genetic sampling.

Ursus: 11:253–260

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Since 1990, molecular genetic research on bear species has generated an impressive amount of new data. A decade ago, very little genetic data existed for natural populations because of the extreme technical difficulty and expense of molecular genetic analyses. Because of recent developments in molecular techniques, genetic analyses of any organism can be performed cost-effectively with relative ease, and researchers can use this genetic information to address questions about the biology, ecology, and behavior of free-ranging species. Recent genetic studies of bears have provided an increased understanding of evolutionary relationships (Zhang and Ryder 1993, 1994; Talbot and Shields 1996a; Waits 1996), population structure (Paetkau et al. 1995, Paetkau and Strobeck 1996, Talbot and Shields 1996b, Wooding and Ward 1997, Waits et al. 1998b), genetic variation (Paetkau and Strobeck 1994, Paetkau et al. 1998b, Waits et al. 1998a), and reproductive success of bears (Craighead et al. 1995). The goal of this paper is to summarize recent significant advances, discuss research in progress, and suggest directions for future study. Five main areas of research will be discussed: (1) forensics, (2) population structure, (3) genetic diversity, (4) paternity and maternity analyses, and (5) non-invasive genetic sampling methods.

GENETIC METHODS AND MARKERS

Molecular methods that are used to analyze DNA include a wide range of techniques such as the polymerase chain reaction (PCR), restriction enzyme analysis, DNA sequence analysis, minisatellite analysis, and microsatellite analysis (for review see Avise 1994, Parker et al. 1998). Detailed descriptions of each technique are beyond the scope of this article, but it is important to note that each technique has inherent strengths and weaknesses and provides differing levels of resolution. Thus,

careful consideration is required to couple a particular research question with the optimal molecular method. One important distinction among markers (i.e. DNA regions used in genetic analysis) is the differences among mitochondrial DNA (mtDNA) markers, Y chromosome markers, and nuclear DNA markers. Mammalian cells contain 2 types of DNA: nuclear DNA and mtDNA. mtDNA is a circular DNA molecule of the mitochondrion, a cellular organelle found in hundreds to thousands of copies in the cytoplasm of each cell. mtDNA is inherited uniparentally from mother to offspring (Brown et al. 1979). Nuclear DNA resides in the nucleus of cells and is inherited biparentally. Thus, cells have 2 copies of each nuclear chromosome — one from the mother and one from the father. For bears, the sex chromosomes, X and Y, are nuclear chromosomes with XX denoting a female and XY denoting a male. The Y chromosome is inherited uniparentally from father to son.

These differences in inheritance patterns have important implications for the interpretation of results from genetic studies. Nuclear DNA markers can provide information about both maternal and paternal evolutionary history, gene flow, genetic diversity and relatedness; mtDNA markers can provide only information concerning female evolutionary history, gene flow, and genetic diversity; and Y chromosome markers can only provide information about paternal evolutionary history, gene flow, and genetic diversity.

FORENSICS

Forensics is the application of laboratory analyses to legal issues or cases. Currently, DNA analysis can provide 4 types of information to forensic studies: species identification, identification of geographic origin, sex identification, and individual identification. Species identification is accomplished by using PCR amplification to

examine mtDNA regions with defined genetic identifiers unique to each species (Waits 1996). Forensic identification of species has been used for 2 types of studies: (1) identifying illegally harvested bears, and (2) differentiating American black bear (*Ursus americanus*) and brown bear (*U. arctos*) samples collected using non-invasive sampling of hair and scat. Large numbers of bears are killed throughout the world because of the value of their body parts (Servheen 1990). For some species, this source of mortality may be the most important threat to survival. Reducing or eliminating the poaching of bears will require complex cultural, economic, and legal changes and forensic methods to identify the species and geographic region of origin. Different groups have reported genetic methods to differentiate bear species, and the U.S. Fish and Wildlife Forensics Laboratory reports that confiscated gall bladders have been identified to species using such methods (S. Fain, U.S. Fish and Wildlife Service, Ashland, Oregon, personal communication, 1998). Species identification has become a very important forensic tool for projects using non-invasive genetic sampling methods to count bears in geographic areas where the range of brown bears and black bears overlap (Woods et al. 1996, 1999).

In addition to identifying the species, the geographic region of origin can also be determined. This information could be used to determine if a forensic sample originated from a region closed to hunting or to determine

geographic regions where many bears are illegally harvested. The major requirement for determining the geographic origin of samples using genetic methods is the collection of genetic data from a large number of individuals across the range of a species. Large data sets of mtDNA sequences from brown bears (Taberlet and Bouvet 1994, Kohn et al. 1995, Talbot and Shields 1996b, Waits 1996, Waits et al. 1998b) and American black bears (Byun et al. 1997, Wooding and Ward 1997) have identified discrete phylogeographic groupings (Figs. 1, 2) that can be used to classify geographic origins of individuals. Preliminary analyses of sloth bears (*U. ursinus*) and sun bears (*U. malayanus*) (Fain et al. 1995) suggest that more phylogeographic groupings can be assigned for these species after additional analyses. Using several nuclear markers also can provide information about the origin of samples at a finer geographic scale. For example, a population genetic survey of polar bears (*U. maritimus*) in the Canadian arctic using nuclear DNA microsatellite markers traced the origin of an individual to the eastern or western side of the Canadian arctic with 93% accuracy (Paetkau et al. 1995). Large nuclear DNA microsatellite data sets now exist for the polar bear and North American and Scandinavian brown bear (Paetkau et al. 1995, 1998a, 1998b; Waits et al. 2000); additional baseline data are needed for Asian and European brown bear populations and all other bear species.

Molecular forensic identification of sex is accomplished using PCR to amplify DNA regions specific to the Y

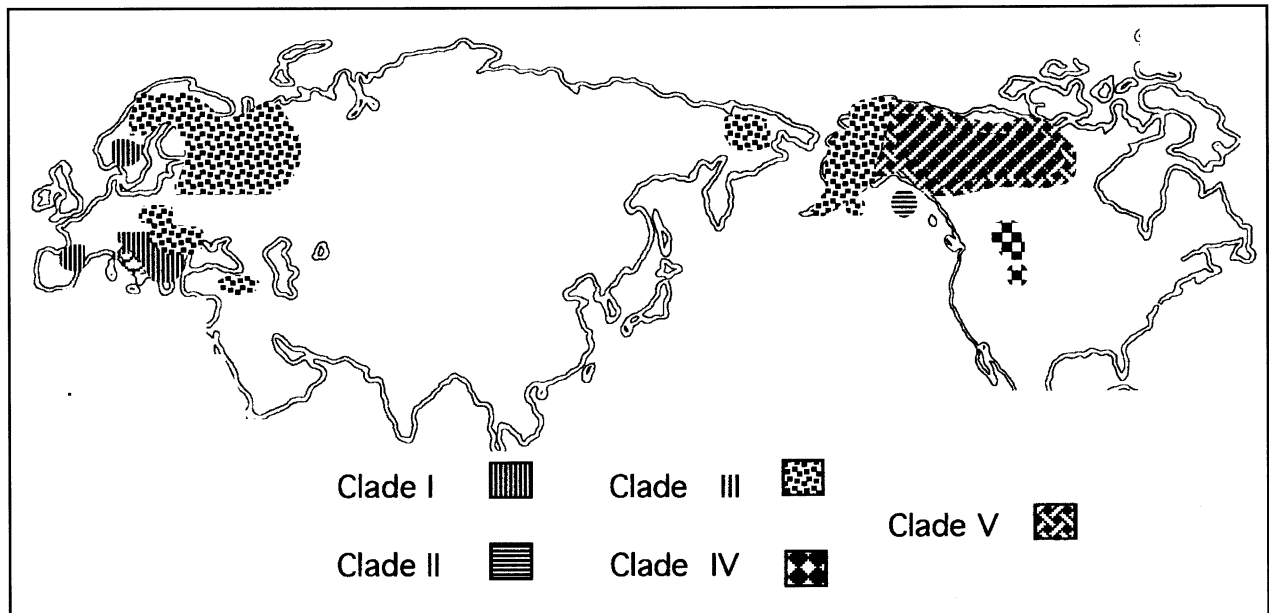


Fig. 1. Geographic distribution of 5 clades (genetic groups) of brown bears defined by mitochondrial DNA sequence analysis ($n = 455$; Waits 1996).

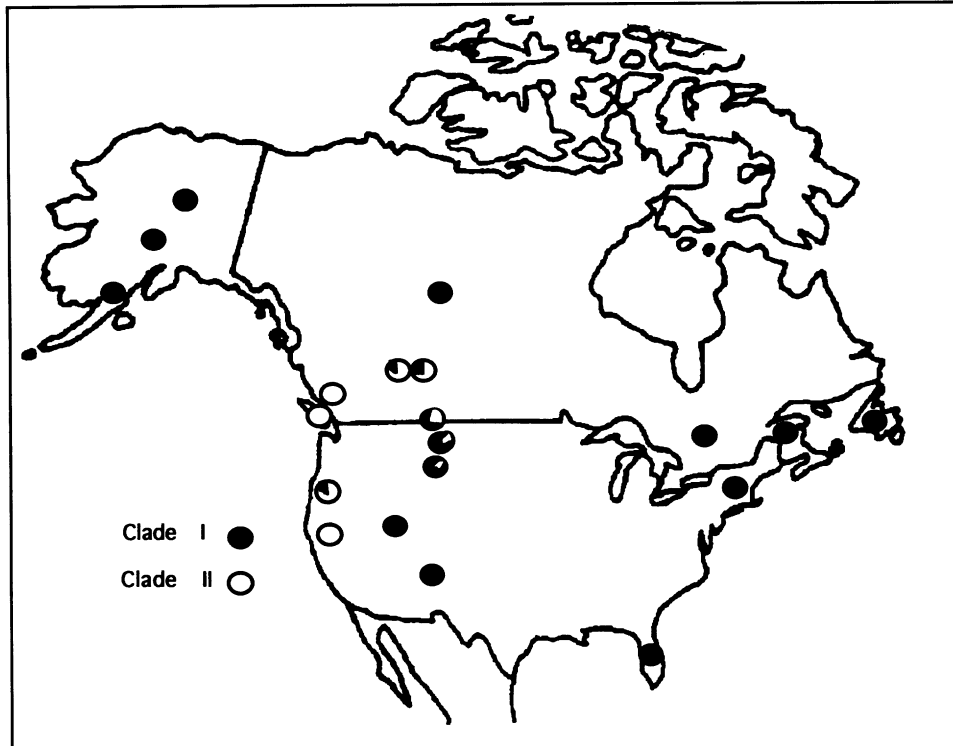


Fig. 2. Geographic distribution of 2 clades (genetic groups) of the American black bear defined by mitochondrial DNA analysis ($n = 140$; from Wooding and Ward 1997, Byun et al. 1997). Each circle is a pie graph describing the proportion of clade I (black) and clade II (white) bears found in each geographic area.

chromosome. Numerous methods have been used to identify sex in mammalian species (Akane et al. 1991, 1992; Serrat and De Herreros 1993). Similar methods have been used to identify sex in polar bears (Amstrup et al. 1993), brown bears (Taberlet et al. 1993), American black bears (Woods et al. 1999), and sun bears (Wasser et al. 1997).

Another application of molecular genetics to forensic studies is identifying individuals. Twenty nuclear DNA microsatellite loci have been identified for bears (Paetkau and Strobeck 1994, Paetkau et al. 1995, Taberlet et al. 1997), and genotypic data from 6 to 10 loci are generally sufficient to identify individual bears except on island populations with low heterozygosity (Fig. 3). There are 2 main uses for individual identification in the conservation and management of bear populations: (1) identifying illegally killed animals for law enforcement, and (2) determining the number of individuals sampled using non-invasive sampling of hair and scat. Identifying illegally killed individuals using molecular genetic techniques is frequently used in Canada (J. Coffin, University of Alberta, Edmonton, Alberta, Canada, personal communication, 1998) and the United States (S. Fain, U.S. Fish and Wildlife Service, Ashland, Oregon, USA, personal communication, 2000). Genetic analyses of scat and hair collected in the field have been used to obtain minimum population estimates, mark-recapture esti-

mates, and general home range estimates in brown bear populations (see section entitled Non-invasive Genetic Sampling).

POPULATION GENETIC STRUCTURE AND GENE FLOW

Genetic structure of a population is defined by the geographic distribution of genetic diversity within a species and describes the genetic relationships between individuals that are distributed across the landscape. This complex area of study can be examined at many levels. For example, we can examine the historical population genetic structure across the range of a species using mtDNA genetic analyses or we can examine breeding units within a geographic region such as the Yellowstone ecosystem. For some geographic regions, such as in Alaska and the Canadian arctic, it is difficult to delineate where one population starts and another begins. Numerous studies have addressed patterns of historical population genetic structure using mtDNA genetic analyses in brown bears (Cronin 1993, Randi et al. 1994, Taberlet and Bouvet 1994, Kohn et al. 1995, Talbot and Shields 1996b, Waits 1996, Waits et al. 1998b), American black bears (Cronin et al. 1991, Byun et al. 1997, Wooding and Ward 1997), and polar bears (Cronin et al. 1991).

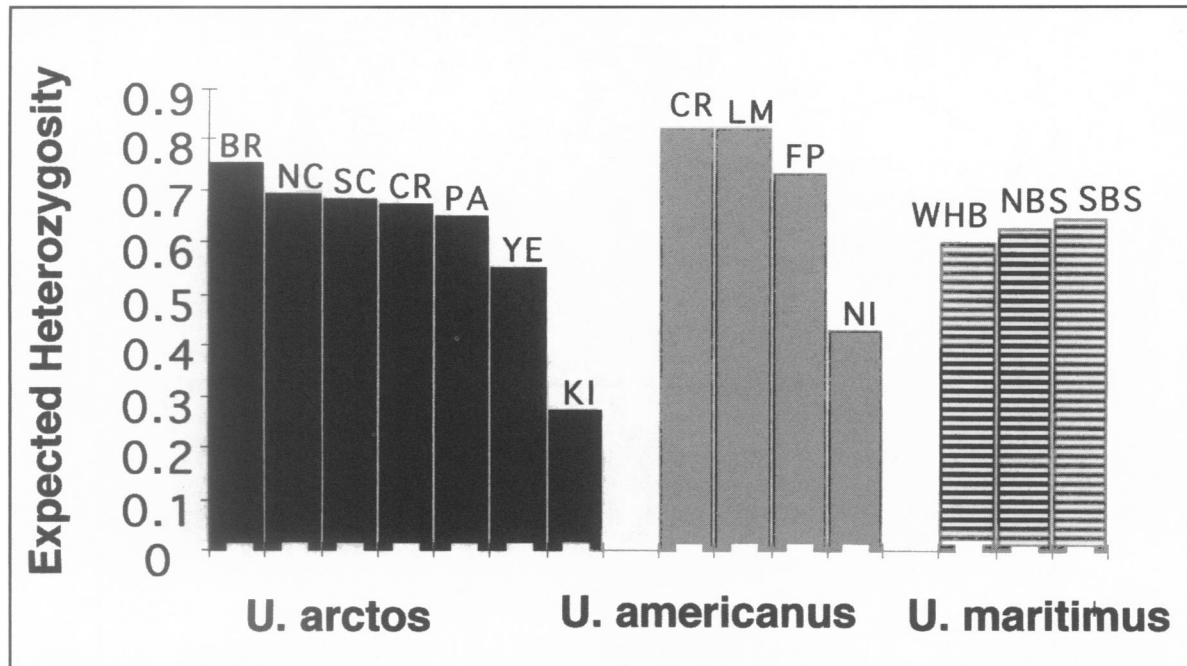


Fig. 3. Average expected heterozygosity of brown, American black, and polar bear populations estimated using 8 nuclear DNA microsatellite loci (from Paetkau and Strobeck 1998, Paetkau et al. 1995, Paetkau et al. 1998b). Abbreviations (sample sizes): BR — Brooks Range (153); NC — Northern Continental Divide Ecosystem (51); SC — Scandinavia (328); CR — Canadian Rockies (55); PA — Paulatuk (58) NWT; YE — Yellowstone Ecosystem (54); KI — Kodiak Island (34); LM — La Mauricie National Park (32); FP — Fundy National Park (11); NI — Newfoundland Island (23); WHB — West Hudson Bay (30); NBS — North Beaufort Sea (30); SBS — South Beaufort Sea (22).

One of the major goals of such studies is to identify evolutionary significant units (ESUs) to provide a guide for prioritizing populations for conservation and for transplantation or reintroduction efforts (Ryder 1986; Moritz 1994, 1995). ESUs are populations that are genetically distinct because they have evolved independently for many generations. The results from comprehensive studies in brown bears (Waits 1996) and American black bears (Byun et al. 1997, Wooding and Ward 1997) are summarized in Figures 1 and 2, respectively. mtDNA analyses of the polar bear have not revealed any population structure. Preliminary analyses of the sloth bear and sun bear (S. Fain and L. Waits unpublished data) suggest that such structure may exist, but additional analyses of samples from a broader geographic range are necessary. It is important to note that these studies reflect female population structure only; thus, additional analyses using nuclear or Y chromosome markers are necessary to understand the complete population structure.

Nuclear DNA microsatellite data also can be used to address questions of population structure and gene flow, and these data sets provide information concerning male and female gene flow. Using 8 highly polymorphic microsatellite loci, Paetkau et al. (1995) observed sig-

nificant differences in allele frequencies among 4 polar bear populations collected in the Canadian Arctic, indicating that population structure does exist. Additionally, assignment tests based on an individual's 8-locus genotype placed individuals in the correct region 94% of the time and in the correct population 60% of the time.

Historical population structure identified in the mtDNA analyses also has been examined using nuclear DNA microsatellite markers, and some interesting differences were detected. mtDNA analyses suggested that coastal Alaska brown bears and the bears of the Admiralty, Baranof, and Chichagof islands have not interbred (Talbot and Shields 1996b, Waits et al. 1998b), but nuclear DNA microsatellite data indicate that gene flow (presumably male-mediated) has occurred (Paetkau et al. 1998a). Similarly, mtDNA analyses indicated that gene flow was not occurring between the southern subpopulation of brown bears and three northern subpopulations in Scandinavia (Taberlet and Bouvet 1994, Taberlet et al. 1995), yet nuclear DNA data indicated that gene flow has occurred (Waits et al. 2000). In contrast, nuclear DNA microsatellite analyses indicate that the Kodiak Island population has been isolated from the mainland population since the last glaciation (Paetkau et al. 1998a,

1998*b*), yet mtDNA analyses did not detect this isolation (Talbot and Shields 1996*b*, Waits et al. 1998*b*). These results demonstrate the importance of addressing questions of population genetic structure using different types of genetic markers. This also demonstrates that the classification of mtDNA clades as ESUs without considering the morphological, behavioral, or nuclear DNA data may be premature.

GENETIC DIVERSITY

Molecular genetic analyses can play a major role in bear conservation by addressing questions concerning loss of genetic diversity in isolated populations. Genetic diversity levels are generally compared among populations using the average number of alleles per locus and average expected heterozygosity. Concerns with genetic diversity have both short- and long-term implications for conservation and management. A short-term concern is that inbreeding depression will cause fitness problems that will threaten the immediate survival of the population. Also, long-term population viability and evolutionary potential will decrease as genetic diversity decreases. These concerns are particularly important for bears because populations generally have low densities with small effective population sizes compared to other mammals.

Early attempts to assess genetic diversity in bear populations were largely ineffective because of the low levels of diversity detected using protein and mtDNA markers (Allendorf et al. 1979, Larsen et al. 1983, Cronin et al. 1991). Recent analyses of nuclear DNA microsatellite loci in brown bears (Paetkau et al. 1998*b*; Waits et al. 2000), polar bears (Paetkau et al. 1995, Paetkau et al. 1998*a*), and the American black bear (Paetkau and Strobeck 1994, 1998) have revealed much higher levels of genetic diversity, allowing evaluations of genetic variation in bear populations at higher levels of resolution. These data were collected using the same set of 8 loci; thus, 2 indicators of diversity, average number of alleles and average expected heterozygosity, can be compared across populations and species (Fig. 3). Expected heterozygosity is the probability of obtaining 2 different alleles when sampling 2 alleles from a particular locus at random from the population (Nei 1978).

The results from these analyses indicate that genetic diversity at the species level can be summarized as follows: on average American black bear populations are more diverse than brown bear populations, which are more diverse than polar bear populations (Paetkau and Strobeck 1998). These differences may reflect differences in evolutionary age, density, abundance of each species, as well as other life history traits. Within species, levels

of genetic diversity may depend greatly on connectivity to other bear populations and degree of human impact. Thus, maintaining current levels of genetic diversity in the face of increasing human development will require corridors for gene flow or genetic augmentation through transplanting animals (Paetkau et al. 1998*b*, Craighead et al. 1998). The extremely low levels of genetic diversity detected on Kodiak Island are interesting because this population has apparently persisted and thrived in isolation for approximately 1,000 bear generations. However, the persistence of 1 brown bear population with low genetic diversity does not mean that any brown bear population can thrive under such conditions. The loss of genetic diversity on Kodiak Island represents a loss of genetic options for response to future environmental change, and each brown bear population will have a different combination of environmental challenges to face. From a sample size of one population, it is difficult to predict such persistence in ecosystems such as Kodiak Island, and it is impossible to extrapolate these results to the vast majority of brown bear populations that inhabit drastically different ecosystems.

PATERNITY–MATERNITY STUDIES

Long-term conservation and management of bears requires an understanding of the reproductive success and mating strategies of males and females. Estimates of female reproductive success are possible through systematic collection of demographic data and tracking animals using radiotelemetry. However, assessment of male reproductive success was not possible until the development of DNA fingerprinting techniques. Using these techniques, it is now possible to identify the father of known mother–offspring pairs and to identify parents of unmarked offspring. The first study of male reproductive output and success in brown bears was completed in the Western Brooks Range of arctic Alaska (Craighead et al. 1995). This study demonstrated that a large number of males were producing offspring, and no male was siring more than 11% of the known offspring (Fig. 4). Additionally, many fathers (37%) were unsampled despite 6 years of intensive field work, suggesting that the effective population size is larger than indicated by field data. Craighead et al. (1995) also identified 1 unequivocal and 3 putative cases of multiple paternity in 30 litters. Results from the Brooks Range can be compared to a recent 10-year study of reproductive success in Scandinavia (Waits unpublished data) where fewer males produced more of the offspring (Fig. 4), and one male sired as many as 22% of all offspring. Multiple paternity also seems to occur at a lower frequency in

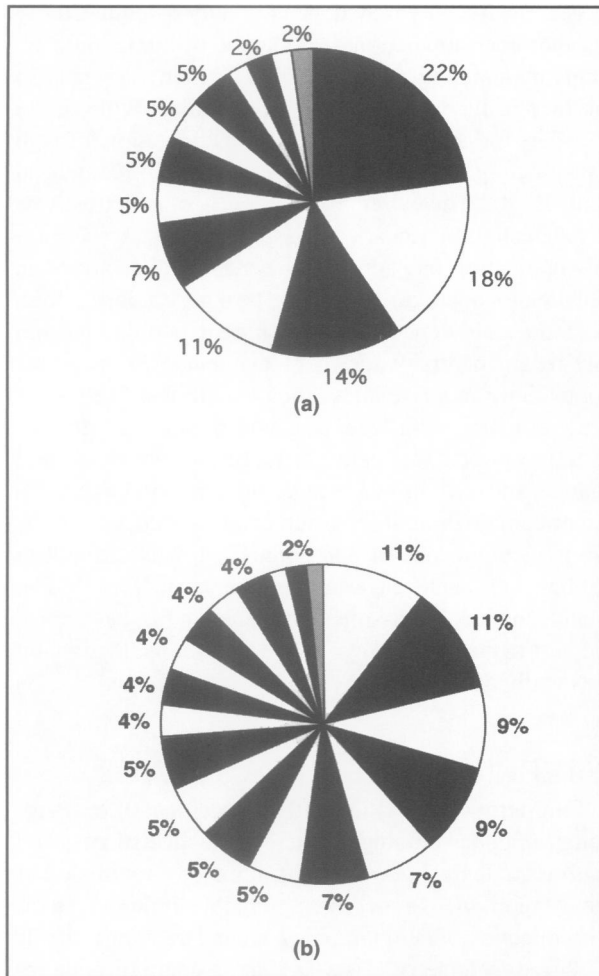


Fig. 4. Pie graphs of male reproductive output in 2 brown bear populations. Each slice of the pie represents a different male in the population: a) Percent of cubs ($n=62$) sired by 13 males in the southern-most subpopulation in Scandinavia; b) Percent of cubs ($n=57$) sired by 19 males in the Brooks Range population of Alaska (Craighead et al 1995).

Scandinavia (1 unequivocal and 0 putative cases out of 31 litters) than arctic Alaska. Age of first reproduction for male brown bears was 3 to 5 years earlier in Scandinavia than in arctic Alaska. Scandinavian data also indicated that an approximately equal number of males and females produced offspring. Genetic analyses of male and female reproductive success in bear populations is a relatively untapped source of data that holds great promise to answer important questions concerning mating strategies and effective population sizes.

NON-INVASIVE GENETIC SAMPLING

One promising application of molecular genetic techniques to bear research is non-invasive genetic sampling.

Recent studies have obtained DNA samples from hair snags (Woods et al. 1996, 1999; Foran et al. 1997a), shed hairs (Morin and Woodruff 1992, Taberlet et al. 1993, Morin et al. 1994, Taberlet et al. 1997), and feces (Höss et al. 1992, Constable et al. 1995, Gerloff et al. 1995, Kohn et al. 1995, Foran et al. 1997b, Kohn and Wayne 1997, Paxinos et al. 1997, Reed et al. 1997, Taberlet et al. 1997, Wasser et al. 1997) collected in the field. An array of genetic markers can then be used to address questions concerning population structure, gene flow, relatedness, sex identification, and individual determination. The potential of using non-invasive DNA collection methods to obtain genetic samples from rare and elusive species or populations or individuals with a particular behavior, such as feeding on livestock or moths, is particularly exciting. Such samples can provide genetic data previously discussed plus minimum population estimates (Taberlet et al. 1997), mark-recapture population estimates (Woods et al. 1999), and home range estimates (Taberlet et al. 1997). In one recent application, Taberlet et al. (1997) determined the minimum number of brown bears remaining in the French Pyrenees and estimated home ranges for males and females. Although the methods hold much promise, these methods are technically difficult, and only a few studies have carefully examined limitations and problems such as genotyping errors (Gerloff et al. 1995, Taberlet et al. 1996, Goossens et al. 1998, Taberlet and Waits 1998) and violations of assumptions (Waits unpublished data). Thus, in the promising field of non-invasive genetic sampling, researchers should rigorously test assumptions, identify limitations, and proceed with caution before implementing large-scale studies or drawing management conclusions.

CONCLUSIONS AND FUTURE DIRECTIONS

We are just beginning to explore applications of molecular genetics to bear research, conservation, and management. Tremendous progress has been made in the field of bear genetics, but there is much left to explore. The range of genetic diversity and intraspecific phylogeography needs to be evaluated for the giant panda (*Ailuropoda melanoleuca*), Andean (spectacled) bear (*Tremarctos ornatus*), sun bear, sloth bear, and Asiatic black bear (*U. thibetanus*). Using genetic techniques, we can now address questions such as: What is the phylogenetic placement of extinct species such as the giant short-faced bear (*Arctodus simus*)? How do bear mating strategies differ at the intra- and interspecific levels? How does social organization such as congregation at salmon (*Oncorhynchus* spp.) streams affect reproductive success

of males? What is the degree of relatedness among individuals within a population that perform a specific behavior such as feeding on clover (*Trifolium* spp.) patches? How do historical levels of genetic diversity in threatened populations compare to current levels? The possibilities are exciting and stimulating, but additional progress will require more than developing new genetic techniques and analytical methods. To realize the full potential of genetic applications to bear research, conservation, and management, there must continue to be an open and cooperative interchange of ideas, skills, and knowledge among bear biologists, ecologists, and geneticists.

LITERATURE CITED

- AKANE, A., S. SEKI, H. SHIONO, H. NAKAMURA, M. HASEGAWA, M. KAGAWA, K. MATSUBARA, Y. NAKAHORI, S. NAGAFUCHI, AND Y. NAKAGOME. 1992. Sex determination of forensic samples by dual PCR amplification of an X-Y homologous gene. *Forensic Science International* 52:143–148.
- _____, H. SHIONO, K. MATSUBARA, Y. NAKAHORI, S. SEKI, S. NAGAFUCHI, M. YAMADA, AND Y. NAKAGOME. 1991. Sex identification of forensic specimens by polymerase chain reaction (PCR): two alternative methods. *Forensic Science International* 49:81–88.
- ALLENDORF, F.W., F.B. CHRISTIANSEN, T. DOBSON, W.F. EANES, AND O. FRYDENBERG. 1979. Electrophoretic variation in large mammals. 1. The polar bear, *Thalarctos maritimus*. *Heredity* 91:19–22.
- AMSTRUP, S.C., G.W. GARDNER, M.A. CRONIN, AND J.C. PATTON. 1993. Sex identification of polar bears from blood and tissue samples. *Canadian Journal of Zoology* 71:2174–2177.
- AVISE, J.C. 1994. *Molecular markers, natural history and evolution*. Chapman and Hall, New York, New York, USA.
- BROWN, W.M., M.J. GEORGE, AND A.C. WILSON. 1979. Rapid evolution of animal mitochondrial DNA. *Proceedings of the National Academy of Sciences, USA* 76:1967–1971.
- BYUN, S.A., B.K. KOOP, AND T.E. REIMCHEN. 1997. North American black bear mtDNA phylogeography: implications for morphology and the Haida Gwaii glacial refugium controversy. *Evolution* 51:1647–1653.
- CONSTABLE, J.J., C. PACKER, D.A. COLLINS, AND A.E. PUSEY. 1995. Nuclear DNA from primate dung. *Nature* 373:393.
- CRAIGHEAD, F.L., M.E. GILPIN, AND E.R. VYSE. 1998. Genetic considerations for carnivore conservation in the Greater Yellowstone Ecosystem. Pages 110–125 in P. Karieva and S. Minta, editors. *Carnivores in ecosystems*. Yale University Press, New Haven, Connecticut, USA.
- CRAIGHEAD, L., D. PAETKAU, H.V. REYNOLDS, E.R. VYSE, AND C. STROBECK. 1995. Microsatellite analysis of paternity and reproduction in Arctic grizzly bears. *Journal of Heredity* 86:255–261.
- CRONIN, M.A. 1993. Mitochondrial DNA in wildlife taxonomy and conservation biology: cautionary notes. *Wildlife Society Bulletin* 21:339–348.
- _____, S.C. AMSTRUP, G.W. GARNER, AND E.R. VYSE. 1991. Interspecific and intraspecific mitochondrial DNA variation in North American bears (*Ursus*). *Canadian Journal of Zoology* 69:2985–2992.
- FAIN, S., P. JARRELL, C. MORSE, A. NICHOLSON, S. GILL, D. COLLINS, AND J. BALL. 1995. DNA analysis and wildlife law enforcement: Efforts to stop international commerce in bear products can benefit greatly by the valuable resources of zoo breeding programs. Page 94 in D.A. Rose and A.L. Gaski, editors. *Proceedings of the international symposium on the trade of bear parts for medicinal use, 9–11 September 1994, Seattle, Washington, USA*. TRAFFIC USA/World Wildlife Fund, Washington, DC, USA.
- FORAN, D.R., K.R. CROOKS, AND S.C. MINTA. 1997b. Species identification from scat: an unambiguous genetic method. *Wildlife Society Bulletin* 25:835–839.
- _____, S.C. MINTA, AND K.S. HEINEMEYER. 1997a. DNA-based analysis of hair to identify species and individuals for population monitoring. *Wildlife Society Bulletin* 25:840–847.
- GERLOFF, U., C. SCHLÖTTERER, K. RASSMANN, I. RAMBOLD, G. HOHMANN, B. FRUTH, AND D. TAUTZ. 1995. Amplification of hypervariable simple sequence repeats (microsatellites) from excremental DNA of wild living bonobos (*Pan paniscus*). *Molecular Ecology* 4:515–518.
- GOOSSENS, B., L.P. WAITS, AND P. TABERLET. 1998. Hair samples as a source of DNA: reliability of dinucleotide microsatellite genotyping. *Molecular Ecology* 7:1237–1241.
- HÖSS, M., M. KOHN, S. PÄÄBO, F. KNAUER, AND W. SCHRÖDER. 1992. Excrement analysis by PCR. *Nature* 359:199.
- KOHN, M., F. KNAUER, A. STOFFELLA, W. SCHRÖDER, AND S. PÄÄBO. 1995. Conservation genetics of the European brown bear — a study using excremental PCR of nuclear and mitochondrial sequences. *Molecular Ecology* 4:95–103.
- _____, AND R.K. WAYNE. 1997. Facts from feces revisited. *Trends in Ecology and Evolution* 12:223–227.
- LARSEN, T., H. TEGELSTROM, R.K. JUNEJA, AND M.K. TAYLOR. 1983. Low protein variability and genetic similarity between populations of the polar bear (*Ursus maritimus*). *Polar Research*. 1:97–105.
- MORIN, P.A., J.J. MOORE, R. CHAKRABORTY, L. JIN, J. GOODALL, AND D.S. WOODRUFF. 1994. Kin selection, social structure, gene flow, and the evolution of chimpanzees. *Science* 265:1193–1201.
- _____, AND D.S. WOODRUFF. 1992. Paternity exclusion using multiple hypervariable microsatellite loci amplified from nuclear DNA of hair cells. Pages 63–81 in R.D. Martin, A.F. Dixson and E.J. Wickings, editors. *Paternity in primates: genetic tests and theories*. Karger, Basel, Switzerland.
- MORITZ, C. 1994. Defining “evolutionarily significant units” for conservation. *Trends in Ecology and Evolution* 9:373–375.
- _____. 1995. Uses of molecular phylogenies for conservation. *Philosophical Transactions of the Royal Society of London Series B* 349:113–118.
- NEI, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*

- 89:583–590.
- PAETKAU, D., W. CALVERT, I. STIRLING, AND C. STROBECK. 1995. Microsatellite analysis of population structure in Canadian polar bears. *Molecular Ecology* 4:347–354.
- _____, G.F. SHIELDS, AND C. STROBECK. 1998a. Gene flow between insular, coastal and interior populations of brown bears in Alaska. *Molecular Ecology* 7:1282–1292.
- _____, AND C. STROBECK. 1994. Microsatellite analysis of genetic variation in black bear populations. *Molecular Ecology* 3:489–495.
- _____, AND _____. 1996. Mitochondrial DNA and the phylogeography of Newfoundland black bears. *Canadian Journal of Zoology* 74:192–196.
- _____, AND _____. 1998. Ecological genetic studies of bears using microsatellite analysis. *Ursus* 10:299–306.
- _____, L.P. WAITS, L. CRAIGHEAD, P. CLARKSON, AND C. STROBECK. 1998b. Dramatic variation in genetic diversity across the range of North American brown bears. *Conservation Biology* 12:418–429.
- PARKER, P.G., A.A. SNOW, M.D. SCHUG, G.C. BOOTON, AND P.A. FUERST. 1998. What molecules can tell us about populations: choosing and using a molecular marker. *Ecology* 79:361–382.
- PAXINOS, E., C. MCINTOSH, K. RALLS, AND R. FLEISCHER. 1997. A noninvasive method for distinguishing among canid species: amplification and enzyme restriction of DNA from dung. *Molecular Ecology* 6:483–486.
- RANDI, E., L. GENTILE, G. BOSCAGLI, D. HUBER, AND H.U. ROTH. 1994. Mitochondrial DNA sequence divergence among some west European brown bear (*Ursus arctos* L.) populations. Lessons for conservation. *Heredity* 73:480–489.
- REED, J.Z., D.J. TOLLIT, P.M. THOMPSON, AND W. AMOS. 1997. Molecular scatology: the use of molecular genetic analysis to assign species, sex, and individual identity to seal faeces. *Molecular Ecology* 6:225–234.
- RYDER, O.A. 1986. Species conservation and systematics: the dilemma of subspecies. *Trends in Ecology and Evolution* 1:9–10.
- SERRAT, A., AND A G. DE HERREROS. 1993. Determination of genetic sex by PCR amplification of Y-chromosome-specific sequences. *Lancet* 341:1593.
- SERVHEEN, C. 1990. The status and conservation of the bears of the world. International Conference of Bear Research and Management. Monograph Series 2.
- TABERLET, P., AND J. BOUVET. 1994. Mitochondrial DNA polymorphism, phylogeography, and conservation genetics of the brown bear (*Ursus arctos*) in Europe. *Proceedings of the Royal Society London B* 255:195–200.
- _____, J.J. CAMARRA, S. GRIFFIN, E. UHRES, O. HANOTTE, L.P. WAITS, C. PAGANON, T. BURKE, AND J. BOUVET. 1997. Non-invasive genetic tracking of the endangered Pyrenean brown bear population. *Molecular Ecology* 6:869–876.
- _____, S. GRIFFIN, B. GOOSSENS, S. QUESTIAU, V. MANCEAU, N. ESCARAVAGE, L.P. WAITS, AND J. BOUVET. 1996. Reliable genotyping of samples with very low DNA quantities using PCR. *Nucleic Acids Research* 24:3189–3194.
- _____, H. MATTOCK, C. DUBOIS-PAGANON, AND J. BOUVET. 1993. Sexing free-ranging brown bears *Ursus arctos* using hairs found in the field. *Molecular Ecology* 2:399–403.
- _____, J.E. SWENSON, F. SANDEGREN, AND A. BJÄRVALL. 1995. Localization of a contact zone between two highly divergent mitochondrial DNA lineages of the brown bear (*Ursus arctos*) in Scandinavia. *Conservation Biology* 9:1255–1261.
- _____, AND L.P. WAITS. 1998. Non-invasive genetic sampling (correspondance). *Trends in Ecology and Evolution* 13:26–27.
- TALBOT, S.L., AND G.F. SHIELDS. 1996a. A phylogeny of the bears (Ursidae) inferred from complete sequences of three mitochondrial DNA genes. *Molecular Phylogenetics and Evolution* 5:567–575.
- _____, AND G.F. SHIELDS. 1996b. Phylogeography of brown bears (*Ursus arctos*) of Alaska and paraphyly within the Ursidae. *Molecular Phylogenetics and Evolution* 5:477–494.
- WAITS, L.P. 1996. A comprehensive molecular study of the evolution and genetic variation of bears. PhD Dissertation, University of Utah, Salt Lake City, Utah, USA.
- _____, D. PAETKAU, C. STROBECK, AND R.H. WARD. 1998a. A comparison of genetic diversity in North American brown bears. *Ursus* 10:307–314.
- _____, S. TALBOT, R.H. WARD, AND G.F. SHIELDS. 1998b. Mitochondrial DNA phylogeography of the North American brown bear and implications for conservation. *Conservation Biology* 12:408–417.
- _____, D. ROON, AND M. MURPHY. 1999. Non-invasive genetic sampling of bear populations. *International Bear News* 8:23–25.
- _____, P. TABERLET, J. SWENSON, P. SANDEGREN, AND R. FRANZEN. 2000. Nuclear DNA microsatellite analysis of genetic diversity and gene flow in the Scandinavian brown bear (*Ursus arctos*). *Molecular Ecology* 9:421–432.
- WASSER, S.K., C.S. HOUSTON, G.M. KOEHLER, G.G. CADD, AND S.R. FAIN. 1997. Techniques for application of faecal DNA methods to field studies of Ursids. *Molecular Ecology* 6:1091–1097.
- WOODING, S., AND R.H. WARD. 1997. Phylogeography and pleistocene evolution in the North American Black Bear. *Molecular Biology and Evolution* 14:1096–1113.
- WOODS, J.G., B. MCLELLAN, D. PAETKAU, C. STROBECK, AND M. PROCTOR. 1996. DNA fingerprinting applied to mark-recapture bear studies. *International Bear News* 5:9–10.
- _____, D. PAETKAU, D. LEWIS, B.N. MCLELLAN, M. PROCTOR, AND C. STROBECK. 1999. Genetic tagging of free-ranging black and brown bears. *Wildlife Society Bulletin* 27:616–627.
- ZHANG, Y.-P., AND O.A. RYDER. 1993. Mitochondrial DNA sequence evolution in the Arctoidea. *Proceedings of the National Academy of Sciences, USA* 90:9557–9561.
- _____, AND _____. 1994. Phylogenetic relationships of bears (the Ursidae) inferred from mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution* 3:351–359.