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Abstract: Microsatellites are short tandem arrays of simple DNA sequences (e.g., $[AC]_n$, where $n \geq 10$), which are often highly polymorphic among individuals. These repetitive elements are rapidly becoming the molecular genetic marker of choice for genetic mapping, parentage analyses, and fundamental population genetics. We have isolated more than 50 microsatellite loci from the whooping crane (*Grus americana*). The microsatellites cloned from whooping cranes are unusually short and infrequent in comparison to other birds and especially so in comparison to mammals or reptiles. However, at least 13 of the 20 primer pairs developed amplify polymorphic loci. Paternity for 2 individuals sharing the same mother and 5 potential fathers was established by investigating 6 polymorphic loci. Additionally, the primers developed yield amplicons of the expected size from other crane species with greater than 90% success. Therefore, the markers we have developed will be useful for addressing questions about the captive management, population structure, and phylogeography of all cranes.

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Managers of captive and endangered species are increasingly aware of the deleterious effects associated with inbreeding and the overall loss of genetic diversity (Ralls and Ballou 1979, Mirande et al. 1991). The use of molecular techniques to study the genetics of endangered species provides useful information for managers attempting to maintain genetic diversity in small isolated populations (Schoenwald-Cox et al. 1983). Information on the genetic structure of wild populations allows managers to mimic that structure in captive breeding programs and reintroduction efforts. Knowledge of the genetic distinctiveness and phylogenetic structure of populations, subspecies, and species promotes informed decisions about the relative importance of those entities when deciding how to allocate scarce resources to conserve threatened or endangered groups (May 1990, Geist 1992).

The crane family, Gruidae, consists of 15 living species (Walkinshaw 1973), 7 of which are endangered or have endangered forms (U.S. Fish and Wildlife Service 1994). It is likely that habitat destruction will continue to threaten many of these birds. Captive propagation from small founding populations has been a crucial component for the rescue of the whooping crane and Mississippi sandhill crane (*Grus canadensis pulla*) from the brink of extinction (Doughty 1989, Gee et al. 1992). Assessment and conservation of the genetic diversity of these and other cranes will play an important role in their successful management and preservation.

Many molecular genetic techniques have been used to assay genetic variation within and among cranes. Isozymes have been used to study genetic variability and phylogenetic relationships in wild and captive populations of cranes (Dessauer et al. 1992). Jarvi et al. (1995) used serological techniques to determine Major Histocompatibility Complex (MHC) genotypes of Florida sandhill cranes (*G. c. pratensis*). Similar serological studies have been completed for the Mississippi sandhill and whooping cranes (S. Jarvi, pers. commun.). DNA fingerprinting has been used to study genetic variation and determine paternity of captive whooping cranes (Longmire et al. 1992). Several studies have used DNA-DNA hybridization to investigate the phylogeny and evolution of cranes (Ingold et al. 1989; Krajewski 1989, 1990). Most recently, Krajewski and Fetzer (1994) sequenced most of the mitochondrial DNA (mtDNA) cytochrome b gene from 1 individual of all crane species for reconstruction of their phylogeny. Several additional studies assaying mtDNA variation within and among species and subspecies have been completed (C. Krajewski, pers. commun.).

The previous genetic analyses have provided much useful information, but additional data are needed to answer remaining questions. For instance, the techniques used previously are limited in their ability to provide precise estimates for several measures of genetic diversity (e.g., allelic diversity, heterozygosity, relatedness, and population differentiation [Lewin 1989]) that would be valuable for management of captive and wild populations of cranes. Furthermore, techniques that avoid handling or invasive sampling would be desirable for endangered cranes. Thus, it would be advantageous to have and use molecular genetic

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markers that (1) provide discrete, single locus data, (2) are highly variable (with many polymorphic loci and many alleles per locus), (3) are present throughout the genome in large numbers, (4) can be screened relatively quickly and inexpensively, and (5) can make use of small and non-traditional tissue samples (such as feathers, droppings, and museum specimens). Microsatellite loci have all these characteristics (Ellegren 1992).

Microsatellites are tandem repeats of simple DNA sequence (1–6 base pairs [bp], e.g., ACAC...AC = $[AC]_n$ where n usually ranges from 10 to 40 [Tautz 1993, Charlesworth et al. 1994]). These small repetitive elements occur more-or-less randomly throughout the genomes of most eukaryotes (Tautz and Renz 1984, Stallings et al. 1991). The total number of unique microsatellite motifs possible is quite small, because apparently distinct motifs are identical, e.g., a repeat of AC is identical to a repeat of CA and, by examining the complementary DNA strand, GT or TG (Fig. 1, Table 1). Microsatellites are related to minisatellites and satellite DNA in that all are tandem arrays of repeated DNA sequence. The size of the repeat unit increases from microsatellites to minisatellites. The number of repeat units usually increases from microsatellite to minisatellite to satellite DNA (Tautz 1993).

In the human genome, microsatellites composed of AC repeats are the most common and are estimated to occur every 30,000 bp (30 kb) in euchromatic regions (Stallings et al. 1991). When all repeat motifs are considered, a microsatellite of some type occurs about every 6 kb (Beckman and Weber 1992). Thus, the microsatellites themselves are a type of interspersed repetitive DNA. Any particular microsatellite can be identified by using the unique single-copy DNA that flanks it. Specific microsatellite loci may be amplified quickly and easily by using the Polymerase Chain Reaction (PCR) with primers that are complementary to the unique single-copy DNA flanking the repetitive array (Fig. 1). More than 8,000 such loci have been identified and characterized in the human genome (Hudson et al. 1995).

Microsatellites tend to mutate by the gain or loss of repeat units at rates of 10^{-5} to 10^{-2} mutations per generation (Di Rienzo et al. 1994). These rates are many orders of magnitude higher than the mutation rate of single-copy DNA, which is about 10^{-9} mutations per year per bp (Li and Graur 1991:69-71). The high mutation rate at microsatellite loci is believed to be primarily due to slipped-strand DNA synthesis, resulting in deletions or additions of repeat units (Levinson and Gutman 1987, Strand et al. 1993). The high mutation rate generates many alleles, which results in a high degree of polymorphism at many loci in large populations. If PCR products (amplicons) for a particular microsatellite locus are amplified from chromosomes carrying alleles with different copy numbers of repeats, the amplicons will differ in size (due to the difference in the number of repeat units). The size differences among amplicons (alleles) can then be detected easily by using standard electrophoretic techniques for DNA (Weber and May 1989, Litt and Luty 1989, Tautz 1989, Ziegle et al. 1992, Morin and Smith 1995).

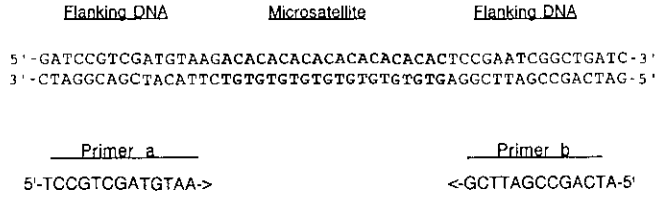

Fig. 1. The sequence of a typical AC microsatellite locus with a pair of oligonucleotide primers that could be used for PCR amplification.

Table 1. List of all unique mono- , di-, and tri-nucleotide repeat motifs. The motifs which make the most useful molecular markers are in bold. The most abundant repeat motifs in vertebrates are underlined.

Mono[a]	Di	Tri
A	**AC**	**AAC**
C	**AG**	**AAG**
	AT[b]	**AAT**
	CG[b]	**ACC**
		ACG
		ACT
		AGC
		AGG
		ATC
		CCG

[a] Alleles of mononucleotide repeats are not easily separated.
[b] The dinucleotide repeats AT and CG are self-complementary and are therefore difficult to isolate.

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METHODS

When we first considered developing microsatellites from

whooping cranes during the summer of 1992, very little was known about microsatellites in birds. The only publication that identified avain microsatellites (Ellegren 1991) described only 2 loci. Our goal was to identify about 50 microsatellite loci from whooping cranes. Our first priority was to estimate the frequency of microsatellites in birds to assess the magnitude of this task. We began by searching all sequences in the Genbank database entered before sequences specifically isolated to contain microsatellites reached large numbers. The vast majority of avian sequences in Genbank at that time were from domestic chicken (*Gallus gallus*).

Samples and DNA Isolation

With the cooperation of personnel of the U.S. Fish and Wildlife Service, the National Biological Service, the International Crane Foundation (ICF), the Canadian Wildlife Service, the Louisiana State University Museum of Natural Science, and S. Jarvi and H. Dessauer, we obtained samples from almost all captive and many wild whooping cranes. Samples from all known crane species were generously provided by the ICF (blood) or C. Krajewski (DNA). Additionally, with the cooperation of many museums, we have obtained samples of skin (~3 cm^2) and, in some cases, a few contour feathers, from more than 100 whooping cranes collected prior to their population bottleneck from throughout the original breeding and migratory range. These samples represent about one-third of all whooping crane specimens in museums (cf. Hahn 1954) and most of the specimens with age and locality data. The museum samples form the basis of continuing research into the amount of genetic variation actually lost during the whooping crane bottleneck.

Prior to 1995, genomic DNA was usually isolated from blood and tissue by standard phenol/chloroform/isoamyl alcohol (PCI) extraction and ethanol precipitation (Sambrook et al. 1989:9.17-9.19). Because bird blood is nucleated, the blood was treated as "tissue," except that it was not homogenized. During 1995, genomic DNA was usually isolated from blood by using guanidine thiocyanate and silica (cf. Boom et al. 1991). The exact protocol used can be downloaded from the LMS Internet server (e.g., with Netscape) by using the URL: ftp://onyx.si.edu/protocols/TSilicaDNA.rtf. The advantages and disadvantages of the silica technique are enumerated in the protocol. Genomic DNA was isolated from museum skins and feathers using a protocol modified from Pääbo et al. (1988). The major modification includes use of a digestion buffer containing 1 mM Ca^{2+} and no EDTA (see Sambrook et al. 1989:B.16). This protocol can also be obtained from the LMS Internet server under the filename MuseumDNA.rtf.

Isolation of Microsatellite Loci

We used small insert plasmid libraries to isolate microsatellites from whooping and sandhill cranes (cf. Hughes and Queller 1993, Ostrander et al. 1992). Briefly, this approach involves digesting total genomic DNA from the species of interest into small fragments with a restriction enzyme. Fragments which were sufficiently long to contain whole microsatellite loci, but still short enough to be conveniently sequenced (300–700 bp), were separated from all other fragments via size selection following agarose gel electrophoresis. Following elution from the agarose, the fragments were then ligated into a plasmid vector and used to transform bacteria. Each resulting bacterial colony contained a random fragment of crane DNA which was screened for the presence of microsatellites by hybridization with radioactively labeled oligonucleotides representing 1 or more simple sequence repeat motifs. Plasmid DNA from the positive colonies was isolated and sequenced. Oligonucleotide primers complementary to the unique sequences flanking the microsatellite were designed and synthesized (Fig. 1). The primers were then used in PCR-based assays as described above. In this paper, we refer to the collection of genomic DNA fragments ligated into plasmids and the resulting transformed colonies as a library. Subsequent collections of plasmids with crane DNA that were isolated from many bacterial colonies are called sub-libraries. Explicit (step-by-step) protocols used to obtain and score microsatellites may be downloaded from the LMS Internet server under the filename MsatManV6.rtf.

Random Libraries.—In random libraries, the microsatellite loci should be detected at a frequency similar to their frequency in the genome (number of colonies screened × average insert size per colony / number of colonies with microsatellites ≈ average number of bp between each microsatellite locus). Many different ligations were used to provide clones for the unenriched libraries.

Enriched Library.—Several library enrichment strategies have been devised which greatly increase the frequency of colonies with microsatellites among those plated and hence reduce the total number of colonies which must be screened (Ostrander et al. 1992, Kandpal et al. 1994, Pulido and Duyk 1994). We used a method derived from Ostrander et al. (1992) which uses the random library we had already made (see also Kunkel et al. 1987, Pulido and Duyk 1994:2.2.1). Briefly, the ligated plasmid vector + crane DNA was transformed into a strain of bacteria that was deficient for uracil DNA glycosylase (*ung*$^-$) and dUTPase (*dut*$^-$). These bacteria incorporated uracil in their DNA at a fraction of the positions (about 0.3%) normally occupied by thymine (Kunkel 1985). Single-stranded plasmid DNA was then

recovered from a large number of these bacterial colonies. This sub-library contains many copies of many different pieces of crane DNA, though each was now present in multiple copies. The single-stranded sub-library was subjected to second strand synthesis primed by an oligonucleotide composed of a simple sequence repeat motif. The primer selects only those plasmids which contain complementary repeats, and the newly synthesized (complementary) strands contain no uracil. The resulting double-stranded plasmids transform normal (*ung*$^+$) bacteria at a rate much higher than the remaining single-stranded plasmids which are destroyed by the bacteria because they contain uracil. Thus, sub-libraries were enriched for several simple repeats.

RESULTS

Genbank Searches

Birds belong to the Genbank group "Other Vertebrates." Although microsatellites were detected in Other Vertebrates about as often as in the groups Primates and Other Mammals, microsatellite motifs were detected in birds much less often than in the remainder of the Other Vertebrates. Microsatellites were detected about half as often in birds as in Primates or Other Mammals (Fig. 2). This result implied that avian microsatellites probably occurred in high enough frequency to proceed with isolation by using standard techniques (cf. Hughes and Queller 1993).

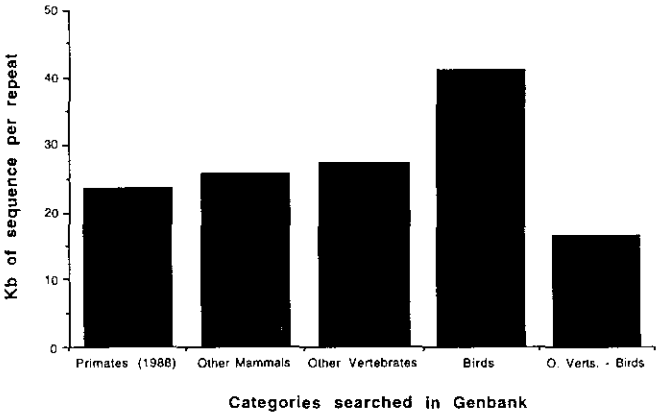

Fig. 2. The frequency of microsatellites in sequences deposited in the Genbank database prior to large-scale isolation of microsatellites (see Devereux 1989 for Genbank categories). The Primate category includes sequences deposited prior to September 1988 and had not been updated through May 1993 (release 79.0). All other categories include sequences deposited prior to October 1992 (release 73.1). "Other Mammals" excludes Primates and Rodents. Birds are included in the "Other Vertebrates" category.

Random Libraries

Our initial experiments estimated a frequency of AC microsatellites in only about 0.06% of all clones from the whooping cranes. If an average insert size of 500 bp is assumed, this frequency would imply that AC microsatellites occur about once every 800 kb. Thus, we estimated that we could isolate our goal of 50 microsatellite loci by screening about 100,000 colonies (about 10 times more than required for mammals). More than 100,000 colonies containing random pieces of whooping crane DNA were screened from 1992 to 1994. This effort, however, yielded only enough clones for 11 primer pairs to be designed.

We also screened a sample of about 10,000 colonies from sandhill cranes and red pandas (*Ailurus fulgens*) to ensure that the low frequency of microsatellites observed in whooping cranes was not an artifact of library preparation. The sandhill crane library yielded positive clones at about the same frequency as the whooping crane libraries. Microsatellites are much more common in the red panda genome with an estimated frequency of AC microsatellites of about 0.42% of all clones. Again, if an average insert size of 500 bp is assumed, this frequency would imply that AC microsatellites occur about once every 100 kb. Although we detected AC repeats in red pandas about half as frequently as we expected (based on the frequency detected in dogs [Ostrander et al. 1992, 1993]), the difference between the pandas and the cranes is similar to the difference detected between other mammals and birds (e.g., Crooijmans et al. 1993).

Subsequent screenings of additional colonies revealed that scaling up reduced efficiency of detection, thus there was a limit of how many colonies could be efficiently screened at once with our equipment. Although more than 100,000 colonies were plated, it is difficult to know how many were actually screened with our procedure. Thus, it was clear that this approach would not effectively yield the desired number of microsatellite loci. Therefore, we sought to obtain additional microsatellite loci by enrichment.

Enriched Sub-Library

About 150,000 colonies from the whooping crane library were subjected to enrichment. From the crane sub-library, 4,058 colonies were replated for screening by hybridization. Following colony hybridization, 439 (about 11%) of the colonies were picked as putative positives. This represents about a 180-fold enrichment relative to the random library.

Sequencing was attempted for a total of 180 clones from the enriched crane sub-library. Of those sequences, microsatellites were detected in a total of 60 unique clones.

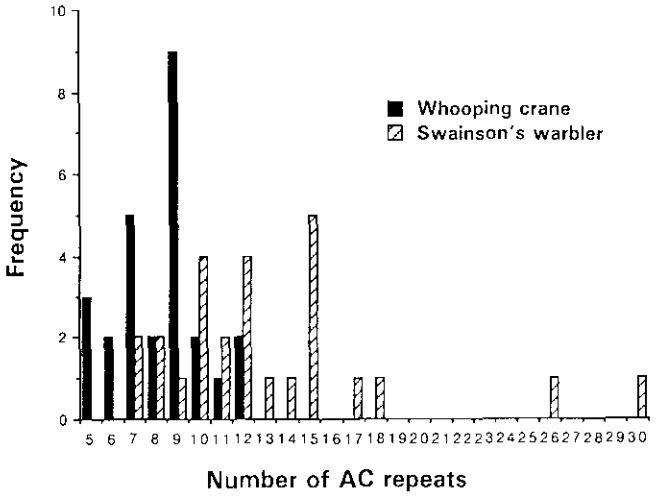

Fig. 3. Comparison of the distribution of the copy number of repeats in microsatellite clones from whooping crane and Swainson's warbler (*Limnothlypis swainsonii*) libraries enriched with use of $(TG)_{15}$. Warbler data are courtesy of K. Winker, G. Graves, and M. J. Braun, Smithsonian Institution.

An accurate determination of microsatellite length was made for 45 clones. The distribution of repeat lengths in the crane clones was dominated by microsatellites with less than 10 repeats, whereas all other libraries we have constructed and enriched from other species (avian, mammalian, and reptilian) are dominated by sequences with ≥10 repeats (e.g., Fig. 3). From these new crane sequences, 26 additional PCR primer pairs were synthesized (24 for AC repeats and 2 for AGG repeats).

Amplification of Microsatellites

PCR amplification conditions have been optimized for 20 of the 37 primer pairs synthesized to date. In an initial survey of cross-species utility, 4 primer pairs were tested on 4 *Grus*, 1 *Bugeranus*, and 1 *Anthropoides* species. Because almost all samples yielded amplicons of the expected size, DNA from at least 1 individual from each of the 15 crane species was subsequently tested for amplification by using 8 primer pairs (Table 2). Amplification was achieved in more than 90% of the species/primer pair combinations with the family Gruidae. However, only 3 of the 8 primer pairs supported amplification in other gruiform birds. Two of these 3 also supported amplification from birds of other orders and American alligators (*Alligator mississippiensis*) (Table 2). Whether these amplicons represent orthologous microsatellite loci in the other taxa remains to be determined, but the fact that they fall in the expected size range encourages optimism. Some loci were screened on acrylamide gels, which allows detection of heterozygotes and thus polymorphism. Polymorphic loci have been detected in red-crowned (*G. japonensis*), sarus (*G. antigone*), sandhill, and Siberian (*G. leucogeranus*) cranes.

The 20 optimized loci were also tested for polymorphism within a small sample of whooping cranes. Thirteen of these loci are polymorphic within a sample of 4 living individuals (Table 3). Genotypes were subsequently obtained for a sample of 16 whooping cranes for 6 of the polymorphic loci. This group contained a known breeding pair with 7 offspring and 2 captive-bred offspring with the same mother and a group of 5 potential fathers. Mendelian inheritance was observed within the known family for all 6 loci (Table 4). Paternity was unambiguously determined for both captive-bred offspring (Table 4). For example, whooping crane 74-001 is the only male not excluded as the potential sire of 85-001 by simply assessing genotypes at locus AGG-2. Initial tests on 4 loci have also shown that successful amplification of DNA isolated from 10 museum specimens could be achieved for about 50% of the specimen/locus pairs.

DISCUSSION

The overall frequency of microsatellites in cranes appears to be similar to that of most other birds (e.g., Croojimans et al. 1993; T. C. Glenn, unpubl. data). Because the frequency is low relative to most mammals, randomly searching small insert plasmid libraries in bacterial colonies is not recommended. Use of enrichment strategies (e.g., Ostrander et al. 1992, Kandpal et al. 1994) or large insert libraries (Margolis et al. 1993, Litt and Browne 1994, Rothuizen and van Raak 1994) is highly recommended when microsatellites are isolated from cranes or other birds.

The average length of the microsatellite loci detected in whooping cranes was quite short relative to other birds (Fig. 3, Crooijmans et al. 1993), mammals, or other vertebrates (e.g., Weber 1990; Serikawa et al. 1992; Ostrander et al. 1993; FitzSimmons et al. 1995; T. C. Glenn, unpubl. data). It is not known if this length bias is shared among other cranes, or is peculiar to whooping cranes. It is possible, though unlikely, that the bottleneck experienced by whooping cranes could have caused the observed bias. Characterization of loci from whooping cranes and additional loci isolated from non-bottlenecked crane species will be needed to elucidate this issue.

Advantages of Microsatellites

Once PCR primers have been designed for particular loci, amplicons are easily synthesized from multiple individuals. When the repeat count in the cloned allele is ≥10, the

Table 2. Ability of PCR primers developed from whooping cranes to amplify other species.

Group	Species[a]	Locus							
		AA-1	AC-1	AT/AC-1	AAC-1	ACC-1	ATC-1	AAGC-1	SH-AG-1[b]
Gruidae	Whooping	+	+	+	+	+	+	+	+
Gruidae	Red-crowned	+	+	+	+	+	+	+	+
Gruidae	Black-necked	+	+	+	+	+	-	+	+
Gruidae	Common	+	+	+	+	+	+	+	+
Gruidae	Hooded	+	+	+	+	+	+	+	+
Gruidae	Sarus	+	-	+	+	+	+	+	+
Gruidae	Brolga	+	+	+	+	+	+	+	+
Gruidae	White-naped	+	+	+	+	+	+	+	+
Gruidae	Sandhill	+	+	+	+/-	+	-	+	+
Gruidae	Wattled	+	+/-	+	+/-	+	+	+	+
Gruidae	Siberian	+	+	+	+	+	-	+	+
Gruidae	Blue	+	+	+	+	+	+	+	+
Gruidae	Demoiselle	+	+	+	+	+	+	+	+
Gruidae	Gray crowned	+	+	+	-	+	-	+	+
Gruidae	Black crowned	+	+	+	-	+	?	+	+
Gruiformes	Trumpeter	+	-	+	-	+	?	-	-
Gruiformes	Rail	+	-	-	-	+	-	-	-
Aves	Brant	-	-	-	-	+/-	-	-	-
Aves	Chicken	+	-	-	-	+	+/-	-	-
Aves	Manakin	+	+	-	-	-	-	-	-
Aves	Cowbird	+	+	-	+	-	?	-	-
Vertebrate	Alligator	+	-	-	-	+	-	-	-
Vertebrate	Raccoon	-	-	-	-	-	+	-	-
	Negative control	-	-	-	-	-	-	-	-
	Expected size	+	+	+	+	+	-	+	+
	Glycerol[c]	-	-	-	+	+	-	-	-

[a] Scientific names of species not mentioned in text: black-necked (*Grus nigricollis*), common (*G. grus*), hooded (*G. monacha*), brolga (*G. rubicunda*), white-naped (*G. vipio*), wattled (*Bugeranus carunculatus*), blue (*Anthropoides paradisea*), demoiselle (*A. virgo*), gray crowned (*Balearica regulorum*), and black crowned (*Balearica pavonina*) cranes; gray-winged trumpeter (*Psophia crepitans*); gray-necked wood rail (*Aramides cajanea*); black brant (*Branta bernicla nigricans*); white-collared manakin (*Manacus candei*); brown-headed cowbird (*Molothrus ater*); raccoon (*Procyon lotor*).

[b] Developed from sandhill crane genomic DNA.

[c] Glycerol composed 10% of PCR reactions designated +.

proportion of polymorphic microsatellite loci exceeds 90% for most taxa (e.g., Weber 1990). Microsatellite loci have been shown to be highly polymorphic, with many alleles per locus, even in organisms with very low allozyme variation (Hughes and Queller 1993, Taylor et al. 1994, Glenn et al. 1996). Because thousands of microsatellites occur within the genomes of most complex organisms, they represent a rich source of genetic markers. Additionally, because the mutation rate is high, each locus is likely to have many alleles, and populations with little gene flow will differentiate quickly. However, the mutation rate is low enough for alleles to be relatively stable within small numbers of generations, allowing for genealogical analysis. Thus, microsatellites are becoming the molecular genetic marker of choice for ecological, human, medical, and population genetics (e.g., Bruford and Wayne 1993, Kuhl and Caskey 1993, Weissenbach 1993, Slatkin 1995).

Microsatellites have several additional benefits. First, the amount of material needed for analysis via PCR is much less than that required for protein studies or Southern blot analysis (i.e., DNA fingerprinting or MHC Restriction Fragment Length Polymorphism [RFLP]), an important consideration for work with endangered species, where destructive sampling and excessive handling are not desirable. For example, the same amount of DNA used for 1 DNA fingerprint can be used to amplify at least 100 microsatellite loci. Second, microsatellites may be amplified from degraded DNA, such as DNA recovered from museum specimens (Ellegren 1991). This was our primary interest in developing these loci for whooping cranes. Other non-traditional sources of tissue have

Table 3. Comparison of the number of polymorphisms detected in whooping cranes by allozymes and microsatellites.

	Allozymes[a]	Microsatellites
Individuals surveyed	34	4
Loci surveyed	24	22
Polymorphic loci	5	13
Polymorphic alleles[b]	11	27

[a] From Dessauer et al. (1992).
[b] Alleles detected at polymorphic loci.

Table 4. Genotypes of captive whooping crane family members at 6 microsatellite loci.

			Locus[b]					
ID no.	Status[a]	Sex	AC-2	AC-5	AC-15	AAT-1	ACC-1	AGG-2
71-001	Dam1	F	ab	ac	bb	bb	ab	-
83-003	Fam1	M	ab	ab	bb	bb	bb	ab
84-003	Fam1	M	aa	ac	bb	-	aa	bb
88-022	Fam1	M	bb	ac	bb	ab	bb	bb
90-018	Fam1	M	ab	cc	bb	bb	aa	bc
90-026	Fam1	F	aa	ac	bb	bb	aa	bc
91-006	Fam1	F	ab	bc	bb	ab	ab	bc
64-001	Sire1,?	M	ab	bc	ab	ab	ab	bc
74-001	Sire?,2	M	ab	aa	ab	-	bb	ac
13-7	Sire?	M	ab	aa	ab	bb	bb	bc
68-001	Sire?,3	M	ab	ab	ab	-	ab	bc
13-17	Sire?	M	bb	ab	aa	ab	bb	bb
85-002	Fam3	F	ab	ac	ab	ab	ab	bc
85-001	Fam2	F	bb	ac	ab	ab	bb	aa
13-16	Dam2,3	F	bb	cc	aa	bb	bb	ab

[a] Numbers refer to the family group. Fam designates offspring of a particular mating pair (e.g., individuals designated Fam1 are offspring of 71-001 and 64-001). Sperm from males 64-001, 74-001, 13-7, 68-001, and 13-17 was used to artificially inseminate 13-16 in 1985. Thus any of those males could be the sire of 85-002 and 85-001. The status of "Sire2" and "Sire3" are determined from the genotypes in this table.
[b] - designates genotypes that could not be unambiguously determined from the first attempt at genotyping.

also been used for PCR based assays (e.g., droppings [Kohn et al. 1995] or feathers [Leeton et al. 1993]). Third, once primer sets for polymorphic loci are available, large numbers of individuals can be analyzed quickly at many loci. Microsatellites are particularly amenable to automation (Ziegle et al. 1992).

A final useful feature of microsatellites is that primer pairs developed from 1 species can often be used to amplify orthologous polymorphic loci in other closely related taxa. Many early attempts at trans-species amplification of microsatellites focused on rather distantly related taxa (e.g., Moore et al. 1991, Schlötterer et al. 1991, Stallings et al. 1991) and had varying levels of success. More recent studies have focused on species within taxonomic families (e.g., Breen et al. 1994, Hanotte et al. 1994, Pepin et al. 1995) and have been highly successful. Our results indicate many of the loci we have developed will be of use in studying other cranes (Table 2). Similarly, it appears that genetic analyses of many threatened and endangered species can make use of primers developed from other species (e.g., Morin et al. 1993, Gotelli et al. 1994). Recent studies also suggest that a small percentage of microsatellite loci are conserved across even very distantly related taxonomic groups (e.g., AA-1, Table 2; FitzSimmons et al. 1995; Stallings 1995; Glenn et al. 1996). Through time, it may be possible to accumulate enough such conserved loci that development of new primers will not be necessary for most studies using microsatellites.

Disadvantages of Microsatellites

Although microsatellites occur in all eukaryotes (Hamada et al. 1982, Tautz and Renz 1984), their distribution, overall frequency, and average length vary widely (Hamada et al. 1982, Langercrantz et al. 1993). Thus, it has been much more difficult to obtain microsatellites from whooping cranes than other taxa, even other birds. In addition to the difficulty of isolating microsatellite loci, the proportion of primers that give unsatisfactory results can vary from taxon to taxon, with whooping cranes among the worst we have studied (T. C. Glenn, unpubl. data). Thus, the difficulty of isolating additional loci and developing subsequent primers for cranes should not be underestimated. However, the primer pairs we have developed usually amplify DNA fragments of the correct size and are often polymorphic in related taxa.

As with other techniques, problems such as null alleles (Pemberton et al. 1995), anomalous signficance of linkage (Knowles et al. 1992), and inconsistency between studies due to small variations in technique (Knowles et al. 1992) can affect microsatellites. These problems, however, have been less likely to affect microsatellites than DNA fingerprinting (Jin and Chakraborty 1995). Additionally, by use of captive cranes with known pedigrees, many of these problems can be detected and dealt with appropriately.

MANAGEMENT IMPLICATIONS

We recognize that ecological and demographic factors are the predominant proximate causes of endangerment (Lande 1988, Caro and Laurenson 1994, Caughley 1994). However, the negative effects of decreased genetic variability and

inbreeding have been documented for several vertebrate species (Ralls et al. 1979, Soule 1979, O'Brien et al. 1985, Wildt et al. 1987, Harvey and Read 1988, Leberg 1990), and we believe the risk of genetic defects will often be additive to other causes of mortality. Thus, small inbred populations experience increased risks of extinction. If a lack of genetic diversity in a population increases its chances of extinction from epidemic diseases or other causes (Harvey and Read 1988), thereby erasing all previous conservation efforts, it is prudent for managers to attempt to preserve genetic diversity when managing species or ecosystems.

Without reserves large enough to maintain viable populations for many vertebrate species, managers have looked for alternatives to increase the effective population size of isolated populations. Methods of increasing gene flow and managing genetic diversity include the use of habitat corridors, translocation, and captive propagation (Foose et al. 1986, Simberloff and Cox 1987, Conant 1988). However, without detailed and accurate genetic information, such practices are difficult to implement and monitor wisely. Microsatellite loci can often provide just such information. The microsatellite loci we have developed for cranes can be used to provide answers to such questions as: who are the parents of a given individual; how related are 2 individuals within a population; how differentiated are populations, subspecies, and species; and what fraction of the original genetic diversity remains in highly endangered subspecies and species. Cranes can be better managed by knowing the answers to such questions.

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