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Microsatellite Markers in Canada Geese (*Branta canadensis)*

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A Canada goose (*Branta canadensis*) genomic library was created and screened for clones containing various microsatellite motifs. Fourteen positive clones were identified and sequenced; five primer pairs were developed and utilized to screen approximately 460 Canada geese for genetic variation. Each of these primer pairs were consistently scorable and polymorphic (average heterozygosity of 55%). DNA was isolated and successfully amplified from 2-year-old goose tail fans which received no special care other than storage at 4°C. Two of the five goose primers also amplified DNA from a partial family of wood ducks (*Aix sponsa; n* = 5), but no variation was detected at either locus. In geese, the number of alleles per locus ranged from 7 to 24. PIC values ranged from 0.41 to 0.91, while average levels of observed heterozygosity varied between 0.34 and 0.78. The high levels of polymorphism exhibited by these markers should be useful in addressing population genetic issues in Canada geese.

Canada geese (*Branta canadensis*) number in the millions across North America. Their populations and natural history have been a source of study for decades (Bellrose 1980; MacInnes 1962; Palmer 1976). Geese are a wildlife management priority from both an economic and biological standpoint. There are 11 putative subspecies of Canada geese based upon morphological differences (Delacour 1954; Johnsgard 1978). These subspecies are grouped according to size into two categories: large- and small-bodied geese. A main objective of North American waterfowl biologists is to identify the population affiliation, if any, of small Canada geese in the Central Flyway (*B. c. parvipes* and *B. c. hutchinsii*).

Initially, analysis of proteins through allozyme electrophoresis was used to detect potential differences among subspecies of Canada geese (Baker and Hanson 1966; Morgan et al. 1977). A major problem with allozyme studies of geese is the low level of variability detectable with conventional protein electrophoresis. From a genetic viewpoint, the available allozyme data suggests that there is no basis for current taxonomic distinctions at the subspecific level. We chose to utilize hypervariable microsatellite loci (which typically have many more alleles per locus than allozymes) to determine if low levels of variability among loci are the primary reason that allozyme studies have failed to delineate populations and subspecies of Canada geese. Our objective was to produce a panel of microsatellite markers that could be used to assess the population structure of Canada geese.

Materials and Methods

Genomic Library

Blood samples were used as a source of high-quality DNA. Our samples came from 13 collecting locales in the Northwest Territories of Canada ranging from Baffin Island in the east to the Mackenzie River delta in the west. Geese were mainly collected on their nesting grounds by driving flightless geese (those that had molted) into an enclosure. Goose blood (0.25–0.50 cc) was drawn from the brachial vein and transferred to a sterile polypropylene tube

Table 1. Molecular properties and population statistics of five Canada goose microsatellites

Locus name	Primer sequence	Product size (bp)	Annealing temperature	Number of geese typed	Average observed heterozygosity	Alleles per locus	PIC values
TTUCG-1F	5′-CCCTGCTGGTATACCTGA-3′						
TTUCG-1R	5′-GTGTCTACACAACAGC-3′	~119	55°C	453	0.4349	7	0.5754
TTUCG-2F	5′-GAGAGCGTTACTCAGCAAA-3′						
TTUCG-2R	5′-TCACTCTGAGCTGCTACAACA-3′	~136	55°C	416	0.7788	24	0.8847
TTUCG-3F	5′-GAGGTCGAATCCAACCTG-3′						
TTUCG-3R	5′-GCACATGATGCATGTGCTG-3′	~79	53°C	377	0.4138	14	0.8562
TTUCG-4F	5′-GGTGTACTCTGCTGAGTGTC-3′						
TTUCG-4R	5′-CTAGAACTAGTGGATCTCTC-3′	~194	55°C	426	0.3427	18	0.4068
TTUCG-5F	5′-GGGTGTTTTCCAACTCAG-3′						
TTUCG-5R	5′-CACTTTCCTTACCTCATCTTG-3′	~206	59°C	402	0.7761	18	0.9090

containing 5 ml of lysis buffer (Longmire et al. 1991). The blood and lysis buffer were incorporated by inversion. Once the tubes arrived at the laboratory, proteinase K was added to the blood-lysis solution to a final concentration of 0.5 μg/ml and incubated at 37°C with rotation overnight. Following a phenol extraction, the aqueous phase was retained and dialyzed against 1× TE (10 mM Tris, 1 mM EDTA) at 4°C overnight. The DNA was then ethanol precipitated and rehydrated with water. DNA concentrations were determined by agarose-gel electrophoresis. Additionally DNA was extracted from feather quills pulled from the tail fan and from muscle associated with the tail fan. These goose tail fans were 2 years old and had no special care given to them other than cold-room storage. The tissues were placed into the lysis buffer and treated in the same manner as the blood samples.

A genomic library was constructed using methods similar to DeWoody et al. (1995). Briefly, genomic DNA from a single goose was digested with *Sau*3A1; small fragments (<500 bp) were collected and cloned into the plasmid vector pBluescript II SK+ (Stratagene Inc., La Jolla, California) and transformed into DH5-alpha competent cells (Stratagene). Different repeat arrays d(GT/CA, CT/GA, GATA/CTAT) were hybridized to resultant colonies according to Janecek et al. (1993); 14 positive clones were isolated and sequenced according to established protocols (Sambrook et al. 1989). Sequences are deposited in GenBank under accession numbers U66089, U66090, U66091, U66092, and U66093.

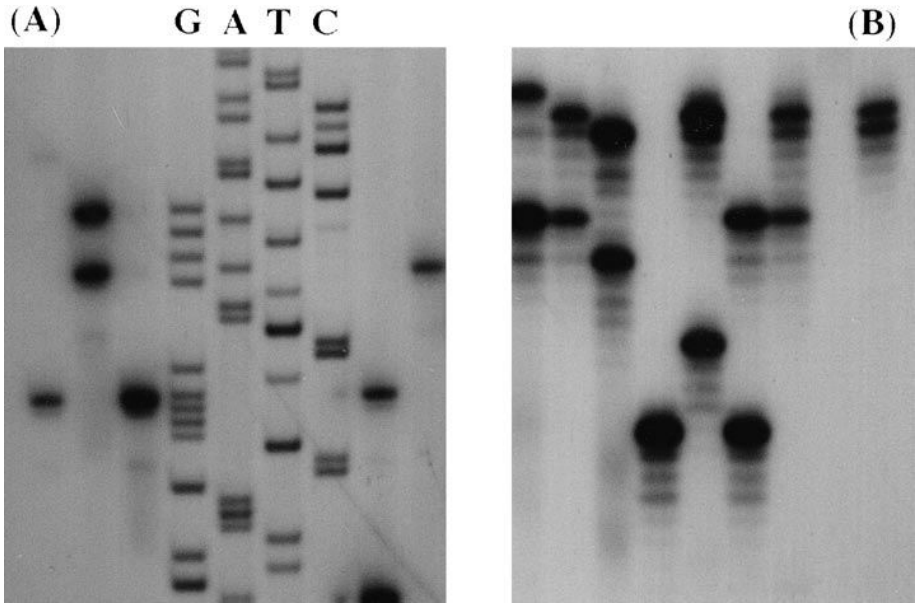

Figure 1. Panels A and B show two microsatellite loci amplified from Canada geese. Panel A shows a pentanucleotide repeat (TTUCG-5) and the sequence of M13. Panel B shows a dinucleotide repeat (TTUCG-2). The numerous slippage bands in the dinucleotide repeat make it more difficult to score than the pentamer, which has fewer and less severe shadow bands. Additionally the size of the dinucleotides are sometimes difficult to discern, as shown in panel B.

DNA Amplification

PCR primers were designed for five of the 14 positive clones. All 14 clones contained repeats; however, 9 of the 14 clones did not allow primer design due to insufficient sequence data. Several of these clones contained microsatellites that were too close to the vector/insert junction to allow primer design. The sequence of each primer, expected product size, and annealing temperature are listed in Table 1.

PCRs were performed in 25 μl volumes containing ~200 ng DNA, 0.25 μM of each primer, 200 μM dNTP, 1.5 mM $MgCl_2$, 1× buffer, and 1.25 units of *Taq* DNA polymerase. Thermal profile consisted of an initial denaturation of 2 min at 94°C followed by 35 cycles of 94°C for 1 min, primer-specific annealing temperature (Table 1) for 30 s, and 72°C for 30 s. PCR products were visualized using a radioactively labeled primer; the forward primer was labeled with ^{32}P-gamma-ATP using T4-polynucleotide kinase (New England Biolabs, Beverly, Massachusetts) according to manufacturer's instructions.

Radiolabeled PCR products were denatured for 4 min at 80°C and electrophoresed in a 6% polyacrylamide gel (Sequagel, Atlanta, Georgia) at 55 W for up to 6 h. After drying, gels were exposed to X-ray film. Autoradiographs were scored utilizing the DNA sequence of M13 as a molecular size standard. Each allele was discrete and defined by size according to this standard.

Results and Discussion

We isolated and sequenced 14 clones that hybridized to three microsatellite repeat motifs from a Canada goose genomic library. We designed PCR primers to amplify five presumably independent loci that contained microsatellite repeats. Four of the five loci (TTUCG-1–4) are each $d(GT/CA)_n$ repeats, while the fifth locus (TTUCG-5) is a $d(GATAA/CTATT)_n$ pentamer. The clone containing the pentamer repeat also contained a d(CT/GA) repetitive element, but our PCR primers amplify only the pentamer repeat. Our results indicate that the pentamer repeat is much easier to score than the dinucleotide repeats because of fewer slippage bands (Figure 1).

Each of the five PCR primer pairs was highly polymorphic in Canada geese, with an average of 15.4 alleles per locus (Table

1). Average observed heterozygosity ranged from 34 to 78%, with an average of 55% over all five loci (Table 1). PIC values (Botstein et al. 1980) indicate that each marker should be highly informative; values ranged from 0.4068 to 0.9090 (Table 1). Two of the five markers (TTUCG-1 and TTUCG-4) amplified DNA from a small sample of related wood ducks (n = 5; mother and four offspring); however, no genetic variation was observed even though genetic variation is segregating within the family according to a duck microsatellite marker (Cathey et al. 1996).

In summary, we created a goose genomic library and developed a panel of five microsatellite markers that are highly polymorphic in multiple populations of Canada geese. Two of these markers were monomorphic in a small family of wood ducks. Additionally we successfully used all five microsatellite markers to amplify goose DNA from tail fans that were collected in the field and donated by hunters. Goose hunters are often surveyed by the U.S. Fish and Wildlife Service to obtain information about their harvest. These surveys offer a convenient and inexpensive way to gather tissue from wild populations that can later be used for genetic analysis. The tail fans were shipped in an envelope at ambient temperature and transferred to a cold room at 4°C at the earliest convenience. No other care and/or preparation was given to these tail fans until some 2 years later, when DNA was isolated as described above. Therefore the markers described herein should be of value to both wildlife managers who study Canada geese as well as geneticists.

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Short Beak: A New Autosomal Recessive Semilethal Mutation in Japanese Quail

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Short beak (SBK) is a new semilethal mutation of Japanese quail (*Coturnix japonica*). The SBK individuals are characterized externally by short beaks, shanks, and digits. The shank is also thicker than the wild type. The shape of the mutant beak does not show a parrotlike appearance, contrasting with that of other poultry chondrodystrophic mutants reported in the literature. Bones in the fore and hind limbs of the SBK mutant are also shorter than the wild types. The humerus and ulna in the wing are significantly thicker than the wild types. Other bones in the wing and leg show approximately the same thickness as the wild types. The majority of the SBK mutants die at the late embryonic stage. Some chicks can hatch with no assistance, but almost all die around 3 days of age. However, a part of the SBK individuals (5.3%, 8/151) reach sexual maturity and can reproduce. Genetic analyses revealed that the SBK mutation is controlled by an autosomal recessive gene. The proposed gene symbol is *sbk.* The *sbk* gene is not allelic to the previously reported stumpy-limb (*sl*) gene that expresses a somewhat similar phenotype to the SBK.

In August 1994 we found unusual chicks having beaks and shanks reduced in size. These were among the progeny from a mating of a black-at-hatch mutant (Minezawa and Wakasugi 1977) female and a wild-type male Japanese quail (*Coturnix japonica*) kept at the Department of Laboratory Animal Science, College of Agriculture, Osaka Prefecture University. All of these unusual chicks died before reaching maturity. Full-sib matings of their normal siblings also segregated such abnormal individuals. Thus this abnormality seemed to be a recessive lethal mutation. In Japanese quail, there are five mutations that show reduced beak and shank lengths and lethality (Collins et al. 1968; Hermes et al. 1990; Hill et al. 1963; Nichols and Cheng 1991; Tsudzuki 1995b). The present mutant seemed to be phenotypically different from all of them. We named our mutant short beak (SBK) and started genetic analysis. This article describes the features of the SBK character and its mode of inheritance.

Materials and Methods

General care of quail is described elsewhere (Tsudzuki 1995a). We compared external appearances between the SBK and wild-type newly hatched chicks. We measured with a caliper the length of the beak, shank, and third digit and the midshaft thickness of the shank in both mutant and wild-type chicks. Furthermore, we compared SBK and wild-type long bones of 15-day embryos by measuring their length and midshaft thickness under a dissecting microscope. For this purpose we stained the bone with alizarin red S (Wako Pure Chemical Industries, Ltd., Osaka, Japan) based on the method described by Tsudzuki and Wakasugi (1988). The resulting data were analyzed by Student's t test or Cochran–Cox test.