Molecular Sexing of Geese Using a Cloned Z Chromosomal Sequence with Homology to the W Chromosome

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At present, sexing of monomorphic birds and of the young of many dimorphic species is limited to surgical techniques (such as endoscopy), to chromosomal analyses, or to cloacal eversion. Surgery carries risks associated with high stress levels, infection, and anesthesia; and, in most cases, it can be performed only on mature birds and by trained personnel (Prus and Schmutz 1987). Chomosomal analysis is tedious and requires identification of the Z and W chromosomes (females are Z/W and males Z/Z) against a background of (often numerous) microchromosomes, as well as the establishment of cell cultures. This can be difficult with samples taken under field conditions. While karyotyping has been used to sex Whooping Cranes (*Grus americana*; Biederman et al. 1982), it is not a very practical technique for a larger number of birds, and the success rate has been shown to be relatively poor in Psittacines (Prus and Schmutz 1987). Cloacal sexing is reliable, but it is generally limited in its applicability to waterfowl.

We report the isolation of a DNA segment of the Snow Goose (*Chen caerulescens*) Z chromosome, a segment that shows homology to the W chromosome and that allows a new approach to sex determination using DNA extracted from small blood samples. Blood is a very convenient tissue to sample and transport, and it consistantly yields high molecular weight DNA, even after storage at room temperature for more than one week (Quinn and White unpubl.). To our knowledge, this is the first report of the cloning of a unique segment of the avian sex chromosome. Related molecular studies have concentrated on repetitive sequences on the avian W chromosome (Tone et al. 1984, Kodama et al. 1987) or on sex-specific fragments detected with M13 DNA (Longmire et al. 1988).

We have described the construction of a genomic library and isolation of DNA probes from the Lesser Snow Goose (*Chen caerulescens caerulescens*) (Quinn and White 1987) and applied such probes to pedigree analyses (Quinn et al. 1987, Quinn et al. 1989). One of these probes (DQSG10, 14 kb) is the focus of this study.

Blood samples (1–5 ml) were taken from 119 live birds captured at La Perouse Bay near Churchill, Manitoba (58°24'N, 94°24'W). We extracted DNA following the procedure of Quinn and White (1987). The sex of each bird was determined by cloacal eversion (Taber 1971) at the time of blood sample collection. Samples of DNA were digested with the restriction endonuclease *Taq*1, electrophoresed, and blotted as described (Quinn and White 1987). The autoradiograph resulting from the hybridization of DQSG10 to a blot which included 5 male and 7 female DNA samples is given as Fig. 1. The surprising feature of this and other autoradiographs produced using this probe was a weak 2.1 kb band present in the female samples but absent in the male samples. In total, 55/57 adult females had this band, while all 26 adult males lacked it. Thus, a 98% concordance was found between the field sexing and the presence/absence of the band in female/male adult birds. We tested an additional 36 juveniles and found a concordance of 92%. Although not verified by dissection, the most likely explanation for the discrepency between cloacal and "molecular" sexing arose from recording or cloacal sexing errors in the field. Banded adults recaptured (and resexed) from one year to the next at La Perouse Bay provided a rough estimate of that error rate. Overall, the recorded sex differed between the first capture and the second capture in 268 of 5,352 (5%) of those cases recorded between 1968 and 1988. There was no evidence of any allelic counterparts to the 2.1 kb band as would be expected if this were a restriction fragment length polymorphism (RFLP). We concluded that this band represented a fragment of (female specific) W chromosomal DNA with homology to DQSG10.

There was evidence that DQSG10 itself was derived from the Z chromosome. Two RFLPs are apparent in Figure 1. One, locus DI, is represented by the 4.4 kb and the 4.0 kb alleles as described previously (Quinn and White 1987). The males appeared homozygous for the 4.4 kb allele (lanes 1, 2, 4, and 8), heterozygous (lane 7), or homozygous for the 4.0 kb allele (not shown). No heterozygous females were found, but rather females appeared hemizygous and carried either the 4.4 kb allele (lanes 3, 5, 6, 10, and 12) or the 4.0 kb allele (lanes 9 and 11). The second polymorphic locus (DIII) was undescribed previously and shows a 2.6 (DIII-1) and a 2.3 (DIII-2) kb allele. The 2.3 kb allele was identical in size to a constant band present in all samples examined. The males were either homozygous for the 2.6 kb band, in which case the 2.6 and 2.3 (constant) bands were of equal intensity (lanes 1, 2, 4, and 7); heterozygous, in which case the 2.3 kb bands were of much higher intensity (lane 8); or homozygous for the 2.3 kb band, in which case the 2.6 kb band was absent (not shown). No heterozygous females were found, and again they appeared hemi-

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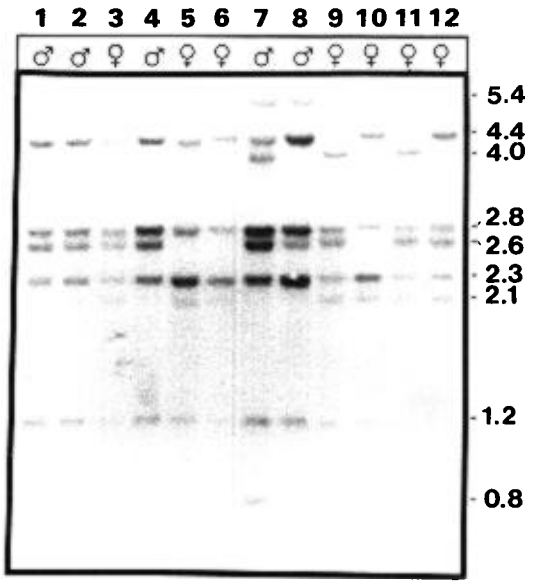

Fig. 1. Autoradiograph of DNA from male and female Snow Geese probed with DQSG10. Genomic DNA (5 μg) extracted from the blood of adult Lesser Snow Geese (*Chen caerulescens caerulescens*) was digested with 15 units of *TaqI* for 3 hours. After electrophoresis in 1% agarose, these were transferred to Genescreen Plus membrane (New England Nuclear) by the method of Southern (1975). This blot was probed with DQSG10 as described (Quinn and White 1987) and washed twice in 2× SSC for 5 min at 20°C, and three times in 0.2× SSC, 0.1% SDS for 30 min at 65°C.

zygous for either the 2.6 kb band (lanes 3, 9, 11, and 12) or the 2.3 kb band (lanes 5, 6, and 10).

By using the presence of the 2.1 kb band to identify females and its absence to identify males, we were able to screen a total of 71 males and 104 females with this probe. The sample included an additional 56 individuals obtained from hunter-killed birds, which were collected originally in British Columbia and the state of Washington for a different study and which had not been sexed in the field. In all cases, the relative band intensities and patterns were consistent with the interpretation of the data of Fig. 1. For both polymorphic regions (DI and DIII), the genotypes of the two sexes were consistent with the male possessing two alleles and the female being hemizygous (Table 1). No cases of females with two different alleles at one locus were found. Out of 41 samples heterozygous for either DI or DIII or both, there were no cases where the 2.1 kb band was present. If the null hypothesis is made that females are homozygous for these RFLPs, then the chance of observing such a deviation from expected Hardy-Weinberg equilibrium is much less than 0.001 for both regions (Table 1). Hence, we conclude that females are hemizygous.

TABLE 1. Distribution of alleles at two loci in males and females. DQSG10-DI and -DIII allele frequencies and genotype distribution within the two sexes were calculated under the assumption (null hypothesis) that both have two copies at this locus and are in Hardy-Weinberg equilibrium. This was evaluated using the *G*-test (Sokal and Rohlf 1969); * = $P \ll 0.001$.

Genotype	Observed	Adjusted[a] (f)	Expected[b] ($\hat{f}$)	G[c]
		Male[d]		
DI-1,1	52	52.5	52.5	
1,2	18	18.5	17.1	3.2 (NS[e])
2,2	1	1.5	1.4	
DIII-1,1	36	36.5	37.8	
1,2	32	32.5	28.0	4.4 (NS)
2,2	3	3.5	5.2	
		Female[d]		
DI-1,1	84	84.5	68.2	
1,2	0	0.5	32.0	101.3*
2,2	20	20.5	3.8	
DIII-1,1	78	78.5	58.5	
1,2	0	0.5	39.0	116.2*
2,2	26	26.5	6.5	

[a] Yate's correction.
[b] Assumes Hardy-Weinberg equilibrium.
[c] $G = 2(\sum^{3} f_i \ln f_i - 2.30259 \sum^{3} f_i \log \hat{f}_i)$.
[d] Sexing based on the presence (♀) or absence (♂) of the 2.1 kb band.
[e] NS: not significant at $P = 0.05$, df = 2.

The second form of evidence of the Z chromosomal origin of DQSG10 was the intensity of the constant 5.4 kb band that, after correcting for the amount of DNA loaded per track, showed a male to female ratio of 2:1 (Table 2).

We conclude that the DQSG10 probe is derived from the Z chromosome and, as described earlier, has homology to the W chromosome. The occurrence of regions of crosshomology between the sex chromosomes has also been noted in humans (Page et al. 1982, 1984; Geldwerth et al. 1985) and may result from either transposition or recombination events between the X and Y chromosomes, or from sequence conservation from an ancestral pair of homologues (Ohno 1967).

These results demonstrate the utility of the general approach of using chromosomal probes for sex determination in birds. Sampling requires minute amounts of blood, as we routinely extract 40 times more DNA than used for this analysis from 50 μl of blood (Quinn and White 1987). Such small volumes of blood carry little risk to the individual sampled, even in the case of small birds (Strangel 1986). In the Snow Goose, a female (W chromosome) specific band

TABLE 2. Densitometric analysis of putative Z chromosomal autoradiographic bands in males and females. Densities were estimated for the 5.4 kb band of Figure 1 by taking the average tracing of three scans (LKB Ultroscan XL Laser Densitometer) and interpolating the area under the curve. As a control for variability in total DNA loaded, another scan was taken of the constant 2.9 kb band produced on an autoradiograph by hybridizing the same blot with the autosomal probe DQSG12.

Track	Sex	DQSG10 area[a] (a)	Control area[b] (b)	Adjusted area (a/b)	Summary
1	M	0.30	2.33	0.13	Male: $\bar{x}_m = 0.134$
2	M	0.28	1.94	0.14	$s_m = 0.0055$
3	F	0.11	1.81	0.06	
4	M	0.26	2.03	0.13	Female: $\bar{x}_f = 0.066$
5	F	0.17	2.70	0.06	$s_f = 0.0079$
6	F	0.09	1.48	0.06	
7	M	0.33	2.57	0.13	
8	M	0.31	2.28	0.14	
9	F	0.14	2.02	0.07	$\bar{x}_m/\bar{x}_f = 2.03$
10	F	0.12	1.86	0.06	$t^c = 16.6$ $(P \ll 0.001)$
11	F	0.17	2.07	0.08	
12	F	0.17	2.43	0.07	

[a] Area under densitometric tracing of the 5.4 kb (constant) band.
[b] Area under densitometric tracing of the 2.9 kb (constant autosomal) band of the same blot probed with DQSG12 (Quinn and White 1987).
[c] Using the pooled standard deviation of $s = 0.0070$, to test the null hypothesis that $\bar{x}_m = \bar{x}_f$ with 10 degrees of freedom (Sokal and Rohlf 1969).

can be scored directly to identify the sex. In this and other species, relative intensities of hybridization signal to the Z chromosome on Southern or dot blots could provide the same information. Preliminary data show that this probe also hybridizes to DNA from members of the Passeriformes, Galliformes, and Charadriiformes.

The DQSG10 probe will allow us to isolate more clones from both the Z and the W chromosomes. Clones from the W chromosome represent DNA molecules that are inherited maternally like mitochondrial DNA but that "travel" in the nuclear rather than mitochondrial cellular compartment. A W chromosome probe would make sex identification possible on the basis of presence or absence of signal on a dot blot. It would then be practical to determine the sex of hundreds of birds over a short time (2 days). Such probes will also allow comparisons of levels and patterns of polymorphisms between the sex chromosomes and autosomal chromosomes over the range of the Lesser Snow Goose, and they will be useful in questions concerning sex determination and rates of molecular evolution on the different chromosomes

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Evidence for Vocal Learning by a Scrub Jay

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The vocalizations of corvids, other than their faintly uttered and seldom heard whisper song, are typically referred to as calls rather than songs (Hardy 1983, Goodwin 1986). This convention reflects a widespread impression that these calls are more simple than the primary advertising songs of many other oscines and are delivered in a different way. Corvids mimic the sounds of other bird species and even the sounds of inanimate objects (Goodwin 1986), but the evidence that they learn their distinctive species-specific calls is sketchy. We present evidence that a captive Scrub Jay (*Aphelocoma coerulescens superciliosa*) from California learned two calls from Florida Scrub Jays (*A. c. coerulescens*).

A California jay was captured near Carmichael, Sacramento County, as a late-stage nestling (23 June 1979) and hand-raised. On 5 August 1979, it was given to the Florida Museum, where it lived in a large aviary with other wild-caught Scrub Jays from Florida and California (Webber and Cox 1987). In the following account, we refer to this California jay as "Red" because of its red leg bands.

In mid-January 1981, Webber noticed that a male Florida Scrub Jay (pink leg bands: "Pink") in the aviary was pairing with a female ("Green") from California. Pink passed food to Green, called in response to her flights, and flew back and forth in front of her while calling loudly. Green gave the rattle call (Webber 1984) in response to Pink's flights. This behavior is typical of Scrub Jays when they form pairs in the wild (Webber 1984). Other jays in the aviary, including Red, often called and flew in response to Pink and Green's pair-forming behavior, as wild Scrub Jays do when a new pair forms in a neighboring territory (Webber 1984). Red gave two kinds of calls (referred to as A [Fig. 1a] and B [Fig. 1d]) that, to Webber, sounded identical to those of Pink (Fig. 1b, e) and another male Florida Scrub Jay ("Yellow") in the same aviary. Webber recorded the calls of Red, Pink, and Yellow on six days from 11 January to 29 March 1981. These recordings include at least 138 calls of type A by Pink and 40 by Red, as well as at least 100 calls of type B by Pink, 36 by Red, and 7 by Yellow (Florida Museum of Natural History Bioacoustics Archives master tapes 905-907).

Webber (1984) found no calls of types A or B in a 12-month study of Scrub Jay calls in Los Angeles County, California (FSM masters 633-637B, 639A-641B). Stefani (pers. obs.) also found no calls of types A or B in a 3-month study of Scrub Jay vocalizations in Davis, California, 28 miles from Carmichael. We think it unlikely that wild Scrub Jays in Carmichael give calls A and B.

Wild Scrub Jays in Florida give calls (FM masters