Enlarged Sex Chromosomes of Woodpeckers (Piciformes)

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The evolution of sex chromosomes in birds is poorly understood. In part, this is because the W chromosome in most species resembles microchromosomes, which are less than 2 μ in length and therefore difficult to characterize. Autoradiographic studies of the chicken by Schmid (1962) indicated that the W chromosome is the last to incorporate tritiated thymidine during the synthesis phase of the cell cycle. Thus, late replication of the DNA of the W chromosome characterizes this element in the karyotype. More recently, the staining of large amounts of constitutive (C-) heterochromatin on W chromosomes of birds by C-banding established another procedure for identification of the W chromosome (Stefos and Arrighi 1971). Neither of these techniques has been used extensively, however. The Z chromosome of birds appears to have been conserved in evolution to the extent that it consistently comprises 8–9% of the chromatin of the entire genome, that it is typically the fourth or fifth largest chromosome of the complement, and that the centromere is consistently mediocentric (Ohno et al. 1964, Ohno 1967). Ohno has referred to this, and a similar chromosome in advanced snakes, as the "original Z" to emphasize its conservative evolution.

We describe a deviation from Ohno's original Z in some woodpeckers (Piciformes: Picidae) and believe that this phenomenon is widespread within the group. Our interest in woodpecker sex chromosomes was stimulated by the unpublished observations of Drs. E. Sexsmith, E. Redrupp, and B. Thorneycroft in the laboratory of Professor K. H. Rothfels of the Department of Botany, University of Toronto from 1965–1967. These researchers studied chromosomes of the Common Flicker (*Colaptes auratus*), the Yellow-bellied Sapsucker (*Sphyrapicus varius*), the Hairy Woodpecker (*Picoides villosus*), and the Downy Woodpecker (*P. pubescens*). They observed that the Z chromosomes of these species are extremely large, nearly twice the size of Z chromosomes of the majority of other birds. Additionally, they demonstrated that the W chromosome of the flicker could be identified by its late replication pattern.

We have now verified the presence of large Z chromosomes in Alaskan flickers and Hairy Woodpeckers. Additionally, we have observed large Z chromosomes in three species of European woodpeckers and have identified the W chromosome of the Lesser Spotted Woodpecker (*Dendrocopos minor*) by C-banding. Enlarged Z chromosomes may characterize not only woodpeckers but also other piciform families.

Collection of specimens for this study has been piecemeal in that, for the most part, we have analyzed the karotypes of various woodpeckers as the birds became available. The Toronto specimens were taken as adults or eggs, and at least three males and three females of each species were used. All karyotypes of these birds were prepared from acetoorcein-stained slides of cultured kidney cells grown in CMRL 1415 +15% fetal calf serum. We verified the presence of enlarged Z chromosomes in Alaskan Hairy Woodpeckers and flickers. These specimens (3 female and 2 male Hairy Woodpeckers and 3 female and 3 male flickers) were taken as embryos and cultured in essentially the same way as the Toronto birds.

Adult lesser Spotted Woodpeckers (3 females, 1 male), 2 female Greater Spotted Woodpeckers (*D. major*), and a male Black Woodpecker (*Dryocopus martius*) were shot near Ås, Norway and processed as above.

The tentative identification by the Toronto group of an enlarged W chromosome in female flickers from the Toronto area made this species the logical choice for autoradiographic identification of the W chromosome. Nestlings of both sexes were used. Kidney cultures were labelled with tritiated thymidine (2 μc/ml, 2,233 μC/μM), and treatment was continuous to fixation at 4, 4¾, and 5½ h. The cells were pretreated with colchicine (1 μg/ml) for 2 h. Metaphase cells were photographed and then autoradiographed with Kodak AR-10 stripping film. After 26 h exposure, the labelled metaphases showed 8–400 grains, depending on the time spent synthesizing DNA in the presence of the isotope. All metaphase cells previously photographed were scored for total grains, grains over the sex chromosomes, and the two pairs of metacentric autosomes. These chromosomes were easily identified and were divided into three or four segments for counting. C-banding of Lesser Spotted Woodpecker cells was done by the method of Sumner (1972).

Partial karyotypes of the four North American woodpeckers studied are shown in Fig. 1. Chromosomes of females are arranged in descending order of size, and Z chromosomes of males of each species are inset.

(a) Common Flicker (*Colaptes auratus*): The Z chromosomes have an arm ratio of 1:3.5. The second and fourth pairs of chromosomes are metacentric. The probable diploid number for this species is 90, with the fundamental number (total number of arms) being 96. Late labelling of centromeres was not observed

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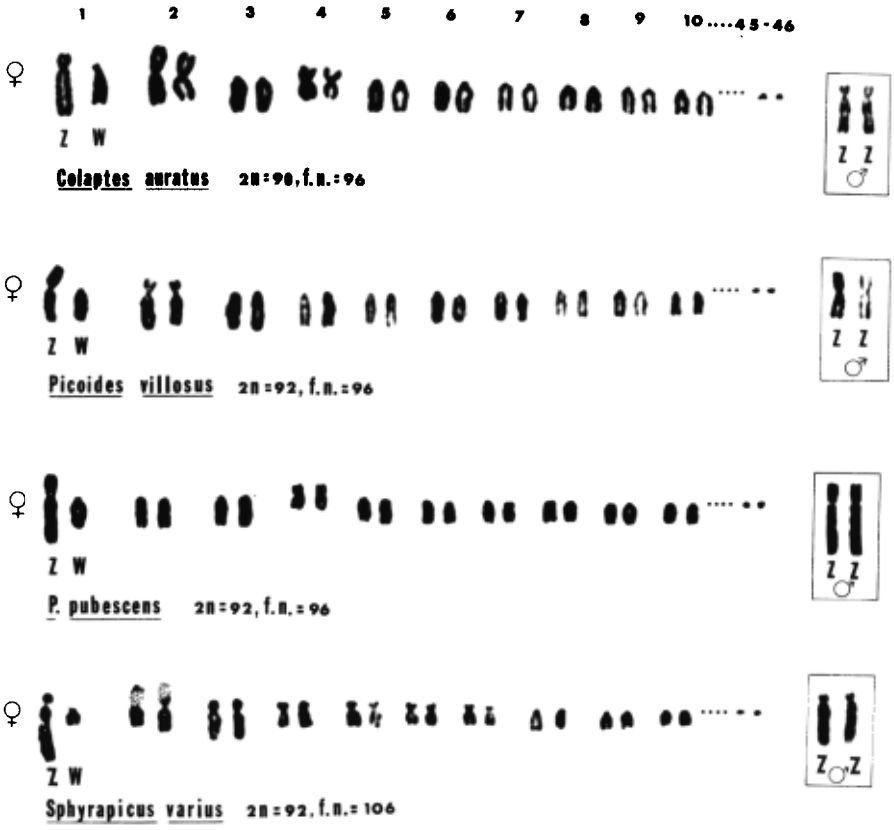

Fig. 1. Partial karyotpyes of female woodpeckers showing enlarged Z chromosomes. Paired Z chromosomes of males of each species are inset.

in the flicker. The W chromosome was late to label in both the short and long arm, but this effect was not as pronounced as in mammalian cells (e. g. late X in Chinese hamster). DNA replication of the Z chromosomes in both sexes was synchronous with the autosomes. There was no difference between labelling patterns of the two Z chromosomes in males.

(b) Hairy Woodpecker (*Picoides villosus*): the Z chromosome of this species has a median centromere with an arm ratio of 1:1.5. The karyotype is also distinguished by the presence of a second large chromosome pair (number 2) with a sub-median centromere (arm ratio 1:2.5). The remainder of the chromosomes are acrocentric. The diploid number appears to be 92, with the fundamental number of 96.

(c) Downy Woodpecker (*P. pubescens*): the Z chromosome of this species is the largest of the complement, being almost twice the size of the next largest pair. The Z has an arm ratio of 1:2. Except for the metacentric fourth pair, all other chromosomes are acrocentric. The diploid number appears to be 92 and the fundamental number 96.

(d) Yellow-bellied Sapsucker (*Sphyrapicus varius*): The Z chromosomes have an extreme arm ratio of 1:5, and the second pair of chromosomes is almost exactly metacentric. This is followed by five sets of chromosomes having various centromere locations. The diploid number appears to be 92, with the fundamental number of 106.

Partial karyotypes of the three European woodpeckers are shown in Fig. 2.

(e) Lesser Spotted Woodpecker (*Dendrocopus minor*): the Z chromosome of this species is again the largest of the complement, its arm ratio being 1:1.4. The W chromosome is conspicuously large and has an arm ratio of 1:1.8. The second pair of chromosomes is sub-metacentric, while the remaining pairs appear acrocentric. The entire chromosome complement of this species is shown in Fig. 3a. The diploid number appears to be 108 while the fundamental number is 112. Figure 3b shows a C-banded cell from the same bird. The W chromosome is heterochromatic, while the negatively staining Z chromosome is unpaired in the complement.

(f) Greater Spotted Woodpecker: (*D. major*): the Z

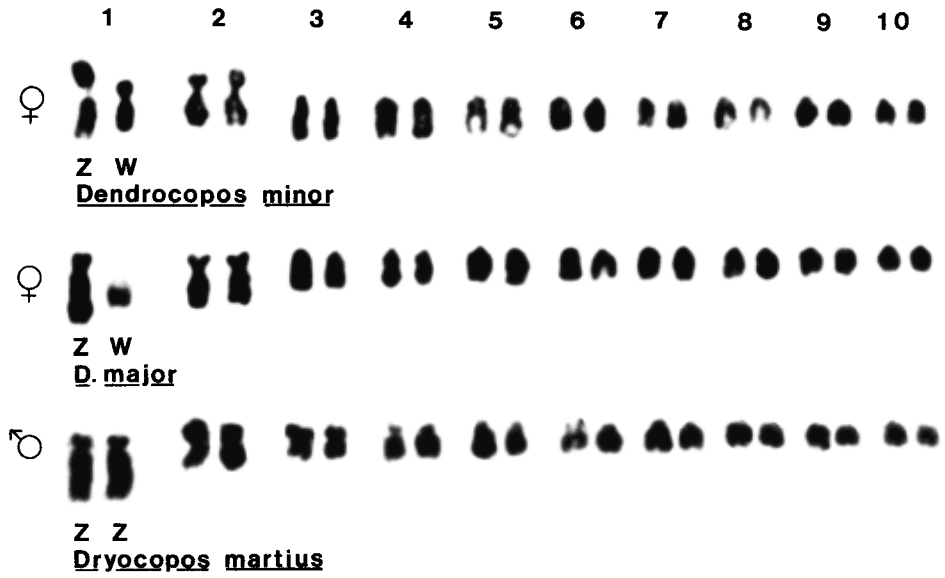

Fig. 2. Partial karyotypes of three species of woodpeckers. Enlarged Z chromosomes are the largest of the complements.

chromosome is large but with a different arm ratio (1:2.5) than the Z of the Lesser Spotted Woodpecker. The remainder of the karyotype is similar to that of the Lesser Spotted Woodpecker.

(g) Black Woodpecker (*Dryocopus martius*): a single male of this species was analyzed, so we cannot unequivocally identify the Z chromosomes. Because the Z chromosomes are the largest chromosomes in the other species, however, it is likely that the largest chromosomes in the Black Woodpecker are also the Z chromosomes; their arm ratio is 1:4.0. The second pair in this species is nearly metacentric (1:1.1). This pair is followed by another nearly metacentric (1:1.2) but smaller pair and by three other pairs that are submetacentric. The remaining chromosomes are acrocentric. The diploid number appears to be 88, with the fundamental number being 98.

Table 1 summarizes the available karyotypic data for piciform birds. All species of Picidae, except the Green Woodpecker (*Picus viridis*) discussed below, possess Z chromosomes that are the largest of the complements. Barbets (Capitonidae) also possess large Z chromosomes. Other piciform families have not been studied.

That large Z chromosomes were described independently by several researchers studying diverse taxa supports our contention that large Z chromosomes characterize all woodpeckers. The karyotype of the Green Woodpecker is a possible exception. Hammar (1970: 44) describes the Z and W chromosomes from this species as acrocentric and as being the fourth and tenth largest in size, respectively. The possibility exists that the karyotype was prepared from an embryo whose sex was either difficult or impossible to determine. If the individual were a male, chromosomes identified by Hammar as Z and W might be paired with a number of autosomes whose structure is similar. As Hammar's description did not include autoradiography or C-banding and as, according to our scheme, the largest chromosomes of his karyotype are similar to the Z chromosomes of other woodpeckers, we propose this alternative interpretation.

The absence of C-bands in the Z chromosome of the Lesser Spotted Woodpecker indicates that enlargement of this chromosome has not been accomplished by the addition of C-heterochromatin. We intend to determine whether or not G- (Giesma) banding sequences in original Z chromosome types also occur in enlarged Z chromosomes of Alaskan woodpeckers.

While the size of Z chromosomes in woodpeckers is generally uniform (Table 1), the position of the centromere is not. Even species of the same genus (*P. villosus* and *P. pubescens* as well as *D. minor* and *D. major*) have Z chromosomes that differ in position of the centromere. This variability is probably the result of intrachromosomal rearrangement (e.g. pericentric inversions or centric shifts).

The enlarged Z chromosome is not unique to Piciformes. The karyotypes of 20 species of the family Accipitridae (Falconiformes) have been described (Takagi and Sasaki 1974, de Boer 1976, Misra and Srivastava 1976, Williams and Benirschke 1976), and these species possess enlarged Z and W chromosomes. The Z chromosomes resemble those of the

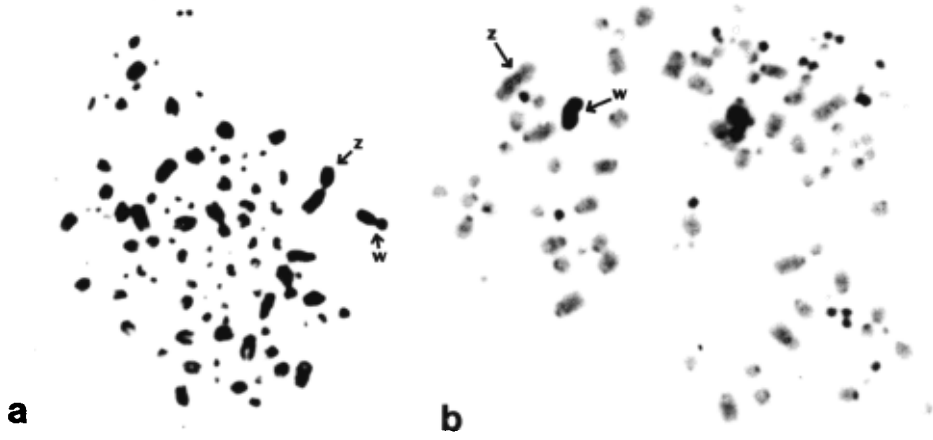

Fig. 3(a). Entire karyotype of a female lesser spotted woodpecker. Z and W sex chromosomes are indicated by arrows. (b) C-banded (constitutive heterochromatin) karyotype of a female Lesser Spotted Woodpecker. The heavily stained W chromosome is obvious as is the unpaired Z chromosome.

woodpeckers in that they, too, are the largest of the complement. Moreover, Bulatova (1973) reported enlarged Z chromosomes in three species of lark (Alaudidae) near Novosibirsk, USSR. We know of no case in birds in which the Z chromosome is enlarged while the W is not. This suggests that a mechanism for enlargement operates on both sex chromosomes in birds. While Ohno's original Z hypothesis characterizes perhaps the majority of avian lineages, derived conditions, such as described here, may be common. Stock and Mengden (1975) did not describe homology between avian Z chromosomes and chromosomes of boid snakes, a primitive family without dimorphic sex chromosomes. The apparent absence of dimorphic sex chromosomes in ratites (Takagi et al. 1972) and in all but the advanced ophidian reptiles complicates Ohno's interpretation of a homologous Z chromosome in these separate classes.

We thank Prof. Klaus H. Rothfels of the Department of Botany, University of Toronto and his students, Bruce Thorneycroft and Elizabeth Sexsmith, for bringing their observations on enlarged sex chromosomes in woodpeckers to our attention. Also, we thank all of these researchers for allowing us to cite and describe their unpublished data. Leonard Peyton located and helped collect eggs and young of Alaskan woodpeckers. Hans Staaland and Knut Rudd helped collect the Norwegian woodpeckers. Finally,

TABLE 1. A summary of sex-chromosome morphology and chromosome numbers in Piciformes.

Species	Z[a]	Z[b]	W[c]	2n	f.n.	Reference
Picidae						
Picoides pubescens	23.3	1:2.0	10.8	92	96	This paper
P. villosus	15.6	1:1.5	10.0	92	96	This paper
P. mahrattensis	16.0	1:3.1	5.1	84	98	Kaul and Ansari (1978)
Dendrocopos minor	19.1	1:1.4	12.0	108	112	This paper
D. major	18.0	1:2.5	9.6	108	112	This paper
Picus viridis	9.2?	n.r.[d]	6.3?	94	102	Hammar (1970)
Dryocopus martius	19.7	1:4.0	?	88	98	This paper
Dinopium benghalense	14.8	1:3.0	?	92	94	Kaul and Ansari (1978)
Colaptes auratus	17.1	1:3.5	11.7	90	96	This paper
Sphyrapicus varius	21.7	1:5.0	6.4	92	106	This paper
Capitonidae						
Megalaima zeylanica	15.6	1:2.6	10.7	96	126	Kaul and Ansari (1979)
M. haemacephala	17.3	1:2.5	2.9	90	106	Kaul and Ansari (1978)

[a] Relative length of Z chromosome in relation to total macrochromosome length.
[b] Ratio of the length of the long arm of the chromosome to the length of the short arm of the chromosome.
[c] Relative length of W chromosome in relation to total macrochromosome length.
n.r.—not reported.

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Individuality of Vocalizations in Dunlin: a Possible Acoustic Basis for Recognition of Parent by Offspring

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Calls of parents can be recognized by the young in several species of the avian order Charadriiformes (Beer 1970a,b; Evans 1970a,b). It can be predicted that parent-offspring vocal recognition may evolve in precocial species with the potential of young of different parentage becoming mixed during foraging activities.

In the eastern Canadian Arctic, I made tape recordings of breeding Dunlin (Charadriiformes, Scolopacidae, *Calidris alpina*). From these I attempted to establish the acoustic properties of a certain vocal signal that may allow young chicks to recognize their parents.

The precocial young Dunlin leave the nest cup within a few hours after hatching and begin pecking at small adult Diptera resting on vegetation. In the first two or three days, a parent broods its offspring, particularly in cold or rainy times. One or both of the parents accompanies the young, often "leading" them by walking ahead and emiting a low-intensity, "purring" vocalization. The purr vocalization is also given by a parent when gathering up its young for brooding. As the chicks wander over the prime feeding areas, two or more broods may mingle and separate.

Observations at this time indicate that adults give purr calls almost constantly and the young move toward an adult that is producing these calls. These observations raise questions about the possible acoustic basis for recognition of the correct parent. It is possible to discount the importance of visual recognition, because the uneven terrain usually prevents the tiny chicks from seeing the parent after a few meters of separation. I did not verify the occurrence of vocal recognition with individually marked birds, but I assumed that the young re-assort with the correct parent. This assumption is parsimonious and logical from an evolutionary point of view. Brooding space under an adult is limited, and the