Cytogenetics and Cell Genetics
Editor-in-Chief: H.P. Klinger, Bronx, N.Y.

Separatum
Publisher: S. Karger AG, Basel
Printed in Switzerland

Cytogenet Cell Genet 53:201–205 (1990)

Rapid identification of sex in birds by flow cytometry

D. Nakamura,[1,2] T. R. Tiersch,[3] M. Douglass,[4] and R. W. Chandler[1,5]

[1] Department of Obstetrics and Gynecology, Division of Reproductive Genetics, and [2] Molecular Diagnostics Laboratory, University of Tennessee, Memphis, TN, [3] Ecological Research Center, Department of Biology, Memphis State University, Memphis, TN, [4] Memphis Zoo, Memphis, TN, and [5] Puckett Laboratory, Hattiesburg, MS (USA)

Abstract. A rapid method to identify sex in birds is described. The method requires microliter volumes of blood, and, under appropriate conditions, results can be available within an hour of sample collection. Samples can be stored at 4 °C or −20 °C without sacrificing the ability to discriminate sex differences in DNA content. The assay will find utility in laboratory, field, and applied studies, in other classes of vertebrates, and in studies on the dynamics of genome size within and among populations.

In most birds the female is the heterogametic sex and carries distinguishable sex chromosomes, the W generally being smaller than the Z (reviewed by Bloom, 1974). It is believed that sequences on the W chromosome have a role in sex determination, although the only evidence to support this notion is the association of female development with the presence of the W chromosome. Possible exceptions to this relationship include the few cases of apparent absence of heteromorphic sex chromosomes or of sex-linked markers among some of the ratites (Takagi et al., 1972; de Boer, 1980; but see Ansari et al., 1988). Clearly though, the lack of heteromorphic sex chromosomes is not incompatible with heterogamety (discussed by Bull, 1983).

Many species of birds do not exhibit pronounced sexual dimorphism, either in youth or as adults (Selander, 1972). In these species, sex can be identified by courtship behavior or breeding history, cloacal examination, or laparoscopy. There are techniques for sex identification that rely on visualizing sex-chromosome heteromorphism or detecting sex-linked markers, including cytological, molecular (e.g., Tone et al., 1984), immunological (e.g., Wachtel et al., 1983), and biochemical (Baverstock et al., 1982) methods. However, these methodologies have shortcomings: variable rates of success, a need for dividing cells, the length and complexity of the procedures involved, and a need for genetic polymorphism. Furthermore, fewer than 10% of the birds have been studied cytogenetically (de Boer, 1984), and, in many cases, only a single specimen or members of only one sex were examined.

If the sex chromosomes are heteromorphic, it is also possible to diagnose sex by DNA measurement. Flow cytometry offers a rapid means of DNA quantification that requires small numbers of nondividing cells (Deaven, 1982; Vindeløv et al., 1983). This approach has been used to distinguish differences in the sex-chromosome complement of leukocytes in man (Deaven, 1982; Elias et al., 1988) and horses (Kent et al., 1988). The purpose of the present study was to develop a flow cytometric assay for identification of sex in birds. The rationale for this proposal was that the presence of a W chromosome is generally diagnostic of female development, that in many species the W is distinguishable in size from the Z, and, in the absence of unusual chromosomal packaging of DNA, that the heteromorphism is related directly to differing amounts of DNA in the Z and W chromosomes. In those birds with heteromorphic sex chromosomes, we were able to identify sex correctly in 119 of 120 cases.

Materials and methods

The scientific name, common name, and source of the birds studied are listed in Table 1. All nomenclature is based on the International Species Inventory System and represents current usage in zoos. If the birds were not sexually dimorphic, they were sexed according to courtship behavior, breeding history, laparoscopy findings, or, when possible, cloacal examination.

Blood was collected in ACD anticoagulant. The samples were stored at 4 °C or at −20 °C prior to analysis. The technique of Krishan (1975), with modifications (Tiersch et al., 1989), was used for DNA analysis. Briefly, an appropriate number of internal reference cells (either porcine [*Sus scrofa*] or channel catfish [*Ictalurus punctatus*] blood cells) and blood cells from the bird under study were suspended in 0.5 ml of lysis-staining buffer, which consisted of 0.1% sodium citrate, 0.1% Triton X-100, and 25 µg propidium iodide. Twenty-five microliters of RNase (1 mg/ml) were added just prior to the addition of cells. The suspension was filtered through a 20-µm nylon mesh, kept at room temperature, and analyzed within 15 min.

Reference cells were analyzed simultaneously with the avian cells to correct for possible fluctuations in laser output or disturbances in sample flow, which can cause spurious DNA readings (Hoehn et al., 1977). Blood cells from channel catfish, used as an internal reference, were diluted in phosphate-buffered saline containing 8% dimethyl sulfoxide. Aliquots were frozen in liquid nitrogen and stored at −70 °C. Each day, catfish cells and similarly cryopreserved cells from mosquitofish (*Gambusia affinis*) and domestic chickens (*Gallus gallus domesticus*) were thawed and analyzed as a mixture with fresh porcine and human leukocytes to calibrate the flow cytometer. An example of a relative DNA content comparison among the four species is depicted in Fig. 1.

Nuclear DNA content was measured with an EPICS V flow cytometer (Coulter Electronics, Hialeah, FL). The argon ion laser was set at a wavelength

Supported in part by a grant from the National Institutes of Health (AI-23 479) and a Van Vleet Memorial Fellowship. D. N.'s present address is The National Law Center, George Washington University, and SMZM&S, 2100 Pennsylvania Avenue, N. W., Washington, DC (USA).

Received 26 October 1989; accepted 6 February 1990.

Request reprints from Dr. Robert W. Chandler, Puckett Laboratory, 4200 Mamie Street, Hattiesburg, MS 39402 (USA).

Table I. Taxonomic classification, scientific and common names, sexes, and sources of 30 species of birds analyzed for nuclear DNA content

Order	Species	Common name	Males	Females	Source
Casuariiformes	*Dromaius novaehollandiae*	Emu	2	3	Memphis Zoo, Memphis, TN
Spheniscifformes	*Spheniscus demersus*	Jackass penguin	1	1	Memphis Zoo, Memphis, TN
Anseriformes	*Anser rossi*	Ross' goose	1	1	Memphis Zoo, Memphis, TN
	Cygnus atratus	Black swan	1	2	Memphis Zoo, Memphis, TN
	Anas castanea	Chestnut-brested teal	2	2	Memphis Zoo, Memphis, TN
	Anas platyrhynchos	Mallard	1	1	Collierville, TN
	Dendrocygna viduata	White-faced whistling duck	1	1	Memphis Zoo, Memphis, TN
	Mergus cucullatus	Hooded merganser	1	1	Memphis Zoo, Memphis, TN
Falconiformes	*Haliaeetus leucocephalus*	Bald eagle	2	2	Two sources[a]
	Aguila chrysaetos	Golden eagle	1	1	Cumberland Wildlife Refuge
Galliformes	*Gallus gallus domesticus*	Domestic chicken (4 varieties)	14	10	
		SC White Leghorn	1	1	K. Geiss, University, MN
		500 New Hampshire	1	1	K. Geiss, University, MN
		Rhode Island Red	1	1	K. Geiss, University, MN
		Rhode Island Red × Barred Rock	11	7	Collierville, TN
	Chrysolophus pictus	Golden pheasant	10	10	J. Price, Fayette County, TN
	Coturnix coturnix japonica	Japanese quail	4	5	B. Gutzke, Memphis, TN
	Guttera pucherani	Kenyan crested guineafowl	1	1	Memphis Zoo, Memphis, TN
Gruiformes	*Balearica pavonina*	West African crowned crane	2	2	Memphis Zoo, Memphis, TN
	Balearica regulorum	East African crowned crane	2	1	Memphis Zoo, Memphis, TN
Psittaciformes	*Aratinga solstitialis*	Sun conure	1	1	Memphis Zoo, Memphis, TN
	Cacatua moluccensis	Moluccan cockatoo	1	1	Memphis Zoo, Memphis, TN
	Cacatua galerita	Sulphur-crested cockatoo	4	2	Memphis Zoo, Memphis, TN
	Lorius garrulus	Chattering lory	1	1	Memphis Zoo, Memphis, TN
	Trichoglossus haematodus	Rainbow lory	1	1	Memphis Zoo, Memphis, TN
	Psittacula krameri	Ringneck parakeet	1	1	D. Christenbury, Memphis, TN
	Nymphicus hollandicus	Cockatiel	1	1	D. Christenbury, Memphis, TN
	Agapornis personata	Masked lovebird	1	1	J. Bullock, Memphis, TN
	Agapornis roseicollis	Peach-faced lovebird	1	1	D. Christenbury, Memphis, TN
	Eclectus roratus	Eclectus parrot	1	1	Memphis Zoo, Memphis, TN
	Anodorhynchus hyacinthinus	Hyacinth macaw	1	1	Memphis Zoo, Memphis, TN
	Ara rubrogenys	Red-fronted macaw	3	2	Memphis Zoo, Memphis, TN
	Ara caninde	Caninde macaw	1	1	Memphis Zoo, Memphis, TN
Piciformes	*Psilopogon pyrolophus*	Fire-tufted barbet	1	1	Memphis Zoo, Memphis, TN

[a] One pair from Cumberland Wildlife Refuge (Mt. Juliet, TN) and one pair from Memphis Zoo.

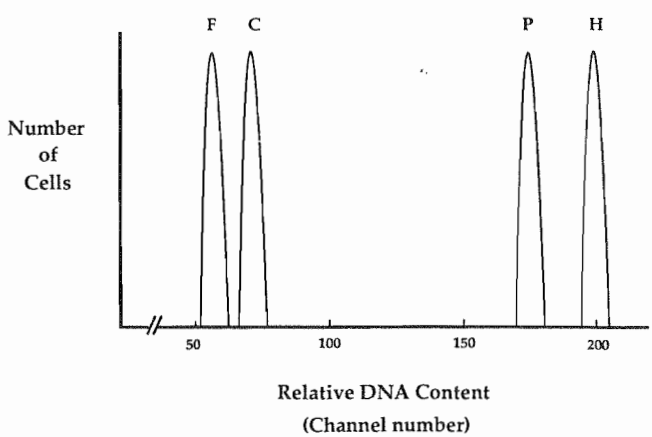

Fig. 1. Graphic representation of DNA fluorescence histogram showing relationship of domestic chicken (C) to channel catfish (F), domestic pig (P), and human (H) cells. Nuclear DNA content is directly proportional to fluorescence intensity, expressed here as channel numbers in a linear scale.

of 488 nm and a power of 300 mW in the light-stabilized mode. Two 515-nm-long-pass barrier filters, a 560-nm dichroic filter, and a 570-nm-long-pass filter were placed before the photomultiplier tube. The propidium iodide–stained nuclei were analyzed at a rate of 200–300 per second. The fluorescence of each nucleus was converted to an analog signal, digitized, and used to generate pulse-height histograms from the analysis of at least 30,000 cells. Fractional mode channels for the fluorescence peaks were calculated to four decimal places in the PARA 1 (Coulter Electronics, Hialeah, FL) program.

The DNA content per cell was expressed in either of two ways. The first was an estimate by mass, relative to an arbitrary standard value of 7.0 pg per leukocyte nucleus for the human male, according to the formula pg DNA = $(A/B) \times (C/D) \times 7.0$, where B and C are the fractional mode channels of the reference cells in the A–B and C–D mixtures, A is the fractional mode channel of the avian sample, and D is the fractional mode channel of a human male (one of us, R. W. C.). DNA content was also expressed as the DNA Index (DI), which was calculated relative to a standard value for a particular avian species according to the formula DI = $(A/B) \times (C/D)$, where B and C are as described above, A is the fractional mode channel of the bird sample, and D is the fractional mode channel of the species standard, which was the female value when only a single female was available. If specimens from multiple females were available, the mean of the ratios of the reference cells to the female cells for that species (C/D) was used to calculate individual values. In the calculation of DI's, the female of each species was assigned a value of 1.0000. Thus, samples with readings of less than 1.0000 indicate cells containing less DNA than the DNA content of cells of the standard female, and readings of greater than 1.0000 indicate cells containing more DNA than the standard female cells.

Results

The nuclear DNA content per cell of each species studied is presented in Table II. The species are ordered according to decreasing DNA content of the male of each species examined. In all

Table II. Nuclear DNA content of males and females from 30 avian species

Common name	Internal reference[a]	DNA content (pg)[b]			
		Male		Female	
Fire-tufted barbet	F	3.41	(1 × 1)[c]	3.36	(1 × 1)
Eclectus parrot	F	3.36	(1 × 1)	3.32	(1 × 1)
Jackass penguin	F	3.31	(1 × 1)	3.20	(1 × 1)
Emu	F	3.26 ± 0.02	(2 × 1)	3.24 ± 0.02	(3 × 1)
Sulphur-crested cockatoo	F	3.22 ± 0.02	(4 × 1)	3.03 ± 0.02	(2 × 1)
Moluccan cockatoo	P	3.12 ± 0.01	(2 × 2)	3.04 ± 0.01	(2 × 2)
Kenyan guineafowl	P	3.09	(1 × 1)	3.04	(1 × 1)
Canindae macaw	P	3.08	(1 × 1)	2.99	(1 × 1)
W. African crowned crane	P	3.05 ± 0.01	(2 × 1)	3.02 ± 0.01	(2 × 1)
E. African crowned crane	P	3.05 ± 0.00	(2 × 1)	2.98	(1 × 1)
Golden eagle[d]	F	3.00	(1 × 1)	2.92	(1 × 1)
Hyacinth macaw	F	2.97	(1 × 1)	2.76	(1 × 1)
Cockatiel	P	2.96	(1 × 1)	2.88	(1 × 1)
Black swan	F	2.94	(1 × 1)	2.87 ± 0.01	(2 × 1)
Bald eagle[d]	F	2.93	(1 × 1)	2.92	(1 × 1)
Mallard	F	2.92	(1 × 1)	2.83	(1 × 1)
Bald eagle	F	2.86	(1 × 1)	2.83	(1 × 1)
Red-fronted macaw	P	2.86 ± 0.02	(3 × 1)	2.78 ± 0.02	(2 × 1)
Japanese quail	F	2.84 ± 0.01	(4 × 1)	2.81 ± 0.01	(5 × 1)
Ross' goose	P	2.79 ± 0.00	(1 × 2)	2.74 ± 0.00	(1 × 3)
White-faced whistling duck	P	2.77 ± 0.01	(1 × 2)	2.71 ± 0.01	(1 × 2)
Ringneck parakeet	P	2.76	(1 × 1)	2.73	(1 × 1)
Sun conure	F	2.76	(1 × 1)	2.66	(1 × 1)
Chestnut-breasted teal	F	2.73 ± 0.01	(2 × 1)	2.68 ± 0.04	(2 × 1)
Chattering lory	F	2.72	(1 × 1)	2.69	(1 × 1)
Masked lovebird	P	2.71	(1 × 1)	2.63	(1 × 1)
Rainbow lory	F	2.68	(1 × 1)	2.62	(1 × 1)
Peach-faced lovebird	P	2.67	(1 × 1)	2.55	(1 × 1)
Hooded merganser	F	2.60	(1 × 1)	2.53	(1 × 1)
Domestic chicken[e]	F	2.49 ± 0.03	(14 × 1)	2.43 ± 0.02	(10 × 1)
Golden pheasant	F	2.44 ± 0.01	(10 × 1)	2.38 ± 0.01	(10 × 1)

[a] F = channel catfish; P = domestic pig.
[b] Mean ± SD in picograms of nuclear DNA content per blood cell in relation to fresh human leukocytes (7.0 pg/cell).
[c] Sample size × number of replications.
[d] Samples were stored at −20 °C prior to analysis.
[e] Some of the samples were stored at −20 °C prior to analysis; see Table VI.

Table III. DNA Index and percent greater DNA content of males vs. females in 30 avian species

Common name	DNA Index	% Difference[a]
Hyacinth macaw	1.0755	7.6
Sulphur-crested cockatoo	1.0611	6.1
Peach-faced lovebird	1.0473	4.7
Sun conure	1.0378	3.8
Jackass penguin	1.0349	3.5
Mallard	1.0325	3.2
Masked lovebird	1.0300	3.0
Canindae macaw	1.0294	2.9
Golden pheasant	1.0286	2.9
Golden eagle[b]	1.0275	2.8
Domestic chicken[c]	1.0266	2.7
Cockatiel	1.0266	2.7
Red-fronted macaw	1.0263	2.6
Hooded merganser	1.0261	2.6
Black swan	1.0248	2.5
E. African crowned crane	1.0238	2.4
Moluccan cockatoo	1.0234	2.3
Rainbow lory	1.0213	2.1
White-faced whistling duck	1.0200	2.0
Ross' goose	1.0182	1.8
Chestnut-breasted teal	1.0172	1.7
Kenyan guineafowl	1.0158	1.6
Fire-tufted barbet	1.0158	1.6
W. African crowned crane	1.0126	1.3
Eclectus parrot	1.0124	1.2
Chattering lory	1.0108	1.1
Ringneck parakeet	1.0106	1.1
Bald eagle	1.0096	1.0
Japanese quail	1.0086	0.9
Emu	1.0058	0.6
Bald eagle[b]	1.0038	0.4

[a] Percent greater DNA mass present in males vs. females.
[b] Sample stored at −20 °C prior to analysis.
[c] Some of the samples were stored at −20 °C prior to analysis; see Table VI.

Table IV. Percent difference in nuclear DNA content between males and females of eight orders of birds

Order	Number of species	% Difference (mean ± SD)
Sphenisciformes	1	3.5
Psittaciformes	13	3.2 ± 2.0
Anseriformes	6	2.3 ± 0.6
Galliformes	4	2.0 ± 1.0
Gruiformes	2	1.8 ± 0.8
Piciformes	1	1.6
Falconiformes	2	1.5 ± 1.0
Casuariiformes	1	0.6

Table V. Correlation of sex-chromosome heteromorphism and percent difference in nuclear DNA content between males and females of selected avian species

Species	% Difference	Hetero-morphism[a]	Reference
Emu	0.6	1	Ansari et al. (1988)
Bald eagle	0.7[b]	1	Au et al. (1975)
Ross' goose	1.8	3[c]	Shoffner et al. (1979)
Moluccan cockatoo	2.3	3	Goodpasture (1989, personal communication)
Hooded merganser	2.6	4	Benirschke and Hsu (1975)
Domestic chicken	2.7	4	Krishnan and Shoffner (1966)

[a] Assessed subjectively from published karyotypes, where 1 represents Z and W chromosomes that are nearly equal in size, 2 represents a W chromosome that is about 75% the size of the Z, 3 represents a W chromosome that is about 50% the size of the Z, and 4 represents a W chromosome that is about 25% the size of the Z.
[b] Mean of readings of frozen samples from birds maintained at the Cumberland Wildlife Refuge and fresh samples from birds maintained at the Memphis Zoo.
[c] Chromosome measurements provided in reference.

30 species, male cells had as much DNA as female cells or had more DNA than female cells. In those species with heteromorphic sex chromosomes, the sex was identified correctly in 119 of 120 animals; the one exception was a female Japanese quail *(Coturnix coturnix japonica)* that was identified as a male. The data are presented as DNA Index values in Table III. The simplest hypothesis to explain the data is that the flow cytometric measurements are monitoring differences between cells with two Z chromosomes and cells with a Z and a W, where the W is equal in size or smaller than the Z.

Differences in DNA content between males and females appear to correlate with the degree of sex-chromosome heteromorphism.

Table VI. Nuclear DNA content, DNA Index, and percent greater DNA content found in the male in four varieties of the domestic chicken

Strain	DNA content (pg)				DNA Index	% Difference
	Male		Female			
Rhode Island Red × Barred Rock	2.50 ± 0.02	(11 × 1)[a]	2.44 ± 0.01	(7 × 1)	1.0272	2.7
500 New Hampshire[b]	2.50	(1 × 1)	2.45	(1 × 1)	1.0228	2.3
SC White Leghorn[b]	2.45	(1 × 1)	2.40	(1 × 1)	1.0190	1.9
Rhode Island Red[b]	2.44	(1 × 1)	2.40	(1 × 1)	1.0150	1.5

[a] Sample size × number of replications.
[b] Samples stored at −20 °C prior to analysis.

The correlation is evident when the species are grouped by order (Table IV) and when selected species are highlighted (Table V). The ratites, in particular, have sex chromosomes with little or no heteromorphism, the DNA values of males and females being found to overlap in the emus studied. The degree of sex-chromosome heteromorphism in the remaining species listed in Table V is graded with respect to the difference in size between the Z and W chromosomes, the most extreme difference being found in the domestic chicken, where the W is about a quarter the size of the Z. In that species, cells of the male contain 2.7% more DNA than do cells of the female. Because most birds that have been karyotyped have W chromosomes that fall into categories 3 and 4 (see footnote of Table V), those species with larger W chromosomes are particularly informative. The sex chromosomes of the female bald eagle *(Haliaeetus leucocephalus)* are nearly identical in size, and, accordingly, the percent difference in DNA content in this species is not large.

Samples from three inbred strains of the domestic chicken and an F_1 hybrid were compared (Table VI). The blood cells of the inbred strains were obtained from adult birds, and the samples were stored at −20 °C for several months. The blood cells of the hybrids were obtained from birds 1–2 d before hatching. As in the other species, there is a sex-specific difference in DNA content in these birds as well.

Discussion

A rapid sexing assay used in mammals (Elias et al., 1988; Kent et al., 1988) was adopted for use in birds. The method relies on quantifying nuclear DNA in the flow cytometer. Mitotically or meiotically active cells are not required, thereby rendering nucleated erythrocytes informative for sexing purposes. Because microliter quantities of blood are sufficient, a single drop of blood offers material for multiple assays. Blood cells collected in ACD and stored at 4 °C for 2 wk remained suitable for analysis, and sex diagnosis remained possible after months of storage at −20 °C. It should be noted that small changes in DNA content were found in frozen samples (unpublished observations). Any nucleated cell is amenable to the assay, and thus the diagnosis of sex in tissues such as feather pulp should be possible (unpublished observations).

Using flow cytometry, Elias et al. (1988) showed sex-specific variability in the DNA content of male and female cells of normal human subjects. These authors also observed that the method was sensitive enough to distinguish trisomy 21, sex-chromosome aneuploidy, and sex-chromosome mosaicism. In the birds, the majority of species in which both sexes have been karyotyped are characterized by heteromorphism of the sex chromosomes. Generally, the W chromosome of the female is smaller than the Z, hence, female cells, in the absence of an unexpected DNA packaging difference between the sexes, contain less DNA than do male cells of the same species. In a cytophotometric study of Feulgen-stained nuclei, Rasch (1976) reported sex-specific DNA content differences in several species of cranes of the genus *Grus*, where males averaged 4–6% more DNA than females. The data obtained by flow cytometry reported here are in accord with these observations.

As a provisional test of the correlation of DNA content difference and degree of heteromorphism, several species in which published karyotypes are available were compared for the degree of heteromorphism and percent difference in DNA content revealed by DNA Index measurements. The degree of heteromorphism was determined subjectively from a visual assessment of the karyotypes. There is little or no heteromorphism of the sex chromosomes of *Dromaius novaehollandiae* (Takagi et al., 1972; Ansari et al., 1988), and analysis of the flow cytometric data indicated a difference of 0.6% and overlapping values of DNA content for the male and female. The W chromosome of the bald eagle is distinguishable from the Z but is not much smaller in size (Au et al., 1975). Our analysis showed a mean 0.7% difference in DNA content between cells of the two sexes. As a species without pronounced sex-chromosome heteromorphism, the bald eagle may represent the lower limit of resolution for sex diagnosis by this assay. The W chromosome of *Anser rossi* is just over half the size of the Z (Shoffner et al., 1979), and we found a 1.8% difference between the male and female of this species. The W is about a quarter the size of the Z in *G. gallus* (Krishnan and Shoffner, 1966), and the mean percent difference in this species was 2.7%. The data of Tables IV and V can be interpreted to indicate that if DNA packaging into chromosomes is uniform, the flow cytometer is monitoring DNA content differences between the sexes whenever the W is distinguishable in size from the Z chromosome.

The data of Table VI, in which there is overlap of male and female values of different varieties of *G. gallus*, provide insight into possible variation of genome size within a bird species. The human Y chromosome varies in morphology and DNA content as a result of different amounts of heterochromatin in the long arm (Wall and Butler, 1989). Additionally, intraspecific variation in genome size as high as 35% has been shown to exist in pocket gophers (Sherwood and Patton, 1982). Thus, variability in heterochromatin

content of the avian W chromosome or in the DNA content of autosomes can result in a level of intraspecific variation in genome size sufficient to obscure sex-specific differences in DNA content. From a practical standpoint, then, there is a need to include appropriate controls when making intraspecific comparisons for sex diagnosis. This is especially true when comparing males and females of a species that is distributed over a broad geographic range, where clinal variation may exist, or when making comparisons among individuals from isolated populations.

The sexing assay should be beneficial in a variety of practical applications, such as in forming breeding pairs of rare or endangered birds that lack sexual dimorphism. A small blood sample can be obtained with ease and little trauma to the bird, and a sex diagnosis can be made within the hour. There are, however, limitations to the assay, and it should not be construed as a general substitute for cytogenetic methods. The method described here relies on size, and hence DNA content difference, between the Z and W chromosomes. The method is limited to comparisons between male and female cells, and thus would have its greatest utility where birds of known sex are available to provide reference values, such as in identifying the sex of chicks in relation to their parents. For rapid results when the status of the sex chromosomes is known and sex diagnosis is all the information required, flow cytometry would appear to be the preferred technique.

Quantification of DNA by flow cytometry has long been used to detect and characterize aneuploid cell populations in cancer patients (Auer et al., 1984; Lovett et al., 1984), and the method may find further use in rapid screening of wild populations for DNA aneuploidy, as in ascertaining whether there has been mutagenic exposure (Deaven, 1982; Bickham et al., 1988). The results of this and other studies show the utility of the method, not only for studies on the dynamics of genome size but also for distinguishing sex in other vertebrates with heteromorphic sex chromosomes.

Acknowledgements

We thank M. Beck, J. Jenkins, R. Mumme, and C. Trost for their comments on the manuscript.

References

Ansari HA, Takagi N, Sasaki M: Morphological differentiation of sex chromosomes in three species of ratite birds. Cytogenet Cell Genet 47:185–188 (1988).

Au W, Fechheimer NS, Soukup S: Identification of the sex chromosomes in the bald eagle Can J Genet Cytol 17:187–191 (1975).

Auer GU, Fallenius AG, Erhardt KY, Sundelin BSB: Progression of mammary adenocarcinomas as reflected by nuclear DNA content. Cytometry 5:420–425 (1984).

Baverstock PR, Adams M, Polkinghorne W, Gelder M: A sex-linked enzyme in birds—Z-chromosome conservation but no compensation. Nature 296:763–766 (1982).

Bernirschke K, Hsu TC: Chromosome Atlas: Fish, Amphibians, Reptiles, Birds, Vol. 3 (Springer-Verlag, New York 1975).

Bickham JW, Hanks BG, Smolen MJ, Lamb T, Gibbons JW: Flow cytometric analysis of the effects of low-level radiation exposure on natural populations of slider turtles *(Pseudemys scripta)*. Arch Environ Contam Toxicol 17:837–841 (1988).

Bloom SE: Current knowledge about the avian W chromosome. Biosci Commun 24:340–344 (1974).

de Boer LE: Do the chromosomes of the kiwi provide evidence for a monophyletic origin of the ratites? Nature 287:84–85 (1980).

de Boer LEM: New development in vertebrate cytotaxonomy. VIII. A current list of references on avian karyology. Genetica 65:3–37 (1984).

Bull JJ: Evolution of Sex Determining Mechanisms (Benjamin/Cummings, Menlo Park 1983).

Deaven LL: Application of flow cytometry to cytogenetic testing of environmental mutagens, in Hsu TC (ed): Cytogenetic Assays of Environmental Mutagens, pp 325–351 (Allenheld, Osmun Publ, Totowa 1982).

Elias S, Chandler RW, Wachtel S: Relative deoxyribonucleic acid content of interphase leukocytes by flow cytometry: a method for indirect diagnosis of chromosomal abnormalities with potential for prenatal diagnosis. Am J Obstet Gynec 158:808–818 (1988).

Hoehn H, Johnston P, Callis J: Flow-cytogenetics: sources of DNA content variation among euploid individuals. Cytogenet Cell Genet 19:94–107 (1977).

Kent M, Chandler R, Wachtel S: DNA analysis by flow cytometry. Cytogenet Cell Genet 47:88–89 (1988).

Krishan A: Rapid flow cytofluorometric analysis of mammalian cell cycle by propidium iodide staining. J Cell Biol 66:188–193 (1975).

Krishnan A, Shoffner RN: Sex chromosomes in the domestic fowl *(Gallus domesticus)*, turkey *(Meleagris gallopavo)*, and the Chinese pheasant *(Phasianus colchicus)*. Cytogenetics 3:53–63 (1966).

Lovett EJ, Schnitzer B, Keren DF, Flint A, Hudson JL, McClatchey KD: Application of flow cytometry to diagnostic pathology. Lab Invest 50:115–140 (1984).

Rasch EM: Use of deoxyribonucleic acid-Feulgen cytophotometry for sex identification in juvenile cranes (Aves: Gruiformes). J Histochem Cytochem 24:607 (1976).

Selander RK: Sexual selection and dimorphism in birds, in B Campbell (ed): Sexual Selection and the Descent of Man, pp 180–230 (Aldine, Chicago 1972).

Sherwood SW, Patton JL: Genome evolution in pocket gophers (genus *Thomomys*). II. Variation in cellular DNA content. Chromosoma 85:163–179 (1982).

Shoffner RN, Wang N, Lee F, King R, Otis JS: Chromosome homology between the Ross's and the Emperor goose. J Hered 70:395–400 (1979).

Takagi N, Itoh M, Sasaki M: Chromosome studies in four species of Ratitae (Aves). Chromosoma 36:281–291 (1972).

Tiersch TR, Chandler RW, Wachtel SS, Elias S: Reference standards for flow cytometry and application in comparative studies of nuclear DNA content. Cytometry 10:706–710 (1989).

Tone M, Sakaki Y, Hashiguchi T, Mizuno S: Genus specificity and extensive methylation of the W chromosome-specific repetitive DNA sequences from the domestic fowl, *Gallus gallus domesticus*. Chromosoma 89:228–237 (1984).

Vindeløv LL, Christensen IJ, Nissen NI: Standardization of high-resolution flow cytometric DNA analysis by the simultaneous use of chicken and trout red blood cells as internal reference standards. Cytometry 3:328–331 (1983).

Wachtel SS, Wachtel GW, Nakamura D, Gilmour D: H–Y antigen in the chicken. Differentiation 23(Suppl): s107–s115 (1983).

Wall WJ, Butler LJ: Classification of Y chromosome polymorphisms by DNA content and C-banding. Chromosoma 97:296–300 (1989).