Chromosomes of two species of woodpeckers (Aves: Piciformes)

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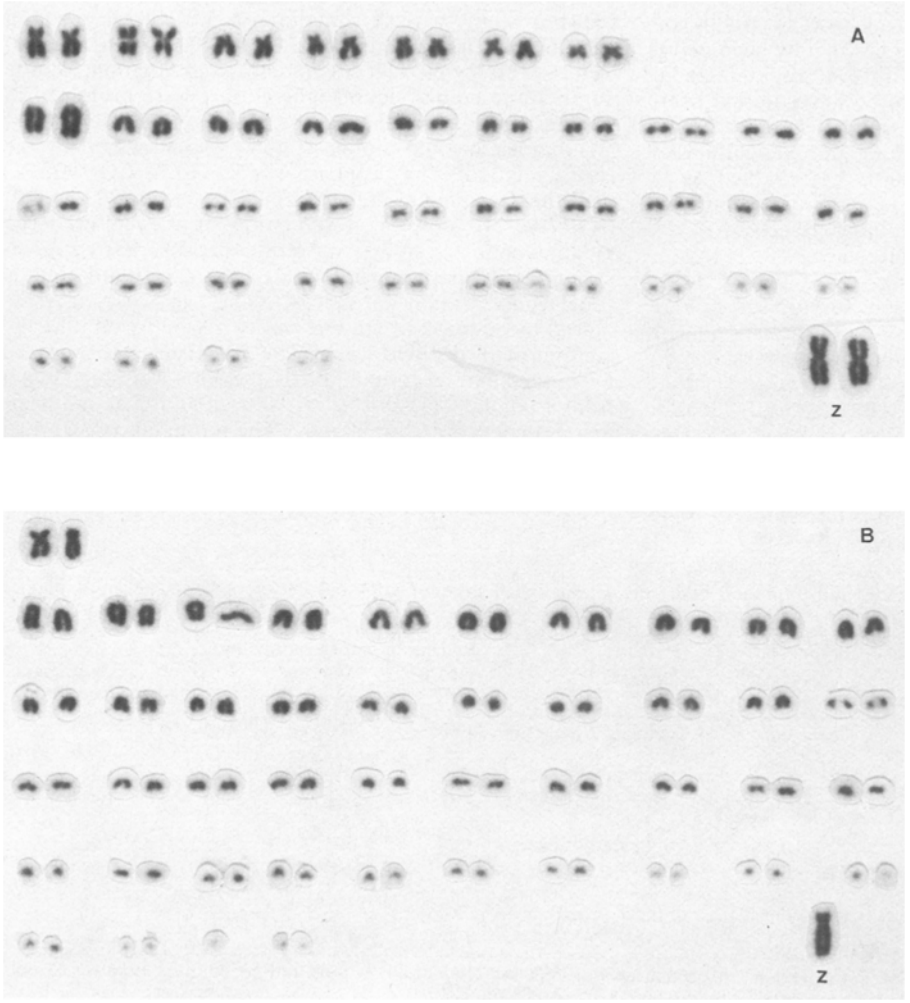
Summary. The karyotypes of *Dendrocopos major* and *D. medius* show a gradual transition from macro- to microchromosomes and a high diploid chromosome number. The Z-chromosome is the largest element.

In only 4 species of Piciformes (1 species of Capitonidae and 3 species of Picidae) have the chromosome complements been studied in detail[1,2]. These studies showed considerable differences in the shape of the macrochromosomes, the sex chromosomes and the total number of chromosomes present. In this communication the results of cytogenetic studies of 2 further species of Picidae *(Dendrocopos major* and *D. medius)* are presented.

Materials and methods. From 4 sibling specimens of *D. major* and 7 sibling specimens of *D. medius* (from woodlands near Basel, Switzerland) the pulpa material of growing body feathers was prepared according to the method suggested by Hoffmann[3] and stained with Giemsa. This method allows cytogenetic studies without sacrifying birds and without risks in applying cytostatica, provided that the birds have growing feathers (specifically nestlings or moulting birds). In our study, about 10 body feathers per specimen provided sufficient material to analyze 5–10 metaphases. Moreover, it was not necessary to work under sterile conditions, making this method useful for field studies. After removal, the pulpa content is incubated for only 3 h in McCoy 5a-Medium (Gibco). The next steps include re-suspension in trypsin solution, in Na-citrate solution and in a fixative.

Results. In both species there was a continuous range of sizes from macro- to microchromosomes. The largest was the Z-chromosome. The chromosome number of *D. medius* was 2n = about 86 and that of *D. major* 2n = about 90. The exact number could not be determined with certainty due to the small size of several microchromosomes. The slightly lower chromosome number in *D. medius* compared to *D. major* seems to be correlated with the higher number of larger metacentric chromosomes in the former. In *D. major,* only 1 pair of larger metacentric autosomes could be found, whereas *D. medius* possesses 7.

The W-chromosome could not be identified clearly (see fig). There was apparently no major variation of the larger chromosomes in all specimens examined.



Karyotypes of *D. medius* (A) and *D. major* (B). The W-chromosome could not be identified with certainty. The loss of single chromosomes is an artefact due to preparation as checked in other metaphases.

Discussion. The gradual transition from macro- to microchromosomes, apparently typical in the Picidae, was also found in *D. major* and *D. medius.* It appears that the Piciformes have among the highest chromosome numbers for birds[2,4,6] and this is supported by our study.

The Z-chromosome is usually not the largest element in the chromosome sets of birds; however, exceptions have been found in the woodpeckers *D. major, D. medius* (this study), *Picoides mahrattensis* and *Dinopium benghalense*[2] as well as in some species of the Alaudidae (Passeriformes)[6].

Among the Piciformes, the Z-chromosome in *Megalaima haemacephala* (Capitonidae) is the 2nd largest and in *Picus viridis* (Picidae) only the 4th largest element[1,2].

In *M. haemacephala,* all larger chromosomes (1-7) are biarmed while in *D. benghalense,* all larger chromosomes (1-11) are telocentric. In the genus *Dendrocopos,* in which *P. mahrattensis* is included by various taxonomists (e.g. Voous[5]), *D. mahrattensis* and *D. medius* show 7 large biarmed chromosomes, but *D. major* only 1. *Picus viridis* has 4 biarmed chromosomes. Kaul and Ansari[2] have suggested that the telocentric chromosomes of *D. benghalense* have been produced by an extensive fission of biarmed chromosomes of the type of *D. mahrattensis.*

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Effects of 5-bromodeoxyuridine on in vitro development of mouse embryos in the early somitic stage

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Summary. The inhibitory effect of 5-bromodeoxyuridine on the early somitic stages of mouse embryos was largely prevented in the presence of excess thymidine but only partially prevented by deoxycytidine.

The teratogenic action of 5-bromodeoxyuridine (BUdR) on the development of rodent embryos has mainly been investigated using pregnant animals[1,2]. However, rapid degradation of BUdR administered to them by the maternal liver[3] did not allow examination of the full potential of BUdR action. Recent progress in the whole-embryo culture of rodent embryos during organogenesis has enabled us to maintain their growth for a limited period of time almost as well as in vivo[4,5]. We report the inhibitory effects of BUdR on mouse embryos at the early somitic stage.

Materials and methods. Mouse embryos of the STD-ddy strain, at the late presomitic to early somitic stage, were extracted from the uterus on day 7 of gestation. The decidua and extraembryonic membranes were removed[4]. 3 embryos were placed in a flask containing 3 ml of medium and rotated at 30-40 rpm at 38 °C. At 0.5-3 h after the initiation of the culture (somitic stages 1-4), each flask was supplemented with 15 µl of nucleoside solution in a Hanks BBS. Untreated controls were supplied only with the same volume of Hanks BBS. After 20 h embryos were transferred into a flask containing 6 ml of fresh medium and cultured for another 24 h. On termination, the heartbeat, visceral yolk sac circulation, axial rotation, somite number and cranial neural tube closure were examined in all the embryos. Some of them were then cleaned of membranes and the protein content was measured by the Lowry method[6]. The gas phase was 5% O_2/5% CO_2/90% N_2 until the exchange of the medium, followed by 20% O_2/5% CO_2/75% N_2 until the termination of the culture. The medium consisted of 80% rat serum and 20% Earle supplemented with streptomycin sulfate (50 µg/ml) and glucose (final concentration 2.0 mg/ml). The osmolarity increase caused by addition of glucose was corrected by diluting the medium with distilled water. The rat serum was prepared by immediate centrifugation of the blood freshly drawn from a female STD-Wistar rat after an 18 h fast and heat-inactivated at 56 °C for 30 min[7]. The serum glucose content was measured by the glucose oxidase method[8].

Results and discussion. The inhibitory effect of BUdR on the mouse embryos of the early somitic stage was dose-

Effects of various concentrations of BUdR and inhibition of BUdR actions by thymidine(TdR) and deoxycytidine(CdR) in explanted mouse embryos of the early somitic stage

	Number of embryos explanted	Somites[a]	Protein[a] (µg)	Percent of embryos showing Heart-beat[b]	Yolk sac circulation[b]	Rotation completed	Open cranial neural tube
Untreated control	21	28.0±0.2	274.3±6.6	100	100	100	0
5 µg/ml BUdR	21	27.5±0.2	259.9±8.7	95.2	95.2	95.2	28.6
25 µg/ml BUdR	21	–[c]	186.4±8.9*	66.7	19.0	19.0	100
100 µg/ml BUdR	21	–[c]	86.7±3.0*	0	0	0	100
TdR control	15	27.7±0.2	262.1±6.0	100	95.2	95.2	0
TdR+BUdR	15	27.7±0.2	243.8±6.1**	100	100	100	6.7
CdR control	15	27.2±0.2	258.1±5.3	93.3	86.7	86.7	0
CdR+BUdR	15	26.9±0.1	203.8±7.6*	100	80.0	80.0	86.7

[a]Values represent mean±SE. [b]Including those with active heartbeat and yolk sac circulation. [c]Could not be counted because of poor definition. *Significant at $p<0.001$. **Significant at $0.05<p<0.02$.