First Karyological Analysis of Black the Crowned Crane (Balearica pavonina) and the Scaly-Breasted Munia (Lonchura punctulata)

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Summary  This research was the first karyological analysis of the black crowned crane (Balearica pavonina) and the scaly-breasted munia (Lonchura punctulata). Two specimens of B. pavonina (one male and one female) and two specimens of L. punctulata (one male and one female) were obtained from Nakhon Ratchasima Zoo, Thailand. After using the standard peripheral blood lymphocyte culture method at 41°C for 72 h in the presence of colchicine, the metaphase spreads were performed on microscopic slides and air-dried. A conventional staining technique was applied to stain the chromosomes. The results showed that the diploid chromosome number of B. pavonina is 2n=80. The chromosomes have two sizes; 11 pairs of macrochromosomes and 29 pairs of microchromosomes were observed. The macrochromosomes consist of 14 metacentric, 6 submetacentric, and sex chromosomes. The Z chromosome is a medium metacentric chromosome and the W chromosome is a small metacentric chromosome. The diploid chromosome number of L. punctulata is 2n=72. The chromosomes have two sizes; 10 pairs of macrochromosomes and 25 pairs of microchromosomes were observed. The macrochromosomes consist of 2 metacentric, 4 submetacentric, 2 acrocentric, 10 telocentric, and sex chromosomes. The Z chromosome is a small metacentric chromosome and the W chromosome is the smallest metacentric chromosome.

Key words  Balearica pavonina, Lonchura punctulata, Chromosome, Karyotype.

The black crowned crane (Balearica pavonina) is a bird in the crane family Gruidae. It is found in the dry savannah region, south of the Sahara in Africa, although it nests in somewhat wetter habitats. This species and the closely related Grey Crowned Crane, B. regulorum, which prefers wetter habitats for foraging, are the only cranes that can nest in trees. This habit, amongst other things, is a reason why the relatively small Balearica cranes are believed to closely resemble the ancestral members of the Gruidae (Johnsgard 1983). The scaly-breasted munia (Lonchura punctulata) is a bird in the family Estrildidae. This estrildid finch is a resident breeding bird in tropical southern Asia, found in India and Sri Lanka in the west to Indonesia and the Philippines in the east. L. punctulata is a small gregarious bird that feeds mainly on seeds. It frequents open woodland and cultivated areas (Restall 1996).

Although many reptiles (e.g. lizards, snakes, and crocodiles) are known to have microchromosomes, and karyotypes of particular species of turtles are quite similar to avian ones, the pattern is a uniquely avian feature. The relatively unchanged nature of the diploid chromosome number among
the majority of avian species further implies that such a karyotype was, and is, a highly successful means of genome organization. Like flight, feathers and a small genome, this characteristic karyotype, once it had appeared in birds, remained relatively constant to the present day (Griffin et al. 2007).

Most avian karyotypes are composed of about 40 pairs of chromosomes. Some notable exceptions are the stone curlew (Burhinus oedicnemus, 2n=20 pairs of chromosomes) and kingfisher (Alcedo atthis, 2n=66 pairs of chromosomes). Seven or eight pairs of the largest chromosomes, the macrochromosomes, are 3–6 μm in length. The remaining 30–32 pairs are 0.5–2.5 μm in length and are known as microchromosomes (Rodionov 1996). Microchromosomes are characteristic of all avian and many reptilian karyotypes, with the notable exception of the genus Crocodylia, a lineage phylogenetically close to the birds with a common ancestor 254 million years ago (Janke and Arnason 1997).

Conventional and banded karyotyping are important taxonomic tools for assessing the diversification between species, subspecies and populations. There is only one previous report on the family Gruidae (crane) by Rasch (2006). From the present study, we show the standardization of karyotypes and idiograms of B. pavonina and L. punctulata. This report describes the first chromosome analysis of B. pavonina and L. punctulata by a conventional staining technique. The knowledge gained here will provide advantageous cytogenetic information for further study on taxonomy and evolutionary relationships. Moreover, it is useful basic information for the conservation, breeding, and chromosome evolution of these birds.

Materials and methods

Blood samples of B. pavonina and L. punctulata were taken from one male and one female specimen each, which were kept in Nakhon Ratchasima Zoo, Thailand, and subjected to cytogenetics studies by lymphocyte culture of whole blood samples (Shoffner et al. 1966; Rooney 2001). The culture cells were examined with the colchicines-hypotonic-fixation-air-drying technique followed by conventional staining with Giemsa’s solution. Chromosomal checks were performed on mitotic metaphase cells under a light microscope. Twenty cells each from male and female with clearly observable and well-spread chromosomes were selected and photographed. The lengths of the short arm chromosome (Ls) and the long arm chromosome (Ll) were measured to calculate the length of the total arm chromosome (LT, LT=Ls+Ll). The relative length (RL) and the centromeric index (CI) were also computed to classify the types and sizes of chromosomes according to Chaiyasut (1989). All parameters were used in karyotyping and idiograming.

Results and discussion

Firstly, the cytogenetic study of B. pavonina and L. punctulata using T-lymphocyte cultures demonstrated that the chromosome number of B. pavonina is 2n (diploid)=80, comprising 22 macrochromosomes (16 metacentric and 6 submetacentric chromosomes) and 58 microchromosomes (Fig. 1). The macrochromosomes include two large metacentric, two large submetacentric, two medium submetacentric, 10 small metacentric, four small submetacentric, and two sex chromosomes (ZW system). The Z chromosome is a medium metacentric chromosome, while the W chromosome is a small metacentric chromosome. The fundamental number (NF, number of chromosome arms) is 102 in both male and female. The chromosome number of L. punctulata is 2n=72, consisting of 20 macrochromosomes (4 metacentric, 4 submetacentric, 2 acrocentric, and 10 telocentric chromosomes) and 52 microchromosomes (Fig. 3). The macrochromosomes include two large metacentric, four large submetacentric, two large acrocentric, 10 small telocentric, and two sex chromosomes (ZW system). The Z chromosome is a small metacentric chromosome, and the W chromosome is
Fig. 1. Metaphase chromosome plates and karyotypes of the male (upper) and female (lower) black crowned crane (*Balearica pavonina*), 2n=80, by a conventional staining technique. The arrows indicate the sex macrochromosomes.

Fig. 2. Standardized idiogram of the black crowned crane (*Balearica pavonina*) from macrochromosomes (11 pairs), 2n=80, by a conventional staining technique. The arrows indicate the sex macrochromosomes.
also a small metacentric chromosome. The NF is 90 in both male and female.

The most complete account of the chromosome number in birds is given by Christidis (1990), including 723 species with relatively accurate chromosome numbers and partial karyotypes. Rodionov (1997) suggested that there are nearly 800 published avian karyotypes in existence and cites several not quoted by Christidis. As we mentioned, the diploid chromosome number is very consistent in birds; about 63% of birds have $2n=74–86$ and about 24% have $2n=66–74$ (Christidis 1990). Our group recently published a complete chicken ($Gallus gallus domesticus$) karyotype with $2n=78$ (Masabanda et al. 2004).

Microchromosomes are a universal characteristic of all avian species and many reptilian karyotypes. The typical avian karyotype contains about 40 pairs of chromosomes and 30 pairs of small to tiny microchromosomes. This characteristic karyotype probably evolved 100–250 million years ago. At one time, the microchromosomes were thought to be a non-essential component of the avian genome. Recent work has shown that even though these chromosomes represent only 25% of the genome; they encode 50% of the genes. Contrary to popular belief, microchromosomes are present in a wide range of vertebrate classes, spanning 400–450 million years of evolutionary
history (Burt 2002). Microchromosomes are also present in the karyotype of many primitive vertebrates (Ohno et al. 1969, Morescaleti et al. 1977a, 1977b, 1979, Burt 2002). These observations have led to the hypothesis that avian microchromosomes may represent an archaic linkage group of ancestral vertebrates (Tegelström and Ryttman 1981, Jones et al. 1997, Fillon 1998).

Newcomer (1955) suggested that microchromosomes are special heterochromatin elements that lack genes and centromeres. However, microchromosomes were found to contain 50% of all avian genes (Smith et al. 2000). They are stable and maintained during mitotic and meiotic cell division (Krishan 1964) and have functional centromeres and telomeres (Solovei et al. 1994). The DNA content of macrochromosomes (800 Mb) and microchromosomes (400 Mb) has also been approximated (Rodionov 1996, Smith and Burt 1998). Many birds however have low numbers of microchromosomes, and some members of the family Coraciiformes have very few. In the family Falconiformes, new world falcons have 84–90 microchromosomes, but old world falcons have only have 24–26 microchromosomes (de Lucca 1985). These differences are likely due to chromosome fusions creating small metacentric chromosomes (Tegelström et al. 1983). The family Coraciiformes is of special interest, having both the lowest, in B. oedicnemus (2n=40), and the highest, in A. atthis (2n=132), number of chromosomes of any avian order (Christidis 1990).

In birds, females are the heterogametic sex, carrying one copy each of the Z and W sex chromosomes. Males are homogametic (ZZ). Although there is a small pseudoautosomal region on Z and W with an obligate crossing over at meiosis, most of the W chromosomes do not recombine (Ellegren 2001). The class Aves has a consistent karyotype (2n=ca. 80) (Takagi and Sasaki 1974) with the Z chromosome always having a similar size (7–10% of the genome) (Schmid et al. 2005) and, depending on the species, representing either the fourth or fifth largest chromosome pair (Ohno et al. 1964). The W chromosome, however, varies greatly in size in different bird families, being virtually the same size as the Z in ratites, but very small and heterochromatic in most carinates (Takagi and Sasaki 1974).

In B. pavonina and L. punctulata, the chromosome markers are the chromosome pair 1, which is the largest metacentric chromosomes pairs. The data of the chromosomal checks on mitotic metaphase cells are shown in Tables 1 and 2. Figures 2 and 4 show the idiograms obtained by a conventional staining technique. The karyotype formulas are as follows:

### Table 1. Mean of the short arm chromosome length (Ls), long arm chromosome length (Li), total arm chromosome length (LT), relative length (RL), centromeric index (CI), and standard deviation (SD) of RL and CI from metaphase macrochromosomes in 10 cells of the black crowned crane (Balearica pavonina), 2n=80.

<table>
<thead>
<tr>
<th>Chromosome pairs</th>
<th>Ls</th>
<th>Li</th>
<th>LT</th>
<th>RL±SD</th>
<th>CI±SD</th>
<th>Types</th>
<th>Sizes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.806</td>
<td>1.174</td>
<td>1.980</td>
<td>0.085±0.007</td>
<td>0.592±0.019</td>
<td>Metacentric</td>
<td>Large</td>
</tr>
<tr>
<td>2</td>
<td>0.703</td>
<td>1.021</td>
<td>1.724</td>
<td>0.074±0.006</td>
<td>0.592±0.044</td>
<td>Metacentric</td>
<td>Large</td>
</tr>
<tr>
<td>3</td>
<td>0.451</td>
<td>0.912</td>
<td>1.363</td>
<td>0.059±0.009</td>
<td>0.669±0.097</td>
<td>Submetacentric</td>
<td>Large</td>
</tr>
<tr>
<td>4</td>
<td>0.385</td>
<td>0.663</td>
<td>1.048</td>
<td>0.045±0.009</td>
<td>0.632±0.043</td>
<td>Submetacentric</td>
<td>Medium</td>
</tr>
<tr>
<td>5</td>
<td>0.345</td>
<td>0.622</td>
<td>0.967</td>
<td>0.041±0.008</td>
<td>0.642±0.028</td>
<td>Submetacentric</td>
<td>Small</td>
</tr>
<tr>
<td>6</td>
<td>0.384</td>
<td>0.540</td>
<td>0.924</td>
<td>0.040±0.003</td>
<td>0.584±0.048</td>
<td>Metacentric</td>
<td>Small</td>
</tr>
<tr>
<td>7</td>
<td>0.314</td>
<td>0.467</td>
<td>0.781</td>
<td>0.033±0.006</td>
<td>0.597±0.089</td>
<td>Metacentric</td>
<td>Small</td>
</tr>
<tr>
<td>8</td>
<td>0.293</td>
<td>0.421</td>
<td>0.714</td>
<td>0.031±0.005</td>
<td>0.589±0.067</td>
<td>Metacentric</td>
<td>Small</td>
</tr>
<tr>
<td>9</td>
<td>0.257</td>
<td>0.332</td>
<td>0.589</td>
<td>0.025±0.003</td>
<td>0.563±0.067</td>
<td>Metacentric</td>
<td>Small</td>
</tr>
<tr>
<td>10</td>
<td>0.226</td>
<td>0.268</td>
<td>0.494</td>
<td>0.021±0.002</td>
<td>0.542±0.036</td>
<td>Metacentric</td>
<td>Small</td>
</tr>
<tr>
<td>Z*</td>
<td>0.504</td>
<td>0.720</td>
<td>1.224</td>
<td>0.056±0.008</td>
<td>0.588±0.046</td>
<td>Metacentric</td>
<td>Medium</td>
</tr>
<tr>
<td>W*</td>
<td>0.366</td>
<td>0.454</td>
<td>0.820</td>
<td>0.035±0.004</td>
<td>0.553±0.058</td>
<td>Metacentric</td>
<td>Small</td>
</tr>
<tr>
<td>12–40**</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Remarks: *sex macrochromosomes (Z and W chromosomes), **microchromosomes (dot like), large macrochromosome LT>1.237, medium macrochromosome LT=0.990–1.237, and small macrochromosome LT<0.990.
B. pavonina: 2n (80)=L\textsubscript{2}^m+L\textsubscript{2}^s+M\textsubscript{2}^s+M\textsubscript{10}^s+S\textsubscript{4}^s+ZW+microchromosome
L. punctulata: 2n (72)=L\textsubscript{2}^m+L\textsubscript{4}^s+L\textsubscript{2}^s+L\textsubscript{10}^s+ZW+microchromosome

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