Genetic Organization Of Motacilla Flava

H. K. Garg, Ashish Shrivastava

Abstract: The Yellow Wagtail, *Motacilla flava* Linnaeus, Motacillidae (Passeriformes : Aves) is a slender 15 - 16 cm long bird that inhabits open country near water such as wet meadows. Cytological investigation depicted the diploid chromosome count between 78 and 85 with 80 as modal value. No structural uncertainty was observed between macro- and micro- chromosomes. Z - W were fourth in order of size, both sub-metacentric. There were sixty four tiny telomeric elements (n=32), scattered all over the cell plates, amounted to 24.11% of the total genome.

Index Terms: Avian cytology, Cell garniture, Chromosomes, Karyotype, Genome, Genetic organization, Yellow wag-tail.

1 Introduction

Birds are characterized by fluff, flight, a petite genome and a distinctive karyotype. Despite large numbers of chromosomes, the diploid count (2n=80) is amazingly constant with 2n = 74 - 86 in 63%, 2n = 66 - 74 in 24% and 2n = 40 & 2n = 142 in the remaining 13% of birds. Of these, chicken chromosomes (2n = 78) have been extensively studied as 1, 2, 3, 4q, 5, 6, 7, 8, 9, 4p and Z representing the ancestral avian chromosomes 1-10 + Z; chromosome 4 being the most ancient. The molecular cytogenetic probes generated from this species have been used to further understand the evolution of the avian genome.

During the past two millennium, 1/5th of the bird fauna have been eliminated worldwide as an aftermath of ecological transformations and human infringement of avian territories. Despite imminent threat for many bird species to join the roll of 'endangered or threatened forms' almost zilch is known cytogenetically about them. Of 8,948 species of extant birds 802 have been karyotyped which supply chromosomal information for less than 9% of the global avian fauna [1]. In order to explore their genetic framework, one species of bird, *Motacilla flava*, has been selected for research investigation.

2 Material & Method

The Yellow Wagtail, *Motacilla flava*, is a small passerine bird of the wagtail family Motacillidae, which includes pipits and long-claws. This insectivorous bird inhabits open country near water such as wet meadows. It nests in tussocks, laying 4-8 speckled eggs. It is a slender 15–16 cm long bird with characteristic long, constantly wagging tail. The breeding adult male is basically olive above and yellow below. The heads of breeding males come in a variety of colours and patterns depending on subspecies. The specimens were traced in nature. Blood cells were drawn from the *vena basilica* at the point where it crosses radius and ulna. Sometimes in long-legged species, it was obtained from the *vena sphanea* approximately midway the tarso-metatarsus.

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Approximately 0.1 ml of a sterile solution of Na-heparin (5000 I.U./ml) is drawn into a disposable syringe and 0.5 to 2.0 ml of blood sample was drawn. Small syringes (up to 2 ml) were preferred since the vacuum produced by larger syringes causes the *vena basilica* to collapse during suck up. The syringe was shaken to mix the blood cells and heparin thoroughly. The cell plates were prepared after Garg [1].

Flame drying and Staining

Commercially available microscopic glass slides (preferably PIC-1) were taken and submerged overnight in 1: 4 sulphuric acid - water. The slides were cleansed under running tap water for an hour, dipped in 70% alcohol for 2 - 4 hr and kept in refrigerator. Now, the cell suspension was taken and a large drop of it was dispersed on a clean wet slide. The slide was then lifted and moved gently over the flame of the spirit lamp or alternatively kept on a hot plate, depending on circumstances, until the fixative got evaporated. The slides were stained in Giemsa (merk) solution (diluted 1:50 with Sorenson's buffer) at pH 6.8 for 15 min. The slides were finally rinsed in tap water, dried in air and mounted in glycerin.

Cytobiometry

Morphometric analysis of chromosomes were carried out from enlarged photomicrographs of five well spread metaphase plates of each sex. The Percentage Relative length ($\%^{R}_{L}$), the Arm Ratio (r) and/or the Centromeric Index ($^{C}_{I}$) were calculated as follows :

$$\%^{R}_{L} = \frac{\text{Length of Macrochromosome}}{\text{Total Haploid Macrochromosomal Length}} \times 100$$

$$r = \frac{\text{Length of the long arm of the chromosome}}{\text{Length of the short arm of the chromosome}}$$

$$^{C}_{I} = \frac{\text{Length of the short arm}}{\text{Total Length of that chromosome}} \times 100$$

3 Results & Discussion

Two males and one female specimens of the yellow wagtail were collected during spring. Of some thirteen-plus metaphase plates examined, nine cells had 40 pairs of chromosomes (2n=80), although in rest of the cells, the diploid chromosome count varied from 78 to 85. There was no morphological ambiguity between macro- and micro-chromosomes.

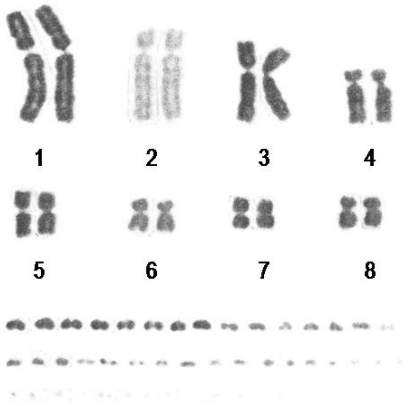

Figure - 1 : *Male karyotype of M. flava*

Macrochromosomes were divisible into two groups : Group I was represented by three large metacentric chromosomes. It was the largest element of the karyotype ($^{R}_{L}$ = 9.54% and $^{C}_{I}$ = 44. 82).

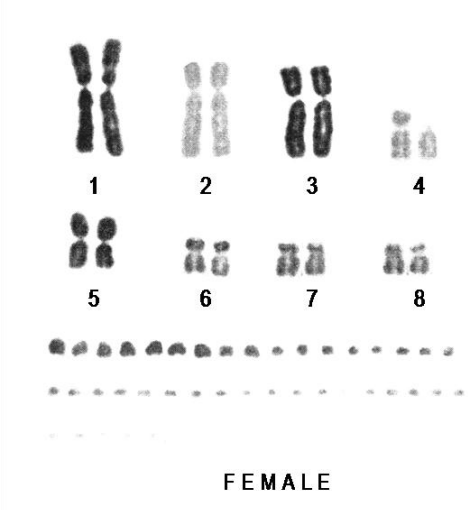

Figure - 2 : *Female karyotype of M. flava*

Group II comprised five pairs of large (≥ 7.5% of TML) to medium (≥ 2.5% of TML) sized sub-median chromosomes (chromosome 4-8). Z-W were fourth in order of size, both sub-metacentric. The last three pairs were difficult to be individualized owing to a regular fall in size, comparable morphology and overlapping array of centromeric indices. Apart from these eight pairs, there were sixty four small dot-shaped telocentric elements (n=32), scattered hither and thither in the cell plates, amounted to 24.11% of the total genome. In the family Motacillidae, Anthus trivialis has been investigated by Bulatova [2]. The karyotype derived from her analysis is quite similar to that observed in the present study. The only exception is that now the Z and W have also been identified. Even its congeneric taxa, Motacilla alba [3], shares a common karyotype. The Z chromosome is of the same size, No. 4 in both species, with centromere in sub-median range whereas, W is a telomeric chromosome in M. alba and a sub-metacentric chromosome in M. flava. The most impressive fact in avian cytogenetics is the conservation of number of chromosomes. A standard karyotype for birds seems to be one encompasses 2n=80 with 8 macrochromosomes and 32 micro-chromosomes. This karyotype is over and over again expressed in the orders Tinamiformes, Anseriformes, Galliformes, Strigiformes and Passeriformes. In correlation studies, between the number of macro- and micro-chromosomes, the two types of chromosomes were found to be negatively correlated within an order. When the number of macrochromosomes increases, the number of microchromosomes decreases. This indicates an evolutionary connection between the numbers of chromosomes of the two different sizes. In birds with a high number of microchromosomes, the smallest macrochromosomes are preferentially telocentric (79.6 %), but in birds with a low number of microchromosomes, the smallest macrochromosomes are metacentric (50.0%) or submetacentric (15.5%). There is also a significant difference among the first six macrochromosomes as they are recurrently telocentric in birds with extremely high microchromosome number. There seem to be arguments supporting the idea that, within a certain order, there is a process of microchromosomal fusion to macrochromosomes, especially giving rise to smaller macrochromosomes. As indicated above, this tendency of chromosomal fusion might be connected with the speciation process and consequently the rapidly evolving orders would show this tendency more than others. Slizynski [4] proposed three hypotheses for the existence of microchromosomes. He proposed that, in the meiotic microchromosomes, the chiasmata are scarce or absent. This probably reduces the amount of genetic variation present in a species. The tendency towards reduction in number of microchromosomes in certain species might reflect an increase in genetic variation caused by microchromosomal fusion. However, we do not know much about the kind of genetic material in the microchromosomes and it remains to be proved that fusion of microchromosomes and increased crossing over between micro-chromosomal genes increase the amount of genetic variation in such a way that it is of selective advantage. The presence of two, more or less, distinct size groups of chromosomes in almost all bird species evokes a thought that there could be functional differences between the large and small chromosomes. But there are several arguments against this opinion. The two size groups of chromosomes are an exception rather than a rule when comparing the karyotypes of bird species belonging to scores of avian orders. Chromosome size forms a continuous series in mitosis in many bird karyotypes [5],[6],[7],[8]. A number of schools opined that there were no structural differences between large and small chromosomes [9] and no differences in DNA synthesis [10]. Thus, there would be no reason to expect functional diversity between large and small chromosomes. All the chromosomes and all differences are, thus, taken into account while comparing the karyotypes of different species.

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