Karyotype and Banding Patterns of Chicken Breeds

H.H. Musa¹,², B.C. Li², G.H. Chen², T.P. Lanyasunya²,³, Q. Xu² and W.B. Bao²
¹Department of Animal Production, Faculty of Veterinary Science, University of Nyala, Sudan
²College of Animal Science and Technology, Yangzhou University, Yangzhou, 225009, China
³Kenya Agricultural Research Institute (KARI), Kenya

Abstract: Traditional karyotyping is invented in animal research for several decades depend on the analysis of characteristic banding patterns along the length of chromosome. In the present study chicken metaphase chromosomes were obtained by peripheral blood lymphocyte culture techniques, G-band patterns were obtained with trypsin and Giemsa, C-band patterns were treated with barium and the nuclear organizer regions (NORs) were identified by silver staining. All species studied presented a diploid number of 78 chromosomes, with 10 pairs of macro chromosomes including the sex chromosome and 29 pairs of micro chromosomes. G-band patterns were found quite different between breeds. The dark stained of C-band was observed on micro chromosome and W chromosome. Karyotype resemblance near coefficient was possible for breeds clustering. The position of centromers, relative length, arm ratio and the evolutionary distance of chicken breeds was estimated. The application of chromosome karyotype and banding techniques was used to study the origin, evolution and relationship of species, also used for gene location and sex determination. While, in the Medical field was used to identify genetic disease. The techniques was consider as a base for further molecular research, for example FISH

Key words: Chicken, karyotype, banding patterns

Introduction
Throughout the amazing diversity of living organisms on the planet there would appear to be little correlation between the number of chromosomes and the organisms themselves (White, 1978). The number of chromosomes appears to vary quite randomly across families, genera and taxa. Certainly there would appear to be no selective advantage in having a greater or lesser number of them generally. This is exactly what would be predicted if one assumed that the only function of chromosomes was for genetic information packaging. Thus, further studies would be needed to determine whether species with the same number of chromosomes actually had fully compatible karyotypes. As we have seen only a few chromosomal aberrations actually lead to chromosome number change. Inversions and translocations might well have been responsible for speciation without affecting the basic number. It is also possible that these species had not yet evolved barriers to prevent gene flow between them at all. Perhaps these are the result of populations that had evolved prezygotic reproductive isolation mechanisms first. If so, they might actually be better classified together as one species. Few studies have been done into the viability of hybrids between primates and this situation is unlikely to change bearing in mind the ethical considerations of such studies. It would appear that the fairly random nature of an organism's karyotype indicates that there is little or no selective advantage in having any particular chromosome number and that these changes therefore occur for other, probably quite random reasons. Although it would appear that changes in chromosome number are practically irrelevant in determining the phenotype and selective fitness of the individual they might be important as postzygotic reproductive isolation mechanisms.

Before sophisticated staining techniques revealed complex banding patterns, however, individual chromosomes were difficult to tell apart. Since then various banding techniques in the early 1970s have revealed a much greater detail of chromosome structure making them much easier to identify (Strachan and Read, 1999). For example G-banding produces a high resolution, high contrast image of chromosomes. They are subject to controlled digestion with trypsin before staining with Giemsa (a DNA binding dye), and Q-banding uses Quinacrine as a dye. Chromosomes treated in this way have to be viewed with UVfluorescence but show essentially the same banding as G bands. R-banding shows the reverse pattern of G banding. Whereas, C-banding produces a number of dark bands are largely confined to areas around centromeres. Nuclear organizer regions (NORs) are
usually found at secondary constrictions. They consist of tandemly repeated 5.8S, 18S and 28S rRNA genes, consists of approximately 80-100 repeats. In most species the 5S rRNA genes are clustered elsewhere in the genome. By carefully applying these dyes, sub-bands and sub-sub-bands can be identified. Along with the improved resolution of detail came new naming standards based on the different lengths of the arms of the chromosome extending away from the centromere, known as the shorter arm p and the longer arm q.

Materials and Methods
The study was conducted at college of animal science and technology university of Yangzhou, china. During the study the old and recent publications and technical report dealing on chicken chromosomes were reviewed. Similarly the protocols used for chicken karyotype analysis in the college were summarized. Chicken were cyogenetically analyzed, metaphase chromosomes were obtained by peripheral blood lymphocyte culture techniques (Moorhead et al., 1960). Constitutive heterochromatin was detected by the technique of Sumner (1972), and the nuclear organizer regions (NORs) were identified by silver staining (Howell and Black, 1980). G-band patterns were obtained with trypsin and Giemsa and C-band patterns were treated with barium hydroxide and Giemsa. The data obtain was analyzed using descriptive statistics, arm ratio was estimated as the ratio of long arm to short arm and relative length (RL %) was estimated according to the following formula:

\[
RL(\%) = \text{The length of one chromosome} \times 100\%
\]

RL (%) = The length of one chromosome \times 100%

The total length of all macro chromosomes

Results and Discussion
Chromosome structure of chicken: The genome of the domestic chicken has a haploid number of 39 chromosomes, the ten largest are referred to as macro-chromosomes, and the other 29 are termed micro-chromosomes (MICs) (Yamashina, 1944). In chickens chromosomes have been numbered in size order, the biggest first. The large number of MICs is typical of avian species (Abbott and Yee, 1975). In comparison to man, the first six chromosomes are of similar size, the largest being 8 Fm. However the MICs are much smaller (the smallest being about 7 Mb) than the smallest human chromosome, which contains about 50 Mb of DNA (Bloom and Bacon, 1987). There is a size difference of 23 times between the largest and the smallest chromosomes in the chicken. Chickens, like other avian species, differ from mammals in that the female is the heterogametic sex (ZW) and the male is the homogametic sex (ZZ), the Z and W chromosomes displaying heteromorphism. The chicken chromosomes are mostly euchromatic with the exceptions of a large terminal C-band (chromatin-band) on the Z chromosome and an almost totally heterochromatic W chromosome, with small C-bands on most of the MICs reviewed by (Feckheimer, 1990). The chicken genome is relatively small, about 1.2 x 10^8 bases (Burt, 2002), less than half that of the mouse and human genomes. This makes the chicken's genomic structure and organization particularly interesting, as evolution appears to have pruned the genome to a minimal size. Alternatively it is possible that mammalian genomes have expanded in the 300 million years since splitting from the avian lineage. However, whatever the mechanism of change between the two lineages, the reason for this difference is still unknown. One theory is that small genome size was favoured by directional selection in birds in order to cope with the metabolic demands of flight (Hughes and Hughes, 1995). However, one aspect of the chicken's unique genome is the relative paucity of repetitive sequences.

In the early 1990s, the development of the chicken molecular genetic map revived the international interest for an updated chicken standard karyotype. The international cytogenetics meeting in 1992 in Netherlands, and then in 1993 at the University of Guelph, indicated that the longitudinal banding patterns obtain from each of techniques describe are differ from each other. Their recommendations of international system for standardized avian karyotype were describe in details by (Ladjali-Mohammedi et al., 1999).

A number of recent studies have now shown that the micro chromosomes are in fact gene rich, with recent estimates suggesting that micro chromosomes contain at least as twice as many genes as the macro chromosomes (Mc Queen et al., 1996; Smith et al., 2000). Heteromorphisms of chromosome banding patterns can be useful markers for gene mapping and other kinds of genetic studies (Akeson and Davisson, 1996; Smith et al., 1999). Recently we studied karyotype analysis for Chinese Native chicken breeds, our comment is that the peripheral lymphocyte culture technique was suitable for avian chromosome preparation. All species studied presented a diploid number of 78 chromosomes, with 10 pairs of macro chromosomes including the sex chromosome and 29
Musa et al.: Karyotype and Banding Patterns

Table 1: Centro mere position of macro chromosome in chicken breeds

<table>
<thead>
<tr>
<th>Position of centromer</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>Z</th>
<th>W</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>10</td>
<td>4</td>
<td>1</td>
<td>6</td>
<td>10</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SM</td>
<td>6</td>
<td>4</td>
<td>7</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>8</td>
<td>8</td>
<td>1</td>
<td>3</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST</td>
<td>2</td>
<td>5</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

M =metacentric, SM =submetacentric, T= telocentric and ST =subtelocentric

In chromosome No.9, 4 chicken breeds were unknown and in chromosome W 2 chicken breed were unknown

Table 2: Relative length (R L) of chromosomes in chicken breeds

<table>
<thead>
<tr>
<th>Number of chromosome</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>Z</th>
<th>W</th>
</tr>
</thead>
<tbody>
<tr>
<td>High R L</td>
<td>23.30</td>
<td>17.75</td>
<td>12.60</td>
<td>10.98</td>
<td>8.24</td>
<td>6.77</td>
<td>5.46</td>
<td>4.95</td>
<td>4.07</td>
<td>10.63</td>
<td>5.44</td>
</tr>
<tr>
<td>Low R L</td>
<td>20.67</td>
<td>16.51</td>
<td>11.73</td>
<td>10.00</td>
<td>7.01</td>
<td>5.05</td>
<td>4.55</td>
<td>4.06</td>
<td>3.28</td>
<td>9.60</td>
<td>4.06</td>
</tr>
</tbody>
</table>

Table 3: The relative length and arm ratio in chicken breeds

<table>
<thead>
<tr>
<th>Number of chromosome</th>
<th>Relative length</th>
<th>Arm ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>22.10±1.74</td>
<td>1.49±0.20</td>
</tr>
<tr>
<td>2</td>
<td>17.28±1.56</td>
<td>1.56±0.26</td>
</tr>
<tr>
<td>3</td>
<td>12.05±4</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>10.77±0.85</td>
<td>2.97±0.68</td>
</tr>
<tr>
<td>5</td>
<td>7.51±4</td>
<td>4</td>
</tr>
<tr>
<td>6</td>
<td>6.39±0.81</td>
<td>2.29±0.54</td>
</tr>
<tr>
<td>7</td>
<td>5.32±0.71</td>
<td>4</td>
</tr>
<tr>
<td>8</td>
<td>4.79±0.68</td>
<td>1.12±0.41</td>
</tr>
<tr>
<td>9</td>
<td>3.70±0.58</td>
<td>4</td>
</tr>
<tr>
<td>Z</td>
<td>9.88±1.06</td>
<td>1.07±0.07</td>
</tr>
<tr>
<td>W</td>
<td>4.77±0.75</td>
<td>1.06±0.07</td>
</tr>
</tbody>
</table>

pairs of micro chromosomes. However, number 4 chromosomes were not completely identical among species. G-band patterns were found quite different. For instance, the macro chromosome of xioshen chickens were divided into 34 zones and 159 bands, while Beijing fatty and langshan were divided into 33 zones and 151 bands. The dark stained of C-band was observed on micro chromosome and W chromosome. Comparison on karyotype and G-banded patterns between domestic chicken and quail showed that they had a diploid number of 78, where the position of macro chromosomes centromers was different. Similarly different was found in number one and two chromosomes when analyzed by G-banded patterns. The higher frequencies G-band and larger number of Ag-NORs was indicating the better performance in Taihe Silkies chicken breed.

Chromosome and heredity disease: In animals karyotype analysis using to identify heredity disease and analyzing the mechanism of pathological change (Huang et al., 1995). Therefore, Standard cytogenetic analysis is used to detect abnormalities in chromosome number or microscopically visible duplications or deletions of chromosomal material. With the advent of molecular cytogenetic techniques, such as fluorescence in situ hybridization (FISH), it is now possible to detect chromosomal rearrangements that are beyond the resolution of light microscopy used for standard cytogenetic analysis. The use of FISH analysis for genetic diagnosis is made possible when a unique sequence of a gene or group of genes is known, and when the disease in question is the result of a deletion of this critical region. This unique sequence, known as a critical region, is synthesized in the laboratory and labelled with a fluorescent marker.

In chicken Solovei et al. (1998) examined Chromomers No. 1 and No. 3 of the chicken W lamp brush chromosome using fluorescence in situ hybridization with genomic probes for each of the two repeat families. Their relative contents of EcoRI and Xhol repeats were determined. There were two types of W chromosome in the chickens (White Leghorn and Rhode Island Red) with respect to the amount of EcoRI repeat, the high-copy-number type has about 4000 copies of the 1.2-kb repeat per Genome and shows a large fluorescence signal on W chromo mere one. A low-copy-number type has about 700 copies per genome and does not have a detectable chromo mere one on W chromosome, nor does it show the lower distance was 0.0012 between Luyuan and Beijing fatty chicken. Comparison on karyotype and G-band patterns between domestic chicken and quail showed that they had a diploid number of 78, where the position of macro chromosomes centromers was different. Similarly different was found in number one and two chromosomes when analyzed by G-banded patterns. The higher frequencies G-band and larger number of Ag-NORs was indicating the better performance in Taihe Silkies chicken breed.
FISH labeling in the region normally occupied by chromo
tume one.
The genome of Fayoumi chickens has about one-sixth
the amount of the Xhol sequence family of White
Leghorns. W lampbrush chromomere 3 is much smaller
and its FISH labelling with the Xhol probe is much
weaker in Fayoumis than in White Leghorns. These
results demonstrate that in the chicken W chromosome,
specific chromomeres are occupied by specific DNA
repeat sequence families. Similarly, Hutchison (1987)
examined lampbrush chromosomes (LBC) prepared
from chicken oocytes of 1-3-mm diam using both light
and electron microscopy. In chicken LBC typical loops
have a contour length of approximately 15 microns,
although some loops range up to 50 microns.

Prospect, innovation and the application of techniques:
All traditional karyotyping are invented in animal
research for several decades depend on the analysis of
characteristic banding patterns along the length of
chromosome. Although synchronization procedures
normally required for high-resolution G-banding were
not needed. Unlike other methods available, only one
round of observation is required using a conventional
fluorescence microscope, the method works without
modification in many species, and in situ hybridization is
not used for chromosome identification (allowing
multiple targets and minimizing background). The banding pattern is probably generated by a combination
of DNA dissolution and heterochromatin reorganization
after enzyme digestion, followed by paraformaldehyde
fixation of the new chromatin structure and incomplete
denaturation. The method is of widespread utility in
comparative genomics and genome organization
programmes. The application of chromosome karyotype
and banding techniques was used to research the origin,
evolution and relationship of species, also used for
gene location and sex determination. While, in the
Medical field was used for the examination of geneti
cdisease. Also, the techniques was consider as a base
for further molecular research, for example FISH

References
Handbook of Genetics, Vol. 4.R. C. King, ed.
polymorphisms in exotic inbred strains of mice: a
method for mapping centromeric ends of
Bloom, E. and D. Bacon, 1987. Linkage of the major
histocompatibility (B) complex and the nuclear
Feckheimer, N.S., 1990. Chromosomes of chicken in Mc
Howell, W.M. and D.A. Black, 1980. Controlled silver-
staining of nucleolus organizer regions with a
protective colloidal developer: a 1-step method.
Experiamenta 36: 1014-1015.
Huang, T.H., J.M. Huang and M. Cai, 1995. Analysis on
chromosome karyotypes of 536 spermatozoa from
9 normal Chinese Male. J. carcinogens is
Teratogenesis and Mutagenesia, 7: 15-19.
Hughes, A.L. and M.K. Hughes, 1995. Small genomes
Hutchison, N., 1987. Lampbrush chromosomes of the
chicken, Gallus domesticus, J. Cell Biol., 105: 1493-
1500.
Ladjali Mohammedi, K., J.J. Bitogood, M. Tixier-Biochard,
for Standardized Avian Karyotypes (ISSAK):
standardized banded karyotypes of the domestic
fowl (Gallus domesticus). Cytogenet Cell Genet., 86:
271-276.
McQueen, H.A., J. Fantes, S.H. Cross, V.H. Clark and
A.L. Archibald, 1996. CpG islands of chicken are
concentrated on micro chromosomes. Nat. Genet.,
12: 321-324.
Moorhead, P.S., P.C. Nowell, W.J. Mellman, D.M. Battips,
and D.A. Hungereford, 1960. Chromosome
preparations of leukocytes cultured from human
Smith, J., C.K. Bruley, I.R. Paton, I. Dunn, C.T. Jones and
D. Windsor, 2000. Differences in gene density on
the chicken macro chromosomes and micro
chromosomes: a tool for gene discovery in
Transmission of C-band variants in Japanese quail.
Solovei, I., A. Ogawa, M. Naito, S. Mizuno and H.
Macgregor, 1998. Specific Chromomeres on the
Chicken W Lamp brush Chromosome Contain
Specific Repetitive DNA Sequence Families.
Strachan, T. and A.P. Read, 1999. Human Molecular
Genetics. Oxford. BIOS.
Sumner, A.T., 1972. A simple technique for
demonstrating centromeric heterochromatin. Exp.
Francisco, W.H. Freeman and Co, M.Y. Yamashina,
1944. Karyotype studies in birds. I. Comparative
morphology of chromosomes in seventeen races of
Yamashina, M.Y., 1944. Karyotype studies in birds. I.
Comparative morphology of chromosomes in
seventeen races of domestic fowl. Cytologia, 13:
270-296.