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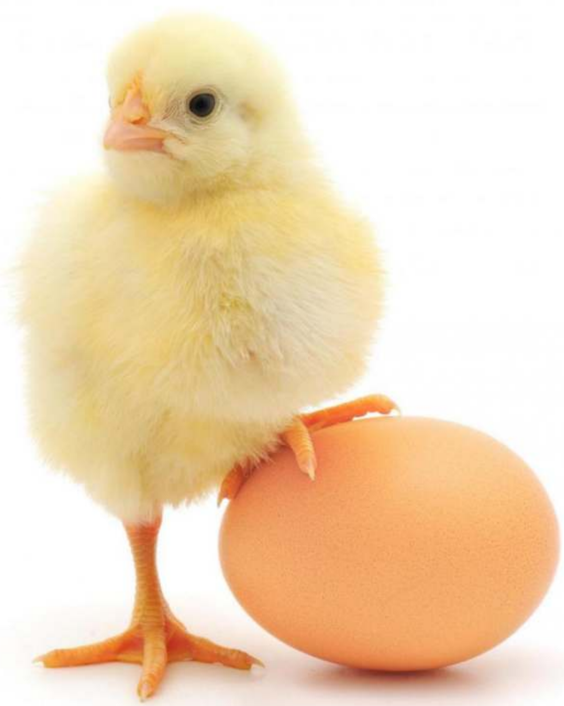

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Karyotype and Banding Patterns of Chicken Breeds

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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

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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 cytogenetically 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 (%) = The length of one chromosome × 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 μm. 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 10^9 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, 1991). On the other hands progress in quail karyotype was done, in Japanese quail the Centro mere region of chromosome No. 4 is the site of heteromorphism. One form of the C-band at this region is relatively small (a form), and alternative form is much larger (b, form). To identify the transmission patterns, all possible mating were made between birds with karyotype a/a, a/b, and b/b. the outcome from all crosses are entirely consistent with the expectation from simple Mendelian transmission. No evidence was found for segregation distortion or gametic selection. This dimorphism, therefore, is a reliable marker (Sohn *et al.,* 1995).

Karyotype and banding pattern of chicken: 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

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

Position of centromer	1	2	3	4	5	6	7	8	9	Z	W
M	10	4		1				6		10	6
SM		6		4		7		1			1
T			8		8	1	8	3	6		
ST			2	5	2	2	2				2

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

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

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

Number of chromosome	Relative length	Arm ratio
1	22.10±1.74	1.49±0.20
2	17.28±1.56	1.56±0.26
3	12.05±∞	∞
4	10.77±0.85	2.97±0.68
5	7.51±∞	∞
6	6.39±0.81	2.29±0.54
7	5.32±0.71	∞
8	4.79±0.69	1.12±0.41
9	3.70±0.58	∞
Z	9.88±1.06	1.07±0.07
W	4.77±0.75	1.06±0.07

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 the domestic fowl and quail showed that they had a diploid number of 78, but their positions of centromer on macro chromosomes were different from each other. Similarly, the difference was found in chromosome No. 1 and No. 2. The position of centromers for the macro chromosome of 10 chicken breeds was presented in (Table 1), the high and low relative length (R L) of chromosomes in (Table 2) and the relative length and arm ratio in chicken breeds was shown in (Table 3).

Karyotype resemblance near coefficient was possible for breeds clustering. Among chicken breeds the higher karyotype resemblance near coefficient was found to be 0.9948 between xiaoshen breed and Langshan, while the lower 0.9651 was estimated between Shouguang and Jiuyuan black. The evolutionary distance of chicken breeds indicated that the high distance was 0.0355 between Jiuyuan black and Shouguang breed and 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. Similar 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 Chromomeres 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 XhoI repeats were determined There were two types of W chromosome in the chickens (White Leghorn and Rhode Island Red) 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

FISH labeling in the region normally occupied by chromo mere one.

The genome of Fayoumi chickens has about one-sixth the amount of the XhoI sequence family of White Leghorns. W lampbrush chromomere 3 is much smaller and its FISH labelling with the XhoI probe is much weaker in Fayoumis than in White Leghorns. These results demonstrate that in the chicken W chromosome, specific chomomeres 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 genetic disease. Also, the techniques was consider as a base for further molecular research, for example FISH

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