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Research Article

USING CYTOGENETIC ANALYSIS FOR DETECTING KARYOTYPE OF PERSIAN CHUKAR POPULATION

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ABSTRACT
Generally karyotype is a feature of chromosome complement of eukaryote species. Karyotypic Studies have displayed important information regarding the taxonomic relationships and evolutionary patterns in various groups of birds. In higher vertebrates, micro-chromosomes exist in each class of most primitive orders. Birds have more micro-chromosomes in their karyotype compared to other vertebrates. So far, there are no cytogenetic data in the literature regarding Persian Chukar. This study presents the initial data on the number and morphology of Karyotype and chromosomes of Persian Chukar. In this species, it contributes with the new data that could help to clarify the evolutionary relations. Bone marrow, tissues of kidney and liver were cultured directly to obtain material for studying the chromosome. Exploring more than 30 metaphase plaques indicated that among 96 observed chromosomes; 18 pairs existed in all spreads; and the others included 30 pairs. They were macro-chromosomes and micro-chromosomes. Chromosome formula of Persian Chukar was determined as 2n=18 MACROCHROMOSOMS, NF=24 and THIRTY PAIRS OF micro chromosomes. The numbers of micro-chromosomes do not seem to be a dependable indicator of species type. Karyogram of the bird has been provided based on the length and the type of chromosomes, length of arms and the location of centromer.

Keywords: *Persian Chukar, Karyotype, Fundamental Number (NF), Chromosome Formula, Metaphase Plaque*

INTRODUCTION
Persian Chukar (Alectoris Graeca Koriakova) is a Eurasian upland game bird which belongs to the Phasianidae pheasant family. This partridge has black and white bars on its flanks and has a black band running that exists from the forehead across the eye and runs down the head. It looks like a necklace that surrounds a white throat.

Figure 1: Persian Chukar image

This bird exists from eastern Persia to Baluchistan and Sind. This race of Chukar breeds between 5,000 and 12,000 feet. In Persia, it breeds from the end of April to June. The number of laid eggs varies from eight to eighteen. This species is comparatively unaffected by loss of habitat or hunting. During the breeding season, its numbers are largely affected by the weather (tripso). Generally Karyotype is the features of chromosome complement of eukaryote species. Karyotypes of lower vertebrates mainly consist of micro-chromosomes. In higher vertebrates, micro-chromosomes are observed in each class of the most primitive orders. Birds have more micro-chromosomes in their karyotype compared to other vertebrates. Accumulation of micro-chromosomes in the avian karyotype probably occurred after separation of birds from reptilians (Radionov, 1996). Avian karyotypes are generally characterized by the high diploid number of chromosomes, ranging from 74 to 86 in about two thirds of species (Takagi and Sasaki, 1974; Tegelström *et al.*, 1983; Belterman and de Boer, 1984; De Lucca and Rocha, 1992; Ebied *et al.*, 2005; Nishida *et al.*, 2008). Chromosome complement of the birds consists partly of these relatively big, 4-8µ, and partly of large number of very small dot-shaped, less than 3µ, chromosomes (Abbott and Yee, 1975; Fillon, 1998; Burt *et al.*, 1999; Harrison *et al.*, 2000). They are often called macro-chromosomes and micro-chromosomes (Hammar 1966, 1970). The micro-chromosomes are so numerous and often so small that it makes it difficult to identify the centromeric position (Goldschmidt *et al.*, 2000; Amaral and Jorge, 2003). The study regarding mitosis and meiosis in Gallus Domesticus revealed that the micro-chromosomes are constant in their number, that they can be followed through complete mitosis and meiosis, and that their behavior does not diverge from them acro-chromosomes (Ohno, 1961). With the help of light microscopy and electron microscopy it was shown that the so-called macro-chromosomes and micro-chromosomes only diverge in size during mitosis and meiosis and not in behavior (Ford and Wollham, 1964). In species with a high number of micro-chromosomes, macro-chromosomes with mono-brachial or acrocentric morphology are prevalent. However, in species with a low number of micro-chromosomes, macro-chromosomes with abi brachial morphology are predominant, suggesting a process of Karyotypic evolution through translocations between macro- and micro-chromosomes, and centric fusions of macro-chromosomes (Gunski *et al.*, 2000; Nieto *et al.*, 2012). Among birds, the sexual chromosomes are designed by Z and W, being the females the heterogametic sex (ZW) and the males, homogametic (ZZ) (Werner, 1931; Charlesworth, 1991; Mazuno and Macgregor, 1998; Amaral and Jorge, 2003).

Figure 2: Karyotype of Persian Chukar

The preparation and study of Karyotypes is a part of Cytogenetic (Derjusheva *et al.*, 2001). In the first complete Karyotype investigation in birds which was done in Gallus domesticus, it was revealed that it is possible to analyze the whole chromosome complement regarding its size and structure (Owen, 1965; Hammar, 1966). Karyotype studies of bird species that were not analyzed yet could offer important information in terms of a better knowledge of the mechanisms of evolution and of phylogenetic connection with the group (Goldschmidt *et al.*, 2000). Karyotypic study provides wealth information for

the animal`s diseases, infertility, tumorigenesis and low resolution of whole genome (Masabanda *et al.*, 2003, 2004). Karyotyping chromosomes and banding techniques is used in order to study the origin of evolution and the relation among species and also for determining the gene situation and animal sex (Musa *et al.*, 2005). It is very difficult to analyze the number of chromosomes and their morphology without making any cell pretreatment before fixation. Hypotonic pretreatment of the cells before fixation caused a considerable improvement in the possibilities of studying the chromosomes in vertebrates. Other chemicals including colchicines have also been combined with the hypotonic pretreatment (Hammar, 1966). For many years, laboratories have been concerned with the production of *in vivo* bone marrow preparations that are made under field conditions. They usually involved animals that are processed immediately in order to produce karyotypic preparations. Such methods have been described in many papers (Hoy and Berlowitz, 1931; Ford and Hamerton, 1956; Patton, 1967; Robbins and Baker, 1978; Lee and Elder, 1980; Baker *et al.*, 1982; Christidis, 1985; Baker and Qumsiyeh, 1988; Hafner and Sandquist, 1989). The putative processes that occur during chromosome preparation were simulated in suspension, methanol, fixative, water and acetic acid. Fixative evaporation was performed under normal atmospheric circumstances. During evaporation of the fixative from the slides, chromosome spreading involves significant water induced swelling of mitotic cells which is a prerequisite for chromosome analysis and the appearance of Giemsa banding patterns. Hypotonic treatment is crucial for well spread metaphase chromosomes because it moves the chromosomes from a central to a more peripheral position in the cell where they can be stretched more effectively during mitotic swelling (Hoy *et al.*, 1938; Claussen *et al.*, 2002).

MATERIALS AND METHODS

Preparation of Slides

An experiment made by sacrificing two specimens (a male and a female) mitotic chromosome preparations were obtained from kidney cells (Bertollo *et al.*, 1978), and bone marrow after being analyzed. The animals were treated with a 0.0125% solution of colchicines, which was injected at a volume of 1ml/100g of body weight at approximately 3 hours before euthanasia and chromosome preparation. The tibia, femur and kidney tissues were dissected, and the cells were dissociated in a hypotonic solution of kcl 0.075 M with a syringe and remained in the solution for 25 min (Valente *et al.*, 2012). The samples were incubated at 37°c for 10-15 minutes. Cell suspension was centrifuged for 8 minutes at 1,500 rpm and the supernatant was discarded. Cooled fresh carnoy`s fixative (3 methanols:1 acetic acid) were added and then followed by homogenization. The fixed cells were centrifuged for 8 minutes at 1,500 rpm. Most of the supernatant was discarded. Five ml of fixative were added and then again followed by homogenization and centrifugation. This procedure was repeated three times and tubes were kept at -20°c until they were required for slide preparations. The fixed material was centrifuged for 8 minutes at 1,500 rpm and the supernatant was discarded. Then, cell pellet was again suspended in 2 ml fixative and dropped over clean slides that had been kept in dried air and cold water (Oliveira *et al.*, 2001). Cells were prepared to be stained with 5%Geimsa solution in phosphate buffer at PH 6.8 for 10 minutes. All the operations were performed *in vivo* in the laboratory of the Department of Biotechnology of Animal Science Research Institute of Iran.

Fixation

For increasing nuclear and chromosomal spreading, the selected cells were treated with 45% acetic acid in water, which was removed shortly and the material transferred to freshly prepared carnoy`s fixative (3 : 1 absolute methanol : glacial acetic acid) and is left for 30 minutes at room temperature (Belterman and Boer, 1990; Christidis, 1998).

Chromosome Spreading and Staining

For the rapid dissociation and good chromosome spreading, the tissue was transferred to a drop of 60% aqueous acetic acid on a warmed slide. Slight maceration was required for proper dissociation (Ashraful, 2012). Conventional and normal Giemsa staining was made. Conventional staining of chromosomes allows detection of numbers and structure. A cover slip was placed over the tissue. Also, in order to

spread chromosomes, tissue was squashed with applying pressure on the cover slip by the thumb. Thus, the slides became ready for chromosomal study (Deny *et al*., 2003).

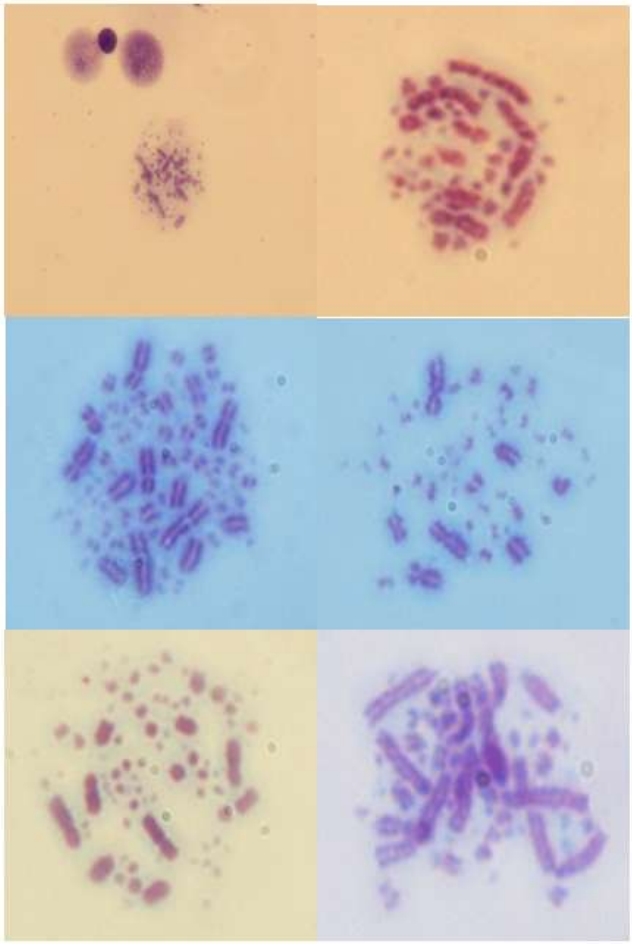
Slide Preparation and Mounting

In order to ensure a maximum spreading, another drop of Carnoy`s fluid was added to the preparation and till it slides in all directions. The slide was then warmed gently over a flame which assists dispersion and evaporation. The dried slides where placed in acetic ethanol (one part of glacial acetic acid in three parts of absolute ethanol) for about three hours to reduce cytoplasmic staining (Ashraful, 2012). The Karyotypic similarities and differences among bird species have been studied morphologically by conventional Giemsa and chromosome banding (Nogeria *et al.,* 2006; Waldrigues and Ferrar, 1982). The stained slides were mounted carefully only with the cover slip; and the tissue was mounted attaching with Enthelen gum. The chromosome spreads were analyzed using an Olympus BX 40 microscope, and the images were captured with the Olympus PM-C 35 digital camera with the software Image-Pro MC 6.0. They analyzed 30 metaphase spreads for all cytogenic procedures carried out on each animal sample. Karyotypes were arranged in the order of decreasing chromosome size, and the chromosomes were classified as meta- telo- and acrocentric. The diploid number was defined as the modal number obtained from numerous counting (Oliveira *et al.*, 2001). The Karyotypes were arranged according to the classification in Levan *et al.,* (1964).

RESULTS AND DISCUSSION

Karyotype of Persian Chukar

To the best of our knowledge, this is the first report of the karyotype of Persian Chukar and a critical step for the completion of Persian Chukar genome map.



Chromosome studies of Fringilla Coelebs identified 80 chromosomes for the animal among which a lot of micro-chromosomes existed (Derjusheva *et al.*, 2001). We studied about 30 metaphase plaque, the number of chromosomes was 90-100 and the most of them were micro-chromosomes (Figure 3). No distinct deviation from the "typical" avian karyotype was shown which displays a high degree of conservation in genome organization of the bird. The difference among macro-and micro-chromosomes is distinguishable enough and could be construed as an evolutionary mechanism of this species by which micro-chromosomes fuse form macro-chromosomes. In previous studies, 92 haplotypes were identified for partridge species among them rock partridge, Chukar partridge and Spanish red-legged partridge had 21, 35 and 36 chromosomes, respectively. The diploid number reported for Persian chukar in this study, is generally in accordance with the conserved 2n=18 chromosomes that is commonly found in red-legged partridge (Alectorisrufa) (Arruga *et al.*, 1996). Although morphological differences are observed between two species, the number of chromosomes is not similar to those of 21 chromosomes in rock partridge. Moreover, in our studies the number of chromosomes, 96 haplotypes, are in contrast with those of Lemakova`s experiment which revealed 80 chromosomes for both pheasant and rock partridge (A. graeca) (Lemakova, 1984). In most species of partridge, the number of micro-chromosomes is 60 to70. The chromosome complement of the bird is composed of 34 autosomes (AA), 30 pairs of micro-chromosomes and a pair of sex-chromosomes (Z and W). Based on MacGregor method (1993), the Karyotypic formula is determined (6AAm + 4AAa + 8AAt) where 6 pairs are metacentric, 4 pairs are acrocentric, and 8 pairs are telocentric. The number of micro-chromosomes does not appear to be a reliable indicator of the type of species. Presently micro-chromosomes are revealed as genetic linkage groups (Lemakova, 1984). The recent research confirmed findings of Kassai which revealed chromosome homology between gallus domesticus and red-Legged Partridge (Kassai *et al.*, 2003). Fundamental Number for Persian Chukar is 24.

Conclusion

A chromosome with its great potential for future achievements has become a completely new synthetic science (Sharma, 1984). The shape and size of the chromosomes seem to be of great importance in the Karyotypic and cytotaxonomy evaluation.

The chromosome number is a key datum for a species compared to any other characteristics that seemed significantly stable to merit taxonomic significance (Garber, 1978). The information could be helpful for species identification and detecting genetic diseases. Moreover, the effects of the findings production performance, management practices, haemato-biochemical and serological parameters, coupled with those on karyotypes in relation to the incidences of Avian Influenza and other diseases in poultry farms are extremely significant (Ashraful, 2012). Determining the numbers and shapes of chromosomes, and also karyotyping of Persian Chukar provides wealth cytological information in terms of the species and is an advantageous tool for recognizing population, determining different populations and identifying probable hybrids and subspecies.

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