Karyotype of chicken-pheasant hybrids

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ABSTRACT

The karyotype of the *Gallus gallus* x *Phasianus colchicus mongolicus* hybrid was studied in mitoses obtained from peripheral blood leucocytes cultures. The culture method provided high numbers of well spread metaphase chromosomes, without overlapping and suitable for chromosome counts. The modal diploid number of chromosomes found was seventy-eight, the same as in the chicken. The hybrid constitution was confirmed by the presence of a macro-chromosomal set derived from each parental species, the chicken and the pheasant. In particular, the hybrid origin of metaphases was easily determined by the morphology of two pairs of homologous chromosomes, number 2 and number 4. The nucleolar organiser regions (NOR) encoding the 18S–5.8S–28S ribosomal DNA, were detected by the silver nitrate staining in one pair of chromosomes, as in the chicken.

Keywords: avian leukocyte culture, diplod number, macro-chromosomes, Giemsa, Ag-NOR.

1. INTRODUCTION

Karyotypes of most birds are remarkably similar. According to Griffin *et al*. (2007), 63% of birds have a haploid number from 37 to 43, 24% from 33 to 37 and extremes are 20 and 71 chromosomes. Bird karyotypes have a few large macrochromosomes, usually from 7 to 8, and a variable number of microchromosomes, usually from 30 to 32. However, the haploid number in some species can vary from 20 in the curlew (*Numenius arcuata*) to 63 in the hoopoe (*Upupa epops*) (Burt, 2003).

Although classification of chicken chromosomes varies in literature, according to Masabanda *et al*. (2004), chicken chromosomes can be divided into four groups. Macrochromosomes, including the sex chromosomes (Z and W) and the nucleolar organiser regions (NOR) bearing chromosome form the first and second groups. In most publications the NOR chromosome, encoding the 18S–5.8S–28S ribosomal DNA, is number 16, so to avoid confusion, several others retain that this chromosome remains the NOR chromosome (Masabanda *et al.*, 2004; Schmid *et al*., 2005). The remaining groups from chromosomes 17 to 38 contain the microchromosomes.

Chromosomes of the domestic fowl were firstly studied by Loyez (1906), but the diploid number of fowl chromosomes was only reported for the first time much later to be 78 in males and 77 in females (Susuki, 1930; and Oguma, 1938). Yamashina (1943) confirmed this diploid number and additionally, described the fifth largest chromosomes as the sex chromosomes, which was always unpaired in females.

Chromosomes of the common pheasant were initially studied by Cutler (1918). Nevertheless, Yamashina (1943) reported for the first time the diploid number of chromosomes, with females and males presenting different chromosome numbers, 81 and 82, respectively. This author also described the fifth largest chromosomes as the sex chromosomes, which were always unpaired in females. However, now it is well known that sex chromosomes are composed of a set of two chromosomes. Moreover, differences in morphology between the domestic fowl and the common pheasant were observed when comparing chromosomes 2 and 4. In Stock and Bunch (1982), chromosomal rearrangements were also seen in these two chromosomes as well as in the Z chromosome from Galliformes. These differences were additionally confirmed in the ring-necked pheasant by Shibusawa *et al*. (2004).

Regarding chromosomes of the hybrid between the domestic fowl and the common pheasant, studies were performed by Cutler (1918), who failed to accuratly determine the chromosome number. Further studies were performed by Yamashina (1943), who reported two distinct size groups of larger (macro) and smaller

(micro) chromosomes. He also reported the diploid chromosome number of spermatogonial complex to be 80, and oogonial complex to be 79. Basrur and Yamashiro (1972) also reported a diploid number of 80. More recent studies by Castillo *et al*., (2007), demonstrated that the sex chromosomes are easily distinguished, and this difference was directly related to the evident sexual dimorphism in these birds due to the live body weight.

In the present study, we present data on chromosomal analysis of *Gallus gallus* x *Phasianus colchicus mongolicus* hybrids by means of classical cytogenetic techniques, comprising Giemsa, G-banding and Ag-NOR staining.

2. MATERIALS AND METHODS

2.1. Animals

At the age of 3 years, 16 chicken-pheasant hybrids were karyotyped by chromosome spreads obtained from leukocytes culture. These hybrids originated from the mating between New Hampshire cocks and female ring-necked pheasants. The birds were hatched and bred at the Experimental Avian Station of the Department of Animal Production of Pisa. The breeding and experimental protocol was approved by the Ethics Committee of the Faculty of Veterinary Medicine of Pisa, Italy. Hybrids were bred according to standard pheasant breeding conditions.

2.2. Luekocyte culture

Blood was taken from the ulnar wing vein from each bird. Blood samples were transferred immediately to a 5-ml glass tube containing heparin-lithium. Samples were taken to the laboratory and leukocytes were separated by a brief centrifugation and cultured for 72 hours at 39°C in an RPMI 1640 medium containing 20% fetal calf serum, antibiotics and 100µg/ml concanavalin-A. Hypotonic treatment was performed with KCl 0.075 M for 15 minutes followed by fixation with methanol/acetic acid.

2.3. Chromosome preparations

Chromosome slides were stained with 10% Giemsa in a phosphate buffer, pH 6.8, and then examined under a light microscope with 100x oil immersion. Metaphasic mitotic plates with clear and well-distributed chromosomes were photographed with a digital photo camera. At least 12 pictures per each bird sample were taken at 100x oil immersion and chromosomes counts were performed on at least 12 metaphase plates per bird with the aid of Image software (Rasband, 2005). One representative metaphase was chosen to build up the karyotype. Chromosomes were arranged according to morphology and size. Arrangements were performed using Image software (Rasband, 2005). Results obtained by Shibusawa *et al*. (2004) and Ryttman and Tegelstrom (1983) were of aid to perform these arrangements.

G-banding chromosome preparations were also performed on two slides with the highest number of metaphase plates. G-bands were obtained by modifications of the trypsin and Giemsa staining procedure as described by Seabright (1971). G-banded chromosomes were also arranged as described above to form the karyotype.

Ag-NOR staining was performed on one sample with good metaphase plates following the method of Howell and Black (1980) with some modifications. Metaphase plates were identified under the light microscope using the 100x oil immersion magnification. More than 30 pictures were taken and the most representative was chosen.

3. RESULTS

Results of chromosome counts in cells of chicken-pheasant hybrids showed a variation from 72 to 82 in somatic chromosome number, and percentage counts concentrated mainly between 76 and 79 chromosomes.

Table 1. Diploid chromosome numbers in female and male chicken-pheasant hybrids.

Table 1 reports modal cells with 78 chromosomes in females and 77 in males. When considering data of females and males together, the most frequent number of chromosomes found for this hybrid was 78.

The distinction between paternal (chicken) from maternal (pheasant) eight largest macrochromosomes was performed by comparison of the structural differences of the parental karyotypes, and therefore the proposed karyotype of this hybrid is reported in figures 1 and 2.
Karyotypes of male and female chicken-pheasant hybrids are shown in figures 1 and 2, respectively. Male karyotype differs from that of the female for the sex chromosomes: males have the ZZ condition, while females are heteromorphic with Z and W chromosomes. In males (figure 1), the first Z chromosome originated from the set of chicken chromosomes, is a medium metacentric element lying between chromosomes number 4 and number 5, while the second Z chromosome originated from the set of pheasant chromosomes, lies between chromosomes number 3 and 4. In females (figure 2), the W chromosome is a smaller and metacentric chromosome when compared to chromosome number 6 in chickens.
Regarding the eight biggest macrochromosomes, an evident difference in morphology can be seen on two homologous chromosomes in figures 1 and 2: chromosomes pair 2, with one submetacentric and one telocentric and chromosomes pair 4, with one submetacentric and one acrocentric. Chromosomes number 1 are both metacentric. Chromosomes number 3 both are telocentric. Number 6 chromosomes one is telocentric and the other is acrocentric. Chromosomes numbers 5 and 7, each presents one acrocentric chromosome and the homologous telocentric. Finally, chromosomes number 8, one submetacentric and the homologous acrocentric, just like chromosome number 4, even if less evident.

Figure 1. Karyotype of male chicken-pheasant hybrid.

Figure 2. Karyotype of female chicken-pheasant hybrid.

The karyotype of a female chicken-pheasant hybrid obtained after staining the chromosomes for G bands is shown in figure 3. The almost entirely G band negative in microchromosomes numbers 17 to 38 is well evidenced. Regarding the sex W chromosome, it appears highly heterochromatic.

Figure 3. G-banded karyotype of female chicken-pheasant hybrid.

A silver stained metaphase of a chicken-pheasant male hybrid is shown in figure 4. Two macrochromosomes are easily visualised for their darker colour.

Figure 4. Silver stained metaphase of chicken-pheasant male hybrid. Arrows indicate Ag-NORs.

4. DISCUSSION

The method used in this study to prepare chromosome slides permited us to obtain quiet high numbers of well spread metaphase chromosomes, without overlapping and suitable for chromosome counts and morphology. Even if the state of contraction of the chromosome due to the mitosis stopper (Colcemid) exposure yielded not optimum results for the G banded karyotype. More extended chromosomes might have been helpful to consider the inter chromosomal differences observed specially in chromosomes pairs 2 and 4, since it does not mean that species specific chromosomes should match up the G bands.
The most frequent diplod chromosome number (78) we found in this hybrid is the same of the chicken. This contrasts with results from previous authors reporting for this kind of hybrid that the chromosome complement consisted of the total sum of the half sets of the parental complexes, thus 80 (Yamashina, 1943; Basrur and Yamashiro, 1972). In mammals as well, horse x donkey hybrid carries half set of chromosomes of each parental species (Benirschke *et al*., 1962).
Considering the morphology of chromosomes number 2 and 4 (figure 1), it is in agreement to what reported by previous authors for the set of chicken (Stock and Bunch, 1982; Ladjali-Mohammedi *et al*., 1999; Shibusawa *et al*., 2004) and pheasant chromosomes (Stock and Bunch, 1982; Ryttman and Tegelstrom, 1983; Shibusawa *et al*., 2004).
The morphology of chicken number 3 chromosome differs with studies from Ladjali-Mohammedi *et al*. (1999), who reported this chromosome as acrocentric. Eventhough, in the G banded karyotype (figure 3) this chromosome tends to show that there is a small p-arm. On the other hand, on all metaphases stained with

Giemsa, our observations are in agreement with Stock and Bunch (1982), who retain that chromosome number 3 is telocentric or, at best, it possessed a very small extension beyond the centromere.
The acrocentric morphology of chromosome number 7 of the chicken we found, agrees with reports from Ladjali-Mohammedi *et al*. (1999), while it contrasts with the telocentric form reported by Stock and Bunch (1982). Observations on chicken submetacentric chromosome number 8 are in agreement with Stock and Bunch (1982), but disagree with Ladjali-Mohammedi *et al*. (1999) reporting this chromosome as metacentric.
The hybrid bird presents only one pair of NOR chromosomes, just like in the chicken (Masabanda *et al*., 2004; Schmid *et al*., 2005). In fact, most bird karyotypes appear to contain one NOR encoding chromosome pair, for example in Meleagris (Chaves *et al*., 2007), in the order Gruiformes (Nishida and Sasaki, 1980), in the families Columbidae (Gunski et al., 1995) and Tinamidae (Garnero, 1996).
It is interesting to observe in figure 4 the different size between the NOR-bearing chromosomes. The size of the NOR is very frequently variabile. Actually, a small but clearly visible difference in the size of these chromosomes was observed in Rheiformes (Gunski and Giannoni, 1998) and other birds (Rocha and Lucca, 1988).
In summary, we found that the karyotype of chicken-pheasant hybrids consists of the same haploid number as that of the chicken. And the most evident characteristic which identifies the hybrid origin of the bird resides in chromosomes number 2 and 4, due to the evident difference of each chromosome inside each pair. In fact, rearrangements in these two chromosomes are good landmarks for representing the process of karyological evolution in several Galliformes species (Shibusawa *et al*., 2004).

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Table

Table 1. Diploid chromosome numbers in female (n=6) and male (n=10) chicken-pheasant hybrids.

metaphase plates	number of diploid chromosomes		
	mode	*min*	*max*
females (n = 76)	78	*73*	*82*
males (n = 147)	77	*72*	*82*