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Chromosomes and heterochromatin in the Italian sparrow, *Passer italiae*, a taxon of presumed hybrid origins

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A b s t r a c t . The Italian sparrow (*Passer italiae* Vieillot, 1817) was karyologically studied. All the specimens examined showed 2n = 76 chromosomes, 22 of which were macrochromosomes with apparent morphology and 54 were microchromosomes. The sex-chromosome system was of the ZZ/ZW type; chromosome Z, the 4th in size, was metacentric, while W, the 11th, was acrocentric. Heterochromatin appeared, was G+C rich and Alu I resistant, and was distributed over the pericentromeric areas of all the macrochromosomes and almost on of the microchromosomes. Chromosome W, as well as the short arms of the 7th and 9th autosomal pairs, was entirely heterochromatic. Besides centromeric heterochromatin, chromosome Z also displayed a large C band on the peritelomeric region of the short arm. A comparison with the chromosomes of the presumed parental sparrow species, *P. domesticus* and *P. hispaniolensis*, suggested: 1) a diversification between *P. italiae* and *P. domesticus* concerning both the sex chromosome pair and the distribution of heterochromatin blocks along autosomes, as well as 2) kinship between *P. italiae* and *P. hispaniolensis* on the basis of chromosome-W morphology.

Key words: chromosomes, heterochromatin, *Passer italiae*, Italian sparrow, Aves

Introduction

Unlike in plants, speciation by hybridization is assumed to be insignificant in animals (B u l l i n i 1985, K i n g 1993). In fact, this unusual evolutionary process occasionally occurs in various unrelated animal taxa, such as insects, snails, planarians, fish, amphibians and reptiles (reviewed by B u l l i n i 1985). The endemic Italian sparrow, *Passer italiae* Vieillot, 1817 has been reported only on the basis of morphological evidence (M a i s e 1936, M a y r 1963). Its presumed parental species are *P. domesticus* (a north-European sparrow) and *P. hispaniolensis* (a south-Mediterranean African species). According to J o h n s t o n (1969), hybridization between these two species dates back approximately 5,000 years, when they colonized the Italian peninsula.

Conversely, S t e p h a n (1986) considered *P. italiae* to have originated by diversification of a peripherally isolated founding population of *P. domesticus*. As a consequence of such unresolved systematics, the taxonomic status of *P. italiae* is still unclear; it is considered a subspecies either of *P. domesticus* (C h i g i 1904,1914, M a r t o r e l l i 1906, S c h i f f e r l i & S c h i f f e r l i 1980, P a r k i n 1987), or of *P. hispaniolensis* (B a u m g a r t 1984, S u m m e r s - S m i t h 1988) (for a review see M a s s a 1989).

Many studies have focused on the phenotypic traits of *P. italiae* (B e r t a n i 1944, J o h n s t o n 1972, C o v a 1977, S t e p h a n 1986, S u m m e r s - S m i t h 1988, F u l g i o n e

et al. 1998b), but only a few were concerned with genetic analysis (Fulgione et al. 1998a), and karyological data are completely lacking.

On the other hand, the karyotypes of *P. hispaniolensis* and *P. domesticus* have been extensively studied (Bulatova et al. 1972, and reference there in De Boer (1984)). These two species possess very similar karyotypes with 2n = 76 chromosomes (pairs 1 to 11 are macrochromosomes, including the 4th pair corresponding to sex chromosomes ZZ/ZW, and pairs 12 to 38 are microchromosomes), differing only in the morphology of the W sex chromosome, which is acrocentric in *P. hispaniolensis* and submetacentric in *P. domesticus*. Furthermore, distribution of heterochromatin blocks is also known in the latter species (Christidis 1986), stet are located on the pericentromeric regions of all chromosomes, including the Z chromosome, which displays a polymorphic prominent peritelomeric band on its long arm. Chromosome W, as well as the short arms of chromosome pairs 7–9 is completely heterochromatic.

In this paper, we report on the results of a chromosomal study of *P. italiae* performed by means of standard and banding methods.

Material and Methods

Seven (2 females and 5 males) free-living sparrows of *P. italiae* were sampled (permit of catching of no. 7285, released on 18 November 1994) in mist nets (6×100 m^2) in Campania (Italy), during spring 1997. The specimens were stimulated with two doses of a mitogen solution (0.1 ml/10 g body weight of pokeweed solution, GIBCO), at a 24-hour interval, before sacrifice (through deep exposure to chloroform vapour). One hour before sacrificing, the sparrows were injected with a dose (0.1 ml/10 g body weight) of a colchicine solution (0.5 mg/ml).

Chromosome preparations were obtained by the direct air drying method from bone marrow and spleen cells, using hypotonic solution KCl 0.075 M.

Conventional staining (5% Giemsa at pH 7) and the following banding methods were used:

- C-banding, employing $Ba(OH)_2$ at 45 °C (according to Sumner 1972);
- staining with the A+T specific fluorochrome DAPI and chromomycin A_3 (according to Schweizer 1976).
- Digestions with restriction enzymes Alu I (following the indications of Mezzanotte et al. 1983).

Results

All the *P. italiae* specimens studied had 2n = 76 chromosomes, the first 11 pairs of which were macrochromosomes, and the remaining 27 pairs microchromosomes (Fig. 1a). Macrochromosome pairs 1 to 3 were submetacentric; the next pair consisted of the ZZ/ZW sex chromosomes, of which chromosome Z was metacentric, while W was acrocentric and the 12th in size. Autosomal pairs 4 to 6 and 10 were acrocentric and, finally, pairs 7 to 9 were metacentric. After C-banding (Fig. 1b), heterochromatic blocks were evident on the pericentromeric area of all the macro- and almost all the microchromosomes. As is generally observed in birds (Belterman & De Boer 1984, Christidis 1986), the acrocentric W chromosome appeared entirely heterochromatic, even though it showed a euchromatic band in the pericentromeric region in less condensed chromosomes. The short arm of the 7th and 9th autosomal pairs appeared heterochromatic. In addition, a centromeric C-band, chromosome Z showed a large peritelomeric heterochromatic band on the short arm. Heterochromatin was Alu I resistant and G+C rich, being Chromomycin A_3 positive (Fig. 2).

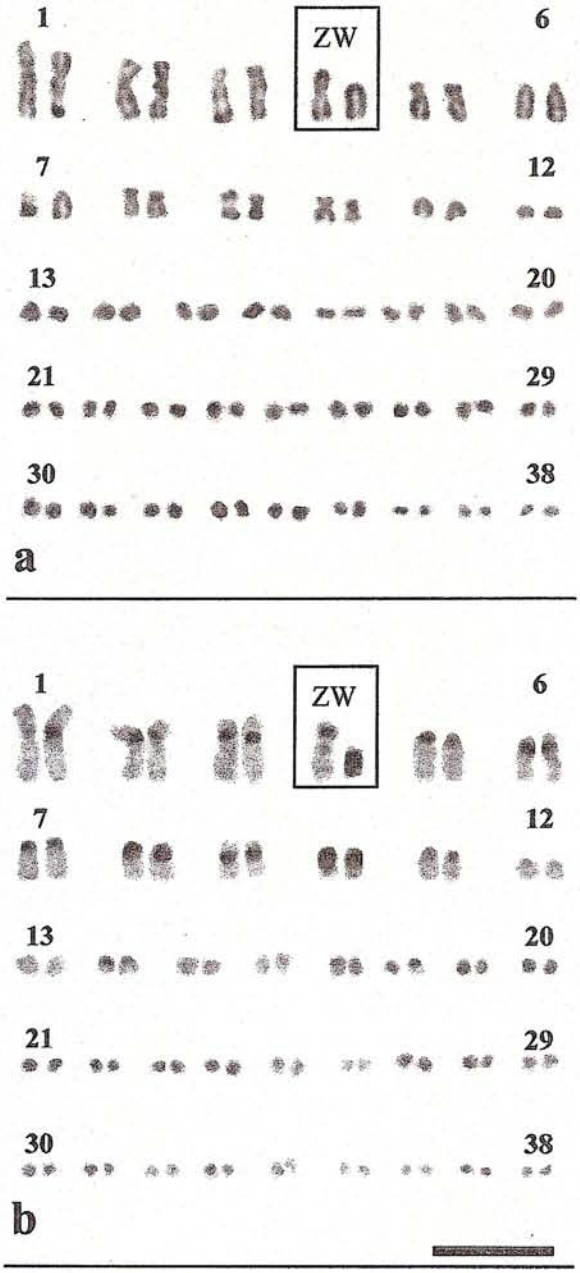

Fig. 1. Giemsa stained (a) and C-banded (b) karyotype of a female of Italian sparrow (*P. italiae*). The bar equals 10 µm.

Discussion

Morphologically, the chromosome set of *P. italiae* differs from that of *P. domesticus* only in chromosome W, which is acrocentric in the former and submetacentric in the latter. A pericentric inversion might account for this difference.

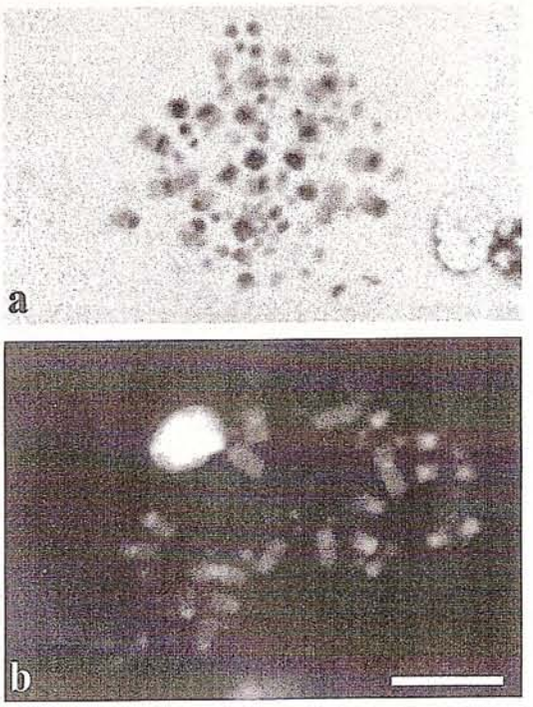

Fig. 2. Alu I digested (a) and Chromomycin A_3 stained (b) metaphases plates of *P. italiae*.

A comparison of the C-banding patterns between the two species revealed other remarkable differences. In fact, the short arms of the eighth pair of autosomes are euchromatic in *P. italiae* but entirely heterochromatic in *P. domesticus*. The involvement of simple chromosomal rearrangement such as inversion, fusion and translocation must be ruled out, since the morphology of these and other autosomes remains unchanged. The transformation of euchromatin into heterochromatin has been invoked in similar cases observed in several amphibian species (King 1980, 1991). The comparison of the C banding patterns between *P. domesticus* and *P. italiae* also displayed a difference in chromosome Z, in particular in the location of a heterochromatic peritelomeric band, which is on the short arm in *P. italiae* and on the long arm in *P. domesticus* (when present). The simplest mechanism that might be responsible for such a difference is a pericentric inversion (Fig. 3). In fact, pericentric inversions differentiate Z chromosomes of closely related avian taxa, and in all these cases (also in *P. italiae*) the same type of chromosome rearrangement also occurs in chromosome W (Christidis 1990).

It is well known that crosses between species cause genomic stress, very often leading to remarkable chromosomal rearrangements, such as deletion, amplification, inversion, translocation (McClintock 1984). The lack of data on heterochromatin distribution in *P. hispaniolensis* did not allow us to establish whether the chromosomal differences observed in *P. italiae* are the result of the cross between *P. domesticus* and *P. hipsaniolensis*. However, the differences in the eighth autosomal pair and in the pair of sex chromosomes between *P. italiae* and *P. domesticus* appear considerable, and some remarks should be made on their role in operating the mechanisms involved in reproductive isolation. Generally, such variations in heterochromatin as those observed in the eighth autosomal pair are considered neutral or

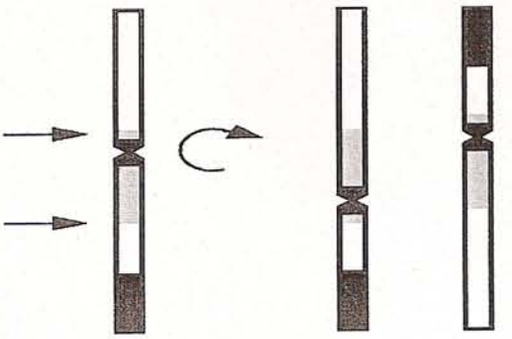

Fig. 3. Schematic representation of a probable inversion involving the chromosome, according to *P. domesticus* vs *P. italiae* transaction. On the left, arrows indicate two breaks occurred in the Z-chromosome of *P. domesticus* (grey area); the following inversion of this areas leads to the Z-chromosome of *P. italiae*.

adaptive (John 1988, King 1993). Of greater importance might be the differences observed in sex chromosome Z. In fact, the rearrangements in the X (Z) chromosome might represent a barrier for reproductive isolation by altering sex-determining mechanisms (King 1993), as observed for instance in the Indian mole rat *Nesokia indica* (Thelma et al. 1988). As regards chromosome W, due to the suppression of crossing-over (Ohno 1967), variation in DNA sequences and/or in the morphology of this chromosome tend to become quickly fixed, differentiating very closely related taxa. For these reasons, chromosome W is considered a good taxonomic marker in several taxa, including birds (Shields 1982, 1983) and several reptilian groups (Mengden & Stock 1980, Moritz 1990, Odierna et al. 1996, 1998). In the three species considered by us, chromosome W separates *P. italiae* from *P. domesticus*, and shows close affinity between *P. italiae* and *P. hispaniolensis*. This prompts us to carry out a C-banding study in *P. hispanoliensis*, in that it might provide useful information on both the presumed hybrid origin and the taxonomic state of the Italian endemic species, *P. italiae*.

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