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A centric fission and heterochromatin polymorphism in *Equus asinus* Spanish breeds

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Summary

In this work a chromosomal study in *Equus asinus* is presented. Forty-two specimens, belonging to five different Spanish breeds in risk of disappearing, have been analysed. Metaphases have been sequentially stained (uniform, G-banded and C-banded) and analysed. All specimens, except one, presented 2n = 62 chromosomes. Variation in chromosome number (2n = 63) is because of a fission in one chromosome of pair no. 3, resulting in two acrocentric chromosomes. Polymorphisms affecting the size of heterochromatic C+ bands and the presence of hetrochromatic 1p and 4p arms are also analysed. The results show no polymorphisms which can be attributed to a particular breed. From the chromosomal point of view, all Spanish asinine specimens correspond to a single population. Rearrangements detected suggest a fission tendency in the homologous *E. asinus* no. 3 chromosome in Equidae species.

Zusammenfassung

Eine zentrische Spaltung und heterochromatische Polymorphismen in spanischen Rassen von Equus asinus

In dieser Arbeit wird eine chromosomale Studie von *Equus asinus* vorgestellt. Zweiundvierzig Probanden, von fünf verschiedenen, vom Aussterben bedrohten spanischen Rassen, wurden analysiert. Die Metaphasen wurden sequentiell gefärbt (uniform, G-Bänderung und C-Bänderung) und ausgewertet. Alle Probanden zeigten 2n = 62 Chromosomen, mit Ausnahme eines Probanden. Die Variation der Chromosomenzahl (2n = 63) ist die Folge einer Spaltung in einem der Chromosomen von Paar 3, die zwei akrozentrische Chromosomen zur Folge hat. Polymorphismen, die die Größe der heterochromosomalen C+ Banden beeinflussten, sowie die Anwesenheit von heterochromosomalen 1p und 4p Armen wurden analysiert. Die Ergebnisse zeigen keine Polymorphismen, die auf eine spezifische Rasse zurückzuführen wären. Aus chromosomaler Sicht sind alle spanischen Rassen eine Population. Die nachgewiesenen Rearrangements deuten auf eine Spaltungstendenz im homologen *Equus asinus* Chromosomenpaar 3 in Spezies von Equidea hin.

Introduction

According to the FAO, the Spanish asinine populations are at a critical risk of disappearing; consequently, a plan for the survival of these endangered domestic breeds is required. A pluridisciplinary study to determine differential characteristics of Spanish asinine breeds is in progress (Jordana et al. 1999; Aranguren-Méndez et al. 2001, 2002a, b). The cytogenetic study presented here is part of that programme.

Cytogenetic characteristics of Equidae species have been described (Benirschke et al. 1962, 1965; Trujillo et al. 1962; Benirschke and Malouf 1967; Buckland et al. 1976; Ryder 1978; Ryder et al. 1978; Romagnano and Richer 1984; Ryder and Chemnick 1990; Bowling et al. 1997; Houck et al. 1998; Raudsepp et al. 2000). All published studies are based on a small number of animals. Descriptions of intraspecific variations are scarce

in *Equus asinus* (EAS); only one report, studying variations in the heterochromatin in six animals, has been published by Kopp et al. (1988).

Fusions/fissions have been described in Equidae species: Trommershausen-Bowling and Millon (1988) reported two specimens of *E. asinus* (mother and daughter) with a centric fission occurring in chromosome pair no. 3. A centric fission in a large metacentric chromosome of a Somali wild ass (*E. asinus somalicus*) has been reported by Benirschke and Ryder (1985). Whitehouse et al. (1984) reported a centric fission in *E. burchelli* pair no. 5, in three animals. Houck et al. (1998) documented a centric fusion rearrangement 19;21 in (*E. a. somaliensis*), Ryder (1978) reported a Robertsonian translocation in 23;24 in

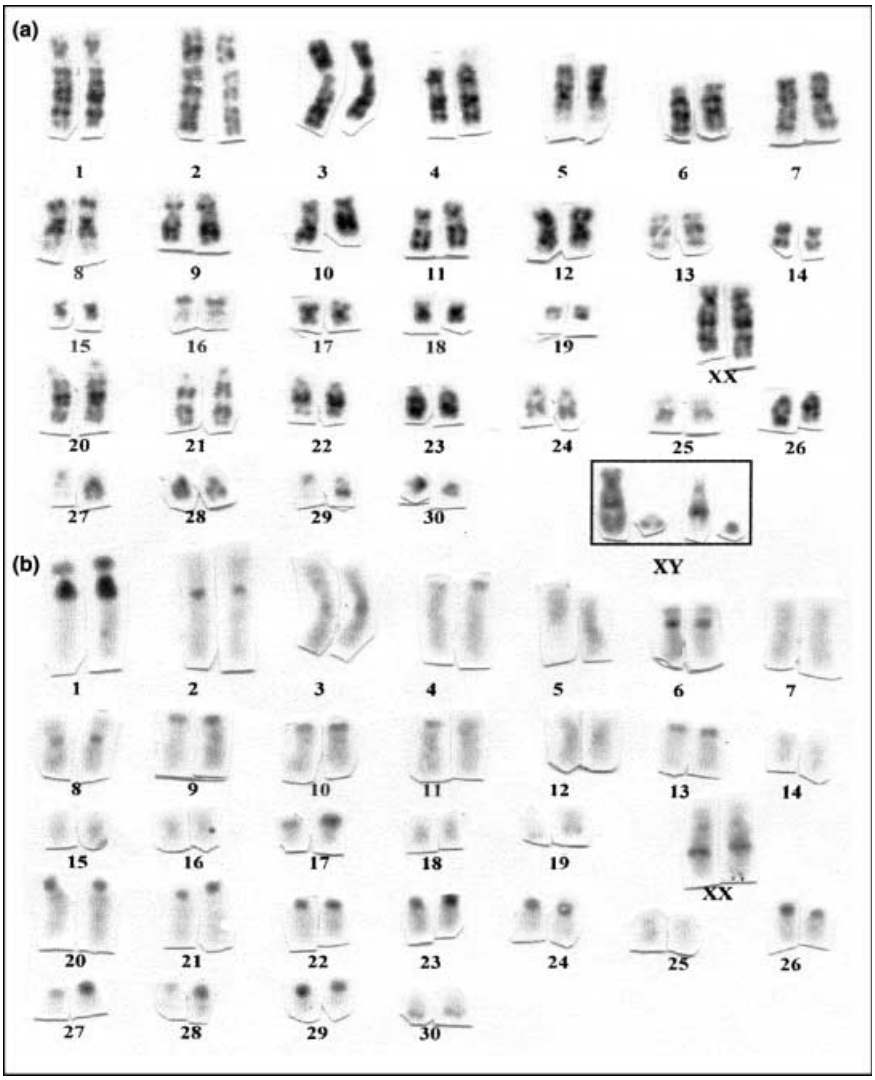

Fig. 1. Sequentially stained G-C female karyotype of *Equus asinus*. (a) G-banded, (b) C-banded. Encircled, an XY pair of a male specimen can be seen

(*E. hemionus*) and a Robertsonian translocation in 22;23 in (*E. kiang*) has been documented by Ryder and Chemnick (1990).

Materials and methods

The cytogenetic study was performed in forty-two specimens of *Equus asinus* (EAS) from five different Spanish breeds with no direct familiar relationship among them; one male and five females from the Andaluza breed, six males and four females from the Catalana breed, two males and seven females from the Encartaciones breed, three males and four females from the Mallorquina breed, and seven males and three females from the Zamorano-Leonesa breed.

Peripheral blood was obtained and cultured in standard conditions using RPMI (Gibco) culture media of 25% foetal calf serum and Pokeweed mytogen in a 1% final concentration.

Metaphase chromosomes were sequentially U-G-C stained/banded (U, uniform stain with Leishman solution; G, G-banded with Wright adapted from Seabright (1971); and C, C-banded adapted from Sumner (1972). A number of 25–30 metaphases per animal has been studied and at least10 sequentially G-/C- banded metaphases have been karyotyped for each animal. Chromosomes were arranged following the karyotype ordering proposed by Raudsepp et al. (2000), and the nomenclature proposed by Bowling et al. (1997).

Results

All specimens presented 2n = 62 (Fig. 1), except one female from the Catalana breed which presented 2n = 63. Variation in the 2n number is because of a fission in one chromosome of pair no. 3 resulting in two acrocentric chromosomes (Fig. 2). This fission was present in all metaphases analysed.

Sequentially stained metaphases from 30 specimens allowed for the identification of C-banded chromosomes and the band position related to G bands (Fig. 1). Centromeric C+ bands were detected in all chromosome pairs and polymorphisms can be seen in Table 1 (because of the small size of chromosome pair no. 30, its C+ band was difficult to analyse). Polymorphic juxtacentromeric C+ bands were observed in chromosomes 1q, 3q. No heterochromatin was observed in the fissioned no. 3 chromosome (Fig. 2).

Telomeric polymorphic C+ bands were present in 6, 9, 10, 11, 13 and 17p arms, and in the 19q arm (Table 1). A heterochromatic polymorphic p arm was observed in pairs 1 and 4;

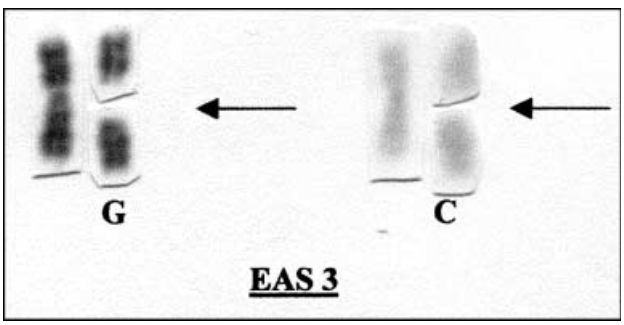

Fig. 2. Fission in pair no. 3 (G-C sequentially banded) present in one specimen of *Equus asinus*, Catalana breed. No centromeric C+ band is observed in this chromosome pair. Arrow shows the fission point

Table 1. **C-band polymorphisms in *Equus asinus* breeds. Symbols represent the absence (–) or the size of heterochromatin band, (+, ++, +++). Numbers in brackets indicate the number of specimens**

Breeds chromosome	Andaluza (3)	Catalana (7)	Mallorquina (7)	Encartaciones (5)	Zamorano-Leonesa (8)	Heterochromatin localization
1	(3) +/+	(6) ++/+, (1) ++/++	(1) +/–, (6) +/+	(2) ++/+, (2) ++/++, (1) +/+	(2) +/–, (1) –/–, (5) +/+	p Terminal
	(2) +++/++, (1) +++/+++	(4) +++/++, (3) +++/+++	(5) +++/++, (2) +++/+++	(5) +++/++	(4) +++/++, (4) +++/+++	Juxtacentromeric
2	(3) +/+	(7) +/+	(7) +/+	(3) ++/+, (2) +/+	(8) +/+	Centromeric
3	(1) –/–, (2) +/+	(1) –/–, (6) +/+	(2) +/–, (5) +/+,	(2) +/–, (2) –/–, 1) +/+	(1) +/–, (7) +/+	Juxtacentromeric
4	(1) +/–, (2) +/+	(4) +/–, (3) +/+	(7) + /+	(5) +/–	(4) +/–, (4) +/+	p Terminal
6	(1) +/–, (2) ++/+	(2) ++/+, (5) +/+	(2) ++/+, (5) +/+	(4) ++/++, (1) ++/+	(8) +/+	Centromeric
	(3) –/–	(7) –/–	(7) –/–	(1) +/+, (4) –/–	(5) –/–, (3) +/+	p Terminal
9	(1) +/+, (2) ++/+	(2) ++/+, (5) +/+	(2) ++/+, (5) +/+	(1) ++/+, (4) +/+	(2) ++/+, (6) +/+	p Terminal
10	(3) ++/+	(6) ++/+, (1) +/+	(3) ++/+, (4) +/+	(5) ++/+	(8) ++/+	p Terminal
11	(3) ++/+	(2) ++/+, (5) +/+	(3) +/–, (1) ++/+, (3) +/+	(5) ++/+	(3) ++/+, (5) +/+	p Terminal
13	(3) ++/+	(6) ++/+, (1) +/+	(3) ++/+. (4) +/+	(5) ++/+	(5)++/+, (3) +/+	p Terminal
17	(3) ++/+	(5) ++/+, (2) +/+	(6) ++/+, (1) +/+	(5) ++/+	(5) ++/+, (3) +/+	p Terminal
19	(2) ++/+, (1) +/+	(6) ++/+, (1) +/–	(4) ++/+, (1) +/–, (2) +/+	(1) +/–, (4) +/+	(5) ++/+, (3) +/+	q Terminal
20	(3) +/+	(5) ++/+, (2) +/+	(7) +/+	(1) ++/+, (4) +/+	(6) ++/+, (2) +/+	Centromeric
21	(3) +/+	(7) +/+	(7) +/+	(5) +/+	(2) ++/+, (6) +/+	Juxtacentromeric
24	(3) +/+	(7) +/+	(7) ++/+	(5) +/+	(2) ++/+, (6) +/+	Centromeric
25	(3) –/–	(7) –/–	(2) +/–, (5) –/–	(5) –/–	(1) +/–, (7) –/–	Centromeric
26	(2) ++/+, (1) +/+	(7) +/+	(7) +/+	(5) +/+	(4) ++/+, (4) +/+	Centromeric
27	(2) ++/+, (1) –/–	(2) ++/+, (5) +/+	(7) ++/+	(5) +/+	(8) +/+	Centromeric
28	(2) ++/+, (1) –/–	(7) +/+	(7) +/+	(5) +/+	(2) ++/+, (6) +/+	Centromeric
29	(2) ++/+, (1) –/–	(7) +/+	(7) ++/+	(5) +/+	(8) +/+	Centromeric
30	(2) ++/+, (1) –/–	(3) +/+, (4) –/–	(6) +/+, (1) +/–	(1) +/+, (4) –/–	(2) +/+, (3) –/– (3) +/+	Centromeric q Terminal

these bands were absent in some specimens, resulting in a decrease of the fundamental number (FN) (Fig. 3a,b, Table 1).

The X chromosome presented an interstitial C+ band in the q arm and the Y chromosome was completely heterochromatic, no polymorphisms have been observed in

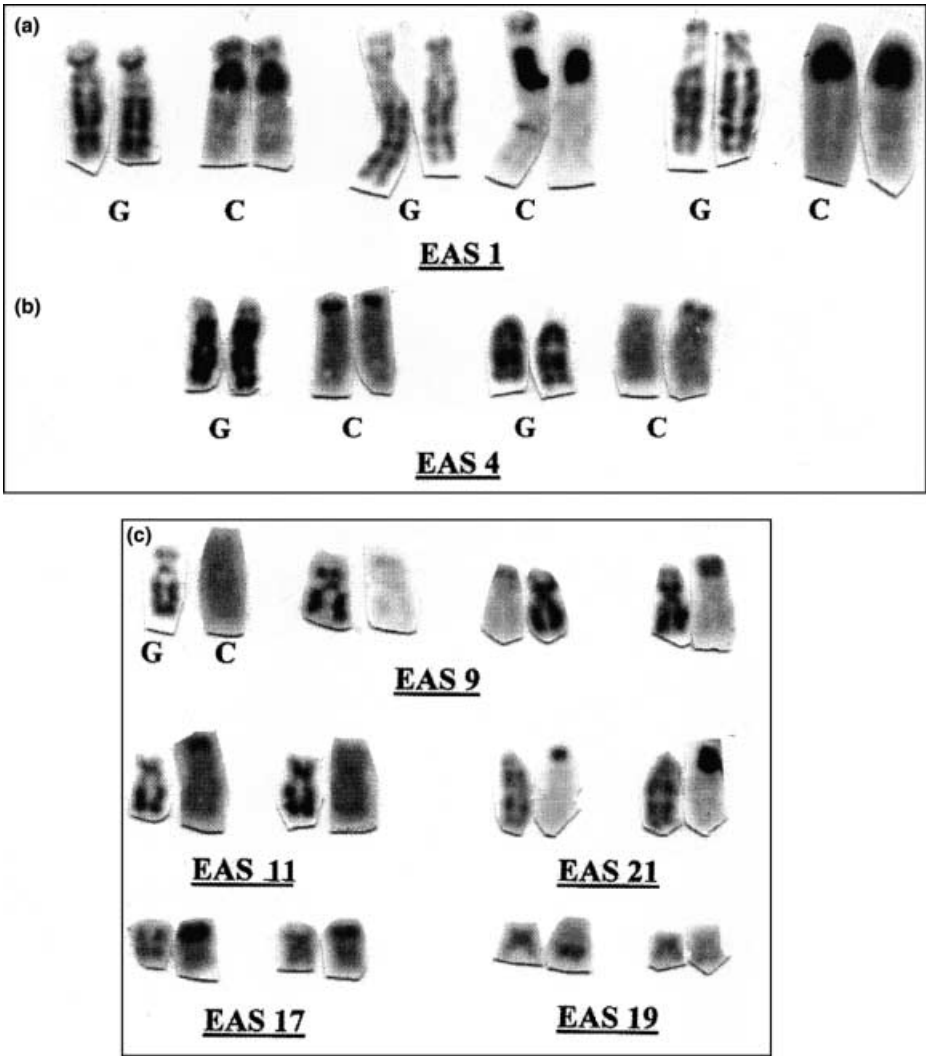

Fig. 3. Polymorphisms in C+ bands (a) pair 1, (b) pair 4 and (c) quantitative polymorphisms sequentially G-C stained in chromosomes 9, 11 and 17 (telomeric in p arm), 19 (telomeric in q arm) and 21 (juxtacentromeric) observed in *Equus asinus*

XY chromosomes. All C+ bands were G negative except the centromeric band in pairs no. 6 and 8.

No polymorphisms could be attributed to a particular asinine breed.

Discussion

Karyotype characteristics (2n and chromosome morphology) are in agreement with previous data published for the *E. asinus* (Ryder et al. 1978; Rong et al. 1988; Raudsepp et al. 2000). The fission found in one chromosome of pair no. 3 is equivalent to that described by

Trommershausen-Bowling and Millon (1988) in a mother and offspring of *E. asinus*, and to that described in a 2n = 45 specimen of *E. burchelli* by Whitehouse et al. (1984).

Comparing G-bands of the donkey chromosomes and other published equid species; the EAS 3 chromosome is homologous to pairs 19 + 21 of *E. a. somaliensis* (Houck et al. 1998), to pairs 23 + 24 of *E. hemionus onager* and of *E. hemionus kulan* (Ryder et al. 1978) and to pairs 22 + 23 of *E. kiang* (Ryder and Chemnick 1990). In *E. burchelli* and *E. grevyi* (Ryder et al. 1978) the homologous chromosome is also metacentric with the same G-band characteristics. Even Raudsepp and Chowdhary (1999) detected partial homology between *E. caballus* 3 and *E. asinus* 3 chromosomes. Using *E. caballus* Zoo-FISH probes in *E. asinus* chromosomes, we could not find this homology when comparing G-banded chromosomes of horses (*E. caballus* and *E. przewalskii*) and *E. zebra* with *E. asinus* G-banded karyotypes, suggesting complex evolutive rearrangements relating both morphologies. Results obtained by FISH with the (TTAGGG)n telomeric probe show that all telomeres present telomeric signals and no signs of interstitial telomeric sequences were observed in the fission band of the EAS 3 chromosome (Raudsepp et al. 2000; Lear 2001, and our results not included). If interstitial telomeric sequences are signals of previous telomere internalization (Ruiz-Herrera et al. 2002), the absence in the EAS 3 fission band probably indicates that the ancestral form in Equidae was metacentric and the forms observed in *E. somaliensis*, *E. hemionus onager*, *E. hemionus kulan* and *E. kiang* are as a result of a posterior fission event, actually the fission tendency is maintained in species with the metacentric form (Whitehouse et al. 1984; Trommershausen-Bowling and Millon 1988, this paper).

Rearrangements and polymorphisms affecting the homologous heterochromatic EAS 1p region were also observed in *E. a. somaliensis* (Houck et al. 1998).

Heterochromatin locations do not coincide with data observed in six animals from a Sicilian *E. asinus* population (Kopp et al. 1988). The number of metacentric and submetacentric chromosomes with a centromeric C+ band is higher in Sicilian populations (eight of 19 chromosomes versus four of 19 in our study). Telomeric heterochromatin is present in four pairs in the Sicilian population versus nine pairs in our study. Heterochromatin in the sex chromosomes coincides in both studies.

In conclusion, the cytogenetic characteristics and polymorphisms observed in five Spanish breeds of donkeys can be considered as corresponding to the species. The results obtained in the present study show no polymorphisms which can be attributed to a particular breed. From the chromosomal point of view, all Spanish donkey breeds correspond to a single population. Rearrangements observed in this study (fission in pair no. 3 and 1p deletion) are probably cytogenetic evolutive tendencies in the Equidae species.

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