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Chromosome C-banding in *Mus musculus* L.1766 strains shows a fixed position for the centromere and variable amounts in different populations

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Abstract
Three subspecies of *Mus musculus* have been recognized in Iran so far. The house mouse (genus *Mus*, species *Mus musculus*) are recognized for their highly conserved morphology and chromosomal structure, but some chromosomal characters offer accurate taxonomic markers in this species that has been shown any unambiguous diagnostic morphological traits. Among the chromosomal characters, centromeric heterochromatin is more useful to identify mouse subspecies and populations. In this study, Samples were collected from 27 stations in Iran and study was performed by the centromeric heterochromatin banding (C-banding). Results indicated that all samples had 40 acrocentric chromosomes and all chromosomes had fixed position for the centromere. The strains had the same amount of C-banding material on homologous chromosomes but showed variation in the amount on different populations.

Key words: Rodentia; Muridae; Karyology; House mouse; Karyotype; C- Banding; Iran

نواربندی سانترومری گستره های موش های خانگی (*Mus musculus* L.1766) ایران، نشان دهنده موقعیت ثابت سانترومر با مقادیر متغییر هتروکروماتین سانترومری در جمعیت های مختلف است

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چکیده
مدت ها است که سه زیرگونه از گونه موش خانگی در ایران شناسایی شده اند. موش خانگی (جنس *Mus*، گونه *M. musculus*) با ساختار کروموزومی و ریختی پایدار خود شناخته می شوند. اما برخی از صفات کروموزومی به عنوان نشانگرهای مورد اعتماد در این گونه شناخته شده اند. در میان این صفات کروموزومی، ساختار هتروکروماتین سانترومری جز این نشانگرها است که برای شناسایی جمعیت ها و زیرگونه های موش خانگی استفاده شده است. در این مطالعه، ۲۷ جمعیت از ایران مورد بررسی قرار گرفتند و ساختار هتروکروماتین آنها با هم مقایسه شد.نتایج نشان داد که در تمام افراد مورد تحقیق سانترومر جایگاه یکسانی در کروموزوم ها دارد. و با اینکه اندازه هتروکروماتین سانترومری در بین کروموزوم های هومولوگ یکسان است ولی بین جمعیت های نختلف تفاوت اندازه دارند.

واژه های کلیدی: جوندگان؛ موش؛ کاریولوژی؛ موش خانگی؛ کاریوتایپ؛ ایران

Introduction

Muridae species despite high morphologic similarity display much higher variability of karyotypes (Romanenko *et al.*, 2007). The centromeric domain of most mammalian chromosomes contains repeated DNA sequences (Britten and Kohne, 1968). In the mouse, *Mus musculus*, the major repetitive DNA component is the satellite DNA sequence family first isolated by Kit (1961). As shown by Pardue and Gall (1969) and Jones (1970) this major satellite is present on all *M. musculus* chromosomes with the exception of the Y chromosome, and constitutes around 10% of the *M. musculus* genome. In 1983 Pietras *et al.* isolated a second repetitive DNA family from *M. musculus*, which comprised a much smaller proportion (<1%) of the genome and called it the minor satellite. By *in situ* hybridization Wong and Rattner (1988), Joseph *et al*. (1989) and Broccoli *et al*. (1990) have shown that the minor satellite DNA sequences within *M. musculus* appear to be physically very close to the primary constriction (*i.e.* the centromere) in these chromosomes. In comparison, the major satellite DNA sequences, although localized to the pre-centromeric heterochromatin in *M. musculus*, occupy a separate non-overlapping domain to that of the minor satellite DNA sequences (Joseph et al., 1989). This domain, together with its associated C-band, can be deleted from a mouse chromosome without affecting centromere function, which argues strongly that major satellite DNA sequences are not directly involved in centromere function (Broccoli et al., 1990).

Materials and Methods

40 specimens were captured from 27 stations of Iran (Table 1 and Fig. 1). Sample codes of specimens are available in the Rodentology Research Department of Ferdowsi University of Mashhad, Iran. Chromosome spreads were obtained from bone marrow cells according to Yosida (1973). About 50 to 100 metaphase plates from both male and female specimens were examined and at least 30 good chromosomal spreads were photographed using a 100x zoom digital CCD camera. The karyological characteristics of all specimens were prepared by Karyological Analysis software (version 1.2, 2010). Chromosomes were classified according to Levan (levan et al,1964), and each chromosome was placed next to its presumed homologue to determine the diploid chromosome number (2n).
C-banding: Barium hydroxide - Saline - Giemsa (BSG) method was performed according to Summer (1972).

Figure 1. Map of specimen collection sites for *M. musculus* strains in Iran (black dots are stations)

Results

In this study, 40 specimens of *M. musculus* including all 3 subspecies present in Iran were studied. The karyological characteristics of these species are described in Figure 2. In most of the strains every chromosome except the Y chromosome was found to have C-banding material (Fig. 3). The size of the C-banding region was the same for both homologous chromosomes in each strain (Figure 4), but the size of this region on some chromosomes varied from one population to another (Fig. 5 and 6). In Mashhad populations, the amount was approximately the same on each chromosome and we have used this strain as a standard for comparison. The centromere region size was variable in populations. For example, in Mashhad population, chromosomes 8 and 14 had smaller amounts of C-banding material than Gonabad population; whereas, chromosomes 3 and 19 had larger amounts of that (Fig. 4). Moreover, the Zahedan strain differs from Zabol in having very little C-banding material on chromosome 10 and 13 and

having larger amount of C-banding material on chromosomes 5 and 12.

Table 1. Sampling localities, geographical coordinates, and sample codes of *M. musculus* from different localities of Iran.

Row	Taxon	Locality	Longitude	Latitude	2n
1	*M. m. bacterianus*	Kerman	30° 17′ 46″ N	57° 05′ 23″ E	40
2	*M. m. musculus*	Gonabad	34° 21′ 10″ N	58° 41′ 1″ E	40
3	*M. m. musculus*	Torbate Jam	35° 14′ 38″ N	60° 37′ 21″ E	40
4	*M. m. musculus*	Sarakhs	36° 32′ 42″ N	61° 9′ 28″ E	40
5	*M. m. musculus*	Dargaz	37° 26′ 40″ N	59° 6′ 29″ E	40
6	*M. m. musculus*	Kalat	36° 59′ 33.01″ N	56° 45′ 23.83″E	40
7	*M. m. Isatissus*	Isfahan	32° 63′ 35″ N	51° 65′ 36″ E	40
8	*M. m. bacterianus*	Zahedan	29° 29′ 47″ N	60° 51′ 46″ E	40
9	*M. m. bacterianus*	Khash	28° 13′ 16″ N	61° 12′ 57″ E	40
10	*M. m. bacterianus*	Zabol	31° 1′ 43″ N	61° 49′ 4″ E	40
11	*M. m. domesticus*	Chabahar	25° 17′ 31″ N	60° 64′ 35″ E	40
12	*M. m. Isatissus*	Shiraz	29° 61′ 0″ N	52° 54′ 0″ E	40
13	*M. m. Isatissus*	Yazd	31° 53′ 50″ N	54° 22′ 4″ E	40
14	*M. m. musculus*	Esfarayen	37° 73′ 03″ N	57° 50′ 72″ E	40
15	*M.m. domesticuss*	Eizeh	31° 50′ 48″ N	49° 50′ 36″ E	40
16	*M. m. domesticuss*	Mamasani	30° 7′ 0″ N	51° 31′ 0″ E	40
17	*M. m. bacterianus*	Sabzevar	36° 70′ 35″ N	59° 6′ 44″ E	40
18	*M. m. musculus*	Tabadkan	36° 29′ 0″ N	59° 40′ 58″ E	40
19	*M. m. musculus*	Mashhad	36° 18′ 0″	59° 36′ 0″ E	40
20	*M. m. bacterianus*	Rask	26°13' 47"N	61°13' 4"E	40
21	*M. m. bacterianus*	Birjand	32° 87′ 0″ N	59° 20′ 0″ E	40
22	*M. m. bacterianus*	Qasregand	26° 15′ 60″ N	60° 45′ 14″ E	40
23	*M. m. bacterianus*	Negor	25° 38′ 15″ N	61° 12′ 33″ E	40
24	*M. m. musculus*	Kardeh	36° 16′ 51″ N	59° 34′ 59″ E	40
25	*M. m. bacterianus*	Zahedan2	37° 33′ 19″ N	45° 04′ 21″ E	40
26	*M. m. musculus*	Neyshabour	36° 20′ 48″ N	58° 10′ 48″ E	40
27	*M. m. domesticus*	Bandar Mahshahr	30° 54′ 32″ N	49° 11′ 58″ E	40

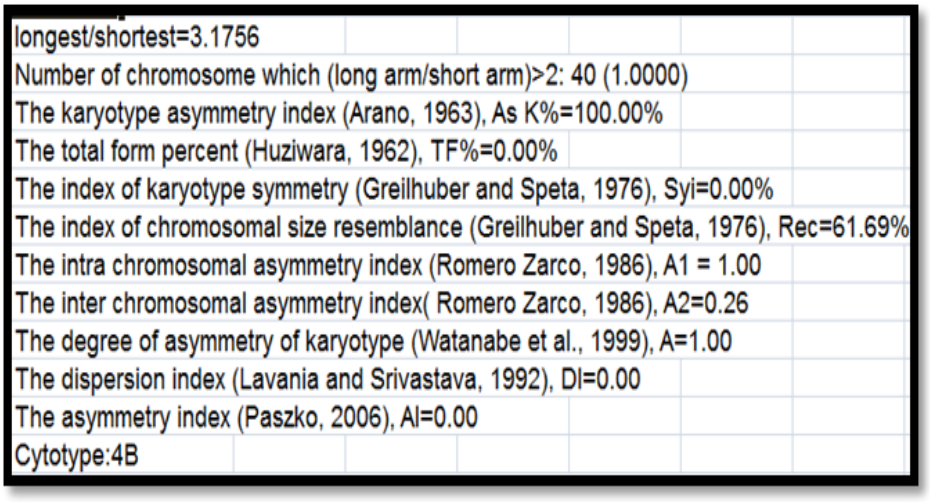

Figure 2. A example of the Karyological Analysis software output of *Mus musculus*.

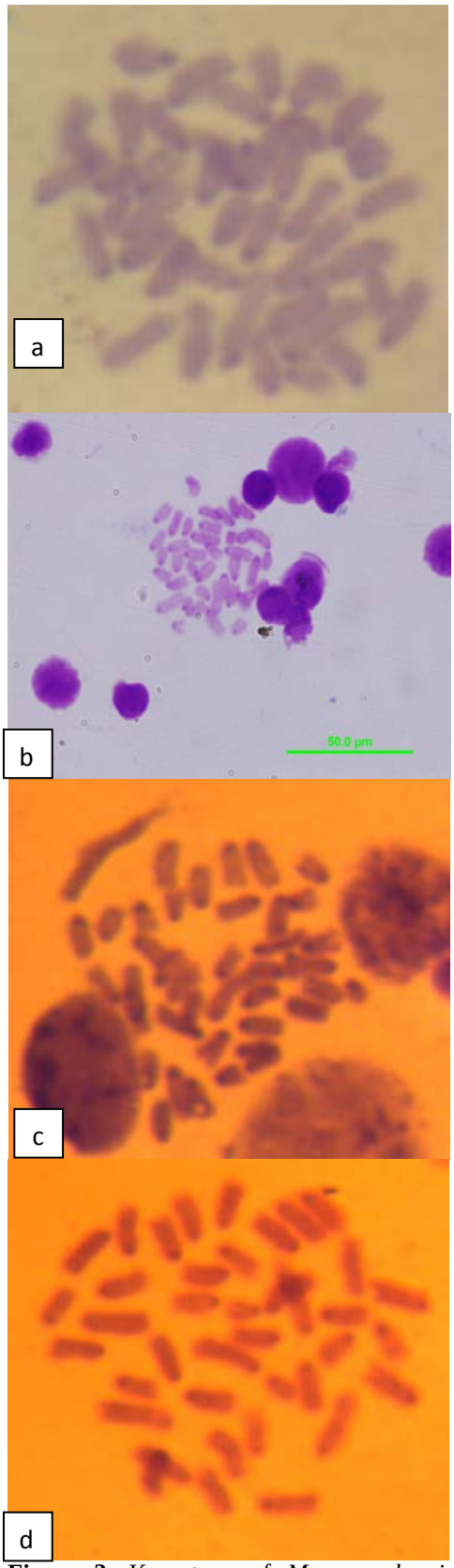

Figure 3. Karyotype of *M. musculus* in Mashhad (a),Yazd (b), Zahedan (c) and Bandar Mahshahr (d)

Figure 4. The same size of the C-banding region of *Mus musculus* for both homologous chromosomes in Mashhad strain.

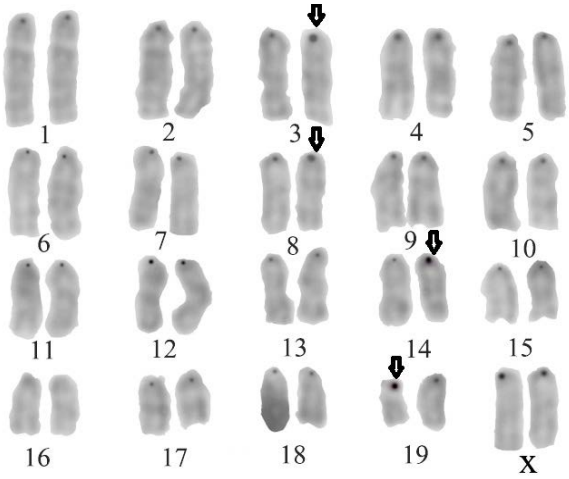

Figure 5. The size of the C-banding regions on chromosomes of *M. musculus* strains captured in Mashhad (left) and Gonabad (right).

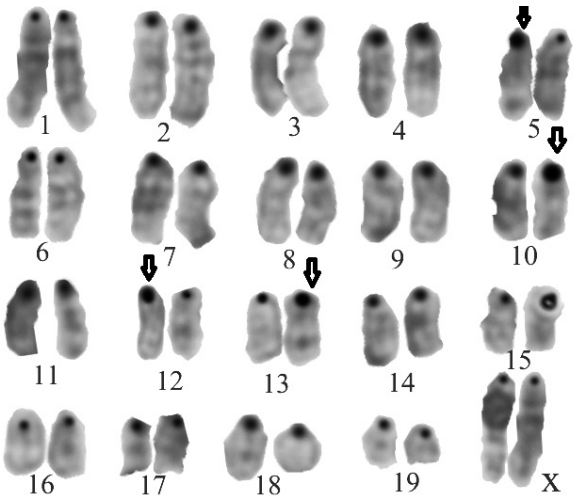

Figure 6. The size of the C-banding region of *Mus musculus* for Zahedan (left) and Zabol (right) chromosomes .

Discussion

Chromosomal evolution in house mouse show reticulated evolution and speciation (Gunduz, 2010).Sub species of *M. musculus* are parapatric (Boursot et al, 1989) Despite the reduction of meiotic fitness in hybrids (Sharma et al, 2003), We Have been reported many hybrids of them in contact zones (Guenet, 2003).Information about highly polymorphic minisatellite markers suggested past and present genetic exchanges among house mouse subspecies (Bonhomme et al, 2007). Rajabi-mahan, et al. (2012) showed *Mus musculus* castaneus is a polytypic subspecies of this species. In 2006, a transition zone of the house mouse have been reported from eastern Iranian plateau by Darvish in 2006. Malcon et al. in 2007 showed chromosomal characters are very useful for recognizing species in rodentia. The primary and secondary constrictions have already been used for comparison between populations in mice by Dev *et al*. (1971). They showed that almost all chromosomes could be distinguished by the presence or absence of large secondary constrictions located near to the C-banding region. In 1971 Eicher used the variant amount of secondary constriction region on chromosome 19 for calculating the degree of diversity in *Mus musculus*. He found that almost every chromosome of *M. m. mollossinus* was different from that of *M. musculus* in the size of C-banding region. Since *M. m. mollossinus* is interfertile with *M. musculus*, markers for every mouse chromosome except the Y chromosome are potentially available. The C-banding regions of mouse chromosomes are composed mainly of highly repetitious DNA, called satellite DNA (Pardu and Gall 1970). It is consisted of millions of copies of very short nucleotide sequences, or minor variants of them (Southerne, 1970). Polymorphic variants have been reported within the chromosomes of laboratory mice as well as in different species of wild mice (Forejt, 1973). Dev *et al*. (1973) looked at C-band variants within *M. musculus* strains and differences in the constrictions of chromosomes in these species using quinacrine fluorescence. Both Dev *et al*. (1973) and Davidson (1989) referred to a C-band variant on chromosome 1, although no reference was made to the type of DNA sequences involved. Vig and Richards (1992) have argued that the formation of the primary constriction in some mouse chromosomes (chromosome no. 1) does not always require the presence of mouse minor satellite DNA sequences. Our results show that the amount of C-banding material on each chromosome is characteristic for each population of *Mus musculus*. The existence of obvious differences in the distribution of C-banding material among the chromosomes in different populations, suggests that the amount of C-banding material on a chromosome is a polymorphic trait that is inherited in a simple fashion.

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