

A New Cytotype of the Iranian House Mouse, *Mus musculus* Linnaeus, 1758 (Rodentia: Muridae), in Birjand Region, Eastern Iran: Implications for the Evolution of Sex-Autosome Translocations

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The highly conserved morphology and chromosomal structures of house mouse, *Mus musculus*, provides a good model for chromosomal studies. Sex-autosome translocations is one of the rarest chromosomal rearrangements among mammals and therefore sex-autosome translocations has been offered as accurate taxonomic markers to identify species with similar morphological traits. In this study, we described the outcome of a comprehensive cytogenetic survey of the house mouse, *Mus musculus*, in the eastern Iran using G banding method. Interestingly, results showed the presence of a new cytotype of X-autosome translocation of house mouse which was found in 18 specimens of Birjand region in the eastern Iran. Larger size of chromosomal translocation was observed in Chromosome X. We used karyotype asymmetry method as a powerful statistical parameter to extract coefficient of variation of chromosome length. Based on the data of this study, the results, Birjand population did not show asymmetry in all Asymmetry indexes (AI, DI, As%, A, A2, A1 and Syi %), and this result confirmed translocations in Birjands' chromosomes as well.

Key words: Cytotaxonomy, systematic, Chromosome structure, House Mouse, Karyology, Iran, Middle East.

INTRODUCTION

The genus *Mus* has been subjected in several chromosomal evolution studies (Britton-Davidian *et al.*, 2000; Veyrunes *et al.*, 2006; Piálek *et al.*, 2005). This genus composed of at least 40 species which classified to four subgenera. However, one of the subgenera is house mouse, *Mus musculus*, which has four subspecies in Iran, including *M. m. domesticus*, *M. m. musculus*, *M. m. castaneus* and *M. m. bactrianus*. These subspecies have the same standard karyotype with 20 pairs of acrocentric chromosomes (Silver, 2001). In spite of extensive study of molecular markers have been used on subspecies of house mouse, still relationships between the subspecies of house mouse are unclear (Vanlerberghe *et al.*, 1986; Orth *et al.*, 1996; Darvish *et al.*, 2006; Rajabi-Maham *et al.*, 2007). With respect to rare genomic changes of chromosomal rearrangements, probably has been considered as ideal candidates method to elucidate the taxonomic issue (Rokas and Holland, 2000; Murphy *et al.*, 2004; Wienberg, 2004). However, chromosomes could be distinguished using various staining protocols such as

banding patterns (Iaca, 2001). Hence, several methods of chromosomal painting have been suggested, among them Fluorescence in situ hybridization (FISH) is considered powerful method of comparative chromosome painting to detect chromosome homologies between species and subspecies (Li *et al.*, 2004). FISH techniques and chromosome-specific multicolor techniques are spectral karyotyping (SKY) improve the characterization of aberrant chromosomes that are not recognized using conventional banding methods. Giemsa-banding staining or G-banding Methods is rapid and economical way to study chromosomes of house mouse. This method is based on the principal of chromatin denaturation or mild enzymatic digestion that is followed by staining with a DNA-binding dye (Craig and Bickmore, 1993). Previous studies of wild house mice showed some non-standard karyotypes in house mice from some regions of Europe, South America and Northern Africa, this results suggested chromosomal differentiations were powerful marker for recognition this subspecies and species (Adolph and Klein, 1981; Wallace, 1981; Searle, 1982). All of the non-standard karyotypes have arisen from simple fusion events that led to bind two standard chromosomes of house mouse in the centromeric region (Adolph and Klein, 1981). Generally, a few sex-autosome translocations are known in mammalian so far (Dobingny *et al.*, 2004; Mudry *et al.*, 2001 and Fredga, 1976).

In comparative karyology, karyotype asymmetry has been proposed on the basis of predominance of chromosomes with terminal/sub-terminal centromeres (intra-chromosomal asymmetry) and making karyotype more heterogeneous (inter-chromosomal asymmetry). This comparative study has been introduced for the first time by Levitsky (1931). Stebbins (1971), proposed a quali-quantitative method to estimate karyotype asymmetry in twelve categories which taking them in to four class, from 1 to 4, the different class were indicated with respect to the largest and smallest chromosome of the complement (A-C) as well as four class (1-4) determined by the proportion of chromosomes with arm ratio more than 2:1. Concerning inter-chromosomal asymmetry, which is due to heterogeneity among chromosome sizes in a complement, other researchers proposed quantitative estimation methods in the following years. This is the case of the Rec index (Greilhuber and Speta, 1976; Venora *et al.*, 2002), the A2 index (Romero Zarco, 1986), the R ratio (Siljak-Yakovlev, 1996) and the CVCL (Lavania and Srivastava, 1992; Watanabe *et al.*, 1999; Paszko, 2006). The latter, coefficient of variation actually is a statistically correct parameter and is able to capture even small variations among chromosome sizes in a complement (Lorenzo and Eroğlu, 2013).

The present study was aimed to use chromosomal analysis in order to clarifying the taxonomic status of the house mouse in the eastern Iran. We described a new cytotype of *Mus musculus* from Birjand in the eastern Iran. The findings provided new insights of evolution of sex-autosome translocations in the species.

MATERIAL AND METHODS

ANIMALS

During three years of field works in 2012- 2015, a total of 126 specimens of house mouse from 18 localities of Iran were examined (Table 1, and Figure 1). Mice were caught using Longworth live-traps in farm buildings. Six morphometric characters including the length of body, tail, ear, hind foot, the zygomatic index ($ZI = \text{width of molar process} / \text{width of upper part of zygomatic arch}$) and the ration between tail length to head and body length ($\text{tail length} / \text{head body length} = T / HB$) were measured and the four subspecies were identified following Gündüz *et al.*, 2000; Darvish, 2004; Darvish *et al.*, 2006; Bonhomme *et al.*, 2007; Darvish *et al.*, 2008; Shabani *et al.*, 2010; Siah sarvie *et al.*, 2012; Rajabi-Maham *et al.* 2012 (for more details, see Table 1).

TABLE 1. Locality and taxon name of studied individuals of *M. musculus* in this study.

Locality	No.	Longitude	Latitude	Taxon	Ref. used for identification
Torbate Jam	5	60° 37' 21"	35° 14' 38"	<i>M. m. musculus</i>	Siahsarvi <i>et al.</i> (2012), Rajabi-Maham <i>et al.</i> (2012)
Kakhk	8	58°38'21"	34°08'59"	<i>M. m. musculus</i>	Rajabi-Maham <i>et al.</i> (2012)
Dargaz	5	59° 6' 29"	37° 26' 40"	<i>M. m. musculus</i>	Darvish <i>et al.</i> (2006)
Sarakhs	5	61° 9' 28"	36° 32' 42"	<i>M. m. musculus</i>	Siahsarvi <i>et al.</i> (2012), Rajabi-Maham <i>et al.</i> (2012)
Mashhad	18	59° 36' 0"	36° 18' 0"	<i>M. m. musculus</i>	Siahsarvi <i>et al.</i> (2012), Shabani <i>etal.</i> (2010), Bonhomme <i>et al.</i> (2007), Rajabi-Maham <i>et al.</i> (2012)
Esfarayen	5	57° 50' 72"	37° 73' 03"	<i>M. m. musculus</i>	Darvish <i>et al.</i> (2006) , Rajabi-Maham <i>et al.</i> (2012)
Gonabad	4	58° 41' 1"	34° 21' 10"	<i>M. m. musculus</i>	Shabani <i>et al.</i> (2010) , Rajabi-Maham <i>et al.</i> (2012)
Kalat	7	56° 45' 23"	36° 59' 33"	<i>M. m. musculus</i>	Darvish <i>et al.</i> (2006) , Rajabi-Maham <i>et al.</i> (2012)
Birjand	15	59° 20' 0"	32° 87' 0"	<i>M. m. bacterianus</i>	Darvish(2008),Shabani <i>et al.</i> (2010)
Tabas	8	56°55'29.0"	33°36'10.9"	<i>M. m. castaneus</i>	Rajabi-Maham <i>et al.</i> (2012)
Kerman	8	56° 31' 23"	31° 30'10"	<i>M. m. castaneus</i>	Rajabi-Maham <i>et al.</i> (2012)
Rask	3	61° 23' 56"	26° 14' 13"	<i>M. m. castaneus</i>	Siahsarvi <i>et al.</i> (2012)
Qasr-e Qand	3	60° 45' 9"	26° 14' 54"	<i>M. m. castaneus</i>	Siahsarvi <i>et al.</i> (2012) & Darvish (2008) & Bonhomme <i>et al.</i> 2007
Zahedan	5	60° 51' 46"	29° 29' 47"	<i>M. m. castaneus</i>	Rajabi-Maham <i>et al.</i> (2012)
Zabol	4	61° 49' 4"	31° 1' 43"	<i>M. m. bacterianus</i>	Rajabi-Maham <i>et al.</i> (2012)
Chabahar	9	60° 64' 35"	25° 17' 31"	<i>M.m.domesticus</i>	Gündüz <i>et al.</i> (2000), Rajabi-Maham <i>et al.</i> (2012)
Khash	4	61° 12' 57"	28° 13' 16"	<i>M. m. castaneus</i>	Rajabi-Maham <i>et al.</i> (2012)
Neyshabour	5	58° 10' 48"	36° 20' 48"	<i>M. m. musculus</i>	Darvish <i>et al.</i> (2006), Rajabi-Maham <i>et al.</i> (2012)



FIGURE 1. Map samples of *M. musculus* used in this study. Black boxes were referred to locality. Details of the samples are given in Table 1.

KARYOTYPE AND CHROMOSOME IDENTIFICATIONS

Chromosome preparations of all specimens of the Iranian house mouse were made from bone marrow cells of yeast-stimulated animals with some modifications (Lee and Elder, 1980). Identification of chromosomes was accomplished by G-banding (Seabright, 1971). The nomenclature of chromosome morphology was followed Levan (1964) and Guerra (1986). The chromosomes were numbered on the basis of euchromatic long arms as per recommendations of the Committee on Standardized Genetic Nomenclature for mice (1972).

KARYOTYPE ANALYSIS

A total of 50 to 100 metaphase spreads from each specimen were examined and at least 10 good chromosomal spreads were photographed using a 100x zoom digital CCD camera. Generally, 18 karyological characteristics of all specimens were prepared by Karyological Analysis software (version 1.2, 2010), all the data is given in Table 2. The relative length of each chromosome pair was expressed by the percentage of the absolute length of each chromosome pair which is divided to the sum of the absolute length of total chromosomes.

INDUCTION OF G-BANDS

Metaphase slide preparation was made 10 to 14 days before banding then remove and bring them to room temperature just prior to banding. Then, grasp the slide with forceps and immerse it in a Coplin jar containing the 0.025% trypsin working solution for 8 to 10 sec. after that, They were kept in a mixture of sodium chloride and sodium citrate solutions (12 x SSC) at 60°C for 1 hr. Then, at the end of the incubation periods the slides were rinsed in several changes of 70% ethanol and then in 90% ethanol. Finally, the slides were then stained with Giemsa solution (pH = 7.0) for 8-10 min, washed briefly with distilled water, air dried and mounted in Permount. The arrangement of chromosomes was checked according to the Committee on Standardized Genetic Nomenclature for Mice (1972).

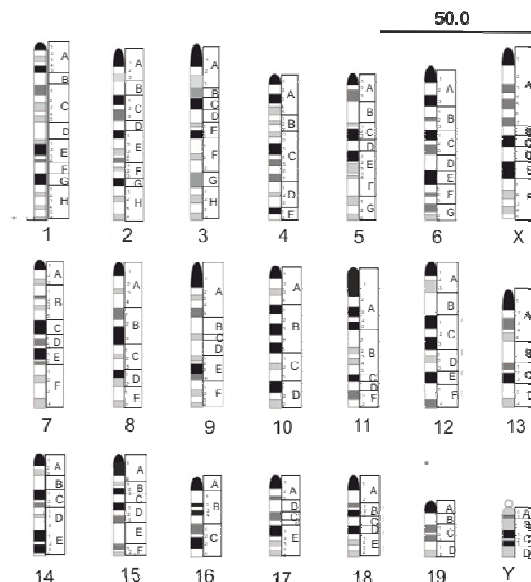


FIGURE 2. Chromosome ideogram of the Iranian house mouse was made according to G-banding method.

TABLE 2. List of characters used for chromosome analysis.

Name	Definition	Formula	Range	Reference	Description	
1	2n	Diploid number of chromosomes	sum of chromosomes	>2	Nägeli, 1842	-
2	Fn	Fundamental number	number of visible major chromosomal arms per set of chromosomes	$F_n \leq 2 \times 2n$	Matthey, 1945	-
3	Fna or An	Autosomal fundamental number	number of visible major chromosomal arms per set of autosomes (non-sex-linked chromosomes).	$F_{na} \leq 2 \times 2n$	Matthey, 1945	-
5	A1	The intra chromosomal asymmetry index	$[1 - \sum_i^0 (p/q) / n]$	0-1	Romero Zarco, 1986	P: long arm, q: short arm, and n: total of chromosome
6	A2	The inter chromosomal asymmetry index	Scl/Xcl	0-1	Romero Zarco, 1986	Scl: Standard error of total chromosomal length. Xcl: Mean of total chromosomes
7	A	The degree of asymmetry of karyotype	$(\sum_i^0 (P_i - q_i) / (p_i + q_i)) / n$	0-1	Watanabe et al., 1999	P: long arm, q: short arm, n: total of chromosome
8	DI	The dispersion index (is a normalized measure of the dispersion of a probability distribution)	$D = \sigma^2 / \mu$	>0	Lavana and Srivastava, 1992	σ^2 : variance μ : mean
9	AI	The asymmetry index	$(\mu_x - \mu_y) / (\sigma_x^2 + \sigma_y^2)^{1/2}$	0 < x ≤ 2.0: The asymmetry is weak. The distribution is relatively symmetrical. 2.0 < x ≤ 4.0: The asymmetry is moderate. The distribution is relatively asymmetrical. x > 4.0: The asymmetry is strong. The distribution is asymmetrical.	Paszko, 2006	$V = (R - L) / (R + L) \equiv X/Y$; $X = R - L$ and $Y = R + L$ μ_x : Means X μ_y : Means Y σ_x^2 : variances
10	Cytotype	An individual of a species that has a different chromosomal factor to another (e.g. haploid versus diploid)	-	-	-	-
11	All.ch.L.	-	All chromosome s length	-	-	-
12	As%	The karyotype asymmetry index	(Length of long arm in chromosome complements/Total sum of chromosome length in a set)x100	50-100	Arano, 1963	-
13	TF%	The total form percent	Total of short chromosomal lengths/ Total of chromosomal lengths	0-50	Huziwarra, 1962	-
14	Syi%	The index of karyotype symmetry	Ms/Ml	-	Greilhuber and Speta, 1976, Venora et al. 2002	Ms: Mean length of the shortarms Ml: Mean length of long arms
15	Rec	The index of chromosomal size resemblance	i=1-40, n=40	0-100	Greilhuber and Speta, 1976, Venora et al. 2002	CLi: Length of total of chromosome LC: Length of longest chromosome
16	SC	Length of shortest Chromosome	micron	-	-	-
17	LC	Length of longest Chromosome	micron	-	-	-
18	Mpq	Mean of chromosomal Length	micron	-	-	-
19	L/S	-	Longest chromosome/ shortest chromosome	-	-	-

TABLE 3. Comparison of karyological records of the studied house mouse populations. Black color : *M. m. musculus*, Red color : Birjand (*Mus musculus bacterianus*.), Blue color : *M. m. castaneus* and *M. m. bacterianus*.

	Localities	No.	2n	Fn	Fna	All ch. L.	L/S	AsK%	TF%	Syi%	Rec	A1	A2	A	DI	AI	Cytotype	Karyotype	SC	LC	Mpq
1	Torbat Jam	5	40	40	38	19876.54	2.8071	100	0	0	64.76	1.00	0.00	0.26	0	0.00	4B	40Acro	3.58	6.42	3.96
2	Kakhk	8	40	40	38	18558.32	2.4739	100	0	0	65.01	1.00	0.00	0.27	0	0.00	4B	40Acro	3.48	6.41	4
3	Dargaz	5	40	40	38	19342.33	2.9631	100	0	0	65.75	1.00	0.00	0.27	0	0.00	4B	40Acro	3.58	6.42	4.09
4	Sarakhs	5	40	40	38	19876.35	3.0152	100	0	0	64.69	1.00	0.00	0.28	0	0.00	4B	40Acro	3.31	6.91	4.03
5	Mashhad	18	40	40	38	18935.39	2.5987	100	0	0	64.98	1.00	0.00	0.22	0	0.00	4B	40Acro	3.21	6.81	4.28
6	Esfarayen	5	40	40	38	19032.65	2.4021	100	0	0	73.42	1.00	0.00	0.28	0	0.00	4B	40Acro	3.43	6.07	3.43
7	Gonabad	4	40	40	38	19997.78	3.6131	100	0	0	60.68	1.00	0.00	0.29	0	0.00	4B	40Acro	3.11	7.01	4.23
8	Kalat	7	40	40	38	197658.3	2.7846	100	0	0	64	1.00	0.00	0.28	0	0.00	4B	40Acro	3.19	6.71	4.14
9	Birjand	15	40	40	38	19786	5.63	97.4	2.6	2.7	47.7	0.97	0.29	0.96	0	0.77	3C	40Acro	3	6.88	4,767
10	Tabas	8	40	40	38	27986.43	2.6521	100	0	0	68.21	1.00	0.00	0.22	0	0.00	4B	40Acro	3.34	6.37	5.01
11	Kerman	8	40	40	38	280213.4	2.6435	100	0	0	66.31	1.00	0.00	0.24	0	0.00	4B	40Acro	3.29	6.57	4.99
12	Rask	3	40	40	38	26405.13	2.6415	100	0	0	68.87	1.00	0.21	0.21	0	0.00	4B	40Acro	3.57	6.55	5
13	Qasr-e Qan	3	40	40	38	26993.98	3.1735	100	0	0	61.68	1.00	0.00	0.26	0	0.00	4B	40Acro	3.13	6.37	4.92
14	Zahedan	5	40	40	38	27854.13	2.6875	100	0	0	62.05	1.00	0.00	0.25	0	0.00	4B	40Acro	3.34	6.43	4.96
15	Zadul	4	40	40	38	27566.13	2.6576	100	0	0	65.43	1.00	0.00	0.25	0	0.00	4B	40Acro	3.45	6.54	4.88
16	Chahbahar	9	40	40	38	15467.65	2.2314	100	0	0	65.48	1.00	0.00	1.00	0	0.00	4B	40Acro	2.67	6.07	4.47
17	Khash	4	40	40	38	14269.67	4.5862	90.95	9.05	0.95	42.32	0.91	0.35	0.87	0	4.21	3C	40Acro	2.98	6.12	4.46
18	Neyshabou	5	40	40	38	18935.39	2.5767	100	0	0	64.98	1.00	0.00	0.22	0	0.00	4B	40Acro	3.31	6.71	4.28

RESULTS

(The G-banded karyotypes of the Iranian house mouse subspecies were determined for the first time in this study. All chromosomes of house mouse painted successfully, the comparison of G-banded patterns of all 126 individual of populations of house mouse showed the common pattern in chromosomal bands (Figure 2). All individuals of house mouse have totally a diploid number of $2n=40$ with acrocentric chromosomes. Results showed no variation in the number and combination of Robertsonian fusions (i.e. fusion by the centromere of two non-homologous chromosomes).

However, six mice chromosomes (3, 6, 9, 12, 19 and X) showed complete conservation of their bands and four chromosome (1, 4, 10 and 15) represented variations in the bands. Results represented specimens from Birjand region, the eastern Iran, which has one fusion involving the sex chromosomes and chromosome 1. The fusion could be represented a diagnostic signature for this special population (Figure 3). The karyotypic analyses indicated the presence of a large chromosome X in this locality and comparative cytogenetic map of the specimens showed that the translocation between band H in chromosome 1 and distal part of chromosome X (Figure 4). Asymmetry indexes indicated asymmetry karyotype in populations of Birjand; the finding confirmed disturbance in chromosomes sizes (Table 3). Chromosome 1 is the largest chromosome of the house mouse with 24 band and is relatively easy to recognize on the basis of the characters as follows: Regions C and H of chromosome 1 are characterized by clusters of almost equal size bands and similar colures, region E containing one gray band, regions B and D are light and region G is Dark. Chromosome X is similar to chromosome 2 in size and has 16 band with two dark bands (C and E) and one light band between them (Kazumi *et al.*, 2003)

DISCUSSION

In perspective of karyology, *Mus musculus* is usually stable with little or no variation in diploid number and chromosomal morphology. Standard karyotype of the house mouse is $2n=40$, $NF=40$ and $NFa = 38$ (Baydemir and Karoz, 2014; Silver, 2001; Mirabzadeh, 2001). Karyotype formulas and quantitative analyses have a great uniformity among populations of this species with exception populations of *Mus musculus domesticus* (Cazaux, 2014). This species formed a homogeneous group and mainly differed in the length of chromosome Y (Levan, 1962; Nesbitt and Francke, 1973). However, the G-banded chromosomes mentioned that many of the major bands contained minor bands and homologous chromosomes were paired according to this banding pattern (Nesbitt and Francke, 1973). **FIGURE 3.** Phenotype, Karyotype and Homologous banded chromosomes of the Iranian house mouse (A) Birjand (*Mus musculus bacterianus.*), (B) Mashhad (*Mus musculus musculus*), (C) Zabol (*Mus musculus bacterianus*) and (D) Kerman (*Mus musculus castaneus*).

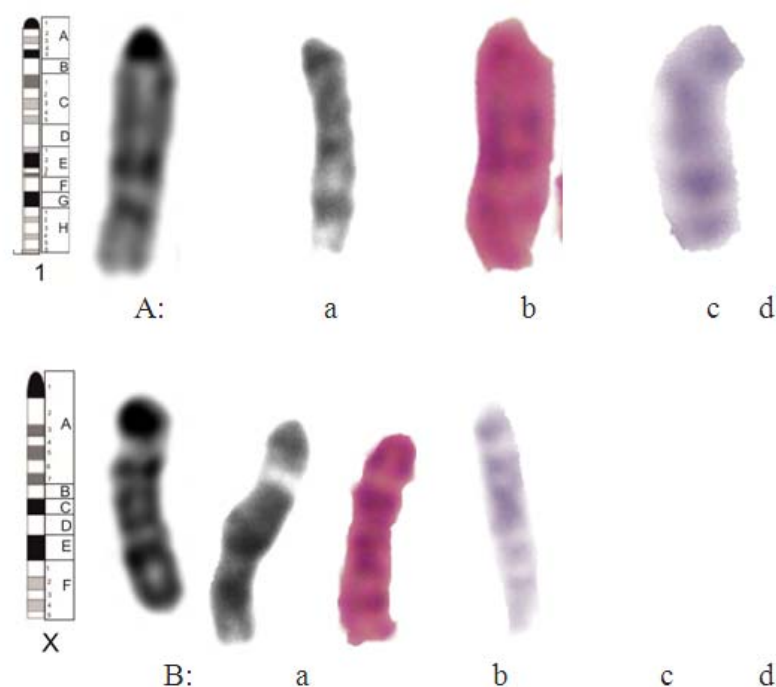


FIGURE 4. (A) G-banded chromosome 1 of the Iranian house mouse with comparative cytogenetic map between the specimens from a. Mashhad (*Mus musculus musculus*), b. Zabol (*Mus musculus bactrianus*), c. Kerman (*Mus musculus castaneus*) and d. Birjand (*Mus musculus bactrianus*). (B) G-banded chromosome X of the Iranian house mouse with comparative cytogenetic map between the specimens from a. Mashhad (*Mus musculus musculus*), b. Zabol (*Mus musculus bactrianus*), c. Kerman (*Mus musculus castaneus*) and d. Birjand (*Mus musculus bactrianus*).

1973; Cowell, 1984; Veyrunes *et al.*, 2006). The chromosome X is one of the longest chromosomes that could be easily paired (Cowell, 1984; Graves *et al.*, 2002; Levan, 1962; Mirabzadeh, 2001). The chromosome Y was small and constantly dark and the centromeric chromatin was not obvious (Cowell, 1984; Nesbitt and Francke, 1973; Sawyer *et al.*, 1987; Mirabzadeh, 2001). According to our results, the chromosome numbers of the Iranian house mouse are the same with previous studies (Mirabzadeh, 2001; Silver, 2001).

Although the house mouse is the most widely studied mammal in terms of chromosomal evolution but comparisons studies between the subspecies of the house mouse are very scarce (Britton-Davidian *et al.*, 2000; Capanna and Castiglia, 2004; Piálek *et al.*, 2005). Our study is the first attempt to establish comparative chromosome maps in the Iranian house mouse. On one hand, some authors attributed the eastern populations of the Iranian house mouse to subspecies *M. m. bactrianus* (Boursot *et al.*, 1996; Boissinot and Boursot, 1997). On the other hand, the others used the name *M. m. castaneus* or "*M. (m.) castaneus*" for the populations (Prager *et al.*, 1998; Siahsarvie *et al.*, 2012; Rajabi-Maham *et al.* 2012). The studies emphasized challenging of taxonomic status in the eastern populations of the Iranian house mouse (Gündüz *et al.*, 2000; Darvish, 2004; Darvish *et al.*, 2006; Bonhomme *et al.*, 2007; Darvish *et al.*, 2008; Shabani *et al.*, 2010; Siahsarvie *et al.*, 2012; Rajabi-Maham *et al.* 2012). High heterozygosity of *M. m. castaneus* or "*M. (m.) castaneus*" was observed in various markers like allozymes, nuclear gene sequences (Din *et al.*, 1996; Bonhomme *et al.*, 2007). Rajabi *et al.* (2012) described "polytypic" subspecies for *M. m. castaneus*. The house mouse population from the eastern Iran formed a complex of biogeographic scenario with the presence of

three important mitochondrial clades that are probably results of human activities (Rajabi-Maham *et al.*, 2012). Additionally, *M. musculus* subspecies of Iran has also been documented secondary contacts with each other (Boursot *et al.*, 1996).

Based on our data, differences in karyotype formulae and asymmetry indexes were found among the Iranian subspecies of house mouse and suggested structural changes that could be contributed the diversification of the populations. Infact, species formed groups with common major karyotype characteristics. Hence, if the mechanisms of speciation within each group involved chromosome rearrangements, these might not be included only structural mutations but included small or cryptic changes. Alternatively, if speciation has occurred as a consequence of large chromosomal modifications, these could not be changed karyotype morphology, such as paracentric inversions or reciprocal translocations with segments of the equal size (Guillermoseijo and Fernandez, 2003). The existence of a similar karyotype in some species suggest that chromosomal evolution in this section may be constrained to non-random changes such as occurrence or fixation of structural rearrangements.

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