

## Comparative Cytogenetic Analysis in the Populations of House Mouse Group, *Mus musculus* L.1766 (Cytotype $2n = 40$ ) (Rodentia: Muridae) in Iran

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### Abstract

Cytotaxonomy is a branch of cytogenetics, devoted to the comparative study of karyological features for systematic and evolutionary purposes. Surely, awareness of chromosomal characters increases our knowledge in different fields of studies. In this study, cytogenetic analyses were performed in 92 *Mus musculus* specimens from 26 localities in Iran. Cytogenetic characteristics of the house mouse, *Mus musculus*, in Iran show that the chromosome number is  $2n=40$  and the arm number is  $NF=40$ . The karyotyping results indicated the presence of 20 Acrocentric (A) chromosome pairs. The L/S (r ratio) was between 2.0621 and 4.5862. The length of shortest chromosome, length of longest chromosome and mean of chromosomal length in different populations were between 2-3.58, 6.07-7.01 and 3.43-5.05 ( $\mu\text{m}$ ), respectively. The results showed two distinct karyotypic formulae, namely cytotype B and cytotype C. Asymmetry indexes (AI, DI, As%, A, A2, A1 and Syi%) in all population except Birjand and Khash showed symmetry in chromosomes. In clustering methods using the matrix of symmetrical indexes similarities, four clusters were revealed, one for specimens of central and east of Iran, the second cluster for specimens from south and west of Iran, the third cluster was related to the eight specimens of Birjand and finally, the fourth cluster for two specimens of Khash locality.

**Keywords:** Cytotaxonomy, Systematic, Chromosome structure, House mouse, Karyology, Iran, Middle East

### Introduction

Now a day, nine species have been recognized in the genus *Mus*. This taxon arose within the last 4 Myr (Bonhomme and Guénet, 1996). *Mus musculus* was originally a Palearctic species, but now it has been spread throughout the world by humans and lives as a human commensal (Musser and Carleton, 2005). Genetic studies have revealed three peripheral geographic populations of house mouse as *Mus musculus musculus*, *M. m. domesticus* and *M. m. castaneus* (Vanlerberghe et al., 1986; Orth et al., 1996; Darvish et al., 2006; Rajabi-Maham et al., 2007). Cytotaxonomy is a branch of cytogenetics, devoted to the comparative study of karyological features for systematic and evolutionary purposes (Siljak-Yakovlev and Peruzzi, 2012). Today, a number of data can be obtained by chromosome studies including chromosome number, karyotype structure, karyotype asymmetry, chromosome banding, FISH (Fluorescence in situ hybridization), Genomic In

Situ Hybridization (GISH) and chromosome painting (Graphodatsky et al., 2011; Cazaux et al., 2012). Among karyotype asymmetry is, one of the most popular, cheap and widely approaches which is used for determining of karyotype asymmetry (Peruzzi and Eroğlu, 2013). The concept of karyotype asymmetry, i.e. a karyotype marked by the predominance of chromosomes with terminal/subterminal centromeres (intrachromosomal asymmetry) and highly heterogeneous chromosome sizes (interchromosomal asymmetry), was developed for the first time by Levitsky (1931). Stebbins in 1971 proposed a quali-quantitative method for the estimation of karyotype asymmetry in twelve categories, by taking into account four classes (from 1 to 4), defined according to the increasing proportion of chromosomes with arm ratio  $< 2:1$ , to be combined with three classes (from A to C) defined according to the increasing ratio between

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largest and smallest chromosomes in a complement. Concerning interchromosomal asymmetry, which is due to heterogeneity among chromosome sizes in a complement, other researchers proposed quantitative estimation methods in the subsequent 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, actually a coefficient of variation, is a statistically correct parameter and is able to capture even small variations among chromosome sizes in a complement (Peruzzi and Eroğlu, 2013). Cytological investigations have shown that the basic chromosome number is constant among the *Mus* species. Despite this stability in chromosome number, large variations in chromosome size have played a major role in the evolution of some species (Guillermoseijo and Fernandez, 2003; Peruzzi and Eroğlu, 2013). Many molecular studies have been performed on house mouse (Rajabi-Mahan *et al.*, 2007; Darvish *et al.*, 2006), but there is a paucity of data for chromosomal morphological characters. From the available information, we do not have any claim of morphological uniformity of chromosomes and homogeneous karyotype arrangements. From a karyosystematic point of view, until now, no group has been classified according to their karyotype morphology. Thus, in this study the karyotypes of specimens captured from 26 localities are analyzed with the following objectives: (1) to clarify the morphology of chromosomes in house mouse of Iran, (2) to examine the patterns of chromosome variations in populations of this taxon.

## Materials and Methods

During field excursions in 26 stations of Iran, 92 rodent species were captured (Fig. 1 and Table 1). Mice were caught using Longworth live-traps in farm buildings. Four morphometric characters including the length of body, tail, ear and hind foot were measured and the animals were karyotyped according to the conventional bone marrow method. The zygomatic index ( $ZI = \text{width of molar process} / \text{width of upper part of zygomatic arch}$ ) and the ratio between tail length to head and body length ( $\text{tail length} / \text{head body length} = T / HB$ ) were determined to identify *Mus* species. Voucher specimens were skinned and stuffed in the standard museum manner. The skin, skulls and karyotype preparations were deposited to Rodentology Research Department, Ferdowsi University of Mashhad.

Mitotic chromosome preparations were made with a modification of the technique described by Summer (1972). To do so, 1 ml of colchicine solution (0.25 mg/ml) per hundred grams of body weight was injected intraperitoneally to each specimen. One hour after colchicine injection, animals were anesthetized with Ether. Bone marrow was separately transferred to a small watch glass containing 8-10 ml hypotonic solution (0.085 M KCl) at 37°C, lasting time for hypotonization treatment was 20-25 min.

## Fixation

The swollen cell suspensions were fixed in 3:1 cooled Carnoy's fixator (3:1 methanol/acetic acid glacial) for 20 min, then the old fixative was replaced with the fresh solution and repeated for three times.

## Slide preparation

The slides were prepared by dropping two drops of the fixing solution containing the cell suspension onto the clean slides from 60 cm height. The slides were stained in a 5% Giemsa (Merck) for ten min. At least 10 metaphases were analyzed for each population (including 2-8 individuals) using a ax100 zoom digital CCD camera, selecting the five best for measurements. Fourteen karyological characteristics (Table 2) of all specimens were prepared by Karyological Analysis software (version 1.2, 2010) and CIP Software. The relative length of each pair was expressed by the percentage of the absolute length of each chromosome pair divided by the sum of the absolute length of total chromosomes.

The chromosome pairs were classified according to Levan *et al.* (1964). The pair numbers were definitely attributed following this classification and in decreasing length order within each class. Means were compared by one-way ANOVA after Bartlett's test of homogeneity. Also, Tukey's test was carried out to measure differences between each pair of means. A cluster analysis of the karyotypic data was carried out to examine karyotype similarities among populations. A data matrix of 26 OTUs (operational taxonomic units) including 14 variables was constructed. Statistical analysis was performed using the SPSS program, version 16.0 (2011). Clustering was performed using the unweighted pair-group method (UPGMA).



Figure 1. Collecting localities for *M. musculus* analyzed in this study (black boxes are stations)

Table 1. The coordinates of sampling localities

	Taxon	Localities	Latitude	Longitude
1	<i>M. m. musculus</i>	Kakhk	34° 8' 59" N	58° 38' 21" E
2	<i>M. m. musculus</i>	Gonabad	34° 21' 10" N	58° 41' 1" E
3	<i>M. m. musculus</i>	Torbate Jam	35° 14' 38" N	60° 37' 21" E
4	<i>M. m. musculus</i>	Sarakhs	36° 32' 42" N	61° 9' 28" E
5	<i>M. m. musculus</i>	Dargaz	37° 26' 40" N	59° 6' 29" E
6	<i>M. m. musculus</i>	Kalat	36° 59' 33.01" N	56° 45' 23.83" E
7	<i>M. m. castaneus</i>	Isfahan	32° 63' 35" N	51° 65' 36" E
8	<i>M. m. castaneus</i>	Zahedan	29° 29' 47" N	60° 51' 46" E
9	<i>M. m. castaneus</i>	Khash	28° 13' 16" N	61° 12' 57" E
10	<i>M. m. castaneus</i>	Zabol	31° 1' 43" N	61° 49' 4" E
11	<i>M.m. domesticus</i>	Chabahar	25° 17' 31" N	60° 64' 35" E
12	<i>M. m. castaneus</i>	Shiraz	29° 61' 0" N	52° 54' 0" E
13	<i>M. m. castaneus</i>	Yazd	31° 53' 50" N	54° 22' 4" E
14	<i>M. m. musculus</i>	Esfarayen	37° 73' 03" N	57° 50' 72" E
15	<i>M. m. domesticus</i>	Eizeh	31° 50' 48" N	49° 50' 36" E
16	<i>M. m. castaneus</i>	Mamasani	30° 7' 0" N	51° 31' 0" E
17	<i>M.m. domesticus</i>	Uromia	37° 33' 19" N	45° 4' 21" E
18	<i>M. m. domesticus</i>	Khalkhal	37° 37' 8" N	48° 31' 33" E
19	<i>M. m. musculus</i>	Mashhad	36° 18' 0" N	59° 36' 0" E
20	<i>M. m. domesticus</i>	Zanzan	36° 40' 0" N	48° 29' 0" E
21	<i>M. m. musculus</i>	Birjand	32° 87' 0" N	59° 20' 0" E
22	<i>M. m. domesticus</i>	Ahvaz	36° 40' 0" N	48° 29' 0" E
23	<i>M. m. castaneus</i>	Qasr-e Qand	26° 14' 54" N	60° 45' 9" E
24	<i>M. m. castaneus</i>	Rask	26° 14' 13" N	61° 23' 56" E
25	<i>M. m. castaneus</i>	Kerman	30° 17' 0" N	57° 5' 0" E
26	<i>M. m. domesticus</i>	Mahshahr	30° 54' 32" N	49° 11' 58" E

**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	$\left( \sum_i^n \frac{q_i}{p_i} \right) / n$	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	$\frac{\sum_{i=1}^n \frac{p_i - q_i}{p_i + q_i}}{n}$	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	Lavanaia and Srivastava, 1992	$\sigma^2$ : variance $\mu$ ,: mean
9	AI	The asymmetry index	$(\mu_x - \mu_y V) / (\sigma_x^2 + \sigma_y^2 V^2)^{1/2}$	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.	Paszko, 2006	$V = (R - L) / (R + L) \equiv X / Y$ ; X:= R - L and Y : R + L $\mu_x$ : Means X $\mu_y$ : Means Y $\sigma_x^2$ : variances

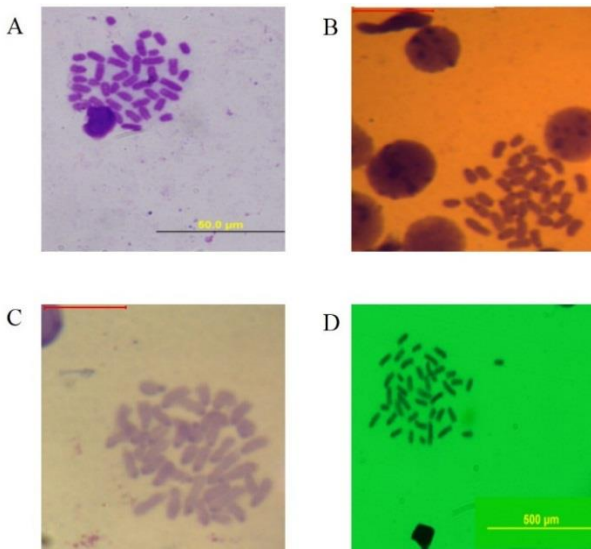
				x > 4.0: The asymmetry is strong. The distribution is asymmetrical.		
10	Cytotype	An individual of a species that has a different chromosomal factor to another (e.g. haploid versus diploid)	-	-	-	-
11	L/S	arm ratio(r)	long arm/short arm	-	-	-
12	As%	The karyotype asymmetry index	(Length of long arm in chromosome complements/Total sum of chromosome length in a set)x100	50-100	Arano, 1963	-
9	TF%	The total form percent	Total of short chromosomal lengths/ Total of chromosomal lengths	0-50	Huziwara, 1962	-
10	Syi%	The index of karyotype symmetry	$\frac{Ms}{Ml}$	-	Greilhuber and Speta, 1976, Venora et al. 2002	Ms:Mean length of the shortarms Ml:Mean length of long arms
11	Rec	The index of chromosomal size resemblance	$= ((\sum_{i=1}^n CLi/LC)/n) * 100$ 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
12	SC	Length of shortest Chromosome	micron	-	-	-
13	LC	Length of longest Chromosome	micron	-	-	-
14	Mpq	Mean of chromosomal Length	micron	-	-	-

**Result:**

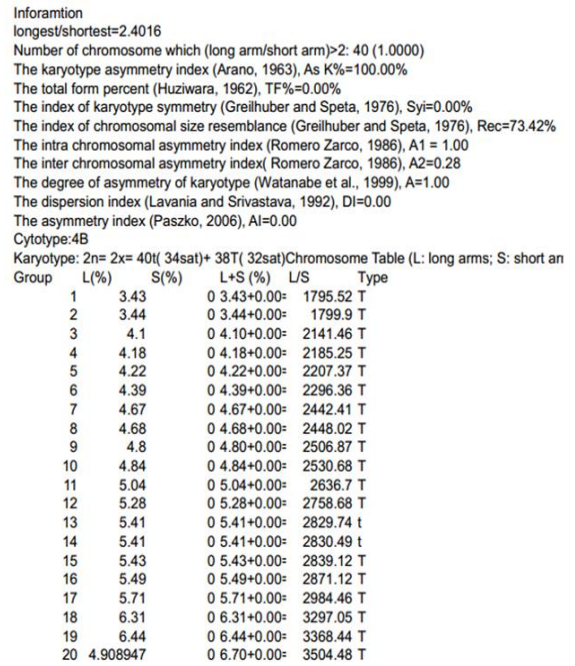
Analyzing chromosome numbers of all specimens from 26 localities showed that they all had 40 chromosomes (2n=40). This result was observed for over 90% of cells in metaphase. Observation of cells lacking a normal number of chromosome (2n=36-38) was probably due to chromosome

losses during preparation or mixing with nearby cells. All chromosomes in prepared karyotypes had a homologous pair. Homologous pairs of chromosomes were arranged according to size decrease and centromeric indexes. The Y chromosomes could be distinguished by small size

and dark color. The representative karyotypes for *M. musculus* captured from different localities are shown in Fig 2.



**Figure 2.** Chromosome spreads of *Mus musculus*, all specimens with  $2n=40$ . Animals were captured from A: Mashhad, B: Chabahar, C: Uromia and D: Mahshahr



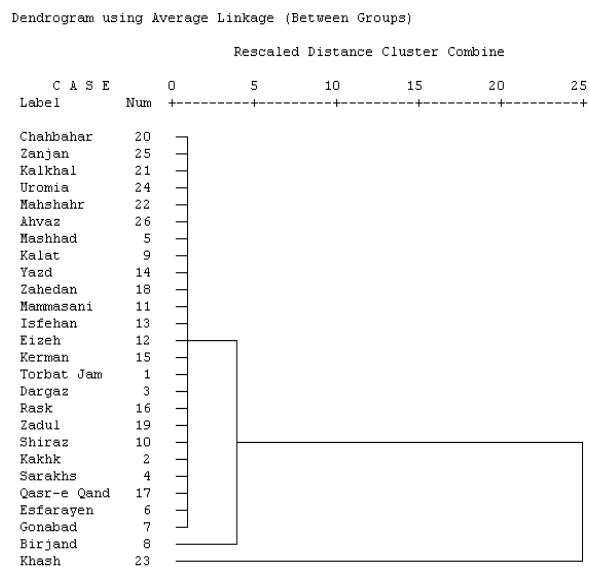
**Figure 3.** An example of the output in karyotype analysis software, this output belongs to a specimen from Torbat-Jam.

Fourty acrocentric chromosome pairs were observed in all preparations. The number of chromosome arms was determined as  $NF=40$ . All chromosomal criteria were calculated for each specimen in karyotype analysis software separately (Fig. 3). All results are summarized in Table 3.

The L/S index was between 2.0621 to 4.5862. The length of shortest chromosome, length of longest chromosome and mean of chromosomal length in different populations were between 2-3.58 , 6.07-7.01 and 3.43- 5.05 ( $\mu m$ ), respectively The UPGMA dendrogram was constructed on the basis of seven symmetrical indexes (Fig. 4).

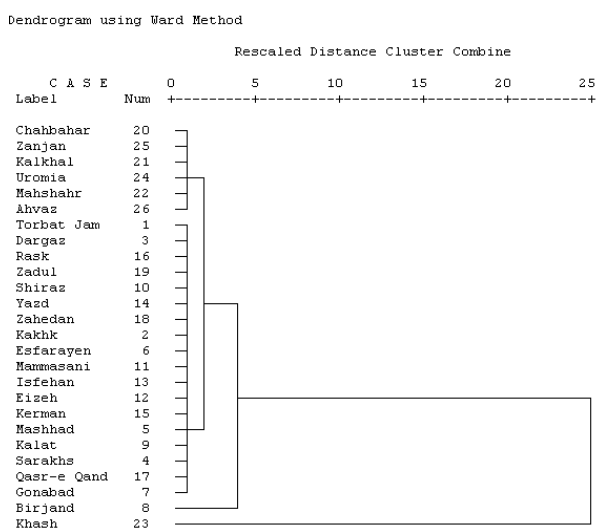
**Table 3.** Comparison of karyological records of the given house mouse populations.

	Localities	2n	Fn	Fna	L/S	AsK%	TF%	Sy <sub>i</sub> %	Rec	A1	A2	A	DI	AI	Cytotype	SC	LC	Mpq
1	Torbat Jam	40	38	38	2.8071	100	0	0	64.76	1.00	0.00	0.26	0	0.00	4B	3.58	6.42	3.96
2	Kakhk	40	38	38	2.4739	100	0	0	65.01	1.00	0.00	0.27	0	0.00	4B	3.48	6.41	4
3	Dargaz	40	38	38	2.9631	100	0	0	65.75	1.00	0.00	0.27	0	0.00	4B	3.58	6.42	4.09
4	Sarakhs	40	38	38	3.0152	100	0	0	64.69	1.00	0.00	0.28	0	0.00	4B	3.31	6.91	4.03
5	Mashhad	40	38	38	2.5987	100	0	0	64.98	1.00	0.00	0.22	0	0.00	4B	3.21	6.81	4.28
6	Esfarayan	40	38	38	2.4021	100	0	0	73.42	1.00	0.00	0.28	0	0.00	4B	3.43	6.07	3.43
7	Gonabad	40	38	38	3.6131	100	0	0	60.68	1.00	0.00	0.29	0	0.00	4B	3.11	7.01	4.23
8	Birjand	40	38	38	3.6289	97.38	2.62	2.69	47.65	0.97	0.29	0.96	0	0.77	3C	3.02	6.88	4.767
9	Kalat	40	38	38	2.7846	100	0	0	64	1.00	0.00	0.28	0	0.00	4B	3.19	6.71	4
10	Shiraz	40	38	38	2.7475	100	0	0	65.27	1.00	0.00	0.23	0	0.00	4B	3.54	6.62	4.98
11	Mammasani	40	38	38	2.7475	100	0	0	65.27	1.00	0.00	0.22	0	0.00	4B	3.14	6.54	4.9
12	Eizeh	40	38	38	2.6378	100	0	0	61.2	1.00	0.00	22.00	0	0.00	4B	3.15	6.59	4.95
13	Isfehan	40	38	38	2.6998	100	0	0	66.11	1.00	0.00	0.27	0	0.00	4B	3.21	6.46	4.97
14	Yazd	40	38	38	2.6521	100	0	0	68.21	1.00	0.00	0.22	0	0.00	4B	3.34	6.37	5.01
15	Kerman	40	38	38	2.6435	100	0	0	66.31	1.00	0.00	0.24	0	0.00	4B	3.29	6.57	4.99
16	Rask	40	38	38	2.6415	100	0	0	68.87	1.00	0.21	0.21	0	0.00	4B	3.57	6.55	5
17	Qasr-e Qand	40	38	38	3.1735	100	0	0	61.68	1.00	0.00	0.26	0	0.00	4B	3.13	6.37	4.92
18	Zahedan	40	38	38	2.6875	100	0	0	62.05	1.00	0.00	0.25	0	0.00	4B	3.34	6.43	4.96
19	Zadul	40	38	38	2.6576	100	0	0	65.43	1.00	0.00	0.25	0	0.00	4B	3.45	6.54	4.88
20	Chahbahar	40	38	38	2.2314	100	0	0	65.48	1.00	0.00	1.00	0	0.00	4B	2.67	6.07	4.47
21	Kalkhal	40	38	38	2.1754	100	0	0	64.87	1.00	0.00	1.00	0	0.00	4B	2.69	6.1	4.38
22	Mahshahr	40	38	38	2.0921	100	0	0	63.26	1.00	0.00	1.00	0	0.00	4B	2.03	6.08	4.59
23	Khash	40	38	38	4.5862	90.95	9.05	0.95	42.32	0.91	0.35	0.87	0	4.21	3C	2.98	6.12	4.46
24	Uromia	40	38	38	2.1756	100	0	0	68.07	1.00	0.00	0.27	0	0.00	3C	2.45	6.4	5.05
25	Zanjan	40	38	38	2.2414	100	0	0	65.43	1.00	0.00	1.00	0	0.00	4B	2.69	6.05	4.45
26	Ahvaz	40	38	38	2.0621	100	0	0	61.26	1.00	0.00	1.00	0	0.00	4B	2	6.06	4.53



**Figure 4.** Dendrogram showing the phonetic relationship among the studied localities of *Mus musculus*, constructed using the matrix of symmetrical indexes similarities with UPGMA.

In general, karyotypes were symmetric, except eight specimens from Birjand and two specimens from Khash. The UPGMA dendrogram constructed on the basis of karyotype similarities (Fig. 5) showed four major clusters. The first cluster is comprised of specimens captured from south and west of Iran, characterized by the smallest size of their chromosomes.



**Figure 5.** Dendrogram showing the phonetic relationships among the populations of *Mus musculus* captured from different localities, constructed using the matrix of karyotype similarities with UPGMA

All specimens from central and eastern Iranian house mouse are separated by the greater phenetic distance and they are placed in second cluster.

The third and fourth clusters contain eight samples from Birjand and two samples from Khash, respectively, which are characterized by asymmetry in their symmetry indexes and their different cytotype. Sizes of chromosomes in Birjand specimens are more similar to eastern and central specimens and sizes of chromosomes in specimens from Khash are more similar to western and southern specimens of Iran.

In summary, on the basis of the morphology of their chromosome complement and quantitative parameters, two main groups of karyotypes can be distinguished in this study: one of them is related to populations living in south and west of Iran and the other is related to mice captured from the east and central parts of Iran.

### Discussion

*Mus musculus* is usually stable from karyotypic point of view, 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). However, variations in the chromosome numbers have been reported in *Mus musculus domesticus*, for example, in Chile (Berríos, 2010), Turkey (G.zcelioŪlu *et al.*, 2005; Gündüz 2000 and Yigi, 2006) and Thailand (Badenhors, 2009). Karyotype formula and quantitative analyses have a great uniformity among populations of this species, except populations of *Mus musculus domesticus*. These studies support the hypothesis that claims intraspecific stability of karyotypes in house mouse subspecies (Baydemir and Karoz, 2014).

Our results indicate that the chromosome numbers of species captured from Iran are the same with those published previously (Mirabzadeh, 2001; Silver, 2001). Although the diploid chromosome number is considered as  $2n=40$  in this study, several incomplete metaphases were also encountered in these preparations. The differences observed in the number and types of chromosomes in different studies may have various reasons. For example, differences in kind of techniques may lead to differences in the type, number of chromosome arms and even number of chromosomes. High concentrations or long treatment period of colchicine could also be the cause of conflict as concentrations or treatment periods of non-compliance can lead to a shortening of chromosomes and therefore the difference between the measurements and the arms of the chromosomes. According to Matthey (1954), Nadler and Lay (1967) and Vorontsov and

Korobitsina (1970), some species in this genus have different FN (Fundamental number). Our findings are not different for the FN or Fna (Autosomal fundamental number) of Iranian house mouse, and do not support the idea that there is a chromosomal variation in the FN of this species. Ruty, 1772, Orsini *et al.* (1983), Auffray *et al.* (1990) and Cucchi *et al.*, (2005) reported the same conclusion for the *Mus* species in Turkey.

Analysis of karyotypes showed that in general, the chromosomes were acrocentric (Nanda *et al.*, 1995; Manna, 1974) and of similar size (Goleman, 1996; Padilla-Nash, 2006) in all mice. They formed a homogeneous group and differed mainly in the length of the Y chromosome (Levan, 1962; Nesbitt and Francke, 1973). The X chromosome, which is one of the longest chromosomes could be easily detected (Cowell, 1984; Levan, 1962; Mirabzadeh, 2001). The Y chromosome was dark and the centromeric chromatin was not obvious (Cowell, 1984).

**Chromosomes and evolution**— Differences in karyotype formulae and asymmetry indexes found among species of different locations suggest that structural changes may have contributed to the diversification of the genus. On the other hand, the fact that species formed groups that share major karyotype characteristics may indicate that if the mechanisms of speciation within each group involved chromosome rearrangements, these may not include structural mutations, but small or cryptic changes. Alternatively, if speciation has occurred as a consequence of large chromosomal modifications, these may have been changes that did not modify the 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 suggests that chromosome evolution in this section may be constrained to non-random changes with particular restrictions for the occurrence or fixation of structural rearrangements. The stability of complements among a group of species was first explained by ortho selection, which considers the occurrence of random chromosome mutations, but with the fixation of a restricted type of rearrangement (White, 1978). An alternative hypothesis was offered by King (1993), who considered the non-random nature of chromosomal evolution. This model contemplates that structural characteristics of the genome restricts the position and number of breaks that could occur and the type of rearrangements that could form. Even though

both mechanisms would have similar results, a bulk of molecular and chromosome data is accumulating in favour of the position that claims that chromosomal mutations are not only non-random but are constrained by the chromosome structure to the type of change that can be produced (Peters, 1982; Shaw *et al.*, 1983; King, 1993; Narayan, 1988). Guillermoseijo and Fernandez (2003) showed that when the size of chromosomes varies without significant changes in karyotype formula, those changes in genome size may have been non-random and that the variations in DNA amounts are equally distributed among all chromosomes of the complements. Moreover, Seijo (2002) showed that the data obtained from banding patterns also support the non-randomness of genomic changes in some species because bands with similar base composition tend to have equilocal disposition in the karyotypes.

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