



Both Environment and Genetic Makeup Influence Sexual Behavior of House Mouse

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Abstract

We examined changes in behavioral elements of *Mus musculus musculus* and *Mus musculus domesticus* while encountering with conspecific, allospecific animals. To do this, pure (*senso lato*) *M. m. musculus* and pure (*senso lato*) *M. m. domesticus* were obtained from the north east and the west of Iran, respectively, and mating experiments were conducted for both sub. Our study of the behavioral elements of opposite sex as the mate preference factors, within and between the two subspecies, showed that females represent more specific behavior than males in all the test groups. Also both females and males showed non-social behavior during inter-subspecies opposite sex encounters, but the aggressive behavior was just observed more when females encountered the other subspecies males. Although further studies on recognition signals (soiled bedding and urine) as a subspecies recognition system will be necessary to show inter-subspecific behavioral differences that may shape pre-mating isolation between subspecies in Iran.

Keywords Behavioral elements · House mouse subspecies · Iran · Mate preference

1 Introduction

Rodents, including mice, commonly create social structures in natural conditions. They often form a community of individuals where each individual is in a series with a specific social status (Hurst 1990; Singleton 1983). Discernment of different levels of kinship and communication status between individuals has played an important role in different taxa. Pre-mating isolation among male and female conspecific recognition systems is more considerable (Laukaitis et al. 1997; Singleton 1983). The choice of potential mates allows the most protected way to pass on genes to the next generation. Species- or subspecies-specific recognition systems are among the most important ones for sexual selection (Christophe and Baudoin 1998; Talley et al. 2001). This is because offspring of inter-species mating may have low fitness and do not make a

positive contribution to the next generations. Behavioral components being the most important interaction systems in pre-mating and pre-zygotic isolating mechanisms have separated different taxa which are related to sexual preferences and have protected species genetic integration (Laukaitis et al. 1997; Piálek et al. 2008). Complete reproductive isolation may not occur between newly forming populations, since their divergence time is not long enough. In essence, populations can exchange genes and produce hybrids in a narrow region known as a hybrid zone (Barton and Hewitt 1985; Boursot et al. 1993).

The three allopatric *Mus Musculus* subspecies (*Mus musculus domesticus*, *Mus musculus musculus* and *Mus musculus castaneus*) spread after their origin and nowadays they form three parapatric subspecies in some areas all over the world (Boursot et al. 1996; Rajabi-Maham et al. 2012; Siahsharvie et al. 2012) A hybrid zone between *M. m. domesticus* and *M. m. musculus* has been observed across central Europe in their secondary contact (Boursot et al. 1993).

Mate preference, as a sexual selection factor for separating different taxa, was seen in behavioral studies of the European hybrid zone between the two subspecies (Smadja and Ganem 2002; Talley et al. 2001). In addition, genetic

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studies on the two subspecies' hybrids have shown asymmetric genetic incompatibility between the two subspecies, both in Europe and Iran, such that male hybrids of *M. m. musculus* maternal origin were sterile because of the maternal sex chromosome introgression (Hashemian et al. 2017).

Studies on laboratory and wild strain encounters, based upon sex and ontogeny, described 23 behavioral elements for mice in simplified laboratory conditions (Grant and Mackintosh 1963; Mackintosh 1981; Terranova et al. 1993). Iran has unique geographical conditions and has provided a pathway for subspecies global distribution and has also been a shared habitat for all the three subspecies. Hybrid zones between *M. m. domesticus* and *M. m. musculus* have also been found in Iran (Siahsarvie et al. 2012). So, it seems to be important to study the divergence of the two subspecies in Iran.

In the present study, we evaluate the behavioral elements of the two *Mus musculus* subspecies in opposite sex encounters. Our aim was to examine the differences between the two subspecies' behavioral elements in the subspecies of their own and inter-subspecies opposite sex encountering. We used the two subspecies reciprocal encounters of the opposite sex to assess the subspecies and examine the differences of gender behavioral elements that could be effective in pre-mating isolations.

2 Materials and Methods

2.1 Mice

Mice were live trapped from Northern Khorasan Province in North Eastern Iran to obtain the pure subspecies of *sensu lato M. m. musculus* and in the Ilam Province of Western Iran to obtain the pure subspecies of *sensu lato M. m. domesticus* (Darvish et al. 2006; Rajabi-Maham et al. 2008). The third generation strains of the two subspecies were used.

Different subspecies were separated from the beginning of the project and housed based upon gender differences in 33 × 13 × 14 cm cages with clean bedding sawdust and metal top in an air-conditioned room. Food and water were available ad libitum, under a constant photo-period (12:12 h light/dark cycle). After 10 days of holding one male and one female (from the same subspecies) together for mating to produce the next generations, mice were weaned after 21 days of birth. Males and females of the two subspecies were separated 40 days before experiments were started.

2.2 Procedure

Behavioral elements were tested in opposite sex encounters in four experimental conditions groups:

- | | |
|---------------------|---|
| Group 1
(DD): | a female <i>M. m. domesticus</i> encountered with a male from <i>M. m. domesticus</i> (♀ <i>M. m. domesticus</i> × ♂ <i>M. m. domesticus</i>); |
| Group 2
(MM): | a female <i>M. m. musculus</i> encountered with a <i>M. m. musculus</i> male (♀ <i>M. m. musculus</i> × ♂ <i>M. m. musculus</i>); |
| Group 3
(Dom-M): | a female <i>M. m. domesticus</i> encountered with a male <i>M. m. musculus</i> (♀ <i>M. m. domesticus</i> × ♂ <i>M. m. musculus</i>); |
| Group 4
(Mus-D): | a female <i>M. m. musculus</i> encountered with a male <i>M. m. domesticus</i> (♀ <i>M. m. musculus</i> × ♂ <i>M. m. domesticus</i>). |

A total of five replicate encountering courses were performed for each group. Each course was conducted using experimentally naïve animals; mice from the same age underwent a 30-min encounter of the opposite sex in a room with air conditioning. One hour before the beginning of the observations, mice were transferred to the experimental room. A test cage was supplied with sawdust and covered with wire mesh top. The duration and a repeat of each behavioral elements in time were measured at 30-min before the beginning of mates encountering. At the end of every experiment, the next one was supplied with a cleaned cage with fresh bedding and sawdust, after 5 min intervals. Behavior was videotaped using a Panasonic AG-6200 and a Hitachi VT-L30E apparatus equipped with AVC-1450CE video cameras for red lights.

Eighteen behavioral elements in females were recorded in four groups (Table 1). The observed behavioral elements, classified with the categories and subcategories described in Table 1, are based principally upon the ethological profile of mouse behavior described by Grant and Mackintosh (Grant and Mackintosh 1963).

2.3 Data Analysis

Independent *t* tests were used to assess the effects of subspecies type and gender on social–nonsocial behavior ratio. To test the effects of encountering treatments, data were first log-transformed to insure normality and homogeneity of variances. A one-way ANOVA followed by Tukey post hoc test was then used to examine the effects treatment on social–nonsocial (social:nonsocial) behavior ratio of males or females. To test similarities, differences and relationships between groups based upon the behavioral elements, Principal Component Analyze (PCA), was used in software PAST v2 (Hammer et al. 2001). To analyze statically of the

Table 1 Behavioral elements observed in females and males in the four groups and their (sign) index

Behavior elements	Index	Investigate behaviors	Social behaviors
Anogenital sniff	A		
Nose sniff	B		
Body sniff	C		
Follow	D		
Squire	E		
Mutual circle	F		
Social inactive	G	Affiliative behaviors	
Allogroom	H		
Crawl under [#]	I	Soliciting behaviors	
Crawl over [*]	J		
Bite	K	Aggressive bouts	
Aggressive groom	L		
Inactivity	M	Activity and exploration	Nonsocial behaviors
Explore	N		
On-bars	O	Escape-oriented behaviors	
Jump	P		
Dig	Q		
Eat	R	Maintenance activities	
Selfgroom	S		

[#]and ^{*}, respectively, in males and females was not seen in four groups

subspecies difference effects on behavioral performance between groups, we used Doornik–Hansen omnibus in PAST v2 to assess normal distribution of data and PERMDISP (Permutational Dispersion) for the homogeneity of multivariate variance between groups. Because the behavioral data analysis do not follow a normal distribution ($P < 0.05$) and also data variance among groups is heterogeneous ($P < 0.05$); and there is a probability of different behavioral elements correlations with each other, we used One-factor PERMANOVA and Bonferroni test to evaluate presence or absence of difference among all the groups in terms of the individuals behavioral variables. The SIMPER (similarity percentage) (PAST v2) was used to evaluate each behavioral element percentage contribution on intra-groups observed difference.

3 Results

3.1 Females' Behaviors

In females' behavioral study, 18 behavioral elements were observed (Table 1). The effect of treatment on the female's social:nonsocial behavior ratio was significant ($df = 3$, $F_{3,16} = 83.94$, $P = 0.00$). Based on the results of Tukey post hoc tests, highest social–nonsocial behavior ratio was achieved when *M. m. musculus* females encountered conspecific males and the lowest ratio recorded in females of

M. m. domesticus encountering with males of the other subspecies (Fig. 1a). Principal Component Analysis (PCA) showed that the first three principal components (PCs) accounted for ca. 84.11% of the total variance. The experimental groups were moderately separated, except in PC2*PC3 plot (Fig. 2). Results of PERMANOVA indicated significant effects of encountering scenario on females' behavioral ($df = 3$, Pseudo- $F = 15.7$, $P = 0.00$); further pairwise tests showed significant differences among all test groups (Table 2).

SIMPER analyzes revealed three behavioral elements as the weightiest elements to account for between-group differences in females' behavior (Table 2).

3.2 Males' Behaviors

Males' behavioral study also indicated 18 behavioral elements (Table 1). The effect of encountering situation was also significant on social:nonsocial behavior ratio of males ($df = 3$, $F_{3,16} = 59.82$, $P = 0.00$). There was no significant difference between male *M. m. domesticus* and *M. m. musculus* encountering with conspecific females. This was also true for those mice encountering with females of different subspecies (Fig. 1b). PCA analysis showed that the first three components explained 81.09% of the total variance. When the scatter plots were created, a rather weak separation could be detected among males of different experimental groups (Fig. 3). Yet, the males' performance

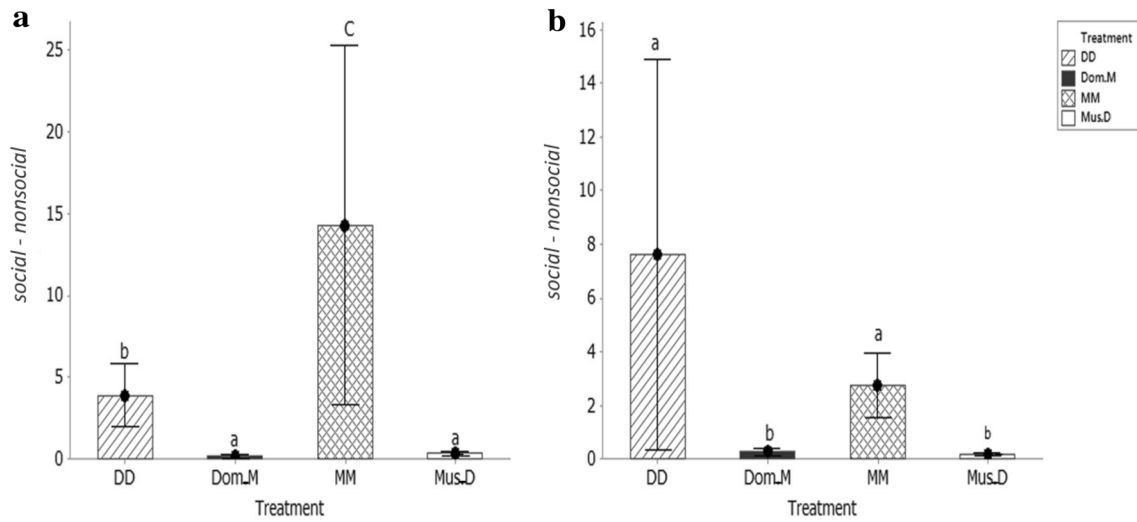


Fig. 1 The effects of treatments on social–nonsocial (social:nonsocial) behavior ratio of **a** females and **b** males. Note: different letters are significantly different

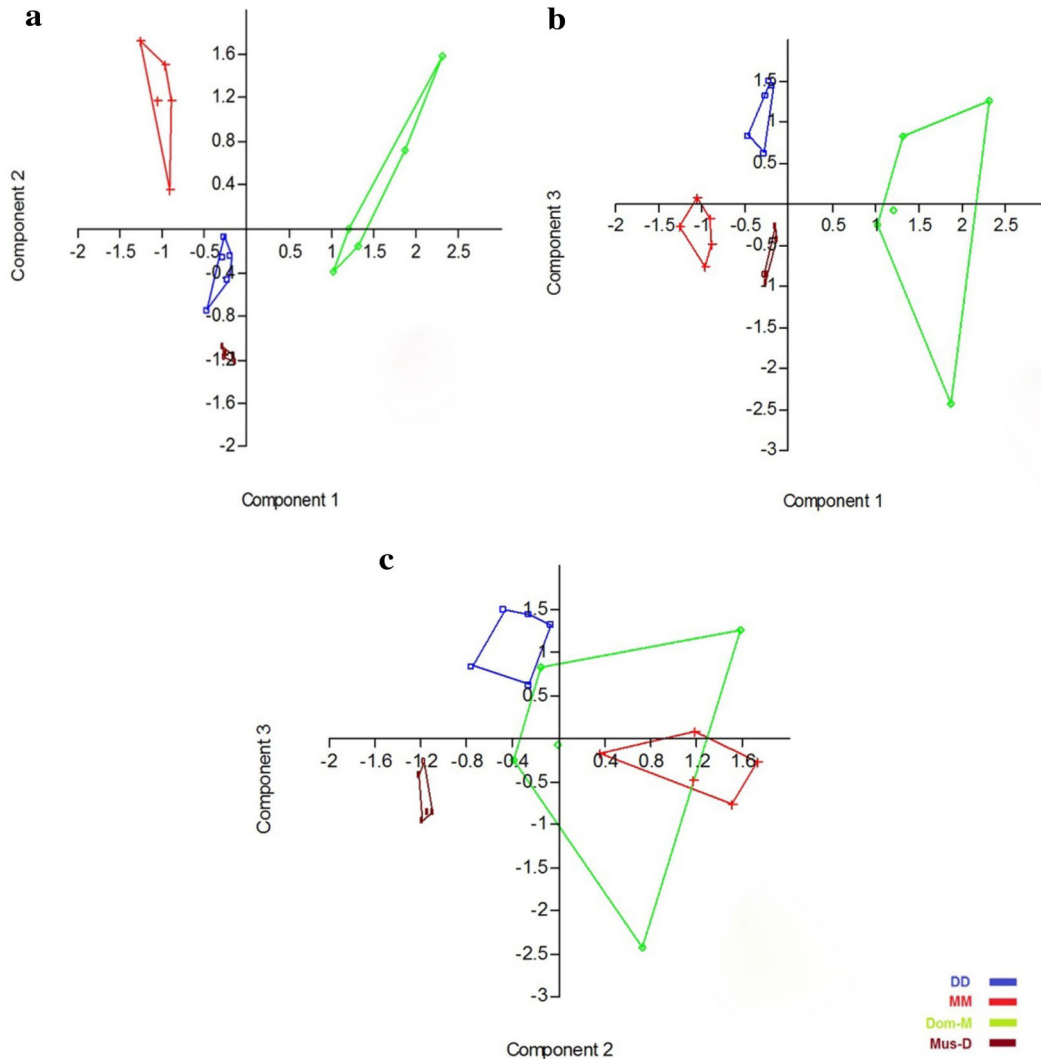


Fig. 2 Female’s scatter plot based on **a** the first and the second; **b** the first and the third; **c** the second and the third components in the PCA test

Table 2 Results of PERMANOVA analysis of female behavior examining significant differences between the all groups, along with contribution of the most responsible behavioral element to significant group pairwise difference of female

G1 (Group1) vs. G2 (Group)	P (PERM)	Most responsible behavioral element	Average element intensity in G1	Average element intensity in G2	Contribution
DD vs. MM	0.0082	D (follow)	2.4	10.6	11.07
DD vs. Dom-M	0.0064	N (explore)	3.8	21.2	23.48
DD vs. Mus-D	0.0065	K (bite)	7.2	0.4	16.83
MM vs. Dom-M	0.0072	N (explore)	1	21.2	22.27
MM vs. Mus-D	0.0073	D (follow)	10.6	0.4	18.21
Dom-M vs. Mus-D	0.0057	N (explore)	21.2	1.2	35.91

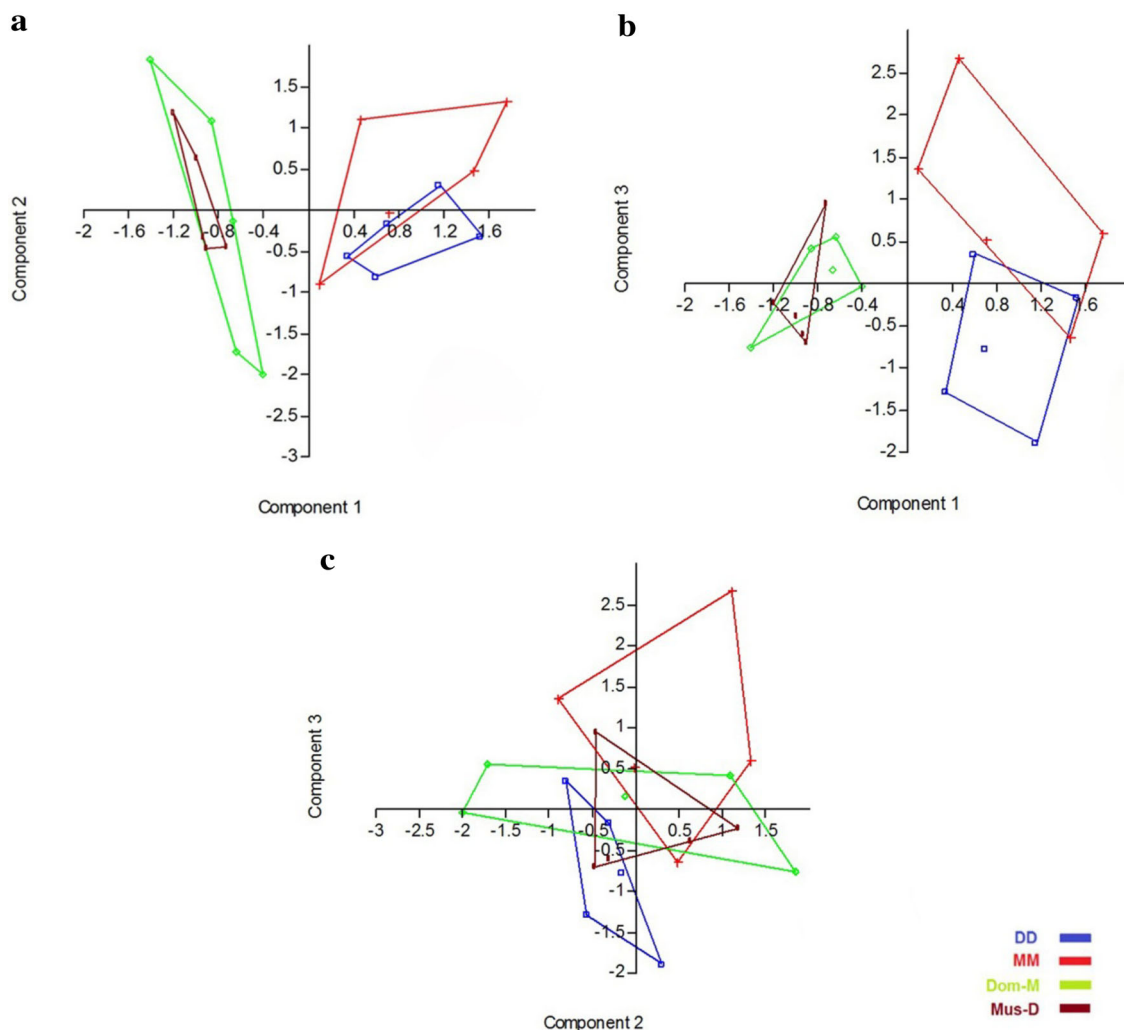


Fig. 3 Male’s scatter plot based on **a** the first and the second; **b** the first and the third; **c** the second and the third components in the PCA test

significantly differed among experimental encountering groups ($df = 3$, Pseudo- $F = 4.76$, $P = 0.00$), in the PERMANOVA model. Pairwise differences were

significant for DD vs Dom-M, DD vs Mus-D, MM vs Dom-M, and MM vs Mus-D trials (Table 3).

Table 3 Results of PERMANOVA analysis of male behavior examining significant differences between DD vs. Dom-M; DD vs. Mus-D; MM vs. Dom-M; MM vs. Mus-D, along with contribution of the most responsible behavioral element of male to significant group pairwise difference

G1 (Group1) vs. G2 (Group)	P (PERM)	Most responsible behavioral element	Average element intensity in G1	Average element intensity in G2	Contribution
DD vs. MM	0.1004	A (anogenital sniff)	8.8	8	7.21
DD vs. Dom-M	0.0064	A (anogenital sniff)	8.8	1.6	13.19
DD vs. Mus-D	0.0065	A (anogenital sniff)	8.8	1.4	14.42
MM vs. Dom-M	0.0072	B (nose sniff)	11.2	1.8	15.19
MM vs. Mus-D	0.0073	B (nose sniff)	11.2	1	17.22
Dom-M vs. Mus-D	0.4824	O (on-bars)	8.6	9.8	17.9

Similarity percentage tests (SIMPER) described three behavioral elements as the most responsible ones for differences among test groups (Table 3).

4 Discussion

Behavioral studies on *M. m. domesticus* and *M. m. musculus* in the European hybrid zone suggest that females of both subspecies have a possible role in subspecies separation and sexual preferences (Laukaitis et al. 1997; Smadja and Ganem 2002; Talley et al. 2001). According to our study, based upon the one-factor PERMANOVA test results, behavioral differences between males and females are approved. Our results show that the behavioral elements of females in all four groups are different from each other, while differences of males in the four groups are only significant between the first two groups (DD, MM) and also in the other two groups (Dom-M, Mus-D) but the latter is more notable. Results suggest that all females in the four groups of *M. m. domesticus* and *M. m. musculus* exhibit specific behavior when males are either in the other subspecies or even in their own. While the behavior of females in each group is different, the behavioral elements of males of *M. m. domesticus* and *M. m. musculus*, are more similar when females are in their own subspecies and another subspecies, therefore it suggests more specific behavior in females when they encounter males. This means the difference between intra-breeding and inter-breeding does not seem to be as important for males as it does for females. Comparison of the SIMPER tests results and PCA analysis for females has indicated that when females of both subspecies encounter males of their own,

the social behavioral elements contribute to the highest participation and that separates female groups from each other. When, both subspecies of females encountered males of another subspecies, the non-social and the aggressive behavioral elements contributed the highest participation. The aforementioned comparison tests and analysis of male behaviors have shown that when males of both subspecies encounter females of their own subspecies, the social behavioral elements are more important in the groups' separation, but in the other subspecies encounters, the non-social behavioral elements are more considerable to separate the groups. Additionally, the female seems to be an important target for studying sexual preference in the house mouse (Coyne and Orr 2004; Smadja and Ganem 2002; Talley et al. 2001). So, we discuss the results of social–non-social behavioral elements analysis for both females' subspecies. Talley et al. (2001), studied *M. m. domesticus* and *M. m. musculus* house mouse subspecies female preferences for males of the both subspecies in a Y-maze. They had used males' salivary androgen-binding protein (ABP) on their pelt, which performs as an act of sexual isolation. Their results indicated that females prefer males of their own subspecies. In general, results of our study support that idea as well, considering the fact that females of the both subspecies presented relatively more social behavioral elements when encountered their own males. According to Smadja and Ganem (2002) who had evaluated females preference for soiled bedding of the both subspecies' males—containing urine, faces, saliva, and other products—in Y-maze apparatus, females of *M. m. musculus* had a preferential signal of their own male while *M. m. domesticus* had shown no particular preference (Smadja and Ganem 2002; Talley et al. 2001).

Our behavioral studies on *M. m. musculus* females are the same when *M. m. musculus* females have display more social behavioral elements when they encounter their own males; but in our results, the *M. m. domesticus* females' ratio of social–nonsocial behavioral elements has been the lowest ratio when they encountered a male of the other subspecies. We suspect that the deprivation of males' bedding (urine, faces, saliva, and other products) in our study was the cause of that. In our experimental encounters, female preference is likely tested based on males' salivary androgen-binding protein (ABP) on their pelt—according to Laukaitis et al. (1997), the ABP plays role as a subspecies specific recognition signal in the both subspecies—as Talley's and their colleagues. Since hybrid male sterility with maternal origin has also been identified in Iran (Hashemian et al. 2017), it seems that more behavioral studies are needed. We especially suggest experimental tests on the subspecies' recognition signals containing bedding of males of *M. m. musculus* and *M. m. domesticus* and assessing the effect on their sexual selection. These suggestions explain the importance of pre-

mating mechanisms which would tend to inhibit genetic incompatibility between the two subspecies.

In conclusion, our descriptive study on the behavioral elements illustrates that females have shown non-social and aggressive behavioral elements when they encounter males of the both subspecies. Males of both subspecies have only shown non-social behavior, and this may be because individuals, especially the individuals of females, have preferred assortative mating.

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Appendix

The percentage of contribution of each female in studied behavioral factors (referable to Table 1) in paired group segregation (described in Sect. 2).

DD × MM	DD × Dom-M	DD × Mus-D	MM × Dom-M	MM × Mus-D	Dom-M × Mus-D						
D	11.07	N	23.48	K	16.83	N	22.27	D	18.21	N	35.91
K	9.17	B	6.94	B	13.01	D	12	B	17.14	S	6.41
B	5.94	K	6.48	N	6.62	B	10.61	A	9.23	O	5.77
A	5.42	S	5.95	D	5.25	S	5.93	H	5.85	K	4.45
N	3.99	O	5.46	S	4.57	A	5.59	C	5.4	Q	3.68
C	3.65	D	3.32	M	3.51	O	4.42	S	4.41	R	3.5
H	3.42	R	3.12	O	3.45	H	3.83	F	4.4	B	1.98
O	3.23	Q	2.98	A	3.16	C	3.57	E	4.2	M	1.67
E	3.16	M	2.1	F	3.1	R	3.17	O	3.79	A	0.97
F	2.74	F	1.71	L	3.03	F	2.92	G	2.76	P	0.83
G	2.36	L	1.69	G	1.99	E	2.91	R	2.54	D	0.78
L	1.53	A	1.64	H	1.99	K	2.82	M	2.52	E	0.4
S	1.5	G	1.12	R	1.96	Q	2.53	N	1.7	G	0
R	1.08	H	1.12	C	1.62	G	1.79	K	1.1	F	0
I	0.22	C	0.88	E	1.09	M	1.72	L	0.63	C	0
P	0	P	0.61	P	0	P	0.49	I	0.27	L	0
M	0	E	0.55	I	0	L	0.42	P	0	I	0
Q	0	I	0	Q	0	I	0.19	Q	0	H	0

The percentage of contribution of each male in studied behavioral factors (referable to Table 1) in paired group segregation (described in Sect. 2).

DD × MM	DD × Dom-M	DD × Mus-D	MM × Dom-M	MM × Mus-D	Dom-M × Mus-D
A	7.21	A	13.19	A	14.42
B	6.81	O	13.04	O	12.09
E	5.46	B	10.04	B	11.81
D	4.73	E	8.16	E	8.57
O	4.17	Q	7.43	D	7.57
N	3.2	N	7.36	M	6.13
F	2.9	D	6.8	N	4.84
S	1.98	M	2.85	F	2.58
H	1.37	F	2.46	R	2.21
J	1.21	P	0.95	S	0.84
R	1.19	S	0.79	Q	0.7
Q	0.96	R	0.67	L	0.61
G	0.92	L	0.59	G	0.46
K	0.82	G	0.44	K	0.31
L	0.77	K	0.29	P	0
C	0.48	C	0	C	0
P	0.23	J	0	J	0
M	0	H	0	H	0
B				B	15.19
A				A	10.49
O				O	7.97
N				N	5.52
M				M	5.3
R				R	5.2
F				F	3.88
S				S	2.46
R				R	2.06
E				E	1.88
H				H	1.62
J				J	1.48
R				R	1.38
Q				Q	1.17
G				G	1.11
K				K	0.89
C				C	0.55
L				L	0.54
P				P	0.27
H				H	0

The first three principal components (PCs) for female and male.

	Eigenvalue	%variation	Cumulative %variation
Female			
PC1	106.77	56.65	56.65
PC2	37.52	19.91	76.56
PC3	14.21	7.54	84.11
Male			
PC1	50.38	46.67	46.67
PC2	24.45	22.65	69.32
PC3	12.71	11.77	81.09

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