Determination of *Meriones* Species (Rodentia, Gerbillinae) by RAPD-PCR

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Meriones crassus, *Meriones persicus*, and *Meriones libycus* from different areas of Khorasan provinces and Yazd province were studied by use of Random Amplified DNA polymorphism (RAPD) technique. DNA samples were analyzed by use of nine primers. Genetic distance among samples varied between 0.24 and 0.78. Dendrogram designed on the base of genetic distance shows separation of these species. In this research, RAPD was used for analyzing the genetic variations among these species.

Key words: *Meriones crassus, Meriones persicus, Meriones libycus*, RAPD-PCR, genetic variation, Khorasn, Yazd.

INTRODUCTION

Genus *Meriones* (Illiger, 1811) belongs to Gerbillinae subfamily. Their distribution range is from North Africa to China and they have adapted with different environments. Jirds are distinguished from other Gerbillinae genus by having incisors with longitudinal groove and big auditory capsule. This genus consists of 17 currently recognized species which eight of them are distributed in Iran (Musser and Carleton, 2005). Four of them are found in Northern, Razavi and Southern Khorasn provinces which are *M. libycus*, *M. persicus*, *M. Crassus*, and *M. meridianus* (Etemad, 1977).

According to Corbet (1978), Harington (1976), Etemad (1978), Ziaei (1978), Lay (1967), Robert (1997), Darvish and Khosravi (1999) and Wilson and Reeder (2005), these species are classified through distinct characters and can be recognized correctly.

  In spite of the presence of distinct characters for classification and distinguishing of these species, they are not constant and some times are ambiguous. In this study we captured three specimens from Tabas; Yazd province which was different from other *Meriones* species in some traits, so we could not classified them in these four groups by use of morphological characters. Because of many morphological similarities between these species, uncertainty of some character states and intraspecific variations, the aim of this study is to know that wich species they belong to; therefore, it is essential to know more about the existing differences between species, genetic similarities such as relationships, and molecular markers. For this reason the molecular analysis methods have been chosen (Darvish, 2003). Methods which are based on using molecular markers are useful (Almeida et al., 2000; GonzalezItting et al., 2002). Some of these methods are: Restriction Fragment Length Polymorphism (RFLP) (Juskeviciute, 2003), Random Amplified Polymorphism DNA (RAPD) (Almeida, 2000), Simple Sequence Repeats (SSRP), Amplified Fragment Length Polymorphism (AFLP), Arbitrarily Primed PCR (AP-PCR), DNA Amplified Fingerprinting (DAF).

  RAPD is a multilocus technique which allows obtaining information on the general polymorphism of a genome. Low expense, efficiency in developing a large number of DNA markers in a short time and requirement for less sophisticated equipment, the simplicity and

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applicability, requirement of small amount of DNA without the requirement of cloning, sequencing or any other form of the molecular characterization of the genome has made the RAPD technique valuable (Bardakci et al., 2001).

MATERIAL AND METHODS

Different species from different localities of Khorasan were sampled (Figure 1). Genomic DNA was extracted from 100% ethanol preserved liver of one *M. crassus*, six *M. persicus*, 13 *M. libycus* and three unknown samples from Tabas, Yazd province, using a genomic DNA purification kit (DNA tissue kit, BILATEST). The isolated DNA was amplified using nine primers (Table1) (Juskevieiute et al., 2002). PCR reactions were performed in a volume of 25 μl containing 2.5 μl PCR buffer (10x), 25 mM MgCl$_2$, and 10 mM dNTPs, and 10μM primer, 14 ng of genomic DNA and 1 unit of *Taq* polymerase (Sinagene, Iran). Amplification was done with a programmable Thermal Cycler (Primus 96 advanced Gradient, Peqlab, Germany) under following conditions: 90˝at 94° C, 45 cycles (30˝at 94° C, 60˝at 42° C, 120˝at 72° C), with a final extension of 10 min at 72° C (Kohler et al., 2000). The amplified fragments were separated on 2% agarose gels and stained with ethidium bromide. The bands were distinguished by Labworks software and analyzed by PopGene for determining genetic distances. Dendrogram was constructed by Neibor-Joining, UPGMA method.
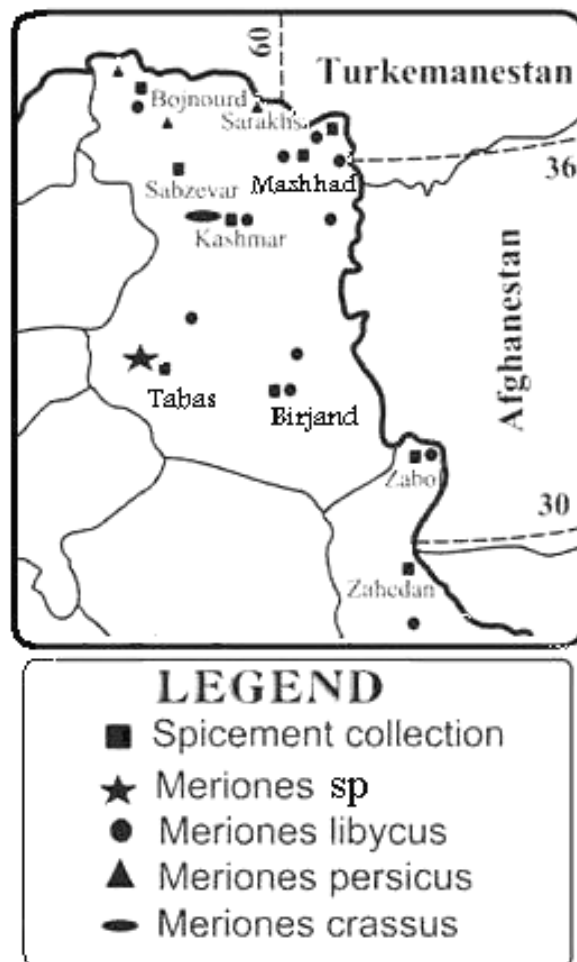

FIG.1. - Sampling localities.

TABLE 1- Primer sequences

Name of primer	Sequence
ROTH-180-01	5'-GCACCCGACG-3'
ROTH-180-02	5'-CGCCCAAGC-3'
ROTH-180-03	5'-CCATGGCGCC-3'
ROTH-180-04	5'-CGCCGATCC-3'
ROTH-180-05	5'-ACCCCAGCCG-3'
ROTH-180-06	5'-GCACGCCGGGA-3'
ROTH-180-08	5'-CGCCCTCAGC-3'
ROTH-180-09	5'-GCACGGTGGG-3'
ROTH-180-10	5'-CGCCCTGGTC-3'

RESULTS

DNA extracted from *Meriones* species was amplified using nine random primers. The amplified DNA of *Tatera indica* was used as an out-group. Each primer provides a distinct pattern of amplified fragments. The number of fragments and the amount of intraspecific polymorphism were varied among the primers (Figure 2). However, there were several common bands between these species.

Genetic distance on the base of Nei (1978) between the species based on RAPD data has been varied between 0.24 and 0.78 (Table 2). Genetic distance from RAPD data was used to draw the dendrogram on the basis of the UPGMA (Figure 3).
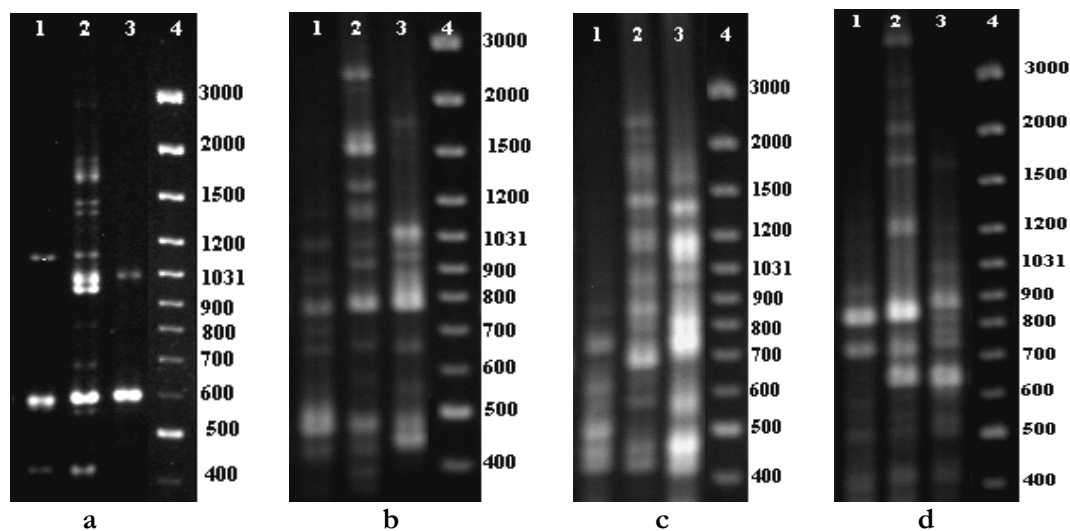
FIG.2.- Ethidium bromide- stained 2% agarose gel with RAPD products (a- Roth-180-02, b-Roth-180-04, c- Roth-180-05, d-Roth-180-06. 1-M. crassus, 2- M. persicus, 3- M. libycus, 4-Gene ruler 100bp DNA ladder.

DISCUSSION

Different kinds of studies have been done on genus *Meriones.* Yigit et al. (1996,1998,1999) analyzed the morphometric and morphologic characters of *M. Persicus*, *M. Meridianus*, and *M. crassus.* Chromosomal studies on these species showed the differences in the number of chromosomes (Yigit et al., 1996, 1998, 1999). Such studies have been done on jirds of Mashhad and Sabzevar by Khosravi and Darvish (1999). The enzymatic studies on the blood serum proteins of *M. meridianus*, *M. persicus*, *M. crassus* and *M. tristrami* have been shown no diagnostic differences between them. It has been shown that the RAPD procedures are useful tools for assessing the genetic variability (Colak et al., 2001).
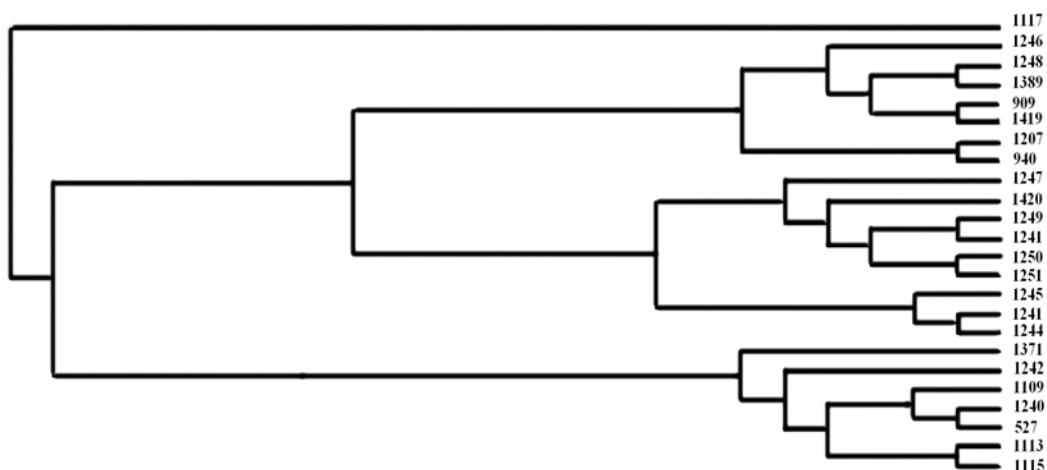
FIG.3.- Dendrogram showing relationship between *Meriones* species. (1117) *T. indica*, (1246 to 1244) *M. libycus*, (1371) *M. crassus*, (1424 to 1115) *M. persicus*.

In this study, morphological characters did not help us to identify these three specimens. Characters such as bushy tuft, thicker tail and longer tail hair than other *Meriones* species made it difficult to make a decision. This dendrogram shows that unknown specimens (1419, 1420, and 1421) were placed with *M. libycus* cluster. Therefore, they can be identified as *M. libycus*.

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TABLE 2 - Distance matrix (lower triangle), similarity matrix (upper triangle).

	1246	1247	1207	940	1241	1244	1245	1249	1250	1251	1248	909	1389	1419	1420	1421	1371	1240	1242	527	1109	1113	1115	1117
1246	****	0.6857	0.64	0.6343	0.6571	0.6857	0.6514	0.7257	0.7314	0.6686	0.7029	0.76	0.7429	0.6971	0.6743	0.7257	0.5657	0.5714	0.6229	0.5657	0.5886	0.6229	0.56	0.56
1247	0.3773	****	0.6343	0.5943	0.72	0.7371	0.7257	0.7314	0.7143	0.7657	0.6171	0.6514	0.6571	0.6114	0.7257	0.7543	0.6629	0.6229	0.6171	0.6057	0.6629	0.6971	0.5886	0.52
1207	0.4463	0.4553	****	0.7886	0.6514	0.6457	0.6114	0.6286	0.6571	0.6743	0.6857	0.6857	0.68	0.68	0.6114	0.6971	0.6171	0.6457	0.64	0.6171	0.6171	0.6171	0.52	0.4971
940	0.4553	0.5204	0.2375	****	0.5543	0.5714	0.5029	0.5886	0.6286	0.6	0.7257	0.68	0.7429	0.6514	0.6057	0.6114	0.5771	0.5943	0.5657	0.5771	0.5771	0.5657	0.4571	0.56
1241	0.4199	0.3285	0.4286	0.5901	****	0.8229	0.7771	0.7029	0.72	0.7029	0.6343	0.6	0.64	0.6171	0.7086	0.7371	0.6686	0.6286	0.6114	0.6229	0.6343	0.6457	0.6057	0.56
1244	0.3773	0.305	0.4374	0.5596	0.195	****	0.7714	0.6857	0.7143	0.6743	0.5943	0.6286	0.6686	0.6229	0.68	0.6857	0.6743	0.6229	0.6057	0.5943	0.6514	0.6286	0.6	0.5543
1245	0.4286	0.3206	0.492	0.6874	0.2521	0.2595	****	0.7886	0.7486	0.7543	0.5829	0.5943	0.6229	0.6114	0.7143	0.7314	0.6857	0.6229	0.5714	0.6057	0.6286	0.6514	0.6114	0.4743
1249	0.3206	0.3128	0.4643	0.5301	0.3526	0.3773	0.2375	****	0.7771	0.7829	0.68	0.7029	0.6629	0.72	0.7314	0.8286	0.6457	0.6514	0.6	0.6686	0.6343	0.6571	0.5943	0.5029
1250	0.3128	0.3365	0.4199	0.4643	0.3285	0.3365	0.2896	0.2521	****	0.8229	0.7314	0.7543	0.7143	0.7714	0.7143	0.8229	0.6171	0.6686	0.64	0.6629	0.6629	0.6857	0.6571	0.52
1251	0.4026	0.2669	0.3941	0.5108	0.3526	0.3941	0.282	0.2448	0.195	****	0.68	0.68	0.6629	0.6971	0.7657	0.8171	0.6686	0.6057	0.6343	0.6343	0.6343	0.7029	0.6057	0.5143
1248	0.3526	0.4827	0.3773	0.3206	0.4553	0.5204	0.5398	0.3857	0.3128	0.3857	****	0.7829	0.7886	0.7657	0.6057	0.7029	0.5429	0.5257	0.5771	0.5657	0.52	0.52	0.5029	0.5257
909	0.2744	0.4286	0.3773	0.3857	0.5108	0.4643	0.5204	0.3526	0.282	0.3857	0.2448	****	0.7543	0.7771	0.6286	0.7371	0.4857	0.5714	0.5771	0.5771	0.5657	0.5771	0.5143	0.48
1389	0.2973	0.4199	0.3857	0.2973	0.4463	0.4026	0.4734	0.4112	0.3365	0.4112	0.2375	0.282	****	0.76	0.6571	0.7086	0.6057	0.5771	0.6057	0.6171	0.5829	0.6057	0.5543	0.5314
1419	0.3608	0.492	0.3857	0.4286	0.4827	0.4734	0.492	0.3285	0.2595	0.3608	0.2669	0.2521	0.2744	****	0.6457	0.7657	0.56	0.5886	0.5943	0.5943	0.5829	0.5829	0.5543	0.5314
1420	0.3941	0.3206	0.492	0.5013	0.3445	0.3857	0.3365	0.3128	0.3365	0.2669	0.5013	0.4643	0.4199	0.4374	****	0.7657	0.7086	0.68	0.6286	0.6857	0.6857	0.6971	0.6229	0.5086
1421	0.3206	0.282	0.3608	0.492	0.305	0.3773	0.3128	0.1881	0.195	0.2019	0.3526	0.305	0.3445	0.2669	0.2669	****	0.6686	0.6743	0.6343	0.6457	0.6457	0.68	0.6171	0.5486
1371	0.5697	0.4112	0.4827	0.5497	0.4026	0.3941	0.3773	0.4374	0.4827	0.4026	0.6109	0.7221	0.5013	0.5798	0.3445	0.4026	****	0.6971	0.6571	0.6686	0.6571	0.7143	0.6743	0.56
1240	0.5596	0.4734	0.4374	0.5204	0.4643	0.4734	0.4734	0.4286	0.4026	0.5013	0.643	0.5596	0.5497	0.5301	0.3857	0.3941	0.3608	****	0.7543	0.8	0.7543	0.7429	0.7143	0.5543
1242	0.4734	0.4827	0.4463	0.5697	0.492	0.5013	0.5596	0.5108	0.4463	0.4553	0.5497	0.5497	0.5013	0.5204	0.4643	0.4553	0.4199	0.282	****	0.7029	0.7257	0.7371	0.64	0.5714
527	0.5697	0.5013	0.4827	0.5497	0.4734	0.5204	0.5013	0.4026	0.4112	0.4553	0.5697	0.5497	0.4827	0.5204	0.3773	0.4374	0.4026	0.2231	0.3526	****	0.7714	0.7714	0.7543	0.5943
1109	0.5301	0.4112	0.4827	0.5497	0.4553	0.4286	0.4643	0.4553	0.4112	0.4553	0.6539	0.5697	0.5398	0.5398	0.3773	0.4374	0.4199	0.282	0.3206	0.2595	****	0.7714	0.7429	0.56
1113	0.4734	0.3608	0.4827	0.5697	0.4374	0.4643	0.4286	0.4199	0.3773	0.3526	0.6539	0.5497	0.5013	0.5398	0.3608	0.3857	0.3365	0.2973	0.305	0.2595	0.2595	****	0.7886	0.5829
1115	0.5798	0.5301	0.6539	0.7828	0.5013	0.5108	0.492	0.5204	0.4199	0.5013	0.6874	0.665	0.5901	0.5901	0.4734	0.4827	0.3941	0.3365	0.4463	0.282	0.2973	0.2375	****	0.5543
1117	0.5798	0.6539	0.6989	0.5798	0.5798	0.5901	0.7459	0.6874	0.6539	0.665	0.643	0.734	0.6322	0.6322	0.6761	0.6004	0.5798	0.5901	0.5596	0.5204	0.5798	0.5398	0.5901	****