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Population Structure of the Yemeni Honey Bee (*Apis mellifera jemenitica*) Entails an Urgent Conservation Strategy in Saudi Arabia

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ABSTRACT

Spread and use of exotic honeybee subspecies into Saudi Arabia bear significant risk on the conservation of the indigenous honeybee *Apis mellifera jemenitica*. The fallout of imported honeybee on population structure and diversity of *Apis mellifera jemenitica* populations have been investigated using microsatellite markers. Results demonstrated high genetic diversity within the native honeybee population compared with other related subspecies. Through the Bayesian approach of microsatellite variations, two groups can be distinguished with high level of introgression between imported and native subspecies. High levels of introgression and Intensive hybridization entail urgent conservation strategy of the native honeybee to be implemented.

Key words: Yemeni honeybee, conservation, population structure, introgression, genetic diversity

INTRODUCTION

The indigenous honeybee of Saudi Arabia, the focus of this publication, has been characterized by Ruttner in 1975 as *Apis mellifera jemenitica*, a honeybee race that has been evolved and adapted to adverse climatic conditions of the region (Ruttner, 1976). In this country beekeeping is in the course of development; the majority of bee colonies are kept in log hives, productivity is too low and beekeepers managing practices such as disease monitoring, feeding and re-queening are nominal or absent. Although, Saudi Arabia represents a wide range of variable climates and habitats, overall drought conditions, short flow seasons and long hot summers are the most obvious obstacles of modern beekeeping in this country (Al-Ghamdi *et al*., 2013; Alqarni *et al*., 2011). Consequently, significant annual losses occur during the summer seasons. To compensate these annual losses, beekeepers of Saudi Arabia introduce massively exotic honeybee packages into the country. In 2012, about 200000 package bees were introduced, most of them were imported from Egypt representing the carniolan hybrids of *A.m. lamarckii* (MoEP, 2012). These packages lack generally quality control parameters and may include disease agents and parasites. Now a days, growing number of local beekeepers depend entirely on seasonal beekeeping based on imported honeybees to construct and run their apiaries shortly before the flow season. The huge importations of exotic package bees, *A. mellifera*, may lead to dramatic changes in the genetic pool of the native honeybee race and may endanger its conservation. Accordingly, during the last few years, critical investigations on the native honeybee populations, has been focused (Al Ghamdi, 1990, 2002, 2005; Al-Ghamdi *et al*., 2012, 2013; Alqarni, 1995, 2006; Alqarni *et al*., 2011). In relation to geographical

variability, Al-Ghamdi *et al.* (2012), reported significant morphometric variations in the native honeybee populations, which were defined into three distinct clusters. In addition to natural selection, intensive hybridization with introduced honeybee subspecies may explain partially these variations. Intensive hybridization entails a conservation strategy of the native honeybee to be implemented. In this study, microsatellite markers were used to detect levels of gene introgression of the imported honeybee and its influence on population structure of the Saudi honeybee.

MATERIALS AND METHODS

Honeybee worker samples were collected from 185 native honeybee colonies representing six native beekeeping areas (Albaha 20°16'15"N: 41°26'25"E, Almadinah 24°33'28"N:39°43'42"E, Altaif 21°41'3"N: 40°27'21"E, Asir 18°59'41"N:42°51'6"E, Jazan 17°22'49"N: 42°44'29"E and Najran 17°28'12"N:44°35'5"E). In addition, 10 samples of imported honeybees were collected from importers apiaries. The distance between selected locations ranged between 153 km (Altaif- Al-baha) to 884 km (Almadinah-Asir) (Fig. 1). Geographical barriers such as wide arid areas of very low plantations separates the sampling locations, we assume that, different localities may resemble distinct subpopulations of the native honeybee.

DNA extraction and genotyping: Total genomic DNA was extracted from single adult worker bee using DNeasy Blood and Tissue Kit (Qiagen), according to Bogaerts *et al.* (2009). Eight specific

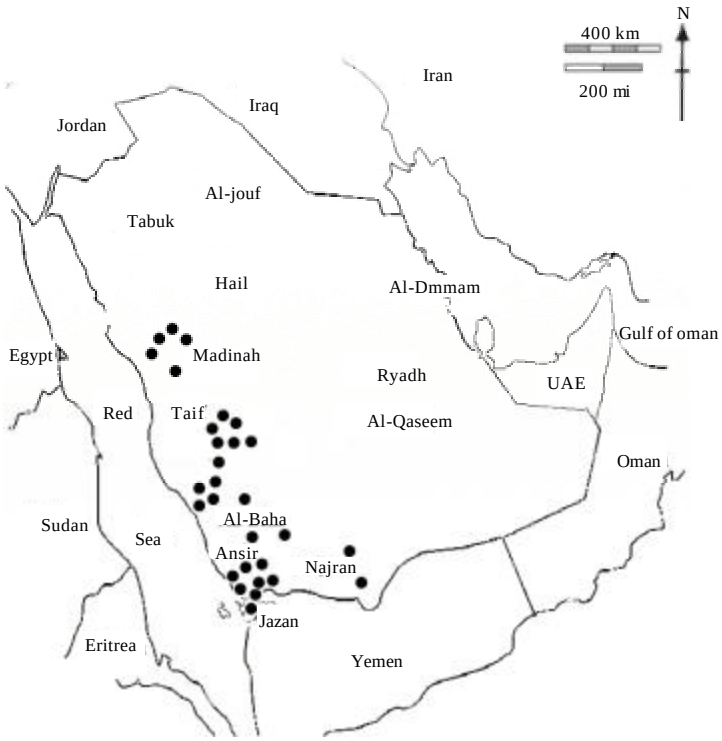

Fig. 1: Outline map of Saudi Arabia that show sampling locations. A total of 195 (185 local and 10 imported) honeybee colonies were sampled

microsatellite loci (A7, A24, A28, A88, A113, B124) (Estoup *et al*., 1995), Ap43 (Garnery *et al*., 1998) and Ap81 (Michel *et al*., 2003) previously reported as polymorphic, were amplified separately using 6-FAM-labeled primer. PCR was carried out in a total volume of 25 μL containing 10 ng DNA, 1X reaction buffer (Promega), 0.25 mM dNTPs mixture, 0.5 mM primer, 1.25 units Taq DNA polymerase (Promega), 2.75 mM $MgCl_2$ and the final volume was adjusted by adding sterile distilled water. The PCR profile consists of: one (2 min) cycle at 95°C, 30 cycles of 95°C for 30 sec, 54°C for 30 sec, 72°C for 30 sec and a 10 min at 72°C. PCR was performed using GeneAmp® PCR system 9700 version 3.10 (Applied Biosystems). The separation of fragments was carried out on automated ABI Prism® 3130 (Applied Biosystems) using® LIZ 500 internal standard. Electrograms were scored using GeneMapper® software version 4.0 and Scanner Software v1.0 (Applied Biosystems).

Genetic differentiation: Population genetic parameters were calculated with Genepop 4.2. (Raymond and Rousset, 1995). Genetic diversity within assumed populations was evaluated by computing allele frequencies, observed heterozygosity (Ho), expected heterozygosity (He), F statistics (Fst, Fis and Fit) and gene flow. The presence of null alleles were verified using Micro-Checker version 2.2.3 (Van Oosterhout *et al*., 2004). Hardy-Weinberg equilibrium was tested with Genpop (Raymond and Rousset, 1995). Population structure and assignments of individuals to populations, probabilistically based on their multilocus genotypes, were inferred using the Bayesian model-based clustering and thereby estimates of the posterior probability for a given number of genetic populations (K) were obtained with the software STRUCTURE v2.2 (Pritchard *et al*., 2000). An admixture model assuming correlated allele frequencies was used. The results were based on simulation of 50000 burn-in-steps and 500000 MCMC (Markov Chain Monte Carlo alogorithm) iterations. Five runs for each K-value (K = 1-8) were used to estimate the most probable value of K. The number of populations was defined using the value of ΔK as described in Evanno *et al*. (2005).

RESULTS

The number of the scored alleles within the native honeybee samples was 215 (Table 1). The average allele number per locus was 4.5 (Table 1). Gene diversity measured as expected heterozygosity (He) ranged from 0.53 (Najran) to 0.58 (Asir and Jazan) (Table 2).

Genetic differentiation, both genic and genotypic resulted in highly significant differentiations among all populations ($p \leq 0.005$). A slight heterozygote deficiency within subpopulations and a higher heterozygote deficiency within the total population were revealed by positive F_{IS} and F_{IT}

Table 1: No. of alleles, total No. of alleles and mean No. of alleles per locus in different sampling locations

Locations	A7	A88	A113	A24	A28	B124	A143	A81	Um	Mean
Albaha	5.0	4.0	6.0	3.0	2.0	10.0	2.0	4.0	36.0	4.5
Almadinah	4.0	4.0	4.0	3.0	2.0	8.0	2.0	4.0	31.0	3.9
Altaif	5.0	4.0	6.0	4.0	3.0	11.0	2.0	4.0	39.0	4.9
Asir	5.0	3.0	6.0	3.0	3.0	12.0	2.0	4.0	38.0	4.8
Jazan	5.0	3.0	6.0	4.0	3.0	13.0	2.0	3.0	39.0	4.9
Najran	4.0	3.0	5.0	4.0	2.0	9.0	2.0	3.0	32.0	4.0
Mean	4.7	3.5	5.5	3.5	2.5	10.5	2.0	3.7	35.8	4.5
Total	28.0	21.0	33.0	21.0	15.0	63.0	12.0	22.0	215.0	27.0

Table 2: Expected heterozygosity (He) and observed heterozygosities (Ho) based on eight microsatellite markers

	A7		A88		A113		A24		A28		B124		A143		A81		Mean	
Locations	He (%)	Ho (%)	He (%)	Ho (%)	He (%)	Ho (%)	He (%)	Ho (%)	He (%)	Ho (%)	He (%)	Ho (%)	He (%)	Ho (%)	He (%)	Ho (%)	He (%)	Ho (%)
Albaha	53	54	58	44	56	36	55	46	43	36	83	72	46	36	64	6	57±12	48±0.13
Almadinah	65	55	53	33	53	50	56	33	39	50	87	91	46	50	37	25	54±16	48±20
Altaif	61	42	60	45	42	40	53	41	57	39	88	78	35	29	62	52	57±16	46±14
Asir	58	47	64	63	34	31	59	58	63	42	87	83	36	26	67	61	58±17	51±19
Jazan	64	47	65	70	28	25	59	62	52	44	89	80	43	30	65	68	58±18	53±20
Najran	54	60	62	60	31	27	57	80	48	47	85	69	30	21	58	67	53±18	54±21
Mean	59	51	60	53	41	35	57	53	50	43	86	80	44	36	61	55	57±0.05	50±0.05

Nei's expected heterozygosity by Nei (1987)

Table 3: Assignment (%) of 195 honeybees individuals from six native populations and one imported reference using structure analysis into two clusters

Population	Individuals assigned to cluster1 (%)	Individuals assigned to cluster 2 (%)	Total No. of individuals
Albaha	63	37	25
Almadinah	45	55	12
Altaif	58	42	48
Asir	66	34	32
Jazan	68	32	55
Najran	70	30	13
Mean	62	38	
Imported	5	95	10

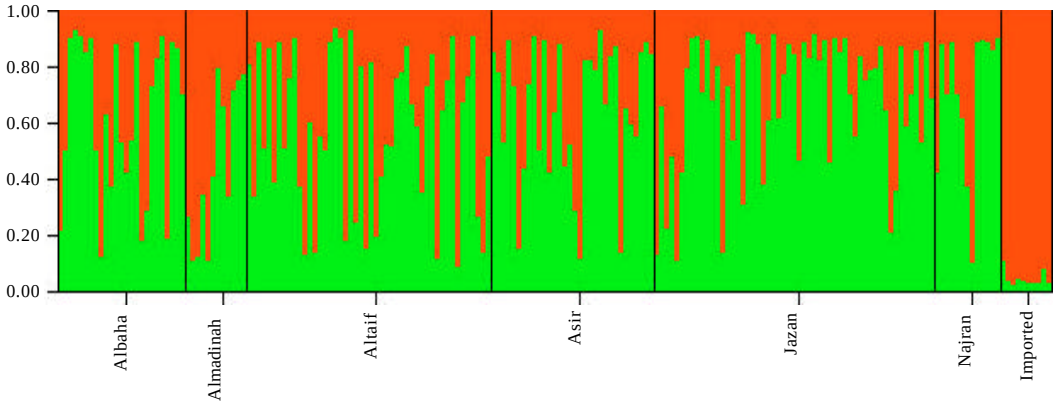

Fig. 2: Population structure obtained by structure analysis. Proportions of membership of six native honeybee populations and one imported reference population in two inferred clusters. Each of the 195 individual honeybee is represented by thin vertical line that is divided into colored segments representing the individual's membership in the cluster of the corresponding color

values (F_{is} = 0.123, F_{st} = 0.009 and F_{it} = 0.13). Clustering using Bayesian method was performed on the entire data set with increasing numbers of inferred clusters. First, we tested the assumption that the collected honeybee samples represent 7-distinct predetermined populations (K = 7: 6 local and one imported). The results of multiple analyses with structure were incompatible with this assumption; in all cases, only 2 populations were identified (Evanno *et al*., 2005). Assignment test allocated many native bee individuals to the second cluster including almost all imported bee individuals (Table 3). The introgression was bidirectional and severer in some locations than the others (Fig. 2).

DISCUSSION AND CONCLUSION

Results provide insights into the genetic diversity and structure on the native honeybee of Saudi Arabia. It also focuses on the gene flow between exotic and local honeybee populations. The assessment of present population structure is essential for an effective conservation policy of the native honeybee. Al-Ghamdi *et al*. (2012) described the presence of three morphologically distinct ecotypes of the local honeybee, *A.m. jemenitica* within Saudi Arabia with an ecoline from the south

to the north along the red sea coast. In this study, based on the geographical origin of the samples, genetic diversity parameters, different allele frequencies and significant variations among assumed populations were demonstrated. Bayesian model-based clustering was highly distinctive to separate the imported honeybees in one cluster including many of the native honeybee individuals. However, structure analysis did not recognize the different subpopulations from the various native honeybee samples. This may be explained by the originally high diversity among native honeybee samples compared with other related populations (Franck, 2000a, b; Franck *et al.*, 2001) and to the human mediated movement of the native honeybee colonies among these locations. Introgression from imported honeybee genes is apparently clear in native honeybee samples with little variation among different geographical regions. This putative gene flow could happen in both directions as demonstrated by the Bayesian analysis of the microsatellites and the miss-assignment of many native honeybee individuals. In conclusion, the analysis of Saudi honeybees with microsatellite markers provides evidence on the high genetic diversity of the native honeybee and entails the necessity for an urgent conservation policy.

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