



Barcoding of the honeybee
Apis mellifera of Saudi Arabia

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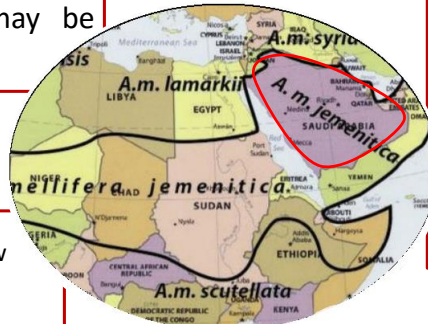


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Why to Barcode *A. mellifera* of SA:

- Population structure may be endangered

- Possibility to characterize new subspecies or ecotypes!



- The bee is adapted to extremes, and many thought it is tolerant to Varroa mite!

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Who I am:

I KNOW YOUR NAME, BUT NOT MUCH MORE

Do you know who I am?

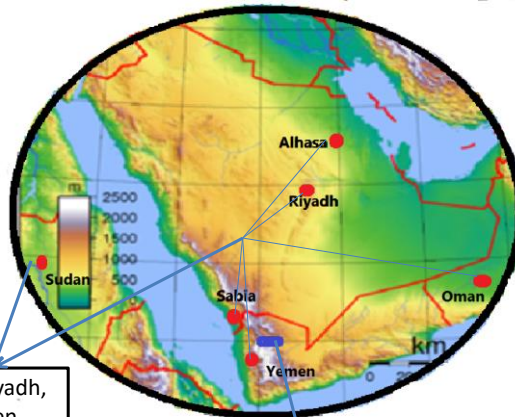
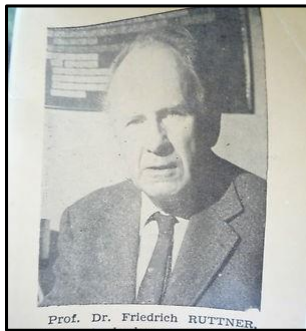


Did you come from Socotra?
Are you originally Asian or African?
Maybe Ethiopian or Somalian!
Are you really Mediterranean?.



Historical Background

2.2 m-km, highly diverted with extremes



He collected samples from Sabia, Riyadh, Alhasa, (Saudi Arabia), Oman, Yemen, Sudan. (1975).

it was first thought to be *A. c. indica*

But Ruttner (1975) correctly identified these bees as small *Apis mellifera* and called *Apis mellifera yemenitica*

Since then, Many Saudi researchers worked on its morphometry, biology and behaviour.

However:

- Samples taken by Prof. Rutter (1975) were not representative to whole SA (N=2)
- Morphological characteristics which, although it is very important in this aspect, is not well suited to characterize honeybee subspecies and study phylogenetic relations. (Frank et al., 2000).
- New approaches are available and more data is now available to study honeybee populations.
- Lack of reference genetic data for this subspecies in the gene banks.

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Approach:

Sample collection:

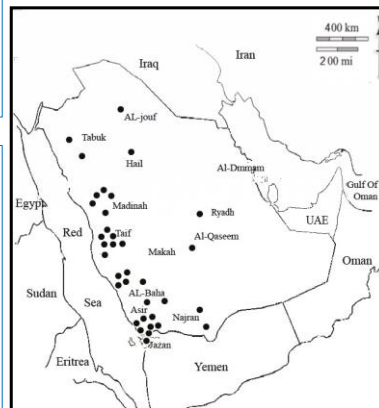
Comprehensive (198 colonies, 1980 bee worker, 45000 readings)

Approaches:

- Morphometry (24 characters) (Ruttner 1988)
- Genetic (*mtDNA* COI-COII) Sequences (N=179) . (Cornuet and Garnery, 1991)

Microsatellite Analysis (195 native + 10 imported)

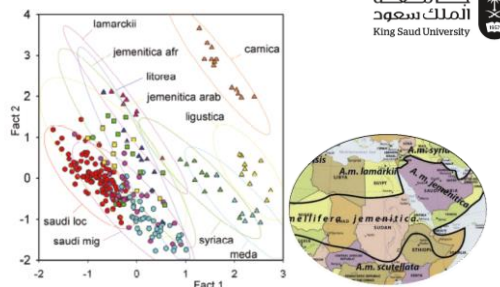
(Estoup et al., 1995), (Garnery et al., 1995) (Michel et al., 2003)



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Results:

Bees from Saudi Arabia clustered with the Reference Yemeni samples (Arabian yemenitica) (24 characters + 9 wing angels)



Squared Euclidian distances between Z-standardized character group fro Saudi Arabia (Sa) and the Arabian reference samples of *A. m. jemenitica* from the respective reference subspecies.

Group	SA south-local	SA south-migratory	SA west-local	SA west-migratory	SA north-migratory	jemenitica-arab	liorea
<i>carnica</i>	218.89	185.02	207.21	180.72	167.38	156.55	131.29
<i>ligustica</i>	143.47	113.14	129.45	106.43	98.94	99.91	81.14
<i>meda</i>	100.79	73.18	90.53	69.14	62.75	68.14	44.78
<i>syriaca</i>	65.27	45.72	61.06	44.17	38.01	38.28	28.35
<i>lamarckii</i>	48.19	35.95	49.57	44.02	31.38	32.48	21.81
<i>jemenitica-arab</i>	10.41	7.60	9.60	9.76	4.78	.000	10.27
<i>liorea</i>	21.75	13.51	20.13	14.45	9.90	10.27	0.00
<i>jemenitica-afric</i>	25.63	18.53	20.95	20.81	13.27	13.36	8.88

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Alattal et al., 2014 : Zoology in the Middle East

Results:

In silico *Dra*I restriction analysis revealed seven different haplotypes; six of them belonged to the O lineage, and one belonged to the A lineage. Three of these haplotypes were novel

Haplotype based on fragment length of *mtDNA* COI-COII intergenic region.

<i>Dra</i> I Saudi haplotype	Location	Haplotype and percentage	Fragment length	N	Haplotype percentage (= N/the entire sample size)
Haplotype 1 (A1)	Najran	O1(Z7) (100%)	29,108,67,371 (PoQ)	122	67
	Jazan	O1(Z7) (87%)			
	Altaif	O1(Z7) (49%)			
	Almadinah	O1(Z7) (50%)			
	Asir	O1(Z7) (84%)			
	Albaha	O1(Z7) (39%)			
Haplotype 2 (A2)	Alqaseem	O1(Z7) (100%)	29,108,65,371 (PoQ)	6	3
	Jazan	O1d (4%)			
Haplotype 3 (A3)	Altaif	O1d (9%)	29,112,67,371 (PoQ)	3	2
	Almadinah	New 1 (50%)			
Haplotype 4 (A4)	Asir	O1' (Z2) (16%)	30,108,67,129,375 (PoQQ)	29	16
	Albaha	O1' (Z2) (42%)			
Haplotype 5 (A5)	Altaif	O1' (Z2) (21%)	34,108,67,129,66,357 (PoQQ)	15	8
	Albaha	New2 (21%)			
Haplotype 6 (A6)	Albaha	New2 (5%)	34,108,66,129,67,357 (PoQQ)	4	2
	Jazan	New3 (9%)			
Haplotype 7 (A7)	Albaha	New3 (9%)	12,108, 67, 129, 357 (PoQQQ)	4	2

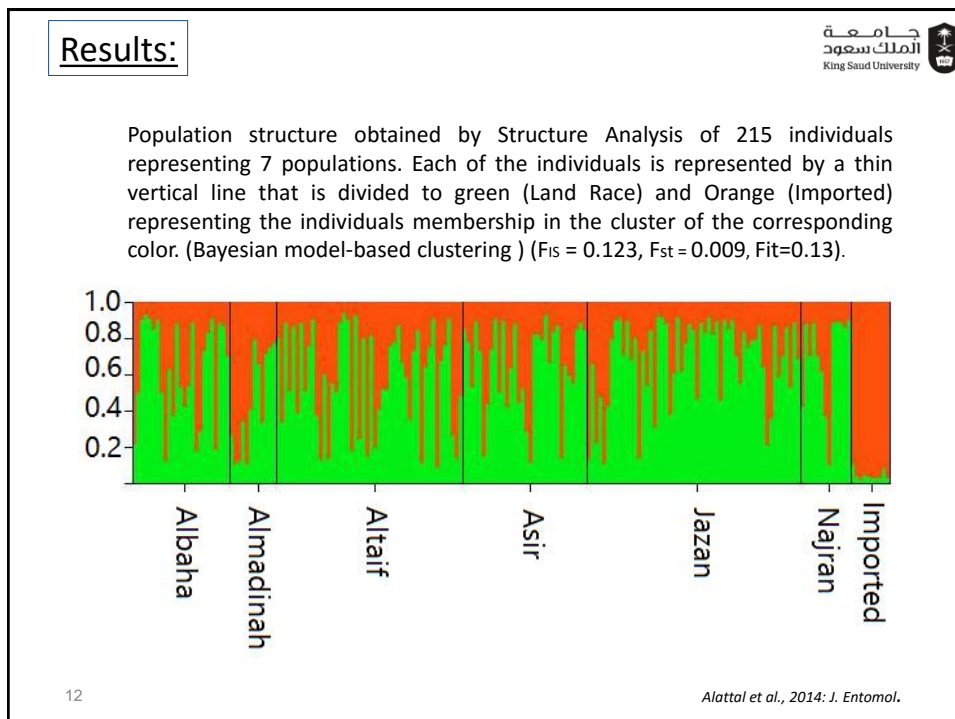
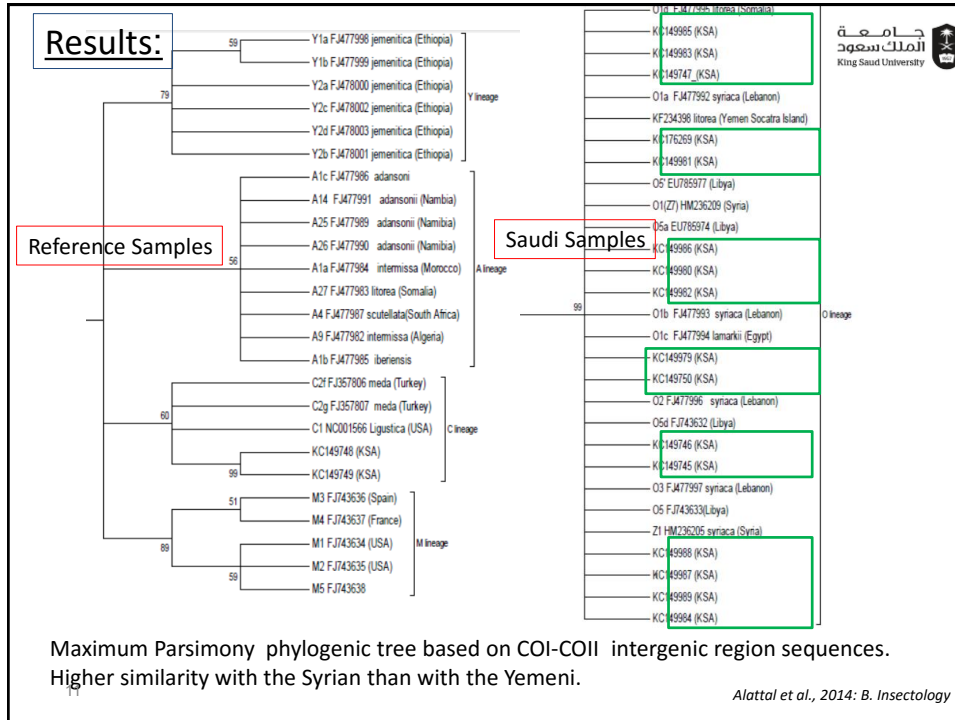
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Alattal et al., 2014: B. Insectology

مؤتمر الجمعية العربية لتربية النحل الأول

5 - 6 فبراير 2018م

مركز أبوظبي الوطني للمعارض - دولة الإمارات العربية المتحدة





Summary – morphometry:



- Bees of Saudi Arabia resemble Yemeni bees *A. m. jemenitica*
- Results indicate significant morphometric variation and a cline of factor one (characters associated with body size) from the north (cluster one) to the south (cluster three).
- Most Saudi honey bee samples clustered with *Apis mellifera jemenitica* reference group, but few were more similar to *Apis mellifera litorea* reference group.
- Morphometric variation among the clusters of this study exceeds the variation between the Syrian and the Yemeni Honeybee reference samples (RUTTNER 1988)
- Socotran sample clustered with the *litorea* group

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Summary of Molecular part:



- Eighteen new and clearly separated haplotypes were characterized for the first time, three of them were novel.
- Sixteen haplotypes belonged to the O lineage and are very close to the Syrian haplotypes.
- Two haplotypes belonged to the A lineage but clearly different from the Ethiopian haplotypes of *A.m. jemenitica*
- Two groups can be distinguished with high level of introgression between imported and native subspecies.

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Conclusion

- Both the morphometric and genetic analyses provide evidence that the Saudi honey bee population from the Arabian Peninsula belongs to the O lineage
- Presence of overlapping and transitional state between the Syrian and the Yemeni Honeybees.
- Geometric results confirmed that samples from Saudi Arabia are very similar to the samples from the subspecies *A. m. jemenitica* (Ruttner, 1967). previously described from Oman, Yemen and Saudi Arabia. However molecular results goes with the Syrian.
- Hybridization of the local bee race with other bee races should be considered.
- Similarities with the Syrian sequences and high genetic diversity in the *mtDNA* COI-COII region should be discussed.
- Intensive hybridization entail urgent conservation strategy of the native honeybee to be implemented.

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Results:

Number and distribution of Saudi honey bee haplotypes according to sequences of the COI-COII intergenic

Haplotype Accession No.	NCBI haplotype	Identical %	Najran	Asir	Jazan	Albaha	Altaif	Alqaseem	Almadinah	N.	Haplotype %
KC149745	O1	99.8	7	11	27	10	13	1	3	72	40.2
KC149747	O1a	99.8	2	12	8	3	10	3	-	38	21.2
KC149979	O1'	99.5	-	5	-	7	-	-	-	12	6.7
KC149746	O4b	99.1	3	3	3	1	-	-	-	10	5.6
KC149984	O1'	99.4	-	-	-	-	11	-	-	11	6.1
KC149989	O1'	99.3	-	-	-	-	7	-	-	7	3.9
KC149749	M4	97.5	-	-	-	-	4	-	-	4	2.2
KC149750	O1	99.3	-	-	-	-	-	-	3	3	1.7
KC149983	O1'	99.4	-	-	-	-	3	-	-	3	1.7
KC149985	O1'	99.3	-	-	4	-	-	-	-	4	2.2
KC176269	O1''	99.3	-	-	-	4	-	-	-	4	2.2
KC149748	A1	97.5	-	-	2	-	-	-	-	2	1.1
KC149987	O1'	99.4	-	-	-	-	2	-	-	2	1.1
Syrian(9)HM236209	O1	100	1	-	1	-	-	-	-	2	1.1
KC149980	O5a	99.5	-	-	-	1	-	-	-	1	0.6
KC149981	O1'	99.7	-	-	-	1	-	-	-	1	0.6
KC149982	O1'	99.6	-	-	-	-	1	-	-	1	0.6
KC149986	O1'	99.4	-	-	-	1	-	-	-	1	0.6
KC149988	O1'	99.2	-	-	-	-	1	-	-	1	0.6
Total			13	31	45	28	52	4	6	179	100