Saudi Journal of Biological Sciences 28 (2021) 578–581

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Mating frequency of *Apis mellifera jemenitica* under desert conditions of Saudi Arabia

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ARTICLE INFO

Article history:
Received 17 November 2019
Revised 21 October 2020
Accepted 21 October 2020
Available online 4 November 2020

Keywords:
Apis mellifera jemenitica
Desert conditions
Mating frequency
Saudi Arabia

ABSTRACT

Queen mating frequency is an important reproductive trait of the western honeybee *Apis mellifera*. Yet, it demands more attention when investigated under extreme or confined ecosystems. Queen mating frequency of the Yemeni Honeybee *A. m. jemenetica* was estimated under Saudi Arabia desert conditions, Riyadh (24°71′36″N, 46°67′53″E). Mating of queens took place after 8–13 days from emergence. Duration of mating flight ranged between 26 and 39 min. Subsequently, six microsatellite loci were used to genotype queen's progeny (n = 30 workers/queen). The average number of drone alleles using workers genotypes ranged between 5.83 ± 0.31 and 6.33 ± 1.09. However, effective paternal allele number was extremely low and ranged between 3.35 ± 0.34 and 3.60 ± 0.40. This relatively low mating frequency of the Yemeni honeybee, *A. m. jemenetica*, might have striking effect on the overall colony survival. Providentially, this relatively low mating frequency does not impact colonial heterozygosity, shown in this study (0.66 ± 0.07–70 ± 0.04), adversely. These results may affect hive survivability and entails distinctive management practices under such conditions.

1. Introduction

The unique ecosystem impact on honeybee fitness within the Arabian Peninsula should be focused. Colony losses during long summer spikes are routinely reported by Saudi beekeepers. Although losses are much higher in introduced bee subspecies of European and Mediterranean origin, colonies of the local bee subspecies *A. m. jemenetica* bear considerable losses (Alattal and Al-Ghamdi, 2015). In addition to pests and diseases (Chauzat et al., 2006; Chen et al., 2008), the main causes of colony losses in Saudi Arabia are related to drought (47 mm/decade), high ambient temperature (>40 °C; PoMEP, 2014), and non-effective management practices (Alattal, Alghamdi, & Alsharhi, 2014; Al-Ghamdi et al., 2013; Ali, 2011; Alqarni et al., 2011). Although many researchers investigated and documented the direct impact of such factors on colony survival and fitness within this region (Alattal and Al-Ghamdi, 2015), the impact of these factors on reproductive traits of the colony such as drone fitness and queen mating number is not very well studied. A recent study indicated inferior reproductive traits of Yemeni honeybee queens and drones compared to other *A. mellifera.* subspecies (Al-Sarhan et al., 2019). This could be related to smaller queen body size of the Yemeni honeybee compared to other bee subspecies (Schluns et al., 2003). However, this could be of minor importance if the queen achieved perfect mating with sufficient number of drones (Gerula et al., 2014). Thus its colony consists of adequate genetically distinct subfamilies, the workers in each subfamily is being derived from its respective mates, which can be detected using molecular markers (Nielsen, Tarpy and Reeve, 2003; Tarpy and Nielsen, 2002). Queen mating numbers as a reproductive trait may affect queen attractiveness to workers and their pheromone profile (Richard et al., 2007), which may increase the supersedure rates with poorly mated queens (Niño et al., 2012). Molecular markers has enabled ecologists to better understand male mating success (Milligan & McMurry, 1993; Nielsen et al., 2001), reproductive tactics (Neff, 2001; Rico et al, 1992), reproductive skew (Nonacs, 2000; Reeve et al., 2000) and population gene flow (Devlin and Ellstrand, 1990; González-Martínez et al., 2002; konuma et al., 2000). The aim of the study is to examine mating frequency of the native

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2020.10.045
1319-562X/

honeybee queens *A.m. jemenitica* and genetic relatedness among workers under desert conditions of Saudi Arabia.

2. Material and methods

The study was conducted at the bee research unit apiary at King Saud University, located in the northern part of Riyadh (24°71′36″N, 46°67′53″E) in the period between February and June 2018. Around the university apiary no beekeeping activities are performed, the nearest possible apiary is about 7 km faraway in Al-duriyah region (16°35′03″N 42°50′35″E). In late march (mid-season in Riyadh), experimental colonies were constructed by splitting five honeybee colonies, *A. m. jemenitica*, containing eight brood frames each to form five new splits in total. As the queen cells started to grow in the splits, only one queen cell was left for final development. Three days after virgin queen emergence, mating was monitored daily from 10:00 to 16:00 for 14 days. Final confirmation of successful mating was verified after two weeks of mating by observing egg laying of newly mated queens. To ensure adequate drone number, five standard colonies were selected at the beginning of the season (1st of Feb) for drone rearing and were kept in the same apiary. Two drone combs were placed within the brood area of each colony. Then drone development and emergence were observed during the study period. Maximum and minimum ambient temperatures were monitored throughout the course of the study. Colonies with successfully mated queens (n = 4) were then used to investigate mating frequency. Thirty newly emerged workers and one drone pupae were collected from each colony 40 days after queen started egg lying. Workers were collected directly from brood frames while emerging directly from colonies using forceps. The observed mating number for each queen was calculated following Tarpy et al. (2010). Initially, whole genomic DNA was extracted from individual workers (n = 30/-colony) using Chelex 100 resin® (Walsh et al., 1991) and subject it to polymerase chain reactions (PCRs: Applied Bio-system 9700) using six microsatellite loci (Am046, Am052, Am061, Am098, Am128, and Am491). PCR Products were then submitted to electrophoresis on 5% polyacrylamide gels for 6 h using 100 bp DNA sizer. Alleles were scored as fragment length in base pairs using UV detector (Genius System). The maternity fragment length was determined by analyzing one drone pupa from each colony and the maternal allele was then removed from the scored alleles prior to analysis. Number of mates of each colony was determined as the number of paternal alleles.

3. Statistical analysis

Analysis was carried out on 120 workers from 4 colonies (30/colony). We tabulated a paternal marker set for each worker to estimate the genetic structure within each colony using COLONY 1.2 (Wang, 2004). The total number of different marker sets within a colony signified the observed paternity frequency of the queen (*No*), or the total number of drone fathers that are represented in the offspring. We also determined the proportion of each subfamily within a colony so that we could calculate the effective paternity frequency (*Ne*) using the sample statistic proposed by Nielsen et al. (2003). Results were expressed as mean and standard deviation (M ± SE). Wright's fixation index (Fis.) as a measure of heterozygote deficiency or excess (Hartl and Clark, 1997), expected homozygosity and heterozygosity were computed following Levene (1949) based on colony program outcomes. SAS software was used to prove significance among colonies.

4. Results

Mating of queens took place after 8–13 days from emergence (Colony 1:11 days; colony 2: 13 days; colony 3: 9 days; colony 4:8-days). Duration of mating flights ranged between 26 and 39 min (Colony 1:29; colony 2: 33; colony 3: 26; colony 4:39 minuets). During mating flights average maximum and minimum temperatures ranged from 29 to 39 and from 19 to 26, respectively. All queens were able to lay eggs and rear brood. A total of 120 individual workers (30/colony) were genotyped. Amplified fragment lengths resembled the expected fragment range. Total number of drone alleles were 48 (Table 1) The average number of drone alleles based on Colony® program analysis using workers genotypes ranged between 5.83 ± 0.31 to 6.33 ± 1.09 (Table 2). However, effective drone allele number was extremely low and ranged between 3.35 ± 0.34 and 3.60 ± 0.40. Results revealed no significant differences among tested colonies (F = 0.16; P. > F = 0.92). Intra-colony heterozygosity ranged between 66 ± 0.07% and 70 ± 0.04% (Table 3). Three loci (Am46, Am128 and Am52) were relatively more polymorphic (Table 1).

5. Discussion

This is the first report of queen mating number for the Yemeni honeybee *A. m. jemenitica*. Overall, the mating numbers of the tested queens were extremely lower compared with previously documented results for all other *A. mellifera.* subspecies. The mean queen mating number in different *Apis mellifera* subspecies ranged between 5 and 34, where the lowest was reported for *A. m. lamarckii*, and the highest reported for *A. m. capensis* (Franck et al., 2000) In this study, the low mating number in Yemeni honeybee queens could be related basically to tow main reasons; firstly to queen body and spermathecal sizes, an adaptive trait of *A. m. jemenitica*, which is the smallest among *all A. mellifera* subspecies, and secondly to drought and elevated ambient temperatures during

Table 1
Identity of microsatellites; gene bank accession no., locus name, microsatellite primer sequence, annealing temperature (Ta.), used genotyping *A. m. jemenitica* workers in Riyadh region (24°71′36″N, 46°67′53″E).

Gene acc. No.	Locus	Primer sequences	Ta. (°C)	Size (Bp)	Repeat Motif
AJ509277	Am46	f-CGAAGGTTGCGGAGTCCTC r-GTCGTCGGACCGATGCG	56	114–130	(GA)14
AJ509283	Am52	f-CGAATTAACCGATTTGTCG r-GATCGCAATTATTGAAGGAG	53	148–178	(CT)10(GGA)7
AJ509292	Am61	f-GCAACAGGTCGGGTTAGAG r-CAGGATAGGGTAGGTAAGCAG	60	244–256	(CT)8 (CT)14(GGCT)8
AJ509329	Am98	f-GGCGTGCACAGCTTATTCC r-CGAAGGTGGTTTCAGGCC	58	135–143	(TA)6GATA(GA)10
AJ509359	Am128	f-GATCAAACACACAAACGAAAGC r-ACCGGAAGCCTAATCAAGG	62	194–218	(GA)6(GA)11
AJ509722	Am491	f-TGTTCCGGCAAGCTGAAG r-GTGCTCCGCAACAACGTG	56	100–112	(A)8G(A)6G(A)5

Table 2
Allele frequencies of drone fathers from each colony (Q1, Q2, Q3, Q4) for 6 variable microsatellite loci. Unique alleles for each colony were underlined.

	Alleles	Q.1	Q.2	Q.3	Q.4	Alleles	Q.1	Q.2	Q.3	Q.4	Alleles	Q.1	Q.2	Q.3	Q.4
Loci	Am46					Am128					Am491				
	116	0.00	0.00	0.00	0.07	196	0.10	0.17	0.13	0.17	100	0.13	0.37	0.00	0.00
	118	0.03	0.20	0.27	0.30	198	0.27	0.27	0.30	0.20	102	0.13	0.03	0.00	0.00
	120	0.13	0.17	0.13	0.07	200	0.43	0.13	0.27	0.00	104	0.30	0.27	0.17	0.00
	122	0.47	0.20	0.23	0.33	202	0.00	0.37	0.10	0.50	106	0.37	0.10	0.53	0.30
	124	0.13	0.17	0.10	0.13	204	0.20	0.07	0.13	0.00	108	0.07	0.13	0.27	0.47
	126	0.03	0.27	0.07	0.10	206	0.00	0.00	0.03	0.00	110	0.00	0.10	0.00	0.23
	128	0.07	0.00	0.20	0.00	208	0.00	0.00	0.03	0.00	112	0.00	0.00	0.03	0.00
	130	0.13	0.00	0.00	0.00	218	0.00	0.00	0.00	0.13	100	0.13	0.37	0.00	0.00
Loci	Am52					Am61					Am98				
	148	0.10	0.00	0.00	0.00	246	0.00	0.07	0.00	0.00	135	0.13	0.13	0.10	0.10
	150	0.00	0.00	0.03	0.00	248	0.03	0.03	0.00	0.13	137	0.00	0.23	0.27	0.17
	152	0.30	0.03	0.23	0.13	250	0.13	0.07	0.10	0.10	139	0.13	0.07	0.40	0.10
	154	0.30	0.20	0.27	0.20	252	0.67	0.77	0.83	0.47	141	0.13	0.30	0.10	0.50
	156	0.00	0.53	0.07	0.20	254	0.10	0.07	0.03	0.20	143	0.60	0.27	0.13	0.13
	158	0.07	0.13	0.07	0.13	256	0.07	0.00	0.03	0.07					
	162	0.00	0.10	0.30	0.13	264	0.00	0.00	0.00	0.03					
	172	0.13	0.00	0.03	0.20										
	176	0.07	0.00	0.00	0.00										
	178	0.03	0.00	0.00	0.00										

Table 3
Colony genetics statistics for drone fathers based on alleles from six variable microsatellite loci. Drone alleles inferred from worker genotypes using colony 1.2.

Colony	N	Na	Ne	Ho	He	F
Q1	30	6.33 ± 1.09	3.35 ± 0.34	0.08 ± 0.05	0.68 ± 0.04	0.89 ± 0.07
Q2	30	5.83 ± 0.31	3.59 ± 0.44	0.13 ± 0.05	0.70 ± 0.04	0.82 ± 0.06
Q3	30	6.17 ± 0.87	3.52 ± 0.64	0.44 ± 0.06	0.66 ± 0.07	0.29 ± 0.10
Q4	30	6.17 ± 0.61	3.60 ± 0.40	0.41 ± 0.06	0.70 ± 0.03	0.40 ± 0.10
Total	30	6.13 ± 0.36	3.52 ± 0.22	0.27 ± 0.04	0.69 ± 0.02	0.60 ± 0.07

N = Sample size, Na = No. of Different Alleles, Ne = No. of Effective Alleles = 1 / (Sum pi^2), Ho = Observed Heterozygosity = No. of Hets/ N, He = Expected Heterozygosity = 1 - Sum pi^2,F = Fixation Index = (He - Ho) / He = 1 - (Ho / He). Where pi is the frequency of the *ith* allele for the population & Sum pi^2 is the sum of the squared population allele frequencies based on colony® analysis.

sexuals development and mating flight. Duration of mating flights, which is higher than usual for European subspecies (Koeniger and Koeniger, 2007) is also affected by drones abilities to mate one after another with no complications (Woyke, 2016), whether drones of A. m. jemenitica need more time for mating or it is the impact of sample size and climatic conditions? is a question for research. Low queen mating number was also reported for island populations compared with main land population of the same honeybee subspecies, A. m. carnica (Neumann et al., 1999). Nevertheless, reported mating number in this study (~4) is still smaller compared to some previously documented low numbers (~6), which is stated as an inflection point of average intra-colony relatedness by Page (1980) and Palmer and Oldroyd (2000). Mating numbers documented in this study was also lower than the cutoff point of 7 mates per queen, which is reported to be relevant to colony survival rates and suggested that intra-colony genetic diversity as a consequence of queen mating number has significant impact on overall colony phenotype and longevity (Tarpy et al., 2013). However, increasing sample number will overcome any probable allele un-detectability.

Acknowledgements

The authors extend their appreciation to the Deputyship for Research and Innovation, "Ministry of Education" in Saudi Arabia for funding this research work through the project number IFKSURG-1442-126.

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