606. Evaluating the potential for mating control in honey bee breeding in three SE European countries (preliminary results)

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Abstract

The study was conducted to explore alternatives for mating control as a part of honey bee breeding programs for genetic improvement & conservation of local honey bee (*Apis mellifera*) populations in Croatia (HR), Macedonia (MK) and Slovenia (SI). We observed nuptial flights of 87 virgin queens (30 in HR, 35 in MK and 22 in SI) on potential sites. Mating success was related to the presence of drone producing colonies, but even at locations chosen for their isolation from known apiaries, mating success of 70% was achieved. On average, queens performed nuptial flights on 1.8 days in HR, 1.6 to 4.3 days in MK and 1.6 to 2.4 days in SI. Unsuccessful nuptial flights were two/threefold shorter than successful flights.

Introduction

Breeding programs for genetic improvement and conservation of local honey bee (*Apis mellifera*) populations have been in place for at least a decade in Croatia (HR), Macedonia (MK) and Slovenia (SI). However, the selection progress has been slower than expected due to the lack of efficient mating control as one of the crucial elements of the breeding programs (Uzunov *et al.*, 2017, Plate *et al.*, 2019). The mating behaviour of honey bees is complex: the queen performs nuptial flights and mates with 7-17 drones that can originate from many different colonies within a 10 km radius (Koeniger *et al.*, 2014). High colony density and lack of geographically isolated areas are recognised as the main factors for the unsatisfactory employment of mating control. However, by scouting possible isolated places or utilizing other means of mating control, such unfavourable situations may be counteracted. Such studies include identifying isolated locations by employing on-field observations and complementary laboratory investigations, investment in technical and human capacities in case of instrumental insemination, or intensive cooperation with surrounding beekeepers if an overflow with selected drones is the strategy. The project BeeConSel, funded by EEA & Norway Grants Fund for Regional Cooperation (2020-2023) provides an opportunity for investigation of alternatives for overcoming the deficiency of existing mating control. The preliminary results from the field investigations by observations on the queens' nuptial flights in the project's initial year are presented and discussed in detail in the next paragraphs.

Materials & methods

In June and July of 2021, the field investigations and data collection were conducted by direct observations. Virgin queens were placed into drone-free mini mating boxes (nucs) with around 700 worker bees and solid food, and kept 72 h in a dark and cool room. Forty-eight hours before the observations began, they were placed in the observation sites with an installed queen excluder on the entrance (Büchler *et al.*, 2013; Scheiner *et al.*, 2013). Nuptial flights were observed on 87 queens: 30 in Croatia (*A. m. carnica*), 35 in Macedonia (*A. m. macedonica*) and 22 in Slovenia (*A. m. carnica)*. The candidate locations were

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selected in each country based on the following main criteria: expected low density or absence of managed and/or feral colonies, geographical isolation, good logistical accessibility, and suitable weather/climate. In Croatia, observations were done on a flatland mating station (Batina, 240 m a.s.l.) flooded with drones originating from 96 drone producing colonies (DPC), headed by sister queens. No other managed colonies were expected to be present withing a diameter of 6 km. In Macedonia, the observations were done on three micro-locations in the frame of the mountainous region of Mavrovo plateau (>1,680 m a.s.l.). At the location MK-Belicki, 17 managed colonies were identified 2.5 km away at the end of observations. The locations MK-Crkva and MK-Cavkarnik were at 3 km air-distance from the next same apiary, that was lower in altitude by 400 m. Following the observations, the queens in the last two locations were allowed to freely fly for two additional days. Three locations were investigated in Slovenia in deep Alpine valleys: Vrata (1,020 m a.s.l.) with direct observation and no known managed/feral colonies present, Krma 0 (960 m a.s.l) with five DPC at the location and Krma 1 which was 1 km further in the valley at 1,015 m a.s.l. Three queens in Krma 0 were observed by video surveillance. The field study was done following a tailored protocol for direct observation (Uzunov *et al.*, *in preparation*). For at least 5 days from 11:00 to 17:00 queens' nuptial flight parameters were monitored and recorded: flight frequency, flight duration, observed mating sign and weather conditions. The direct observations were done by installing an extension on the mating box entrance with a transparent cover and queen excluder allowing observation and control of any queen flight attempt (Koeniger, 1981). At the time of the field studies, the weather conditions were suitable in all sites, although with noticeable variations (particularly wind) between the micro-locations in Macedonia.

Results

The number of observed, active and mated queens, the average number of flying days and flight frequency, as well as the queen's average flight duration with or without the mating sign per country and location are presented in the Table 1.

A mating success of over 76% was achieved in HR-Batina where 96 DPCs were placed and over 91% in MK-Cavkarnik where an apiary was at a distance of 3 km. In contrast, the mating success in SI-Krma 1, where 5 DPCs were present 1 km away from the locations with mating boxes, was only 56%. In SI-Vrata,

Table 1. Descriptive statistics of the main parameters from the observations. The minimum and maximum values are given in round brackets.

	Number of queens		Aver. per queen		Aver. flight dur./queen**	
Country	Observed [Flew]	Mated* [%]	Flying days	Flight frequency	w/o mating sign (min)	with mating sign (min)
HR-Batina[1]	30 [28]	23 [76.7]	1.8 (1-3)	3.8 (2-6)	8 (1-28)	14 (4-22)
MK-Belicki[2]	12 [12]	5 [41.7]	4.3 (2-6)	8.8 (2-16)	6 (1-21)	22 (8-32)
MK-Crkva[2]	11 [9]	6 [54.5]	1.6 (0-3)	2.6 (0-7)	7 (1-55)	19
MK-Cavkarnik[2]	12 [11]	11 [91.7]	2.2 (0-3)	3.9 (0-10)	6 (1-20)	12 (3-18)
SI-Vrata[3]	10 [7]	7 [70.0]	2.4 (1-5)	2.8 (0-5)	11 (1-32)	26 (18-34)
SI-Krma 1[1]	9 [5]	5 [55.6]	2.4 (2-4)	2.6 (0-10)	10 (2-29)	20 (17-23)
SI-Krma 0[1,4]	n.a.	n.a.	1.6 (1-2)	2.3 (0-3)	5 (3-9)	11 (3-18)

[1] With the presence of DPC at the mating station.

[2] Managed/feral colony(ies) found at the location.

[3] No managed /feral colony(ies) found at the location.

[4] Video observed, video analysis incomplete, *Values based on sampled sealed worker brood, **Values based on partial or complete observations of queens' nuptial flights.

where no managed or feral colonies were known to exist 70% of the virgin queens succeeded in mating. In the remaining two Macedonian locations MK-Belicki and MK-Crkva, the mating success was below 55%.

The average number of flying days per queen and the average flight frequencies were in the expected biological range except the high values in MK-Belicki (average of 4.3 flying days and 8.8 flights per queen). The lowest values for average number of flying days were recorded in HR-Batina (1.8) and SI-Krma 0 (1.6). Almost on all locations, the average flights' duration per queen returning to the mating box without a mating sign were two to threefold shorter than the flights with a mating sign. The highest average differences of 3.7 times, between flights with and without observed mating sign, was recorded in MK-Belicki.

Discussion

In HR-Batina, where the location was saturated with a sufficient number of drones, the results of high mating success (76.7%) show the importance of the availability of a high number of mature airborne drones. Under such conditions, the queens flew on a low number of days (1.8) and had a regular flight frequency (3.8) per queen, reducing the risk of additional flights and engendering possible losses, clearly listing the location as a good candidate for setting up a mating station, and making the approach of 'drone flooding' appear promising. With a queens' flight speed of around 20 km/h (Koeniger *et al.*, 2014) and an average flight duration of 14 minutes for those returning with a mating sign, we estimated an average distance of 1.5 km to the potential mating site(s), taking into account the time of around 5 min for the mating *rendezvous* with the drones (Koeniger *et al.*, 2014). Still, a successful flight as short as 4 minutes indicate that the queen was mated close by.

With five DPCs employed at the locations SI-Krma 1, partial mating success was obtained with 56%. According to Tiesler *et al.* (2016), the required number of DPCs ranges from 8 for 50 virgin queens to a minimum of 20 DPCs for 500 virgin queens. Nevertheless, due to the small sample size, the results from SI-Krma 0 needs to be interpreted with caution even though the flight activities indicate that the queens' mating was efficient. On the other hand, the unsatisfactory results from SI-Krma 1 might be a consequence of the ambiental conditions with brief sunny periods due to high mountain walls or rough transportation to the location. Finally, the results from the supposedly drone-free location SI-Vrata, show that there seems to be a significant drone 'noise' from the surrounding area; however, the average duration of mating flights – both with or without a mating sign, was the longest of all studied locations. This may indicate that distance alone is not always sufficient to predict the suitability of a candidate location.

In Macedonia, we recognised the possibility of using MK-Belicki as the most prosperous location for establishing a reliable mating station. All queens were flying but only 42% of queens (n=5) were producing worker brood. The above-average number of flying days and high average frequency of 8.8 flights per queen indicate a queen's excessive efforts for finding partners (Uzunov *et al.*, 2014) even under the fact that 17 managed (but not DPC) colonies were later discovered 2.5 km away. At MK-Crkva, almost 82% of the queens flew but 55% of the queens (n=6) successfully produced worker brood even with the existence of an apiary in the vicinity of 3 km. However, the queens' flight activities were lower and similar to the locations saturated with drones. The persistent wind occurrence recorded during the observations might affect queens flight performance at this location (Koeniger *et al.*, 2014). MK-Cavkarnik, with the highest mating success (91.7%) among the Macedonian locations, even it is on the same distance from the next apiary as MK-Crkva is (but in different direction), seems an unsuitable choice for setting up a mating station. Such prediction is also based on the queens' flight activities and in particular, the short duration of 3 minutes for the performed successful flight in MK-Cavkarnik. Koeniger and Koeniger (2007) and Heidinger *et al.* (2014) found shorter mating flight duration in an area saturated with drones, while in the area with a low number of colonies, mating flights were longer (Koeniger *et al.*, 1989). The obvious relationship between

the duration of mating flights and the number/vicinity of potential mating partners, also observed in the present experiment, suggest that this parameter can be used as an additional indicator for judging of the suitability of a location for isolated mating.

In order to answer the question of the achievable mating purity with greater certainty, an analysis of the drone's origin and semen composition are indispensable. This information will be available before the beginning of the next beekeeping season. Therefore, in the following two seasons, the activities will be focused on verifying the current locations using sufficient DPCs or exploring new sites by observations on the queens and drones' nuptial flights.

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