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BeeConSel - Joint Effort for Honey Bee Conservation and Selection

DELIVERABLE 5

Pilot mating control in situ

BeeConSel is funded by Iceland, Liechtenstein and Norway through the EEA and Norway Grants Fund for Regional Cooperation.

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BEECONSEL Joint Effort for Honey Bee Conservation and Selection

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EXECUTIVE SUMMARY

Mating control is a complex and important element in any honey bee breeding program. The countries from SE Europe face major obstacles for systematic use towards genetic improvement and conservation of the native or local stock. Accordingly, the BeeConSel project provided great opportunities for *in situ* tests of all models of mating control, considering environmental, socio-economic and beekeeping-related aspects.

Thorough on-field studies, which included 1184 mating boxes with virgin queens and 459 drone-producing colonies on 16 locations, were undertaken to overcome the lack of mating control in the honey bee breeding programs in the beneficiary countries, Croatia, N. Macedonia and Slovenia.

The results from the field test and direct observations of the queens' mating (nuptial) flights, including testing of the avantgarde Jo Horner method, indicate a breakthrough towards feasible solutions for establishing mating control as a systematic tool in the breeding programs of the beneficiary countries. Therefore, in each partner country, a few locations (2 in Croatia, 3 in N. Macedonia, and 2 in Slovenia) were suggested as suitable for establishing isolated mating stations with a low risk of genetic "pollution" from drones of unknown origin. In addition to selecting a suitable, isolated location, a key point seems to be the number of prolific drone-producing colonies, also indicated by the long mating flights observed in Norway where the density of colonies is low, a conclusion that is of great importance to beekeepers.

The results from the test of the delayed time mating flight models (Jo Horner cooling and labyrinth) in N. Macedonia, Norway and Slovenia show a great potential for utilizing of this novel approach under conditions where no bee-free locations are available.

An additional massive specimen collection, including queens, their brood, and samples from the drone-producing colonies, was established to substantiate the obtained results.

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FIELD TESTING

The genetic improvement and conservation of honey bee (*Apis mellifera* L.) populations crucially depend on the systematic utilization of mating control (Du et al., 2021, 2023; Uzunov et al., 2022). Mating stations, instrumental insemination and the delayed time mating flight models are feasible and, at the moment, available approaches for achieving mating control in honey bees (Musin et al., 2021; Uzunov et al., 2022). Still, there are significant differences between these approaches concerning their utilization. Nevertheless, implementing and managing these breeding instruments requires extensive time, resources, and capacity building.

Croatia, N. Macedonia and Slovenia, the beneficiary countries of the project, are characterized by similar beekeeping practices and traditions but also by different environmental and logistical aspects that bridge the challenge of lacking functional mating control practices in the respective honey bee breeding programs. Therefore, in the course of the BeeConSel project, massive field, laboratory and desk research activities were conducted for:

i. Identification of suitable isolated areas for establishing a mating station with reliable mating control
    a. Geographical (G)
    b. Biological (Saturation) (S)
ii. Testing of novel delayed time mating flight model
    a. Cooling (TC)
    b. Labyrinth (TL)
iii. Capacity building for utilization of instrumental insemination (II)

The labyrinth model was tested in Norway as an expert country and the most northern region within the project.

The field testing was preceded by intensive training of the research and technical staff and the involved targeted groups (beekeepers and students). In the spring of 2021 in N. Macedonia, NBA, in cooperation with CARPEA, organized a customized on-field training for identifying isolated areas, drone congregation areas (DCA), direct observations on nuptial flights and data recording and analysis (Deliverable 2). In addition, a detailed protocol for observation of nuptial flight, including a data recording sheet and a digital file, was developed and used in the course of all testing seasons (Deliverable 3). Finally, the protocol was published as a scientific paper (Uzunov et al., 2023; Annex).

Table 1. Total data of the number of used mating boxes/queens, DPC, observations and locations by mode of mating control, country, and year from 2021 to 2023.

	Year	Mode of mating control*	Location	Concept	Micro-locations	Mating boxes/ queens	DPC	Observations (No/Direct/Video)
HR	2021	G/S	Batina	Flatland	1	120	96	Direct
		G	Gorski kotar	Deep forest	1	40	0	No
	2022	G/S	Batina	Flatlands	1	80	96	No
		G	Gorski kotar	Deep Forest	1	30	6	Direct
	2023	G	Batina	Flatland	1	30	96	No
	Total					300	294	
MK	2021	G+TC+TL	Mrshevci		1	40	0	Direct**
		G	Krivolak	Flatland	3	30	0	No
		G	Krivolak	Flatland	2	16	0	No
		G	Galicica	Highlands	1	16	0	No
		G	Mavrovo	Highlands	3	36	0	Direct
		G	Mavrovo	Highlands	2	18	0	No
		TC+TL	Mrshevci	Horner	1	42	0	Direct
	2022	G	Ravna Reka	Highlands	1	23	0	No
		G	Nikiforovo	Highlands	1	31	35	No
		TC+TL	Radishani	Horner	1	41	13	Direct
		G	Toranica	Highlands	1	29	20	No
	2023	TC+TL	Radishani	Horner	1	28	10	Direct
		G	Toranica	Highlands	1	30	10	No
		G	Snake Island	Island	1	24	14	No
	Total					404	102	
SI	2021	G	Krma	Alpine	5	30	5	Direct+Video
		G	Krma	Alpine	5	30	5	Direct+Video
		G	Vrata	Alpine	5	30	0	Direct
		G	Vrata	Alpine	5	30	0	Direct
	2022	G	Krma	Alpine	3	30	5	Direct+Video
		G	Krma	Alpine	3	30	5	Direct+Video
		G	Vrata	Alpine	2	30	4	Direct
		G	Vrata	Alpine	2	30	4	Direct
		TL	Ljubljana	Horner	1	30	0	Direct
		TL	Ljubljana	Horner	1	30	3	Direct
	2023	G	Vrata	Alpine	2	30	8	Direct
		G	Vrata	Alpine	2	30	0	Direct
	Total					360	39	
NO	2022	TL	Ås	Horner	1	30	6	Direct
		TL	Ås	Horner	1	30	6	Direct
	2023	TL	Aurskog	Horner	1	30	6	Direct
		TL	Aurskog	Horner	1	30	6	Direct
	Total					120	24	
					Grand Total	**1184**	**459**	

**Mode of mating control: G: geographical isolation, S: biological isolation with many DPCs, TC: delayed mating flight with cooling method, TL, delayed mating flight with labyrinth method.*
***Training session.*

In total, 1184 mating boxes/queens, 459 drone-producing colonies (DPC) and 16 locations (28 micro-locations) were used for training and field testing in 2021, 2022 and 2023 in Croatia, N. Macedonia, Slovenia, and Norway. Table 1 shows the total data by mode of mating control, country, and year.

The *survival rate* is a parameter that may provide information about the overall suitability of the given mode under specific environmental conditions; for instance, low survival rates can occur due to the presence of harsh weather conditions, predators, lack of food sources etc. In addition, this parameter is affected by drone saturation. Higher survival rates are expected when enough vital airborne drones are available (sufficient number of DPC). When testing a location for the presence of feral or drones from a not-known origin, queens' survivability shows the efforts acquired in finding mating partners.

The *mating success* is a more exact parameter indicating the suitability of the employed mating mode or location. When no drones from the known origin are present, lower mating success promotes the tested location as drone-free, which is the required condition. When sufficient DPCs are supplied on a selected location or a particular mating mode is used, a higher mating success indicates location/mode suitability.

The *flight duration* average and rank of the successful matings contribute to a better understanding of the potential mating place's distance (but not direction). The average queen's flight speed is considered to be twenty km per hour and is used to index the observed flight duration (Table 2) and to calculate the possible flight distance.

Croatia

In Croatia, two concepts for mating control were tested: “flatland” and “deep forest” (Figure 1). The "flatland" mating station, located near the village Batina, is partly isolated with 10-20 documented honey bee colonies within a 5-10 km distance. Controlled mating was tested by saturating the area with drones. To achieve this, 96 drone producing colonies, headed by sister queens from the same mother queen, were installed. In each colony, six to eight drone brood frames were inserted throughout the season to produce a large number of drones.

The average survival rate of queens at 94% over three seasons demonstrates the high success of the preparation and manipulation of mating boxes with virgin queens. The average mating success rate of 73 % aligns with expectations for this setup and is consistent with the average success among breeders in Croatia (Deliverable 1). The average mating flight duration of 14 minutes indicates that the queens could perform several matings in a short time period. This is further confirmed by the shortest successful mating flight, which lasted only 3 minutes.

Figure 1. Mating station in flatland (left) and deep forest (right).

The "deep forest" location was chosen for its isolation. During winter and spring, this area is less favoured by beekeepers due to a lack of food for colonies, which affects their development. Consequently, there are no beekeepers in this area until the fir trees pasture at the start of summer.

This location was tested as a potential mating station over two seasons.

In 2021, 40 mating boxes were installed without any drone-producing colonies. The resulting average survival rate of queens was 55 % and the mating success rate was 27.5 %. The low mating success suggests that the area lacked a sufficient number of drones for the queens to mate with. The low survival rate and the number of virgin queens that failed to mate imply that some queens were lost during extended mating flights.

In 2022, 30 mating boxes and 6 drone producing colonies were installed. This arrangement increased the survival rate to 77 % and the mating success rate to 87 %. Some queens were lost due to transportation over approximately

450 km. However, the mating success achieved was significantly higher than that in the flatland, where the success rate was 68 % in the same season.

Based on these results, the flatland location, which is partly isolated and saturated with many drones of known origin, could be effective as a mating station. This mating station in the deep forest could be used early in the season, around May, when there are no other honey bee colonies present. Installing at least 6 drone producing colonies is recommended, but more colonies would most probably increase the success of mating control.

N. Macedonia

In N. Macedonia, the test locations (Figure 2) Krivolak, Mavrovo, Galicica and Ravna Reka (where no DPCs were provided) are considered unsuitable for establishing or use as mating stations. This is based on the results in which the higher survival rates and relatively good mating success were observed although no DPCs were provided. However, with some exceptions for micro-locations in Mavrovo, mating control is possible with sufficient drone saturation.

Figure 2. DPC on a Snake Island in 2023.

Toranica and Nikiforovo locations were tested in the presence of DPCs and with a minimum of 3 km air distance from known neighbouring apiaries. In Toranica, the high survival and mating rates show that this location could be exploitated as a mating station. The lower survival rate in Nikiforo, which has a very high altitude and northern exposition, could be a consequence of bad weather conditions during the mating period. However, the high mating success of the surviving queens is a positive outcome. Therefore, considering the advantageous logistical conditions, both locations have great potential for establishing mating stations.

Snake Island, as the most isolated location geographically (2 km of water belt) and with an unexpectedly high survival rate, is a prosperous alternative location. Still, the lower mating success shows a need for increased drone saturation (more DPCs). DPCs, particularly adult drones, may suffer from unfavourable environmental conditions such as low pollen availability and wind.

Using the labyrinth and cooling approach, we also tested the novel delayed time mating flight model in N. Macedonia. Although significant progress was achieved in managing and the technical improvement of the methods, the lower mating success needs further improvement. This method can be a reliable, although costly, alternative, as confirmed by the survival rate, which is relatively similar to other methods, and the expected lower mating success during periods when mating is not natural.

Finally, due to the highly collaborative environment and exchange of experiences with other project partners, instrumental insemination was introduced and established for the first time in N. Macedonia as a systematic approach for achieving mating control.

Slovenia

Slovenia used the orographical features of the Julian Alps to investigate the concept and value of the »Alpine« mode of geographical isolation. Within the idea is the use of natural deep alpine valleys surrounded by high slopes, which should at least hypothetically prevent the drone »cross-talk«. After careful consideration with the assistance of a Norwegian partner, two valleys were chosen. In the first valley, Krma, a close cooperation with a resident beekeeper and queen breeder was established; a local agricultural cooperative assisted with the selection of locations in the second valley, Vrata.

The two locations are very similar in physical features, giving the possibility of comparing the different experimental situations. In the first year, we kept valley Vrata without drone-producing colonies (DPCs), while in Krma DPCs are permanently present due to the activities of the above-mentioned queen breeder. We have noted that in Vrata - despite the DPC absence - queens in the experimental mating nuclei got mated in an unexpectedly large proportion (67 % of survivors) compared to Krma, with a known presence of DPC and 75 % of survivors got mated. These results indicate that despite the remoteness and low human population in Vrata, this valley is not isolated in terms of presence of honeybees. However, the time queens invested on risky flights to get mated in the valley without DPCs was more than double compared to the valley with DPCs (27 min vs 12 min on average), indicating that drone density and likely the foreign colony presence wasn't high.

In the following year, DPCs were also installed in the valley of Vrata, which cut down the flight time and exposure of queens to predation to 16 minutes and increased the success rate of mating to 86 %. This manipulation demonstrates that queens do not engage on lengthy and likely risky long-distance flights in the presence of drones. Further increasing the number of DPCs in Vrata in the last season further decreased the flight time (13 minutes on average), indicating that our assumptions were correct. Accepting that changes in the mating behaviour of queens are a result of our simple manipulation with number of installed DPCs, this marks the possibility of future use of Vrata valley as a mating station.

Figure 3. Future leaders: frame with queen cells.

The avant-garde method of delayed time mating flight model using a labyrinth was tested in rural area of Ljubljana marsh as a part of a student thesis and in close cooperation with other consortium partners and the advisory group. In this region, queens have their normal peak of mating activity around 15:00. In order to move this peak to a different time frame, specially adapted nucleus hives were used to achieve the restraining of queens' instincts. Two different later time intervals were used, one at 17:17 and the second at 18:17. In the first round without time-manipulated DPCs, the second test group had lowest mating success rate compared to the control (60 % vs 83 %). In the second round, we added three time-manipulated DPCs, which were opened at same time as the first test group. The success rate improved slightly (70 % vs 86 % in control). The fact that the success rate was high - in the range of normal mating success rate - in the first round without added DPCs, clearly demonstrates the presence of satisfactory number of drones in the environment. The location in Ljubljana doesn't have geographical features such as high mountain faces; therefore, we expect that drones spread out evenly. Domination of manipulated drones is not guaranteed at this number of DPCs used.

Norway

In Norway, we tested the avant-garde method of delayed time mating flight to obtain mating control. In 2022, the experiment was carried out in an area with a high density of surrounding colonies and 6 DPCs. Based on the absence of drones following the bait with queen pheromones after 17:30, queens in the mating boxes and drones in the DPCs were released to fly from 18:00. Cold and wet weather during the experiment probably contributed to the low mating success both in the experimental group of queens and in the group of control queens that were used, for the first time in Norway, to obtain data on natural mating behaviour of honey bees. The labyrinth in the set-up used in 2022 was not optimal as also worker bees in several of the mating boxes failed to show normal flight activity.

In 2023, we modified the placement of the labyrinth in the mating boxes and repeated the experiment in an area with low density of honey bee colonies and using 6 DPCs. The first round of the experiment was performed during optimal weather conditions for honey bee mating and survival and mating success was 100 % in the control group. The experimental group had lower survival (69 %), but the mating success of the surviving queens was 100 %. The second part of the experiments was performed in cold and wet weather, but both survival (100 %) and mating success (92 %) was high in the control queens. In the experimental group, survival was also high (100 %) but only 33 % of the queens performed nuptial flights and mating success was 8 %.

Figure 4. Experimental apiary for testing delayed time mating control in Norway.

Molecular analyses will reveal to what extent the queens that were released to mate after 18:00 actually mated with drones from the DPCs. However, in Norway, honey bee mating success is susceptible to harsh weather conditions in general, and restricting the matings to the early evening further decreases the odds to obtain high mating success. The method can be applied successfully under optimal weather conditions, and the mating mode can easily be changed to open mating if the weather is sub-optimal for delayed time mating.

Overall, the literature (Uzunov et al., 2014) and the project results concerning the flying time duration of the successful matings are reversely proportional to the density of available airborne drones. Therefore, an increase in the number of available and well-prepared DPCs leads to shortened flight durations, expected lower queen loss rates and higher mating success, and the overall economic feasibility of the controlled mating as a service for the beekeepers. The scientific outcomes from the field data generated in WP3 in detail will be presented and discussed in the forthcoming article by Uzunov et al., in preparation. In general, the successful mating flights in Norway were longer than those recorded in N. Macedonia, Croatia and Slovenia. This might reflect the lower density of colonies and thereby drones in Norway.

The results from the molecular analysis will verify the findings reported here from the field and observations study. For more details, please check chapter Sampling from this report and the subsequent Deliverable 6.

Table 2. The successful mating rate by mode. The table contains data from the direct observations and data from the inspections (without observation). NO – No observations. Mode of mating control: G: geographical isolation, S: biological isolation with many DPCs, TC: delayed mating flight with cooling method, TL, delayed mating flight with labyrinth method.

	Year	Mating control	Location	Mating boxes	DPC	Survival rate (%)	Mating success (%)	Ave flight time (min)
HR	2021	S	Flatland	165	96	96	75	14 (3 - 22)
		G	Deep Forest	40	0	55	27,5	NO
	2022	S	Flatland	130	96	92	68	NO
		G	Deep Forest	30	6	77	87	16 (7 - 26)
	2023	S	Flatland	40	96	95	75	NO
NMK	2021	G	Krivolak	30	0	83	100	NO
		G	Krivolak	16	0	94	60	NO
		G	Galicica	16	0	81	92	NO
		G	Mavrovo	36	0	81	45	16 (3-32)
		G	Mavrovo	18	0	61	100*	NO
		TC+TL	Mrshevci	42	0	79	36	17 (13-24)
	2022	G	Ravna Reka	23	0	83	84	NO
		G	Nikiforovo	31	35	57	95	NO
		TC+TL	Radishani	41	13	42	53	18 (8-28)
		G	Toranica	29	20	90	100*	NO
	2023	TC+TL	Radishani	28	10	75	48	19 (17-20)
		G	Toranica	30	10	83	88	NO
		G	Snake Island	24	14	88	48	NO
SI	2021	G	Krma	60	5	88	75	12 (0 - 35)
		G	Vrata	60	0	100	100	27 (18 - 34)
	2022	G	Krma	60	5	82	75	15 (4 -21)
		G	Vrata	60	4	90	86	16 (6 - 22)
		TL	Ljubljana	30	3	100	80	16 (6 - 36)
		TL	Ljubljana	30	0	90	90	26 (7 - 26)
	2023	G	Vrata	30	8	88	75	13 (4 - 21)
		G	Vrata	30	0	91	91	22 (19 - 26)
NO	2022	Control	Ås	10	0	50	100	28 (23-40)
		TL	Ås	20	6	90	0	NO
		Control	Ås	15	0	83	100	24 (15-40)
		TL	Ås	15	6	75	17	24 (17-30)
	2023	Control	Aurskog	15	0	100	100	21 (14-32)
		TL	Aurskog	15	6	69	100	25 (12-43)
		Control	Aurskog	15	0	100	92	26 (14-48)
		TL	Aurskog	15	6	100	8	19 (15-25)

*an additional dead queen was found and sampled from the mating box.

MAPPING

On the following maps (Figure 5 to 7), all tested locations are marked with indications of suitability for conducting mating control.

Figure 5. The locations of field testing in Croatia and suitability assessment for mating control. Red – unsuitable, yellow – possible, green – suitable.

Figure 6. The locations of field testing in N. Macedonia and suitability assessment for mating control. Red – unsuitable, yellow – possible, green – suitable.

Figure 7. The locations of field testing in Slovenia and suitability assessment for mating control. Red – unsuitable, yellow – possible, green – suitable.

SAMPLING

In addition to the above-reported results from the field testing and sampling, the molecular analysis will provide further critical insight (D6). The following tables (Tables 3 to 6) summarize the number of samples (where each sample represents one colony with many individual specimens - worker or drone brood, queen) provided by year, country, mating control mode, and location. More detailed information regarding sampling will also be provided in D6.

Table 3. Total data of the number of sampled colonies by year and country for the period from 2021 to 2023.

Country	Count of sampled colonies
2021	
Croatia	268
N. Macedonia	121
Slovenia	44
2022	
Croatia	224
N. Macedonia	133
Norway	46
Slovenia	185
2023	
Croatia	126
N. Macedonia	91
Norway	40
Slovenia	68
Grand Total	**1342**

Table 4. Total data of the number of sampled colonies by mating control mode for the period from 2021 to 2023.

Mating control mode	Count of sampled colonies
Alpine	258
Deep Forest	37
Flatlands	580
Highlands	234
Horner	213
Island	24
Grand Total	**1343**

Table 5. Total data of the number of sampled colonies by year, country and mating control mode for the period from 2021 to 2023.

Location & sample type	Count of sampled colonies
2021	
Croatia	
Deep Forest	
mating_nuc	11
Flatlands	
DPC	137
mating_nuc	120
N. Macedonia	
Highlands	
mating_nuc	93
Horner	
mating_nuc	28
Slovenia	
Alpine	
DPC	5
mating_nuc	39
2022	
Croatia	
Deep Forest	
DPC	6
mating_nuc	20

Location & sample type	Count of sampled colonies
Flatlands	
DPC	100
mating_nuc	98
N. Macedonia	
Highlands	
DPC	40
mating_nuc	64
Horner	
DPC	12
mating_nuc	17
Norway	
Horner	
DPC	5
mating_nuc	41
Slovenia	
Alpine	
DPC	26
mating_nuc	54
observation_nuc	67
Horner	
mating_nuc	39

Location & sample type	Count of sampled colonies
2023	
Croatia	
Flatlands	
DPC	30
mating_nuc	96
N. Macedonia	
Highlands	
DPC	15
mating_nuc	22
Horner	
DPC	10
mating_nuc	21
Island	
DPC	13
mating_nuc	11
Norway	
Horner	
DPC	6
mating_nuc	34
Slovenia	
Alpine	
DPC	26
mating_nuc	42
Grand Total	**1343**

Table 6. Total data of the number of sampled colonies by mode of mating control and locations for the period from 2021 to 2023.

Mating control mode & Location	Count of sampled colonies
Alpine	
Krma	75
Lučka Bela	62
Vrata	121
Deep Forest	
Gorski kotar	37
Flatlands	
Batina	580
Highlands	
Galichica	13
Kovanec	5
Krivolak 1	17
Krivolak 2	13
Krivolak 3	9
Mavrovo 1	12
Mavrovo 2	14
Mavrovo 3	11
Mavrovo 3/2	1
Mavrovo B	1
Mavrovo LB	1
Mavrovo Y	1
Nikiforovo	40
Ravna Reka	17
Toranica	79
Horner	
Ås	46
Aurskog	40
Ljubljana	39
Mrshevci	28
Skopje	60
Island	
Golem grad	24
Grand Total	**1343**

ANNEX

Aleksandar Uzunov, Sreten Andonov, Bjørn Dahle, Marin Kovačić, Janez Prešern, Goran Aleksovski, Magdalena Jovanovska, Borce Pavlov, Zlatko Puškadija, Jakob Wegener, Egoitz Galartza, Ricarda Scheiner & Ralph Büchler (2023) Standard methods for direct observation of honey bee (*Apis mellifera* L.) nuptial flights, Journal of Apicultural Research.

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Standard methods for direct observation of honey bee (*Apis mellifera* L.) nuptial flights

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ABSTRACT
Honey bees (*Apis mellifera*) have a peculiar and complex reproductive biology, with queens being polyandrous and mating with several drones during one or more mating (nuptial) flights in so-called drone congregation areas. Observing the virgin queens' and drones' flight behaviour provides data to understand and interpret a portion of the honey bees' complex reproductive process. Observing the behaviour of the virgin queens on the hive entrance also serves to estimate the distance from the mating place or potential drone congregation areas (DCAs) as well as to detect the presence of airborne drones in the area.
In this paper, we provide a detailed description of the methodology used for observing queens' and drones' flights during the period of expected mating. In addition, we provide information about required equipment, tools as well as step by step description of the observation and recordkeeping process.

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Honey bee; mating; observation

Introduction

Honey bees (*Apis mellifera*) have a peculiar and complex reproductive biology, with queens being polyandrous and mating with several drones during one or more mating (nuptial) flights in so-called drone congregation areas (Woyke, 1955; Estoup et al., 1994; Palmer & Oldroyd, 2000; Tarpy et al., 2004; Tarpy et al., 2010). Young virgin queens leave the colony for three different behaviours: orientational flights, nuptial flights, or leaving the colony with an "after-swarm", i.e., a swarm that leaves after the fertile queen has left with a primary swarm (Winston, 1987; Kryger & Moritz, 1997). In parallel, drones also leave the colony to orient themselves, perform cleansing flights or perform nuptial flights (Reyes et al., 2019; Ayup et al., 2021). In case of successful mating, the respective drone dies. However, the sperm he passed to the queen is kept alive within the queen's spermatheca and used for worker or queen production. Unsuccessful drones have a short lifespan, which rarely exceeds six weeks (Page & Peng, 2001). The availability of young queens and drones corresponds to the swarming period, which occurs in the season(s) with abundant food resources, i.e., nectar and pollen. The swarming seasons and in some regions the so-called supersedure seasons vary among regions, reflecting seasonal resource availability.

Observing the virgin queens' flight behaviour provides data to understand and interpret a portion of the honey bees' complex reproductive process. In addition, observing the behaviour of the virgin queens on the hive entrance also serves to estimate the distance from the mating place or potential drone congregation areas

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