Evaluation of Drone Migration on Mating Station Torfhaus (DE-6-14) in 2020 by Cubital Index Analysis

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Abstract

The western honeybee Apis mellifera can be subdivided into different subspecies, like carnica, and breeding lines, i.e. "Carnica-Troisek" (C-T). In order to safe this lines and subspecies, typical characteristics are determined for each. To prevent mating of queens with unspecific males, restricted mating stations for breeding lines and subspecies exist. These mating stations are registered and protected by the local authorities. Consequently the setting of unfamiliar colonies in the protection zone of mating stations is prohibited, to prevent the mating of virgin queens with unfamiliar drones and. We analyzed drones of 13 colonies, selected for the mating station Torfhaus (DE-6-14) in Lower Saxony by their cubital index. During the summer 2020 a shift in the drones' characteristics was obtained. The shifts dynamic suggests the migration of external drones. Our results underline the expressiveness of an easy, reliable method to detect migration of external drones in selected drone hives of mating stations. Moreover, we could show, that migration of external drones decreases during the summer, according to the abundance of drones in typical hives, which are not especially prepared for mating stations. Finally, we assume that early and late periods in the beekeeping season may promise a higher safety on emerged land mating places.

Keywords — *Cubital index, Apis mellifera subsp. carnica, breeding, mating station, morphometric analysis*

I. INTRODUCTION

The western honeybee (*Apis mellifera* L.) is the most famous bee in beekeeping all over the world. Its use for mankind is indispensable, because of its rule in pollination [1]. According to its distribution area, many different subspecies have evolved among Europe, Asia and North Africa [2, 3].

Within these subspecies, beekeepers focused on selection of specific behaviors and characteristics [4, 5]. However, queens are able to produce fertilized eggs, which develop into female, diploid individuals (worker and queens) due to heterozygous sex alleles. Unfertilized eggs develop into males, called drones due to the hemizygous sex allele. Sexually mature drones from different colonies fly to drone congregation areas (DCA) in the circumference of its colonies. Virgin queens are mated at these DCAs in the flight by up to 30 different drones [6, 7].

In order to prevent mating of selected virgin queens from specific subspecies or breeding lines with unspecific drones, official mating yard are established. These mating stations are surrounded by a protection zone, which in beekeeping is restricted by the local authorities. DCAs in protection zones should only be visited by selected drones of the respective mating station [5].

Mating stations differ by the rules of its protection zone. Some are used for the breeding of a specific line; in these protection zones the keeping of bees is strictly forbidden. Other are used for the breeding of a subspecies; in these protection zones the keeping of this specific subspecies is allowed. However, breeding stations on mainland are less safe than islands, which are less safe than instrumental insemination [8].

Typical characteristics for subspecies and breeding lines of *A. mellifera* are listed in the rules of specific organizations (i.e. Deutscher Imkerbund e.V.). Typical characteristics were conducted [2] and used for breeding rules in extracts. Mainly the cubital index (CI) of the fore wing is used to distinguish between the subspecies of capital importance (Fig. 1). Moreover, characteristics of the abdomen can be used.

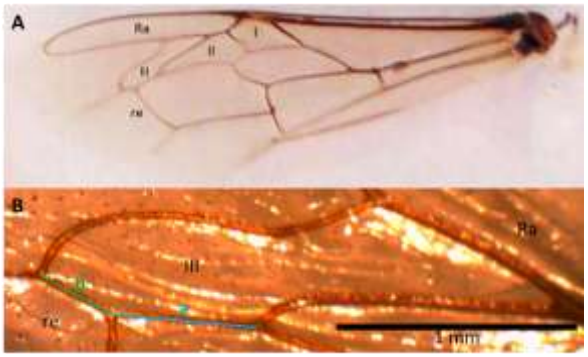

Fig. 1: Measurement of cubital index. The cubital index (CI) is measured at the third cubital cell on the fore wing. A, fore wing of an *A. mellifera* drone, showing the radial cell (Ra), nervus currens (re) and the first, second and third cubital cell (I, II, III). B, detailed view on the third cubital cell. CI is calculated by the ratio of a:b.

The Lower Saxony State Office for Consumer Protection and Food Safety (LAVES), Institute for Apiculture is responsible for three mating stations: (i) Neuwerk (DE-6-2) on an island in the North Sea, (ii) Torfhaus (DE-6-14) in the mountain Harz (Fig. 2) and (iii) Rehwinkel (DE-6-10) in a normal country side. A specific population of *A. melifera* subsp. *carnica* breeding line Troisek, called *Celler Linie* ("C-T Celle") is bred by the Institute for Apiculture with the ID DE-6-1-####-YEAR.

Fig. 2: Schematic-geographical location of DE-6-14. Two mating stations are located next to each other in the average mountain Harz in Lower Saxony. T, mating yard Torfhaus (DE-6-14), surrounded by a theoretical flight radius of 5.0 km (yellow) and the theoretical protection zone of 8.0 km (red). N, mating yard Lautenthal (DE-6-17), surrounded by a theoretical flight radius of 5.0 km (yellow) and its theoretical protection zone with a radius of 6.0 km (red).

In order to an increase of beekeeping in Germany and reports on unspecific successors of queens, mated on DE-6-14, we analyzed its drones in 2020 by their CI, during the breeding seasonThis document is a template. An electronic copy can be downloaded from the conference website. For questions on paper guidelines, please contact the conference publications committee as indicated on the conference website. Information about final paper submission is available from the conference website.

II. MATERIAL AND METHODS

Colony and mating station management. Torfhaus is an official mating station (code DE-6-14) for Apis mellifera subsp. carnica in Lower Saxony's mountain Harz. It is used for the breeding line "C-T Celle" only by the LAVES Institute for Apiculture. Thirteen hives (1b) (Tab. 1), daughters of one selected queen (DE-6-1-0332-2017) (4a), were chosen as drone hives for DE-6-14 in 2020. Queen DE-6-1-0332-2017 (4a) was mated with f1 drones (4b) of DE-6-1-0462-2014 (12a), in 2017 on Neuwerk (code DE-6-2), the island mating station. Each colony was prepared with two drone combs and 20 combs of worker cells in the brood section. The honey section consisted of 11, optional 22, combs. Beekeeping was performed in Segeberger hives with 11 combs/module and a frame size of 370 x 222 mm. Specific drone excluders were installed in April in front of the hives to prevent migration of unselected drones. In May 2020, these hives ware relocated to the mating station and the excluders were removed.

Sampling. Young emerged workers and drones (each n=50) of DE-6-1-0332-2017 (4a) were sampled in 2019 to ensure about their typical characteristics [9]. Males of the selected drone hives (1b) (n=50) were sampled for statistics, according to the guidance [9] in May 2020. During the summer, ⩾15 drones were sampled in June, July and August 2020.

Cubital index. Forewings of sampled individuals were separated and prepared for determination of the cubital index (CI) of the third cubital cell [2]. Samples according to the guidance were analyzed by Ingrid Müller, Merkmalsuntersuchung [Eicklingen, Germany]. Other samples were analyzed with 2x magnification on a stereo microscope [Müller-Optronic, Germany], using ScopePhoto 3.0, version x64, 3.1.312, in combination with a ScopeTek DCM130E camera [ScopeTek, Germany]. The CI was transferred into classes 1-30, according to RUTTNER [5]. Mean, standard error of the mean (SEM) and significance (unpaired Student t test) were calculated using R, version x64 3.6.1 [R Core Team, Austria].

III. RESULTS

Characterization of the drone colonies ancestors. In order to breeding procedure in beekeeping, *4a* (DE-6-1-0332-2017) was analyzed in 2019. Worker were characterized with a typical formula of *A. mellifera* subsp. *carnica* (100O/e / 94k:6m / 88F:12ff / I=2.91 (2.23-4.20)). The drones were also typical for *A. mellifera* subsp. *carnica* (100O/i / 100gr / I=2.23 (1.71-3.19)).

Queen *4a* was mated with f1 drones of *12a* (DE-6-1-0462-2014), which was typical for *A. mellifera* subsp. *carnica* (worker: 100O/e / 96k/4m / 96FF:4ff / I=3.29 (2.54-4.95); drones: 100O/I / 100gr / I=2.25 (1.57-3.69)) (Fig. 3).

Queen *4a* was reared from *7a* (DE-6-1-2149-2015). Successors of *7a* were not typical for *A. mellifera* subsp. *carnica* (worker: 100O/e / 96k:4m / 96FF:4ff / I=2.64 (2.06-4.50); drones: 100O/I / 92gr:8lgr/rbr / I=2.18 (1.25-3.35)), according to the recommended breeding rules [Deutscher Imkerbund e.V., 2017].

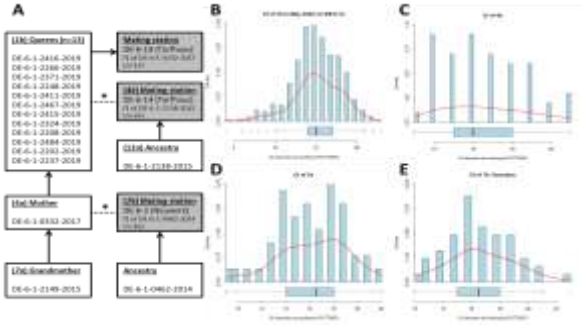

Fig. 3: Schematic map of the drones ancestry and its cubital index (CI) on mating yard Torfhaus (DE-6-14) in 2020. A, Schematic map of drones hives (*1b*) on DE-6-14 with their ancestry. B, CI of the drone hives (*1b*), measured

in May 2020; C, CI of the drone hives mother (*4a*); D, CI of the drone hives grandmother (*7a*) with untypical characteristics for *A. mellifera* subsp. *carnica* (containing CI classes <10); E, CI of the drones mothers father (*12a*). Arrows indicate a direct ancestry, * visualize the mating of virgin queens with specific drones of the mating stations.

Cubital index on DE-6-14. The CI was determined during the mating season 2020 among *1b* on the male individuals.

Tab. 1: Analysis of cubital index (CI) on mating station Torfhaus (DE-6-14) in 2020. Drones of the drone hives (*1b*) were sampled during the mating season 2020 in May, June, July and August. Mean, standard error of the mean (SEM), minimum (Min) and maximum (Max) of the CI data were calculated.

Queen (*1b*)	CI data: mean ±SEM Max:Min			
	May (n=50)	June	July	August
DE-6-1-2411-2019	2.40 ±0.060 1.70:3.53	n=17 2.16 ±0.108 1.22:2.80	n=17 2.16 ±0.090 1.43:2.80	n=20 2.32 ±0.128 1.54:3.23
DE-6-1-2335-2019	2.05 ±0.063 1.14:3.15	n=17 1.93 ±0.128 1.03:2.68	n=17 2.08 ±0.118 1.48:2.91	n=20 2.10 ±0.107 1.44:3.25
DE-6-1-2416-2019	1.69 ±0.074 0.89:3.09	n=16 1.61 ±0.086 1.19:2.39	n=18 1.67 ±0.091 1.20:2.56	n=21 1.83 ±0.102 1.22:3.36
DE-6-1-2371-2019	1.90 ±0.045 1.40:2.70	n=17 1.81 ±0.103 0.93:2.91	n=18 1.88 ±0.077 1.38:2.47	n=20 1.89 ±0.084 1.23:2.65
DE-6-1-2484-2019	1.64 ±0.043 1.00:2.43	n=21 1.94 ±0.078 1.14:2.78	n=16 1.81 ±0.112 1.21:2.88	n=20 1.85 ±0.080 1.21:2.38
DE-6-1-2324-2019	1.80 ±0.039 1.00:2.60	n=18 1.80 ±0.054 1.37:2.20	n=17 1.82 ±0.070 1.42:2.35	n=20 1.87 ±0.066 1.47:2.39
DE-6-1-2415-2019	2.07 ±0.046 1.60:2.86	n=18 1.70 ±0.075 1.31:2.32	n=18 2.05 ±0.082 1.65:3.12	n=20 2.05 ±0.082 1.65:3.12
DE-6-1-2266-2019	2.07 ±0.051 1.53:3.00	n=21 1.74 ±0.084 1.27:2.57	n=17 1.97 ±0.090 1.39:2.78	n=20 2.21 ±0.078 1.68:2.74
DE-6-1-2248-2019	2.22 ±0.057 1.62:3.70	n=22 2.23 ±0.111 1.28:3.41	n=17 2.35 ±0.128 1.60:3.36	n=21 2.11 ±0.087 1.38:2.75
DE-6-1-2202-2019	2.33 ±0.055 1.75:3.31	n=17 2.28 ±0.065 1.90:2.77	n=17 2.22 ±0.048 1.72:2.52	n=20 2.09 ±0.105 1.18:3.41
DE-6-1-2467-2019	1.88 ±0.065 1.19:3.03	n=19 1.98 ±0.081 1.35:2.55	n=18 1.89 ±0.096 1.30:2.87	n=21 2.03 ±0.076 1.44:2.71
DE-6-1-2208-2019	2.10 ±0.053 1.45:2.91	n=16 1.97 ±0.093 1.35:2.87	n=18 2.21 ±0.110 1.54:3.56	n=23 2.11 ±0.107 1.49:3.41
DE-6-1-2237-2019		n=19 2.08 ±0.079 1.50:2.65	n=20 2.22 ±0.117 1.37:3.68	n=21 1.95 ±0.087 1.39:2.57

First samples were taken in May 2020, before colonies were transferred to DE-6-14, when the CI was at 15.21 ±0.12. In June, the CI differed significantly (p=0.02758) with a mean of 14.7 ±0.20. The CI increases up to the initial level in July (15.50 ±0.19) and August (15.15 ±0.18) without any significant difference to the results from May (p_{July}=0.8411, p_{August}=0.7806) (Fig. 4).

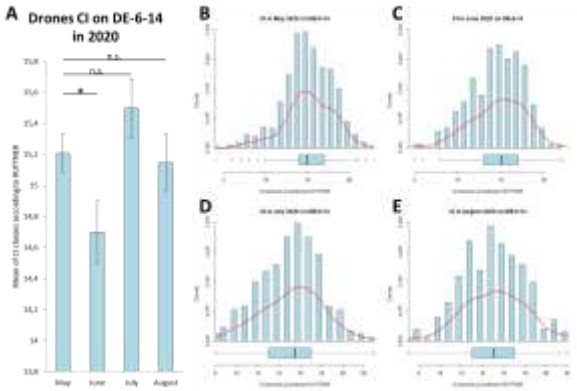

Fig. 4: Shift of the drones cubital index (CI) on mating yard Torfhaus (DE-6-14) in 2020. A, mean of measured CI with its standard error of the mean (SEM). *, significant differences (p<0.05); n.s., no significance (p>0.1). B, distribution of drones CI in May (15.21 ±0.12); C, distribution of drones CI in June (14.7 ±0.2); D, distribution of drones CI in July (15.5 ±0.19); E, distribution of drones CI in August (15.15 ±0.18).

IV. DISCUSSION

Climatic change and an upcoming interest in nature and ecology lead to an increase of public focus on sustainable agriculture and nature, such as biodiversity. However, also the beekeeping increases. In Lower Saxony the number of beekeepers increases by approximately 25% in the last four years [10]. The majority of beekeepers are well organized in beekeeping associations, like the Deutsche Imkerbund e.V., but numerous beekeepers are not organized and registered by the authorities. Often, these beekeepers generate their knowledge by incomplete statements and reports in social media, which leads to lacks of knowledge. This may result in migration and founding of unregistered apiaries within the protection zone of mating stations.

The LAVES Institute for Apiculture, mates >2,000 virgin queens per year on its three mating stations. However, some customers complained about unspecific characteristics of queens,

mated on Torfhaus (DE-6-14), like rings on the abdomen of breeded workers. According to these reports and observations of our beekeepers, who take care on DE-6-14, we analyzed CI of its drones in 2020.

The drone's population was analyzed in May 2020, when the migration of unfamiliar drones was impossible, because of the excluders. Analyzed individuals showed typical characteristics for *A. mellifera* subsp. *carnica* drones in their color of thorax hair (98.5gr:1.5lgr/rbr) and their abdomen muster (100O/i). The CI was at 15.21 ±0.12, but the minimum was out of the typical range [9] in four of twelve colonies (DE-6-1-2416-2019, DE-6-1-5467-2019, DE-6-1-2324-2019, DE-6-1-2484-2019). These out of range results could be explained by the genetic material of *7a*, which was used in ancestry in spite of an unspecific drone CI (I=2.18 (1.24-3.35)).

In defiance to *1b* colonies with unspecific CI, a significant decrease in the CI was detected in June 2020. *A. mellifera* subsp. *carnica* drones are characterized with a CI mean of >1.8, but the minimum in the data set should be >1.4. *A. mellifera* subsp. *mellifera* drones typically have a CI mean of 1.2-1.5, but the maximum in the dataset shout not exceed 1.5 [5]. The typical CI classes according to RITTER result in classes <12 for drones of subsp. *mellifera*. Subspecies *mellifera* is known under the pen name "Dark Bee", which mainly decreased in the early 20th century in Europe, because of Acarapsis [11, 12, 13]. Nevertheless, the Buckfast Bee represents a cross-breeding of different subspecies of *A. mellifera*, especially of subspecies *mellifera* and subspecies *ligustica* [14, 15]. A CI downtrend my also indicate migration of drones with characteristics of subsp. *mellifera*, which can also be founded in the Buckfast Bee.

According to a "back to the roods" trend in beekeeping and the upcoming interest in the "Dark Bee", a migration of Dark Bees drones could be discussed, but the low portion of beekeepers handling this bee and the abdominal rings and islands, which could be observed on the sampled drones abdomen in June and July (data not shown) indicate typical characteristics subsp. *ligustica*, which are transported by the Buckfast Bee [14, 15].

Mating stations can be differentiated by their breeding program. DE-6-14 is used for "C-T Celle", a breeding line of subspecies *carnica*. In order to ensure a safe and effective mating of virgin queens without inbreeding, it is not allowed to keep any bees in the protection zone of DE-6-14, even not colonies of "C-T Celle". Mating yard DE-6-17 (Lautenthal) is used for breeding the race Buckfast Bee, without any line specificity. According to the rules of DE-6-17, beekeepers are allowed to keep colonies of the Buckfast Bee within its protection zone.

Moreover, Torfhaus is known by the beekeepers as mating station. The mating of virgin queens in the mating station is linked with a kind of fee and certificates of the beekeepers apiary to be free of *Paenibacillus larvae*, the causative agent of AFB [16]. Additionally, only nucs (small hives, containing a virgin queen and workers without drones) are allowed on DE-6-14. It can be assumed, that somebody tries to hide these facts by the illegal placement of colonies with virgin queens in the protection zone next to Torfhaus. Although in this case no or only a few drones would be expected in the nucs.

Another fact might be the increase of the regions' attractant in honey production. In the last years lots of forest clearings appeared in the Harz, because of damages in the spruce cultures (*Picea abies*), leading to a massive spread of European raspberry (*Rubus idaeus*) and blackberry (*Rubus* spec.). These may attract beekeepers to migrate their colonies into this bloom.

V. CONCLUSION

Taken together, we assume an accumulation of drones from DE-6-14 and unspecific drones on DCAs in the valleys next to DE-6-14, resulting in a migration of these drones into the drone hives of DE-6-14. A significant shift in the CI can be found in June in the drone hives. This shift decreases during the summer. Drones are only present in the summer month, during the breeding season [17]. Moreover, the varroa mite (*Varroa destructer*) prefers the drone brood for its reproduction. Therefore, drone frames are removed by the beekeepers to interrupt the mite's reproduction [18, 19]. Colonies, managed for the production of honey reduce their drones in the end of summer or when the provision of nectar and pollen decreases. With the scope on mating virgin queens, special prepared drone hives are managed to nurse as many drones and as long as possible. Therefore, we are able to hold drones available even when unprepared colonies do not have drones anymore. This colony management declares reduction of the Buckfast Bees' characteristics in the CI of drone hives on DE-6-14 during July and August. Moreover, the late time slots for mating virgin queens on mating station Torfhaus seem to increase the safety of mating with selected drones.

However, there should be investigations of the authorities and organizations to inform beekeepers about the protection zones of mating stations, in order to secure the organized and focused breeding of honeybees on mating stations on continental Europe.

ACKNOWLEDGMENT

We thank Stefan Lembke and Paula Markwitz for excellent assistance in beekeeping and providing the colonies material. This work received financial support from the Lower Saxony State Office for Consumer Protection and Food Safety, within a project on detection and analysis of viruses in honeybees.

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