GENETIC COMPOSITION AND PHENOLOGY OF MATING DRONE CONGREGATIONS IN THE HONEY BEE *APIS MELLIFERA*

Errol Benstead

RÉSUMÉ. — Composition en lignées génétiques et phénologie des congrégations nuptiales de mâles chez l’abeille *Apis mellifera*. — *Apis mellifera* s’est diversifiée en 25 sous-espèces ou races géographiques, comportant des caractéristiques éco-éthologiques adaptées à leur habitat. Aujourd’hui sur le territoire français, l’abeille indigène *Apis mellifera mellifera* est introgressée, en particulier par les sous-espèces *ligustica*, *caucasica* et par une lignée synthétique, la Buckfast. Afin de mieux comprendre les mécanismes mis en œuvre lors des accouplements inter-races et, éventuellement, proposer dans l’avenir des outils de sauvegarde de la diversité de l’abeille domestique, cette étude vise à répondre à la question: existe-t-il des spécificités éthologiques particulières à la constitution des congrégations de mâles en fonction des races? Les résultats montrent que, dans le cas d’*Apis mellifera mellifera*, il y a un isolement reproducteur comportemental basé sur un décalage temporel du vol nuptial des mâles selon les lignées génétiques auxquelles ils appartiennent bien que tous se rassemblent sur les mêmes sites.

SUMMARY. — Genetic composition and phenology of mating drone congregations in the honey bee *Apis mellifera*. — *Apis mellifera* is diversified into 25 subspecies or geographic races with eco-ethological characteristics adapted to their environment. Today, on the French territory, the native bee *Apis mellifera mellifera* is eroded, in particular by subspecies *ligustica* and *caucasica*, and by a synthetic lineage, the Buckfast. For a better understanding of the mechanisms acting during inter-race matings and, possibly, to propose, in the future, tools for the protection of the variety of domestic bees, this study addresses the question whether particular behavioural specificities exist in the constitution of the drone congregations according to the race. Results show that, in the case of *Apis mellifera mellifera*, there is a behavioural reproductive isolation based on a nuptial flight time-lag depending on the drones lineages. This time-lag is present although all congregate on the same sites.

There are four distinct evolutionary lineages within the *Apis mellifera* species (A, M, C, O) regrouping 25 subspecies or geographical races (Arias & Sheppard, 1996; Cornuet & Garnery, 1991; Franck *et al.*, 2000; Garnery *et al.*, 1993; Garnery *et al.*, 1998a,b; Ruttner, 1988a,b; Sheppard *et al.*, 1997). The A lineage includes the African races; M is represented by the *mellifera* race located in Western Europe, from Spain up to Scandinavia; C is to be found in Central and Eastern Europe; the O lineage regroups the subspecies of the Caucasus as well as those of the Middle-East.

1 EPLEFPA Précieux-Montbrison - Saint-Genest-Malifaux, BP 204. F-42605 Montbrison Cedex. E-mail: errol.benstead@educagri.fr

Every geographic race generally has the eco-ethological characteristics adapted to the geographical areas where it lives. Those subspecies themselves include ecotypes which are adapted to climatic conditions or food resources. One of the best described ecotypes is that of the *mellifera* race from the Landes area, which is specialized in living off heather, *Calluna vulgaris* (Louveaux *et al.*, 1966; Strange *et al.*, 2007).

Human activity has in many cases caused colonies of various races to move from one area to another, sometimes over great distances. In France, for instance, the transhumance and the importation of queens and swarms aiming at obtaining more productive colonies but above all at replacing the colonies which die in great number each year, have modified the geographic distribution of the native race *mellifera*. Today, on French territory *Apis mellifera mellifera* is being subject to introgression by subspecies *ligustica* and *caucasica*, and by a synthetic lineage, the Buckfast. In the way, inter-race matings are likely to speed up the erosion of the genetic diversity of the *mellifera* race.

In the heart of the native subspecies to be found in France, *Apis mellifera mellifera*, there is an ecotypic form which is particularly well adapted to the environmental conditions of the Landes de Gascogne, the Landes ecotype (Louveaux *et al.*, 1966). Indeed, the development cycle of the broods of its colonies follows the availability of the floral resources of the Landes forest and particularly during the blossoming time of the heather *Calluna vulgaris* and *Rhamnus frangula* (Strange *et al.*, 2007). Thus, in that area, beekeepers have at their disposal, with this ecotype, a first class genetic inheritance, particularly for the production of heather honey with its important economic outlets on the Northern European market. Yet, when it comes to cope with the aggressiveness of that honey bee, some producers have chosen a more gentle, artificial stock, the Buckfast (lineage C) whose colonies compensate their lack of adaptation with an important production of workers. As a result there is an important risk of introgression of that ecotype as well as coming of hybrids in the same way as what has been observed in the Canary Islands by De la Rúa *et al.* (2002).

The honey bee *Apis mellifera* mates on specific sites known as drone congregations. Those sites are relatively constant at mating time and remain the same over a span of several years (Koeniger, 1990a). A large number of drones gather between 10 and 40 m in altitude in those areas and wait for a queen to arrive. When the queen is here, the drones then try to mate with her while in flight (Gary, 1963). In favorable weather conditions, the noise made by the drones flying may help to locate a congregation (the gathering of males). Whether the congregation is there can then easily be verified by putting up a lure, moistened with royal pheromone set in trap in order to catch the drones. However the precise eco-ethological mechanisms concerning the making of a congregation are not known, though various hypotheses have been made on the subject. Most of the researchers have defined the topographic characteristics of the sites as the main factor in the making of a congregation (Koeniger, 1990a). The general consensus is that those drones’ congregations take shape above a clearing or in an open field (Koeniger, 1990a; Koeniger *et al.*, 2000; William, 1987; Woyke, 1955). However, although it is taken for granted by many researchers on honey bee reproduction, it has actually not been much tested. Thus, no study has really gone closely into the hypothesis of a differentiated making of those congregations according to the original subspecies of their drones, which could prove to be a significant variable that could be used by the queen to select its sexual partners.

The current study has been carried out within the general framework of the analysis of inter-race sexual competition in the *Apis mellifera* species among which several races were used: *Apis mellifera mellifera*, *Apis mellifera ligustica*, *Apis mellifera caucasica*, and Buckfast. The purpose was to detect a possible influence of the drone behaviour on the making and the composition of he congregations among which the matings will take place and particularly to try and find out the existence of ethological characteristics belonging to the drones of two different evolutionary lineages, *Apis mellifera mellifera* (lineage M) and the synthetic race Buckfast (lineage C).

From a practical point of view, the results could prove to be a precious help in the decisions that need to be made during conservation operations of races or honey bee ecotypes.
MATERIALS AND METHODS

SITES OF STUDY

The study was carried out in the South West of France, in the heart of the Parc Régional Naturel des Landes de Gascogne close to the village of Daugnague in the Landes department.

Site N°1 is set above a thick plantation of *Pinus pinaster*. The trees are approximately 12 m high, laid out in rows with a 10 m space between paths. The drones have been caught by raising the balloon (as you will see further) in a little clearing created by a 7 m wide forest road, and by allowing the balloon to drift above the trees. The drones were trapped between 20 and 35 m above ground level but were never caught before the trap actually rose above the tree tops.

Site N°2 has all the features of a wetland with an important density of *Molinia caerulea* and shrubs of *Rhamnus frangula*. It is located in an approximately 20 m wide clearing and follows a stream on a 200 m distance. The trees living the site are 25 m high mature *Pinus pinaster*, several shrubs of *Quercus robur* and *Salix alba* are also present on the site. The congregation (the gathering of males) is active on a small part of the site, which is lined by *Pinus pinaster* on three sides and a more open clearing on the fourth. However, the drones show some interest to the lure (see further on) a little further, on the largest clearing, and that congregation may be but a part of a complex which includes several active sites.

MATING BEHAVIOUR OF THE HONEY BEES

A typical colony of honey bees is composed of only one queen which can lay eggs, over a 4 to 5 year span, of up to several thousands of drones according to the season, and of 10 000 to 60 000 workers (Moritz & Southwick, 1992). Virgin queens mate on 1 to 5 mating flights performed within the drone congregations (Roberts, 1944) sometimes located at a distance of 16 km from their own colonies (Peer, 1957). During the flight, the queen may mate with numerous drones coming from different colonies or apiaries (Roberts, 1944). The diversity of sexual partners of a queen modifies the genetic composition of the population of workers in a colony. The colonies of honey bees are composed of several paternal lineages (Adams et al., 1977; Arnold et al., 1996; Estoup et al., 1994) as result of the mixing of the various semen of drones in the queen’s spermatheca (Page & Metcalf, 1982). The estimated level of polyandry among honey bees goes from 1 to 37 according to the various researchers and methods used to study the phenomenon (Adams et al., 1977; Arnold et al., 1996; Estoup et al., 1994; Gary, 1963; Moritz et al., 1995; Oldroyd et al., 1997; Taber, 1954; Woyke, 1960).

Little is known so far about the impact of the various ethological factors (such as the drone and queen behavior, the climatic conditions) on the number of efficient matings for each queen.

METHODS USED FOR THE STUDY

Time schedule of the drones

The two sites of congregation (N°1 and N°2) were studied in June 2002 for four days at different times of the day from 9:00 am till 8:00 pm. Table I gives the number of caughts during time-slots of 30 minutes (only are indicated periods with a size superior to 0). The purpose of that first stage, carried out when the drone congregations started to take shape, was to optimize the time spent handling it with the trap afterward (see further on).

<table>
<thead>
<tr>
<th>Time-slots of capture</th>
<th>Site N°1</th>
<th>Site N°2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date of capture</td>
<td>June 03</td>
<td>June 05</td>
</tr>
<tr>
<td>3:30 pm – 4:00 pm</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>4:00 pm – 4:30 pm</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>4:30 pm – 5:00 pm</td>
<td>9</td>
<td>12</td>
</tr>
<tr>
<td>5:00 pm – 5:30 pm</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td>5:30 pm – 6:00 pm</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>6:00 pm – 6:30 pm</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>6:30 pm – 7:00 pm</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>7:00 pm – 7:30 pm</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>
The catching of the drones

The samples of drones were taken from site N°1 from June to August 2002, and from site N°2 from June to September 2002, following the William technique (1987) (see Tab. II). The trap is made of a plastic film, in the shape of a 75 cm high cone, with, as its base a 45 cm wide opening. That opening is reinforced with a thin metal ring in order to keep it open. A piece of cotton, moistened with a synthetic mixture of royal pheromone [(E)-9-oxo-2-Decenoic-acid] as well as 3 lures representing drones are hung inside the trap. The trap is closed at the top and tied to a thread under a balloon, inflated with helium and with a circumference of 170 cm. The trap is then raised up to the congregation for several minutes during which the drones go inside it. It is then taken down and the drones which have been caught are placed in ethylic alcohol (95°).

<table>
<thead>
<tr>
<th>Site of capture</th>
<th>Date of capture</th>
<th>Lineage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C</td>
</tr>
<tr>
<td>N°1</td>
<td>June 21</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>June 26</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>August 03</td>
<td>7</td>
</tr>
<tr>
<td>N°2</td>
<td>June 11</td>
<td>82</td>
</tr>
<tr>
<td></td>
<td>June 13</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>June 21</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>June 26</td>
<td>133</td>
</tr>
<tr>
<td></td>
<td>July 19</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>August 13</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>September 19</td>
<td>21</td>
</tr>
</tbody>
</table>

Genetic determination of the drones

The DNA of the drones was extracted by using the chelex extraction protocol (Garnery et al., 1998a,b). As for the mitochondrial analysis, the intergenic area COI-COII was examined according to the garnery protocol (Garnery et al., 1993). The COI-COII region was PCR-amplified with primers E2 (5’GGCAGAATAAGTGCATTG3’) and H2 (5’CAATATCATTGATGACC3’) located in the tRNAleu and COII genes, respectively (for PCR conditions, see Garnery et al., 1993). A part of the products from the amplification then migrated on agar gel (1.4 %) in order to determine their size. The rest undergoes a digestion by the Dra I restriction enzyme, then on to acrylamide gel (7.5 % or 10 %) to determine the haplotype.

The mitochondrial DNA analysis enables us to classify the individuals by their lineage because the Q sequence is characteristic of the C lineage to which the Buckfast stock belongs, the sequences PQ, PQQ and PQQQ are specific of the M lineage to which the Apis mellifera mellifera race belongs and the sequences POQ, POQQ and POQQQ are proper to the African lineages (Garnery et al., 1993).

Data processing

A Chi-square test was made on the time data of table I to make sure of the independence between the catching dates, the sites (N°1 and N°2) and the time-slots of catching. The level of significance was set at $P < 0.05$.

From table II, the respective sum of the number of the drones of lineages C and of lineages M monthly caught was tested by Chi-square method to verify dependence existing between the genetic composition of the congregations of drones and the period of process of formation of the congregations. The level of significance was set at $P < 0.05$. Table III presents the chi-square by compartment.

TABLE II

Distribution of the population of Apis mellifera caught in the Landes de Gascogne and the absolute frequency of the various lineages revealed by the mitochondrial DNA
TABLE III

Table of the Chi-square results presenting the difference between the actual and theoretical samples sizes

<table>
<thead>
<tr>
<th>Month of capture</th>
<th>Lineage C</th>
<th>Lineage M</th>
</tr>
</thead>
<tbody>
<tr>
<td>June</td>
<td>(+) ***</td>
<td>(-) ***</td>
</tr>
<tr>
<td>July</td>
<td>(-) ***</td>
<td>(+) ***</td>
</tr>
<tr>
<td>August</td>
<td>(-) ***</td>
<td>(+) ***</td>
</tr>
<tr>
<td>September</td>
<td>(-) ***</td>
<td>(+) ***</td>
</tr>
</tbody>
</table>

(+): higher actual sample size than theoretical sample size. (-): lower actual sample size than theoretical sample size. ***: significant chi-square result, with a threshold of alpha = 0.01.

RESULTS

TIME SCHEDULE OF THE DRONES PRESENCE

Drones have an identical activity on the two sites and during all the period of study ($P < 0.05$, df = 21, $\chi^2 = 2.746$, $\chi^2_{cv} = 32.671$). The activity started at 3:30 pm with a peak density of drones of 83% between 4:30 and 6:30 pm (Tab. I). This time slot has been respected afterwards so that the largest number of drones could be caught.

DRONES CONGREGATION FORMATION

The June congregations correspond to the C lineage. Conversely, the congregations from July to September correspond to the M lineage ($P < 0.05$, df = 3, $\chi^2 = 258.965$, $\chi^2_{cv} = 7.815$).

On site N°1, in June ($n = 89$), the congregation is composed of 88% drones of C lineage, in other words of Buckfast stock. The native drones of the M lineage represent only 7% of the catchings. This trend is inverted in August ($n = 32$) when *Apis mellifera mellifera* becomes the major race with 78%. Another noticeable fact is the low proportion of African drones of the A lineage (Fig. 1).

![Figure 1. — Genetic composition of the drones congregations on site N°1 from June to August (in %).](image)
In the congregation on site N°2, most of the individuals belong to lineage C in June (78%) whereas (n = 408), from July to September, the congregation is composed of a majority of native honey bees (M lineage). The catch operated on August 13th is composed of 100% of native honey bees (n = 52). As for all the other captures, from July to September, over 75% of the drones belong to the M lineage. We notice a very low proportion of African drones from the A lineage (Fig. 2).

![Figure 2. — Genetic composition of the drones congregations on site N°2 from June to September (in %).](image)

**DISCUSSION - CONCLUSION**

Thanks to the genetic analysis allowing the identification of the race of the drones in the congregations, two new notions can be drawn about congregations. First, there is a time-lag when the drones go out, according to the lineages, and secondly, the congregations can gather above wooded areas.

In order to create a reproductive isolation, it is necessary that the queens and drones of the same race should meet at the same time, and preferably, at another period of time than the other races. The current study shows that the drones of each of the two races that we tested, the Landes ecotype *Apis mellifera mellifera* and the synthetic breed Buckfast, made up their congregations at two different times in the summer period. It turns out that those two races present different eco-ethological characteristics.

One may suppose that if the queen performs her mating flight at the same time as the making of drone congregations of her own race, then the chances for homogamy are at a maximum. This would lead to the beginning of a reproductive isolation between the two races thanks to the season time-lag. Whether the virgin queens’ outings followed the same time pattern or not, has not been looked into. The correlation of dates cannot thus be verified. Yet, since a long time, we know that a time-lag affects the birth rate of the workers of ecotype (Louveaux *et al.*, 1966). Moreover, the delay of the congregations’ formation of the Landes ecotype in comparison with the one of the Buckfast is probably the result of the existing time-lag between the births of the drones of the two races. We can suppose that this time-lag in the cycles of the broods of reproductives between the two lineages (Strange *et al.*, 2007) goes with a time-lag concerning the mating flight of the queens of these two lineages.

The genetic composition of the congregation in site N°1, a thickly wooded area, is not different from that in site N°2, located in a clearing. Thus, it seems that a congregation including
drones of various races can gather above the canopy in a thickly pine-planted site. This may show that the presence of *Apis mellifera* drones congregations has been underestimated, so far, in the wooded areas. In the present case, it seems that the environmental and geographical characteristics have no influence on the reproductive isolation that could occur between those two races.

REFERENCES


