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A CASE OF SIBLING SPECIES AMONG SOCIAL BEES [1]

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(With 2 text-figures)

MAYR (1942), who coined the expression, calls sibling species "groups of related species which are so similar that they are considered as belonging to one species until a more satisfactory analysis clears up this mistake".

Morphological and bionomical characteristics sometimes vary enormously among different populations of bees of the same species. For instance, *Apis mellifera ligustica* Spin., the Italian honeybee, is yellow banded, swarms very seldom, starts dancing the "wagg-tail" dance (with which a bee indicates to her companions the distance and direction of a certain food source with high precision) at 50 meters; its workers have tiny spermathecae, complete their development in 21 days, and are very gentle. These data for other subspecies of *A. mellifera* L. are quite different as shown in Table I.

TABLE I

Some Characters of Four Subspecies of Apis mellifera L.

Subspecies	Minimum distance to start precision dances	Swarm impulse	Days from egg to adult stage (worker)	Color of abdomen	Temper	Size of worker sperma-thecae
A.m. ligustica	50 m	weak	21	yellow	gentle	small
A.m. mellifera	100 m	average	21	gray-black	gentle	small
A.m. adansonii	5 m	strong	19	gray-yellow	aggressive	small
A.m. capensis	—	average	—	black	gentle	enormous

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In spite of these differences, the subspecies intercross freely if hives are put in the same mating ground which, according to PEER (1957), has a radius of about 13 km. These differences presumably developed either because their native territories are quite distant or because of different selection operating in different environments. There is so much difference between these subspecies that only interbreeding, combined with their allopatric distribution justifies the inclusion of all of them in one species.

The various subspecies of *Melipona marginata* Lep., native in Central and South America, are equally distinct in many characters, but we found that they intercross if maintained in the same mating ground.

In Central Africa, South of the Sahara, especially in Angola and Tanganyika, there is a very tiny bee that occupies small cavities in tree trunks, specially in dead and dry ones, a habit which facilitates its existence in cities and villages. Specimens of these bees where sent to Mr. H. F. Schwarz, Dr. C. D. Michener, and Prof. J. S. Moure, specialists in the systematics of the meliponids. Both Mr. Schwarz, who would be more at the "lumper" side and Prof. Moure, who has "splitting" tendencies, agreed that these bees belong to the same species, which has been identified as *Trigona (Hypotrigona) braunsi* Kohl.[2] Dr. Michener, even being aware of our problem, said when he first studied them that he would be reluctant to give them taxonomic recognition on morphological grounds alone.

It happens, however, that the native negros of several tribes of Angola, probably using as a basis the nest entrance size, refer to this "species" under two names. On the Kimbundu dialect these are: *"cassusso"*, the most common bee, and *"landula"*, rarer than the former and a little larger, but sympatric. Impressed by this different nomenclature, PORTUGAL-ARAÚJO (1955) studied the bionomics of both forms and found one important bionomic difference: *"cassusso"* has its brood cells organized in clusters (fig. 1) and *"landula"* has its brood cells organized in more or less vertical single layer combs (fig. 2). Latter, studying the African robber social bee, *Lestrimelitta cubiceps* Friese, PORTUGAL-ARAÚJO (1958) found one more striking difference: "cassusso" constitutes the most common prey of *L. cubiceps* Friese, while "landula" is very seldom disturbed. Also, "cassusso" workers defend the hive by pouring honey on the attackers, while "landula" workers do not use these tactics, rather fighting the robbers from the very start of the attack. "Cassusso" nests in tree trunks, either alive or dead, but "landula" is more often found in living trunks. It could be that these differences are due to moderate genic differences

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[2] Cited by PORTUGAL-ARAÚJO (1955) as *Trigona (Hypotrigona) gribodoi* Magretti.

such as those which separate the subspecies of *Apis mellifera* L. and *Melipona marginata* Lep. To determine if cassusso and landula participate in a common gene pool, the following experiment was carried out.

MATERIAL

The experiment itself was done in the apiary of PORTUGAL-ARAÚJO, in Luanda, Angola (Africa), and we used 3 colonies of *Trigona (Hypotrigona) braunsi* Kohl variety *landula,* collected in a region 90 km Northeast of Luanda, and 6 colonies of *T. (H.) braunsi* Kohl variety *cassusso* collected both in the same region as the others and in Luanda itself.

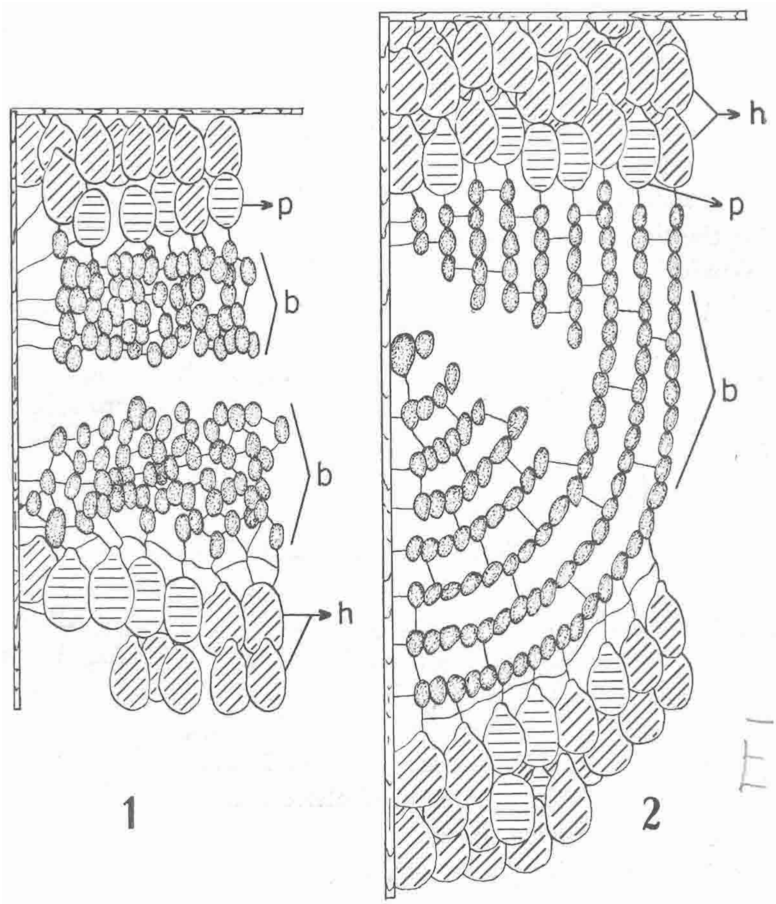

Fig. 1 — Nest of "cassusso". Brood cells (b) are arranged in cluster; pollen and honey are stored in pots (p and h). Fig. 2 — Nest of "landula". Brood cells (b) are organized in single layer combs. Pollen is deposited in pots (p) close to the brood (horizontally striped) and honey somewhat more distant (h). This arrangement is common to all meliponids and Apis.

A colony of *landula* contains about 2,000 to 2,500 bees, and a colony of *cassusso* averages 400 to 600 individuals. Within a radius of 100 m there were about 20 native colonies of *cassusso* and none of *landula.*

METHODS AND RESULTS

Two colonies of *landula* were dequeened (Oct. 14, 1956 and March 17, 1957) at times when both of them contained queen cells. One hive of *cassusso* was dequeened (Jan. 1, 1957) and all the unemerged brood was replaced by brood of *landula,* including several queen cells. This last operation was done to avoid the remote possibility that virgin queens exhibit preferential mating with drones of their own colony.

In September, 1957, the first two hives remained landulas; the third gradually changed to *landula* with the emergence of new bees. Therefore, none of the three *landula* queens produced *cassusso* offspring.

One colony of *cassusso* was divided (March 24, 1957) and the queenless fragment was given an occupied queen cell: this hive developed *cassusso* bees. The unmerged brood of one dequeened colony of *landula* was removed and replaced by brood of a colony of *cassusso* including a *cassusso* royal cell. This colony gradually changed to *cassusso* as the old *landula* workers were replaced by newborn *cassusso*.

During the time when we had *landula* hives in the bee yard, 4 swarms of *cassusso* occupied some of our empty boxes. All four swarms of *cassusso* developed into *cassusso* colonies. It should be mentioned that in swarms of meliponids it is not the old inseminated queen that follows the swarm, but it is a virgin newly emerged queen that does so (NOGUEIRA-NETO, 1954). Therefore, the four queens of these natural swarms flew in their nuptial flights from our yard and had opportunities to mate with either *cassusso* or *landula* males.

DISCUSSION AND CONCLUSIONS

The size of these bees is quite comparable to that of the Indian species, *Trigona (Tetragona) iridipennis* Smith. LINDAUER (1957) found that the maximum distance of flight of this Indian bee is about 150 meters. Therefore, we accept as a reasonable mating ground for this species the area 100 meters in radius around the nest. In this area, we had 5 hives of *landula* and 32 of *cassusso* (20 outside the yard, 6 mentioned above and 6 in original tree trunks that were cut and transported to our bee yard). In the experiments described above three *landula* virgin queens and 6 *cassusso* virgin queens mated in this yard.

If we estimate the number of males per hive in relation to the number of total individuals per hive, we find that about half of all the males belonged to each variety. In this case, the probability that the three queens of *landula* would mate with only *landula* males would be $(0{,}50)^3 = 0.125$. This probability for our variety *cassusso* would be $(0{,}50)^6 = 0.015625$. The probability that these two events would happen together by chance would be practically $P = 0.002$. If one assumes an equal number of males in each hive, this final probability would be $P = \left(\frac{5}{37}\right)^3 \times \left(\frac{32}{37}\right)^6$, which gives about $P = 0.001$. However, whatever be the number of males, the minimum value for our P would be $P = 0$, that is, when for lack of their own males either *landula* or *cassusso* did not copulate; and the maximum value of P would be $P = 0.03075$. This maximum value would be reached if in the mating ground 1/3 of the males would be *landula* and 2/3 would be *cassusso*.

Therefore, with our data, the probability that these two bees had not mated due to random causes will be always smaller than 1%. We can safely conclude, then, that *landula* and *cassusso* do not interbreed; therefore, they are two different species.

These two cryptic species (see the description of them in MICHENER, 1958) are obviously very close and presumably they differ by few genes. One of the main bionomical differences lies in the brood cells arrangement (see figs. 1 and 2). From this we conclude that the differences between a cluster type arrangement of brood cells and a vertical single layer comb are due to few genes. This is consistent with the idea of considering horizontal combs as evolving from cluster of cells independently in different groups of stingless bees. It is reasonable therefore, to find horizontal combs in *Trigona (Meliponula) beccarii* Gribodo and cluster type in *Trigona (Melipona) bocandei* Spin.; horizontal combs in *Lestrimelitta limão* F. Smith and cluster type in *Lestrimelitta cubiceps* Friese, etc. For several reasons the cluster type is considered the primitive one. In this same line of thought, *Dactylurina staudingeri* Gribodo[3] may, but does not necessarily figure in the evolutionary line of *Apis mellifera* L.

SUMMARY

Two varieties of African social bees, known by the native Kimbundu negros under two names, *landula* and *cassusso*, were both known by systematists under the name *Trigona (Hypotrigona) braunsi* Kohl. In one mating ground containing 5 colonies of *landula* and 32 of *cassusso*, studies were made dequeening 3 colonies of *landula* and 6 of *cassusso*. Also, the development of four swarms of *cassusso* were studied. All hives containing *cassusso* royal cells and the four swarms, developed *cassusso* offspring, and the ones containing *landula* queen cells developed *landula* offspring. The frequency of males of both types was unknown, but the greatest value of P would be when, in the mating ground, one would find 1/3 of *landula* and 2/3 of *cassusso* ($P = 0.003$). This probability being smaller than 1%, it is safe to conclude that the varieties are two different species. It was also concluded

[3] *Dactylurina staudingeri* Gribodo has pots for honey and pollen storage, as meliponids do, but has vertical double sided combs for the brood similar to these of *Apis mellifera* L.

that horizontal combs and cluster arrangements of brood cells do not differ in so many genes as was thought before, and therefore might well have independent origins in different groups of meliponids.

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