STING GLANDS IN STINGLESS BEES—A VESTIGIAL CHARACTER (HYMENOPTERA: APIDAE)[1]

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ABSTRACT

The problem of what has occurred to the sting accessories of the stingless bees is considered. Primitive Meliponini retain the sac homologous to the sac of the acid gland of stinging bees; evolved stingless bees do not have this sac in the workers but do have it in the queens. Since *Apis mellifera* lose their stings as a result of use of the sting in defense, the authors suggest that new methods of defense evolved which do not exact a heavy toll of the colony. The fact that there are at least thirteen ways in which stingless bees protect themselves, makes stings obsolete. In 24 species of Apinae it has been possible to correlate different levels of evolution with: nests, communication systems, mandibular glands and cytology. A diagrammatic representation of evolutionary levels reached by each species is presented.

Social Apidae belong to three different tribes, namely, Bombini, Apini and Meliponini. This last group is known as stingless bees because the workers have no sting. It has conservatively, 5 genera (*Melipona, Trigona, Lestrimelitta, Dactylurina, Meliponula*). Sting accessories (sting acid gland, poison sac and basic gland) of *Apis mellifera* L. have been known since 1841 (Bordas 1895 and Snodgrass 1956). The problem we want to solve is: what has happened with the sting accessories in the stingless bees since they no longer possess a sting. The only stingless bee with a poison gland is *Trigona (Oxytrigona) tataira* F. Smith the poison glands of which are located in the base of the mandibles (Kerr and Cruz 1961) and not in the abdomen.

MATERIAL AND METHODS

We used the following species of bees: *Meliponula bocandei* (Spinola) from Luanda, Angola, Africa: *Dactylurina staudin-*

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geri (Gribodo)[2], collected in Morogoro, Tanganyika, during the trip of senior author to Africa in 1956; *Apis florea* Fabricius and *Apis dorsata* Fabricius, sent from Ceylon to Kansas and from Kansas to Rio Claro through kindness of Dr. Domiciano Dias; *Melipona (Melipona) rufiventris* Lepeletier and *Trigona* (*Frieseomelitta*) *freiremaiai* Moure, collected in Guarapari, E.S., Brazil; *Melipona (Micheneria) marginata* Lepeletier, *Melipona (Melipona) quadrifasciata* Lepeletier, *Trigona (Tetragonisca) jaty* F. Smith, *Trigona (Plebeia) droryana* Friese, *Trigona* (*Plebeia*) *schottkyi* Friese, *Trigona* (*Nannotrigona*) *testaceicornis* (Lepeletier), *Trigona (Partamona) cupira* F. Smith, *Trigona* (*Trigona*) *amalthea* (Olivier) (= *Trigona trinidadensis*), *Trigona* (*Trigona*) *hyalinata* (Lepeletier), *Trigona* (*Trigona*) *spinipes* (Fabricius) (= *Trigona ruficrus*), *Trigona (Scaptotrigona) postica* (Latreille), *Trigona (Scaptotrigona) bipunctata* (Lepeletier), *Trigona (Scaptotrigona) xanthotrycha* (Moure) all collected from colonies found in a maximum radius of 100 kilometers from our bee yard (Rio Claro, S.P., Brazil). Specimens of *Apis mellifera adansonii* Latreille were collected in our apiary. This subspecies was introduced in Brazil in 1956 from queens collected in Tabora (Tanganyika) and Pretoria (South Africa). *Bombus (Fervidobombus) atratus* Franklin was found in a colony near our laboratory. Specimens of *Trigona (Trigona) fulviventris guianae* Cockerell were obtained in Ilha Comprida, S.P., Brazil, in a semi-subterraneous nest. *Trigona (Oxytrigona) tataira* F. Smith and *Trifona (Cephalotrigona) capitata* F. Smith were given by Dr. Paulo Nogueira Neto (both colonies found in this same region). *Lestrimelitta* (*Lestrimelitta*) *limão* F. Smith was collected in Poços de Caldas, M.G., Brazil.

The use of the names *T. spinipes* and *T. amalthea* is in accordance with the recent revision of Moure (1960). The use of the subgenus *Melipona* (*Micheneria*) is in accordance to the paper of Lopes and Kerr (1962).

Workers and, when possible, queens were dissected under a Zeiss stereoscope; drawing were made with a Zeiss camera lucida, and photographs with a Zeiss photomicroscope. The fixative used was Dietrich's; the sac and atrophied glands (when exist-

[2] According to Moure (1961) the subspecies which inhabits Tanganyika is *Dactylurina staudingeri schmidti* (Stadelmann)

ing) were stained with hematoxylin-eosin, Nile green, methyl green, aceto-orcein. Sections (10μ) and whole mounts were made. Weighing was done in a torsion balance with precision of 0.1 mg.

OBSERVATIONS

Observations on three groups of bees are listed, each in a phylogenetic order.

Bombus (Fervidobombus) atratus (workers). We dissected three workers, one of which had functional ovary, full of eggs in its eight ovarioles (four in each ovary). All three workers had the spermatheca without spermatozoa. The sting accessories, Dufour gland (or basic gland), acid gland and sac of the acid gland, do not differ consistently from the description made by Bordas (1895) for *Bombus (Agrobombus) muscorum* Fabricius and *Bombus (Pyrobombus) pratorum* Linné.

A schematic drawing of *B.* (*F.*) *atratus* showing the sting apparatus is seen in Fig. 1.

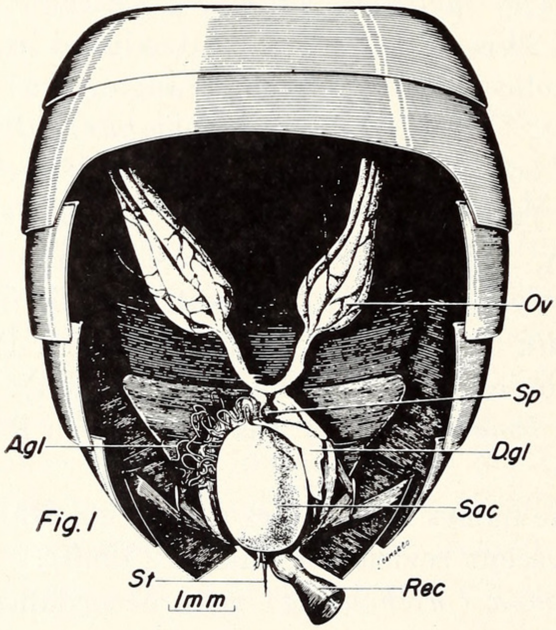


Fig. 1—Sting glands of *Bombus* (*Fervidobombus*) *atratus* Franklin. A.gl. = Acid glands, D.gl. = Dufour glands, Ov = ovaries, Sac = poison sac, St = sting, Sp = spermatheca, Rec = rectum.

According to Bordas (o.c.) the acid gland is composed of two thin cylindric and filiform tubes, that unite in a short common trunk. In our material this acid gland was more branched, i.e., four tubes unite in two and these two in one short trunk. Sometimes one or two very short branches are found laterally attached to these four tubes (in one bee we counted nine branches). Histologically these tubes are composed of secretory cells around a canal lined by a thin membrane (Fig. 2). Several canalicules

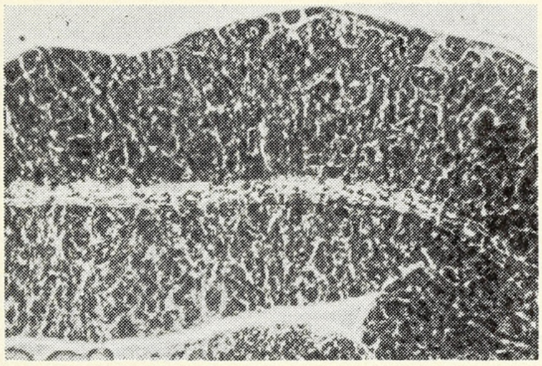


Fig. 2—Acid gland of *Bombus (F.) atratus* Franklin. Longitudinal view of small section of acid gland, showing central canal which collects the secretion (Photographed with phase contrast).

collected the secretion of the cells and released it in the central canal (Fig. 3). These canalicules in the anterior part of the

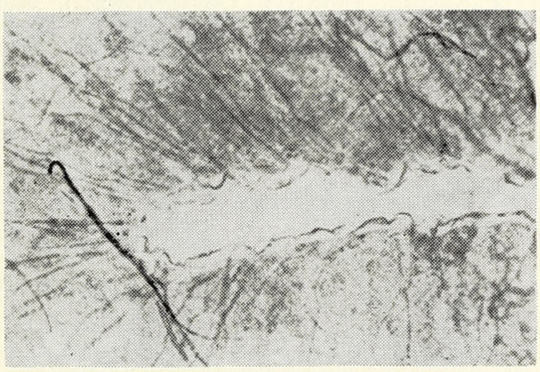


Fig. 3—Anterior end of the acid gland of *Bombus (Fervidobombus) atratus* Franklin. Region of greater secretory activity. Taken without phase contrast to show enormous quantity of canalicules opening into central canal (aceto-orcein smear).

sac are very thick and short. However, in the tubular acid gland they become thinner and longer toward the anterior part of the gland. The most active secretory cells are the ones in the distal end where one can see thousands of secretory cells, each with its respective thin canalicule (Fig. 3). The inner membrane shows the perforations of the canalicules.

Histologically the ovoid poison sac of *Bombus (Fervidobombus) atratus* is made by an external transparent membrane, an intima membrane and between these two, in about $1/3$ of the sac, a glandular epithelium, formed by a group of secretory cells different from the acid gland. To increase the secretory surface these cells are assembled in outfoldings (Fig. 4).

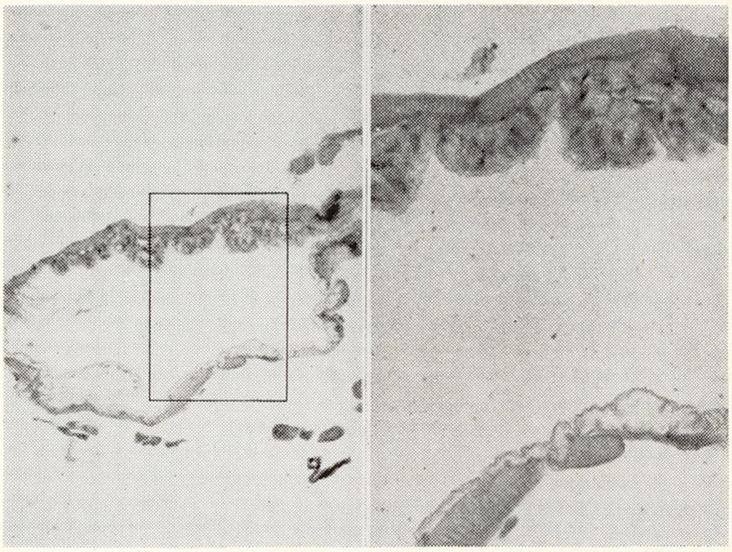


Fig. 4—Transverse section of poison sac *Bombus (Fervidobombus) atratus* Franklin. Approximately the section which passes in middle of sac. Note secretory epithelium in upper part. Left side shows the section practically complete. Right side shows framed part for easy comparison of secretory with the non secretory membranes.

The Dufour gland is also full of outfoldings, but distinct from those of the acid sac (Fig. 5). These villosities are much longer

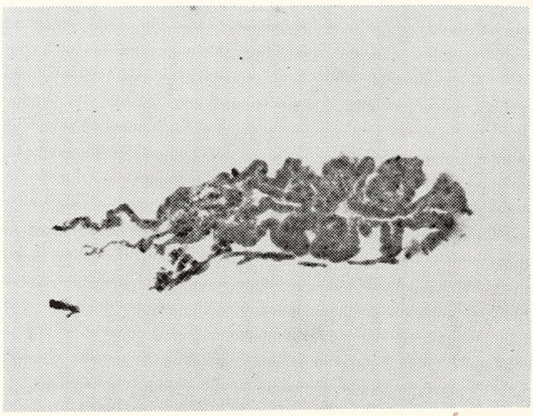
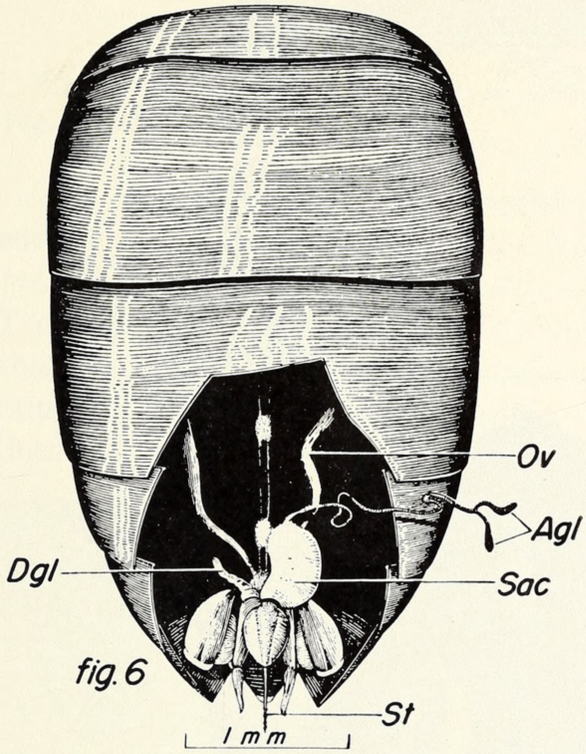


Fig. 5—Dufour gland of *Bombus* (*Fervidobombus*) *atratus* Franklin, transverse section, showing outfoldings distinct from villosities found in poison sac (Ref. Fig. 4).

and formed by smaller and more uniform cells. This gland of *B. atratus* does not differ from descriptions of other authors.

Apis florea (workers). The acid gland and poison sac do not differ consistently from those already described for *Bombus,* the differences being that there are only two branches in the acid gland and no villosities in the sac. The Dufour gland, however, is more developed than in *Apis mellifera.* (Fig. 6.)



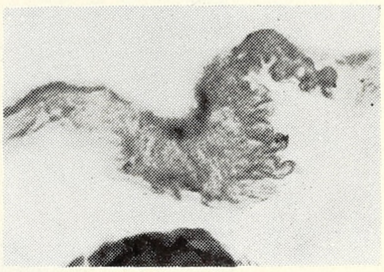


Fig. 6 (left)—Sting glands of *Apis florea* Fabricius. A.gl. = acid gland, D.Gl. = Dufour gland, Ov. = ovary, Sac = poison sac, St = sting. The Dufour gland more developed than in *Apis mellifera* (definitely more Bombus-like than *mellifera*).

Fig. 7 (right)—Small portion of cross section of poison sac of *Apis dorsata,* showing two membranes, the internal with anucleated villosities, having no secretory activity. The darker spot is dense secretion stored in sac.

Apis dorsata (workers). The sac is full of a dense secretion, darker than *A. florea* or *A. mellifera.* This may be the answer as to why its sting hurts more than other *Apis* species. In sections it was seen that the secretion absorbed more eosin than the secretion of other species. Higher absorption is also observed in every section of the acid gland in the central canal. The

Dufour gland is also well developed. No secretory villosity was seen in the sac of the acid gland (Fig. 7). Anatomy and histology do not differ from *Apis mellifera*. (Fig. 8).

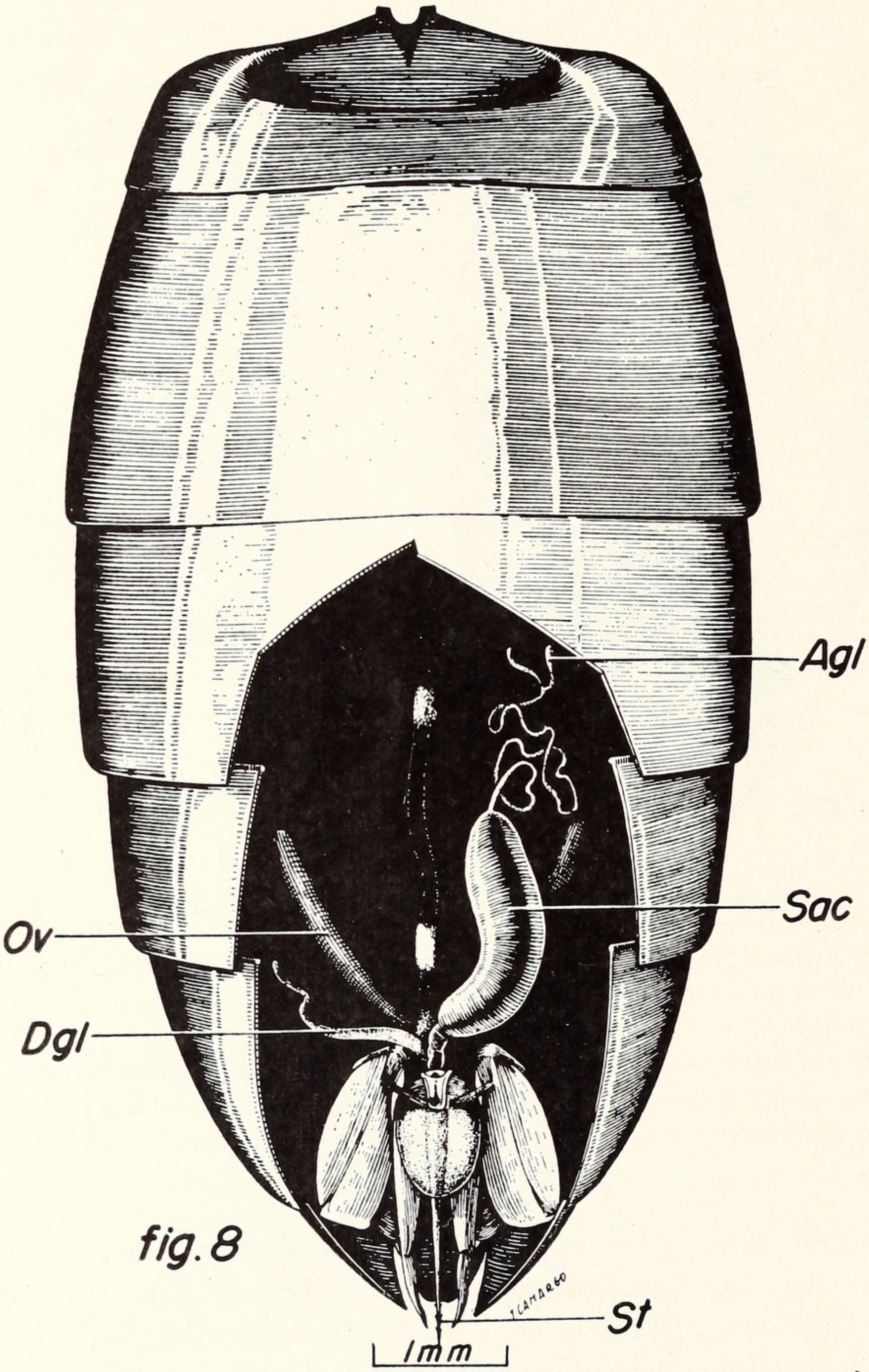


Fig. 8—Sting accessories of *Apis dorsata* Fabricius. Legend as in Fig. 1 and 6. The Dufour gland is also Bombus-like when compared with *mellifera*.

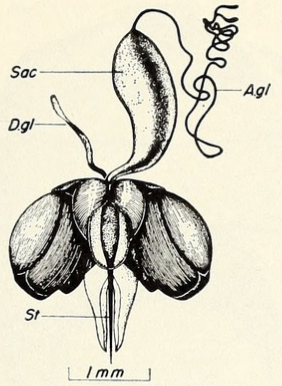
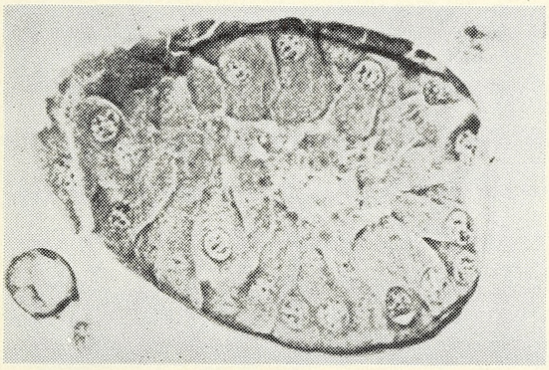


Fig. 9—Sting accessories of *Apis mellifera adansonii.* Legend as in Fig. 1 and 6.

Apis mellifera adansonii (workers and queens). The sting accessories do not differ (Fig. 9) from the description of *Apis mellifera mellifera* and *Apis mellifera ligustica* by Carlet (1890), Trojan (1930), Snodgrass (1956) and others. It is interesting that the acid gland, from the poison sac to the bifurcation, is much shorter in the queen than in the worker. The histology does not differ in principle (Fig. 10) from what is seen in



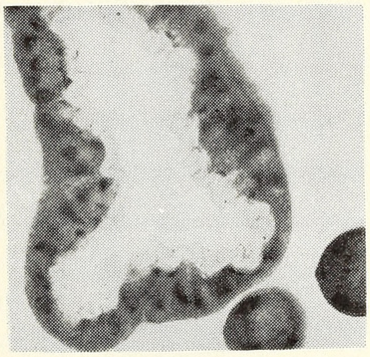


Fig. 10 (left)—Cross section of acid gland of *Apis mellifera adansonii* showing secretory cells of region with low secretory action.

Fig. 11 (right)—Cross section of the poison sac of *Apis mellifera adansonii* Latreille in anterior region, nearer acid gland. Poison sac merely a dilatation of acid gland. External membrane shown with layer of cells of the secretory type between.

Bombus atratus. The sac does not have secretory villosities (Fig. 11).

Meliponula bocandei (workers). All workers used were either field bees or guard bees.

It has an enormous u-shaped sac (Fig. 12). Six of the dis-

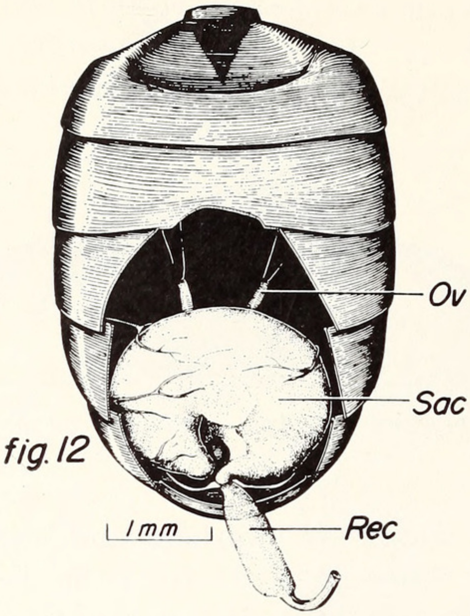


Fig. 12—Abdomen of *Meliponula bocandei* (Spinola) showing huge sac. Acid gland and Dufour gland lacking. Picture does not show sac at maximum volume, which can be about ⅓ greater. Ov = ovary, Rec = rectum, Sac = sac corresponding to poison sac of *Apis* and *Bombus*.

sected bees had this sac completely full of an oily liquid, nonmiscible with water. However 3 workers had it only partially full. The position of the exit of this sac is the same as in *Apis mellifera*, i.e., between the rectum and the main oviduct.

Histological sections showed that the sac had basically the same organization as that of *Bombus atratus*, i.e., a thin external membrane, a median layer of secretory cells and an internal intima membrane. The secretory epithelium increased its surface considerably, forming outfoldings, to compensate for the lack of more specialized secretory cells (Fig. 13). Several of

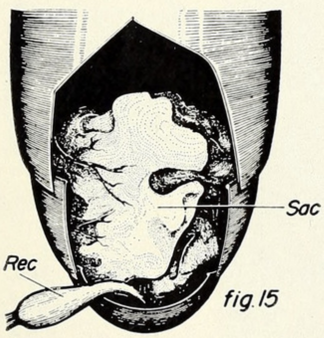
Fig. 13—Small portion of section of sac of *Meliponula bocandei* (Spinola) showing external membrane and villosities of secretory epithelium. Epithelium similar to *Bombus (Fervidobombus) atratus* Franklin shown in Fig. 4, only more developed.

the secretory cells have large vacuoles (Fig. 14), indicating secre-

Fig. 14—One outfolding of sac of *Meliponula bocandei* (*Spinola*) showing secretory cells full of vacuoles and nuclei. The intima membrane penetrates among the cells, possibly to increase transfer of secretion to lumen.

tory activity. The internal membrane is more delicate than the ones found in *Bombus* or *Apis* and of variable thickness.

Trigona (Frieseomelitta) freiremaiai (workers). Six field bees were used. The sac is well developed, and bent to the left (Figs. 15 and 16). The gland is not as developed as in *Melipon-*



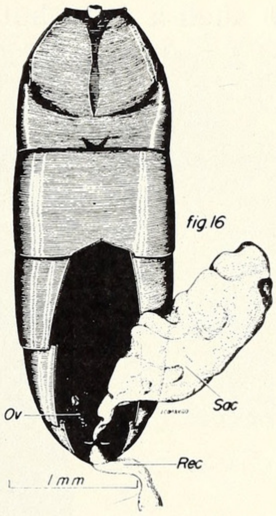


Fig. 15—Sac of *Trigona (Frieseomelitta) freiremaiai* Moure in natural position. (Refer. 1 sac unfolded in Fig. 16). Rec = rectum, Sac = sac corresponding to poison sac of *Apis* and *Bombus*.

Fig. 16—Sac of *Trigona (Frieseomelitta) freiremaiai* Moure unfolded to show enormous size. Legend as in Fig. 1 and 12.

ula, but it had more foldings.

The histology is similar to that of *Meliponula bocandei,* but the villous processes were smaller and less frequent than in that species (Fig. 17). There are no canalicules linking the cells to

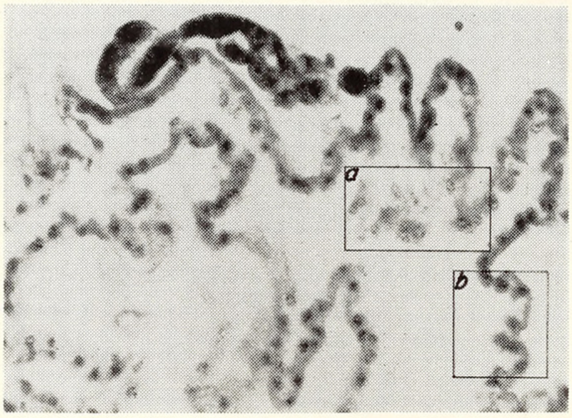


Fig. 17—Cross section of sac of *Trigona (Frieseomelitta) freiremaiai* Moure showing a great number of foldings and one villosity. Villosity shown in detail Fig. 18. Sac possesses two membranes, one external and an intima. Among them secretory cells seen surrounded by intima membrane. Refer: Fig. 19.

the lumen, however the membrane involves each secretory cell in such a way that diffusion of the liquid is easy. Note villosity of secretory cells (Fig. 18) and secretory cells in the wall (Fig. 19).

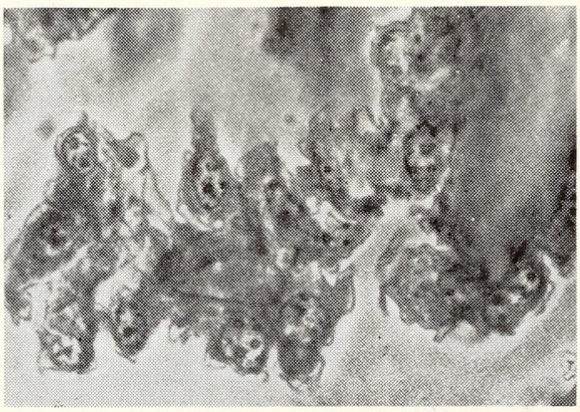


Fig. 18—Villosity within the upper square (a) of Fig. 17. (Phase contrast).

Trigona (Nannotrigona) testaceicornis (workers and two queens). Workers had a large sac filled with an oily liquid.

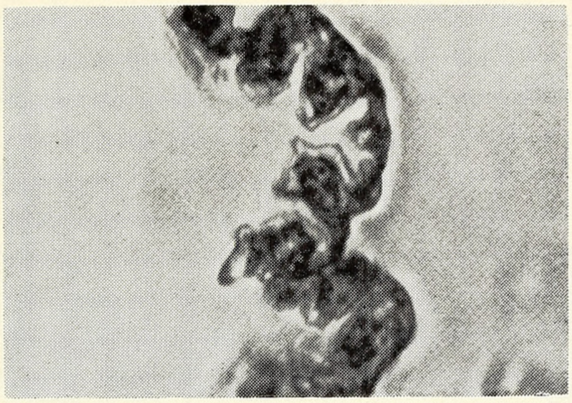
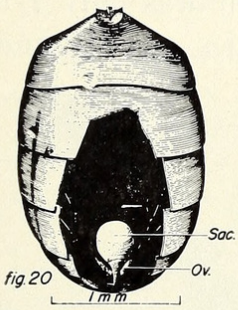


Fig. 19—Secretory cells in lower square (b) of Fig. 17. (Phase contrast).

Two virgin queens examined also possessed a large sac. These queens had not yet emerged but were about to do so in two to three days. Both queens had empty sacs. No villosities were found in the sacs of workers or queens, although secretory cells could be seen in between the sac membranes.

Trigona (Plebeia) droryana (workers). The workers examined did not differ from the *T.* (*N.*) *testaceicornis*, i.e., they had a large sac (Fig. 20). The secretory cells are not specialized (Fig. 21).



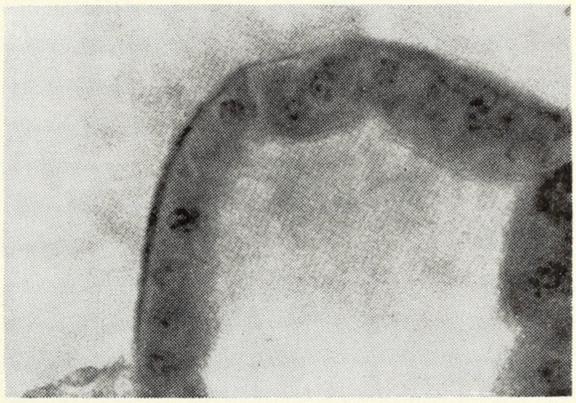
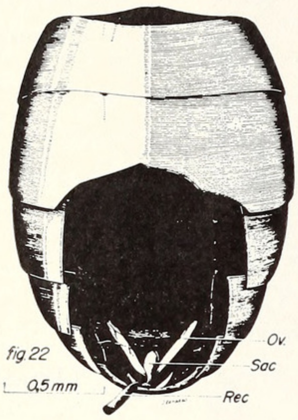


Fig. 20—Sac of *Trigona (Plebeia) droryana* Friese. When completely full can be twice as large.

Fig. 21—About 1/5 of cross section of sac of *Trigona (Plebeia) droryana* (Frieseomelitta.) Between dark external membrane and light intima membrane is layer of secretory cells that do not seem as specialized as in Fig. 13 and 17.

Trigona (*Plebeia*) *schrottkyi* (workers). The workers possessed sacs similar to *T.* (*P.*) *droryana*. (Fig. 22).



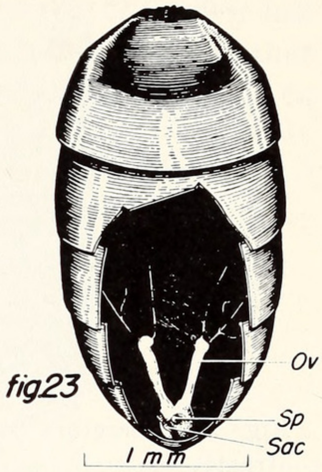


Fig. 22—Sac of *Trigona (Plebeia) schrottkyi* Ov = ovary, Rec = rectum, Sac = sac homologous to poison sac of stinging bees.

Fig. 23—Vestigial sac of *Trigona (Oxytrigona) tataira* F. Smith. Ov = ovary, Sac = vestigial sac, Sp = spermatheca.

Trigona (*Partamona*) *cupira helleri* (workers). The workers showed either no sac or a small vestigial sac, with little inside.

Trigona (Tetragonisca) jaty (workers). The workers possess a small sac, smaller than *T.* (*P.*) *droryana,* but not vestigial as *T.* (*O.*) *tataira.*

Dactylurina staudingeri (workers). The workers of this species possess a relatively large sac (homologous to the poison sac) but with a few patches of secretory layer. In fact in the section where the secretory epithelium was larger was only 1/9 of the total section (Fig. 24). A few villosities were found

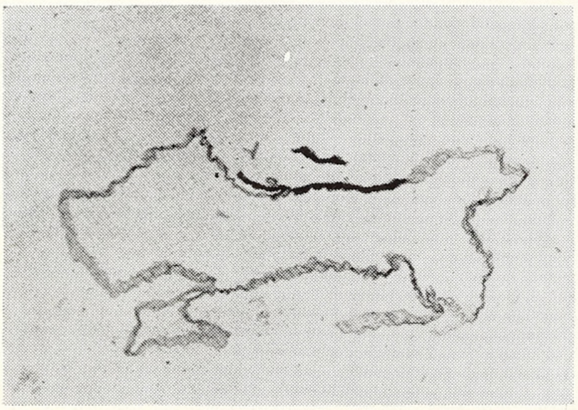


Fig. 24—Cross section of the sac of *Dactylurina staudingeri* (Gribodo) showing secretory epithelium (dark) covering 1/9 of whole section.

Fig. 25—Villosity of sac of *Dactylurina staudingeri* (Gribodo). Type of sac and of villosity differ from all others found in meliponids.

(Fig. 25) but of a type different of *M. bocandei* or *T. feiremaiai.*

Trigona (Oxytrigona) tataira (10 workers). The vestigial sac is smaller than the vestigial spermatheca (Fig. 23).

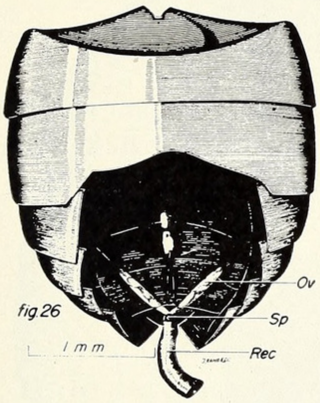
Trigona (Trigona) hyalinata (workers). The workers do not possess sting accessories.

Trigona (Trigona) amalthea (workers). The workers do not possess sting accessories.

Trigona (Trigona) fulviventris subspecies *guianae* (workers). The workers do not possess sting accessories.

Trigona (*Trigona*) *spinipes* (workers). Two workers showed a vestigial sac, about 350μ long and 40μ wide. One worker did not have a sac.

Trigona (Scaptotrigona) postica (workers, virgin queens, old queen). All workers examined showed no sign of gland or sac (Fig. 26). However the virgin has a well developed sac (Fig.



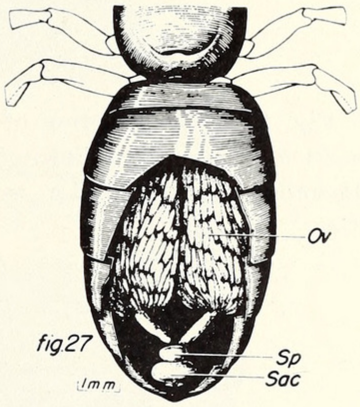


Fig. 26—*Trigona (Scaptotrigona) postica* Latreille shows no sign of poison sac. Ov = ovary, Rec = Rectum, Sp = spermatheca.

Fig. 27.—Queen of *Trigona (Scaptotrigona) postica* Latreille with developed sac. In virgin queen sac 4 × bigger than in gravid queen as above. Ov = ovary, Sac = sac homologous to the poison sac, Sp = spermatheca.

27). This queen was about 25 days old and had come from a mother hive to a new location, where bees were founding a new home (for swarming in stingless bees see Nogueira Neto 1950). The sac was full of an oily liquid. The old queen had this sac much smaller than the virgin, being no greater than the spermatheca.

Trigona (Scaptotrigona) xanthotricha (workers and queen). Workers had no sac nor glands. A virgin queen (17 days old just being killed by her sisters, workers of a queen-right colony) was fixed and dissected. She had the same structures as *T.* (*S.*)

postica, except that the sac was not completely filled.

Trigona (Scaptotrigona) bipunctata (workers and queen). Workers with no sting accessories. A virgin queen, 10 days old, had structures like *T.* (*S.*) *postica*, with its sac full of an oily liquid. The histology shows the sac full of villosities similar to the *Meliponula bocandei* (Figs. 28, 29, 30).

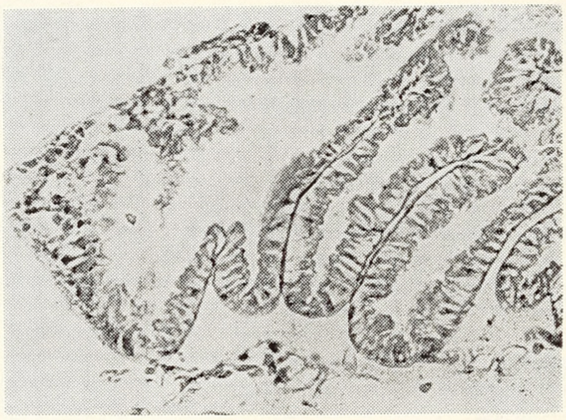


Fig. 28—Cross section of sac of virgin queen of *Trigona (Scaptotrigona) postica* Latreille, showing primitive condition, similar to *Meliponula bocandei* (Gribodo), i.e., secretory epithelium formed by numerous villosities.

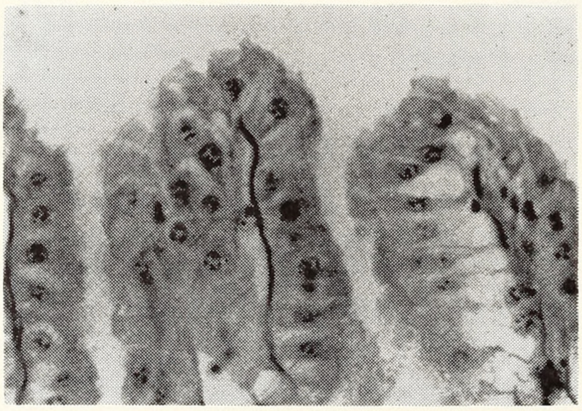


Fig. 29—Two villosities of sac of *Trigona (Scaptotrigona) postica* virgin queen in left, cells in no great secretory activity and in right cells with vacuoles full of secretion. These groups of cells are shown in Fig. 30.

Trigona (*Cephalotrigona*) *capitata* (workers). Workers show no sac.

Melipona (Micheneria) marginata (workers). Workers with very small sac.

Fig. 31—Vestigial poison sac of *Melipona (Melipona) quadrifasciata* Lepeletier. Ov = ovary, Rec = rectum, Sac = vestigal poison sac, Sp = spermatheca. →

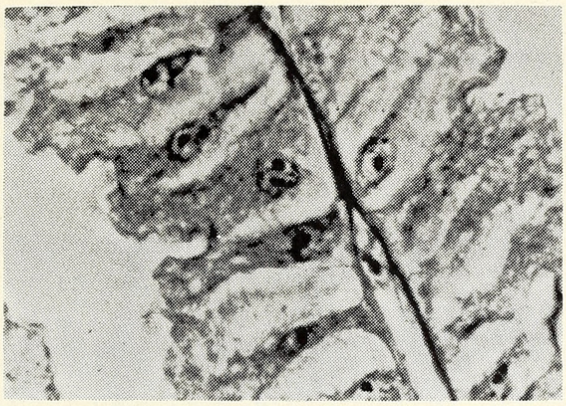


Fig. 30—Villosity of secretory cells with vacuoles full of secretion of sac of virgin queen of *Trigona (Scaptotrigona) postica.* Nuclei compressed to one side of cell. (Phase contrast).

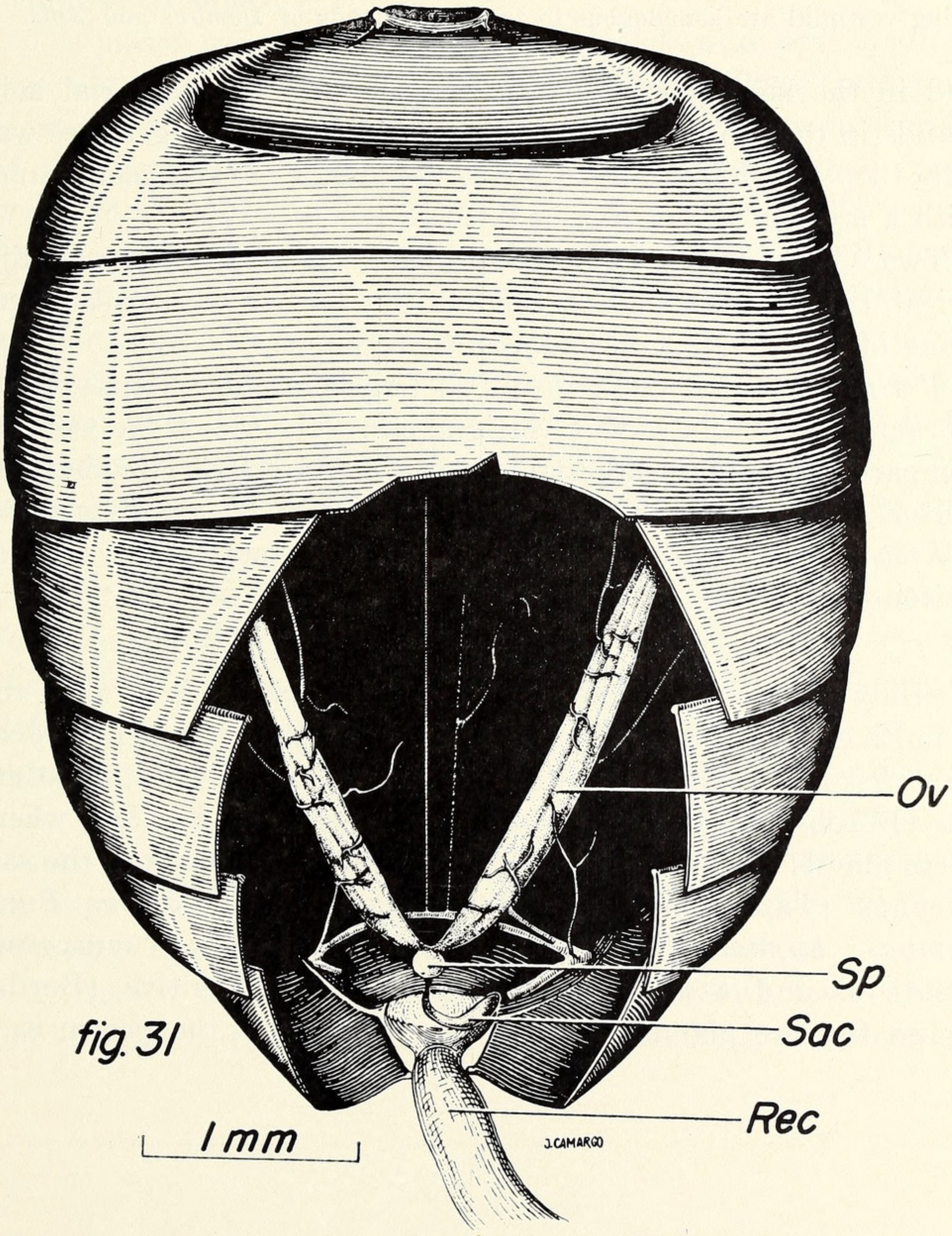


fig. 31

Melipona (*Melipona*) *quadrifasciata* (workers and virgin queen). Workers have many variable structures although all of them small and vestigial. In about ⅓ of the workers the sac was extremely small (Fig. 31) in another ⅓ it was a little larger,

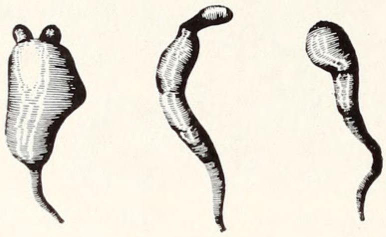


Fig. 32—Three types of vestigial sacs found in *Melipona (Melipona) quadrifasciata* Lep. First sac has two protuberancies which in spite of being vestigial are homologous to two acid glands of *Bombus* and *Apis.*

and in the last ⅓ it was pear shaped with two vestigial acid glands in the top (Fig. 32). The liquid in these larger sacs was jelly-like. Histologically, the sac was made of two membranes with a layer of cells between them.

Two virgin queens were examined. Their sacs were cylindrical, two to four times bigger than the largest worker sac seen. None of the queen sacs had vestigial glands.

Melipona (Melipona) rufiventris (workers and queens). The workers and queens have a vestigial sac. The sac of the workers compares with the smallest of *M.* (*M.*) *quadrifasciata,* but the queens have the vestigial sac equal to that of *M. quadrifasciata.*

Lestrimelitta (Lestrimelitta) limao (workers). No sign of poison sac.

Discussion

Study of the morphology of the acid gland shows that the primitive condition for Apidae is the one preserved in *Psithyrus rupestris* in which each acid gland opens directly into the poison sac (Bordas 1895). The second step is found in *Bombus,* where these glands are united in a common trunk opening into the sac. *Bombus (Lapidariobombus) lapidarius* Linné, *Bombus Pyrobombus*) *hypnorum* Linné, *Bombus* (*Agrobombus*) *muscorum* Fabricius and *Bombus sylvarum* are more primitive (Bordas found the two glands uniting 3 to 4 mm from the poison sac) and *Bombus (Hortobombus) hortorum* Linné, *Bombus (Pyrobombus) pratorum* and *Bombus (Pomobombus) pomorum* Panzer are more evolved (Bordas found that the union occurs 10 to 20 mm from the sac) in relation to the acid gland. The primitiveness of this characteristic is strengthened by the fact that the *Apis* queen has a shorter common trunk for the acid gland than the workers (it is common to see primitive characters possessed by queens of social bees). Finally, the worker of *Apis mellifera* has the longest common trunk (and the shortest individual branches of all). The two protuberances found in the poison sac of *Melipona quadrifasciata* indicate that meliponid of the primitive type may have been similar to *Psithyrus* or to some primitive *Bombus.*

The poison sac of *Apis florea, Apis dorsata* and *Apis mellifera* does not show secretory cells assembled in a villous epithelium. However, they were found by us in *Bombus atratus,* in workers of *Meliponula bocandei* and *Trigona (Frieseomelitta) freiremaiai* and in queens of *T. postica.* The secretion of *Meliponula bocandei* is not caustic which suggests that this secretion has some other function. In *Trigona (Nannotrigona) testaceicornis* and *Trigona (Plebeia) droryana* these cells no longer form outfoldings but still exist in great quantity in a non-stratified epithelium. In other groups of stingless bees few cells exist in the sac due to its small size.

Bees of the genus *Apis* lose their stings after they use it in a victim, therefore an enemy attack can deplete a colony greatly, leaving it weak. Weak colonies fail to reproduce. It is, therefore, conceivable that new methods of defense have evolved that would not take a heavy colony toll. Stingless bees are not defenseless but have according to Kerr (1950, 1951) at least thirteen different means of keeping enemies out. These include:

1. Deposits of wax mixed with vegetable gums or gums and resins, to adhere to the enemy in a fight or to plaster an enemy getting inside the hive. This system is used by the subgenera *Plebeia, Tetragona, Trigona, Tetragonisca,* and others.

2. Robust mandible sometimes reinforced with teeth able to cut an enemy apart are characteristic of the *Melipona* genus and

of the *Trigona* subgenus (species: *spinipes, amalthea, hyalinata*).

3. Unpleasant taste and smell. This method is considered the most important by Marianno Filho (1910). Species of the subgenera: *Trigona, Scaptotrigona, Geotrigona* and many *Melipona* species have this property.

4. Massive attack to repel an intruder, penetrating nostrils and ears. Such a method is used by *Scaptotrigona, Oxytrigona, Trigona, Partamona,* and other subgenera.

5. Large number of workers. Huge colonies of more than 200,000 workers are found in many species such as *T.* (*T.*) *jaty, T.* (*S.*) *pectoralis* Dalla Torre, *T.* (*T.*) *hyalinata, T.* (*T.*) *cupira,* and others.

6. Entrance fitted to allow only one bee in or out. Such a method increases the defense system considerably, and it is used by almost all *Melipona* and many *Trigona.*

7. Closing the entrance as sunset with wax and reopening it in the morning. Such a system is used by weak colonies in general.

8. Blocking the entrance with wax or resins. According to Fiebrig (in Maidl, 1934) workers of *Lestrimelitta limao* F. Smith placed in an elaborated entrance tube small pieces or blocks of wax and resin to keep ants from getting in. When an attack is over the bees remove them. Maidl (1934) cites that *Trigona* (*Lophotrigona*) *canifrons* Smith workers build a ring of resin surrounding the entrance, which is constantly renewed. We noted that some species of the subgenus *Plebeia* have the same behavior.

9. Some species of stingless bees such as *T.* (*F.*) *silvestri* Friese appear dead when touched by a strong enemy (personal communication of Dr. Nogueira Neto).

10. Camouflage of the nest or of the nest entrance. This is done by several species, such as *Melipona* (*M.*) *quadrifasciata, Trigona* (*F.*) *silvestrii, Trigona* (*Plebeia*) *cupira,* and others.

11. Mimicry. Kerr (1951) cites 9 cases of mimicry where the stingless bee copies a bee or a wasp possessing a strong sting.

12. *Trigona* (*Oxytrigona*) *tataira* uses its mandibular glands to inflict on the enemy, especially mammals, a terrible "burning," due to the action of a caustic secretion (Kerr and Cruz 1961).

13. *Trigona* (*H.*) *braunsi* defends itself against a *Lestrimelitta*

(*Cleptotrigona*) *cubiceps* attack by pouring honey on the invaders (Portugal-Araújo 1958).

These methods, made stings obsolete and mutations to diminish or abolish stings become established in the primitive populations. However, the sting glands and accessories had no strong selective disadvantage aside from the space they occupied and so were selected against much more slowly.

The small basic gland (or gland of Dufour) disappeared completely. Histologically the sac that remains in some stingless bee species is homologous to the acid gland sac. This sac is merely a dilatation of the acid gland's posterior part.

The drawing of Dufour (1841), Carlet (1890) and Bordas (1895) are in error concerning the external opening of the Dufour gland (basic or alkaline gland). Our slides show that Trojan (1930) is correct, in that this gland opens into the sting chamber, below the sting (Fig. 33). Trojan suggests that this

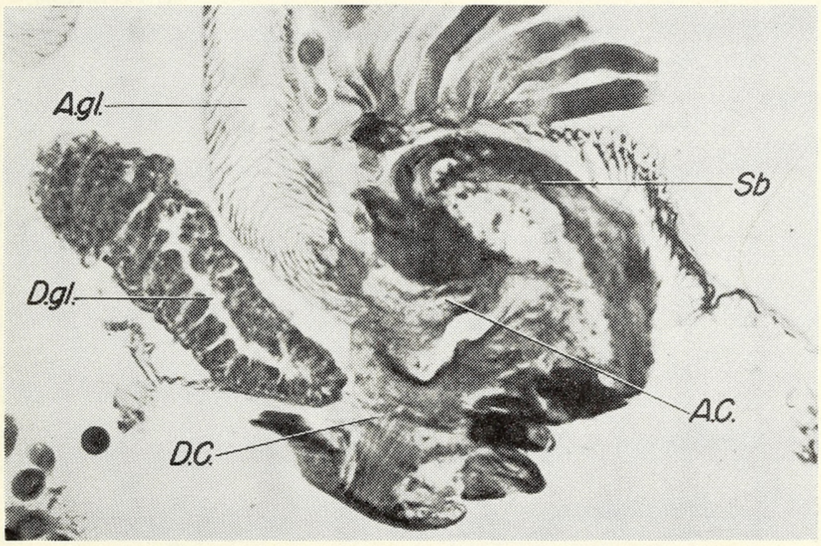
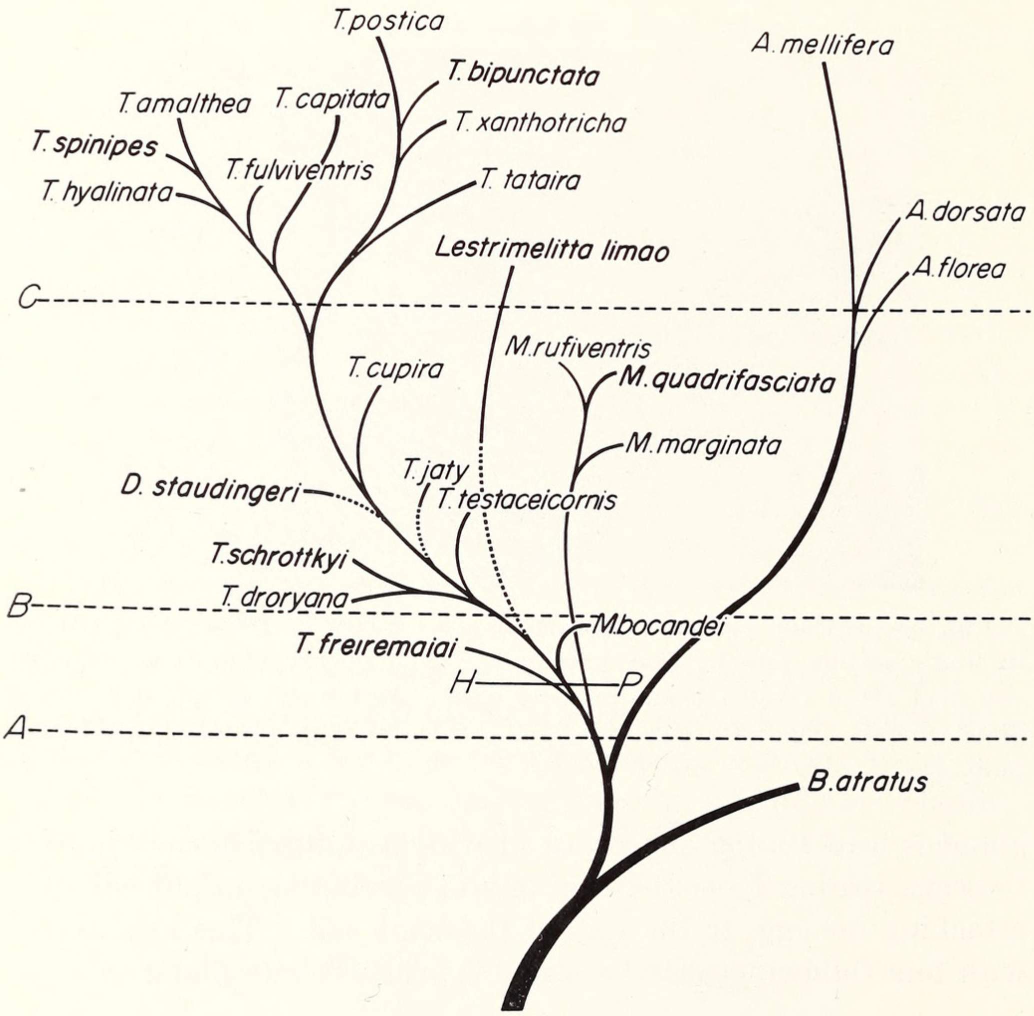


Fig. 33—Dufour and acid glands of *Apis mellifera adansonii* Latreille, in single section (10μ). Dufour gland (D.gl.) opens below sting chamber and acid gland (A.gl.) opens in sting bulb. A.gl. = acid gland, A.C. = Acid gland canal, D.gl. = Dufour gland, D.C. = Dufour gland canal, Sb = sting bulb.

gland is used by the queens, to provide a protective covering for the eggs issuing from the vagina and serving as an adhesive for attaching the eggs to the wall of the comb cell. This idea agrees with our following observations: a) The Dufour gland is more developed in *A. florea* and *A. dorsata* than in *A. mellifera*. This may be due to the fact that these two species build combs in the open air, subjected to the direct impact of the rain. Notwithstanding these bees provide a good defense of the alveoli, covering them with their water repellent bodies. It is more likely that such eggs need more protection and a stronger adhesive than *A. mellifera*. b) The Dufour gland completely disappeared in the meliponids (stingless bees). It is completely unnecessary in these bees, because the workers fill an alveolus with food (liquid in all species except *Meliponula bocandei*) and the queen lays an egg on it. Immediately the bees close the alveolus with wax. Therefore no need to fasten the egg to any surface exists.

There are over 300 species of stingless bees (according to Prof. J. S. Moure's files, added with some *Melipona* subspecies that we found to be good isolated species) distributed in groups



with different levels of evolution. According to their nests (Kerr and Laidlaw 1956), their system of communication (Kerr 1961), their mandible glands (Cruz 1960, Kerr and Cruz 1961), and their cytology (Kerr 1962, unp.) a diagrammatic representation (Fig. 34) shows the relative evolutionary levels reached by each species. The relative size of the sting poison sac closely follows this diagram. In fact, *Trigona freiremaiai* and *Meliponula bocandei* have the largest sacs (these two bees are so primitive that they do not make combs but have their brood cells arranged in clusters, similar to some *Bombus* species); *Trigona* (*Nannotrigona*) *testaceicornis*, *Trigona* (*Plebeia*) *schrottkyi* and *Trigona* (*Plebeia*) *droryana* have well developed glands, but not very large. *Dactylurina staudingeri* has a large sac but provided with few secretory patches. *Melipona* species and *Trigona* (*Tetragonisca*) *jaty* have vestigial sacs; it is evident that in *Melipona quadrifasciata* there is a great variation in size and shape indicative of an unstable genetic constitution; *T. jaty* has glands somewhat larger. Vestigial sacs, and sometimes no sacs are found in bees like *Trigona* (*Partamona*) *cupira*, *Trigona* (*Trigona*) *spinipes*, *Trigona* (*Trigona*) *fulviventris*, *Trigona* (*Trigona*) *amalthea*, *Trigona* (*Trigona*) *hyalinata*, *Trigona* (*Oxytrigona*) *tataira*, *Trigona* (*Cephalotrigona*) *capitata*, *Lestrimelitta* (*Les-*

Fig. 34—*Tree of evolutionary level and possible relationship.* Below line A no communication exists and each queen found new colony with no help of workers. Between lines A and B species have primitive communication system; the *Frieseomelitta* group has tubes for pollen like superior *Bombus*, and chromosome number in n = 9; the *Meliponula* species has dried food. Below line B only cluster type of cell arrangement is found. Line H – P indicates that branches originating above it have n = 18 chromosomes (or 17 when reduction through translocation took place). Between lines B and C species with good alarming system (for communication) are found, and some, like *T. cupira*, and superior *Melipona* have some means of directional communication. Some species still have cluster type of cell arrangement and may or may not have involucrum. Species above C line have precise type of communications, and nests are quite evolved.

Degeneration of sting gland sac follows: Below line A acid gland and Dufour gland are well developed. Sting sac of *Bombus* has few villosities of secretory cells. Above line A no meliponid found with Dufour gland. Between lines A and B meliponid species have well developed sting sac full of villosities which contain secretory cells. Between B and C several degrees of degeneration of sac homologous to acid gland sac are found. Above line C vestigial sac or no sac at all is found among meliponids, and no secretory villosity is found in sac of *Apis* species.

trimelitta) *limao, Trigona* (*Scaptotrigona*) *bipunctata* do not show signs of these sting accessories in the worker. However in *T.* (*S.*) *bipunctata*[3], *T.* (*S.*) *postica* and *T.* (*S.*) *xanthotricha,* whose queens were examined this sac was well developed. Why would the queen show a well developed gland and the worker none? Bees constitute the best didactic example against Lamarckism and Lisenkoism. The genic constitution of the queen is mainly selected for genes that will benefit the workers so the entire colony becomes more fit. Extra weight of an unnecessary poison sac which would not be a serious burden to a queen because of her domestic life would mean a lot of extra work to a worker which has to fly an average of 10 to 20 kilometers a day. A worker of *Apis mellifera adansonii* weighs an average of 67.0 mg (s.d. 5.5) and can carry in its honey crop as much as 54.8 mg (s.d. 7.2) of nectar (personal communication of Mr. D. Beig); its acid gland, sac and sting weigh 4.0 mg, which is 6% of the body weight. A worker of *Meliponula bocandei* weighs 39.4 mg has a honey-sac capacity of 31.4 mg and its sting sac when full weighs 9.1 mg which is 29% of the total body weight. Therefore, genes will be selected that affect the worker sac. Whether these same genes do or do not affect the queen also, is irrelevant. In queens of *Melipona quadrifasciata* the size of the sac is only two to five times greater than the average worker, but in the *Scaptotrigona* the difference is enormous (compare Fig. 26 and 27).

The evolution of the stingless and poison-gland-less condition in social bees provides an example of an orthogenetic series, in which evolution left alive all intermediate types and substantiates our explanation of a vestigial character.

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[3] The virgin queen of *T.(S.)bipunctata* examined by us was secreting wax through her three last tergite connections. It was known (Drory 1873, 1874, 1877, Kerr 1951) that males of stingless bees secrete wax (unlike drones of *Apis mellifera*) but it was not known that a virgin queen can also do it.

A.m.adansonii taken from his experimental data; Dr. S. F. Sakagami, Prof. J. S. Moure and Dr. Carminda da Cruz Landim for valuable suggestions. The subgenus of *Bombus* was named according to Dr. S. F. Sakagami; All drawings were done by Mr. J. M. F. Camargo.

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Delayed Publication

The response from the South American co-workers of the late Herbert F. Schwarz in contributing to this issue of the *Journal* has been overwhelming. The magnificant cooperation and the fact that many papers were received late in the year, however, created a problem of publication. Since it was not possible to include all of the papers in this issue, the Editors and the Publication Committee reluctantly made the decision to carry over the following papers for Volume LXXI:

Araujo, Virgilio de Portugal	Subterranean Nests of Two African Stingless Bees
Kerr, Warwick E., Amilton Ferreira and Neide Simões de Mattos	Communication Among Stingless Bees—Additional data
Landim, Carminda da Cruz	Evaluation of the Wax and Scent Glands in the Apinae
Sakagami, Shôichi	On the Male of *Trigona* (*Tetragona*) *fimbriata* Smith
Kerr, Warwick E. and Vilma Maule	Geographic Distribution of Stingless Bees and its Implications.

Upon publication, each paper will be accompanied by a note indicating that it was submitted for inclusion in the HERBERT F. SCHWARZ MEMORIAL VOLUME.