Original article

Dwarf gynes in *Nannotrigona testaceicornis* (Apidae, Meliponinae, Trigonini). Behaviour, exocrine gland morphology and reproductive status

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Summary — The behaviour and morphology of dwarf gynes produced in worker-sized cells of normal colonies in *Nannotrigona testaceicornis* (Meliponinae, Trigonini) were studied. The behaviour of these dwarf virgin queens was the same as observed for normal Trigonine gynes. The glandular equipment is also the same: Dufour glands, fat bodies and spermathecae are present. Despite these similarities, their ovaries are different. The functional significance of dwarf gynes is unknown, but may be a basis for an alternative reproductive strategy.

dwarf gynes / larval food / reproductive strategies / stingless bees / Trigonini / *Nannotrigona testaceicornis*

INTRODUCTION

Currently two main systems of raising queens are known in stingless bees. In Meliponini, queens, males and workers originate from cells which are of the same size. This implies that larvae of both castes ingest almost similar amounts of food. Kerr (1950) and Kerr et al (1966) suggested that in Meliponini caste is genetically determined, although environmental factors and food quantities may have an additional influence in caste determination. In Trigonini, a trophic system occurs and it was assumed that caste is exclusively determined by the amount of food offered. Larvae producing queens are superfed, although the strategies to reach superfeeding may vary (Terada, 1974). Most of the bees belonging to the Trigonini construct large royal cells, which contain much more food than cells for workers and males.

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In *Schwarziana quadripunctata*, a regular production of gynes (in a range of sizes) is normally observed throughout the year. As many as 35 gynes can be simultaneously found in the nest (Imperatriz-Fonseca and Zucchi, 1995), emerging from cells of a similar size as cells for workers and males. Camargo (1974) verified that combs with pupae giving rise to queens of various sizes, presented larger cells in an irregular spatial arrangement. Cells with slightly larger pupae, which produce medium-sized queens, and a few large royal cells which produce large queens or giant males, are recognized by their lower bottom, but differences among them are not apparent when cells are constructed. Some of the gynes have the same size as the workers, others can be smaller and, therefore, are called dwarf or miniature gynes. These gynes have less ovarioles (Camargo, 1974) than the larger ones. In *Schwarziana*, the ovariole number in gynes varied from six to nine per ovary, although most of them present eight ovarioles per ovary.

Other Trigonini besides *Schwarziana* also produce dwarf gynes; these are reported in *Cephalotrigona femorata* (Nogueira-Neto, 1951), *Plebeia juliani* (Juliani, 1962), *P remota* (Imperatriz-Fonseca et al, 1975) and *P emerina* (Kleinert, personal communication). However, these dwarf gynes are rarely found, suggesting a different reproductive strategy compared to *Schwarziana*.

Recently, the process of cell construction and oviposition in *Nannotrigona testaceicornis* was studied by Sakagami et al (1993), but they do not report on virgin queens, differently sized cells, nor on differently sized workers. The occurrence of dwarf gynes is an unpredictable event. This paper compares dwarf gynes in *Nannotrigona (Nannotrigona) testaceicornis* with workers and normal gynes.

MATERIALS AND METHODS

A mature comb was taken from a swarming colony of *N testaceicornis*, and kept in a Petri dish, without any attendant workers. Among the emerged bees, workers and males, eight dwarf gynes and a normal gyne were found. One of the dwarf gynes died inside the cell; it was weighed upon removal from the cell. The other gynes and 17 workers were weighed as soon as they emerged; gynes were also weighed on following days. The behaviour of some of these gynes was observed in the Petri dish. After these observations, four dwarf gynes were separated in boxes containing cerumen, sugar water and some pollen, where each dwarf gyne was attended by 20 workers that emerged from the same comb the same day as the gynes. Each gyne was randomly observed during 5 days.

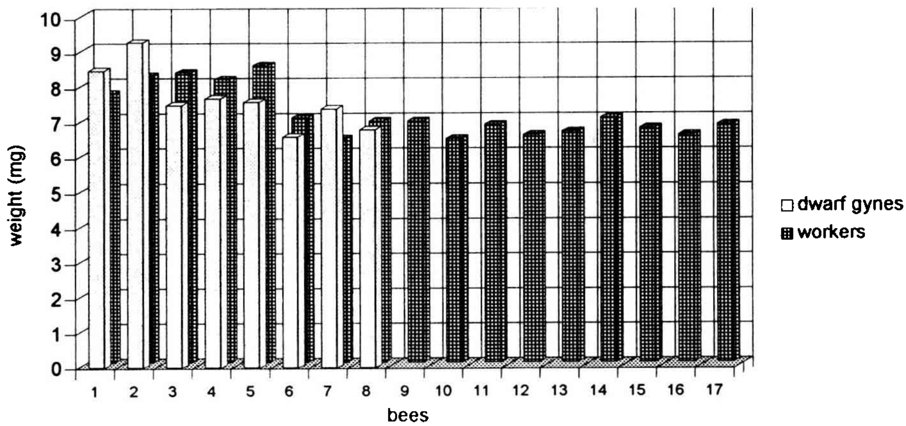
Three of the gynes used for behavioural observation were subsequently collected for morphological studies, as well as the normal gyne that hatched later at the edge of the same comb, ten workers 5-days-old and a physogastric queen from another colony. To study some of their glands (mandibular, tergal and Dufour) and ovaries, they were fixed in 4% paraformaldehyde and embedded in JB4 historesin. Sections were stained with hematoxylin and eosin. All of the analyzed gynes remained unmated. Workers were studied for comparison with gynes.

The other dwarf gynes were deposited in the JMF Camargo bee collection (Universidade de São Paulo, Ribeirão Preto, Brazil).

RESULTS

In the studied comb, the size of workers differs from cell to cell. One possibility for detecting such variability is to consider their weight. Variation in gynes weight is expected, as it occurs among workers. Figure 1 shows the weight of workers and dwarf gynes, just after emergence.

Dwarf gynes' weight at emergence varied from 6.5 to 9.3 mg (n = 8, $\bar{x}$ = 7.67 mg, *SD* = 0.87) whereas workers' weight varied from 6.4 mg to 8.5 mg (n = 17, $\bar{x}$ = 7.13 mg, *SD* = 0.72), all of them from the same piece of comb. Development time of males, dwarf gynes and workers was apparently similar, although gynes raised in royal cells hatch later, and often can be found in isolated cells from a comb from which all bees



Workers: Av=7.13 SD=0.72; Dwarf Gynes: Av=7.67 SD=0.87

Fig 1. Weight of workers and dwarf gynes of *N testaceicornis* raised in similar brood cells.

already emerged, and where the cells have been taken down by the workers.

Similar to the normal *N testaceicornis* workers and gynes at emergence, the dwarf gynes were not pigmented and did not attract workers. When observed together in the Petri dish, differences in attractiveness appeared and the behaviour of a more attractive dwarf virgin queen suggested aggression because she often tried riding over her sister queens. The behavioural repertoire of attaining attractiveness by these dwarf gynes involved the inflation of the abdomen, rapid movements through the box, soliciting trophallaxis with workers and frequent exhibition of the abdominal tip to the surrounding workers. Aggression involved strong abdominal movements by which the nearby workers were pushed away, and the attack of the other dwarf gynes. During some periods dwarf gynes also rested, as do gynes that have a normal size. Although attractive dwarf gynes generally presented a distended abdomen, suggesting an increase in size, they lost weight as they got older (for instance, one of the marked gynes weighed 9.3 mg after hatching, and 8.5 mg 3 days later; another had a weight of 7.5 mg after hatching, and 6.6 mg the next day). The nature of the frequent trophallaxis that occurred among workers and gynes deserves special attention: workers often offered food to the gynes; nevertheless sometimes gynes also offered a yellowish liquid to the workers.

A comparative survey of the exocrine glands of gynes (normal and dwarf), fertilized queen and workers of *N testaceicornis* is presented in table I. The mandibular and the epithelial glands of the third tergite were more developed in the dwarf gynes than in the workers. All gynes had Dufour glands, which were absent in workers. Hypopharyngeal glands were present in all queens (poorly developed) and in workers (well developed). Tarsal glands in the legs were absent in mated queen, but present in the gynes and workers.

Figure 2a shows the head glands of a dwarf gyne (salivary glands, mandibular glands with their reservoirs, intramandibular unicellular glands). Figure 2b shows the tergal glands of third, fourth and fifth tergites, as well as fat bodies. Besides morphological differences in the glands and ovaries, the gynes had more fat body and

Table I. Exocrine glands in *N testaceicornis*.

			Queens			
			Normal virgin	*Normal mated*	*Dwarf virgin*	*Worker*
Salivary system	Head (labial)		+	+	+	++
	Thorax (labial)		++	++	++	+++
	Mandibular		+++	+++	++	++
	Intramandibular	unicellular	+	+	+	+
		epithelial	+	+	+	+
	Hypopharingean		+	+	+	++++
Tergal glands	Unicellular		+++	++++	+++	+
	Epithelial		+	+++	++	++++*
Sting	Dufour		+	+	+	-
	Venom		-	-	-	-
Legs (tarsal)			+	-	+	+

+ = present; ++ = poorly developed; +++ = developed; ++++ = well developed; – = absent; * wax glands.

urate cells than the workers. The amount of fat body in dwarf queens was slightly lower than in the large one.

The number of ovarioles varied, ranging from four to 12 per ovary. The ovarioles were long in gynes and short in workers. The small gynes had four to eight long ovarioles (fig 3 a, c, d), while the normal gyne, coming from royal cell, had 12 (fig 3b). All workers had four short ovarioles (fig 3e). The spermathecae of dwarf gynes were empty.

DISCUSSION

The problem of caste determination in stingless bees has been also discussed by Darchen and Delage-Darchen (1977), Hartfelder (1986), Hartfelder and Engels (1989, 1992), Engels and Imperatriz-Fonseca (1990), Velthuis and Sommeijer (1991). Darchen and Delage-Darchen (1977) discussed the nature of hypopharyngeal gland products, suggesting that the different amounts of this component given to larvae could play a role in queen-worker differentiation. Hartfelder (1986) analyzed the larval food composition in *N testaceicornis* and other species, but did not find any special feature in it. Velthuis and Sommeijer (1991) discussed data from mixed colonies [consisting of a queen of one species, laying eggs in cells constructed and provisioned by workers of another species, studied by Nogueira-Neto (1950), and Silva (1977)], considering that even if the food composition deviates from its specific pattern, larval development can still occur. But they also mentioned the intra-specific variation in the food factor, and that cell provisioning by workers should be studied in detail. Hypopharyngeal gland secretion could function as an enzyme, facilitating food digestion, rather than as an alimentary food component (Velthuis, 1993).

Queens of the Trigonini usually emerge from royal cells and most of them are larger than workers. In *N testaceicornis,* the occurrence of eight dwarf gynes in a small piece of comb, combined with the fact that five dwarf gynes were also found by Lucio Oliveira Campos (unpublished data), sug-

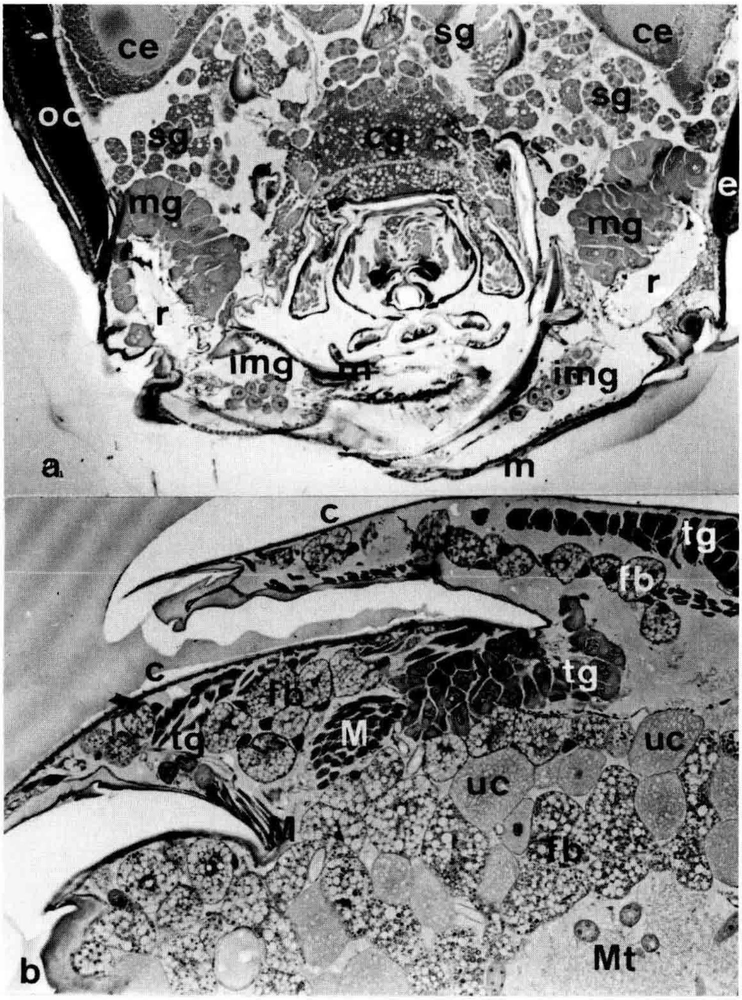


Fig 2. Exocrine glands of a small gyne of *N testaceicornis*.

a. Head glands: sg = salivary glands; mg = mandibular glands; r = mandibular gland reservoir; img= unicellular intramandibular glands; m = mandibles; fb = fat body; ce = cerebral ganglion; e = compound eye. **b.** Abdominal glands: tergal glands (tg) in III, IV and V tergites; c = cuticle; fb = fat body; uc = urate cells; Mt = Malpighian tubules.

gest that under certain conditions, several dwarf gynes can appear in the colonies. Their rarity could be linked to production during a short period. Some questions arise: what are the morphological and behavioural differences among workers, dwarf gynes and normal gynes? What could be the colonial advantages of producing dwarf gynes? Who controls their production: the queen or the workers?

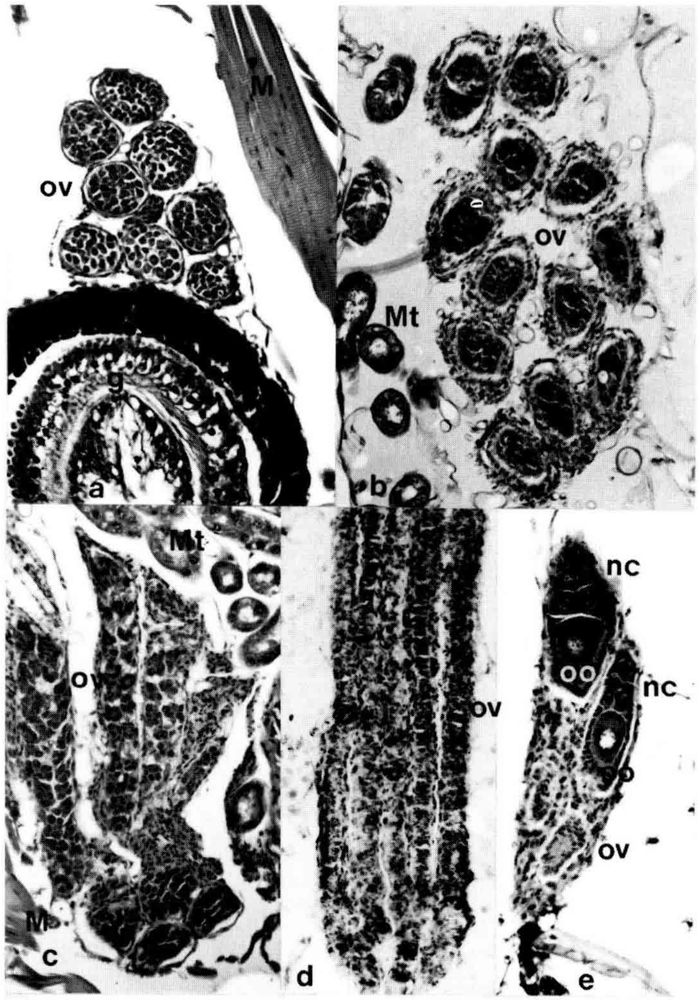


Fig 3. Ovaries of *N testaceicornis*.

a. Cross section of an ovary (ov) of a dwarf queen showing eight ovarioles. **b.** Cross section of an ovary (ov) of a queen reared in a royal cell showing 12 ovarioles. **c.** Oblique section of an ovary (ov) of a dwarf queen showing four ovarioles. **d.** Longitudinal section of an ovary (ov) of a dwarf queen showing five long ovarioles. **e.** Developed ovary (ov) of a worker: oo = oocytes; nc = nurse cells; Mt = Malpighian tubules; M = muscles; g = gut.

The dwarf gynes were produced during swarming time, certainly a short period during which a colony needs gynes for swarm preparation and for its completion. Other rare behaviours related to reproductive strategies in stingless bee colonies, such as male production by the workers, also involve short cycles and conflicts among workers and the queen.

The bases of gyne production in unspecialized cells in some Trigonini may include different larval food quantity in some cells, different composition of food among cells. Caste determination may have also a genetic basis.

Concerning the brood cell sizes, the small differences that occur among them make it impossible to know which cell will produce a male or a worker. If we compare the weight of dwarf gynes and workers, some workers are heavier than some gynes, suggesting that they come from larger cells, or from cells with more food, an indication that the quantity of food is not the only factor in caste determination. Campos et al (1986) measured the quantity of larval food consumed by *N testaceicornis* workers: from 15.9 μL to 20 μL. Cells that received 25 μL of larval food produced queens, intercastes and workers.

The quality of larval food could also be responsible for caste determination. However this problem was studied by Hartfelder and co-authors (Hartfelder, 1986; Hartfelder and Engels, 1989,1992), and they did report differences in larval food. Campos (1993) artificially reared queens and workers through manipulations of juvenile hormone and quantities of food. Differentiation in the larval food in normal colonies could be made by the queen, for instance, by adding something to the larval food before oviposition, when waiting at the edge of the cell before laying an egg in it. Workers also have the same possibility when provisioning, controlling the quantity or the quality of larval food in the cell. The question "who controls queen production, workers or the laying queen?" cannot be answered yet. Some evidence for the importance of quantity is found in the system of queen production described by Terada (1974) in *Leurotrigona mulleri* and *Frieseomelitta varia*, where royal cells construction rarely occurs. Apparently, such taxa produce queens in two different ways:

– by the ordinary method, ie, with workers building larger royal cells;

– by a larval behavioural alteration that promotes superfeeding.

After eating the food stored in their cell, some larvae invade neighbouring cells and get access to an extra-feeding tour. As a consequence, their body size enlarges rapidly and produces cracks of their own cell walls. The workers rapidly repair such cracks, and a larger royal cell appears. Both strategies result in queens larger than workers.

The behaviour of dwarf gynes follows the pattern described by Imperatriz-Fonseca and Zucchi (1995) for other stingless bees species. The mandibular glands, as well as epithelial glands, probably influence their attractiveness. The increase in the size of the abdomen seems to be due to the abdominal air sac inflation. Nevertheless, the variation in the number of ovarioles in gynes of the same species, according to their size, is important. Iwata and Sakagami (1966) discussed the possibility that bees with more ovarioles have a more efficient reproductive ability. The number of ovarioles may influence gyne selection by the workers. Besides the reference in Camargo (1974) for *Schwarziana quadripunctata*, our results show the same tendency of variation in ovarioles' number in workers and gynes of *N testaceicornis*. Although *Schwarziana* gynes reared in royal cells are more attractive and most of the colonies have fertilized normal queens, a few colonies contained medium-sized queens, and rarely dwarf queens.

To discuss the advantage for a colony to produce smaller gynes would be speculative. The facts are that gynes reared in worker-sized cells emerge more rapidly than workers or males (in *Melipona* and in *Nannotrigona*), whereas gynes reared in royal cells generally have a larger developmental period. An exception was observed in *Scaptotrigona bipunctata* (Kleinert and Piva, 1992), in which a gyne emerged after 37.5 days, whereas workers emerge after 41.5 and males after 40.3 days.

In the present case the gynes were different in size but similar in morphology (glandular system, ovaries and fat body) and behaviour. Caste determination in Trigonini is still an open subject that needs further studies for its comprehension.

This paper is offered in memory of Roger Darchen.

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Résumé — Les reines naines de *Nannotrigona testaceicornis* (Apidae, Meliponinae, Trigonini). Comportement, morphologie des glandes exocrines et état reproducteur. Un rayon de couvain prêt à éclore a été prélevé dans une colonie de *Nannotrigona testaceicornis* en train d'essaimer et mis dans une boîte de Pétri. Outre des ouvrières et des mâles, sont nées huit reines naines et une reine normale. Le comportement de quelques unes de ces reines a été observé en boîte de Pétri. Puis, quatre reines naines ont été isolées dans des cagettes avec de la cire, du sirop de sucre et un peu de pollen. A chaque reine ont été adjointes 20 ouvrières qui étaient nées le même jour que les reines et issues du même rayon. On a utilisé trois de ces reines naines, dix ouvrières âgées de 5 jours, la reine normale et une reine normale physogastre issue d'une autre colonie pour l'étude morphologique des glandes exocrines (glandes mandibulaires, intramandibulaires, hypopharyngiennes, tergales, de Dufour et tarsales) et des ovaires. A l'émergence, les reines naines pesaient entre 6,5 et 9,3 mg (n = 8, $\bar{x}$ = 7,67 mg, SD = 0,87), les ouvrières entre 6,4 et 8,5 mg (n = 17, $\bar{x}$ = 7,13 mg, SD = 0,72). La durée de développement des mâles, des reines naines et des ouvrières était à peu près semblable, alors que les reines élevées en cellule royale sont nées plus tard. Comme les ouvrières et les reines normales de *N testaceicornis*, les formes naines ne sont pas pigmentées à la naissance et n'attirent aucune ouvrière. Bien que généralement les reines naines attractives présentent par la suite un abdomen dilaté, elles perdent du poids avec l'âge (par exemple, une reine marquée juste à la naissance pesait 9,3 mg et seulement 8,5 mg 3 jours plus tard, une autre pesait 7,5 mg à la naissance et 6,6 mg le lendemain). Le tableau I compare les glandes exocrines des reines normales, des reines naines et des ouvrières. La glande mandibulaire et la glande épithéliale du 3^{e} tergite étaient plus développées chez les reines naines que chez les ouvrières. Toutes les reines possédaient des glandes de Dufour mais celles-ci étaient absentes chez les ouvrières. Les reines possédaient aussi des glandes hypopharyngiennes mais elles étaient peu développées, contrairement à celles des ouvrières. Les glandes tarsales étaient absentes chez la reine qui s'était accouplée, mais ont été identifiées chez les reines vierges et les ouvrières. Toutes les reines avaient un corps gras plus gros et un plus grand nombre de cellules à urates que les ouvrières. La différence entre leurs ovaires était nette. Toutes les ouvrières avaient quatre ovarioles courts; ceux des reines, en revanche, étaient allongés et au nombre de quatre à huit par ovaire chez les reines naines et de 12 chez la reine normale. Les reines étudiées étaient donc différentes par leur taille mais semblables par leur morphologie et leur comportement. La question du déterminisme des castes chez les Trigonini reste ouverte et bien d'autres recherches sont encore nécessaires pour comprendre les relations entre les divers facteurs.

***Nannotrigona testaceicornis* / Trigonini / reine naine / stratégie de reproduction / glande exocrine**

Zusammenfassung — Zwergköniginnen von *Nannotrigona testaceicornis* (Apidae, Meliponinae, Trigonini). Verhalten, Morphologie der exocrinen Drüsen und reproduktiver Zustand. Eine schlupfreife Wabe wurde einem schwärmenden Volk von *Nannotrigona testaceicornis* entnommen und in einer Petrischale gehalten. Außer Arbeiterinnen und Drohnen schlüpften 8 Zwergköniginnen und eine normale Königin. Das Gewicht der Königinnen und Arbeiterinnen wurde bestimmt. Das Verhalten einiger Königinnen wurde in der Petrischale beobachtet. Danach wurden 4 Zwergköniginnen in Kästen vereinzelt, die mit Cerumen, Zuckerwasser und etwas Pollen ausgestattet waren. Zu jeder Königin wurden 20 Arbeiterinnen beigegeben, die am selben Tag aus derselben Wabe wie die Königinnen geschlüpft waren. Für morphologische Untersuchungen wurden 3 dieser Zwergköniginnen, 10 fünftägige Arbeiterinnen, die normale Königin und zusätzlich aus einem anderen Volk eine normale physogastrische Königin genommen. Die Drüsen (Mandibel-, Intramandibel-, Hypopharynx-, Tergal-, Dufour - und Tarsaldrüsen) und die Ovarien wurden untersucht.

Die Zwergköniginnen wogen beim Schlupf zwischen 6,5 und 9,3 mg (n = 8, x = 7,67 mg, SD = 0,87), das Gewicht der Arbeiterinnen variierte von 6,4 - 8,5 mg (n = 17, x = 7,13 mg, SD = 0,72). Die Entwicklungszeit der Männchen, Zwergköniginnen und Arbeiterinnen scheint etwa gleich zu sein, während Königinnen, die in Königinnenzellen gezogen werden, später schlüpfen. Ähnlich wie normale Arbeiterinnen und Königinnen von *N testaceicornis* sind die Zwergformen beim Schlupf nicht pigmentiert und locken keine Arbeiterinnen an. Obwohl später die attraktiven Zwergköniginnen im allgemeinen ein ausgedehntes Abdomen aufweisen, verloren sie während des Älterwerdens an Gewicht (So wog z.B. eine markierte Königin direkt nach dem Schlupf 9,3 mg, aber 3 Tage später nur noch 8,5 mg, eine andere 7,5 mg beim Schlupf und 6,6 mg am nächsten Tag).

Eine vergleichende Übersicht der exokrinen Drüsen der normalen Königinnen, der Zwergköniginnen und Arbeiterinnen ist in Tabelle I dargestellt. Die Mandibulardrüse und die Epitheldrüse des 3. Tergits waren bei den Zwergköniginnen stärker entwickelt als bei den Arbeiterinnen. Alle Königinnen hatten Dufourdrüsen, die bei Arbeiterinnen nicht vorhanden waren. Hypopharynxdrüsen fanden sich in allen Königinnen, sie waren aber im Gegensatz zu Arbeiterinnen mit gut entwickelten Drüsen nur schwach ausgebildet. Die begattete Königin hatte keine Tarsaldrüsen, aber bei den unbegatteten Königinnen und den Arbeiterinnen konnten diese nachgewiesen werden. Königinnen hatten einen größeren Fettkörper und mehr urate Zellen als die Arbeiterinnen. Ein deutlicher Unterschied bestand zwischen ihren Ovarien. Alle Arbeiterinnen hatten 4 kurze Ovariolen, dagegen waren die Ovariolen der Königinnen lang und ihre Anzahl variierte zwischen 4 - 8 pro Ovarium in Zwergformen. Die normale Königin hatte 12 Ovariolen.

Die hier untersuchten Königinnen waren zwar unterschiedlich in der Größe, aber ähnlich in ihrer Morphologie und ihrem Verhalten. Die Frage nach der Kastendetermination bei den Trigonini ist immer noch offen und es sind noch weitere Untersuchungen notwendig, um die Zusammenhänge zu verstehen.

***Nannotrigona testaceicornis* / Zwergköniginnen / reproduktive Strategien / stachellose Bienen / Trigonini / exocrine Drüse**

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