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MALE PRODUCTION PATTERN IN STINGLESS BEE, *TETRAGONULA* NR. *PAGDENI* (HYMENOPTERA: APIDAE: MELIPONINI) IN INDIA

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ABSTRACT

Male production pattern in stingless bee, *Tetragonula* nr. *pagdeni* was studied by trapping outgoing bees daily over a year (August 2017 to August 2018) in five colonies at Gandhi Krishi Vignana Kendra Campus of the University of Agricultural Sciences, Bengaluru for the first time in India. Males were produced throughout the year except two major breaks of 5- and 7-weeks during October and December-January in stronger colonies. However, in weaker colonies males occurred only during August, November, March, and June with intermittent long breaks. Stronger colonies produced more males (0 to 13 males/colony/week) than the weaker colonies (0 to 3 males/colony/week). Total number of males produced by stronger colonies ranged from 39 to 53 while in weaker colonies it ranged from 3 to 9 over a period of 52 weeks. At colony level, maximum of 13 male bees while at population level 22 male bees were produced in 36th standard week (September) of 2017. All the five colonies together produced 143 males during the study period. Based on these studies it is concluded that male production pattern in stronger colonies of stingless bee, *Tetragonula* nr. *pagdeni* is almost continuous and similar trend may occur in other species of stingless bees in India.

INTRODUCTION

Stingless bees that belong to order Hymenoptera, family Apidae and tribe Meliponini, are one of the important beneficial insects acting as honey yielders and pollinators (Cortopassi-Laurino *et al.*,2006; Heard, 1999, 2016). There are about 600 described species of stingless bees worldwide belonging to 60 genera (Michener, 2000, Rasmussen and Cameron 2010, Rasmussen, 2013, Rasmussen *et al.*,2017).

Many studies have been conducted in an attempt to determine the ultimate reasons that cause variations in reproductive patterns in stingless bees. These studies are concerned with the mechanisms of caste determination. Greater knowledge about these factors would enable us to better understand the mechanisms of maintenance and growth of the colony (Alves *et al.,* 2009). Female production and behaviour have been the major focus of these studies. Although male production is an investment that is in conflict with investment in colony size, males play a vital role in colony reproduction.

In India, 14 species of stingless bees belonging to three genera namely *Lepidotrigona, Lisotrigona* and *Tetragonula* have been reported so far (Jobiraj and Narendran, 2004; Rasmussen, 2013; Rathor *et al,* 2013; Vijayakumar and Jeyaraj, 2014; Viraktamath and Jose, 2017). Among these, only description of males is reported for two species *viz. Tetragonula iridipennis* (Smith) and *Lisotrigona chandrai* Viraktamath and Sajan Jose (Vijayakumar and Jeyraj, 2014; Viraktamath and Sajan Jose, 2017). Males are particularly important for identification of species correctly as they have more diagnostic characters than females (Sakagami, 1978; Rasmussen, 2013; Attasopa *et al*, 2018). However, there are no studies or reports about male production pattern for any stingless bee species from India mainly because of the assumption that males are highly seasonal, and they are produced only during the breeding period. To test the veracity of this assumption, the present studies on male production pattern in stingless bees was made and the results are presented in this paper.

MATERIALS AND METHODS

Present studies were made on the campus of the University of Agricultural Sciences, Gandhi Krishi Vignana Kendra, Bengaluru. Five feral stingless bee colonies nested in different places were selected and numbered consecutively from 1 to 5. (Table 1). As the number of outgoing bees depends on the strength of the colony (Sajan Jose, 2015 and Singh and Khan, 2015), these colonies were categorized as strong if outgoing foragers were more than 10 bees and weak if they were less than 10 bees/minute. As per this criteria Colony 1, 4 and 5 fell under strong colonies while Colony 2 and 3 weak colonies.

Previous study by Alves *et al.* (2009) on production of workers, queens, and males in *Plebia remota* required intense manipulation of the colony as it involved periodical sampling of brood cells and opening each cell to identify the sex of the pupa. However, when we adopted this method, it was not possible to identify the sex by examination of the head of the pupa in Indian stingless bee species. Hence, we followed an indirect method descried by Viraktamath *et al.* (In press) to collect males and to study male production pattern in this study. A water trap which consisted of a plastic container with about 100 ml water was installed below the entrance of each of the five selected colonies (Figure 1) throughout the study period. All the bees trapped in water were collected separately from each colony in 95% alcohol vials daily and brought to the laboratory. Each bee was examined under a stereoscopic binocular microscope for determining the sex. Male bees were identified by the presence of genitalia at the tip of abdomen and recorded separately for each colony. Later, these observations were pooled for every standard week for each colony for the entire study period. Total number of males per standard week for five colonies was also recorded separately.

RESULTS AND DISCUSSIONS

Weekly observations on number of males collected from each colony depicted the male production pattern at each colony level while the total male bees for all the five colonies together represented the male production pattern at population level and this data are presented in Table 2.

At colony level, maximum number of males produced in stronger colonies was 13, 07 and 05 in colony 1, 4 and 5, whereas in weaker colonies it was 2 and 3 in colony 2 and 3 in a standard week, respectively (Table 2). The range of male production in stronger and weaker colonies varied from 0 to 13 and 0 to 3, respectively. Similarly, total number of males produced during entire study period varied from 39 to 53 and 3 to 9 in stronger and weaker colonies, respectively.

Males were produced in distinct periods of the year in stronger colonies which was similar in all the three colonies. Males were recorded during August, September, November and from last week of January to August with intermittent short breaks in October and December. However, in weaker colonies, such trend was not apparent, and males were produced only in August, November, March, and June with long breaks in between.

At population level when the data of all the five colonies were combined, male production was found throughout the year. Highest peak of male production (22 males) was observed during 36th standard week of 2017 (first week of September) followed by second peak (14 males) during 34th week (fourth week of August) (Figure 2). Four smaller peaks (5 to 8 bees) were observed during 47th standard week of 2017 and 10th, 11th 12th, 27th and 33rd standard weeks of 2018. Stronger colonies produced 39 to 53 males while weaker colonies produced 3 to 9 males during the entire study period. All the five colonies together produced 143 males from 33rd standard week of 2017 to 33rd standard week of 2018 (Table 2).

There were three periods when males were recorded during the study period (Figure 2) which indicated three distinct male producing periods. The first male production period was from 33rd to 39th standard week of 2017 (second week of August to last week of September), the second the shortest from 46th to 48th standard weeks (first week of November to first week of December) and the third the longest from 4th standard week of 2018 (last week of January) to 33rd standard week (third week of August). During the entire study period, four breaks were observed when no male bees were recorded. The first two major breaks occurred from 40th (October first week) to 44th (first week of November) and from 49th (first week of December) to 3rd (third week of January) standard week of 2018. Two minor breaks were of very brief periods of two weeks each and occurred during 25th to 26th (third and last week of June 2018) and 28th to 29th (third and last week of July 2018) standard weeks.

Male stingless bees are extremely important for species identification as emphasized by several workers (Sakagami, 1978; Rasmussen, 2013; Attasopa *et a.,* 2018). However, male bees are extremely like females and are believed to be highly seasonal.

Male bee production is an investment for a stingless bee colony. However, they are vital for growth and reproduction of the colonies. It has been documented at colony level of a number of stingless bee species, that most of the males are produced in periods with a restricted duration termed as Male Producing Periods (MPP). These periods may be the effect of a synchronous production of reproductive eggs by a number of workers or sometimes with the production of haploid eggs by the queen, over a short period of time (Velthuis *et al.*, 2005). Even though individual colonies may produce their males during certain time periods, these periods may not be synchronous among colonies and thus, at the population level, male production is usually not restricted to certain periods of the year (Chinh *et al.*, 2003).

In Meliponini the male bees are known to form congregations often close to the colony in many species (Cortopassi-Laurino, 1979; Engels and Engels, 1984; Engels and Imperatriz-Fonseca, 1990; Roubik, 1990; Imperatriz-Fonesca *et al.*,1998; Nogueira-Ferriera and Soares, 1998; Veen and Sommeijer, 2000; Paxton, 2000; Cameron *et al.*,2004). Some male bees may also be found flying around the entrance. In the current study, though congregation of male bees was not observed throughout the study period, but they were found hovering around the nest entrance of the colonies on several occasions. However, Sajan Jose (2015) has reported male bees' congregation in

Figure 1: Trapping of outgoing stingless bees in water from a colony

Table 1: Details of colonies of *Tetragonula* nr. *pagdeni* selected for studying male activity at GKVK, Bengaluru

Colony no.	Nesting place	Outgoing foragers	Strength of colony
1	Cement wall cavity	>10 bees/minute	Strong
2	Water drainage pipe	<10 bees/minute	Weak
3	Stone wall cavity	<10 bees/minute	Weak
4	Palm tree cavity	>10 bees/minute	Strong
5	Electric pipe	>10 bees/minute	Strong

Table 2: Male production pattern in five stingless bee colonies of *Tetragonula nr. pagdeni*

Standard week	Period	Number of male bees recorded in					
		Colony 1	Colony 2	Colony 3	Colony 4	Colony 5	Total
33	Aug 13-Aug 19	1	0	0	0	0	1
34	Aug 20-Aug 26	5	1	3	4	1	14
35	Aug 27-Sep 02	9	0	0	0	1	10
36	Sep 03-Sep 09	13	0	0	7	2	22
37	Sep 10-Sep 16	7	0	0	0	0	7
38	Sep 17-Sep 23	0	0	0	0	0	0
39	Sep 24-Sep 30	1	0	0	0	0	1
40	Oct 01-Oct 07	0	0	0	0	0	0
41	Oct 08-Oct 14	0	0	0	0	0	0
42	Oct 15-Oct 21	0	0	0	0	0	0
43	Oct 22-Oct 28	0	0	0	0	0	0
44	Oct 29-Nov 04	0	0	0	0	0	0
45	Nov 05-Nov 11	0	0	0	0	1	1
46	Nov 12-Nov 18	1	0	1	0	2	4
47	Nov 19-Nov 25	7	0	0	0	0	7
48	Nov 26-Dec 02	1	0	0	0	0	1
49	Dec 03-Dec 09	0	0	0	0	0	0
50	Dec 10-Dec 16	0	0	0	0	0	0
51	Dec 17-Dec 23	0	0	0	0	0	0
52	Dec 24-Dec 31	0	0	0	0	0	0
1	Jan 01-Jan 07	0	0	0	0	0	0
2	Jan 08-Jan 14	0	0	0	0	0	0
3	Jan 15- Jan 21	0	0	0	0	0	0
4	Jan 22-Jan 28	0	0	0	0	2	2
5	Jan 29-Feb 04	1	0	1	0	2	4
6	Feb 5-Feb 11	0	0	0	1	1	2
7	Feb 12-Feb 18	0	0	0	2	0	2
8	Feb 19-Feb 25	0	0	0	1	0	1
9	Feb 26-Mar 4	0	0	0	0	1	1
10	Mar 5-Mar 11	1	0	1	1	5	8
11	Mar 12-Mar 18	0	0	0	5	2	7
12	Mar 19-Mar 25	0	0	0	4	2	6
13	Mar 26-Apr 1	0	0	0	0	0	0
14	Apr 2-Apr 8	0	0	0	1	1	2
15	Apr 9-Apr 15	0	0	0	0	2	2
16	Apr 16-Apr 22	0	0	0	3	2	5
17	Apr 23-Apr 29	0	0	0	1	0	1
18	Apr 30-May 6	0	0	0	1	3	4
19	May 7-May 13	1	0	0	0	2	3
20	May 14-May 20	0	0	0	1	1	2
21	May 21-May 27	0	0	0	1	1	2
22	May 28-June 3	0	0	0	1	1	2
23	June 4-June 10	0	2	0	0	0	2
24	June 11-June 17	0	0	3	0	0	3
25	June 18-June 24	0	0	0	0	0	0
26	June 25-July 1	0	0	0	0	0	0
27	July 2-July 8	1	0	0	2	3	6
28	July 9-July 15	0	0	0	0	0	0
29	July 16-July 22	0	0	0	0	0	0
30	July 23-July 29	0	0	0	0	1	1
31	July 30-Aug 5	1	0	0	0	0	1
32	Aug 6-Aug 12	0	0	0	1	0	1
33	Aug 13-Aug 19	3	0	0	2	0	5
Total		53	3	9	39	39	143

T. iridipennis from Kerala. There are reports of males of different species occurring together in congregation (Velthuis *et al.*, 2005). However, occurrence of males of different species flying around the nest entrance was not noticed in the present studies.

In temperate regions male production is influenced by outside factors (climatic factors) but in tropical regions factors inside the colony (colony strength, demographic composition) play an important role (Velthuis *et al.*, 2005). In the present study, at colony level, male production period clearly differed in stronger and weaker colonies. Stronger colonies produced more males over a longer period with shorter intermittent breaks as evidenced in collection pattern of males. In contrast, the

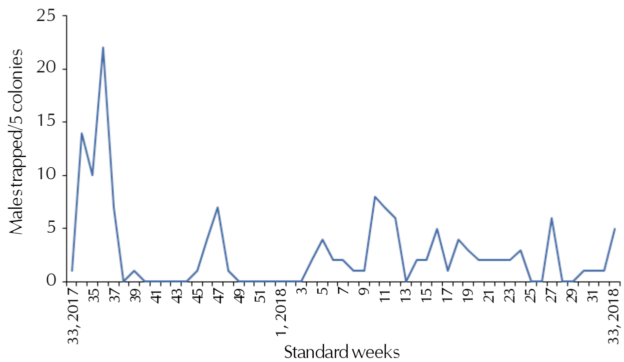

Figure 2: Variation in total male bee production in five stingless bee colonies of *Tetragonula* nr. *pagdeni*

weaker colonies produced few males with exceedingly long breaks in between. In *Melipona* species, male production is regulated by constantly maintaining a ratio which increases with colony size and cell numbers. Males are produced as an outburst once optimum strength of colony is reached (Velthuis *et al.,* 2005). Similar regulatory mechanisms may also be operating in *Tetragonula* species in India. There was a synchronicity in production of males within stronger or weaker colonies.

At population level, males were produced throughout the year with brief gaps of five (October-November) and seven weeks (December-January). Two short gaps of two weeks each occurred during June and July. Similar pattern of male production is also found at population level in *Melipona favosa* (Chinh *et al.,* 2003) and *M. bicolor* (Alves, 2004). Current results point out that fluctuation in male production periods within individual colonies has no impact on the availability of males at population level. As Velthuis *et al.,*(2005) propose, it could be colony strength and demographic factors regulating the production of males. This view is strongly corroborated in the present studies as the male production pattern clearly differed among strong and weaker colonies.

Based on the results of the present studies we conclude that at population level, male production pattern in stingless bee, *Tetragonula* nr. *pagdeni* is almost continuous which contradicts the common assumption that males are highly seasonal. Similar trend may occur in other species of stingless bees in India. This is the first detailed account of male production pattern for any stingless bee species from India. Further detailed studies are needed to find out whether the ratio between the sexuals and males or colony strength or climatic factors regulate the production of males in Indian stingless bee species.

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