UNIVERSITI PUTRA MALAYSIA

SOME ASPECTS OF THE REPRODUCTIVE BIOLOGY AND BEHAVIOUR OF THE ASIATIC GIANT HONEYBEE,APIS DORSATA FABRICIUS

NGUYEN QUANG TAN

FP 1997 15

SOME ASPECTS OF THE REPRODUCTIVE BIOLOGY AND BEHAVIOUR OF THE ASIATIC GIANT HONEYBEE, *APIS DORSATA* FABRICIUS

By

NGUYEN QUANG TAN

Thesis Submitted in Fulfilment of the Requirements
for the Degree of Master of Agricultural Science
in the Faculty of Agriculture, Universiti Putra Malaysia

June 1997

ACKNOWLEDGEMENTS

I would like to thank my supervisor, Assoc. Professor Dr. Makhdzir Mardan, for his invaluable help and guidance during the study. My appreciation of thanks are also due to other members of the Supervisory Committee, Prof. Dr. Mohd. Yusof Hussein and Dr. Mohamad Muid for their contribution to my research plan and thesis. I would also like to thank Dr. Trinh Cong Thanh (University of Agriculture and Forestry, Ho Chi Minh City) for his interest and care of my study and Mr. Azhar Zainal for his kind help throughout the study.

It is my pleasure to acknowledge the following people without whose helps this study could not have been completed: Mr. Pham Hong Thai, Mr. Nguyen Hoang Vu, Mr. Nguyen Van Le, Mr. Nguyen Van Binh and Mr. Ngo Van Duong. Also I would like to express my gratitude to Dr. Joop Beetsma (Wageningen Agricultural University) for kindly sending some materials for the experiments and my colleagues in the university, Mr. Duong Nguyen Khang and Dr. Luong Van Huan for lending the equipment for the various field experiments.

My acknowledgment must be sent to the Bee Network for Asia / International Development Research Center (BEENET ASIA / IDRC) for sponsoring my study in Malaysia and research project in Vietnam. I also thank the Faculty of Agriculture, Universiti Putra Malaysia and the Faculty of Veterinary Medicine & Animal Science, University of Agriculture & Forestry, Ho Chi Minh City for providing the facilities and personnel for the research.

Last but not least, my deepest appreciation is to my dear wife, Tran Minh Huynh Nhung, for not only her moral encouragement but also contributing ideas during the study.

TABLE OF CONTENTS

APPENDIX

LIST OF TABLES

Table **Page**

LIST OF FIGURES

Figure **Page**

LIST OF PLATES

Abstract of thesis submitted to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Master of Agricultural Science.

SOME ASPECTS OF THE REPRODUCTIVE BIOLOGY AND BEHAVIOUR OF THE ASIATIC GIANT HONEYBEE, *APIS DORSATA* FABRICIUS

By

NGUYEN QUANG TAN

JUNE 1997

Chairman: Assoc. Prof. Dr. Makhdzir Mardan

Faculty: Agriculture

ABSTRACT

Up to now, little is known about the reproductive biology of the Asiatic wild giant honeybee *Apis dorsata*, so a comprehensive study on its reproductive biology is needed to improve the beekeeping technique.

This study was implemented in the U-Minh *Melaleuca* forest, Vietnam and the Campus of Universiti Putra Malaysia, Selangor between July 1995 and January 1997. It was aimed to determine the following:

i) The drone flight time, sperm number of a sexually-mature drones and age of sexual maturity of drones

ii) The number of queen cells and mating flights of virgin queens.

The observation on four colonies of *A. dorsata* showed that drones started to fly at 3.4 ± 2.4 (n = 16) minutes after sunsets and stopped after a duration of 24.7 ± 1.0 (n = 16) minutes. The sperm count of drones during days after emergence indicated that *A. dorsata* drones became sexually-mature after eight-days-old and a sexually-mature drone had 1.24 ± 0.39 (n = 31) million spermatozoa..

The number of natural queen cells raised per colony (9.5 ± 1.6, n = 6) and that of emergency queen cells (9.4 ± 1.8, n = 5) were not significantly different (P>0.05). All queen cells were at the lower edge of the combs. A virgin queen of *A. dorsata* took her first flight at 6 ± 1 (n = 5) days after emergence. She started mating flights at 12.5 ± 4.1 (n = 14) minutes after sunset and flew for 15.4 ± 4.3 (n = 14) minutes. A queen demonstrated 2-4 mating flights and the fully and newly-mated queen had 5.5 ± 0.9 (n = 5) million spermatozoa in her spermatheca. The number of sperm in a queen and a drone showed that *A. dorsata* queen practiced multiple-mating. Upon on the five investigated queens, mating signs were not observed on *A. dorsata.*

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk mendapatkan ijazah Master Sains pertanian.

ASPEK BIOLOGI PEMBIAKAN DAN TABIAT

LEBAH TUALANG, *APIS DORSATA* FABRICIUS

Oleh

NGUYEN QUANG TAN

JUNE 1997

Pengerusi: Profesor Madya Dr. Makhdzir Mardan

Fakulti: Pertanian

ABSTRAK

Sehingga kini, tidak banyak maklumat berkaitan biologi pembiakan Apis dorsata diketahui. Kajian perlu dijalankan bagi tujuan memperbaiki teknik pemeliharaan lebah ini.

Kajian ini telah jalankan di hutan *Melaleuca* U-Minh, di Vietnam dan kampus Universiti Putra Malaysia, Selangor antara Julai 1995 dan Januari 1997. Kajian ini bertujuan untuk menentukan:

i) Masa penerbangan, bilangan spermatozoa dan umur kematangan dron

ii) Bilangan sel ratu dan penerbangan mengawan ratu

Pemerhatian terhadap empat koloni *A. dorsata* menunjukkan dron mula terbang pada 3.4 $\pm$ 2.4 (n = 16) minit selepas matahari terbenam dan tamat 24.7 $\pm$ 1.0 (n = 16) minit kemudiannya. Pengiraan spermatozoa pada dron menunjukkan ia mula matang pada umur 8 hari selepas muncul dewasa dan mempunyai 1.24 $\pm$ 0.39 (n = 31) juta spermatozoa.

Tiada perbezaan beerti (P> 0.05) antara bilangan sel ratu semulajadi (9.5 $\pm$ 1.6, n = 6) dan sel ratu kecemasan (9.4 $\pm$ 1.8, n = 5). Semua sel-sel ratu dibina di pinggir komb. Ratu dara mula terbang 6 $\pm$ 1 (n = 5) hari selepas muncul dewasa. Penerbangan ratu untuk mengawan bermula 12.5 $\pm$ 4.1 (n = 14) minit selepas matahari terbenam dan berterusan selama 15.4 $\pm$ 4.3 (n = 14) minit. Ratu lebah terbang 2-4 kali untuk mengawan dan ratu yang baru mengawan sepenuhnya mempunyai 5.5 $\pm$ 0.9 (n = 5) juta spermatozoa di dalam spermathecanya. Bilangan sperma pada seekor ratu dan dron menunjukkan ratu mengawan dengan lebih dari satu ekor dron. Tiada penanda mengawan ditemui pada kelima-lima ratu yang di kaji.

CHAPTER I

INTRODUCTION

Background

In Mesolithic times, probably about 7000 BC, primitive man learned to rob honey from the honey bee (Crane, 1975). Then beekeepers have used hives to keep bees and harvested honey from them for at least 4500 years (Crane, 1975, 1990). In Ancient Egypt, the technique of migratory beekeeping evolved, beekeepers moved their bees up and down the Nile river to take advantage of the changing flora (Morse, 1975).

Major technological developments have taken place on beekeeping with the European honey bee, *Apis mellifera* and were similarly adopted or adapted for the Asiatic hive honey bee, *Apis cerana*. However, little progress has taken place with the technology of the open nesting bees, including the Asiatic giant honey bee, *Apis dorsata*. With the global changes in environmental awareness and perception on sustainable development, *A. dorsata* beekeeping technology will have to embrace the new values in development strategy. In the future, beekeeping could be one of the activities for environmental and ecological conservation (Bradbear, 1993; Kevan, 1993; Mardan, 1994). Nevertheless, the need for understanding basic knowledge of the species remains the same. Fundamental knowledge on the reproductive biology of many Asian bees are lacking and it hampers to the development of the technology for sustainable beekeeping.

Reproductive Biology

The growth of knowledge in reproductive biology of the honey bee from 1500s to 1800s was summarized by Crane (1990) in her book *Bees and Beekeeping: Science, Practice and World Resources* as the followings:

Nickel Jacob (1568) discovered that a queenless colony with eggs or young larvae can rear a new queen. Luys Méndez de Torres (1586) first stated in his book that the leader bee in the hive is a female that lays the eggs from which workers, drones and future queens develop. In 1609, Charles Butler reported drones were male and Richard Remnant (1637) showed workers female. Function of sperm storage of the queen's spermatheca was first described by Arthur Dobbs (1750). Anton Janscha (1771) described mating between the queen and drone. Francois Huber (1792) reported that workers have (undeveloped) ovaries and can lay eggs.

Major achievements in beekeeping technology have been experienced in the nineteenth and twentieth centuries. Langstroth (1851) introduced an effective movable-frame-hive (Waine, 1955; Morse, 1978; Howe & Howe, 1980; Crane, 1990). Mass queen rearing with artificial queen cells was practiced by Doolittle (1889) (Morse, 1978; Laidlaw, 1979; Crane, 1990). Watson (1926) instrumentally inseminated queens successfully (Cale & Rothenbuhler, 1975; Morse, 1978; Woyke, 1979; Crane, 1990). These advantages have motivated beekeepers and bee scientists to the development of programs on bee breeding and genetics to increase honey yield. That pattern of development are to be developed in the beekeeping with the Asiatic honey bees, including *A. dorsata*. Currently, across species studies on honey bees were achieved in the last three decades, especially those conducted by the Koenigers and co-workers in Asia.

Mating flights of drone bees of *A. mellifera* were observed as early as in 1928 by Mikhailov (cited by Gary, 1975), Gary (1963); Zmarlicki & Morse (1964); and then studied on other species, too (Koeniger & Wijayagunasekera, 1976; Koeniger et al., 1988, 1994b; Underwood, 1990; Rinderer et al., 1993;

etc.). Day of sexual maturity of drones was reported by Ruttner & Tryasko (1979), Crane (1990). Number of sperm in a drone was informed by many authors (Ruttner et al., 1973; Woyke, 1975; Koeniger et al., 1989, 1990, 1994a; etc.). In queen bees, mating flight, number of sperm, sperm transfer mechanism from drones to a queen, and number of drones mating with a queen were studied (Ruttner et al., 1973; Woyke, 1975; Koeniger et al., 1979, 1989, 1990, 1994a).

However, most of the studies done so far have been on the European honey bee, *Apis mellifera*. Crane (1993) reported that the research on the Asian honey bees amounted to only a few percent of that on *A. mellifera*. Among the Asian honey bees, sixty percent of the publication is on *A. cerana* and nearly forty percent is for *A. florea* and *A. dorsata*. Initially, more attention was given to *A. dorsata* but since 1979 there have been more work on *A. florea*, because the *A. dorsata* was very defensive and/or inaccessible for people to do research .

I have chosen the subject on reproductive biology of *A. dorsata* for my research because the technique of *rafter* beekeeping with *A. dorsata*, provide ample opportunities for hive manipulation and also the abundance of low-nesting colonies in the *Melaleuca* forests in the South of Vietnam (plates 3 & 4)

Study Objectives

A comprehensive study on the reproductive biology of *A. dorsata* is fundamental to the future development of artificial insemination and breeding programme. Moreover, a reasonable comparative study on reproductive biology among the four species-groups of *Apis* (*A. mellifera*, *A. cerana/koschevnikovi*, *A. florea/andreniformis* and *A. dorsata/laboriosa*) can not be conducted until information on *A. dorsata* is documented.

In the breeding programs of honey bee, the following questions on reproductive biology have to be answered before the artificial insemination technique could be applied:

1) Can people rear queen cells and how many queen cells are raised per colony?

2) At what age do the virgin queen and drones become sexually mature?

3) When and how long they will fly to mate?

4) How many spermatozoa are there in a sexually-mature drone and a laying queen?

5) How many drones does the virgin queen mate with to have enough spermatozoa stored in her spermatheca?

Hypothesis: Being a species in the genus *Apis*, *A. dorsata* has the same reproductive biology as other honey bee species. Nevertheless, because it is a separate species, *A. dorsata* also has its own unique characteristics.

Therefore, in this study on the reproductive biology of both *A. dorsata* queens and drones, my hypothesis is that the reproductive behaviour of *A. dorsata* does not deviate much from the general reproductive behaviour of the genus *Apis*. This hypothesis is tested by studying:

i) the time of occurrence of drone mating flights and its duration; the number of sperm in a sexually-mature drone; and the fluctuation of sperm number in drones during days after emerging

ii) the number of queen cells reared per colony; the day of the first mating flight of virgin queens; the time of occurrence of queen mating flight and its duration; and the number of sperm in a newly mated-queens in comparison with that in an age-unknown mated-queen.

CHAPTER II

LITERATURE REVIEW

Since most of the research on honey bee have been done on the European honey bee, *Apis mellifera*, this literature review here is derived from *Apis mellifera* and hopefully insights can be drawn from the findings. Otherwise, findings from other *Apis* species are gathered to gain perspective on the finer aspects for the study.

General Biology of a Honey Bee Colony

Castes of the Honey Bee Colony

A bee colony normally has one queen, up to tens of thousands of adult workers, brood and in the active season, some hundreds of adult drones (Butler, 1975; Morse, 1975). In some cases, a colony may be temporarily broodless during dearth periods or during winter or if it is a swarm with a virgin queen (Morse, 1978; Laidlaw, 1979).

The main functions of the queen bees are laying eggs and governing the colony (Butler, 1975; Gojmerac, 1980; Moritz & Southwick, 1992). Those of drones are mating with the queen (Butler, 1975; Gojmerac, 1980) and partly rising the temperature (Crane, 1990). All of the other activities in the colony; such as, cleaning cells and hive, feeding brood, attending the queen, capping brood, building comb, receiving food, guarding and foraging, etc. are performed by workers (Gary, 1975; Tingek, 1987)

Life Cycle and Development Time

The development of a honey bee goes through four stages: egg, larva, pupa and adult (Butler, 1975; Gojmerac, 1980). The queen lays an egg in a cell from which larva hatches by the third day later. The larva is fed intensively to increase its weight. The cell is sealed by worker about 5 days later and the larva then becomes pupa. Finally, the pupa changes into adult and the adult bee comes out from the cell. The development stages of queen, worker, and drone are similar, but occupy different lengths of time (Butler, 1975; Morse, 1975).

The time of development stages (days after egg is laid) of some honey bee species in different places are reported as follows:

		Egg	Hatch	Sealed	Emerge	Place, Author
Worker	*Apis mellifera*	0	3	8	21	Europe, Butler (1975)
		0	2.9	8.7	20.5	South Vietnam, Tan et al. (1993)
	Apis cerana	0	3.75	10.75	22.75	Malaysia, Muid (1987)
		0	2.7	7.5	18.5	South Vietnam, Tan et al. (1993)
	Apis dorsata	0	2.5 - 3	6.5 - 8	16 - 20	Pakistan, Qayyum & Ahmad (1967)
Drone	*Apis mellifera*	0	3	10	24	Europe, Butler (1975)
		0	3.0	9.7	24.4	South Vietnam, Tan et al. (1993)
	Apis cerana	0	3.75	10.75	24.75	Malaysia, Muid (1987)
		0	2.8	8.7	22.8	South Vietnam, Tan et al. (1993)
	Apis dorsata	0	2.5 - 3	6.5 - 8	20-23.5	Pakistan, Qayyum & Ahmad (1967)
Queen	*Apis mellifera*	0	3	8	16	Europe, Butler (1975)
	Apis cerana	0	3.75	9.75	18.75	Malaysia, Muid (1987)
	Apis dorsata	0	2	6.5	13-13.5	Pakistan, Qayyum & Ahmad (1967)

Very little is known about the life cycle of *A. dorsata* because of the strongly defensive and migratory behavior of the species. Qayyum and Ahmad (1967) showed that the total development times of worker, queen and drone of *Apis dorsata* is 1 to 3 days faster than those of *A. cerana* in India.

Drone Bees

Function and Origin

Drones develop from unfertilized eggs that are laid by the queen in drone cells. The drone cells are similar to the worker ones in shape and orientation but the hexagons are wider. In the case of laying workers or drone-laying queens, drones can be reared in worker cells (Butler, 1975; Gojmerac, 1980).

Drones are haploid but if the queen mates with a drone which shares with her a sex allele, some fertilized eggs will develop into diploid drones. However, the diploid drones are eaten by worker bees a few hours after hatching (Woyke, 1963).

The main function of drones is mating with the queens and their presence plays a small part in rising the temperature. Number of drones in a colony is controlled by the workers. Most of them are reared during the reproductive phase of the colony's seasonal cycle. When the dearth season approaches, the workers finally drive the drones out of the colony, where they die (Butler, 1975; Gary, 1975).

Reproductive Organs

Snodgrass (1975) described the details of reproductive organs of an *A. mellifera* drone as follows:

The organs where the primary reproductive cells develop into spermatozoa are the *testes*. They are a pair of small flattened bodies lying in the sides of the abdomen. From each testis there proceeds posteriorly a duct, the *vas deferens*, at first coiled , but soon enlarging into a long slender sac, the *seminal vesicle*. The narrowed posterior ends of the two vesicles enter the lower ends of a pair of huge *mucous glands*, lying side by side, and the two glands open together