THE DRONE HONEY BEE

Lovleen Marwaha

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FOREWORD

Honey bees play an important role in the sustainability of our agro-ecosystem. Bees not only provide pollination services but also provide hive products and thereby act as a crucial source of income for the masses. A thorough understanding of the biology of honey bee queens, workers and drones and the factors affecting their quality and performance helps in improving the productivity of honey bee colonies. A fertile quality queen bee is essential for the long-term survival and growth of a honey bee colony. Further, the productivity of the honey bee colony is also highly dependent on the quality of the queen bee. Therefore, the rearing of queen bees from the most productive honey bee colonies having the desired traits leads to the path to stock improvement. Drones in the honey bee colonies contribute by fertilizing the queen bees and thereby enabling them to lay fertilized eggs resulting in a huge population of worker bees. Therefore, the rearing of a large number of good quality drones from the selected best-performing honey bee colonies is equally important in the bee breeding program. However, for rearing good-quality drone bees, addressing the nutritional requirements of drones is also very important. Understanding the drone maturity, synchronization of drone maturity and queen maturity, mating flight, drone congregation area, and weather conditions suitable for queen bee mating success help us in better planning and execution of bee breeding. This book provides very good information on drone development, pheromonal communication, and the reproductive system of drones. The chapter on the mating and reproduction in *Apis mellifera* provides detailed information regarding drone congregation area and reproductive potential of drones and the factors which affect these parameters. This book also throws light on the significant role drones play in stock quality improvement. This book will provide useful information to the students and researchers. My best wishes to the author.

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PREFACE

'The Drone Honey Bee' has been written especially for B.Sc., M.Sc. and Ph.D. students, highlighting various aspects of the drone honey bee's life cycle. Books on honey bees are easily available in the market which gives immense knowledge about colony organization, different castes of the colony, communication in the colony, the productivity of the colony, genomics, proteomics, and others. But, drone honey bee is very less explored in comparison to other castes, being not directly involved in colony productivity and hence in economic benefits. However, drones can be used in the bee quality improvement of the colony. Drones can be reared artificially in the colony or outside the colony up to certain stages that can be larval, pupal or adult. The drone pupae are good protein supplements for human consumption. The current book elucidates the available details of the specific caste of honey bees.

The present book elaborates on general introduction, morphology, development, pheromonal profile, mating, reproduction, artificial drone development and genomic contribution of drones in colony improvement. The drone honey bee provides patrilineal genomic contributions to the honey bee colony that influence colony productivity, colonial behavior, adaptability, and others. Although the prevalence of drones is seasonal and as per the availability of food resources, the specific caste is an integral part of the colony with a chief role in mating and thermal regulation. The current book highlights information about drone honey bees as per the availability of literature.

Regards,

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CHAPTER 1

Comprehensive Overview of Apis mellifera Drone Development, Biology, and Interaction with The Queen

Abstract: The male honey bees, the reproductive caste of the colony, develop through haploid/diploid parthenogenesis. The drones develop from haploid/ diploid unfertilized eggs produced by parthenogenesis or from diploid fertilized eggs having identical sex alleles, formed after sexual reproduction, with more probability when the queen honey bee mates with the drones of the same hives. Therefore, two types of drone honey bees, based on ploidy, are common in colonies, *e.g.* haploid or/and diploid. The number of drone honey bees staying in the colony varies according to protein resources and the strength of the worker honey bees. Generally, the haploid drone eggs/larvae laid by workers are removed by the nurse bee due to cannibalism. The above-mentioned eggs/larvae are marked with certain specific hormones that act as markers for cannabalic removal of the same.

Further, the development of drones is influenced by colony temperature; hence overall development can be completed within 24-25 days. The purpose of drone life is to produce sperm and mate with the queen. The queen attracts the drone's honey bees toward herself with pheromones 9-ODA, 9-HDA and 10 HDA. The drone number and fertility depend upon the colony's environmental conditions, genomic possession and available food in the colony. The specific chapter provides deep insight into the development of drones, the biology of drones, the reproductive system, and the mating behaviour of particular castes. Subsequent chapters highlight morphometric characteristics of drones, development, mating, reproduction and artificial drone production.

Keywords: Haploid and Diploid Drones, Parthenogenesis, Developmental Synchronicity.

INTRODUCTION

The drone honey bees perform the function of mating and temperature regulation. Further, the concerned caste does not forage, maintain the hive, defend the colony or perform other functions. During the nuptial flight, the polyandrous honey bee

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queen usually mates with 6-17 drones (Peer *et al*., 1956; Renner and Baumann, 1964; Adams *et al*., 1977; Santomauro *et al*., 2004), and post-mating death of drones is inevitable (Witherell, 1956). Unfortunately, the procurable scientific literature for drone honey bees is not vastly explored. Therefore, limited information is available on drones' contribution to agricultural pollination, apicultural production, or the protection of colonies. Further, drones can enhance honey bee colonial productivity, can make colony disease and swarm-resistant, can control overall behaviour and organization through genomic contribution, and others.

THE DEVELOPMENTAL SYNCHRONICITY OF DRONE HONEY BEES

The haploid drone honey bees usually carry maternal inheritance due to their formation from unfertilized eggs laid in drone/worker wax cells by the queen or from a haploid egg laid by the pseudo queen or egg-laying honey bee workers in queen-righted or queen-less colonies(Kerr, 1974a,b; Herrmann *et al*., 2005; Brutscher *et al*., 2019). The haplodiploid sex-determination mechanism is well exemplified in eusocial insect honey bees.

Even diploid drones can develop from fertilized eggs in case of the queen mates with drones of the same colony (Page and Laidlaw, 1985). Such diploid drones can carry inheritance from both maternal and paternal sides. Furthermore, egg-laying workers can lay diploid eggs, with two sets of chromosomes coming from one polar body and an ovum. The specific process is known as thelytoky, a type of parthenogenesis. Other workers sense such diploid eggs through coated pheromones; therefore, such diploid drones are eaten by workers within a few hours after the eggs hatch, which highlights the phenomenon of cannibalism in honey bee colonies (Woyke, 1965).

The developmental duration of drone honey bees varies according to temperature. The temperature is different in the hive's centre and periphery, so the drone's development varies. For the development of drones from egg to adult, about 24 days are required (Jay, 1963), whereas, in the peripheral areas of the hive, more time is usually needed, which could be up to 25 days (Fukuda and Ohtani, 1977). In other words, drone development is correlated with brood nest temperature variation (Free, 1967; Jay, 1963; Fukuda and Ohtani, 1977; Santomauro *et al*., 2004).

For the general development of drones, about three days are required for the egg to hatch, six days for larval development, and 15 days for the pupal phase.

SOME FACTS ABOUT THE DIPLOID DRONES

Haploid drones develop from unfertilized haploid eggs laid by queens or workers. In contrast, some drones develop from diploid eggs formed by the fusion of the ovum with one of the polar bodies or from fertilized eggs that are homologous at the sex locus (Woyke *et al*., 1966; Herrmann *et al*., 2005). Diploid drones can have uniparental origins or biparental origins. Further, the bi-parental origin diploid drone can create by matchmaking a queen and drones with identical sex alleles from the same colony.

Generally, brood-attending worker honey bees eliminate the false diploid drones (Woyke, 1962; Woyke, 1965; Woyke, 1963 a, b, c, d; Herrmann *et al*., 2005). Additionally, the diploid drones produce more cuticular hydrocarbons than the workers (Santomauro *et al*., 2004). The diploid drones produce diploid spermatozoa, having twice the DNA and an elongated head.

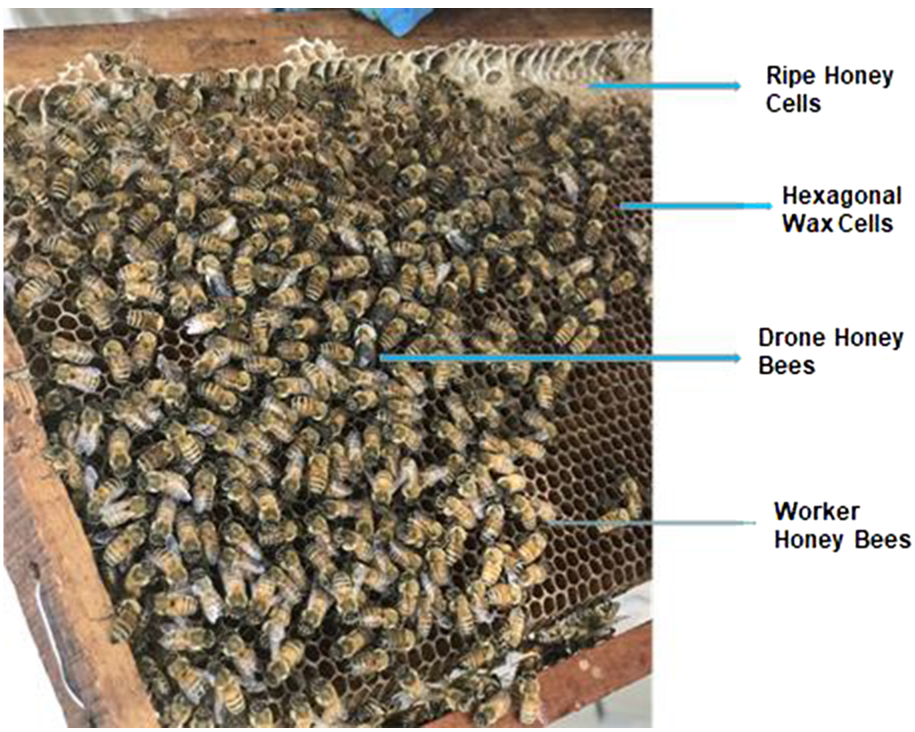

Fig. (1). Hexagonal Wax Cells, Worker Honey Bees, Drone Honey Bees and Ripe Honey Cells are depicted in this image. Worker honey bees perform different duties like the exchange of information, honey processing, and adding worker jelly to developing worker larvae.

The diploid drone larvae secrete certain substances known as cannibalization substances that act as the highlighter of diploid drones that attract other workers

CHAPTER 2

The Drone Honey Bee Morphometric Character

Abstract: The Drone caste exhibits specific diagnostic morphometric characteristics that facilitate its differentiation from other colony castes. Drone formation gets completed in about 24-25 days, with the first ten days in an open cell and the final 14 days under capped conditions. Furthermore, the drone caps are convex in shape. The Drone egg is measured about 1.49±0.12 (range 1.12–1.85) mm, the width is 0.35±0.02 (range 0.30–0.40) mm, and the volume is 0.10±0.02 (range 0.06–0.15) cubic mm. After hatching for the first three days, the larval weight is 0.11 mg, the 7th-day-old larva is 120 mg, 11th-day-old larva reaches a weight of 350 mg. An adult has a mean body length of about 1.5 cm. Further, the drone character varies as per ecological conditions, species, genotype, and other environmental conditions.

Keywords: Diploid drones, Haploid drones, Pheromones, Reproductive system.

INTRODUCTION

In a strong honey bee colony, usually with one queen, thousands of workers and a few hundred drones live harmoniously (Fig. **1**). The queen honey bee is polyandrous as she mates with multiple drones. When the queen of a colony is not reproductively active, workers start laying eggs and acting as pseudo-queens. Pheromones from the queen suppress the development of the workers' reproductive systems; therefore, workers cannot mate. As a result, workers can lay only unfertilized eggs, which can develop into drone honey bees (De Groot & Voogd, 1954; Butler, 1957; Jay, 1968; Pettis *et al*., 1997). When a colony becomes queenless and workers cannot rear another queen, in that case, workers undergo ovarian development and become reproductively active to lay unfertilized haploid eggs, which results in drone production (Winston, 1987; Page & Erickson, 1988; Visscher, 1998).

In general, in honey bee colonies, two distinct-sized hexagonal cells are common, including small cells (5.2–5.4 mm in diameter) in which the queen lays fertilized eggs and large cells (6.2–6.4 mm in diameter) in which the queen lays unfertilized eggs (Winston, 1987). Even in queen-headed colonies, some workers lay unfertilized eggs in worker cells (Page & Erickson, 1988). Generally, such eggs

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are eaten by the other nestmate workers, and about 0.1% of drones are reared to adulthood by workers (Ratnieks & Visscher, 1989; Visscher, 1989). Typically, pseudo-queen formation occurs in queenless colonies, which begin laying unfertile eggs within 1-4 weeks, depending on the geographic region (Ruttner & Hesse, 1979). Hexagonal cell size determines the body size of emerging drones. Drones that have been reared in worker cells are smaller than those which emerge from other drone cells (Berg, 1991; Berg *et al*., 1997; Schlüns *et al*., 2003).

Drones are male honey bees without stings, and they do not perform hive duties. Structurally, drones are incapable of collecting food in the field. Food supply is stopped during late autumn when the colony experiences a dearth period. Drones are fed by worker honey bees in a colony. Due to food scarcity during winter, drones are not maintained in the colony. When drones become weak, their wings are torn off. Their legs are pulled and eventually dragged out of the hive. Drone larvae and pupae are removed from the hive sometimes. Their function is to mate with virgin queens on a mating flight.

The presence of healthy drones is a prime requirement of the colony for queen mating. The drone production depends upon the season and other environmental conditions of the colony and on the young and foraging workers' population. Furthermore, drone quality is influenced by divergent factors like species or subspecies of honey bees, age of drones, rearing season, food supply, size of comb cell, the infestation of comb, and colony strength. The queen honey bee mates with an average of 12 drones and can store 4.3-7.0 million spermatozoa in her spermatheca. Although the life span of a drone is about 55-90 days, and it gets sexual maturity at 16 days, it grows less suitable for mating. The prime role of the drone is to fertilize queens.

A ten-day-old drone is capable of impregnating a queen honey bee. Mating takes place in the air, and after mating, drones die. Copulation occurs about 12.8 kilometres from the hive in a drone congregational area. Drone produces different pheromones in drone congregational sizes. Therefore, producing viable drones is a limiting factor in successful queen rearing. Furthermore, the drone drifts to the colony when the colony possesses enough nectar, and pollen is permitted to stay.

Weight at maturity is considered a competitive advantage to drones over smaller males when fighting for access to females (BERG *et al*., 1997). Therefore, Schluns *et al*. (2003) hypothesized that the low reproductive success of smaller drones is due to a low success rate in competition for accessing the queen.

Fig. (1). *Apis mellifera* strong colony with one exposed comb.

MORPHOMETRIC CHARACTERS

Haploid or diploid drones of honey bees possess certain specific morphometric features. The body is divided into a head, thorax, and abdomen, with three pairs of legs and two pairs of wings. Further explorations witnessed different morphometric features of drones. It can be easily speculated from these studies that drone body size varies as per ecological conditions. A few reports which deal with the morphometric analysis of drones are: Taha *et al*. (2012) studied *Apis mellifera jemenitica* (AMJ) colonies and drones' morphometric features of Carniolan and Yemeni. They reported that the body weight and cell size of Carniolan and Yemeni drones were 190.90 and 0.40 v.s 227.22 mg and 0.43 cm^3, respectively. Further, they speculated that the length and width of the right forewing and the number of hamuli on the right-hand wing of Carniolan drones were significantly higher than in Yemeni subspecies. Taha and Alqarni, 2013 compared the reproductive potential of Yemeni drones and Carniolan.

CHAPTER 3

The Development of the Drone Honey Bees: The Parthenogenesis

Abstract: The drone honey bee develops from unfertilized or fertilized eggs depending on the homozygosity of the sex alleles in inherited genomic content. In the honey bee colony, if the polyandrous queen honey bee mates with the drone honey bees of the neighbouring colonies, then the drones develop from the unfertilized eggs, confirming the haploid parthenogenesis. However, the mating of the queen with the drones of the same colony accelerates the feasibility of the development of drones, even from fertilized eggs. In the above-mentioned former case, the drones are known as the haploid drones, whereas in the latter case, the drones are referred to as the diploid drones. Generally, the diploid drones are removed by worker honey bees by recognizing the pheromones coated on the egg surface. The worker honey bees can remarkably distinguish the queen's drone eggs and the workers acting as pseudo-queens' drone eggs. The pseudo-queen develops if the colony is queen-less or the queen is not carrying the required reproductive potential and pheromonal emission. Drone development takes 24-25 days in total, with four distinct phases: egg, larval, pupal, and adult, with durations of 3 days, six days, 15-16 days, and about 1-3 months, respectively. The present chapter is attributed to the drone honey bees' developmental synchronicity, haploid inheritance, parthenogenesis, and patrilineal genomic contribution to the colony that influences colonial behaviour, productivity, life span, immunity, and others.

Keywords: Drone honey bee, Ecdysis, Holometabolous, Haploid parthenogenesis.

INTRODUCTION

The haploid/diploid drones (n/2n=16/32) of *Apis mellifera* (*A. mellifera*), a hymenopteran insect, develop following a typical holometabolous pathway of development with the inclusion of stages such as eggs, larvae, pupae, and adults. The inheritance is exclusively maternal as they develop from the unfertilized eggs in the natural populations (Figs. **1-6**). Under optimal environmental conditions, about 24–25 days are required for complete synchronous developmental events. Furthermore, the duration of development can vary depending on genomic

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possession, transcriptional expression, protein functionality, pheromonal communication, food resource availability, temperature, disease/pest infection/ infestation, and other factors. About three days are required for the haploid eggs to hatch; after that, five larval instars develop. The larval phase undergoes moulting to convert to the pupa that completes organogenesis within an enclosed compartment walled with bee wax. After pupation, imagoes emerge by breaking the seal of the capped wax cells. For the first three days, the drones are fed by the worker honey bees; after that, they can take care of their diet. They prefer to feed on honey and stored pollen grains. The average life span of drones can be up to 55-90 days, which further varies as per environmental cues. The purpose of the male caste in the colony is to mate with queens of other honey bee colonies to contribute patrilineal genomic content. The drone's genomic contribution can influence the general behaviour, strength, productivity, disease resistance, and other characteristics of the honey bee colony.

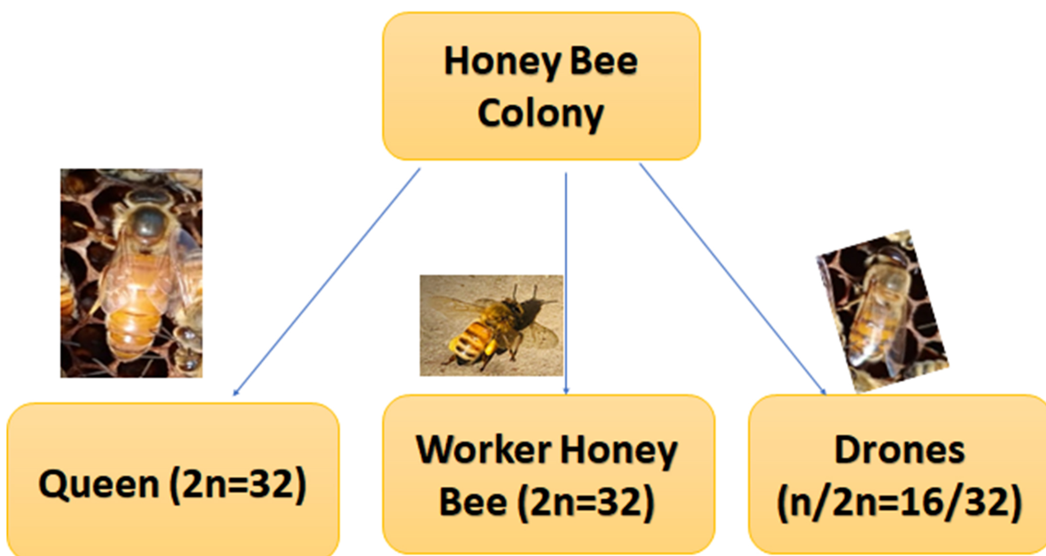

Fig. (1). Schematically elucidating the development of three different honey bee castes with different morphology, anatomy, physiology, proteomics, reproductive potential, life span, functionality, immunity, and others.

Furthermore, every honey bee colony needs to rear and maintain healthy drones because it is essential for successful queen bee mating. A polyandrous queen honey bee usually mates once with about 12–20 drones in her life span. A properly mated queen carries about 4.3 to 7 million spermatozoa in her spermathecae, sufficient to lay fertilized eggs during her reign in the colony (Rhodes, 2002). After mating, the queen stores sperm in her spermathecae, keeping it viable even after the drone that produced the male gametes dies (Klenk *et al*., 2004; Phiancharoen *et al*., 2004). A drone must find the queen in the drone congregation area and compete with hundreds of other drones from the same or different colonies to mate with her (Gary, 1963; Ruttner and Ruttner, 1972; Page, 1986; Koeniger, 1988; Berg *et al*., 1997). Hence, drone honey bees exhibit long anatomical and physiological adaptations for strong and forceful flight (Radloff *et al*., 2003).

A colony can raise a new queen if the existing queen is not mated correctly and does not carry sufficient sperm storage for egg laying. Generally, a queen with 3 million sperm after mating can be superseded within 12 months under commercial conditions, which is not acceptable for beekeeping as it accelerates swarming in the colony. A normal, reproductively active queen honey bee uses 2 million stored sperm to oviposit and fertilize eggs annually. A 16-day-old, reproductively healthy drone honey bee can produce about 5-10 million sperms. Further, the suitability of drones for mating decreases after 28 days of age.

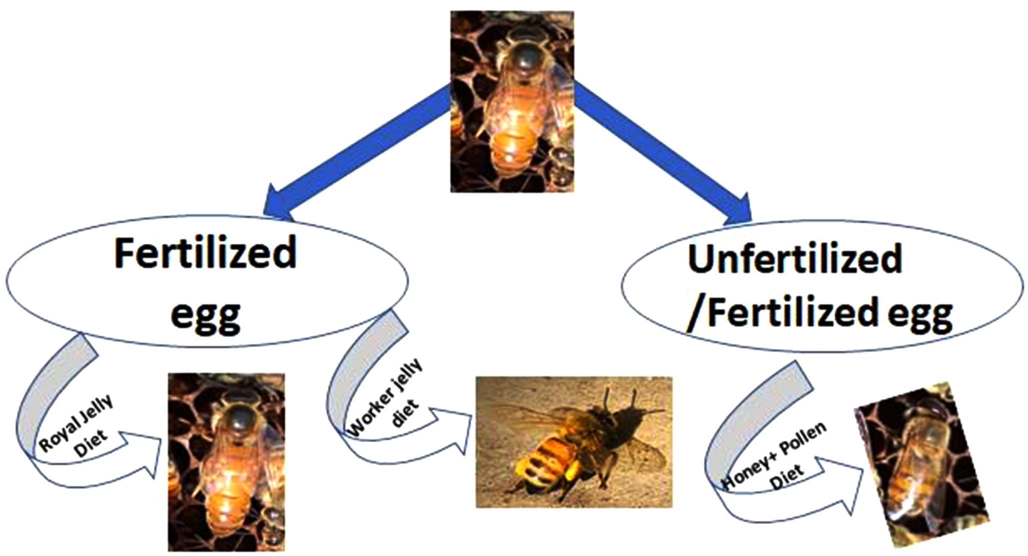

Fig. (2). Description of the polyandrous queen's ability to lay fertilized and unfertilized eggs, which can develop into female castes (the queen and the workers). The phenomenon of haploid parthenogenesis can be easily exemplified in the case of drone honey bees. In contrast, queen and worker honey bees witness polyphenism, the developmental plasticity of the same genomic contents expressed differentially per environmental cues.

In short, the purpose of the drone honey bee's life is the production of sperm and mating with the queen. Drones possess elaborative mating organs, powerful sense organs, comparatively larger eyes with numerous omatids, and long antennae with

CHAPTER 4

The Pheromonal Profile of the Drone Honey Bees Apis mellifera: The Volatile Messengers

Abstract: The drone honey bee produces volatile chemicals during developmental and adult stages that facilitate chemical interaction between drone larvae and workers, drone to drone, and drone to the queen. For example, the drone larvae solicit larval food from nurse bees through chemical messengers; further, adult drones attract other drones to drone congregation areas (DCA) through pheromones; the drones attract queen honey bees to DCA through volatile chemicals. The mandibular drone gland secretes volatile chemicals, a mixture of saturated, unsaturated, and methyl-branched fatty acids. In the drone honey bee of *Apis mellifera*, about 18,600 olfactory poreplate sensilla per antenna are associated with receptor neurons. The current chapter highlights the role of chemical volatiles in Drone honey bee's life and overall influence.

Keywords: Chemical communication, Colonial cehaviour, Pheromones.

INTRODUCTION

The volatile chemicals and pheromones play a critical role in intra-specific communication in insect societies, including honey bees. Chemical communication governs interactions between larvae and adult workers, workers to workers, queens to queens, and queens to drones (Gary and Marston 1971; Pankiw *et al.* 1998; Slessor *et al.* 2005; Thom *et al.* 2007; Grozinger 2015). The honey bee colony is quite suitable in speculating the significance and complexity of pheromonal communication.

Due to their limited role in colonial productivity, the males remained relatively little in their exploration of chemical production and the involvement of specific chemicals in social communication. Therefore, few investigations are available that elaborate on the quantity, types, and function of the male sex-specific chemicals in the honey bee. Nevertheless, for a long time, drone honey bees have been assumed to play a role in colony resource consumption and reproduction (Boomsma and Ratnieks 1996; Tarpy and Nielsen 2002).

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A few studies indicate that drones are engaged in various social interactions inside and outside the colony. According to Lensky *et al.* 1985; Boucher and Schneider 2009 studies, worker-drone and drone-drone interaction are facilitated by drone pheromone production. Further, the drones interact with the workers and the queen in the colony through chemical communication. As the drones get food from the nurse workers, they regularly engage in trophallaxis (Free 1957; Naumann *et al.* 1991).

Villar *et al.* (2018) reported that drones find and tightly aggregate with each other while inside a colony. The reason and implication of this specific aggregation are uncharacterized, but possibly drone-specific olfactory cues may support such an aggregation within a colony.

For mating, sexually mature drones fly out of the colony and aggregate at specific drone congregation areas (DCA) with other drones, waiting for the virgin queen to arrive (Ruttner and Ruttner 1972). The drone perceives and responds to queen pheromones 9-ODA (9-oxo2-decenoic acid) inside the colony, and specific chemicals further influence drone physiology and behaviour (Villar and Grozinger 2017). As the location of DCA is stable over time, it is probably due to the fact that the drone uses particular landscape features (GalindoCardona et al., 2015).

Further, according to Boucher and Schneider (2009), a specialized worker nursing stage preferentially targets sexually immature drones with a vibratory signal. Drones with specific signals solicit food from workers. The pathway for nurse bee particular behaviour for specific drone age groups is unknown but probably could be facilitated with a drone-produced, odour-mediated mechanism (Wakonigg *et al.* 2000).

THE PHEROMONAL COMMUNICATION IN THE COLONY

Pheromonal secretion regulates the colony's foraging, defensive, and brood-rearing activity (Free and Winder 1983; Free 1987; Vallet *et al.* 1991; Breed *et al.* 2004; Hunt 2007; Stout and Goulson 2001; Slessor *et al.* 2005; Maisonnasse *et al.* 2010). Slessor *et al.* 1990; Slessor *et al.* 2005; Grozinger 2015). The communication of the sex pheromones has been demonstrated by Gary (1962) in *Apis mellifera*. The queen's mandibular gland secretion includes 9-ODA (9-ket--2(E)-decenoic acid), due to which drones get attracted toward the queen. Further, other studies, including Butler *et al.* 1967; Sanasi *et al.* 1971, scrutinized other honey bee species, including *Apis florea, Apis cerana*, and *Apis dorsata* using the same type of pheromones.

Further, the communication between workers and developing larvae is regulated by pheromonal communications. Developing drone larvae are dependent upon the worker honey bees. Larvae release capping pheromones from their salivary gland containing ten methyl or ethyl fatty acid esters (Le Conte *et al.* 1989, 1990, 2006; Trouiller *et al.* 1991). The composition and quantity of pheromonal secretion vary by the age and sex of larvae. The workers adjust their behavioural responses to larvae accordingly (Free and Winder 1983; Le Conte 1994; Slessor *et al.* 2005). At the end of the final larval stage, workers secrete a thin wax cap over the cell to facilitate the pupation process within a clean and stable environment.

Le Conte *et al.* 1990 speculated that four components trigger the behaviour of workers, which include methyl palmitate (M.P.), methyl oleate (MO), methyl linoleate (ML), and methyl linolenate (MLN) (Le Conte *et al.* 1990). The pheromonal secretion reaches to peak when larvae are about to enter the pupation phase (Trouiller *et al.* 1991). Qin et al., 2019 analyzed the change in gene expression across the cell capping. Further, they analyzed the biosynthetic pathways for M.P., MO, ML, and MLN and a "housekeeping gene" (β-actin). For a specific analysis, larval samples had been taken from three different colonies and were stored in frozen liquid nitrogen at −80 °C until used. First, they analyzed the relative expression level by formula $2^{-\Delta\Delta Ct}$ (Liu and Saint (2002), and after that, the square root was transformed to normalize the distribution of the data before ANOVA analysis. They found that M.P., MO, ML, and MLN acted as essential signals for honey bee capping behaviour. Similarly, Trouiller *et al.* (1992) concluded that drone honey bee larvae increase the amount of capping pheromones at the stage of wax capping. Consistently, Yan *et al.* (2009) also reported that in *Apis cerana* workers and drone larvae, there is a similar trend of hormonal release.

The drone cells are generally located at the edge of the bee hive, so Qin et al., 2019 took samples for study from the edges of the bee hive. Varroa mite is a global pest of honey bees (Le Conte *et al.* 2010). Varroa mites get attracted toward colonies with the influence of M.P., E.P., and ML (Le Conte *et al.* 1989). Acetyl-CoA is a precursor of different metabolic pathways which result in the synthesis of fatty acids and esters (Mcgee and Spector 1975; Cripps *et al.* 1986; Moshitzky *et al.* 2003). Insects can synthesize fatty acids de novo or get fatty acids in their diet. Yan *et al.* (2009) demonstrated through 13C and 2H isotope tracing that capping pheromone components were de novo synthesized by larvae. Furthermore, they have proposed that about 12 genes facilitate the processes of M.P., MO, ML, and MLN in honey bee larvae.

THE DRONE MANDIBULAR GLAND PHEROMONES

The mandibular drone glands secrete a blend of saturated, unsaturated, and methyl-branched fatty acids primarily, with chain lengths of nonanoic to

CHAPTER 5

The Mating and Reproduction in Apis Mellifera: The Role of Drone Honey Bee

Abstract: Mating in honey bees occurs in the drone congeration area, where the queen and drones gather for reproduction. Sexually mature drones from all colonies congregate there, and the queen mates with multiple drones, increasing patrilinear inheritance variation. The variation in genomic content results in specific colonial behaviour, productivity, and strength. The current chapter discusses mating in honey bee colonies.

Keywords: Haploid drones, Diploid drones, Pheromones, Reproductive system.

INTRODUCTION

Apis mellifera colony consists of a polyandrous queen, several thousand facultative sterile female workers, and a few thousand seasonal males (drones), constitute a colony. Multiple mating increases genetic variability within a colony, which may have a selective value. Such colonies might cope better with changing environmental conditions (Crozier and Page 1985; Robinson and Page 1988). Polyandry further reduces the chances of parasites or pathogens destroying the colony to the extent of survival and reproduction (Sherman *et al.* 1988). In a colony with several sex alleles in a population, there is a high brood survival rate (Page 1980; Page and Metcalf 1982).

Further, polyandry conditions have resulted in complex flight behaviour. Polyandry in honey bees is not caused by insufficient sperm production by drones; a single drone produces more sperms than the spermatheca can hold. Instead, polyandry in honey bees occurs due to some other advantages like genetic variation.

Mating of queen and drone occurs in the drone congregation area, a location away from the hive, further increasing flight time and associated survival risk. During the nuptial flight, drones compete with one another, and the fastest drone will be able to mate. The mechanism for the queen's preference for her mate is unknown

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(Kerr and Bueno 1970). The drones, after mating, support the succeeding drones to mate with the queen. As such, a drone marks the queen with conspicuous colour to reduce the mating-flight time (Koeniger, 1990).

In a colony, drone rearing occurs during the reproductive season when resources are plentiful and a large workforce is ready for rearing (Winston 1987; Rowland and McLellan 1987; Rangel *et al.* 2013; Rangel *et al.* 2013; Smith *et al.* 2014). As a result, male production occurs with the construction of comb cells with comparatively larger sizes than worker-destined cells (Seeley and Morse 1976; Boes 2010; Smith *et al.* 2014). Further, there is a variation in drone cells, which further results in variation in the adults' size (Berg 1991; Berg *et al.* 1997; Schlüns *et al.* 2003; Berg *et al.* 1997; Couvillon *et al.* 2010).

Generally, drones are produced about 3–4 weeks before the production of a new queen so that a new queen formed during the reproductive season would be able to get sexually mature drones for reproduction (Page 1981). Drones mate once, but the queen exhibits extreme polyandry by mating with 12 to 14 drones, which can go up to 50 or more drones (Estoup 1995; Tarpy and Page 2000; Rhodes 2002; Abdelkader *et al.* 2014; Palmer and Oldroyd 2000; Koeniger *et al.* 2005a; Amiri *et al.* 2017; Brutscher *et al.* 2019). After a few days of hatching, the young queen honey takes several nuptial flights to drone congregation areas, where she mates in the air with an average of 12 drones from other colonies (Tarpy and Nielsen, 2002). The exact number and origin of drones are difficult to observe (Koeniger et al., 2015). Artificial insemination has been developed as a practical tool for economic breeding (Laidlaw, 1987; Nolan, 1932).

Drone development needs 24 days, with queen development 16 days and 21 days. Further, this developmental time can vary depending upon factors like haplotype, temperature, and overall colony conditions (DeGrandiHoffman *et al.* 1998; DeGrandiHoffman 1993; Biekowska *et al.* 2011; Sturup *et al.* 2013; Winston 1987; DeGrandi-Hoffman 1993; Collison 2004). Few reports witness that in the case of honey bees, spermatogenesis starts at the larval stage and is completed at the pupal stage (Holldobler and Bartz 1985; Bishop 1920; Hoage and Kessel 1968). A drone ejaculates between 0.91 and 1.7 μL per drone, containing about 3.6 to 12 million sperm cells (Woyke 1960; Nguyen 1995; Collins and Pettis 2001; Rhodes 2008; Rousseau *et al.* 2015; Mackensen 1955; Woyke 1962; Duay *et al.* 2002; Schlüns *et al.* 2003; Rhodes *et al.* 2011). Further, sperm count is influenced by size, larval diet, and season (Nguyen 1995; Schlüns *et al.* 2003; Rhodes 2011).

During mating, the drone honey bee congregates in specific areas independently of the presence of the queen (Jean-Prost 1958; Ruttner & Ruttner 1963, 1965;

Zmarlicki & Morse 1963). After the entry of a queen honey bee in that area, drones just pursue her like a comet-like group due to queen sex pheromones and visual cues (Gary 1962, 1963; Pain & Ruttner 1963; Strang 1970; Gerig 1971).

For DCA (**Drone Congregation Area**), about 11,000 drones gather in mid-air, about 10 to 50 m above ground (Free 1987; Baudry *et al.* 1998; Koeniger *et al.* 2005a). Drones emit specific volatiles, which modulate their social interaction and further assist in forming DCAs (Villar et al., 2018; Brandstaetter et al., 2014). When virgin queens enter the congregation area, they attract drones with pheromones, in particular, 9-oxo-2-decenoic acid, or with visual cues at short range, which focus on finding and matching with the queen (9-ODA; Brandstaetter *et al.* 2014; Gries and Koeniger 1996; Baudry *et al.* 1998; Jaffé and Moritz 2010; Goins and Schneider 2013).

Virgin queens visit DCA on one or several mating flights, which can happen in one or several days (Roberts 1944; Tarpy and Page 2000). DCA comprises drones from colonies located 5 km away from each other (Free 1987; Baudry *et al.* 1998; Koeniger *et al.* 2005a). The drone hone bee gathers away from the colony to avoid inbreeding (Winston 1987). Factors that promote drones' gathering to DCA are poorly understood (Koeniger *et al.* 2005b).

Mating in honey bee colonies occurs in the drone congregation area. Further, properly exploring these areas is very important for keepers to get queen mating. Abou-Shaaraa and Kelany, 2021) explored DCA using remote sensing and geographical information systems. Mating of honey bee queens occurs in the air with numerous drones (Cobey, 2007; Moritz et al., 1996; Neumann & Moritz, 2000). These areas possess specific characteristics which is why the same site is chosen repeatedly (Ruttner & Ruttner, 1972).

During mating, at the beginning of the eversion of the membranous endophallus, drones become paralyzed. With the queen's movements, eversion of the endophallus proceeds, and sperm injection occurs in the oviduct. After mating, drones drop to the ground and die. The mating signs include mucus from male accessory glands, expelled chitin plates from drone endophallus, and a sticky orange layer from cornual gland cells (Koeniger 1988). The honey bee colony size is positively proportional to the number of queens produced for reproduction and the number of swarms (Winston 1979; Winston & Taylor 1980).

DRONE CONGREGATION AREA

Drone honey bees and queens gather in the Drone Congregation Areas (DCAs). DCA is formed in places with characteristic features. Galindo-Cardona, 2012, studied DCA with the help of a helium balloon equipped with queen-se-

CHAPTER 6

Artificial Methods of Drone Rearing in Apis Mellifera and the Role of Drones in Quality Improvement

Abstract: A few protocols are available for artificial drone rearing under controlled conditions within or outside the honey bee colony. The initial development phase of the drone development can be completed on royal jelly exclusively. Still, on worker jelly, complete drop development can be carried out, as witnessed by a few researchers. Sugars accelerate the drone's growth and support the passage of the drone through certain embryonic and post-embryonic stages. The drones contribute patrilineal genomic content that influences the overall strength of the colony, colonial behaviour, productivity, and other characteristics. The current chapter is attributed to some available artificial haploid/diploid drone-rearing methods and the influence of multiple patrilineal genomic content contributions to other honey bees.

Keywords: Artificial drone rearing, Genetic quality improvement.

INTRODUCTION

Artificial drone rearing is not a common practice in beekeeping as the male honey bees are not directly involved in the colony's productivity. Therefore, very limited explorations are available that are attributed to the development of the drone honey bee, although male honey bees are essential for genetics, embryology, and insect nutrition. Different researchers had tried to rear the drone outside the colony, but minimal information is available about the drones. Takeuchi et al., 1972 reared the diploid drones from the larvae fed on royal jelly mixed with extra sugar, and they succeeded in getting adults at age 2, 4, or 5 days. In total, they could rear about 13 adults artificially. However, the royal jelly could not sustain the complete development of the drone bees.

Rhein (1951) tried to rear the drones on the worker jelly and could get pupae and adults artificially. They concluded that the chemical composition of the drone jelly is similar to that of worker jelly. Jay (1959) attempted to feed the drones royal jelly but did not obtain any drone adults. Woyke (1965) also used royal jelly

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for diploid drone development. He concluded that drones were challenging to rear compared to queens and further reported that drone larvae pupate if fed on mixed food of older larvae rather than royal jelly exclusively (Woyke, 1963a, 1963b). During the drone larval development, the chemical composition of the drone jelly changes like that of the worker jelly (Gontarski, 1949; Haydak, 1957; Matsuka et al., 1973).

The older drone larvae's diet contains a considerable quantity of sugar. Furthermore, in worker jelly, sugar is known to promote pupation in worker larvae (Shuel and Dixon, 1968). Takeuchi et al., 1972, tried to feed 5-day-old drone honey bee larvae with royal jelly modified with extra sugar to study if sugar could enhance pupation rate and adult emergence. They tried to alter drone jelly by adding 0.8 parts of a 60% sugar solution. They kept the modified diet below 5 degrees Celsius until it was used. For artificial rearing, first, they selected eggs laid in an empty comb for 24 hrs. and kept the comb within the colony for 2–5 days. Then, twenty-five drone larvae were reared by putting them in a plastic vessel containing an experimental diet. The plastic vessel had been placed in a desiccator with controlled humidity. Woyke (l963b) found that the frequency of diet renewal was beneficial to the growth of drone honey bees. At the pre-pupal stage, larvae were transferred to a dish made up of paper for pupation after washing with lukewarm water at 35°C. The relative humidity was maintained at 82% with the help of a supersaturated solution of Na2SO4. They followed techniques described by Sasaki and Okada (1972). Further, Takeuchi *et al.* (1972) speculated that drones could be reared in incubators during the latter part of their larval life. Royal jelly can provide the required nutrients for drone development. They further reported that adding sugar increases larvae's body weight and can influence pupation (Shuel and Dixon, 1968). Some components of royal jelly induce inhibition of larval growth. Takeuchi et al., 1972 did not get adults if they tried to feed drone larvae on royal jelly exclusively and, further, they noticed a remarkable reduction in weight in the pre-pupal and pupal phases. Dietz (1966) tried to rear the drones on royal jelly diluted with diet from an older larval stage. According to Jay (1965), young drones were sensitive to the environmental conditions maintained for rearing (Takeuchi et al., 1972).

Woyke, 1962, tested drone-rearing techniques with the best results when diploid drone larvae of 2-3 days old were reared in the incubator. Generally, the diploid drones are eaten after a few hours of hatching by worker honey bees (Woyke, 1962; Woyke, 1963a; Woyke, 1963b). However, the diploid drones are viable and can be reared easily outside the colony in an incubator up to pre-pupal and pupal stages (Woyke, 1963c, 1965b; Woyke, 1965a). The workers eat the diploid drones if laid in worker and drone cells but are more protected in queen cells (Woyke, I965c; Woyke, 1965d). Nevertheless, no diploid drone cell is reared in queen cells as most of them fall out of the cells.

Woyke, 1962, tried to rear the drone by different methods. The first method includes the artificial rearing of drones for the first two days in an incubator and transferring them to the colony. For that, the worker comb with hatching eggs laid by the queen has been taken out. Afterward, the comb is wrapped in a moist towel or inserted in an isolator with water at the bottom. The rearing had been carried out at a temperature of 34.5°C. Each brood has been examined every 3 hours. After hatching, newly hatched larvae have been grafted into drone cells. The larvae can be placed in an incubator at 34.5°C with a relative humidity of 95–100%. The 2-3 day-old larvae had been transferred to the drone cells. The survival rate was checked two days later, and sex was determined after capping. The sealed brood had been protected with wire gauze.

The second method they have adopted is rearing in colonies throughout the larval life. They had maintained other conditions similar to the first method. Worker bees have been observed preparing convex seals on worker cells containing drone larvae. It has been further noted that bees seal only isolated cells. It has also been speculated that no adult diploid drone emerged from worker cells. The diploid drone larvae can survive for one day or more. They had speculated that a relatively low percentage of larvae reared in incubation could survive in the colony. After being cultivated for 2-3 days in incubators, they concluded that drone cells exhibit a low survival rate.

Their findings indicated that young diploid larvae could be separated and transferred back to a colony where they could be reared like normal diploid females. Woyke, in 1962, assessed the normal survival rate of haploid and diploid drones in different environmental conditions. They compared the haploid drone survival rate during the spring, summer, and autumn seasons. They had considered a haploid drone in a queened and queen-less colony. Woyke (1965) demonstrated that many diploid drones are not eaten by workers when they are located in queen cells.

Polaczek *et al.* (2000) conducted artificial insemination of queens of *Apis mellifera carnica* with the semen of their sons. They also attempted to rear diploid drone offspring using two established techniques, including elaborate laboratory manipulations and small mating nuclei, which facilitate drone raising to adulthood. The ploidy level of drones has been determined by using DNA microsatellites. Furthermore, they reported that elaborative techniques reared all drones, and 90% of drones raised in a small mating nucleus were diploid. The abovementioned method makes it easy to get diploid drones without sophisticated

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