

CHAPTER 1 - INTRODUCTION

Introduction

The life of the honey bee, *Apis mellifera* Linnaeus (Hymenoptera: Apidae) begins from the egg. All eggs hatch into larvae approximately three days after oviposition. Several factors, including internal characteristics of an egg, metabolic processes and environmental variables influence the exact number of hours to eclosion. Egg hatching is known to be a function of the temperature in the brood area and the genetic make-up of the population (Caron 1995). Harbo et al. (1981) estimated a developmental time difference of 3.7 hours between the eggs of Africanized honey bees and the eggs of European honey bees, with the former developing earlier than the latter. Similarly, Harbo and Bolten (1981) recorded a developmental time difference of 3 hours between artificially incubated female (fertilized) eggs and male (haploid and diploid) eggs at 34.8⁰C (60 to 80 RH). Since temperature fluctuations usually occur inside hives (Owens 1971, Seeley 1985), large variations should be observable in developmental times of eggs laid over the same period on any one comb. Eggs oviposited at the center of a comb are exposed to ideal temperature for development compared to those laid toward, or at the periphery of the comb. A large proportion of fertilized eggs are distributed at the center where temperature variations are minimized by cluster formations, while unfertilized eggs (also known as drone eggs), a few fertilized eggs and peripheral queen cells, which also contain fertilized eggs, are most affected by any drastic changes of temperature in the hive. Since there are insufficient data and information on the development of honey bee eggs under artificial incubation (Harbo and Bolten 1981), and only few studies have compared respiratory rates of eggs oviposited by mated queens and those of laying workers, there is a need to study the effects of different temperatures on honey bee eggs and the changes in the levels of nutrients during embryonic development. This study therefore provides information on respiration rates of eggs from queens and those of laying workers exposed to temperatures within, as well as outside the optimal range for honey bee egg development. It also provides comparative information on the levels of nutrients in fertilized eggs from queens and unfertilized eggs from laying workers collected from the hives during the three days of embryonic development.

The objectives of this study were:

1. Determine the respiratory rate of honey bee eggs by measuring the amount of CO₂ released when eggs are artificially incubated at different temperatures. Differences were also observed among queens' eggs, as well as differences between the eggs of queens and laying workers in order to relate the results to what may occur under natural conditions.
2. Determine the types and amount of lipids and carbohydrates in eggs of *A. mellifera*.
3. Compare metabolic activities of fertilized and unfertilized (drone) eggs produced by queens to those of unfertilized eggs from laying workers.

The investigation is divided into three parts: The first part examines the measurement of respiratory rates in the eggs of queens and those of laying workers incubated at different temperatures. The second part introduces the extraction and analysis of carbohydrates by HPTLC to determine the levels of sugars in the eggs of queens and those of laying workers on day one, two and three, respectively. Finally, the third part outlines the use of HPTLC to extract and analyze lipids in the eggs from queens and laying workers in order to determine changes in the levels of these nutrients as eggs advance in age.

CHAPTER 2 - LITERATURE REVIEW

The Origin, Distribution and Importance of the genus *Apis*

The origin of *Apis* is obscure; the exact number of species in the genus remains a major controversy. *Apis mellifera* L. is one of seven possible members of the genus known to date. The other species are: *Apis andreniformis* Smith, *A. cerana* Fabricius, *A. dorsata* F., *A. florea* F., *A. laboriosa* Sakagami et al., and *A. koschevnikovi* von Buttel-Reepen (Ruttner 1988, Engels and Schultz 1997). In 1862 Gerstäcker proposed the existence of four species (Alexander 1991). Ruttner (1968) hypothesized a tropical origin for *Apis* with migration into temperate regions of western Asia, southern and central Europe.

Of the four well known species, three (*A. cerana*, *A. dorsata* and *A. florea*) are confined to Asia and Indian subcontinent, while *A. mellifera* is naturally distributed in Africa, Europe and the Middle East. It was introduced into North America, South America, Australia, Japan, Taiwan and Indonesian archipelago (Michener 1974, Seeley 1982). Smith (1977) confirmed the introduction of *A. mellifera* into North America in 1622. In the continents of origin and initial dispersion, several subspecies of *A. mellifera* evolved which are commonly referred to as “races” of honey bees. von Buttel-Reepen introduced a trinomial nomenclature to indicate geographical race or distribution of subspecies in Africa, Europe and Asia. Africa has approximately eleven subspecies (Ruttner 1975b, 1988); while Europe may have seven (Ruttner 1988). Winston (1987) showed the possible present day geographical distributions of the subspecies.

Honey bees are humans’ most beneficial insects. They are the major producers of honey, which was widely used as an earlier form of natural sweetener and wax for making candles. Other hive products of economic importance include royal jelly, propolis, pollen and bee venom (Crane 1990). Brood, generally consisting of eggs, larvae, and pupae have been consumed in honey hunting societies for centuries (Pager 1973, Crane 1983). But the introduction of modern beekeeping techniques has greatly improved the harvesting of honey bee products in areas where honey hunting and forest destruction often took place (Kigatiira 1976, Nightingale 1976). Economic impact of honey bees and their products in human societies is enormous. Standifer and McGregor (1977) estimated an income

greater than \$100 million annually from the sales of honey and wax harvested from approximately 4 million colonies in the United States alone. Along with honey bee products, many wild plants and crop plants benefit from cross pollination by honey bees (Standifer and McGregor 1977, Robinson et al. 1989). Standifer and McGregor (1977) also reported that honey bees pollinate in excess of \$1 billion worth of agricultural crops. For scientific investigations, honey bees have been used as major subjects in many areas of research, particularly in the study of genetics where they provided excellent specimens for the study of chromosomes (Kerr 1951, Kerr and Laidlaw 1956, Kerr 1972a). The introduction of *Drosophila melanogaster* by Castle and Morgan into genetics research in the early 1920s removed the honey bee from this prestigious position. However, there has been sporadic interest in honey bee genetics, particularly after the use of artificial or instrumental insemination on queens became a reality in the mid-1940s (Moritz 1994). Honey bees have also been used as a means of biomonitoring the harmful effects of chemicals used in the environment, mainly in agroecosystems (Bromenshenk et al. 1988, Celli et al. 1996). In art and literature, honey bees have provided societal models which have been a source of admiration, inspiration, speculation, as well as great controversy since antiquity (Crane 1983). However, the honey bee/human interaction has not always remained peaceful, romantic and revenue-laden. Many people, especially those who have had nasty encounters with honey bees, have a great dislike for them. Some people are also highly allergic to bee venom. *A. mellifera* belongs to a group of hymenopterans called aculeates, which are notorious for causing terror with their painful, poison-laden and occasionally lethal stings (Schmidt 1992). A sub-race of Africanized honey bees from South America have been widely described in literature and nicknamed “killer bees” (Winston 1992). These bees have also been negatively publicized in Hollywood films and the print media (Potter 1977, Nunamaker 1979), a true indication of apiphobia. Although African races of honey bees protect their hives by stinging intruders in large numbers, often chasing them over long distances, and may remain agitated for a long time in the hive; a trait necessary to survive the harsh environments in Africa, they often peacefully cohabit among humans and different animals found on the continent.

Honey bees have enjoyed an association with humans dating from prehistoric times (Harpaz 1973, Pager 1973, 1976, Crane 1983, 1990). They are therefore regarded as one of the most studied insects (Winston 1987, Moritz 1992), with a study period extending from the days of Aristotle to the present time (Peck 1965, Crane 1983, Pellegrin and Preus 1986). During this period many aspects of honey bee biology and social life have been investigated and described, producing enormous amounts of literature. Surprisingly, in spite of these endeavors, several parameters of honey bee life remain inadequately investigated or understood.

Reproduction is one such area of honey bee biology which deserves thorough study, if the origin of eusociality is to be understood. The enormous reproductive capacity of queen bees (queens) contrasts with the sterility (infertility) and reproductive altruism in worker bees (workers). The complexity of the situation prompted Darwin to re-examine his theory of natural selection relating to sociality as a way of life (Darwin 1859). This phenomenon has baffled sociobiologists for a long time (Wilson 1971). Consequently, several questions have been posed seeking to establish the existence of evolutionary differences between the queen and the worker (Starr 1987). Although Starr elaborately described morphological, physiological and behavioral differences between the queen and the worker, the answers to these complex questions may lie in unwrapping the mystery surrounding the reproductive strategy in eusocial insects in general, and in honey bees in particular.

Biology of the honey bee, *Apis mellifera*

Honey bees are eusocial insects living in communities called colonies, where the duties performed by each member is based on its caste and age (Michener 1969, 1974, Wilson 1971, Southwick 1988). Depending on the season and weather condition, one reproductive female, the queen, is assisted by from 20,000 to 80,000 sterile females or workers to care for eggs, larvae, and pupae (brood). The workers find nesting sites, build combs, gather food from floral and non floral sources, store the food, maintain favorable hive temperature, clean the hive, and guard it from intruders. On a routine basis, they process honey, synthesize wax for comb construction and repair, synthesize royal jelly and worker jelly to feed larvae. Queens are also fed on royal jelly. The bases for allocation or

performance of tasks and division of labor in a colony are not completely understood, even though they have been studied over a long period. Lindauer (1952) provided the first detailed description of workers' activities. Seeley (1985) outlined age polymorphism as the basis for performance of different duties by individual workers in a colony. Robinson and Page (1989) used allozyme markers to determine the genetic basis of the division of labor among workers and concluded that workers of specific genotypes tended to perform certain duties more frequently than others. Trumbo et al. (1997) partitioned responsibilities of undertaker specialists and other middle-aged workers in honey bee colonies with the conclusion that advances in adult development may keep some workers in specific duties more frequently than others. Harmony in the colony is maintained through chemical communications using pheromones, which are distributed throughout the hive by trophallaxis and contact (Korst and Velthus 1982, Winston and Slessor 1992, Pettis et al. 1995). Other methods of communication include visual or tactile cues, dances (von Frisch 1967) and auditory cues, such as piping in queens (Wood 1956), buzzing in the drones (male honey bees), and humming in workers. These sounds are usually produced by wings or by body vibrations (Spangler 1986).

Drones have a sole duty of mating with virgin queens during nuptial flights (Koeniger et al. 1979). Mating is highly competitive which ensures only aggressive males will mate. Page and Metcalf (1984), and Henderson (1993) estimated a 1/2000 probability of a male ever mating with a queen. The mating process results in the immediate death of successful drones. Koeniger et al. (1979) photographed and described the mating process of honey bees. The queen mates with 10 to 12 males (Henderson 1993), with a reported maximum of 20 drones (Seeley 1985, Page 1986, Estoup et al. 1994, Oldroyd et al. 1997). The queen therefore obtains an adequate supply of spermatozoa to store in her spermatheca for use during her entire reproductive life. The fate of unsuccessful drones in the colony depends on their geographical location. In temperate regions they are driven out of the hive at the end of fall to perish from starvation and cold temperatures. Those in tropical areas shift or drift to other hives where they are usually accepted with little resistance.

Multiplication of colonies occurs through swarming, a natural method of exploiting new habitats and increasing colonial survival. In tropical and subtropical areas, swarming

seasons coincide with periods of long and short rains, a time when nectar flows are common. Swarming takes place more often among African (*Apis mellifera scutellata* Lepeletier) and Africanized sub-races of honey bees than it occurs in European honey bees. Fell et al. (1977) studied the cyclic swarming behavior of *A. mellifera*, and observed a bi-modal swarming pattern with the main swarm emergence in mid-June and the second but smaller swarms between August and September. Absconding and migration of honey bees are also common, especially where pest attacks, diseases and adverse climatic conditions persist (Kigatiira 1984). Kigatiira (1984) also defined the difference between swarming, migration and absconding in honey bees. He emphasized that swarming is characterized by a sudden departure of a proportion of workers, the old queen and some drones, to form a new colony elsewhere. Migration is an adaptive seasonal departure of a whole colony which has been made broodless due to forage-poor habitat and has therefore moved to a new, forage-rich habitat. He defined absconding as a departure of all adult bees of a colony from a nest caused by factors other than shortage of forage. Such factors as water shortage, damage from overheating of the nest, or damage from pests or predators, may cause absconding.

Nutrition of the honey bee, *Apis mellifera*

Honey bees are strict vegetarians. Nectar is the main source of carbohydrates while pollen provides proteins, lipids, vitamins, minerals and some carbohydrates. Haydak (1970) exhaustively described nutritional requirements of honey bees, depending on caste, age, and physiological status. Turunen and Crailsheim (1996) called this dependence on nectar and pollen, a “specialized herbivory”. Different floral or non-floral sources produce nectar or honeydew of different sugar concentrations, usually ranging between 5% to 80%, depending on environmental conditions (Moritz 1994). Pollen grains vary in texture, color, taste and may contain protein (6-28%), lipids (1-20%), sterols \leq 0.5%, some carbohydrates, vitamins and minerals (Winston 1987). Worker bees feed on honey and pollen directly. The queen can feed directly on pollen or honey but is usually fed on glandular secretions by nurse bees, a secretion which consists predominantly of royal jelly (Haydak 1970, Moritz and Southwick 1992). Royal jelly is composed of 66% water, 12.3% protein, 5.5% lipids, 12.5% reducing substances (carbohydrates), 0.8% ash and

2.8% undetermined substances (Gojmerac 1980). Larvae are also attended by nurse bees but are fed the glandular secretions in amounts and frequency designed to influence the final caste status. There is an intricate interplay between the quality of food, amount consumed, age of larvae, and hormonal secretions from corpora allata, which determines the process of queen and worker differentiation (Beetsma 1979). Newly emerged drones are fed on glandular secretions resembling a modified worker jelly, honey and pollen (Haydak 1970). Later they feed on honey and pollen (Winston 1987). Queen larvae are fed on royal jelly throughout their immature life, while worker larvae feed on royal jelly for the first 2.5 days, then they continue on worker jelly up to pupation. Honey bees bring water into the hive mainly for temperature regulation and dilution of food for young, but direct intake of water may take place among field bees when they are out foraging. The amount brought to the hive is regulated by a number of factors including thirst among foragers or hive bees, as well as reception of water by hive bees from foragers (Kuhnholz and Seeley 1997). Temperature and the relative humidity in the hive may also influence water collection. In addition, water is also derived from metabolic processing of lipids in the food consumed (Chapman 1982).

Reproduction and Egg Development in *Apis mellifera*

Reproduction in *A. mellifera* is generally a royal privilege. Normally, only the queen lays eggs in a hive. Reproduction in workers is suppressed through production of pheromones by an actively laying queen. The best studied pheromone, queen substance, contains a blend of substances, especially 9-oxo-decenoic acid (9-ODA), and 9-hydroxy-2-decenoic acid (9-HDA) which partially inhibit ovarian development in workers (Winston and Slessor 1992). Egg-marking pheromones on queen laid eggs (Ratnieks 1995), brood pheromones (Jay 1968, 1970, Jay and Nelson 1973), as well as worker policing and aggressive behavior toward laying sisters (Visscher 1989) are all known to assist in maintaining worker sterility. But in spite of these reproduction suppressing mechanisms on workers, approximately 0.1% of drones in a normal colony are produced by laying workers. In queenless colonies up to 30% of workers will lay eggs (Visscher 1989). Ratnieks (1993) confirmed egg laying activities by workers and worker policing in colonies having reproductive queens.

In general, the roles of hormones in controlling reproduction in the queen or worker still raise questions. The activities of juvenile hormone (JH) in regulating oogenesis in queens are not clearly understood (Wheeler 1996). Fluri et al. (1981) demonstrated that there is no difference in the JH titer of a laying queen and a non-laying queen or worker, and that the titers of protein and vitellogenin in a laying queen were higher than in the worker, but similar to a non-laying queen. They concluded that the process of oogenesis in honey bee queens may not be under the direct influence of JH.

A typical honey bee egg is about 1.5-1.7 mm long and 0.3-0.4 mm wide (DuPraw 1967, Dade 1977), and weighs 120 to 130 g (Taber and Roberts 1962). Woyke (1994) compared the sizes of eggs from queens and those from laying workers. He calculated the coefficient of variation for the length of eggs deposited by laying workers to be 3.5 times greater than the eggs from queens.

An egg forms a link between generations of honey bees. The maturation of an egg depends on materials absorbed from the hemolymph and other substances produced within the ovaries themselves (Bast and Tefler 1976, Irie and Yamashita 1983). The major materials include proteins, lipids and carbohydrates. The ovary provides a site where nutrients are absorbed from the circulatory system into the oocytes. In an immature female of *A. mellifera* there are 70 ovarioles in the ovary. The number in a developing queen increases during development while the number in developing workers declines [Snodgrass (1956), exhaustively described the process following Meier (1916)]. This developmental process produces the differences between a queen and a worker. The ovary of a mature queen may contain 160-180 ovarioles (Snodgrass 1956, Engels 1968). She lays 1000-2000 eggs per day at peak ovulation and oviposition. A laying worker has 3-10 ovarioles in an ovary and lays 5-15 eggs a day (Velthuis 1990, Seeley 1992). Before the onset of vitellogenesis an ovariole in virgin queens increases in length from 1 mm to 6 mm (Bier 1954). The growth is required to facilitate the synthetic activity of nutrients as well as sequestration of the nutrients from the hemolymph into the ovaries. Egg production is accompanied by ovarian expansion and rapid growth of oocytes and nurse cells. Such rapid changes in size ensure that oogenesis will take place efficiently. Since mating provides signals for oogenesis and oviposition to commence in some insect orders

(Engelmann 1970), and will result in rapid expansion of the ovaries in mated queens, the ovarian activities taking place in the mated queens should be different from those of the virgin queens or laying workers. A method of determining any differences in the eggs produced by members of the castes may provide information on the reproductive strategies in honey bees. Many questions relating to the exact mechanisms involved in nutrient uptake from the hemolymph during oogenesis, including their incorporation into the oocytes, remain unanswered.

Although syntheses of components needed for egg growth and development in insects are species specific (or caste specific in social insects), basic processes for egg production are common to all species. First, the process of eucytoplasmic growth or euplasm takes place, followed by vitellogenetic growth (production of macromolecules). These macromolecules are stored in vacuoles (lipid and proteinaceous yolk spheres). Glycogen, which occurs in aggregates, is mainly synthesized as the last component during oogenesis (Yamashita and Hasegawa 1970; Huebner and Anderson 1972). Landim da Cruz (1985) studied parietal and perivisceral fat bodies of queen bees during oviposition and observed that parietal fat bodies had lipid deposits, as well as some β -glycogen during oogenesis and oviposition which were mobilized periodically as oviposition took place. The trophocytes showed well developed Golgi apparatus and granular endoplasmic reticulum. Similar events were not observed in the perivisceral fat bodies at the time, which therefore indicated that parietal fat bodies were involved in vitellogenin synthesis. Most insect eggs have high amounts of lipids (mainly triacylglycerides) which provide energy for the developing embryo as well as a source of phospholipids for membrane synthesis.

Honey bees have meristic (polytrophic) ovarioles. Egg development starts at the terminal filament end of each ovariole. The process is initiated when primordial sex cells develop into oogonia (Chapman 1982). In the germarium, oogonia start the maturation process as they move down the ovarioles toward the oviduct. At this stage meiotic divisions take place and differentiation in the cells to oocytes and trophocytes (nurse cells), as well as follicle cells, take place. The oocytes with their trophocytes remain in contact but orient in the ovarioles such that the oocytes are moving toward the oviducts in relation to their trophocytes. The follicle cells form a layer around each oocyte except at

the point of contact with the trophocytes, a site which later becomes the micropyle (Dade 1977). From the germarium, maturing oocytes accompanied by their trophocytes move into the vitellarium where the process of vitellogenesis takes place. Trophocytes absorb nutrients from the hemolymph through the ovarian wall as they increase in size, before incorporating the nutrients into the oocytes. As vitellogenesis continues the oocytes increase in size while trophocytes reduce in size and number, until the last trophocytes are absorbed by their respective oocytes. The follicle cells degenerate leaving the chorion (Snodgrass 1956, Dade 1977). The eggs are finally ready for oviposition in the order of maturation. Delays in oviposition or resorption can occur in some insects (Chapman 1982), but this has not been documented in honey bees.

Oviposition and Environmental Variables

Egg laying behaviors in queen bees and those in laying workers are quite different. Even though individual variations are observed, queens start laying individual eggs in each cell at the center of a comb and move toward the outside, with each egg attached (glued) to the base of a cell. They move in circles and work their way methodically followed by a group of workers (retinue). The queens lay fertilized eggs in hexagonal worker cells of 5 mm in diameter, or in cups which are expanded into thimble shaped queen cells, approximately 10 mm in diameter and 20 mm long. Workers can also modify worker cells to queen cells in emergency queen rearing. The unfertilized eggs are placed in hexagonal drone cells of 6 mm in diameter. Laying workers are less orderly. They lay more than one egg in a hexagonal cell, and will move from the center or lower parts of the comb upward with each laying worker 'guarding' her cell, as depicted by the general reluctance to move out of the cells during comb inspection and egg sampling (Lucas, personal observation). They place eggs in any available cell, often scrambling for space. Page and Robinson (1994) studied competition for reproduction among workers in queenless colonies and concluded that conflicts occur among subfamilies as well as individuals. Since the workers are not mated, any eggs that hatch usually become drones (DeGrandi-Hoffman and Martin 1993, Caron 1995, Mangum 1996). DeGrandi-Hoffman et al. (1998) observed that a small number may become females.

Queens are methodical in ovipositing. They first scrutinize each cell by dropping their antennae inside it, possibly to gauge its size and cleanliness, then they turn and place the tips of their abdomen into the cell, pushing up to 3/4 of the abdomen into the cell before depositing eggs (Koeniger 1970). Mangum (1996) photographed egg laying as performed by laying workers, and observed that they are not as efficient as the queens in depositing eggs in the cells. Their wings often get entangled on the edge of the cells as they struggle to move their abdomens into the cells. During egg oviposition, laying workers are literally lying full length inside the cells.

Sander et al. (1985) described an insect egg as a “self-sustaining developmental system capable of functioning autonomously within a wide range of environmental conditions”. Consequently, it is possible to infer that an egg serves as a transmitter of genetic information vital for the production of an organism’s features, a provider of nutrients necessary to produce morphological structures, and a protector of the developing embryo from environmental hazards. Furthermore, efficient, accurate development, and safety of developing embryos are the primary functions of eggs.

Temperature is an important environmental factor for the survival of living organisms (Hoffmann 1985). Honey bees are poikilotherms, and workers utilize energy generated from body metabolism to regulate hive temperatures (Seeley 1982, Heinrich 1996). Even though honey bees endeavor to maintain temperatures in the brood area at $34.5^{\circ}\text{C} \pm 2^{\circ}\text{C}$, significant fluctuations take place between the core and peripheral regions of the cluster formed by the workers (Owens 1971, Seeley 1985, Southwick 1988). In temperate climates where winter and fall temperatures vary over short periods, wide temperature differences may be realized. Seeley (1985) recorded a 60°C difference between brood chamber and ambient temperatures. Such changes may significantly affect the energy budget of a colony and the ability of workers to maintain hive temperatures which may in turn affect the duration of brood development, the survival of developing embryos, and colony population. Schnetter (1934) found that less than 50% of honey bee eggs incubated at $29\text{-}30^{\circ}\text{C}$ developed, and malformations were common in the bees that developed, as opposed to 35°C which constantly prevails in the hives. These low temperatures, if present in a hive, will affect the strength of a colony.

Other environmental parameters affecting embryonic development include humidity and photo-period. Doull (1976) reported no egg hatch in honey bees at 35⁰C and 50% relative humidity (RH) but eggs hatched normally at the same temperature at 80-100% RH. Chapman (1982) described the effects of temperature and relative humidity, indicating that low RH of $\leq 30\%$ at 20⁰C will slow development in *Ptinus* while high humidity ($\leq 90\%$) at the same temperature reduces development period by five days. Low relative humidity causes water loss in eggs. Du Praw (1967) reported weight loss in honey bee eggs of up to 30% during incubation, mainly due to water loss. Naturally the relative humidity is kept above 60% in the broodnest, which protects eggs from desiccation. The effects of light as an environmental variable may be different for different species. The choice of nesting sites determines the amount of light falling on the hive. Sites with small openings are chosen to facilitate guarding of a hive. Colonies living inside box-type hives are in dark environments at all times, while honey bee colonies under large tree branches may be exposed to varying amounts of light and darkness. The overall effect of light on the development of honey bee eggs may be minimal compared to other insect eggs.

Embryogenesis and Post-Embryonic Development

Sex determination in honey bees, as in all Hymenoptera, is by haploid-diploid system. A naturally mated or artificially inseminated queen lays fertilized eggs as well as unfertilized eggs depending on the needs of a colony. The queen has a regulatory cue for deposition of eggs in suitable cells. Fertilized eggs develop into queens if reared in queen cells or workers if placed in worker cells. Drones are produced from unfertilized eggs in drone cells. The process of embryogenesis begins soon after fertilization and egg activation. It has been argued since Warburg first proposed in 1911, using sea urchin eggs, that the entry of sperm into the egg increases oxygen consumption and subsequently leads to egg initiation. But this idea was set aside when it was found out that not all eggs behaved in the same way after fertilization took place. But whatever may be involved, fertilization plays a major role in triggering embryogenesis. In the fertilized egg, sperm entry starts a series of biological activities in which the nucleus of the male gamete unites with the nucleus of the female gamete to produce the full chromosomal complement.

After fusion several nuclear divisions take place to produce energids. These migrate from the cytoplasm to the periplasm forming the blastoderms. This is followed by formation of blastula, as a conglomeration of cells gathered at one pole called the germ anlage becomes a point at which the center of embryonic division, enlargement, and differentiation takes place. Gastrulation (also known as cavity formation and the initiation of organogenesis) starts followed by embryonic growth together with an increase in fluid pressure which assists in splitting the chorions thus propelling the insect larvae from eggs. The chorions in honey bee eggs do not split open, but dissolve enzymatically to release the larvae (Du Praw 1961, 1967). In unfertilized eggs, however, events which trigger parthenogenesis are generally unknown. Several hypotheses have been proposed to explain the initiation procedures in parthenogenesis, including the pressure exerted by the ovipositor during egg laying (Hepburn 1985). Once the egg initiation process has occurred, the developmental patterns in unfertilized eggs are the same as those described in the fertilized eggs.

During larval development, the queen larvae are fed on large amounts of royal jelly at more frequent intervals than worker larvae. They are both fed on royal jelly during the first 2.5 days of their larval development. After that period, worker larvae are fed on worker jelly up to pupation. Queen larvae continue to feed on royal jelly. They are also fed differently, as outlined above, during adult life.

The virgin queens have spherical and functional spermathecae compared to rudimentary, non-developed, non-functional spermathecae in the workers. After mating the ovaries in the queens attain full size while those in the workers remain small and grossly undeveloped since they are not mated (Winston 1987). Such phenomenal changes raise a number of interesting questions. Does mating trigger a developmental process that further makes the queen's reproductive system different from that of the worker? Are queens typically workers transformed by the quality and quantity of food they consume, coupled with the differential development of the reproductive system, or are workers simply queens with degenerate body parts? Is the difference between the queen and the worker an evolutionary event or a form of division of labor designed to enhance reproduction and overcome the problems of predation that may have effected the survival of honey bees? Or can the phenomenon be an "on-going" evolutionary activity?

Biochemistry of nutrients in honey bee eggs

The fate of nutrients stored in the eggs of honey bees is determined by the genetic information inherent in the egg at oogenesis. During oogenesis the nutrients required for storage in the oocytes are mobilized from the hemolymph or the midgut or the fat body through hydrolysis by enzymes to simpler molecules suitable for absorption and incorporation (Beenackers 1983, Steele 1983). The genetic “blueprint” will guide the patterns of biosynthetic processes (Postlethwait and Giogi 1985), but the interaction between the genotypic characters and the environment influences the phenotypic manifestations in an individual.

Eggs of most insects have glycogen as the main source of carbohydrates, since they are incorporated in the eggs at the end of oogenesis (Yamashita and Hasegawa 1970, Huebner and Anderson 1972). Glycogen is known to predominate in the body tissues of honey bees, although a large amount also exists in the fat body. Trehalose is a predominant blood sugar in insect hemolymph (Mullins 1985), but is also synthesized in the fat body. Woodring et al. (1993) established a high hemolymph concentration of trehalose, glucose and fructose in honey bees. A metabolic homeostasis exists between glycogen and trehalose in insect eggs.

Large amounts of lipids have been reported in insect eggs (Chino et al. 1977, Beenackers 1983). Lipids are hydrolyzed through activities of lipase. The polymeric forms of lipids like triacylglycerol, phospholipids and glycosylglycerides are hydrolyzed into the monomeric fatty acids and glycerols (Turunen and Crailsheim 1996). Proteins occur as yolk proteins (Huebner and Anderson 1972), which are incorporated into the eggs as vitellogens.

The biochemical processes that occur in the eggs of honey bees may be those involved in the intermediate metabolism of nutrients. Chippendale (1978) argued that digestive processes in insects are similar to those found in the vertebrates. Turunen and Crailsheim (1996) showed the fate of glucose molecules after crossing the gut wall. Most of it is converted to glycogen and released to the hemolymph and tissues in general. Some glucose is immediately converted to fructose, sucrose and trehalose before being released into the hemolymph. Some may be converted to fat, glycogen and trehalose for storage in

the fat body. The fat body is therefore a reservoir for lipids, carbohydrates (glycogen) and proteins (Southwick 1992). Enzyme systems exist in the fat body and other tissues to hydrolyze these food reserves as required by the body. Since the level of development in the reproductive system of the queen differs from that of a laying worker, it is therefore of interest to measure respiration rates and determine changes in the levels of nutrients in honey bee eggs during embryonic development, in order to find out if differences exist between queen-laid and worker-laid eggs.