Discussion
DISCUSSION

The resemblance of hoverflies to wasps and bees led to a queer belief that persisted from the dawn of history right up to the 17th century. People believed that a swarm of honeybees could be engendered by leaving the carcase of a large animal to rot. An ox was usually recommended and in the Samson legend it was a lion. The truth is that flies Eristalis breed on a decaying, liquefying carcase and after a time large number of these bee-like flies emerge from it. Another classic example of this occurred when aphids that were threatening the Long Island (U.S.A.) potato crop were brought under control largely by syrphids. At that time many people complained of the inordinate number of 'wasps' that filled the region not knowing that these were saving the potato crop. These extremely interesting flies thus mimic bees and wasps and match with them in behaviour in many ways.

Behavioural studies

Many insects search mates near resources exploited by
members of the opposite sex (Parker, 1970, 1978). Syrphid flies are one group which utilizes resources (e.g., flowers) as sites for sexual rendezvous. Male syrphids exhibit many different mate-seeking behaviors including patrolling blossoms, waiting on foliage of flowering plants or near oviposition sites and hovering near flowers and other resources exploited by females (Gruhl, 1924; Collet and Land, 1975, a; Maier, 1978; Maier and Waldbauer, 1979; Sareen et al., 1986).

Patrolling, often alternated with waiting on foliage in all the species studied. This has also been observed in two major syrphid subfamilies, the milesiinae and syrphinae (Collet and Land, 1975, a). Males mate with females that visit blossoms to feed on pollen or nectar, the former a requisite for ovarian development (Schneider, 1948; Maier, 1978). Parmenter (1944) hypothesized that patrolling males of *Syritta pipiens* were territorial because they chased conspecific males and other insects away from their immediate vicinity at flowers. Nielsen (1966) reported that *Helophilus* sp. defended sunning spots, but it is unknown if this territorial behavior is related to mating.

During the present studies males have been observed to hover singly or in groups and pursue passing insects. If they do not
encounter a receptive female during a chase, they return to their original hovering spot. Hovering or aerial station-taking is also found in other species of *Eristalis* (Collet and Land, 1975a), *Vollucella* (Blickle, 1959), *Melanostoma* (Grul, 1924) and *Syphus* (Heinrich and Pantle, 1975) but it is known to be widespread in syrphinae. Some syrphids have been reported taking waiting stations on summits apparently to mate with females (Chapman, 1954).

The aggressive interactions between males of *Maliota posticata* and/or *Spilomyia decora* indicated that they compete not only for mating territories near rot cavities but also for patrol routes (floating territories) at flowers (Maier and Waldbauer, 1979). During the present studies rapid interactions were seen in all the species; thus, with unaided eye, it was difficult to ascertain whether or not males simply attempted to mate with all conspecific flies which enter their immediate vicinity. But they have definitely been observed to drive away competing males (and unreceptive females) while hovering and thereby have the opportunity to mate with receptive females entering their immediate area.

Interactions between males of syrphids and butterfly, *Pararge aegeria* are examples of asymmetric contests (e.g., contests in which participants receive unequal payoffs or have differences in fighting ability; Maier and Waldbauer, 1979), also discussed
in detail by Maynard Smith and Parker (1976). Findings of Davies (1978) and the present ones apparently support the theoretical predictions of Maynard Smith and Parker (1976); that is, the evolutionary stable strategy in asymmetric contests is to permit the asymmetric cue to settle the contest quickly.

In cyclorrhaphous Diptera, females that mate repeatedly usually have the majority of eggs fertilized by spermatozoa obtained in their last mating (Boorman and Parker, 1976). Males, therefore, gain a distinct selective advantage by mating last with females about to lay eggs (Parker, 1970). According to Maier and Waldbauer (1979), if this type of spermatozoa competition occurs in syrphids and if females are receptive most of the time, then males that hold territories of high quality near rot cavities should usually mate last with gravid females and thereby have a higher fitness than other males.

Dual mate-seeking strategies also exist in male bees e.g., in the anthophorid Centris pallida (Alcock, 1976; Alcock et al., 1977) and in the megachilid Osmia rufa (Raw, 1976). Territoriality has been documented in many bee species and most bee families (Cazier and Linsley, 1963; Barrows, 1976; Eickwort, 1977). Parallels in bee and syrphid ecology may largely be responsible for the evolution of dual mate-seeking strategies, particularly territorial
behaviour in these taxonomically distant groups. Both bees and syrphids exploit two well-defined resources, flowers for pollen and nectar and sites for larval developments. Since females have to utilize these resource areas, males that search or wait there probably have increased mating success.

Periodic activity, a characteristic of most natural populations, is a behavioural adaptation that can reinforce or maintain reproductive isolation and can reduce competition, hygrothermal stress or predation pressure (Mitchell and Epling, 1951; Hardeland, 1972; Cloudsley-Thompson, 1975). Activity rhythms of economically, genetically and medically important Diptera have been extensively studied in nature. Notably, many species of Nematocera have crepuscular biting, egg-laying, flying and swarming activity (Nielsen and Greve, 1950; Haddow, 1961; Bidlingmayer, 1974). Many Drosophilae, especially desert inhabiting species, are crepuscular, an apparent behavioural adaptation to avoid hygrothermal stress (Pavan et al., 1950; Mitchell and Epling, 1951; Dyson-Hudson, 1956). Diurnal activity, however, is the norm for most higher diptera inhabiting temperate regions.

Most syrphids as also observed during present investigations feed on blossoms in the morning and early afternoon (Sareen et al., 1986), but Volucella vesicularis is crepuscular, visiting
Cephalanthus occidentalis flowers shortly before and after sunrise and sunset (Waldbauer, 1963). Kikuchi (1963, 1965, a), Nielsen (1966) and Schneider (1958) discussed the effect of various meteorological factors on syrphid activity but during present studies important biotic variables like availability of pollen or nectar and colour of the flowers were also taken into consideration which strongly influence their activities. The ubiquitous syrphid, Eristalis tenax has been reported to have a single peak in oviposition activity during moderate weather but also has two peaks in hot weather (Campan, 1973), in the latter case, a midafternoon lull in egg-laying corresponds to the hottest part of the day. Such behavioural plasticity allows flies to avoid times of severe stress not only when ovipositing but also when engaged in other activities.

The species studied were observed to arrive at flowers early morning near the beginning of pollen dehiscence. As a result, they can feed on pollen and perhaps also nectar before most other foragers can arrive and deplete these resources. On certain mornings they were seen to fly before the air temperature was high enough to heat their flight muscles to an efficient operating temperature. The dronefly, E. tenax visits blossoms when the air temperature is only 4\(^\circ\) C - 10\(^\circ\) C but not until the radiant temperature is above 15\(^\circ\) C (Kato, 1943). Syrphus adults
elevate their thoracic temperature more than 10°C above ambient by basking and shivering and therefore, are able to fly at low temperature (Heinrich and Pantle, 1975). Behavioural thermoregulation, commonly observed in syrphids, or physiological thermoregulation may account for their early appearance at flowers, particularly on cool mornings.

High temperature, either acting in concert with humidity or alone, limits syrphid activity at some point. Present investigations and those by Kikuchi (1965), Nielsen (1966) and Maier and Waldbauer (1979 a) suggest that flies leave flowers and seek shade when the air temperature becomes high near mid day. This adaptive behaviour allows flies to avoid excessive heating and water loss. But it has also been reported by Maier and Waldbauer (1979 a) that Mallota bautias and M. posticata adults persist at flowers even in the afternoon which may be attributed to both their adaptive behaviour and morphology. They have been known to fly less and avoid direct sunlight in the hot afternoon. They also have the lowest surface : volume ratio (i.e., they are more robust) and the greatest pilosity. Pilosity and robustness are common morphological adaptations of organisms occupying extreme environments such as deserts and Arctic areas (Sladen, 1919; Downes, 1965; Hadley, 1972). A smaller surface : volume ratio can reduce water loss and decrease heat flow between an organism and the environment or vice versa (Church, 1960; Hadley, 1972). Hairs also
reduce the amount of radiant energy reaching the body surface and therefore, delay heating.

Hygrothermal conditions near flowering plants improve slightly near the end of the day, but the syrphids investigated here do not exhibit another burst of activity at flowers. This is surely due to the absence of pollen and perhaps nectar. Syrphids may leave flowers in open fields and enter shady areas or forests in response to changes in light intensity, temperature or humidity. Dolley and Golden (1947) reported that *E. tenax* is photosensitive from $10^\circ - 30^\circ$ C but photonegative outside those limits. Similarly, when the temperature reached $30^\circ$ C, the muscid *Glossina moritans* moved from lighted to the dark side of a container (Jack and Williams, 1937). Furthermore, *Drosophila subobscura* which is usually crepuscular in forest-edge or field habitats, oriented to dark areas and restricted its locomotor activity when exposed to bright colour or high temperature (Koch, 1967). These studies supporting the present investigations also indicate that highly adaptive avoidance responses, triggered by increased temperature or light intensity, may be widespread in Diptera.

The present evidence suggests that flowers such as *Chrysanthemum* species, *Iberis amara*, *Brassica campestris*, *Copeopsis tinctoria* and *Helichrysum bractiatum* have coevolved with syrphids (Knuth, 1906) and Eyde and Morgon (1973) recognized that certain
flowers have adaptations to facilitate pollination by syrphids. The large aggregated flowers of *Iberis* and other perennial shrubs frequented by large syrphids, produce copious qualities of pollen, a prerequisite for rapid ovarian development (Svensson and Janson, 1984) in the four species studied and probably in most other syrphid species (Schneider, 1948; Maier, 1978). The temporal synchrony between pollen dehiscence and syrphid activity at flowers further strengthens this fly-flower relationship.

The present observations suggest that the effectiveness of syrphids as pollen vectors ultimately depends not only on flower morphology but also on the morphological and behavioural capability of flies to pick up pollen and transfer it to receptive stigmas. Syrphids can definitely be considered second to bees in pollination. Syrphids visit and often pollinate numerous entomophilous flowers (Knuth, 1906, 1908, 1909; Robertson, 1928) as well as some anemophilous flowers (Goot et al., 1970; Leereveld *et al.*, 1976; Stelleman and Keeuse, 1975).

Many syrphids have also been studied for their migratory nature (Heydeman, 1967; Nielsen, 1968; Gatter, 1975; Aubert and Goeldlin de Tiefenan, 1981; Svensson and Janzon, 1984). In favour of the present investigations even Svensson and Janzon (1984) have also reported that low pollen content in females indicates migration phase, as females have to feed on pollen for development of their ovaries.
Histomorphological studies

In diptera the ovariole is known to be of polytrophic meroistic type. Henning (1973) recognized two types of ovaries which are different in the manner of attachment of ovarioles to the lateral oviducts. The ovary of the first type consists of many ovarioles that are attached like a bunch of grapes (traubenformige ovarien) as found in species studied and in the second type ovarioles are attached to the cup shaped anterior ends of the oviducts (buschelformige ovarien) which has been reported only in Cyclorrhapha.

Highly variable numbers of polytrophic ovarioles form an ovary in diptera. The number of ovarioles ranged from 110 to 152 in the species studied. It has been reported that there is a tendency for the number of ovarioles per ovary to be greater in lower diptera than in higher diptera (Matsuda, 1976). Among higher diptera, Musca autumnalis (Valder, 1969) the number varies from 5 to 15 while in lower diptera number can go even upto 400 to 500 (Christophers, 1945).

Polytrophic ovarioles are characterized by follicles consisting of one oocyte together with a well defined number of trophocytes or nurse-cells, the whole complex being surrounded by a sheath of follicle-cells (Rockstein,
The egg follicles in the flies selected for the present studies belonging to subfamily Eristalinae, consists of a single oocyte and seven trophocytes enclosed in the follicular epithelium. The number of trophocytes in the dipteran egg varies from species to species (Koch and King, 1966; Klug et al., 1968; Cumminns and King, 1969; Dapples and King, 1970; King, 1970).

The development of egg follicles in the present species has been divided into 15 stages. Previtellogenesis occurs upto stage 5 while vitellogenesis from stage 6 onwards. The follicle cells undergo a series of mitotic divisions resulting in the formation of cystocytes enclosed by follicular epithelium. One of the cystocyte becomes oocyte and others trophocytes. The oocytes and trophocytes have been observed to be interconnected by intercellular canals which pick up Hg-BPB stain. In accordance with the present observations Koch and King (1966, 1969), Koch et al. (1967) reported that these are protein-rich ring canals which allow the transport of RNA (ribosomes) and mitochondria to the oocyte during vitellogenesis. The cystocytes have also been reported to be interconnected by intercellular pores (Verma and Ishikawa, 1984).

During previtellogenesis the nuclei of the trophocytes have been seen to undergo change in size and DNA contents. The
size of the trophocyte nuclei and DNA contents reach maximum level at stage 10. In the germinal vesicle the entire chromosomal compliment has been observed in the diffused form throughout suggesting the long period of prophase.

The germinal vesicle has a highly basophil nucleolus and it has been seen to bud off nucleolar extrusions in *E. tenax*. The trophocyte nucleoli release basophil granules in the trophocyte cytoplasm which has also been reported by Nath (1968) in polytrophic ovary. Electrophoretic and autoradiographic analysis have also revealed that nurse cells supply the oocyte through intercellular bridges with RNA including ribosomal transfer and other kinds of RNA's (Kawaguchi and Fujii, 1983). According to ultrastructural observations of Yamauchi et al. (1981) the cytoplasmic organelles found in the nurse cells and in the lamina of the intercellular bridges vary in kind and quantity during stages 1–8. Cytoplasmic transportation from the nurse cells to the oocyte has been reported to terminate at stage 9 in polytrophic ovary of *Bombax mori* because of the collapse of the nurse cells and the closure of the intercellular bridges (Yamauchi and Yoshitake, 1984).

The increase in DNA contents of trophocyte nucleus observed in all the species is due to endomitotic divisions which has also been reported by Telfer (1975) that nurse-cell nuclei in polytrophic meroistic ovarioles undergo asynchronus endomitosis,
producing endopolyploidy. With the advancement of oogenesis the nurse cells are noticed for the first time at stage 6 to be actively involved in the synthesis of glycogen granules which flow into the oocyte through intercellular bridges, the rounding-up and increased volume of nuclei at stage 7 and the nuclear breakdown at stage 8. It seems that these graded differences are related to the asynchrony of polyploidization in the seven nurse cells. Thus, the nurse cells strongly support oocyte development by contributing cytoplasmic components. These observations are strongly supported by Yamauchi et al. (1961) and Yamauchi and Yoshitake (1964).

During stage 4, the trophocyte primary nucleolus multiplies into secondary nucleoli which are rich in proteins and RNA. The ribonucleoprotein granules pass on to the trophocyte cytoplasm through the nuclear membrane, later they pass to ooplasm where they pick up same dark stain (toluidine blue). This has been reported by various workers in the polytrophic ovary (Sharma, 1968; Cummings and King, 1969; Krzystofowicz, 1971). Bier (1963, a) clearly demonstrated that in polytrophic ovarioles a directed flow of RNA proceeds from the anterior nurse cells through the posterior one into the oocyte, as the injected uridine(\(^3\)H) was first observed in the nuclei of nurse cells, subsequently in their cytoplasm and
later in a distinct stream entering into the oocyte while
nurse cells became emptied.

Electron microscopic studies reveal that RNA was delivered
from the trophocytes to the oocytes as ribosomes. Oocyte
receiving ribosomes are described in *Drosophila* (Dapples and
King, 1970) and *Bombyx* (Miya et al., 1969, 1972; Yamauchi and
Yoshitake, 1984).

The trophocytes are the major source of proteins and
carbohydrates in the species studied which has also been reported
by other workers (de Loof and de Wilde, 1970; Favard-Sereno,
1971). Proteid yolk bodies are PAS-reactive and salivary amylase
resistent, suggesting incorporation of carbohydrates in glyco-
proteids on mucoproteids. It has been accounted that the
vitellogenic female protein, the most important proteid yolk
precursor in the hemolymph, is also a glycoproteid in Colorado
beetle (de Loof and de Wilde, 1970) and in *Gryllus* (Kunz and

During vitellogenesis the follicular epithelial cells are
very active synthetically, they help in yolk synthesis, form
vitelline membrane, endochorion, exochorion and micropylar
apparatus (Cummings and King, 1969; Hinton, 1981; Junquera,
1983). Upon descending into the vitellarium, the follicle cells
increase in number by mitotic divisions and have been observed to form monolayer in all species studied. Their number seems to be stationary but steady increase in the volume of the oocyte results in a tangential stretch of the follicle cells. Consequently, these cells are first seen to be columnar, then cuboidal and at last flattened, i.e., squamosal. The major contribution of the follicle cells have been observed in the formation of the vitelline membrane which appears in stage 10 and the chorion during the present studies. Matsuzaki (1968) reported through electron microscopic studies that follicle cell cytoplasm is characterized by markedly developed r-ER, Golgi bodies and secretory vesicles. While it has been reported by Yamauchi and Yoshitake (1984) that as the development of the follicle proceeds, the follicle cells lay down an electron-lucent homogeneous layer, a trabecular layer and a lamella composed of successive stacks of helicoidally arranged fibrils, so chorion deposition starts at the anterior end of the follicles and proceeds laterally and posteriorly.

4 to 8 border cells migrate at the beginning of stage 8, from the anterior pole of the egg chamber, between nurse cells to the oocyte surface when they later function in the synthesis of the micropylar apparatus. It has been reported that in
Diptera, the micropyle is often complex and consists of an endomicropyle penetrating the vitelline membrane (King, 1964).

The structure of vitelline membrane and the chorion is such that respiration remains possible while water evaporation is kept to a minimum. Much debate has been raised around the question whether the oocyte itself or the follicle cells secrete the vitelline membrane. Ultrastructural investigations in Gryllus (Favard-Sereno, 1971), Leptinotarsa (de Loof, 1971), Drosophila (King, 1964, Quatropiani and Anderson, 1969) clearly demonstrated that the follicle cells synthesize the precursor material for the vitelline membrane. The precursor material is known to be synthesized in the Golgi complexes and is a polysaccharide-protein complex, as observed in present studies (Favard-Sereno, 1971).

However, contrary to the present findings, Geerity et al. (1967) has reported that vitelline membrane is synthesized by the oocyte. The chorionic layers, inner and outer, are synthesized by the follicle cells (de Loof, 1971; Rockstein, 1973).

Favard-Sereno (1971) found that the precursors of the two egg envelopes are synthesized within the follicle cells through three successive secretory cycles and that at last they are released in the intercellular space between follicle cells and oocyte, which strongly supports the present observations.
According to Rockstein (1973) the activity of the follicle cells is undoubtedly one of the most remarkable and complicated features of oogenesis. If the vast variety of forms and structures of insect eggs, adapted to a great many oviposition media and substrates, their micropylar apparatus, ridges and caps, their finely sculptured outer surface, it is difficult to realize that this is accomplished by one layer of cells.

Light and transmission electron microscopic studies have shown that the micropylar channels extend from the external gateway through the chorion and end in the vitelline membrane (Salked, 1973; Chaurin et al., 1974). As reported by Miya (1978) Margaritis et al. (1980) regional modifications of the vitelline membrane and chorion are particularly conspicuous at the micropylar region.

Follicle cells have also been reported to synthesize yolk sphere components in some insect species (Ono et al., 1975; Irie and Yamashita, 1983). It has also been shown that the follicle cells synthesize proteins which are incorporated into the yolk proteins of the oocyte during vitellogenesis (Anderson and Telfer, 1969; Chia and Morrison, 1972; Brennan et al., 1982). In addition, the follicular epithelium seems to play a role in the incorporation of hemolymph proteins in the oocyte (Abu-Hakima and Davey, 1977; Kessel and Ganion, 1979; Rubenstein, 1979; Huebner and
Injeyan, 1980; 1981; Koeppe et al., 1980). However, no direct evidence was found for this function of follicular epithelium during the present studies.

Studies by means of the transmission and Scanning electron microscope have shown architectural features in the eggshell in all the species studied. According to Hinton (1981), the crucial problem for eggs laid in the dry environments is to minimize water evaporation and at the same time allow the free exchange of $O_2$ and $CO_2$. Specialized area of the chorion are aeropylar, hydropylar, micropylar structures and the shell has been observed to have layers of fine meshwork with different patterns in the flies studied, hexagonal in *E. arvorum*, polygonal in *E. lataeus* and pentagonal in case of *E. quinquilineatus* and *E. tenax* which are endochorionic spaces and probably filled with gas. In dipteran eggs, the whole surface or a restricted area functions as a plastron, 'a gas layer of constant volume and extensive water-air interface' (Hinton, 1981). In the flies studied during the present investigation, whole surface of the egg was covered by plastron as observed with scanning electron microscopy. According to Hinton (1960) the structure of the shell is similar in two species of Eristaliinae; *Helophilus pendulus* and *Eristalis intricaria* and consists of a continuous inner sheet of chorion and outer sheet is broken up into many small 'islands' or ridges of meshwork.
Only the plastron of *Rhingia campestris* (Syrphiidae) has been tested in water in a pressure chamber by Hinton (1960). At an excess pressure of 20 cm Hg (72.8 dyn/cm; 18°C) the plastron was not wetted in 6 hours. When small drops of ethanol or oil are applied to plastron, meshwork are wetted and clearly visible. Similar meshwork has been observed in species *E. latexus* and *E. ovatopunctatovus*.

As evident by SEM trachea are seen penetrating the spermatheca and are primarily concerned with maintaining the adequate respiratory exchange for the epithelial wall. Efficient gaseous exchange would be important to wall integrity since it would support maximum life to the cell in this tissue where mitotic cell replacement does not appear to occur perhaps leading to furrows and ridges on the surface of mature spermatheca. Although tracheoles are seen to penetrate cuticle of spermatheca of all the flies investigated, there are no direct respiratory channels connecting the spermathecal lumen with the tracheal network outside the wall which strongly suggests that under the afore-mentioned conditions respiratory supply to spermatozoa within the spermathecae would be of secondary importance and limited to that quantity which could diffuse across dense wall (Poole, 1970). Similarly, Koeniger's (1969) results can be reinterpreted as suggesting that removal of the tracheae altered the integrity of the spermathecal wall and thus produced, indirectly...
a decline in the fertilizing capacity of the spermatozoa contained within.

There is no demarcation of secretory cells and collecting cells. The lumen is not lined by microvilli as also described by Grodner (1976) after mating, the spermatozoa swim up the spermathecal duct in response to the secretions of the spermathecal gland (Grodner and Steffens, 1978). These support present observations where young virgin females have secretions filling the lumen of the spermatheca. In mature flies the spermatozoa were observed in the spermathecal duct and are stored in the spermathecal gland which functions to maintain them. In mature flies the spermatozoa were observed in the spermathecal duct (Suzzoni, 1972; Filosi and Perotti, 1975; Borisova, 1981, 1982, 1985). Villavaso (1975) has shown that after the spermathecal gland is removed from older females, spermathecal filling occurred but the spermatozoa gradually lost their motility and fertilizing capacity and spermathecal emptying did not occur, demonstrating the necessity of the secretions for their maintenance. These observations indicate that either the young females had not stored enough secretions within the spermatheca for spermatozoa to respond or the spermathecal gland secretions of the young females was not same as found in older females.
The secretions of spermathecal gland is glycoproteinous and aids in the nutrition of spermatozoa to induce their motility, whereas Sareen and Sood (1985) reported that secretions of spermathecal gland in Caryedon serratus is lipoidal and glycoproteinous. Similar observations have been made by Monga (1972) and Thukral (1976).

The secretions of the female accessory glands are mainly proteinous in the flies investigated. They contain only traces of 1:2 glycol groups and glycogen. It is, therefore, concluded from the observations that these glands have only minor or supplementary function in storing the spermatozoa in spermathecae but they might be helping to form the glue for the attachment of the eggs to their substratum because the glands were empty after oviposition, their secretions being utilized in covering the eggs. The accessory glands are highly branched structures and all the gland cells are of one type. Each gland cell acts as a unit gland and the presence of large reservoir in the gland cells is its most obvious adaptation to the secretory function and their branched unique structure might be helping to increase the surface area. Similar type of accessory glands but unbranched have also been reported in other dipterans (Sareen et al., 1985)
The efferent ductules are undoubtedly chitinized since they are confluent with the cuticular lining of the gland lumen. According to various workers (Clements and Potter, 1967; Gupta and Smith, 1969). The structural proteins of feltwork and of the tubules is resilin. It has also been reported that efferent ductules pick up the same stain as by chitinous intimal lining of the lumen of the gland of other dipterans (Sareen and Pannu, 1983, Sareen et al., 1985)

The present results are in concurrent with many other reports that accessory glands in female dipterans perform two main functions:

1) Protecting the eggs as visible by their state after oviposition;
2) Nutritive for spermatozoa which have been received by the female after copulation (Nayar, 1965; Chapman, 1972; Sareen and Sood, 1985; Sareen et al., 1985)

**Male Reproductive System**

In all the dipterans, the testis has been known to be a single sperm-tube (Matsuda, 1976). The studies dealing with histology and development of testis (including spermatogenesis) have been reported in various insects, *Glossina* (Itard, 1970);
Musea (Gassner et al., 1972); Drosophila (Lange, 1969, a; Rassmusseu, 1973). The literature available so far on the male reproductive system is by Keuchenius (1913) and Nayar (1965).

Testis can be described in three zones, apical, middle or intermediate and basal zones or terminal zones in subfamily Eristalinae. The testicular wall consists of two layers, the outer pigmented and inner layer with flat cells. These observations are in confirmation with another dipteran, Drosophila (Tokuyasu, et al., 1972).

The spermatogonia are seen to be arising from the stem cell divisions at the tip of the testis. According to Hannah-Alava (1965) and Tokuyasu et al. (1972, a), spermatogonia undergo four synchronous mitotic divisions, resulting in 16 primary spermatocytes which enter meiosis in the middle zone of the testis.

Meyer (1961) described the intercellular bridges between spermatogonia or spermatocytes in D. melanogaster. Such intercellular bridges were observed only in D. arvorum. According to King and Akai (1971) the inter-spermatocyte bridges are due to incomplete cleavages of the mitotic divisions preceding meiosis. The subsequent differentiation of spermatids into flagellated spermatozoa takes place in the
basal part of the follicle in all the four species investigated. According to Rokstein (1973) it involves the concentration of nuclear material in the head of the spermatozoa and the characteristic distribution of spermioplasm to form the acroblast.

The morphology of insect male accessory glands is known to differ from species to species (Chen, 1984). In spite of the variations, one common feature is that the ducts of the glands, together with vasa deferentia, open into the ampullary part of the ejaculatory duct also observed in the present investigations. During copulation the spermatozoa stored in the seminal vesicle, a dilated portion of the vas deferens, enter the ejaculatory duct where they are mixed with the glandular secretions and transferred to the female genital canal. In many insects their main function is the production of spermatophore which is absent in subfamily Eristalinae, the glands may fulfill a variety of functions (Hinton, 1974; Bernt and Pruss, 1979). In Drosophila it has been suggested that the accessory secretory granules may serve to assist sperm mobility in the female genital canal, as energy substrate to maintain sperm activity and to support sperm penetration into the egg (Bairati, 1968). As vacuolizations has been observed during the present studies in these glands it can be postulated that the cells are of holocrine type. In Drosophila funebris, Federer and Chen (1982) reported the presence of two
types of secretory cells. According to Perotti (1971) the main cells are of merocrine type whereas secondary cells exhibit holocrine secretions which strongly support the present observations. In addition to Drosophila, information is available on the reproductive functions of the male accessory glands in several other dipterans. In mosquitoes the gland secretions stimulate oviposition and induce monogamy (Leahy and Craig, 1965; Chen et al., 1977).

**Biochemical Studies**

It is apparent that number of factors such as pre-imaginal and adult nutrition, genetic background as well as environmental factors such as temperature, photoperiod, humidity and population density of related species can influence ovarian development and total egg production in insects which is well documented during the present studies (Engelmann, 1970).

The development of polytrophic egg chambers may be divided into 3 major phases. In the first phase (stages 1 to 7) the oocyte and nurse cells grow at identical rates, but in the nurse cell nuclei an endomitotic replication of DNA occurs (Cummings et al., 1971). Since the ovary of young females contains only egg-chambers in stage 1 to 7, the level of DNA in the ovaries represents that of previtellogenic ovary in the flies investigated. In the second phase of development (stages 8 to 10)
vitellogenesis begins and the oocyte grows rapidly at the expense of the nurse-cells. The beginning of this phase (stages 7 and 8) is marked by an increase in DNA content per nurse-cell nucleus. According to various workers during this phase some nurse-cell nuclei reduplicate their DNA (Jacob and Sirlin, 1959; Cummings et al., 1971; Hall et al., 1976). From stage 7, the last pre-vitellogenic stage, there is many fold increase in the amount of total DNA in the ovaries which varies seasonally in the species studied during the year.

In the post-vitellogenic stage of development (stages 11 to 14) the endomitotic replication of DNA ceases and the nurse cell nuclei are broken down and sloughed off with the concomitant reduction in the DNA content per egg chamber as evident by cytological studies. Consequently, the amount of DNA per ovary drops off sharply as a number of mature oocytes and post-vitellogenic stages per ovary increase. This is in concurrent with the previous observations of King and Vanoucek, 1960; Muckenthaler and Mahowald, 1966; Cummings and King, 1970).

Ovarian content has been observed to be correlated with the appearance of vitellogenic and post-vitellogenic egg-chambers. Although increase in ovarian RNA is seasonal in the four species it follows the similar trend which is preceded by an increase in DNA content. It is, therefore, seen to be associated with the endomitotic replication of nurse-cell DNA in vitellogenic
chambers. According to Cummings et al. (1971) a minimum number of DNA replication must occur before synthesis of RNA (90% ribosomal) can take place.

Ovarian protein content begins to increase rapidly and vitellogenic stages 8 to 10 show increasing amount of proteins in both nurse cells and oocyte. The nurse cells rapidly incorporate tritiated aminoacids mostly into cytoplasmic proteins (Chia and Morrison, 1972). It has been seen that cytoplasm of the nurse cells stain intensely with alkaline fast green while the nuclei stain only lightly. This staining for basic proteins parallels that for the cytoplasmic RNA in both distribution and intensity. It seems lightly, therefore, that ribosomal proteins constitute a significant fraction of the nurse cell cytoplasm which is subsequently transferred to the oocyte. Consequently, the mature oocyte contains large amounts of protein in the form of yolk spheres and ribosomal components which is concomitant with increase in proteins during vitellogenic and post vitellogenic stages (Nagabhushnam et al., 1983).

Acid and alkaline phosphatases were maximum during the vitellogenic stages and they have been associated with insect development especially in relation to nutrition and egg maturation (Rokstein and Inashima, 1953; Ludwig et al., 1962; RayChaudhuri and Butz, 1965; Nath and Butler, 1971, 1973).

Insect flight muscle contains a limited amount of carbo-
hydrate reserves which can meet the energy requirements of the muscles only during the first few minutes of flight, extra carbohydrates from other sources must, therefore, be mobilized. This carbohydrate is supplied to the muscles via haemolymph, mainly in the form of trehalase - the principle blood sugar in most insect species (Wyatt, 1967; Bailey, 1975). It has also been reported to serve as an indispensible substrate for energy production and for synthesis of macromolecules in many insects throughout their life cycles (Chippendale, 1978; Steele, 1981). In the mature ovary a major yolk protein (vitellin) is organized into yolk granules and easily dissolved at a high concentration of NaCl or KCl (Izumi et al., 1980). That was the reason that isolation medium with NaCl was selected. Seasonal variations in trehalase activity in the four species suggested that there was concomittant increase in trehalase along with glycoproteins. Thus, trehalase could react directly with haemolymph trehalase at the surface of oocyte which supports the idea of previous authors that hydrolysis of trehalose by trehalase at the surface is an obligatory step before the transport of haemolymph carbohydrates into the oocyte can take place (Shimada and Yamashita, 1979; Azuma and Yamashita, 1985).

Fractionation of trehalase by differential centrifugation in E. tenax indicate that ovarian trehalase is associated with
microsomes but not with mitochondria which is in accordance with the observation of Yamashita et al. (1972) in the polytrophic ovary of *Bombyx mori*.

Electron microscopic observations of Akutsu and Yoshitaki, (1977) and Telfer et al. (1982) demonstrated that trehalase is exclusively localized in the oocyte cortex where endoplasmic reticulum and Golgi are present in very small quantities which leads to the proposition that trehalase is situated on the plasma membrane.

**Toxicological Studies**

The perfect pesticide would be one that selectively kills only those organisms which are considered pests. Such compounds are indeed the goal of Scientists and laymen alike, but in some cases, it is the non-target organisms which are most sensitive to insecticides.

The toxicity of carbaryl to honey bees is perhaps the best known example of carbamate adversely affecting a non-target species (Kuhr and Dorough, 1976). The symptoms produced by poisoning insects with carbamate esters are neither as complex nor as dramatic as those evidenced by mammals. However, there exists an unmistakable carbamate intoxication syndrome of hyperactivity, incoordination (ataxia), clonic and tonic con-
vulsions, paralysis and death. The remarkable aspect of insect poisoning is the ability of these animals to withstand extended periods of paralysis without dying. Complete recovery can take place after hours and hours of motionless prostation as observed in both the species investigated. This has been reported to be due to the infusion-type tracheal respiratory system and primitive unstructured circulatory system, both of which are relatively unaffected by AChE inhibition (Kuhr and Dorough, 1976). Presumably, with time, the carbamate is detoxified and/or the acetylcholinesterase is spontaneously reactivated, allowing recovery.

Although the combination of contact and stomach poisoning broadens the scope of possible toxic action of the carbamate insects, stomach poisoning probably plays minor role in efficacy of most of these materials. If applied as contact poisons their rapid action provides little opportunity for susceptible exposed insects to continue feeding in a normal manner. Carbamates have been observed to be highly toxic when topically applied. The period of paralysis was longer with lower doses of carbofuran and carbaryl in E. quinquilineatus than E. tenax. Maximum inhibition of AChE was observed after 6 hours in E. quinquilineatus with 3.5 μg/g and recovery took around 18 hours, while in
E. tenax maximum inhibition of AChE with 3.5 μg/g was after 2 hours of treatment and recovery took only 14 hours. But the symptoms in both the species with the insecticides were seemingly concurrent with AChE inhibition.

The experiments also showed that carbofuran caused greater fly head AChE inhibition with all the doses and showed severe cholinergic symptoms than did carbaryl with minimum to maximum dose.

Carbaryl and pirimicarb when given orally to honey bees did not result in the inhibition of head AChE while when topically applied caused significant inhibition (Westlake et al., 1985). Carbaryl (LD50) when topically applied to house flies caused 60% maximum inhibition and recovery took about 21 hours (Mengle and Casida, 1958).

The appearance, intensity and duration of symptoms are dependent on dose, chemical structure of the toxicant, length of exposure, mode of administration and site of application. There have been repeated failures through the years to relate insect toxicity of carbamate insecticides with in vitro cholinesterase inhibition (Casida et al., 1960; Metcalf et al., 1960; Weiden and Moorefield, 1965; Reay and Lewis, 1966; Green and Dorough, 1976; Singh and Aggarwal, 1982; Pandey and Aggarwal, 1982, a; Westlake et al., 1985).
An elaborate experiment with two species of flies and several carbamate insecticides indicated no consistent relationship between in vivo head cholinesterase inhibition and toxicity (Respicio and Sherman, 1972). However, there was a general trend of increased inhibition with time until paralysis, while in present studies the inhibition of AChE was observed even after paralysis. Most of the methyl- and dimethyl carbamates are known to adversely affect the reproductive potential, food intake and survival rate of the housefly (Georghiou, 1965). The remarkable aspect observed in the ability of these insects to withstand the extended periods of paralysis without dying. Lindane and carbofuran have been seen to affect the central region of the mesothoracic ganglion directly when applied topically and their thoracic ganglion revealed some holes in the neuropile in house flies (Collins et al., 1979).

The extent to which insects can metabolize and thereby degrade toxic or otherwise detrimental chemicals is of considerable importance to their survival in a chemically unfriendly environment while all the insects probably possess detoxicative capacity, the amount can be expected to vary among species with developmental stage and with the nature of the insect's recent environment. Recent studies of detoxication in insects have revealed that further versatility in the adaptation of insects
to their environment is provided by the phenomenon of induction. This is the process in which a chemical stimulus enhances the activity of the detoxication system by the production of additional enzymes. The induction of detoxication systems is not limited to insects and because of equally important implications for drug and xenobiotic metabolism in higher animals, it has received much attention in the medical and environmental health fields (Terriere, 1984). According to Nebert et al. (1981) microsomal enzymes, located in the endoplasmic reticulum of most cells but especially rich in liver cells, insert an atom of oxygen into a variety of functional groups of lipophilic molecules, thereby preparing them for more rapid excretion. This function has been reported to function through the haemoprotein cytochrome P-450, which forms a tertiary complex with a molecule of the xenobiotic to be oxidized and a molecule of oxygen (Terriere, 1984). On receiving two electrons from another constituent of the system, cytochrome P-450 reductase and/or cytochrome b_{5} reductase, an atom of oxygen is activated in a manner not yet fully understood and is inserted into the xenobiotic molecule. The oxidized xenobiotic is then released alone with a molecule of water and the cytochrome P-450 is free to bind another molecule of the substrate (Hodgson, 1985).

As in the case with mammals and other higher animals, the
Key component of the microsomal oxidase system in insects is cytochrome P-450. The majority of the work on insects has been with the housefly and blow fly (El-Oshar and Dauterman, 1979; Yu, 1982; Glickman et al., 1982).

It has been observed during present studies that both carbamate insecticides significantly increase the microsomal proteins and cytochrome P-450 perhaps to eliminate the toxic metabolite formed by these chemicals. Many insecticides have been shown to induce cytochrome P-450 and associated mono-oxygenase activities in mammals (Hodgson et al., 1980). Induction of cytochrome P-450 by dieldrin and carbaryl on the expression of carcinogenicity has been reported in higher animals (Madhukar and Matsumura, 1979; Tennekes et al., 1981; Triolo et al., 1982).