CHAPTER I

INTRODUCTION

1. PROBLEMS AND RESEARCH RATIONALE

Chrysomya megacephala (Fabricius, 1794) is a blow fly species that has a wide distribution covering many parts of the world. In Thailand, it is extremely abundant in urban areas and the most commonly collected blow fly species. *C. megacephala* has been demonstrated to be a mechanical vector of numerous pathogens infectious to humans, as well being a source of annoyance. Problems arising from *C. megacephala* in the country is a result its rapid development capability under warm temperatures as well as the wide variety of filth sources that this fly can take advantage of as breeding sites. Therefore maintenance of populations of this species below disease transmission thresholds is of critical importance.

Control strategies for pest fly populations currently rely heavily on insecticides and some problems have been encountered. These problems include environmental pollution, insecticide resistance and increased pesticide costs. With the current movement towards using decreased amounts of any kind of insecticide, alternative control methods that can be included in an integrated control program for filth fly management should be developed and tested for application to the current filth fly issues in the country.

One such alternative strategy to traditional insecticide application is the suppression of the reproductive success of the flies via disruption of progeny

production or the disruption of metamorphosis during development. To investigate this possibility the ultramorphology of the reproductive organs in certain arthropods of medical importance has been studied to provide basic information that can be applied to developing better control strategies. In this regard, light and/or electron microscopy have been employed to investigate the reproductive organs of many arthropods. Examples of basic research, on the reproductive systems of medically important arthropods using this approach include that on the reproductive systems of the blow fly, *Chrysomya bezziana* (Diptera: Calliphoridae), accessory glands of *Chrysomya putoria* (Diptera: Calliphoridae), female reproductive tract of the fly, *Curtonotum helvum* (Diptera: Curtonotidae), *Pseudacteon wasmanni* (Diptera: Phoridae), the Mediterranean fruit fly, *Ceratitis capitata* (Diptera: Tephritidae), and the ovary of the tick, *Amblyomma cajennense* (Acari: Ixodidae). In the case of male arthropods, studies have included the sperm structure of the fire ant, *Solenopsis invicta* (Hymenoptera: Formicidae), glandular cells at the terminalia of the reduvid bug, *Triatoma rubrofasciata* (Hemiptera: Reduviidae), and accessory glands in many species of fungus-gardening ants (Hymenoptera). Regarding applied research, studies have been reported on the bacterium, *Rickettsiella* in the ovary of the Oriental cockroach, *Blatta orientalis*, insect virus Hz-2V in the reproductive organs of the female corn earworm moth, *Helicoverpa zea* (Lepidoptera), and infection of the endobacterium, *Wolbachia*, in the ovary of the sand flea, *Tunga penetrans*.

During the course of some of the studies just described it was found that application of certain substances induces alteration of the reproductive system of arthropods. For example, precocene II induces abnormalities in the ovaries and accessory glands of the female rich moth, *Corcyra cephalonica*. Oil extracts from the

plant, *Thevetia peruvine*, cause pathological alterations in the ovaries of the mosquito, *Culex pipiens*. Concerning such published information, it is the objective of this study to thoroughly investigate the reproductive system of *C. megacephala*, using light microscopy (LM), scanning electron microscopy (SEM) and transmission electron microscopy (TEM). This information will establish baseline data on the reproductive system of this species.

As an added feature of this investigation, some substance has been tested for its efficacy to suppress the reproductive success of *C. megacephala*. The female human contraceptive, estrogen, was used because it of its close structural similarity to ecdysone which is naturally produced in the insect and is an important hormone involved in development and reproduction. Estrogen has been shown to reduce reproductive success in the tomato moth, *Lacanobia oleracea* (Lepidoptera: Noctuidae), the silkworm, *Bombyx mori* (Lepidoptera: Bombycidae) and the aquatic midge, *Chironomus riparius* (Diptera: Chironomidae). Observations on the effects of a human contraceptive against *C. megacephala* was monitored in flies directly affected (F_0 generation) and in the first (F_1), the second (F_2) and the third (F_3) generations to observed any reduction in egg production and possible deformations in the reproductive organs. This information provided scientific knowledge that can be applied to fly control in the future.

2. LITERATURE REVIEW

2.1 General consideration and medical importance of *Chrysomya megacephala*

Distribution

Chrysomya megacephala (Fabricius), the Oriental latrine fly, is a blow fly species that prevails widely throughout Asia, Australasia, the Pacific Region, South Africa and South America (Zumpt, 1965; Wells and Kurahashi, 1994; Kurahashi and Chowanadisai, 2001). In Thailand, it is the second most abundant fly species, and top ranked among those blow fly collected (Sucharit et al., 1976; Tumrasvin et al., 1978; Sucharit and Tumrasvin, 1981). In other countries such as Egypt, it is one of the most common species as well (Gabre et al., 2005).

Life cycle and morphology

The life cycle of *C. megacephala* is holometabolous (complete metamorphosis), having four stages of development: egg, larva, pupa and adult. During the larval stage, the fly passes through 3 different stages or instars by moulting. Morphologically, the egg is creamy, banana shaped, measuring 1.40×0.40 mm. Under natural temperature in Chiang Mai, northern Thailand, the eggs hatch within one day. The larvae are creamy, muscoid shaped. The mature larvae of the third instar are up to 16 mm in length. The anterior spiracle shows 11-13 branches, while the posterior spiracle has three straight slits, encircled by an incomplete peritreme. As for puparia, the pupal case looks like a barrel, and the mature puparium is mahogany brown. The pupal stage lasts approximate 100 hr after which time the

newly emerged flies break out from the pupal case. The adult is metallic greenish blue with purple reflections. The body length is 8-11 mm, with a noticeably large head. The compound eyes are unusually large and a very prominent shade of red. The upper facets of the male's compound eye are enlarged and sharply demarcated from the small ones in the lower third. In females, the upper facets are neither strikingly enlarged nor demarcated from the lower ones. The face including buccae in both sexes is bright orange, but the frons are predominantly black.

General biology

Wells and Kurahashi (1994) reported the development of *C. megacephala* at 27°C, to be: eggs hatch, 18 hr; first moult, 30 hr; second moult, 72 hr; pupariation, 144 hr; and adult emergence, 234 hr. Adult longevity is dependent on temperature and humidity. At temperatures of 25-29°C and 75% relative humidity, these flies live for an average of 54 days (90 days maximum), but at lower humidity, they appear to live longer (Greenberg, 1973). While being studied in a laboratory setting at 26°C with 60-70% relative humidity, females laid only one batch of eggs averaging 223.7 in number: the mean developmental times were: egg, 1 day; larva, 5.4 days; and pupa, 5.3 days, and of the hatch eggs deposited resulted in 85 adults (44 males, 41 females); with mean adult longevity of males, 25.3 days and females, 25.8 days (Gabre et al., 2005). Females laid their eggs in batches of 150-300 eggs.

Larvae of *C. megacephala* are primarily carrion feeders, while adults show a preference for the fresh remains of a corpse and/or other carrion (Bohart and Gressitt, 1951). Adults are commonly found near human dwellings. Females deposit their eggs on suitable oviposition sites (breeding sites), for example, in highly humid areas of

garbage, meat, carrion, sweet food, urine, excrement, and decaying animals or corpses.

The behavior of adult *C. megacephala* is hemisynanthropic to eusynanthropic and exophilic in nature. Adults have a pronounced activity peak during the heat of the afternoon. It is one of the first species to become active in the early morning and one of the last to depart carrion at nightfall (Byrd and Castener, 2000). *C. megacephala* seldom oviposits on isolated human feces, but rather accumulates its eggs in large masses on fresh rather than old carrion (Bohart and Gressitt, 1951).

Medical importance

The greatest medical importance of *C. megacephala* is its effect as a mechanical carrier of various pathogens that may cause disease in humans. It fulfills all the conditions required of a human disease vector, namely: (1) being eusynanthropic maintaining a close existence with humans; (2) consuming both contaminated and non-contaminated food; (3) having great flight activity and dispersal and (4) constantly alternating between feces and food in their feeding behavior (Greenberg, 1971). By crawling over and feeding on filth, flies gather pathogens on their legs and body surface or take them into the digestive tract with food. In later visits to human food, flies leave behind some of these pathogens. Their habit of disgorging some of their food and expelling feces, both of which may contain pathogens, contaminates food used for human consumption. Also, it is evident that larva feeding on infected material can produce infected adults (Smith, 1973).

Diseases spread by flies include viral (poliomyelitis and Coxsackie virus, both via human feces) and bacterial diseases (many diarrheal and enteric fevers, infantile summer dysentery, typhoid and paratyphoid fevers, and bacillary dysentery; all via humans feces); and conjunctivitis, tuberculosis, leprosy and plague (Sukontason et al., 2000; Nayduch et al., 2001). They also spread protozoan parasites (cysts, trophozoites, trypanosomes); tapeworms and nematodes (e.g., *Thelazia* spp.) and other arthropods (egg of *Cordylobia* spp. and nymphs of *Pediculus* spp.) (Greenberg, 1971; Smith, 1973; Monzon et al., 1991).

In addition to transmitting pathogens, adult flies can cause annoyance in slaughterhouses and by landing on meat, fish, sweets, fruit and other foodstuffs in market places (Greenberg, 1973). For humans, flies can disturb work and be a direct nuisance. They have been reported to cause economic loss in poultry farming by decreasing chicken egg production. Fly feces decrease the aesthetic appearance and value of eggs (Eldridge and Edman, 2000). Large swarms of flies that emerge from livestock farms and move to neighboring domestic settlements may result in problems for the farmers (Den et al., 1999).

Further causes of *C. megacephala* being medically important their causing include myiasis, which is a pathogenesis from fly larvae infesting living tissue (Zumpt, 1965). Reports include cutaneous and nasopharyngeal blow fly-caused myiasis (Smith, 1973; Eldridge and Edman, 2000).

Another aspect of *C. megacephala*'s medical importance is currently involvement in forensic entomology. Fly specimens of this species (egg, larvae or puparia) collected from corpses have been used as entomological evidence in forensic investigations. These specimens are not only used for estimating the postmortem

interval of a corpse (Lord, 1990; Goff and Flynn, 1991), but also detecting some toxic substances in a putrefying human body (Gunatilake and Goff, 1989).

2.2 General reproductive system of insects (Snodgrass, 1993; Chapman, 1998)

All of the reproductive organs in insects are bisexual, male and female. The functions of the male reproductive system consist of the production, storage and delivery of spermatozoa. For females, this system has three main functions; producing and storing eggs, receiving and storing spermatozoa, and depositing eggs or larvae.

2.2.1 Male reproductive system

The male reproductive system (Figure 1) is located in the posterior portion of the abdomen and typically consists of paired gonads (testes) connected by various ducts, which ultimately open into the penis or aedeagus. Accessory glands are usually associated with a duct.

Testes: The testes are usually bilateral and pair structured. Basically each testis consists of a varying number of testicular follicles or sperm tubes, which are usually enclosed by a layer of connective tissue. Each follicle is covered by a layer of epithelial cells, which serve to absorb nutrients from the hemolymph for processing the spermatogenesis. In certain insects, such as Coleoptera or Diptera, each testis is a simple sac-like organ, which, in most cases, is a single sperm tube, though in some Diptera it is said to be partially subdivided (Snodgrass, 1993).

Ducts: The follicles connect to a common duct, the vas deferens, through individual vas deferens tubes. A portion of the vas deferens may be enlarged as the seminal vesicle that serves as a storage reservoir for sperm before it is transferred to

the female. The two vasa deferentia connect to the ejaculatory duct, which is composed of cells that are ectodermal in origin and produce a lining of cuticle. The terminal portion of the ejaculatory duct opens into the external reproductive organ, the aedeagus. Both the vas deferens and ejaculatory duct are invested with a layer of muscles that are involved in the propulsion of semen.

Accessory glands: A pair of accessory glands is present. These glands can open into either the vas deferens or the ejaculatory duct. Male accessory glands serve a variety of functions, including the production of seminal fluid, which serves as a transporter and activation medium for sperm, the vaginal mating plug that temporarily blocks sperm entering from another male, and the formation of spermatophores that are proteinaceous secretions of the male accessory glands that enclose the sperm. Additionally, peptides that are produced by the male accessory glands and transferred to the female during mating can also affect several physiological systems of the female. A common effect is the prevention of subsequent mating by the female, either temporarily or permanently.

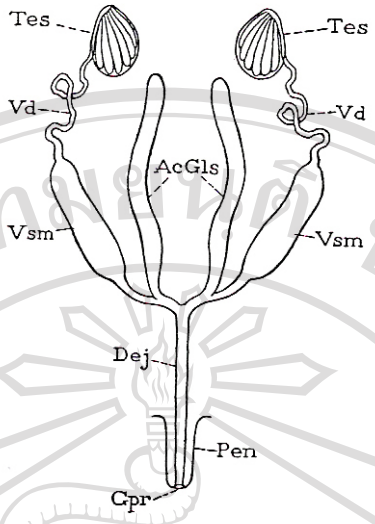

Figure 1 General diagrammatic structure of the male reproductive organs. AcGls, accessory glands; Dej, ductus ejaculatorius; Gpr, gonopore; Pen, penis; Tes, testis; Vd, vas deferens; Vsm, vesicular seminalis (Snodgrass, 1993).

2.2.2 Female reproductive system

The female reproductive system (Figure 2) is located in the posterior part of the abdomen. It typically consists of a pair of ovaries connected by a series of tubes to the vagina, which opens to the exterior in the eighth or ninth abdominal segment and receives the penis during copulation. A variety of accessory glands is also present.

Ovaries: The ovaries are bilateral and suspended in the hemocele by a terminal filament at their anterior end, and the lateral oviducts are at their posterior end. Each ovary comprises a series of tapering egg tubes, the ovariloes, and the functional units that contain a progression of oocytes. Each ovariole produces oocytes that develop and grow within it. The number of ovarioles in each ovary varies tremendously depending on the size and reproductive strategies of the particular

insect species, and although it is genetically determined, it can be regulated by the diet of the immature stages. Ovariole number largely determines fecundity and typically ranges from 4-8 per ovary, although many dipterans have about 50 ovarioles in each ovary.

Ducts: At the base of each ovariole is a small duct, or pedicel, which joins the other ovariloes together in a bulbous calyx; and in turn, into the lateral oviduct. The lateral oviducts are joined to form the common oviduct, which serves as a communicating tube between the lateral oviducts and the bursa copulatrix or vagina, with an opening to the outside.

Accessory glands: Most insects generally have one or two pairs of accessory glands, which usually open into the apical portion of the bursa copulatrix, vagina, or common oviduct. These glands vary in structure and function. They are commonly involved in the secretion of adhesive materials and serve to cement eggs to the substratum or hold them together in masses.

Spermathecae: The spermathecae are used for the storage of sperm once the females are inseminated. They generally open into the common oviduct and release sperm as the fully formed eggs pass by. The spermathecal duct may contain glycogen deposits that can serve as an energy source for the sperm as they pass through to the egg.

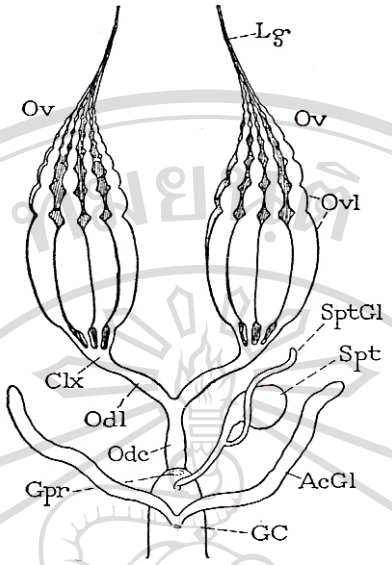

Figure 2 General diagrammatic structure of the female reproductive organs. AcGls, accessory glands; Clx, calyx; Gc, genital chamber; Gpr, gonopore; Lg, ovarial ligament; Odc, oviductus communis; Odl, oviductus lateralis; Ov, ovary; Ovl, ovariole; Spt, spermatheca; SptGl, spermatheca glands (Snodgrass, 1993).

2.3 Previous studies involving the morphology of the reproductive organs of insects

2.3.1 Male reproductive organs:

The internal male reproductive organs of flies in the family Calliphoridae consist of a pair of testes, vas deferens and a pair of accessory glands with a single sperm duct and sperm pump. The external male genitalia are basically composed of the aedeagus. Male reproductive morphology of the blow fly, *Lucilia cuprina* and *Chrysomya bezziana* (Diptera: Calliphoridae), has been previously investigated by Clift and McDonald (1973) and Spradbery and Sands (1976), respectively. The fine

structure of the internal organs is the testes, deferens ducts, accessory glands, and ejaculatory duct. The external reproductive organ has not been recorded. However, the ultramorphology of male reproductive organs in other insects has been reported as follows:

Testes: The fine structure of testes has been described by Snodgrass (1993). A characteristic feature of the testicular tubes is the presence of a large cell or nucleated mass of protoplasm in the apex of the germarium, which is the apical cell. The apical cell consists of a large mass of cytoplasms containing a nucleus. Surrounding the apical cell are several concentric rows of spermatogonia, in which the nearest the apical cell is seen to connect with the latter by protoplasmic strands containing dark granules that appear to originate in the former. Each spermatogonial group in most insects soon becomes enclosed in a cellular envelope, known as a sperm cyst.

Dallai et al. (1997) observed the testes in the fine structure of thrip, *Frankliniella occidentalis* (Thysanoptera: Thripidae). Cross sections showed the external layer pigment cells, with cytoplasms full of pigment granules. A thin layer of circular muscle fibers and an internal epithelium were visible, consisting of fat cells, which extended up to the basal region of the cell contact. They contained numerous spermatozoa among which some late spermatid was visible, showing a flagellum having an axoneme with a 9+0 microtubule pattern.

Testis ultrastructure has been studied in two species of *Elenchus tenuicornis* and *E. japonicus* (Strepsiptera: Elenchidae) using light and electron microscopy (Carpupino et al., 1998). In both species, the testes are paired and consist of several large irregularly shaped follicles, each consisting of a single clone of germ cells surrounded by a thin epithelium. The cross section of the testis indicated the presence

of spermatozoa surrounded by a fat body. In the centriolar region, the mature sperm is 9+9+2 axoneme flanked by mitochondrial derivatives.

Dallai et al. (2000) reported the fine structure of testes in *Allacma fusca* (Collembola: Symphypleona). A semi-thin section showed the germinal cells at different stages of maturation, from spermatogonial cysts to mature spermatozoa. The epithelial cells, rich in endoplasmic reticulum and a huge Golgi system, have large cytoplasmic lobes, which extend into the central region of the testis to surround the clusters of germinal cells. In the proximal part of the testes, which contains the rolled up sperm, the epithelial cells also produce long peduncles, which temporarily connect spermatozoa to the epithelial wall.

Moreover, the ultrastructural investigations of spermatozoa in four Plecoptera species: *Taeniopteryx stankovitchi*, *T. kuehtreiberi*, *Brachyptera risi* and *Leuctra fusca* were carried out using LM, SEM and TEM (Fausto et al., 2001). SEM micrographs showed that testes consist of a number of separated follicles. In Taeniopterygidae, the two testes are united in the median proximal portion, forming a single arc: In *B. risi*, the follicle are ovoid, and in *L. fusca*, each of two testes consists of 9-10 follicles, almost tubular in shape. Spermatozoa of all species have a complex acrosome, are filiform and flagellate, with a 9+9+2 axoneme flanked by two mitochondrial derivatives.

TEM investigation in the halacarid mite, *Thalassarachna basteri* and *Halacarellus thomasi*, revealed that the testes were composed of a glandular and a germinal part. The testicular lumen is filled with a very complex secretion that contributes to sperm cell aggregates and mature sperm cells. The somatic tissue of the germinal layer consists of extensive processes that surround clusters of developing

germ cells. Apically, these processes are provided with some microvilli. The cytoplasm contains heterogeneous inclusions, presumably lysosome, extensive cisternae of rough endoplasmic reticulum, some variable sizes of mitochondria and Golgi bodies (Alberti and Meyer-Rochow, 2002). Additionally, the fine structure of spermatozoa in other insects, *Xenos vesparrum* (Strepsiptera) and *Mantispa perla* (Neuroptera, Planipennia) were examined (Dallai et al., 2003; Dallai et al., 2005). These spermatozoa are also 9+9+2 axoneme, flanked by two mitochondrial derivatives.

Vas deferens: The vas deferens cells vary from squamous to cubical according to reproductive condition. The ultrastructure of the vas deferens of *F. occidentalis* and *Melipona bicolor bicolor* (Hymenoptera: Apinae) was recorded by Dallai et al. (1997) and Dallacqua and Cruz-Landim (2003), respectively. The epithelial layer is surrounded by circular muscle fibers and pigment cells. Their walls have the structural organization of an external layer of pigment cells, intermediate layer of circular muscle fibers, among which the ends of nerve fibers are often present, and an epithelium lining the lumen. This layer consists of flat cells with elongated nuclei, mitochondria, and scattered dense droplets. Spermatozoa are seen in deferens ducts.

Accessory glands: The fine structure of male accessory glands of *F. occidentalis* has been studied by Dallai et al. (1997). In cross sections, the epithelial cells were visible, showing long elliptical nuclei and a cytoplasm rich in free ribosomes, endoplasmic reticulum, Golgi complex and mitochondria. The secretion in the lumen was finely granular with scattered small dense bodies. The epithelial cells overlay a very thin basement lamina, beneath which a thin layer of circular muscle

fibers was visible. Likewise, the ultrastructure of the male accessory glands of *Ceratitis capitata* (Diptera: Tephritidae) was investigated using LM, fluorescence microscopy, SEM and TEM (Marchini et al., 2001). There are two types of accessory glands in the reproductive apparatus. The ejaculatory duct, lined by the cuticle, has epithelial cells with limited involvement in secretory activity. In blow fly, the secretory activity of the accessory glands of *L. cuprina* can be used as an age indicator because of its change with physiological age (Clift and McDonald, 1973).

Ejaculatory duct: The male ejaculatory duct of *F. occidentalis* was ultrastructurally investigated by Dallai et al. (1997). The epithelial cells of the ejaculatory duct are joined by a sinuous septate junction. The cytoplasm is rich in rough endoplasmic reticulum, often disposed in a parallel fashion. Golgi complexes, mitochondria, vesicles of different sizes and contents are evident. The epithelial cells overlie a thin basement lamina, beneath which a thick layer contains intermingled pigment cells and muscle fibers. Many spermatozoa are embedded in the homogeneously dense secretion in lumen. Subsequently, Dallai et al. (2000) described the fine structure of the ejaculatory duct of *A. fusca* using TEM. The epithelial wall of the ejaculatory duct consists of flat cells lined by a very thick cuticular intima. The duct lumen of the proximal part of the ejaculatory duct is divided into two parts by a transverse cuticle septum. The dorsal region retains a thick cuticular intima, with very long cuticular spines, which avoid sperm clusters. However, investigation using TEM showed that the ejaculatory duct of *M. bicolor bicolor* was not presented on the outer muscular layer (Dallacqua and Cruz-Landim, 2003). The epithelial cells are flat and covered in the luminal surface. The basal side of the cell is covered by a thin basement membrane and presented deeper in a folding plasmic membrane. Also, the

ejaculatory duct of *C. capitata*, examined by Marchini et al. (2001), showed a series of epithelial folds corresponding to longitudinal cords, giving it a star-like appearance. The epithelium cells are lined by a continuous thin cuticle beneath which a rich system of microvilli is evident. The nucleus is located in the basal position. The cytoplasm is rich with the endoplasmic reticulum, mainly in the basal region; while the mitochondria are preferentially located in the apical part. Beneath the epithelium, several scattered inner longitudinal and a few outer circular muscle fibers are evident.

External reproductive organs: The surfaces of body segments and appendages of the male and female human bot fly, *Dermatobia hominis*, were studied using SEM (Fernandes et al., 2004). Sternites II to V of the male abdomen were longer than those in females. Circus and tergum X were covered with bristles.

2.3.2 Female reproductive organs:

The female reproductive organs of blow flies consist of a pair of ovaries, lateral oviducts, a single common oviduct, 3 spermathecae and a pair of accessory glands. The female reproductive organs of *L. cuprina* and *C. bezziana* have been studied by Clift and McDonald (1973) and Spradbery and Sands (1976), respectively. In *C. megacephala*, only the ovary, spermatheca and accessory glands were investigated using LM (Bansal and Murad, 1987); while other organs as well as external reproductive structures have not been previously recorded. However, the ultrastructure study of female reproductive organs in other insects has been documented as follows:

Ovaries: Each ovary of *L. cuprina*, *C. bezziana*, and *C. megacephala* is composed of 100, 75-115, and 100-110 polytrophic ovarioles (Clift and McDonald,

1973; Spradbery and Sands, 1976; Bansal and Murad, 1987). Ultrastructurally, the fine structure of the ovary in *Cryptostemma alienum* and *C. carpaticum* (Heteroptera: Dipsocoridae) has been described by Štys et al. (1998). The tropharia are composed of 30-50 mononucleate nurse cells that are connected with a centrally located trophic core by means of broad cytoplasmic strands. The anterior nurse cells are markedly smaller and often reveal signs of degeneration. The trophic core is surrounded and penetrated by elaborate F-actin meshwork. Arrested oocytes and prefollicular cells are localized at the base of the tropharium.

Szklarzewicz (1998) determined the ovary of *Porphyrophora polonica* (Heminoptera: Coccinea) using LM and TEM. Ovaries are composed of ≈300 ovarioles of the telotrophic type. Within the ovarioles, oocytes are connected by nutritive cords to the trophic core. In cytoplasms of arrested oocytes, abundant free ribosomes, bacterial endosymbionts and numerous mitochondria exist. Nuclei of the oocyte are irregular in shape. Trophocytes are characterized by their giant sizes, located nuclei and cytoplasm filled with numerous free ribosomes and mitochondria.

The structure of the ovaries of *Orthezia urticae* (Hemitera: Coccinea) has been investigated by Vogelgesang and Szklarzewicz (2001). Paired ovaries are composed of 20-30 ovarioles of a telotrophic-meroistic type. Each ovariole is subdivided into an apical tropharium and vitellarium that contains a single developing oocyte. This oocyte is surrounded by a mono-layer follicular epithelium that is responsible for the synthesis of egg envelope precursor. In addition, Zelazowska and Bilinski (2001) described the ultrastructure and function of nurse cells in the ovaries of the pig louse, *Haematopinus suis* (Anoplura) and bird lice, *Eomenacanthus stramineus* and *Columbicola columbae* (Mallophaga). All three species have polytrophic-meroistic

ovaries, which means that each oocyte remains connected with a group of nurse cells via specialized cytoplasmic canal-intercellular bridges (ring canals). The nurse cells are equipped with large, highly extended (irregularly lobed) nuclei. The inner nuclear membrane is lined with a relatively thick layer of nuclear lamina.

In 2003, Koteja et al. (2003) reported the morphology of the ovary of *Steingelia gorodetskia* (Sternorrehyncha: Coccinea). Paired ovaries are composed of ≈100 telotrophic ovarioles devoid of filament. In the longitudinal section, a tube-shaped tropharium is composed of a small, somatic prefollicular cell and large germ cells, a trophocyte and young oocyte. The central area of the tropharium is devoid of cells and connected with both the oocyte and trophocyte. The trophocyte possesses large nuclei located with accumulations of chromatin and a single nucleoli. The nuclear envelope is perforated by numerous pores. The cytoplasm is filled with ribosomes, mitochondria and endosymbiotic microorganisms. Likewise, the histology and SEM observations of the *Pseudacteon wasmanni* (Diptera: Phoridae) ovary, presented with a spherical shape, were examined by Zacaro and Porter (2003). The total number of eggs found was ≈31-280. Each ovary is enclosed by a thick muscular sheath in which muscle fibers appear to be perpendicular to the longitudinal axis of the ovary. The peritoneal sheath is seen beneath this muscular sheath as a thin epithelium. The muscular sheath that covers each ovary is contiguous with the lateral and common oviducts. The external muscle layer of the ovaries consists of ventral sutured muscle fibers forming a depression, with the common oviduct running out of this suture. Moreover, the histology and ultrastructure of the ovariole have been described in the bee, *Apis mellifera* (Tanaka and Hartfelder, 2004). Early follicles in the ovariole of a virgin queen shows follicle epithelium cells migrating between the

trophocyte. TEM analysis demonstrated that putative germ cells in the terminal filament of a virgin queen ovary are separated from the thick basal lamina by at least one layer of somatic cells. Small cystocyte clusters indicate initial intercellular bridge structures in the upper germarium region of a virgin queen ovary.

Aside from their described above, the developmental stage morphology of female reproductive organs during oogenesis has been examined in many insects. The stage of terminal follicle development of *L. cuprina* ovarioles can be used as an age indicator in females (Clift and McDonald, 1973). Adams and Reinecke (1979) examined oogenesis, which was divided into a series of 10 stages in the screwworm fly, *Cochliomyia hominivorax* (Diptera: Calliphoridae). The nurse cell, with a maximum volume of nuclei was found at stage 6 before it started to decrease, while maximum cytoplasmic volume was observed in the follicle stage. The nurse cells degenerated during stage 9. The number of follicle cells increased during stage 2, 3, and 4 and decreased during stages 6-10. The follicle cells over the nurse chamber were squamous during stage 6 through 9. During stage 6 and 7, the follicle cells over the oocyte were columna; at stage 8, they were cuboidal; and during stage 9 and 10, they became elongated. Subsequently, Rosciszewska (1995) presented the first LM and TEM studies of follicular epithelium development in the stone flies *Perla marginata* and *P. pallida* (Plecoptera: Perlidae) during oogenesis. The major function of the follicular epithelium in *Perla* sp. is the secretion of eggshells. The follicular epithelium passes through several district stages of oogenesis. Furthermore, the chronological development of the egg chorion of the beetle, *Listronotus oregonensis* (Coleoptera: Curculionidae), was studied using SEM and TEM (Nenon et al., 1995). A cross section of the ovary revealed that the oocyte membrane comprised a single

layer of follicular cells. The follicular cells have a large nucleus, Golgi apparatus occupying the external periphery of the cell, and a cytoplasm that has a dense and granular endoplasmic reticulum.

Spermathecae: The fine structure of spermatheca of *F. occidentalis* was described by SEM and TEM (Dallai et al., 1996). It is a small sphere, 27-28 µm in diameter, located at the base of ovipositor. A cross section near the middle of the spermatheca showed an irregular epithelium surrounding a large spermathecal lumen, filled with dense homogeneous secretion. In the spermatheca lumen a compact mass of spermatozoa is evident. No muscle fibers were observed outside the epithelium, which consists of a few scattered secretory cells and duct forming cells of unusual appearance. The duct-forming cells are polymorphic, the cavity is bordered by microvilli and contain a dense secretion. An extension of duct-forming cells originate from a vault.

Winterton et al. (1999) examined the histology of the spermathecal sac in female *Agapophytus albobasalis* and *Ectinorhynchus variabilis* (Diptera: Asiloidea). The spermathecal sac of the former is an elongated one located approximately halfway along the abdominal cavity. Its wall is 125 µm thick and highly reticulate, with a complex labyrinth of large lacunae and smaller interconnecting canals running along the length of the sac. The lumen and lacunae are lined with a cuticular intima. The epithelium encompassing the lacunae and lumen is interspersed with nuclei. A thin meshwork of muscle fibers surrounds the sac, in which sperm is evident. There are three ovoid sacs in *E. variabilis*, each comprising two distinct regions. The sacs have a narrow lumen and thick intima near their junction with the duct. No sperm is evident in the sac. Additionally, details of the fine structure of female *Anastrepha*

suspense (Diptera: Tephritidae) spermathecae were resolved by UV-light microscopy, tissue sectioning, SEM and TEM (Fritz and Turner, 2002). Three spermathecae are pear shaped and similar in size. SEM micrographs of the spermathecae capsule showed exterior mounds that terminate in secretory ducts, and the duct surfaces are highly convoluted. The interior of the spermathecae is sculptured intricately with hollow, sclerotized, tubular processes, into which glandular ductules empty their content. Cross sections of spermathecae from inseminated females demonstrated unicellular gland cells, with a large vacuole of the secretory ducts containing filamentous or laminar secretions. The spermathecae lumen was filled with spermatozoa and glandular secretion.

Accessory glands: Tirone and Avancini (1997) determined the morphology and histology of the accessory glands of the blow fly, *C. putoria*. They have two accessory glands that are tubular and more dilated at the distal extremity. Two morpho-physiological regions are distinguishable in the longitudinal semi-thin sections of the glands. The secretory region is constituted by three layers: the acuticular intima lining the lumen, followed by a layer of small cells, and then a layer of very large secretory cells. The ductal region presents two layers: the cuticular intima and cellular layer. In both regions, a basement membrane is present. Similarly, Bene et al. (1998) observed the ultrastructure of the accessory gland in *Heliothrips haemorrhoidalis* (Thysanoptera: Thripidae). It consists of an apical bulb and a fine gland duct. The former consists of an epithelium with secretory and duct-forming cells surrounding a large gland lumen lined with a thin cuticle and filled with dense secretion. Spent secretory cells degenerate and are eliminated from the epithelium.

The gland duct is characterized by irregular, branched lumen surrounded by a very flat epithelium.

Vagina: Fritz and Turner (2002) studied the fine structure of the vagina of *A. suspense* by using UV-light microscopy and TEM. The signum corresponds to a pair of long, cuticular structures located in the dorsal wall of the vagina. Each structure's thickest cuticle is furrowed and broadens towards its anterior end. Cross sections of the vagina revealed the signum, surrounding muscle fibers, and globular secretion.

2.3.3 External reproductive organs

The external female genitalia of *C. bezziana* is composed of sclerites from abdominal segments VI-VIII, modified to form an ovipositor (Spradbery and Sands, 1976). The genitalia of three female phlebotomine sand flies *P. argentipes*, *P. papatasi*, and *P. major major* were examined using SEM (Mukhopadhyay et al., 2003). The individual genital plate of these three species is triangular in shape. The inner border of the plates in *P. argentipes*, *P. papatasi*, and *P. major major* make the external genital opening heart, cylindrical, and dumbbell shaped, respectively. The external views of the female reproductive system of the decapitating fly, *P. wasmanni* were also analyzed using LM and SEM (Zacaro and Porter, 2003). The external abdomen morphology showed two sets of three sensilla on the sternite just before the external genitalia. These sensilla may function as an extension of the ovipositor and/or for the injection of an egg.

Furthermore, studies of the important external female reproductive organ, the ovipositor, include one on the ovipositor of the parasitoid wasp *Trybliographa rapae* (Hymenoptera: Cynipidae) using SEM (Brown and Anderson, 1998). Two types of

sensilla were observed on the ovipositor; namely basiconic sensilla and coeloconic sensilla. (Yu-Tong et al., 1998), investigated the ovipositor of *Plutella xylostella* (Lepidoptera: Yponomeutidae) and found that it is densely covered with long sharp tipped sensilla, presumably mechanosensory in nature.

2.4 Morphological changes of the reproductive organs of insects exposed to substances

The efficacy of some chemicals, oil extracts, insect intracellular bacteria or viruses on structural changes in the reproductive system of insects has been tested. Martoja et al. (1983) suggested that mercury (Hg) has a direct effect on the germinal and somatic cells of the reproductive system of *Locusta migratoria* (Orthoptera) while cadmium (Cd) inhibits the differentiation of the fat body cells and, therefore, the synthesises yolk products. Maiza et al. (2004) tested the effect of RH-0345 (ecdysteroid agonist), methoprene (juvenile hormone analogue) and carbamate, benfuracarb (insecticide) in the reproduction of the German cockroach, *Blatta germanica*. The results showed that treatment with RH-0345, methoprene, and benfuracarb significantly reduced the number of oocytes and size and volume of basal oocytes in the ovaries during sex maturation. It is obvious that flucycloxuron (FCX), a chithin synthesis inhibitor, affects the reproduction of mealworm (Hami et al., 2004).

Efficacy of oil extracts against the insect reproductive system has been investigated. Oil extracts from *Thevetia peruvine*, *Datura stramonium* and *Acacia* sp. were tested on larvae of the mosquito, *Culex pipiens* (Hussein, 1999). Histological and histochemical analysis of the ovaries indicated obvious changes in oocytes of adult females derived from the treated larvae. Mukhopadhyay et al. (2003) explored

the reproductive toxicity of argemone oil in the transgenic fruit fly larvae of *Drosophila melanogaster*. The ultrastructural morphology of the male accessory glands reared from treated larvae showed acute signs of necrotic cells, as evidenced by necrotic nuclei and higher vacuolization. Disorganized endoplasmic reticulum decreased the number of Golgi vesicles, and disorganized, loosely packed filamentous structures in the gland lumen were also found.

The pathology and ultrastructure of intracellular bacteria infecting the reproductive system of insects has been examined. Bacterium, in particular the genus, *Wolbachia* (Rickettsiales), has been studied a great deal recently. It was detected in the eggs, sperm cells and ovaries of their insect hosts; and is of special interest, as it may alter host reproduction. The bacteria occur predominantly in the oocytes of the stored-product pest, *Liposcelis bostrychophila* (Psocoptera), mosquito *C. pipiens* and sand flea *T. penetrans* (Yusuf et al., 2000; Mahilum et al., 2003; Heukelbach et al., 2004). Two different forms of *Wolbachia* were observed, rod and cocci. In addition, the fine structure of *Wolbachia* has been examined in the testes of the almond moth, *Ephestia cautella* (Kellen et al., 1981). The rod shaped structures are abundant in the cytoplasm of the hypertrophied spermatids. Moreover, the ultrastructure of *Rickettsiella* in the fat body cells and ovarioles of the cockroach, *B. orientalis*, was recorded by Radek (2000). In the fat body, bacterial vacuoles contain four stages of *Rickettsiella*: rod-like elementary bodies, a flat body, condensing sphere, and large spherical initial bodies. However, in the oocytes, the rod shaped bacteria are lined between the follicle cells and microvilli while the cytoplasm of oocytes show flat and elementary bodies of bacteria.

The pathology and ultrastructure of insect virus, Hz-2V [a.k.a. gonad specific virus (GSV)] infection in the agonadal female and male corn earworm, *H. zea*, was studied (Rallis and Burand, 2002a; Rallis and Burand, 2002b). The ultrastructure of the grossly malformed agonadal female reproductive tissues from insects aged 3-10 days post-pupation (dpp) revealed an absence of cuticular lining in these infected oviduct tissues. The cuticular lining is found in the oviducts of normal moths, and proliferation of epithelial cells. Large quantities of virus were found aggregated into a large mass in the lumen of the malformed cervix bursa of 10 dpp agonadal female pharate adult moths. In male reproductive tissues at 7 and 10 dpp, virus particles appeared as a small bulb-shaped structure. Large quantities of rod shaped, enveloped virus particles were organized into arrays dispersed throughout these infected nuclei. Moreover, virus particles were also found in the lumen and membrane vesicle in the lumen of the malformed tissues.

In addition, Fausto et al. (1997) displayed the ultrastructure of the accessory glands of fed and unfed *P. perniciosus* females using LM, SEM and TEM. Female accessory glands are paired and tube-shaped. SEM investigation revealed that the female accessory glands were small in size and had walls that were constituted by globular-shaped cells. After a blood-meal, the glands increased in size and the cells constituting the wall stretched upwards. TEM investigation showed that the accessory glands of newly emerging females lacked the central lumen. The epithelial cells had a large basal nucleus with dense heterochromatin clumps and a cytoplasm rich in mitochondria and ribosome. After the blood-meal, the cells increased in size, forming a simple columnar epithelium. The glandular lumen was full of secretory material consisting of many granules. The cytoplasm of glandular epithelial cells was

characterized by a rough endoplasmic reticulum, abundant free ribosomes, mitochondria and many large Golgi complexes associated with a lot of condensing vesicles.

2.5 Endocrine control of the reproductive system of male and female dipterans

The reproductive system of insects is involved with juvenile and 20-hydroxyecdysone hormones.

Juvenile hormone (JH): JH is biosynthesized by the corpora allata of adult female insects. The techniques of chemical derivation followed by electron capture detection have been used to confirm the existence of three hormones (JH I, JH II and JH III) in the blood of several larval and adult insects (Rockstein, 1978). Nation (2002) showed structures of six JH-type compounds that had been isolated from insects (Figure 3). JH III may be the most common in insects. JH I, II, III, JH 0, and Iso JH 0 have been found in some insects, particularly Lepidoptera. JH bisepoxide is the principle of the fly, *D. melanogaster*, and it also occurs in the blow flies, *Phormia regina*, *Calliphola vomitoria* and *L. cuprina*, as well as four species of mosquitoes.

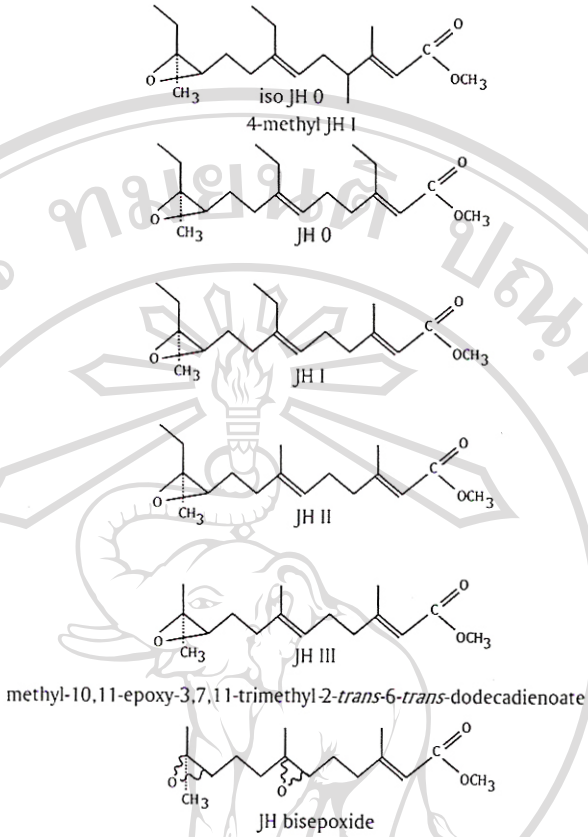

Figure 3 Naturally occurring molecules with juvenile hormone (JH) activity. JH III may be the most common in insects. JH I, II, III, JH 0, and Iso JH 0 have been found in some Lepidoptera. JH bisepoxide is synthesized by the ring gland of some Diptera (Nation, 2002).

20-hydroxyecdysone (20E): The prothoracic glands sequester cholesterol from the circulating haemolymph and convert it into ecdysone or a closely related ecdysteroid (Nation, 2002). They do not store ecdysone, but secrete it into the haemolymph as it is made. Ecdysone appears to have low hormonal activity itself, although this is difficult to gauge because it is rapidly converted to 20-hydroxyecdysone by enzyme 20-hydroxymonooxygenese present in most insect

tissues. In older literature, 20-hydroxyecdysone is also known as *β*–ecdysone, ecdysone, and crustecdysone (Nation, 2002). It has been isolated and crystallized in pure form from larvae of the blow fly, *Calliphora*, and pupae of the silkworm (Wigglesworth, 1984). From the chemical characteristic of 20E, evidence was obtained of the steroid nature of ecdysone, when one oxy-group and five hydroxyl-groups were found (Novak, 1975), as shown in Figure 4. Ecdysone was the first water-soluble steroid discovered. Subsequently, it was found that many similar steroids, some of them identical with ecdysone, occur in plants, notably in the leaves of yew and the rhizomes of bracken. In the insect they are synthesized from cholesterol or sitosterol in the diet (Wigglesworth, 1984).

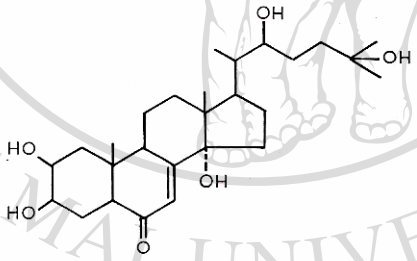

Figure 4 Structure of 20-dihydroxyecdysone (Wigglesworth, 1984).

In female reproduction, the production of vitellogenins, which are synthesized during the process of vitellogenesis for producing and developing oocytes in ovarioles, is dependent on both JH and 20E (Rockstein, 1978). JH regulates the formation of new endoplasmic recticulum in the fat body and sequestration of the vitellogenin produced, while 20E regulates the rate of production. After the adult female emerges, JH, which is released by paired corpora allata during the first few

days of life, prepares both the fat body and the ovary for vitellogenesis. In response to an early peak of JH, the follicle cells that surround the oocyte cell are initiated to differentiate and increase in size. The 20E peak also stimulates the follicle cells of the ovary to synthesize the vitellin envelope and inner layer of the chorion, and act on the germarium to cause the chorion of follicle.

Little is known about the endocrine regulation of male reproduction compared with that in females. The mitotic division rate of spermatogonia, which form a spermatocyte, is increased by high levels of 20E, but high titers of the JH abolish this increment. JH may also control the development and secretion of the male accessory glands. The accumulation of some secretory peptides in the glands is enhanced by JH, while inhibited by 20E.

2.6 Ovulation, fertilization and oviposition of insects

Once egg development has been completed, the mature ones are moved into the oviduct by the process of ovulation. This is only possible once the follicle cells that surround the oocyte degenerate, leaving the egg free to move out of the ovariole. The presence of eggs in the oviduct triggers stretch receptors which stimulate the spermathecal muscles to contract and release the sperm that is stored within spermatheca. As the egg is passed from the lateral into the common oviduct, it travels past the spermathecal duct and the released sperm enters the egg through the micropyle. After ovulation and fertilization, the egg is usually deposited outside of the female's body in the process of oviposition. It moves down the common oviduct by a peristaltic wave of muscular contraction and out of the body through the ovipositor to the habitate of the species.

2.7 Human estrogen hormone

The major endocrine products of follicles are estrogens, which are the steroids that produce female characteristics. Estrogens are synthesized from the primary source of cholesterol. All naturally-occurring estrogens are C-18 steroids with an aromatic A-ring and hydroxyl group on the 3-carbon (Hedge, 1987). There are three physiologically significant estrogens: estrone, which has a ketone group at the C-17 position; estradiol (Figure 5), which has hydroxyl groups at both the C-3 and C-17 positions; and estriol, which has hydroxyl groups at the C-3, C-16 and C-17 position. These estrogens are often referred to as E_1, E_2 and E_3, respectively, with the subscripts indicating the number of hydroxyl groups attached to the steroid ring. In the nonpregnant adult woman, estradiol is the principal ovarian estrogen. The ovary secretes twice as much estradiol as estrone, and estrone has only 10% of the estrogenic potency of estradiol. Estriol is the least potent of the three estrogens and in nonpregnant women derives only from the metabolic degradation of estrone and estradiol (Hedge, 1987).

Some human contraceptive techniques act at one of three major steps in the reproductive process: ovulation, sperm transportation to the ovum, or implantation. Oral contraceptive methods, which usually contain an estrogen-progestin combination, block ovulation in the female reproductive system. The activity of these pills also decreases sperm transportation by decreasing muscular contraction in the female reproductive tract and increasing the viscosity of cervical mucus.

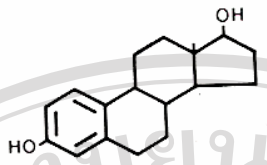

Figure 5 Structure of human estradiol (Hedge, 1987).

2.8 Studies involving effects of human steroid hormones on insects

Interestingly, testosterone- and progesterone-like substances have been isolated from several invertebrate groups, including insects. For example, testosterone- and progesterone-like immunoreactivity has been found in the flesh fly, *Sarcophaga bullata* (Diptera: Sarcophagidae), and phytophagous insect, *Leptinotarsa decemlineata* (Clerck et al., 1983; Clerck et al., 1988). In addition, estradiol has been identified in the silkworm, *Bombyx mori* (Ohishi et al., 1985).

Very few records have been found in the literature concerning the effect of vertebrate steroids in insects. Ohishi et al. (1985) reported that injected estradiol at various doses into the whole pupae of *B. mori* considerably reduced oviposition rate in adult females, although estradiol had no effect on ovarian and embryonic developments. Three vertebrate hormones (estrogen, testosterone and thyroxine) were tested for their effects on growth, development and reproduction of the tomato moth, *Lacanobia oleracea* (Lepidoptera: Noctuidae) (Kirkbride-Smith et al., 2001). Larvae exposed to estrogen or thyroxine caused a significant effect in larval length of the third, fourth, fifth and sixth instars. Larvae exposed to estrogen reduced mean weight of the fifth and sixth instars. Additionally, exposure of larvae to testosterone

significantly increased the number of deformed pupae; while this steroid caused a significant decrease in egg production and viability. The effect of 17-ethinylestradiol (EE), a synthetic estrogen used as a female contraceptive, and bisphenol A (BPA) on the development and reproduction of the aquatic midge, *Chironomus riparius*, was determined over two generations in chronic sediment exposure assays (Watts et al., 2001). The results indicated that emergence time and percentage adult emergence was affected by EE and BPA exposure. At very low concentrations (1 ng/l) of EE, both the first and second generations of adults emerged significantly earlier than those in a control group. The number of egg-ropes produced by the first generation females varied in treatment; but no dose-response pattern was evident. (Watts et al., 2003) investigated the effects of the endocrine-disrupting chemicals EE and BPA on development of the aquatic life-cycle stages (egg, larva and pupa) of *C. riparius*. The moulting period was delayed and larval wet weight significantly reduced at the highest treatment concentration (1 mg/L) of both chemicals. *C. riparius* exposed to EE at a very low concentration provided a greater incidence of deformities in the mouthparts than that exposed to BPA.

3. PURPOSE OF THIS STUDY

The main purpose of this study concerns the reproductive system of the blow fly, *Chrysomya megacephala*, including the ultramorphology of its reproductive organs, and effect of a human contraceptive on the fly reproductive system. The scope of this study was divided into two parts as follows:

Part I: To investigate the ultrastructure of the reproductive organs of the male and female fly using SEM and TEM**.**

Part II: To determine the efficacy of the human contraceptive on the reproductive system of *C. megacephala*.

4. SIGNIFICANCES OF THE RESEARCH

1) This study will establish the basic knowledge of male and female reproductive organs of *Chrysomya megacephala*. Basically, this will enable a clear understanding of this complex system, and it may be useful for further studies on the biology, ecology or control of this fly.

2) The data resulting from the investigation of the efficacy of the human contraceptive on the male and female reproductive system of *C. megacephala* may be useful for further development of a new fly control strategy that is simple and environmentally safe.