CHAPTER II

REVIEW OF RELATED LITERATURES

1. *Ae. aegypti* mosquitoes

Mosquitoes are classified in the family Culicidae, suborder Nematocera. They are living in the tropical and sub-tropical regions of the world.

1.1 Biology of *Ae. aegypti*

Ae. aegypti mosquito is small in comparison to others, usually between three to four millimeters in length discounting leg length. It is totally black apart from white 'spot' on the body and head regions and white rings on the legs. The thorax is decorated with a white 'lyre' shape of which the 'chords' are two dull yellow lines.

Ae. aegypti life cycle have 4 distinct stages: Egg, Lava, pupa and Adult as show in Figure 2.1

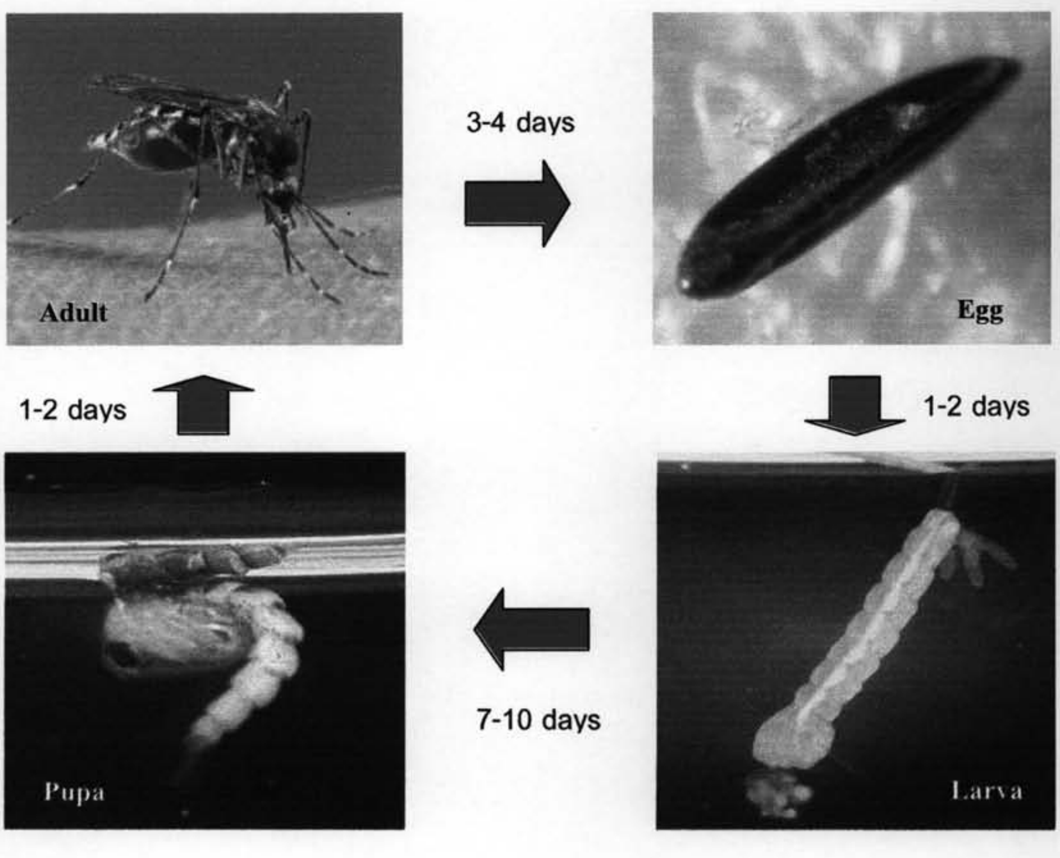

Figure 2.1 Life-cycle of *Ae. aegypti* Mosquito (23).

1.1.1 Eggs

The eggs are typically laid directly singly between 50 to 200 eggs and are produced by the females 2-3 days after a blood meal near water on the inside of containers or tree holes at or above the waterline or less often on the surface. *Ae. aegypti* prefers clean water for the development of the larva. The eggs are laid in areas that will flood with water (24). The eggs can survive for long periods in dry state, often for more than a year (14).

Mosquito eggs assume a number of structural forms. *Aedes* species, lay eggs that are shaped like elongated U.S. style footballs (25). They are generally elongate and protected by a rigid, proteinaceous shell that minimizes water loss but permits gas exchange. Eggs are soft and white when first deposited becoming dark within an hour or two (24).

1.1.2 Larvae

The larvae were hatched quickly when the water level rises (14) and feed by mouthparts that in most mosquitoes are adapted to either collecting and filtering microorganisms and detritus from the water commonly the air /water interface or for scraping material off surface. Initially very small but rapidly grows to size of ¼ inch or longer. The larva lives in the water and comes to the surface to breathe through their air tubes and feed on microorganisms and organic matter in the water.

Mosquitoes pass through 4 larval stages (instars) take 5 and 10 days for development. The larvae are legless, vermiform and although they breathe air, are uniform aquatic. The larval body is divided into 3 distinct regions (24).

The head is formed by a rigid capsule and bears the antennae, eyes, and mouthparts. The antennae are located to either side of the head, towards the front. The eyes are behind the antennae near the hind margin of the head. The mouthparts are at the underside of head near the front.

The thorax is broader than the head or abdomen. The thorax has several groups of hairs useful for identification of species.

The abdomen is composed of 10 segments; the first 7 segments are roughly identical and form a long cylinder. The posterior 3 segments are modified and bear the siphon, 4 anal papillae, a variety of scales, sclerotized plates, and hair tufts. The siphon ends in a pair of spiracles that are the respiratory openings to the air.

1.1.3 Pupae

The pupa is aquatic and unlike the pupae of most insects is motile and active. It will drive and move rapidly with a jerking motion when disturbed. The pupa head and thorax are fused into a large cephalothorax that bears 2 large respiratory trumpets. The trumpets maintain contact with the air when the papa is at the water's surface. The abdomen is a long, muscular cylinder that is used to propel the pupa in the water. The abdominal segments curl under the cephalothorax and terminate with 2 paddles. Abdominal muscles remain functional throughout the pupal stage, and contraction of these muscles provides effective rapid movement for the pupa. Because air is contained in the region of the cephalothorax in which the adult appendages develop, the pupae are buoyant. When placed in cold water to reduce activity, the pupae float to the surface whereas the larvae sink to the bottom, a difference that is often used in separating larvae and papae in colonized mosquitoes.

1.1.4 Adults

The adult mosquitoes are small, fragile and slender body files with long legs. They were divided into 3 regions.

1.1.4.1 The head

The head are small and almost spherical (14). The head bears a large pair of compound eyes, a pair of antennae, a pair of palpi, and the elongate proboscis. The head were joined to the thorax by a narrow membranous connection. The antennae have a whorl of hairs which are short and sparse in the females but long and bushy in the males. The palpi have 5 segments and originate at the lower margin of the head near the proboscis. The proboscis consists of a labium enclosing a group of six stylets, adapted for piercing and sucking. The proboscis is long, slender, projects downwards and forwards from the lower front margin of the head. The labium encloses paired maxillae, mandibles, an epipharynx and a hypopharynx. The hypopharynx carries a salivary duct, through which an anticoagulant is delivered into the hosts tissues. The roof of the proboscis is formed by the labrum.

1.1.4.2 The thorax

The thorax bears the 2 wings, halters and 3 pair of long legs. The overall of the thorax is wedge-shaped and the dorsal is broad end. The upper surface of the thorax is covered with coarse hairs or scales which are variously colored. The sides of the thorax may be covered with scale bristles and bear several groups of the hair or bristles which are useful for identification. The legs is arise from the lower sides of the thorax and consists of a short, conical coax, a small hinge-like trochanter, a stout femur, a long slender tibia and a 5 segmented tarsus. The first segment of the tarsus is the longest in length and is often equal to the tibia. The fifth tarsal segment bears a small pair of claws. The legs are covered with dark or colored scales that form patterns which are often useful in identifying species. The wings are long and narrow with leaf-like, elongate scales on the veins and which project as a fringe along the posterior margins. The varying colors of the scales sometimes form definite patterns which can assist with species identification. The wings folded over abdomen at rest. Two small knobbed structures are located behind and slightly below the wings.

1.1.4.3 The abdomen

The abdomen is elongate and nearly cylindrical. The cylindrical abdomen consists of 10 segments, 8 of which are visible; 2 terminal segments (the ninth and tenth segments) are greatly modified for reproductive functions. The terminal segments of male abdomen are greatly modified for mating. *Ae. aegypti* have a pair of long, filamentous, segmented antennae; conspicuous and plumose in the males and pilose in females.

The females are generally larger and possess mouthparts adapted to piercing. The females is the biting insect everyone is familiar with because generally requires a blood meal as a protein source for egg development before laying eggs that will hatch.

The males have mouthparts that are adapted to feeding on liquid sugar sources such as plant nectars and posses long feathery antennae that are used to detect females prior to mating. Usually they are found around the breeding site and lives on plant juices.

The life span for adult mosquitoes is between 2 weeks to a month or more depend on the temperature and can be as little as three or less during the summer. A female mosquito will live 2-3 weeks but the male's lifespan is shorter. Male mosquitoes may mate many times, whereas female generally mate only once (24).

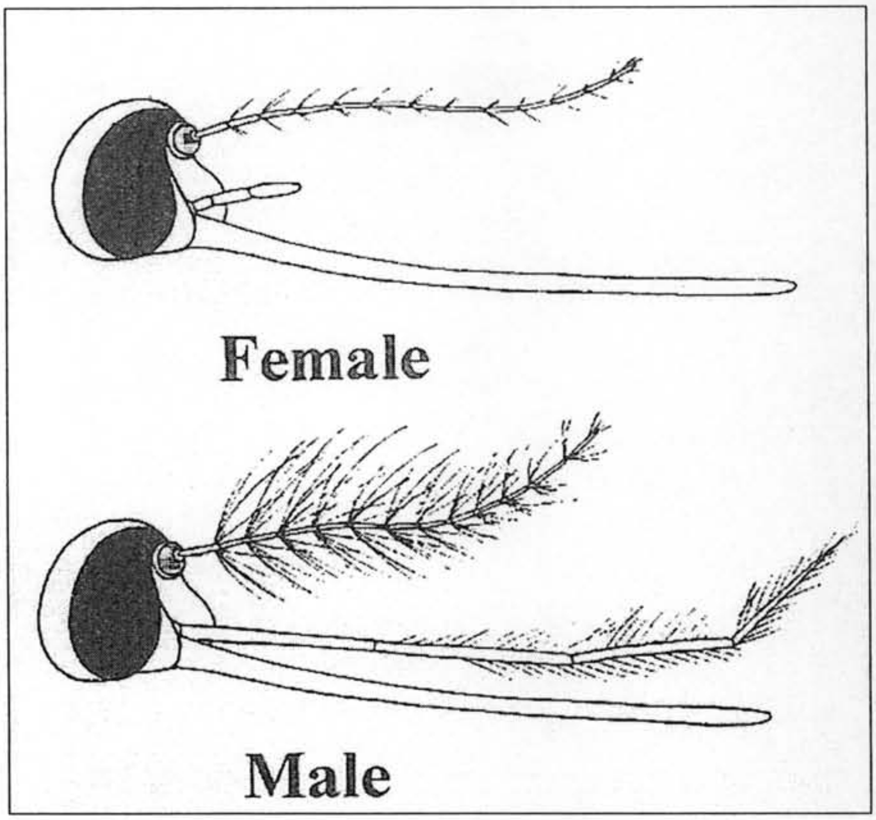

Figure 2.2 Sexual dimorphism in the morphology of antennae. Adult male has dense hairs antennae, whereas adult female has sparse hairs (24).

2. *Wolbachia* spp.

Wolbachia is a gram negative obligate intracellular bacterium (22) and maternally inherited bacteria (21) endosymbionts in the α-proteobacteria (18, 19, 27), that are found in reproductive tissues (ovaries and testes) of a wide range of arthropods species (27, 28, 29). The first description of *Wolbachia* was made in 1924, when it was detected in the ovaries of the mosquito *Culex pipiens* and classified as an unnamed *Rickettsia*. *Wolbachia* has been limited to laboratories with insect-rearing facilities due to the inability to cultivate these bacteria outside of invertebrate hosts and cannot be cultured in defined media and detection within infected gonadal cells (29) but can be simply established and stably maintained in vitro by using standard tissue cell cultures in shell vials (30). *Wolbachia* is maternally transmitted and can rapidly invade insect population through the reproductive distortions that it generates in hosts (22). These maternally inherited symbionts cause various alterations in the reproduction of their hosts which can enhance the spread of the infection in arthropod populations (31), including cytoplasmic incompatibility (CI) (15), parthenogenesis induction (PI), and feminization of genetic males or male killing (32, 33, 34). CI results in a high rate of embryo mortality when the sperm of infected males fertilized oocytes that are uninfected or infected by another bacterial variant (31). Failure of karyogamy, perhaps by delaying nuclear envelope breakdown of the male pronucleus (35), and consequently may promote *Wolbachia* invasion of uninfected populations because infected females are able to mate and produce offspring successfully with both infected and uninfected males, whereas uninfected females are unable to produce offspring when they mate with infected males (15). There are two types of CI: unidirectional or bidirectional. Unidirectional CI is typically occurs when an infected male mates with an uninfected female (36). The reciprocal mating is fully compatible, as are mating between infected individuals (Figure2.2). Bidirectional CI usually occurs in matings between infected in individuals harboring different strains of *Wolbachia* (Figure2.3) (28, 37, 38). CI apparently involves a two component system; bacterial "modification" of the sperm and a bacterial "rescue" in the fertilized egg. These bacteria present in the testes modify the developing sperm. The same bacterial strain must then be present in the egg to rescue this modification. If rescue does not occurs then incompatibility between the egg and sperm results. The model is consists with unidirectional incompatibility; modified sperm from infected males are not rescued by uninfected eggs and bidirectional incompatibility; different bacterial strains use somewhat different modification rescue system. These modifications of host reproduction impart a selective advantage for the bacteria (28). Various surveys have found these bacteria estimated that 16-76% of insect species of infected with *Wolbachia* (15).

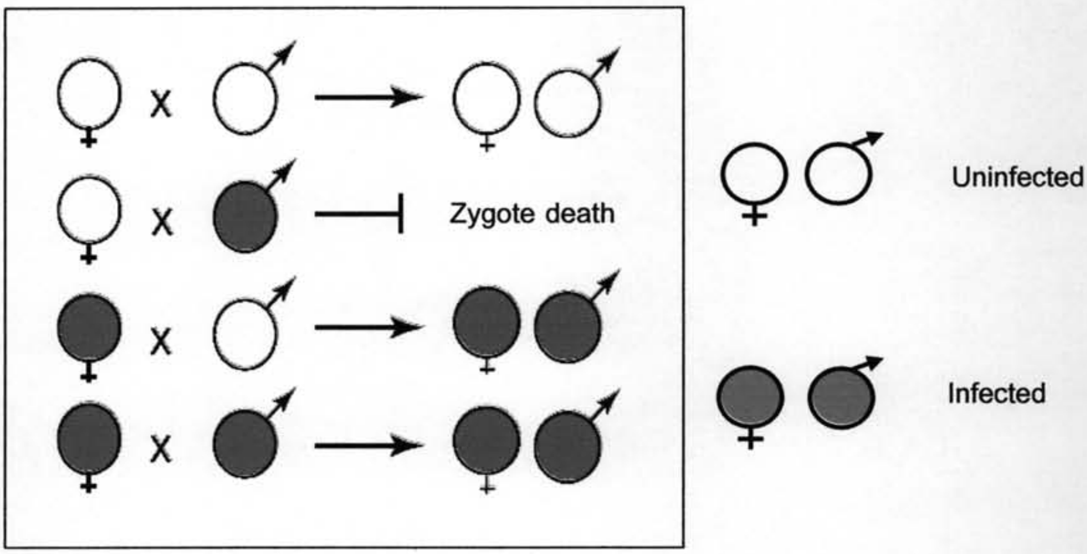

Figure 2.3 Unidirectional CI (37)

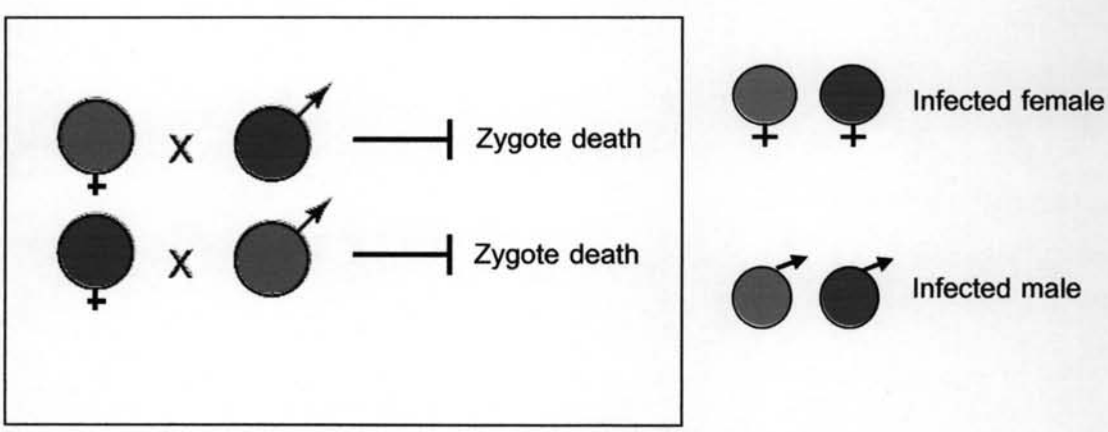

Figure 2.4 Bidirectional CI (37)

They have also been found in isopods, mites and nematode. Eight major *Wolbachia* "supergroups" (A-H) exist based on phylogenetic clustering of *FtsZ* and 16 S rDNA gene sequences. A, B and E infect diverse arthropods; C and D infect nematodes; G infects spiders; H infects termites and F infects both arthropods and nematodes (19).

Although CI expression and other forms of reproductive parasitism have made *Wolbachia* an evolutionary success, with an estimate that infections occur in ~20% of infect species (28), *Wolbachia* infections do not naturally occur in *Ae. aegypti* (15, 39) but in *Ae. albopictus* is known to represent a true superinfection "*w*AlbA and *w*AlbB" and not multiple copies of diagnostic genetic loci in a single *Wolbachia* type. There fore, the establishment of *Wolbachia* infection and induction of CI expression in *Ae. aegypti* would be an essential prerequisite to possible application of *Wolbachia* as a transgene drive mechanism to aid in genetic control of this important vector species (15).

3. Bed bugs (*Cimex hemipterus*)

The *C. hemipterus* is insects in the family Cimicidae are obligatory hematophagous ectoparasites of birds, bats and humans (18, 19). *C. hemipterus* is considered as the common tropical bed bug (40). They were once a common public health pest worldwide. Bed bugs are wingless insects, roughly oval in shape, 4-5 mm long when fully ground and fast runners. They are not evenly distributed throughout the environment but are instead concentrated in harborages (40). They can hide in narrow cracks and crevices in walls, furniture, behind wallpaper and wood paneling or under carpeting (41). Although, they are not commonly considered as vectors of disease but bed bugs infestations can cause considerable distress to humans since bed buds bites are caused probably by vasodilatory substances in the saliva and the subsequent allergic response is caused probably by other xenogenic constituents of bed bug saliva injected during feeding and they are usually only active during night but will feed during the day when hungry (40).

4. *Wolbachia* transformation (transinfection)

In 1994, Henk R. et al. (42): to determine bidirectional CI. They could be successfully to transfer *Wolbachia* from the Asian tiger mosquito: *Ae. albopictus* into naturally populations to replace the naturally infected of *Drosophila simulans* by embryonic microinjection and capable to induce CI expression by determined crossed with uninfected files.

In 2000, Tetsuhiko Sasaki and Hajime Ishikawa (43): to determine the difference of CI levels depends on *Wolbachia* or the host. They could be performed transinfection in Mediterranean flour moth *Ephestia kuehniella* by embryonic microinjection and successfully transferred *Wolbachia* from the Yokohama strain into the Tsuchiura strain and CI expression have been a level near the Yokohama strain. Since both Yokohama and Tsuchiura strains were infected with *Wolbachia* that belonged to the A supergroup but Yokohama strain expression CI at a higher level than the Tsuchiura strain. Before establish transinfection, *Wolbachia*-uninfected of Tsuchiura strains were reared on a diet containing tetracycline. The ooplasm of Yokohama ovaries was injected into the eggs of Tsuchiura by using the microinjector. After adults emerged, the males that infected were mated with uninfected females to determine CI.

In 2004, Markus Riegler et al. (44): could be successful to transfer of two *Wolbachia* (*w*Cer1 and *w*Cer2) from the cherry fruit fly *Rhagoletis cerasi* eggs into uninfected *Drosophila simulans* eggs by using cytoplasmic injection with a microneedle at the posterior part. They can perform to high levels of *Wolbachia* transmit into offspring and fitness costs but at a low levels of the CI expression. In addition, individual of *w*Cer1 cannot to develop in the new host because its raid loss after successful injection. In the other hand, *w*Cer2 can establish in the new host but low level of transmission rates.

In 2004, Sofia Zabalou et al. (45): could be successful to establish *Wolbachia* infected lines in uninfected of the medfly *Ceratitis capitata* from naturally infected of the cherry fruit fly *Rhagoletis cerasi* by using embryonic cytoplasmic microinjection to transfer embryo cytoplasm of mature oocytes at the posterior pole of the preblastoderm embryos by a microcapillary needle. They can support *Wolbachia* infections and express complete CI in the novel host.

In 2005, Zhiyong Xi et al. (21): could be successful to applied mosquito control strategies and the evolution to transferring *Wolbachia* (transinfection) in a medically important mosquito species by using embryonic microinjection to transfer embryo cytoplasm from a double-infected (*w*AlbA and *w*AlbB) *Ae. albopictus* line into an aposymbiotic line is single-infected with the *w*AlbB *Wolbachia* type. The researcher could be to generate artificial *Wolbachia* infections and new CI crossing types represent an important advance toward implementation for suppression and replacement of mosquito vector populations. They are capable to experiment of the bidirectional CI but can not to induce complete CI.

In 2006, Ruang-areerate T and Kittayapong P (15): could be successful *Wolbachia* transfer and establishment of double strains of *Wolbachia* from *Ae. albopictus* (*w*AlbA and *w*AlbB) via directly microinjection into newly emerged adult females of naturally uninfected *Ae. aegypti*. In this study, the researcher clearly shows that crosses of AegW males with uninfected *Ae. aegypti* females result in significantly lower hatch rate than other crosses and that CI expression is not affected by multiple matings. The level of CI expression corresponds with *Wolbachia* density within the AegW host. Furthermore, they can demonstrate that there is potential for using *Wolbachia* as a gene-driving system by integrating *Wolbachia* with transgenic mosquitoes it may be possible to manipulate vector populations genetically. But in this research, they are only to capable incomplete CI.

Since, some of the research at previous described on above could be establish the transinfection of *Wolbachia* from same approach species and successful of incomplete CI expression only. So, we would like to test the CI expression in *Ae. aegypti* by using *Wolbachia* from the *C. hemipterus*, because bed bug has been infected with *Wolbachia* in many regions of the world, it may produce high CI expression in *Ae. aegypti* mosquitoes and to determine cross transinfect from different insect in order to verify that this bacteria can be adapt to live in the novel hosts.