Insects in Scientific Research Advancement

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Learning Objectives

The present chapter focuses on the advancement of the scientific research in insects. Considering the space constraint, following are the major objectives, which will help to build up the general concept of the chapter:

1. To highlight the role of insects in various fields of science and technology.
2. To help the readers realize the role of non-model insects in the fundamentals of agriculture, microbiology, molecular biology.
3. To comprehend the concept of Sterile Insect Technology (SIT) and its role in Integrated Pest Management (IPM).
4. To make the readers to comprehend the endosymbionts and their role in ecology.
5. To understand the underlying mechanism of the diversity in the wing patterns of butterflies.
6. To decipher the underlying mechanisms and signalling involves in the limb regeneration of insects.
7. To underscore the concept of sexual selection, this is an important evolutionary process.

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1   Introduction

Before the start of the chapter, let us answer a question "what is an insect and do we actually need to care about these creepy crawlies?" According to the words of American biologist Edward O Wilson "yes, they are little things that run the world" Insects make up more than half of the living organisms on the earth, which accounts for more than 10 lakh species. Insects inhabit almost all habitats across the globe except for the deep sea seven in the harshest of environments. Insects provide many ecosystem services, in their role as pollinators, decomposers, recyclers and much more. Their presence on dead bodies have enabled the resolution of crimes and developed into an entire field of forensic entomology. Insect contribution to education over the past four decades has been unparalleled thanks to the small but mighty fruit fly, *Drosophila melanogaster*. Other than fruit flies, another beetle i.e., red flour beetle, *Tribolium castaneum*, has also been useful to understand the body plans during development. This can be easily manipulated by the use of RNA interference (RNAi) technique. There are other insect models which have opened the paths for novel scientific studies. The present chapter is an attempt to throw some light on the recent scientific advances using insects as the model organism.

2   Insects in Microbiology

The close bond between insects and microbiota usually in the form of symbiotic associations is recognized as a result of evolutionary selection. Many insects are associated with several microbes during their lifecycle. The harbouring of bacteria by insects marks the onset of microbial symbiosis. This is followed by the coevolution of the bacterial species and the insect, till the insect gut makes a comfortable home for the bacteria so as to enable prolonged sustenance and successful transmission across generations. Studies revealed the major role of evolutionary processes within the insect body leading to the conversion of free-living microbe to an obligate and facultative symbiont resulting in the diversification of symbiotic relationship.

2.1   Male Killing Bacteria and Insects

Male killing bacteria are known to derive from different clades. Molecular systematic studies involving DNA sequencing of bacteria or polymerase chain reaction of infected and uninfected lines have supported this line of though. For example, male killing bacteria predominantly belongs to the genus *Spiroplasma* (Mollicutes), the Flavobacteria-bacteroids group and the gamma and alpha subdivisions of proteobacteria.

Those male killing bacteria which are associated with arthropod bacterial clades, are not themselves male killers. On the basis of their transmission, these clades can be separated into two types i.e., complete horizontal transmission or a mix of horizontal and vertical transmission and second is vertical transmission. *Spiroplasma* and *Rickettsia* form the first type. In *Spiroplasma*, transmission is either horizontal or mixed vertical or horizontal. *Rickettsia*, most commonly has a mix of horizontal and vertical transmission. Earlier *Rickettsia* was regarded as one of the vertebrate pathogens borne by arthropods, but now it has been recognised to be transmitted after feeding on the plant host. In second type of clade, vertical transmission rate exceeds the horizontal transmission. *Wolbachia* and flavobacteria lineage are associated with this group. *Wolbachia* is mainly maintained by the manipulation of their host's reproduction. This trait of bacteria is maternally inherited in arthropods via multiple modes of transmission.

2.1.1 Role of Different Insects in the Study of Male Killing Bacteria

a. In Butterflies

There is low to intermediate prevalence of male killing bacteria in natural population with only mild effects on the sex ratio of host population. There are many arthropods that act as the host to intracellular bacteria transmitted vertically from hosts to their progeny via egg cytoplasm (Buchner, 1965). Sex ratio distortion induced microorganism is now well known in arthropods including the induction of parthogenesis, feminization of genetically male hosts and selective killing of sons (O'Neill et al., 1997). There are five insect orders in which male killers are well known and they are not restricted to particular host genetic systems despite varying sex determination mechanisms. In butterflies, scientists have analysed the entire DNA sequence of the bacteria and female butterflies' chromosomes. They revealed that the Neo-W chromosomes alters the colour patterns and has spread rapidly through the population aided by the male-killing bacteria. In *Hypolimnas bolina* extreme prevalence of *Wolbachia* has been recorded (Charlat et al., 2005) (Table 1). The female butterflies are heterogametic and have the chromosomes Z and W. A neo-W chromosome in African Queen monarch butterfly, *Danaus chryippus*, is formed by the fusion of an autosome and W-chromosome. This chromosome is widely found in the majority of females in hybrid zone of *D. chrysippus* (Smith et al., 2016). These females arise from the infection by *Spiroplasma bacterium* (Jiggins et al., 1999) which causes the fatality of males as eggs. Another family of butterfly which is known to be infected by highest number of *Wolbachia* strain is Lycaenid butterfly. *Wolbachia* may play important role in mitochondrial structure, possible hybridization and even evolution of these butterfly species (Bartoňová et al., 2021).

b. In Drosophila

Studies in *Drosophila* with respect to male killing bacteria *Spiroplasma,* begun in the 1950s. The many studies since then have eventually led to the development of understanding of the genetic basis of male killing. The two bacterial symbionts of

Table 1 Prevalence of *w*Bol1 in male and female *Hypolimnus bolina* from different populations as detected by PCR assay

| Country	Population	Map	Prevalence in females (%)	*n*	Prevalence in males (%)	*n*	References
Malaysia	Kota Kinabalu	1	96	25	100	19	This study
Vietnam	Mekong Delta	2	100	8	100	10	This study
China	Hong Kong	3	100	3	100	3	This study
American Samoa	Tutuila	4	0	6	0	10	Dyson and Hurst 2004
	Olosega	5	0	23	0	25	This study
Fiji	Wayalailai	6	54	76	0	8	Dyson et al. 2002; this study
	Viti Levu	7	59	34	0	9	Dyson et al. 2002
	Taveuni	8	25	24	–		Dyson et al. 2002
Independent Samoa	Savaii	9	100	35	50	2	Dyson and Hurst 2004
	Upolu	10	99	257	33	3	Dyson and Hurst 2004
French Polynesia	Rurutu	11	73	62	0	57	This study
	Tubuai	12	0	19	0	31	This study
	Ua Huka	13	86	43	12	25	This study
	Moorea	14	96	25	0	18	This study
	Tahiti	15	100	14	17	6	This study

Sample sizes are indicated in brackets. (–) indicates no sample obtained. Map (Map reference, Fig. 1)

Drosophila, *Wolbachia* and *Spiroplasm* have independently evolved male killing ability in an organism. Two phenotypes of *D. bifasciata* with male killing bacteria *Wolbachia* strain were used to conduct the comparative studies with the *Spiroplasma* strain found in *D. melanogaster*. The studies has revealed that DNA damage and its segregation in *Spiroplasma* is activated by its infection which causes the defects on the male X-chromosome, thereby activating male specific apoptosis in *Drosophila*.

2.2 Sterile Insect Technique (SIT)

Sterile insect technique, which deals with the release of laboratory bred sterile males into the wild so as to out compete the fertile males was first proposed in the1930s and 1940s by A.S. Serebrovskii at Moscow State University, F.L. Vanderplank at a

Fig. 1 Logistic population model
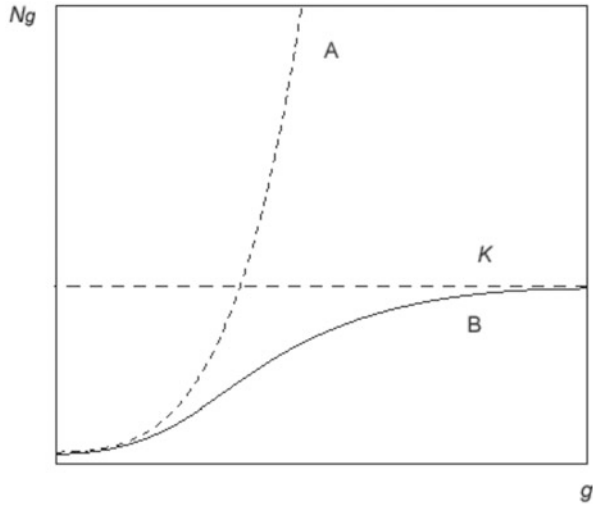
Tsetse Field Research in Tangangiska and E.F. Knipling of the United States Department of Agriculture. This technique involves rearing of target species, subjecting them to gamma irradiation for inducing sterility and then introducing them into the target population. The introduce sterile males compete with potent males in the wild for access and mating success with females, which may all likelihood cause reduced or disrupted insect reproduction (Knipling, 1955). But, this technique is effective only in those insect species who mate only once in their life time.

Sterile insect technique is a very potent device for mapping of sparce and isolated pest populations. It is a highly targeted form of pest management by regulation of natality. One of the major strategies of SIT includes the Area Wide Pest Management for coping with pest problems (Kogan, 2000), which was strongly supported by Knipling as a strategy independent to SIT (Klassen, 2003).

Area wide integrated pest management (AW IPM) is defined as a kind of integrated pest management technique that applied against an entire population of pest within a delimited geographical area with a minimum protection size of a buffer zone so that natural dispersal of the population occurs only within this area.

Technical Components of the SIT

The important principles of population and behavioural ecology in relation to application of SIT include:

1. **Logistic population model** estimates the fluctuation in the population of a targeted organism (Fig. 1).
2. **Mark and recapture** estimates the density and mortality rate of the target population.
3. **Models of dispersal** to assess the dispersal distance of target population.

4. **Equations** for estimating the decrease of sexual competitiveness of mass reared strains under field conditions (Fig. 2).

2.2.1 Mass Rearing for SIT

Sterile insect techniques majorly depend on the population with the high proportion of sterile matings that helps to reduce the population below the level of maintenance.

Therefore, the sufficient production of insects in is one of the principle requirements for this technique. The use of SIT into an area wide IPM is economical as compared to the other techniques. Large quantities of insects are required for the use of SIT into AW IPM programme. Some key publications for the main SIT pest target groups are:

1. Moths—Zethner (1980), Davis (2009), Dyck (2010), Xsit (2018), Boersma (2021).
2. Screwworms—Brown (1984), Scott et al. (2017).
3. Fruit flies—Schwarz et al. (1985), Cáceres et al. (2014)
4. Weevils—Shimizu et al. (2007)

Mass rearing of insects is done for many reasons i.e. for bioassays, physiological research, post harvest treatment testing etc. For all the above mentioned purposes, cost of rearing is not critical and once a diet is developed, very little work is further pursued. However, for SIT we need to give a special attention to the vital factors affecting quality, fecundity and cost. The rearing for the SIT is distinguishable from the insect rearing as it requires large area for rearing for example EI Pino factory in Guatemala (Fig. 3) has the capacity to produce more than 2000 million Mediterranean fruit flies.

2.2.2 Strategy for Using SIT in IPM

There are basically four strategies to use SIT for area wide pest management i.e. suppression, eradication, containment and prevention; pros and cons of each of which are addressed below.

1. *Suppression*—The main objective of this programme is to maintain the pest population below the economic injury level. In recent years, pest population suppression is increasingly being considered as a suitable strategy for SIT in AW-IPM for some pests (Hendrichs et al., 2007). This is due to: (1) Improvements in rearing and releasing techniques for some pests leading to cost effectiveness of SIT (Caceres et al., 2004), (2) Enhanced restrictions on insecticides (Whalon & Mota-Sanchez, 2008), and (3) preference for organic products (Greene et al., 2017).
2. *Eradication*—It involves phytosanitary measures for localised pest elimination. This also reduces insecticide usage. This strategy is usually deployed in two situations:
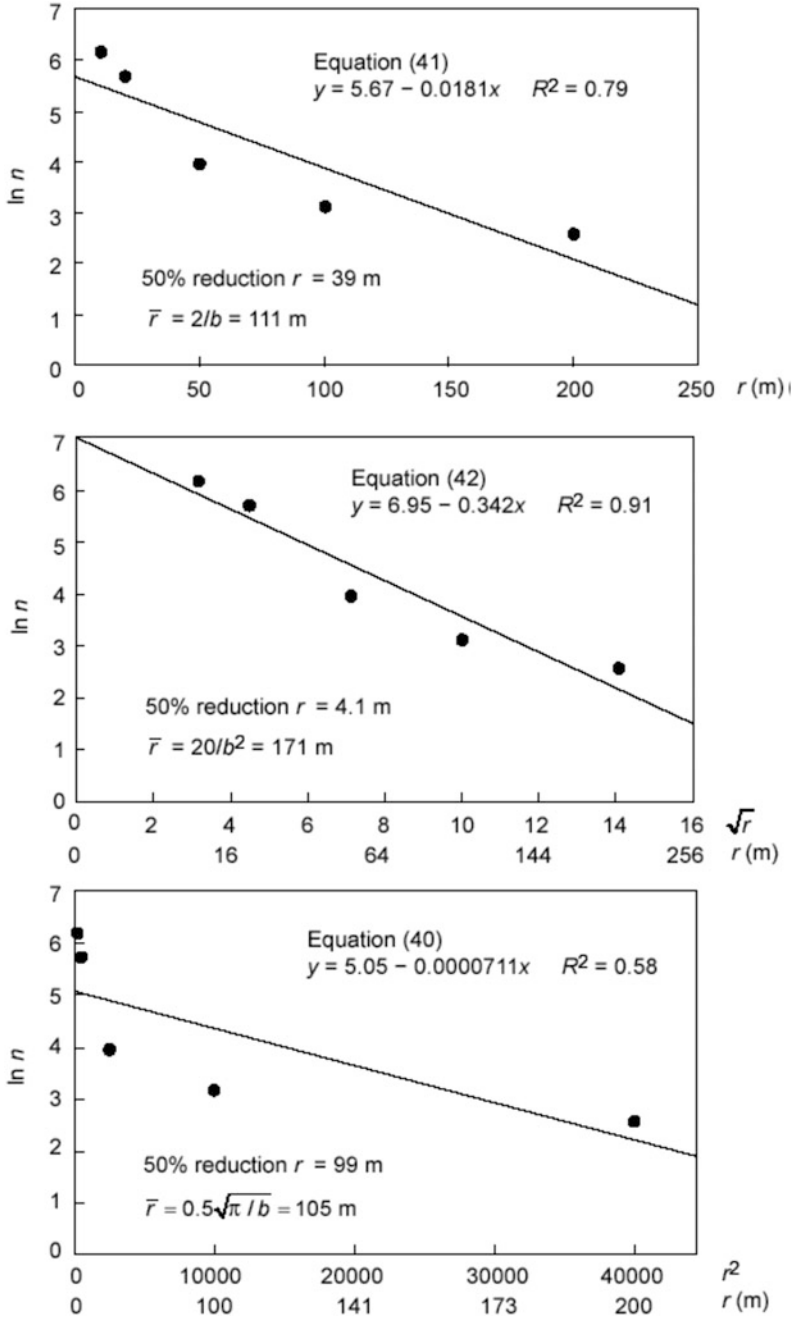
Fig. 2 Distance-density curve of number of marked adult sweetpotato weevils recaptured in traps. Equation is fitted by linear regression. (Data from Miyatake et al. 2000)

Fig. 3 EI Pino factory in Guatemala for mass rearing required for SIT

  (i) Eliminating the established population, for example, tsetse fly in Unguja island (Zanzibar) (Vreysen et al., 2000).
 (ii) Eliminating the outbreak of exotic species before full establishment of the pest can occur. In order to declare eradication of pest: (1) releases of sterile insects should continue after the detection of last wild insect, and (2) continued monitoring after releases should have stopped.

3. *Containment*—It is a measure that prevents the spread of a pest in and around the infested area. Various programmes are used to contain the spread of the pests that have invaded and established in part of an area where the eradication programme is ongoing. Out of these programmes some are largely stationary and became permanent containment efforts, whereas others successfully advance or gradually retreat and eventually collapse. Various other population control tools have been integrated in areas and hotspots where pests are too high to implement SIT.

2.3 Endosymbionts

Insects possess extremely efficient immune responses which help them in managing pathogenic onslaught. Endosymbionts are found in 95% of insect species. Endosymbionts such as *Wolbachia* and *Spiroplasma*, inhabit the haemolymph where they interact with humoral immune components (Haselkorn, 2010). These symbionts can be used for management of pests via manipulation of symbionts and its association

with the host. This can be attained via high throughput sequencing, functional and comparative studies and gene editing technologies. The application of these techniques on mutualistic symbionts have added extra properties to their hosts, facilitating them to survive in the novel ecological conditions, having novel impact on the enemies, pesticides and also affecting the other trophic levels. *Buchnera* is one of the deeply investigated cases of endosymbionts providing the nutrition in aphids and *Wolbachia* as protecting symbionts in the flies.

2.3.1 Symbiosis and Its Types

Symbiosis was first defined by German botanist and mycologist Heinrich Anton de Bary in De Bary, 1879 in his monograph "Die Erscheinung der Symbiose". According to him, symbiosis was a phenomenon in which "dissimilar organisms live together" (Oulhen et al., 2016). In 1952, Paul Buchner gave the modern concept of symbiosis in his book "Endosymbiose de Tieremitpflanzlichen Mikroorganism" (Steinhaus, 1953). In his book he described different symbiosis of insects with the main focus on the anatomy of the microorganisms (Feldhaar, 2011). Symbiotic relationships ranges from mutualism to commensalism or parasitism on basis of effect of symbiont on host (Baumann, 2005).

2.3.2 Diversification in Symbiotic Relationship in Insects

Several microbial symbionts live within or on the body of insects (Baumann, 2005; Su et al., 2013). Those on the outsides usually assist with the breakdown of complex substrates into the simpler forms, which are easy for consumption (Sudakaran et al., 2017). Such symbionts are commonly found in gardens or galleries of fungi farming insects. The symbionts residing within insects are called gut microbes (Engel & Moran, 2013), extracellular symbionts, and intracellular symbionts (also called endosymbionts). More deep the microbes reside, the more intimate and close relationship it develops with the host insect. Thus, it can be concluded that the endosymbiosis the most intimate relationship between the two organisms which is maintained through several generations of the host (Su et al., 2013).

A wide range of symbioses occur across the insect taxa on the basis of biology and evolutionary history (Sudakaran et al., 2017). There are many obligatory symbionts (Primary or P-) which are essential for the survival and reproduction of the host (Haine, 2008) by supplying them the essential nutrients which are either rare or absent in the host's diet (Engel & Moran, 2013). Such symbionts usually live in a special organ called a bacteriome and show vertical transmission (Moran et al., 2008).

The best-known P-symbionts are *Buchnera aphidicola* in aphids, *Portieraal eyrodidarum* in whiteflies, *Carsonella ruddii* in psyllids, and *Tremblaya princeps* in mealybugs (Su et al., 2013).

Facultative symbionts (secondary or S-) do not affect their hosts significantly, but may affect the important traits of the hosts, such as reproductive ability, defense mechanism, thermal regulation, nutrition, etc. (Moran et al., 2008). There are many subdivisions of S type symbionts whose functions are facultative mutualistic, facultative reproductive, etc. (Moran et al., 2008; Wernegreen, 2012; Su et al., 2013). These symbionts are irregularly distributed within various tissues either intra-or extracellularly (Sudakaran et al., 2017). They are mostly vertically transmitted but few are transmitted horizontally also between individuals as well as species (Haine, 2008; Ferrari & Vavre, 2011).

The third category consists of extracellular microbes which infect the guts of insects and are present in the gut lumen or special posterior side of the midgut structure known as crypts or caecae (Fukatsu & Hosokawa, 2002; Sudakaran et al., 2017). These symbionts are either orally or vertically transmitted to the nymphs by post hatching mechanisms (Buchner, 1965; Fukatsu & Hosokawa, 2002; Kashkouli et al., 2021a, 2021b). Members belonging to this category may contribute in making the fitter host by providing sufficient nourishment, cycling of nitrogen or detoxification of plant defence chemicals (Sudakaran et al., 2017).

Sex Ratio Distortion

The distortion in the population sex ratios by male killing bacteria may influence reproductive strategies of their hosts. Generally, the operational sex ratio is male biased in the sexually reproducing organisms, females being the limiting sex. This results in the male competition for access to mates, while females are choosy. In case of female biased sex ratio, the selection for males to compete and females choosiness will reduce. Such the reversal in sexual roles has been reported in the sibling species of *Acraea* butterflies (Jiggins et al., 1999). The population in which the ratio of sexes is more than 20:1 has been reported in favour of females. Females are less promiscuous than the males but can achieve more number of copulations as compared to the males. The result of the studies in both the species revealed that female compete on lekking sites and males visit the aggregations of females to mate.

2.3.3 Significance of Insect Symbionts

The endosymbionts play a very vital role in ecology of insect as they facilitate digestion of food, providing nutrition, plant-insect interactions, heat tolerance, detoxification of pesticides and the protection against the natural enemies (Feldhaar, 2011).

There are many insects which depend on the single source of food and other supplementary nutrients (Akman et al., 2002; Engel & Moran, 2013). There are many blood-feeding arthropods which are obligatory symbionts providing vitamins B such as *Wigglesworthia glossinidia* found in the tsetse flies (Akman et al., 2002), *Rhodococcus rhodnii* in *Rhodnius prolix* (Eichler & Schaub, 2002). Insects feeding on the plant sap are associated with the symbionts which provide them those nutrients which are lacking in vitamins and amino acids (Sasaki et al., 1996). The

role of symbionts in nutrition has been extensively studied in aphids. With few exceptions, most species of aphid, possess intracellular bacteria of the genus *Buchnera* (Brownlie & Johnson, 2009). The complete genome sequence annotation of *B. aphidicola* revealed that the genetic capacity of these bacteria to synthesise several amino acids are known to be essential for hosts (Shigenobu et al., 2000; Douglas, 2009; Smith & Moran, 2020). In *Carsonella rudii* and *Sulciam uelleri* it has been reported that similar gene content and order has been maintained among strains for biosynthesis of amino acids (McCutcheon & Moran, 2012; Hansen & Moran, 2014).

Furthermore, plataspid bug *Megacopta punctatissima* and pentatomid stinkbugs *Acrosternumarabicum* and *Halyomorpha halys* harbor crypt-associated symbionts 'Candidatus Ishikawaella capsulata', 'Ca. Pantoeapersica', and "Ca. Pantoeacarbekii", respectively (Kashkouli et al., 2021a, 2021b; Nikoh et al., 2011; Kenyon et al., 2015). These symbionts feature reduced genomes that encode the biosynthesis of essential amino acids and vitamins (Kashkouli et al., 2021a, 2021b; Nikoh et al., 2011; Kenyon et al., 2015; Sudakaran et al., 2017).

2.3.4 Impacts of Symbionts on the Host Population

The impact of symbionts have been studied on the dynamicity of population of the host, their demography, and their genetic diversity (Feldhaar, 2011). There are several ecological models which have focused on the population dynamics of the symbionts in the host transmitted vertically or horizontally (Haine, 2008). There are different endosymbionts such as *Wolbachia, Arsenophonus, Spiroplasma* and *Cardinium* which significantly influence the population dynamics through different ways like cytoplasmic incompatibility (CI), parthogenesis induction (PI), feminization, and male-killing (Su et al., 2013). In cytoplasmic incompatibility, eggs which are not infected, are fertilized by the sperm of infected male resulting in its death. Thus this benefits the females and also facilitates the spread of symbiont through the host populations (Zug & Hammerstein, 2015a, 2015b). In parthogenesis induction, viable diploid females were formed from the unfertilized haploid host eggs which may result in a rapid decline of genetic diversity and increased percentage of the transmitting hosts (Su et al., 2013; Zug & Hammerstein, 2015a, 2015b). In feminization, infected males reproduce as the females. The endosymbionts may also have the impact on traits which are relevant for the dispersal and influence the population of the host insects (Feldhaar, 2011). Pea aphids containing *R. insecticola*, produce only half the number of winged offspring in response to crowding than those lacking this endosymbiont (Feldhaar, 2011). In contrast to this, Goodacre et al. (2009) demonstrated a link between the presence of *Rickettsia* in a pest controlling money spider, *Erigoneatra*.

2.3.5   Application of Symbionts in Pest Control

Symbionts are known to be the demanding novel pest management strategy. Four main approaches for this strategy includes: (1) utilization of heterologous microorganisms, (2) paratransgenesis, (3) insect incompatibility technique (IIT), and (iv) the disruption of microbial symbionts required by insect pests.

(i) **Heterologous associations**

In the recent, numerous studies in *Wolbachia* have been reported that infection has an anti-pathogenic effect in the host (Zug & Hammerstein, 2015a, 2015b). *Wolbachia* strains can also enhance the resistance against various insects viruses in *Drosophila*, *Aedes aegypti, Aedes gambiae*. Thus, microbial symbionts can be helpful in containing various devastating diseases such as malaria, dengue, yellow fever, and Chagas. These relations are the result of the experimental transfer of microorganisms from one species to another species. For example *An.* species and *Ae. aegypti* do not harbour *Wolbachia* naturally, so the transinfection of *Wolbachia* into mosquitoes is required (Iturbe-Ormaetxe et al., 2011; Walker et al., 2011).

(ii) **Paratransgenesis**

It is alteration in the traits of insect by genetic manipulation of the associated microorganisms (Beard et al., 2002). The technology of paratransgenesis was proposed back in early 1990s especially in relation to the mosquito vectors of human diseases (Arora & Douglas, 2017). It is widely known to be the alternative method of transgenesis (direct genetic manipulation of the insects). It could help to overcome the problems of transgenesis without any genetic drive system which is required for transgenes in insects and also the instability of transgenes in insect genomes (Arora & Douglas, 2017; Qadri et al., 2020). The key requirements for paratransgenesis are the microbial partners, which can be cultured, transformed and can be transferred to the host insects to facilitate their dissemination (Beard et al., 2002). Best suited microbes for paratransgenesis are gut bacteria due to the aforementioned features. Example of such microbe is genetically amenable gut bacteria, i.e. *Sodalis glossinidius*, *Asaia* sp., and *Pantoeaag glomerans*, represent a valuable resource for paratransgenesis (Engel & Moran, 2013).

(iii) **Incompatible insect technique (IIT)**

It is a potential *Wolbachia* based approach to control the insect vectors (Stouthamer et al., 1999). This mechanism of *Wolbachia* based on the induced CI, manipulates the natural populations of pests through embryonic lethality. CI can be unidirectional, i.e. crosses between infected males and uninfected females) or bidirectional i.e. target population is uninfected. On the basis of the unidirectional CI, there is the method called "population replacement" (Bourtzis et al., 2014) where infected females are introduced in the targeted population to suppress it. In such case, *Wolbachia* plays an important role to limit the transmission of pathogens, directly or indirectly (Bourtzis et al., 2014).

3 Insects in Morphological Studies

In previous chapter, insects in the developmental biology have been focused due to their peculiar development such as presence of centrolecithal eggs undergoing the superficial cleavage. The current studies of developmental biology have given the comprehensive knowledge of the *Drosophila* model. However, there are morphological developments, which make the insects peculiar in one or the other. Here we will be discussing the role of science in discovering the mechanisms involved in patterning the wings of butterflies and also we will be discussing the limb regeneration in insects, which have recently attracted the attention of various scientists.

3.1 Wing Pattern in Butterflies

There are variety of patterns on the wings of butterflies. About 1800 species of butterflies can be distinguished from the pattern of their wings. The elements that make up the complete colour pattern in butterflies are different from those of the vertebrates as these elements in butterflies can be traced from species to species and typically across genera and families. These patterns in the butterflies are not the object that can be dissected out but it is rather a product of localised event of pigment synthesis which results due to the activation of various enzymatic pathways.

3.2 Structure of Wing Pattern

The organising principle which influences the wing patterns in the butterflies is the symmetry system (Fig. 4). This symmetry system includes pigment bands that are
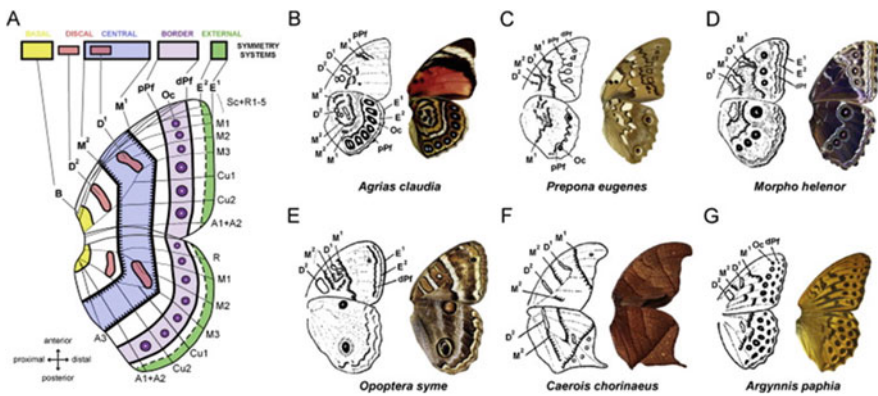

Fig. 4 Symmetry systems in butterflies

parallel to each other and runs from anterior to posterior end. The basic wing pattern are made up of the three system of bands i.e., basal symmetry, central symmetry and the border symmetry system.

Along the midline of the border and central symmetry system, there is often a set of pigmented marks. The veins present in the wings act as boundaries that compartmentalize the wing surface with respect to colour pattern formation. This results in the in the three compartmentalization which leads to the three symmetry systems into parallel series of isolated and independent pattern elements.

Genetics and Developmental Basis of Wing Pattern in Butterfly

As mentioned in the previous section that the major impediments to evolutionary innovation is the constraints on genetic change imposed by existing function.

Studies on the butterflies' wing pattern are good connecting link between the genetic changes in the developmental processes that leads to the diversity (McMillan et al., 2002). Wing development is known to be highly conserved process in insects showing the similar expression of genes between flies and butterflies (Weatherbee et al., 1998). As mentioned previously, butterfly wing patterns are derived from the conserved pattern elements known as symmetry systems.

The first symmetry system with its molecular characterization of was developed by showing the expression of Wnt A genes, which are in association with the major basal, discal, central and external symmetry system patterns of Nymphalid butterflies (Martin & Reed, 2014). Several recent studies have narrowed down the genetic basis of wing pattern variation to single gene explaining phenotypic switches involved in mimcry and sexual selection (Reed et al., 2011; Martin et al., 2012; Gallant et al., 2014). Thus, wing pattern in butterflies combine several unique properties which helps to understand the evolutionary development through genetic basis of phenotypic variation between populations (Fig. 3). Wnt family was considered as the important candidate for symmetry system due to the following reasons: Techniques such as grafting ablation and pharmacological manipulations are known to be involved in determining symmetry system colour pattern in some moths and butterfly (Monteiro et al., 2001; Toussaint & French, 1988; Serfas & Carroll, 2005).

In butterflies, wing pattern belongs to the class of intervein. These patterns occur in range of shapes including eyespot ellipses and midlines and are known to evolve through developmental shifts along a midline to eyespot. At the molecular level it has been studied that Notch (N) upregulation followed by activation of the transcription factor Distal less (DII) leads to the early development of eyespot and intervein midline pattern (Fig. 4) (Reed & Serfas, 2004). Another morphogen, which is associated with the early determination of major basal, discal and marginal strip patterns in Lepidoptera is wingless (Wg). Wg gained interest after it was recently identified as the first known morphogen involved in colour pattern (Werner et al., 2010). In following section, we will be discussing about the eyespot which is considered as important evolutionary tool to study wing pattern in butterfly.

3.3 Eyespot in Butterfly

3.3.1 Origin of Eyespot

Butterfly eyespot is an important example to study the evolutionary recruitment of the developmental gene leading to the rise of novel character system and their diversity under the influence of natural selection (Nijhout, 2001; Mcmillan et al., 2002) (Fig. 5). Eyespots are known to be originated from Nymphidae present at the base of its sister Danainae (Fig. 5). Eyespot is defined by the presence and absence of the defined circular spots with light and dark coloured concentric rings of different
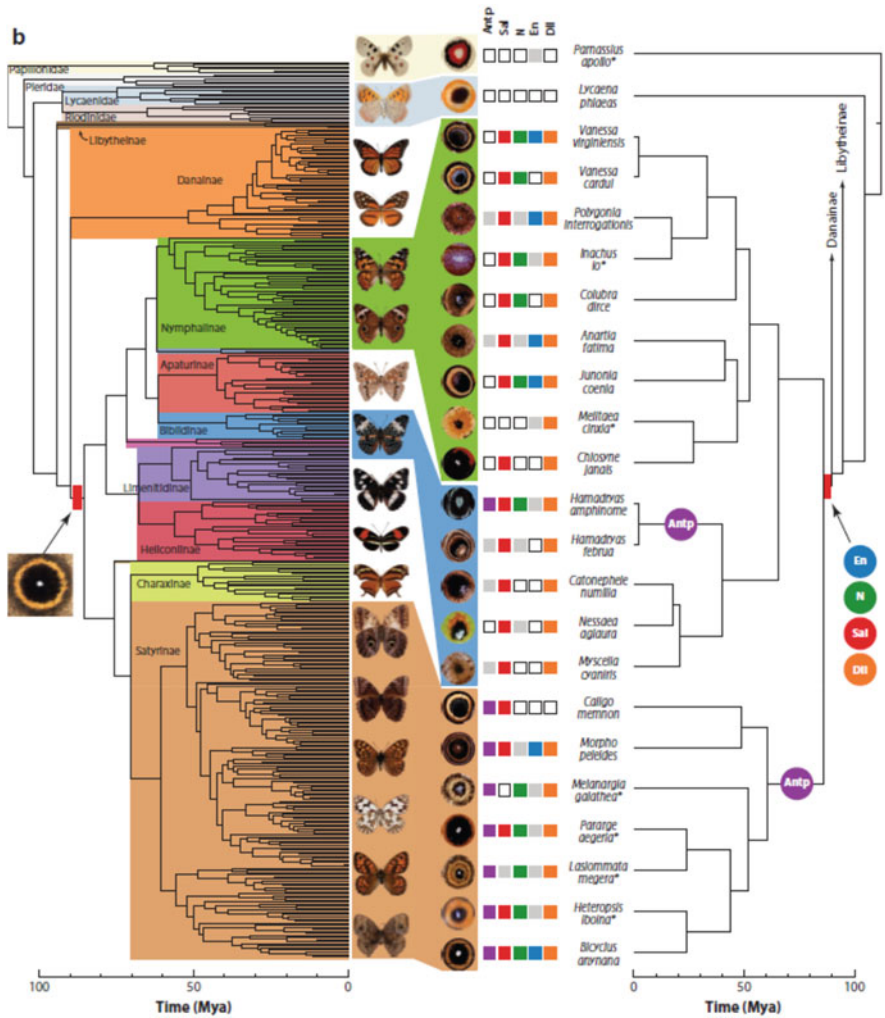

Fig. 5 Evolution of eyespot in butterflies (Monteiro, 2015)

colours. These spots are were found nearly in 400 species of Nymphalid butterflies. However, for more extensive information detailed phylogenetic analysis of eyespot distribution other than the Nymphalids will be required to understand the independent origin of the eyespot in the Lepidoptera as a whole. The homology of the origin and the pattern of the eyespot in butterflies are similar to that of the fins in vertebrates which were originally found to develop along the dorsal midline as a dorsal fin appearing as a pair of anterior pectoral fins and leading towards the posterior region of the body forming the pelvic fin (Ruvinsky & Gibson-Brown, 2000). Development of eyespot involves the patterning of pigments induced by the long range signal originated from a group of focal cells at the centre of the eyespot (Nijhout, 2001).

The different models of eyespot differentiation has described in detail in the following section.

3.3.2 Models of Eyespot Differentiation

The cells located at the centre of the eyespot differentiate to complete eyespot around them during the process of pupation. What is known through transplantation experiments in *Funonia coenia* and *Bicyclus anyana* (French & Brakefield, 1995) is that, the cells at the centre of the eyespot can differentiate complete eyespot wherever they are placed on the early pupal wing. Different models of eyespot differentiation are well explained in the following table (Monteiro, 2015):

Model	Gradient model	Induction model	Data that support either model
Signal properties	Fusion of different morphogens from central to surrounding cells	Signals from central cells are produced as waves and they travel at different times and different distances during the process of development	Two known morphogens i.e., Wingless and TGF-β are present in the center of eyespots during the early pupal development
Properties of the surrounding cells—differentiation	Response of surrounding cells to the central morphogens at different concentration and at the same time (approx.) Surrounding cells responding to the central morphogen(s) at different concentration thresholds and at approximately the same time	Interpretation of the wave signals roughly at the same time for the differentiation of the dark bands around the eyespot	Expression of multiple transcription factors in different coloured rings present in the eyespot at same time including light and dark rings. This supports the gradient model
Number and type of inducing cells	Sources of diffusible signals are assumed to be only eyespot centers	The secondary sources of signals are assumed to be dark rings inducing the inhibitory effect on the light rings	Candidate morphogens are known to be present in the eyespot centers. However, no molecular evidence currently

Model	Gradient model	Induction model	Data that support either model
			supports secondary induction signals required for the induction model
Boundaries between colour rings	Sharp boundaries between different rings are assumed to be due to cross regulatory interactions among adjacent transcription factors present in some species	Sharp boundaries between dark and light rings are assumed to be to the result of a reaction-diffusion interaction between short-range activating and long-range inhibitory signals	No molecular mechanism is to support the either model

3.3.3 Genetics of Eyespot Development

The developmental and genetic basis for the formation, plasticity and diversity of eyespot patterns in butterflies are well examined and are of great interest for researchers till date. Several molecular, genetic and transplantation studies have focussed on the eyespot position, number, size, and colour. More detailed studies on the gene expression profiles are needed so that it can reveals some differences between the traits found at the centre of spots and eyespots during their development. Survey of the expressions during the larval stage of wing development in Nymphalid species with eyespots found that at least two genes (Spalt and Distallus were expressed in most of the centres (Oliver et al., 2012)) (Fig. 6). However, in many species these genes are not expressed at the respective developmental stage. Post expression of these genes, multiple genes expression was lost from eyespot centre without loss of eyespot. The eyespot patterns found on the wings of Nymphalid butterflies are novel traits and originate first in hindwings and subsequently in forewings. Hindwings differ from the forewings due to the differential expression of *Ultrabithorax* (*Ubx*). However, the function of this Hox gene in eyespot
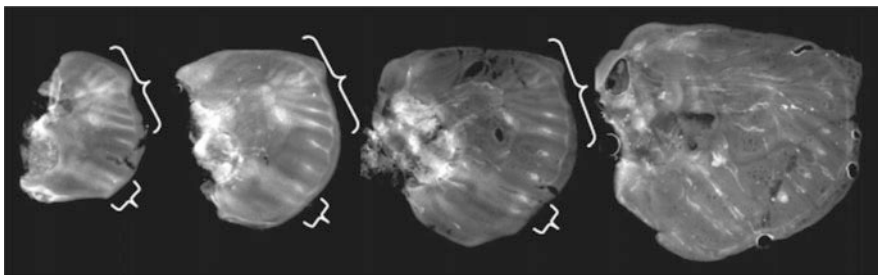

Fig. 6 Genetic expression in the particular wing of Nymphalid butterfly during the larval stages (Monteiro, 2015)

development and that of another Hox gene, *Antennapedia* (*Antp*) were still not clear in *Bicyclus anyana*. To study the role of *Antennapedia*, CRISPR-Cas 9 were used to target both genes and it was concluded that *Antp* is essential for the eyespot development on the forewings as well as for the differentiation of the white centres and larger eyespot on the hindwings while *Ubx* gene is essential not only for the development of atleast some hindwing eyespots but also for repressing the size of other eyespots (Matsuoka & Monteiro, 2021).

3.4   Limb Regeneration Studies in Insects

The ability of regeneration in animals has been discovered along history dating back to the eighteenth century. It has fascinated both scientists and non-scientists. The ability to regenerate limb was considered to be a fundamental developmental property of biological systems. Earlier, it was considered as a simple reiteration of normal limb development which is generally blocked after certain developmental stages. It was in the salamanders that the unique regenerative abilities might be linked to local evolution of their genome suggesting the process in response to the natural selection. In the following sections, we will be discussing about the types of regeneration, diversity of the regeneration, mechanism of regeneration.

3.4.1   Process of Regeneration

Regeneration in organisms takes place through epimorphosis and morphallaxis. Epimorphosis involves cell division to recreate the lost structures. One classic example of this type of regeneration is the limb amputation in Urodela (amphibian). Here the cells dedifferentiate to form structure known as the blastema and continue replicating to generate enough cells to reform the limb. After the blastema formation the cells continue to grow enough to reform the limb. In contrast to this mechanism, in morphallaxis not all division is involved in regeneration. Here the remaining tissue is remodelled to rebuild the missing structure(s) resulting in a smaller but functional structure. Such kind of regeneration is observed in Hydra. In arthropods the regeneration occurs by the process of epimorphosis.

3.4.2   Diversity of Limb Development

1. *Differentiated limb/Direct development*
   In spiders, crustaceous species, ametabolous insect limb development is completely differentiated at the time of hatching of the eggs. Legs and antennae are formed of newly hatched nymphs of hemimetabolous insects that undergo incomplete metamorphosis.

2. **Larval limbs**

   In holometabolous insects, the insect goes under complete metamorphosis and regardless of the theories, limbs of larvae are neither identical to limbs present in embryo nor completely differentiated. Studies in tobacco hornworm *Manduca sexta* have demonstrated that the larval legs in Lepidopterans consist of three distinct cell population i.e. imaginal cells, polymorphic cells and larval specific cells (Tanaka & Truman, 2005). Imaginal disc are precursor cells that proliferate extensively during metamorphosis and make up much adult legs.

3. **Imaginal discs**

   These are the precursors cells of the appendages. They are no involve in the development of larval structures but they contribute in the development of the adult appendages. In many lepidopterans and dipterans including *Drosophila*, imaginal discs are formed during embryogenesis. These early developing structures have served as the model for studying imaginal disc regeneration (McClure & Schubiger, 2007).

3.4.3   Signalling Involve in Limb Regeneration

Limb regeneration in insects is regulated by the grafting experiment by using the imaginal disc of *Drosophila* and the legs of cockroach (French, 1976, 1978). Studies from the mid 90s have identified the key molecular regulators which are involved in the regeneration.

Here the molecular signalling involve in various insects has been discussed, summary of which has been represented in the Fig. 7.

1. **JNK Signalling**

   It is one of the key signalling pathways which is triggered due to wounding or genetically induced cell death. This includes the activation of the c-Jun N-terminal kinase (JNK) pathway, a mitogen-activated protein kinase (MAPK) pathway. Recently, studies have shown that the JNK pathway is triggered from the signals received from the epidermal wound exposure of cells to the cytokines or any other environmental stress (Galko et al., 2004; Rämet et al., 2002). In *Drosophila* the pathway involves the role of four major kinases which helps in phosphorylation cascade. This includes JNKKKK Misshapen, the JNKK Slipper, the JNKK Hemipterous (Hep) and the JNK Basket (Bsk) (Alfonso-Gonzalez & Riesgo-Escovar, 2018). JNK Basket phosphorylates the AP-1 transcription factor Jra along with Dfos/Kayak (the vertebrate homolog of Fos) to activate the early genes.

2. **JAK-STAT Signalling**

   This pathway is activated when cytokines and the growth factors bind to the membrane receptors associated with the JAK tyrosine kinases (Rawlings et al., 2004). When ligand binded to the receptors, it is dimerize bringing together the cytoplasmic domain of the receptor subunits leading to the phosphorylation and activation of the JAKs. The activated JAKs then activates the signal dependent transcription factors (STATs) which then enter into the nucleus for the regulation

Fig. 7 Signalling involve in insect limb regeneration
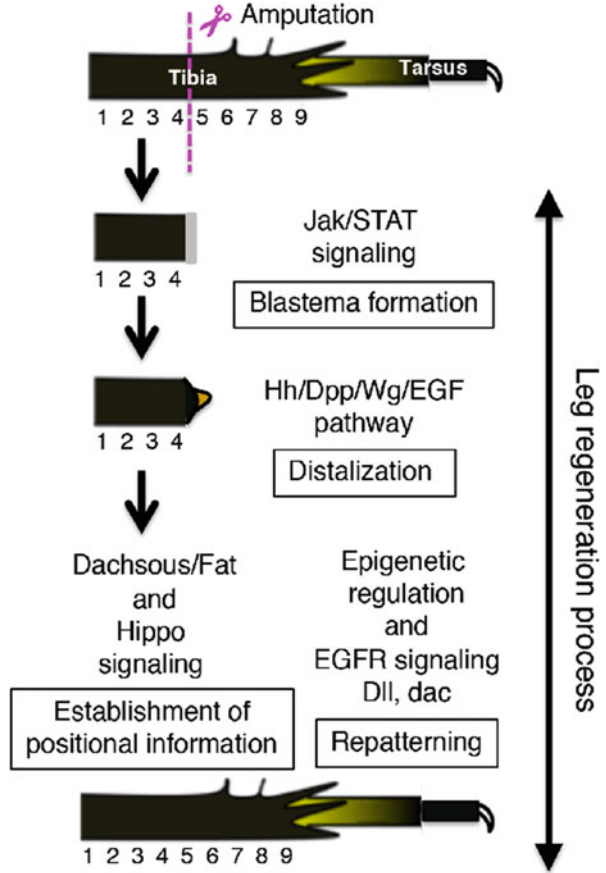
of the gene expression (Rawlings et al., 2004). In *Drosophila*, three ligands (Upd1, Upd2, Upd3) and one receptor (Domeless (Dome)) have been identified (Zeidler & Bausek, 2013). Binding of Upd and Dome leads to the phosphorylation of Hopscotch (Hop) which further activates the STAT transcription factor.

3. ***Hippo Signalling***

The main components of the Hippo pathway includes kinase Hippo, the WW domain-containing protein Salvador (Sav), the NDR family protein kinase Warts (Wts), the adaptor protein Mob-as-tumor-suppressor (Mats), the transcriptional coactivator Yorkie (Yki), and the transcription factor Scalloped (Sd) (Pan, 2010). The mutation in any one of the above Hippo proteins may lead to the outgrowth of the tissues. The Hippo-Sav kinase complex activates Wts-Mats kinase complex through phosphorylation. This further phosphorylates Yki which acts as an oncoprotein (Huang et al., 2005). On other hand if Hippo is inactivated, Yki accumulates in the nucleus and binds with transcription factors for the regulation of the downstream target genes (Goulev et al., 2008; Wu et al., 2008; Zhang et al.,

2008). Other than contributing in the normal development, Hippo pathway also contributes in the formation of proper shape and dimension of regenerating appendages (Bando et al., 2009).

4. ***Wnt Signalling***

Variety of developmental processes is regulated by Wnt Signalling by promoting cell proliferation and differentiation (Swarup & Verheyen, 2012). In addition to this, Wnt signalling pathway has also a critical regulator of the metazoan regeneration (Whyte et al., 2012). In canonical type of Wnt pathway, ligand binds to the Frizzled receptor which leads to the phosphorylation of the Disheveled and membrane tethering of Axin through LRP5/6 (Clevers, 2006).

When Axin is bound to the membrane, β-catenin, a homolog of *Drosophila* Armadillo (Arm), is stabilized and becomes nuclear-localized, where it promotes transcription of target genes by interacting with Tcf/Lef transcription factors, or the *Drosophila* homolog, Pangolin (Behrens et al., 1996; Brunner et al., 1997; Clevers, 2006; Molenaar et al., 1996; Willert & Nusse, 1998). In the absence of Wnt, Axin initiates the formation of a destruction complex, which phosphorylates β-catenin/Arm, and ultimately leads to its destruction by proteasomes.

4 Insects in Ethology

Ethology is a rapidly evolving branch of life sciences that deals with the various behavioural patterns of living organism. Ethological studies in insects involves various activities such as locomotion, grooming, feeding, communication, reproduction, dispersal, flight, learning, migration, host or prey selection, diapause, and various responses to environmental hazards such as temperature, humidity, parasites, and toxins (Alcock, 1984; Tauber et al., 1986; Gatehouse, 1997; Bazzett, 2008). Understanding the behaviour of pest and beneficial insects could improve pest-management programs (Renou & Guerrero, 2000; Bendena, 2010). Sexual selection is one of the most important behavioural aspect of organism. The vividness of the male characters in comparison to that of female is now known to lead to the preferred selection of the male. Many secondary sexual characters such as towering antlers of stags, magnificently colourful tails of peacocks, melodious chirping of birds, etc. posed a challenge to morphology based classification for several years. Darwin first noticed that usually such sexual dimorphic males had some attractive characters which were negatively correlated with their survival rate and this was contradictory to his theory of natural selection. It was then that Darwin (1871) proposed the theory of sexual selection in his book,—*The descent of man and selection in relation to sex* (1871) as an answer to the dichotomy observed in the appearance of sexes and the constraint on survival in males, bearing prominent secondary sexual characters. This theory proposes that extravagant secondary sexual characters, usually in males, increase their chances of being selected as a mate. In the following section we will be understanding the concept of sexual selection in social and non-social insects and also discussing the different types of sexual selection.

4.1 Sexual Selection

Sexual selection is a driving component of the evolutionary biology and it is defined as a struggle for the existence but the possession of the females and this results in few or no offspring. Genetically, sexual selection is the extent to which a given allele is under selection marginally changes the allele frequency associated with non-random access to gametes for fertilization. Therefore, sexual selection is one way of partitioning allele frequency change. There are several other points, which are important to understand the definition of the sexual selection: first competition is for access to gametes. Recognition of mate is a typical first step towards obtaining access to gametes and we indeed expect two broad classes of sexual selection fitness components i.e. those associated with precopulatory mating success (Hardy & Briffa, 2013) and those associated with postcopulatory fertilization success (Parker, 1970; Simmons, 2001).

Relationship Between Natural Selection and Sexual Selection
The new advancements in the field of behavioural studies have lead to the better understanding of the relation between the natural and sexual selection which has been long debated from Darwin onwards (Klug et al., 2010; Alonzo & Servedio, 2019). Theorists have considered that the natural selection opposes the sexual selection during the development of the sexual ornaments in terms of fecundity and viability. According to the Endler's view, sexual selection sets within the broad sense evolution by natural selection.

4.1.1 Sexual Selection in Social Insects

In general, females are more or less choosy in relation to what type of partner to mate. Traditionally social insects have been more targeted for sociobiological studies, but it is interesting to study the sexual selection in social insects, such as ants, bees and wasps and how it shapes the reproductive behaviour and their social organisation. To understand the mechanism of sexual selection in social insects is a notorious task and the evidences related to them are limited. In social insects, mating usually occurs in the mid air under the precise environmental conditions making the control mating impossible. Other than this, females generally disperse after the mating making the quantification of the fitness difficult or impossible. In *Apis mellifera*, the mating is instantaneously fatal to males and their fitness is entirely linked to the queens, which return to the nest postmating. The males in honeybees (drones), gather in the special areas known as the "drone congregation areas" which consist of the hundreds and thousands of males from different colonies (Baudry et al., 1998). During mating, queen bees join the mating area singly followed by the "comet" of males with the male in front of the comet get chance to mate with the queen (Gary, 1963; Koeniger et al., 2005). Unlike the other social insects which are monandrous in nature (Hughes et al., 2008), queen bee mates with 12 males on average over 1–3 mating flights (Tarpy et al., 2004). Queen bees store these sperms

and use them randomly through her remaining life (Franck et al., 2002, 2004). The bees colonies generally produce thousand of males with few number of queens leading to the few successful matings of males with a queen and therefore leading to the extreme male-male competition.

Males of honeybees live in the colonies where they are protected and provided with food by the sister workers. Males themselves do not do any work and their main role is to participate in mating. The main benefit to the males is they are gifted with the strong flight muscles which helps them to gain maximum matings. Indeed, males show considerable variation in the number of spermatozoa they possess (Schlüns et al., 2003; Koeniger et al., 2005). The fitness of males may potentially differ between different colonies (Kraus et al., 2004). The major reason behind the variation in the mating success is the male size. Males of honeybees are normally reared in special "drone" cells which are about 6.2 mm in diameter as compared to the cells used for rearing of workers (5.2–5.8 mm diameter). However, in some cases males can also be reared in the workers' cells. Such males are smaller in size as compared to the normal-sized males. These small size males may make up 9% of the males in a drone congregation area and are successful at winning mates and siring of offspring (Berg et al., 1997; Schlüns et al., 2003). In social insects, males sire only the female offspring and therefore, they contribute genetically to the next generation through the queen offspring. This leads to the conflict in fathers with their mates as well as with their worker offspring about the preferred sex ratio. In such case, males prefer high investment into female offspring while queen prefers an equal sex ratio investment. However, the workers invest in 3:1 female biased sex ratio (in a monandrous colony) (Trivers & Hare, 1976). Consequently, males are more in agreement over the sex ratio with their worker offspring as compare to their mates (Boomsma & Ratnieks, 1996).

4.1.2 Sexual Selection in Other Insects

Intensive form of sexual selection or the mate choice can facilitate the evolution of alternative condition dependent reproductive strategies. In tree cricket, males produce long-distance acoustic signals to localised the females and the mate choice (Deb et al., 2020). The amplification of the acoustic call by male tree crickets is a classic example of the condition dependent alternative strategy. Though most males can baffle, smaller and low amplitude callers predominantly use the alternative strategy. Baffling allows the males to amplify their call and increase their range to attract higher number of females. Baffling also indicates the quality of males providing them the benefit to mate with the females for longer duration. Another study in domestic cricket *Acheta domesticus* have shown the relationship among the signal components fit within the systems (Hebets et al., 2016).

In this study he proposed the systematic approach to understand the communication. This stresses the entire system to be evaluated simultaneously (Hebets et al., 2016). This proposal in relation to the sexual selection clearly recognises the entire

signal repertoire of an organism and how the all of it components are simultaneously affecting the reproductive success and therefore the overall fitness.

The system approach formalizes how the signals interact both for a single mode as well as within the multimodel communication framework (Hebets & Papaj, 2005). According to this, approaches can be classified into four different designs (Hebets et al., 2016; Rosenthal et al., 2018) i.e., (1) Redundancy, where structure and function are shared among signal components, (2) Degeneracy, where different signals serve similar functions, (3) Pluripotentiality, one signal or component of a signal serves multiple functions in one display, (4) Modularity, where different signals formed linked structural and functional clusters (Hebets et al., 2016; Rosenthal et al., 2018).

In *Drosphila*, males show no parental care and benefit from a high mating rate (Bateman, 1948), yet there is evidence for male mate choice (Byrne & Rice, 2006). The evolution of male mate choice in this species are likely to be high costs of ejaculate production or limits to courtship, which can reduce the capacity of a male to mate with multiple females (Wedell et al., 2002). In nightingale grasshoppers, *Chorthippus biguttulus*, the traits of the calling song are under the influence of sexual selection (Klappert & Reinhold, 2003). Two main functions of acoustic signalling in this context can be distinguished. Male song or advertisement calls can be used by females both for species recognition, that is, to identify conspecific males, and to choose between them. Sexual selection has been also studied in much loved ladybird beetles (Coleoptera: Coccinellidae). Evidences of mate choice on the basis of morph has been studied in two-spot ladybird *Adalia bipunctata* (Linnaeus, 1758) (Majerus et al., 2006), *Propylea japonica* Mulsant, *Coccinella septempunctata* L. (Srivastava & Omkar, 2005), *Propylea dissecta* Mulsant (Mishra & Omkar, 2014) and *C. septempunctata* and *C. transversalis* Fabricius (Bista & Omkar, 2015). Other than morph, there are several abiotic factors such as seasonal changes in temperature which can act as modulating factors for the mate choice (Wang et al., 2009; Dubey, 2016). In *A. bipunctata*, female biased sex ratio was more prominent owing to the increased male bacterial infection and thus role reversal was hypothesized where males are limiting sex and indulge in mate choice (Majerus, 2006).

The advancement in science has also lead to understand the mechanism at the post copulatory level, which was earlier not possible to study by the scientists. Postcopulatory sexual selection is often considered important to measure the strength of selection acting on sexual traits or phenotypes. Most often post-copulatory sexual selection is studied with reference to male reproductive success; however, post-copulatory sexual selection acting on females has till recently gained very little attention (Ah-king et al., 2014; Arnqvist, 2014; Eberhard, 1996). A frequently used method to measure postcopulatory sexual selection is competitive fertilization when subjected to multiple matings with the reproductive success of the males being determined by phenotypic and genetic markers (Simmons, 2001). Besides this, there are several other measures, which can be used in non-competitive situations. In many species males sometimes fail to fertilize females (García-González, 2004; Greenway et al., 2015) hence, proving that postcopulatory sexual selection may rise from the differential selection of females. Single mating

experiments can also be designed to investigate the proximate outcomes of the postcopulatory sexual selection, such as factors influencing the number of sperms reached in the female reproductive tract and stored in the storage organ (spermathecae) (Holwell et al., 2010; Tadler, 1999).

4.2 Postcopulatory Sexual Selection

Postcopulatory selection process can be broadly categorised into sperm competition (direct male-male competition) (Simmons, 2005; Snook, 2005; Eberhard, 2009; Manier et al., 2010) and cryptic female choice (female modulation of male-male competition) (Eberhard, 1996, 2009).

Sperm competition was initially defined as the competitive ability of the sperms of two or more males in order to fertilize the ova (Parker, 1970). Males use several mechanisms in sperm competition. Males can dilute the sperm of the previous males with their own voluminous ejaculate, which is also called 'raffle competition' (Parker et al., 1997). This tactic is generally reported in vertebrates, where ejaculate size is correlated with the degree of polyandry (Birkhead & Moller, 1992). Males often increase the size of their ejaculates in presence of sperms of other males (Gage & Baker, 1991) and also perform the copulations followed by mate guarding, when there are other males in the vicinity. Other tactics involve modification the sperm morphology, such as loss of flagellum, multiple flagella, gigantism, polymorphism and conjugation. Males are also known to use their genitalia or spermatophores to physically displace sperms of other males (Simmons, 2001; Gack & Peschke, 1994). These mechanisms will be discussed in details later in the chapter. Several other mechanisms of sperm competition include "kamikaze" sperm that kill or dissect the sperm of other males.

On the other hand, while sperm competition is about male competition for paternity, females also have a control on the paternity share of their offspring and the mechanism to achieve this postcopulation selection is known as cryptic female choice (CFC, hereafter). It emphasizes on the female selection, which is invisible in respect to the Darwinian criteria for reproductive success. Traits of males, which are associated with such kind of selection include morphology, behaviour and difference in the ejaculate composition. Offspring will be sired by the father who better in eliciting such responses (Eberhard, 2009).

1. *Sperm competition*

In majority of sexually reproducing species, the ejaculate of different males can compete to fertilize the ova of female and this process is called sperm competition. This is especially true in females with sperm storage organs, as these can generate intense selection for males' adaptations that can promote fertilization success. Sperm competition is a powerful selective force that favours male traits, which can maximize competitive fertilization success. Across a diverse range of taxa comparative and experimental studies have been conducted,

which demonstrate response to sperm competition, such as increase in testis size (Moller, 1991; Harcourt et al., 1995; Hosken, 1997; Hosken & Ward, 2001; Byrne et al., 2003). Furthermore, inter and intraspecific studies have also revealed that the sperm competition is positively correlated with sperm numbers (Briskie & Montgomerie, 1992; Dunn et al., 2001). This is because large testes produce large number of sperms (Amann, 1970; De Reviers & Williams, 1984; Møller, 1988a, 1988b).

Sexually reproducing females need viable sperm to reproduce. During ejaculation, sperm is transported through the female reproductive tract and is subjected to various physical and chemical stresses. Since in the initial phase sperm is deprived of many defense mechanisms, therefore female provides protection against the degenerative changes. Conditions which prevent the sperm from reaching the eggs may include: (1) sperm ejection by females (Pizzari & Birkhead, 2000), (2) physical barriers, such as cervix and long ducts, and (3) chemical barriers, such as low pH, viscous mucous. Thus, only a small proportion of the sperms have the opportunity to encounter the egg. To understand the ejaculate and female interactions in depth, four non-mutually exclusive hypotheses have been proposed (Birkhead & Møller, 1993).

Firstly, environment which is selective on sperm may safeguard against the pathogens that may enter the female reproductive tract, especially during the time of mating (Birkhead & Møller, 1993). Secondly, adaptations to discriminate sperms of abnormal morphology (Birkhead & Møller, 1993) act as safeguards for future offspring fitness. Thirdly, high sperm mortality which may results in fewer sperms at the site of fertilization and may have evolved to prevent polyspermy. Fourth, conditions of the female tract may be of two types, i.e. by posing challenges to the sperm so that ova could be fertilized by the best quality sperm (Birkhead, 1998; Birkhead & Møller, 1993; Eberhard, 1996) or by favouring male adaptations which increase the probability of sperms to fertilize the eggs.

2. **Cryptic Female Choice**

Sexual selection is the component of differential reproduction of individuals that results from differential access to mates. In 1966, Eberhard had given the idea of the cryptic female choice, which ignites the theory that sexual selection is also a female driven process. Female insects may choose their mates either before or after mating. Premating females' choice include the termination of mating before the insemination or refusal to mate with an unhealthy male. Postmating choice may occur in two contexts: i.e. female primarily use sperm of preferred mates to sire their eggs or towards zygotes by superior mates. The result of female postmating choice includes ability of female to reduce or eliminate availability of sperms of inferior males by digesting them or by moving them to such location, where they cannot be accessible to eggs. The term cryptic here is used in the sense that it is hidden decision made by female after copulation. In cryptic female choice genital coupling does not necessarily result in successful intromission or insemination, which results in successful storage of sperm in spermatheca. The best example of this is the female of cassidine plant beetle *Chelymorpha*

alternans, which has a long coiled spermathecal duct. When the different males mate with same female, the male with long sclerite sire significantly more offspring. However, the exact mechanism of this biasness is not known yet.

To study cryptic female choice, we first need to understand the traits of females or the behaviour of the sperm that affect the uptake or utilization for fertilization. Secondly, female show the differential response such that the sperm of particular males are predictably favoured or disfavoured based on genotypic or phenotypic factors. This relationship between male traits and pattern of sperm utilization by females can be demonstrated by manipulating male phenotype experimentally or excluding the other factors. Any change in sperm utilization or fertilization success is in consistency with cryptic female choice (Pilastro et al., 2004). Artificial insemination or in vitro assays of sperm utilization and fertilization can be used to control ejaculate traits and eliminate the effect of premating mechanisms (Martin-Coello et al., 2009; Løvlie et al., 2013; Firman & Simmons, 2015).

Females might influence paternity by controlling the timing and order of insemination. For example, in moth *Ephestia kuehniella*, the paternity share of second male is influenced by remating of females (Xu & Wang, 2010). The outcome of sperm competition is often mediated by the number of sperms inseminated by different males. While the ejaculate size is largely under the control of males, the acceptance and ejection of spermatophores depends on the female. In several species the loss of ejaculate depends on the several female processes, such as sperm digestion, ejection, and incapacitation. The sperm ejection depends on male size (Ala-Honkola et al., 2011), identity of species (Manier et al., 2013) and copulation duration (Peretti & Eberhard, 2010).

Another mechanism influencing cryptic female choice is the differential sperm storage. If the sperm is not able to be stored, it can get ejected, digested or incapacitated. Eberhard (1996) was the first who suggested that the complexity of fertilization tubule increases the female control of the sperm storage and paternity. In dungflies, *Scathophaga stercoraria*, the four sperm storage controls paternity better that those with the three sperm storage organs. In *D. melanogaster*, sperms longer in length, stored in seminal receptacle (Miller & Pitnick, 2002) due to their ability to replace the sperm with shorter length. This facilitates the cryptic female choice and thus, proves that the sperm competition is not exclusively male dependent but work with male-female interactions.

5   Conclusion

Insects are important in the ecosystem in several ways. They are not only restricted to benefit as pollinators of crops, decomposers of decaying organic materials, pests biocontrol, food for other wildlife organisms. Insects are also the inspiration for new inventions or improvements. Even in today's era of digitalization, we extract ideas and other sources from the insects and their relatives. In this technological era, we

have recognised the importance of insects and the achievements of the insect evolution after we have accidently duplicated them with our own devices. Insects' equivalent to the radar and sonar helps the insects such as moths, mantids and other arthropods to avoid bats. In ancient time, dung-rolling scarab beetles act as the GPS navigated by the celestial objects. The emerging science in the field of biomimicry has led to the creation of robotic insects. In some cases it also allows humans to operate live insect as if they are robot. Studies dealing with the eyes and brains of the dragonflies have led to the development of new algorithms for the visual tracking that are 20 times faster than previous generations of such software. Our knowledge on interaction of insects with other species and surrounding environment is very narrow and not sufficient and must requires the intensive studies. The one of the greatest qualities of entomology is its potential for discovery, and the blossoming fields of citizen science and science communication allow anyone to have an impact on our collective knowledge. Observations have led to a better understanding of the geographic distribution of different species, led to the discovery of new species, and documented previously unknown behaviours and relationships thanks to videos taken on cell phones. All of this can be shared instantly through social media and online platforms.

The section on microbiology is aimed at summarizing the major breakthroughs of the field. It is hoped that the study of male killing bacteria, sterile insect techniques, endosymbionts will lead to the better understanding of the mechanisms and their importance in the agriculture. The study of wing pattern of butterflies is also important as it serves the three big purposes, i.e. flight, camouflage and mate selection. This section will make the readers to understand the activity of the signalling pathway responsible for the diversity in the wings of butterflies. The study of limb regeneration in insects has its significance as it is one of the important aspects of growth and it provides a direct selective advantage.

The section dealing with the behavioural studies of insects helps us to know and understand the interactions of the insects and their surrounding environment. The study of sexual selection has emerged as one of the most fascinating and important evolutionary processes. The study of sexual selection is important not only to fill the lacunae in information, but also helps to understand a comparative evolutionary significance for different reproductive strategies which can provide insight into differences and similarities between species by encouraging rigorous examination of the adaptive problems and evolved solutions.

Thus, we should be grateful to insects for their inspiration in the field of science and research. We must do so, however, with a degree of reverence, rather than an overriding desire for monetary windfalls.

Points to Remember
- Insects are ecological important and have amazing diversity.
- The role of insects in the microbiology is revolutionizing in agriculture by using male killing bacteria, endosymbionts and SIT.
- The colorful patterns of the butterfly wing are an exceptional example of evolutionary innovation and adaptation.
- The studies on the signalling involve in eye spot provide an elaborate view of their development and the variation in their shape, size and number.
- Wnt A plays an important role in the patterning of wings in butterflies.
- Limb regeneration in insects involves networks of gene regulation mediated by secreted morphogens play key roles in controlling development, repair and regeneration.
- Sexual selection is an important evolutionary process and it shapes the reproductive behaviour of insects.
- Study of sexual selection is difficult in social insects as compared to other insects since the mating takes place in mid air in social insects.

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