REPRODUCTIVE STRATEGIES IN INSECTS

Edited by

Omkar

Geetanjali Mishra

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Preface

'Let us investigate more closely this property common to animal and plant, this power of producing its likeness, this chain of successive existences of individuals, which constitutes the real existence of the species'. – *Comte Georges-Louis Leclerc de Buffon*

Reproduction is one of the most primitive, yet essential function that is fundamentally vital to the very existence of life and furtherance of generations. It takes myriad forms and mechanisms depending on the taxa and the complexity of the living being, ranging from asexual to sexual, each of which comes in numerous variants, but it is always there; a sustained mechanism that forms the primal basis of existence and sustenance of all organisms. Or as Louis Schwartzberg said, 'Nature has invented reproduction as a mechanism for life to move forward. As a life force that passes right through us and makes us a link in the evolution of life'.

Reproduction is so primitive and fundamental that the mechanism involved is highly complex and not yet clearly understood. An overview of all taxa from prokaryotes to eukaryotes, plants to animals, the simplest to the most complex, provides an astonishing range of mechanisms that have evolved to ensure the successful propagation of each living organism. These mechanisms have evolved to enhance the way organisms mate, produce offspring or raise them, thereby increasing their direct or indirect fitness levels. However, if there is a single group of organisms that encompasses the most diversity in reproductive mechanisms and strategies, that would be insects.

Insects form one of the most varied and eccentric taxon across the entire range of organisms. Not only are they immensely diverse in shape, size, habitat, and ecosystem services; they employ an equally diverse range of reproductive strategies; encompassing almost all possible modifications across the animal world. Reproduction in insects ranges from purely sexual to parthenogenetic, which itself comes in a wide number of variants.

The search for mates, number of mates, display of mate quality, assessment of mate quality, acceptance of mate, rejection of mates, forced copulations, the fight for paternity pre, during, and post copula, the modulation of paternity, ovipositional strategies, and parental care together form the basis of reproductive strategies that are employed to successfully transmit the genome to future generations. As these myriad steps interconnect for successful reproduction of individuals, it is essential to understand that at each of these steps, the male and female usually do not 'hold hands' but are rather at antagonistic warring ends with each other, a process scientifically known as sexual conflict. In fact it is sexual conflict that plays a major role in the evolution of reproductive strategies.

Further to add to the complexity of the entire situation, most of these behaviours are not fixed in nature but are modulated by environmental factors, both abiotic and biotic. For example, a darker or more melanic individual would turn out to be an exemplary mate in colder climates as it would provide its offspring the warming cloak of darkness. However, if the weather turned warm, the selection of a melanic mate would be a folly. Furthermore to limit oneself to one mate when multiple mates are easily accessible would be as big a folly as scrambling to find more mates, when they are too widely dispersed.

While there are multiple books on animal behaviour, there are relatively fewer on reproductive strategies. Those that concentrate on reproduction are usually detailed volumes on any one aspect such as mating systems, sperm competition, sexual selection, or parental care. This book deals with the morphological, physiological, and ethological aspects of the entire gambit of reproductive strategies, and also deals with their ecological and evolutionary aspects. Further being inspired and focused on insects make this a niche book for ethologists, biologists studying behavioural evolution, and entomologists. Also because of being concentrated on insects, it presents a wide ranging diversity of reproductive strategies in one place.

In this endeavour we were supported by a whole bevy of people. We are really grateful to our research team members; Dr. Shashwat Singh, Dr. Swati Saxena, Ms. Apoorva Shandilya, Ms. Priya

Singh, Ms. Chandni Verma, Ms. Tripti Yadav, Ms. Shriza Rai, Ms. Gaurvanvita Singh, Ms. Lata Verma, and Ms. Dipali Gupta for their constant support throughout the planning and execution of this project. I (Geetanjali) am especially indebted to my parents, Mrs. Munni Mishra and Lt Col. Ramesh Chandra for their unwavering faith and support. We are also thankful to our spouses, Ms. Kusum Upadhyay and Dr. Ravi Shanker Verma for constant encouragements and sacrifice for sparing me for this work. We are also thankful to Dr. Renu Upadhyay and her entire team from CRC press for their constant encouragement and follow-up that helped us complete the work in time.

Editors

Editors

Prof. Omkar, FNASc, has been associated with teaching for about 35 years and research for more than 42 years. He is Former Head, Department of Zoology, University of Lucknow, Lucknow-226007, Former Coordinator, UGC-SAP (DRS-II), DST-FIST, DST-PURSE, Centre of Excellence, Govt. of UP, programs. He has worked on more than 10 projects of state/central funding agencies. He specializes in Environmental Toxicology and Entomology, with particular reference to the Insect Pest Management. He is also an Associate Editor, *International Journal of Tropical Insect Science*, Springer Nature and Chief Editor, *Journal of Applied Bioscience*, besides being President of International Society of Applied Biology.

He is a recipient of several awards, such as Saraswati Samman by the Department of Higher Education, Govt. of UP; Prof. T N Ananthakrishnan Foundation Award, Rescholar Award of Excellence in Agricultural Entomology by Association of Entomologists, Prof. G. S. Shukla Gold Medal by The Academy of Environmental Biology, Dr. S. Pradhan Memorial Lecture Award by The Division of Entomology, IARI & The Entomological Society of India, ACCLAIM and Uddeepan awards from University of Lucknow. He is a Fellow of The Entomological Society of India, Zoological Society of India, Society for Biocontrol Advancement, and Fellow of The National Academy of Sciences India (FNASc), besides many others. He has 16 books to his credit, such as Pesticides, Man & Biosphere, Ecofriendly Pest Management for Food Security, Elsevier, Industrial Entomology, Springer Nature, Pests & Their Management, Springer Nature, Sucking Pests of Crops, Springer Nature and Polyphagous Pests of Crops, Reproductive Strategies of Insect Pest Management, etc. He has published 235 research papers, 10 reviews, 40 reviews as book chapters, and 17 popular science articles. Prof. Omkar has guided more than *two* dozen PhD students. He has visited the School of Biological Sciences, University of East Anglia (2006) for 3 months and Department of Zoology, University of South Bohemia and Czech Academy of Sciences, Ceske Budejovice, Czech Republic (May 2016).

Dr. Geetanjali Mishra, has been associated with teaching for about 14 years and research for more than 21 years. She is a passionate behavioural ecologist, evolutionary biologist and an ardent entomologist. She has been working on insect behaviour and biology for the past 21 years using ladybird beetles as her model of choice. She is Member, Executive Council, Indian Society of Evolutionary Biology. She is also an Associate Editor, *Journal of Applied Bioscience*, and reviews extensively for journals of repute in the field of entomology, behaviour, and ecology.

She has been a Commonwealth Academic Fellow at the Centre for Ecology & Conservation, University of Exeter, Penryn Campus, UK. She has also visited California State University, Long Beach, USA as a part of a select team of teachers to brainstorm over teacher education quality. She has been a university Gold Medalist during her post graduation in Zoology and has also been awarded Prof. T N Ananthakrishnan Young Scientist Award and Prof. B.V. David Women Scientist Award. She has published 1 proceeding, 1 technical bulletin, 85 research papers, 12 reviews as book chapters, and 5 popular science articles. She is also an avid popular science speaker and panelist in radio and TV shows. Dr. Mishra has guided 5 PhD students.

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Various Modes of Reproduction

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1.1 Introduction

Reproduction is a fundamental biological process for propagation of species. Asexual and sexual are two different forms of reproduction. In asexual reproduction, an organism can reproduce without involvement of another organism, creating a genetically similar or identical copy of itself. In sexual reproduction, two reproductive cells (male and female gametes) are created by meiosis and by fertilization of a female gamete with a male gamete, which leads to formation of a zygote. The zygote develops into an embryo to give rise to offspring, whose genetic make-up is derived from both of their parental organisms.

Insects are one of dominant life forms on earth; a state that can partly be attributed to their diverse reproductive strategies. They are equipped with high reproductive rates and adopt a number of behavioural and physiological adaptations for survival in diverse ecological habitats.

Sometimes, eggs are retained by females after fertilization and eggs continue to grow before they are laid. If this internal period of retention of eggs is longer, eggs develop into larvae and female gives birth to larvae (Viviparous). Viviparity assumes different forms, such as ovoviviparity, pesudoplacental, haemocoelous, and adenotrophic viviparity. Sometimes, more individuals develop from a single egg (polyembryony). Certain species of insects mature precociously and start producing young ones even when they are still at larval or pupal stage (paedogenesis). At times, whole of their life cycle or in alternate generations, some insects undergo asexual mode of reproduction, as eggs develop without being fertilized (parthenogenesis).

Different modes of reproduction in insects have earlier been reviewed by several workers: Blackburn (1999) on viviparity and oviparity, Hagan (1951) on viviparity, Suomalainen (1962) and White (1964) on parthenogenesis, Ivanova-Kasas (1972) on polyembryony, and Kerr (1962) on sex determination. These studies provide an overview of reproductive and functional significance, role of reproductive symbionts on manipulation of sex of insects, and as such on mode and physiology of reproduction.

1.2 Modes of Reproduction in Insects

1.2.1 Oviparity

Most insects are oviparous in nature, i.e. female insects lay eggs and embryonic development occurs after oviposition by utilizing yolk in deposited eggs. The process of egg laying consists of ovulation followed by fertilization and oviposition. Females usually deposit their eggs either on or near food source through posterior abdominal segment modified as ovipositor or an egg-laying organ (Figure 1.1). In Lepidoptera, Coleoptera, and Diptera, posterior abdominal segments of female abdomen itself function as ovipositor, as they can be protracted into a telescopic tube in which opening of egg passage is close to the distal end.

1.2.2 Viviparity

Some species are viviparous in that the egg is retained by mother after fertilization and hatching of eggs and development of young ones takes place within mother. The nutrition required for development of embryos is directly received from the parent. Generally, viviparous species produce fewer offspring than oviparous species and this may be due to lesser number of ovarioles.

There are four distinct types of viviparity found in different insect groups.

1.2.2.1 Ovoviviparity

Eggs are incubated inside the reproductive tract of a female and hatching of eggs occurs just prior to or soon after oviposition; young ones are released immediately after hatching. As eggs contain yolk for nourishment, no special nutritional structures are present. Ovoviviparity occurs across several insect orders, such as Ephemeroptera, Dictyoptera, Psocoptera, Hemipera, Coleoptera, etc. Fecundity is

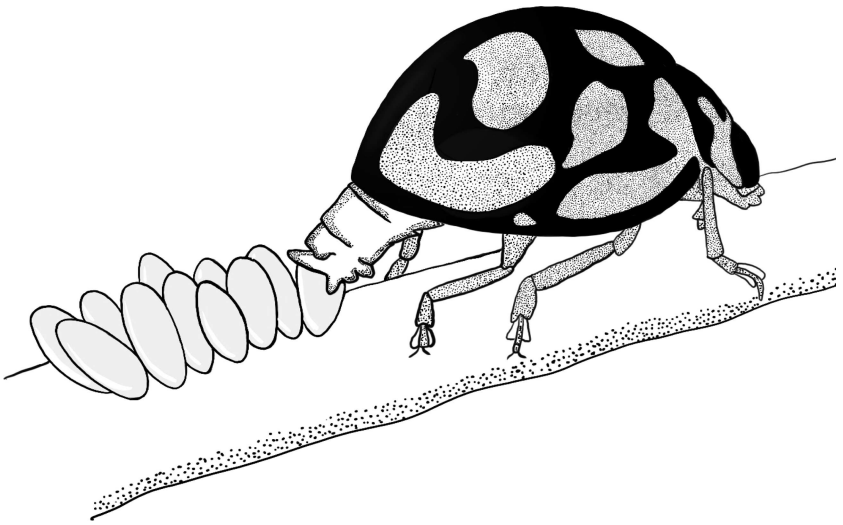

FIGURE 1.1 Oviposition by South African ladybird beetle (Oviparous type of reproduction).

relatively low when compared to oviparity as greater protection is afforded to eggs carried by females. Size of eggs is also relatively larger as eggs contain more nutrients to support embryo development beyond normal hatching and larvae are born in late stage of development.

1.2.2.2 Pseudoplacental Viviparity

The eggs contain little or no yolk. Yolk-deficient eggs develop inside the genital tract of a female. Nutrients are transferred to developing embryos through special maternal structures called pseudoplacentae. It does not involve any oral feeding and larvae are laid upon hatching. Oocyte is accompanied by a single nurse cell and enclosed by a follicular epithelium, which differentiates into a form maternal pseudoplacenta. The embryo develops in pseudoplacental cavity and is connected with pseudoplacentae through cytoplasmic processes extending from cells of amnion and serosa of egg. It is presumed that nutrients pass from pseudoplacentae to fluid surrounding the embryo, thus allowing developing young ones to obtain nutrients (aphids, earwigs, psocids, and polytenid bugs).

1.2.2.3 Haemocoelous Viviparity

Embryos develop freely in a female's haemolymph with nutrient uptake taking place by osmosis. This occurs in Strepsiptera and some gall midges. Haemocoelous viviparity differs from other forms of viviparity, as development of young ones takes place in haemocoel of a female adult. In Strepsiptera, no oviducts are present and mature oocytes are released into haemocoel by rupture of ovarian cells. Sperm enters through genital canal present on ventral midline of female adults and fertilize eggs in haemocoel. Development of young ones occurs in haemocoel with nourishment from haemolymph. The eggs hatch and larvae find their way out through genital canals.

1.2.2.4 Adenotrophic Viviparity

When a poorly developed larva hatches from an egg, it feeds orally on accessory (milk) gland secretion within the uterus of mother's reproductive system. The embryonic development follows as in ovoviviparity, but when larva hatches, it remains in uterus and receives nutrition from special maternal glands. The full-grown larva is deposited and pupation occurs immediately. In tsetse flies, oocytes are fertilized in uterus and embryonic development occurs within. The larva hatches inside uterus, on the ventral wall of which there is a small pad of glandular cells with a cushion of musculature.

This structure is known as choriothete and is responsible for removing chorion of eggs. The larvae feed in uterus on secretion from specialized accessory glands known as milk glands; nutrients to which are derived from blood meal taken by parent fly.

1.2.3 Parthenogenesis

Parthenogenesis is common in animal kingdom. Charles Bonnet was the first to discover the parthenogenesis phenomenon in aphids in 1740. During the next hundred years, further experiments with aphids, bagworm moths, drone bees, and silkworm moths verified this phenomenon.

There are different types of parthenogenetic forms based on occurrence, such as facultative (bees), obligate (sticky insects), and cyclic or sporadic (aphids). Based on sex produced by parthenogenesis, it can be classified into three forms, such as Arrhenotoky (bee), Thelytoky (aphids), and Amphitoky or deuterotoky (cynipid wasp). Based on cytological basis, it can be categorized as Apomictic (no meiosis) or Automictic (meiosis occurs but diploidy is maintained). Detailed description of parthenogenesis is available in later part of this book.

1.2.3.1 Parthenogenetic Forms Based on Sex

1.2.3.1.1 Thelytoky

There are two distinct forms of thelytokous parthenogenesis in insects: facultative and obligate. Hymenopteran insects (ants, bees, and wasps) are characterized by a haplodiploid sex-determining system, wherein females arise from unfertilized eggs. However, in ant, *Cataglyphis cursor,* unmated females may produce diploid daughters from unfertilized eggs through thelytokous parthenogenesis (Pearcy *et al.*, 2006). Thelytoky allows females to pass along their successful genotypes to all of their offspring so as to produce only daughters, thereby maximizing rate of increase and eliminate need for finding or attracting a mate.

Facultative or obligate thelytoky occurs sporadically in over 80 families of super class Hexapoda. Thelytoky is present in Thysanoptera, Psocoptera, Hemiptera (especially suborder Sternorrhyncha) and Phasmatodea, while it is of low occurrence in orders Lepidoptera, Diptera, and Coleoptera. However, thelytoky occurs at high rate in weevils (Coleoptera: Curculionidae), bagworm moths (Lepidoptera: Psychidae), and chironomid midges (Diptera: Chironomidae). In Hymenoptera, it has been documented in some taxa, including Cynipidae, Tenthredinidae, Aphelinidae, Ichneumonidae, Apidae, and Formicidae.

1.2.3.1.2 Arrhenotoky

Arrhenotoky is often referred to as "haplodiploidy" with males developing from unfertilized haploid eggs, while females develop from fertilized diploid eggs. The entire order Hymenoptera and many species in Homoptera, Thysanoptera, and Coleoptera are arrhenotokous. It also occurs in some scale insects (Hemiptera: Margarodidae), whiteflies (Hemiptera: Aleyrodidae), some bark beetles, and *Micromalthus* sp. of monotypic family Micromalthidae.

1.2.3.1.3 Deuterotoky

In deuterotoky, both males and females are developed parthenogenetically. In some species that usually reproduce by thelytoky, a very small percentage of individuals develop into males, which may or may not be able to mate. The term deuterotoky is also sometimes applied to cyclic and facultative parthenogenesis, which is seen in aphids (Simon *et al.*, 2002). Deuterotoky involves development of unfertilized eggs into either males or females, and at least one insect, a mayfly, is reported to exhibit facultative deuterotoky.

1.2.3.2 Parthenogenetic Forms Based on Cytology

In parthenogenetic forms, eggs develop into new organism without fertilization and this absence of fertilization causes alterations cytologically and genetically. Parthenogenetic forms are often

accompanied by lack of chromosome conjugation or reduction, when compared to normal ones having undergone fertilization. The genotype of gametes in parthenogenetic forms is not haploid as in sexual reproduction but it is diploid set of chromosomes as in somatic cells. In some parthenogenetic forms, diploidy is also restored albeit in an abnormal manner. Hence, an understanding of cytological basis of parthenogenetic forms gives insight into genetic nature of parthenogenesis. Parthenogenesis can be divided into three main types on cytological basis: apomictic, automictic, and generative or haploid parthenogenesis.

1.2.3.2.1 Apomictic

In apomictic parthenogenesis, meiotic features are lacking fully or in part. Only one maturation division takes place in eggs and division is an equatorial one. No chromosome reduction takes place during maturation division, and zygoid chromosome number and whole genome is thus maintained (Suomalainen, 1962). Apomictic parthenogenesis is most common in Homoptera (Suomalainen, 1962), Diptera (White, 1964), and Hymenoptera (Peacock & Sanderson, 1939).

It is the simplest type of parthenogenesis. Meiosis is totally absent and, consequently, genetic recombination is not possible; offspring retains exact genetic constitution as mother. Genotypically new forms may arise only through mutations.

1.2.3.2.2 Automictic

Early stages of meiosis in egg are quite normal in this type of parthenogenetic forms, i.e. it is similar to those of forms with fertilization. The chromosomes pair at zygotene, crossing over occurs between them, and they form bivalents. As a result of chromosome reduction during meiosis, gametes are haploid in nature. However, it is restored soon to diploid status via fusion of two zygotic nuclei. The fusion of nuclei occurs due to some kind of endomitosis. The developed animals always have a diploid soma and are homozygous. This mode of parthenogenesis has been recorded in Phasmidae (Nakano *et al.*, 2019), Homoptera (Nur, 1971), Thysanoptera, Diptera, and Hymenoptera (Suomalainen *et al.*, 1976).

1.2.3.2.3 Generative or Haploid Parthenogenesis

Regular chromosome conjugation and reduction take place in eggs in this type of parthenogenetic forms. In spite of this, eggs may develop either through fertilization and give rise to a diploid female or without it resulting in production of haploid male. The eggs of a single female may thus develop into both females and males; latter being of parthenogenetic origin. Generative parthenogenesis is, therefore, always facultative and arrhenotokous (male-producing). This type of parthenogenesis has been reported in Homoptera, Thysanoptera, and Hymenoptera (Whiting, 1945).

1.2.3.2.4 Cyclical Parthenogenesis

It is characterized by a heterogeneous alternation of generations, one or usually several parthenogenetic generations alternating with a bisexual generation. This kind of alternation of generations is favourable for organism, as species is able to reproduce quickly through thelytoky. The speed of reproduction is also accelerated in certain forms of paedogenesis (*Micromalthus*) and by viviparity (many aphids). In addition, favourable gene combinations may be widely spread in animals with cyclical parthenogenesis.

1.2.4 Paedogenesis

Sometimes immature insects that mature precociously are able to reproduce by a phenomenon known as paedogenesis. Life cycles are reduced by loss of adult and pupal stages. In this precocious stage, gonads develop and give birth to young ones by parthenogenesis or by viviparity.

Larval paedogeneis or larval giving birth to larvae or even oviposition is more common in *Miaster* (Phasmidae) and *Micromalthus* sp. (Coleoptera). Young larvae are set free in body cavity of paedogenetic larvae and they feed on maternal tissues and eventually emerge out from body wall of parent. In *Micromalthus* sp., besides adult male and female, there are larvae producing males or females or both. The form emerging from egg is called a triungulin, which moults into apodous larvae that can develop

into pupae and into an adult female; or to male-producing paedogenetic larvae or female-producing paedogenetic larvae.

Pupal paedogenesis sporadically occurs in gall midges. The embryos are formed in haemocoel of paedogenetic mother pupa termed Hemipupa, which differs from a normal pupa. A brood of larvae is released into haemocoel of parent larvae and they survive in Hemipupa for up to 18 months, from whereafter they escape.

1.2.4.1 Paedogenesis and Neoteny

Paedogenesis appears to have evolved to allow maximum use of locally abundant but ephemeral larval habitats, such as a mushroom fruiting body. When a gravid female detects an oviposition site, eggs are deposited, and larval population builds up rapidly through paedogenetic development. Adults are developed only in response to conditions adverse to larvae, such as food depletion and over-crowding. Adults may be female only, or males may occur in some species under specific conditions.

There are no reproductive adaptations beyond precocious egg development in true paedogenetic taxa. In contrast, in neoteny, a non-terminal instar develops with reproductive features of adult, including ability to locate a mate, copulate, and deposit eggs (or larvae) in a conventional manner. For example, scale insects (Hemiptera: Coccoidea) have neotenous females. In coccids, final nymphal instars moult into a winged adult male; whereas one or more instars are omitted in development of female that is sedentary nymph-like or larviform with a vulva and developing eggs. Neoteny also occurs in Strepsiptera with female development ceasing at puparium stage.

1.2.5 Polyembryony

This form of asexual reproduction involves production of two or more embryos from one egg by subdivision, mostly observed in parasitic insects (*Platygaster*). Nutrition for a large number of developing embryos cannot be supplied by original egg and is acquired from host's haemolymph through a specialized enveloping membrane called trophamnion.

Many insects are terrestrial organisms that expose their eggs to atmosphere. Adaptations to develop under these conditions require presence of a strong chorion that protects embryo against desiccation and an abundant yolk supply to provide nutrients required for growth. For this type of egg morphology, most insects undergo syncytial cleavage, resulting in several thousand nuclei residing in a specific cytoplasm before cellularization. Then these nuclei migrate to egg surface, creating a cellular blastoderm. This blastoderm forms a single-layered epithelium above yolk, forming primordium for future embryonic development. Future epithelial invaginations may form insect's mesodermal and endodermal derivatives. This mechanism is surprisingly conserved in insects, although size of initial embryonic primordium from blastoderm can differ greatly in individual species (Sander, 1976).

Embryogenesis of polyembryonic wasps greatly varies from that of other insects, as thousands of progenies would come from a single egg to clonally develop. Unlike other insects, for example, polyembryonic species are subject to holoblastic cleavage such that they are embryogenized from an early stage in cellularized environments (Grbic *et al.*, 1996). Another interesting characteristic associated with insect polyembryony is that during its development embryonic mass increases in size (Ivanova-Kasas, 1972). This developmental characteristic is related to mammals (Davidson, 1990; Gurdon, 1992) and is considered unusual in other metazoans. Because insect polyembryony emerges solely from endoparasitic taxa, developmental changes in endoparasitic lifestyles are likely to lead to conditions that favour development of polyembryony (Strand & Grbic, 1997).

Marchal (1898) reported occurrence of polyembryony in insects (1898) and since then it has been described in Hymenoptera (bees, wasps, and ants) and Strepsiptera (Ivanova-Kasas, 1972). In Hymenoptera, most extreme examples of obligatory polyembryony are those of some species that contain more than 2,000 embryos from each of their laid eggs (Strand & Grbic, 1997). Since polyembryony in strepsipterans is so little known, most discussions are focused on polyembryony in wasps (Noskiewicz & Poluszynski, 1935).

Polyembryony was initially identified in parasitic wasps using light microscopy in early 20th century (Baehrecke & Strand, 1990). *Copidosoma floridanum*, polyembryonic wasp, is perhaps the most extreme form of polyembryony that has been recorded in literature. Usually, up to 2000 embryos develop from a single egg (Baehrecke *et al.*, 1993). *C. floridanum* oviposits its egg into *Trichoplusia ni* egg. The wasp egg is tiny (60 μm), without yolks, and is cleaved into a single embryo (Grbic *et al.*, 1996). After host egg hatches, *C. floridanum* embryo extends to thousands of embryos in host larva. When host larva enters its final (fourth) position, these embryos begin morphogenesis and ultimately grow into adult wasps. It remains unclear how embryonic polarity in each of 2000 clonal progenies is formed following such large cell division. A gene that controls late stage embryonic patterns in cellular environment in *C. floridanum* is expressed as a preserved periodic pattern in relation to *Drosophila* (French, 1996). The mechanism by which this regulation occurs within individual embryos remains unknown.

Polyembryony likely has spread over Hymenoptera four independent times (approximately 40 million years is divergence time) and polyembryonic species are fairly closely related in each case to monoembryonic species (Strand & Grbic, 1996). This repeated and very rapid occurrence of this extreme development shift indicates that improvements required for transition to polyembyrony can be found in comparing morphogenesis and patterning mechanisms of different hymenopteran species. A full course of polyembryonic development from current literature cannot be reconstructed. For an understanding of embryonic patterning processes in polyembryonic wasps and developmental changes leading to polyembryony, precise knowledge of cellular events during embryogenesis needs to be collected.

1.2.5.1 Polyembryony in Hymenoptera

In a comparative review (Strand & Obrycki, 1996), an analysis of basic life history characters revealed that polyembryonic wasps in all families have similar life history. All polyembryonic wasps lay small, yolkless eggs, which appear holoblastic. Polar bodies form an enveloping membrane, proliferation occurs in an increasing number of embryos, when extraembryonic membrane partitions are rounded, loosely aggregated embryonic cells. As in *C. floridanum*, germ-band extension is generated by an extension of embryonic primordium in polyembryonic platygasterids, emphasizing that despite apparent lack of any syncytial stage, at least a few other polyembryonic wasps are likely to be long germ-band insects. This conservation of polyembryonic wasps in their external characteristics is remarkable, although there are differences in early development of these species (Anderson, 1972). Such a punctuated alteration in early development reveals a shift in history of life in polyembryony in Hymenoptera. It is thus instructive to look at what this change may have been and how embryological processes that regulate polyembryony may have come from their closest ancestors.

1.2.5.2 Evolution of Polyembryony

When considering evolution of polyembryony, we must first evaluate direction of development of Hymenopteran history. The majority of hymenopterans are of the sub-order Apocrita, which consists of free living and parasitic species. The free-living apocritans, like honeybees and ants, are confined to a single group (Aculeata) while rest are parasitic. Parasitic wasps exhibit two different strategies for development (Strand, 1986). Some species are ectoparasitoids, which deposit their eggs on outside a host and feed their larvae by rasping a hole through cuticle of host. Other species develop as endoparasitoids by injecting their eggs into haemocoel of host, which feeds blood and tissue of their progeny. The ectoparastic Orussoidea is considered as sister group to Apocrita (Whitfield, 1992; Dowton & Austin, 1994) based on molecular and morphological data. This is consistent with an observation of ectoparasite basal groups in most apocritan superfamilies. It also indicates that: (1) all apocritans and free living species have probably evolved from an ectoparasitic ancestor, and (2) endoparasitism has evolved independently from different ectoparasitic ancestors at least eight times.

1.2.6 Hermaphroditism

The occurrence of male and female gonads in same individual is called hermaphoditism. It may be a functional hermaphroditism as in case of cottony cushiony scale, *Icerya purchasi* (Hemiptera: Margarodidae). Several species of *Icerya,* that have been studied cytologically are gynomonoecious hermaphrodites, as they are female-like but possess an ovo-testis (a gonad that is a part testis and a part ovary). In these species, occasional males arise from unfertilized eggs and are apparently functional, but normally self-fertilization is assured by production of male gametes prior to female gametes in body of one individual (protandry of hermaphrodite). Non-functional hermaphroditism is noticed in case of stonefly, *Perla marginata.* In this species, testes are fused anteriorly and as soon as testicular follicles are differentiated, oogenesis does not proceed further. Oogenesis is not completed and partially grown eggs degenerate.

1.3 Alternation of Generations

Some insects are reproduced by alternating sexual and asexual generations. Females typically produce males and females, but at some point, females cease producing males and only produce females parthenogenetically. Subsequent generations of males start development again, allowing sexual reproduction before parthenogenetic process begins again. This occurs most commonly in Hymenoptera and Hemiptera.

1.3.1 Aphids (Hemiptera: Aphididae)

Aphids are remarkable as different female morphs can reproduce parthenogenetically and become virginopara/vivipara without mating as males do not exist. At other times, females may sexually reproduce and be oviparous. The development of female embryo depends on whether it is meant to become a vivipara (live birth) or ovipara (lays eggs) and is influenced by genetic and environmental factors. The aphid life cycle is discussed in much greater detail in Chapter 13.

1.3.2 Gall Wasps (Hymenoptera: Cynipidae)

Many cynipids have a dynamic life cycle that requires heterogeny or alternation of generations. As a result, a bisexual (both male and females) generation alternates with a unisexual generation (all females). By parthenogenesis, female generation reproduces and unfertilized eggs become sexual offspring. Due to haplodiploid genetics of wasps, all males will develop from unfertilized eggs. Females are, therefore, product of chromosome replication in unfertilized nucleus of cell. The wasps from two generations also look different morphologically and target same or different plant structures and create very different galls. As a result, insects of both generations were sometimes misrepresented as separate species.

1.4 Castration

The destruction or alteration of gonad tissues is called castration. It is one of the strategies used by parasites to modify host tissues and is also called parasitic castration. Parasitic castration is an infectious strategy that requires eventual intensity-independent elimination of host reproduction as primary means of acquiring energy (Lafferty & Kuris, 2002). The parasite responsible for reduction or suspension of fecundity is termed as parasitic castrator. In parasitic castration, there will be modification of host secondary sexual characteristics and may also have physiological and behavioural effects. Parasitic castration is one of the reproductive disturbances induced by parasites of insects, where there is widespread occurrence of parasite induced reduction in host fecundity via manipulation of hosts by influence of parasite genes. Suppression of host reproduction by parasitic castration falls into one of the

three following categories: inhibition of host mating due to morphological or behavioural changes, destruction of host reproductive tissue, and reduction of egg output due to malfunction of ovarian yolk sequestration. Parasitic castrators involve some strespiterans and *Rodolia* sp.; they consume adult hosts of braconids and Diptera (Kuris, 1974). In doing so, they consume reproductive tissues early, a cautious step in lengthy and precise process of fully consuming an adult host (Hall *et al.*, 2007). Apart from insects, fungi (Microspora), flat worms (Dilepididae and *Acanthocephala*), and horse hair worms (Nematomorpha) also act as parasitic castrators (Lafferty & Kuris, 2009).

1.5 Genetic Systems of Diverse Reproductive Forms

Most insects are diploid (2n) in their somatic cells and haploid (n) in their gametes. Other systems can be found; some insect groups are parthenogenetic and may be polyploid, including species in Orthoptera (Blaberidae, Tettigoniidae), Homoptera (Coccidae Delphacidae), Embioptera (Oligotomidae), Lepidoptera (Psychidae), Diptera (Chamaemyiidae, Chironomidae, Psychodidae, Simuliidae), Coleoptera (Ptinidae, Chrysomelidae, Curculionidae), and Hymenoptera.

Thelytokous insect species have females only. Thelytoky may be the only mode of reproduction in a species, or it may alternate with sexual reproduction in regular manner (cyclical thelytoky) in some cases of thelytoky; eggs only develop after penetration by a sperm (pseudogamy or gynogenesis). In Homoptera, both arrhenotoky and thelytoky occur but even more complex genetic systems can be found. For example, in some mealybugs (Pseudococcidae), both males and females develop from fertilized eggs, but in embryos that develop into males, paternally derived chromosomes become heterochromatic, genetically inactive, and are not transmitted to male progeny. This genetic system has been called parahaploidy.

1.5.1 Ploidy in Insects

The discussion of ploidy is confusing because, in most insects, some of the somatic tissues exhibit high levels of endopolyploidy. Haploid male honey bees have about same amount of DNA, as females in some of their somatic tissues because nuclei of male undergo compensatory endomitosis so that equal amounts of DNA are present. Polyploid insects usually are 3n or 4n, but exceptions include curculionid weevil species that are 5n and 6n. Owing to lack of fertilization, there is no gene recombination and subsequent segregation, and if conjugation and reduction are also absent, new gene combinations cannot rise even by crossing over.

1.6 Role of Reproductive Symbionts

1.6.1 Direct Manipulation of Host Sex Determination

Sex determination can be affected by inherited bacterial endosymbionts (See Table 1.1. For bacterial endosymbionts associated with sex manipulation in insects). Disrupting mode of sex determination of their hosts could be advantageous for endosymbionts because they are predominantly transmitted vertically through female egg cytoplasm, and not via male sperm. Thus, males represent dead ends for such microorganisms. Consequently, any effect of endosymbiont that distorts host sex ratio towards females will be selectively advantageous for endosymbiont. Recent surveys showed that >30% of sampled arthropods is infected by most common reproductive parasites. Among them, *Wolbachia* is not only the most common bacterial endosymbiont involved in reproductive parasitism but is also the only one known till date to induce all four commonly recognized types of reproductive manipulations:

i. Cytoplasmic incompatibility: Sperm–egg incompatibility leading to post-zygotic sterility between infected males and females that are uninfected or infected with a different endosymbiont strain.

TABLE 1.1

Bacterial endosymbionts associated with sex manipulation in insects

Endosymbiont	Infected Arthropod Host Groups	Manipulation Phenotypes
Wolbachia	Insects, crustaceans, mites, spiders	F, PI, CI, MK
Cardinium	Insects, mites, spiders	F, PI, CI
Rickettsia	Insects, spiders	PI, MK
Spiroplasma	Insects	MK
Flavobacteria	Insects	MK
Arsenophonus	Insects	MK

F: feminization of genetic males; PI: parthenogenesis induction; CI: cytoplasmic incompatibility; MK: male killing.

ii. Male killing (MK): Sex-ratio distortion towards females through targeted death of male progey.
iii. Feminization of genetic males: Sex-ratio distortion towards females through conversion of genetic males into functional females.
iv. Parthenogenesis induction: Sex-ratio distortion towards females through induction of asexual daughter development.

1.6.2 Cytoplasmic Incompatibility

It favours endosymbiont transmission without host sex-ratio distortion. Among different reproductive manipulations induced by bacterial endosymbionts, cytoplasmic incompatibility (CI) is most widespread phenotype: it has been described in mites, isopods, and insects and is caused by *Wolbachia* and *Cardinium*. Unlike feminization, parthenogenesis induction and MK, CI does not induce host sex-ratio distortion. Instead, CI favours infected female reproduction and, thereby, transmission of maternally inherited endosymbionts. CI has been particularly studied in *Wolbachia*.

CI *Wolbachia* is thought to cause sperm modification in infected males. Although modified sperm can be rescued by infected oocytes and lead to viable progeny, uninfected females fail to rescue modified sperm and abort. CI-induced sterilization of uninfected females, thus leads to spread of females carrying *Wolbachia* in uninfected populations.

Bidirectional CI is also observed when both sexes are infected by different *Wolbachia* strains, which are not able to rescue sperm modification induced by other strain. Several studies have shown that bidirectional CI between diverging populations could promote speciation that can be reinforced by premating isolation.

Although CI molecular mechanisms remain largely unknown, cytological studies in different insects and isopod species have described asynchrony in development of male and female pronuclei, leading to defects of first mitotic division of embryo. Delay of histone H 3.3 phosphorylation in male pronucleus, which is required for initiation of mitosis, induces late male chromosome condensation during first metaphase and exclusion during following anaphase, leading to embryonic lethality. If both male and female insects are infected with *Wolbachia* – progeny will be infected. If female is infected and male is not infected, progeny will all be infected. If female is not infected and male is infected, there will not be any progeny (See Figure 1.2 for schematic illustration of *Wolbachia*-induced cytoplsmic incompatibility).

1.6.3 Male Killing: Sex-Ratio Distortion without Manipulation of Host Sex Determination

MK is an adaptation to maternal transmission used by several bacterial endosymbionts, such as *Wolbachia, Rickettsia*,,, and *Flavobacteria* that are found in five insect orders and acari. Embryonic male killers (i.e. early male killers) are found in host species with environmental, XO, XY, ZW, and haplodiploid sex-determination systems. Contrary to feminization and parthenogenesis induction, which

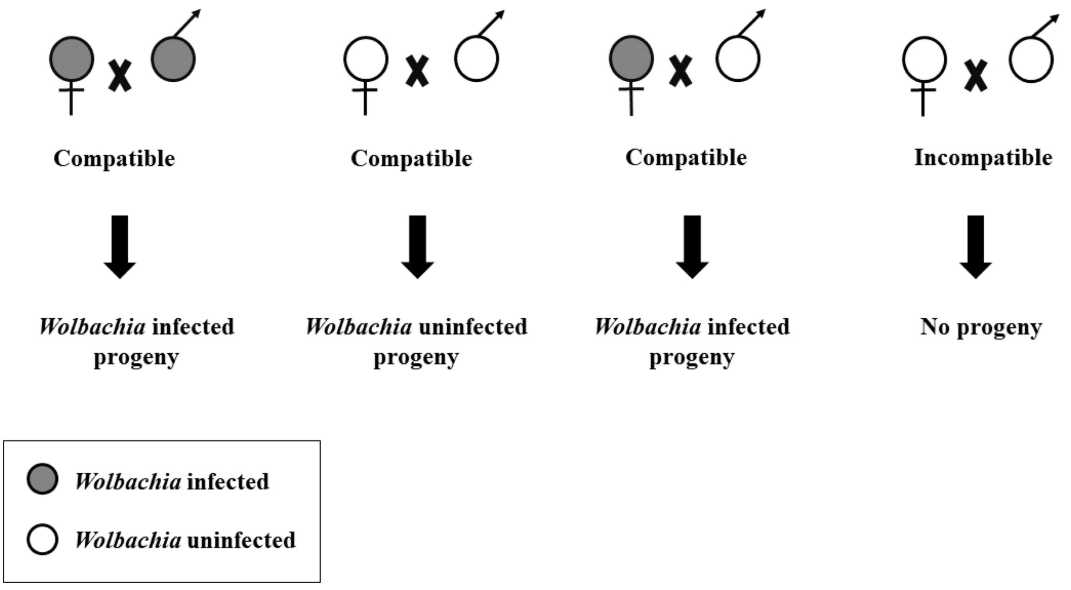

FIGURE 1.2 Cytoplasmic incompatibility influenced by reproductive parasite *Wolbachia* in insects.

both consist of converting non-transmitting males into transmitting females, MK involves death of sex that does not vertically transmit endosymbionts (i.e. males). In ladybird beetles, which are 'hotspots' for male killers, MK-induced death of males benefits their infected sisters by sibling egg consumption, decreased intensity of antagonistic interactions between siblings, and reduced levels of inbreeding. As a result of this fitness compensation, infected females produce daughters with a higher probability of survival than uninfected ones, allowing endosymbionts to spread in population. However, fitness compensation is generally imperfect (death of male progeny commonly increases sister host survival probability by 10% or less). Selection for an MK endosymbiont is, therefore, much smaller than that for a feminizing endosymbiont, such that male killer prevalence in majority of hosts is lower than 40%, although high-prevalence infections do occur. The low drive also increases sensitivity of male killer prevalence to environmental conditions.

The mechanism by which sex specificity of virulence is achieved has been demonstrated in association between *Spiroplasma poulsonii* and *Drosophila melanogaster*. A functional dosage-compensation complex, a major component of sex-determination pathway in *Drosophila,* is required for MK by *S. poulsonii*. Endosymbionts failed to kill males lacking any of five protein components of dosage-compensation complex. This result can be exploited to yield further insights into MK mechanism, which still remains unknown.

1.6.4 Feminization

Sex determination in insects is a cellular process. Consequently, endosymbionts have to infect all host cells and interact with genetic control of sex determination in all somatic cells for feminization to be effective. This view changed, however, with discovery of *Brevipalpus phoenicis* mites feminized by bacterial endosymbiont *Cardinium*. Cases of feminization in insects have also been discovered recently, mediated by *Wolbachia* in butterfly *Eurema hecabe* and leafhopper *Zyginidia pullula* and by *Cardinium* in wasp *Encarsia hispida*.

In *E. hecabe* and *Z. pullula*, *Wolbachia*-infected females produce all-female broods in laboratory conditions and antibiotic treatment leads to male-biased progenies, which suggested that these phenotypic females possess a male genotype. In *E. hecabe*, genetic sex determination follows female heterogamety (ZZ males and ZW females) and cytological observations have confirmed that *Wolbachia*-infected females are in fact ZZ genetic males inverted into phenotypic females.

1.6.4.1 Mechanisms of Feminization

In insects, *Wolbachia* could interact with key genes that control somatic sex determination, such as *Drososophila melanogaster doublesex* or transformer homologs. *Doublesex* (a switch gene at bottom of sex-determining cascade) and transformer (responsible for female-specific splicing of *doublesex*) have been identified in several insect orders. In moth *Ostrinia scapulalis*, *Wolbachia* endosymbionts typically induce MK but, at lower density, they also have a feminizing effect on genetic males. It was recently shown that *Wolbachia* can manipulate *O. scapulalis* sex determination by interfering with sex-specific splicing of *doublesex* homolog or with an upstream gene in sex-determination cascade. In addition, *doublesex* is not regulated at level of pre-mRNA splicing but it rather exhibits different expression levels between males and females. Alternatively, it has been hypothesized that *Wolbachia* could modulate host sexual phenotypes by interacting with hormonal pathways involving ecdysteroids. It is suspected that bacterial endosymbionts are able to interact with several different molecular pathways to achieve feminization of their arthropod hosts.

Endosymbiont-mediated reproductive manipulations directly impact host sex determination:

a. Feminization of genetic males, characterized by sex-ratio distortion towards females through conversion of genetic ZZ males into phenotypic ZZ females.
b. Parthenogenesis induction characterized by sex-ratio distortion towards females through conversion of genetic (haploid) males into genetic (diploid) females. Black (white) coloration: individual carries (does not carry) endosymbionts. ZZ/ZW, homo/heterogametic status of individual. n/2n, haploid/diploid status of individual. To simplify, endosymbiont transmission rate from mother to offspring is assumed to be 100% (See Figure 1.3 for schematic illustration of endosymbiont mediated feminization in insects).

1.6.5 Induction of Parthenogenesis in Insects

Feminizing endosymbionts drive female development to enhance their vertical transmission. However, successful endosymbiont transmission not only requires production of males and females but also mating. From an endosymbiont perspective, ultimate manipulation would consist in driving female development while making males superfluous. This strategy is used by at least three endosymbionts: *Wolbachia*, *Cardinium*, and *Rickettsia*. In all cases, these endosymbionts induce parthenogenesis in haplodiploid insects (hymenopterans and thrips) and acari. In these taxa, sex is normally regulated by ploidy of embryo: males develop from unfertilized haploid eggs and females develop from fertilized diploid eggs.

Parthenogenesis-inducing (PI) endosymbionts are able to convert non-transmitting males into transmitting females by enabling unfertilized eggs to develop as females. This is achieved through doubling of chromosome number in unfertilized eggs, rendering them diploid. As a result, infected parthenogenetic females are in turn able to produce endosymbiont-transmitting female progeny without need for sexual reproduction, egg fertilization and, thus, males as for feminization; precise molecular mechanisms underlying parthenogenesis induction are unknown. However, cytogenetic observations have shown that parthenogenesis is induced by endosymbionts in at least three different ways (See Figure 1.4 for an overview of endosymbiont mediated parthenogenetic induction in insects).

In hymenopterans, endosymbiont-mediated diploidization is caused by disruption of cell cycle during early embryonic development in two different ways:

i. In *Trichogramma* wasp species, two haploid sets of chromosomes do not separate during anaphase of first mitotic division, resulting in one diploid nucleus with two identical sets of haploid chromosomes instead of two haploid nuclei.
ii. In wasp *Muscidifurax uniraptor*, first mitotic division is normal, leading to two cells with haploid nuclei, and diploidy is restored by fusion of two cell nuclei after completion of first mitotic division.
iii. A third mechanism occurs in *Bryobia* mites, in which *Wolbachia* induces parthenogenesis by meiotic modification in infected eggs, resulting in diploid gametes.

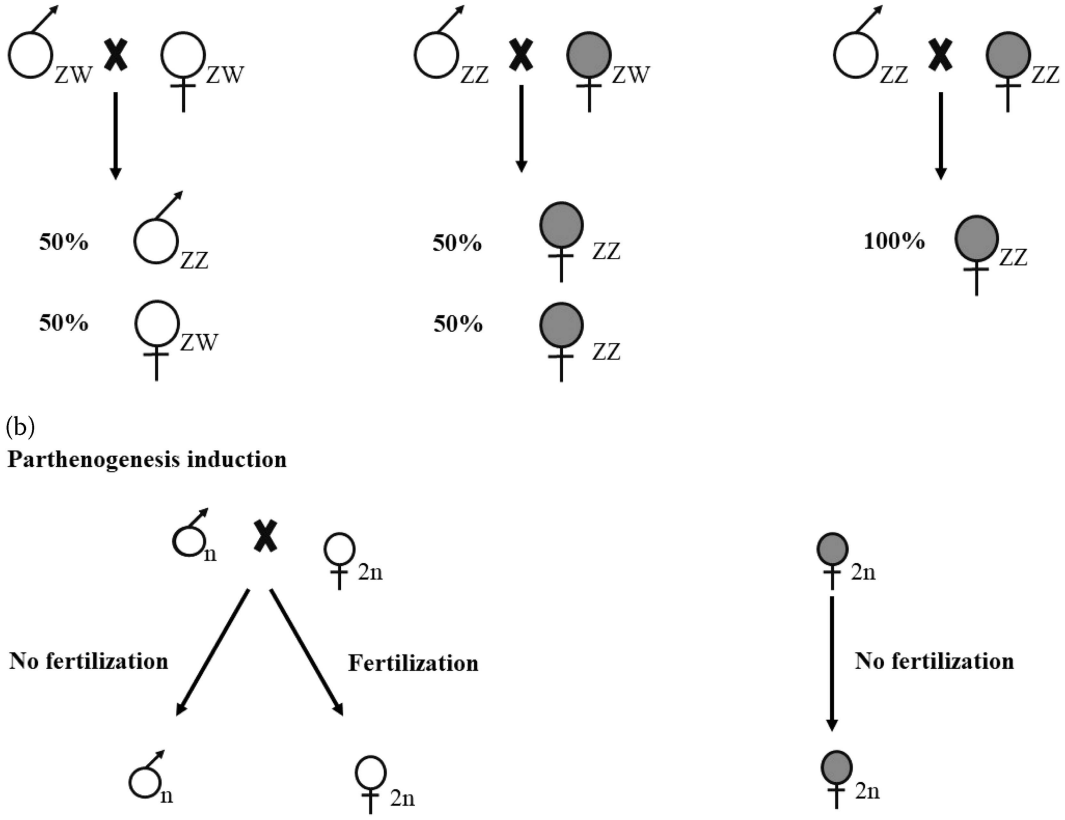

FIGURE 1.3 Endosymbiont-mediated feminization in insects: (a) Feminization of genetic males by sex-ratio distortion. (b) Feminization of induction of parthenogenesis induction accomplished through conversion of genetic (haploid) males into genetic (diploid) females.

Similar to feminizing endosymbionts, PI endosymbionts sometimes produce intersex phenotypes when some tissues become diploid, whereas others remain haploid during embryogenesis. This is influenced by rearing temperature of mothers, which is thought to affect density of endosymbionts (which are thermosensitive).

For example, in *Trichogramma* species infected by *Wolbachia*, all-female progenies are produced at temperatures below 26.8°C because endosymbiont density is high enough to diploidize unfertilized (haploid) eggs. By contrast, higher temperatures (>30.8°C) eliminate *Wolbachia* endosymbionts. As a result, unfertilized (haploid) eggs are not diploidized, which leads to production of all-male progenies. At intermediate temperatures, *Wolbachia* titer is moderate and many infected females produce males, females, and gynandromorphs (i.e. individuals in which some tissues are male whereas others are female) from unfertilized (haploid) eggs. In gynandromorphs, gender of tissue is determined by level of ploidy of cell from which tissue is derived. In these individuals, *Wolbachia*-mediated diploidization could be somewhat repressed, and this does not take place during first mitotic division but at a later stage in a subset of cells.

Overall, outcomes of parthenogenesis induction and feminization of genetic males are fairly similar in many respects. They both convert males into females and occasionally produce intersexes. The main difference is that feminizing endosymbionts invert genetic males into phenotypic females, whereas PI endosymbionts invert genetic males into genetic females.

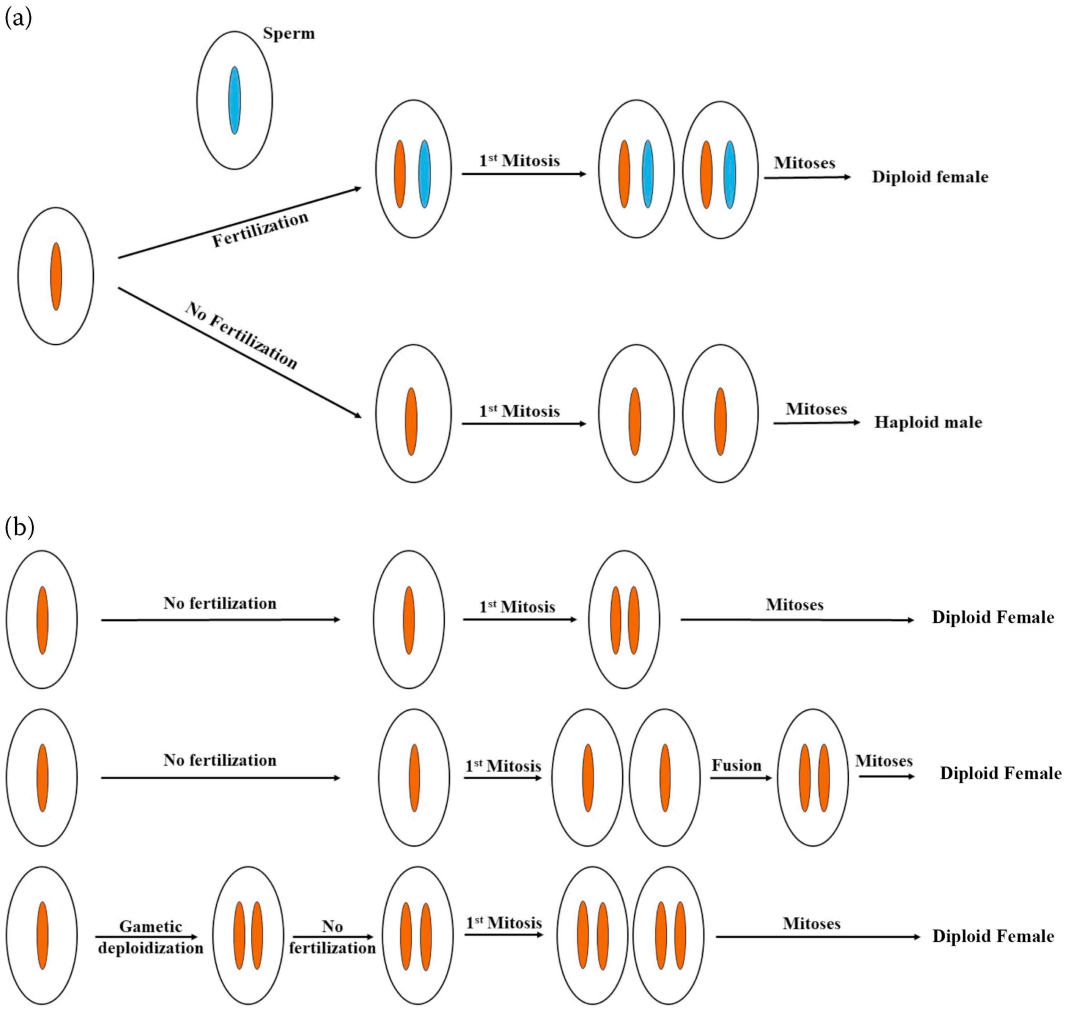

FIGURE 1.4 Schematic illustration of mechanisms of male and female development in haplodiploid species lacking or carrying parthenogenesis-inducing endosymbionts. (a) Reproduction in absence of endosymbiont. (b) Reproduction in presence of parthenogenesis-inducing endosymbionts. Maternal (red) and paternal (blue) sets of chromosomes.

1.7 Physiological Regulation of Insect Reproduction

The initiation and termination of some reproductive events often depend on environmental factors, such as temperature, humidity, photoperiod, or availability of food or a suitable egg-laying site. Additionally, these external influences may be modified by internal factors, such as nutritional condition and state of maturation of oocytes. Copulation also may trigger oocyte development, oviposition, and inhibition of sexual receptivity in female via enzymes or peptides transferred to female reproductive tract in male accessory gland secretions. Fertilization following mating normally triggers embryogenesis via egg activation. Regulation of reproduction is complex and involves sensory receptors, neuronal transmission, and integration of messages in brain, as well as chemical messengers (hormones) transported in haemolymph or via nerve axons to target tissues or to other endocrine glands. Certain parts of nervous system, particularly neuro-secretory cells in brain, produce neurohormones or neuropeptides (proteinaceous messengers) and also control synthesis of two groups of insect hormones – the ecdysteroids and juvenile hormones (JH).

TABLE 1.2

Role of neuropeptides in insect reproduction

Sl. No.	Neuropeptides	Function
1	Antigonadotropin (e.g. oostatic hormone, OH)	Suppresses oocyte development
2	Ovarian ecdysteroidogenic hormone (OEH = EDNH)	Stimulates ovarian ecdysteroid production
3	Ovary maturing peptide (OMP)	Stimulates egg development
4	Oviposition peptides	Stimulate egg deposition
5	Prothoracicotropic hormone (PTTH)	Affects egg development
6	Pheromone biosynthesis-activating neuropeptide	Regulates pheromone production (PBAN)

JH and/or ecdysteroids are essential to reproduction, with JH mostly triggering functioning of organs, such as ovary, accessory glands, and fat body, whereas ecdysteroids influence morphogenesis as well as gonad functions. Neuropeptides play various roles at different stages of reproduction, as they regulate endocrine function (via corpora allata and prothoracic glands) and also directly influence reproductive events, especially ovulation and oviposition or larviposition. The role of neuropeptides (Table 1.2) in control of reproduction is an expanding area of research, made possible by new technologies, especially in biochemistry and molecular biology. To date, most studies have concentrated on Diptera (especially *Drosophila,* mosquitoes, and houseflies), Lepidoptera (especially tobacco hornworm, *Manduca sexta*), locusts, and cockroaches.

1.8 Conclusions

Understanding evolutionary perspectives of insect's modes of reproduction may explain phylogenetic relationship between different groups of insects. Detailed investigations on impact of ecological factors on adaptation of insects and their influence on modes of reproduction is an interesting subject matter for investigation in context of climate change scenario or global warming. Understanding molecular basis of sex manipulation by endosymbionts and especially parthenogenetic induction by reproductive parasites offers a great scope for applied pest control in a variety of ways.

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2

Sexual Reproduction

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CONTENTS

2.1 Introduction

The wide range of reproductive strategies in insects alongwith their physiological systems has resulted in their enormous success on earth. Most insects have very few reproductive opportunities due to a short lifespan (Arnqvist *et al.*, 2000), for which they compensate by displaying numerous reproductive strategies (parthenogenesis, pedogenesis, polyembryony, functional hermaphroditism, viviparity, or sexual reproduction) facilitating production of phenomenal numbers of offspring under varied ecological circumstances (Klowden, 2013). The basic organization of reproductive systems of both male and female insects is quite similar (Figure 2.1).

The germ cells of reproductive organs arise from pole cells, which are amongst foremost to be differentiated during embryogenesis. Along with mesodermal tissues, germ cells develop into adults' reproductive organs. A pair of gonads is connected by individual ducts to a common duct. This common duct is both mesodermal as well as ectodermal in origin. The activities of both male and female

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reproductive systems are coordinated by hormones and transcription factors, and are eventually regulated by physiological and environmental factors (Klowden, 2013).

The male reproductive organs consist of a pair of testes, with a series of testicular tubes/follicles where spermatozoa are produced. The testes open separately into sperm duct or vas deferens of mesodermal origin, which extends posteriorly and forms seminal vesicle or sperm-storage organ (Snodgrass, 1957). Most insects have a number of accessory glands that open into the ejaculatory duct. These glands are either formed as diverticula of vasa deferentia, or vasa deferentia themselves become glandular and carry out the functions of accessory glands. The paired vasa deferentia unite and lead into ectodermally derived ejaculatory duct, the tube that transports semen or sperm package to female gonopore/genital pouch (Snodgrass, 1957; Gullan & Cranston, 2014).

The female reproductive system consists of a pair of ovaries, which are connected with a pair of postero-lateral oviducts that join to form a median/common oviduct. This median oviduct opens posteriorly into a genital chamber that sometimes forms a tube, vagina, and is often developed to form a bursa copulatrix for reception of penis/aedeagus. The genital chamber (vagina) opens into a spermatheca that stores sperms, and, frequently, a pair of accessory glands (Klowden, 2013) (Table 2.1).

In this chapter, attempts have been made to provide a detailed description of male and female reproductive systems of insects.

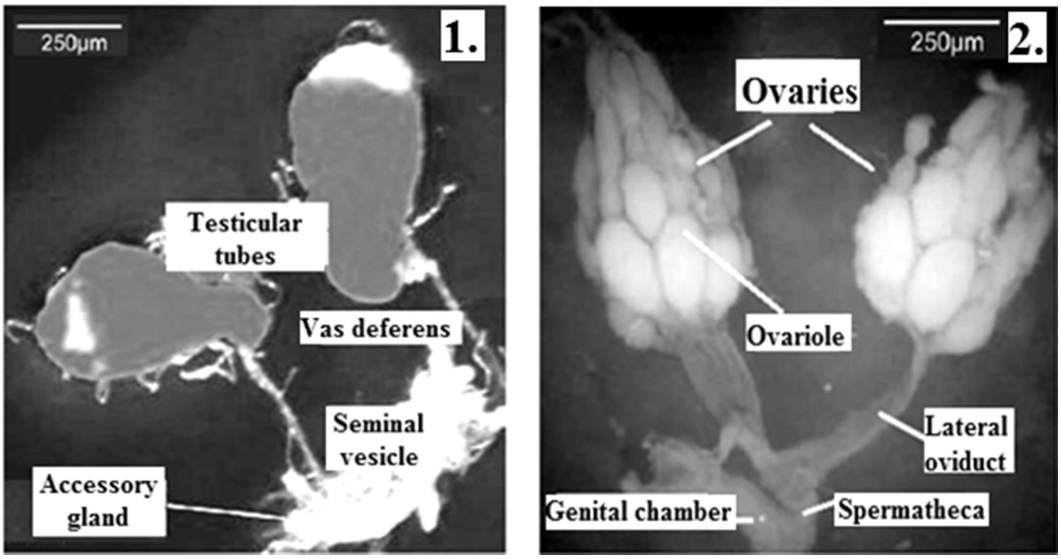

FIGURE 2.1 (1) Male and (2) female reproductive systems of an insect.

TABLE 2.1

Comparison between male and female reproductive systems (Richards & Davies, 1979; Gullan & Cranston, 2014)

Female Reproductive System	Male Reproductive System
Paired ovaries composed of ovarioles	Paired testes composed of follicles
Paired oviducts	Paired vasa deferentia
Egg calyces (reception of eggs)	Seminal vesicles (sperm storage)
Common/median oviduct and vagina	Median ejaculatory duct
Accessory glands (ectodermal origin) colleterial or cement glands	Accessory glands (ectodermal or mesodermal in origin)
Bursa copulatrix (copulatory pouch) and spermatheca (sperm storage)	No equivalent
Ovipositor	Male genitalia/aedeagus

2.2 Evolution of Sex

Previous studies (Maynard Smith, 1978, 1982) have predicted that ancestral gametes were small and isogamous. The evolution of anisogamy was an important transition in evolution, as it caused separation of sexes (Maynard Smith & Szathmary, 1997). Physiologically, males produce sperms and females produce ovum. Anisogamy or sexual asymmetry in gamete size plays central role in regulating all pre- and postcopulatory processes of Darwinian sexual selection. Parker *et al.* (1972) proposed a theory, often known as PBS (Parker, Baker, and Smith) model (Bell, 1978; Lessells *et al.*, 2009) for evolution of anisogamy and two sexes by gamete competition. Based on PBS model, Charlesworth (1978) and Bell (1978) suggested that anisogamy may evolve only if there is sufficient size variation in ancestral population to generate sexual asymmetry in gamete size or when gamete size mutations have sufficiently large effects (Maire *et al.*, 2002).

Parker *et al.* (1972) proposed two selection pressures that act on gamete production, i.e. number of gametes and fitness of resulting zygotes. They further argued that whatever gamete size ancestral isogamous population showed, selection generally pushes gamete size towards a zone of zygote fitness versus size ratio relationship. Anisogamy will occur depending on zygote fitness versus size ratio relation for ancestral state. The analysis of Bulmer and Parker (2002) suggested that ancestral stage would have been isogamous unicells (microgametes). As adult size increased through evolution of muticellularity, zygote fitness versus size ratio increased away from its early position near gamete survival. The isogamous microgamete size initially increased and at some threshold, isogamy became unstable, and was replaced by anisogamy through invasion of macrogametes (Parker, 2011).

The PBS model (Parker, 1979) also suggests that once anisogamy evolved, disassortative fusions of ova with sperm further led to loss of motility in ova. Due to vast numerical predominance of sperm arising through size-number trade-off at production, selection quickly favoured avoidance of sperm-sperm fusions, while ova fused much quickly with sperm. Although loss of motility in ova interfered with ability of ova to fuse with other ova, it allowed energy spent on motility to be concentrated on to increased productivity (Parker, 1978). The PBS model further argued that gamete competition between sperm of different males over fertilization of a given set of ova was responsible for evolutionary origin and maintenance of anisogamy (Parker, 1970, 1982). As multicellularity increased, increased size of zygote further led to an evolutionary increase in size of ovum. Simultaneously, sperm competition, maintained sperm at a minimal size with increased fertilization success. Consequently, anisogamy ratio (ovum size/sperm size) became very large and became the fundamental reason why sperm are small and adapted solely to function in succesfull fertilization without making any contribution to zygote provisioning (Parker, 2011).

The production of a sperm attraction pheromone is another adaptation that has effectively favoured increased size of egg. The large size and extra volume of egg is not only solid matter, but also a zone of attraction around egg, which increases its probability of fertilization. Dusenbery (2000) assumed that rate of pheromone production is proportional to egg volume. Pheromone-releasing eggs show greater than 50-fold increase in fertilization success (Jantzen *et al.*, 2001). Gamete motility is also a possible factor in determining anisogamy, with faster sperm having fertilization advantage under sperm competition (Levitan, 2000). Dusenbery (2000) predicts that anisogamy offers advantage of increased gamete encounter rates, and evolution towards anisogamy occurs by creating one gamete smaller and motile and the other larger and non-motile.

Theory of disruptive selection by gamete competition is the most powerful explanation of origin of anisogamy that has resulted in sexual selection and sexual conflict (Lessells *et al.*, 2009).

2.3 Evolution of Sexual Reproduction

The term ‘mating system’ includes nature of gametes, mate-choice strategies, patterns of male–female pairings, and behavioural strategies displayed by individuals to attain reproductive success and mate, even if they are not chosen (Breed & Moore, 2016); all dimensions of sexual reproduction. While an

asexually reproducing animal passes all of its genes to each offspring, however, in sexual reproduction only half are passed on to each offspring. Despite the cost associated with producing male and female offspring during sexual reproduction (Otto, 2008), yet selection favours sexual reproduction (Hartfield & Keightley, 2012). This mismatch between theoretical prediction and empirical reality is generally called as 'the paradox of sex' (Maynard Smith, 1978). Selective advantages from genetic recombination and genetic diversity, i.e. non-accumulation of deleterious genes among offspring outweigh costly nature of sexual reproduction (Lehtonen *et al.*, 2012).

In a strict sense, sexual reproduction refers to mixing of genetic material, either within an organism (e.g. recombination) or between organisms (Alcock, 2001). While meiosis eliminates harmful changes and mutations, and favours repair of damage to DNA; it also promotes reorganization of accumulated epigenetic signals that regulate gene expression (Dugatkin, 2014). The main function of sexual reproduction could possibly be genetic editing. In addition, sexual reproduction produces offspring that are better suited for changing environment.

When Valen introduced 'Red Queen hypothesis' to evolutionary biology, it initially emphasized on the concept of co-evolution, stating that when a prey species evolves a defense, predator evolves to defeat that defense and therefore evolves another defense (Alcock, 2001). However, the hypothesis can be equally applied to evolution of sexual reproduction.

Sexual reproduction evolves more quickly when a species' environment changes rapidly, such as a shift in community of interacting species, especially host and parasite species (Dugatkin, 2014). The genetic associations built up by past selection, along with sex and recombination improve fitness of offspring, thereby turning recombination load into an advantage. Genetic recombination gained from sexual reproduction produces new defenses against diseases and parasites (Alcock, 2001). When disease and parasite pressures are low, cost of meiosis and benefit of proven genetic combinations favour asexual reproduction, however, in case of a surge sexual reproduction is favoured (Dugatkin, 2014).

In animals, meiosis and gametogenesis occur sequentially in germline cells and sex differentiation occurs in somatic gonads into which the germline cells migrate, which is in contrast to plants, where meiosis and gametogenesis occur separately from somatic cells of sporophyte and gametophyte, while sex differentiation occurs in them (Bai, 2015). Meiosis, gametogenesis, and sex differentiation seem to have evolved independently, integrated later by chance, and genetically fixed as a program in protists. Each cell can thus behave independently for emergence of meiosis and gametogenesis, and can also live together closely enough to make cell fusion and cell–cell recognition possible. Integration of these three events have brought selective advantages together and the integration is selected for and referred to as 'sexual reproduction' (Bai, 2015).

During sexual reproduction, individual animals combine their genes with genes of another animal, via gametes of different sizes, to give their offspring greatest possible genetic advantage. Sexual reproduction creates both a social atmosphere and competition among individuals as each strives to maximize its genetic contribution to subsequent generations (Alcock, 2001). Males usually make multiple small, relatively inexpensive gametes and try to fertilize as many eggs as possible, while providing little or no care for their offspring. On other hand, production of an egg by a female is more costly than that of sperm, leading to production of few individually expensive gametes (Breed & Moore, 2016). The value of these gametes suggests that perhaps most eggs should be fertilized, while sperm may go waste (Dugatkin, 2014), thereby making females more protective of eggs. Because females make fewer, larger gametes and often also provide parental care, they usually have a lower potential rate of reproduction than males (Alcock, 2001). As a result, receptive females are scarce, and males typically compete for access to them, while females choose among many potential mates. Mate choice and parental investment follow from these differences in investment in gametes (Dugatkin, 2014).

2.4 Male Reproductive System

In males, paired testes produce spermatozoa. Each testis comprises a series of tubular follicles that may vary from one in some apterygotes and Diptera to over 100 in Orthoptera and 300 in Hymenoptera.

The follicles are enclosed by a peritoneal sheath. Within each follicle, developing sperm in successive stages of maturation are present (Phillips, 1970). Each follicle is divided into a series of zones: (i) *Germarium:* contains primordial germ cells (spermatogonia) that multiply; (ii) *Zone of growth:* where spermatogonia increase in size and develop into spermatocytes after repeated mitosis; (iii) *Zone of division and reduction:* where spermatocytes undergo meiosis, giving rise to spermatids; (iv) *Zone of transformation:* where spermatids get transformed into spermatozoa (Richards & Davies, 1979). The follicles are connected to a main duct, *vas deferens*, through individual *vas deferens* tubes. A portion of *vas deferens* is enlarged as *seminal vesicle*, and serves as a storage reservoir for sperm before being transferred to female reproductive system. The two *vasa deferentia* are enclosed by circular muscles and are connected to *ejaculatory duct*, which is ectodermal in origin (Klowden, 2013; Gillot, 2018).

The terminal portion of ejaculatory duct forms intromittent organ known as *aedeagus*. Aedeagus is absent in Apertygota males, and in Odonata a secondary copulatory structure at anterior of abdomen performs its function. Some ancestral pterygotes bear a pair of intromittent organs. Other accessory structures, known as claspers/parameres, maybe present on neighbouring segments and are used to grasp female during copulation. A pair of male accessory glands may also be present that can open into either vas deferens or ejaculatory duct (Richards & Davies, 1979; Chapman, 2009). Male accessory glands perform a variety of functions, viz. (i) production of seminal fluid that transports and/or activates sperm, (ii) as vaginal mating plug that temporarily blocks entry of another males' sperm, and (iii) formation of spermatophores that enclose sperm (Leopold, 1976). Apterygote males produce and deposit spermatophores on moist ground, which are taken up by females (Mann, 1984). However, in more advanced insect orders, sperm are transferred directly in seminal fluid by internal fertilization, without production of spermatophores (Hinton, 1974) (Figure 2.2).

Male accessory gland proteins (AGPs) can supplement nutritional reserves of females and stimulate egg production/ripening, post mating (Happ King, 1984). AGPs are known to remove physiological block preventing ovulation and egg deposition until mating takes place, in many insect species (Gillott, 2003; Klowden, 2013). AGPs reduce oxidative stress in females and sustain sperm quantity necessary for fertilization. Antibacterial proteins present in AGPs even protect sperm, and male and female reproductive tracts from infection (Lay *et al.*, 2004). Although male accessory glands cannot synthesize juvenile hormone (JH), they are capable of methylating already-synthesized JH acid and subsequently transferring active JH to females (Engelmann, 1970; Koeppe *et al.*, 1985). This transferred JH may facilitate patency of developing oocytes and thereby increase female fecundity (Lay *et al.*, 2004; Chapman, 2009; Klowden, 2013).

2.4.1 Spermatogenesis

In male insects, a small number of stem cells give rise to cells having two different fates: a daughter spermatogonial cell that subsequently differentiates into spermatozoa and pluripotent stem cell that is

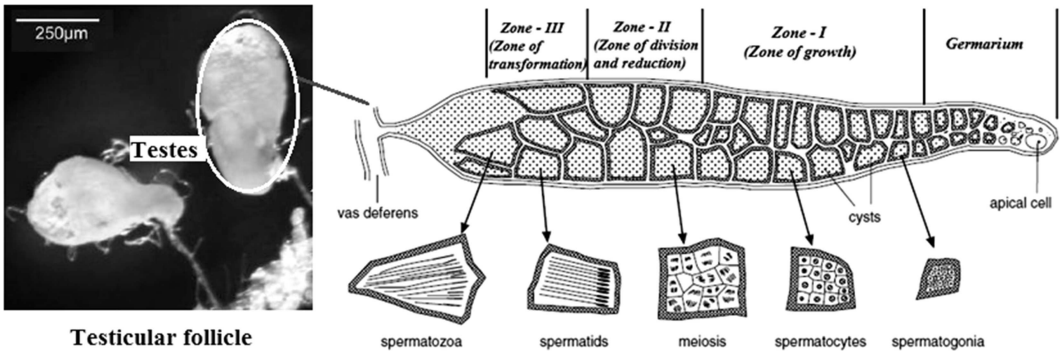

FIGURE 2.2 Testicular follicle of an insect showing different stages of development of sperm (modified from Chapman, 2009).

capable of regenerating. The latter allows male insects to continue producing mature sperm throughout its reproductive life. The spermatogonial cell undergoes several mitotic divisions to produce spermatocytes and subsequently divide meiotically to produce spermatids that further differentiate into spermatozoa. The production of spermatozoa occurs within testes follicles (Klowden, 2013). Two primary cell types, viz. primordial germ cells and somatic precursor cells, occupy microenvironment of a stem cell niche at its apical end (Chen & McKearin, 2005). Constituting niche are 10–15 specialized nondividing somatic cells that comprise hub. The somatic cells are surrounded by 6–9 apical germline stem cells (GSCs), which are further surrounded by somatic cyst stem cells. Both germline and somatic cyst stem cells directly contact hub. JAK-STAT signaling pathway from niche regulates renewal, maintenance, and survival of stem cells and provides cues that prevent differentiation and self-renewal of stem cells (Klowden, 2013).

Insulin-like peptides are also involved in division of both germline and somatic cyst stem cells. After receiving niche signal, somatic cyst stem cells divide mitotically to produce one gonialblast and another stem cell. The daughter cells that remain within niche post stem cell division continue to receive signals and self-renew. However, daughter cells that move out of niche do not receive signals, and began to differentiate as gonialblasts. The gonialblasts initiate four mitotic divisions with incomplete cytokinesis. These divisions result in a syncytium of 16 spermatogonia connected by ring canals (lined with anillin, an actin-binding protein that attaches it to membrane). The spermatogonia divide mitotically, grow in volume, and become primary spermatocytes. While in *Rhodnius* spermatocyte stage is reached at eight divisions (128 cells per cyst), in *Drosophila* four divisions result in cysts with 64 cells (Chapman, 2009; Klowden, 2013).

The subsequent meiotic division of spermatocytes yields haploid spermatids. Gene transcription ceases and morphogenesis of spermatids continues within syncytium, where all spermatid nuclei that are descended from a primary spermatocyte remain connected by cytoplasmic bridges. The mitochondria of these postmeiotic spermatids aggregate alongside each haploid nucleus and fuse into two large mitochondrial derivatives, forming spherical *nebenkern*. These mitochondrial lobes begin to unfold and elongate parallelly to develop axoneme and become the source of sperm motility (Klowden, 2013).

The sperm maturation further involves individualization of syncytium into individual spermatozoa. One meiotic and four mitotic divisions lead to four haploid spermatids for each diploid spermatocyte within cyst. The spermatids further differentiate into mature spermatozoa through developmental events called spermiogenesis. Events of differentiation through individualization process occur as a cytoskeletal membrane complex assembles at end of cyst containing nuclei and moves down its length. The individualization complex moves down cyst, becomes visible as a cystic dilation, encases each spermatid in its own cell membrane and extrudes ring canals and syncytial cytoplasm (Klowden, 2013). These components are eliminated from cyst tail as complex detaches at the end of individualization process. The acrosomal complex develops at sperm head and is associated with enzymes acrosin and hyaluronidase. The individualized bundles of sperm become coiled before their release. As differentiation proceeds, cysts elongate and eventually rupture, releasing spermatozoa into vas efferens. The spermatozoa move to seminal vesicles where they remain until mating takes place (Chapman, 2009; Klowden, 2013).

2.4.1.1 Spermatozoa

Insect sperm comprise a head region and a long flagellum that is used for locomotion. Within head are a haploid nucleus and acrosomal complex at tip that arises from Golgi apparatus during differentiation. The acrosome contains enzyme acrosin that dissolves egg membranes for fertilization. Sperm are only insect cells that bear flagella, which may be absent in proturans (disc-like sperm). The motor portion of flagellum is known as axoneme. The axoneme consists of microtubules originating from centrosome that forms flagellum at the base of sperm nucleus (Phillips, 1970; Baccetti, 1972). The centrosome is the organizer of microtubular network and influences formation of mitotic spindle and consists of two centrioles surrounded by a mass of protein, with a centriolar adjunct forming a collar around it. Although centrosome is inactive in female gamete, it persists in sperm to organize first mitotic spindle in fertilized zygote.

The arrangement of microtubules in collembolans consists of a cylindrical array of doublets connected by cross-bridges of motor protein dynein and a pair of single microtubules running up the center, known as 9 + 2 arrangement. However, other insect sperm have a modified 9 + 2 arrangement with an additional ring of nine accessory microtubules, a 9 + 9 + 2 arrangement. A 9 + 9 + 3 pattern, with three central tubules, is found in some dipterans and neuropterans (Geyer, 1951; Baccetti *et al.*, 1973).

The microtubules are made of dimeric protein tubulin, and dynein arms that form cross-bridges between doublet fibers allowing them to slide past each other in a sliding filament model to cause bending of flagellum. Accessory tubules further strengthen axoneme and amplify its motion. Comparative studies indicate that 9 + 9 + 2 insect sperm show helicoidal pattern compared to planar beat of species with 9 + 2 sperm. The tail length of flagella may vary within a species (Baccetti *et al.*, 1973). In *D. melanogaster,* sperm ranges from 1.6 to 2 mm, but males of *D. bifurca* produce sperm over 58-mm long. *Drosophila* species that produce longer sperm also take longer to reproductively mature (Parker, 1993). This variation in individual size of sperm may result from cross-linking and sliding of microtubules against mitochondria during individualization process (Klowden, 2013; Gillot, 2018).

Lepidopteran males produce two different types of sperm, apyrene and eupyrene, with former having no nuclei and being genetically non-functional. Both are transferred to females, but only eupyrene sperm fertilize oocytes, while apyrene sperms assist former to move through female reproductive tract, and/or provide essential nutrients (Friedländer, 1997). About 70–90% of transferred sperm may be apyrene, and may displace eupyrene sperm from previous matings. In *Bombyx mori,* absence of apyrene sperm results in lack of fertilization. Thus, both types of sperm are necessary for fertilization to occur (Friedländer, 1997; Chapman & Davies, 2004; Klowden, 2013).

2.4.2 Endocrine Control of Male Reproductive System and Spermatogenesis

Testes of several lepidopteran species produce ecdysteroids when stimulated by an ecdysiotropin, produced by medial neurosecretory cells of brain. In addition, insulin signaling stimulates production of gonial blasts from GSCs and stimulates growth of spermatocytes (Engelmann, 1970). In some insects, rate of mitotic divisions of spermatogonia to form spermatocytes increases with 20-hydroxyecdysone; however, the increase is restricted by high titers of JH (Gullan & Cranston, 2014; Hardie, 2018). Spermatocytes then begin meiotic divisions that are arrested at prophase until end of larval development. The post-wandering peak of 20-hydroxyecdysone unblocks meiosis and subsequently allows cells to proceed to metaphase. In many insect species, JH stimulates protein synthesis in male accessory gland and enhances the process of spermatogenesis (Wigglesworth, 1964; Gillott & Gaines, 1992). However, in some lepidopterans stimulation of protein synthesis is done by 20-hydroxyecdysone. During mating, JH is also transferred to females and augments vitellogenesis (Chapman, 2009; Klowden, 2013).

2.5 Female Reproductive System

Female reproductive system consists of a pair of ovaries, which are connected to a median oviduct by a pair of posterolateral oviducts. Ovaries develop from splanchnic mesoderm during embryogenesis (Hoffmann, 2018). The portions that bear germ cells develop into ovary, while other anterior cells give rise to a suspensatory ligament, which unites all ovarioles and anchors ovary to body wall. At posterior ends, ovaries are connected to posterolateral oviducts that combine to form ectodermally derived common oviduct. Ovarioles are functional units of ovary-containing developing oocytes. These oocytes grow sequentially within ovarioles in an assembly line fashion (Cruickshank, 1973). The number of ovarioles per ovary varies enormously and depends on size and reproductive strategies of insect (Chapman, 2009). While apterygotes have single ovariole per ovary; some social insects may have over 2,000 ovarioles per ovary. However, the most common range of ovariole number in majority of insect species is between 4 and 10 per ovary (Martoja, 1977; Klowden, 2013).

2.5.1 Germarium

The separation of somatic and germline cells occurs during early embryogenesis (Williamson & Lehmann, 1996). At the tip of mature ovariole, 2–3 GSCs reside within a special microenvironment, stem cell niche (Chen & McKearin, 2005). Signaling within niche acts on GSCs and promote either their self-renewal or differentiation.

Three types of somatic cells that surround GSCs, i.e. terminal filament, cap, and escort stem cells provide signaling to GSCs through physical contact and molecular signaling. The GSCs contain a cytoplasmic organelle known as spectrosome (Telfer, 1975; Williamson & Lehmann, 1996). The spectrosome is composed of cytoskeletal proteins α- and β-spectrin and ankyrin, and is attached to cap cells by E-cadherin adherens junctions.

The branching of spectrosome in later stages of development determines which differentiating cell will become oocyte (Chapman, 2009; Klowden, 2013). During asymmetrical divisions within GSC, daughter cell closest to terminal filament and cap cells remains attached to cap cells as a stem cell. However, other cell differentiates into a cystoblast that begins oogenesis, ultimately forming a 16-cell cluster of interconnected cystocytes surrounded by escort cells (Chapman, 2009; Hoffmann, 2018) (Figure 2.3).

Although JAK-STAT signaling pathway is required to maintain self-renewal, other signals from surrounding cells also play crucial roles. Ecdysone modulates intensity of signaling, while insulin-like peptides regulate the rate at which GSCs divide. With continuation of mitotic division, spectrosome becomes branched and gives rise to fusome of daughter cells that anchor mitotic spindle during cell division. The escort stem cells produce follicle cells. The latter surround newly formed cystoblast, as it undergoes cell division with incomplete cytokinesis (Gullan & Cranston, 2014; Klowden, 2013).

The cystoblast ultimately gives rise to a cyst consisting of 16 cystocytes that are connected to each other by cytoplasmic bridges or ring canals. The fusome continues through ring canals as cyst enlarges, and is distributed asymmetrically to daughter cells. As cystocyte complex moves down ovariole and is completely surrounded by follicle cells, it enters vitellarium region (Telfer, 1975; Klowden, 2013). Follicle cells surround cystocyte complex and provide cell signaling interactions that determine polarity of developing oocyte. In certain insect orders, follicle cells also express vitellogenin gene. Ring canals are means for a number of proteins and mRNAs that provide a pattern for development of oocyte. One of the two cystocytes that has inherited more fusome and contains four ring canals subsequently becomes oocyte (Spradling *et al.*, 1997). The developing oocyte enters meiosis-I, but is arrested at prophase-I until fertilization occurs. The remaining cystocytes develop as nurse cells at anterior end of oocyte (Chapman, 2009; Klowden, 2013).

2.5.2 Additional Structures of Female Reproductive System

The calyx joins the posterior end of ovariole to lateral oviduct. The common oviduct is lined with cuticle and is ectodermal in origin. However, lateral oviducts are mesodermal in origin, similar to follicle cells and ovariole wall. A thin acellular membrane, commonly known as tunica propria, covers ovariole from terminal filament to calyx. The female accessory glands or collaterial glands produce a cement that allows deposited eggs to be attached to substrate or glued together (Mercer & Brunet, 1959). In certain insect species that retain their eggs after hatching, such as tsetse fly, *Glossina*, and cockroach, *Diploptera*, accessory glands produce a nutritive secretion that nourishes larvae during their development. The poison glands of Hymenoptera are also modified accessory glands (Richards & Davies, 1979). However, female accessory glands of cockroaches and mantids also produce hard egg case, or ootheca. In insects, females also contain capsule-like spermathecae that temporarily store sperm for subsequent fertilization.

Spermathecae are ectodermal in origin and open into common oviduct of female to release stored sperm. Spermathecae are often present as multiple storage organs and allow females to better segregate and manipulate sperm. Depletion of stored sperm may limit fertility of female. Under such circumstances, multiple matings in females are necessary to replenish sperm numbers. Presence of spermathecal duct, containing glycogen deposits, serves as an energy source for sperm when they pass

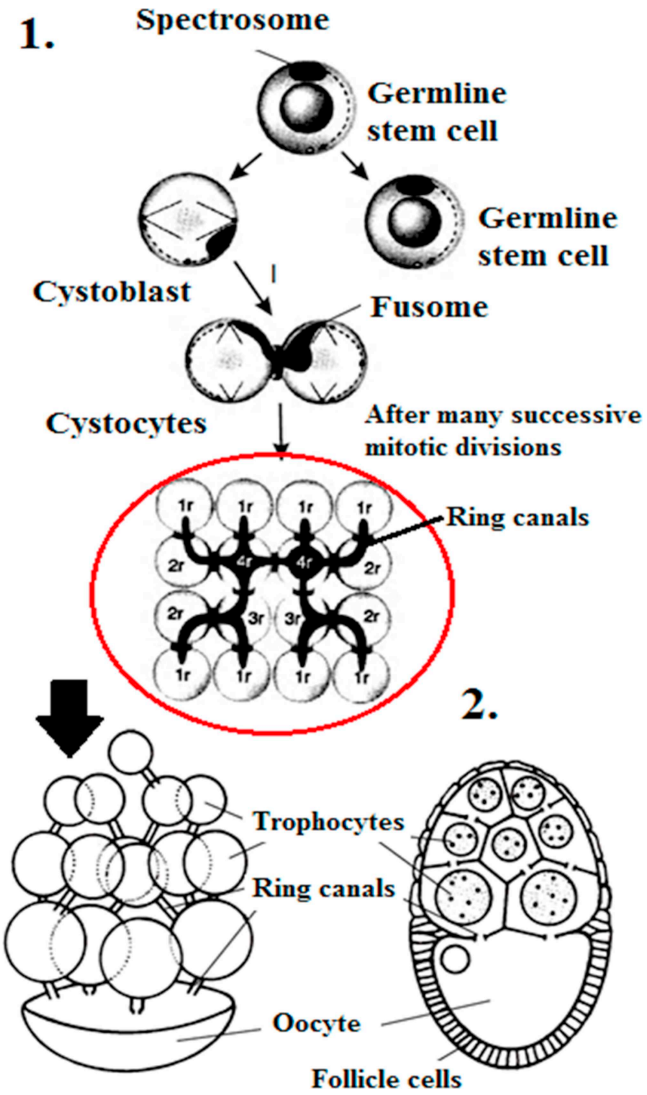

FIGURE 2.3 (1) Division of germline stem cell and formation of cystocytes. (2) Differentiation of cystocytes into trophocytes and an oocyte (modified from Klowden, 2013).

through to egg. The secretions of spermathecal gland, which are rich in proteins and carbohydrates, are responsible for maintaining sperm viability within spermathecae (Gillott, 1988). A honey bee queen can store about 6 million sperm for up to 7 years, and presence of high levels of antioxidative enzymes within spermathecae protects sperm from oxidative damages. While opening to outside, common oviduct becomes modified to a genital chamber, which is capable of incubating eggs internally. The bursa copulatrix is an additional pouch present within genital chamber. It is the place where sperm are first deposited after mating. Thereafter, sperm leave bursa copulatrix, move to spermatheca, and are stored permanently (Klowden, 2013).

In primitive insects that produce spermatophore to enclose sperm, bursa copulatrix bears a series of tooth-like structures that disrupt spermatophore and facilitate release of sperm. Bursa copulatrix secretes certain chemicals into haemolymph when it is filled with sperm so as to signal that mating has occurred. In certain tephritid flies, an additional sperm storage organ, fertilization chamber, is present as a cuticular extension of ventral wall of bursa copulatrix. The chamber is initially filled with sperm, but