1

An Introduction to the Reproductive Biology of Flowering Plants

Reproduction is a very important stage in the life-history of a species, being essential for its survival and sustenance. Different organisms adopt different strategies as they attempt to maximize their reproductive success and produce a favourable number of new individuals. Reproduction in plants can be achieved by either vegetative or sexual means or a combination of both. The seeds and propagules produced by asexual and sexual modes of reproduction have differing implications on the perpetuation of the species. Asexual means (such as vegetative reproduction) in plants is a quicker reproductive strategy that leads to production of new individuals genetically identical to parents. However, there is a limitation of genetic variability in vegetative reproduction and this may affect the long-term survival of a species. On the other hand, reproduction by sexual means brings genetic heterogeneity in progeny resulting in their wider adaptability and better survival. Sexual reproduction in angiosperms is a complex process involving several sequential events which take place in different organs of a flower. Thus, flower is a unit of sexual reproduction in angiosperms.

Plant reproductive biology is the study of the mechanisms of both sexual and asexual reproduction in plants. It involves the study of interactions of plants with biotic factors (such as pollinators, seed dispersal agents) and abiotic components (such as soil, space, climate) in the environment. With the integration of the many aspects of ecology, reproductive biology of flowering plants is now also known as *Reproductive Ecology of Flowering Plants*.

1.1 Different aspects of Reproductive Biology of Flowering Plants

Study of reproductive biology of plants broadly includes observations on phenology, structural and functional floral biology, sexual system, pollination biology, mating system, pollen–pistil interactions, fertilization, embryo-endosperm development, seed formation,

seed dispersal and seed recruitment. These events may also be considered as the series of steps neccessary for the formation of a perfect new sporophyte. These aspects being interconnected, each of these is discussed sequentially in the subsequent sections.

- **Phenology:** Phenology is the timing of recurring biological phases in response to seasonal variations. In the life-cycle of flowering plants various events such as appearance of leaves, onset of flowering, fruit initiation and seed dispersal occur in consonance with seasonal changes and are termed as *phenoevents*. The timing of these recurring and periodic life-cycle events plays a significant role in interaction with other species in the ecosystem. Therefore, the phenological behavior of any species is important for insights into its long-term survival under its ecological conditions. Variations in the phenoevents are very good indicators of climate change; and can be used for predicting such changes in coming years. For these predictions, recordings of phenoevents is conducted over several years in a row at various levels, namely, individual flowers, individual plants as well as the population as a whole. At the level of flower, the timing of flower opening (floral anthesis), anther dehiscence, stigma receptivity, and pollination are recorded. This leads to a detailed understanding of pollination syndromes and breeding strategies of a particular species. All these events are species specific and may happen at any time in a twenty-four hour cycle.
- **Floral Biology and Sexual System:** Floral biology studies provide an understanding of the structural and functional features of a flower. The structural (morphological as well as anatomical) features include the details of all four whorls of a typical flower, viz. calyx, corolla, stamen, and pistil. It may also include the study of development (ontogeny) of the whorls. To understand the functionality of a flower one needs to study the functional features of all the four whorls with special emphasis on pollen, pistil and their interactions. Floral biology also includes study of morphology of pollen grains, stigma and style, estimation of pollen production, pollen viability, pollen fertility, stigma receptivity, and number of types of ovules. These provide an indication of the pollen: ovule ratio, ovule: seed ratio, type of pollination and breeding system of the species. Floral biology studies most importantly help in the identification of the sexual system of the species; it being hermaphrodite, monoecious or dioecious. Structural features of flower also include the spatial and temporal arrangement of its sex organs. The synchronization or asynchronization in the maturity of anther and stigmatic receptivity indicate the mating strategy operating in a species. Asynchronization in maturity is indicative of presence of dichogamy in a species while spatial separation marks the herkogamy. This information is essential for the technique of hybridization in crop breeding experiments.
- **Pollination Biology:** The transfer of pollen grains (male gametophyte) to conspecific stigma (female sporophyte) by the plant itself or through biotic or abiotic means is known as pollination. Abiotic pollination is through either wind or water; biotic pollination is carried out by pollinators such as insects, birds, bats, and others. These pollinators in turn are rewarded with pollen and nectar, fulfilling

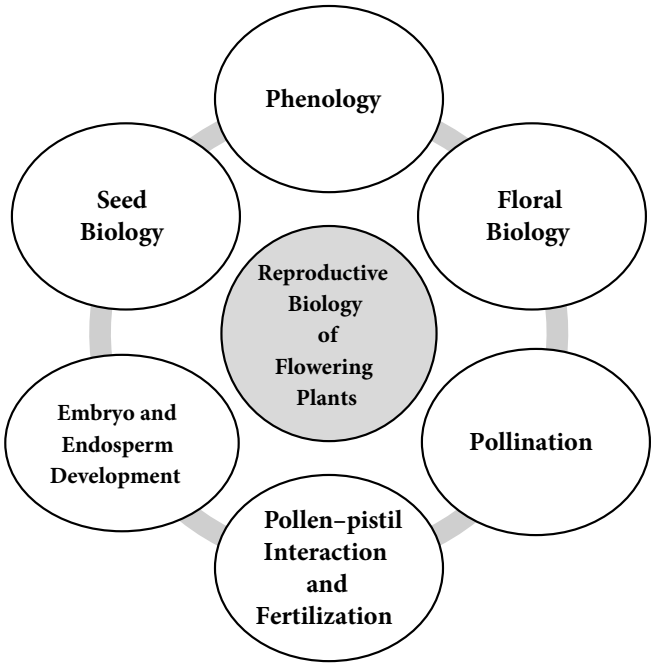

Figure 1.1 Various aspects of reproductive biology of flowering plants.

their nutritional requirements. Pollination biology is studied in the field by direct observations; with the help of photography and video recordings. Pollination biology needs thorough understanding of the floral biology of the species. The floral biology suites observed in a species constitute the pollination syndrome which offers clues of prospective pollinators of the species. Similarly, critical observation of the time of flower opening (anthesis) in a day is also important for studying the pollination mechanism as there is a correlation seen between the time of anthesis and the type of pollinator. For example, the flowers which exhibit anthesis during the day are usually pollinated by bees and other insects, while flowers opening in the evening or night are primarily pollinated by moths and bats.

- **Mating System:** Flowering plants display a remarkable diversity of mating patterns. Species in which ovules are predominantly fertilized by pollen from the same flower are known as autogamous, while allogamous species are those in which the ovules of a flower are fertilized by pollen from another conspecific flower present on the same plant or from another conspecific plant. Apart from the different mating systems, certain plants have the ability to develop seeds without fertilization (and even without pollination) through a phenomenon known as apomixis. The mating system of a species provides an insight about the gene flow and an estimation of the genetic makeup of the population. The mating system of a species can be deciphered through manual pollinations, bagging experiments, and by genetic marker studies (parent-progeny analysis). Many species avoid self-pollination which is achieved

either by virtue of their sexual systems (namely dioecy, monoecy, etc.) or through specific genetic mechanisms such as self-incompatibility, dichogamy, herkogamy. Self-incompatibility may be investigated by manual pollination experiments, and by molecular studies, while dichogamy and herkogamy can be studied by exploring structural and functional floral biology.

- **Pollen–pistil Interactions and Fertilization:** The landing of pollen grains on conspecific stigma initiates a series of events (like pollen adhesion, hydration, germination and pollen tube growth) which are unique to angiosperms. Following pollination, conspecific pollen grains germinate on stigma giving out pollen tubes which then travel through the style to reach the ovary where the male gametes are discharged into the ovules. Throughout these events there is constant interaction between pollen and the stigma of pistil; and then between the pollen tube and style and ovary of the pistil. These pollen–pistil interactions are essential for determining compatibility/incompatibility between pollen and pistil and ensuring successful fertilization. The pollen–pistil interactions can be elucidated by means of histochemical staining, sectioning and molecular tools. Various other features like presence of callose plugs in pollen tubes of angiosperms, tip-oriented pollen tube growth, pollen tube competition, and pollen tube growth rate are important aspects of pollen–pistil interactions. Information on structural details of the pistil is crucial for understanding pollen–pistil interactions. This information has helped in understanding the evolution of angiosperms and their successful establishment on the earth.
- **Embryo-Endosperm Development:** Pollen tubes carry two male gametes, out of which one fuses with the egg cell and the other fuses with the polar nuclei. These two events together constitute 'double fertilization' which is a unique feature of angiosperms. The fusion product of a male gamete and egg cell is the zygote which develops into an embryo. The fusion of two polar nuclei with the second male gamete is called the triple fusion; and results in the formation of an endosperm. The study of development of embryos is known as embryology. The development of an embryo and its nutritive tissue, the endosperm, follows different developmental pathways in different angiosperms. Embryological studies are an important component of reproductive biology as these studies help to understand the development of a new sporophyte within ovule and ovary.
- **Seed formation, Dispersal and Recruitment:** The ultimate goal of reproduction in flowering plants is to produce seeds for perpetuation. Many of the seeds are of economic and commercial importance. Except in apomictic plants, seeds are formed from the fertilized ovules, representing new genotypes. The establishment of new genotypes is crucial for the maintenance of populations.The study of the development, dispersal and recruitment of seeds is known as seed biology which is an integral part of the reproductive biology of plants. Angiosperms exhibit a huge diversity in seed types and in their development mechanisms. The knowledge of seed dormancy, measures to break dormancy, seed viability, seed storage, dispersal mechanism and recruitment pattern is necessary for the conservation of a species.

1.2 Scope of Reproductive Biology of Angiosperms

Understanding plant reproduction and pollination of plants has been an area of interest for naturalists and scientists for centuries. During the course of the past several years considerable progress has been made in understanding the reproductive biology of angiosperms and its scope has widened with the integration of several disciplines such as ecology, population biology, genetics, physiology, molecular biology, biotechnology, and conservation biology. Depending on the problem at hand, reproductive biology can be integrated with other arenas for a stronger and more focused research. The integrated nature of the subject which involves field and laboratory based observations and hypothesis testing makes plant reproductive biology one of the most dynamic field of research. Some important research themes based on reproductive biological studies are summarized here:

- **Reproductive Biology and Conservation Biology:** Loss of biodiversity is one of the major challenges that has risen in the last century. Uncontrolled collection from the wild (plants or plant parts), deforestation, habitat fragmentation, climate change and anthropogenic interference have threatened biodiversity. These challenges have induced changes in the reproductive behavior of species with detrimental consequences on production of new individuals and hence sustainance of natural populations. These factors affect reproduction in various ways, viz. low/no availability of pollen grains (the situation becomes even more critical if the species is cross pollinated), loss of services offered by pollinators, reduced pollination (both cross or self) leading to decrease in fruit set, high inbreeding, diminished fitness and reduced regeneration. The changing temperature and abiotic factor regimes, adversely affect plant distribution and their reproduction. Due to rise in temperature, many species are showing altitudinal shifts which along with increase in concentration of CO_2 are affecting the phenology of species. Several plants are showing early flowering in a short duration which is becoming a threat to both the host plant and the pollinators relying on that particular plant for food or other requirements. Uncertain reproduction may be the major reason for the rarity of medicinal plants especially tree species in the wild. Thus, there is an inevitable need for planning conservation and restoration of biological diversity. One of the most important aspects of conservation and management of plants is the collection of baseline data on their reproductive biology. In the absence of such data, any conservation effort will remain ineffective. Thus, detailed information on the reproductive biology of plants is essential for developing effective strategies for their conservation and sustainable utilization. With this aim, elaborate studies on plant phenology, pollinators and plants distribution are gaining prominence.

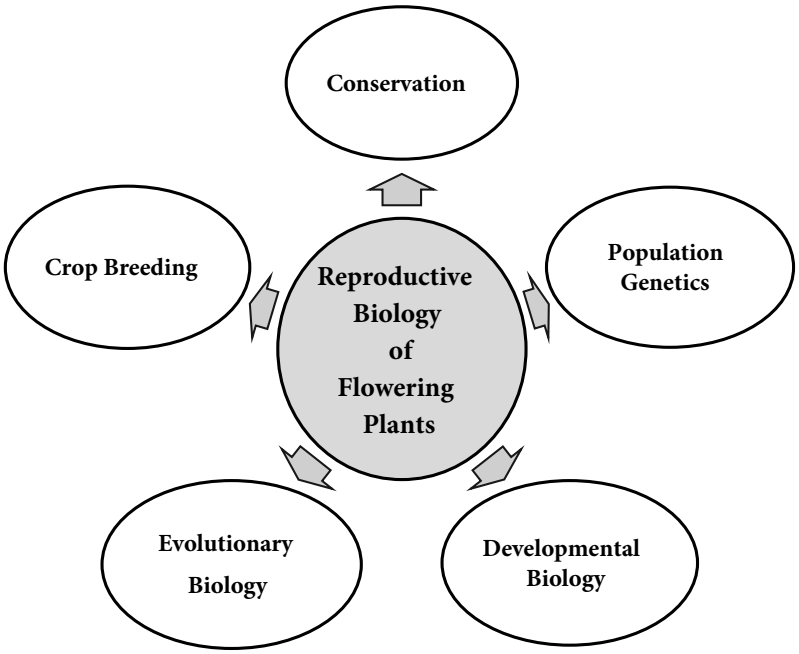

Figure 1.2 Various fields requiring data on reproductive biology of flowering plants.

- **Reproductive Biology and Developmental Biology:** Plant reproduction represents a highly coordinated and complex developmental process. In the past few decades molecular biology, genetics and biotechnological tools have been variously employed to elucidate underlying mechanisms, pathways and allied phenomena in the various aspects of plant reproduction. These studies have led to the discovery of several families of genes involved in flower formation (homeotic genes), development of anther and pollen, viability of pollen, embryo sac development, molecular genetics and the biochemical basis of pollen–pistil interactions, genetic control of self-incompatibility, embryo and endosperm patterning and apomixis. Understanding these developmental aspects of plant reproduction not only sheds light on how plants reproduce but can also be exploited in agriculture, especially the genes involved in male sterility and self-incompatibility. However, it is important to have basic knowledge of reproductive events for studying different aspects of developmental biology.
- **Reproductive Biology and Population Genetics:** Flowering plants display a diverse array of mating strategies and sexual systems. These reproductive systems have a profound influence on the patterns of genetic variation within and among plant populations. Principles of population genetics and phenotypic selection models are used to understand the adaptive significance of these mating strategies. Using molecular techniques, outcrossing rates, inbreeding coefficients, and paternity data, the success of mating system of a species can be evaluated. Gene flow is the transfer of genetic information within and between populations

of a species. The genes in plants are exchanged in the form of pollen grains and seeds. The distance traveled by pollen grains (pollen flow) via biotic or abiotic means in a population or between populations has demographic consequences for a species. Therefore, it is imperative to study pollination biology of a species to have a better understanding of genetic structure of a population and its genetic diversity. Such information on pollen flow and consequent gene flow is also mandatory before the release of any transgenic crop species for commercial cultivation. It helps in planning the strategy to prevent unwanted introgression of transgenes into the wild and its ill-effects on pollinators. Studying reproductive biology at the population level can also provide important taxonomic insights into the delimitation of species even at the infraspecific level.

- **Reproductive Biology and Crop Breeding:** Genetic improvement of crops has been a constant endeavor of agriculturists for ensuring food security. Developing new varieties that are higher yielding, disease resistant, drought tolerant or better adapted to different environments and growing conditions, can be achieved through hybridization. This approach requires information on reproductive events for planning and carrying out breeding programs. To carry out manual pollinations, one must know the stage and duration of anther fertility and stigma viability, so that emasculation can be performed before the attainment of maturity of either stigma or anther. The studies on floral biology and embryo-endosperm development are also helpful for carrying out various *in vitro* protocols, viz. anther culture, pollen culture, embryo culture and such-like.
- **Reproductive Biology and Evolutionary Biology:** Research in plant evolutionary biology aims to understand the basis of adaptation and speciation in different plant systems. Today, reproductive systems of flowering plants are the focus of considerable research in plant evolutionary biology. This is primarily due to the ecological and evolutionary consequences of these reproductive adaptations which are often cited as the reason for the success and diversification of angiosperms. Therefore, the understanding of evolution of diverse sexual systems and different modes of pollination is crucial in understanding the general evolution of angiosperms. Application of molecular genetic approaches to understand the mechanisms responsible for changes in reproductive system among different lineages have added an altogether new dimension to reproductive biological studies. For example, genetic and molecular studies on dioecious model systems have shown significant variation in the inheritance pattern of sex, ranging from single locus to multiple loci to sex-specific chromosomes. Nearly 39 plant taxa have been examined for sex chromosomes among the angiosperms. Molecular genetic approaches can also help to decipher reproductive transitions which have evolved in a particular angiosperm lineage over the years.

Reproductive Biology of plants is a focal theme and understanding it is of utmost importance for managing the environmental challenges, for maintaining biodiversity and genetic resources, for crop improvement and understanding the ecology and evolution of plants.

Bibliography

Mangla, Y., Khanduri, P. and Tandon, R. (2016). Gender determination mechanisms in plants: foundation for unisexuality. *The International Journal of Plant Reproductive Biology* 8:103–114.

Mangla, Y., Tandon, R. Goel, S., and Raina, S. N. (2013). Structural organization of the gynoecium and pollen tube path in Himalayan sea buckthorn, *Hippophae rhamnoides* (Elaeagnaceae). *AoB PLANTS* 5: 1–11. plt015; doi:10.1093/aobpla/plt015.

Shivanna, K. R. and Tandon, R. (2014). *Reproductive Ecology of Flowering Plants: A Manual.* Springer-India.

Tandon, R., Shivanna, K. R. and Koul, M. (2020). *Plant Reproductive Ecology: Patterns and Processes.* Springer-India.

Mangla, Y. and Gupta, C. K. (2014). Love in the air-wind pollination: ecological and evolutionary considerations. In R. Kapoor, I. Kaur and M. Koul, eds., *Plant Reproductive Biology and Conservation* Delhi: I. K. International, pp. 234–45.

2

The Flower

In angiosperms diverse forms of flowers are accompanied by an array of mating strategies and sexual systems.

2.1 Introduction

Angiosperms possess a vast diversity of flowers which serve various purposes for the different groups of living beings, including humans. Due to their color, fragrance, and beauty flowers have always occupied a special place in human lives. Flowers are considered sacred across most cultures and have inspired much artistic expression. Apart from their aesthetic value, flowers possess myriad medicinal properties that further enhance their value to humans. Describing from a botanist's perspective though, the flower is a unit of reproduction in angiosperms. A flower may be defined as a modified determinate shoot system with four distinct whorls, viz. calyx, corolla, androecium and gynoecium arranged on a receptacle. Outer whorls, calyx and corolla are leaf-like structures which are not directly involved in reproduction. The two inner whorls, the androecium and the gynoecium harbor the reproductive organs of the flower and are the ones involved in reproduction. Flowering plants exhibit enormous diversity in size, shape, color, symmetry and the other morphological features (Fig. 2.1). This diversity in floral forms plays a huge role in ensuring pollinator services by different groups of pollinators. The diverse forms of flowers are accompanied by an array of mating strategies and sexual systems in angiosperms.

The timing of flowering in plants is critical for their reproductive success as both late and premature flowering can limit proper seed development. Plants also attempt to realize their reproductive potential by synchronizing their flowering to match pollinator availability. Floral induction is promoted by distinct environmental cues such as photoperiod, vernalization and endogenous regulators like phytohormones. These signalling cues are perceived in the leaves and the shoot apical meristem (SAM) for induction of flowering. Plants use genetic machinery to control all events starting from induction of flower to development of different whorls. Research in the last few decades has identified numerous genes which are involved in floral induction, floral meristem formation, and

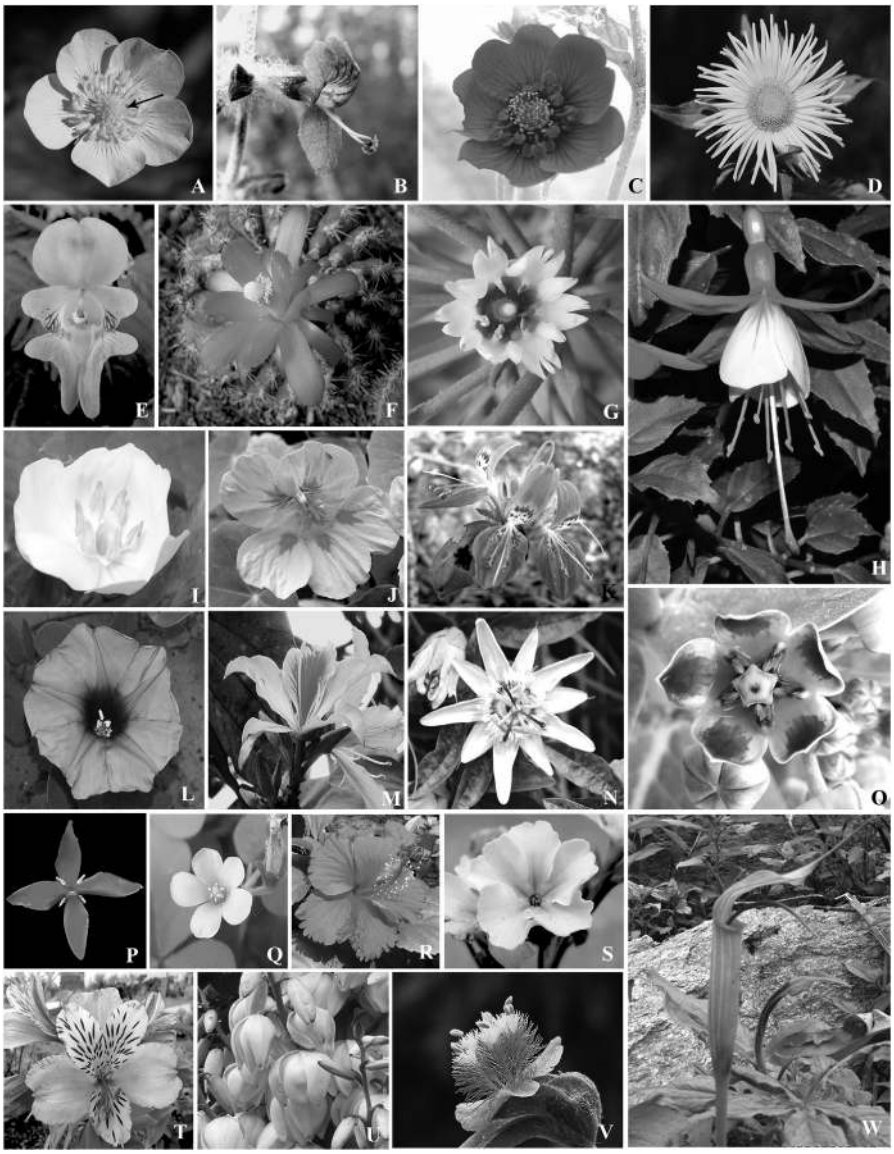

Figure 2.1 Flower diversity in angiosperms. **A.** *Ranunculus* sp. Arrow indicates carpels. **B.** *Lavendula* sp. (Lamiacaeae) with bi-lipped corolla. **C.** *Potentilla* sp. (Rosaceae). **D.** *Innula racemosa* (Asteraceae), a capitulum. **E.** *Impatiens* sp. (Balsaminaceae), a zygomorphic flower. **F.** Cactus flower. **G.** *Manilkara zapota* (Spotaceae). **H.** *Fuchsia* sp. (Onagraceae), Note the colorful sepals. **I.** *Podophyllum hexandrum* (Berberidaceae). **J.** *Tropaeolum majus* (Tropaeolaceae). **K.** *Peristrophe* sp. **L.** *Ipomea* sp. (Convolvulaceae), funnel shaped flowers. **M.** *Bauhinia variegata* (Fabaceae). **N.** *Passiflora incarnata* (Passifloraceae). **O.** *Calotropis procera* (Apocynaceae). **P.** *Ixora* sp. (Rubiaceae). **Q.** *Oxalis* sp.(Oxalidaceae). **R.** *Hibiscus rosa-sinensis* (Malvaceae). **S.** *Primula* sp. (Primulaceae). **T.** *Alstroemeria* sp. (Alstroemerieae). **U.** *Yucca* sp. (Asparagaceae), bell shaped flowers. **V.** *Cynotis* (Commelinaceae), note the stamens with colorful hairs on filaments. **W.** *Arisaema* sp. (Araceae), also known as cobra plant, stigma extend from perianth tube like the tongue of a cobra. **See Color Plates (page 473).**