

K.R. Shivanna · Rajesh Tandon

# Reproductive Ecology of Flowering Plants: A Manual

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## Foreword

Tropical countries, including India, are rich in biodiversity with a high incidence of endemism. For the sake of short-term gains, vast areas of wilderness have been destroyed. However, the sustainability of whatever biodiversity is still available in various ecosystems is seriously threatened by continuing human need. Unless effective measures are taken to salvage biodiversity, a large number of species would become irretrievably lost. According to some conservation biologists, we are soon heading towards the sixth mass extinction in evolutionary history. Successful reproduction is the basis not only for the stability of the species in their natural habitat but also for the productivity of our crop plants on which humans and their domesticated animals are dependent. Studies on reproductive ecology are, therefore, important for the effective management of our dwindling biodiversity and improvement of yield in the crop plants. Unfortunately, knowledge of reproductive ecology of wild and crop species in the developing countries is meagre. This constitutes a major limitation that hampers our efforts to conserve biodiversity and optimize crop yields and animal production.

Contrastingly, there is enormous information available on reproductive ecology of wild and cultivated species growing in countries with developed economies. Therefore, they have been able to manage their biodiversity and optimize crop yields in a substantial way than their counterparts in developing nations. Also because of the availability of baseline data on reproductive ecology on their plant resources, most of their present studies on reproductive ecology are aimed at formulating hypotheses, testing their validity and preparing mathematical models on reproductive events. Tropical countries do not have even the baseline data on most of the species. Therefore, there is urgent need to initiate and emphasize research on reproductive ecology in tropical countries, where population pressure is acute and malnutrition is rampant, for effective management of biodiversity and to improve the yield and nutritional quality of the crops.

One of the limitations of initiating research in this area is lack of mentors and more importantly authentic manuals to motivated and enterprising young plant biologists for conducting research on various aspects of reproductive ecology. *Reproductive Ecology of Flowering Plants: A Manual* is the first attempt in this direction. K.R. Shivanna and Rajesh Tandon have been working in this field throughout their research careers. This handbook covers the total range of reproductive ecology starting with a thorough study of plants and sites of their occurrence, phenology, breeding system, record of

reproductive events including dispersal of diaspores and ending with seed viability, dormancy and finally seedling recruitment, the ultimate step in the sustainability of populations. This manual will be extremely useful to those who are residing in areas with rich flora but lacking in essential laboratory facilities. I am confident that the manual would encourage the younger generation to take up research in this important discipline of production and utilization of plant resources.



Sriram Institute for Industrial Research  
Delhi, India  
6 June 2014

H.Y. Mohan Ram

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## Preface

The stability of any species in its natural habitat is dependent on its successful reproduction and recruitment of new individuals to sustain populations. Erosion of biological diversity is one of the most challenging issues of our times. Although overexploitation, habitat destruction and climate change are the proximate causes for erosion of biological diversity, the ultimate driving force for pushing any species to endangered category is its inability to produce enough number of seeds and to recruit new individuals to sustain populations. Tropical countries are rich in biodiversity with a high proportion of endemic elements, and presently many plant species in tropical rain forests are at maximum risk of reproductive failure. One of the major problems in conservation and management of tropical forests is lack of baseline data on their reproductive ecology. This is particularly true for a large number of endemic, endangered and economically important species. This lack of knowledge is largely because very few laboratories in tropical countries, particularly in south and south-east Asia, are working on reproductive ecology. In the absence of such data, any conservation efforts are arbitrary and remain ineffective. Therefore, it is necessary to initiate extensive studies on reproductive ecology of our plants as a requirement for their effective conservation and sustainable utilization. Also, in most of our crop plants, fruits and seeds are the economic products and any constraint in reproduction reduces their production. For sustenance and improvement of yield in the crop species, an understanding of reproductive ecology is an essential prerequisite. Thus, reproductive ecology has direct relevance to conservation biology, agri-horticulture, forestry and plant breeding.

Several workshops and training programmes conducted in India during the last few years on reproductive ecology have shown an enormous enthusiasm amongst young researchers to initiate research. A major limitation for the beginners to take up research is absence of simple work manuals that provide the basic steps used in studying reproductive ecology. A few manuals available so far deal largely with pollen biology and pollination biology, and there is no manual covering the whole spectrum of reproductive ecology including seed biology, seed dispersal and seedling recruitment which are important in sustaining the populations and species in their natural habitat.

The major aim of this manual is to fill this void and provide the essential working steps involved in studying all aspects of reproductive ecology. The intention is to keep the volume small, and therefore the methods described have been selective (based on the need and our own experience) and does not



aim to give a monographic coverage of all the available techniques. Each chapter gives a concise conceptual account of the topic before describing the protocols. Important relevant references are cited to help the user to get more theoretical information in the subject. The manual covers over 60 protocols. Each protocol lists special requirements followed by detailed working steps so that the researchers do not find any difficulty in conducting investigations. Appendices provide the details of preparation of fixatives, stains and a few other relevant requirements. Most of the protocols presented in the manual require minimum facilities which are generally available in colleges, university departments and research institutions. This is important since most of the biodiversity in developing countries in the tropics is located where only a limited number of well-established laboratories are present.

The manual caters to teachers, students and researchers who deal at any level of reproductive ecology of flowering plants – botanists, zoologists, entomologists, ecologists, agri-horticulturists, foresters, plant breeders and conservation biologists. We hope that this manual would stimulate youngsters to undertake studies on reproductive ecology and generate useful data over the years which would be invaluable for conservation and management of our plant diversity.

We are grateful to Prof. H. Y. Mohan Ram, for his collaboration in some of our studies on reproductive ecology over the years, encouragement throughout our career and also for writing the foreword for the manual. We sincerely thank Prof. N. S. Rangaswamy for encouragement and counsel, which have been invaluable. Palatty Allesh Sinu, Department of Animal Science, Central University of Kerala, Kasaragod, has critically gone through the chapters on seed dispersal and seedling recruitment. We express our indebtedness to him. Dr. Mamta Kapila, Publishing Editor, Springer (India) Pvt. Ltd., took special interest in the publication of this manual.

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KRS thanks the Indian National Science Academy for the award of positions as INSA Senior Research Fellow (2003–2008) and INSA Honorary Scientist (2009–to date), and the Ashoka Trust for Research in Ecology and the Environment (ATREE), Bengaluru, for providing facilities. It is a pleasure to thank Giby Kuriakose and Manju Vasudevan Sharma who were associated with research activities of KRS during the past 10 years.

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Reproductive ecology covers all aspects of reproductive events and their interactions with biotic and abiotic components of the environment. Although reproductive strategy of all organisms is to maximize reproductive success and genetic diversity, they differ in the means of achieving these goals. The main reproductive strategies in plants are largely dictated by their immobility. They have to use other agents particularly animals to perform some of their reproductive functions. A large number of flowering plants follow a dual strategy of reproduction through seeds as well as through vegetative propagules (asexual/vegetative reproduction); this dual strategy maximizes fitness by combining the advantages of both the types of reproduction. Important means of natural vegetative propagation are the production of: bulbs (onions, lily and tulip), corms (*Gladiolus*, *Crocus* and *Freesia*), stem tubers (potato), tuberous roots (sweet potato, dahlia, *Canna* and turmeric), rhizomes (iris and ginger), suckers (chrysanthemums), runners (strawberry) and bulbils (*Agave*). Vegetative reproduction facilitates colonization and local dominance of the population in well-adapted niches by rapid lateral expansion. However, it limits genetic variability due to lack of gene exchange and also dispersal benefits. Increased homozygosity in the species also becomes an impediment for long-time survival, as it hinders the competitive ability of the plants to adapt to new niches in changing environments and thereby restricting the populations to specialized habitats.

In many commercial crops, vegetative reproduction is induced through human intervention. Horticulturists have been able to propagate desired varieties through various methods such as stem cuttings, grafting, layering and budding in a number of crops (McKey et al. 2010). In micropropagation, tissue culture technology is used to produce a large number of plants by culturing practically any part of the plant body and inducing organogenesis (root and shoot formation) or embryogenesis (formation of somatic embryos). Micropropagation at a commercial scale is being practised in a number of species such as orchids and bananas (Kozai 1991; Chugh et al. 2009; McKey et al. 2010; Mudoj et al. 2013). In all the above methods of asexual reproduction, seeds are not the units of reproduction. However, in a few species, there is another unique method of asexual reproduction, termed apomixis, which results in seed formation without fertilization (Richards 1986, 2003; Koltunow 1993; Ozias-Akins and Van Dijk 2007; Barcaccia and Albertini 2013). The progeny resulting from all methods of asexual reproduction, including apomixis, are the clones of the parent plant.

Sexual reproduction is the only method which permits genetic recombination (through meiosis and fertilization) and results in heritable variations, the raw materials for evolution. Sexual reproduction in flowering plants is more complex than in the other groups of plants. Flowers are the units of sexual reproduction. Therefore, the first event in sexual reproduction is the initiation and

development of the flower. Although they show a high degree of variability in structural features, flowers of all species essentially bear the stamens and/or the pistil, representing the male and female partners, respectively. Stamens are differentiated into the filament and the anther. Anthers produce pollen grains, the male partners in sexual reproduction. Pollen grains represent highly reduced male gametophytes. Mature pollen grains are shed from the anthers following their dehiscence. The pistil is divided into the stigma, style and ovary. The stigma is the recipient of pollen grains, the style is the conduit for the growth of pollen tubes carrying male gametes, and the ovary is the container of ovules which harbour the female gametophyte.

Pollination is the transfer of pollen grains from an anther to the stigma. Flowering plants have developed amazing adaptations to achieve pollination. Following compatible pollination, pollen grains germinate on the stigma, and the resulting pollen tubes grow through the tissues of the stigma and style and enter the ovary and eventually the ovule. These events from pollination up to the entry of pollen tubes into the ovule are termed pollen–pistil interaction. The pollen tube eventually enters the embryo sac, the female gametophyte, located in the ovule, and releases the two sperm cells. One of the sperms fuses with the egg to give rise to the zygote, and the other fuses with the fused polar nuclei (secondary nucleus) to give rise to the primary endosperm nucleus. Following incompatible pollinations (self-pollen in self-incompatible species and cross-pollen from the reproductively isolated species), pollen grains are unable to complete pollen–pistil interaction, and thus pollen tubes fail to reach the ovary resulting in the failure of fertilization. Some interspecific crosses show post-fertilization barriers; fertilization occurs, but the zygote or young embryo aborts and no viable hybrid is realized.

The zygote develops into an embryo, and the primary endosperm nucleus (resulting from the fusion of one of the sperms with secondary nucleus) produces the endosperm, a nutritive tissue for the developing embryo. The details of the development of the embryo and endosperm have been well investigated (Maheshwari 1950, 1963;

Johri et al. 1992; Raghavan 1997; Ramawat et al. 2014). Fertilized ovules develop into the seeds and the ovary into the fruit. Following the maturation of the fruits, the seeds are dispersed by various agents. Eventually the seeds land on the soil and, under suitable conditions, germinate and produce seedlings. A large number of seedlings perish due to predation, competition or abiotic stress, and only a very small proportion of them eventually grow into new adult plants, completing the cycle of sexual reproduction.

In cultivated species, seed is generally considered as the end product of sexual reproduction. However, in wild species, sexual reproduction also includes events leading to natural recruitment (seed biology and seedling recruitment). Thus, sexual reproduction in flowering plants broadly involves the following sequential events:

- Flower initiation and development
- Production of functional pollen grains and ovules
- Transfer of pollen grains from the anther onto a receptive stigma (pollination)
- Pollen–pistil interaction and fertilization
- Development of seeds and fruits
- Dispersal of seeds and their germination leading to the establishment of viable offspring

Any break in these sequential events results in the termination of reproductive events. Various aspects of reproductive ecology may be pursued interdisciplinarily by integrating techniques employed in several major disciplines such as ecology, population biology, genetics, physiology, molecular biology, biotechnology and conservation biology. Depending on the rationale, relevant techniques from these areas may be appropriately used in studies on reproductive ecology for achieving both fundamental and applied objectives. However, for generating baseline data on reproductive ecology, field studies with limited laboratory facilities are satisfactory.

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## 1.1 Reproductive Ecology and Conservation Biology

Effective management of biological diversity is going to be a major challenge in the coming decades (Sodhi and Ehrlich 2010; Shaanker et al.