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REPRODUCTIVE STRATEGIES IN ORCHIDACEAE

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Abstract

Survival of a species in the wild essentially relies on its reproductive performance. Orchidaceae, with nearly 25,000 species is one of the most successful families of angiosperms, essentially by virtue of the floral contrivances that have evolved to maximize their reproductive efficiency. Among these, the shape of flower, rewards, fragrance and pollination have immensely contributed to their speciation and adaptive radiation. With enormously high incidence of one-to-one specialization in pollination (60-70% orchids), many orchids have become endangered (~10%) in the wild due to habitat modification. Reproductive strategy in orchids is full of lure and deceit. Nearly one-third of the known orchids offer no rewards and yet outwit insects in a variety of ways to successfully effect in pollination. Deception for food or sex leading to successful pollination is the key strategy. In sexually deceptive orchids, the floral fragrance intercepts the pheromonal communication by luring the male insect pollinators to visit the flowers that mimic their female counterpart. The scent of each orchid may be specific for one type of insect pollinator. It is not yet completely understood that how this strategy engenders speciation, maintains the amazing co-adaptive traits and helps in the reduction of costs, which is usually incurred on realizing fitness in, otherwise, flowers with rewards. Reproductive isolation is mandatory to maintain the species boundaries. In orchids, the pollinator mediated pre-fertilization barriers are considered sufficient to result in speciation, as a great majority of wide-hybrids succumb to hybrid sterility manifested due to their divergent karyotypes. There is paucity of data on the genetic architecture of reproductive barriers in orchids, which perhaps requires the mapping of loci involved, onto the chromosomal locations. Owing to obligate reliance on community level symbiotic interactions (pollination and mycorrhizal association), survival and speciation in orchids essentially operates in the ecological context. Knowledge on the present status of these interactions is urgently required for consolidating the base-line data on the biology of Indian orchids. Recent advances in molecular tools can be suitably integrated in the much needed reproductive and demographic studies to have a meaningful rationale on conservation of orchids in the country. In this paper, we mainly present an ecological perspective of reproductive strategies crucial for pollination and seed set in orchids.

Introduction

THE ORCHIDACEAE, ranked second in numbers (~25,000 species including the subspecies) and yet second to none, is one of the most successful families of flowering plants. The astonishing diversity in the occurrence-range attests to the ability of orchids to occupy a variety of niches that are conquered by millions of the tiniest of seeds known among the seed plants. Likewise, the floral architecture of orchids, though have some unique natural apomorphies shared by the group (such as zygomorphy, occurrence of labellum), also exhibits enormous variation in their display. Natural reproduction in the family is mainly attributed to outcrossing which is mostly mediated by the biotic agents.

Pollination, though appears to be simple transfer of a pollen from an anther to a stigma, is a complex ecological phenomenon and irreplaceable by any gadget. Coherence of ecological events such as timing of flowering/blooming and the availability of a suitable/legitimate pollinator is the essential prerequisite for pollination success. For an in-depth knowledge of species biology, study of species specific pollination strategy and pollinator behaviour are necessary in acquiring better understanding of the processes that generate enormous morphological and genetic variation (Vereeckan *et al*., 2010), as shown by orchids. Incidentally, dating of the extinct and fossilized pollinator (*Proplebeia dominiciana*) carrying the orchid pollinia (*Meliorchis caribea* gen. *et. sp. nov.*) on its back has indicated that Orchidaceae has an archaic (~84 Myr ago) origin (Ramirez *et al*., 2007)

The genesis of concepts such as 'mutualism', 'specialization vs generalization' in plant-pollinator interactions and 'co-adaptations' among plants and pollinators can be traced back to the seminal work by Charles Darwin entitled *The Various Contrivances by which British and Foreign Orchids are Fertilized by Insects* (1862). Further, identification of a predicted moth species with 22 cm long proboscis (*Xanthopan morgani pradaecta*) in 1903 as the pollinator of a 1½ ft long-spurred orchid, *Angraecum sesquipedale*, has engendered a vast gamut of information on pollination biology and inspired several naturalists to become pollination biologists. Ever since this startling observation was made by Darwin (1862), the saga of exploring the plant-pollinator interaction appears to be a never ending endeavour (Tandon *et al*., 2009). The discovery of pseudocopulation, the mechanism by which floral mimicry entices copulatory behaviour in male insects, was discovered much later (Coleman, 1927; Pouyanne, 1917). By and large, there is familial concentration of pollination by deceit in Orchidaceae (Ellis and Johnson, 2010) and is believed to have played a key role in maximising the reproductive fitness in the family (Micheneau *et al*., 2009). It is now believed that deception strategy has played a cardinal role in the diversification of orchid taxa as well (Cozzolino and Widmer, 2005).

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The reproductive features including specialization of floral parts, dispersal of pollen grains in units (pollinia), constancy with specialized pollinators and the refined cues (mostly scent) for attraction to one functional group or a type of pollinator (Van der Pijl and Dodson, 1966), make natural study of orchids a challenging task in terms of prolonged and repeated monitoring as an essential requisite in the field. Although cosmopolitan in distribution, most of the orchid species exhibit restricted distribution and require extraordinary efforts along with suitable equipment to unravel their survival strategies.

Orchids have been categorized as protected species under the Convention on International Trade in Endangered Species (CITES), as many orchid species are threatened or endangered. For sustainable utilization of orchids and sustenance of floriculture, conservation of natural germplasm and gene pools is undeniably essential. As the reproductive success of orchids majorly involves their pollinators, any conservation effort is likely to involve an ecosystem approach. The universal threats to conservation encompassing habitat fragmentation, destruction/modification and the continuous plundering of germplasms from the wild have to be countered with both in *situ* and *ex situ* approaches.

Regrettably, in contrast to its diversity, only a small proportion of orchids have been studied. The information from the tropics is, appallingly, almost negligible (Neiland and Wilcock, 1998); only 100 orchid species have been looked into so far! In India, there are 1200 species and our knowledge is primarily confined to demographic endeavours. Our effort to produce hybrids are also the least (~50) in the world market. Several aspects of reproductive biology covering phenological records, floral biology, plant pollinator interaction, assessment of breeding system and fruit and seed biology provide essential baseline data for any judicious conservation program or generation of marketable hybrids. Among these, pollination ecology is crucial, as orchids are known to exhibit astonishingly higher degree of specialization with pollinators. In evolutionary and ecological terms, specialization may also lead to higher levels of species endangerment (Swarts and Dixon, 2009). Thus, it becomes pertinent to intensify reproductive biological studies in future endeavours of pursuing orchid biology in the country.

Threats from environmental stochastic events and other anthropogenic perturbations has infused realization to conserve the natural stocks with an interdisciplinary and integrated approach. There are sufficient guidelines available to undertake conservation and reproductive biological studies of plants. These methods can be suitably applied to reproductive biology of orchids. The general guidelines and methods to pursue plant reproductive studies are amply detailed in several compendia (Dafni, 1992; Dafni and Kevan, 2003; Faegri and van der Pijl, 1971; Jones and Little, 1983; Kearns and Inouye, 1993; Real, 1983; Shivanna and Rangaswamy, 1992).

Flowering Phenology

Under natural conditions, an endosperm-less orchid seed requires nearly 6 months of mycorrhizal association to produce the first leaf and then passes through a prolonged juvenile phase (up to 16 years) to become reproductive. Subsequently, flowering becomes a strongly seasonal affair. The peak flowering period, at least, in the South Indian orchids has been identified to reside either during August/September or March/April (*https://sites.google.com/site/orchidkerala*). Incidentally, the former period is predominantly shown by the terrestrial orchids whereas the latter by the epiphytic species. Whether or not these peak flowering periods coincide with the availability of their pollinators has not been ascertained. Detailed information on the seed-set patterns during the entire stretch of flowering period in a species would be required to explain if there is any stabilized evolutionary action on either the growth-forms or place of their occurrence. The pattern of peak distribution (modality) with respect to seed-set percentage across the distribution range of a species will likely explain if there is any shift in the pollinator preference or flowering period as well. Unfortunately, there is insufficient data from other regions to draw any generalization on flowering phenology of orchids in India. In other parts of the world, staggered flowering occurs throughout the year with a couple of observable peaks in the rewarding flowers, whereas the non-rewarding flowers bloom before the onset of peak flowering in the rewarding orchids (Table 1). This could be a strategy to avoid competition for pollinators. Low temperature along with sugar accumulation through photosynthesis appears to be the key environmental cues that control flowering in several orchid genera including *Dendrobium*, *Miltoniopsis*, *Phalaenopsis* and *Zygopetalum* (Hsiao *et al*., 2011).

Floral Biology

Structural diversity of orchid flowers and their longevity have direct bearing on the reproductive success. The longevity of flowers is affected by pollination and is largely a resource allocation strategy (Primack, 1985). Unpollinated orchid flowers remain in bloom for a very long period (1-2 months). For example, the shelf-life of

Table 1: The generalized and contradistinctive reproductive features among the rewarding and non-rewarding orchids.

Feature	Rewarding orchids	Reward-less orchids
Habit	Mostly epiphytic	Mostly terrestrial though some epiphytes are also known, e.g.-*Tolumnia variegata*
Flowering period	Throughout the year (Nov/Dec and April to June)	Coincides with the availability of naïve pollinators and before the onset of peak flowering in the rewarding orchids
Floral Biology		
Size of flower	Ranges from small to big	Generally very small
Morphology of lower lip	Not much hairy	Mostly hairy, have spots alike those on insects body etc., pattern varies according to their pollinator.
Presence of spur and nectaries	Prominently visible	True ones are absent and some show extra-floral nectaries for to deceiving
Pollinarium bending	Slow	Fast
Tactile stimulatory structures	Absent and not much required	Floral hairs on corolla lip and papillae
Rate of fertilization	Greater	Lower than the rewarding flowers
Pollination		
Visitation rate	Low, (~17%)	Significantly higher, (~50%)
Reward Type	Pollen/nectar	None
Amount of reward (nectar)	3.3 to 4.0 μl	Almost nil
Pollination rate	High, up to 98%	Low ~50%
Overall variation in floral scent	Low	High
Response of pollinators to Olfaction	Strong	Weak
Mimicry (pheromonal/floral shape)	Absent	May mimic the rewarding orchids or the female of insect pollinators
Age and experience of pollinators	Mature and naïve both bring about pollination	Mostly the inexperienced and naïve pollinators are effective
Cues for attraction	Showy flowers, fragrance in some and nectar spur in others	Mimicry of both food rewarding and pheromones.
Effect on pollinator fitness	Increases in some by providing fragrances that help in luring females\males;	Reduces; male wasps can't discriminate between insect and orchid chemical cues; pseudo-copulation drains out the energy.
Flower handling time	Short	Long
Breeding System		
Self-compatibility and inbreeding depression	Highly prevalent	Absent
Incidence of Cross-pollination	Low	High
Kind of selection mechanism responsible for low variation in floral signals	Stabilizing selection	Relaxed selection
Fruit and Seed Biology		
Production of seeds	High, (~70%)	Low, (10-15%)

Table 1: The generalized and contradistinctive reproductive features among the rewarding and non-rewarding orchids. (contd.)

Feature	Rewarding orchids	Reward-less orchids
Seed viability	Low, (20-30%)	High
Natural Fruit Set		
North America	49.3%	19.5%
Europe	63.1%	27.7%
Temperate southern hemisphere	74.4%	41.4%
Tropics	24.9%	11.5%

(Ackerman *et al*., 1994; *http://www.teara.govt.nz/en/orchids; http://www.beautifulorchids.com/orchids/*.Johnson *et al*., 2003; Kull *et al*., 2002; Neiland and Wilcock, 1998; O'Connell and Johnston, 1998; Pellissier *et al*., 2010; Smithson and Gigord., 2001)

Paphiopedilum druryii is estimated to be nearly three months (Dr. C. Satheesh Kumar, TBGRI, pers. comm.). As a great majority of orchids are pollinator-limited due to specialization, 'sit-and-wait strategy' with extended receptive period is considered to be advantageous for reproductive success (Ashman and Schoen, 1994). In *Cymbidium* spp., the life span of unpollinated flowers is 5-6 weeks whereas after pollination the flowers are retained only for 3-4 days. Distinct floral changes are induced within four hours after pollination and such flowers are avoided by the pollinators. The longer shelf-life is the key floricultural trait and an inventory of such Indian orchids would be beneficial and may give a boost to the floriculture industry in the country.

Architecturally, orchid flower is the most complicated among the angiosperms and perhaps is an outcome of the key developmental changes accompanied with variability. An elaborate median lip of the inner whorl of perianth (the labellum), column (adnation of style and filaments) and rostellum (a projected part of the column) are characteristic to Orchidaceae besides the pollinia; the latter are found in other flowering plants also. All these features have undergone modification in several advanced tribes of the family and there has been gradual simplification of some of these structures with an increase in the complexity of flowers (Holttum, 1960). Functionally, they contribute to precision in presentation of pollinia to pollinators and prevent selfing.

Orchids exhibit unusual embryological developmental events also (Arditti, 1992; Dahlgren *et al*., 1985). First, there is coordinated timing of megagametogenesis along the entire length of placenta. This harmonized developmental event obviously maximises the simultaneous seed-set, as the pollinia supply pollen *en masse*. Secondly, the endosperm usually perishes after forming a few cells and the embryo development takes a pause at globular stage. The dispersed seed virtually carries a young impoverished sporophyte which must associate symbiotically with a suitable mycorrhizal fungal species to establish into a new plant. According to Sorenson *et. al*., (2009), Orchidaceae could be a model family to elucidate the molecular basis of these developmental embryological inconsistencies.

At the molecular level, the major endeavour has been to isolate the homologous genes and establish their role either by overexpression or silencing (Hsiao *et al*., 2011). Mondragon-Palomino and Theiβen (2009) using their 'orchid code' hypothesis have postulated that various types of floral modification in orchids can be explained by monogenic gain or loss of functional mutants that altered the expression of *DEF*-like or *CYC*-like gene. They argue that the four *DEF* paralogues - *PeMADS2*-like; *OMADS3*-like, *PeMADS3* and *PeMADS4*, have largely shaped the floral diversity of orchids and probably were instrumental in eliminating the evolutionary constraints to cause different kinds of co-adaptive changes in perianth in response to either pollinators or genetic drift.

Hsiao *et al*. (2011) have recently reviewed the molecular mechanism of flower development in orchids, based on information gathered from the roles of MADS-box genes in *Phalaenopsis equestris*, *Oncidium* Gower Ramsey, and *Cymbidium ensifolium*. In *Phalaenopsis*, *PeMADS4* is the key regulatory gene in lip development whereas in *Oncidium*, *OMADS5* is essential (Chang *et al*., 2010; Tsai *et al*., 2008). According to 'Homeotic Orchid Tepal (HOT) model' based on expression patterns of B-Class genes (Pan *et al*., 2011), *PI* and *AP3B* clades determine the sepals, a combination of *PI* and *AP3A1* along with *AP3B* clades regulate the formation of lateral perianth lobes in the inner whorl; and *PI* and *AP3A1*, *AP3A2* clade genes along with *AGL6*-like genes result in lip morphogenesis at the floral bud stage.

Pollination Ecology

Conservation of pollination system and its network in ecologically fragile areas is an emerging discipline. So far, there are no reports of restoring pollination system or its network in any species reclamation program (Swarts and Dixon, 2009). In orchids, a variety of pollinators ranging from tiny wasps to rodents and birds pollinate the flowers. Among these, the Hymenopterans predominate - the relatively primitive terrestrial taxa are pollinated by the solitary bees and the large flowered epiphytic orchids of the tropics are adapted for pollination by *Xylocopa* spp. (Stebbins, 1970). The shape, size and colour of the tepals, size of the foraging agent, foraging behaviour of the pollinator, the precision in removal of pollinia and their deposition onto the stigma of other conspecific flowers, often contributes to specialization of their pollinators. Floral constancy and fidelity is achieved by specialized mode of pollination-by-deceit or the pheromone-like volatile compounds that are collected by the male pollinators (Dressler, 1981).

Orchids, in general, suffer from pollinator limitation (Calvo, 1993; Robert, 2003; Tremblay, 2005). Finding the difference between supplementary pollinations and natural fecundity is a useful conventional method to establish if there is any pollination limitation in the species. Nevertheless, the pollination requirement, pattern and processes vary among the different species of orchids in different regions of their occurrence (Pemberton, 2010). Some of the recent findings having significance in conservation and management of orchids are mentioned below.

Floral Deception - an Evolutionary Stable Strategy (ESS)

The exceptionally high incidence (~40%) of pollination by deceit (food/sex) in orchids is an intriguing feature and its evolutionary maintenance is equally perplexing. There are 10,000 species of orchids that deceive the pollinators for food and nearly 400 species that mimic the females of their insect pollinators. Some of the deceptive orchids are listed in Table 2. As the evolutionary stability of the deception has direct bearing on the fitness components, reproductive studies become pertinent in identifying the evolutionary pathways that could be maintaining the mechanism. A few studies, so far, have shown that taxa with deceptive pollination strategy incur higher costs on seed set than those which offer rewards to pollinators (Table 1). Genetically, low seed-set in the deceptive orchids is not disadvantageous; as slower utilization of the available resources within a plant allows continuous production of seeds with greater viability (Table 1) for several seasons (Jersáková *et al.*, 2006).

There are many correlated features in deceptive orchids and it is difficult to identify any one of these to have majorly involved in evolving deception strategy. However, some data suggest pollinator limitation to be the key feature which promoted floral deception. Phylogenetic analysis based on plastid DNA sequence (Kocyan *et al.*, 2004) has demonstrated that nectar-less but pollen rewarding Apostasioideae represent an archaic condition. Pollination by deceit is a derived condition and the direction of evolution appears to be from the rewarding flowers to the deceptive ones (Cozzolino and Widmer, 2005). Among the deceptive orchids, sexually-deceptive species seem to have been derived from the food-deceptive species (Johnson *et al.*, 1998). Singer (2002) is of the view that nectar rewarding orchids have evolved from the food-deceptive ones.

Pollinator Specialization

There is indeed very high incidence of plant-pollinator specialization in orchids (Gravendeel *et al.*, 2004; Tremblay, 1992). Nearly 70% orchids are believed to exhibit specialization with single pollinators which obviously make them vulnerable to mutual extinction too! Considering the complexity of pollination mechanisms in orchids, specialization in evolutionary context may have different consequences in maintaining the boundaries among the sympatric taxa. Among the deceptive orchids, the sexually deceptive orchids are able to maintain the species boundaries through male pollinator insects (Soliva and Widmer, 2003) whereas in food deceptive orchids, the boundaries may likely break by allowing more than one pollinator to forage their flowers (Cozzolino *et al.*, 2004). However, it has also been shown that the orchids with shared pollinators have more divergent karyotypes that cause meiotic abnormalities in inter-generic hybrids. Floral isolation (isolation due to morphological variations in the flower) may also result in differential placement of pollinia (Schiestl and Schlüter, 2009). For example, variability in the structure of the column among *Platanthera chlorantha* and *P. bifolia* lead to placement of pollinia on the proboscis and eyes of moth, respectively (Darwin, 1862; Nilsson, 1983). Thus the sympatric orchid species may remain reproductively isolated even if they share the same pollinator or the functional group (Kay, 2006). Pollinator specificity is far more crucial as the key prezygotic mechanism in reproductive isolation of orchids (Cozzolino and Widmer, 2005; Schiestl and Schlüter, 2009).

Many orchids do not have a specialized relation with pollinators. In a study comprising ~550 species of orchids, it was found that ~34% species had more than one species of bee pollinator carrying out pollination

Table 2. Some of the deceptive orchids with specific mode of deception and their pollinators.

Species	Region	Type of deception	Pollinator	Class of pollinator	Type of attractant	Composition	Reference
Barlia robertiana	Southern Spain and Northern Europe	Food	*Bombus lucorum*	Hymenoptera	-	-	Smithson and Gigord (2001)
Ophyrus sp.	Europe	Mating	Aculeate hymenopteran insects	Hymenoptera	Pheromones, trichomes on corolla	Aliphatic 1-,2-alcohol and terpenes;alkanes and alkenes	Nilsson (1992)
Drakea sp.	Europe	Mating	Thynnid male wasps	Hymenoptera	pheromones	?	Nilsson(1992)
Anacamptis morio	Southern France	Food	*Bombus lucorum*	Hymenoptera	nectar	?	Smithson (2002)
Epidendrum ibaguense		Mating	*Danaus plexippus* (Monarch butterfly)	Nymphalidae (sub family= Danainae)	Aphrodisiac Pheromones	Pyrrozidine	
Orchis mascula *O. pauciflora*	Europe	Food	*Anthophora* sp., *Eucera* sp.;(bees)	Hymenoptera	Chemical composition of flowers	Monoterpenes, FA derivatives, sesquiterpenes, benzoids, ocimene, myrcene, limonene, linalool	Salzmann *et al.*, (2007)
Epicatis helleborine	Europe		*Vespula vulgaris* (social wasp)	Hymenoptera	Alarm signals (prey mimicry)	Green leaf volatiles (GLV)	Whitman and Eller (1990)
Ophyrus speculum	Europe	Mating	Wasps	Hymenoptera	Floral morphology	Size of the Flowers and Hairy labellum.	Proctor *et al.*, (1996)
Dactylorhiza sambucina	Europe	Food	*Bombus lucorum* (bumble bees)	Hymenoptera	Floral morphology	Flower colourdimorphism (yellow and Purple flowers)	Kropf and Renner (2005)
Ophyrus insectifera	Europe	Mating	*Argogorytes nigroaenea*	Hymenoptera	14 pheromones	?	Jeziorny (2008)
Ophyrus fusca	Europe	Mating	Male bees	Hymenoptera	Pheromones	Cuticular hydrocarbons	Schiestl 2005
Ophyrus exaltata	Europe	Mating	*Colletes cunicularis*	Hymenoptera	Pheromones	Cuticular Hydro-carbons	Mant *et al.*, *(2005)*

Table 2. Some of the deceptive orchids with specific mode of deception and their pollinators. (contd.)

Species	Region	Type of deception	Pollinator	Class of pollinator	Type of attractant	Composition	Reference
Ophrys sphegodes	Austria	Mating	*Andrena nigroaenea*	Hymenoptera	Visual cues and volatile semiochemicals	Saturated nonterpenoic esters, n-alkanes, n-alkenes, and nalkadienes with chain lengths of 19-33 carbons and aldehydes with chain lengths of 9-22 carbons	Ayasse *et al.*, (2000)
O. lutea	Austria	Mating	*Andrena fuscipes*	Hymenoptera	Pheromones	Aliphatic 1-alcohols, 2-alcohols, and terpenes	Ayasse *et al.*, (2000)
Dactylorhiza romana	Southern Italy	Food	*Bombus lucorum* (bumble bees)	Hymenoptera	Colour polymorphism and floral odour difference among the distinct colour morphs	Monoterpenes (b-pinene, sabinene, limonene, b-phellandrene, (E)-ocimene), trans-sabinene hydrate	Salzmann and Schiestl (2007)
Neotinea sp.	Southern Italy	Food	Bees	Hymenoptera	Blooms along with the rewarding plants	-	Cozzolino *et al.*, (2005)
Cypripedium acaule	North America	Food	*Bombus lucorum*	Hymenoptera	Bright flower colour, sweet scent	-	Davis (1986)
Tolumnia guibertiana	Western Cuba	Food	*Centris poecila* (female)	Hymenoptera	In search of the oil	?	Vale *et al.*, (2011)
Tolumnia variegata	Puerto Rico	Food	*Centris poecila*	Hymenoptera	Floral features	-	Ackerman *et al.*, (1997)
Oncidium sp.	Puerto Rico	Food	*Centris poecila*	Hymenoptera	Floral features	-	Dodson and Frymire (1961)
Lepanthes glicensteinii	Cerro Plano, Costa Rica	Mating	*Bradysia floribunda* (male fungus gnats)	Diptera	Pheromones	Heptadecane and other long-chain saturated hydrocarbons	Blanco and Barboza (2005)
Lepanthes stenorhyncha	Cerro Plano, Costa Rica	Mating	*Bradysia floribunda* (male fungal gnats)	Diptera	Pheromones	?	Blanco and Barboza (2005)

Table 2. Some of the deceptive orchids with specific mode of deception and their pollinators. (contd.)

Species	Region	Type of deception	Pollinator	Class of pollinator	Type of attractant	Composition	Reference
Chiloglottis trilabra	Australia	Mating	*Neozeloboria proxima* a thynnine wasp (Male)	Hymenoptera	Pheromones	-	Peakall and Handel (1993)
Cyanicula gemmate	Australia	Food	*Adalia bipunctata* (Beetles)	Coleoptera	Floral morphology	Bright purple colour	Sutherland (2010)
Drakaea glyptodon	South-West Australia	Mating	*Zaspilothynnus trilobatus*	Hymenoptera	Pheromones	?	Peakall(1990)
Caladenia tentaculata	Australia	Mating	*Neozeleboria cryptoides*	Hymenoptera	Pheromones (chemical present on the tip of glandular petals	?	Peakall (2007)
Leporella fimbriata	Southern Australia	Mating	*Myrmeci aurens* (Winged males of the ant)	Formicidae	Pheromones	?	Peakall (1989)
Cryptostylis ovate	Australia	Mating	*Lissopmila excelsa*	Ichneumonid	Floral features	Colour of corolla with yellow pollinia spotted prominently	Coleman (1927)
Chiloglottis trapeziformis	Australia	Mating	*Neozeleboria cryptoides* (Thynnine wasps)	Hymenoptera	Pheromones	2-ethyl 5-propyecyclohexan -1,3	Schiestl (2003)
Diuris sp.	Australia	Food	Australian native bees	Hymenoptera	Flowers mimic fabaceous corolla		
Disa pulchra	South Africa	Food	*Philolichea ethopica*	Diptera	Mimicry with sympatric Watsonialepida	Sucrose and nectar	Jersakova and Johnson(2006)
Satyrium pumilum	Leliefontein Kamiesberg Region	Mating	Carrion flies	Blow flies (Calliphoridae) House flies (Muscidae) Flesh flies (Sarcophagidae)	Carrion scent	Oligosulfides, 2-heptanone, p-cresol and indole	Van der Niet *et al.*, (2011)
Epidendrum paniculatum	Tropics	Mating	Lepidopterans	Lepidoptera	Pheromones	Pyrolizidine alkaloids	De Vries and Stiles (1990)
Dendrobium sinense	Chinese Island Hainan	Mating	*Vespa bicolor*	Hymenoptera	Alarm signal of honey bee, wasps are predators (prey mimicry)	(2)-11-eicosen-1-ol	Broadmann *et al.*, (2009)
Steveniella satyrioides	Anatolia, North Iran	Food	*Vespula vulgaris, Dolichovespula sylvestris* (social wasps)	Hymenoptera	Prey mimicry	Mimicry of the piece of meat (wasps feed their youngones with meat)	Nazarov (1995)

(Additional sources: *www.david.element.ukgateway.net/speciesindexscientificnames; http://www.how-to-grow-orchid.com/types-of-orchid.*)

with almost equal efficacy. In *Epipactis palustris*, a terrestrial orchid with sexual mimicry, 103 pollinators have been identified (Nilsson, 1978) comprising mainly the nectar feeders (van der Pijl and Dodson, 1966). According to Jakubska-Busse and Kadez (2011), the pollinator type largely depends upon the population size of the orchids and also the ambient temperature and precipitation levels during the vegetative season. Therefore, species-specific pollination ecology would be more meaningful if explored for 3-4 years in continuation from different populations to generate a complete spectrum of pollination system.

Local Specialization in Widespread Species

Specialization may be greatly influenced by phenology and decline in individuals of an orchid or its pollinator. For example, in the non-rewarding and outbreeding *Cypripedium* sp. there is enormous seasonal variability in fruit set which is attributed to decline in the demography of pollinators which eventually influences the natural rates of pollination and fruit-set (Bernhardt and Edens-Meier, 2010). In *Diuris maculata*, the southern Australian population show better fruit set as they are exposed to more pollinators than that in the northern population where they are exclusively pollinated by the male bees of *Trichocolletes calceolus*. Thus, it is possible to establish a mutualistic interaction only if a species is surveyed and information on pollination ecology is gathered from its entire occurrence range.

Cues for Attraction

Floral presentation traits such as olfaction and display of pigmentation are the major cues for attraction and each of these traits may contribute in different proportion to the reproductive success of orchids. For example, in *Ophyrus*, the lablellum and the lateral tepals are believed to have greater role in attracting the male wasp pollinators than the scent alone (Vereecken and Sciestl, 2009). Studies involving biochemical dissection of volatile compounds emanating from the orchid species and their involvement in pollinator attraction have been recently taken up. Large genome size, prolonged juvenility and lack of efficient transformation systems in orchids impose difficulties in scent research.

In general, the compounds involved in floral fragrances is a complex mixture of low molecular mass volatile compounds represented by terpenoids (monterpenes), simple aromatics (vanillin, methyl salicylate, methyl benzoate, eugenol, 2-phenyl-acetate or-phenol), aminoid compounds (indole, 3-methyl skatole), fatty acid derivatives and hydrocarbons. The composition of mixture may differ between the species with scent or without scent. For example, in scented flowers of *Phalaenopsis bellina*, monterpenes, linalool, and its derivatives and trans-Geraniol are predominant whereas they are not detectable in the scentless *P. equestris*. The latter species emits volatiles with phenylpropanoid and benzenoid, which are not detectable by humans (Hsiao *et al.*, 2006). So far not many genes are known for scent production in orchids. In *P. bellina*, *PbGDPS*, a flower specific gene has been identified which is involved in maximal monoterpene production (Yu and Goh, 2001; Hsiao *et al.*, 2008).

Floral scents in many orchids mimic the pheromones of female insects to sexually deceive the male insect pollinators (Kullenberg, 1961; Ledford, 2007). The prolonged process of such a co-adaptation, obviously, might have resulted in a biochemical mutualism. This may involve a single odour compound to attract a specific pollinator. For example, in an Australian orchid, *Chiloglottis trapeziformi* (Schiestl, 2003), chiloglottone is believed to mimic the pheromone of the female wasp (*Neozelboria cryptoides*) in sexually deceiving the male wasps. In *Ophyrus sphegodes*, by employing gas chromatography-electromagnetic detection methods, Schiestl *et al.*, (2003) have shown that there are as many 15 compounds which elicit electroantennographic responses in male antennae and copulation attempts in the male bees (*Andrena nigroaenea*). The blend of scent may vary in composition from geographically distinct populations (Vereecken, 2010). Salzman *et al.*, (2007) reported that scent variation is greater in non-rewarding orchids than the rewarding ones.

The beauty of orchids lies in the array of pigmentation patterns on perianth lobes. There is too little information on species-specific patterning of pigments in orchids. However, some of the regulatory genes identified in orchid flower pigmentation such as dihydroflavanol 4-reductase (DFR), Chalcone synthetase, flavanone 3-hydroxylase and Phe ammonia-lyase encode enzymes in the flavonoid pathway (Liew *et al.*, 1998, Johnson *et al.*, 1999).

Autopollination

Nearly 20% of the orchids are known to exhibit ability to reproduce without a pollinator (Catling, 1990). In epiphytic *Holcoglossum amesianum*, the bisexual flowers have been noticed to turn their anthers to nearly 360° to lodge pollinia into their own stigmatic cavity (Liu *et al.*, 2006). This mechanism serves as means of reproductive assurance and compensates for pollinator limitation, usually observed in orchids (Jakubska-Busse and Kadez, 2011). This feature may be common in several pollinator depauprated populations because of the increased female fitness and greater likelihood of spread of the allele for selfing (Fisher, 1949).

Environmental Fluctuation may Hamper Pollination and Fertilization

Many natural ecosystems are being adversely affected due to regional climate change (IPCC, 2007). It has been realized that because of frequent fluctuations in the climatic pattern, particularly temperature and moisture (Primack and Hall, 1990), timing of blooming of a plant species may become altered and the crucial pollinator or the pollinators may locally disappear causing loss in the extent of natural fecundity. Most of the orchids will be affected due to habitat modification and significant shift in the ambient conditions. In some orchids with prolonged pollen-pistil interaction (*Phalaenopsis* sp, O'Neill, 1997; *Cypripedium parviflorum*, Bernhardt and Edens-Meier, 2010), climatic fluctuation may adversely affect the fertilization process and result in poor or no seed years. However, it is also argued that some orchids may also co-adapt with changing climatic regimes as their physiological performance may not get altered with increase in CO_2 (Seaton *et. al*., 2010). Presently, we do not have reliable phenological records that explicitly indicate the date of onset of flowering and their period. In the absence of such a crucial data it has become difficult to correlate whether the lack of pollinator or low fruit-set is the result of environmental variation at the regional level. Thus, long-term phenological observations in species rich areas of orchids may help in preparing a monitorable record.

Community Level Studies for Conservation

As many threatened orchids do not offer rewards to foragers, pollination activity, even by deceit, may depend upon the availability of pollinators in the vicinity of orchids (Bernhardt and Edens-Meier, 2010). Bernhardt (2005) has recommended generation of area-specific long-term pollen records from the body of insects that visit the orchid flower to establish (i) if a particular orchid species is in competition for pollinators with any co-blooming plants; (ii) if low fruit set in reward-less orchid flowers occur even in the presence of pollinators and (iii) if the co-blooming species impart any 'magnet effect' (Thomson, 1978) and accelerate the proportion of fruit set in rewardless orchid flowers (Indsto, 2006). Bernhardt and Edens-Meier (2010) have recommended that in addition to the population survey of orchids, a record of demography and natural history of pollinators is also essential in ensuring in situ conservation. Although a difficult exercise, capture and release of the pollinators in addition to localization of oviposition sites is suggested to be immensely useful in managing the magnet species. There is paucity of studies which emphasizes the role of co-flowering plants in increasing the availability of pollinators in regions inhabited by the threatened orchids. The only study from India carried out at the farmer managed and fragmented Sopinabetta forest of Western Ghats has shown that the fragmented forest can suitably support diversity of epiphytic orchids comparable to the pristine forests provided appropriate management practice are employed like controlled lopping of branches and avoiding litter-forming coppicing trees (Sinu *et al*., 2011).

Breeding System

A majority of orchids are self-compatible, although with greater incidence of seed abortion than those obtained from cross-pollination (Dressler, 1993; Tremblay *et al*., 2005). Increased dependence on pollinators for seed-set in combination with greater incidence of self-compatibility in Orchidaceae is puzzling. Moreover, the combination is expensive in term of resource investment in floral features. It is argued that the evolution of such elaborate outbreeding devices have been possibly driven by the occurrence of pollinia. Compound pollen units are known to increase the pollination efficiency, as the usually greater number of pollen than the ovules, are able to sire all the available ovules in one act of pollination (Kress, 1981). This is also in accordance with the observed evolutionary trend in increased adaptive value of (i) reduction in the number of fertile anthers (ii) production of pollinia in the derived taxa compared to that of free or mealy pollen observed in archaic taxa of orchids (Dressler and Dodson, 1960).

Self-incompatibility is reported from a fewer (~10%) orchids and it largely operates at the post-zygotic level. For example, Tremblay (2005) demonstrated in nearly 75 orchid species, 49 species were self-incompatible, as controlled self-pollinations lead to post-zygotic inhibition and siring of a fewer seeds. The extent of compatibility may vary over the distribution range of the species as documented for *Galearis spectabilis* (Tremblay, 2005). In smaller populations, a strong pre-zygotic barrier will reduce the fecundity due to the presence of limited number of S-alleles dispersed by the plants. In *Coelogyne fimbriata*, Cheng *et al*., (2009) showed that although 69% of the stigma received the pollinia deposited by *Vespula* wasp, fruit set ranged between 1-6%. Thus, an assessment of allelic variation is crucial before attempting the reintroduction of micropropagated genotypes or increasing the fruit set through manual pollinations by using pollen from within the population. Marker assisted tools such as allozyme variation, AFLP and RAPD, may provide useful insight for assessing the breeding system. Information on breeding systems of orchids at the population level is meagre and is indeed, required for self-sustaining population of orchids in the wild.

Although adventitious embryogeny, a trait closely related to apomictic plants, has been cited for a number of orchid genera (Naumova, 1993; Sun and Wong, 2001; Swamy, 1943, 1948, 1949), there are not many cases of obligate apomixis. A recent study on *Corunastylis apostasioides*, an Australian midge orchid, has clearly shown the occurrence of fertilization independent obligate apomixis (Sorensen *et al.*, 2009). In this species, the megaspore mother cell bypasses meiosis (apomeiosis) and undergoes diplosporic apomixis. Several additional embryos are formed from the integument and nucellus (sporophytic apomixis). A similar combination of apomixis and polyembryony has been earlier reported in *Epidendrum nocturnum* (Strot and Pavanelli, 1986).

The fact that besides 25,000 species of orchids, there are over one lakh registered orchid hybrids in the world, also suggests the occurrence of weak reproductive barriers. It is interesting to note that the inter-generic hybrids are conveniently formed within the subfamily whereas wide hybridization across the subfamilies results in severe hybrid sterility (Scopece *et al.*, 2007). This is an interesting aspect of reproductive strategy and perhaps the absence of the usual 'prolonged' cross-talk between the developing embryo and endosperm which essentially happens in other families of flowering plants (except the Podostemaceae and the apomicts with autonomous endosperm development), helps sympatric species of orchids to form primary hybrids in nature. According to Lenz and Wimber (1959), evolution in Orchidaceae appears to be too rapid for the establishment of effective reproductive barriers.

Orchids represent a specialized domain of mutualistic interactions in the architecture of ecosystem services. Although there is necessary realization, commitment and available pool of expertise in India, conservation biologists have yet to begin comprehensive studies on the reproductive ecology of orchids. Pollination ecology and assessment of breeding system along with a record of demography, phenology and genetic variation would be a constructive step in conservation of orchids in their natural habitat. However, in spite of greater species diversity, small population size of many orchid taxa is a challenge. Interdisciplinarity and research on focussed issues would be a practical approach not only in furthering orchid biology in the country but also in reaping the benefits through sustainable utilization of taxa important to floriculture and other economic benefits.

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