POLLINATION DYNAMICS IN A CHANGING TROPICAL FOREST LANDSCAPE

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Abstract

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Little is known of pollination in the canopies of tropical trees or plants in which flowering is a rare event. I investigate reproductive success as a function of floral resources, pollinator guilds and the breeding habit in two tree species – *Nothapodytes nimmoniana* and *Myristica dactyloides,* and one monocarpic shrub *Strobilanthes kunthianus,* each representing a different forest type in the Western Ghats of India. Using three distinct approaches, I ask if pressures on the plant populations (from harvesting of plant resource or habitat degradation) have an impact on pollination outcomes.

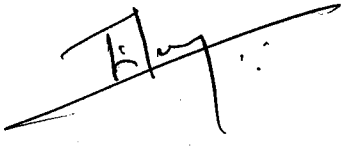
In the two canopies, the pollinator assemblage consisted of small diverse insects with some degree of specialisation (Coleopterans on *Myristica* and Dipterans on *Nothapodytes*). Bees were abundant in the montane grassland mass bloom of *Strobilanthes*, assuring 100% pollination efficiency. There was no pollen limitation in the three species, but in *Nothapodytes*, I provide evidence for a shift in insect diversity and a possible decrease in fruiting in populations where floral density is altered either naturally or from disturbance. An indirect measure of fruiting success in *Myristica* highlighted the influence of non-forest matrix and over-harvesting on mutualisms.

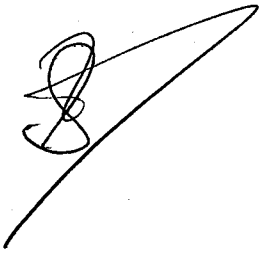
In two species, I examine the evolutionary setting in relation to pollination dynamics: polygamous individuals seem to guard against decline in mates or pollinators in *Nothapodytes* and a set of adaptive floral traits render *Strobilanthes* protection from fruiting failure.

I suggest that species with multiple pollinators may not be resilient to fluctuations, since generalists are just as sensitive to disturbances as specialists. Also, in forest canopies, there is a frequent overlap of functional guilds: a change in faunal assemblage could have a cascading effect on pollination processes. Habitat conservation is the key to maintaining connectivity between pollinator foraging resources that may be located in different forest types.

Declaration

The work in this thesis is my own, with the exception of Chapter VII, which was a collaborative research project initiated with Giby Kuriakose and Dr K. R. Shivanna at the Ashoka Trust for Research in Ecology and the Environment, India.

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CHAPTER I

Introduction

There is growing evidence for disruption of plant - pollinator interactions and reduced reproductive success in plants thriving in human altered ecosystems. Often, in the biodiversity rich tropics, it is difficult to know whether low fruiting and poor seedling regeneration result from pollination limitation or from reduced plant population sizes from disturbance, or both. This might partly be because the processes involved in the pollination mechanism, the plant's evolutionary adaptations and the factors affecting habitat alterations are seldom studied as a whole. This dissertation takes a cross-scale approach to demonstrate that reproductive success is a function of the interplay of floral displays in the population, pollinator types (and specificities), and the breeding system, viewed from the evolutionary setting and current trends in habitat destruction. In this introductory chapter, I present the three study species, each representative of a habitat type and a canopy stratum, and the proximate causes for their exploitation and habitat degradation. The questions addressed to understand pollination ecology and quantify fruiting success in the plant species in a rapidly changing forest landscape are laid out. I review what is known of plant - pollinator mutualisms in anthropogenically disturbed landscapes and introduce the relevance of such studies to conservation biology in the Western Ghats of India.

Introduction

Tropical forests are the most diverse of terrestrial ecosystems and a good understanding of its floral and faunal components is essential to the unravelling of the complex functions they contain (Turner, 2001). They are more productive and complex than most other biomes on earth, owing mostly to the higher diversity they contain (Daniels et al., 1995). Studies on the nature of the interactions between plants and their pollinators have shed light on the structure and dynamics of plant communities in tropical forest ecosystems (Rathcke & Jules, 1993; Bond, 1994; Parra-Tabla et al., 2000; Ghazoul, 2004), and have led to clarifying issues of regeneration and conservation of tropical forest resources (Bawa & Hadley, 1990).

Pollination systems in tropical forests of the Western Ghats

The Western Ghats, locally known as the 'Sahyadri', are a long chain of hills along the west coast of India extending as long as 1600 km. They provide one of the best examples in the world (together with Madagascar, and to a lesser degree, Queensland in Australia) of a belt of tropical evergreen forests spread over more than 10 degrees in latitude, not centred over the equatorial zone (Pascal, 1991). The wide variation of rainfall patterns in the Western Ghats, combined with geographical features, produces a variety of vegetation types, from scrub forests in the low-lying rain shadow areas and the plains, to deciduous and tropical rainforests up to about 1,500 m., and a unique mosaic of montane forests and rolling grasslands above 1,500 m. (Champion & Seth, 1969). Together with Sri Lanka, the Western Ghats form a global biodiversity hotspot with an abundance of endemic flora and fauna (Myers et al., 2000) and has been identified as an area of high conservation significance (Ramesh et al., 1997). Much of the forest cover of the region is heavily degraded and fragmented owing to tremendously growing pressure from human sources (Daniels et al., 1995; Anonymous, 2007). A region that can potentially support 64750 km^2 of evergreen forests and 103000 km^2 of deciduous forest (Daniels et al., 1995) has witnessed a 20% reduction in forest cover between 1972 and 1982, and a further low rate of 4-32% between 1982 and 1990 (Forest Survey of India, 1989, 1992). Gadgil and Meher-Homji (1986) estimate a 22000 km^2 reduction in the evergreen forest cover of the Western Ghats.

Of the estimated 250000 species of the planet's angiosperms, a large percent are distributed in the tropics, where insects dominate the pollinator community (Buchmann & Nabhan, 1996a). And yet, pollination requirements of most wild species in the tropical forests remains unknown (Kearns & Inouye, 1997). Tropical plants are committed to sexual reproduction; outcrossing is almost obligate (Bawa & Hadley, 1990), making them dependent on a variety of vertebrates and invertebrate pollen vectors. The intricacies in pollen ovule compatibility are proof that the quality of pollination is key to the success of plant reproduction (Wiens et al., 1987). Finding the right pollinator partner that will deposit the right quantity and quality of pollen is a challenge plant systems have lived with, and evolved, over centuries.

At the community level, plant - pollinator interactions have attracted considerable attention, frequently coupled with studies on the breeding systems in entire assemblages of forest trees (Bawa et al., 1985a; Bawa et al., 1985b). There is a great extent of generalist pollination and loose niches in the south - east Asian forests and the neotropics (Bawa et al., 1985a; Momose et al., 1998b). It is believed that canopy and emergent trees constitute a foraging environment rather different from sub-canopy and under storey (Janzen, 1971; Stiles, 1978; Endress, 1994b; Kress & Beach, 1994; Momose et al., 1998b). Community phenology studies in Malaysia reported sub - canopy flowers to have minimum visual lures (Appanah, 1981) and a Costa Rican study showed that pollination systems exhibit maximum diversity in the sub-canopy and under-storey in comparison to the upper canopy (Bawa et al., 1985a). Another study of the under-storey plants in Sarawak revealed 70% of the plants with specialist interactions (pollinated by trap-lining bees) and the rest, by birds, flies, wasps, butterflies and beetles (Kato, 1996).

A long - term community pollination study (Devy & Davidar, 2003) at a mid elevation forest site is a crucial reference point for pollination research in the Western Ghats. They found a high diversity of pollination modes, about 75% of the tree species being specialised to a single group of insects such as bees or beetles, and the rest, pollinated by insects from diverse groups. Findings of another study on the under-storey shrubs in an evergreen site of southern Western Ghats pointed out that most plants are generalists, using diverse pollinator groups (Krishnan, 1994).

The aim of this dissertation is not to make conclusions at the large scale or community level pollination mechanisms, but to draw out a sample set of species and consider the importance of pollination dynamics in relation to fruiting success in the context of disturbance. Each target species forms a case study from a particular habitat, and a distinct stratum in the forest structure and each represents a vulnerable state in a changing forest landscape.

Global concern about pollinator decline

World over, there is talk of pollinator decline in agricultural and other landscapes. The argument of food crisis from lack of pollination originates from an anthropogenic view and concerns some very significant aspects of agricultural practices such as non-native bees, use of pesticides, etc (Kearns et al., 1998). In the context of tropical forests and biodiversity, such an argument does not accommodate the need for conserving an entire realm of wild plant and pollinator species that inhabit forest ecosystems, all fascinatingly inter - connected in a complex and dynamic loop. Hence, a distinction ought to be made between declines of pollinators of cultivated plants and pollinators of wild plants (Borges, 2003). Pollination systems in the tropics are under increasing threat from anthropogenic forces, including fragmentation of habitat, changes in land use, and invasive species (Kearns et al., 1998). Plants occurring in these species-rich tropical communities may be more prone to pollen limitation simply because of interspecific competition for pollinators. As a consequence, being in biodiversity hotspots (such as the Western Ghats), they face a higher risk of extinction, since the combination of higher pollen limitation and habitat destruction probably presents a dual risk (Vamosi et al., 2006).

Specialised plant - pollinator systems are believed to be more sensitive to disturbances (Kunin, 1993), and the pollinator and the plant may be influenced if there is a change in abundance in either of the two (Janzen, 1974; Gilbert, 1980; Cunningham, 2000). Habitat fragmentation modifies species richness and composition, altering the mating structure in populations, and can influence diversity and behaviour of pollinators (Aguilar et al., 2006), and strictly self-incompatible and dioecious species being dependent on pollinating agents, are more susceptible to pollen limitation from these disturbances

(Bawa et al., 1985b; Jennersten, 1988; Aizen & Feinsinger, 1994a; Bond 1994; Didham et al., 1996; Kearns et al., 1998; Steffan-Dewenter et al., 2002; Ghazoul, 2005). There are also many species that reduce their risk of pollinator loss via compensatory or 'insurance' mechanisms such as multiple or generalist pollinators (Bond, 1994), wind pollination, self - pollination, or decreasing dependence on seeds via vegetative reproduction or long life spans (Spira, 2001).

For over a decade now, the extent of dependence and linkage in pollination systems has been under investigations, as an issue affecting long - term integrity of ecosystems and biodiversity conservation (Saunders et al., 1991; Murawski et al., 1994; Aizen & Feinsinger, 1994a; Buchmann & Nabhan, 1996a; Kearns & Inouye, 1997; Kearns et al., 1998; Allen-Wardell et al., 1998; Kremen & Ricketts, 2000; Ghazoul, 2005).

There is now considerable evidence for disruption of plant - pollinator mutualisms in fragmented landscapes. The causes can be varied, from loss of native pollinator diversity (Aguilar et al., 2006; Chacoff & Aizen, 2006) or lack of nesting sites for key insect pollinators (Aizen & Feinsinger, 1994b; Cane, 2001), to a decline in pollinator visits when plant population sizes are reduced by fragmentation and habitat alteration (Bond, 1994). Pollinator behaviour and number and quality of pollen grains transferred could be influenced by the size, density and shape of the plant population (Handel, 1983; Ghazoul et al., 1998; Cunningham, 2000; Ghazoul, 2004; Chacoff & Aizen, 2006; Cheptou & Avendaño, 2006; Tylianakis et al., 2008). In fact, shifting plant abundances caused by disturbance may even lead to a restructuring of the plant – pollinator interaction web (Ghazoul, 2004). When populations are fragmented or altered, one of the results could be fewer pollinator visits, causing less pollen to reach flowers and fewer seeds to be produced (Jennersten, 1988; Aizen & Feinsinger, 1994a; Aizen et al., 2002; Burgess et al., 2006). Reduced fruit and seed production, in turn, must have a negative effect on seedling recruitment (Cordeiro & Howe, 2003) and research has warned that such populations may become unviable eventually and enter a spiral towards extinction (Kearns & Inouye, 1997). Then there is also an expected cascading effect of reduced pollination on seed dispersal by frugivores (Harrison, 2000; Tylianakis et al., 2008), thus adding to the negative effects of reduced dispersal, particularly in habitat fragments (Cordeiro & Howe, 2003; Garcia & Chacoff, 2007).

In the southern Western Ghats study on community pollination modes (Devy & Davidar, 2003), a very low number of pollinator species was encountered within each guild compared with other tropical lowland forest sites across the world. They also found the relationship between the pollinators and the food plants here more intricate than the other sites, and pointed out that such sites could face drastic consequences of disturbance, distorting such relationships and leading to cascading local extinction of the food plants and their pollinators. The exceptionally low fruit set observed in certain tree species during many mass flowering years of their long-term study was also suspected to be due to pollinator deficit (Ganesh & Devy, 2000).

Threatened plants – is there a pollination crisis?

Even at a time when conservation had become a buzz word, there were very few studies that examined the role of habitat change on plant pollinator interactions (Rathcke & Jules, 1993; Bond, 1994), but the last decade has seen considerable contribution to the literature and evidence in this field. Yet, there is a dearth of research on threatened tree species (or plant communities) and their reproductive and regeneration success from the Indian tropics. There is but one study from a highly fragmented seasonal cloud forest the Western Ghats that examined the effect of plant resource extraction and related population alteration on wild pollinators and fruit set (Somanathan & Borges, 2000). Although the threat to plant populations is severe from habitat degradation, and more specifically non-timber forest product (NTFP) harvest, there has been a surprisingly low research focus on the question of how plant and pollinator populations fare under these anthropogenic forces.

Very small shifts in the phenology of individual plant species are capable of causing large impacts on community-wide pollination mutualisms (Memmott et al., 2007). Added to this is the risk of small populations suffering from reduced pollen delivery. Rare and endemic plants that depend on specific pollinators could be especially vulnerable if fewer pollinator visits result in fewer seeds, higher selfing rates and increased inbreeding depression (Spira, 2001). Due to their small populations and sparse floral displays, endangered species may attract fewer pollinators or have greater variation in pollinator visitation than more common species (Jennersten, 1988;

Husband & Barrett, 1992; Campbell & Halama, 1993; Spira, 2001).

The study takes a case study approach with two endangered tree species of the Western Ghats - *Nothapodytes nimmoniana* and the endemic *Myristica dactyloides,* and one monocarpic shrub *Strobilanthes kunthianus* – an endemic species that flowers once in twelve years. The three species are from three distinct habitats across an elevation gradient – they are by no way representative of a habitat or elevation, but present a case each of a threatened species in a given forest type and a given canopy stratum, with a potentially distinct pollinator niche. What the three endangered species do represent is a threatened state in a disturbed habitat, and this unfortunately is not uncommon in tropical systems today.

The two tree species – *Nothapodytes nimmoniana* and *Myristica dactyloides* are commercially exploited as NTFPs, a subset from six species shortlisted at the Ashoka Trust for Research in Ecology and the Environment (ATREE), India, for a project on "reproductive biology and species enrichment program of critically endangered species of the Western Ghats". *Strobilanthes kunthianus* is an endangered semelparous shrub, flowering once in twelve years, its habitats facing severe degradation and land use change. When the species flowered in August 2006, it was a perfect opportunity to study its pollination ecology in the most accessible population.

Nothapodytes nimmoniana Graham.(Family Icacinaceae), formerly *Mappia foetida* and *Durvasanemara* in the vernacular, is a small spreading, deciduous, sub - canopy tree distributed in the dry and moist deciduous, and evergreen forests of the Western Ghats of India, and in the eastern states of Assam and West Bengal in a wide range of elevation (600 – 1400 m. asl.).

Myristica dactyloides Gaertn. (Family Myristicaceae – the Nutmeg family) or *Jatipatri* in the vernacular, is a characteristic component of the wet evergreen forest canopy in mid and high elevations, preferring riparian habitats. It occupies the intermediate stratum – just below the canopy (Giriraj et al., 2008), growing up to 20 - 35 metres in height. The species is seen to inhabit high altitude evergreen forests (850 - 1400 m. asl.) and is endemic to the Western Ghats and Sri Lanka.

Strobilanthes kunthianus Nees. (Family Acanthaceae) is a small shrub in which flowering is a gregarious event. It is a 'narrow endemic' inhabiting the montane wet grasslands of the subtropical high ranges of the Western Ghats (1500 - 2600 m. asl.), called the Nilgiri Hills. The plant is a small shrub reaching a height of 0.5 - 3.0 m.

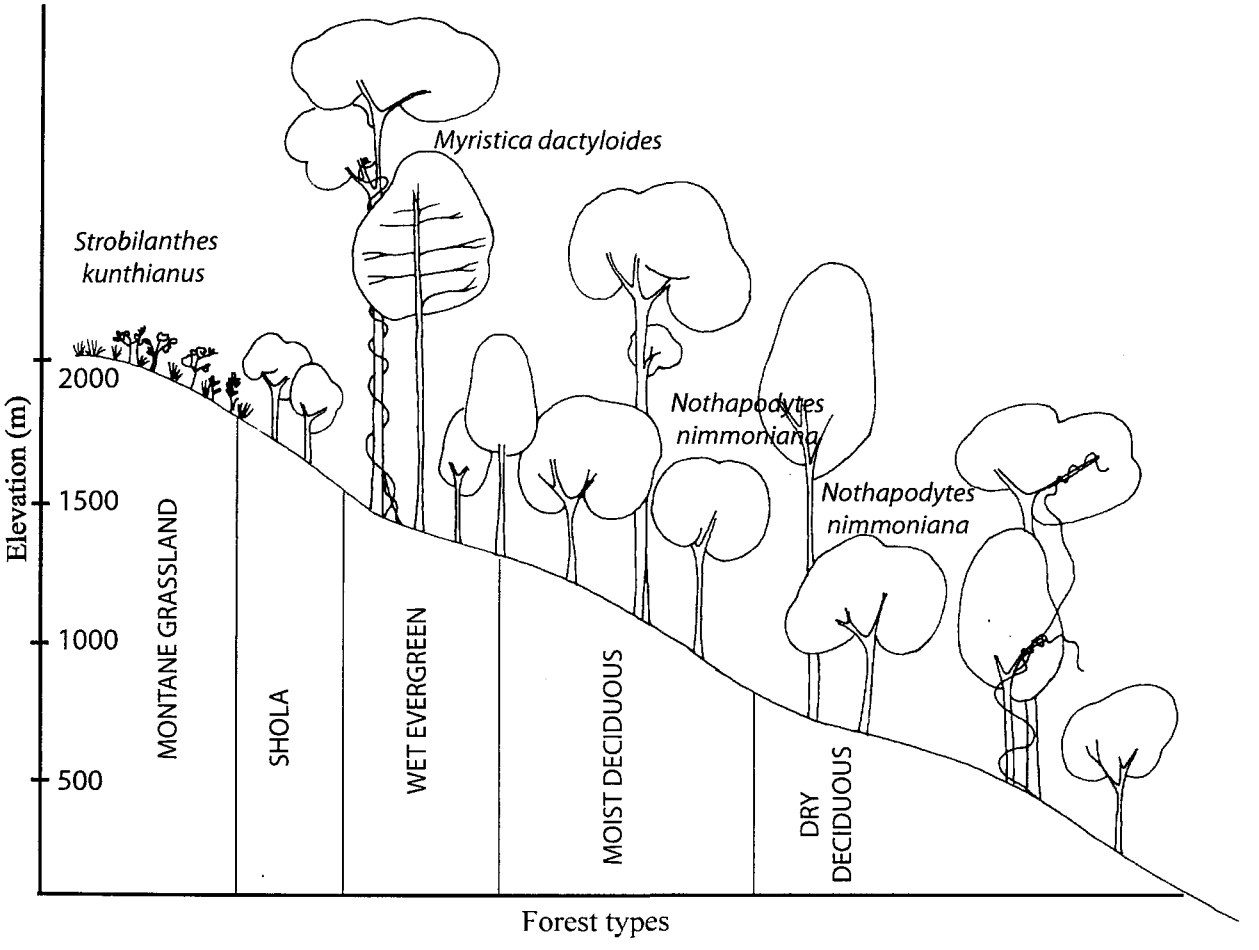


Figure 1.1 A depiction of the distribution of the three study species across the forest types and elevations in the Western Ghats of India. This is re-constructed from a vertical forest profile (adapted from Pascal, 1988) and not strictly to scale. It also does not represent all the forest types contained in the region, for instance, the semi-evergreen forest, lowland evergreen forest and the scrub forest are not drawn here. *Strobilanthes kunthianus* is a semelparous shrub growing exclusively in the subtropical montane grassland-Shola habitats. *Myristica dactyloides* occupies the intermediate canopy (below the emergent stratum) of high altitude wet evergreen forests. *Nothapodytes nimmoniana* is in the sub-canopy with a wider range of distribution, from dry deciduous to moist deciduous to semi-evergreen types.

There is a diversity of forest types in the Western Ghats – wet, semi-evergreen and dry evergreen, moist and dry deciduous forests, littoral and swamp forest, a thorn forest type and even a subtropical montane Shola forest type in higher altitudes sheltered in the slopes of rolling grasslands (Champion & Seth, 1969). The wet evergreen forests at high, medium and low elevations in the Western Ghats house a great diversity of angiosperm species, many of which are localised endemics (Krishnan & Ramesh, 2005). The dry evergreen forests that occur in some patches on the eastern slopes of the Ghats are also unique in their species composition (Ramesh, 2001). The evergreen vegetation covering the Biligiri Rangan Hills in the state of Karnataka and the Nilgiri Hills (spread over Tamil Nadu and Kerala states) are an important floristic link between the Eastern and Western Ghats (Legris & Meher- Homji, 1982; Ramesh, 1989). The moist deciduous forests form a band that surrounds the montane evergreen rain forests in the southern part of the Western Ghats. They do not host as many endemic species as the rain forests, but are richer in species, harbouring more mammal and bird species (Rawat et al., 2001).

This study spans across three distinct forest types in the central and southern Western Ghats. The three species were not studied in a single Protected Area but they are characteristic of distinct forest types and canopy strata over an elevation gradient (Figure 1.1).

Nature of threat

In 1997, the International Union for Nature Conservation (IUCN) Red List of Threatened Plants listed 34,000 species (about 12.5% of the world's higher plants) as facing extinction. Pollination ecologists have alerted that it is important to ask how far pollination failure is linked to the threat of extinction in these species (Bond, 1994; Kearns et al., 1998; Wilcock & Neiland, 2002).

The Western Ghats have been subject to intense exploitation and a large part of the climax vegetation has disappeared. They form a critical zone for ecological research on the variations in their structure and floristic composition (Pascal, 1991). Large areas of forested land are lost to development projects such as dams and mining (Rawat et al., 2001). The region is home to several conservation regimes - community based

protection of natural resources, agro-forestry techniques and organisations promoting sustainable NTFP harvest practises. Yet there are many challenges concerning conservation of plants and their habitats (Godoy & Bawa, 1993). NTFPs have provided subsistence (Ticktin, 2004) and income (Iqbal, 1993) for thousands of forest - dependent communities in the tropics over thousands of years. Commercially there has been an increase in trade in natural products for commercial purposes (Iqbal, 1993; SCBD, 2001) and herbal medicine (Uniyal et al., 2000), leading to increased pressure on wild plant populations (Kuipers, 1997; Lange, 1998). Depletion of populations (Nepstad et al., 1992) and impacts on growth and reproduction of harvested individuals (Ticktin, 2004) are probably the most direct ecological consequence of NTFP over-exploitation (Rawat, 1997; Tiwari, 2000).

The three species I study represent tropical plant populations in a forested landscape of the Western Ghats, affected by anthropogenic pressures from NTFP over-harvesting or land use change such as fire and plantation.

Natural populations of *Nothapodytes nimmoniana* face indiscriminate harvest for trade of their wood chips, a source of camptothecin which is an anti - cancer drug component (Govindachari & Viswanathan, 1972). About 20% of the wild populations in India have declined in the last decade alone (Ravikumar & Ved, 2000). Branches and often entire tree trunks are lopped in vulnerable populations for extracting wood chips.

Fruits of *Myristica dactyloides* have seeds covered with an aril. Seeds (nutmeg) and aril (mace) are both NTFPs highly valued in spice and medicinal trade. Large quantities of fruits are collected to obtain a few grams of the seed coat. Forest dwelling communities have easy access to populations of these trees in high elevation evergreen forests.

Strobilanthes kunthianus habitats are the high altitude subtropical montane grasslands that suffer from severe degradation in the form of fire, grazing and conversion to plantation (Robinson, 1935; Rajkumar, 2008). The mass flowering that occurs once in twelve years attracts thousands of tourists and the impact of irresponsible tourism is extremely harsh.

The nature and level of threat faced by each study species is different. While both *N.*

nimmoniana and *M. dactyloides* are commercially exploited, the former has a rather recent history of exploitation when compared with the latter; and the case of *Strobilanthes* is not relevant to plant resource exploitation, but habitat destruction and land use change are a serious cause for conservation attention. Understanding the mechanisms of pollination and the causes of any pollination limitation or failure in plants can lead to finding answers to the conservation and recovery of rare and threatened plants and also perhaps offer some pointers to sustainable use of wild plant resources (Wilcock & Neiland, 2002). The thesis addresses pollinator responses and pollination outcomes in human-dominated landscapes and where possible, compares results with populations in undisturbed habitats.

Research Questions

Pollinator limitation is best assessed ideally by experimental manipulation of pollinator populations, but a less direct approach is commonly used as evidence. Pollen supplement experiments have suggested that fruit set is limited by pollination in over 50% of the plant populations studied by Burd (1994) and Knight et al. (2005). The magnitude of pollen or pollinator limitation is also believed to be rather high: a 75% higher fruit set in hand pollinated flowers in comparison to those natural pollinated (Knight et al., 2005; Corlett, 2007). I consider the importance of pollination phenomena in limiting fruit set in the three endangered plant species and start with investigating their reproductive ecology in detail. I ask if disturbance from over - harvesting of plant parts or habitat degradation has a negative effect on pollinator assemblages and movement, and on the reproductive success of the species.

Pollination ecology has its roots in descriptive natural history (Richards, 1978) and the relevance of this must not be overshadowed by its advanced techniques and wide applications to conservation science. I allow myself to dwell into the natural history of the species, highlighting aspects of floral biology and its adaptive significance. I also attempt to place the preference for outbreeding or selfing in the given species in an evolutionary perspective. In these aspects, the work will appear to be a study on autoecology. Although I make no direct observations on pollinator nesting habitats or their depletion, much of the findings give an insight to the importance of habitat connectivity in keeping pollinator populations viable and active.

The thesis is not hypothesis driven in the conventional sense. In the light of growing evidence that depressed pollination in altered or fragmented populations leads to decreased fruit set (Jennersten, 1988; Saunders et al., 1991; Kearns & Inouye, 1997; Steffan-Dewenter & Tscharntke, 1999; Cunningham, 2000; Ghazoul & McLeish, 2001; Murren, 2002), I speculate that disturbed sites in comparison with undisturbed sites, will show signs of pollinator decline and a dip in reproductive success. Isolation of flowering individuals created in populations under pressure from over-harvesting of plant parts in *Nothapodytes nimmoniana* and *Myristica dactyloides* could alter the mating structure of the species, thus having a negative influence on pollinator movement and consequently reproductive success. In the case of *Strobilanthes kunthianus,* degraded habitats present a situation where pollinator behaviour and reproductive success can be explored in a rare, mass flowering species, under a changing landscape facing risks of fire, grazing and a non-forest surrounding matrix.

To summarise the research questions, –
(i) In natural undisturbed populations of the three species, what are the floral display trends and pollinator guilds, and what is the reproductive success?
(ii) Does altering the population through unsustainable resource harvest and other habitat disturbance alter the floral resource and mating structure of the population?
(iii) Is there depressed pollination in disturbed study sites with anthropogenically degraded habitats? In other words, is there a marked difference in pollinator assemblage and percent fruit set in such populations when compared to less disturbed populations?

One of the underlying questions is whether sexuality and pollination in the species can be explained from an evolutionary perspective. Are the floral parts and sexual system of the study species, both known to play a role in the integrity of pollination systems (Bawa & Beach, 1981), 'best adapted' for optimum reproductive efficiency? Do we know how effective and resilient these traits are in human disturbed populations? Another pressing question is whether we can protect over-harvested populations with out knowledge on breeding system and pollination mechanisms.

The relevance of species specific studies, particularly of rare and threatened, has been underestimated, when it comes to plants and insects (Spira, 2001; Wilcock & Neiland,

2002). This research addresses plant habitats, pollinator responses and pollination outcomes from the perspective of individual plant species located in forested landscapes that are human dominated.

Reproductive ecology in tropical forest trees is a little investigated field (Perry & Starrett, 1980; Roubik et al., 2003; Roubik et al., 2005). The two tree species give me an opportunity to explore the young, frontier science of canopy biology and the exciting world of interactions hidden up in the tropical forests. Tropical forest canopies may be the most species rich habitat on earth (Erwin, 1983) and yet the fauna of tropical canopies remain largely unexplored (Basset et al., 2003, Leather, 2005). Similarly, reproductive ecology is highly unstudied in semelparous or monocarpic plant species in which flowering is a rare event, separated by huge gaps that last for years. *Strobilanthes kunthianus*, my third study species is one such species with a twelve year flowering cycle.

The two chapters following the section on methodology are dedicated to *Nothapodytes nimmoniana*; the next two, to *Myristica dactyloides*. Chapter VII covers the *Strobilanthes kunthianus* study. During the three years of the study to understand tropical plants and insects and their highly variable nature, some new ideas evolved that further shaped the research, mostly triggered by my preliminary observations. One such turn in the study was the evolutionary aspect of the sexuality in *Nothapodytes nimmoniana* based on observations on the floral morphs, population structure and breeding system (Chapter IV). Another development in the thesis was the introduction of measuring fruit crop size in *Myristica dactyloides* on a disturbance gradient across several populations (Chapter VI). The final chapter is a synthesis of all the results that have been separately discussed in the chapters based on the three species. Therein, I place the main findings of the thesis and emphasise the significance of studying plant animal interactions in tropical forests and the substantial challenges such studies offer to biodiversity conservation in disturbed landscapes.

To borrow an expression from Professor Inouye, renowned pollination ecologist, "there is enduring relevance in rainforest research. Maintaining the human birthright - the preservation of nature's masterpieces while fulfilling the true goals of our lives and histories - is still the primary purpose of science".

CHAPTER II

General Methodology

This chapter broadly encompasses the materials and approaches that are common to all the three species of my study, detailing some of the theory of certain methodologies. Individual species chapters incorporate further qualifications to the particular methods discussed here, but this general chapter is placed here to minimise overlaps between following chapters. Flowers in the canopy of *Nothapodytes nimmoniana* and *Myristica dactyloides* were accessed with a locally made bamboo ladder for carrying out experimental pollinations and detailed insect observations. Methods used in the *Strobilanthes kunthianus* study differed from the other two species not only because it is a shrub not requiring climbing techniques, but also because the study was restricted to a single flowering period in one population.

The Sites and Study period

Studies on the two tree species were carried out in the Western Ghats of Karnataka State, mostly concentrated in the Biligiri Rangaswamy Temple (B.R. Hills) Wildlife Sanctuary (WLS), based at the Field Station of the Ashoka trust for Research in Ecology and the Environment (ATREE) within the Sanctuary. The mountain range covers about 38019 km^2 in the State (Forest Survey of India (FSI), 1989,1992), with 5 National Parks, 15 Wildlife Sanctuaries and a part of the Nilgiri Biosphere Reserve. The *Strobilanthes* study was carried out near the Eravikulam national Park located in the Nilgiri Hills in the State of Kerala, bordering the State of Tamil Nadu. The B.R. Hills WLS (540 km^2) holds diverse habitats with the representation of almost all the main forest types within one Sanctuary – from scrub jungle to dry and moist deciduous to wet evergreen and the Shola - grassland type. Dandeli WLS, the largest protected area (1060 km^2) of Karnataka, has contiguous tracts of evergreen and deciduous forests. All my other field sites were located in evergreen forests of the central Western Ghats of Karnataka – Brahmagiri and Talakaveri WLS, Kudremukh National Park and Kemmangudi.

Observations on floral morphology, insect visitors, and hand pollination experiments were conducted during the peak flowering period, towards the second week from flowering initiation in all the three species. The number of flowers or trees (*n*) sampled and time spent observing flowers and visitors varied among the three species.

Nothapodytes sp.

The study was carried out in two populations in the moist deciduous forests of the B.R. Hills WLS (Doddasampige N 11° 57' 079", E 77° 09' 632" and Malkibetta N 11° 57' 221", E 77° 08' 688", elevation: 1210 m.) and one population in Dandeli WLS (N 15° 10' 115", E 74° 28' 919", elevation: 612 m.) and the sites offered the potential of comparison between disturbed and undisturbed populations. Research was initiated during the flowering phase in May and June 2005 in both the Protected Areas, and was repeated in the following annual flowering season in B.R. Hills alone. Fruiting data were collected from end July to early August.

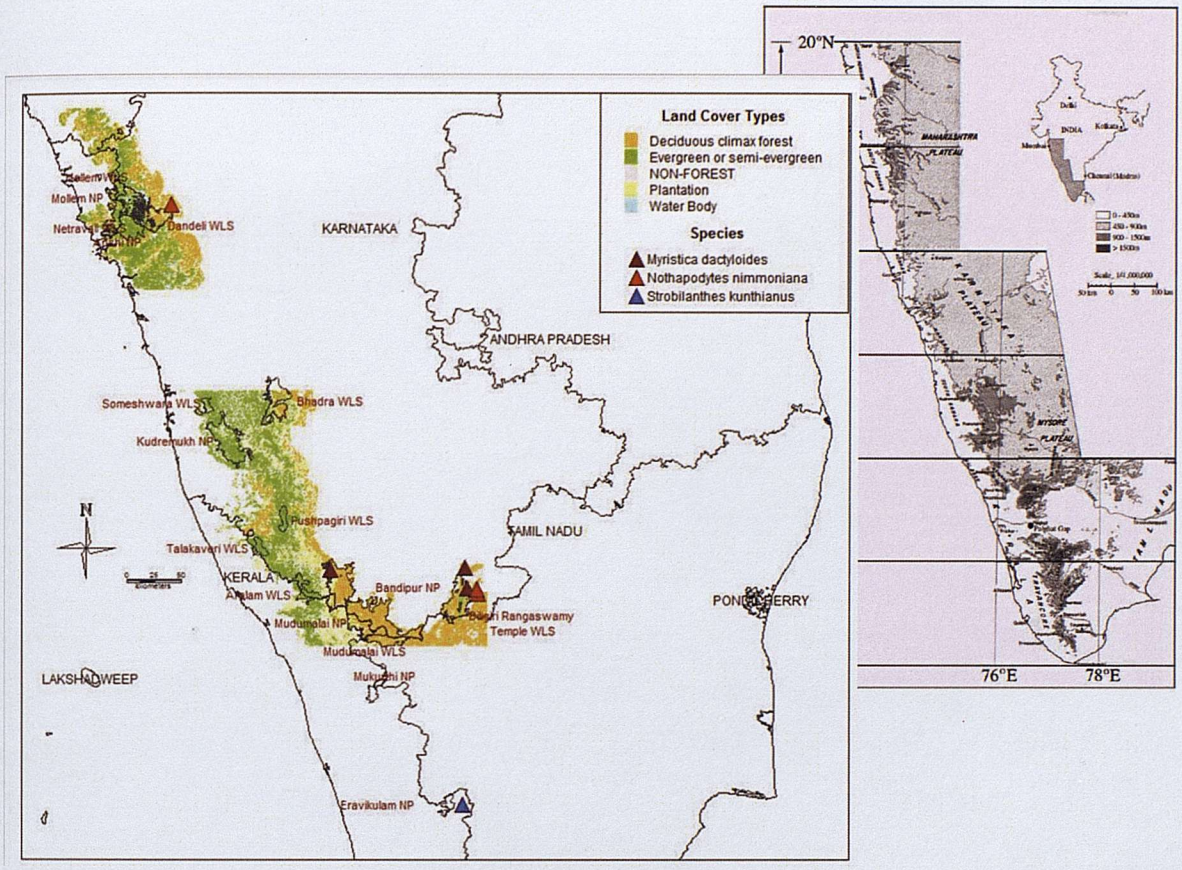


Figure 1.1 Landcover map of some parts of the Western Ghats of India highlighting the study sites of the three study species – *Myristica dactyloides, Nothapodytes nimmoniana* and *Strobilanthes kuntianus*.

Myristica sp.

I selected one site in the evergreen forest range of B.R. Hills WLS (N 11° 54' 442", E 77° 11' 189", elevation: 1366 m.) for detailed studies on the flowering population and insect assemblage in December 2006 and 2007, and collected limited data from Brahmagiri WLS in Coorg District. In addition, I surveyed seven Myristica populations across Karnataka State collecting data on a set of parameters defined to measure the effect of disturbance on the size of fruit crop. These surveys were conducted during the fruiting period in March – April of 2008.

***Strobilanthes* sp.**

A single population was chosen in the grassland ecosystem in the village of Kanthallur (N 10° 13' 12.6", E 77°12'14.9", altitude 1581 m.), delimited by Eucalyptus plantations, and adjoining the Shola forests of the Mannavan Shola National Park. The site was not far from Eravikulam National Park which is well known for its massive *Strobilanthes* bloom every twelve years. This site would have been ideal to study the system in an undisturbed habitat, but for the limitation of not having sufficient time to apply for a Forest Department permit. Research was conducted in the flowering season of September 2006 in the Kanthallur population that covered an area of about 70 acres.

Plant population surveys

Floras of the region (Gamble, 1935; Sharma et al., 1984; Keshavamurthy & Yoganarasimhan, 1990) were used as reference before setting out to locate populations of the two tree species. In the summer of 2005 (March - May), preliminary surveying of the two tree species was conducted with local help. Tribal inhabitants in the area are usually familiar with plant population locations, once given the plant vernacular name and description. The following surveys were made during the flowering period of the target species, this time based on guidance from local informants and collaborative research groups of ATREE (Bangalore, India) at the Colleges of Forestry in Sirsi and Coorg Districts of Karnataka. The population of *Strobilanthes* sp. in Kanthallur village was not pre-surveyed; it was visited when the mass flowering was reported from local newspapers and research colleagues.

Floral display

I marked mature, ready-to-open buds (n=30) on different individuals using numbered plastic tags, to observe the time of anthesis the following morning. Anthesis is defined as beginning when anthers and stigmas are exposed to the pollinating agent(s), either because the flower opens or because the organs protrude from a closed flower in such a manner as to expose themselves to the same agents (Faegri & van der Pijl, 1979). I also observed in the marked flowers, anther dehiscence and other floral changes until they senesced.

Flower colour, shape and odour were recorded and used for 'blossom type' categorisation (Faegri & van der Pijl, 1979). Flowers of the different sexes were scored for morphological measurement of floral parts (*n* varied across the three studies); the average length of stamen and pistil, and the length of the ovary and anther were measured. To record the phenology of the species, I tagged flowering inflorescences marked with indelible ink and monitored them at weekly intervals over the entire flowering period.

I estimated inflorescence sizes on the different sexes from counting individual flowers and buds in a given inflorescence (n=20). In the polygamous individuals of *Nothapodytes* sp. and the dioecious *Myristica* sp., I used a two sample t-test to test the difference between the number of flowers in male and female inflorescences to be correlated to visitation frequency of pollinators to male and female floral resources. Flower production differences suggest that males in dioecious species, and hermaphrodites that function as males in gynodioecious species are more attractive to pollinators than females (see Wilson, 1979). *Strobilanthes* sp. is bisexual with male and female reproductive organs on one flower.

Flower function and reward for pollinators

Pollen production and pollen viability are measures of male function in angiosperms, and ovule number and stigma receptivity, of female function. To estimate pollen production, a mature but undehisced anther from a male (or bisexual) flower (*n* varied across the three studies) was placed in a drop of safranin or auromine O and teased with needles to eject all the pollen grains. After removing the debris, the number of pollen grains was carefully counted under the compound microscope. Pollen viability was assessed on alternate days beginning from the day of anthesis, using the fluorescine diacetate test (Heslop-Harrison & Heslop-Harrison, 1970).

Transverse sections of ovaries were taken under the stereo microscope in order to determine the number of ovules in an ovary. To determine the period of stigma receptivity, I implanted pistils on agar medium and performed hand pollinations on the stigmas. Pollen is brought on a needle tip from male or bisexual flowers and carefully placed on the stigma. These pollinations were carried out at 6-hour intervals

(n=10 at each interval) starting with anthesis. The stage that permits maximum pollen germination and pollen tube growth would be the receptive stage. The length of stigma receptivity usually coincides with the period until which effective pollination can be achieved in a flower. The pistils were fixed 24 hours after hand pollination in FAA (formalin-acetaldehyde-acetic acid) for later observations on pollen germination and pollen tube growth using aniline blue fluorescence (Linskens & Esser, 1957; Shivanna & Rangaswamy, 1992). This technique allowed precise assessment of stigma receptivity and pollen germination results of hand pollinations.

I also used DAB (Sigma *Fast*™ 3,3'-diaminobenzidine; Sigma D-4) tablets - a reagent that indicates at the same preparation, the receptivity of the stigma as well as the viability of the pollen grains that had reached it (Dafni, 2007). The stigmas for this test were scanned under a hand microscope (Magiscope, USA) designed for field use.

I made nectar measurements from fresh flowers using 1µl or 10µl microcapillaries (Microcaps, Drummonds). In *Strobilanthes* sp., flower buds were bagged before they opened to prevent insect visitation. Bagged flowers were excised at 4 hour intervals (n= 10 at each interval), starting from flower opening until 1800h on the first day, and again from 0600h-1800h on the second day, and the quantity of nectar was measured. The differences in nectar quantity at different time intervals were tested using a generalised linear model with a quadratic term fitted for time to describe the non-linear distribution.

What pollinates?

When flowers of one plant are visited by more than one insect species, the different sorts of insects may have contrasting effects on pollen movements, in part because of differing pollen load capacity but also because of different flight and foraging behaviours (Handel, 1983). Not all pollinators are equal, as species vary in their pollination efficiency, and not all flower visitors are pollinators (Tepedino et al., 2006), and even a minimum experience on field observing insects visiting flowers is sufficient to realise this. Pollen vectors have different efficiencies in transporting pollen (Faegri & van der Pijl, 1979). Some visitors are pollen and nectar robbers that do not contribute towards pollination of the plant, a phenomenon known as 'floral larceny' which is a widespread phenomenon in interactions (Irwin et al., 2001).

I use pollination syndromes (Baker & Hurd, 1968; Faegri & van der Pijl, 1979) to predict the pollinator type, but merely as a pointer to what might be the potential pollinator; the actual pollinator might not be as expected. Time of anthesis is also a useful cue in predicting pollinator type (Bawa et al., 1985a). Ultimately it is long hours of direct observations on floral visitation and foraging behaviour that confirm the pollinators. Additionally stigmas from flowers are scanned for pollen deposition data. Insects seen foraging were collected using a sweep net or a bottle and immobilised with ethyl acetate vapours. They were identified by Dr Sinu, sometimes with reference collections at the Entomology Laboratory at ATREE, to the lowest taxonomic unit possible, usually the level of Family, and where possible Genus. To narrow down a list of potential pollinators from the array of visitors, I checked for pollen load on the insect body under the stereomicroscope back in the laboratory.

I used Yellow Sticky traps (5 X 10 cm., Growing Success Ltd., Wiltshire, UK) for sampling canopy insects (Young, 2005) on the assumption that the chance of capture is directly related to the frequency and foraging activity of the species (Armstrong, 1997). I hung them near flowering twigs from dusk to dawn to look for nocturnal insect visitors in particular. To check for the possibility of wind pollination, I hung vaseline - coated standard microscope slides on the branches of the plant for 24 hours and scanned them under the Stereo Microscope for observing pollen deposition.

Patterns of insect visitation

***Nothapodytes* sp.**

Observations were made in May – June 2005. To calculate frequency of floral visits by insects, observations were made in 15 minute pulses from 0600h to 1800h on marked male, female and polygamous trees. In each observation unit, 4-6 inflorescences were observed simultaneously. I pooled this into three time periods: morning (0600h-1000h), midday (1000h-1400h), and afternoon (1400h-1800h). Since data comprised of over-dispersed count data of insect visits, a generalised linear model with a quasipoisson distribution of the error term was fitted in R 2.7 (Ihaka & Gentleman, 1996; R version 2.7.1 (C), 2008). The same model was used to identify the main factors affecting foraging pattern – time of the day preferred by the pollinators and the most frequently visited inflorescence type.

***Myristica* sp.**

In the flowering season of December 2006, I surveyed floral visitors. Insects coming in contact with the flower anthers and stigma were classified as potential pollinators; close observations were possible using a pair of Pentax 6 X 2.1 binoculars. I performed censuses in 30 minute observation periods starting from 0600h to 1800h. In each observation unit, 4-6 inflorescences in their flowering peak were observed simultaneously. The number of visits recorded per census was drastically low making it difficult to sort data at the single flower level. Visitation rates were recorded at the individual tree level with data pooled from direct observations and sticky trap captures. As in the *N. nimmoniana* study, a generalised linear model was fitted in R 2.7 test the difference of visitation of the pollinators alone on female and male inflorescences, but with a poisson distribution of the error term as the data was not over-dispersed. In November 2006, I collected limited data on insect foragers from a population in the Brahmagiri Wildlife Sanctuary (N 11° 57' 790", E 75° 58' 380", elevation: 1169 m.).

***Strobilanthes* sp.**

During the mass bloom in September 2006, I monitored flower inflorescences for insect visitors from 0600h to 1800h (15 minutes in each hour) for three days, covering a randomly selected site on each day. For analyses, the data from the three days were pooled and the observation period was divided into six 2-hour intervals. In another experiment, insect visits to the same set of flowers on the first and second day were monitored. For this, the flowers (n=20) were tagged in the morning of the day of opening and kept under continuous observation (30 minute pulse at each hour) for visits from 0600h to 1800h on the first and second day of opening. In both experiments, I fit a generalised linear model with a poisson or quasipoisson distribution of the error term (depending on whether or not there was over-dispersion) in R 2.7 to analyse the difference in visitation rates across time slots during the lifetime of the flower.

Flower success

Quantifying pollinator frequency and **pollination efficiency** is a measure of pollen or pollinator limitation in the population. To look for any constraints in the plant's reproduction, I compare the **fruit set** data from hand pollinated and open pollinated

flowers. If hand pollinated plants produce more seeds than naturally pollinated controls, it is an indication that reproduction is being limited by pollination, although care must be taken in their design and interpretation (Thomson, 2001; Wilcock & Neiland, 2002).

Pollination efficiency

It was possible in *Strobilanthes* sp. to expose tagged flowers to a single insect visit since the flowers were not too small and allowed individual bagging. I collected stigmas soon after insect visit for censusing pollen grains. In the other two species, stigmas from two or three - day old flowers were scanned under the stereo-microscope to observe pollen deposit. The pollen grains were then counted to calculate pollination efficiency. It is an estimate in percentage derived from the number of stigmas with pollen load as against the number of stigmas that carry no pollen load. The use of stigmas rather than artificial samplers when censusing pollen grains, eliminates the possible sources of error that mechanical devices such as artificial samplers of pollen create (Raynor, 1979; Handel, 1983).

I also captured insects seen foraging for pollen or nectar on the target flowers and scanned their body for presence of pollen. This is used as evidence for 'pollinator efficiency'. One needs to carefully examine if the pollen are from the contiguous species or a confluent population (Handel, 1983). This I assured by studying the pollen structure of target species and having it ready for comparison when stigmas and insect bodies were scanned.

Fruit / Seed set

I calculated natural or open fruit set, a direct measure of reproductive success, by tagging a known number of flowers with indelible ink until fruit set. I conducted hand pollination experiments on emasculated female and bisexual flowers and left them bagged to exclude insect visitors. During the unripe fruiting stage, which was chosen to avoid data distortion as an effect of fruit consumption by frugivores, I counted fruits from open pollinated and hand pollinated inflorescences. All percent fruit set were square root transformed before further analysis.

***Nothapodytes* sp.**

In May – June 2005, I counted the total number of flowers on 24 inflorescences on four

female trees and 24 mixed inflorescences on three polygamous trees and left them tagged with indelible ink until fruit set. To estimate percent fruit set, I monitored these tagged inflorescences and counted raw, immature fruits after 2-3 weeks, and compared with results from hand pollinated flowers (also see next section on 'breeding habit'). This was repeated in the flowering season of 2006. A two sample t-test was employed to test the difference between fruit set from hand pollinated and open pollinated flowers.

***Myristica* sp.**

In December 2006, 35 inflorescences on two female trees were tagged for recording natural/ open fruit set in February 2007. This was repeated in December 2007 on 49 inflorescences on the same two female trees were tagged for recording natural/ open fruit set in February 2008. Hand pollinations were carried out in 2006 on 24 inflorescences of the female trees using pollen from two different male trees in the vicinity. Here too, I used a two sample t-test to compare the fruit set from the two treatments.

***Strobilanthes* sp.**

In September 2006, I tagged 30 inflorescences with a record of the number of flowers on each. Hand pollinations (with pollen from same plant as well as from different plant) were carried out on select flowers on 30 inflorescences. Mature fruits were harvested before dehiscence and the number of seeds was counted. Unlike in the two tree species with smaller flowers, it was possible in *Strobilanthes* sp. to monitor fruit set in individual flowers and therefore I used a generalised linear model with a binomial distribution of the error term to test for difference in fruiting success between open and hand pollinated flowers.

The breeding habit

A plant produces thousands of pollen grains and ovules in a lifetime and one of the cardinal lessons in plant ecology is that reproductive efforts fail more often than succeed (Howe & Westley, 1997). Plant breeding systems circumvent the possibility of reproductive failure through evolutionary strategies that promote male fitness through both pollen (male function) and ovules (female function). Outcrossing species and their

populations are more dependent on pollinators than those that are self – compatible (Bawa & Hadley, 1990). I perform controlled pollinations in order to determine the breeding system of the three species.

Controlled pollinations involved emasculating mature buds (if the flower has male reproductive parts), tagging them and leaving them bagged with butter paper bags (8 x 12 cm) overnight to prevent insect visitation. The next morning I unbagged them and hand pollinated the stigmas. Pollen or entire anthers/ androecium were brought from a male (or a bisexual flower) on a needle tip and carefully daubed on the receptive stigma and the flower(s) re-bagged. Bags were removed a week after hand pollination. In *Nothapodytes nimmoniana* and *Myristica dactyloides,* flowers were too small to be marked or tagged individually, so I covered the whole inflorescence after snipping away younger buds.

In all the three species, the control is natural or open pollination on flowers left unbagged. Fruit set data were collected after three to four weeks. Some stigmas are also removed after 24 hours of experimental pollination to be fixed in FAA, followed by alcohol, and then processed in NaOH to scan under the fluorescence microscope for presence of germinating pollen.

***Nothapodytes* sp.**

The preliminary survey revealed male, female and polygamous trees with flowers that looked like pin and thrum morphs but were either functionally female or functionally male respectively. To understand sexual variation, I scored six male, six female and six polygamous trees for recording sex of flowers on tagged inflorescences. During peak flowering phase in June 2005, I designed the experimental pollination with the following treatments (n=12 flowers under each).

(i) Geitonogamous selfing: pollinate female flowers with pollen from male flowers of same plant,

(ii) Pollinate intermediate flowers with male pollen from male flowers of the same plant,

(iii) Crossing: Pollinate female flowers with male pollen from a different plant,

(iv) Pollinate intermediate flowers with male pollen from a different plant,

(v) Pollinate thrum flowers with pollen from thrum flowers on the same plant,

(vi) Pollinate thrum flowers with pollen from thrum flowers on a different plant.

(vii) Spontaneous selfing: thrum, pin and intermediate flowers were bagged before anthesis without further manipulation.

In this species alone, I examined the breeding preference and its evolutionary relationship with pollinators.

***Myristica* sp.**

Being dioecious with separate male and female trees, the experiments were restricted to the female trees. During peak flowering phase in December 2006, I applied two treatments (n=15 each): (i) with xenogamous pollen (pollen from the male flowers on male trees), and (ii) autogamy (bagged flowers and opened after 4-5 days – just after the period of stigma receptivity).

***Strobilanthes* sp.**

Flowers of the species are bisexual (hermaphrodite). In September 2006, I applied three pollination treatments (n=50 each) on randomly selected plants: (i) geitonogamous pollen (from a different flower on the same plant), (ii) from xenogamous pollen (from a bisexual flower on a different plant), and (iii) autogamy or spontaneous selfing (bagged flowers and opened after 4-5 days, just after the period of stigma receptivity). To test the prevalence of apomixis, emasculated flowers (n=50) were bagged and scored for fruit set.

Disturbance in the plant habitat

To understand how habitat disturbance and resource exploitation plays into the dynamics of pollination and reproduction, two different approaches were used for the two annual flowering tree species, while the question of disturbance was not addressed in the same manner in the *Strobilanthes* study, given a single study site.

In *N. nimmoniana,* I used two sites of visibly varying exploitation levels to compare two measures – pollinator assemblage and reproductive success. The sites are referred to in the study as Site UE or unexploited and Site E or exploited. Using genuine counts of individuals (insect abundance), I calculated Inverse Simpson diversity indices for the two sites; I chose this index against the Shannon index because it emphasises the more

common species (Hill, 1973), and due to its ability to avoid bias in small samples (Lande, 2000) and consistently rank assemblages when sample size varies (Magurran, 2004). I compared the Inverse Simpson diversity indices (vegan package in R 2.7) for insect morpho-species encountered in the unexploited site with that of the exploited site using a two sample t-test. I also tested fruit set between female trees in exploited and unexploited sites with a two sample t-test.

In *M. dactyloides,* I sampled fruiting trees from a total of nine populations across Karnataka State (on a disturbance gradient). Two of these were in B.R. Hills WLS, two in Brahmagiri WLS, two in Talakaveri WLS, two in Kudremukh National Park, and one in Kemmangudi. I used 'absolute fruit set' or 'size of fruit crop' (response variable) as a function of disturbance, independent of flowering and pollination success. Disturbance was measured as five potentially influencing factors (explanatory variables) – (i) distance from road, (ii) method of harvest, (iii) habitat matrix – forest or plantation, (iv) Protected Area or not, (v) seedling regeneration. To estimate the influence of the measured explanatory variables on the size of fruit crop, a linear model was fitted to the data.

CHAPTER III

In the sub-canopy of a moist deciduous forest - *Nothapodytes nimmoniana*

The floral biology and pollination ecology of a commercially exploited tree species is detailed in this chapter. *Nothapodytes nimmoniana* is a sub-canopy tree inhabiting the moist deciduous forests of India. I compare the pollinator assemblage and reproductive success in two sites, one unexploited, and the other, facing severe anthropogenic pressure and a resulting decrease in the population's mating structure and floral resource. A discussion on generalist pollination systems thriving in exploited habitats is presented as a pointer for conservation of endangered species and habitats.

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SUMMARY

The pollination ecology of a commercially exploited tree species from the Western Ghats of India - *Nothapodytes nimmoniana* was studied during two flowering seasons. Wood chips from the tree are a source of camptothecin, a globally sought after alkaloid with cancer treating properties, and are harvested unsustainably in some forests. I asked two questions - 1. In an unexploited population, what is the floral resource availability and are there any constraints in pollination and fruit set? 2. Does over-harvesting of wood chips in *N. nimmoniana* reduce the pollinator activity and percent fruit set in the exploited population as against unexploited sites? Flowers belong to the fly pollination syndrome and are visited by Calliphorid flies and *Apis dorsata* and *Trigona iridipennis*. Fruit set did not vary between exploited and unexploited sites, but there was a significant difference in pollinator assemblage. In a lean male flowering year, female fruit set was low, suggesting pollen limitation in the population. There was no fruiting in the second year at the site where trees were felled. When floral density is altered in exploited populations, pollinators of generalist nature may show a shift in foraging pattern. I argue that generalist plants are as susceptible to pollination loss as are specialist plants. The need for outcrossing would probably exacerbate this vulnerability for the plant.

Keywords: Calliphorid flies, camptothecin, *Apis dorsata, Trigona iridipennis*, floral resource, fruit set, generalist, pollen limitation, unsustainable harvest

Introduction

Studies on the nature of the interactions between plants and their pollinators are important to understand the structure and dynamics of tropical forest ecosystems, and have helped clarify issues of regeneration and conservation of tropical forest resources (Bawa & Hadley, 1990). In the past couple of decades, much research has been carried out on the changing pollination dynamics of wild plant populations in the context of habitat change and fragmentation (Bond, 1994; Ghazoul & McLeish, 2001; Murren, 2002; Lennartsson, 2002). Yet, apart from a handful of studies (Rathcke & Jules, 1993; Somanathan & Borges, 2000; Pandit & Choudhury, 2001), there is lack of literature

from the Indian tropics on work examining pollinators affected by fragmentation and more specifically, the effect of a specific exploitation threat on the pollination ecology of a single tree species. I address this question with regard to a tree species from the Western Ghats of India that has come under a recent and sudden threat of declining population due to commercial exploitation.

Nothapodytes nimmoniana Graham. (Family Icacinaceae), formerly *Mappia foetida* (threat status: endangered/ vulnerable (IUCN, 2003) is a small spreading sub - canopy tree distributed in the dry and moist deciduous, and evergreen forests of the Western Ghats of India, and in the eastern states of Assam and West Bengal. Natural populations face indiscriminate harvest for trade of their wood chips, from which an anti - cancer drug component, camptothecin (CPT), is derived (Govindachari & Viswanathan, 1972; Vasudeva et al., 2004; Suhas et al., 2007). CPT is the third most important alkaloid sought after by the pharmaceutical companies around the world (Hombegowda et al., 2002). Over 20% of the wild populations of *Nothapodytes* in India have declined in the last decade or two now (Ravikumar & Ved, 2000) and the method of harvest is often unsustainable - lopping branches or often, the main trunk (Vasudeva et al., 2004). Apart from a report on sexuality (Hombegowda et al., 2002), there is no information on its reproductive ecology. I speculate that floral resource depletion and increased isolation of individuals from such exploitation may have a negative influence on pollinator abundance and movement (Ghazoul & Uma Shaanker, 2004) and consequently reproductive success in the study species. Pollination studies in fragmented and anthropogenically exploited plant populations elsewhere have suggested that decreased fruit set (Jennersten, 1988; Saunders et al., 1991; Kearns & Inouye, 1997; Steffan-Dewenter & Tscharntke, 1999; Cunningham, 2000; Ghazoul & McLeish, 2001; Murren, 2002) and a decline in population viability (Ghazoul, 2005) are likely outcomes of depressed pollination in such cases.

I consider the importance of pollination mechanisms in constraining fruit set in *N. nimmoniana*. I examine the flowering phenology, breeding system and the mode of pollination and quantified reproductive success in two populations in Biligiri Rangaswamy Temple (B.R. Hills) Wildlife Sanctuary, an unexploited habitat with no history of extraction from the species (either in the Forest Department records or local

NTFP harvest record) and one population in the north west of Karnataka State, Dandeli Wildlife Sanctuary that faces indiscriminate harvest of wood chips. The sites thus offered the potential for comparison between exploited and unexploited populations. Pollinator assemblage and reproductive success (measured as percent fruit set) were compared between the exploited and unexploited sites, the hypothesis being that there would be a decline in pollinator activity and in percent fruit set in the exploited population. The study was initiated in May 2005 and was repeated in the following annual flowering season.

Study species and Study sites

Nothapodytes nimmoniana is dioecous, but some individuals are polygamous - with male, female and bisexual flowers (Vasudeva et al., 2004). Trees range from 12-20 m. in height. Flowering in this deciduous tree initiates in end May or early June soon after leaf bud initiation (Figure 3.1A); it coincides with the south west monsoon showers and lasts for over six weeks. Fruits mature in mid - July into red-purple single seeded berries (Figure 3.1B) and are bird dispersed (pers. obs.).

The study was conducted in the moist deciduous forests of B.R. Hills Wildlife Sanctuary (Site UE or unexploited) and Dandeli Wildlife Sanctuary (Site E or exploited) during the flowering period of *N. nimmoniana* from end May to late July. Observations and field experiments were concentrated in two populations in Site UE (Doddasampige N 11° 57' 079", E 77° 09' 632" and Malkibetta N 11° 57' 221", E 77° 08' 688", elevation: 1210m.) separated from each other by approximately 4 km, and one population in Site E (N 15° 10' 115", E 74° 28' 919", elevation: 612m.). The natural history and reproductive ecology in the species were studied in the unexploited populations of B.R. Hills Wildlife Sanctuary and the population in Dandeli was used only for comparing data on pollinator assemblage and fruit set.

Methods

A locally made bamboo ladder was used to climb the trees (Figure 3.2) and once in the canopy, a pair of Pentax 6X2.1 binoculars was used for observing insect foraging from

a close range. Most observations and hand pollination experiments were also conducted in the canopy during the peak flowering period, towards the second week from flowering initiation.

Floral display

I tagged 10 ready- to-open buds on two trees, to observe anthesis the following day. The size of inflorescences was estimated from counting individual flowers and buds in



Figure 3.1 **A.** Young flower buds of *Nothapodytes nimmoniana* appear on terminal nodes soon after leaf bud initiation. **B.** Ripening fruits in the canopy, July 2005. B.R.Hills Wildlife Sanctuary, Western Ghats, India.



Figure 3.2 Accessing the canopy of *Nothapodytes nimmoniana* to bag flowers for hand pollinations in the B.R.Hills population, Western Ghats, India.

a given inflorescence (n=25 of three female, three male and three polygamous trees each). A two sample t-test was used to test the difference between the number of flowers in male and female inflorescences to be correlated to visitation frequency of pollinators to male and female flowers.

Floral reward

Pollen was counted under a compound microscope stained in safranin or auromine O (n=20 anthers each 3 male and 3 polygamous trees). Pollen viability was assessed from anthesis with the fluorescine diacetate (FDA) test (Heslop-Harrison & Heslop-Harrison, 1970). Nectar measurements were made directly from open flowers on the branches using 1µl microcapillaries (Microcaps, Drummonds).

Floral visitors

To calculate frequency of floral visits by insects, observations were made in half - hour blocks from 0600h to 1800h on eight trees of which three were male, two female and three polygamous. In each observation unit, 4-6 inflorescences were observed simultaneously. I pooled this into three time periods: morning (0600h – 1000h), midday (1000h – 1400h), and afternoon (1400h – 1800h). Since the data comprised of count of insect visits, a generalised linear model with a quasipoisson distribution of the error term was fitted in R 2.7 (Ihaka & Gentleman, 1996) to identify time period of the day most preferred by pollinators and the most frequently visited inflorescence type, the hypothesis being that males attract more visitors. Insects seen foraging were collected using a sweep net and immobilised with ethyl acetate vapours. They were identified at the Entomology Laboratory at the Ashoka Trust for Research in Ecology and the Environment (ATREE, India) to the level of Order or Genus. To detect potential pollinators, insect bodies were scanned under the stereomicroscope for pollen load.

5 X 10 cm Yellow Sticky traps (Growing Success Ltd., Wiltshire, UK) were hung near flowering twigs from dusk to dawn to look for nocturnal insect visitors (Armstrong, 1997; Sakai, 2001). To check for the possibility of wind pollination, I hung vaseline-coated standard microscopic slides in the canopy of the tree for 24 hours and scanned them under the stereo microscope for presence of pollen.

I compared the Inverse Simpson diversity index (Vegan package in R) calculated for insect morpho-species encountered in the unexploited site with that of the exploited site and then used a two sample t-test to compare the mean diversity indices in the two sites.

Reproductive success

To determine the period of stigma receptivity (which coincides with the period until which effective pollination can be achieved in a flower), I conducted hand pollinations on female and bisexual flowers at 6-hour intervals starting with anthesis. The pistils were fixed 24 hours after pollination in FAA for later observing pollen germination and pollen tube growth using aniline blue fluorescence (Shivanna & Rangaswamy, 1992).

Natural fruit set (in %) was estimated by counting the total number of flowers on 24 inflorescences on 4 female trees and 24 mixed inflorescences on 3 polygamous trees, they were left tagged until I counted fruit set 2-3 weeks later. Hand pollination experiments were carried out on (bagged) female and (emasculated and bagged) bisexual flowers using pollen from male flowers of the same tree or a different tree. In polygamous trees, I also pollinated female flowers with pollen from bisexual flowers to test for self-compatibility. As flowers were too small to be marked or tagged individually, I covered the whole inflorescence with butter paper bags (8 x 12 cm) after snipping away younger buds.

I transformed percent fruit set data using square root-arcsin transformation before analysis. A two - sample test was used to test the difference between fruit set in hand pollinated and open pollinated flowers. To compare reproductive success in exploited and unexploited populations, I tested fruit set between female trees in site E and site UE, again with a two sample t-test.

Results

Floral display

Flowers of *N. nimmoniana* are creamish, bowl shaped and foetid smelling. The ovary is monocarpellary with 2 ovules. All the three sex types have a disc shaped nectary

between the petals and the ovary. Stamens of varied in length making flowers appear pin, thrum and intermediate types. Flowers open during the day – ranging from 0600h up to 1400h. The small flowers are arranged in large terminal corymbose panicles of 20 - 400 flowers, intensifying advertisement. The difference in the mean number of flowers per inflorescence in male and female inflorescences was statistically significant (Two Sample t-test, $t = -3.64$, d.f. = 33, $p < 0.001$); male inflorescences had a larger display (mean no. of flowers = 182.8 ± 51.3 SE) than the females (mean no. of flowers = 68.9 ± 17.7 SE). In polygamous trees, female and bisexual flowers were negligibly few in number and they made their appearance in select inflorescences towards the end of the flowering season (see Chapter IV for details on phenology).

Floral reward

A single male flower remained open for one day, while a female flower for 1-4 days depending on pollination success: if pollinated on the first or second day of opening, the flower begins to senesce and if pollen has not landed on the stigma, the flower remained open longer. Nectar secretion varied from 0.88 ± 0.02 µl. (n=8) at 0700h, to 0.25 ± 0.06 µl. (n=6) at 1600h. Male flowers also offer more reward than females in terms of pollen in addition to nectar. Mean pollen production in male anthers was 1009.6 ± 61.91 (n=15) and in bisexual anthers 448.4 ± 185.9 (n=12); females had sterile anthers. Pollen viability lasted for more than 10 days from the day of opening.

Visitor assemblage

There were nectar hunters and pollen foragers on the flowers. Seventeen species were encountered across the two sites, with flies being the most frequent visitors (Figures 3.3A – B). I found two species of Calliphoridae, one species of Syrphidae, the short - tongued Halictid bee, the stingless bee *Trigona iridipennis, Apis dorsata* (Figure 3.3C), *Apis florea*, sepsid flies, Lycaenid butterflies (Figure 3.3D) and 5 species of ants. The Empididae, Scathophagidae, Mirid bug, and Pompilid wasp appeared to be predators foraging on flower parts. Of these, only *Trigona iridipennis*, *Apis dorsata*, and two Calliphoridae members showed pollen load on their body (Figure 3.4A & B) and were treated as the four potential pollinators.

Pollen grains on these species were confirmed to be of *N. nimmoniana* by comparing with pollen from squashed anthers under the microscope. Data on visitation were fitted to a generalised linear model with a quasipoisson distribution of the error term and the simplified model revealed a highly significant influence of floral display ($Z = 6.31$, $p<0.001$) – female inflorescences attracted significantly fewer number of pollinators compared to male and mixed inflorescences. Pollinators also showed a weakly significant preference for foraging during the morning ($Z = 2.29$, $p = 0.023$). Male flowers and morning hours showed a highly significant interactive effect ($Z = - 4.2$, $p<0.001$) (Table 3.1, Figures 3.5 & 3.6). Insect abundance and mean Inverse Simpson index were higher in site E (Two sample t-test, $t = -3.66$, d.f. = 48, $p<0.001$) than in site UE (Figure 3.7 & 3.8). Four species encountered in the unexploited site were not encountered in the exploited site – *Trigona iridipennis*, Halictid bee, Syrphid fly and the Pompilid wasp, one of them a potential pollinator (*Trigona* sp.). Twenty - two % of the vaseline – coated slides hung in the canopy of polygamous trees showed pollen on them, suggesting some role of wind in pollination within such individuals. No insects were captured on the sticky traps laid on tree branches from dusk to dawn, thus ruling out nocturnal pollinators.

Reproductive success

There was considerable variation in the extent of fruit set between years in site UE. In 2005, mean natural fruit set under open pollination in mixed inflorescences (on polygamous trees) was 40.94 % and that in female inflorescences (on female trees) was 46.87%, with no significant difference (Student's t-test, $t = 1.35$, d.f. = 46, $p = 0.18$). The mean fruit set in hand pollinated female flowers in UE site was 49.16 %, with no significant difference with that in open pollination (46.87%) (Two - sample t-test, $t = 0.51$, d.f. = 46, $p = 0.61$).

During the second year (2006) however, fruit set in female inflorescences was as low as 6.91% in comparison with 20.02 % in mixed inflorescences with a significant difference in the means (Student t-test, $t = -3.32$, d.f. = 48, $p = 0.002$). Five of the seven target female trees in 2006 did not have flowering male trees in the radius of about 50 metres, thus causing a decline in the male floral resource in the population. Eight of the

30 buds (26.67%) on polygamous trees with mixed inflorescences netted to keep out visiting insects set fruit, indicating some natural self-pollination (geitonogamy).

In site E, fruit set results in 2005 showed a weakly significant difference between hand pollinated (46.4 %) and open pollinated (40.17%) flowers (Student t-test; t = 2.26, d.f. = 56, p = 0.02). During the second year of study, many of the trees I had marked were harvested soon after the flowering observations and thus no data on reproductive success for these trees were available. Several individuals were chopped back to the trunk leaving no branches to flower in the third year. The test for open fruit set compared between exploited and unexploited populations resulted in a non- significant difference (Two sample t-test; t = - 0.45, d.f. = 80, p = 0.65).

Table 3.1 The minimal model derived for the number of pollinator visits as a response to the variables inflorescence type and time of day.

	Estimate	Std. Error	z value	Pr(>|z|)
Inflorescence Female	0.69315	0.10977	6.314	< 0.001 ***
Inflorescence Male	0.26236	0.13616	1.927	0.0558
Inflorescence Mixed	0.15415	0.11934	1.292	0.1984
Time Evening	0.20972	0.12888	1.627	0.1057
Time Morning	0.29725	0.12989	2.289	0.0234 *
Inflorescence Male: Time Evening	-0.30503	0.17201	-1.773	0.0781 .
Inflorescence Mixed: Time Evening	-0.09724	0.14351	-0.678	0.4990
Inflorescence Male: Time Morning	-0.76029	0.18092	-4.202	< 0.001 ***
Inflorescence Mixed: Time Morning	-0.22139	0.14619	-1.514	0.1320

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for quasipoisson family taken to be 0.151932)

Null deviance: 29.200 on 160 degrees of freedom, Residual deviance: 24.729 on 152 degrees of freedom, AIC: NA;

Number of Fisher Scoring iterations: 4

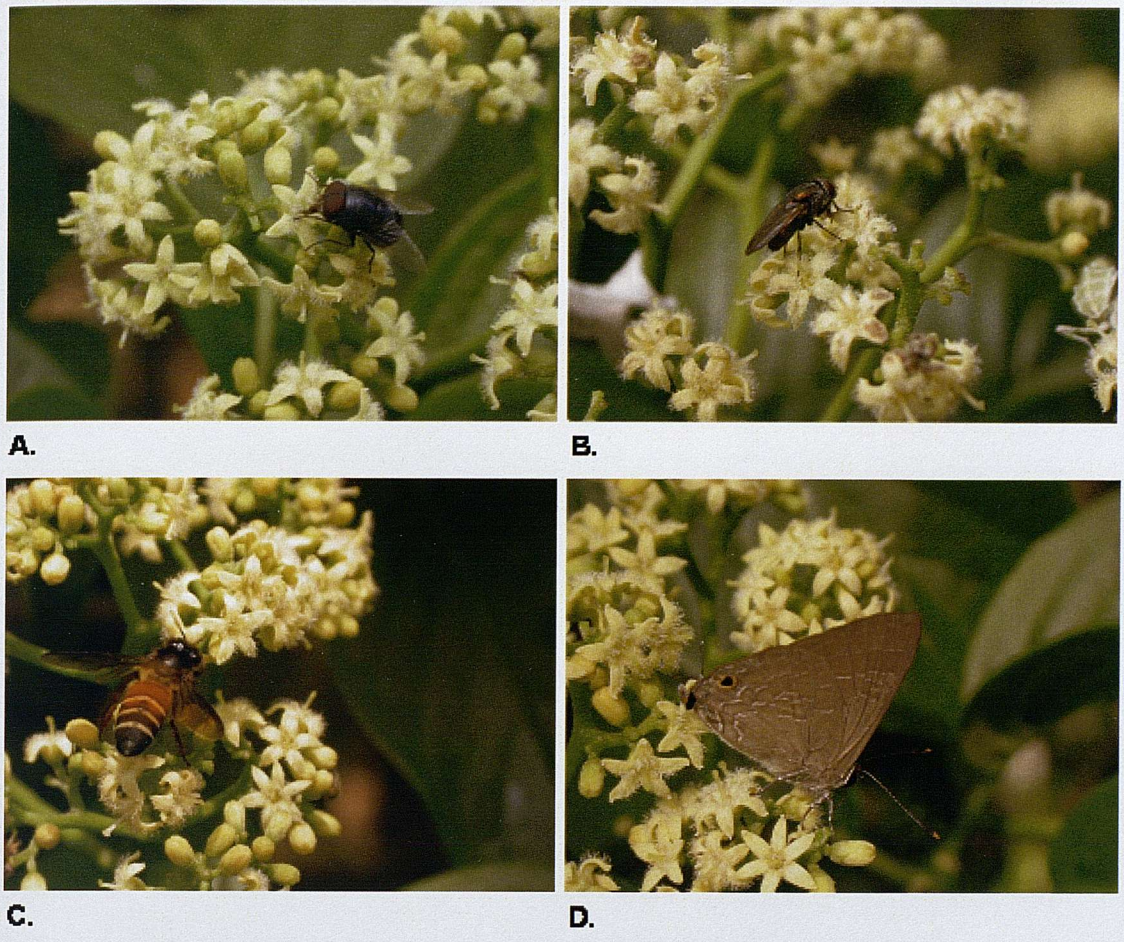


Figure 3.3 Insects foraging on flowers of *N. nimmoniana,* B.R. Hills Wildlife Sanctuary, Western Ghats, India: A & B. Calliphorid flies, C. *Apis dorsata,* D. Lycaenid butterfly

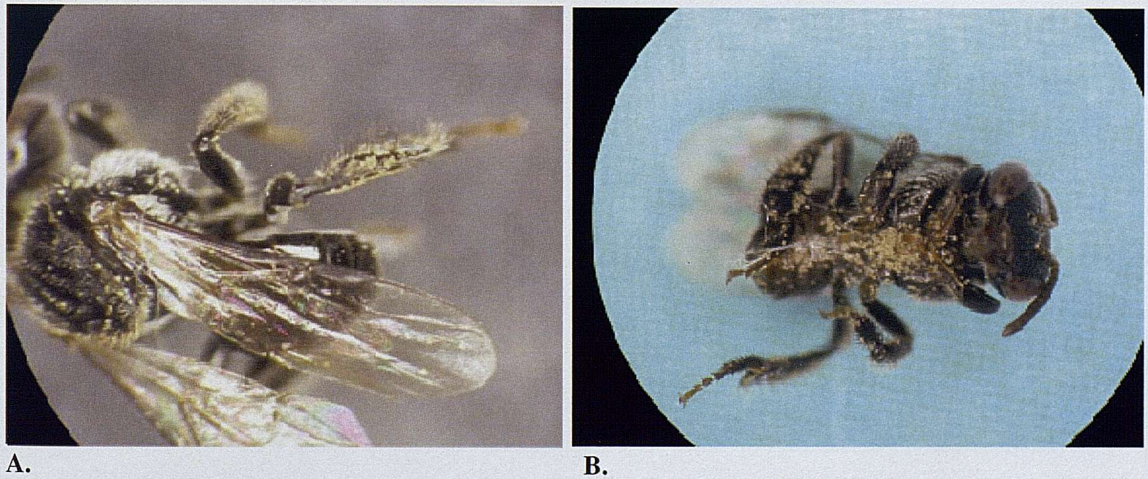


Figure 3.4 Trigona bee captured at a female *Nothapodytes nimmoniana* tree as seen under the stereo microscope; pollen load visible on its legs (A) and ventral side (B).

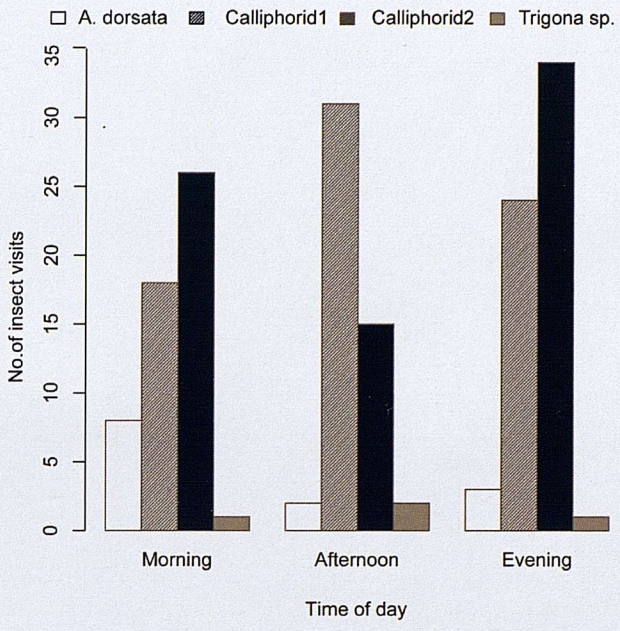


Figure 3.5 Insect foraging preference against time of day in *Nothapodytes nimmoniana* as recorded in B.R. Hills Wildlife Sanctuary (the unexploited population), Western Ghats, India.

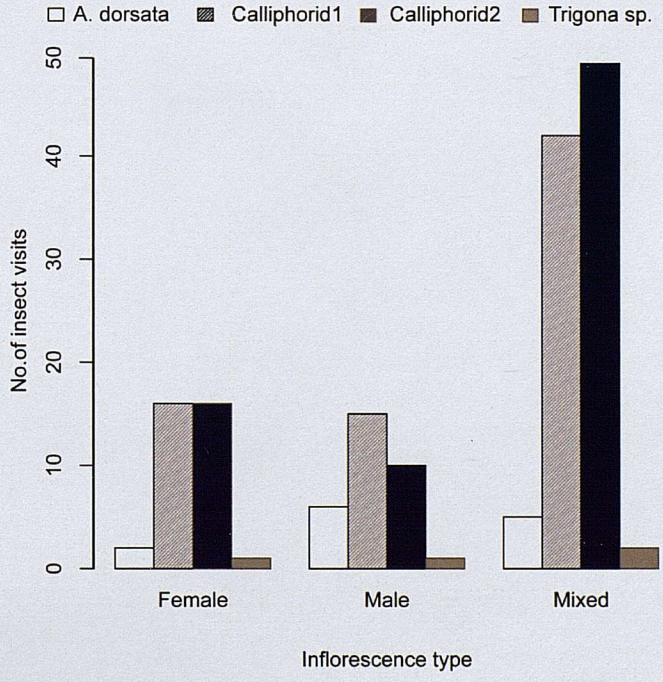


Figure 3.6 Insect foraging preference against inflorescence type in *Nothapodytes nimmoniana* as recorded in B.R. Hills Wildlife Sanctuary (the unexploited population), Western Ghats, India.

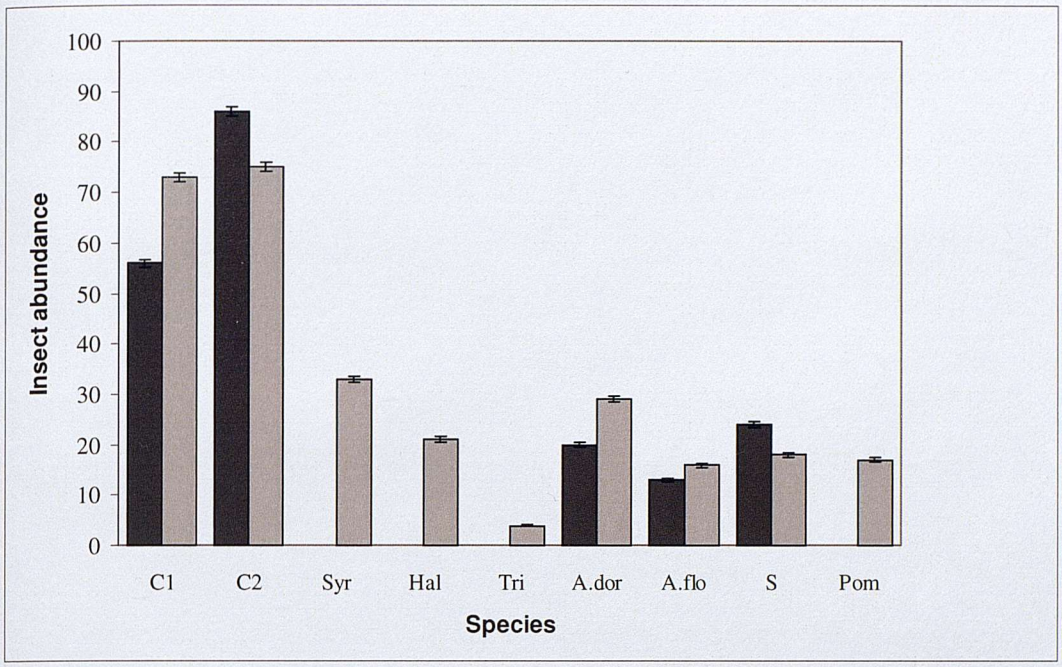


Figure 3.7 Insect abundance in exploited (dark bars) and unexploited (light bars) populations of *Nothapodytes nimmoniana* in the Western Ghats, India. Insect families and species represented as C1 & C2: two Calliphorid species, Syr: Syrphid fly, Hal: Halictid bee, Tri: *Trigona iridipennis,* A.dor: *Apis dorsata,* A.flo: *Apis florae,* S: Sepsid fly, P: Pompilid wasp.

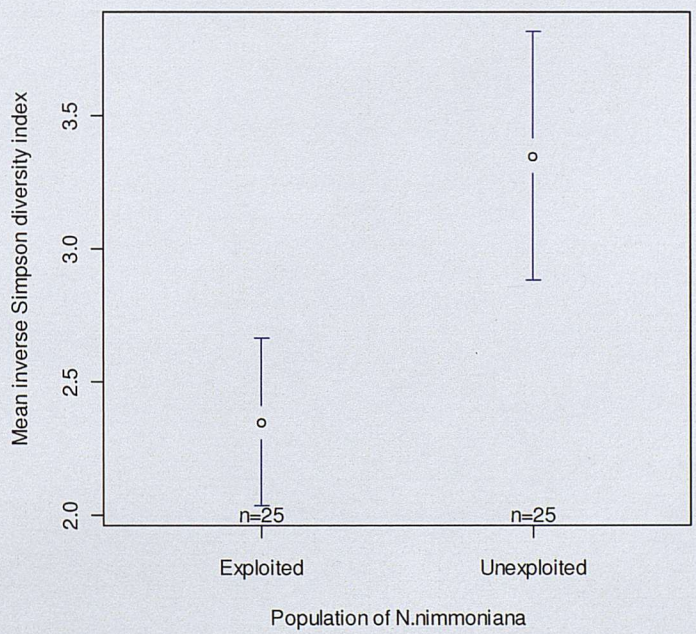


Figure 3.8 Mean Inverse Simpson diversity indices of exploited and unexploited sites. The diversity index at site E (Dandeli Wildlife Sanctuary) was significantly lower (Two sample t-test, t = -3.66, d.f. = 48, $p<0.001$) than site UE (B.R.Hills Wildlife Sanctuary).

Discussion

Natural history

Most of the findings of the study contribute to the natural history and pollination ecology of a threatened species. The hypothesis that visitors may be more attracted to male inflorescences with higher floral reward was borne out. Male inflorescences of *Nothapodytes* were larger than female inflorescences and hence perhaps more attractive to foragers (Eckhart, 1991; Grindeland et al., 2005; Huang et al., 2006). Males also offer more reward (pollen and nectar) while females offer only a minute amount of nectar. This could be considered a strategy to increase visitation and assure pollen transfer (Bertin, 1987; Harder & Barrett, 1996; Ohashi & Yahara, 1998; Hirabayashi et al., 2006). There might be a trade-off between lesser flower number and investment per flower; often, plants save their resource cost in pollinator attraction for investment in ovules or seeds (Schemske & Agren, 1995; Vaughton & Ramsey, 1998; Sato & Yahara, 1999). In female inflorescences, many flowers in close bunches open at one time, while in male inflorescences, flowering is more spread out. This too may be a strategy for higher and longer visual attractivity. The higher floral productivity of males is consistent with the pattern observed for many tropical dioecious species (Bawa, 1980; Armstrong & Irvine, 1989b; Percy & Cronk, 1997; Osunkoya, 1999). Male *Nothapodytes* trees are a huge floral resource for insects and thus when a disappearance of male flowering branches/ whole trees is encountered in the exploited study site, it points to the potential decline in pollinator activity.

Unspecialised visitors and wind are both characteristic of tropical dioecious trees (Charlesworth, 1993). Dipterans and *Apis dorsata* were the main pollinators observed in the current study on *Nothapodytes* along with typical generalists the Halictid bee and *Trigona* sps. The flowers conform to the non-specialised bees and fly pollination syndrome: small bowl/ dish shaped flowers, large inflorescence, dull colour and strong foetid odour (Baker & Hurd, 1968; Faegri & van der Pijl, 1979). Little research has been done to determine effectiveness of flies as pollinators although there is literature that suggests flies as regular visitors or pollinators (Moldenke & Lincoln, 1979; Primack, 1983; Kearns & Inouye, 1994).

The disturbance factor

Nothapodytes nimmoniana is reported to be facing severe decline in reproduction and regeneration in many populations across the country (Ravikumar & Ved, 2000; Vasudeva et al. 2004). The Dandeli population in my study presents a case of unsustainable harvest of wood chips from the species by cutting tree branches from almost the base of their trunk, in effect not very different from timber extraction. During my first year of study, all the trees in the population were lopped leaving no fruits behind, and consequently no seeds on the forest floor to establish a new cohort. This population, in spite of being located in a Protected Area, was close to the highway and two human settlements. The B.R. Hills population with no history of harvest of wood chips provided an ideal sample for studying pollination and reproductive success in the species in an unexploited forest. The Sanctuary is 540 km^2 in area and has large contiguous stretches of undisturbed forests.

While the fact that there was only one population under the exploited category as against two unexploited populations might be seen as a limitation, I resolve replication issues by taking into account the role played by provenance (geologically separated populations). Just as the two populations in site UE fall under a single region of provenance, I do not expect that another population if sampled in site E would show any variation. In other words I assume that a second or third population in site E is 'within the provenance' since they share similar ecological conditions (Kleinschmit et al., 2004). Although not all factors that contribute to variation within and among provenances are accounted for by geological and climatic parameters, in studies concerning pollen dispersal (Bergin et al., 2008), this assumption is justifiable as long as regular environmental conditions exist (Schoppa & Gregorius, 2001).

I make valid comparisons between the samples from exploited and unexploited areas, suggesting that the differences that have emerged in pollinator assemblage may well represent patterns of exploited vs. unexploited. Although it cannot be concluded that any difference is down to disturbance, I suggest that over-exploitation in site E may be part of the explanation of any difference observed.

A generalist system in a degraded forest

It has been raised that habitat conversion can reduce the abundance and diversity of insect pollinators (Rathcke & Jules, 1993) and my results provide some evidence in this direction. Two generalist species Halictid bees and *Trigona iridipennis* were not encountered at target trees in the exploited population. Although the lack of these insects in the exploited site does not seem to have a visible impact on fruit set, it might be a pointer to the depression in the pollinator community might face when there is a sharp floral resource decline, and in particular to the likely habitat loss of solitary and stingless bees in exploited forest systems. Many floral generalists are actually specialists for high density resources; low intensity flowering and spatial isolation of flowering individuals can make even the generalists vulnerable to extinction (Pimm & Pimm, 1982). In times of floral resource decline, generalist pollinators such as flies and bees might show a decreased foraging preference for the target species and switch to floral rewards from other flowering species in the forest (Murcia, 1996; Somanathan & Borges, 2000), leading to reduced visitation, less pollen reaching flowers, fewer fruits/ seeds produced and in turn, poor recruitment (Jennersten, 1988; Aizen & Feinsinger, 1994b; Harris & Johnson, 2004).

Ashworth et al. (2004) use asymmetry in plant – pollinator systems (i.e., a specialist interacting with a generalist) in forest communities as a plausible explanation of why specialist and generalist plant species respond similarly to habitat fragmentation (Aizen et al., 2002) as against the expected pattern of specialised interactions being more prone to reproductive failure (Waser et al., 1996). Recent studies (Bascompte et al., 2003; Vazquez & Aizen, 2004) have demonstrated that generalist plants are often pollinated not only by specialists, but also by generalist pollinators, while specialist plants tend to be pollinated mostly by generalist animals, as against the expected trend. Thus, according to Ashworth, loss of many or all of their specialist pollinators, places generalist plants in as susceptible a position as specialists. In the same vein, being a generalist does not make *N. nimmoniana* less vulnerable to impacts of habitat disturbance, especially when the pollinator assemblage appears a mix of specialist and generalist and when little is known about the community level plant – pollinator networks. My study re-asserts that there is a need for the plant and animal sides of

mutualistic interactions to be assessed, to better understand reproductive vulnerability of plants to habitat disturbance.

Fruiting success

The fruit set in experimentally pollinated flowers was not higher than open pollinated flowers, suggesting no pollinator limitation in the species. Comparison of percent fruit set between exploited and unexploited populations showed no significant difference; yet, I discuss here some relevant aspects of the two sites studied. When floral resources are scarce as in the lean-male-flowering year, even the unexploited population with zero level of extraction suffered a drastically low fruit set (6.91%). The situation would not be different in a population like Dandeli, where more than 60% of the trees were felled; there would be no floral resources if at all in spatially isolated trees causing severe pollen limitation. In the same year, the polygamous individuals of site UE (those carrying flowers of male, female and bisexual flowers) showed a higher fruit set (20.02 %) than female trees (6.91 %), evidently because there was self - pollination in these individuals. In the entire population only about 7% of the trees encountered were polygamous and the population is predominantly dioecious (Sharma et al., in review), making the species prone to the effect of lower flowering density and distribution (Bawa, 1990). Comparing fruit set in the lean and good flowering seasons in site UE gives an insight into the possible effect of spatial isolation of male individuals from cutting trees on the reproductive success in a dioecious species.

Concluding remarks

There are at least three other populations of *Nothapodytes* in the deciduous forests of Dandeli Wildlife Sanctuary where exploitative harvest has been recorded (Vasudeva, pers. comm.). This pattern of harvest of tree trunks in vulnerable sites leading to huge gaps in flowering between years is cause for conservation concern. The habitats of *Nothapodytes* are the dry and moist deciduous forests of the Western Ghats - unique in vegetative characteristics and holding a diversity of birds and mammals, larger than evergreen forests. These habitats are under an alarming rate of fragmentation, facing pressures from fire, grazing and large projects of mining and dams (Rawat et al., 2001).

Pollinator limitation (Rathcke & Jules, 1993; Kearns & Inouye, 1997) and reduced outcrossing rates in low flowering density populations (Murawski et al., 1994) have been reported from logged and fragmented populations elsewhere in the tropics. Changes in floral resource intensity from exploitation of plant resources, the kind encountered in *Nothapodytes nimmoniana,* could affect the mating structure of species and lead to a negative effect on seedling recruitment (Somanathan & Borges, 2000). It may be argued that frequent selfing in *Nothapodytes* may have an advantage in coping with pollinator decline (Chapter IV), but that is no reason for complacency since my study clearly shows that these plant populations are highly vulnerable to anthropogenic pressure and that disturbance does have an effect on the flowering intensity in the population and on insect diversity. Further, different pollinator species may respond to land use changes such as lack of habitat connectivity at different spatial scales (Steffan-Dewenter et al., 2001) and possibly varying time scales too.

Nothapodytes nimmoniana remained an obscure tree from the biodiversity rich tropics until it became economically valuable to the human society, endangering native populations from exploitative harvest of plant parts. A single species approach addressing the process of reproductive ecology of a threatened tree species in its natural habitat and considering its ecological traits (breeding system, pollination dynamics, level of generalisation or specialisation) can provide answers to intrinsic questions related to reproduction in a population and help predict plant species responses to fragmentation particularly when carried out at a community level (Ashworth et al., 2004). Such information, combined with studies on regeneration, is potentially valuable to recommend and develop strategies such as enrichment *in situ* or *ex situ* conservation (Shivanna et al., 2008), depending on the context. With much sought after modern drug components such as camptothecin with a soaring market value, the challenge remains to discuss with local stakeholders, the possible approaches to sustainable harvest and options of creating alternative propagation sites of *N. nimmoniana* for extraction of wood chips (Vasudeva et al., 2004). It is left to us as conservation biologists to evaluate the case for conserving tropical plant and insect species by taking into account the intricate, often many-layered dynamics of interactions in forest ecosystems.

CHAPTER IV

Sex and evolutionary choices in *Nothapodytes nimmoniana*

The study populations of *Nothapodytes nimmoniana* in the Biligiri Rangan Hills and Dandeli Wildlife Sanctuaries showed great sexual variation, being largely dioecious with a frequent polygamous individual. This motivated a further investigation of the functional gender and sexual fidelity in the species. The breeding system of a species represents different patterns of resource allocation - pollen and nectar - in time and space. Here, I examine the evolutionary pathways that may have led to the current sexual system and find explanations for the possible selective pressures.

Manju V. Sharma, R. Uma Shaanker, Vasudeva R. and K.R. Shivanna. **Sexuality in *Nothapodytes nimmoniana*: Evolution of dioecy from heterostyly.** In review with *Plant Systematics and Evolution*. Reprinted here with modifications.

SUMMARY

Preliminary studies to quantify reproductive success in *Nothapodytes nimmoniana* revealed intriguing sexual variation in the population. Functional gender of the different flower morphs was determined using pollen fertility and germination techniques. Flowers are morphologically comparable to thrum and pin morphs of dimorphic species and are borne on different trees. I establish through hand pollinations and observations on stigma receptivity and pollen viability that thrum flowers and plants are functionally males and pin flowers and plants are females. Thirty five % of the trees in the population produce a proportion of female flowers and intermediate (bisexual) flowers with functional pollen and stigma. These polygamous individuals are self-compatible. Sexual system in *Nothapodytes* appears to be a stage in evolution between heterostyly and dioecy, morphologically female and male (both functionally bisexual) forms trying to achieve functional dimorphism. I explain the purpose of the remnant stamens in bisexual and female flowers from a pollination perspective, and suggest that incomplete dioecy in the species evolved from an ancestor with reciprocal herkogamy without diallelic self-incompatibility.

Keywords: functional gender, incomplete dioecy, polygamous, reciprocal herkogamy, self-compatible, sexual variation

Introduction

Nothapodytes nimmoniana Graham. (Family Icacinaceae) is a small spreading tree distributed in Indo-Malesian region and China. In the Indian sub-continent, it is distributed in the Western Ghats of Maharashtra, Karnataka, Kerala and Tamil Nadu as well as in the eastern states of West Bengal and Assam. Natural populations of *Nothapodytes* are vulnerable to exploitative harvest for trade in camptothecin, an active cancer treating component (Govindachari & Vishwanathan 1972, 1974; Suhas et al., 2007) extracted from its wood chips. The species has a threat status of endangered/ vulnerable (Ved, 1997).

Hombegowda et al. (2002) reported the population of *N. nimmoniana* growing in

Dandeli Wildlife Sanctuary in Karnataka to be polygamous with a wide array of gynomonoecious and trimerous conditions co-existing in individuals. Sexual differentiation in dioecious flowering plants in understood to be often incomplete (Ross, 1982). Work on angiosperm breeding systems involving two, sometimes variable sex forms has emphasised the value of a functional rather than a morphological approach to their definition (Vaarama & Jaaskelainen, 1957; Connor, 1973; Arroyo & Raven 1975; Lloyd, 1976). During my intensive study to understand the reproductive ecology of two populations in the Western Ghats, I identified three floral forms which led me to further investigate aspects of functional gender and sexual fidelity in the species. I examine here, the evolutionary pathways that may have led to this sexual system and possible selective pressure.

Study site and Study period

The study was conducted in the moist deciduous forests of Biligiri Rangaswamy Temple (B.R. Hills) Wildlife Sanctuary in the central Western Ghats of India for three consecutive years (2005 - 07), during May and June. Observations and experiments were concentrated in two populations (Doddasampige N 11° 57' 079", E 77° 09' 632" and Malkibetta N 11° 57' 221", E 77° 08' 688", elevation: 1210 m.) separated from each other by a distance of approximately 4 km. The altitude of the study area is 1210 m. and it receives a mean annual rainfall of 1800 mm. Trees of *Nothapodytes nimmoniana* grow up to 10 to14 m. in height.

The study essentially involved: (i) recording the phenology and sexuality of flowers on the basis of their morphology, their distribution on different individuals in the population, and any transitions in sex across the years (ii) verifying the functional gender of the flower, (iii) establishing the breeding system of polygamous trees, and (iv) quantifying fruit set.

Methods

Floral morphology and phenology

Flowers in the canopy were accessed using a bamboo ladder. Preliminary studies

identified three types of flowers: male, female and bisexual based on their morphology. For studies on floral biology, a total of 30 flowers of each of the three morphs were scored for measurement of floral parts using digital callipers; and the average length of stamen and pistil, and the length of the ovary and anther were measured. Transverse sections of ovaries from 20 flowers were taken to determine the number of ovules in an ovary. A one-way ANOVA compared mean stamen and mean pistil lengths of bisexual flowers with that of female and male flowers.

To study inflorescence phenology, I tagged 8 -10 inflorescences each on ten trees, selected to cover all floral morphs. I made daily visits to the marked trees during the peak of flowering season for 2 weeks, to score the number and morphological sex of the flowers opening everyday. This gave me data not only on the intensity and pattern of flowering, but also helped to know if there is a shift in the sex expression of individual trees during the flowering season. To study sexual fidelity of individual trees, I marked 40 trees with metal tags and their sexuality was recorded each year during the three year study, by scanning flowers from several branches. Each tree was scored as 'male' or 'female' or 'polygamous' (trees that bore mostly male flowers but also a small proportion of bisexual and female flowers).

Functional gender of flowers

Male sexuality of flowers of different morphs was verified by estimating pollen fertility by staining them with a drop of acetocarmine and counting the number of fertile and sterile pollen. Fertile pollen take up the stain and sterile pollen are generally shrunken and do not take up deep stain and thus can be easily distinguished. To estimate pollen production, mature but undehisced anther (n=15 thrum morphs, n=15 pin morphs, n=12 intermediate morphs from 4 different individuals of each sex type) was placed on a glass slide in a drop of safranin and teased with a needle to extrude pollen. After removing the debris, the number of pollen grains was carefully counted under a compound microscope. Pollen viability was assessed on alternate days beginning the day of anthesis, until there was no viable pollen left, using fluorescine diacetate test (Heslop-Harrison & Heslop-Harrison, 1970). Pollen function was studied by carrying out manual pollinations on the stigma of pin and intermediate morphs and studying pollen germination and pollen tube growth following aniline blue fluorescence

technique (Linskens & Esser, 1957; Shivanna & Rangaswamy, 1992). Female function of flowers was analysed through studying its ability to support pollen germination and pollen tube growth in manually pollinated pistils. I also assessed female function on the basis of fruit set. Many enzymes, particularly non-specific esterases, have been shown to be present on the surface of functional stigma (Heslop-Harrison & Shivanna, 1977). To test if there are any differences between thrum and pin morphs in stigma surface esterases, I conducted the cytochemical test for non-specific esterases on stigmatic surface following the method of Mattson et al. (1974).

Breeding system of polygamous trees

Both populations comprised of three kinds of trees: male, female and polygamous. To study the breeding system on female and bisexual flowers developed on the polygamous trees, I carried out hand pollination experiments using the emasculation (for bisexual flowers) and bagging method. For bisexual flowers, I used pollen from the same plant (self-pollination) and from male flower of another plant (cross-pollination). As flowers were too small to be marked or tagged individually, I bagged the whole inflorescence after snipping off younger buds and older flowers.

To check for natural selfing in inflorescences containing male, female and bisexual flowers in polygamous trees, I bagged some polygamous inflorescences (n = 30) (after snipping off open female and bisexual flowers that could have already been pollinated) and opened after a month to record fruit set. Resulting fruit set would be an evidence for natural self-pollination in polygamous trees.

Fruit set

To estimate percent fruit set, I tagged 30 inflorescences each on 4 female trees and 4 polygamous trees and counted the number of female and/or bisexual flowers. These inflorescences were monitored until fruit maturity and the number of fruits on each inflorescence counted. Natural fruit set in female inflorescences (female trees) and mixed inflorescences of polygamous trees was compared and the difference tested with a Student's t-test.

Sexual fidelity

Forty trees were marked for recording sexuality during the flowering period in 2005. Each individual was monitored in the following year to note the sex of flowers and also to observe fruit set.

Results

Floral morphology and phenology

Nothapodytes nimmoniana flowers from the end of May to late July. The flowering period lasts 4-6 weeks in a population. Flowers are arranged in large terminal corymbose panicles of 20 – 400 flowers. They are foetid smelling. Each inflorescence lasts for 2-3 weeks. Petals are creamish and villous within. Five lobed sepals (hairy on outer surface similar to those on the petals) are present.

Morphologically all floral morphs appear bisexual with typical dimorphic flowers – thrum type with long stamens and short pistil and pin type with long pistil and short stamens (Figures 4.1A,B; 4.2 A,B). The two morphs are generally borne on different plants. Thrum morphs have turgid anthers that produce pollen grains, and have small, green stigma on a shortened style. Pin morphs have shrunken, non-dehiscent anthers that contain no pollen, and a prominent stigma. On the basis of these morphological features, I term thrum type as male flowers and pin type as female flowers. Both male and female flowers bear normal ovary with two ovules. Further observations on 40 marked plants led to the identification of a third floral morph on a few individuals, with the lengths of stamens and the pistil intermediate to those of thrum and pin morphs (Figures 4.1C, 4.2C). Flowers of this morph produce pollen grains and have two ovules in the ovary. Such flowers I refer to as bisexual. They have medium sized pistil and their stamens and anthers are of varying sizes (also see scatter plot - Figure 4.3).

Mean stamen length of bisexual flowers was measured to be significantly longer than female flowers (one-way ANOVA, $F = 22.4$, d.f.= 2, 87, $p < 0.001$) and not significant ($p = 0.151$) when compared to male flower stamens. Pistils of bisexual flowers were intermediate in length from male and female flowers and when tested with a one-way ANOVA showed high significance ($F = 63.18$, d.f.=2, 87, $p < 0.001$). All the three sex

types have a disc shaped nectary between the petals and the ovary and it surrounds the ovary about one third of the lower part. Measurements of floral parts of the three morphs are presented in Table 4.1.

Table 4.1 Mean lengths (± SD) in mm. of flower parts of *Nothapodytes nimmoniana*. (n = 30 for each morph).

Flower type	Diameter	Petal	Stamen	Anther	Pistil	Ovary	Stigma	No. of pollen/ anther
Male	7.48 (±0.96)	6.46 (± 0.8)	5.39 (± 0.69)	1.29 (± 0.22)	5.39 (± 0.63)	1.57 (± 0.29)	0.49 (± 0.8)	1030 (± 156)
Female	7.33 (±0.44)	4.78 (± 0.47)	2.06 (± 0.36)	0.98 (± 0.19)	6.78 (± 0.27)	1.4 (± 0.17)	1.22 (± 0.08)	0
Bisexual	6.44 (±0.46)	5.01 (± 0.53)	5.18 (± 0.66)	1.69 (± 0.31)	6.2 (± 0.35)	1.68 (± 0.22)	0.69 (± 0.16)	448 (± 329)

In polygamous trees, female and bisexual flowers made their appearance in a few (<10%) inflorescences towards the second week of flowering. During the entire flowering period of 15 -20 days, a total of 4.41 % (± 3.2) female flowers and 4.47 % (± 3.1) bisexual flowers are produced on an inflorescence (n=24), majority of the flowers (91.12% ± 5.8) being male.

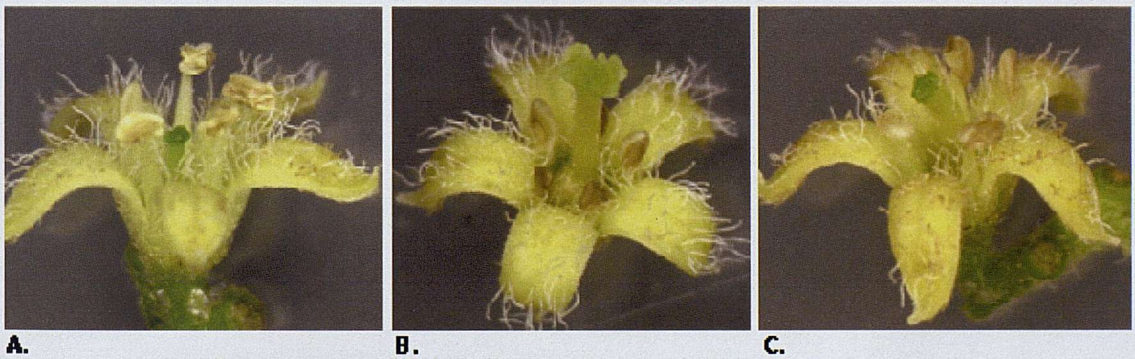


Figure 4.2 The three floral morphs of *Nothapodytes nimmoniana*. **A.** Male flower with tall stamens and dithecous turgid anthers and small, green stigma on a shortened style; **B.** Female flower with expanded stigma on a tall style and shrunken, non-dehiscent anthers on short stamens; **C.** Bisexual flower with style and stamens of intermediate lengths.

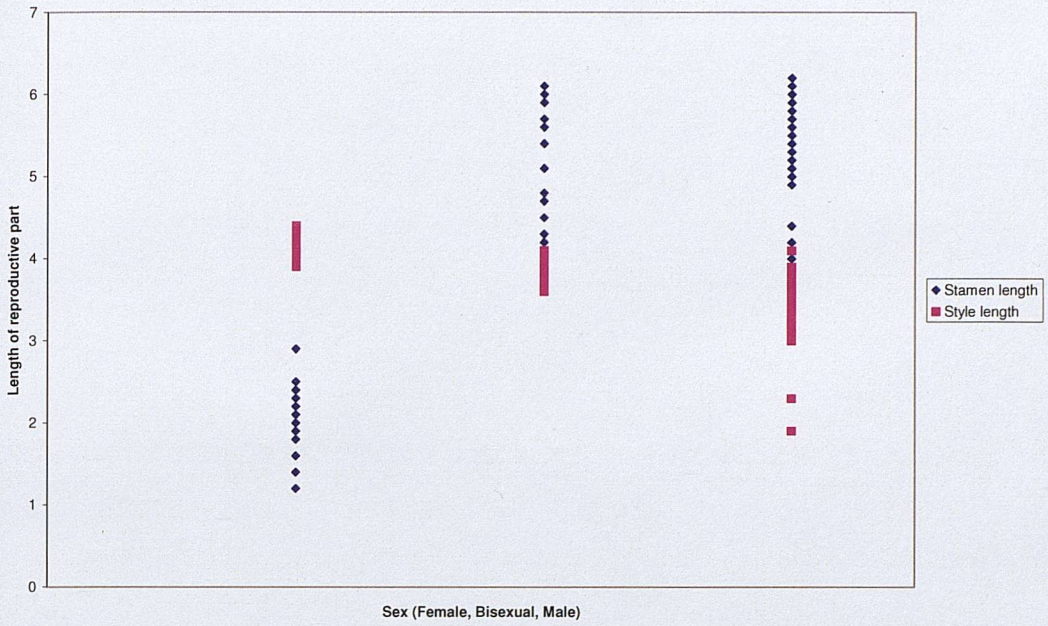


Figure 4.3 Variation in stamen and style length in female (measured in millimeters), bisexual and male flowers of *Nothapodytes nimmoniana* (n=30).

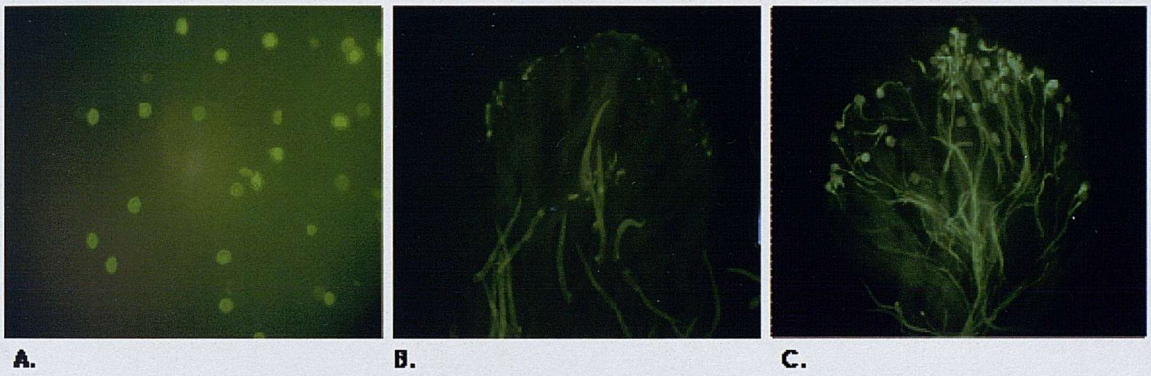


Figure 4.4 Pollen and stigma function in *Nothapodytes nimmoniana* **A.** Male and bisexual flowers produce functional pollen, seen here are viable pollen fluorescing with FDA test; **B.** and **C.** Fluorescent micrographs of the stigma and upper part of the style. The stigma of thrum flowers did not support pollen germination. Stigmas of pin and intermediate flowers supported pollen germination and pollen tube growth. Few hair like, slightly fluorescing structures on the stylar region are surface trichomes.

Functional gender of flowers

To further confirm sexuality of flowers, I determined pollen production, their fertility and ability to germinate on the stigma and the ability of the style to support pollen germination and pollen tube growth. Both male and bisexual flowers produced functional pollen (Figure 4.4A). The number of fertile pollen grains differed significantly ($p < 0.005$) between male and bisexual flowers (Table 4.1).

In bisexual flowers, only 2 or 3 of the total five anthers, which are longer produced fertile pollen; the shorter anthers contained a few (32 pollen per anther, n=12) functional or all sterile pollen. The stigma of male flowers did not support pollen germination (Figure 4.4B) in manually pollinated flowers and they did not set fruits. Female flowers did not produce functional pollen. The stigmas of both female and bisexual flowers supported good pollen germination (Figure 4.4C) and pollen tube growth and resulted in fruit set.

The stigmatic surface of female and bisexual flowers developed pinkish colour in the cytochemical test indicating the presence of esterases. The stigma of male flowers did not develop any colouration indicating absence or very low amount of esterases.

Breeding system of polygamous trees

Hand pollination of female and bisexual flowers with pollen of male and bisexual flowers of the same tree as well as male flowers of other trees showed good pollen germination and set fruits (Table 4.2), suggesting that the female and bisexual flowers produced on polygamous trees are self-compatible. Eight of the 30 bisexual and female buds (on mixed inflorescences of polygamous trees) netted to keep out visiting insects set fruit, indicating natural self-pollination to a certain extent.

Table 4.2 Results of hand pollination on bisexual and female flowers borne on polygamous trees.

Flower type	Pollinations using pollen from (n=12)	Average fruit set (%)
Bisexual	Males of same plant	33.2
Bisexual	Males of different plant	37.5
Bisexual	Bisexuals of same flower/ same tree	33
Female	Males of same tree	41
Female	Males of different tree	39
Female	Bisexuals of same tree	43.4

Fruit set

During the first year of study, in mixed inflorescences (on polygamous trees), mean natural fruit set under open pollination was 40.67% (± 28.8) and in female

inflorescences (on female trees) was 46.83% (± 15.03). In 2006, fruit set in mixed inflorescences was 20.02 % and 6.91% on female inflorescences. Five of the seven target female trees in the second year of study (2006) did not have flowering male trees in the vicinity.

Sexual fidelity

Of the 40 trees marked for recording sexuality each year, 35% were polygamous individuals. Transitions from one sex type to another during the flowering period in 2005 and 2006 were observed in a few individuals. The study was based on results on phenotypic gender and fruit set. Male trees remained male across the years. 3 out of 14 (21.4%) polygamous individuals became male in the second year. I also recorded in the third year (2007), female to polygamous transition in 3 out of 17 (17.6%) individuals.

Discussion

Intriguing floral sexuality – a closer investigation

In many species, herkogamy (separation of floral sexual parts in space, to reduce the likelihood of self fertilization) is incorporated in a polymorphism for stigma and anther heights, referred to as reciprocal herkogamy. It results in pollen from the two morphs being carried on different locations on pollinators' bodies, which in turn promotes inter - morph pollination (Lloyd and Webb, 1992). Flowers of *Nothapodytes nimmoniana* are described bisexual in various floral descriptions (Keshavamurthy & Yoganarasimhan 1990; Ravikumar & Ved 2000). On superficial observation, the flowers are comparable to typical dimorphic floral morphs. Functionally separate sexes in morphologically hermaphrodite flowers are well known (Fernald, 1950; Arroyo & Raven, 1975; Bawa & Opler, 1975). I confirmed the sexuality of the three types of flowers by determining the functional gender of flowers through analyses of pollen and pistil function. The thrum type flowers had normal ovaries and completely non-functional stigma. They seem to have developed from bisexual flowers having achieved complete female sterility. The pin type flowers had shrunken anthers with no pollen and their stigma supported pollen germination. They appear to have evolved from bisexual flowers having achieved complete male sterility. The bisexual flowers produced on polygamous trees revealed intermediate function for their anthers; only two or three of the total five anthers

produced fertile pollen, the rest had a few functional or all sterile pollen in them; thus exhibiting incomplete male sterility. The stigma of bisexual flowers also permitted good pollen germination. Further confirmation of sexuality came from fruit set data: pin floral morphs on female trees set fruit, but thrum floral morphs on male trees never set fruit, while pin and intermediate flowers of polygamous trees produced fruits. These detailed studies on the function of male and female organs demonstrated that *N. nimmoniana* exhibits incomplete dioecy.

The surface of functional stigma has been shown to contain a range of components made up of several enzymes and non-enzymic moities referred to as extracellular matrix (ECM) (Heslop-Harrison & Shivanna, 1997; Lord & Russell, 2002), that are known to play an important role in pollen germination and pollen tube growth (Lord & Russell, 2002). Studies on localisation of a group of enzymes, non-specific esterases, on the stigma surface of male, female and bisexual flowers clearly indicated the presence of esterases on stigmas of female and bisexual flowers but surface enzymes could not be localised on male flowers. Non-functioning of the stigma of male flowers is probably due to the absence of esterases or any other components of ECM, which are required for pollen function. Thus female sterility in male flowers seems to be the result of inactivation of gene(s) involved in the secretion of ECM component(s) required for pollen function on to the surface of the stigma. Similarly male sterility in female flowers appears to be the result of inactivation or mutation of gene(s) required for pollen development as has been reported in several species (Kaul, 1988; Sawhney, 1997).

In a population of *N. nimmoniana* studied in north western Karnataka (Hombegowda et al., 2002), the species is reported to demonstrate a wider range of variation in morphology. This population has been subjected to more physical disturbances such as chopping of branches (also see Chapter III). This leads me to believe that the perturbations and flexibility in sexuality may be magnified when the plants are subjected to severe disturbances (Pritchard 1916).

In my study population, the number of female and bisexual flowers on polygamous trees is very limited - around 7%. I therefore consider this species as predominantly dioecious. Marked individuals monitored for sexual fidelity over three flowering

seasons suggest that males remain male and some females become polygamous. Some polygamous individuals became male in the second year, suggesting that they are in fact 'inconstant male' trees (a term borrowed from Lloyd's study, 1975) not producing female and bisexual flowers in some years. The system seems similar to the report on *Fraxinus excelsior* L. (Wardle, 1961), where there are (a) trees predominantly male, but always a few female and hermaphrodite flowers on some branches, and (b) trees purely male in some years, but in other years producing some female and hermaphrodite flowers. Grant (1975) also indicated that many species possess intermediate breeding systems, providing a mix of outcrossing and selfing.

Evolution of dioecy

As many as five pathways have been suggested for the evolution of dioecy (Ross, 1982). Of these, evolution of dioecy from distyly or heterostyly is one (Baker 1958; Ornduff, 1966; Opler et al., 1975). In dioecious species derived from heterostyly, long-styled plants are typically female and short-styled plants are male. In *N. nimmoniana* also, the morphology of female and male flowers resemble typical pin and thrum morphs respectively of a typical dimorphic species which differ reciprocally in stigma and anther height. The sterility of male and female sexes is so cryptic that it cannot be readily identified without studying the functional attributes of the flowers. Because of this many taxonomists have described the species as hermaphrodite (Keshavamurthy & Yoganarasimhan, 1990; Ravikumar & Ved, 2000). It is tempting, therefore, to suggest that dioecy in this species has evolved from heterostyly as has been documented in several other families – Rubiaceae (Baker, 1958), Boraginaceae (Philipp & Schou, 1981) and Menyanthaceae (Ornduff, 1966). A major difficulty in considering the origin of dioecy in *Nothopodytes* from heterostyly has been the absence of any heterostylous taxa in Icacinaceae. Icacinaceae is not a monophyletic family; it includes several evolutionary lineages (Karehed, 2001). There are however three instances where a reference is made to polygamous individuals in Icacinaceae - *Leretia* sp. where flowers are described as 'perfect, rarely unisexual by abortion'; *Discophora* sp. where flowers are 'possibly unisexual' and *Citronella* sp. where there are fertile and sterile stamens, and fertile and sterile pistils (Woodson et al., 1976).

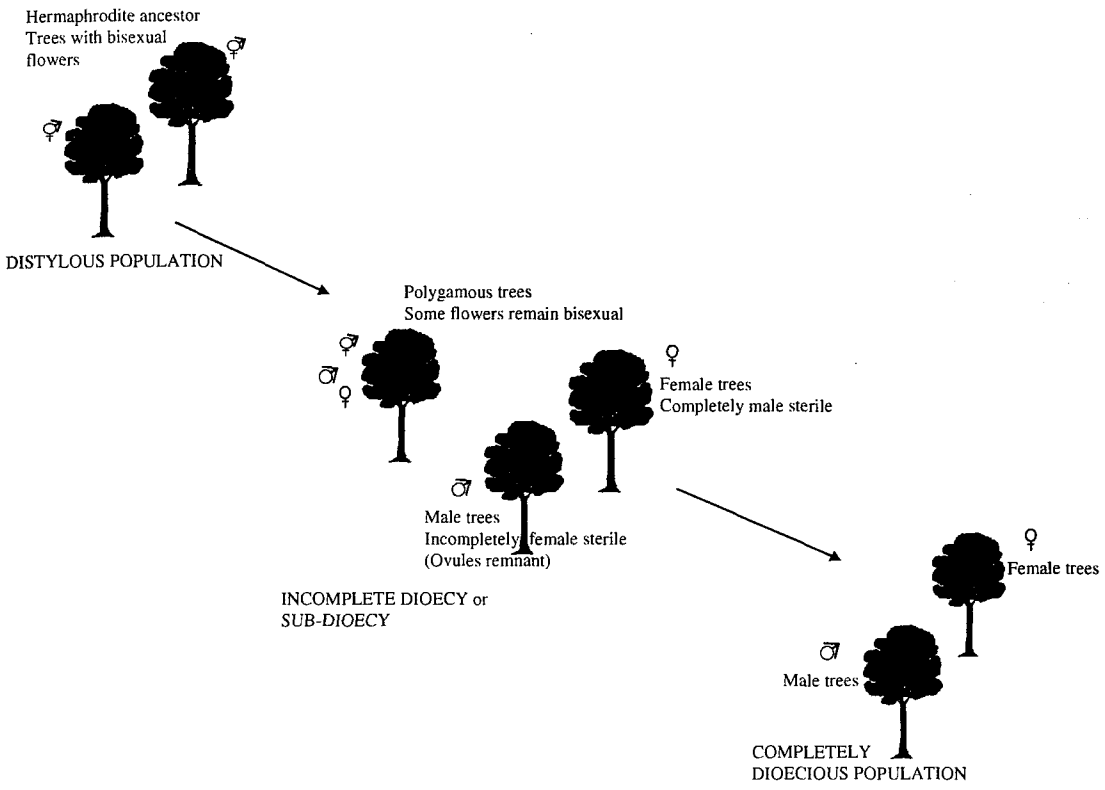


Figure 4.5 Illustration depicting the present sub-dioecious populations of *Nothapodytes* sp. as a stage in the evolution of dioecy from a hermaphrodite ancestor.

The populations of my study are clearly not gynodioecious or androdioecous; therefore I eliminate the pathway from hermaphroditism to dioecy via gynodioecy (Ross, 1982). What is seen is something similar to an andromonoecious individual, more often exhibited as a trimonoecious individual with more male and a few female and bisexual flowers. Thus I propose the following model for the evolutionary pathway of the species: Heterostyly- Trimonoecy/ Subdioecy - Dioecy (Figure 4.5). I use the term 'incomplete dioecy' to describe the sexuality seen in *N. nimmoniana* and suggest that the sexual system is a stage of evolution between heterostyly and dioecy, morphologically female and male (both functionally bisexual) forms trying to achieve functional dimorphism.

Selection forces

The discussions so far on the evolution of dioecy from heterostyly have been largely about evolution from a typical heterostylic and self-incompatible species. It is generally agreed that heteromorphic form of self-incompatibility is polyphyletic; but the pathway(s) of evolution of reciprocal herkogamy and its integration with self-

incompatibility to form an efficient outbreeding system is not clear (see de Nettancourt, 2001). According to one hypothesis diallelic self-incompatibility arose first and reciprocal herkogamy evolved later (Charlesworth & Charlesworth, 1979). Another hypothesis considers that morphological dimorphism of floral parts and diallelic incompatibility arose simultaneously (Mather & de Winton, 1941). According to the third hypothesis, reciprocal herkogamy evolved first and diallelic self-incompatibility occurred later (Vuilleumier, 1967; Webb & Lloyd, 1986). On the basis of this last hypothesis, one could argue that dioecy in *Nothapodytes* has evolved from a species in which reciprocal herkogamy evolved without diallelic self-incompatibility. The prevalence of self-compatibility in bisexual and female flowers developed on male plants of *Nothapodytes* supports such an argument. I speculate that in such a species, inbreeding depression may have acted as selection pressure for the evolution of dioecy.

Pollination as a selection force

The factors associated with the transfer of pollen to stigma should strongly influence the way different types of flowers are distributed in time and space. Yet, the evolution of breeding systems, which also represent different patters of resource allocation (pollen and nectar) in time and space, has rarely been examined from the pollination perspective (Bawa, 1980).

In several angiosperm genera distyly has evolved into dioecy (Beach & Bawa, 1980). In every case they examined, male plants are believed to have evolved from short-styled and females from long-styled plants. Consequently, short-styled plants only donate pollen while long-styled plants only receive pollen. Where female and male functions are separated, female flowers are spared the male costs and can afford to carry a higher proportion of fertilised fruits. Pollen emission and pollen receipt must become more efficient when separated on to different flowers or different trees (Richards, 1978). Males in *Nothapodytes* have larger inflorescences, and attracted more insects than female inflorescences (Chapter III). I believe that the retension of stamens in bisexual and female flowers acts as an attractant for pollinators; it is understood that there is strong selection pressure against loss of stamens in some species due to its role in integrity of the pollination system (Bawa & Beach, 1981).

A high proportion of unidirectional rather than reciprocal pollinations by insects may have been responsible for the development of dioecism from distyly (Baker, 1958). Although Baker did not allude to changes in pollinator vectors, it is obvious that disruption of bi-directional pollen flow in distylous species must be caused by a change in the pollination systems. Beach and Bawa (1980) hypothesised that dioecy has evolved in these taxa as a result of the disruption of the pollen flow complementarity that had permitted individuals in distylous species to function as simultaneous hermaphrodites. They proposed that a specific change in the pollination biology of these species is ultimately responsible for the evolution of unisexuality. A study by Lewis (1973) suggested that periodic population crashes in the outbreeding *Clarkia* sps. created small, isolated populations in which self-fertilisation evolved because of inadequate pollination of outcrossing flowers.

The shift from obligate crossing to self-fertilisation is known to occur chiefly in species that occupy temporary, pioneer habitats. Often these habitats are on the margins of the area of distribution for the species and its genus, and may be subject to adverse conditions for cross- pollination such as absence of suitable pollen vectors (Stebbins, 1970). Baker (1955) associates the shift to transoceanic migration of species and suggests that in order to be successful, the change must be gradual, via an intermediate stage of facultative selfing. According to the reproductive assurance hypothesis too, selfing has been suggested as an evolutionary consequence of rarity (Baker 1955, Carpenter & Recher, 1979). The selective advantage of self pollination lies in assured seed production when pollinators are insufficient for full pollination of ovules, e.g. due to poor climatic conditions or following long distance dispersal to areas where pollinators or mates are absent.

A stage of facultative selfing, the kind encountered in *Nothapodytes nimmoniana* is likely to be an intermediate one and may have adaptive implications. Self - compatibility may be of advantage in the species, ensuring there is minimum fruit set even during a year when flowering is erratic and uneven, or when there is a pollinator limitation.

CHAPTER V

In the canopy of a tropical wet evergreen forest – *Myristica dactyloides*

I investigate the floral characteristics and pollinator assemblage and quantify fruit set in *Myristica dactyloides,* a climax species of the wet evergreen forest type in the higher reaches of the Western Ghats. The chapter brings together reviews of generalist and specialist pollination systems to better understand the pollinator niche in the canopy of a threatened tree in the context of continually disappearing forest cover in the Western Ghats. It also highlights the importance of understanding canopy faunal assemblages and their functions in the conservation of tropical forest communities.

Manju V. Sharma, Simon R. Leather and K.R. Shivanna. **Pollinator guild and fruiting in the evergreen canopy of a Western Ghats endemic, *Myristica dactyloides*.** Submitted to *Journal of Tropical Ecology.*

SUMMARY

Myristica dactyloides is a dioecious tree, endemic to the mid and high elevation wet evergreen forests of the Western Ghats of India. I studied the pollinator assemblage in two relatively undisturbed populations in the region and quantified pollination efficiency and fruit set in one of them. I used Yellow Sticky traps to sample insects in the canopy during the flowering season of December 2007 in addition to direct observations of insect activity in 2006. Myristicaceae members from other tropics have been reported to be specialised to beetle pollination, but my observations indicated that the pollinator guild of the species is composed of diverse small insects – Thysanopterans (thrips), Coleopterans (beetles), Halictid bees, and Dipterans - Syrphid flies and Phorid flies. The system appears a continuum of specialist to generalist pollination. I found no pollination limitation in one of the populations, but recorded a 29% flower abscission and possibly fruit abortion. I briefly discuss how natural gaps between female flowering individuals and years can be aggravated by unsustainable harvesting of fruits in the species. I also highlight the importance of studying insect functional guilds in the canopy of these vulnerable rain forest trees and their habitats.

Keywords: canopy, Coleoptera, Diptera, endemic, flower abscission, specialist and generalist pollination, *Myristica dactyloides,* Thysanoptera, wet evergreen forest

Introduction

Myristica dactyloides Gaertn. is a tree endemic to India's Western Ghats and Sri Lanka, two regions that together form a global biodiversity hotspot. It grows to about 20-35 m., occupying the intermediate stratum just below the emergent canopy (Giriraj et al., 2008), and inhabits high altitude wet evergreen forests (850-1400 m.). The Family Myristicaceae belongs to Order Magnoliales and consists of canopy and sub-canopy trees. The species is dioecious, as are many tropical trees (Bawa, 1990). It has been classified as 'endangered' (IUCN, 2003) and its aril (seed coat) are harvested from evergreen forests for trade.

This study aims to determine the pollinator assemblage in *Myristica dactyloides* and

investigate if there is a pollinator limitation in the population. The study was carried out in two rain forest communities in the Western Ghats of Karnataka State, both high altitude evergreen forests. The only study from the Western Ghats on pollination in Myristicaceae was on *Myristica fragrans,* a cultivated nutmeg and was carried out in a low elevation plantation (Armstrong & Drummond, 1986). It revealed an Anthicid beetle to be the pollinator but might have left out a larger pollinator community that could have been encountered if the research was carried out in a forested landscape (Armstrong, pers. comm. 2008). A study on the Australian *Myristica insipida* reported nine species in five Coleopteran families as floral visitors (Armstrong, 1997). Curculionids, Staphylinids and Chrysomelids came up as pollinators of three species of *Knema* and one of *Gymnacranthera* in Sarawak (Momose et al., 1998b; Momose, 2005). Thrips have almost always been encountered on Myristicaceae but have been reported as pollinators only in *Horsfieldia grandis* in Sarawak (Momose et al., 1998b) and the neotropical *Compsoneura sprucei* (Bawa et al., 1985a). Small diverse insects were reported in a single study on *Virola koschnyi* (Bawa et al., 1985a). Although it has been suggested from examining flowers of Myristicas that they may have a non-beetle pollinator syndrome (Armstrong & Drummond, 1986), most research so far has reported beetles as pollinators, often dismissing other visitors as inefficient pollinators (Armstrong & Drummond, 1986; Armstrong & Irvine, 1989a; Armstrong, 1997), and there is an accepted view that the system in all Myristicaceae is that of specialist beetle pollination (Chandran & Sivan, 1999).

Study sites

A large part of the study was carried out in a population within the Biligiri Rangan Hills (B.R. Hills) Wildlife Sanctuary (N 11° 54' 442", E 77° 11' 189", elevation: 1366 m.), in the flowering season of December 2006 for over 10 days during the flowering peak. Some observations and pollination experiments were repeated in December 2007. In November 2006, I collected limited data on insect foragers from another population in the Brahmagiri Wildlife Sanctuary (N 11° 57' 790", E 75° 58' 380", elevation: 1169 m.). Both the populations were spread either along or very close to perennial streams. In the B.R. Hills population, flowering trees were identified and ladders were constructed (Figure 5.1A, B) using indigenous material along the bole of two male and

two female trees to access the canopy; one of the female trees did not flower in the second year of my study.

Methods

Floral resource

Floral display and reward in male and female trees were recorded as the size of the inflorescence and quantity of pollen or/and nectar. Flowers on 38 inflorescences from 3 female trees and 25 inflorescences on 4 male trees were counted. A two sample t-test was used to test the difference between the number of flowers in male and female inflorescences to be correlated to pollinator frequency in the canopy of male and female trees. Female flowers were dissected to examine the number of ovules. Nectar measurements were made using 10 µl. microcapillaries (Microcaps, Drummonds). To estimate pollen production, a mature but undehisced anther was torn off the fused androecium of a male flower (n=20 from six male trees) was placed in a drop of safranin or auromine O, pollen grains teased out, debris removed and pollen counted under a compound microscope. Pollen viability was assessed on alternative days beginning from the day of anthesis using the fluorescine diacetate (FDA) test (Heslop-Harrison & Heslop-Harrison, 1970). To estimate the pollen-ovule ratio for the two populations, I multiplied the number of pollen grains per flower by the ratio of male to female flowers and by the sex ratio of each population (see Table 5.1)

Insect partners

To calculate frequency of floral visits by insects, observations were made in half- hour blocks from 0600h to 1800h on two female trees and two male trees. In each observation unit, 4-6 inflorescences were observed at one time. In the B.R. Hills population, Yellow Sticky traps (Growing Success Ltd., Wiltshire, UK) were hung near flowering inflorescences for 24 hours to determine the visitor assemblage that might have escaped observation. This also allowed continuous monitoring (Armstrong, 1997; Sakai, 2001). I hung 10 sticky traps each, on one male and one female tree. They were retrieved after left hanging in the canopy for two consecutive days to scan the arthropods trapped on the yellow sticky surface.

Pollination

Insects seen foraging were collected using a sweep net and immobilised with ethyl acetate vapours. They were identified in the Entomology Laboratory at the Ashoka Trust for Research in Ecology and the Environment (ATREE, India) to the level of Family or Genus. My data comprised of a negligible number of insect visits directly observed; most insects were captured on sticky traps. Sticky traps were used with the assumption that chance of capture is directly related to the frequency and foraging activity of the species (Armstrong, 1997). I thus combined the insect visitation data from direct observations and the sticky trap method for the purpose of analysis. Insects were scanned under the stereomicroscope and those seen with pollen grains on body parts were considered the potential pollinators and the others as non-pollinating associates. A generalised linear model with a poisson distribution of the error term was fitted using R 2.7 (R version 2.7.1 (C), 2008) to test the difference of visitation of the pollinators alone on female and male inflorescences, the hypothesis being that males attract more visitors.

Stigmas from two or three-day old flowers were scanned under a hand microscope (Magiscope, USA) to observe pollen deposit. Forty - one female flowers, 0-5 days old, were examined under the stereomicroscope for the presence of pollen grains on the stigma. Pollen grains were then counted to calculate pollination efficiency: the percentage derived from the number of stigmas with pollen load as against the number of stigmas that carry no pollen load:

No. of stigma with pollen load x 100 / Total. No. of stigma scanned

I also used DAB (Sigma *Fast*™ 3,3'-diaminobenzidine; Sigma D-4) tablets to ascertain the viability of pollen grains that had reached the stigma (Dafni, 2007). Non-viable pollen grains remain light coloured, whilst viable ones turn a brown - purple - red in DAB.

Fruiting

Data on fruit set were collected from the B.R. Hills population alone in February 2007 and February 2008. As flowers of *M. dactyloides* were held on short peduncles, they could not be tagged individually; before anthesis, they were covered with butter paper

bags to exclude insects. The following day, when one or two buds were open, a full androecium was brought to the receptive flower (n=24) and pollen daubed on the stigma, younger buds were snipped away and the inflorescence was re-bagged. Unbagged inflorescences (n=35 in 2006 and n=49 in 2007) were tagged as open – pollination controls. For fruit set data, developing fruits were scored from both types of pollination about 4 weeks later and compared to check if there was a pollination limitation. Percent fruit set data were transformed using square root-arcsin transformation before employing the two sample t-test.

Results

The flowering canopy

In the Brahmagiri population, I marked 10 male and 7 female trees with an average girth class of 81.75 cm and 81.33 cm respectively. In the B.R. Hills population, I marked out 25 male and 16 female trees with the mean girth class being 178.8 cm and 174.5 cm respectively. Male trees had an average of 9.84 (± 3.27) flowers per inflorescence (n=25) and female inflorescences (n=38) were usually smaller with a mean number 6.42 (± 2.2) flowers. Flowers were urceolate, with males being narrower and females more rounded (Figure 5.2 A,B). An average male flower lasted 7 - 9 days and offered no nectar and only pollen as reward; pollen production was estimated to be 785.75 (± 97.6) per anther (anthers were 6-9 in number, fused to form an androecium). The anthers dehisced at least 24 hours before flower opening, as detected when mature buds were dissected. Pollen viability on day one was 28.5 and reduced to 15.5 on day 2 (FDA test). Female flowers remained fresh for about 6 – 7 days and had a wet stigma that remained receptive for 4 – 5 days and produced no nectar. The microcapillaries collected a fluid enveloped in the corolla tube of both sexes that was mistaken to be nectar before confirming for absence of sugar content with a refractometer.

Pollen ovule ratios (P/O) for *M. dactyloides* in the two populations in the Western Ghats were almost three times higher (Table 5.1) than the average P-O ratio for other obligate outcrossing plants (Cruden, 1977). As Armstrong and Irvine (Armstrong & Irvine, 1989b) argue, the exclusive pollen reward system may be part of the reason for

this high P/O; most other tropical dioecious plants tend to have female individuals that offer some amount of floral reward.



Figure 5.1 Ladder constructed on female (**A**) and male (**B**) trees using indigenous material provided access to the canopy of *Myristica dactyloides* in the B.R. Hills population

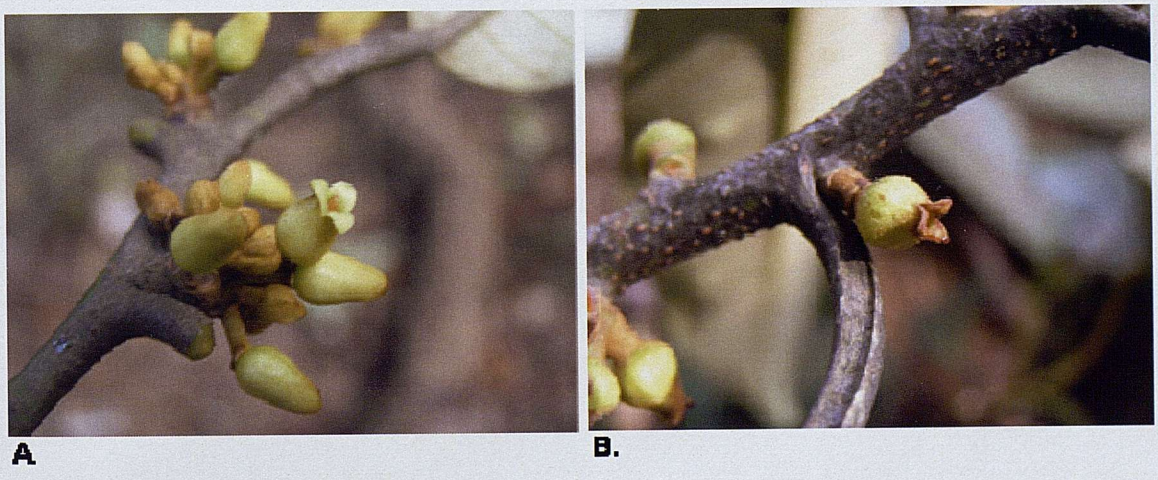


Figure 5.2 (**A**) Male and (**B**) female inflorescence in *Myristica dactyloides*, Brahmagiri Wildlife Sanctuary, Western Ghats, India.

Table 5.1 Pollen –ovule ratios calculated for the two Myristica populations at B.R Hills WLS and Brahmagiri WLS, Western Ghats, India

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Female flowers
1 Ovule / Flower (n=38)
Male flowers
(1) 6-9 Anthers/ Flower, mean = 7.5 (n=25)
(2) x 785.75 (± 97.6) Pollen grains/ Anther SD (n=20)
(3) = 5893.1 Pollen grains/ Flower
(4) x 1.53 Male flowers/ Female flower
(5) = 9016.4 P,O (if sex ratio were 1:1)
(6) x 1.56 Male trees/ Female trees at B.R. Hills
(7) = 14065 P/ O at B.R. Hills
(6') x 1.43
(7') = 12893 P/ O at Brahmagiri

Pollination

In B.R. Hills, where I used an additional insect capturing technique of sticky traps during the second year of study, I recovered a greater diversity of insects than in the Brahmagiri sampling which consisted of direct observations alone. A large number of beetles belonging to three families were captured; in the Brahmagiri sampling, the Staphylinid beetle was detected with a pollen load and in B.R. Hills I found Curculionidae and Cleridae members, of which only Curculionidae showed pollen on their body. Of the two bees seen foraging for pollen, the Halictid bee carried a large pollen load (Figure 5.3) and no pollen was detected on the body of *Apis cerana.* Two families of thrips were encountered frequently inside male and female flowers (Figure 5.4). Three Dipterans emerged in the sampling – Syrphidae and Dolichopodidae in B.R. Hills, and a Phorid fly captured at both the study sites. Syrphid flies had pollen all over their body, mainly in the bristles of the thorax near shoulders and the Phorid fly had a relatively low pollen load (Table 5.2A). The two Dipterans were thus included with Curculionid beetles and thrips in the visitation analysis owing to their contribution to pollination. Many of the sticky trap captures were of non- pollinating associates (Table 5.2B). Ant-mimicking wasps (Drynidae) and Vespid wasps were less frequent visitors at male and female trees in the B.R. Hills population. Butterflies were often seen in the upper canopy feeding apparently on the fluid stored inside the urceolate flowers.

Table 5.2A The insect faunal assemblage captured on direct observations (O) and in sticky traps (S) represented in families. Some beetles, thrips and flies were classified as potential pollinators (asterixed) since pollen was detected on their body parts.

Insect Taxa	**Capture method**	**Collected in**
Staphylinidae*	O	Brahmagiri
Curculionidae*	S	B.R. Hills
Cleridae	S	B.R. Hills
Phlaeothripidae* - adult and larvae	O, S	B.R. Hills
Thripidae*	O	Brahmagiri
Phoridae*	S	B.R. Hills, Brahmagiri
Syrphidae*	O, S	B.R. Hills
Calliphoridae	O	B.R. Hills
Dolichopodidae	S	B.R. Hills
Muscidae	O	B.R. Hills
Sacrophagidae	O, S	B.R. Hills
Halictidae*	O	Brahmagiri
Apis cerana	O	B.R. Hills

Table 5.2B The non-pollinating insect assemblage directly observed (O) or captured on sticky traps (S).

Drynidae (ant mimic wasp)	O, S	B.R. Hills
Aphididae (Heteroptera nymph)	S	B.R. Hills
Sciaridae fly (Nematocera div)	S	B.R. Hills
Cecidomidae (gall fly) (Nem)	S	B.R. Hills
Braconidae (Parasitoid)	S	B.R. Hills
Eulophid wasp	S	B.R. Hills
Cicadidillae (Homoptera)	S	B.R. Hills
Vespid wasp	O	Brahmagiri
Ichneumonid wasp	O	B.R. Hills
Camponotus sp (Formicidae)	O	Brahmagiri
Neptis hylas (Lepidoptera)	O	B.R. Hills
Cupha erymanthis (Lepidoptera)	O	B.R. Hills
Lymantrid moth - larva	O	B.R. Hills, Brahmagiri

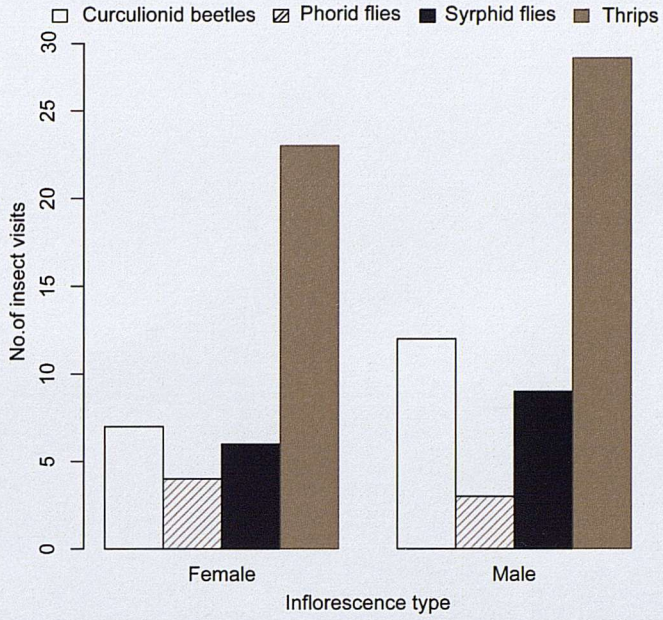


Figure 5.2 Patterns of pollinator visitation on male and female flowering trees, B.R. Hills, Karnataka, India. Data from direct observations and sticky trap collections were pooled in to generate this.

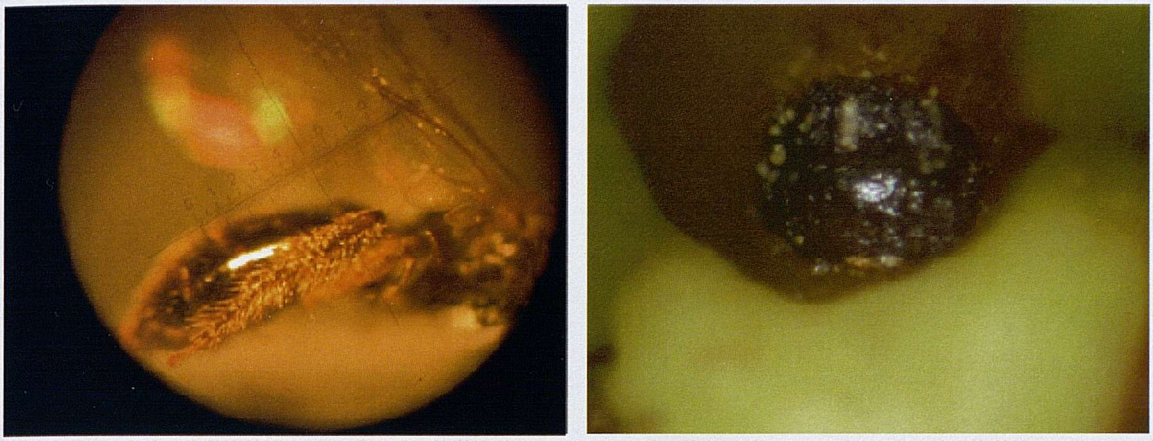
Data on insect visitation (pooled from direct observations and the number of individuals captured on sticky traps) of four potential pollinators were fitted to a generalised linear model with a poisson distribution of the error term and it revealed a highly significant influence of floral display (Z value = 12.91, $p<0.001$). Female inflorescences attracted fewer number of pollinators compared to male inflorescences (Figure 5.2).

Pollination efficiency

In the B.R. Hills population, I recorded a high pollination efficiency of 58.53 % in December 2006. There seemed to be a correlation between the presence of pollen grains on the stigma and the age of the flowers. Among the 41 flowers examined, the maximum number of pollen grains (>40) were located on the stigmas of four-day old flowers.

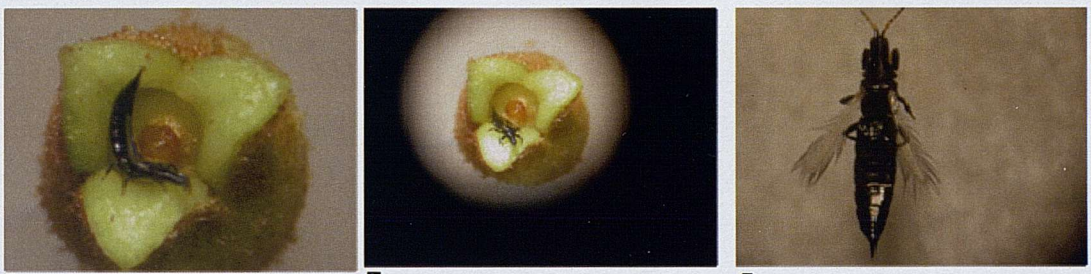
Increasingly smaller numbers of pollen grains (> 10) were found on the stigmas of two-day old flowers (Figure 5.3B). Pollen grains were almost always absent on the stigmas

of freshly opened and one-day old flowers. Results of the DAB test gave a measure of the percentage of viable pollen landing on the stigma: 67.13 % (± 8.9) of the pollen scanned on the stigmas stained deep brown in DAB.



A. B.

Figure 5.3 A. Halictid bee (Family Halictidae) collected from *Myristica* flowers, being scanned under a stereo microscope for detecting pollen load. **B.** Three day old stigma of a *Myristica* flower with pollen grains, as seen under a Magiscope field miscroscope.



A. B. C.

Figure 5.4 A.& B. A thrip (Family Phlaeothripidae) emerging out of a female Myristica flower, **C.** A winged thrip collected from a female flower. Not all thrips showed pollen load. B.R.Hills Wildlife Sanctuary, Western Ghats, India

Fruiting success

In the B.R. Hills population, fruit production ranged from 1–6 per inflorescence, with an average of 2.1 fruits. Mean fruit set in open pollinated flowers was 22.84% (n=178 flowers) in 2007 (from the December 2006 flowering) and 24.39% (n=254 flowers) in 2008 (from the December 2007 flowering). There was no significant difference (t = 0.79, df = 57, p = 0.43) between fruit set in hand pollinated (27.2%, n=81 flowers) and open or natural pollinated flowers (22.84%), suggesting that there is no pollination limitation in this population.

The considerably large difference between the percent pollination efficiency (58.53%) and fruit set (22.84%) in the B.R. Hills population indicates that not all flowers that received pollen resulted in fertilization or they were abscised after fertilization or were aborted further later as immature fruits. The inflorescence peduncle of a typical *M. dactyloides* is dotted with scars from abscission of flowers and this I noticed during the second and third week of flowering during consecutive surveys. There was a noticeable difference in the total number of buds and flowers originally produced as noted at the time of tagging the inflorescences for fruit set monitoring (mean = 6.42/ inflorescence) and the number noted about 2 weeks later (mean = 4.52/ inflorescence), 29.4% of the total flowers having abscised. Apart from flower abscission, at least 20% of the hand pollinated flowers that had formed young fruits dropped off during the first few weeks of fruit development, although I did not quantify this. Thus, most of the shortfall in fruit set could be attributed to flower abscission; the rest appeared to have been caused by fruit abortion.

Discussion

Looking beyond beetles

Although thrips have been established as pollinators in many Dipterocarps of the south east Asian forests (Appanah & Chan, 1981; Ashton et al., 1988; Momose et al., 1998a), they have been dismissed as pollinators in almost all Myristicaceae studies so far (Armstrong & Drummond, 1986; Armstrong & Irvine, 1989a; Armstrong, 1997; Corlett, 2004), except in *Horsfieldia* grandis (Momose et al., 1998b) and *Campsoneura sprucei* (Bawa et al., 1985a). I found pollen on some individuals of the two thrip species of Families Phlaeothripidae and Thripidae collected at the female trees in B.R. Hills and Brahmagiri, contradictory to earlier studies on Myristica (Armstrong & Drummond, 1986; Armstrong & Irvine, 1989a) that found no pollen on thrips that were seen abundantly on female trees (Williams et al., 2001a). Flowers and buds of Myristica seemed to act as brood sites for thrips, similar to reports in other species (Moog et al., 2002), as larvae were often discovered inside the urn shaped flowers/ buds. In a review of thrip pollination in Australian rainforests, Williams et al. (2001a) discuss how thrips have the habit of regularly cleaning the pollen off their body when preparing their wings for flight. They warn that this may lead to incorrect conclusions

concerning the effectiveness as pollen transporters. In my B.R. Hills observations, a great density of thrips was observed at female trees, possibly because one of my target trees had its branches very close to those of a male tree nearby. Intimate vegetative contact could have resulted in thrips emerging from male flowers to result in pollen export to female flowers (Armstrong, 1997). From my observations on foraging activity and abundant pollen load on Syrphid flies, they appear to be significant contributors to pollination in *M. dactyloides.* This Family of Dipterans has been reported to be a key pollinator group in the temperates (van der Goot & Grabandt, 1970) and in orchids (Pansarin, 2008) and palms (Schmid, 1970) of the tropical forests, usually seen along with beetles (Ervik & Feil, 1997). Halictid bees were seen foraging at male and female trees. If a bee could gather the pollen then they might get deceived into visiting a female flower and delivering pollen just as beetles were reported to in a classical study on 'mistake pollination' on the Australian *Myristica inspida.* It may be worth noting that while in *M. insipida*, Armstrong (1997) observed the the perianth opening of the flower filled by the stigma, essentially preventing insect entry (Armstrong, 1997), flowers of *M. dactyloides* were seen easily visited by bees, flies and butterflies.

Beetles have been confirmed to be the most efficient pollinators in some species of Myristicaceae (Armstrong & Drummond, 1986; Armstrong, 1997; Momose et al., 1998b; Momose, 2005). In this study, two species of beetles (belonging to Families Staphylinidae and Curculionidae) emerged as potential pollinators, and they were encountered in abundant numbers in comparison to other insects at male as well as female trees. This, however, does not nullify or discount the efficiency of the thrips and Dipterans and Halictid bees as pollinators, all of which were seen to carry pollen load in the present study. An investigation of the functional guilds in the canopy of *Myristica fatua,* a lowland swamp specialist tree, revealed two thrip families and five beetle families – Cucujidae, Silvanidae, Cantharidae, Curculionidae and Cleridae. Some beetles in the study initially thought to be strict predators and herbivores were later classified as potential pollinators (Sinu & Sharma, in review). In fact, the chewing mouth-parts of many flower beetles have for long perplexed botanists and entomologists (see Grimaldi, 1999; Bernhardt, 2000) because some beetle species are known to combine the roles of floral predator and pollinator. The use of female flowers as suitable sites to complete agonistic and mating activities (Gottsberger, 1977;

Gottsberger, 1988; Goldblatt et al., 1998) has been written about; some beetles also enter flowers because the blossom produces a physical temperature preferable to the external environment (Bay, 1995; Seymour & Schultze-Motel, 1997; Bernhardt, 2000), others are known to feed on stigmatic secretion or petals (Momose, 2005). This appears true in my study, where the wet stigma and urn shaped flower might be providing food and shelter for the beetles.

I conclude that pollination in *Myristica dactyloides* is a case of loose mutualistic niche, where there is no single pollinator, but a generalist range of them, including possibly a specialist group of beetles too, and that if the sampling intensity were to be increased, I might find a more diverse pollinator assemblage. Early research (Givnish, 1980; Bawa et al., 1985b) has suggested that *Myristica* conforms to a generalised, small insect pollinator. Corlett (2004), in his review of beetle pollination in the south-east Asian forests, emphasises on an overlap between families of Coleoptera in specialist *vs.* generalist flowers. He also points out that taxa belonging to the beetle families Cerambycidae, Curculionidae, and Scarabaeidae are most likely to pollinate both specialist and generalist systems. More recent research (Bascompte et al., 2003; Vazquez & Aizen, 2004) has brought in evidence that generalist plants are often pollinated by specialists and generalist pollinators, while specialist plants tend to be pollinated mostly by generalist animals; such asymmetry in interactions is only natural considering the complex nature of processes in tropical systems. We must be careful in categorising plants as 'cantharophilous', or 'chiropterophilous' or any such strict syndrome, and must not become so entranced by jargon that we forget that plant - pollinator interactions are biologically variable (Wyatt, 1983), as are most things in nature. In addition, the pollinator assemblage of individual species is known to vary on a temporal and spatial scale (Horvitz & Schemske, 1990; Gomez & Zamora, 1999; Fenster & Dudash, 2001; Price et al., 2005; La´zaro et al., 2009). It is essential not to make prior assumptions about rigidly specialised syndromes since it can be counterproductive to field studies, since generalist pollination appears to be the rule, not the exception, in floral evolution (Waser et al., 1996; Corlett, 2004) and the generalisation – specialisation is more a continuum that varies across plant communities (La´zaro et al., 2009).

Fruiting

The high pollination efficiency and low percent fruit set in the B.R. Hills population, combined with observations on flower abscission and fruit abortion, clearly suggest that there is no pollination limitation in the population, although there appears to be a level of resource limitation (Howe & Westley, 1997). A high level of abortion of female flowers and raw fruits was found in *Myristica fragrans* (Armstrong & Drummond, 1986) and reports of female flower and immature fruit abortion troubling nutmeg growers in the sub-continent are not recent (Flach, 1966). Armstrong and Irvine (Armstrong & Irvine, 1989b) also recorded a 50% abortion of immature fruits during the initial period of fruit development in *M. insipida* and remarked that the failure could not be explained.

The abscission of female flowers and immature fruit has been encountered in other rain forest trees, cross - pollinated flowers often facing abortion (Bawa et al., 1985b). Such abortion could be for varying adaptive reasons, either bet hedging against environmental uncertainties, pollination, fruit/ seed predation, or resource availability, or it could just be selective abortion to improve offspring quality, or a result of enhancing male function via hermaphrodite flowers (Stephenson, 1981; Armstrong, 1997). The primary function of non - fruiting flowers does appear to be that of enhancing female display to a level of least equal to minimum male display; so these flowers cannot be considered redundant or excess. Female flowers might avoid the cost of reward, but they apparently cannot afford to cut down on the cost of display.

Conserving insects in the evergreen canopy

I found a great diversity of non-pollinating associates of the flowering phase and this only reflects the diversity of insects in the microhabitats provided by the Myristica canopy in a wet evergreen forest. The *Myristica dactyloides* pollinator guild was composed of insects that showed flower constancy at varying levels, with thrips appearing to be predominantly present during all instances of sampling, and beetles, bees and flies being less constant and bound to spatial variation, as suggested by the site differences in my study (Table 2a). Plant species specialised on pollinators showing fixed specialisation or high flower-constancy behaviour (such as bees in general) are

expected to be less affected by aspects such as diversity of floral neighbourhood in terms of pollinator composition and competition (Waser, 1986; Chittka et al., 1999; Moeller, 2004; Ghazoul, 2006), than plant species specialised in less flower-constant pollinators (La´zaro et al., 2009).

Of concern is the fact that evergreen forests are facing the threat of fragmentation all over the planet and much of the climax vegetation has disappeared in regions such as the Western Ghats (Pascal, 1991). Many populations of *Myristica dactyloides* are victims of unsustainable harvest of fruits for trade in non-timber forest products (NTFP) and this often leaves large gaps between flowering individuals and flowering years (see Chapter VI). This kind of variation due to anthropogenic disturbances in the canopy stratum is additional to any temporal variation some female trees in the population are known to show such as non-flowering years (as in this study), and floral resource depletion in the canopy can potentially affect pollinator diversity and movement (Somanathan & Borges, 2000) and alter the functional guilds dependent on the floral resource base. Canopy insect studies elsewhere in the tropics have pointed out the vulnerability of beetle families such as Curculionidae and Chrysomelidae are particularly susceptible to anthropogenic disturbances (Floren & Linsenmair, 2003, Speight et al., 2003). These studies have clearly demonstrated how canopy faunal diversity was distinctly lower in disturbed forests when compared to primary forests. The Western Ghats landscape offer forests of several vegetation types and provides a suitable zone to study the limits and conditions necessary for development and maintenance of wet evergreen forests (Pascal, 1991). We have no studies from the region that address canopy fauna at a forest community level, and not knowing enough about what species might be at risk, and what processes (including pollination) might be facing threats up in the evergreen canopies, puts us in a dangerous situation while making conclusions on conservation of trees and insects.

In the B.R. Hills Sanctuary, Myristica forms a characteristic component in the canopy in the *Mesua ferrea - Palaquium ellipticum – Olea glandulifera* vegetation type (Ramesh, 1989), which is structurally and floristically different from other formations in the B.R. Hills. The species is also a dominant in some other parts of the Western Ghats (Giriraj et al., 2008). Flowering and fruiting in the canopy of evergreen forests is a huge resource for a great variety of insects and vertebrates. In *M. dactyloides,* the

pollinator guild was composed of a range of insect Families and during the fruiting period, hornbills, giant squirrels and macaques are seen frequenting the canopy (Chetan & Ganesh, 2006). Both my study sites – Brahmagiri and B.R. Hills had a reasonable extent of habitat connectivity and thus offered a suitable template to understand the flower - pollinator dynamics of a threatened species, away from anthropogenic disturbances - with no recent recorded history of harvest of the fruits for NTFP trade. Such studies are important in the context of ecological conservation (Devy & Ganesh, 2006) and contribute to our understanding of how natural forests function.

CHAPTER VI

Fruiting success in a thinning canopy

The seeds and aril of *Myristica dactyloides* are harvested from evergreen forests and are traded along with the widely cultivated *Myristica fragrans.* In many parts of the Western Ghats, they contribute to the income generated from non-timber forest products (NTFPs) in local communities. In nine populations of *M. dactyloides* (including the two selected for pollination studies), I measured fruit crop as a function of disturbance defined by a set of variables, and independent of flowering intensity or pollination success. Here, I discuss the implications of harvesting techniques and habitat fragmentation for the dispersal and regeneration dynamics of these tree populations.

Manju V. Sharma, Catherine M. Collins and Simon Leather. **Anthropogenic influences on fruit crop in a wild nutmeg from the evergreen forests of the Western Ghats, India.** Submitted to Biological Conservation.

SUMMARY

The mid and high elevation wet evergreen forests of the Western Ghats are composed of several vegetation types, one of which is characterised by a climax species *Myristica dactyloides*. The aril of the fruits is a valuable non-timber forest product and the trees face indiscriminate harvest in many parts of the mountain range. We made visual estimates of the size of the fruit crop on female trees across nine populations with varying levels of disturbance. Disturbance was assessed using five variables: distance from road, method of harvesting fruits, matrix around the population, level of seedling regeneration and whether the population is within a Protected Area. Unsustainable methods of harvest and proximity to a road had a negative impact on fruit crop, as did a non-forest matrix surrounding a population. The study demonstrates that while high protection status and strict measures against the harvest of fruits of *M. dactyloides* for NTFP trade have kept some populations safe from exploitation, the method of harvesting plays the greatest role in determining the fruiting success in *M. dactyloides*.

Keywords: climax species, disturbance factors, fruit crop, harvest, *Myristica dactyloides*, NTFP, Western Ghats, wet evergreen forests

Introduction

The Western Ghats of India form a non-equatorial belt of tropical forest and along with Sri Lanka are recognised as a global biodiversity hotspot (Myers et al., 2000; Nayar, 1996). *Myristica dactyloides* (Gaertn.) is a dioecious tree endemic to this region and has been recently included as Endangered/ Vulnerable in the IUCN listing (IUCN, 2003). It has been described as a 'climax species' in the *Mesua ferrea - Palaquium ellipticum – Olea glandulifera* vegetation type (Ramesh, 1989), and as a 'primary species' (Parthasarathy, 1999) and 'dominant species' (Pascal, 1988) in the *Cullenia exarillata – Mesua ferrea – Palaquium ellipticum* vegetation type. *Myrsitica dactyloides* is clearly a characteristic component of the evergreen forest canopy in mid-elevation and high elevation zones and also seems to prefer riverside habitats.

Like other Myristicaceae, *M. dactyloides* fruits have a high lipid and protein rich aril (Manasse & Howe, 1983) and are a preferred food source for large bodied birds and

primates (Howe, 1981; Leighton & Leighton, 1983; Howe et al. 1985; Galetti et al. 2000; Holbrook, 2003; Yasuda & Miura, 2006). The aril is also the source of wild mace and the seeds are traded as nutmeg, both important non timber forest products (NTFP); fruits from wild populations are frequently harvested, often unsustainably (Borges, 2003). The entire Myristica genus in the Western Ghats, composed of six tree species including one cultivated, is a victim of commercial exploitation. NTFPs are a significant source of income to local communities (Iqbal, 1993). Market samples reveal arils from the cultivated *M. fragrans* along with produce from *M. dactyloides* and *M. malabarica* and every kilogram of dried seed coat can fetch up to Rs 750/ kilogram (= $ 15.4) and the seed is priced Rs 450/ kilogram in the local market (Indulal, pers. comm.) and the estimated consumption by the domestic herbal manufacturing units for the year 2005-2006 was assessed at 180 metric tonnes (in dry weight) (Anonymous, 2002). As a consequence, large quantities of fruit are collected to harvest a few grams of the seed coat.

During the mature fruiting period, fruit crop in *Myristica dactyloides* was measured in relation to habitat disturbance. The nine *M. dactyloides* populations selected varied in disturbance levels.

Study sites

In July and August 2008, I carried out a broad survey of Myristica populations across Karnataka's Western Ghats based on information from the region's Floras (Gamble, 1935; Keshava Murthy & Yoganarasimhan, 1990) and peer references, and identified nine appropriate fruiting populations. Two of these were in BR Hills WLS, two in Brahmagiri WLS, two in Talakaveri WLS, two in Kudremukh National Park, and one in Kemmangudi (Figure 6.1, Table 6.1).

Methods

Estimation of fruit set

In each identified population, fruiting trees were located and a visual estimate was made of total fruit crop. Fruit of *M. dactyloides* is a drupe, 5-8 X 3-5 cm in size and are

borne in singles or clusters of two or three at one node (Figure 6.2 A & B). The number of fruits on a single branch was counted (N1) and multiplied by the total number of fruiting branches on the tree (N1 x total number of fruiting branches = total fruit on the tree). These observations were made from ground level with a pair of binoculars (Bushnell, 8 X 42) without accessing the canopy. Like most Myristicaceae, *M. dactyloides* has characteristic radial branching – making it easy to count without obstruction from foliage. I use the term 'size of fruit crop' to describe absolute fruit set, i.e., fruiting not in relation to the number of flowers produced or pollinated, but as an independent measurement in space. This is the response variable in the analysis that follows. The estimates were made post- harvest, but when fruit would remain on the tree in the wild. Fruit crop was estimated on 108 fruiting female trees found across the nine populations.

Estimation of disturbance

The explanatory variables selected and measured were:

(a) Harvest method – Categorical with four levels, i) chopping entire fruiting branches, ii) climbing the tree to collect fruits, iii) selective harvest, i.e only some trees targeted and not repeat harvesting from same tree/ population every year, or iv) no harvest.

(b) Matrix immediately surrounding the target population – Categorical with two levels, i) forest or ii) non-forest (plantation, degraded or encroached land).

(c) Distance from road – Continuous and estimated in metres.

(d) Protection status – Categorical with two levels, i) within a protected area (PA) or ii) outside a PA.

(e) Regeneration – Categorical with two levels – i) good (more than 5 seedlings seen) or ii) bad (fewer than 5 seedlings seen). Regeneration is an indirect measure of disturbance, being itself dependent on the extent of harvest and the number of seeds in the site.

Table 6.1. A glimpse of the harvest method in the nine populations of *Myristica dactyloides* across the Protected Areas of Karnataka's Western Ghats.

Site	Co-ordinates N	E	Elevation (m.)	Harvest method
BR Hills	11° 55' 988"	77° 11' 131"	1211	No harvest
BR Hills	11° 54' 442"	77° 11' 189"	1366	No harvest
Brahmagiri	11° 57' 790"	75° 58' 380"	1169	Climb and collect fruits
Brahmagiri	11° 57' 786"	75° 48' 312"	1220	Climb and collect fruits
Talakaveri	12° 23'	75° 29'	1230	Chop entire branches
Talakaveri	12° 13'	75° 29'	980	Chop entire branches
Abaskat	13° 16' 65"	75° 08' 29"	440	Selective harvest
Abaskat	13° 15' 54"	75° 08' 19"	440	Climb and collect fruits
Kemmangudi	13° 32'	75° 45'	1450	Chop entire branches

Analysis

To estimate the influence of the measured explanatory variables on the size of fruit crop, a linear model was fitted to the data. Log transformation (ln) improved the model fit. To account for variation between populations these were also initially fit as a main effect. There was insufficient independent data to estimate interaction terms, so model simplification was performed by removing main effects in order of least significance until the minimally adequate model was identified. Additionally a regression tree was generated to better understand the influence of each explanatory variable on the fruit crop. All analyses were performed in R 2.7 (R version 2.7.1 (C) 2008).

Results

Disturbance that mattered

Harvest method, the matrix around the population and the distance from road all affected the abundance of fruit in the samples (Table 6.2). Populations subject to the chopping and climbing methods of fruit harvesting had the smallest fruit crop size remaining on Myristica trees (Figure 6.3).

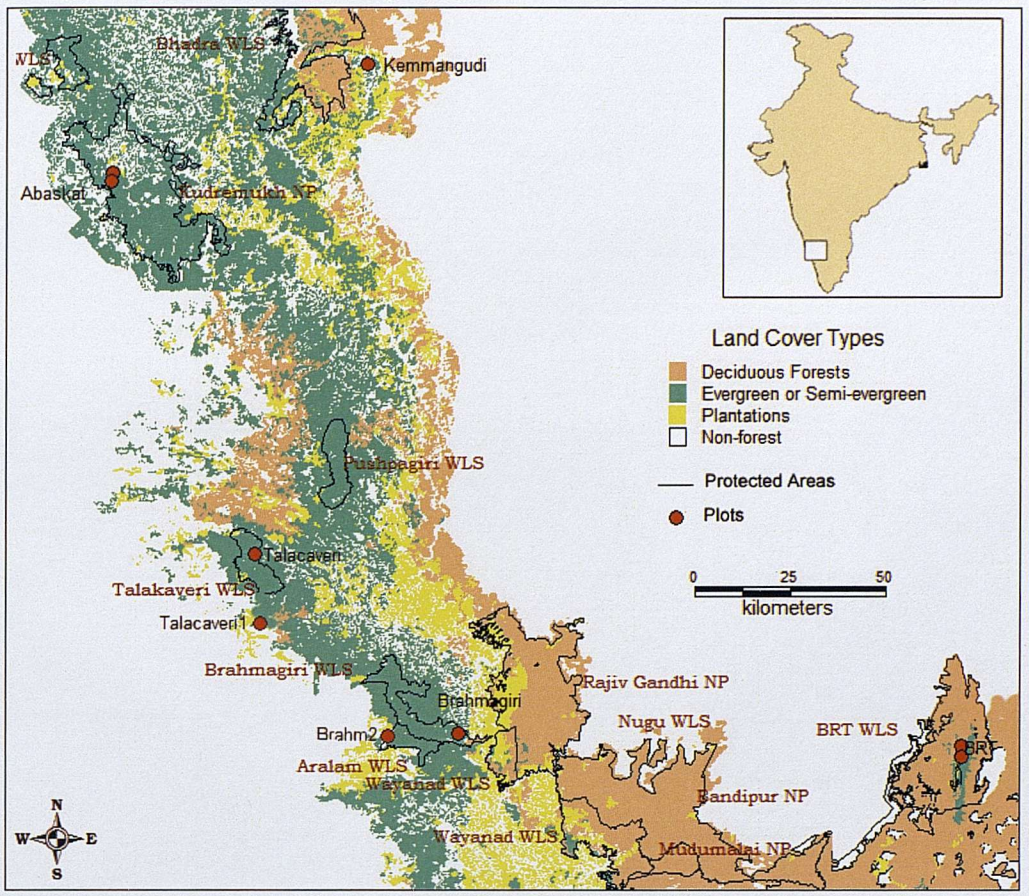


Figure 6.1. Landcover map showing the distribution of the nine *Myristica dactyloides* populations selected for the study. Central Western Ghats, India.



A. **B.**

Figure 6.2 A. Mature fruits in the canopy of *Myrsitica dactyloides;* and **B.** a dehiscing fruit, slightly revealing the orangish red aril inside. Brahmagiri Wildlife Sanctuary, Western Ghats, Karnataka, India

Selective harvest or no harvesting allowed the greatest Myristica fruit survival (Figure 6.3, Table 6.2). Chopping entire branches was the most exploitative method of harvesting and led to the lowest fruit remaining. The nature and extent of harvest was

confounded with decreasing distance from road (Figure 6.4), although generally trees over 3.5 km. from the road had a large fruit crop, while those close to the road (easier access for harvesters), had a low fruit crop. Also not suitable for a good fruit crop was a non-forest matrix; these usually lay outside Protected Areas (Table 6.2).

Table 6.2 Model summary of anthropogenic disturbance factors affecting the (ln) fruit crop size in *Myristica dactyloides*, Western Ghats, India.

	Estimate	**Std. Error**	**t value**	**Pr(>|t|)**
'Chop' harvest, matrix 'Forest'	4.312e+00	2.400e-01	17.97	< .001 ***
Harvest by climbing	6.950e-01	2.280e-01	3.049	0.0029 **
No harvesting	1.635e+00	2.116e-01	7.725	< .001 ***
Selective harvesting	2.575e+00	2.300e-01	11.199	<0.001 ***
Non-forest matrix	1.594e+00	2.273e-01	7.012	<0.001 ***
Distance from road	2.440e-04	8.267e-05	2.952	0.0039 **

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.5051 on 102 degrees of freedom

Multiple R-squared: 0.6517, Adjusted R-squared: 0.6346

F-statistic: 38.17 on 5 and 102 DF, p-value: < 2.2e-16

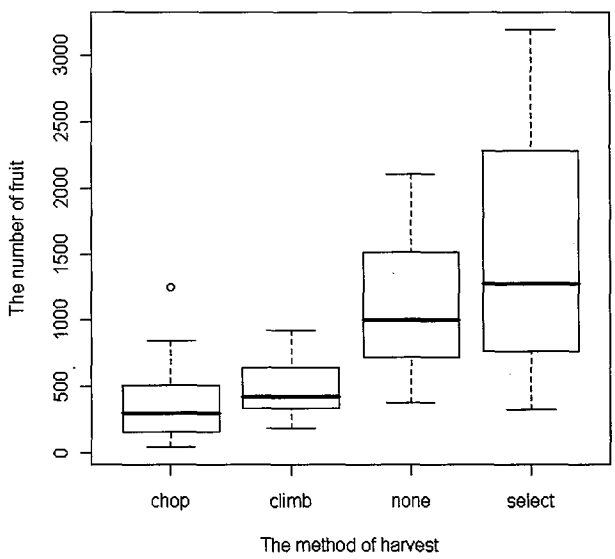


Figure 6.3 Fruit crop size on *Myristica dactyloides* in nine populations of the Western Ghats of India as a function of harvest method.

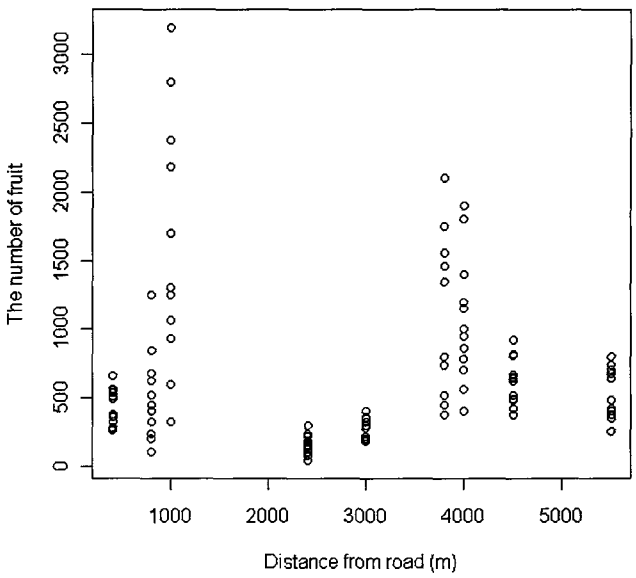


Figure 6.4 The size of fruit crop on *Myristica dactyloides* in nine populations of the Western Ghats of India as a function of distance from road (m).

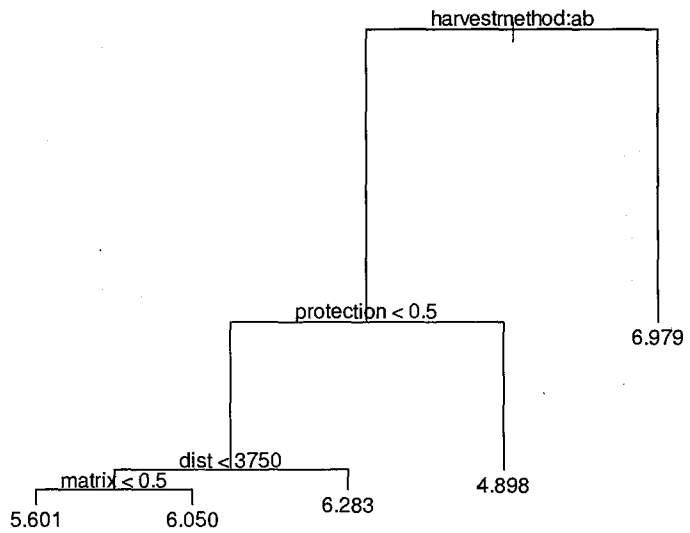


Figure 6.5 A regression tree summarising the influence of the measured anthropogenic disturbance variables. Mean fruit set values (log transformed) (n=108 trees across 9 populations) are at the terminal nodes of branches. The most important explanatory variable is 'harvest method'.

The regression tree indicated harvest method as the most important explanatory variable, as did the linear model; populations with no harvest or those facing selective harvest were least disturbed and had the largest fruit crop (Figure 6.5). For populations facing unsustainable harvest practices (lopping entire branches or climbing up the tree to collect fruit), protection status is the most important variable determining fruit abundance. Those outside protected areas were the ones with the lowest fruit crop size measured in this investigation.

Distance from road was the most important influence for populations within Protected Areas (PAs): trees more than 3.5 km. from the road had high fruit crops, while those close to the road and providing easy access for harvest, in spite of being located within a forested matrix, had a lower fruit crop (also see Figure 6.3).

Discussion

Patterns of harvest

Lopping entire fruiting branches in *Myristica* sps. has been recorded in the past (Menon, 2002) and emerged the most damaging method of collecting fruits in this study. This method would lead to substantially reduced flowering potential for those trees the following year. Selective harvest, the most productive harvesting category, includes sites with no harvest in some years and harvest from selected trees in other years. Thus, high fruit set in areas with selective harvest may be an artefact of the sampling year; the population surveyed in this sampling effort was possibly one that did not witness any harvesting in the year of study. Climbing trees for fruit collection involved opportunity costs which harvesters seemed to minimise in various ways. They usually target one population at one time; when they avoid one area for a given reason (sometimes to escape the Forest Department law or for 'sustainability' as some harvesters claim), then all trees in that population escape harvest. Similarly, trees that do not bear heavy fruit crops were left unharvested because of the high investment involved.

Although the model suggests a pattern of lower fruit crop near roads, some trees close to the road did have a large fruit crop. These were populations inside Protected Areas

and it may be that harvesters were avoiding road-side trees in these locations to be safe from the Forest Department watchers.

Regeneration as measured by seedling presence did not reflect crop size in my model, but a couple of sites, inside Kudremukh National Park and Talakaveri WLS emerged with low seedling regeneration. Often, seeds of Myristica are seen piled close to the trees after the aril have been peeled off (Figure 6.5B). At other times the collectors carry the fruits back to their hamlet where they are cut open to remove aril. Both are destructive practices from the perspective of seedling regeneration, the net effect being a loss of seeds from the forest floor and reduction in seedling recruitment in the population.

Three other sites (including one in B.R. Hills) showed high fruit set and good regeneration. Although an older study in the area recorded low regeneration of *M. dactyloides* (Ramesh, 1989), a recent survey at Ashoka Trust for Research in Ecology and the Environment (ATREE) for seedling recruitment studies in *Myristica dactyloides* populations indicated adequate seedling recruitment, but a low importance value index (IVI) compared to other species - an IVI index of eight 2.89 in 2006 and 5.59 in 2007 (Shivanna et al., 2008). It also reported a low number of female fruit bearing trees with a low seed production. This appears to agree with the findings of my study. A survey by Murali et al. (1996) on regeneration and population structure as influenced by NTFP extraction, listed 15 tree species (including one liana), as sources of NTFP collected and marketed at the local co-operative 'Large Scale Adivasi Multipurpose Societies', and the list did not contain fruits or aril of *Myristica dactyloides.* They found poor regeneration potential of the NTFP species and attribute it mostly to over – extraction of the respective plant resources.

In over-harvested populations, young seedlings and saplings of the species might deplete to the extent of leaving none to replace the adult stand in the forest – the 'living dead' phenomenon that Janzen (1986) described. Even where regeneration does occur, heavy harvesting and selective removal of the best fruit over time may affect the genetic composition of the exploited population to leave a fruit tree population dominated by trees that produce less fruit of lower economic value (MacKinnon, 1998).

Fruits in the canopy, if left unharvested

The impacts of harvesting plant parts can be manifested in various ways depending on what is being harvested and the environmental context (Avocèvou-Ayisso et al., 2009). Intuitively, indiscriminate harvesting of seeds or fruits could have long-term detrimental effects on recruitment of new individuals (Shackleton et al., 2005; Avocèvou-Ayisso et al., 2009). It can also affect animal populations by reducing their food resource availability (MacKinnon, 1998) and this includes both seed dispersers and predators in the canopy of the forest community. The seed disperser assemblage in large - seeded and arillate fruits of Myristicaceae is composed of large bodied birds such as toucans and aracaris in the neotropics (Howe, 1981; Howe et al., 1985; Holbrook, 2003) and four species of hornbills south east Asian forests Asia (Leighton & Leighton, 1983; Galetti et al., 2000; Kitamura & Poonswad, 2006). In the Western Ghats, the endangered Malabar pied hornbill (*Anthracoceros coronatus*), Malabar grey hornbill (*Ocyceros griseusi*), Nilgiri wood pigeon (*Columba elphinstonii*) (Figure 6.5A), and mammals such as the endangered lion tailed macaque have been reported to be feeders of the aril (pulpy red seed coat) in *Myristica malabarica, Myristica dactyloides, Myristica fatua* and *Gymnacranthera canarica* (Chetan & Ganesh, 2006; Sharma et al. in preparation). A standing crop of large fruits in the canopy of a mature forest is capable of satiating the disperser assemblage (Howe & Estabrook, 1977). *Myristica dactyloides* occurs at



A. B.

Figure 6.2 A. Nilgiri wood pigeon in the fruiting canopy of a *M. dactyloides*, B.R.Hills Wildlife Sanctuary, **B.** A pile of seeds left behind on the forest floor after the aril has been removed, Talakaveri Wildlife Sanctuary, Western Ghats of Karnataka, India.

varying high and low densities in the Western Ghats (Parthasarathy, 1999; Giriraj et al., 2008) and has obligate relationships with seed or fruit dispersers, characteristics that make it more susceptible to over exploitation (Peters, 1996; MacKinnon, 1998). Added to this is the fact that few tropical forest species produce reliable fruit crops at well-defined intervals: mast fruiting in Dipterocarps (MacKinnon, 1998) and inconstant fruiting in Myristicaceae (Vasudeva, pers comm.) are examples. And when the resources being harvested are fruits and seeds, the unpredictability of flowering and fruiting is a key issue in sustainability. It has been raised in the past that there is no knowledge of the pollination in fruit crop trees of Myristica, an upstream process that must be necessarily conserved to ensure continued fruit production on existing trees (Borges, 2003). We now have this information for *M. dactyloides* (detailed in Chapter V).

A climax species is a component of a mature ecosystem or climax community (Turner, 2001) that will remain essentially unchanged in terms of species composition for as long as the site remains undisturbed (Nix, 2008) and such species usually have a narrow ecological range and low phenotypic plasticity (Osborne, 2000). *Myristica dactyloides* is not only a climax species characteristic of mid and high elevation wet evergreen forests of the region, but also often the singular representative of the primitive angiosperm family of Myristicaceae in these communities (Parthasarathy, 1999). This study assumes conservation significance when combined with the fact that members of Myrsiticaceae are known to have a very restricted distribution across the tropics (Chandran & Sivan, 1999). Many populations of *M. dactyloides* stand in disturbed habitats and fragmented evergreen forests across the Western Ghats, and as these habitats face fragmentation and degradation (Gadgil & Meher-Homji, 1986; Nadkarni et al., 1989), the species is under severe threat.

Research needs

Although the approach used in this particular study does not take into account the biological factors such as fruiting in disturbed sites as a function of flowering and pollinator activity, or fruit removal by birds and mammals, it would nevertheless be valuable to combine these strands of information for the species and look at a more comprehensive picture.

There has been great focus on studies concerning extraction and regeneration in *Phyllanthus* sps., oil palms, brazil nut and other major NTFPs of the tropics. This study urges a shift of research resources to minor NTFPs such as aril from endangered Myristica sp. to protect existing populations. One approach that would prove particularly useful is a long-term monitoring, the kind carried out on *Phyllanthus* sps. in BR Hills (Setty et al., 2008). It could provide data on extraction rates from Myristica populations (fruits harvested per tree) and a more accurate estimate of regeneration. People who collect these fruits to be sold in the local market are one of the stakeholders that must be approached to seek co-operation in developing sustainable techniques of harvest, using already existing practises from other forest dependent communities. At the base of the chain are the growing demand and market forces that influence harvesting pressure that must be addressed, as social and economic factors are often a problem in forest management and long - term utilisation of natural resources (Vasquez & Gentry, 1989).

Conclusion

Plant populations growing in forests subject to varying levels of anthropogenic pressure may respond to harvest in very different ways just like other tropical trees (Ticktin, 2004) and this was demonstrated in my study comparing nine populations of *M. dactyloides.*

The study suggests that while high protection status and strict measures against harvest of fruits of *M. dactyloides* for NTFP trade have kept some populations safe from exploitation, harvest methods play the most significant role in determining the fruiting success in *M. dactyloides.* It is important that more rational harvesting techniques are used. As Vasquez & Gentry (1989) suggest, this may be achieved through incorporating a conservation ethic into local educational and economic systems, the kind that is known to exist in some parts of the Western Ghats such as the BR Hills Sanctuary (Uma Shaanker et al., 2004). Sustainable harvesting is contingent on harvesting without branch cutting, since the latter even with lower levels of harvesting can be unsustainable (Sinha & Bawa, 2002). Selective practises of harvest that have emerged in this study can be used as models to replicate in community based conservation

protocol. Ultimately sustainable harvesting is a sensitive and often ambiguous term because it depends on selection of sites, co-operation of communities dependent on forests and adjusting harvest levels to allow regeneration and growth of species being harvested (MacKinnon, 1998). The information that is crucial to the viability of these exploited populations is the level of regeneration and only long term monitoring of several sites would reveal if overharvesting has a real impact on seedling regeneration. Ecological studies combined with forest management might hold the key to sustainable resource extraction in these forests of the Indian tropics.

CHAPTER VII

Out in the montane grasslands - *Strobilanthes kunthianus*

In trying to understand the natural history of the pollination process and fruiting success in the mass flowering species *Strobilanthes kunthianus*, a fascinating story of evolutionary floral traits unravelled itself. This chapter is composed mainly of a manuscript that was developed from observations of the adaptive traits that I call 'reproductive strategies'. I present here the results of the observations on pollination experiments, insect activity and fruiting success. I also examine aspects of rarity in the plant populations, pollinator movement and the mast seeding habit, in the face of the current scenario of the threats faced by the high elevation montane grassland – Shola ecosystem, a unique element of the tropical forest landscape of the region.

Manju V. Sharma, Giby Kuriakose and Kundaranahalli R. Shivana. 2008. **Reproductive strategies of *Strobilanthes kunthianus*, an endemic, semelparous species in southern Western Ghats, India**. *Botanical Journal of the Linnean Society*. **157**, 155 – 163. Re-printed here with minor alterations.

SUMMARY

Strobilanthes kunthianus (Acanthaceae) is a monocarpic species endemic to the subtropical montane grasslands of the southern Western Ghats, displaying synchronised flowering and mast seeding once in twelve years. As semelparous plants have only one chance to reproduce, they are expected to develop effective strategies to prevent reproductive failure. The reproductive strategies of *S. kunthianus* were investigated by studying the floral traits, pollination ecology, and breeding system that are critical for reproductive success. The species exhibits a series of floral traits: (1) gregarious flowering attracts a large number of *Apis cerana indica*, the major pollinator; (2) the stigma is sensitive to touch by the pollinator; in fresh flowers, the receptive surface faces the entry path of the incoming bee, facilitating pollen deposition; as an immediate response, the stigma curves backwards moving the receptive surface away from the path of the exiting bee, thus preventing autogamy and interference in pollen transfer; (3) flowers remain fresh for 2 days with receptive stigma and nectar and pollen reward. These traits render the species 100% pollination efficient to ensure a high seed set. As the species is self-compatible, the prevailing high degree of geitonogamous pollinations does not interfere with fruit set. The evolution of the adaptive floral traits has facilitated mast seeding in the species.

Keywords: *Apis cerana indica,* floral traits, monocarpic, pollination efficiency, reproductive success, sensitive stigma, self-compatible

Introduction

Semelparous or monocarpic species are perennial flowering plants that flower once in their lifetime and die. The frequency of flowering of successive generations in such species is highly variable. Such species invest all their resources into seeds and generally produce a larger seed crop (referred to as mast seeding) in comparison with most iteroparous species (flowering and fruiting at multiple times during their life span), which retain a major part of their reserves for continued growth. Semelparous plants have only one chance to reproduce; they are committed to a reproductive event at a specific time regardless of the environmental conditions. A long-lived gregariously

flowering semelparous plant is even more committed to this single event because all of its kin will flower and die at the same time. Obviously, then, a major reproductive strategy of a semelparous species is to evolve devices to facilitate reproductive assurance.

Semelpary is found in a wide range of plant species across at least 20 families (Young & Augspurger, 1991). Bamboos are well known semelparous plants and the flowering frequency ranges from 3 to 120 years in different species (Janzen, 1976; Keeley & Bond, 1999). Several species of Strobilanthes (Acanthaceae) distributed in the Indian subcontinent and south-east Asia are also semelparous and flower at intervals of 3 to12 years. *Strobilanthes kunthianus* (Nees.) T. Anderson, called *'kurinji'* or *'neelakurinji'* in the vernacular, is a species endemic to the high altitude subtropical montane grassland – Shola system in southern Western Ghats (Figure 7.1A). The flowering periodicity of this species has been well documented. Robinson (1935) recorded a twelve-year flowering cycle for this species from the Nilgiri Hills between 1838 and 1934, based on the records of one of the earliest European families to settle in the Hills. Matthew (1971) also reported a twelve-year flowering cycle for this species in the Palni Hills. According to earlier reports, mass flowering of this species attracted a large number of jungle fowl to feed on the large quantity of seeds produced (Matthew, 1971; Janzen, 1976). With the area under the plant fast diminishing owing to the denudation of land for cultivation, mass migration of jungle fowl has not been seen in recent years.

Strobilanthes kunthianus flowered during August - October 2006 (Figure 7.1B), covering the grassland slopes with a bluish purple carpet from its dense massive bloom, and attracting a large number of tourists. The reproductive strategies of *S. kunthianus* were investigated during its flowering season in 2006 in order to answer the question: how does the floral biology of *S. kunthianus* serve to prevent catastrophic reproductive failure? The aim was to identify the features of the floral traits, pollination biology, and breeding system that are critical for reproductive success.

Study Site

The plant is a small shrub reaching a height of 0.5-3.0 m., inhabiting the high-altitude (1500–2600 m.) subtropical montane grasslands of the Western Ghats of southern



Figure 7.1 **A.** The Shola-grassland forest type characteristic of the subtropical upper montane in the higher altitudes of the Western Ghats. **B.** Mass flowering of *Strobilanthes kunthianus* during 2006 at Eravikulam National Park, Kerala, India. Photo: Sajan Varma.

India. The study was conducted in a grassland ecosystem in Kanthallur village (10°13′12.6″N, 77°12′14.9″E, altitude 1581 m.), located a few miles outside Eravikulam National Park in the state of Kerala (map on page 16, Chapter II). The *S. kunthianus* population was dense (31.96 individuals per $4m^2$, n=25) and covered an area of about 70 acres. The area was close to human habitation and delimited by Eucalyptus plantations. The surrounding Shola forests form part of the Mannavan Shola National Park.

Methods

Flowers

The flowering phenology of the species was recorded through field visits at weekly intervals. Studies on floral phenology and pollination biology were carried out during the peak of the flowering period. To study the floral phenology, flower buds (n= 30) that would open the next day were tagged and kept under observation to record anthesis, anther dehiscence, and other floral changes until they senesced. For studies on floral morphology, details of the floral whorls were studied and all the floral parts were measured (n=25). The number of ovules in each ovary was counted through dissections under a stereomicroscope.

Stigma receptivity and pollen viability

To determine the period of stigma receptivity, hand pollinations were conducted on

bagged flowers at six-hour intervals (n= 10 at each interval) starting with anthesis until 48 hours. The pistils were fixed 24 hours after pollination in formalin–acetic acid–alcohol (FAA; 5: 5: 90, v/v), and were later employed to study pollen germination and pollen tube growth using the aniline blue fluorescence method (Shivanna & Rangaswamy, 1992). The stage that permitted pollen germination and pollen tube growth was considered receptive. Pollen viability was assessed through fluorescein diacetate test (Heslop-Harrison & Heslop-Harrison, 1970).

Rewards

To estimate pollen production, mature but undehisced anthers (n= 10) were taken in a drop of safranin and teased with needles to eject all the pollen grains. After removing the debris, the number of pollen grains was carefully counted under a compound microscope. Microcapillaries (1 and 10 µl.) (microcaps, Drummonds) were used to measure the amount of nectar present in the flower. Flower buds were bagged before they opened to prevent insect visitation. Bagged flowers were excised at 4 - hour intervals (n=10 at each interval), starting from flower opening until 1800h on the first day, and again from 0600h - 1800h on the second day, and the quantity of nectar was measured. The differences in nectar quantity at different time intervals were tested using a generalised linear model with a quadratic term fitted for time to describe the non-linear distribution (R version 2.7.1, 2008).

Floral visitors

Floral visitors were monitored from 0600h (starting at flower opening) to 1800h for 15 minutes in each hour for three days, covering a randomly selected site on each day. As preliminary studies showed that *Apis cerana indica* was the only frequent visitor to the flowers, the frequency of visits, foraging behaviour (for pollen/nectar), and foraging time were recorded for *A. cerana indica*. For statistical analyses, the data from the three days were pooled and the observation period was divided into six two-hour intervals. To analyse the difference in visitation rates across time slots during the lifetime of the flower, I fit a generalised linear model with a quasipoisson distribution of the error term to correct over-dispersion. A sample of foraging insects was collected using a sweep net, and the insects were immobilised by transferring them to a vial containing a piece

of filter paper dipped in ethyl acetate. Immobilised insects were observed under a stereomicroscope to check for pollen load on their bodies. In another experiment, I monitored *A. cerana indica* visits to the same set of flowers on day one and two. Flowers (n=20) were tagged in the morning of the day of opening and kept under continuous observation (30 minute pulse at each hour) for bee visits from 0600h - 1800h on the first and second day of opening. The number of flowers visited during the day was calculated. In this experiment, a generalised linear model with a poisson distribution of the error term was fitted to analyse the difference in visitation rates across time slots during the lifetime of the flower. To check the possibility that wind may play a role in pollination, vaseline-coated slides were hung on the branches of the plants. They were collected 24 hours after exposure and observed under a microscope for pollen deposition.

Breeding system

I carried out controlled pollinations to study the breeding system. Flower buds were emasculated and bagged one day before anthesis. On the day of anthesis, the bags were opened in the morning hours and the flowers were pollinated with either self- (pollen from another flower of the same plant) or cross- (pollen from another plant) pollen and re-bagged (n=50 each). The bags were removed after three days when the stigma was no longer receptive and the flowers kept under observation for fruit development. To assess seed set under open pollination, 100 flowers were tagged randomly and monitored for fruit development. To look for apomixis, emasculated flowers (n=50) were bagged and scored for fruit set. Mature fruits were harvested before dehiscence and the number of seeds was counted. Since individual flowers could be monitored in the species, I used a generalised linear model with a binomial distribution of the error term to test for difference in fruiting success between open, self and cross pollinated flowers. All quantitative values are represented as the mean ± standard error.

Results

Flowers

Flowering was initiated at the end of the second week of August 2006 and reached its

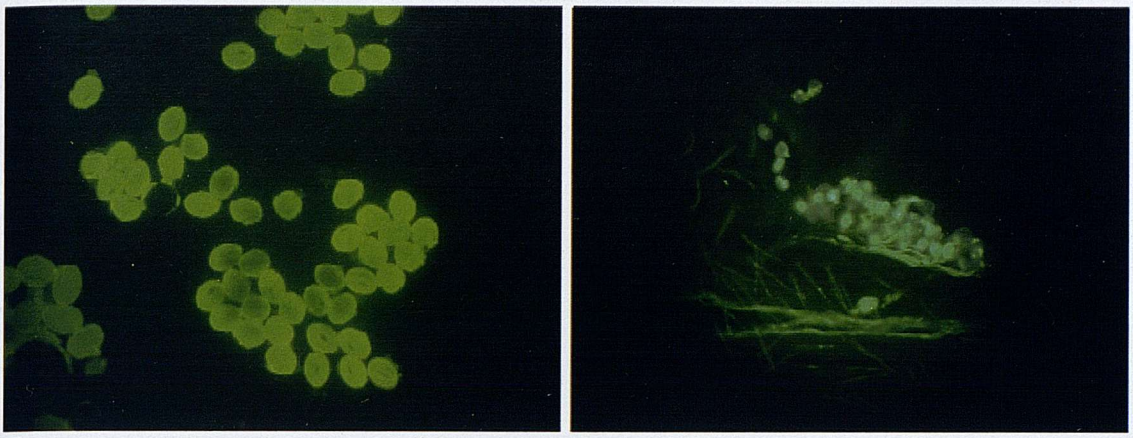
peak in the second and third weeks of September. Flowering declined towards the end of September and ceased towards the end of October. Flowers opened between 0600h and 0900h. They remained fresh on the second day and senesced on the third day.

The flowers are hermaphrodite and are borne on compactly arranged racemes (Figure 7.8A). Each plant produces an average of 82.5 ± 62.5 (n=100) inflorescences and each inflorescence (n=50) bears an average of 23.8 ± 8.8 flowers. During the peak of flowering, two to four flowers open on each day in each inflorescence. An interesting feature of the anthers is that they do not dehisce until touched by the visiting insect. The anthers can be induced to dehisce by a gentle touch with a needle. In bagged flowers, the anthers failed to dehisce by the evening of the first day, and even on the second day they showed a thin slit along the anther but pollen grains were not exposed from the anther until physical disturbance. Lack of dehiscence in bagged flowers as a result of changes in microclimate brought about by bagging cannot be ruled out. Following dehiscence, the anthers turned blue.

The corolla is tubular and five lobed at the tip, measuring 2.94 ± 0.12 cm in length. The two stamens are epipetalous. Each stamen is 2.15 ± 0.1 cm in length, and the anther length is 2.22 ± 0.25 mm. The anthers are bilobed and each anther produces 2880.3 ± 659.4 pollen grains. The pollen grains are elliptic, cream coloured, and sticky; they are trizonoporate and vertically banded as described previously (Valsaladevi & Mathew, 1985). The pistil is long (2.24 ± 0.13 cm) and the basal part of the ovary is covered with a conspicuous, yellow nectary. The style is 1.86 ± 0.14 cm in length and is held in place at the tip by a narrow groove on the posterior petal. The stigma is narrow, 1.91 ± 0.25 mm in length, and sharply curved. The receptive surface is pink facing the front of the flower with a shallow longitudinal groove at the centre (Figure 7.8C). Each ovary bears four ovules. The pollen to ovule ratio is 720: 1.

Stigma receptivity and pollen viability

Fresh pollen grains showed 82.7 ± 13.2% viability. The viability decreased steadily during the second (57.8 ± 17%) and third (37.4 ± 19.9%) days, and was lost completely by the fourth day. The stigma was receptive soon after anthesis and continued to remain receptive until 48 hours after anthesis.



A. B.

Figure 7.2 A. Fertile pollen grains stained in fluorescein diacetate (FDA) on day one. **B.** Germinating pollen stained with aniline blue seen under the UV filter of the fluorescent microscope.

Pollination ecology

Flowers offer both pollen and nectar as reward. The amount of nectar was limited in freshly opened flowers (Figure 7.3). It increased steadily during the day and reached over 2 µl. by evening. The amount of nectar increased up to 4 µl. by the morning of the next day in bagged flowers, indicating continued secretion of nectar during the night. The amount of nectar decreased gradually on the second day and, by 1800h, it was only about 1.5 µl.). The generalised linear model with a quadratic term fitted for time revealed a significant difference in nectar quantity across time (t-values=4.950 & -4.359, $p<0.001$). There was a non- linear, humped effect of time. Nectar, the response variable first increased with time, reached a peak and then decreased (Figure 7.3), which is indicated by significant coefficients for both linear and quadratic terms for time (Table 7.1). In unbagged flowers, most of the available nectar was foraged during the first day of opening and there was hardly any nectar in the evening.

Table 7.1 The minimal model derived for the number of pollinator visits as a response to the variable time of day.

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	-2.40942	0.83070	-2.900	0.00465 **
Time	0.42455	0.08577	4.950	< 0.001 ***
I(Time^2)	-0.00767	0.00176	-4.359	< 0.001 ***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
Null deviance: 442.07 on 95 degrees of freedom, Residual deviance: 325.52 on 93 degrees of freedom, AIC: 397.66. Number of Fisher Scoring iterations: 2

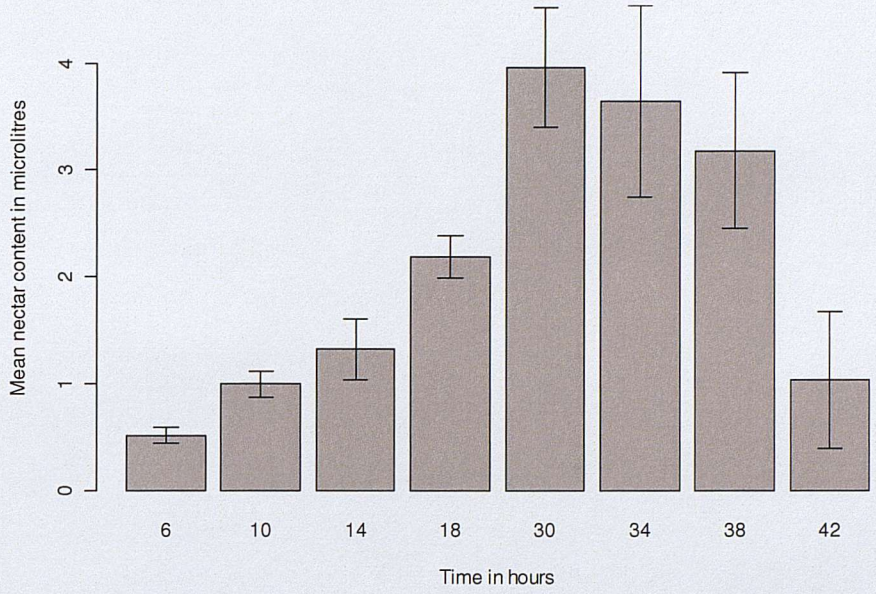


Figure 7.3 Amount of nectar in bagged flowers on the first (06-18 h) and second days (30-42 h) of flower opening (mean ± SE) in *Strobilanthes kunthianus*, Western Ghats, India.

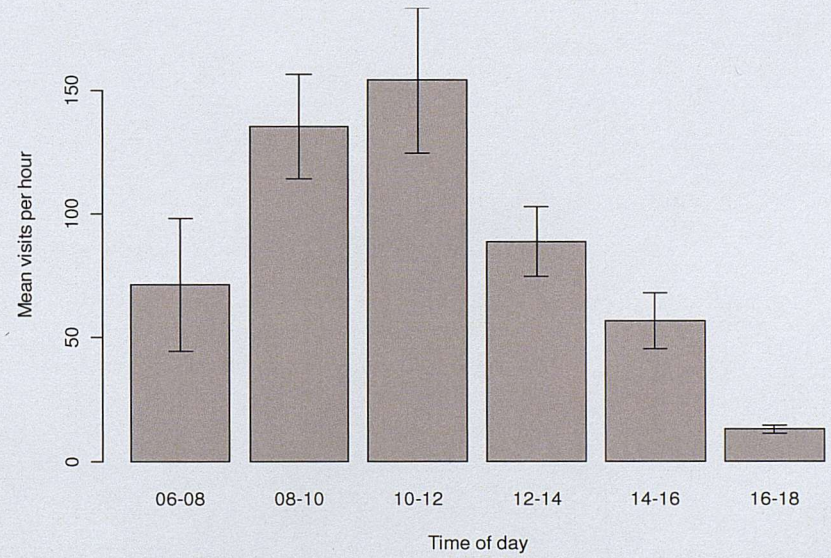


Figure 7.4 Visitation rates for *Apis cerana indica* (mean ± SE) on *Strobilanthes* inflrorescences, Western Ghats, India

The most frequent floral visitor was *A. cerana indica.* The density of the bee population in the study area was high. Bee activity coincided with flower opening and reached its peak between 1000h and 1300 h (Figure 7.4); the activity declined towards the evening.

A generalised linear model with a quasipoisson distribution showed insect visitation frequency to vary significantly throughout the day, reaching a maximum at pre-noon or 1000-1200h (t-value= 3.191, p = 0.003), dropping again in the early evening or 1400-1600h (t-value = 15.071, p <0.001). The foraging time (15–20 s per flower) did not vary markedly, except in the last hour (1800 h) when it was drastically reduced (2.5 s).

The bee remained near the mouth of the corolla tube during pollen foraging (Figure 7.6A) and entered deep into the corolla tube to forage nectar (Figure 7.6B). It was therefore possible to record whether the bee foraged pollen or nectar during each visit (Figure 7.5). Pollen foraging was observed only in the morning hours (0700–1100h), whereas nectar feeding was recorded throughout the day. As the pollen was heavily foraged during the morning hours, there was hardly any left in the afternoon on the first day of opening. Bees foraged nectar exclusively on the second day.

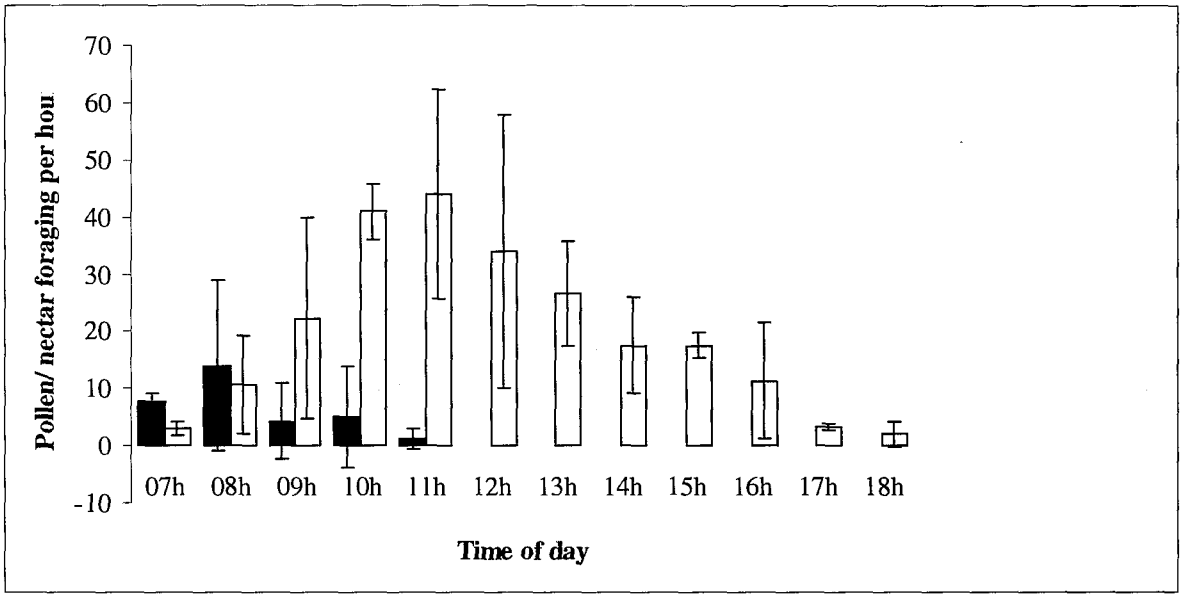


Figure 7.5 Mean foraging visits of *Apis cerana indica* for pollen (filled bars) and nectar (open bars) during the day (mean ± SE) on *Strobilanthes* inflorescences, Western Ghats, India.

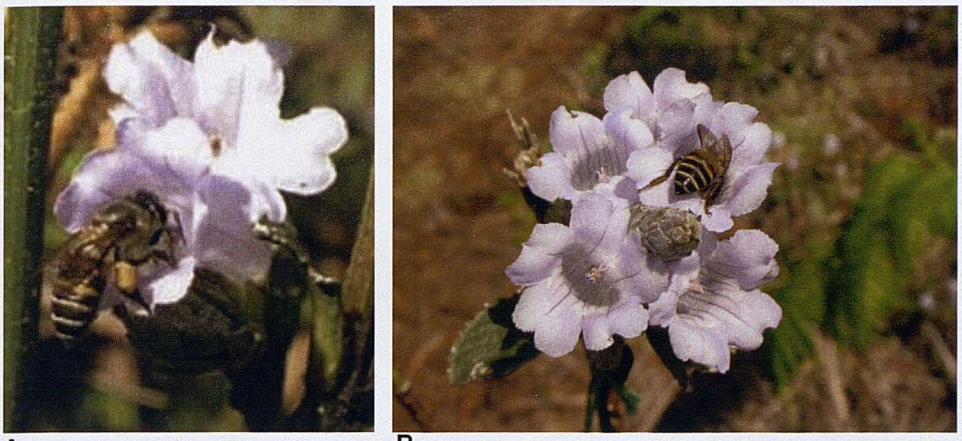


Figure 7.6 *Apis cerana* at the mouth of the flower foraging pollen (A) and entered inside the corolla tube feeding on nectar (B). *Strobilanthes kunthianus* population, Kanthallur, Western Ghats, India.

In the second experiment, the difference in insect visitation per flower between time intervals on day one and day two was tested with a generalised linear model with a poisson distribution of the error term and found highly significant (Table 7.2, Figure 7.7). Although the maximum number of visits occurred on the first day of flower opening (15.4 ± 4.7 visits per flower per hour), a considerable number of bee visits (3.2 ± 3.5 per flower per hour) were recorded on the second day as well.

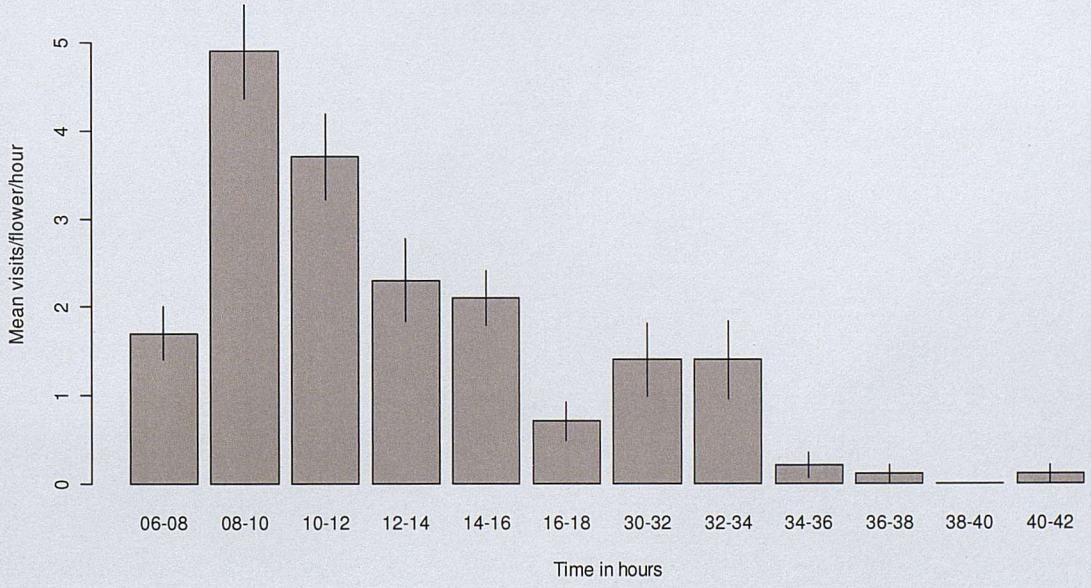


Figure 7.7 Visitation rates per flower per hour on the same set of tagged flowers of *Strobilanthes kunthianus* over the first and second days of opening (mean ± SE).

Table 7.2 The generalised model derived for the number of pollinator visits as a response to time of day on day 1 and day 2 in the *S. kunthianus* population, Kanthallur, Western Ghats, India.

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	0.5306	0.1715	3.094	0.001974 **
Time08-10h	1.0586	0.1990	5.319	<0.001 ***
Time10-12h	0.7777	0.2072	3.754 0	0.000174 ***
Time12-14h	0.3023	0.2262	1.337	0.181371
Time14-16h	0.2113	0.2307	0.916	0.359689
Time16-18h	-0.8873	0.3176	-2.794	0.005203 **
Time30-32h	-0.1942	0.2552	-0.761	0.446773
Time32-34h	-18.8332	1278.4791	-0.015	0.988247
Time34-36h	-18.8332	1278.4791	-0.015	0.988247
Time36-38h	-2.8332	0.7276	-3.894	<0.001 ***
Time38-40h	-18.8332	1278.4791	-0.015	0.988247
Time40-42h	-2.8332	0.7276	-3.894	<0.001 ***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
(Dispersion parameter for poisson family taken to be 1)
Null deviance: 675.77 on 239 degrees of freedom, Residual deviance: 242.03 on 228 degrees of freedom, AIC: 568.42, Number of Fisher Scoring iterations: 16

When a bee enters the flower, it first brushes the receptive surface of the stigma and reaches the anthers for foraging. Bee visit induces two immediate responses in the flower. First, the stigma, which is curved in a fresh flower, exposing the receptive surface to the path of bee entry (Figure 7.9A), straightens and curves slightly backwards within seconds before the bee departs the flower. This movement orients the stigma, particularly the receptive surface, away from the path of the exiting bee (Figures 7.8C & 7.9B). Second, the anthers dehisce immediately on bee contact, exposing the pollen grains; they stick to the ventral surface of the bee during foraging. Observations of immobilised bees showed a considerable number of pollen grains on the lower surface of the body in addition to the two pollen baskets. When exiting the flower, the body of the pollen- loaded bee does not come into contact with the stigma. When the pollen-loaded bee enters another fresh flower, it brushes the outward-facing receptive surface of the curved stigma and thus causes pollination. As the bee tends to move from one flower to another of the same inflorescence before moving to the

neighbouring inflorescence of the same or another plant, there is a high level of geitonogamy. After the bee visit, the stigma eventually comes back to its original position in 30–60 minutes. Movement of the stigma induced by the bee visit can be mimicked by touching the stigma with a needle.



Figures 7.8 **A.** An inflorescence at the peak of flowering. **B.** Freshly opened flowers to show the position of the stigma in an undisturbed virgin flower. **C.** The stigma touched by a needle to induce the backward curvature of the stigma away from the path of the pollinator. Arrows indicate the path of bee entry and exit; arrowheads point to the receptive surface of the stigma.

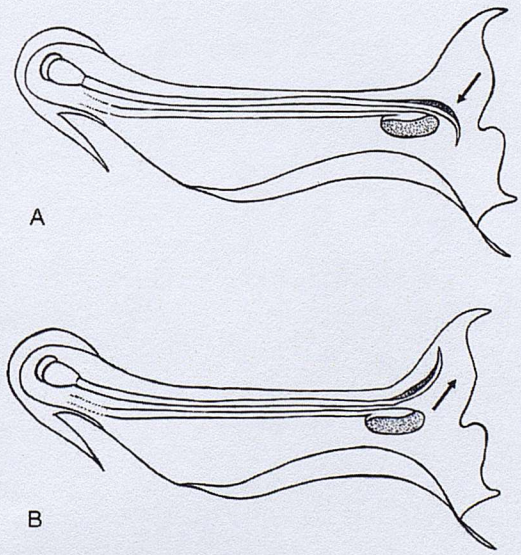


Figure 7.9 Diagrammatic representation of the longitudinal sections of the flower to show the position of the anther and stigma in fresh flower (**A.**) and its changed position in response to bee visit (**B.**). Arrows indicate the path of the incoming and outgoing bee. The receptive surface of the stigma is indicated by dark shading.

The stigmas of flowers exposed to a single bee visit were examined for pollen load under a stereomicroscope; 75% of the flowers with an average pollen load of 8.45 ± 6.1 pollen grains were deposited by a single bee visit (n=20). This pollen load was considered to be adequate for full seed set as it was twice the number of ovules present in the ovary. As most of the flowers receive repeated visits, the pollination efficiency is very high. Microscopic examination of the stigmas collected randomly from senescing flowers (on the third day, n=25) showed 100% pollination.

Apart from *A. cerana indica*, other floral visitors encountered were a Halictid solitary bee, the rock bee (*A. dorsata*), a Scoliid wasp, two species of Hesperid butterfly, and ants. Of these, the Halictid bee carried a pollen load on its ventral surface and legs. *A. dorsata* also carried some pollen on its legs. However, their frequency was low: I encountered four visits of a Halictid bee and two *of A. dorsata* during three days of observation, when compared with 775 visits by *A. cerana indica* during the same period. No pollen load was found on the body of other visitors. A beetle and a grasshopper were seen feeding on the petals; they did not come into contact with the anthers or the stigma. The vaseline- coated slides hung on the branches showed no pollen deposition, suggesting no role of wind in the pollination of this species.

The breeding habit

Fruit set and seed number from the different pollination treatments are summarised in Table 7.3. There was no apomixis, as none of the emasculated and bagged flowers set fruit. A very high proportion of fruit set was observed in all pollination treatments. Although two or three seeds developed in each fruit, some of them remained small and shrivelled in mature fruits.

The generalised linear model with a binomial distribution of the error term showed a slightly significantly higher fruiting success in cross pollinated flowers in comparison to self – pollinated and natural or open pollinated flowers (z-value=2.69, p=0.0071).

Table 7.3 Fruit set (in %) and seed set (± SE) in different pollination treatments in the montane grassland *Strobilanthes kunthianus* population, Kanthallur, Kerala, southern India.

Pollination treatment	No. of flowers	No. of fruits developed	Fruit set (%)	Mean no. seeds/ fruit
Open pollination	100	90	90.00	2.63 ± 1.35
Self pollination	19*	17	89.47	2.60 ± 0.5
Cross pollination	17*	16	94.12	2.71 ± 0.74
Apomixis	21*	0	–	–

*Number of flowers that could be relocated for scoring fruit and seed set. The remaining flowers (from 50 each) could not be relocated due to disturbances by tourists or cattle in some areas of the study site.

Discussion

There are many hypotheses, mostly with reference to bamboos, on the origin of semelpary: predator satiation, fire cycle hypothesis, increase in resource availability, and a mechanism for escaping parasites (Janzen, 1976; Keeley & Bond, 1999). Although the present investigation does not throw any light on the origin of semelpary, it does provide interesting data on the evolution of existing reproductive strategies in semelparous plants to achieve mast seeding.

Floral traits that facilitate pollination efficiency

It is well-established that the floral traits of species influence most aspects of reproduction (Ashman & Majetic, 2006). My observations have highlighted several floral features of S. kunthianus that facilitate pollination efficiency. The extension of floral longevity for two days enables pollination on the second day of flowers that remain unpollinated on the first day.

Although most of the pollen is foraged in the morning hours on the first day, the flowers offer a considerable amount of nectar to the visitors, and the stigma remains receptive for the second day. Pollinators visit the flowers on the second day, although the number of visits is much lower than on the first day. A considerable proportion (60%) of pollen grains also remain viable on the second day, although this may not be a

critical factor for pollination as the bee can bring viable pollen from its visits to fresh flowers.

An interesting feature of the flower of *S. kunthianus* is the dehiscence of anthers only in response to touch by a visiting insect. This keeps the pollen fresh until insect visitation and prevents pollen wastage. It also ensures that most of the available pollen comes into contact with the ventral surface of the pollinator during foraging. Although there are reports of explosive anther dehiscence in several anemophilous species (Bianchini & Pacini, 1996; Taylor et al., 2002), I am unaware of any other species in which anthers dehisce only in response to touch by the pollinator. Reports of the movement of the stamen when touched by the pollinator to bring the anthers into contact with the body of the insect (Faegri & van der Pijl, 1979; Meeuse, 1996) are not comparable with the situation in Strobilanthes.

The stigma in *S. kunthianus* is sensitive to touch by the pollinator and removes the receptive surface away from the path of the exiting pollinator. The change in position is instantaneous and occurs even when the pollinator is still foraging the pollen, thus effectively preventing intrafloral selfing. After 30–60 minutes, the stigma returns to the original position to expose the receptive surface ready to receive pollen from the next bee visit. This curvature cycle of the stigma occurs irrespective of the pollen load and also continues on the second day. Apart from preventing intrafloral selfing, the sensitive stigma in this kind of 'movement herkogamy' (Webb & Lloyd, 1986) effectively removes interference between pollen receipt and pollen export. It however does not prevent geitonogamy.

There are several examples of sensitive stigmas in members of Scrophulariaceae, Bignoniaceae, Martyniaceae, Lentibulariaceae, and Lobeliaceae (Newcombe, 1922, 1924; Stephenson, 1982; Fetscher & Kohn, 1999). The stigma in these species is bilobed and the two lobes spread out at about 180° in fresh flowers. The stigmatic lobes close together in response to touch by the pollinator. Several hypotheses – improves the capture and germination of pollen, prevents intrafloral selfing, and reduces the interference with pollen dissemination – have been put forward to explain the adaptive significance of stigma closure (Webb & Lloyd, 1986; Fetscher & Kohn, 1999; Fetscher, 2002). Detailed studies on *Mimulus aurantiacus* (Fetscher & Kohn, 1999; Fetscher,

2001) have shown that the main adaptive significance of stigma closure is to reduce interference with pollen dissemination. In general, protandry has been attributed to the avoidance of interference in pollen transmission (Bertin & Newman, 1993; Routley et al., 2004). In *S. kunthianus*, the stigma sensitivity is equally effective in avoiding interference in pollen transmission without reducing the period of stigma receptivity.

Two other species of Strobilanthes, *S. sessilis* and *S. ciliata*, also flowered together with *S. kunthianus* in 2006 in other parts of the Western Ghats, and limited data were collected on the floral traits of these species. *Strobilanthes sessilis* is a semelparous species reported to flower once in seven years. The stigma of this species is also sensitive to touch by pollinators, similar to *S. kunthianus*. *Strobilanthes ciliata* is non-semelparous and the stigma is not sensitive to touch (pers. obs.). These features suggest that stigma sensitivity evolved later than semelpary.

Pollen limitation (inadequate quantity or quality of pollen deposition on the stigma for optimal fruit and seed set) is quite common in flowering plants (Burd, 1994; Dudash & Fenster, 1997; Wilcock & Neiland, 2002; Knight et al., 2005). However, in *S. kunthianus*, reproductive synchrony, attracting an adequate number of pollinators, together with other floral traits facilitating pollination resulted in a very high level of pollination efficiency. The species achieves 100% pollination efficiency during the peak of flowering.

Evolutionary constraints and compromises

Bamboos are the only other semelparous species for which some scattered information is available on the breeding system (Janzen, 1976). Studies on bamboos have not involved controlled pollinations and thus no conclusions can be drawn on the breeding system. However, reports of a lack of fruit set in isolated clumps of several species indicate the prevalence of self-incompatibility. In *Bambusa vulgaris*, flowering has been reported from different countries, including India, during the last 150 years; there is no record of seed set in any region (John & Nadgauda, 1997; Koshy & Pushpangadhan, 1997; Koshy & Harikumar, 2000). The lack of seed set in clumps flowering in India has been attributed to pollen sterility and stigma abnormality.

Controlled pollinations on *S. kunthianus* clearly showed that the species is self-compatible. Fruit and seed set were high, even under open-pollination conditions, and was comparable with that realised in controlled pollinations. Self-compatibility seems to have adaptive significance in mast seeding of this species. Although reproductive synchrony, a characteristic feature of semelparous species, is known to enhance the plant's ability to attract pollinators (Janzen, 1976; Augspurger, 1980; Stephenson, 1982), the large number of fresh flowers available on each plant results in a high degree of geitonogamy. As has been reported in many self-incompatible species, pollen quality may be limiting despite adequate pollination if pollinators deposit geitonogamous pollen on the stigma before cross-pollen (Burd, 1994; Knight et al., 2005). This interference by self-pollen can potentially result in low fruit and seed set (Byers, 1995; Ramsey & Vaughton, 2000). If *S. kunthianus* happened to be self-incompatible, its reproductive success would have been significantly reduced because of the prevalence of geitonogamy and the resulting interference of self-pollen. The evolution of self-compatibility has removed this major constraint, although this may have involved evolutionary compromise to a certain extent.

Several cumulative floral traits – the life of the flower extending for two days with adequate floral rewards and stigma receptivity, sensitive stigma to expose the receptive surface to the path of the incoming pollinator and away from the outgoing pollinator, dehiscence of anthers in response to the pollinator's visit, and self-compatibility – have enabled *S. kunthianus* to achieve the mast seeding habit.

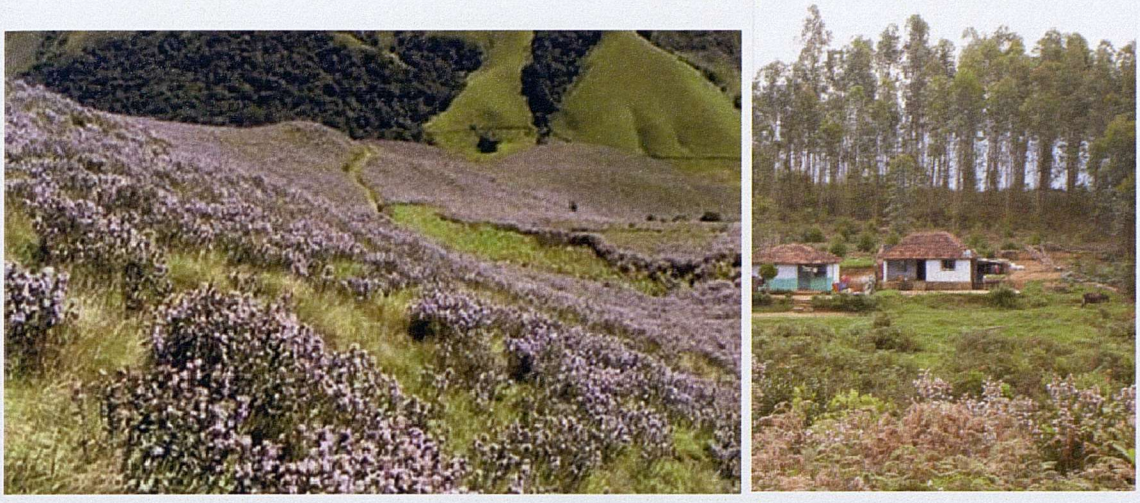
POSTSCRIPT TO CHAPTER VII

While the thrust of the manuscript published was investigating the reproductive strategies in *Strobilanthes kunthianus* (vernacular name: '*Kurinji*' or '*Neelakurinji*'), the underlying objective of the study remained understanding the natural history of the species and examining its reproductive success in the face of the current scenario of threatened habitats in the tropical forest landscape. It describes from the perspective of floral adaptations and pollination efficiency, the strategies developed by a semelparous species to avoid a mass reproductive failure. Floral traits are known to have a significant impact on pollination success (Wilcock & Neiland, 2002) and the study encompasses the ecological and evolutionary aspects to a great extent. The question of whether these rare plant populations will survive the threat of anthropogenic pressure and how habitat connectivity might affect bee movement patterns was beyond the realm of the paper, and this I discuss here.

Vanishing montane grasslands

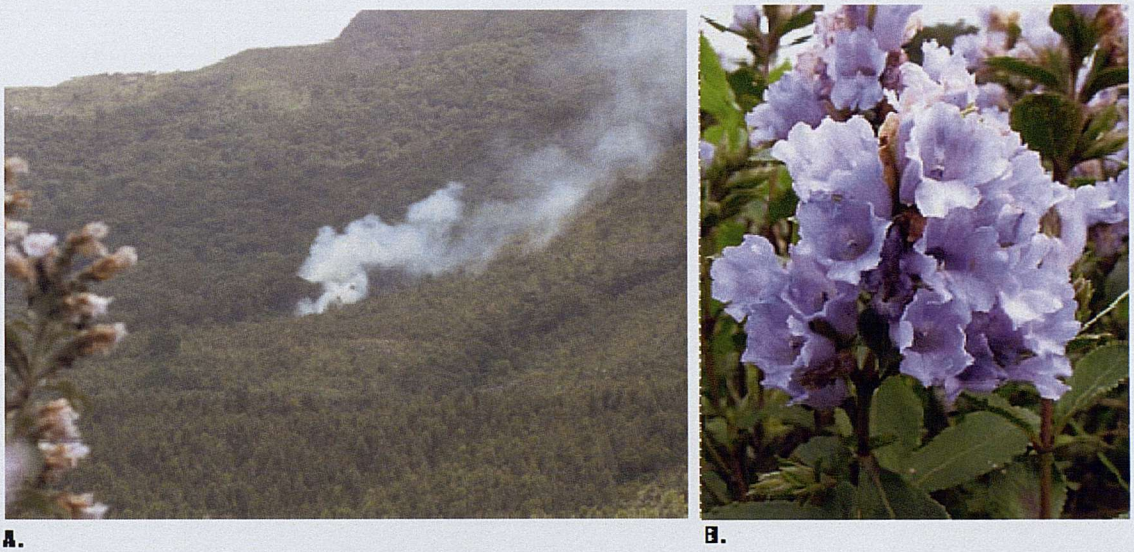
Strobilanthes kunthianus (earlier *Nilgirianthus kunthianus* and *Strobilanthes kunthiana*) exclusively inhabits the montane wet grasslands of the tropical high altitudes of the southern Western Ghats (1500 - 2600 m.). Besides the Western Ghats, it is also seen in the Shevroy Hills of the Eastern Ghats (Rajkumar, 2008). These grasslands form a mosaic with the Shola forests, the stunted evergreen forests in the high-altitude montane rain forests ecoregion, forming a Shola-grassland complex (Figure 7.10A) found only in the south central and southern Western Ghats mountains of Karnataka, Kerala and Tamil Nadu states. They are also the habitat of the endemic mountain goat Nilgiri Tahr (*Hemitragus hylocrius*), which enjoys a high protection status. The earliest reports of the twelve-year flowering cycle for *Strobilanthes kunthianus* in the Nilgiri Hills are from 1934 (Robinson, 1935) and 1970 (Matthew, 1971). The mountain range of Nilgiri (*Neel* meaning blue and *giri* meaning mountain) is known to have derived its name from the gregarious flowering of *S. kunthianus* (Gamble, 1923) that turned the rolling grasslands into a carpet of purple blue flowers every twelve years.

The Genus Strobilanthes is composed of 74 species in southern India and Sri Lanka and



A. B.

Figure 7.10 **A.** Mass bloom of *Strobilanthes kunthianus* in the Nilgiri Hills with the Shola forests visible in the background, **B.** The study population of *S. kunthianus* surrounded by Eucalyptus plantations that have replaced the mosaic of Shola forest.



A. B.

Figure 7.11 The endemic *Strobilanthes kunthianus* (B) in which flowering is a rare event, thrives in habitats affected by stark changes in land use, such as fire and plantation (A).

is often the dominant shrub in upland forests in this region (Scotland, 2000). At altitudesbove 2000 m. exposed to incessant wind, Shola forest trees are typically stunted (Matthew, 1971). Large areas of such vegetation are reported to have been cleared for tea estates, fruit or vegetable culture and buildings during the last century (Robinson, 1935; Matthew, 1971). Evergreen forest species of Strobilanthes were

treated like weeds and cut down to encourage the growth of young tree seedlings (Gamble, 1923). *S. kunthianus* was exterminated as a pest in the high altitude grasslands (Robinson, 1935) and concern has been raised that destruction of habitats, and the selective cutting of Strobilanthes may have led to some species becoming extinct. Anitha and Prasad (2007) warn that mass flowering species of *Strobilanthes* might face the risk of reduced or failure of seed set under unfavourable environmental conditions and unavailability of pollinators.

The study site at Kanthallur was surrounded by heavily degraded land mostly dominated by Eucalyptus plantation (Figure 7.8B) and adjoining them were the Shola forests belonging to the Mannavan Shola, the largest Shola patch in Asia (South Indian Spiders, 2008). The greatest threat to the population was apparently from encroachment and conversion to plantation. The mass flowering attracted thousands of tourists to remote populations such as the one in Kanthallur village, as a spill - over of visitors who could not find access into Eravikulam, a National Park with highly restricted access to public and researchers alike. I witnessed in the study site, signs of irresponsible tourism such as uprooting of *Strobilanthes* plants and littering the landscape. Researchers in the past have raised their concern about Shola - grassland mosaics being continually destroyed since the 1930s from fragmentation, fire and land use change such as grazing and conversion to plantations (Kumar, 1993; Rajkumar, 2008). During the 1970 bloom, Matthew recorded the conspicuous absence of mass migration of jungle fowl, the major seed disperser (Janzen, 1976) and attributed it to "the area under the plant diminishing owing to denudation of virgin land for cultivation". Forty years since then, the pressures on these Strobilanthes habitats have only grown, causing stark negative changes in the land use pattern (e.g. Figure 7.11A).

Such severely degraded habitats present an ideal setting for studying pollinator foraging habits and reproductive success in a mass flowering species under a changing landscape with a non-forest habitat matrix, particularly if they can be compared with more undisturbed flowering populations. The largest and most intact flowering population of Strobilanthes in 2006 was reported from Eravikulam National Park and during the peak blooming season, 3000 - 4000 visitors trekked up the Park's slopes (Mustafa, 2006) on a given day. Eravikulam is one of the most pristine montane grassland - Shola stretches in the Western Ghats (Nair et al., 2001) with high protection status.

Pollinator responses to disturbance

Four decades ago, Matthew (1971) reported the migration of *Apis dorsata* and *Apis cerana indica* during the mass bloom of *Strobilanthes kunthianus,* a huge resource of food for the bees, leading also to an increase in honey yield. *A. dorsata* is the rock bee and *A. cerana indica,* the cavity nesting species, a tropical ecotype of *A. cerana* (Mattu and Verma 1984), both species having large colonies (Koeniger et al., 1975). Robinson (1935) wrote that in 1922, about 28 hives of *Apis dorsata* were found hanging from one Eucalyptus tree in the Nilgiris and 32 were counted on an over hanging rock. Contrary to the expectation, a negligible number of *A. dorsata* visits were encountered during the present study. Research on social bees in Malaysia (Itioka et al., 2001) and India (Devy, 1997) have shown that *A. dorsata* migrate locally and seasonally in response to floral resource availability. An outburst in a mass flowering event such as that of numbers in *Strobilanthes kunthianus* is expected to have normally caused a rapid increase in the and activity of these bees. Recruitment behaviour of social bees is known to increase the quick exploitation of mass flowering trees (Roubik, 1989; Roubik et al., 1995). *A. dorsata* is capable of timing its arrival to specific mass flower resources; Devy's study (1997) reported this migrant behaviour and role in pollination in during the prolonged flowering of an evergreen forest tree *Pallaquium ellipticum.* On the other hand, there is also evidence from an assessment of the presence and relative abundance of bee species by vegetation type in the Nilgiri Biosphere Reserve, that *A. cerana indica* may possibly be associated with the grassland and Shola forest type (Keystone Foundation, 2007a), possibly with their nests in the cavities of trees in the Shola forests. The species is also understood not to migrate (Crane, 1990; Thomas et al., 2009). So it may after all not be surprising that the study population was visited predominantly by *Apis cerana* and not *Apis dorsata.*

Although there were no negative pollination outcomes in the study site owing to an abundance of *Apis cerana* activity and sufficient fruit set, there was a general expectation in the research community we interacted with, that *Apis dorsata* is the potential pollinator of Strobilanthes. In retrospect, it is not clear if the near absence of *A. dorsata* in the Kanthallur population was a result of competition from the *A. cerana indica* that was present in large numbers using up all the available floral resource in the grassland, or due to other community level factors related to flowering intensities of

other angiosperms inside the evergreen forests at lower elevations. If there is an association of bee species by different vegetation types as suggested by the Keystone study, then there is surely more complexity to the "montane grassland *Strobilanthes* and its pollinator story" to what we already know and what we speculate as researchers. It is well-established that there are important linkages between flowering and bee abundances (e.g., (Potts et al., 2003)). Yet, not enough is known about the migratory routes and patterns of *A. dorsata* to make conclusive remarks (Keystone, 2007b). Although *A. dorsata* migration is thought to be influenced by biotic factors such as food plant flowering cycles (Devy, 1997), its migration into montane grasslands and role in pollination of *S. kunthianus* has not been demonstrated since Matthew's study in 1971.

On the one hand, tropical bee communities appear to have a certain degree of resilience (Brosi et al., 2008) and can persist in substantial numbers in human altered habitats (Cane, 2001; Williams et al., 2001b), and on the other, they are known to have suffered losses in fragmented and degraded habitats (Aizen & Feinsinger, 1994a). Less fragmented primary forests are understood to be crucial in maintaining viable bee populations (Liow et al., 2001). If there is in real such a loss of bees in the forests of Western Ghats, it would have direct impacts on forest biodiversity, due to their irreplaceable role as pollinators to several species, and also as a source of livelihood with many local communities depending on honey and wax. In the Nilgiris, *A. cerana* is managed by beekeepers: the Toda tribe look after colonies that are nesting in tree cavities, other communities keep these bees in the walls of their houses and basket hives. *Apis cerana* has been reported to be declining in some regions due to the introduction of *Apis mellifera*, disease and pesticide use (Partap, 1999).

It would have to be an extreme situation if a scene of pollen or pollinator limitation emerged in *S. kunthianus,* where synchronous flowering attracts gregarious pollinators and 100% pollination efficiency is achieved during peak flowering. It does not however seem improbable if the degradation of bee nesting habitats in the evergreen and Shola forest types continues, given the pressures on the entire Western Ghats. I draw attention to the importance of habitat connectivity for pollinator movement, and equally on the viability of the plant populations as their unique grassland habitats rapidly disappear.

CHAPTER VIII

Synthesis

In this concluding chapter, I re-assert the importance of understanding upstream pollination processes in plant species conservation in the tropics. Summarising the main findings of the thesis, the three case studies are linked together, to fit into a 'protecting the Western Ghats' picture within the larger context of pollination systems in tropical forests under severe anthropogenic pressure. I also present justifications and limitations of the research, and then go on to briefly discuss the substantial challenges the study imposes on plant and insect conservation in these landscapes.

The scene for the three studies is set in the Western Ghats, known for their rich biodiversity and endemism (Pascal, 1991; Ramesh, 2001). The region's vegetation is a mosaic of different stages of degradation (Daniels et al., 1995), owing to land uses changes over the past few decades that is believed to cause local species extinctions and erosion of biomass (Pascal, 1988; Nadkarni et al., 1989; Gadgil & Subash Chandran, 1989). In the face of such habitat loss, beyond the risk of species extinctions, is the extinction of ecological interactions, which Janzen (1974) describes as the insidious kind. Using a species - centred view to explore the private world of plant – pollinator interactions in a changing tropical landscape, I find several answers, some conflicting, to the pressing question of 'Will the pollination crisis drive already endangered species to extinction?'.

Pollination guilds in tropical plant communities

My work offers a window into the different strata in the forest structure (Figure 1.1, Chapter I), with *Nothapodytes nimmoniana* representing the under-canopy, *Myristica dactyloides* occupying the intermediate level between the canopy and emergent, and *Strobilanthes kunthianus* a shrub outside the montane Shola forest. A large part of the study contributes to the natural history and autoecology of the three species. Tropical flowers are known to be a basis for the study of floral evolution (Endress, 1994a) and the *Strobilanthes* bloom provided an ideal setting for an exploration of adaptive floral traits in addition to understanding its pollination mechanisms. In the three species, I provide a detailed assessment of the floral display, pollinator reward structure, and the plant breeding system (summarised in Table 8.1), all expected to have an effect on pollination outcomes in disturbed (Smithson & Macnair, 1997; Ghazoul, 2005) and undisturbed systems (Waser, 1983; Chittka & Thomson, 2001).

In spite of the high conservation value, canopy research is a relatively young field in the Indian sub-continent (Devy & Ganesh, 2003). The past decade has seen a whole new push to explore the world of the tree tops and there have been a range of studies from entomological arrays (Srinivasa et al., 2004) and epiphytic compositions to bird nesting patterns (Ramachandran & Ganesh, pers comm.). Tropical flowering trees are a huge resource of food for insects (Roubik, 1990; Kirmse et al., 2003) and many

foraging activities in the canopies lead to pollination. Our perceptions of pollination mechanisms in the inaccessible canopies are often based on crude assessments of floral morphology. The impacts of disturbance on the reproductive output of tropical forest trees remain unquantified partly due to the complexity of plant-pollinator interactions and partly due to the practical difficulties of quantifying interactions in the forest canopy (Ghazoul & McLeish, 2001) and the problems associated with sampling canopy insects (Roubik et al., 2003; Speight, 2005; Leather, 2005) have been talked about. Many simple questions remain unanswered in this arena: how many species of arthropods live in the canopy of various tree species and forest types, what their resource base is, and how their ecological niches have evolved (May, 1994). Other than a few social bees, the pollinating organisms in tropical systems are poorly known, which impedes further studies on their populations and foraging movements (Borges, 2003). Even in the most studied bee groups, the extent of negative effects of habitat change is not fully understood (Liow et al., 2001; Brosi et al., 2008).

Bees are understood to be the dominant pollinator group in the tropics (Endress, 1994a; Liow et al., 2001), owing to their wide foraging range and preference for high floral densities (Roubik, 1990). Two species of *Apis* were encountered in all my three studies: *A. dorsata* were recorded at the moist deciduous site on *N. nimmoniana* and also in the grassland on flowers of *S. kunthianus*, while *A. cerana indica* were seen foraging voraciously on *S. kunthianus* and in smaller frequencies in the evergreen canopy of *M. dactyloides*. Apidae – in particular, *Trigona* – are seen everywhere in rain forests (Appanah et al., 1986) and offer important pollinator services (Sakai et al., 1999), especially in the understorey (Bawa & Opler, 1975; Appanah, 1981). In my study, they were seen at flowers of the sub-canopy tree *N. nimmoniana* in a moist deciduous forest type. The short-tongued Halictid bee revealed a pollen load when captured on *M. dactyloides*. On *N. nimmoniana,* its visits were limited and the single bee that was captured did not show a pollen load and it was not possible to conclude its role in pollination. Calliphorid flies emerged as the most important pollinator in the *N. nimmoniana* study, and Syrphid and Phorid flies contributed to the pollinator assemblage in *M. dactyloides*. Flies are efficient pollinators (Endress, 1994b), but lack of appreciation of their importance in pollination systems (Kearns, 2001) has led to Dipteran populations being neglected in forest entomology studies (Borges, 2003).

Table 8.1 A summary of the main findings of the study, listing the populations, the breeding system of the species and the pollinator assemblage observed. The threats faced by the species and the populations in particular are highlighted.

Plant Species and Form	**Forest Type**	**Site(s)/ Coordinates**	**Study Season**	**Pollinator Assemblage**	**Breeding System**	**Threats**
Nothapodytes nimmoniana Sub - canopy tree	Dry & moist deciduous; Evergreen	B.R. Hills WLS N 11° 57' 079" E 77° 09' 632" Dandeli WLS N 15° 10' 115" E 74° 28' 919"	May – Aug 2005 May – Aug 2006	Dipterans (Calliphorid flies) *Apis dorsata* *Trigona iridipennis* Halictid bees (limited visits)	Male, female and polygamous trees - Predominant outcrossing	Over-exploitation of wood Populations declining Depleting floral resources
Myristica dactyloides - Intermediate tree	Evergreen	B.R.Hills WLS N 11° 54' 442" E 77° 11' 189" Brahmagiri WLS N 11° 57' 790" E 75° 58' 380"	Dec 05 – Apr 2006 Dec 06 – Apr 2007	Beetles Thrips Dipterans (Syrphid and Phorid flies) Halictid bees *Apis cerana* (limited visits)	Male and female trees - Strict outcrossing	NTFP harvest causing gaps in flowering years and a possible decline in regeneration
Strobilanthes kunthianus - Shrub	Montane grassland	Kanthallur (near Mannavan Shola National Park) in the Nilgiri Hills N 10° 13' 12.6" E 77° 12' 14.9"	Aug – Sept 2006	*Apis cerana* *Apis dorsata* (limited visits)	Plants with hermaphrodite flowers – Outcrossing and Self-compatible	Habitats facing severe degradation – fire, plantations, grazing

Thrips and beetles are some of the lesser known insect taxa, both apparently significant pollinators at the community level in the evergreen forests (see Chapter V and references therein).

The high density blue flowers of *Strobilanthes* sp. present a case of bee specialised pollination, as has been observed in other high montane habitats (Feinsinger, 1983). Both tree species reveal a continuum of generalist to specialist pollination composed of a diverse group of small insects, although *N. nimmoniana* appeared predominantly fly pollinated and *M. dactyloides* largely beetle preferred. Such loose niches (Roubik et al., 2003) and asymmetry in interactions (specialist interacting with generalist) (Vazquez & Aizen, 2004) are more common in tropical systems than earlier thought to be. Many floral generalists are actually specialists for high density resources; low intensity flowering and spatial isolation of flowering individuals can make even the generalists vulnerable to extinction (Pimm & Pimm, 1982). Similarly when a plant is a generalist, it implies pollination by specialist and generalists alike (Bascompte et al., 2003; Vazquez & Aizen, 2004), making them susceptible to loss of a particular pollinator in adverse conditions (Ashworth et al., 2004, also see Discussion in Chapter IV). I argue that being generalist in nature as against the expected and more romantic - 'specialised' does not make these interactions (and so the plants and insects) less susceptible to negative impacts of disturbance. This contradicts the conventional belief that species with multiple pollinators are more resilient to fluctuations (Bond, 1994).

Disturbance and the intricacies of interactions

Human - induced forces exert severe pressures on the plant and animal populations inhabiting diverse forest types in the Western Ghats – conversion to plantation, harvest of non-timber forest produce (NTFP) and fire are just a few examples. The *N. nimmoniana* study was replicated in a disturbed population with altered flowering intensities caused due to over exploitation of wood chips from the tree trunk and branches; this gave the potential for examining likely changes in the response of pollinators. Although there was no evident pollination limitation, there was a shift in pollinator assemblage - my sampling in the disturbed site showed an absence of the stingless bee *Trigona iridipennis*. Having short foraging ranges of less than 200 m (Bawa & Opler, 1975) and finding suitable cavity nest sites in large trees (Koeniger &

Vorwohl, 1979) are both limiting factors for the density of some Trigona (Inoue et al., 1990). This obviously raises concern about the habitat quality and connectivity for such insects in parts of the Western Ghats that are severely fragmented. There was low fruit set in a lean male flowering year in the undisturbed site, pointing towards pollen limitation in the population in the given year. This is a clear illustration of how insufficient floral densities in a population with altered mating structure (due to lopping of entire flowering tree branches) can have a direct influence on reproductive success. In *M. dactyloides,* observations were carried out in undisturbed populations, and my findings suggest ample pollination and fruit set. There was variation in flowering and fruiting intensities of female trees within populations and across study years. My data on faunal assemblage is preliminary and limited to a short sampling period and is bound to be more comprehensive if a long - term monitoring of insects in the Myristica canopy is carried out. Beetle assemblages elsewhere in the tropics are reported to respond to anthropogenic disturbance (Floren & Linsenmair, 2003; Speight et al., 2003) and a comparative study of the kind initiated in *Nothapodytes* may hold answers to significant questions on pollination mechanisms in human dominated landscapes.

In the context of such comparisons of canopy insects in primary forests and their degraded or managed counterparts, Speight et al. (2003) recommend a 'functional niche' or 'trophic groups' approach for a better understanding of ecosystem functioning and community structure, as against the 'species richness and abundance' approach. Indeed, pollination studies demand a full inventory of species composition (to compare sites, for instance) and I use this method in my study species. In two recent studies from the Western Ghats carried out on *Myristica dactyloides* (Chapter IV) and *Myristica fatua* (Sinu and Sharma, in review), the functional guilds approach was used and it clearly added the dimension of community and functional niche to a pollination study.

In the mass blooming *S. kunthianus*, I only had access to one study site, which was surrounded by Eucalyptus plantations and encroached land. Such a huge floral resource in the open grassland – Shola ecosystem, was naturally reason enough for the abundant activity of the bees (*Apis cerana indica*) encountered in the study, although no bee hives were observed around in the habitat. Encroachment, conversion to plantation, fire, grazing and irresponsible tourism emerged as the predominant threats in this exclusive montane grassland habitat of *S. kunthianus.* It is crucial that we see

pollination systems as part of the larger habitat and ecosystem. Foraging sites may be spatially separated, occupying different forest types, and this is why researchers have emphasised the size of preserves as an important factor in preserving plant – pollinator interactions (Spira, 2001).

Endangered plants, such as my study species, seem to exist in 'ecological traps' surrounded by contrasting habitat matrices with varying degrees of disturbance (Stebbins, 1979), and Kearns & Inouye (1997) point out that like plants, pollinators may also persist in such 'traps' as a result of disturbance - induced loss of nesting sites or food plants. Plants under the threat from human induced changes could face increased selection pressure to specialise on certain pollinators or diversify into different phenological niches (Vamosi et al., 2006). From the pollinator perspective, habitat connectivity is more important than we ever understood - bees must find suitable nesting sites, butterflies enough larval food plants, and they must all be able to locate sufficient floral resources for their pollen and nectar intake. Habitat conversion can reduce the abundance and diversity of insect pollinators (Powell & Powell, 1987; Rathcke & Jules, 1993; Aizen & Feinsinger, 1994a). On the other hand, some insects such as bees (Brosi et al., 2008) and some pollination systems are quite resilient to anthropogenic changes, and pollen flow between trees in fragments separated by several kilometres has been reported recently (White et al., 2002; Harris & Johnson, 2004). Also, certain insect groups are known to flourish well in logged forests and plantations (Chung et al., 2001).

Understanding the plant perspective of pollination requires a shift from the rather obvious subject of pollinator diversity, specificity, movement and foraging patterns, to the more subtle aspects of plant reproduction. Plant breeding system, which is studied in most reproductive ecology research, is but one attribute. Some of the questions that arose during this research provided more clarity to my findings. Does the cost of reproduction differ between sexes in dioecious species? What are the advantages of separating the pollen and nectar rewards between individual trees at a spatial scale, with the added cost of tapping the right pollinator for outcrossing? How important are environment cues in determining plant sexuality; do severe disturbances cause the perturbations and variation in the morphology of *Nothapodytes nimmoniana*? Why do

some plants delay flowering; does it help them achieve assured reproductive efficiency?

My case studies may have demonstrated a certain degree of flexibility in pollination interactions such as generalist pollination and self - compatibility. In some other tropical systems, however, a compensatory mechanism for pollinator loss may be lacking. Since this is the first study on pollination success for each of the study species, it might be too early to conclude whether there is a decline in pollinator assemblages and a consequent dip in fruit set and seedling recruitment, without having an earlier reference point. Only consistent, long - term monitoring would give us any evidence on 'change' in the interaction web or in the regeneration pattern (Roubik, 2001). It is important to bear in mind though that pollination failure can occur at all stages and different levels in the pollen dispersal in a plant's life cycle (Wilcock & Neiland, 2002) and many a times, results of particular studies are an artefact of the several possible combinations of plant traits, e.g., breeding systems, degree of specialisation in pollinators, rarity, and ecosystem properties. Several factors may have influenced the outcome of my pollination studies, both biological and environment related. Floral resource availability and plant breeding systems are important determinants of pollination success in altered landscapes; yet, the scale at which pollinator services are affected by disturbance could vary for different pollinator taxa and may depend on pollinator movements between plants (foraging sites) and nesting sites (Somanathan & Borges, 2000). Additionally there are inherent variations in flowering intensities (MacKinnon, 1998) and marked year to year shifts are common in insect populations, particularly bees (Roubik, 1996; Roubik, 2001).

Detours? Not really.

To predict the reproductive vulnerability of plants to habitat disturbance, it is useful, as demonstrated in the three studies, to assess the plant and animal sides of mutualistic interactions. What might appear like an occasional detour (into evolution of the breeding system in *Nothapodytes* sp. (Chapter IV) or into disturbance factors independent of the pollination phenomena in the study on *Myristica* sp. (Chapter VI), will seem justified only if we realise the significance of the evolutionary setting of the plants, and of the history of anthropogenic disturbance in questions addressing plant

reproductive success. Taking into account other realms in the dynamics of plant reproduction also adds an exciting dimension to the study.

The intriguing sexuality in *Nothapodytes* sp. when observed closely by examining the functional gender of the sex types and by experimental pollinations, helped develop the possible evolutionary pathway of dioecy from heterostyly in the species. A literature search on the correlation between entomophily and dioecy opens out a set of theories and arguments about the evolution of dioecy in heterostylous taxa as a result of disruption of the pollen flow complementarity that had permitted individuals in heterostylous species to function as simultaneous hermaphrodites. Thus a possible change in pollination interactions in these species is perhaps responsible for the evolution of dioecy. The frequent selfing in polygamous individuals may, in fact, be of selective advantage in *Nothapodytes* sp. acting as a reproductive assurance during times of pollinator depression or absence of mates (Baker, 1955). It is definitely true that plant - pollinator interactions in tropical forests offer unique opportunities to address several problems of current evolutionary and ecological interest (Bawa, 1990).

When the *Myristica* study was initiated, the protocol on pollination ecology was meant to be replicated in a disturbed site to compare with data from the undisturbed BR Hills population. As it turned out, when new populations were identified across the State of Karnataka, a suitable female flowering tree was not always located. This temporal variation is characteristic of tropical forest phenomenon (Roubik, 1990; MacKinnon, 1998). Devy et al (1998) also talk of the unpredictability of yearly flowering in a southern Western Ghats forest. When flowering trees were located, it was not always possible to plan the logistics of constructing a canopy access ladder and finding a base camp close enough for field work. Only the site in BR Hills provided the leverage to set up the canopy ladder using help from the indigenous Soliga tribals; and a study spanning two consecutive flowering years was set up. To overcome the shortfall in the study due to absence of disturbed sites for comparative studies on pollination success, a new design was developed, where 'absolute fruit set' in nine *Myristica* populations was recorded as a response to a set of defined disturbance factors, independent of flowering intensities or pollination success. In Chapter VI, I demonstrate that fruit crop size was negatively influenced by unsustainable methods of harvesting fruits of *Myristica* and in the model, a non-forest matrix surrounding the population showed a negative

interactive effect with destructive harvest method on fruit crop size. It also highlighted the potential impact of thinning fruiting canopies on frugivore activity and on the evergreen forest composition as a whole. The species and the Family Myristicaceae have been reported to be under-represented in seedling recruitment studies from primary forests of Western Ghats (Ramesh, 1989) and south-east Asia (Ercelawn et al., 1998), and this part of my study on fruit crop size in *M. dactyloides* at a population level holds some insight into the long - term impacts of overharvesting on regeneration in evergreen forests.

Why a species - centred approach?

For the maintenance of biodiversity, mutualisms or interaction networks, be it predation, parasitism or pollination, are central (Bascompte et al., 2003; Tylianakis et al., 2008). In the context of pollination, maintenance of a particular plant species in an ecosystem may be contingent upon the presence of the right pollinators and also on other plant species that serve as a continuing resource for its pollinator. Howe and Westley (1988) call mutualisms uneasy partnerships and point out that the disappearance of key flowering and fruiting plants is pushing animals dependent on them to the brink of extinction. At the same time, the survival of the pollinating agents is fundamental to the viability of angiosperm populations (Havens, 1999). These interactions also are crucial to maintain the resilience and resistance of 'ecosystem services' (Ives & Carpenter, 2007; Tylianakis et al., 2008).

Plants exist in communities. Despite large number of species sharing similar ecology in the biodiversity rich rainforests, there is strict niche partitioning in these systems (Turner, 2001). Complete community-level characterisation of pollination was not known to have been carried out in any tropical forest site in the world (Shatz, 1990). Evidently it is a huge task that has been attempted but a few times. Bawa and others (Bawa et al., 1985a) and later Kress and Beach (Kress & Beach, 1994) conducted extensive studies on the pollination mechanisms of over a hundred species occupying distinct canopy strata in a neotropical lowland forest in Costa Rica. From the Old World tropics, Momose et al. (1998b) studied pollination systems of 270 species in a lowland Dipterocarp forest of Sarawak. A ten - year long survey of pollination modes in over 80 species in the southern Western Ghats by Devy and Davidar (2003) reported

a low diversity of pollinator species and low fruit set in some tree species due to pollinator deficit.

Admittedly, a community wide study was not feasible to carry out with the limited manpower and resources an 'independent' doctoral research offered. The next plausible and answerable question of interest to my mind was asking if plant - animal interactions are negatively influenced by disturbance in individual species already facing threat. Different species of tropical plants adopt unique solutions to complex environmental problems (Turner, 2001) including habitat change, and if we look carefully, there are patterns in plant – animal interactive systems that have adaptive significance. The species specific approach gave me the advantage of asking detailed questions on floral displays, pollinator types and specificities, breeding system in the population, all in the context of disturbance – aspects that need to be considered together (Somanathan et al., 2004) to determine what factors influence reproductive success.

Species are a measure of biodiversity and to make reliable judgements on the conservation value of a species, it is crucial to know its habitat requirements, its geographical range and also its evolutionary and taxonomic importance in terms of its uniqueness in the evolutionary lineage (Padmanabhan & Gadgil, 2000). In a world where habitats are being transformed into what does not favour biodiversity and there are reported losses and shifts in species and their distribution (e.g. Simberloff, 1988), it is a constant struggle to keep up with the ever changing definitions and demands of 'conservation'. When species are considered as resources, then conservation takes on a different meaning, the emphasis shifting to resource sustenance for prolonged extraction of the resource (Milner-Gulland & Mace, 1998). In this anthropogenic sense, species are also like the touchstone of whether or not we are using the planet sustainably (Kunin & Lawton, 1996).

The future

There are several exciting hypotheses that can be tested in the evergreen forest canopy when addressed beyond the single species level. Do dominant species drive the pollinator dynamics in a forest habitat (Devy & Ganesh, 2006)? Are the pollination networks in the forests of Western Ghats strictly specialist or generalist? Does there

exist some degree of asymmetry in plant pollinator interactions? Intensive studies at the community level are bound to throw light on pollinator dynamics at the canopy level, and will help find answers to questions related to plant and pollinator populations, habitat connectivity and broadly conservation.

Meanwhile, prospects for the three species of the study are rather discouraging when projected at a long - term scale. *Nothapodytes nimmoniana* inhabits the unique moist deciduous forests of the Western Ghats. The species has no recorded history of NTFP harvest but the past twenty years have seen a 20% decline in its populations across India, due to a sudden market demand in the pharmaceutical industry for its wood chips extract. *Myristica dactyloides* is a critical canopy component in riparian stretches of evergreen forests and the threat it faces from over harvesting of fruits for trade in spice and traditional medicine has made populations extremely vulnerable. We must be particularly alert to the status of canopy inhabitants as tropical forests face rapid habitat loss across the world (Basset et al., 2003). In *Strobilanthes kunthianus*, studies from as early as 1974 report habitat degradation and absence of seed dispersers. Flowering in the species being a rare event makes the case for preserving the vanishing *Kurinji* habitats even stronger: the question is whether we can afford to wait another twelve years to study and make interpretations and the necessary policy recommendations. Studies in other tropical forests have proven that declining populations of bees (Aizen & Feinsinger, 1994b; Liow et al., 2001) and canopy beetle families (Floren & Linsenmair, 2003) in disturbed landscapes are no more a speculation or myth. We need a better understanding of these processes and the only way to achieve this is to gather more empirical results to detect the effects of habitat fragmentation on critical ecological processes such as the reproductive performance of plant species and functional dynamics of their insect partners.

Each part of the thesis could stand alone as a contribution to pollination studies in the Western Ghats, especially since the species are either endemic, represent a dominant or climax component of the community, or are unique in their life history and breeding system. But the real message is for them to be held not in isolation as separate individuals in separate forest types or habitats, but rather to comprehend the Western Ghats as one large living system with a unique geographic and evolutionary history, with thousands of species and patterns and processes, all inter - connected. Along with

urging not to underestimate the value of individual species, the purpose of the thesis is to loop back into the significance of maintaining natural pollination systems in tropical forests and for slowing the rate of species loss, through habitat preservation (Spira, 2001). This, in the context of the Western Ghats, means establishing stricter conservation guidelines to assure habitat connectivity, long - term monitoring of regeneration patterns of whole communities in different forest types, and if required species enrichment programs to supplement the efforts. Trade in non - timber forest products is an inseparable element of the socio- economic fabric in forest dependent communities. The recent paradigm in environmental conservation in the region has been of community participation. The Western Ghats are home to several conservation regimes, some of them with success stories of community mediated conservation. The data generated though my research on pollination systems in the natural populations of the two tree species could be used to design tree recruitment programs and alternative propagation sites for extraction of NTFP through establishing a rapport with local communities. Spira (2001) also recommends a community or ecosystem based management for protecting pollination mechanisms given the multiple species assemblages and large landscapes that support plants and pollinators.

Tropical forest ecosystems are among the most vulnerable to climate change variability and long - term changes in temperature and rainfall. This in turn could foster reduction of suitable natural habitats (Centre for International Forestry Research (CIFOR), 2009) and cause phenological shifts in plant species. According to recent research, plant – pollinator networks are extremely susceptible to global environment change, with up to 50% of pollinator species expected to be unfavourably affected (Memmott et al., 2007). Added to this are threats from increased exposure of pollinators to invasive competitors (Chittka & Schurkens, 2001; Ghazoul, 2004) to parasites and diseases, to pesticides (Buchmann & Nabhan, 1996b) and to changing climate, issues not addressed in my work.

The findings and further questions raised in this thesis clearly have application beyond the study circumstances. The precise causes of pollinator decline have always been hard to identify, with a suite of factors influencing it - fragment size, variation and distance between flowering individuals and the environment of surrounding matrix (Jennersten, 1988; Rathcke & Jules, 1993). Nevertheless, this study along with several

others (mostly reviewed in Ghazoul, 2005) in the past decade provide evidence that pollination failure can occur in varying levels and magnitudes in different populations and habitats. Different pollinator species respond to changes in land use at varying spatial scales (Steffan-Dewenter et al., 2001; Somanathan et al., 2004). The relevance of this for conservation in the tropics is more than obvious – isolated plant populations may become reproductively unviable, or head towards extinction owing to reduced reproductive capacity. Most recent research (Steffan-Dewenter & Westphal, 2008) on disturbance related pollination has emphasised on the need for incorporating landscape components of pollinator diversity (such as food and nesting sources). The fact that plant - pollinator interaction dynamics in unprotected habitats are least predictable (Roubik, 2001) presents itself as a compromise and challenge for future studies. Considering two or more ecological traits of the species (e.g. breeding system, pollination and specificity) as explanatory factors, the manner in which addressed in this thesis, has been recommended for better predictions of plant reproductive vulnerability to habitat fragmentation (Ashworth et al., 2004). As Corlett (2007) warns, only time-consuming, species-by-species experiments can perhaps detect if there are actual pollinator declines and if changes in pollinator abundance or behaviour affect offspring quality. The broader question remains how committed we are as field ecologists and conservation biologists to face the challenges imposed by the relentless pressures from ill-conceived development projects in the region and how can nature conservation be aided through inculcating a conservation ethic in the society and through actively influencing policy in the region.

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