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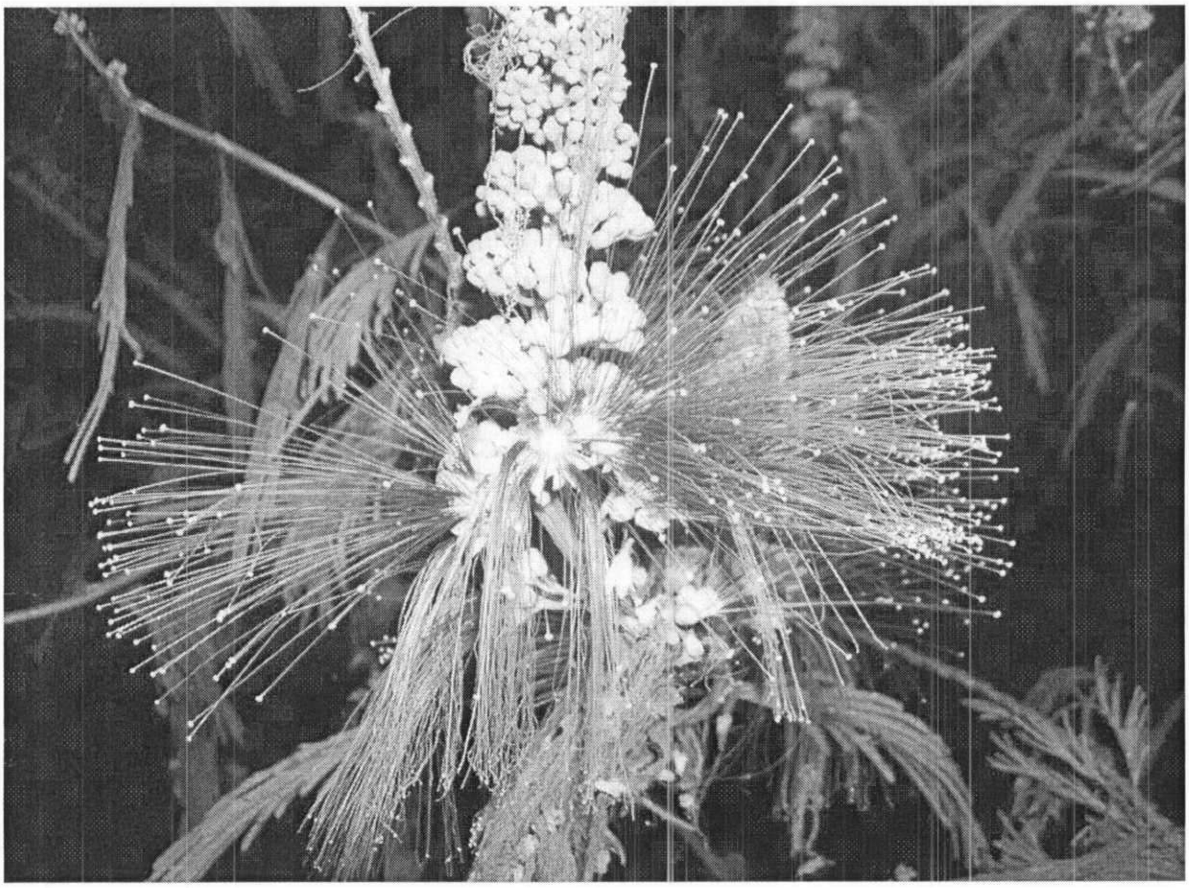
Generalisation and specialisation in tropical pollination systems



MSc Thesis

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Contents

Photos and drawing by Kim Jensen

Resumé

I dette studie har jeg undersøgt bestøvningssystemet hos to små tropiske træer, *Calliandra calothyrsus* i Kenya og *Micromelum minutum* i Vietnam. En stor del af feltarbejdet har været baseret på observationer af bestøverne, når de besøgte blomsterne for at hente nektar. Bestøvernes adfærd og antal under blomsterbesøg er af stor betydning for, hvor meget pollen de enkelte arter overfører fra blomst til blomst.

Calliandra calothyrsus indføres for tiden til skovagerbrug i store dele af troperne. I Østafrika har man haft problemer med, at træet ikke producerer frø nok, så det kan blive fordelt effektivt rundt til landbrugerne. En grund til den lave frøproduktion kan være, at træet ikke bliver bestøvet så godt i Østafrika som i Centralamerika, hvor det stammer fra. Observationer af blomsterne viste, at de besøges af et stort antal honningbier og mange fuglearter om morgenen og sidst på dagen. Hverken fugle eller bier kom dog i kontakt med støvknapper og støvfang, og de kan derfor kun regnes som nektartyve. Om natten blev blomsterne i nogle områder besøgt af flyvende hunde. De kom på det tidspunkt, hvor blomsternes nektarindhold var højest, støvknapperne havde åbnet sig og støvfangene var mest modtagelige for pollen. Samtidig rørte de støvknapper og støvfang for hvert blomsterbesøg. Kun flyvende hunde så ud til at bestøve blomsterne, hvilket tyder på, at træet er specialiseret til flagermusbestøvning. Desværre var de ikke så flittige bestøvere, som man kunne ønske sig. For at tiltrække flere flyvende hunde til de frøproducerende træer foreslår jeg, at blomsterstandene gøres lettere tilgængelige. Det kan gøres ved at plante træerne i rækker og lade dem blive høje og åbne. Så kan de flyvende hunde nemmere komme til blomsterne uden at baske deres vinger imod løv og grene, der ellers forhindrer dem i at besøge de fleste blomsterstande.

I Vietnam undersøgte jeg blomsterbesøgerne til *Micromelum minutum* i Huu Lien naturreservat nord for Hanoi. Undersøgelsen foregik i december, hvor vejret var køligt, og kun på varme dage blev blomsterne besøgt af insekter. *Micromelum minutum* blev besøgt af et stort antal arter inden for flere insektordener. De mest talrige blomsterbesøgere var sommerfugle, men også bier, hvepse og fluer besøgte blomsterne. Halvdelen af sommerfuglene og bierne bar pollen på kroppen eller snabelen, og det samme gjorde nogle af hvepsene og fluerne. Sammenholdt med observationer af blomsterbesøgernes adfærd tyder det på, at *M. minutum* kan blive bestøvet af forskellige typer insekter. Træet har derfor et generaliseret bestøvningssystem, hvilket kan være en fordel i den kolde blomstringsårstid, hvor kun få dage bringer insekter på vingerne.

General introduction: are tropical pollination systems generalised or specialised?

Flowering plants are visited by different numbers of pollinator species. Some flowers are visited by a high diversity of pollinators, while others receive visits from only a few or maybe a single pollinator species. Likewise, some pollinator species visit a wide range of flower species, while others visit only few flower types. The levels of specialisation or generalisation have evolved in complex evolution between the plants and their pollinators. Selection pressures may be conflicting and changing over time as species abundances fluctuate and ranges increase or decrease.

Generalisation and specialisation is not a dichotomy but a continuum of higher and lower levels (Waser et al. 1996, Olesen 2000), which makes most sense in comparison between species. The specialisation level of a plant species can be defined by the number of animal species visiting and pollinating the plant. Another approach is to count the number of pollinator families. The most informative approach, maybe, is to lump the visiting animals into functional groups or guilds, which possess similar morphological and behavioural traits. Often, the taxonomical groups also form the most applicable functional groups, but this is not always the case. For example, a bee and a fly species may belong to the same functional group because their body features are alike, e.g. they may both possess a long proboscis. They therefore may perform a uniform selective pressure on the flowers. The difficulty in using functional groups is where to set the limits. The specialisation level of a pollinator is found in a similar way, by counting the number of species, families, and functional groups of flowering plants it visits. When measuring the level of generalisation or specialisation, the randomness by which pollinating animals are attracted to the flowers should also be taken into consideration (Olesen 2000). If a plant is pollinated by few species, but these constitute a large proportion of the flower-visiting animals in the area, the plant is not necessarily a specialist.
The pollination efficiency of different flower visitors is an important trait to include. Following Stebbins' (1970) "most effective pollinator principle", the most effective pollinator species are those that transfer most viable pollen to the stigmas. This is influenced by the abundance of the pollinator, but also by its pollination efficiency, i.e. the number of pollen grains transferred to stigmas per flower visit. A plant may receive most pollen from less abundant flower visitors, and the floral specialisation level to these pollinators should be considered higher.

Whether a specialised plant-pollinator interaction is the result of coevolution is often hard to say, as species may be pre-adapted to each other. Often, coevolution is asymmetric. For example, a specialised plant that is pollinated by a generalised pollinator has adapted more to the pollinator, than the pollinator has adapted to the flowers of the plant.

Floral adaptations to special groups of pollinators are sometimes described as pollination syndromes (Faegri and van der Pijl 1971). Plants with flowers that fit into a pollination syndrome should not automatically be regarded as specialised, though, as these plants are normally much more generalised than the pollination syndrome suggests (Waser et al. 1996). But still, pollination syndromes are good indicators of the most efficient functional group of pollinators, as these are the ones that exert the highest selective pressure on floral traits (Fenster et al. 2004).

Tropical forests are known to contain a higher diversity of species than anywhere else (Whitmore 1998). Generally, the number of both plant and animal species increases towards the tropics (MacArthur 1972). The higher species density in tropical forests entails that each species is relatively rarer. Populations or individuals are more scattered, inferring longer distances of pollen flow for cross pollination. Probably for this reason, wind pollination in the tropics is rare compared to temperate zones, as too few pollen grains would reach the stigma of a conspecific individual. For pollen to reach the stigma of another individual, transference must be precise, eventually involving specialised pollinators. The higher species density in the tropics might also have effected a higher niche differentiation driven by interspecific competition, leading towards higher levels of specialisation. In the tropics, pollination systems seem more finely grouped, and additional pollination systems, which do not exist in the temperate zone, are found here. The most conspicuous examples are pollination by birds and bats, but also deceit pollination, presentation of oil or resin rewards, brood rearing sites and others are examples of specialised pollination systems only found in the tropics (Fenster et al. 2004). It is a general consensus amongst biologists that tropical pollination systems are more specialised than temperate systems (Johnson and Steiner 2000).

For precise pollen transference to occur, a pollinator should be restricted to visit only a few flowering plant species at a time. Big bang and steady state flowering are at the extremes of a continuum of flowering strategies (Gentry 1974). Plants, which flower at a steady state during a prolonged period, are able to attract specialised and efficient pollinators and exclude more generalised but less efficient pollinators. Traplining pollinators can rely on the nectar of a steady state flowering plant and will be a loyal pollinator. At the other extreme, mass flowering, the nectar supply is so large that pollinator species will restrict their visits to this species during its short time of flowering. A large amount of generalised pollinators will be attracted, and if they stay loyal to the species during the short but highly rewarding flowering period, they will all aid in effective pollination of the species.

In the tropics, where the period suitable for flowering is long, plant species may flower in succession, taking turns in attracting the available pollinators. This kind of flowering keeps both flowers and pollinators at a generalised level. Network analyses show that large networks contain higher amounts of extreme generalists and extreme specialists (Bascompte et al. 2003, Vázquez and Aizen *in press*), suggesting that the flowering phenologies of more plants are at the extreme ends of the steady state – big bang continuum. Their results also show that large networks are highly asymmetric, meaning that specialists interact mainly with generalists. It should be remembered here that a species may be more or less generalised within a functional group, as its niche within the functional group may be broad or narrow.

Comparing plant-pollinator networks from different latitudes supports the hypothesis of higher specialisation towards the tropics, at least concerning the plants (Olesen and Jordano 2002). Pollinators were not found to be more specialised at low altitudes. If tropical plants flower in succession over a prolonged flowering season, this finding makes sense. Because of niche differentiation, plants may have adapted to defined functional groups, but the pollinators in the functional groups have to survive throughout the year and thereby must visit a high number of flowering plants. Pollinators, for this reason, appear more generalised. The lower connectance found in large networks supports the hypothesis that tropical plants and pollinators are divided into more restricted functional groups, as interactions occur at a less random level. The connectance is the number of observed interactions in relation to the total number of possible interactions, i.e. interactions/(pollinator species × plant species). Bawa (1990) and Kress and Beach (1994) recognised well-defined functional groups of pollinators with an associated set of host plants in tropical lowland rain forests.

Overall, plant-pollinator interactions seem more specialised in the tropics, although generalisation is prevailing (e.g. Kanstrup and Olesen 2000), and some authors argue that tropical plant-pollinator interactions are no more specialised than elsewhere (Ollerton and Cranmer 2002, Corlett 2004).

Knowledge about the pollination system and its generalisation level is useful when growing a species outside its natural habitat, e.g. as an agroforestry tree. A plant with a generalised pollination system normally produces abundant fruit and seed in the new environment, as it does not require special pollinators. The more specialised a plant is, though, the higher is the risk that efficient pollinators do not exist in the new surroundings, and fruit and seed set will be restricted.

In this study, the pollination system of two tropical trees, *Calliandra calothyrsus* and *Micromelum minutum*, were investigated in Kenya and Vietnam, respectively. Both species are small trees with little shading, ideal in agroforestry. The pollination efficiencies of flower visitors and the levels of generalisation and specialisation are discussed in order to infer their ecological needs for pollination and seed production. Recommendations and concerns about planting are discussed. The two investigations are written in the form of article manuscripts.

Pollination of *Calliandra calothyrsus* (Leguminosae, Mimosoideae) in Western and Central Kenya

Abstract

The flower visitors to *Calliandra calothyrsus*, a small mimosoid tree, were investigated in seed orchards and small-scale farms in Western and Central Kenya, where *C. calothyrsus* is introduced. Timing of nectar production, anther shedding, stigma receptivity and floral morphology were compared to timing and behaviour of the flower visitors to determine which animals contribute to pollination. Floral characters clearly imply that the flowers are adapted to nocturnal pollination. Optimal time for pollination was around two hours after dusk, and chances for pollination were generally higher throughout the night than at daytime. During daytime, the flowers were visited by a large amount of honeybees (*Apis mellifera*) and a wide range of primarily passerine birds, half of which were sunbirds (Nectariniidae). At night, at least two species of fruit bats were observed visiting the flowers. Both timing and behaviour of the fruit bats indicate that they pollinate *C. calothyrsus*. In contrast, timing and behaviour of diurnal visitors exclude them as pollinators. A constraint for *C. calothyrsus* seed production may be a limited visitation rate by fruit bats. One condition limiting fruit bat visits could be the accessibility to inflorescences. It was generally observed that inflorescences within the canopy of seed orchards were not visited by bats while inflorescences on branches protruding above the canopy were visited repeatedly. While fruit bats are the best pollinators in East Africa, they are presumably not as effective as the nectarivorous bats in the native range, as fruit bats have a more generalised feeding ecology and visit flowers more as a dietary supplement or when fruit sources are low. Pollination therefore should be expected to be less efficient in East Africa, where true nectarivorous bats are absent.

Keywords: Calliandra calothyrsus, *Epomophorus* spp., Kenya, nectar, pollination efficiency.

Introduction

Introducing a new species

When a plant species is introduced to an area outside its natural range, barriers may appear which block or reduce its reproductive success. These barriers may be caused by abiotic factors such as climate or soil conditions, or they may have biological causes like competition, herbivory or lack of mutualistic partners. An example of the latter could be the absence of efficient pollinators. Mutualists are often not introduced together with the new species, especially not when they do not live in close symbiosis. When introduced to a new area, plants therefore often lack pollinators from their native range, where they may have adapted to specific pollinators through coevolution. If a plant has specialised pollinators in its natural range, pollination in exotic areas may be insufficient or lacking, resulting in a reduced seed set and reproduction. In by far the most cases, though, plants pollination systems are more or less generalised, and do not rely on a single pollinator species. Even plants that are specialised on a small set of pollinators in their home range are often able to interact with new species in a new area, as other species in the new area may be pre-adapted to utilize the flowers. Besides, generalised pollinators in the new area will visit the flowers and may contribute to pollination, depending on the flower's complexity and level of specialisation. Species with generalised pollination systems in their home range rarely have problems with pollination in a new environment. As specialised flowers are normally specialised for pollination by a group of pollinators, often taxonomically close or ecologically and behaviourally alike, rather than a single species, the chance that other species belonging to the same group are present in the new area is high.

Study species

In this study, the pollination ecology of *Calliandra calothyrsus* Meissner (Leguminosae, Mimosoideae) was investigated in Kenya. The species is a large multiple stemmed shrub or a small single stemmed tree of up to 12 metres in height (Macqueen and Hernández 1997). Its natural range is from the western Pacific coast of Mexico to the north coast of central Panama (Macqueen 2001), where it is found in riparian and secondary forests. It was introduced to Indonesia as an agroforestry tree in the 1930's (Riswan 1996) and has more recently been distributed to a wide range of tropical countries. In Kenya, over 30.000 farmers have planted the species since it was seriously promoted and introduced in the 1990's (Franzel et al. 2004). In developing countries, it is highly promoted for small-scale farms because of multiple purposes and easy handling (National Research Council 1983, Gunasena et al. 1997). It is a fast-growing tree which in its natural range occurs in early succession on disturbed soils, especially along river banks, and it demands high amounts of light. Its potential as a weed is therefore not presumed to be critical.

On small-scale farms, *C. calothyrsus* is planted in rows along boundaries, especially across sloping land as barriers to soil erosion. Most trees are pruned for cattle fodder at a height of one meter and do not get the chance to flower. Farmers are advised to leave at least three trees for seed production. In seed orchards, trees are typically planted in 3×3 metre square plots, as recommended by Chamberlain (2000).

Calliandra series *Racemosae* has unusually elongated, racemose, penicillate inflorescences and compact flower heads in sub-umbels (Macqueen 2001). Inflorescences are borne on terminal branches at the top or the side of the trees, often protruding from the foliage. Flowering is primarily nocturnal with a few flowers opening per inflorescence per night. Flowers open acropetally over a period of months. In *C. calothyrsus*, flowers unfold in the late afternoon, remain open throughout the night, and wilt the next morning before noon. Each night, the inflorescence forms a ring of open florets around the inflorescence axis.

Flower morphology

Flowers of *C. calothyrsus* are borne on 1 cm long pedicels. Corollas are 6-8 mm long, widely campanulate and pale green with 3-5 mm long acute lobes (Macqueen and Hernández 1997). The calyx is 1.5 - 3 mm long. At the base of the corolla is a nectariferous disk secreting a drop of yellow nectar. Stamens are red or pink and numerous, fused for 2-3 mm at the base. The nectar drop is held by cohesion forces well beyond the base of the fused stamens. Flowers possess an average of 37 (31-41) stamens at a length of 64 (57-68) mm (Boland and Owour 1996). Anthers are yellow and centrally attached at the end of the stamens. Each anther contains 8 polyads, comprising 8 pollen grains (Boland and Owour 1996, Macqueen 1996). The basal cell of the polyad is sticky and points outwards when anthers have dehisced. Many individuals present a small amount of staminate flowers in addition to the hermaphroditic flowers. The level of andromonoecy varies and probably functions to balance reproduction and limited nutritional resources (Chamberlain and Hubert 2001). Hermaphroditic flowers have one white style of 74 (66-79) mm (Boland and Owour 1996), protruding about 10 mm beyond the anthers. At the end of the style is a cup-shaped stigma. The ovary contains an average of 12 (8-13) ovules (Boland and Owour 1996), producing 8-12 seeds per pod (Hernández 1991, Macqueen and Hernández 1997) and Matthews and Hopkinson (1998) found an average of 8.7 and 8.8 ovules per ovary, respectively. Macqueen (1992) reported a pollen to ovule ratio of 320, but the ratio is even lower using the numbers given above. Two to three polyads need to be transferred to the stigma for full pollination, as not all pollen grains contact the stigmatic surface and germinate (Chamberlain 1998). The breeding system is partially self-incompatible, which is evident by a low fruit set after controlled self-pollination compared to cross-pollination (Rajaselvam et al. 1996, Chamberlain and Hubert 2001), so cross-pollination is important for fruit and seed production. Flowers are reported to be slightly protandrous, anthers dehiscing about half an hour before the stigma is receptive (Boland and Owour 1996). Anthesis begins by late afternoon and flowers wilt the next morning before noon.

Pollinators of C. calothyrsus *in Central America*

The pollinators of *C. calothyrsus* have been studied before, both within its native range and in some exotic areas. In all investigations, honeybees (*Apis mellifera*) are reported as very frequent flower visitors, but their contribution to pollination is doubtful, as they do not touch the reproductive organs when entering the flowers (Chamberlain and Hubert 2001). The copious nectar suggests adaptation to pollinators with high energetic requirements (Heinrich and Raven 1972), and the long stamens and protruding style suggest that efficient pollinators are larger animals.

This was indeed found to be the case by Chamberlain and Rajaselvam (1996b), who found *C. calothyrsus* pollen on 265 bats and six hawkmoths (Sphingidae) caught during 23 nights in Honduras. Nearly 80% of the bats belonged to the nectarivorous genus *Glossophaga* (Glossophaginae). The rest belonged to the genera *Phyllostomus* (Phyllostominae), *Sturnia* (Stenoderminae), *Uroderma* (Stenoderminae), *Artibeus* (Stenoderminae) and *Carollia* (Carollinae), all primarily frugivorous. Individuals of *Glossophaga* spp. carried small amounts of other pollen types and were the only recaptured bats, indicating that they were reliable pollinators. They are also seen visiting four other *Calliandra* spp. in the series *Racemosae* (Macqueen and Hernández 1997). *Glossophaga soricina* is found to pollinate other *Calliandra* spp. in Central America (Dobat and Peikert-Holle 1985) and was suggested as a regular visitor and pollinator in Nicaragua and Guatemala (Macqueen 1992). In addition to bats, Hernández (1991) observed the hawkmoths *Eumorpha vitis* and *Erinnyis ello* as pollinators in Mexico. The two moths *Palpita flegia* (Pyralidae) and *Letis vultura* (Noctuidae) were observed only as nectar robbers. Four other species of *Calliandra* are shown to be pollinated by hawkmoths in Mexico (Cruden et al. 1976). *Calliandra haematocephala* has been predicted to be ornithophilous on the basis of floral characters (Nevling and Elias 1971), but further evidence is needed.

Pollinators of C. calothyrsus *in exotic areas*

In the Old World, the most efficient pollinators of *C. calothyrsus* are likely to be nectarivorous bats from the subfamily Macroglossinae (Chamberlain and Hubert 2001). *Macroglossus minimus* is mentioned as a common pollinator in Indonesia (Chamberlain 1998, 2000), where seed production is often high enough for export (Chamberlain and Rajaselvam 1996a). The species in Macroglossinae have evolved morphological and behavioural traits similar to those of Glossophaginae (Mickleburgh et al. 1992), but the subfamily is not represented in East Africa (Chamberlain and Rajaselvam 1996b). In Sri Lanka, *C. calothyrsus* is pollinated by *Rousettus* *seminadus* and *Cynopterus sphinx* (Rajaselvam et al. 1994). In North Queensland, Australia, *Pteropus conspicillatus* was observed as a common pollinator (Matthews and Hopkinson 1998). Six species of honeyeaters (Meliphagidae) were abundant flower visitors.

As summarized by Chamberlain and Hubert (2001), *C. calothyrsus* is most efficiently pollinated by nectarivorous bats, but less specialised fruit bats and large moths will also visit and pollinate *C. calothyrsus*.

Seed set problems

A typical problem of *C. calothyrsus* in East Africa is a low seed set, and seed orchards in general do not produce the amount of seed needed by the seed distributors (Chamberlain and Rajaselvam 1996a). To meet the demands, seed dealers buy seed from local farms where seed production is often higher. The causes of the low seed production is unknown but might be influenced by poor pollination.

Objective

The flower visitors to *C. calothyrsus* were studied in Kenya to interpret which animals aid in pollination of *C. calothyrsus* in East Africa. A wide range of study sites were used to include as many flower visitors as possible, as the composition of flower visitors was expected to vary spatially (Herrera 1988). Both small-scale farms and orchards for seed production were included to investigate likely differences between the two. The efficiencies of visitors as pollinators were estimated by relating visitor behaviour and visit time to flower morphology and timing of nectar presentation, receptivity and pollen shedding. Reasons for low seed set in Kenya are discussed.

Methods

Study sites

Studies were performed over a large area in the highlands of Western and Central Kenya (fig. 1). Locations and study periods are shown in table 1. Two study sites in Western Kenya and one in Central Kenya were seed orchards, the rest were small-scale farms with many *C. calothyrsus* left for seed production. The study sites are described shortly in appendix 1. Field studies were performed from the middle of March until the end of June 2003. Timing was based on seed harvest outputs from the *C. calothyrsus* seed orchards in Embu during the preceding years (appendix 2). The amount of seed harvested during each year peaks abruptly around September and October, but there is high variation in timing and seed production between years. Seed maturation time after pollination is three to four months (Chamberlain and Hubert 2001), so pollination prior to peak seed set occurs around April and May, which is during the period of the long rains in East Africa. This period is also observed to be the time of peak flowering in Kenya. In the year of the study, the dry season was prolonged and the long rains began in April instead of March.

Measurements

Measurements of nectar content, stigma receptivity and polyad shedding were performed in Western Kenya. Standing crop of 20 flowers was obtained with 20 μl capillary tubes once per hour during periods of observation. Flowers were chosen randomly amongst as many trees as possible. Furthermore, the nectar content of ten flowers in a bagged inflorescence was extracted each hour. A new inflorescence on a new tree was used each hour. Inflorescences were bagged with 1 mm meshed bags before anthesis to ensure that no nectar was removed by flower visitors. Because nectar dropped easily, flowers examined were from chest height up to two metres above the ground to facilitate easy access.

The proportion of flowers that were receptive to pollen over time was estimated by touching 20 stigmas with a finger each hour. If stigmas stuck to the finger, flowers were presumed to be receptive. In the mornings and evenings the estimate was repeated each half hour. Flowers were chosen from as many trees as possible and new flowers were used in each examination.

Time of polyad shedding was estimated by touching the anthers of 20 inflorescences each hour and checking the hand for polyads. New inflorescences from as many trees as possible were used each hour.

The number of open flowers within 20 inflorescences was counted during five nights.

Temperature and relative humidity was measured each hour, and an estimate of wind and light intensity was noted. In the orchards and in some of the farms in Western Kenya, the height of at least 30 randomly chosen trees was measured, and the number of inflorescences per tree was counted.

Observations

In Western and Central Kenya, flower visitors were observed from anthesis until wilting of the flowers, in Western Kenya concurrently with the measurements. Observations were sometimes performed continuously throughout the night, but often only a part of the flowering period was chosen. A total of 316 hours were spent on observations (table 1). Out of these, 52 hours were spent before darkness, 160 hours during night and 104 hours after daybreak. A spot with a good view of trees and inflorescences, around 4 to 7 metres from the nearest tree, was chosen. Flower visitors were identified in the field if possible, and their behaviour and abundances were observed. All nocturnal flower visits were recorded, and their durations were estimated in whole seconds at some of the sites. Nocturnal visitors were observed as silhouettes against the sky, which was never totally dark. Sometimes a flashlight was used in order to see the behaviour at the inflorescences more clearly.

During rain, measurements and observations stopped, as pollinators were assumed not to be active. Besides, the rain increased the nectar volume and diluted the nectar. Most nights without observations were rainy nights, and rain was quite prevailing. Observations and measurements were sometimes performed in the morning although it had been raining in the previous evening or early night.

Results

Light/darkness, temperature, humidity

Daybreak came at 6.00 am and it was bright at 6.15 am. Direct sunshine appeared at 7.00 am. The twilight fell at 6.45 pm, and at 7.00 pm it was dark. There was no marked difference between timing in Western and Central Kenya. It was rarely windy, besides shortly before rain. At dusk and during the night, the wind was almost always calm. Temperature typically varied from 25°C in the afternoon to 17°C before sunrise. Humidity during night was often high, ranging from 50 to 100%.

The flowers

Flowers opened around 4 pm and stamens and style were fully unfolded around 5 pm. Half an hour after darkness, stamens and style had obtained full turgidity forming a brush around the inflorescence axis (fig. 2). Turgidity of the stamens and style was held throughout the night but was lost at daybreak, and flowers wilted around 10 am to noon. A mean of 12 flowers were open per inflorescence (1-32, SD = 6.78, N = 100 inflorescences) per night at the old Maseno orchard. After darkness the large bipinnate leaves folded, creating more space around the inflorescences.

Nectar

Nectar production was initiated soon after anthesis, and standing crop increased steadily until midnight, when it stabilized around 50 μl per flower (fig. 3a,b). Inflorescences then roughly contained 500 μl nectar each. There was no observable difference in the nectar content of bagged and unbagged flowers during flowering. Nectar volume means for both bagged and unbagged flowers were normally distributed from 9 pm to 7 am ($P>0.05$). Within this time span, there was no difference in the nectar content of bagged and unbagged flowers ($0.015 < t < 0.964$; $0.099 < P < 0.96$), except at 2 am and 3 am ($P<0.05$). The nectar volume was held throughout the night and dropped in the morning in both bagged and unbagged flowers, but the decline was faster in unbagged inflorescences exposed to flower visitors.

Stigma receptivity

The percentage of sticky stigmas increased steeply from 10% at 5.30 pm until 8.30 pm, when 96% of stigmas were sticky and therefore regarded as receptive (fig. 4). Average receptivity declined throughout the night but variation between nights was high. Some nights stigmas were receptive until the morning, whereas receptivity decreased markedly before midnight at other nights. At daybreak, the number of sticky stigmas increased slightly, but fell abruptly after 6.30 pm.

Anther dehiscence and polyad shedding

Anthers dehisced after 6.00 pm and all anthers were open at 9.00 pm (fig. 5). Polyad shedding was highest during the first part of the night, i.e. in the first hours after the anthers had dehisced. The polyad content remaining in the anthers during the nights was highly variable. Sometimes anthers held polyads throughout the night, but in general the polyad content decreased. Few polyads were left in the anthers by the late morning.

Insects

Honeybees (*Apis mellifera*) visited *C. calothyrsus* flowers in very high numbers and were by far the most abundant flower visitors. They were observed visiting *C. calothyrsus* in all areas, except at a few farms. In most cases beehives were nearby, as these were promoted with *C. calothyrsus* for honey production. The bees came in an increasing number from anthesis until darkness, when they suddenly disappeared. In the mornings they came in high numbers around 6.20 am and stayed until the nectar was depleted around 10 am. Normally, activity started levelling off after 8 am when bees were in very high numbers. After rainy nights only few bees visited the flowers in the morning. Bees almost never touched the anthers or stigma when searching for nectar (fig. 6). Anther contact was in rare cases observed when a bee arrived or left moving through the stamens, but by far the most flower approaches were from the sides where no stamens impeded accessibility. Sucking the nectar of one flower took minutes, and the next flowers to visit were in most cases other flowers of the same inflorescence. If previously unvisited, the nectar content of a single flower was enough to fill the gut capacity of a bee. After 10 am, a few bees sometimes climbed to the tip of the stamens and collected pollen from the anthers. Stigma contact was not seen in these cases.

In many farms, a red-coloured species of potter wasp (Eumeninae, Vespidae) was a regular flower visitor in the late morning, when the number of honeybees decreased. It was not a constant forager like the bees, and numbers were low. No anther or stigma contact was observed.

In a tree at the Nairobi ICRAF station, up to ten individuals of the large beetle *Pachnoda ephippiata* were observed at a time eating stamens in the morning hours. Beetles of different species were occasionally observed in other areas as well. One evening in Kahaiini, around one hundred beetles were swarming in the trees just after darkness. Like other insects observed, beetles did not touch anthers or stigma.

Ants were also abundant flower visitors, but most pronounced before the rainy season set in. Their activity was highest at night. At least four species of ants ranging in size from 3 to 20 mm were found in the flowers. They approached the flowers via the stem and were only observed at the inflorescence basis.

After darkness, very few, small moths were observed visiting flowers. However, no hawkmoths were seen.

Birds

A wide range of bird species were observed drinking nectar from *C. calothyrsus* flowers (table 2), often in high numbers. The time of bird visits was the same as that of bee visits, in the evenings before darkness and in the mornings. Some birds were seen in many areas and others were only observed at a single site. About half of the species recorded were sunbirds (Nectariniidae), and they constituted about half of the birds at each site. The most ubiquitous flower-visiting bird was *Nectarinia kilimensis*, other species of sunbirds varied between sites and areas. Amongst other passerines, one or two stationary pairs of *Pycnonotus barbatus* were normally present at each farm, and two *Ploceus* spp. visited farms and seed orchards in small groups. Birds in general foraged in mixed species groups, only *Ploceus* spp. and *Colius striatus* showed a true group structure, and they obviously preferred foraging in the larger trees. At the farms in Busia and Teso, *C. calothyrsus* was not visited by birds at all, or only rarely. In Embu, no bird visits were observed.

Sunbirds were very elegant foragers. They perched on the twig beneath the inflorescence and inserted the tip of their bill into the flowers without touching anthers or stigmas. Only one anther and stigma contact was seen during all the observed sunbird visits. In each inflorescence, more than one flower was normally searched for nectar. Sunbirds mostly visited inflorescences that were near to each other and on the same tree and visited a high number of inflorescences over time.

Ploceus spp. and *P. barbatus* were larger and less elegant in their foraging than sunbirds. As their bills were shorter, they raised their heads nearer to the nectar and came closer to the stamens while drinking. Although they touched stamens at times, anthers and stigma were still out of reach and clear anther and stigma contacts were not observed. *Ploceus* spp. visited many inflorescences, but their movements were slowed down by their heavy weight on the inflorescence-bearing twigs. They were not systematic in their foraging as the sunbirds and visited fewer inflorescences in a tree before they went to the next or disappeared.

Bats

Fruit bats (Pteropodidae) visited the inflorescences of *C. calothyrsus* during the night in some areas (table 3), adding up to a total of 689 recorded bat visits. The time period of bat visitation extended throughout the night, starting just after darkness (fig. 7). Visits came in bursts, and during most of the night hours nothing happened. Fruit bats of two clearly distinctive size classes visited the inflorescences to drink nectar. During flower visits, bats landed directly on inflorescences and clung to them (fig. 8). This approach involved anther and stigma touches in every visit. While the bat clung to it, the inflorescence was pulled down by the weight of the bat. Most visits were very short, lasting from less than one second to three seconds. For the small fruit bats in Nyambula, an average visit took 1.8 seconds (SD = 1.38, N = 263), while the large bats in Embu used 2.8 seconds (SD = 3.38, N = 221) per visit, although a few visits lasting more than one minute were observed. In both cases, mean visit time was overestimated, as visits of less than a second were recorded as one second.

Bats visited inflorescences protruding about one metre above the closed canopy at much higher rates than inflorescences inside the canopy. Only one inflorescence was visited at a time, and the bat

flew away to return later. The same few protruding branches were visited by bats repeatedly while by far the most other inflorescences received no bat visits at all. It was noteworthy that pods developed at a higher extent on the same kind of branches that the bats favoured visiting.
A roost of fruit bats were observed in large cypress trees about one kilometre from the *C. calothyrsus* farm in Kegoye, where the frequency of bat visits was often high during night. In Akites, fruit bats rested in the mango trees right next to the planted *C. calothyrsus* in one farm (appendix 1), but only few bat visits were observed during the week of observations in the area.

Discussion

Optimal and possible times for pollination

Nectar secretion, stamen turgidity, anther dehiscence, and stigma receptivity clearly indicate that the flowers of *C. calothyrsus* are adapted to nocturnal pollinators. Based on the measurements of stigma receptivity and pollen shedding, the optimum time for pollination is in the early night around 8.30 pm, when nearly all flowers were both receptive and shedding pollen. Stigma receptivity patterns are in high correspondence to patterns revealed by hydrogen peroxide and Nile blue tests, and by seed set after controlled crosses (Rajaselvam et al. 1996, Matthews and Hopkinson 1998). Although more stigmas are receptive at night, a proportion of receptive stigmas are also exposed to diurnal flower visitors. High amounts of pollen are only available at night, but as pollen is available in small amounts both in the late afternoon and in the morning, the possibility of diurnal pollination must be considered. The little wind at dusk and during the night makes efficient cross pollination by wind unlikely, which is also confirmed by Rajaselvam et al. (1996).

Which flower visitors contribute to pollination?

Behavioural observations of the diurnal flower visitors did not indicate that they contribute to pollination. Because of their small size, chances that honeybees and wasps pick up polyads and deposit them on stigmas are very low. Polyad loads on the surfaces of honeybees visiting *C. calothyrsus* are shown to be generally very small to undetectable (Boland and Owour 1996, Matthews and Hopkinson 1998), and inflorescences exposed only to bee visits have had very little fruit set (Rajaselvam et al. 1996). So although honeybees and wasps are very abundant visitors, they must be disregarded as pollinators. In any case, the low number of flowers visited per bee before returning to the hive would involve little pollen exchange among flowers and mainly lead to geitonogamy.

Birds could be expected to contribute in pollination because the red colour of the stamens, the faint scent, the copious nectar, and the penicillate flower shape are traits often associated with ornithophily (Faegri and van der Pijl 1971), as is coloured nectar (D. M. Hansen, pers. comm.). Sunbirds are specialised nectar-feeders, while the other birds are more generalised foragers which feed on nectar occasionally when it is easily accessible (Nicolson and Fleming 2003). The avoidance of contact to the anthers by all birds prevents them from picking up polyads, and they therefore do not contribute to pollination. This was also concluded for honeyeaters visiting *C. calothyrsus* in Australia (Matthews and Hopkinson 1998) and hummingbirds in Central America (Hernández 1991, Macqueen 1992). The absence or low rate of bird visits to flowers in Busia, Teso and Embu, which are areas of high seed production, supports the view that flowers are not pollinated by birds. The traits associated with bird pollination, however, suggest that the current pollination system may have evolved from an ornithophilous pollination system.

Since no hawkmoths were observed during the period of peak flowering, the possibility that hawkmoths are important contributors to *C. calothyrsus* pollination in Kenya is low. Hawkmoth activity might be higher in the dry seasons, but in a study in Costa Rica, flowering of sphingophilous plants peaked in the wet season coinciding with the highest hawkmoth abundance (Haber and Frankie 1989). This suggests that hawkmoths are most active during the wet seasons.

Bats visited the flowers at the time when they were most rewarding, which was also when the chance of picking up pollen was highest and when flowers were most receptive to pollination. Traits like nocturnal anthesis, copious easily accessible nectar, penicillate flowers and flowers lasting only one night are typical for chiropterophilous plants (van der Pijl 1961). Although bat flowers are most often white or drab in colour, dark red bat flowers are also found (Marshall 1983). Bats clearly touch anthers and stigmas when they land on inflorescences and most likely both deposit and receive polyads during visits. Polyads from *C. calothyrsus* were found on the bodies of flower-visiting bats in Honduras and Sri Lanka (Rajaselvam et al. 1994, Chamberlain and Rajaselvam 1996b). The observed flight pattern of foraging fruit bats in *C. calothyrsus* in Kenya corresponds to patterns observed elsewhere (Gould 1978), and makes fruit bats highly efficient vectors for outcrossing. Fruit bats prefer prominently exposed inflorescences and visit them repeatedly (Baker and Harris 1957, Chamberlain and Rajaselvam 1996b), and the higher pod formation observed on tall branches free of the foliage supports the hypothesis that the *C. calothyrsus* flowers are pollinated by bats.

Foraging behaviour of flower-visiting bats

The preference of prominently exposed inflorescences in the top of the canopy was also observed for phyllostomid frugivorous bats visiting *Bauhinia pauletia* (Caesalpiniaceae) in Costa Rica (Heithaus et al. 1974). In addition to flowers in the high canopy, the smaller, nectarivorous *Glossophaga soricina* also visited flowers on the lower branches. Whereas *G. soricina* hovered briefly while extracting nectar with its long, papillous tongue, the larger, frugivorous bats clung to inflorescences bending the thin inflorescence branches, and it was observed that they only visited inflorescences that would not be pulled into the foliage during visits. Because of their smaller size and high agility, *G. soricina* were able to visit less prominent inflorescences and utilized a wider range of inflorescences. These abilities of the nectarivorous bats are important for the pollination efficiency in species like *C. calothyrsus*, where most inflorescences do not protrude markedly from the foliage and the inflorescence-bearing branches are too thin to hold the weight of alighting bats. The folding leaves, the thin inflorescence-bearing branches and the stamen length, fitting the tongue length of nectarivorous bats, indicate that pollination in *C. calothyrsus* has evolved in adaptation to hovering, nectarivorous bats.

Fruit bats in Kenya

In the East African region, common pteropodid bats consist of *Epomophorus* spp. and *Rousettus aegyptiacus* (Kingdon 1974). In contrast to other East African fruit bats, *R. aegyptiacus* roosts in caves and uses a primitive sonar system of low frequency tongue clicks in addition to sight (Kwiecinski and Griffiths 1999). Probably, only *Epomophorus* spp. were observed in this study, as fruit bats were sometimes observed resting in trees nearby and no clicking sound was heard from the foraging bats. If present, rousettine fruit bats are likely pollinators as well, as observed in Sri Lanka (Rajaselvam et al. 1996). Epomophorine bats differ in size from the smallest weighing 40 gram to the large *E. gambianus* of up to 155 g (Boulay and Robbins 1989). The distribution of *E. gambianus* ranges into Kenya but it mainly occurs in West Africa. In East Africa, *E. wahlbergi* dominates the niche of large epomophorine fruit bats (Acharya 1992). *Epomophorus wahlbergi* is sometimes found to be rather tame, resting in farm trees like palms, mango and cypress or banana plants, e.g. in the suburbs of Nairobi, and it is often found in co-existence with the small dimorphic *E. labiatus* (Kingdon 1974). In a bat survey in Meru National Park in Central Kenya, *E. labiatus* and *E. wahlbergi* were the only megachiropterans present in the area (Webala et al. 2004). Whereas *E. wahlbergi* was most pronounced in natural habitat, *E. labiatus* was a common species in both natural habitat and farmland. On this basis, the most likely pollinators of *C. calothyrsus* in this study are considered to be *E. labiatus* and *E. wahlbergi*. *Epomophorus minor* also occurs in the region and might be confounded with *E. labiatus*.

Fruit bat effectiveness as pollinators

Considering the amount of flowers and the overall nectar presentation, nocturnal visitors observed in this study were very few. Only a small proportion of the flowers were visited during night, which is also indicated by the equal amounts of nectar in flowers in bagged and unbagged inflorescences. Even on the nights of the highest visitation frequencies, far the most flowers were obviously not visited. Overproduction of nectar is sometimes obtained in night-flowering plants to increase attraction, as night-flowering plants are often pollinator-limited (Ramirez 2004).

Where *C. calothyrsus* is pollinated only by fruit bats, the trees not only need to compete with other flowering plants for pollination services, but they also must compete with trees offering ripe fruit. Fruit bats prefer fruit as their main food source and may disregard floral products if ripe fruit is available in satisfying amounts (Fleming 1993). This may explain the surprisingly low visit rate in Akites, where fruit bats were present and seed production was high. As mango and other fruit trees in the area bore ripe fruit at this time, fruits may have constituted a more preferable food source for the bats at the time of the study. The eager feeding in mango by *E. gambianus* is described by Ayensu (1974). Because fruit bats alter their feeding habits according to seasonal food availability and feed opportunistically on floral products, they should be regarded as less reliable pollinators than the specialised, nectarivorous bats. In a study by Heithaus et al. (1975) in Costa Rica, frugivorous bats only visited flowering trees during high abundance flowering, and only *G. soricina* visited flowers during the period of low flowering. On the other hand, frugivorous bats appeared to rely heavily on nectar as a food source in the dry season, when fruit availability was low. The flowering phenology of *C. calothyrsus* is a combination of big bang and steady state flowering (Gentry 1974). Probably, fruit bats are most attracted during peak flowering when the overall rewards are highest (Fleming 1982). The steady production of flowers throughout the year makes it a reliable nectar source for traplining, nectarivorous bats, but the high flower production during peak flowering also attracts the more generalised fruit bats, which take part in pollination during the mass flowering period.

Rajaselvam et al. (1996) concluded that fruit bats in Sri Lanka are effective pollinators when they visit *C. calothyrsus* flowers, but that they are much less frequent visitors than the chiefly nectarivorous bats pollinating in the native range. Likewise, frugivorous bats within the native range were concluded to be effective pollinators of *C. calothyrsus* when visiting, but they were less reliable pollinators than bats of the subfamily Glossophaginae (Chamberlain and Rajaselvam 1996b).

Rain

Although rain enhances flower production, it is also a serious factor limiting pollination, as pollinators are not active during rain. During the season of the long rains, a high proportion of the nights are rainy and the number of nights optimal for pollination thereby diminishes. After rain, flowers are less attractive to pollinators, as the nectar is diluted and the stamens clasp. Diluted nectar loses the sweet taste, the yellow colour, and the high viscosity. Moreover, the rain depletes the anthers of polyads (Boland and Owour 1996, Chamberlain 1998). Even bees and birds visited at low frequencies after heavy rain in the night.

Edge Effects

In the older seed orchard at Maseno and the seed orchard in Malava, where trees had uniform heights and overlapping canopies (appendix 1), it was noted that trees at the edges produced more flowers and more pods than trees inside the orchards. Matthews and Hopkinson (1998) documented a significant edge effect on the fecundity of *C. calothyrsus* in a seed orchard in North Queensland. Trees at the edge of the orchard produced more pods than trees within the orchard, coinciding with more inflorescences and more flowers per inflorescence in edge trees. They ascribed the effect to differences in competition either for light, water or nutrients. Better light conditions at the edge seems a reasonable explanation of a higher inflorescence and flower production in edge trees, but they also found a higher production of pods per inflorescence in edge trees than in trees within the orchard.

A likely explanation for a higher pod production per inflorescence at the edge may be a more successful pollination caused by free accessibility to inflorescences for the pollinating bats. An important trait in the bat flower syndrome is presentation of flowers free of the foliage (van der Pijl 1961). In dense seed orchards, trees typically have the same height and form a continuous canopy. Bats only visit inflorescences which have space enough around them to allow free flight, both when approaching but also when leaving inflorescences. Alighting bats that are pulled into the foliage during visits have problems when leaving. Fruit bats besides need more space, because they are often larger than nectarivorous bats and their wings are built for fast but less agile flight (Fleming 1993). In East Africa, where hovering nectarivorous bats are missing, the need of space around the inflorescences might therefore be more pronounced than elsewhere.

Recommendations for planting C. calothyrsus *for seed production*

Planting trees in rows like performed in most farms makes bat access easy, as trees do not form a closed canopy and the bats can approach the trees from both sides. If trees are tall and the branches open as observed in some farms where *C. calothyrsus* grew amongst other trees, bat access is optimized. In square plots of 3×3 metres between stems, bat access to the inner trees is impeded. Bat visits are therefore restricted to trees at the edge and tall branches within the orchard, where they are not pulled into the foliage during visits. Matthews and Hopkinson (1998) recommended a wider spacing between rows within orchards to make all trees behave like the edge trees. Following their calculation, this would more than double the overall seed set for an area of the same size, although it includes fewer trees. Planting taller but not too shading trees in between might increase bat visitation frequency and pod production, if *C. calothyrsus* trees stretch for the light and become taller and more open. This growth form was seen at a farm of high seed production near Embu. Seed orchards should be placed as near to fruit bat roosts as possible. Although fruit bats commute several kilometres in search of food (Fleming 1982), food sources near roosts will be more heavily exploited.

In conclusion, the pollination system of *C. calothyrsus* is inferred to be chiropterophile, i.e. specialised in pollination by bats. New World nectarivorous bats have probably performed the most important selective pressure in the evolution of the pollination system, and nectarivorous bats are thought to constitute the most efficient and reliable pollinators, although frugivorous bats are also efficient pollinators. In both morphological and phenological floral traits, *C. calothyrsus* resembles the pantrophic, mimosoid genus *Parkia*, which is pollinated by pteropodid bats, e.g. *Epomophorus gambianus*, in the Paleotropics and phyllostomid bats in the Neotropics (Baker and Harris 1957, Dobat and Peikert-Holle 1985). *Calliandra calothyrsus* is a good example in support of the view that the bat flower syndrome is universal and that bat flowers from one part of the world will be visited and pollinated by bats in other parts of the world, if introduced (Faegri and van der Pijl 1971). Bat flowers are rarely specific to a certain bat species as they have evolved in diffuse coevolution (or reciprocating evolution) with a range of flower-visiting bats, which are likewise generalistic within flowers performing traits of the bat flower syndrome (Baker 1973, Heithaus 1982).

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Table 1. Places and times of the study.

Period	11-13/3	14-15/3	19–23/3	19-20/3	21/3	25-27/3	28/3	29/3	10-14/4	14-15/4	17-24/4
District	Nairobi		Nyeri			Maragua			Kisumu		Vihiga
Sublocation			Gakindu			Kandara			Maseno		Mbihi
Village	Gigiri	suburb	Kagarii			Kahaiini			Maseno		Magui
Farm/Orchard	ICRAF	Karanja	Wanyinyi	Gichohi	Victoria	Gichuna	Murera	Irungu	Old orchard	Young orchard	Ndenga
Afternoon hours	0	3	3	2½	2¾	1½	1½	2	4	4	3
Night hours	0	0	6½	11	1	6	3	2	17	4	3
Morning hours	1	2	4	2¼	0	6½	6	0	10½	2	8¾

Table 1. *Continued*

Period	24/4-2/5	4-10/5	11-12/5	12-13/5	13-17/5	17-18/5	19-22/5	22-25/5	24-28/6	25-28/6	26-27/6
District	Vihiga	Kakamega	Busia				Teso		Embu		
Sublocation	Kegoye	Malava	Bukhalalire				Aludeka		Embu		
Village	Mbale	Malava	Nyambula				Akites		KARI Embu		
Farm/Orchard	Otiende	Orchard	Lyala	Odock	Ogola	Dick	Masai	Sikoyo	Patulul stand	Embu stand	San Ramon
Afternoon hours	5	3¼	2	½	4	1½	3¾	1	¾	1½	1¾
Night hours	30¾	5½	9½	2	16½	0	12	5½	9½	8¼	7
Morning hours	20¼	14¼	2	4	2½	2¼	7	4	2	3½	0

Table 2. Bird species observed drinking nectar from *C. calothyrsus* flowers in areas of Western and Central Kenya. Numbers indicate intensity of flower visits from low (1) to high (3) based on abundance and activity.

Birds (Aves)	Common names	Maseno	Magui	Kegoye	Malava	Nyambula	Akites	Nairobi	Kagarii	Kahaiini	Embu
Coliiformes											
Coliidae											
Colius striatus	Speckled Mousebird			2							
Passeriformes											
Cisticolidae											
Prinia subflava	Tawny-flanked Prinia			1							
Fringillidae											
Serinus mozambicus	Yellowfronted Canary			2	2						
Serinus striolatus	Streaky Seedeater								2	2	
Nectariniidae											
Cinnyris cuprea	Copper Sunbird				2	1	1				
Cinnyris preussi	Northern Double-collared Sunbird								2		
Cinnyris venusta	Variable Sunbird			1						2	
Chalcomitra amethystina	Amethyst Sunbird				3		1		2		
Chalcomitra senegalensis	Scarlet-chested Sunbird			3	3						
Cyanomitra cyanolaema	Green-headed Sunbird			1							
Hedydipna collaris	Collared Sunbird				2			2	2		
Nectarinia kilimensis	Bronze Sunbird	3	3	3	3			3	3	2	
Nectarinia reichenowi	Golden-winged Sunbird								1		
Ploceidae											
Ploceus baglafecht	Baglafecht Weaver		1	2				2	3	3	
Ploceus cucullatus	Blackheaded Weaver	3	2	3	2	1			2		
Pychnonotidae											
Pycnonotus barbatus	Common Bulbul/Garden Bulbul		3	3	2	1		3	3	3	
Turdidae											
Turdus olivaceus	Olive Thrush								2		
Zosteropidae											
Zosterops abyssinicus	Abyssinian Whiteeye								3		
Zosterops senegalensis	Yellow Whiteeye		3	3	2						

Table 3. Bats pollinating *C. calothyrsus* in areas of Western and Central Kenya. Numbers indicate intensity of visits from low (1) to high (3).

Fruit bats (Megachiroptera)		Maseno	Magui	Kegoye	Malava	Nyambula	Akites	Nairobi	Muburoine	Maragua	Embu
Small fruit bats	e.g. *Epomophorus labiatus*	1		3		3	1				
Large fruit bats	e.g. *Epomophorus wahlbergi*			2	1						3

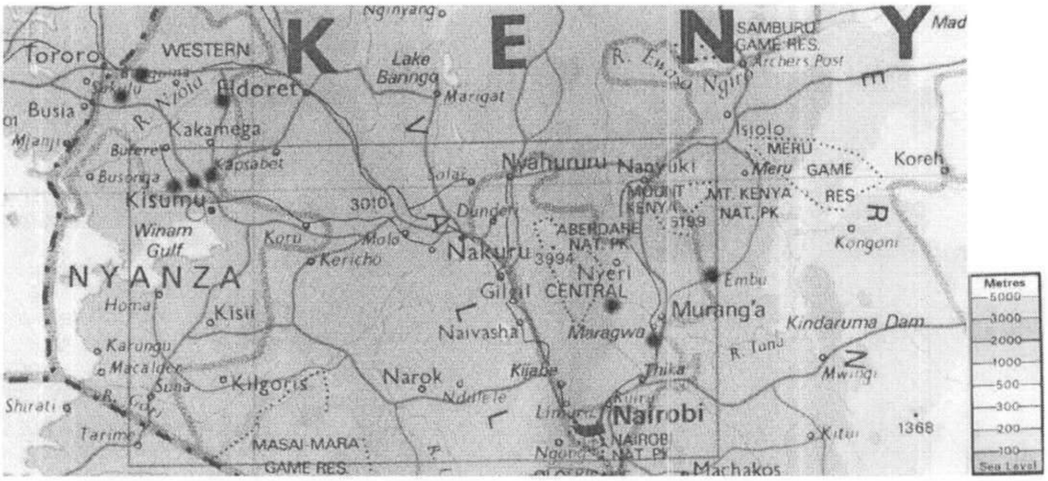

Fig. 1. Map of Western and Central Kenya. Study areas are marked with blue dots. Source: http://www.blissites.com/kenya/map.html. Scale: 1:4.400.000.

Fig. 2. Inflorescence of *C. calothyrsus* 5, 15, and 30 minutes after darkness.

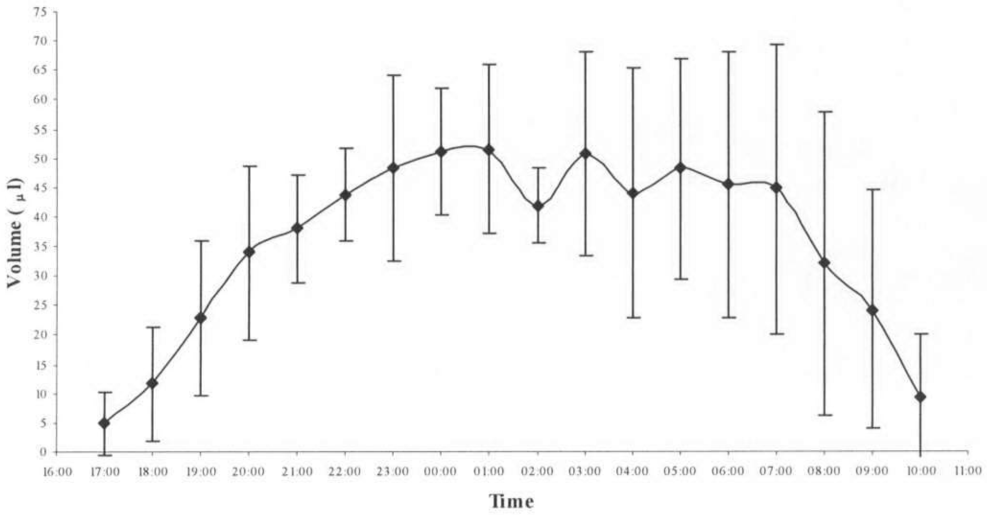

Fig. 3a. Nectar volume of unbagged flowers from anthesis to wilting measured in Western Kenya. The number of measuring events per clock hour from 5 pm to 10 am are 16, 16, 18, 16, 14, 9, 10, 7, 6, 6, 7, 7, 7, 20, 23, 22, 12, and 13, respectively.

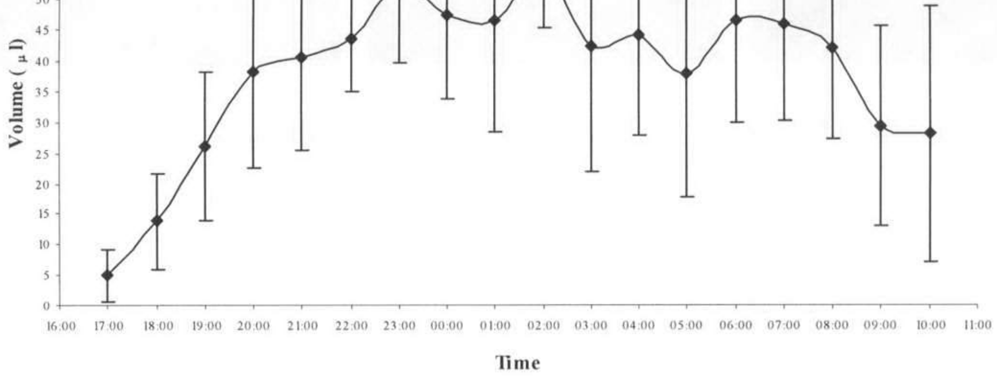

Fig. 3b. Nectar volume of bagged flowers from anthesis to wilting in Western Kenya. The number of measuring events per clock hour from 5 pm to 10 am are 16, 16, 17, 15, 13, 8, 9, 6, 5, 5, 6, 6, 6, 19, 22, 22, 13, and 12, respectively.

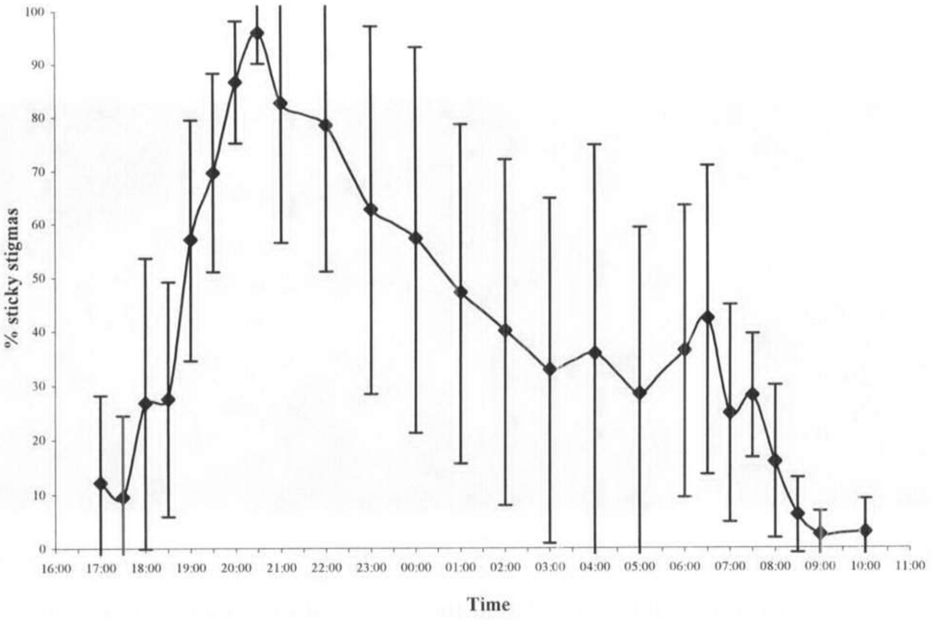

Fig. 4. Stigma receptivity measured as percentage sticky stigmas during flowering in Western Kenya. The number of measuring events per half or whole clock hour are 18, 11, 17, 14, 20, 14, 18, 11, 17, 13, 11, 9, 7, 8, 7, 6, 6, 18, 9, 22, 10, 23, 9, 10, and 12, respectively.

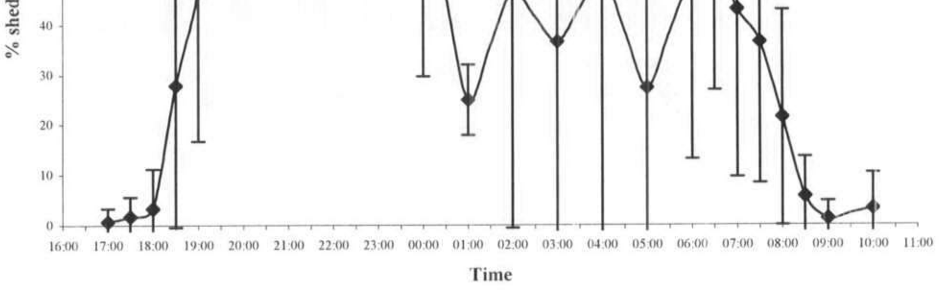

Fig. 5. Proportion of shedding anthers during flowering in Western Kenya. The number of measuring events per half or whole clock hour are 14, 12, 12, 14, 15, 13, 13, 12, 10, 8, 5, 4, 2, 3, 3, 4, 4, 13, 8, 14, 9, 13, 7, 7, and 9, respectively.

Fig. 6. Honeybees (*Apis mellifera*) visiting *C. calothyrsus* flowers. Bees have free access to the nectar from behind the flowers and rarely get near the small yellow anthers and the single stigma. Honeybees can therefore only be regarded as nectar thieves. But who can blame them, when the nectar is so little protected at daytime? The pictures were taken in the early morning, and large bubbles of nectar are visible in the flowers beneath the bees.

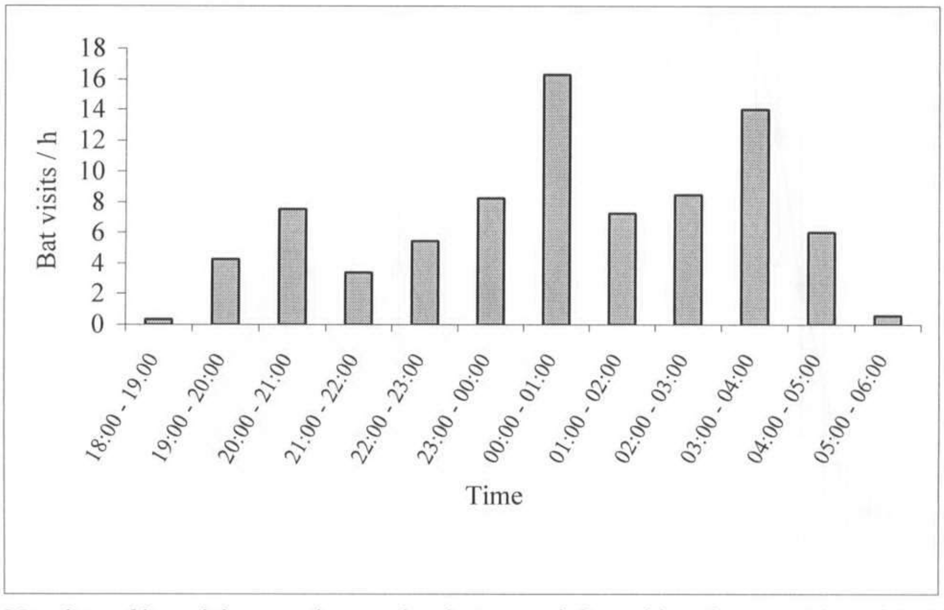

Fig. 7. Number of bat visits per observation hour on nights with at least one bat visit. Numbers of observation hours per clock hour were 12, 12, 11, 8, 9, 12, 10, 7, 7, 6, 6, and 5, respectively.

Fig. 8. Fruit bat drinking nectar from a *C. calothyrsus* inflorescence in the "Embu" stand at KARI Embu (see appendix 1). The bat clings to the inflorescence to the right. Inflorescences here were at the ends of very long branches; ideally suited for bat visits. Trees were rather tall and open, perhaps because they were grown in a low area surrounded by other trees which forced them to stretch for the light. This stand grew around a small stream, very much like the preferred natural habitat, which also explains why they thrived so well at this spot. Note how the bat sits directly on the anthers, bending them down. The whole inflorescence is dragged down from its original position above the other inflorescences by the weight of the bat. In the adjacent inflorescence, a ring of shining nectar and light petals is visible. Stamens are stiff and pointing, ready to receive a visitor. The bat activity in this seed orchard was very high.

Appendix 1

Nairobi

Nairobi ICRAF station

Three *C. calothyrsus* trees at a height of 5 to 6 metres were planted singly in the park outside the main offices. One tree was multiple-stemmed and chosen for observations, the other two were single-stemmed. The tree was visited by a high number of insects. Five to ten *Pachnoda ephippiata* foraged eagerly on stamens during both periods of observation in the late mornings. Besides the honeybees and the red potter wasps also observed in other areas, the flowers here were also visited by two additional wasp species; a small grey wasp and a yellow and black potter wasp. Collared sunbirds were the only flower visiting birds observed.

Karanja's farm

Karanja's farm in the Nairobi suburbs contained around 50 *C. calothyrsus* trees of around four metres height. Trees were planted in parallel rows for seed production. The many trees caused a high activity of honeybees and red potter wasps, bronze sunbirds, baglafecht weavers, and common bulbuls.

Kagarii

The area in Kagarii was hilly and extensively cropped with banana (*Musa* sp.), coffee (*Coffea arabica*), avocado (*Persea americana*), passion fruit (*Passiflora edulis*), napier grass (*Pennisetum purpureum*) and blue gum (*Eucalyptus* sp.). *Calliandra calothyrsus* was planted in many farms around the village. Three farms were visited for studies.

Wanyinyi's farm

At Wanyinyi's farm, five trees of 7 to 10 metres height were planted along the path from the garden gate to the house, and eight others of 4 to 5 meters were planted outside the garden gate along the road. Besides honeybees, the tall trees were visited by bronze sunbirds, amethyst sunbirds, goldenwinged sunbirds, common bulbuls, and olive thrushes. The smaller trees along the road were only visited by bees.

Gichohi's farm

Gichohi's farm was placed on a hill slope. Thirty *C. calothyrsus* trees were left for flowering, most of them in two rows of 5 to 7 metres height as erosion barriers across the slope. Flowers were visited by honeybees and a high number of birds including bronze sunbirds, blackheaded weavers, common bulbuls, and Abyssinian whiteeyes.

Victoria's farm

In Victoria's farm, three multi-stemmed trees of six meters and one of four meters grew in a row exposing around a hundred inflorescences. No bees visited the flowers, but birds were numerous. Nectar-foraging birds included bronze sunbirds, amethyst sunbirds, collared sunbirds, northern double-collared sunbirds, baglafecht weavers, streaky seedeaters, and Abyssinian whiteeyes. A few small butterflies and a small moth visited flowers shortly.

Kahaiini

In Kahaiini, the area was quite dry as the rainy season came late. The area was densely packed with small scale farms cropping maize (*Zea mays*), coffee, avocado, blue gum, silky oak (*Grevillea robusta*) and mango (*Mangifera indica*). Three farms were chosen for the studies.

Gichuna's farm

In Gichuna's farm, seven *C. calothyrsus* trees were left for flowering. Two single trees at a height of 2½ and 3 metres, bearing 17 and 16 inflorescences, were observed. The trees were planted on the boundary between two bare maize plots. Flowers were visited by honeybees and red potter wasps, bronze sunbirds, baglafecht weavers, common bulbuls, and streaky seedeaters at daytime and by 2 cm large ants and a single stamen-eating beetle at night.

Murera's farm

A row of 12 trees at a height of three metres was observed at Murera's farm. Flowering was sparse, probably because of the prolonged dry season. No bees visited at all. Bronze sunbirds and variable sunbirds visited for nectar. A female variable sunbird was seen touching a stigma during 14 visits to inflorescences.

Irungu's farm

At Irungu's farm, a group of eight *C. calothyrsus* trees at a height of 3 to 5 metres with 29 inflorescences grew in an open spot. Few honeybees, a red potter wasp and a bronze sunbird visited the flowers before darkness. At 7.00 pm, a swarm of about one hundred yellow 2 cm long beetles possessed the group of trees eating stamens and mating. After settling mainly at inflorescences, only few beetles moved to other inflorescences.

Maseno

The study in Maseno was carried out at ICRAF Maseno in two seed orchards, an older orchard planted in 1999 and a younger orchard planted in 2001. The two seed orchards were about one kilometre apart. Both seed stands were planted in the Veterinary farm, a wide grassland for cattle. The older seed orchard was planted right next to the road from Maseno to Luanda, a few hundred metres from ICRAF Maseno. In the Veterinary farm, only few trees such as *Acasia* sp. and *Spathodea campanulata* were found. The surrounding area is densely populated by small-scale farmers. Studies at Maseno coincided with the beginning of the rainy season. Later in the season, flowering intensity was observed to be much higher, whereby more pollinators might also have been attracted.

The 1999 seed orchard

The older seed orchard consisted of 352 trees planted at 3×3 meters. Trees in the orchard had an average height of 6.67 m (SD = 0.74, N = 30) and 6.5 (SD = 2.75, N = 30) leading stems per tree. Inside the stand, most of the leaves and flowers were in the top, but at or near the edges flowers and leaves were also produced on the lower branches. Average number of flowering inflorescences per tree was 17.23 (SD = 15.86, N = 30).

Honeybees visited the orchard in high abundance, but also bronze sunbirds and large groups of blackheaded weavers visited occasionally to drink nectar. Ants of 1 cm length were found in many flowers during night. Three short bat visits were observed.

The 2001 seed orchard

The young stand consisted of 489 trees planted at 3×3 metres. Their average height was 2.26 m (SD = 0.46, N = 49). Average number of flowering inflorescences per tree was 3.94 (SD = 5.53, N = 49). Trees had up to 23 flowering inflorescences, but many trees presented no flowers. Only honeybees and a few beetles visited the flowers.

Magui

Ndenga's farm

The Magui location was a curved landscape densely populated by small scale farmers. Ndenga's farm was the only farm in the nearby area growing *C. calothyrsus*. His farm contained around 200 trees within an area of about 80×20 m^2. Trees had an average height of 3.44 m (SD = 0.89, N = 30), the tallest growing along the boundaries. In between, young trees grew with napier grass. Only an average of 3.63 inflorescences flowered per tree (SD = 4.13, N = 30), with the highest number of flowers in the tall boundary trees. It was noticeable that a few single trees had a high pod production, while the rest of the trees produced only few pods. Half of his trees had no pods at all.

Flowers were visited by honeybees and red potter wasps, bronze sunbirds, baglafecht weavers, blackheaded weavers, common bulbuls, and yellow whiteeyes. Bats were in the air at night, but none of them landed on the inflorescences.

Mbale

Otiende's farm

Also the area around Mbale was densely populated by small scale farmers, some of them growing a few flowering *C. calothyrsus*. In Otiende's farm, *C. calothyrsus* was planted along boundaries and in rows across the land, which was slightly sloping. A total of 185 trees were counted. All trees were left to flower and produce fruits although branches were harvested for the cow. Trees had an average height of 4.98 m (SD = 0.79, N = 32) and bore an average of 20.38 (SD = 15.82, N = 32) flowering inflorescences.

Flowers were visited by honeybees and red potter wasps, but the abundance of birds was striking. Visiting bird species included speckled mousebirds, bronze sunbirds, scarlet-chested sunbirds, variable sunbirds, greenheaded sunbirds, baglafecht weavers, blackheaded weavers, common bulbuls, yellowfronted canaries, and yellow whiteeyes. A total of 114 bat visits were counted.

Malava

Malava forest orchard

In Malava forest, a fragment of the Kakamega forest, ICRAF had a site where *Grevillea robusta*, *Makemia lutea* and *Calliandra calothyrsus* were grown in orchards for seed production. Next to the ICRAF site was an agroforestry area with maize, and a secondary forest area. The primary forest was a few hundred metres away, across the main road.

The *C. calothyrsus* orchard contained 394 trees at a spacing of 2×2 – 4×5 metres. Trees had an average height of 7.20 m (SD = 1.29, N = 34) and bore an average of 32.56 (SD = 29.79, N = 34) flowering inflorescences. Seed production was largely limited to trees in the edges. Edge trees on average bore 29 pods (SD = 22.55, N = 5), while trees inside the stand bore only 0.72 pods (SD = 2.15, n = 29).

Flowers were visited by honeybees and a wide range of birds, including bronze sunbirds, amethyst sunbirds, collared sunbirds, scarlet-chested sunbirds, copper sunbirds, blackheaded weavers, common bulbuls, yellow-fronted canaries, and yellow whiteeyes. Three visits by large fruit bats were observed.

Nyambula

In Nyambula, farms had more space than in the previously visited areas, and there were even patches left without cropping. *Calliandra calothyrsus* was widespread and well-known in the area, but few farmers left trees for flowering. Some farmers collected *C. calothyrsus* seed and sold them to the seed dealer from ICRAF Maseno. Four farms were visited for studies.

Lyala's farm

Lyala had thirty trees of up to four metres height in one row across a maize field.

Honeybees were the only diurnal flower visitors. During one night, inflorescence visits by small fruit bats were observed 178 times. Almost all visits were restricted to the upper branches of the two highest trees. Bats came one or two at a time, and it was difficult to tell how many bats that interacted. Visit intensity ranged from 0 to 23 visits every 15 minutes throughout the night.

Odock's farm

In Odock's farm were 27 *Calliandra calothyrsus* of up to four metres height. They were planted in a horseshoe formation at a site with surrounding taller trees but little shading. Only honeybees were observed visiting the flowers.

Ogola's farm

In Ogola's farm, a large number of *C. calothyrsus* were planted on a slope. Five rows were planted across the slope and along one boundary down the slope. The trees were planted densely and were allowed to form thickets. Most trees were about three metres high, the tallest reaching about five metres.

Diurnally, flowers were visited only by honeybees. During two nights, 112 visits of small fruit bats were observed. Again, most visits were restricted to the upper branches of the highest tree.

Dick's farm

At Dick's farm, diurnal pollinators of two single *C. calothyrsus* trees of three to four metres height were observed. They grew in the boundary to an open agroforestry landscape and near two rows including about twenty larger *C. calothyrsus*.

Besides honeybees, the flowers were visited by blackheaded weavers, common bulbuls, and copper sunbirds.

Akites

Akites was the least intensively cropped area visited. Spacing between farms was high, and many plots lay fallow, where the grass was allowed to grow tall. Except from a recently cleared hill nearby, the landscape was flat agricultural land with scattered trees. *Calliandra calothyrsus* was observed elsewhere in the area, e.g. at the local well. Two farms were used for studies. In both farms, the seed dealer from ICRAF Maseno came to buy *C. calothyrsus* seed.

Masai's farm

At Masai's farm, *C. calothyrsus* grew in a thicket with other trees and with beehives inside. A sixty metre long *C. calothyrsus* row was planted on the boundary between a bare maize field and a fallow field. All trees were four to five metres high and produced many inflorescences. A corresponding row grew in the adjoining farm. Small fruit bats rested in the farm's mango trees (fig. 9). The mango and a to me unknown indigenous fruit tree bore ripe fruit at the time of the study. Honeybees were very abundant diurnal flower visitors, but no birds visited flowers. Only two bat visits were observed.

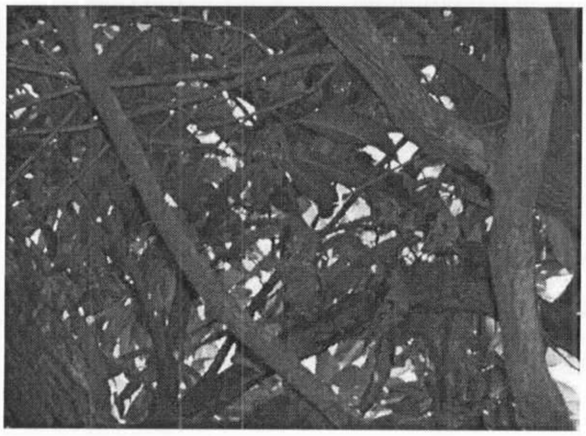

Fig 9. Small fruit bats resting in a mango tree outside Masai's house. The bats had come to rest in the family's mango trees since the nearby hill was cleared of trees a few years ago.

Sikoyo's farm

Sikoyo's farm contained 430 trees planted in two duple rows of 120 metres length and 7 metres height. The purpose of the trees was seed production, which seemed rather high.
Honeybees and a few amethyst sunbirds and copper sunbirds visited flowers at day, but no flower visitors were observed at night.

Embu

Three *C. calothyrsus* seed orchards of the different provenances Embu, Patulul and San Ramon, were planted in the KARI (Kenya Agricultural Research Institute) Embu research area. The Patulul and the Embu orchards were about one hundred meters apart and a few km away from the San Ramon orchard.

The Embu orchard

The Embu seed orchard was planted in 1997 and grew in a low, wet area along a small stream. It was surrounded by taller but open trees. The trees were around six metres tall with a high pod production on branches protruding above the general canopy. The provenance is introduced from Indonesia, but it is closely related to the Patulul provenance.

Honeybees but no birds visited the flowers at daytime. During part of two nights, 221 inflorescence visits by large fruit bats were observed. Around 15 bats were judged to participate, although only one or two visited at a time. Again, only inflorescences on tall protruding branches were visited (fig. 8). Bats visited continuously throughout the nocturnal observation periods at rates of 0 to 17 visits every 15 minutes.

The Patulul orchard

The trees in the Patulul orchard were a bit shorter than those in the Embu orchard, and the soil was drier as the orchard was not planted around the stream.

Honeybees but no birds visited flowers diurnally. Large fruit bats were observed visiting inflorescences 59 times during two half nights. The preference for tall open trees was noted again.

The San Ramon orchard

The trees in the San Ramon orchard did not look as healthy as the other *C. calothyrsus* specimen studied. Trees were rounded and inflorescences were not protruding, leaves were pale green instead of dark green, stamens were pink instead of dark red, and nectar production was sparse.

Only a few honeybees visited the flowers.

Appendix 2

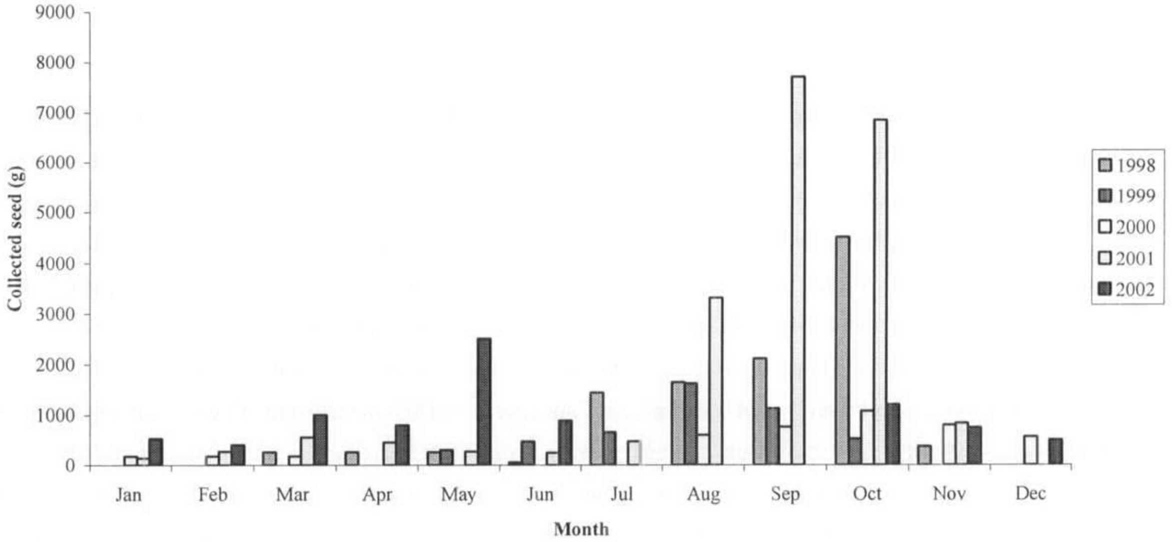

Fig. 10. Monthly *Calliandra calothyrsus* seed harvest in the Embu provenance at KARI Embu research station in the years prior to the study. Data are kindly provided by Paul Tuwei, KARI, Embu. In 2002, no seed collection was performed in July to September.

Generalised pollination in *Micromelum minutum* (Rutaceae) in northern Vietnam

Abstract

The pollination system of *Micromelum minutum* (G. Forst.) Wight & Arn (Rutaceae), a primitive and widespread member of the citroid subfamily Aurantioidae, was investigated in northern Vietnam during December. All observed flower visitors to *M. minutum* were caught and identified, and the pollen load of each insect was measured. Information on visitor abundance, behaviour, body structure, and pollen load was used to propose a flowering strategy for *M. minutum*. The flowers were visited by a diverse assemblage of insects. Thirteen species of butterflies (Lepidoptera) visited the flowers, constituting half of all flower visitors both in abundance and in number of species. The most abundant flower visitor was the butterfly *Appias albina*. Hymenopterans were frequent flower visitors as well, represented by two bee species and seven wasp species. Four species of Diptera visited the flowers, but only *Episyrphus* sp. was abundant. Generally, the most abundant visitors also carried most *Micromelum* pollen. Almost half of all butterflies above a certain size carried *Micromelum* pollen on their proboscis. The highest amount of pollen was found on *Bombus melanurus*, though, which only visited once. Based on pollen loads and behaviour, most flower visitors are thought to contribute in pollination, and *M. minutum* is expected to have a highly generalised pollination system. The pollination system may be an adaptation to flowering during the cool season, when insect activity is restricted to only a few warm days.

Keywords: Micromelum minutum, generalised pollination, Lepidoptera, pollen load, Vietnam, winter-flowering.

Introduction

Pollination interactions are often considered more specialised in the tropics than in the temperate zone (Johnson and Steiner 2000). However, some authors have argued that pollination interactions are not as specialised, even in the tropics, as formerly believed (Ollerton and Cranmer 2002). Plants tend to be more specialised on specific pollinators if pollinator populations are reliable and competition for pollinators is high. In other circumstances, when a pollinator fauna is fluctuating and unpredictable in size and composition, flower morphology more often indicates a generalised pollination mode (Eckhart 1992). In seasonal environments, generalisation levels may be correlated with the flowering season. In the cold season, pollinator diversity and predictability is lower than in the warm season. Therefore, plant species flowering in the winter or early spring are expected to have more generalised pollination systems. The level of generalisation depends on the range of pollinators that visit the flowers. These visitors, though, may not contribute equally to pollination. In some cases, only a small fraction of the flower visitors take part in the pollination of a plant, or the visitors may vary in pollination efficiency, some being more efficient pollen vectors than others (Schemske and Horwitz 1984, Herrera 1987).

Micromelum is considered the most ancestral genus in the citroid subfamily Aurantioideae (Rutaceae) (Swingle and Reece 1967). The genus constitutes 9 species, all small trees or shrubs. *Micromelum minutum* (G. Forst.) Wight & Arn differs from the other *Micromelum* species in having a wider distribution. Its range covers Southeast Asia, Indonesia, the Philippines, and northern Australia. It is an understory specialist which utilizes light flecks efficiently for growth (Watling et al. 1997). Research in *M. minutum* has largely been focused on chemical constituents (Nakahara et al. 2002, Rahmani et al. 2003). It is a winter-flowering tree, and a well-known butterfly attractant in Australian flower gardens (http://www.greeningaustralia.org.au, http://farrer.riv.csu.edu.au). Pollination studies on *Citrus* species report the honeybee (*Apis mellifera*) as the only pollinator of any significance (Moffett and Rodney 1971, McGregor 1976, Malerbo-Souza et al. 2003), but pollination studies on *Micromelum* species are missing.

In this study, the flower visitors to *M. minutum* were investigated in a nature reserve in northern Vietnam. Visitors were identified to lowest possible taxonomic level and their abundances were estimated during the first month of flowering. The pollen load of all visitors was analyzed in order to evaluate their importance as pollen vectors and their flower constancy.

Methods

Study sites

The study was conducted in the Huu Lien Nature Reserve, Lang Son province, Vietnam (fig. 1). Inside the reserve, *M. minutum* was found in forest edges bordering grassland or rice fields. Two study sites were chosen; one at the edge of a grassland clearing surrounded by forest-covered mountains (site 1), and one near a small village with rice fields on one side and cleared hills on the other (site 2). Both were open places with good light conditions. The two study sites were separated by 6 to 7 kilometres. The plains of the area are approximately 100 metres above sea level (Furey et al. 2002), and the surrounding mountains rise abruptly with peaks between 300 and 500 metres above sea level.

Time of study

The study was performed throughout December 2002, in the beginning of the flowering season. The weather conditions were constantly changing and very unpredictable. In the first half of the month, rainy days predominated, and towards the end of the month, the weather became chillier. In general, the sky was overcast throughout the month. Temperatures varied between 8 and 27°C, with an average around 15°C. On dry days, observation started at 10 am. Observations stopped if no insects had visited the flowers after two hours.

Flowers and flower visitors

One tree in each site was chosen for the study on the basis of the amount of flower buds and accessibility. At each tree, about ten open flowers were within our reach during the investigation. Dimensions of ten flowers were measured with a ruler to nearest millimetre.

All visiting insects were caught with a sweep net as they left the flower. Butterflies and moths were killed by a pinch to the thorax and stored in envelopes. All non-lepidopterans were conserved in centrifugation vials containing 70% alcohol. Home, insects were weighed (wet weight for insects conserved in alcohol), and the body length of all insects, the wing span of the lepidopterans and the proboscis length of the large hymenopterans were measured to nearest millimetre with a ruler.

Pollen loads

Total pollen loads from the insect surfaces were counted using a scanning electron microscope (SEM). To isolate the pollen, insects were removed from their vials and washed in another vial containing 70% alcohol. All vials were then centrifuged for five minutes at 2000 RPM, and the supernatant was removed. The remaining alcohol and pollen of each pair of vials was transferred to an SEM stub. After the alcohol had evaporated, pollen was coated with gold in an Edwards Sputter coater 5150B and examined. For lepidopterans, only the proboscis was examined.

Results

Flower morphology

Where it was found in full light conditions, *M. minutum* was a small tree up to four meters with a spherical canopy. When growing amongst other trees, it became taller.

Flowers are arranged in corymbs containing about 40 to 50 flowers. The flower is actinomorphic with 5 petals each 8 mm long, 10 stamens and one style (fig. 2). Flower diameter is about 22 mm. Stamens are 5 mm long central to the petals and 7 to 8 mm long in between the petals. Stamens adjoin towards the base forming a 4 mm long tube. The diameter of the tube is 2.5 mm with a slim space between style and stamens. At the base of the staminal tube is a discus-shaped nectary. The pistil is 2 to 3 mm long. Pistil, style and stigma are collectively 6 mm long. The style is a little less than 1 mm in diameter above the ovary and with a 1.1 mm broad, almost ball-shaped stigma. Sepals are highly reduced. The petals, stamens, and style are white in colour, whereas anthers and stigma are yellow. The ovary is covered with a dense layer of stiff upward pointing hairs. Blooming flowers remain open both day and night and last for several days. Pollen grains are tri-colporate with an elliptical equatorial outline and a circular to almost triangular polar outline (fig. 3). The length is about 45 μm and the width about 35 μm. Flowers had no noticeable fragrance.

Visitor abundance

Although the trees were observed almost daily, flower visiting insects were seen and caught only on seven days during the study period. These were all days with a temperature above 23°C, sunshine, and no wind.

A total of 70 insects comprising four orders, 13 families and 27 species, were caught after visiting the *M. minutum* flowers (table 1). Lepidoptera constituted most species of flower visitors and was also the most abundant order of flower visitors. Half of the visitor species and nearly half of all flower visitors were butterflies, with Pieridae as the most abundant family. *Appias albina* was the most abundant flower visitor constituting almost half of the butterflies. Hymenoptera was well represented, especially with wasps of the family Vespidae. Within Apidae, only two species were recorded. The three species *Orancistrocerus atterianus* (Vespidae), *Rhynchium mellyi* (Vespidae) and *Apis cerana* (Apidae) were abundant flower visitors. Four species of Diptera visited the flowers, but only *Episyrphus sp.* (Syrphidae) was an abundant visitor. Only five visitor species were caught at both sites.

Behaviour

Butterflies sat on top of the flowers with their legs on the petals and the body just above the anthers and stigma while inserting their proboscis to the flower bottom (fig. 4). The wasps *Orancistrocerus atterianus* and *Rhynchium mellyi* were seen forcing themselves into the flower from the top, touching anthers and stigma. *Apis cerana* sometimes inserted its proboscis between the stamens from the side (fig. 5). Members of the family Diptera never approached the nectar. They walked on top of the anthers and stigma in search of pollen (fig. 5). The two individuals of *Dysdercus simplex* (Heteroptera) were caught while mating on a flower.

Pollen load

Micromelum pollen was found on the proboscis of 12 butterflies belonging to nine species (table 1). Few butterflies carried other pollen types. All lepidopterans with pollen on their proboscis had a body length of more than 15 mm and a wing span wider than 40 mm. Pollen carrying hymenopterans all had a proboscis length of 3 mm or more and a body mass above 5 g. One third of *Orancistrocerus atterianus* and *Rhynchium mellyi* carried *Micromelum* pollen. Two thirds and one third of the individuals carried other pollen types, respectively. Half of the *Apis cerana* carried pollen. The one caught individual of *Bombus melanurus* carried the largest amount of pollen grains of any visitor. About 240 pollen grains were sampled from its body surface. Within Apidae, only one *A. cerana* carried another pollen type in addition to *Micromelum* pollen. In Diptera, three of the *Episyrphus* sp. carried pollen. Two of them, however, only carried one pollen grain, while the third carried about 40 pollen grains (table 1).

Discussion

Pollinators

The quality of a pollinator to a plant species depends on its abundance and individual visitation frequency, its tendency to pick up and carry large amounts of pollen and deposit it on stigmas, its flower constancy, and its movement pattern within and between individual plants (Herrera 1987). Only some of these parameters were estimated here. A large number of insect species was observed to visit *M. minutum*, but since flower visitors often fluctuate both temporally and spatially (Herrera 1988, Horvitz and Schemske 1990, Eckhart 1992), probably only a fraction of the flower visitors have been recorded.

As butterflies were highly abundant and often picked up pollen when visiting *M. minutum* flowers, they are expected to constitute a good group of pollen vectors for *M. minutum*. Because of its high visit frequency, *Appias albina* was probably the most important pollinator during this study. The length of the stamens and style fairly fit the length of the butterfly legs, so the butterfly body is just above the anthers and stigma while sucking nectar. In this position, pollen may be deposited in the scales on its ventral side. Since very few species of plants flowered in the area during December, pollen transfer between *M. minutum* individuals is likely to be effective. The few other pollen types found on butterfly proboscises indicated that few other flowering plants were visited.

Hymenopterans above a certain size seem to be good pollinators as well. They must be strong enough to force themselves into the flower and have a proboscis long enough to reach the nectar at the bottom of the staminal tube. The examined bees carried very little other pollen types and are therefore also thought to have mainly visited *M. minutum* before capture. The additional pollen types found on wasps indicate that they had visited more flower types before capture, so their constancy towards *M. minutum* flowers was lower.

Episyrphus sp. carried some *Micromelum* pollen and might transfer pollen to the stigma by the feet. The contribution to pollination is likely to be sparse, though, as the rest of the body does not contact the flowers. Syrphid flies may visit flowers more or less randomly and also have a negative effect as pollen predators.

The analysis of pollen loads revealed that the most abundant groups of visitors also picked up most pollen. These were the butterflies above a certain threshold size, *Apis cerana*, *Orancistrocerus atterianus*, *Rhynchium mellyi*, and *Episyrphus* sp. With the exception of *Bombus melanurus*, less abundant non-lepidopterans did not carry *Micromelum* pollen. It is very likely that *B. melanurus* and other *Bombus* species are the most efficient pollinators of *M. minutum*, as they are large and strong enough to force themselves into the flowers and have special hairs adapted to collect pollen (Barth 1991). Because of these traits, bumblebees are often very effective pollinators. As shown by Schemske and Horwitz (1984), the most efficient pollinator may not belong to the most abundant ones.

Flowering Strategy

The flowering time of *M. minutum* during the cold season of the year causes little overlap to the flowering of other plant species, and competition amongst plants for pollinators may be small. Different pollinator species are therefore likely to concentrate their visits to this species. A broad range of visitor species with eventually fluctuating abundances causes diffuse selection on the flowers, and specialisation for specific pollinators is unlikely. The flowers then remain generalised (Schemske and Horvitz 1989, Eckhart 1992). Contrarily, the high floral generalisation level allows a higher opportunity for pollination if pollinators are unpredictable. In the cold days of winter, the weather is not favourable for insects and only the few warmer days in between allows high insect activity (McCall and Primack 1992). On those days, pollination has to be effective, i.e. as many potentially pollinating insects as possible should be attracted. Since individual flowers remain open for several days, each flower has a chance of being pollinated on a warm day when pollinators are active.

The wide taxonomical range of pollen-carrying insects indicates that *M. minutum* has a generalised pollination system. It is yet another study in contribution to the view that extensive generalisation in pollination systems is prevailing, also at lower latitudes.

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Table 1. Flower visitors to *Micromelum minutum* in Huu Lien Nature Reserve, Vietnam, December 2002.

Species:	**Abundance**	**No. of caught individuals at site 1**	**No. of caught individuals at site 2**	**No. of individuals with *Micromelum* pollen (mean; range)***	**Other pollen taxa found on the species (mean; range)**
Diptera					
Calliphoridae					
Lucilia bazini	1	0	1	0	0
Mydidae	1	0	1	0	1 (1; 1)
Syrphidae					
Episyrphus sp.	8	3	5	3 (14; 1-40)	3 (1; 0-3)
Tachinidae	1	1	0	0	0
Heteroptera					
Pyrrhocoridae					
Dysdercus simplex	2	0	2	0	0
Hymenoptera					
Apidae					
Apis cerana	6	6	0	3 (31.7; 20-55)	1 (0.17; 0-1)
Bombus melanurus	1	1	0	1 (240)	0
Sphecidae					
Lyrodae venusta	1	0	1	0	0
Vespidae					
Antepipona biguttata	1	0	1	0	0
Labus hubertianus	1	0	1	0	0
Orancistrocerus atterianus	6	1	5	2 (38.5; 7-70)	3 (2; 0-4)
Pseumenes depressus	1	0	1	0	0
Rhynchium mellyi	6	1	5	2 (24.5; 9-40)	2 (0.33; 0-1)
Stenodynerus fraenfeldi	2	0	2	0	0
Lepidoptera					
Lycaenidae					
Jamides bochus	1	0	1	0	0
Rapala manea	1	0	1	0	0
Nymphalidae					
Argyreus hyperbitus	1	0	1	1 (2)	0
Cirrochroa tyche	1	1	0	1 (6)	1 (1; 1)
Lebedea martha	1	1	0	0	0
Papilionidae					
Lamproptera curius	1	0	1	1 (13)	0
Papilio bianor	1	0	1	0	0
Pieridae					
Appias albina	13	6	7	5 (12.2; 6-25)	0
Appias lyncida	4	0	4	2 (3; 3)	1 (0.25; 0-1)
Cepora nadina	2	2	0	0	3 (2; 2)
Cepora nerissa	3	2	1	2 (17.5; 8-27)	0
Eurema blanda	1	0	1	0	0
Pyralidae					
Lamprosema indicata	2	2	0	0	0

*Only individuals with *Micromelum* pollen are included.

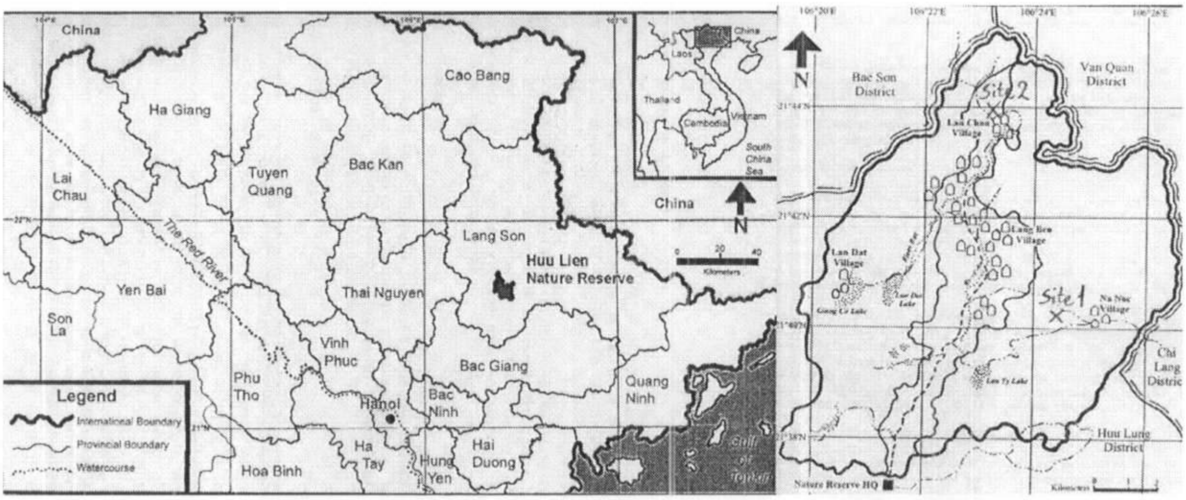

Fig. 1. Map of Huu Lien Nature Reserve. The study sites are marked by crosses. Source: Furey et al. (2002).

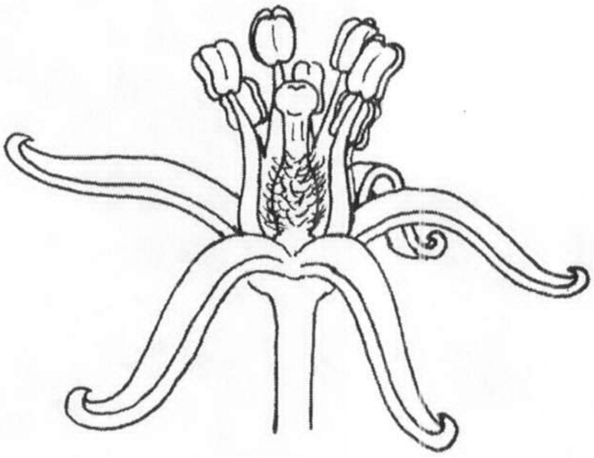

Fig. 2. *Micromelum minutum* flower. Two short stamens and one long have been removed. Five times magnification.

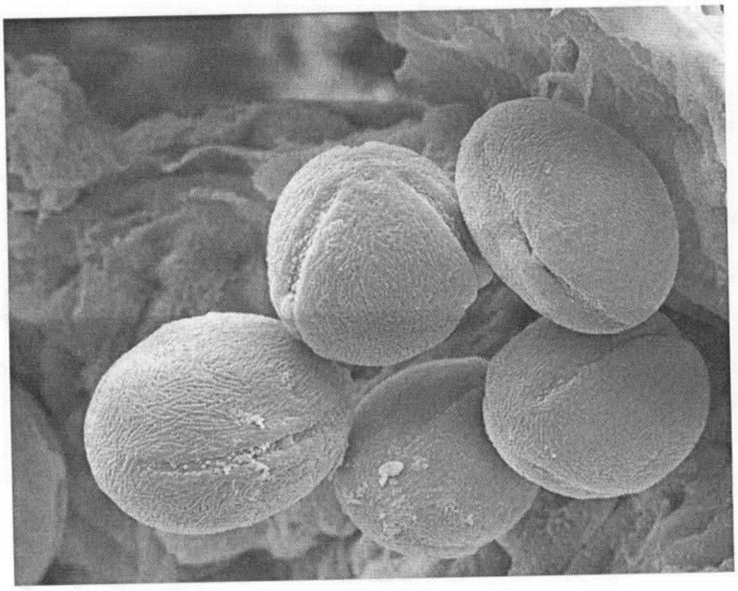

Fig. 3. *Micromelum minutum* pollen at 900 times magnification.

Fig. 4. *Appias albina* on a *Micromelum minutum* flower while sucking nectar.

Fig. 5. *Episyrphus* sp. probing for pollen and *Apis cerana* sidejacking on *Micromelum minutum* flowers.

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Afterword

I just received an e-mail on the 1st of June from Hans-Joachim Esser. He confirmed my suspicion that the tree of my study in Vietnam is not *Micromelum minutum*. It is a Rutaceae, though, but we do not know which. But well, it's all good practice.

Dear Kim,

I am really sorry to reply with so much delay. But there are a few things I can comment on your images of Micromelum of Vietnam.
I was reluctant to reply because Micromelum is a very difficult genus. It has only c. 9 species, but I think that the species boundaries were never really understood. It is the most difficult of the Indochinese genera of Rutaceae.
I worked a bit with Micromelum recently since my last email. Now I am quite confident that the species on your images is not Micromelum minutum. I agree with your opinion. The flowers are much too large. There can be much variation in leaf size and inflorescence size, but the flower size usually is quite invariable in species of all these genera.
Micromelum minutum should have flowers with petals c. 4 mm long, ovary 1.5 mm long, style c. 1 mm long. Ovary, style and stigma together should be 2-2.5 mm long. The whole flower has less than 10 mm diameter. This cannot be true for your species.
For the same reason I am doubtful if it is Micromelum at all. According to the literature, there are three species known from Vietnam:
Micromelum falcatum has very narrow leaflets (4-11 x 1.5-3 cm), long-acuminate. Typical for M. falcatum is also a large oil gland on top of each locule in the ovary. The leaflets in your plant are too wide for this species.
The third species is Micromelum hirsutum. This one has leaves with 10-25 leaflets and is a small shrub, often only 1 m tall. The flowers are slightly larger than in M. minutum, but still much smaller than in your specimen, petals 4-5 mm long.

My impression is that your plant is definitely not Micromelum minutum, perhaps not even a Micromelum. A similar genus with larger flowers is Murraya. Murraya can have flowers similar to your images, but most species have small inflorescences with few flowers only. There are however a few species of Murraya described from Vietnam that are poorly known and unusual. Most species (but not all) of Murraya have a glabrous ovary.
I am not an expert on this group of genera. My guess as determination would be Murraya sp.

I am sorry (again) if this opinion comes late, and I hope that it is nevertheless usueful.
I am working in Thailand, and I know that it is difficult for Thai people to and discuss a mistake.

Best regards

Hajo Esser

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