Effects of forest fragmentation on pollination of *Mesogyne insignis* (Moraceae) in Amani Nature Reserve forests, Tanzania

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Abstract

The efficacy of pollination biology of *Mesogyne insignis* is poorly known in fragmented forests of Amani Nature Reserve. This study was conducted to determine the effect of forest fragmentation on potential pollinators of this endangered species. Three intact forests and three forest fragments were selected for this study, the intact forests serving as control. Visual observation of insects visiting inflorescences, insect trapping and pollinator exclusion experiments were the methods employed in assessing pollination. Most members of the solitary bee genus *Megachile* were found to be potential pollinators of *M. insignis*. Diversity of pollinators was significantly higher in intact forests than in forest fragments. Overall, the total number of fruits set was significantly higher in intact forests than in forest fragments, perhaps a reflection of the higher diversity and abundance of potential pollinators in an intact forest. The pollination system of *M. insignis* is generalist in terms of systematic group of pollinators, and forest fragmentation may have significant impact on this pollination pattern. A similar study should be conducted in other Eastern Arc Mountains where *M. insignis* grows to find out whether the potential pollinators are similar or not.

Key words: Castillea, inflorescence, Moraceae, obligate mutualism, reproductive success

Résumé

On sait peu de choses de l’efficacité de la biologie de la pollinisation de *Mesogyne insignis* dans les forêts fragmentées de la Réserve Naturelle d’Amani. Cette étude a été réalisée pour déterminer l’effet de la fragmentation forestière sur des pollinisateurs potentiels de cette espèce menacée. Nous avons choisi pour cette étude trois forêts intactes et trois fragments forestiers, les premières servant de témoins. Pour évaluer la pollinisation, nous avons employé les méthodes suivantes : l’observation directe des insectes visitant les inflorescences, des pièges à insectes et des expériences d’exclusion de pollinisateurs. On a découvert que la plupart des membres du genre d’abeille solitaire *Megachile* étaient des pollinisateurs potentiels de *M. insignis*. La diversité des pollinisateurs était significativement plus élevée dans les forêts intactes que dans les forêts fragmentées. En général, le nombre total de fruits formés était significativement plus élevé dans les forêts intactes que dans les forêts fragmentées, ce qui reflète peut-être la diversité et l’abondance plus grandes des pollinisateurs potentiels dans une forêt intacte. Le système de pollinisation de *M. insignis* est généraliste pour ce qui est des groupes systématiques des pollinisateurs, et la fragmentation des forêts peut avoir un impact significatif sur ce schéma de pollinisation. Il faudrait réaliser une étude similaire dans d’autres montagnes de l’Arc oriental où pousse *M. insignis* pour voir si les pollinisateurs potentiels sont semblables ou pas.

Introduction

The East Usambara Mountains forests harbour many species that have been geographically separated from their closest relatives for long periods. These mountains are considered to be one of the most important forest blocks in East Africa (Lovett, 1998), with at least 3450 species of vascular plant recorded, one quarter of which are endemic...
or near-endemic, and many of which are threatened by human activity (Iversen, 1991; Burgess et al., 2007; Ger- eau et al., 2008). Notwithstanding of its importance, the East Usambara Mountains has been made to habitat fragmentation since the colonial period in the late 1800s into the early to mid 1900s (Hamilton & Bensted-Smith, 1989; Newmark, 2002). Elsewhere in tropical areas, deforestation produces more spatially isolated forest fragments daily (Quesada & Stoner, 2004). Forest fragmentation and the resulting spatial isolation of tree species can modify the activity of pollinators by reducing the density of potential food materials and decreasing pollinator visitation and the proportion of outcross pollen deposition (Sih & Baltus, 1987; Quesada et al., 2004). The effect of forest fragmentation on pollinator diversity and foraging behaviour may have important implications for both the reproductive success and the mating systems of the plant they pollinate (Quesada et al., 2004). This is because resources in fragmented forests may be little, and the distance between resources tends to increase; as a result, many pollinators invest more foraging within the same plant or flower (Cascante et al., 2002; Fuchs, Lobo & Quesada, 2003).

The pollination mode in the family Moraceae differs among the five tribes of the family. For example, in the well-studied tribe Ficeae, pollination is achieved through a co-evolved, obligate mutualism between figs and fig wasps (Janzen, 1979; Weiblen, 2001). In addition, most members of the tribes Artocarpeae and Castilleae are believed to be insect pollinated at the same time providing brood sites for insect larvae (Bawa et al., 1985; Berg, 2001). Overall, pollination ecology of Castilleae has been studied only in two species, in Castilla elastica Köhler, 1897 (Sakai, 2001) and in Antiaropsis decipiens Pulsford, 1953 (Zeregat, Mound & Weiblen, 2004). Castilleae provides an opportunity to study older insect-plant interactions as this group diverged from the tribe Ficeae c. 59–88 million years ago (Zeregat et al., 2005).

This is the first study to explore the effect of forest fragmentation on the pollinators of Mesogyne insignis Engler in the forests of Amani Nature Reserve. The species is listed as ‘Vulnerable’ on the International Union for Conservation of Nature (IUCN) Red List (IUCN, 2007) because of its restricted distribution and threats from anthropogenic activities to the forest in which it is found. Therefore, there is a need to investigate a reproductive success of this endangered species in fragmented forests of Amani Nature Reserve.

Materials and methods

Study site

The study was conducted in three forest fragments (c.5°06’S, 38°37’E) and three intact forests (c.5°22’S, 38°33’E) of Amani Nature Reserve forests within the East Usambara Mountains (Fig. 1). The forest fragments lie at 900–1100 m in elevation and are surrounded by a matrix of tea plantation. The climate and vegetation of the region are widely influenced by its close proximity to the Indian Ocean. Mean annual rainfall is 1918 mm at Amani and is generally sustained throughout the year by constant flow of moist currents from the nearby Indian Ocean (Hamilton & Bensted-Smith, 1989). Humidity is high, and mean annual temperature at Amani is 20.6°C (Hamilton, 1989).

Study tree: Mesogyne insignis

Mesogyne insignis is a member of tribe Castilleae in the family Moraceae (Rohwer, 1993). It is a small understorey shrub native to the eastern Tanzania (Berg & Hijman, 1989). It grows up to 15 m tall and is locally known in Sambaa as ‘Mkule’. M. insignis is a monocious shrub with

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Fig 1 A map of Amani Nature Reserve and its surroundings to show location of study sites (Fragments: F7, F9 and F12) (Intact forests: S1, S2 and M = Mboomole forest)
leaves pinnately veined and free stipules. The female inflorescences contain only one flower with 1–2 stigmas that are thin strip-shaped and of the same length (Plate 1). The male inflorescence is discoid to hemispherical and has more than one flower each with 1–6 stamens (Plate 2) (Berg & Hijman, 1989).

Visual observation of visitation of inflorescences by insects

A total of eight trees and 64 flowers were observed per site, resulting in 192 observations for 24 trees for the entire study. Visual observations were carried out with binoculars to ascertain insect visitation on both staminate and pistillate inflorescences. Two staminate inflorescences and two pistillate inflorescences were observed separately for 20 min each during four daily time periods (06.00–09.00, 09.00–12.00, 12.00–15.00 and 15.00–18.00 hours), a total of eight staminate and eight pistillate inflorescences per day.

The observations were made twice weekly for 8 weeks from September to December 2008, resulting in eight observations per tree over the duration of the study. Data reordered during the observations periods were (i) the number of insect visitors observed during the 20-min interval; (ii) the type of insects that were feeding on pollen or other flower parts; and (iii) the time spent by each insect under observation on the flower. Some of the observed flower visitors were collected using an aspirator and/or sweep net at the end of each 20 min of observation and were preserved in 70% ethanol except moths that were pinned. The insects were identified first to order level and where possible to family level and stored at the Department of Zoology and Wildlife Conservation, University of Dar-es-Salaam.

Insect trapping

To assess the density and specificity of insect visitors, sticky traps (Petri dishes lined Tangle foot pest barrier) were placed near inflorescences to collect insect visitors to *M. insignis* flowers at anthesis and at stigma receptivity (Plate 3). A total of 120 sticky traps were placed in three sites located in forest fragments and three sites located in intact forests. In each tree, four sticky traps were attached to twigs with wire twists and oriented perpendicular to the inflorescences at a distance of about 3–4 cm from the flowers. Thirty sticky traps each were placed near staminate inflorescences that were not yet receptive (had not
started to shed pollen grains) and near pistillate inflorescences that had not yet developed stigmas. In addition, 30 sticky traps each were placed near mixed inflorescences (those having both staminate and pistillate inflorescences) and on nonflowering branches of *M. insignis* trees to act as control.

Data on insect identity and abundance were recorded. Sticky traps were removed after 6 weeks, and there was no receptivity beyond this period. For staminate inflorescences, this was when no more pollen was observed or when the inflorescences had fallen off. For pistillate inflorescences, this was after a flower had withered or been pollinated and a fruit was developing. After removal, traps were covered and stored in a ziplock bag with silica gel packets to avoid rotting of the specimens. Trapped arthropods were classified into their respective orders in the laboratory.

**Pollinator exclusion experiments**

The experiments were carried out in both forest fragments and intact forests to determine whether or not pollination was affected by fragmentation. Prereceptive pistillate inflorescences were subjected to one of three bagging treatments as follows: (i) coarse-mesh bagging that exclude insects larger than 1.3 mm (Plate 4); (ii) fine-mesh bagging that excludes all insects but not pollen (c. 12.5 μm mesh width); and (iii) an open treatment (no bagging) used as control. Two mesh bags of each type were placed on pistillate inflorescences that had not yet developed stigmas per tree, making a total 60 fine-mesh bags and 60 coarse-mesh bags for the entire study. Another 60 receptive pistillate inflorescences were monitored as control. Starting from the second week of the experiment, the bags were checked after every 2 weeks until the end of experiment. After receptivity had ended (this was when the flower had developed into a fruit or withered), the bags were removed after about 3 months and viable fruits (those with seeds) were counted. In case an inflorescence had produced a fruit, the fruit was removed from the plant and were stored in a labelled coin envelope.

**Data analysis**

The statistical analyses were performed using InStat 3 (Graph Pad Software, Inc., San Diego, CA, USA), and two-tailed probability results <0.05 were considered statistically significant. Chi-square test was used to compare visit frequencies on staminate and pistillate inflorescences. Mann–Whitney U-test was used to test the differences in abundance of insects found on sticky traps in different sites. One-way ANOVA was used to compare the number of fruits set among treatments, and chi-square test was also used to compare the per cent of fruit set between intact and fragment forests (Zar, 1999).

**Results**

**Flower visitors**

The most abundant flower visitors were insects from four orders, Hymenoptera (35.75%), Coleoptera (19.75%), Diptera (13.58%) and Lepidoptera (3.7%) (Fig. 2). Overall, the frequency of insect visitors was significant higher in staminate inflorescences than in pistillate inflorescences (*U = 22.5, P < 0.05*). Most individuals of Hymenoptera were actively either feeding on the flowers or collecting

Plate 4 A coarse-mesh bag used in pollinator exclusion experiment in fragment forest of Amani Nature Reserve (October 2008)

Fig 2 The frequency of insect visitation to *Mesogyne* staminate and pistillate inflorescences in fragmented forests of Amani Nature Reserve

Fig 3 The main behavioural type (feeding and hovering) of *M. insignis* flower visitors in Amani Nature Reserve

pollen. In addition, nearly all individuals of Lepidoptera were actively feeding on pollen. A few individuals of Coleoptera and Diptera fed on *M. insignis* flowers. Overall, flower visitors were most active in late morning between 10:00 and 12:00 hours and in the mid afternoon between 14:00 and 16:00 hours (Fig. 3).

**Trapped insects**

Diptera were the most abundant insects in sticky traps, followed by Coleoptera, Lepidoptera and Hymenoptera (Fig. 4). Of the four abundant arthropod orders, the median abundances of Hymenoptera, Diptera and Coleoptera were significantly higher for sticky traps placed near inflorescences than those on nonflowering branches of *M. insignis*. However, the abundances of these insect orders were not significantly different between intact forests and fragment forests (*P* > 0.05). The respective N, with t values, were: Diptera (*N* = 26 and 35, *t* = 0.82), Coleoptera (*N* = 26 and 29, *t* = 0.58), Hymenoptera (*N* = 16 and 16, *t* = 0.91) and Hymenoptera (*N* = 14 and 12, *t* = 0.68). The intact forest had higher species richness in terms of insect orders (ten) than forest fragments (eight). In addition, both Simpson and Shannon–Wiener diversity indices were higher in the intact forests than in forest fragments (Table 1), and thus, the diversity of insect orders captured by sticky trap was significantly higher in the intact forests than in the forest fragments (*t* = 10.38, *P* < 0.05).

**Pollinator exclusion experiments**

Among the survivors, the mean number of fruits set per inflorescence in forest fragments varied from fine-mesh-bagged inflorescence (two) to coarse-mesh-bagged inflorescence flowers (6.67) and unbagged inflorescences or control (7.67). In intact forests, the mean number of fruits set per inflorescence was 5, 6.67 and 8 for fine-mesh-bagged inflorescences, coarse-bagged inflorescences and unbagged inflorescences, respectively (Fig. 5). The number of fruits set was significantly different between treatments in both forest categories (*F* = 16.36, d.f. = 2, 15, *P* < 0.05).

![Graph showing the mean abundance of potential pollinators of *Mesogyne insignis* captured in forest fragments and intact forests of Amani Nature Reserve.]

![Graph showing the mean number of fruits set per treatment in intact and fragment forests of Amani Nature Reserve.]

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Table 1 Diversity index of trapped insect orders between intact forests and fragment forests in Amani Nature Reserve

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<thead>
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<th></th>
<th>Intact forests values</th>
<th>Forest fragments values</th>
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</thead>
<tbody>
<tr>
<td>Species richness</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>Evenness</td>
<td>0.70</td>
<td>0.68</td>
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<tr>
<td>Shannon–Wiener diversity</td>
<td>1.61</td>
<td>1.57</td>
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<tr>
<td>Simpson diversity</td>
<td>4.18</td>
<td>3.90</td>
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Discussion

Visitation of inflorescences by insects

Few pollination syndromes are understood in the Moraceae family apart from the well-known obligate mutualism between figs and fig wasps (Janzen, 1979; Weihlen, 2001). It was found that insects are the main flower visitors of *M. insignis* in Amani Nature Reserve forests. Insect visitors from different orders visited *M. insignis* flowers. This suggests that pollination of *M. insignis* has got a generalist pollination syndrome. The observation results showed that staminate inflorescences had significantly higher flower visitors than pistillate inflorescences. The higher number of flower visitors noted in staminate inflorescences was probably due to the papiroaceous (a small and conspicuous flower) nature of staminate inflorescences. Most individuals from the genus *Megachile* (one morphotype) and another from the family Vespidae (one morphotype) are considered as potential pollinators of *M. insignis* because of their actively foraging behaviours. These individuals were frequently observed landing on staminate inflorescences and picking pollen.

This is consistent with other studies; for instance, individuals from genus *Megachile* have been recorded elsewhere as efficient pollinator of plants with small and conspicuous flowers (McGuire, 1993; Momose et al., 1998). Furthermore, some of them touched the stigma when searching for their palatable flower parts. A similar pattern of activity was shown by most morphotypes of Lepidoptera, and they were therefore considered also potential pollinators of *M. insignis*. Some of the individuals in the order Diptera and Coleoptera were not considered significant pollinators because their frequency of visitation was low. A similar observation was also explored in palm, whereby beetles showed the least mobility and also rather low values of visitation frequency for the majority of individual taxa (Listabarth, 2001).

Insect trapping

The most abundant insect orders captured by sticky traps were Diptera, Coleoptera, Lepidoptera and Hymenoptera. The abundance of each insect order was significantly greater near flowering branches compared with non-flowering branches. This suggests that individuals of those four insect orders were considered to be potential in pollinating *M. insignis*.

The abundance of each insect order was not significantly affected by forest fragmentation, although the abundances of Diptera and Lepidoptera were slightly higher in the forest fragments than in intact forests. This indicates that these two groups of insect orders might be the most adaptable group of insects in forest fragments. Location of the forest fragment settings is another aspect that might increase the chance of pollinators to be caught by sticky traps. This is because these sites are surrounded by tea plantations, while at the same time, *M. insignis* flowers are visited by diverse insect orders. Thus, there is a possibility that some of the trapped arthropods were also visiting tea flowers, and this could increase catch rates in forest fragments compared with intact forests. However, different species of insects in the same community can show independent fluctuation patterns ranging from low to high diversity, making it difficult to explain a single environmental process that results in changes in abundance (Wolda, 1992).

Generally, the diversity of pollinators was significantly higher in intact forests than in forest fragments because of massive transformation of habitat. Habitat disturbance in Amani Nature Reserve forests started in 1944 when large-scale tea plantations were established (Schmidt, 1989). Deforestation and isolation of remnant forests by agriculture for more than 70 years has left <413 km² of the original 950 km² of natural forest (Newmark, 2002). Because the vast majority of the trees of the Usambara forests bear number of fruits set adapted for animal dispersal, the fragments left by agriculture persist only as relict nonreproductive populations, evidently healthy as adults but incapable of effective recruitment (Newmark, 2002). Forest fragmentation has profound effects on the survival of both plants and animals either by reducing habitat size or by interfering with the availability of food resources (Lindenmayer & Fischer, 2006). Pollinators depend on the availability of various plants throughout a season. Habitat loss can negatively affect timing and amount of food available thereby increasing competition for the limited resources. Similarly, forest fragmentation increases the spatial isolation of a population, and its effect on the dynamics of gene flow may directly influence genetic structure within forest fragments. The impacts of forest fragmentation on the diversity of arthropods in the East Usambara Mountain forests have also been reported in another study: The diversity of beetles was higher in intact forests than in forest fragments of Amani Nature Reserve (Namwanda &
Effects of forest fragmentation on pollination of Mesogyne insignis

It is therefore concluded that pollination system of M. insignis is generalist in terms of systematic group of pollinators. In addition, forest fragmentation may have significant impact on the pollination pattern of M. insignis in Amani Nature Reserve forests.

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