Fortpflanzungsstrategien und Bestäubung zweier afrikanischer *Acanthus*-Arten
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So happy together? Pollination and reproductive strategy of two sympatric species of *Acanthus* L. in Kenya

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In Erinnerung an Silke Stenner

Danke, für alles.

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ABSTRACT

Kakamega Forest represents the last remnant of equatorial rainforest in Kenya. Hence, it is of great importance as last habitat for species specialized on these forests and their associated ecosystems and, consequently, for East Africa's biodiversity. The forest has been divided into several fragments due to anthropogenic habitat change still continuing today. Even though large parts of the forest have been designated protected areas, they are still used for illegal hunting, logging, and firewood collection.

This thesis focusses the pollination ecology of two species of *Acanthus*. *A. eminens* occupies clearings and riversides inside the forest, while *A. polystachyus* grows in copses and hedgerows of the surrounding farmland, as well as at the forest edge. Through forest fragmentation, *A. eminens* continues to loose suitable habitat. In addition, the distance of its populations to the congener is reduced, and the relative abundance of the species shifts towards *A. polystachyus*.

As flowering time and floral morphology of the species are highly similar, it seems likely that habitat fragmentation causes changes in pollination and reproduction of the species. In particular, *A. eminens* may loose pollinators to *A. polystachyus*, and receive more heterospecific pollen. As *A. eminens* is a common species of the natural ecosystem, such effects would indicate that habitat fragmentation causes long-term changes in ecosystem processes of Kakamega Forest, threatening its future existence in its natural state.

In my thesis, I demonstrate that the species flower in synchrony during winter dry season between October and February. There are no negative effects of distance to or habitat availability of the congener on pollination and reproduction of the species. However, I find plants in drier habitats and flowers opening later during dry season to display higher fruit set.

Both species are pollinated by carpenter bees (*Xylocopa*). There is no indication for a partitioning of pollinators through divergent flower morphology, as the species display highly similar flowers. *A. eminens* offers greater quantities of both nectar and pollen, while the total amount of floral rewards is greater in the larger individuals and populations of *A. polystachyus*. Both species probably represent important resources for pollinators in their respective habitats. Differences in pollinator abundance between populations are likely caused by habitat preferences of the pollinators.

Even though the species share most of their pollinators, interspecific pollen transfer does not affect their reproduction. This may be explained by the prevalence of geitonogamy, as bees commonly visit several flowers on the same inflorescence, plant, and neighbouring, likely related individuals. Moderate visitation rates with low pollination success indicate that carpenter bees are ineffective pollinators, and that the greatest proportion of pollen is lost between visits. Consequently, most pollen is transferred between close, potentially closely related neighbours, reducing the incidence of heterospecific pollen transfer.

These findings indicate that reproduction of either species may be limited by pollen quantity or quality. However, hand-pollination experiments show that both species are limited by pollen quantity but not pollen quality, as fruit set nearly doubles when supplementary pollen, regardless of its source, is provided. As there is no difference in seed set and seed viability between treatments, I conclude that pollen quality is generally high.

There is convincing evidence that *A. eminens* and *A. polystachyus* are adapted to flowering during dry season, which offers favorable conditions for fruit and seed development as well as for seed dispersal and germination. Differences in humidity are strongly linked to reproduction in either species, and these effects are strong enough to mask all putative effects of competition for pollination and heterospecific pollen transfer.

ZUSAMMENFASSUNG

Dem Kakamega Forest als letztem Rest äquatorialen Regenwalds in Kenia kommt eine hohe Bedeutung als Refugium für an diese Wälder und ihr Umfeld gebundene Arten und entsprechend für den Erhalt der Biodiversität der Region zu. Durch den noch andauernden anthropogenen Einfluß des dicht besiedelten Umlandes ist der Wald in mehrere Fragmente unterschiedlicher Größe zerfallen. Trotz Schutzmaßnahmen werden Teile des Waldes noch immer zur illegalen Jagd sowie zur Feuer- und Bauholzgewinnung genutzt.

Diese Arbeit thematisiert die Bestäubung zweier im Studiengebiet heimischer *Acanthus*-Arten. *A. eminens* wächst in offenen Waldbereichen, an Lichtungen und an Flussrändern, während *A. polystachyus* am Waldrand, an den Rändern großer Waldlichtungen und in Hecken und Gebüschen vorkommt. Durch die Waldfragmentierung hat *A. eminens* nicht nur an Lebensraum verloren; auch die Distanz zu Populationen von *A. polystachyus* sowie das Abundanzverhältnis der Arten hat sich verschoben.

Auf Grund der augenscheinlich gleichen Blühperiode der Arten sowie der ähnlichen Blütenmorphologie stellt sich die Frage, ob sich die Waldfragmentierung auf die Bestäubung und Reproduktion der Arten auswirkt, und ob hierdurch insbesondere *A. eminens* als besonders durch den Habitatverlust betroffene Art zusätzlich durch eine Konkurrenz um Bestäuber und durch interspezifischen Pollentransfer beeinträchtigt wird. Ein solcher Effekt auf ein häufiges, typisches Florenelement des Regenwaldes würde zeigen, dass die regionalen Veränderungen durch menschlichen Einfluss nicht nur Lebensräume zerstört, sonder auch in den verbleibenden Habitaten Folgen für wichtige Ökosystemprozesse hat, die diese Lebensräume in ihrer typischen Ausprägung auf lange Sicht bedrohen.

In dieser Arbeit zeige ich, dass beide Arten synchron während der Wintertrockenzeit zwischen Oktober und Februar blühen. Weder die Nähe zur noch die Habitatverfügbarkeit für die jeweils andere Art haben einen negativen Einfluss auf die Reproduktion der Arten. Hingegen gelangen Blüten an Pflanzen trockenerer Standorten und Früchte, die sich am Ende der Trockenzeit entwickeln, mit höherer Wahrscheinlichkeit zur Samenreife.

Die Blütenmorphologie der Arten weist zwar Unterschiede auf, ist sich aber so ähnlich, dass beide Sträucher von denselben Holzbienen (Gattung *Xylocopa*) besucht werden. *A. eminens* bietet Bestäubern mehr Nektar und Pollen, *A. polystachyus* bildet hingegen dichtere Bestände, wodurch beide Arten in ihrem jeweiligen Habitat eine attraktive Ressource für ihre Besucher darstellen. Die Besucherfauna beider Arten ist standortabhängig, was vermutlich auf Habitatpräferenzen der Blütenbesucher zurückzuführen ist.

Auch wenn die Ähnlichkeit der Bestäuberfaunen sehr groß ist, findet zu wenig heterospezifischer Pollentransfer statt um selbst in gemischten Populationen einen Effekt auf die Reproduktion zu haben. Eine Erklärung hierfür bietet das hohe Maß an Nachbarbestäubung (Geitonogamie). Die Bienen besuchen häufig mehrere Blüten an derselben Infloreszenz, mehrere Infloreszenzen desselben Individuums, sowie mehrere nah beieinander liegende Individuen einer Population. Die insgesamt niedrigen Bestäubungsraten lassen hierbei vermuten, dass die Holzienen ineffektive Bestäuber sind und dass ein Großteil der Pollenkörner verloren geht. Entsprechend wird allenfalls Pollen von nahen, vermutlich nah verwandten Pollenspendern übertragen, wodurch eine Bestäubung mit heterospezifischem Pollen weitgehend ausgeschlossen ist.

Diese Beobachtungen führten zu der Vermutung, dass die Reproduktion beider Arten durch die Quantität und Qualität des übertragenen Pollens begrenzt ist. Diese Hypothesen ließen sich aber nur teilweise experimentell bestätigen. Zwar ist die Reproduktion beider Arten durch die Verfügbarkeit von Pollen limitiert, da eine zusätzliche Handbestäubung zu einem nahezu verdoppelten Fruchtansatz führt. Eine Auswirkung unterschiedlicher Pollenherkunft und -qualität auf den Frucht- und Samenansatz oder auf die Keimfähigkeit der Samen läßt sich aber in keiner Weise feststellen.

A. eminens und *A. polystachyus* sind beide an die Trockenzeit als Blühperiode angepasst, wodurch eine optimale Frucht- und Samenreife sowie ein günstiger Zeitpunkt für die Samenausbreitung und -keimung sichergestellt wird. Die Auswirkungen von Feuchtigkeit auf die Reproduktion der beiden Arten sind derart stark, dass sie alle möglicherweise durch die Konkurrenz um Bestäuber und heterospezifischen Pollentransfer ausgelösten Effekte überlagern.

GENERAL INTRODUCTION

The multitude of interactions between flowers and their animal pollinators has fascinated researchers and non-scientists alike since the discovery of xenogamy by Sprengel (1793). The precise match between specialized flowers and their pollinators has been, quite literally, a textbook example of co-evolution since the seminal work of Darwin (1862) described the mutual adaption of flowers to their animal visitors and vice versa based on a study of various orchids.

Historically, many studies have focussed highly specialized pollination systems, in most of which mutual dependence between animals and plants is strong due to the nature of their interaction. For example, figs (*Ficus* spp., Moraceae), *Yucca* (Asparagaceae) spp. and *Trollius europaea* (Ranunculaceae) all sacrifice some of their ovules as food for larvae of their pollinators (Janzen 1979; Aker and Udovic 1981; Pellmyr 1989). Special rewards offered by plants, e.g. oil in some Cucurbitaceae (e.g. *Momordica* spp.) and Primulaceae (e.g. *Lysimachia*) and perfume in orchids of the subtribes Stanhopeinae and Catasetinae, as well as the attraction of pollinators through deceptive imitation of food (e.g. in *Arum maculatum* and several other *Araceae* and numerous members of the tribe Stapeliae (Apocynaceae, subfamily Asclepiadoideae)) or mates (e.g. in orchids of the genus *Ophrys*) also are striking examples of adaption (Buchmann 1997). These various mutual adaptions rarely fail to amaze botanists, zoologists and ecologists alike. Though uncommon groups of animals continue to be described as pollinators (e.g. lemurs, Kress 1993; day-geckos, Olesen 2003; and crickets, Micheneau *et al.* 2010), the majority of animal pollinators are birds, bats, and insects, and among the latter, predominantly bees and wasps (Buchmann 1997).

STATE OF THE ART

In all of the large taxa of pollinating animals, the whole range from highly specialized interactions between just one animal and one plant species to generalized interactions between numerous animal species and various flower types has been described. Recently, it has been repeatedly demonstrated that most plant-pollinator interactions are generalistic, and that in most habitats, the majority of flowers is visited by several potential pollinators, and the majority of pollinators visits several different flowers (Waser *et al.* 1996; Bosch *et al.* 1997; see references in Richardson *et al.* 2000). Simultaneously, the classic concept of 'pollination syndromes', a set of floral traits thought to indicate the adaption of a flower to certain animal visitors (Faegri and Pijl 1979; Stebbins 1970), has been repeatedly challenged (Ollerton 1998; Ollerton *et al.* 2009; Smith *et al.* 2009; but see Fenster *et al.* 2004). Numerous studies have demonstrated that plant-pollinator interactions are malleable, allowing animals and plants alike to adept to dynamic environments. Indeed, nothing seems fixed in many of these systems: Pollinator abundance on and their relative importance for each plant species in a system may constantly shift (Herrera 1988; Fleming *et al.* 2001; Alarcón *et al.* 2008), depending on relative abundances (Herrera 1988; Horvitz and Schemske 1990; Ashman and Stanton 1991; Kwak and Jennersten 1991; Gómez and Zamora 1999; Fenster and Dudash 2001; Herrera 2005a; Price *et al.* 2005), climate (Vicens and Bosch 2000; Abrahamczyk *et al.* 2011), nesting site availability (Potts *et al.* 2005) and many more factors and their interactions. This flexibility renders pollination processes to be astonishingly robust, with plants being pollinated by different pollinators in different habitats, and pollinators quickly adapting to different flowers depending on their availability and floral resources (Alarcón *et al.* 2008; Kaiser-Bunbury *et al.* 2010; Hagen *et al.* submitted).

Pollination ecology has experienced an increase in interest during recent years, on the one hand due to breakthroughs in methodology, but also due to the realization of the importance of pollination as ecosystem process sustaining biodiversity, and as an ecosystem service highly relevant for crop production (Kremen *et al.* 2002; Tilman *et al.* 2002; Klein *et al.* 2003, 2007; Aizen *et al.* 2009; Klein 2011). New methods both for collecting and interpreting data have been and continue to be developed to analyze the complex interactions of plant communities with their pollinator faunas. Progress has been made in understanding community-level pollination processes by analyzing plant-pollinator interactions with network methodology (Olesen *et al.* 2006), and efforts are increasing to synthesize studies encompassing wide ranges of habitats and ecosystems to shed light on evolutionary and ecological patterns in pollination ecology (for a list of current areas of inquiry in the field, as well as an excellent overview of the current state of the art, see Mayer *et al.* 2011).

But for the interpretation of these analyses, detailed knowledge of the biology, or at least the reproductive phenology, of all species involved is needed. In many cases, these data are limited, which is both a consequence of the high number of angiosperms being pollinated by animals (299200 species out of an estimated total of 352000 angiosperms, i.e. 87.5%; up to 94% in the tropics (Ollerton *et al.* 2011)), and one of the reasons why current estimates of this number still carry significant degrees of uncertainty. Given the high spatiotemporal variability described above, the collection even of simple lists matching plants and their floral visitors is painstaking work indeed (see the monumental works of Knuth 1898a,b,c).

The simultaneous increase in interest in biodiversity-related topics, leading to the collection of myriads of specimens, and the loss of taxonomic experts during the last decades have led to what has been called the 'taxonomic impediment' in ecology: Ironically, we are able to process and analyze larger and larger amounts of data with sophisticated methods and high computational power, but have partially lost the basic taxonomic and ecological knowledge to collect and interpret these data (Mayer *et al.* 2011).

Pollination and Conservation

As one of the most crucial steps in angiosperm reproduction, the transfer of pollen grains from anthers to conspecific stigmas unsurprisingly is of great importance for conservation. A loss of pollinator species may mitigate reproduction, and threaten the continued existence of populations (e.g. Segal *et al.* 2006). Shifts in plant abundance or the introduction of invasive species may cause competition for pollinators (Levin and Anderson 1970; Campbell and Motten 1985; Brown *et al.* 2002; Ghazoul 2004), as well as heterospecific pollen transfer (Waser 1978; Matsumoto *et al.* 2009). Compatible species interacting via shared pollinators may hybridize, to the extent that one of the parent species outcompetes the other (Levin *et al.* 1996; Burgess *et al.* 2005; Matsumoto *et al.* 2011). Even within a single plant species, pollinator-mediated pollen flow may cause a loss of genetic diversity when barriers preventing gene flow break down through anthropogenic influence (Rhymer and Simberloff 1996; Ackermann *et al.* 2008). Generally, it has been demonstrated that pollinator and floral diversity are closely linked, and that processes reducing one of them also mitigate the other (Biesmeijer *et al.* 2006b). Consequently, pollination is of interest both for the conservation of plants and animals.

In developing and emerging countries, many problems of ecological research converge. Though they harbor a great part of terrestrial biodiversity (Barthlott *et al.* 1996; Mutke and Barthlott 2005), offering innumerable opportunities to study specific and general ecological and evolutionary patterns and processes, they historically have received little attention by scientists (for a visualization of the global distribution of studies on anthropogenic change in tropical forest systems, see Gardner *et al.* 2009). Species inventories often are dated, and may not represent the actual fauna in these rapidly changing areas (compare Farnsworth and Ogurcak 2008). Due to their often delicate economical and political states, many of these countries lack the funds to teach and employ qualified researchers and, hence, ecologists and taxonomists. Therefore, it is quite unsurprising that efforts to analyze global ecological patterns have often found that our current knowledge from all levels of biological organization in many less-developed countries continues to be limited (e.g. Machado and Lopes 2004; Gikungu 2006; Vamosi *et al.* 2006).

On the other hand, rapid changes in land use are threatening the remaining natural habitats in these regions at an unprecedented and accelerating rate. Population growth facilitates demand for arable land, and forestry, mining and changing water regimes alter the face of many landscapes. The areas experiencing the greatest population pressure are those where biodiversity is highest, increasing conflicts between conservation and development (Balmford *et al.* 2001). Moreover, models of climate change predict changing weather patterns for many tropical regions, which may alter water availability, change species phenologies, and cause more and more severe extreme weather events (Hulme *et al.* 2001).

During the last decades, it has been realized that a breakdown of biodiversity may have strong impacts on human affairs, as ecosystem services, e.g. natural pest control, water retention, protection from erosion, but also pollination may be mitigated when species are lost (Chapin *et al.* 2000; Kremen *et al.* 2002; Tilman *et al.* 2002). These findings have increased the public interest in conservation issues, and pollination has been in the focus of media attention since the possible consequences of the so-called pollinator crisis, describing the decline of (crop-)pollinators, hit the news (e.g. Biesmeijer *et al.* 2006b; Biesmeijer *et al.* 2006a; Klein *et al.* 2007, 2008). In general, biodiversity and plant-pollinator mutualisms are closely linked (Fontaine *et al.* 2006), but more studies are urgently needed to assess the risks the disruption of plant-pollinator interactions harbors for the conservation of natural and semi-natural ecosystems and agricultural landscapes alike.

HABITAT FRAGMENTATION

The potential response of biological systems to habitat change likely is as complex as the systems themselves, but even less understood. For example, different functional groups and ecosystem processes may respond differently to habitat fragmentation (García and Chacoff 2007; Farwig *et al.* 2009; Herrera *et al.* 2010; Schleuning *et al.* 2011; see Gardner *et al.* 2009, and citations therein), and even individual species may display different responses (Didham *et al.* 1996; Kirika *et al.* 2008; Tylianakis *et al.* 2008). In addition, the effects of habitat fragmentation on species depend on the surrounding matrix in which the fragments are embedded (Prevedello and Vieira 2009). For predictions of the future development of ecosystems, more – and more detailed – studies of natural and anthropogenic ecosystems focussing all levels of biological organization are urgently needed.

Habitat fragmentation implies habitat loss, and facilitates isolation (Didham *et al.* 1996; Chapin *et al.* 2000; Steffan-Dewenter *et al.* 2002; Kremen *et al.* 2004). Both are major causes of biodiversity loss, and may cause a disruption of ecosystem functions (Chapin *et al.* 2000), further accelerating the decline of species and, consequently, whole ecosystems. Their effects are often difficult to discriminate (Diekötter *et al.* 2007; Haynes *et al.* 2007), and the effects of fragmentation itself on biodiversity are often idiosyncratic (Fahrig 2003). For pollinators, several studies in isolated habitats have found reduced pollinator activity (Didham *et al.* 1996; Steffan-Dewenter and Tscharntke 1999; Aguilar *et al.* 2006) and reduced plant reproduction (Farwig *et al.* 2009). More specialized species are more vulnerable (Rathcke and Jules 1993), but even highly generalistic species may be affected by changes in plant communities. For example, Pimm and Pimm (1982) have suggested that some generalistic species may be specialists for high resource densities, and a reduction of the latter will lead to extinction or at least dramatic population declines. On the other hand, pollinator abundance may remain unaffected by fragmentation (Farwig *et al.* 2009), and fruit and seed set of several plant species have been found to increase in habitat fragments in the study area focussed in this thesis (Bergsdorf 2006). Generalist pollinators may compensate for the loss of specialized taxa (Aizen and Feinsinger 1994), but this possibility is only given for flowers capable of being pollinated by generalists.

RELATED SPECIES

Highly similar, related plant species which occur sympatrically and flower simultaneously present a conundrum which has repeatedly attracted scientist's attention. Since related plants are more likely to hybridize, the fitness costs associated with interspecific pollen transfer are likely to be higher than for non-related species pairs. In addition, being taxonomically close often entails similar flowering times (Ollerton and Lack 1992; Wright and Calderon 1995) and floral similarity, which increases the potential for interspecific pollen transfer. When habitat fragmentation causes shifts in abundance of similar and compatible species, hybrid zones may be formed.

The most drastic effects resulting from the interaction of related species have been observed when invasive and native species interact, and the invader outcompetes natural plant populations, e.g. through competition for pollinators, or through repeated hybridization and introgression (Ghazoul 2004; Burgess *et al.* 2005; Matsumoto *et al.* 2011). On the other hand, there are many examples of related species which do not hybridize under natural conditions, and studies have explored the barriers which separate species and how they are influenced by the biotic and abiotic environment (e.g. Kay 2006). Anderson (1948) showed that hybridization is a consequence not only of compatible species growing in increasingly close vicinity, but also of the availability of intermediate 'hybrid' habitats, which are often formed through anthropogenic influence.

POLLINATION ECOLOGY IN KENYA

In Kenya, only few studies on pollination have been published before the year 2000 (Gikungu 2006). A subproject of the german BMBF(Bundesministerium für Bildung und Forschung)-funded project BIOTA (Biodiversity Monitoring Transect Analysis in Africa) East Africa has, from its start in 2001, addressed several topics of pollination in natural ecosystems, anthropogenically disturbed habitats, and agricultural landscapes, aiming both at increasing scientific knowledge on pollination processes and training of local taxonomists and pollination experts (e.g. Bergsdorf 2006; Gikungu 2006; Kasina *et al.* 2009b,c,a; Hagen and Kraemer 2010; Kasina *et al.* 2010; Hagen *et al.* submitted; Schleuning *et al.* 2011).

This study aims to contribute to the knowledge about pollination processes of two species naturally co-occurring in one of BIOTA East Africa's main study sites, Kakamega Forest in Western Province, Kenya

(Fig. 0.1), which is subject to anthropogenic habitat fragmentation. Today, about 4,000 ha of the northern part of the forest are declared National Reserve under control of the Kenyan Wildlife Service (KWS), while the southern part is managed by the Kenyan Forest Service (KFS, previously Kenyan Forest Department). Five isolated forest fragments are found around the main forest, and the total forest area encompasses 12,000 ha. The forest represents Kenya's last remaining Guineo-Congolian rainforest (Cords and Tsingalia 1982; Kokwaro 1988), and one of the few remaining forest areas of the country. Consequently, the forest is of great importance for the conservation of Kenya's biodiversity, but served or still serves as source of charcoal, fuelwood, gold, honey, medicinal plants, and construction materials for the surrounding population (Wass 1995). The area surrounding Kakamega Forest belongs to the most densely populated rural areas of the world, sustaining 600 people per square kilometer (KIFCON 1994). Currently, charcoal production and firewood collection have a strong impact on the vegetation, and bushmeat hunting and fishing put animal populations under pressure. Just before the presidential election in December 2007, the Kenyan government declared – or was rumored to have declared – firewood collection legal in protected areas, only to ban it again directly afterwards, highlighting the fickle situation of conservation in the area especially during times of political and economical unrest.

Study Species

A. eminens Clarke and *A. polystachyus* Delile var. *polystachyus* (referred to as *A. polystachyus*) are two highly similar shrubs (Fig. 0.2), growing sympatrically in the area of Kakamega Forest. *A. eminens* is a shrub species populating recent clearings in secondary and primary forest (Beentje 1994; Vollesen 2007). It grows up to five meters in height, carries spiny, robust leaves and flowers in decussate spikes with up to 50 flowers. Small individuals carry one and large individuals up to over 100 inflorescences. Flowers are zygomorphic with a showy royal blue lower lip and a short basal tube from which the stigma protrudes. Pollinated flowers develop into fruit capsules with four seeds which are explosively expelled on dehiscence.

Morphologically similar to *A. eminens*, *A. polystachyus* shares most of these traits with *A. eminens* but has pink flowers, softer, hairy leaves and grows slightly larger (up to 6 m). It is found in hedgerows, shrubland, and at forest edges, forming dense populations surrounding grasslands within the forest and the forest itself in Kakamega area, flowering in up to over 700 inflorescences on a single individual.

Both species are capable of vegetative reproduction, mainly in form of branches developing roots when in contact with soil or in humid conditions. Neither species is of any commercial value. Due to their spines and their light, hollow stems, both are of little importance for firewood collectors, and are sometimes removed to allow easier access to more valuable wood sources (own observation). Selective removal may be the reason for the disappearance of *A. eminens* from one forest fragment (Kaimosi Forest) where it was still found in 2003 (C. Analo, pers. comm.). It also provides an explanation for the complete absence of *A. eminens* from another, heavily logged fragment (Malava Forest).

A. polystachyus presumably plays a role in forest regeneration, as it spreads quickly and is a dominant species at the forest edge, capable of re-growing after grassland fires within a few weeks. In farmlands, it may, through its abundance, be an important structural element e.g. for birds which rely on shrubs for nesting (compare Laube *et al.* 2007).

Both species are visited by several species of insects, of which carpenter bees (*Xylocopa*) have been identified as pollinators (Dietzsch 2004). The large bees are capable of triggering the anthers and touch the stigma when entering the flower, while smaller bees are not. Hence, the latter likely are pollen robbers (see Fig. 0.3).

Being natural species of the study area, both species of *Acanthus* are affected differently by anthropogenic habitat change. *A. polystachyus* may benefit from the conversion of natural forest to small-scale farmland, as its habitat increases with the ratio of forest edge to continuous forest area due to forest fragmentation and with the establishment of fallow fields and hedgerows in the area. On the other hand, it is not known whether its pollinators depend on the forest as nesting habitat, and the increase in abundance of the mass-flowering shrub may cause a 'pollinator shortage', as the rising numbers of flowers have to be compensated by a greater abundance of pollinating insects.

Though not a rare species where it occurs, *A. eminens* looses habitat through the conversion of forest areas into farmland and through degrading habitat quality, as it is found in primary and secondary forests, but not in plantations and bushland. Currently, the few protected areas still covered with at least semi-natural forests, like *Kakamega Forest* (National Reserve), *Nandi Forest*, *Saiwa Swamp* (National Park), *Mt Elgon* (NP), Mt Kenya (NP) and Mau Forest (NR), remain the only potential habitats for *A. eminens* in Kenya (populations in *italics* confirmed by the author). The only confirmed populations outside Kenya are on

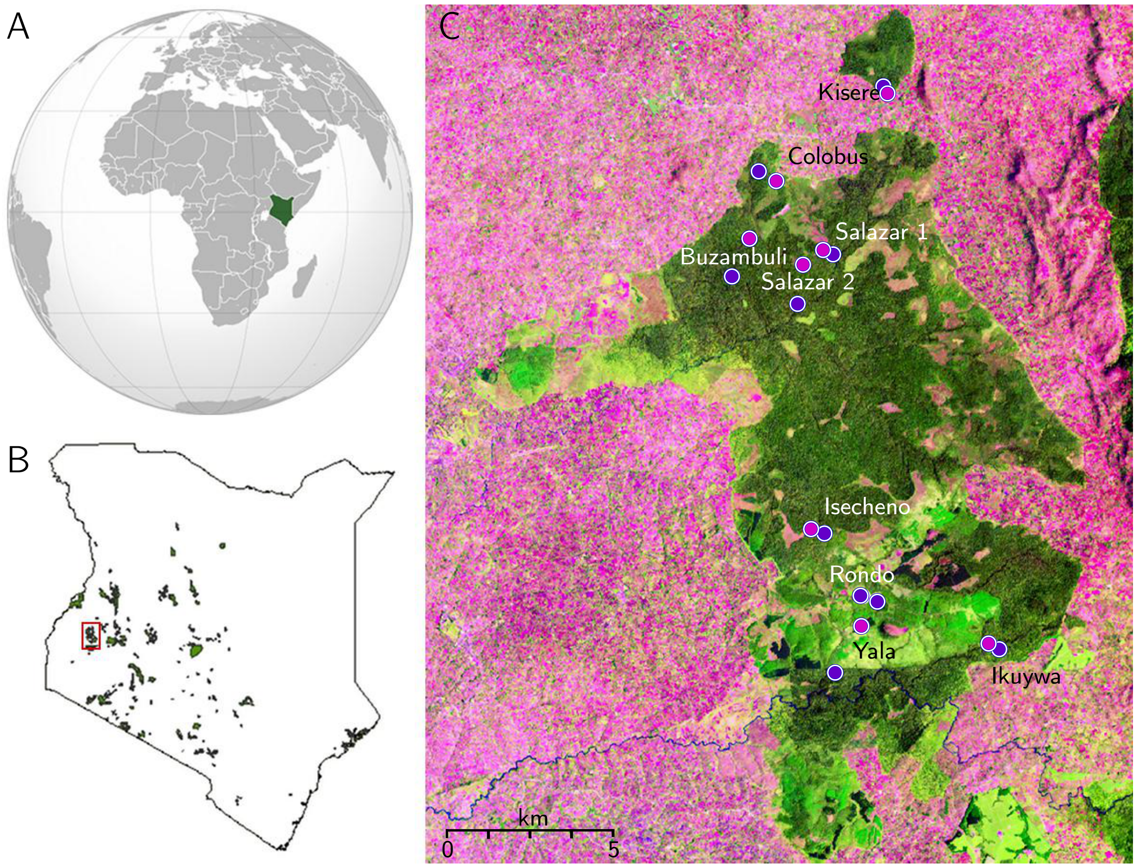

Figure 0.1: A: World map with highlighted location of Kenya (Source: Wikipedia); B: Map of Kenya indicating remaining forest areas, box highlighting Kakamega Forest; C: Satellite image of Kakamega Forest (green) and the surrounding farmland (pink). Blue and pink dots indicate populations of *A. eminens* and *A. polystachyus* included in this study, respectively (Landsat ETM +(7); 05 Feb 2001, spectral bands 5/4/3, contrast enhanced; B and C courtesy of G. Schaab, Karlsruhe University of Applied Sciences).

the Ugandan side of Mt Elgon, very likely spreading to north Uganda, the Imatong Mts in Sudan, and populations in Ethiopia (Vollesen 2007).

A. eminens' life history traits may be affected by anthropogenic habitat change: As the species colonizes recent clearings, rapidly spreading from few founder individuals both vegetatively and from seeds, genetic diversity in its populations may be limited. This may affect reproduction, and it is of interest whether forest fragmentation further increases isolation of populations, e.g. by rendering isolated populations to be visited by fewer pollinators, or by decreasing the amount of high quality (outcross) pollen reaching stigmas (Cunningham 2000). For example, the agricultural matrix surrounding forest fragments in my study area may constitute a barrier which pollinators do not cross, either because they only occur inside the forest or will not leave the forest habitat due to habitat preferences, or because the flowers offered in the matrix are highly attractive, reducing the range of foraging bouts (Bartomeus and Winfree 2011).

Reproductive interference between plant species is strongly frequency dependent (Kuno 1992), and if the relative abundance of the species shifts towards an even greater abundance of *A. polystachyus*, *A. eminens* may receive fewer pollinator visits and increased amounts of heterospecific, congeneric pollen (Gibson *et al.* 2006; Vamosi *et al.* 2006). Several *Acanthus* species are known to hybridize, and pollinator sharing could facilitate hybridization between the species (McDade *et al.* 2005).

This combination of traits renders *Acanthus* to be highly interesting for studies on the evolution and ecology of pollination systems: If the species are partly compatible, an analysis of patterns of hybridization may facilitate understanding of evolutionary and ecological

Figure 0.2: *A. eminens* (left) and *A. polystachyus* (right) in habitat (A+B); inflorescences (C+D); fruit capsules (E+F).

processes in a changing environment, and will further highlight the need to keep in mind plant-pollinator mutualisms when trying to predict the effects of habitat fragmentation. If they are not, the reproductive barriers between the species and the putative impact of anthropogenic habitat change on these barriers and *A. eminens* itself provide interesting study cases on their own, as an analysis may contribute to our understanding of ecosystem processes in Kakamega Forest, which are still largely unknown.

Previous Studies

Gikungu (2006) found a high bee species diversity in Kakamega Forest, describing 243 species in 36 genera during a two-year survey. Pollination processes in the study area were found to be resilient against habitat fragmentation in a summary of several of the findings of Biota, as bee species richness increased with decreasing fragment size and selective logging increased pollination success (Schleuning *et al.* 2011). A recent analysis of plant-pollinator networks in Kakamega Forest indicates that pollinator spillover from the surrounding structurally rich agricultural matrix supports pollination in forest habitats (Hagen and Kraemer 2010). However, the latter two studies are based on the analysis of common herbaceous species, e.g. *Justicia flava* (Forssk) Vahl (Acanthaceae, see Hagen 2008). This species grows in in a variety of more open habitats, and may generally benefit from forest fragmentation. The response of herbaceous plants to ecosystem fragmentation may be fast due to the fact that relatively short periods of time may encompass several of their generations. On the other hand, the effects on plants belonging to different functional groups may differ, and it is impossible to infer general patterns from studies on single species or vegetation types. Lifetime effects on trees and shrubs are more difficult to detect, as data take more time and effort to collect, populations may be less dense and individuals spread over large distances. Analyses of several consecutive generations are nearly impossible given limited time and funding. As from many tropical forest systems, detailed knowledge of the pollination systems of woody plants in Kakamega Forest is rare, as is detailed knowledge of specific reproductive traits (but see studies on *Prunus africana* Hook f. (Rosaceae), e.g. Farwig *et al.* 2005; Farwig *et al.* 2008a; Farwig *et al.* 2008b; Berens *et al.* 2008).

Three previous studies have focussed the pollination of *Acanthus* in Kakamega Forest. Gebhardt (2004) demonstrated that the species offer comparable floral rewards, and that their flower morphology is highly similar. She found flowers of *A. eminens* and *A. polystachyus* to differ in size, and flower size to differ between populations in each species, which may allow for partitioning of pollinators. However, flower morphology and floral rewards commonly vary between years and populations (Maad 2000; Herrera 2005b; Alarcón *et al.* 2008), and any conclusions drawn from the observation of a single reproductive period must be taken with great caution.

Dietzsch (2004) demonstrated that experimental interspecific pollen transfer decreases fruit set in a heterospecific population of *A. eminens* and *A. polystachyus*, and that there may be hybridization between the species. But whether these phenomena reduce reproduction in either species under natural conditions, or whether fertile hybrids exist, remains unknown.

In the same study, pollinators were found to travel freely between flowers of the species, but whether this only applies in heterospecific stands of the congeners remains unknown, as well as the extent to which spatial distance between populations influences pollinator fauna similarity both within and between species.

Finally, Bergsdorf (2006) has demonstrated that reproduction of *A. eminens* in terms of fruit set is increased by habitat fragmentation, and showed that increased pollinator abundance is a likely reason for this finding. However, the study did not include tests of seed viability, which may strongly limit reproductive success even when it seems high when inferred from fruit or seed set. In addition, pollinators were only determined to genus. As all pollinators are carpenter bees (*Xylocopa*), the question whether pollinator faunas differ between populations remains unanswered.

Aims of this Study

The fitness of a plant is determined by the success of every step along the way between the accumulation of resources for reproduction and the establishment of seedlings. Interacting with endogenous constraints, abiotic and biotic factors exert strong influence on the fitness and fecundity of individuals.

Based on the results of the previous studies described above, the main goal of this study is to synthesize the available data on pollination of *A. eminens* and *A. polystachyus*, their putative pollinator-mediated interaction and their reproduction, as well as to fill the blanks and answer open questions pointed out by previous works. In particular, I am interested in the effect anthropogenic habitat change has on reproduction of either species, with an emphasis on *A. eminens*. I try to answer the question whether the species benefits or suffers from habitat change, and which role may

Figure 0.3: *Xylocopa sp.1* cleaning itself after visiting a flower of *A. eminens* (A), *X.sp.2* entering a female-phase flower of *A. eminens* (B, note bend stigma and the bee's pollen load), a pollen-laden *X.sp.1* approaching a flower of *A. eminens* (C), and a non-pollinating *Meliponula bocandei* collecting or feeding on pollen of *A. eminens* (D), as well as a pollen-covered *Xylocopa* approaching (E) and entering (F) a flower of *A. polystachyus*. Note the spreading of the previously clustered anthers.

be played by its congener, *A. polystachyus*, in terms of competition for pollinators or even hybridization and introgression (Fig. 0.4). My study aims to contribute to the understanding of the effects of anthropogenic habitat change on biotic interactions in biodiversity-rich afrotropical forest ecosystems, which have received little attention by scientists so far.

CHAPTER OVERVIEW

The *first chapter* of this thesis (p. 27) focusses the reproductive phenology of the two species. Direct pollinator-mediated interaction is only possible between species flowering in synchrony, and though *A. eminens* and *A. polystachyus* obviously co-flower for some part of their reproductive periods, it is not known whether populations of either species differ in onset and intensity of flowering. Hence, I regularly quantified and compared flowering and fruiting of several populations of each species. I describe which factors may influence the timing of anthesis, and scrutinize whether stigmatic pollen loads and reproduction vary over the course of flowering in either species. All of these inquiries provide necessary background information for an assessment of the potential interaction between the species, as its overall strength depends on the phenology of each species and the synchrony between them.

Figure 0.4: A heterospecific population of *A. eminens* and *A. polystachyus* at the edge of the Kisere Forest fragment.

The *second chapter* (p. 51) deals with the differences between floral traits of *A. eminens* and *A. polystachyus*. As floral morphology and floral rewards strongly influence the abundance and behaviour of pollinators on flowers, I investigate whether floral traits of the species contribute to putative floral isolation. The data are compared with those collected by Gebhardt (2004), and I have a closer look on spatial and temporal variation in flower morphology and floral rewards. In addition, this chapter includes a survey of pollinator abundance and visitation frequency.

The *third chapter* (p. 79) describes floral visitor abundance and visitation frequencies in more detail (Fig. 0.5). Several populations of either species were observed during standardized observation units, and I answer the question whether the species share pollinators, and to what extent the abundance of pollinators depends on population traits like surrounding land use and forest cover.

Figure 0.5: A northern double-collared sunbird (*Nectarinia preussi*) visiting flowers of *A. polystachyus*. Sunbirds are rare visitors of either species at forest edges.

In the *fourth chapter* (p. 107), the focus shifts back towards the plants by addressing their reproductive success. During two reproductive periods, I measured fruit set in either species, systematically harvested fruits, catalogued their seeds, and used all seeds in greenhouse experiments to determine seed viability.

Finally, in *chapter five* (p. 131), I report the results of hand-pollination experiments aimed at discerning the influence of pollen quantity and quality on all levels of reproduction of either species of *Acanthus* (Fig. 0.6).

At the end of this thesis, a synthesis of the chapters summarizes the results of my study (p. 151), using the accumulated data for a description of the reproductive strategy of either species and the effects recent habitat change has on the congeners. In addition, I speculate on the consequences of the findings presented here for the future of the Kakamega Forest ecosystem.

Figure 0.6: Flower of *A. polystachyus* with the straight style protruding just a little from between the anthers during the male phase of the flower. Note that this flower already displays yellow, pollen-covered stigma lobes.

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1 FLOWERING AND FRUITING PHENOLOGY PATTERNS OF TWO AFRICAN SPECIES OF *ACANTHUS* L.

NILS HASENBEIN AND MANFRED KRAEMER

Acanthus eminens CLARKE and *A. polystachyus* var. *polystachyus* DELILE (Acanthaceae) occupy different habitats, with spatially close and sometimes even heterospecific populations in the fragmented afrotropical forest area of Kakamega Forest in Kenya. We demonstrate a strikingly similar flowering phenology of these closely related and morphologically similar shrub species. We reject the hypotheses that this similarity 1) mitigates reproductive success through competition for pollinators and interspecific pollen transfer with decreasing distance between species, and that 2) pollination success in terms of stigmatic pollen loads is reduced in populations where both species grow in close vicinity. Also, we do not find evidence that 3) temporal variability of pollination success explains the co-adaption of the species to the same reproductive period. However, we present data suggesting that reproductive success is linked to progress of dry season and microclimatic conditions, indicating that potential selection on flowering phenology is constrained by adaption to climate and habitat conditions in these species.

INTRODUCTION

Reproductive isolation, which is crucial for both speciation and coexistence of species, may be achieved through several pre- and postzygotic barriers in flowering plants. Because of their value as model systems to help us understand the different mechanisms of isolation and their often complex interactions, pairs or groups of co-flowering, sympatric and often morphologically similar species have repeatedly received attention from scientists (e.g. Anderson and Hubricht 1938; Sinclair 1968; Arnold *et al.* 1993; Chari and Wilson 2001; Ramsey *et al.* 2003; Kay 2006; Martin and Willis 2007; Botes *et al.* 2008).

Stiles (1975) pointed out that it would be "(...) probably very unusual to find in nature simultaneously flowering species that are identical in floral phenology (...)", and it has been shown that co-flowering plant species may compete for the services of shared pollinators (e.g. Levin and Anderson 1970; Brown and Mitchell 2001, but see Laverty 1992; Ghazoul 2006), receive incompatible pollen (Waser 1978), or produce hybrid offspring (e.g. Anderson 1948; Anttila *et al.* 1998; Wilsdon and Richards 2009). All of these reproductive interferences are likely to reduce reproductive success, and many studies have focussed on mechanisms which have putatively evolved to avoid them (e.g. Barone *et al.* 1992; Chari and Wilson 2001; Ramsey *et al.* 2003; Yang *et al.* 2007; Bänziger *et al.* 2008; Botes *et al.* 2008; Wu and Zhang 2010). In a recent review of studies on reproductive isolation in plants, Widmer *et al.* (2008) have concluded that in most scrutinized species pairs, prezygotic isolation is stronger than postzygotic isolation (see also Templeton 1981, but see e.g. Wendt *et al.* 2008). Among prezygotic barriers to pollen flow, separation of flowers by different habitat preferences and segregation of flowering phenology may be the most effective (Stiles 1975, 1977; Waser 1978).

Anderson (1948) noted that divergent habitat preferences are a reproductive barrier likely to be affected by recent anthropogenic habitat changes (see also Anderson and Hubricht 1938; Anderson and Stebbins 1954). A breakdown of spatial isolation may increase hybridization and cause the formation of hybrid swarms in compatible species (Anderson 1948; Arnold 1997). Anttila *et al.* (1998) and Wolf *et al.* (2001) have demonstrated that plants invading new habitats may genetically swamp compatible congeners, to the extent of genetic assimilation of large populations and, potentially, species. Moreover, Rhymer and Simberloff (1996) have argued that anthropogenic loss of genetic traits through reproduction of genetically divergent individuals is not limited to hybridization between native and invasive species or between native species whose abundance patterns are modified, but occurs through the loss of genetically distinct populations of the same species as well (e.g. Ackermann *et al.* 2008).

Divergence in flowering time also has been described as a mechanism of maintaining the integrity of otherwise compatible species in the same habitat (Baker 1961; Stiles 1977; Botes *et al.* 2008), and as a potential path toward speciation (Arnold 1997; Ferriol *et al.* 2008). Flowering at different times reduces competition for pollinators even between generalistic plants (Stiles 1977; Lack 1982; Aizen and Rovere 2010). Hence, it may reduce interspecific pollen transfer, and thereby competition for stigmatic surface, which both have been shown to be detrimental to reproductive success even for species not closely related (Waser 1978). Sequential flowering of similar species may also facilitate continuous resource availability for shared pollinator species, stabilizing their populations and increasing pollination success (Waser and Real 1979).

On the other hand, synchronous flowering of plants may also be beneficial to reproduction of individuals of the same species (Crone and Lesica 2004) and of heterospecific populations of flowers visited by the same pollinators (Thomson 1978; Moeller 2004; Ghazoul 2006). Flowering time of plants may be linked directly to the temporal abundance pattern of pollinators, as demonstrated by Waser (1979) (see also Ashton *et al.* 1988; Sakai 2002), and a change in this synchrony may have dramatic effects on pollination success (Thomson 2010).

Interspecific differences in flowering phenology may be caused by genetic variation, reflecting adaption to different habitats (Primack 1980; Matziris 1994). Recently, interest in temporal and spatial isolation as mechanisms of reproductive isolation between species has been additionally fueled by growing concerns about the potential effects of global changes in climate and land use. Both have strong effects on relative abundances and distances between species, and may alter species composition and flowering phenology within an ecosystem (Lamont *et al.* 2003), affecting the strength of interactions between species in a community. When patterns of phenology are differentially affected by climate change, one possible consequence may be phenological mismatches between plants and their pollinator species (compare Visser and Holleman 2001; Miller-Rushing *et al.* 2010) which may also be caused by divergence of flowering phenology between different microclimates (e.g. Bertin and Sholes 1993; Forrest *et al.* 2010).

Many studies on pollinator-mediated reproductive interaction between closely related, morphologically similar species of flowering plants have focussed on altitudinal gradients in alpine plant communities (e.g. Inouye 2008; Post *et al.* 2008), environmental gradients (Inouye *et al.* 2003) or are based on the analysis of life-cycle traits in temperate ecosystems (Kudo *et al.* 2008). In these systems, strong seasonality clearly limits and shapes flowering phenologies. Pollination studies from warmer climates, especially from tropical areas, are rare though the latter encompass both the most economically and hence environmentally challenged nations and the greatest richness across all levels of biodiversity on the planet's terrestrial surface (Vamosi *et al.* 2006, but see Chapman *et al.* 2005). Climate change in tropical systems may cause dramatic changes in some areas (Hulme *et al.* 2001), potentially affecting all kinds of vegetation, but most studies have focussed on tree species (Wheelwright 1985; Ashton *et al.* 1988; Murali and Sukumar 1994; Chapman *et al.* 2005, but see Opler *et al.* 1980). The basic knowledge of individual species traits, e.g. of reproductive phenology and specific roles in mutualistic and antagonistic interactions, is missing in many taxa. Hence, studies from tropical systems are urgently needed, as this lack of knowledge reduces our capability to design and interpret long-term studies to predict consequences of change in habitat and climate for natural and anthropogenic biological systems alike. It also hinders progress in our understanding of large-scale ecological patterns, due to missing data not only from certain biomes, but also from their respective phylogenetic groups (Larson and Barret 2000).

Habitat and climate change may be linked in various ways, and Lamont *et al.* (2003) have shown that plants in disturbed habitats may display a flowering phenology deviating from that in their natural habitat, facilitating hybridization in species which were both spatially and temporally isolated before. Both processes may lead to changes in, or disruption of, links between plants and their pollinators, and these effects may be cascading, affecting sequentially flowering species connected through shared pollinator species (Saavedra *et al.* 2003). Especially in the case of invasive species, new interactions may be formed (Abbott 1992; Vilà *et al.* 2000).

In disturbed and artificial habitats, competition for pollinators is common (Rathcke and Lacey 1985), and has stronger effects on comparatively less abundant species, as has interspecific pollen transfer through shared pollinators (Levin and Anderson 1970; Burgess *et al.* 2005, 2008; Morales and Traveset 2008). Conspecific pollen may be lost when pollinators visit heterospecific flowers before pollen deposition on a conspecific stigma (see Morales and Traveset 2008 and citations therein), and the amount of interspecific pollinations and of heterospecific pollen on stigmas may increase with the number of flowers of a competing pollen donor (Levin and Anderson 1970; Ghazoul 2002). Thus, these processes may endanger rare species and those found in fragments of increasingly rare habitats when their relative abundance is reduced, either by a reduction of flowers of the species itself or by an increase in the abundance of the competitor.

In this study, we investigate the reproductive phenology of *Acanthus eminens* and *Acanthus polystachyus*, which are found growing in different but interlocked habitats in our study area, Kakamega Forest in Western Province, Kenya. Kakamega Forest has suffered from anthropogenic fragmentation in recent history (Schaab *et al.* 2010), reducing the distance between potential habitats of the species. While *A. eminens* is found in gaps and along riversides in highland forests, *A. polystachyus* is part of succession at forest edges, invading the surrounding open habitat, and is commonly found in hedgerows. Due to forest fragmentation and the abundance of hedgerows in the surrounding farmland, *A. polystachyus* benefits from recent habitat change. *A. eminens* looses potential habitat through loss of forest area, and the relative abundance of the species is shifting towards a higher density of *A. polystachyus*. *A. eminens* flowers only during dry season between October and February. Simultaneously, *A. polystachyus* displays a pronounced peak of mass-flowering, though it also flowers sporadically throughout the year. The species present morphologically highly similar, albeit differently coloured flowers which renders pollen transfer between the species highly likely in populations very close to the respective congener, and especially in heterospecific populations.

We assess the flowering phenology of *A. eminens* and *A. polystachyus* through analysis and comparison of the temporal and spatial patterns of flowering and fruiting in populations of both species, aiming to understand the selective forces shaping the reproductive phenologies of the species. We hypothesize that 1) fruit set of both species is reduced with decreasing spatial distance between congeners, reflecting either competition for pollinators or for stigmatic surface. We use a) a nearest-neighbour approach by analyzing fruit set in relation to the distance to the next heterospecific *Acanthus* population, and, since fruit set should depend not only on the distance to, but also on the abundance of heterospecific plants, additionally assess whether b) increased habitat availability for the congener in terms of land cover decreases fruit set.

Both flowering phenology and fruit set may be linked to other factors, including microclimatic conditions (Jackson 1966), resource availability (Ayre and Whelan 1989) and genetic similarity between plants, some of which may be autocorrelated with spatial distance (Almeida-Neto and Lewinsohn 2004). Since our main interest is to scrutinize whether there is pollinator-mediated reproductive interference between species, we use stigmatic pollen loads as a direct measure of pollination success, allowing to discriminate between pollinator-mediated and other effects. We hypothesize that 2) conspecific stigmatic pollen loads of *A. eminens* decrease with decreasing spatial distance to or decreasing habitat availability of the congener, due to reduced competition between species.

Lower pollination success and fruit set of early and late flowering plants would indicate stabilizing selection on flowering phenology (Primack 1980), and divergence from this pattern might provide clues about other factors influencing fruit set. We therefore test the hypothesis that 3) pollination success and fruit set are reduced at the beginning and the end of the flowering period, which would indicate stabilizing selection.

Together, data on spatial and temporal variations of both fruit set and stigmatic pollen loads allow to discern putative pollinator-mediated reproductive interference from other factors which might influence reproduction in *Acanthus*.

MATERIALS AND METHODS

STUDY AREA

Our study was conducted in Kakamega Forest in Western Kenya (0"170N, 34"540E). Kakamega Forest is considered the easternmost remnant of the lowland Guineo-Congolian rain forest belt, and forest fragmentation has led to the formation of 5 fragments (130 to 1,400 ha) and a remnant main forest (8500 ha, Schaab *et al.* 2010) comprising a total of 12,000 ha of rainforest vegetation at an altitude between 1,500 to 1,700 m above sea level. It is managed by the Kenyan Wildlife Service (KWS) in the north and the Kenyan Forest Service (KFS, former Forest Department) in the south. Mean monthly temperatures range from 11°C to 29°C, with an average temperature of 22°C. Rainfall averages 2,000 mm per year, with two distinct rainy seasons between March and May ('long rainy season') and September and November ('short rainy season') and two distinct dry seasons between December to February ('dry season') and from June to August ('cold dry season'). The forest is under significant pressure due to illegal logging, firewood collection, grazing, and hunt for bushmeat, and large parts are highly disturbed (Schaab *et al.* 2010). The forest is surrounded by densely populated (336 to 746 inhabitants per km^2, Mitchell 2004), highly diverse and structured farmland with 0.2 to 0.7 ha of land per household (Greiner 1991; Kenyan Ministry of Agriculture 2006). 62% of all households generate their income from agriculture, and the district's poverty rate is about 52% (Dose 2007).

PLANT SPECIES

Acanthus eminens CLARKE is a shrub growing in clearings in primary and old secondary mountain rainforest between 1,500 and 2,800 m above sea level in Kenya (Beentje 1994). It is also found on the Ugandan side of Mt Elgon (pers. obs.), as well as in Ethiopia, and in the Imatong Mts in Sudan, very likely spreading to north Uganda (Vollesen 2007). It forms distinct populations of between 65 and 2,574 plants in Kakamega forest area, as well as relatively continuous but less dense stands along riversides. The species grows up to five meters in height, carries spiny, robust leaves and flowers in decussate spikes up to to 50 flowers. Plants carry several inflorescences, with small individuals carrying one and large individuals up to over 100 inflorescences. *A. eminens* shares shape and functionality of its zygomorphic flowers and of its four-seeded fruit capsules which explosively expel seeds on dehiscence with *Acanthus polystachyus* DELILE var. *polystachyus* (referred to as *Acanthus polystachyus*). The latter grows in hedgerows as a common shrub, as well as on the outer edges of forests and around grasslands inside forests between 1,100 and 2,500 m above sea level in Ethiopia, Eastern Sudan, Eastern Uganda and Tanzania. In Kenya, it occurs only in Western Province and is missing on the plains around lake Victoria (Vollesen 2007). Flowers of *A. polystachyus* are slightly smaller than those of *A. eminens*, while plants are larger and may carry up to several hundred inflorescences. Both the violet-blue flowers of *A. eminens* and the magenta/pink flowers of *A. polystachyus* are visited mainly by carpenter bees, as well as, occasionally, by sunbirds (Bergsdorf 2006). Though *Acanthus* species are known to hybridize readily in cultivation (McDade *et al.* 2005), and at least two of the other species of this genus (*A. leucostachyus* WALLICH and *A. longipetiolatus* KURZ) have been suggested to be of hybrid origin (Furness 1996), to our knowledge there is no report of hybridization between *A. eminens* and *A. polystachyus*.

In this study, we analyzed data from five main forest and six forest fragment populations of *A. eminens*, and the eight corresponding nearest-neighbour populations of *A. polystachyus*. In two of the fragments, we analyzed data from two different populations of *A. eminens*. Two fragment populations were heterospecific stands with less than 10 m distance between individuals of both *Acanthus* species. Distances between other populations ranged between 220 and 4,000 m. This study includes data from three different flowering periods, between start of November and end of February 2006/2007, 2007/2008, 2008/2009. Note that, for the sake of readability, we address the flowering seasons by the year they end (e.g. 2006/2007 is reported as flowering season 2007).

Due to high intensity of human disturbance, we were not able to tag individual plants for assessments of phenology and fruit set, and resorted to random sampling. In addition, please note that due to political unrest following Kenya's presidential election, some sample sizes of data obtained between December 2007 and February 2008 are decreased.

FLOWERING PHENOLOGY

During two flowering periods (2008 and 2009) we conducted 6 biweekly surveys in up to 11 populations of *A. eminens* and up to 8 populations of *A. polystachyus*, randomly selecting up to 15 plants for data collection. Surveys were started after anthesis of at least one plant of *A. eminens*. We counted the total number of inflorescences, the total number of flowers of a randomly selected individual inflorescence, the amount of flowering inflorescences, the amount of fruiting inflorescences and, additionally, the amount of fruits of one randomly selected fruiting inflorescence (if present). Inflorescences were considered to be flowering when they had at least one open flower, while only inflorescences which had spent all flowers were considered to be fruiting. From these absolute counts, we calculated the percentages of pre-anthesis, flowering and fruiting inflorescences per plant.

To assess not only the overlap of flowering periods (see Primack 1980; Augspurger 1983) but the overall synchrony in intensity of flowering between populations and species (Freitas and Bolmgren 2008), we calculated the percentage overlap (Bray-Curtis dissimilarity, which equals 1-QS, with QS being the Sœrensen similarity index) between the average percentages of open flowers as well as the percentages of budding, flowering and fruiting inflorescences for populations of both species in both years.

To calculate the phenological overlap between species, we averaged the percentage of flowering inflorescences for each population in each observation interval. Each value was paired with the corresponding value from the closest heterospecific population. Data points where no plant of either species was found flowering were excluded from analyses. The same analysis was repeated for the percentage of open flowers, fruiting inflorescences and inflorescences before anthesis. The degree of correlation between values for *A. eminens* and *A. pubescens* was determined by calculating the Pearson product-moment correlation coefficient (r).

Fruit set

Fruit set per flower was determined during phenology surveys by counting the total number of flowers and fruits of a single, randomly chosen fruiting inflorescence per plant.

We pre-selected three factors which we assumed to potentially influence fruit set: a) forest cover in a 1,000 m radius, centered on the center of the population as a measure of habitat availability of either species (with higher values indicating a higher density of forest as habitat of *A. eminens*, and lower values indicating a more open landscape suitable as habitat for *A. polystachyus*); b) distance to the next heterospecific population, and c) the progress of time measured as count of days since the first of October.

As we assume d) canopy cover to be a strong indicator for microclimatic conditions we included it into analysis for *A. eminens*, but left it out for *A. polystachyus* due to the difficulties arising from determining a similar, meaningful and comparable factor for the populations of *A. polystachyus* in the open landscape and at forest edges. The same applies for e) population size, which we calculated for the localized patches of *A. eminens*, but could not quantify for most of the linear populations of *A. polystachyus* which often extend for several kilometers along the forest edge.

Forest cover and Inter-Population distances

Forest cover and inter-population distances were kindly provided by the team of BIOTA E02 (Prof. Dr. G. Schaab, University of Applied Sciences, Karlsruhe), based on GPS measurements inside populations. Note that we re-calculated models including forest cover for all available scales (radius of 100, 500, 1,000 and 2,000 m around population centers) separately for both species, and used only the scale which provided most explanatory power.

Canopy cover and population size

Continuous measurements of microclimatic data were not possible due to the high intensity of human disturbance inside the forest. To reflect climatic conditions within the forest, we used two measures of canopy cover, which is linked to many abiotic factors, especially to humidity and exposure to sunlight. First, we recorded canopy cover during transect walks. In each population, we used two perpendicular transects of 100 m, subjectively measuring canopy cover from 0 (no cover) to 8 (very dense cover) every ten meters. In addition, we validated our measurements in a subset of populations using digital image analysis, using the Software GapLightAnalyzer (Simon Fraser University, Institute of Ecosystem Studies), corrected for the GPS location of Kakamega Forest. For each population, (10-)25 images were taken with a digital camera (Coolpix 5400, Nikon, Tokyo, Japan) equipped with a fisheye lens (FCE9 with UR-E10 adapter, focal length 5.8 mm, Nikon, Tokyo, Japan), mounted on a leveled tripod. All images were taken in the late afternoon to avoid overexposure, and north-aligned with a compass. Distance between individual images was >10 m, which excluded overlap between image area. We found our subjective measurements confirmed by comparison with canopy openness [%] as calculated by the software (Linear regression, $F_{1,7} = 27.06$, $r^2 = 0.795$, $p = 0.0013$). Analyses presented here are based on transect walks, as not all remote populations could be accessed in time for canopy imaging.

Population size was assessed during the same transect walks as canopy cover, counting all flowering and non-flowering plants of *A. eminens*. Population size was estimated by multiplying the mean number of flowering plants per square meter by the area covered by the population, which was calculated from GPS measurements.

Primary pollination success

We assessed the primary pollination success (PPS) of both *A. eminens* and *A. polystachyus* as the presence of *Acanthus* pollen grains on a stigma as described below. Pollen of the congeners were not discriminable (compare Furness 1996).

We collected styles from flowers of both species which did not show any sign of wilting or physical damage to their stigmas after they had dropped their corolla. The opposing flower on the inflorescence, albeit not collected, was checked for damage or infection to further ensure sample quality. No sampled flower showed presence of nectar, or was observed to be visited by potential pollinators. We therefore assume that these flowers had received all, if any, potential pollinator visits possible during their lifetime. Styles were removed using forceps, and stored individually in labeled tubes containing 70% alcohol. For analysis, stigmas were embedded in fuchsine jelly cubes (based on Kearns and Inouye 1993 p. 289, protocol modified for Agarose by U. Zumkier). The cubes were molten on glass slides, squashed under a glass cover, and pollen grains were counted using a standard light microscope.

Flowers of *Acanthus* have two locules with two ovules each. As reviewed by Cruden (2000), flowers commonly have to receive more pollen than they have ovules for optimal pollination success, with most studies suggesting four to six times more pollen than ovules. McDade (1983) found that *Trichanthera gigantea*, another Acanthaceae, only sets fruit when pollinated with one pollen grain per ovule. We converted absolute *Acanthus* pollen grain numbers into three levels of pollination success: First, stigmas with any adherent congeneric pollen. Second, to account for sample impurities, stigmas with at least four adherent pollen grains (one per ovule, the minimum for fruit set suggested by McDade (1983)), and third, stigmas at least 16 adherent pollen grains (the minimum suggested for maximum seed set as suggested by Cruden (2000)).

STATISTICS

For data analysis and visualization, we used R (R Development Core Team 2011) Version 2.12.2 and newer. Additional packages and references for non-standard procedures are provided in the description of methods below. For tests of normality, we used package 'car' (Fox and Weisberg 2011). Non-parametric statistics were used where required.

Sources of variation in flowering phenologies were partitioned using a permutational multivariate analysis of variance using distance matrices (ADONIS, package 'vegan', Oksanen *et al.* 2011). In our model, we included canopy cover, forest cover in a radius of 1,000 m (*A. eminens*) and 500 m (*A. polystachyus*) around the center of the population, and position along a north-south gradient as potential predictors of variance, due to their potential effects on microclimate. Since variation in canopy cover did not significantly affect phenological traits of either species in both years, we excluded it from the model, focussing solely on the north-south-gradient, forest cover, and their interaction.

It was also used for Mantel tests, which we utilized for comparison of spatial distance and phenological dissimilarity (Legendre and Legendre 1998). The test procedure calculates correlation (Pearson's product-moment correlation coefficient) between to matrices of identical rank, and assesses significance by repeatedly and randomly permuting rows and columns of one of the matrices. p-values represent the proportion of permutations with a higher correlation coefficient than the original data.

To assess which factors contribute to fruit set of the species, we used hierarchical partitioning (HP, package 'hier.part', Walsh and Mac Nally 2008) to quantify the contribution of each measured variable to the variance of the observed data (for a detailed description of HP, and a comparison with other methods of factor selection, see Mac Nally 1996, 2000, 2002; Quinn and Keough 2002). HP partitions variance between predictors included into the model by calculating the independent contribution of each factor alone, and the relative contribution of each predictor to variance of the response variable across all models based on all potential combinations of the factors, weighed by the explanatory power of each model. Statistical significance is based on Z-Scores derived from randomization of the original data, with $Z \geq 1.65$ representing an upper 0.95 confidence limit equivalent to $p \leq 0.05$. All our HP analyses were based on r-square measures of goodness of fit, and 5,000 randomizations. Note that HP partitions the variance explained by the factors included in the model, highlighting its importance relative to the other factors, but not its absolute contribution. Hence, we calculated standard linear regressions for models including the factors found to have significant contribution to data variance based on HP, in order to quantify the percentages of variance explained by each factor in a way compatible with standard r-squared calculations.

RESULTS

In total, we collected 725 (2008) and 842 (2009) phenological records for *A. eminens*, and 563 (2008) and 521 (2009) records for *A. polystachyus*. Several inflorescences started flowering simultaneously (Fig. 1.1A). During the first survey, over 80% of inflorescences of *A. eminens* had not opened their first flower during both reproductive periods. In *A. polystachyus*, more inflorescences opened simultaneously, with up to 60% open during the first survey in the second flowering period. For both species and seasons, the percentage of inflorescences in bud stage dropped to nearly zero in January (*A. eminens* – 2008: 2.3%, 2009: 4.0%; *A. polystachyus* – 2008: 6.5%, 2009: 4.0%). We continued surveying a subsample of these inflorescences, finding their buds dried out before anthesis and moulding after rain. The number of open inflorescences in both species peaked during surveys around new year's eve (Fig. 1.1B). In *A. eminens*, the peak was more pronounced in both years, with up to nearly 80% of inflorescences flowering simultaneously in 2009.

The general pattern observed for inflorescences is reflected in the percentage of open flowers (Fig. 1.1C). At the begin of our surveys, very few flowers were open (*A. eminens* – 2008: 0.7%, 2009: 0.3%; *A. polystachyus*: 2008: 1.4%, 2009: 1.4%). In populations of *A. eminens*, at most 11% of all flowers and in *A. polystachyus* at most 8% of all flowers were open simultaneously during peak flowering.

In *A. eminens* populations, we did not find any fruiting inflorescences during our first survey (Fig. 1.1D). In *A. polystachyus* populations we found a low average number (2008: 4.7%, 2009: 6.2%) of fruiting inflorescences at this time.

In both species, plants with several inflorescences did not open all of them simultaneously. Flowers opened at a mean rate of 0.66 flowers per day, and in most flowering inflorescences there were two to three flowers at any given time (For *A. eminens* mean = 2.65, median = 2.67 and mean = 2.34, median = 2.19. For *A. polystachyus* mean = 3.01, median = 3.00 and mean = 2.94, median = 2.96. Values for 2008 and 2009, respectively).

Factors influencing flowering phenology

The number of open flowers and the percentage of flowering inflorescences of *A. polystachyus* were only influenced by position of the population along a latitudinal gradient, possibly reflecting an environmental gradient (Tab. 1.1). The same effect was found in *A. eminens*, where both traits were also influenced by years. Forest cover was only found to have an effect on the percentage of flowering inflorescences in *A. eminens*. The percentage of budding inflorescences was not significantly influenced by any of the factors included in our models, in either species.

We used Mantel tests to analyze whether spatial distance between populations explains variation in the percentage of open flowers, flowering inflorescences, and budding inflorescences for each species. We did not find any significant correlation for *A. polystachyus*. In *A. eminens*, we found positive correlations between spatial distance and dissimilarity based on open flowers in 2008 (r = 0.471, p = 0.014), Additionally, we found a significant influence of spatial distance on the percentage of open inflorescences in both 2008 and 2009, indicating a higher similarity in flowering phenology for spatially close populations of this species (r = 0.374, p = 0.026; r = 0.333, p = 0.027, respectively. Tests are based on 10,000 permutations. For all Mantel statistics, see Appendix, Tab. 1.A). As a consequence, we focussed on proximate heterospecific population pairs for detailed analyses of synchrony between species.

Table 1.1: Factors influencing flowering phenology of *A. eminens* and *A. polystachyus*. Variance was partitioned by year, position along a latitudinal gradient (LG) and forest cover (FC) in 1,000 m (*A. eminens*) and 500 m (*A. polystachyus*) radius around the population centers. Model statistics based on 10,000 randomizations in a non-parametric analysis of variance ('ADONIS'). Randomizations were restricted within years. Interactions between LG and FC were not significant, and consequently removed from the model.

A. eminens

Predictor	r^2	F-Stat. ($F_{1,14}$)	p-value	
Open Flowers				
Year	0.054	1.102	0.034	*
Lat. Grad. (LG)	0.169	3.472	0.034	*
Forest Cover (FC)	0.099	2.034	0.148	
Flowering Inflorescences				
Year	0.034	0.794	0.012	*
Lat.Grad. (LG)	0.199	4.620	0.019	*
Forest Cover (FC)	0.163	3.773	0.040	*
Budding Inflorescences				
Year	−0.179	−2.412	0.432	
Lat.Grad. (LG)	−0.090	−1.213	0.825	
Forest Cover (FC)	0.232	3.140	0.209	

A. polystachyus

Predictor	r^2	F-Stat. ($F_{1,10}$)	p-value	
Open Flowers				
Year	0.076	1.006	0.395	
Lat. Grad. (LG)	0.249	3.319	0.038	*
Forest Cover (FC)	−0.076	−1.014	0.999	
Flowering Inflorescences				
Year	0.169	2.770	0.173	
Lat.Grad. (LG)	0.258	4.237	0.025	*
Forest Cover (FC)	−0.037	−0.599	0.982	
Budding Inflorescences				
Year	0.000	0.004	0.269	
Lat.Grad. (LG)	0.256	3.355	0.069	
Forest Cover (FC)	−0.020	−0.263	0.923	

Phenological overlap

We found strong correlations between phenological traits of the species when comparing nearest heterospecific neighbour populations (Figure 1.2). With a single exception, our results show a strong linear relationship between values for *A. eminens* and *A. polysta-*

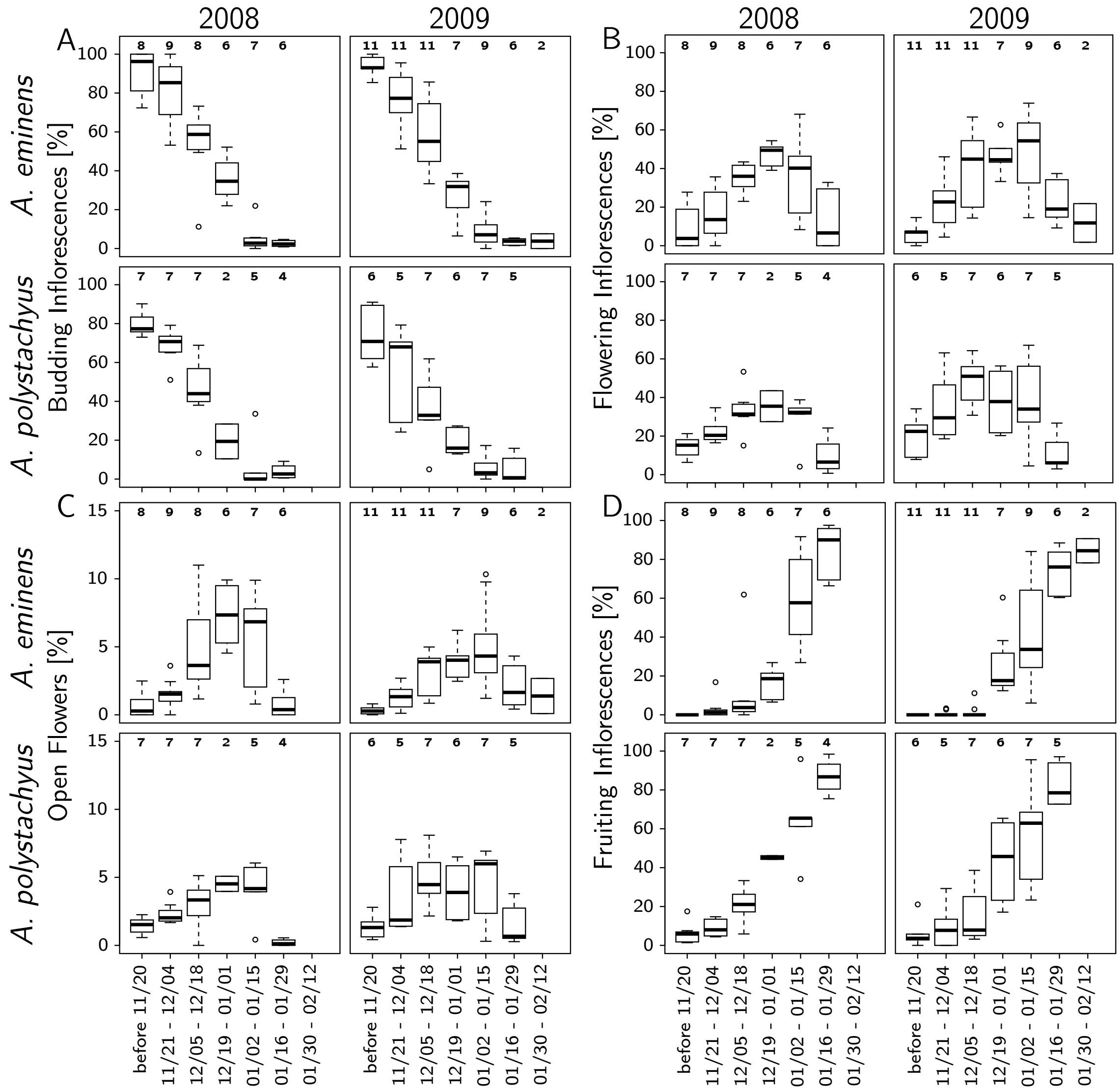

Figure 1.1: Phenology of A) budding inflorescences, B) flowering inflorescences, C) open flowers and D) fruiting inflorescences. Boxplots summarize mean percentages from 6-9 and 6-11 populations of *A. eminens* (upper sub-plots) during each biweekly survey in reproductive periods 2008 (left sub-plots) and 2009 (right sub-plots), respectively, and likewise for 4-7 and 5-7 populations of *A. polystachyus* (lower sub-plots). Black bars represent medians, boxes represent quartiles, and whiskers represent maximum and minimum, or 1.5 times the inter-quartile range in case of outliers. Boxes for two samples do not provide an accurate summary of data, and were included for visualization only. Numbers above boxes inside the plot area indicate the number of populations.

chyus in both observed flowering periods, for inflorescences in bud stage, flowering inflorescences and fruiting inflorescences. We did not find a significant link between the percentage of flowering inflorescences in 2009 (Budding Inflorescences – 2008 (A): r = 0.871, df = 27, p < 0.001; 2009 (B): r = 0.795, df=33, p < 0.001. Flowering Inflorescences – 2008 (C): r = 0.690, df = 27, p < 0.001; 2009 (D): r = 0.071, df=34, p < 0.682. Fruiting Inflorescences – 2008 (E): r = 0.917, df = 27, p < 0.001; 2009 (F): r = 0.780, df=32, p < 0.001)

Fruit set

In many inflorescences, we found some flowers to be infected by mould or underdeveloped. However, this amount did not vary over time in both species, and both reproductive seasons (*A. eminens* – 2008:

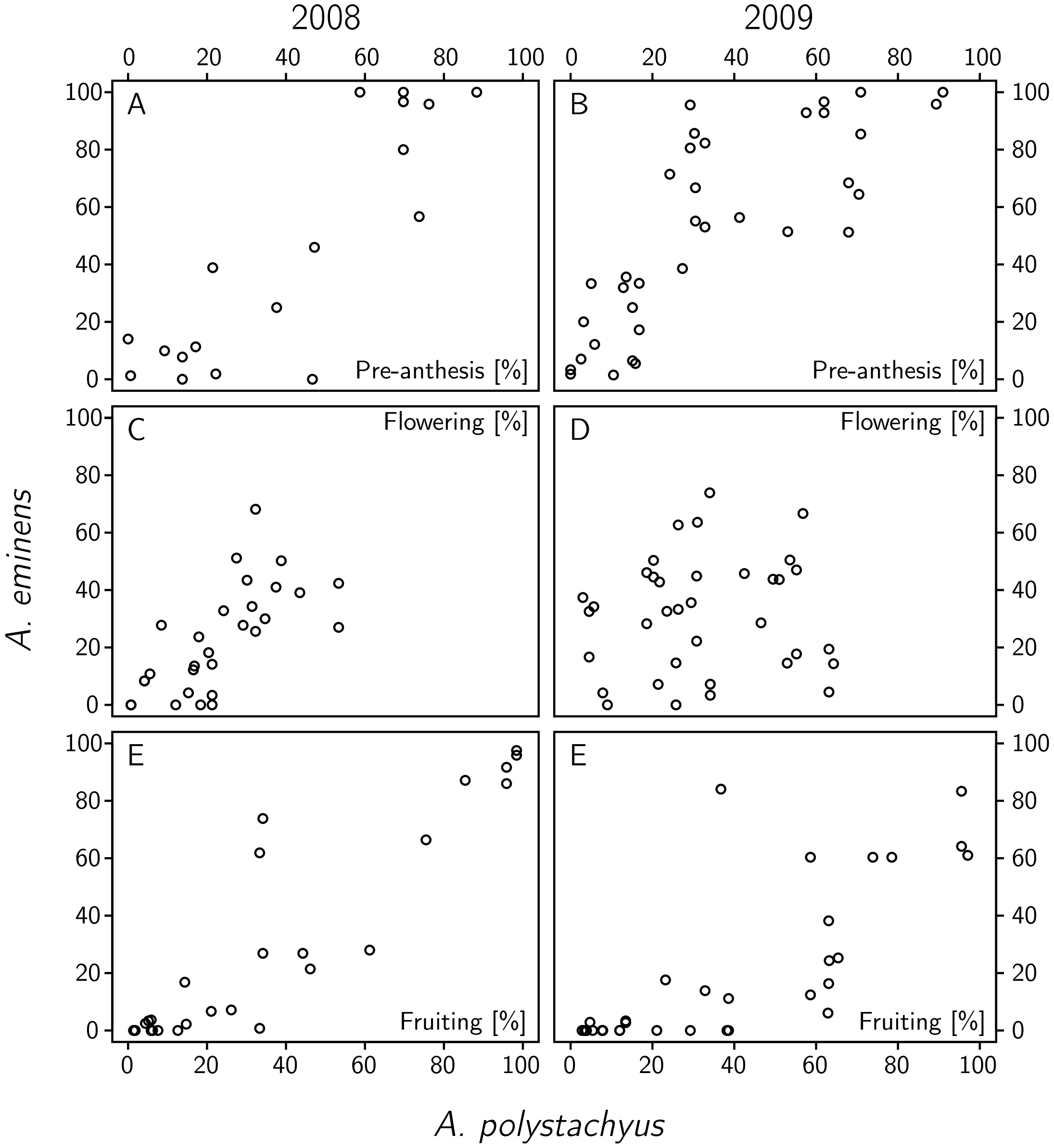

Figure 1.2: Percentages of pre-anthesis (A+B), flowering (C+D), and fruiting (E+F) inflorescences. Points represent population means during surveys of proximate population pairs of *A. polystachyus* (x) and *A. eminens* (y) during flowering seasons 2008 (left column) and 2009 (right column).

$r^2 = 0.030$, $F_{1,41} = 1.278$, $p = 0.265$; 2009: $r^2 = 0.047$, $F_{1,55} = 2.732$, $p = 0.104$. *A. polystachyus* – 2008: $r^2 = 0.007$, $F_{1,27} = 0.1766$, $p = 0.678$; 2009: $r^2 = 0.007$, $F_{1,34} = 0.2446$, $p = 0.624$.).

HP revealed the progress of flowering season to be the most influential of the analyzed predictors in both species. It explains 69.7% and 80.6% of variance across all models of fruit set in *A. polystachyus* and 68.3% and 47.3% in *A. eminens* during flowering periods 2008 and 2009, respectively, and was found to be significantly linked to fruit set in both species and years (For complete HP tables, see Appendix, Tab. 1.B). Distance to heterospecific populations was the only other variable having a significant effect, explaining 15.9% of variance across models in *A. eminens* in 2008, but not in 2009 and not during both reproductive periods of *A. polystachyus*. A simple linear regression revealed a negative trend of fruit set as a function of distance to the next heterospecific population for *A. eminens* in 2008 (Fig. 1.3, *A. eminens* – 2008 (A): $F_{1,7} = 12.84$, $r^2 = 0.647$,

$p = 0.009$; 2009 (B): $F_{1,9} = 0.06$, $r^2 = 0.007$, $p = 0.813$. *A. polystachyus* – 2008 (C): $F_{1,6} = 2.46$, $r^2 = 0.291$, $p = 0.168$; 2009 (D): $F_{1,6} = 0.494$, $r^2 = 0.076$, $p = 0.509$).

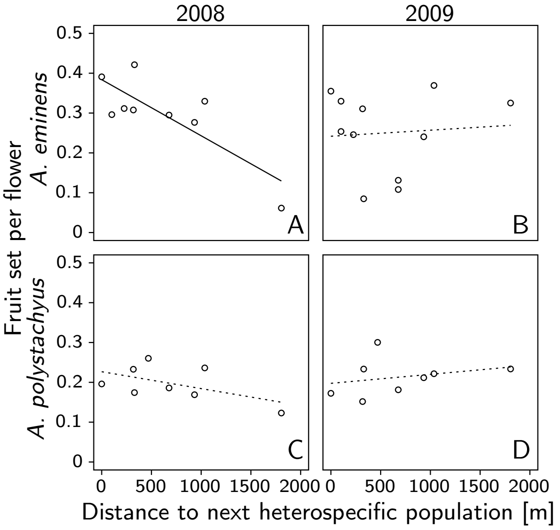

Figure 1.3: Fruit set as a function of distance to the next heterospecific population vor *A. eminens* (A+B) and *A. polystachyus* (C+D), during flowering seasons 2008 (A+C) and 2009 (B+D). Lines indicate model predictions for significant (dashed lines: not significant) linear regression over the range of data.

Fruit set strongly increased over the flowering periods of both species (Fig. 1.4: *A. eminens* – 2008 (A): $F_{1,23} = 16.63$, $r^2 = 0.420$, $p < 0.001$; 2009 (B): $F_{1,19} = 2.76$, $r^2 = 0.127$, $p < 0.113$. *A. polystachyus* – 2008 (C): $F_{1,27} = 10.61$, $r^2 = 0.282$, $p = 0.003$; 2009 (D): $F_{1,29} = 20.17$, $r^2 = 0.410$, $p < 0.001$). This effect is more pronounced in *A. eminens* in 2008, with fruit set quadrupling during flowering in 2008. However, fruit set was not found to be linked with progress of time in 2009. In *A. polystachyus*, we found fruit set to more than double between November and February in both reproductive periods.

Though we were able to collect only limited data on variations in fruit set over different years, regression analysis including preliminary data on fruit set from 2007 shows a strong, but only marginally significant link between log-transformed values of both fruit set and sum/mean of rain during the months of flowering for *A. eminens* ($r^2 = 0.982$, $F_{1,1} = 53.66$, $p = 0.086$), but not for *A. polystachyus* ($r^2 = 0.421$, $F_{1,1} = 0.7261$, $p = 0.551$. Rainfall data averaged from four rain collectors inside the northern part of the forest. Rain data was kindly provided by R. Gliniars, University of Hohenheim. For details, see Appendix, Tab. 1.C).

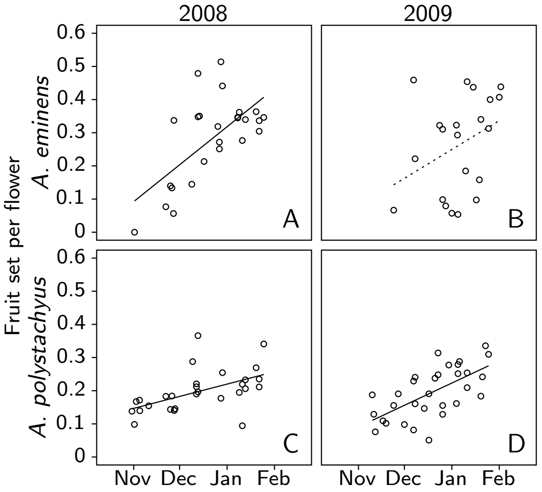

Figure 1.4: Mean fruit set per flower in populations of *A. eminens* (A+B) and *A. polystachyus* (C+D) during flowering periods 2008 (A+C) and 2009 (B+D) as function of date. Lines represent model predictions for significant (dashed lines: not significant) linear regression over the range of data.

Stigmatic pollen loads

Absolute pollen grain numbers differed between surveys in both reproductive seasons, both within species (*A. eminens* – 2008: $W = 56$, $p < 0.001$; 2009: $W = 48$, $p < 0.001$. *A. polystachyus* – 2008: $W = 28$, $p = 0.009$; 2009: $W = 36$, $p = 0.006$) and between species (2008 – First survey: $W = 56$, $p < 0.001$; Second survey: $W = 28$, $p < 0.009$. 2009 – First survey: $W = 36$, $p = 0.003$; Second survey: $W = 48$, $p = 0.003$. All p-values in comparisons of pollen loads are corrected using Benjamini-Hochberg adjustment for multiple comparisons).

There were no significant differences between species across all levels of pollinations in each survey ($p = 0.788$ for all comparisons after adjustment).

In *A. polystachyus* we found statistical differences between all levels of pollination in 2008, suggesting a decrease in the percentage of stigmas receiving *Acanthus* pollen (Tab. 1.2. Any pollen: $W = 26$, $0 = 0.036$, ≥4 pollen: $W = 27$, $p = 0.036$, ≥16 pollen: $W{=}25.5$, $p = 0.037$. For complete statistics, see Appendix, Tab. 1.D). In this species, we did not find significant differences between surveys in 2009, regardless of pollination levels. In *A. eminens*, we did not find any differences in pollination success between surveys in either year and level of pollination success.

We did not find any evidence for a link between

Table 1.2: Percentage of stigmas of *A. eminens* and *A. polystachyus* found with adherent *Acanthus*-pollen during the first and the second half of the flowering period (mean±sd). n-values reflect the number of observed populations, while values in brackets indicate the total number of stigmas. Significance was tested using Wilcoxon rank sum tests, p-values adjusted by Benjamini-Hochberg correction for multiple comparisons. For W and p-values, see Appendix, Tab. 1.D.

A. eminens

2008

	Survey		Sig.
	1^{st}	2^{nd}	
Pollen grains	n = 8 (180)	n = 7 (168)	
any	69.9±14.5	53.7±24.2	
≥ 4	57.2±11.5	40.5±22.6	
≥ 16	23.3±15.4	28.4±20.7	

2009

	Survey		Sig.
	1^{st}	2^{nd}	
Pollen grains	n = 6 (168)	n = 8 (152)	
any	55.1±17.8	63.2±15.3	
≥ 4	47.0±15.8	52.2±11.1	
≥ 16	30.5±10.1	28.1±17.1	

A. polystachyus

2008

	Survey		Sig.
	1^{st}	2^{nd}	
Pollen grains	n = 7 (229)	n = 4 (121)	
any	72.8±11.9	52.8±4.3	*
≥ 4	61.7±10.9	36.9±7.4	*
≥ 16	38.0±19.5	16.4±8.5	*

2009

	Survey		Sig.
	1^{st}	2^{nd}	
Pollen grains	n = 6 (202)	n = 6 (139)	
any	59.1±17.9	64.8±10.2	
≥ 4	44.1±21.9	49.6±13.5	
≥ 16	21.6±14.7	15.6±6.7	

Table 1.3: Results for linear regression of stigmatic pollen loads dependent on distance to the spatially closest heterospecific population.

A. eminens

		Slope $[10^{-4}/m]$	r^2	p-value	
2008	any	−0.03	0.000	0.985	
	≥ 4	0.49	0.007	0.733	
	≥ 16	−0.13	0.001	0.920	
2009	any	−0.02	0.000	0.977	
	≥ 4	−0.06	0.003	0.940	
	≥ 16	0.73	0.069	0.249	

A. polystachyus

		Slope $[10^{-4}/m]$	r^2	p-value	
2008	any	0.96	0.069	0.310	
	≥ 4	1.08	0.067	0.317	
	≥ 16	2.29	0.349	0.013	*
2009	any	1.32	0.136	0.120	
	≥ 4	0.82	0.044	0.386	
	≥ 16	0.92	0.144	0.109	

pollination success and distance to the next heterospecific population in *A. eminens* (Tab. 1.3). For *A. polystachyus*, we found an increase of pollination success for one level of pollination (16 *Acanthus* pollen grains or more) with increasing distance to the congener in 2008 (r^2 =0.412, $F_{1,15}$ = 8.044, p=0.013).

In general, we did not find any differences in levels of primary pollination success (PPS) between surveys in both years for *A. eminens*. Pollen deposition is generally low, as suggested by the large amount of stigmas found containing no pollen at all, with more than 25% and up to about 45% of unpollinated stigmas in both species and years, which corroborates potential pollen limitation of reproduction in both species.

DISCUSSION

Acanthus eminens and *A. polystachyus* occupy closely interlocking habitats and display similar flower morphologies and flowering phenologies.

Individuals of each species show highly synchronous flowering, which is a prerequisite for pollen flow between conspecific populations. Especially for *A. eminens*, which forms isolated populations in forest gaps, synchronous flowering may be an adaption to optimize inter-population pollen exchange (Rathcke and Lacey 1985). Simultaneous mass flowering may on the one hand attract large numbers of pollinators and increase interspecific pollen transfer (Crone and

Lesica 2004), but increased resource density may on the other hand reduce pollinator movements between populations, reducing potential for genetic exchange. For example, Melampy (1987) has demonstrated that in *Befaria resinosa* (Ericaceae), pollen was dispersed over greater distances during periods of little flower production, while it was dispersed over short distances during flowering peaks. Other studies have shown that flowers may receive pollen from more distant paternal plants during early and late flowering season (Elzinga *et al.* 2007).

The species display a strong overlap in flowering phenology, to the extent that the percentage of open flowers and the percentage of budding, flowering and fruiting inflorescences in neighbouring populations is nearly identical. As the species are related, competition for pollinators or stigmatic surface, or hybridization may be expected to reduce reproductive success in at least one of them. Kochmer and Handel (1986) have shown that flowering phenology is subject to phylogenetic and seasonal constraints, demonstrating that closely related species display more similar flowering phenologies than those which are only distantly related. But other studies have demonstrated staggered flowering in closely related species (Anderson and Schelfhout 1980; Henderson *et al.* 2000; Botes *et al.* 2008), and have used character displacement as an explanation for this effect (Snow 1965; Stiles 1977; Wheelwright 1985). Temporal isolation has been judged as a mechanism to avoid competition for pollen flow and interspecific pollen transfer (Levin and Anderson 1970), and divergence of flowering phenologies has been considered a potentially mutualistic interaction between species, as continuous availability of floral resources may sustain larger pollinator communities (Waser and Real 1979).

However, experimental and natural flowering synchrony also may have positive effects on pollination and reproduction in some species (Ghazoul 2006), or no effect at all (Armbruster and McGuire 1991; McGuire and Armbruster 1991; McGuire 1993). Also, flowering synchrony of species as well as divergent flowering may be caused by other factors (Kochmer and Handel 1986; Ollerton and Lack 1992, see also Fox and Kelly 1993 and Ollerton and Lack 1993).

We found no correlation between spatial distance between populations of *A. polystachyus* and its phenological traits. In *A. eminens*, Mantel test analysis reveals close populations to be more similar in the percentage of open inflorescences and the percentage of open flowers. Though we do not find any link between the amount of budding inflorescences and spatial distance, we conclude that overall similarity of reproductive phenology between populations of *A. eminens* is linked to the distance between them. This may be caused by a stronger differentiation of populations of *A. eminens* inside different forest areas compared to those of *A. polystachyus* at forest edges and in hedgerows, e.g. due to differences in microclimate, soil chemistry, or resource availability.

Though there is some variation in flowering phenology between years for *A. eminens*, the position of populations along a north-south gradient is the most consistent source of variance in flower phenological traits of both species. A gradient of rainfall in Kakamega forest with decreasing amounts of rain from north to south has been described (Mutangah 1996), and it seems likely that climatic influence of divergent amounts of rainfall in populations affects flowering. Consistent with this assumption, as flowers should be generally more susceptible to damage from moisture when they are open, position along a latitudinal gradient also has a measurable effect on both the amount of open flowers and of flowering inflorescences. In addition, forest cover reduces radiation on the forest floor and facilitates retention of humidity, and putative effects of forest cover on humidity levels inside the forest provide a possible explanation for the effect of spatial distance to congeneric populations on fruit set found for *A. eminens* in 2008.

However, variation in flowering phenology is small, and though it is likely linked to environmental factors, we do not find evidence for significant temporal isolation of populations both between and within species. On the contrary, the congruence of flowering patterns between neighbouring heterospecific populations provides further evidence that the species share patterns of flowering onset, duration, and intensity. Considering that simultaneous flowering increases the risks of competition for pollinators and reproductive interaction for closely related and morphologically similar species, the flowering synchrony between the species is striking.

Our data do not provide evidence for any negative reproductive interaction between the two species of *Acanthus*, as we cannot demonstrate a negative link between fruit set and habitat availability of and distance to the congener in either species. Contrary to our hypothesis, *A. eminens* populations close to stands of *A. polystachyus* show higher fruit set than those in more remote forest areas in 2008. Though this result may suggest facilitation of pollinator visitation in neighbouring heterospecific populations, this is not corroborated by our data, as pollination success is not linked to distance to the congener in both seasons for *A. eminens*.

Levels of stigmatic pollen loads of *A. eminens* remain constant during both seasons, and are also constant in *A. polystachyus* in 2009, while they decrease between surveys in 2008. Possible explanations include stronger temporal variance in abundance of pollinators at the forest edges and in the surrounding farmland due to greater susceptibility of the open forest edge to seasonal climate compared to the forest interior, but Hagen and Kraemer (2010) found low temporal variability of pollinator species abundance between seasons in Kakamega Forest. However, the abundance patterns of individual bee species during seasons remain unknown. PPS of *A. polystachyus* increases with distance to the congener for the highest level of pollen loads analyzed in 2008. But this effect is not reflected in fruit set, which may respond to lower pollination levels not showing significant spatial variation.

Other factors like plant resource availability and allocation may mask putative effects of stigmatic pollen loads (Wesselingh 2007). Also, simultaneous flowering is only disadvantageous when there is competition for pollinators, and Hagen *et al.* (submitted) have, in a study in Kakamega Forest, attributed differences in pollinator fauna composition between populations of the same plant species to differences in pollinator species abundance between habitats. Hence, we consider partial isolation through divergent pollinator faunas highly likely in *Acanthus*, especially between spatially distant populations.

As we found less than two thirds of stigmas with four or more adherent pollen grains, we consider pollination success to be an important factor limiting reproduction. Pollen limitation, which has been demonstrated to be common in biodiversity-rich ecosystems (Vamosi *et al.* 2006), may be prevalent in both species, and contribute to reproductive isolation. Low pollen loads, divergent pollinator faunas and high ratios of intraspecific pollinator movements may be strong barriers against reproductive interaction. However, for both species, our findings indicate constant visitation rates and relatively constant pollen loads of visiting animals, whereas fruit set increases over time. Hence, temporal variation in fruit set is obviously not caused by a corresponding variation of pollination.

As both PPS and fruit set seem to be largely independent of spatial distance to the congener, we conclude that variance in fruit set is not caused by changes in pollination intensity or by transfer of heterospecific pollen due to pollinator-mediated effects between *A. eminens* and *A. polystachyus*. Therefore, we reject our hypotheses that fruit set and pollination success decrease with spatial distance between congeners in our study system. In addition, constant levels of pollination success in both species, as well as the constant increase of fruit set in both species over the course of their reproductive period do not corroborate our hypothesis that pollination success is a factor of stabilizing selection on synchronous flowering. However, the observed increase in fruit set over time in both seasons and species and the homogeneous levels of primary pollination success point towards other factors than pollinator visitation to exert strong stabilizing influence on reproduction.

As pointed out by Levin (1971), Primack (1987) and Miller-Rushing *et al.* (2010), selection on any life-history event is likely interacting with selection on other life-history events, as later flowering implies later seed set and seedling germination. Apart from being influenced by selection pressures on flowers and pollination, early stage traits of reproductive phenology like budding and anthesis have been found to be adapted to optimized fruiting phenology, ensuring that ripe seeds are released under optimal conditions to facilitate germination and seedling survival (Rathcke and Lacey 1985; Schaik *et al.* 1993).

We exclude the possibility that reproductive phenology of the species could be influenced by zoochorous fruit and seed dispersal, as we did not observe fruit or seed dispersal in our mechanically dispersed species. We did find evidence of fruits being infected by insect larvae, and a detailed analysis of temporal variation in seed predation, though beyond the scope of this study, may give additional insights into selection pressures shaping reproductive phenology (Snow 1965; Levin 1971; Augspurger 1981; Brody 1997; Tokumoto *et al.* 2009).

But most strikingly, flowering in *A. eminens* and *A. polystachyus* coincides with the beginning and end of dry season. Our findings are in accordance with the hypothesis that both species are co-adapted to flowering in dry season, and produce most of their fruits just before the onset of rainy season. Though we are not able to test this hypothesis with our data, it is corroborated by our observation that reproduction in terms of fruit set peaks just before the onset of rainy season, and by the observation that flowers and fruits which have not matured until the start of the rains are aborted. Additional evidence lies in the fact that, due to the peak of flowering around new year, the end of rainy season not only coincides with the highest ratio of fruit set per flower, but also with the highest amount of mature fruits releasing seeds, as fruits need four to six weeks to mature. Susceptibility of flowers and fruits to moisture also provides an explanation for the increase in fruit set with decreasing spatial distance to *A. polystachyus* found in *A. eminens* in 2008.

In species without seed dormancy, seeds should be released just before periods of favorable climatic conditions, reducing seed predation and losses due to premature germination after short-term rain events (Wheelwright 1985). In addition to the described patterns of fruit set, we found germination of about 90% of seeds of both species ten days after a single watering event under greenhouse conditions, pointing towards rapid germination after the onset of rainy season (see Chapter 4), which may be interpreted as adaption to fast seedling establishment at the beginning of rainy season. This is in accordance with studies which have provided evidence that other life-cycle related traits, interacting with flowering phenology, influence the timing of reproduction of plant species (Ågren and Fagerström 1980; Primack 1987).

In a study on phenology of a dry forest in Ghana, Lieberman (1982) found that dry-fruited species principally fruit in dry season, as compared to fleshy fruited species which were found to flower during both wet and dry season. The author concludes that, as dry fruits should need less water for development, this is an adaption to reduce water limitation on fruit set. However, dry fruits may also have evolved as part of the dispersal mechanism for lightweight fruits and seeds, especially in the case of dry and maybe explosive capsules as found in *Acanthus*. Fruiting in dry season may either have evolved together with dry fruits in seasonal habitats, or may be an adaption of already dry-fruited species to seasonality. In either case, excess moisture on fruits developing too early, too late, or under unusual climatic conditions may affect fruit development and seed dispersal (Murali and Sukumar 1994). This also provides a possible explanation for our finding that *A. eminens* fruit set decreased with increasing distance to *A. polystachyus* populations in 2008: If fruit set of *A. eminens* is linked to environmental humidity, its increase in populations near to the forest borders may be due to the drier microclimate to be expected in the edge habitat.

Therefore, an adaption to fruiting in dry season with release of seeds before rainy season seems highly likely in *Acanthus*, and the strength of environmental constraints on reproduction may completely mask other factors which may affect reproductive success of the species. Rathcke (1988) pointed out that plants may flower simultaneously when the gain in reproductive success through a divergence in flowering times is smaller than potential losses associated with it, e.g. through seasonal variation in pollinator abundance. We propose that climatic constraints on fruit development and seed dispersal favour selection towards fruiting during late dry season, when environmental moisture is low and seeds are exposed only for a short time before the onset of rains. As fruit development is stopped by the begin of rainy season, the demonstrated increase in fruit set is 'cut off' by the onset of rains, favoring selection towards earlier flowering. These two strong selective pressures explain the flowering pattern found in our study, and are likely strong enough to mask other putative selection pressures like adaption to divergent flowering phenologies or pollinator abundance.

Most studies on these mechanisms have focussed on annuals and species from alpine, temperate or colder climates (e.g. Kudo 1993; Ollerton and Lack 1998; Kudo *et al.* 2008), likely due to the research opportunities offered by the strong effects of freezing temperatures on reproduction, and we are aware of only one study addressing potential challenges for conservation due to the effect of climate change on different types of fruit in the tropics (Chapman *et al.* 2005).

Several climate change models predict a general, in some models dramatic, increase of rainfall for our study area, which may be bolstered by extreme rain events during *Acanthus*' flowering period caused by El Niño/Southern Oscillation (ENSO) events (Hulme *et al.* 2001), disrupting patterns of rainy and dry seasons. Aseasonal rains may reduce reproductive success in species, changing their population dynamics (Chapman *et al.* 2005). Long-term monitorings of pollinators and keystone plant species are needed to provide fundamental information for policy makers, as many effects of changing temperature and rainfall on herbivores, frugivores, and pollinators remain largely unknown, and warrant increased attention to African ecosystems, which have received little attention by scientists so far.

Though we are able to rule out reproductive interaction as a factor limiting reproduction in either species, the question remains why even in these species with apparently similar flower morphology and reproductive phenology no positive or negative effects of species interaction on reproduction can be found. Distance between *A. eminens* and *A. polystachyus* has recently been reduced through anthropogenic forest fragmentation (Schaab *et al.* 2010), and it is possible that the species have already co-existed in neighbouring habitats before anthropogenic changes, e.g. near the edges of natural glades or at sites where rivers enter the surrounding open habitats, and may have evolved strong barriers to reproductive interference unaffected by habitat change. As studies have repeatedly shown that observed reproductive isolation is often achieved by the interaction of several cumulative mechanisms of isolation (Marques *et al.* 2007), we conclude that additional data are needed to untangle the mechanisms of pollination of and reproductive isolation between *A. eminens* and *A. polystachyus*. Flower visitor observation and an analysis of flower morphology, floral rewards, pollen loads and pollinator efficiency might provide additional clues about the partitioning of pollinators between species and populations, as well as about potential shifts in the abundance and importance of individual pollinator species during the flowering period of the species. Hand-pollination studies on fruit set, seed set and seed viability may be useful to assess the effects of pollen performance from both heterospecific and conspecific, inter- and intrapopulation pollinator movements, revealing potential inbreeding depression, pollen limitation, and potential for hybridization, and may shed additional light on the interlocking processes of reproductive isolation and pollination, revealing their roles in shaping the reproductive phenology of either species.

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Appendix

Table 1.A: Mantel test results for matrix correlation between flowering phenology and spatial distance between populations. Tests are based on Pearson's product-moment correlation and 10,000 permutations.

A. eminens	Mantel r	p-value
2008		
Open Flowers	0.471	0.014*
Open Inflorescences	0.374	0.026*
Budding Inflorescences	−0.120	0.707
2009		
Open Flowers	0.142	0.160
Open Inflorescences	0.333	0.027*
Budding Inflorescences	−0.019	0.510
A. polystachyus	Mantel r	p-value
2008		
Open Flowers	0.289	0.199
Open Inflorescences	0.505	0.070
Budding Inflorescences	0.255	0.198
2009		
Open Flowers	0.263	0.120
Open Inflorescences	0.031	0.417
Budding Inflorescences	−0.010	0.512

Table 1.B: Results of hierarchical partitioning of variance of fruit set. Predictors: progress of flowering season as count of days (date), distance to next heterospecific population, forest cover in 1,000 m and 500 m radius around centers of populations of *A. eminens* and *A. polystachyus*,respectively, position along a latitudinal gradient from north to south, and, for *A. eminens*, population size and canopy cover. Z-Statistics based of 5,000 randomizations, with a Z-score threshold of 1.65 representing a confidence interval of 0.95. * marks significant results.

A. eminens	% Variance	Z-Score
2008		
Date	68.3	9.75 *
Distance	15.9	1.68 *
Forest Cover	4.2	−0.11
Population size	1.5	−0.55
Canopy	2.1	−0.45
Latitudinal Grad.	7.3	−0.37
2009		
Date	47.3	2.52 *
Distance	8.7	−0.18
Forest Cover	4.0	−0.51
Population size	3.8	−0.56
Canopy	26.7	1.02
Latitudinal Grad.	9.5	−0.10
A. polystachyus	% Variance	Z-Score
2008		
Date	69.7	6.40 *
Distance	7.3	−0.01
Forest Cover	11.9	0.47
Latitudinal Grad.	11.0	0.44
2009		
Date	80.6	8.96 *
Distance	2.3	−0.46
Forest Cover	14.5	0.92
Latitudinal Grad.	2.7	−0.43

Table 1.C: Sum of rainfall during the reproductive period of *Acanthus* between begin of November and end of February. Fruit set of populations (mean±sd) during flowering periods 2007, 2008 and 2009.

A. eminens	Rainfall [mm]	Fruitset	n
2007	755	0.064 ± 0.076	10
2008	271	0.288 ± 0.093	11
2009	317	0.287 ± 0.116	11

A. polystachyus	Rainfall [mm]	Fruitset	n
2007	755	0.232 ± 0.063	7
2008	271	0.196 ± 0.044	9
2009	317	0.230 ± 0.045	8

Table 1.D: Percentage of stigmas of *A. eminens* and *A. polystachyus* found with adherent *Acanthus*-pollen during the first and the second half of the flowering period (mean±sd). n-values reflect the number of observed populations, while values in brackets indicate the total number of stigmas. Wilcoxon rank sum test, p-values adjusted by Benjamini-Hochberg correction for multiple comparisons.

A. eminens

2008

	Survey		Significance	
	1^{st}	2^{nd}	W	p
Pollen grains	n = 8 (180)	n = 7 (168)		
any	69.9±14.5	53.7±24.2	43	0.277
≥ 4	57.2±11.5	40.5±22.6	39.5	0.303
≥ 16	23.3±15.4	28.4±20.7	24.5	0.728

2009

	Survey		Statistics	
	1^{st}	2^{nd}	W	p
Pollen grains	n = 6 (168)	n = 8 (152)		
any	55.1±17.8	63.2±15.3	17.5	0.719
≥ 4	47.0±15.8	52.2±11.1	18	0.719
≥ 16	30.5±10.1	28.1±17.1	26	0.852

A. polystachyus

2008

	Survey		Statistics	
	1^{st}	2^{nd}	W	p
Pollen grains	n = 7 (229)	n = 4 (121)		
any	72.8±11.9	52.8±4.3	26	0.036*
≥ 4	61.7±10.9	36.9±7.4	27	0.036*
≥ 16	38.0±19.5	16.4±8.5	25.5	0.037*

2009

	Survey		Statistics	
	1^{st}	2^{nd}	W	p
Pollen grains	n = 6 (202)	n = 6 (139)		
any	59.1±17.9	64.8±10.2	15	0.818
≥ 4	44.1±21.9	49.6±13.5	14.5	0.818
≥ 16	21.6±14.7	15.6±6.7	20	0.818

2 TEMPORAL AND SPATIAL VARIATIONS IN FLORAL TRAITS OF TWO SYMPATRIC SPECIES OF *ACANTHUS* L.

NILS HASENBEIN AND MANFRED KRAEMER

ABSTRACT

Acanthus eminens CLARKE and *Acanthus polystachyus* var. *polystachyus* DELILE grow in different but closely neighbouring habitats in the area of Kakamega Forest National Reserve in Western Province, Kenya. Anthropogenic habitat change has reduced spatial distance between the species and likely altered their relative abundance, which may facilitate reproductive interference and hybridization. In this study, we investigate flower morphology during two and floral rewards during four flowering seasons in several populations of each species. Though there are significant interspecific differences in flower morphology, these are largely masked by a high spatial and temporal variability in either species. This similarity allows for pollinator sharing between species, which we find confirmed by a preliminary investigation of the plants' flower visitor spectrum. However, there is no indication of hybrid individuals growing in the wild, though we do not find evidence of floral morphology playing a role in floral isolation between the congeners. Local abundance of pollinator species and pollinator behaviour are suggested as reasons for reproductive isolation in the congeneric species *A. eminens* and *A. polystachyus*.

INTRODUCTION

Flowers of most animal-pollinated plants are generalistic, allowing for access of several species of pollinators (Waser *et al.* 1996; Bosch *et al.* 1997). Flowering simultaneously, these plant species may compete for pollinators (Levin and Anderson 1970) or facilitate each other's pollination by attracting more visitors together than each species on its own (Laverty 1992; Johnson *et al.* 2003; Feldman *et al.* 2004; Ghazoul 2006).

Though floral structures commonly serve as attractants for pollinating animals (Stephenson 1979; Dudash 1991; Eckhart 1991), floral traits may also act as filters for floral visitors, limiting access to floral rewards to reliable pollinators (Grant 1949; Levin 1971). Consequently, flower morphological traits may be the best predictors of which animal species are capable of visiting flowers of a certain plant species (Stang *et al.* 2006b,a; Alarcón *et al.* 2008). However, evolution of floral traits may be rapid, and an adaption of flower morphology to local pollinator faunas has been demonstrated in several plants (Galen 1996).

If a majority of pollinators is attracted to flowers of a single species, these likely receive more visits transferring conspecific pollen (Levin and Anderson 1970; Inouye 1978; Feinsinger and Tiebout 1991). Relative attractiveness of flowers is mainly determined by the amount of floral rewards they present to visitors and the costs of foraging (Heinrich 1975). Morphological traits determine flower handling costs (Stout *et al.* 1998), and may influence the foraging behaviour of bees (Waser and Price 1983). In turn, they also determine the intensity of pollinator-mediated interaction between heterospecific flowers and, consequently, the rate of interspecific pollen transfer (Hopper and Burbidge 1978).

In addition, flower number and population size strongly influence the relative attractiveness of a species (Schemske 1980; Sih and Baltus 1987; Schemske and Ågren 1995; Ågren 1996; Conner *et al.* 1996). Many-flowered plants or dense stands of flowers provide a high density of resources, attracting many pollinators and reducing their movement distances (Heinrich 1975; Augspurger 1980; Jong *et al.* 1992; Maad 2000). The most abundant flower type receives most visits when pollinators do not show any preference for flowers of a certain species. On the other hand, pollinators may also preferentially visit the most abundant flower type (Hopper and Burbidge 1978).

Consequently, shifts in relative abundance have been found increase the incidence of heterospecific pollen transfer to less abundant species (Kunin 1993). One well-studied reason for shifts in relative abundance of species is the breakdown of spatial barriers due to anthropogenic habitat change, which may facilitate pollinator-mediated interaction between species which did not interact before (Anderson 1948; Levin *et al.* 1996; Lamont *et al.* 2003).

Many plants sharing pollinators are subject to interspecific pollen transfer (Campbell 1985; Waser and

Fugate 1986; Bosch *et al.* 1997; Bell *et al.* 2005; Theiss *et al.* 2007). In addition, and according to a recent review more importantly (Morales and Traveset 2008), the presence of co-flowering plants may decrease male reproductive success and conspecific stigmatic pollen loads through loss of pollen during pollinator foraging bouts on heterospecific flowers (Campbell and Motten 1985; Stout *et al.* 1998; Morales and Traveset 2008). Placement of pollen on pollinators and deposition of pollen on stigmas during pollinator visits strongly depend on the mechanical fit between flower morphology and pollinator body (Schemske and Horvitz 1984; Nilsson 1988; Borg-Karlson 1990; Botes *et al.* 2008). Hence, floral morphology is not only a determinant of conspecific pollen transfer effectiveness, but can also act as barrier to interspecific pollen transfer.

When pollen is transferred between incompatible species, heterospecific pollen grains may block the pistil for conspecific pollination (Palmer *et al.* 1989; Scribailo and Barrett 1994). In related species, heterospecific pollen tube growth may be impaired (Williams *et al.* 1982; Brown and Mitchell 2001, but see Carney *et al.* 1996), and fertilization may be impossible or hybrid seeds aborted (Stephenson 1981). Flowers of congeners often are functionally identical, and their potential for reproductive interference is higher than for unrelated species pairs (Levin 1971). Congeneric species also are more likely to be compatible, and interspecific pollen transfer may lead to the formation of hybrid swarms with intermediate characteristics, which have been used repeatedly to study the evolutionary and ecological aspects and consequences of hybridization, and the formation and breakdown of species barriers (e.g. Neuffer *et al.* 1999; Vilà *et al.* 2000; Emms and Arnold 2000; Lexer *et al.* 2003; Matsumoto *et al.* 2009). Stable hybrid zones may exist in disturbed habitats (e.g. Anderson 1948; Lamont *et al.* 2003), or species may merge into a new hybrid species (Mallet 2007). But more commonly, one of the congeners will outcompete the other through hybridization and introgression (Levin *et al.* 1996; Rhymer and Simberloff 1996; Anttila *et al.* 1998; Mallet 2005).

Kakamega Forest in Western Provice, Kenya, has recently suffered from deforestation, which reduced the original forest area and lead to fragmentation of the original habitat (Schaab *et al.* 2010). In this study, we address the question whether floral traits of two congeneric species, *Acanthus eminens* and *A. polystachyus*, provide an explanation for the apparent lack of reproductive interference between the species, or may reveal putative hybrids between the species displaying intermediate characteristics.

A recent study demonstrated a strong overlap in flowering phenology of the species, but could not provide evidence for reproductive interference (Chapter 1). In fact, co-flowering did not seem incur any fitness loss for either species. There was limited evidence that proximate heterospecific populations show higher levels of fruit set, but stigmatic pollen loads did not support facilitation of pollinators. Co-adaption to climatic conditions provided an explanation for simultaneous flowering, as the loss of reproductive success caused by a putative divergence from the current flowering pattern may be greater than the losses caused by the observed synchronicity, as climatic conditions favour the development of fruits during increasing drought in dry season.

As mechanisms of reproductive isolation, we propose that 1) flowers of *A. eminens* and *A. polystachyus* differ in size, and 2) offer different amounts of floral rewards, facilitating mechanical and ethological isolation of flowers. Additionally, we hypothesize that 3) there are flowers displaying intermediate characteristics, indicating hybridization and introgression between the congeners. We also provide preliminary data on pollinator abundance on flowers of each species.

Materials and Methods

Study area

Our study was conducted in Kakamega Forest in Western Kenya (0"170N, 34"540E). Kakamega Forest is considered the easternmost remnant of the lowland Guineo-Congolian rain forest belt, and forest fragmentation has led to the formation of five fragments (130 to 1,400 ha) and a remnant main forest (8,500 ha, Schaab *et al.* 2010), with a remaining forest area of 12,000 ha between 1,500 to 1,700 m above sea level. It is managed by the Kenyan Wildlife Service (KWS) in the north and the Kenyan Forest Service (KFS, former Forest Department) in the south. Mean monthly temperatures range from 11°C to 29°C, with an average temperature of 22°C. Rainfall averages 2,000 mm per year, with two distinct rainy seasons between March and May ('long rainy season') and September and November ('short rainy season') and two distinct dry seasons between December to February ('dry season') and from June to August ('cold dry season'). The forest is under significant pressure due to illegal logging, firewood collection, grazing, and hunt for bushmeat, and large parts are highly disturbed (Schaab *et al.* 2010). The forest is surrounded by densely populated (336 to 746 inhabitants per km^2, Mitchell 2004), highly diverse and structured farmland with 0.2 to 0.7 ha of land per household (Greiner 1991; Kenyan Ministry of Agriculture 2006). 62% of all households generate their income from agriculture, and the district's poverty rate is about 52% (Dose 2007).

Plant species

We studied two species of *Acanthus* L.. *Acanthus eminens* Clarke is a shrub growing in clearings in primary and old secondary mountain rainforest between 1,500 and 2,800 m above sea level in Kenya (Beentje 1994). It is also found on the Ugandan side of Mt Elgon (pers. obs.), as well as in Ethiopia and in the Imatong Mts in Sudan, very likely spreading to north Uganda (Vollesen 2007). Although not a rare species within these habitats, it is restricted to highland and mountainous forest areas, which have suffered heavily from deforestation in more recent history. *A. eminens* grows up to five meters in height, carries spiny, robust leaves and flowers in decussate spikes. Plants may flower at relatively small size, carrying only one inflorescence, but most carry several, and up to over 100 inflorescences.

Acanthus polystachyus var. *polystachyus* Delile (referred to as *Acanthus polystachyus*) grows along forest edges and grasslands inside forests between 1,100 and 2,500 m above sea level in Ethiopia, Eastern Sudan, Eastern Uganda and Tanzania. In Kenya, it occurs only in Western Province and is missing on the plains around lake Victoria (Vollesen 2007). It is a common shrub in hedgerows, and often found invading grasslands and as part of natural forest regeneration in Kakamega area.

A. polystachyus does not form distinct populations as it grows in more or less loose clusters in small copses and hedgerows, also forming dense, continuous stands along forest edges. Morphologically similar to *A. eminens*, *A. polystachyus* has softer, hairy leaves and is generally larger. Its inflorescences are slightly larger than those of *A. eminens*, and a single plant carries up to several hundred of them. The flowers are magenta/pink in colour, and show the same overall appearence as those of *A. eminens*, which are royal blue.

The zygomorphic flowers of both species are hermaphroditic, with the filaments of their four stamens protruding along the style, the anthers forming a tight cluster around it some mm short of the pistil. Petals are fused to a short basal tube at their base, forming a compartment partly sealed off by a ring of hair-like structures, which encloses the ovary. Nectar is produced by glands around the base of the ovary. Flowering starts at the end of rainy season between October and November, and continues up to the end of dry season in mid-February. Fruits of *A. eminens* are dehiscent capsules with up to four seeds which are explosively expelled from the fruit on dehiscence by a woody central column in the fruit. Fruits and seeds do not show any sign of adaption to dispersal by animals (e.g. clinging hairs, pulp, elaiososomes), and do not germinate after being damaged (pers. obs.). *A. eminens* is capable of vegetative reproduction, and forms distinct populations of between 65 and 2,574 (median: 142) plants in Kakamega forest area, as well as relatively continuous, but less dense, stands along riversides.

In this study, we analyzed data from five main forest and four forest fragment populations of *A. eminens* and *A. polystachyus*. Two fragment populations were heterospecific stands with less than 10 m distance between individuals of both *Acanthus* species. Nearest distances between heterospecific populations ranged between 220 and 4,000 m. We are reporting data from four reproductive periods, between start of november and end of february 2002/2003, 2006/2007, 2007/2008, 2008/2009. Please note that we address the flowering seasons by the year they end (e.g. 2002/2003 is reported as flowering season 2003). Sample sizes for observations of floral visitors and sampling of floral nectar content are reduced in 2008, due to constrained mobility in the wake of the political unrest which followed presidential elections in december 2007.

Flower morphology

Flower morphology characteristics were measured during two flowering periods to account for seasonal variation. Twelve characteristics (see diagram in Appendix, Fig. 2.A) were measured in eight populations (four in the main forest fragment, four in separate smaller forest fragments) during the flowering season in 2003 by K. Gebhardt (see Contributions). As sizes were highly correlated, we reduced the number of measurements to flower width, flower length, length of style and upper/lower stamen length during our second survey in 2007 (measured by N. Hasenbein). Measurements were taken from nine populations of *A. eminens* (five in main forest and four in forest fragment populations) and seven populations (five and two in the main forest fragment and smaller fragments, respectively) of *A. polystachyus*. Flowers were sampled from the middle of each inflorescence and measured with a caliper immediately. All measurements were repeated thrice to increase accuracy, and analyses use means of these replicates.

For an assessment of flower similarity, we determined the common minimum and maximum value for either floral trait. We divided the resulting range into equally sized intervals, and calculated the density of measurements in these intervals separately for each species. For comparison, we calculated the Sœrensen similarity index (equivalent to 1-BC, with BC being Bray-Curtis dissimilarity), ranging between 1 (identity) and 0 (no similarity).

Flower colour analysis

During the flowering period 2007/2008, we measured floral colour of both *A. eminens* and *A. polystachyus* using an 'AvaSpec-2048 Faseroptik spectrometer' (Avantes, Eerbeek, The Netherlands; range 175-1100 nm) with a deuterium-halogen lightsource (DH 2000 Mikropack, Avantes, Eerbeek, The Netherlands). Light was transmitted by a 200 cm fiberglass cable. The light hit the sample in 45°. Reflection was measured against a WS-2 standard white balance (barium sulphate), and reflection values between 300 and 700 nm were extracted using Avasoft v7.0 software. Flowers were collected from seven populations of *A. eminens* and six populations of *A. polystachyus* and measured as soon as possible to avoid wilting. We measured in two positions on the showy lower lip of the flower, as these typically varied in visible colour. The first measurement was taken from the middle of the lip, and the second near the left or right edge of the lip. We used non-metric multidimensional scaling (NMDS) to visualize the differences between the measured colour profiles, excluding the wavelengths below 380 nm and above 650 nm due to high noise levels in the data. We calculated euclidean distances between colour profiles and used a two-dimensional minimum-stress approach, selecting the model with the lowest stress value from 25 iterations (Package 'ecodist', Version 1.2.3 in R 2.11.1).

For visualization, we present means of measurements for intervals of 5 nm.

Floral rewards

We measured nectar content by bagging individual flowers in the afternoon, sampling nectar between 11 a.m. and 1 p.m. on the following day using 30 mm two and five µl glass capillaries and two hand refractometers (range 0-50% and 45-80%). In 2003, empty flowers (which were only found in *A. polystachyus*) were discarded under the assumption that they were parasitized. However, measurements in 2007 found nectarless flowers to be present in both species, and not all of them infected by parasites. Therefore, the amount of nectarless, un-parasitized flowers was noted during all flowering periods starting 2007. Amount of nectar was measured with a ruler, and converted to µl. Sugar concentration was converted to g/l according to the conversion table in Kearns and Inouye (1993). The percentage of nectarless flowers was calculated for each population. Subsequent analyses of nectar concentration, volume, and floral sugar excluded empty flowers. However, we calculated the mean amount of sugar available for animals foraging on the flowers by multiplying the ratio of flowers containing nectar by mean floral sugar content of each flower for each year. Differences in the percentage of nectarless flowers were analyzed using Pearson's χ^2-test for all measured flowers, and with a Kruskal-Wallis rank sum test for differences between population means in single flowering periods.

Stamens were sampled from flower buds due to the early opening of the anthers during floral anthesis, and separately stored in alcohol for analysis. For counting pollen, anthers were squashed in five ml of 60% alcohol and extracted using an ultrasound bath. Ten subsamples per anther were counted using a hemocytometer. Subsequent data analyses are based on the average pollen count from subsamples. Values of pollen counts presented here therefore are not absolute measurements of the anther's pollen content, but an indicator of the amount of pollen in each anther relative to the other measurements using the same methodology and equipment.

Flower visitors

For a preliminary assessment of flower visitation, we observed flowers of both species during standardized observation units. In each unit, ten flowers on different inflorescences were observed simultaneously. Each unit consisted of 30 minutes of observation, and ten minutes of insect catching on all flowers of the population. Observations were started between 9 a.m. and 2 p.m., which was established as time of highest visitation rates by prior observation, and started on the hour to allow for changing position between populations. Reference insect specimens were killed, pinned and stored for identification (species identification by Dr. M. Gikungu, National Museum of Kenya, Nairobi).

Observation units without recorded flower visits were discarded. We then calculated the sum of individuals per species across all observation units of a single population. Relative abundance was calculated by dividing these absolute numbers by the total number of individuals observed in that population. Means for each species were acquired by averaging of these relative abundance values across all populations. These abundances were normalized to values between 0 and 1 by dividing the mean value of each visitor species by the sum of means for each plant species.

Forest cover and Inter-Population distances

Forest cover and inter-population distances were kindly provided by the team of BIOTA E02 (Prof. Dr. G. Schaab, University of Applied Sciences, Karlsruhe), based on GPS measurements inside populations. Note that we re-calculated models including forest cover for all available scales (radius of 100, 500, 1,000 and 2,000 m around population centers) separately for both species, and used only the scale which provided most explanatory power.

Statistics

Graphics, statistical analyses and additional calculations were done using R 2.1 (R Development Core Team 2011) and packages 'beanplot' (Kampstra 2008), 'car' (Fox and Weisberg 2011), 'vegan' (Oksanen *et al.* 2011), 'ecodist' (Goslee and Urban 2007) and additional packages (see 'dependencies' of the aforementioned packages).

To assess which factors influence floral morphology of the species, we used hierarchical partitioning (HP, package 'hier.part', Walsh and Mac Nally 2008) to quantify the contribution of each measured variable to the variance of the observed data (for a detailed description of HP, and a comparison with other methods of factor selection, see Mac Nally 1996, 2000, 2002; Quinn and Keough 2002). HP partitions variance between predictors included into the model by calculating the independent contribution of each factor alone, and the relative contribution of each predictor to variance of the response variable across all models based on all potential combinations of the factors, weighed by the explanatory power of each model. Statistical significance is based on Z-Scores derived from randomization of the original data, with $Z \geq 1.65$ representing an upper 0.95 confidence limit equivalent to $p \leq 0.05$. All our HP analyses were based on r-square measures of goodness of fit, and 5,000 randomizations. Note that HP partitions the variance explained by the factors included in the model, highlighting the importance of each factor relative to the other factors included, but does not provide an absolute measure of factor importance. We calculated standard linear regressions for models including the factors found to have significant contribution to data variance based on HP, in order to quantify the percentages of variance explained by each factor in a way compatible with standard r-squared calculations.

In all cases, we used parametric tests where appropriate after checking for normality and homogeneity of errors using qq-plots (as implemented in package 'car'), and resorted to non-parametric statistics when requirements for parametric analysis were not met.

RESULTS

MORPHOLOGY

We measured flower traits on 388 plants of *A. eminens* and 412 plants of *A. polystachyus* (Tab. 2.1). Most flower characteristics sufficed conditions for parametric analysis, although histograms and qq-Plots suggest a slightly long-tailed distribution of flower sizes in both species.

In *A. eminens*, all measured floral traits showed high positive correlation with flower length (r between 0.389 and 0.717, $p < 0.001$ in 2003; r between 0.287 and 0.466, $p < 0.001$ in 2007) and width (r between 0.381 and 0.628, $p < 0.001$ in 2003; r between 0.228 and 0.374, $p < 0.004$ in 2007. For complete correlation tables, see Appendix Tab. 2.A-2.C), with the exception of ovary size (measured only in 2007, $p > 0.05$). Correlation between flower length and width was high ($r = 0.830$, $p < 0.001$ in 2003, and $r = 0.675$, $p < 0.001$ in 2007). In *A. polystachyus* in 2003, the significance level of correlation with flower length was lower for basal tube diameter ($r = 0.218$, $p = 0.020$), but strong for all other traits highly correlated with it (r between 0.319 and 0.649, $p < 0.001$). For flower width, significance levels were lower for comparison with total gynoecium length ($r = 0.191$, $p = 0.042$) and tube length ($r = 0.218$, $p = 0.020$), but also strong for all other traits (r between 0.353 and 0.504, $p < 0.001$). In 2007, flower length of *A. polystachyus* was positively correlated with all other measured floral traits (r between 0.264 and 0.512, $p < 0.001$), while flower width was positively correlated (r between 0.235 and 0.305, $p < 0.002$) with all traits except ovary size ($r = 0.148$, $p = 0.076$).

In all morphological measurements, both across species and years, we found only one significant correlation to be negative (ovary and style size in *A. eminens* in 2007, $r = -0.220$, $p = 0.005$).

The range of several morphological traits of one species encompasses the whole range of the same trait of the other (Tab. 2.2). For example, in *A. eminens*, we found both smaller and larger flowers than in *A. polystachyus* in terms of flower length in 2007, and in both flowering seasons, the range of width of *A. polystachyus* flowers encompassed the whole range of width of *A. eminens* flowers. Percentage similarity between floral traits of the species was high, ranging between 41.2% (flower length) to 75.9% (length of upper stamen) in 2003 and 77.0% (flower length) and 96.2% (lower stamen length) in 2007. For male and female structures, percentage similarity was ≥60.2% in 2003 and ≥87.0% in 2007.

Due to the strong correlations between traits, we focussed on flower length and flower width for analysis, assessing differences between populations, species and years (nested ANOVA with population nested in species nested in reproductive periods). We found significant differences between species, between years for each species, and between populations in the different years (Tab. 2.3, Fig. 2.1).

Table 2.1: Mean morphological trait parameters [mm] of *A. eminens* and *A. polystachyus* during reproductive periods 2003 and 2008. mean±sd.

	2003			2007		
	A. eminens	*A. polystachyus*	ratio	*A. eminens*	*A. polystachyus*	ratio
n =	165	165		223	232	
Flower						
Length	54.31±4.03	46.53±3.85	1.17	50.44±5.51	44.77±4.18	1.13
Width	40.89±3.53	38.59±3.76	1.06	39.42±4.92	37.89±4.48	1.05
Tube						
Length	6.73±0.83	7.11±0.80	0.95			
Diameter 1	3.03±0.39	3.48±0.57	0.87			
Diameter 2	6.99±0.64	6.62±0.63	1.06			
Lower Stamen						
Complete	23.06±1.68	24.23±2.16	0.95	23.25±1.86	23.90±1.72	0.97
Filament	18.79±1.70	22.00±2.04	0.85			
Stamen	6.95±0.57	4.88±0.52	1.42			
Upper Stamen						
Complete	23.37±1.65	24.09±2.30	0.97	22.85±1.81	23.98±2.00	0.95
Filament	18.61±1.69	21.26±2.02	0.88			
Stamen	7.87±0.54	5.24±0.52	1.50			
Gynoecium						
Complete	39.28±3.50	34.29±3.69	1.15	37.13±4.17	34.42±2.74	1.08
Style				33.05±3.72	30.74±2.69	1.08
Ovary				4.22±0.79	3.68±0.59	1.15

Table 2.2: Minima and maxima [mm] of morphological trait parameters of *A. eminens* and *A. polystachyus* during reproductive periods 2003 and 2007. Values in *italics* indicate the minimum and maximum for each floral trait across species in the separate flowering periods. Similarity between floral traits was expressed using the Sœrensen similarity index (equivalent to 1-BC, with BC being Bray-Curtis dissimilarity), ranging between 1 (identity) and 0 (no similarity).

	2003		2007	
	A. eminens	*A. polystachyus*	*A. eminens*	*A. polystachyus*
n =	165	165	223	232
Flower Length				
min	44.97	*34.75*	*32.20*	33.57
max	*64.50*	58.08	*62.17*	56.23
Similarity	0.412		0.770[a]	
Flower Width				
min	31.22	*28.98*	27.27	*24.13*
max	49.40	*53.25*	59.20	*61.10*
Similarity	0.711[b]		0.921[b]	
Upper Stamen				
min	*18.20*	18.65	17.33	*15.63*
max	28.50	*33.33*	27.63	*30.10*
Similarity	0.759		0.925[b]	
Lower Stamen				
min	*18.03*	18.77	*17.93*	18.50
max	27.65	*33.62*	*28.50*	28.17
Similarity	0.757		0.962[a]	
Gynoecium				
min	26.53	*14.67*	*6.30*	27.03
max	*47.82*	44.20	*45.93*	40.04
Similarity	0.602		0.870[a]	

[a] Note that the range of this floral trait of *A. eminens* encompasses the whole range of *A. polystachyus* during this flowering period.

[b] as [a], with *A. polystachyus* encompassing the range measured in *A. eminens*

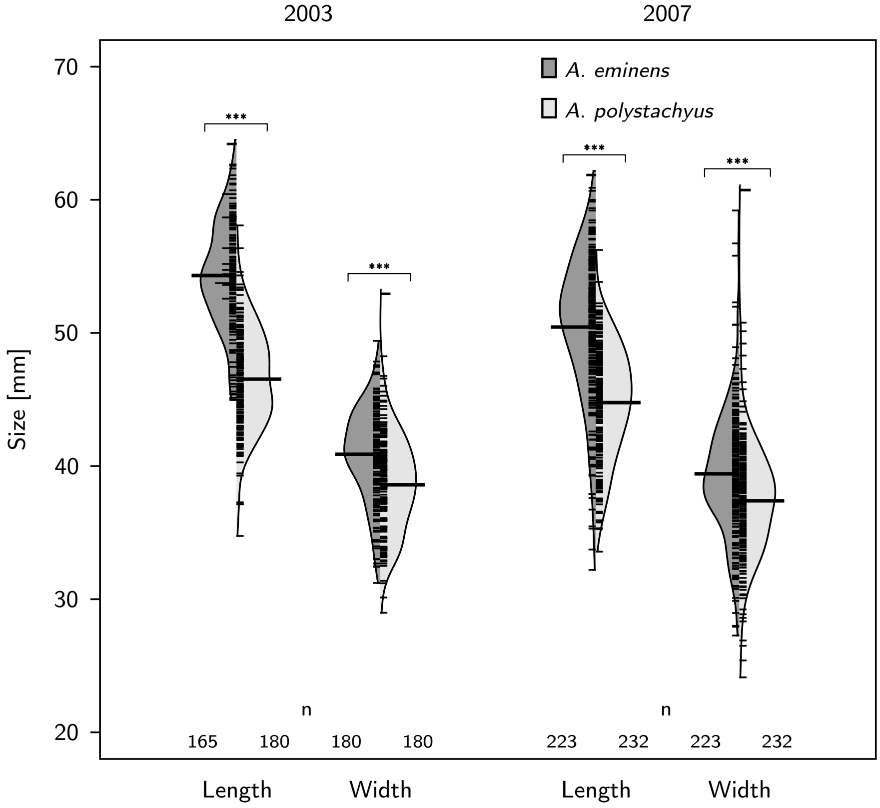

Figure 2.1: Beanplots of flower width (1 and 3) and flower length (2 and 4) size distribution in 2003 (one and two) and 2007 (three and four). Dark grey halves of each bean visualize data for *A. eminens*, light grey halves data for *A. polystachyus*. Long horizontal bars in each subplot represent mean values of subplots, while short horizontal bars represent individual measurements. Halved bean areas visualize smoothed density estimates of measurements, comparable to vertical histograms.

Table 2.3: Nested ANOVA of flower length and flower width of *A. eminens* and *A. polystachyus*.

Flower Length	df	Mean Sq	F	Pr(>F)	
Year	1	1,454.3	89.740	<0.001	***
Species[a]	2	4,422.4	272.895	<0.001	***
Population[b]	31	117.7	7.265	<0.001	***
Residuals	762	16.20			
Flower Width	**df**	**Mean Sq**	**F**	**Pr(>F)**	
Year	1	338.1	21.826	<0.001	***
Species[a]	2	459.4	29.651	<0.001	***
Population[b]	31	86.9	5.611	<0.001	***
Residuals	762	15.5			

[a] nested in Year

[b] nested in Species and Year

Only one factor, forest cover, was found influencing flower width of *A. polystachyus* during one field season (For full results of hierarchical partitioning, see Appendix Tab. 2.E). Linear regression revealed that flowers are broader in habitats with more forest cover in the surroundings. However, this effect was only marginally significant after Bonferroni-Holm correction for multiple comparisons ($r^2 = 0.606$, $F_{1,5} = 7.687$, $p = 0.078$; unadjusted $p = 0.039$). Apart from not being significant, the importance of all factors included strongly varied between floral traits and years.

Matrix correlation revealed flower morphological dissimilarity to be linked with distance between populations in 2003, but not in 2007 (Tab. 2.4).

Table 2.4: Mantel test results for matrix correlation between flower morphology dissimilarities and spatial distance between populations. Tests are based on Pearson's product-moment correlation and 10,000 permutations. Morphology matrix for both years based on euclidean distances calculated from morphological traits measured in both flowering periods (flower length and width, total upper and lower stamen length, and total gynoecium length).

A. eminens	Mantel statistic r	p-value	
2003	0.183	<0.001	***
2007	0.019	0.218	
A. polystachyus			
2003	0.076	<0.001	***
2007	−0.006	0.581	

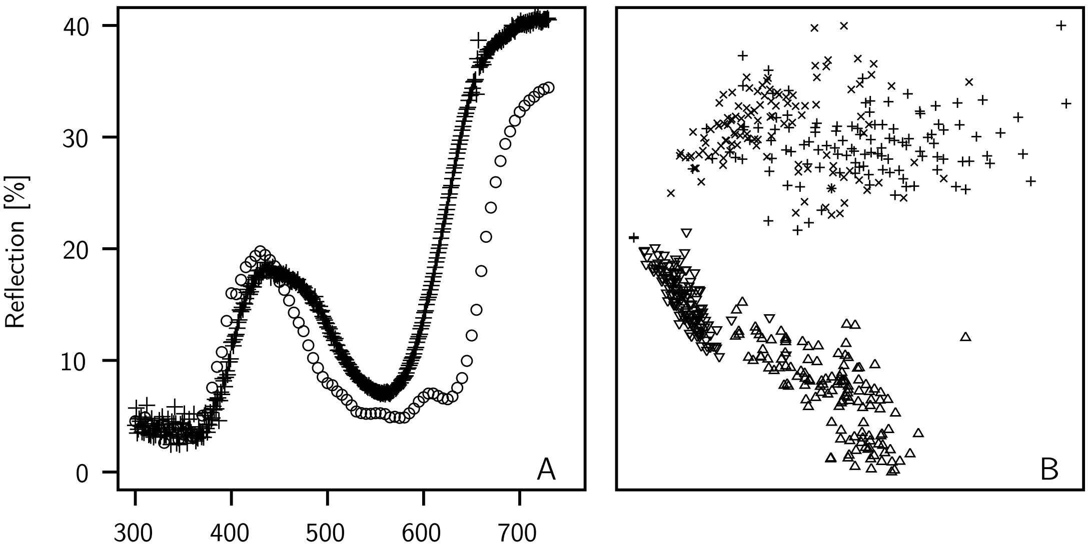

Figure 2.2: A: % reflection per wavelength (in 5 nm steps) of flowers of *A. eminens* (○) and *A. polystachyus* (+) for wavelengths between 300 and 730 nm. B: NMDS plot based on dissimilarities between floral colour spectrums of *A. eminens* (Flower lip center ▽ and flower lip edge △) and *A. polystachyus* (Flower lip center × and flower lip edge +). $R^2 > 0.99$, stress of plotted model < 0.025.

FLOWER COLOUR

Flowers of *A. eminens* displayed a distinct peak of floral light reflection at 424 nm, and a minimum at 571 nm, while *A. polystachyus* displayed a peak at 435 nm and a minimum at 560 nm (Fig. 2.2 A). Reflection values did not differ strongly between species between wavelengths from 300 nm and 560 nm, with at most less than 10 percentage points difference in reflection. The strongest difference was found between 600 and 700 nm, with a maximum at 644 nm, at which point *A. polystachyus* reflected 22.6 percentage points, or three times, more light (32.1%) than *A. eminens* (9.6%).

NMDS visualization based on flower colour data showed that the colour spectrum of the separate samples from each flowers of each species was distinct (Fig. 2.2 B, 2-dimension NMDS with 25 iterations based on reflection values for restricted wavelengths of 350 to 650 nm. $r^2 > 0.99$, stress of plotted model < 0.025). We restricted analysis to wavelengths with low noise levels. Flowers of *A. eminens* showed a pronounced difference between the center of the flower and the outer edges of the flower lip. This difference was also present, but less pronounced, in *A. polystachyus*.

Floral rewards

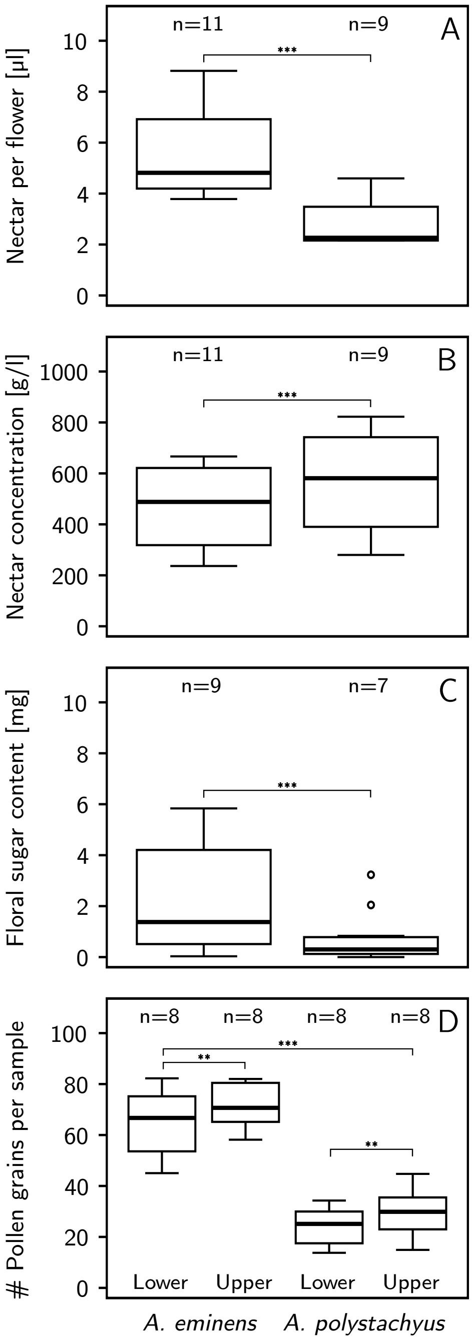

Figure 2.3: Floral rewards of *A. eminens* and *A. polystachyus.* A: Nectar volume per flower [µl]; B: Nectar sugar concentration [g/l]; C: Floral sugar content [mg] (data from 2007, 2008 and 2009, as nectarless flowers were not recorded in 2003); D: Pollen grains per sample from upper and lower anthers (left and right, respectively). Boxplots based on population mean values.

Overall, we found more 'empty' flowers without nectar in *A. polystachyus* (45.5%) than in *A. eminens* (21.2%, Chi-square test: $\chi^2 = 29.175$, df = 1, $p < 0.001$, Tab. 2.5). As nectarless flowers were not recorded in 2003, and sample sizes for nectar content of *A. polystachyus* were small in 2008, reliable statistics could only be calculated for flowering periods 2007 ($\chi^2 = 1.193$, df = 1, $p < 0.275$) and 2009 ($\chi^2 = 8.167$, df = 1, $p = 0.004$), indicating a tendency of *A. eminens* to present less nectarless flowers (2007: 30.6%, 2009: 25.6%) than *A. polystachyus* (2007: 40.9%, 2009: 55.6%).

We used a nested analysis of variance (anova) to analyze the difference in sugar content between populations, species and years (Tab. 2.6, populations nested in species, nested in years). We found significant differences in all levels of analysis, indicating that floral sugar content is spatially and temporally variable, and differs between species (Fig. 2.3). *A. eminens* contained significantly more sugar per flower than *A. polystachyus*. When taking into account the higher ratio of empty flowers in *A. polystachyus*, this difference becomes even more pronounced, with *A. eminens* flowers offering an average of 2.43±1.36 mg of sugar in contrast to 0.76±0.36 mg offered by flowers of *A. polystachyus*. A nested anova on the amount of nectar shows that *A. polystachyus* produced less nectar than *A. eminens*, and that nectar amount varied between years and species (Tab. 2.6). However, Kruskal-Wallis rank sum tests on population nectar concentration means did not reveal conclusive differences between species, as we found nectar concentration to be significantly higher in *A. polystachyus* in 2003 (Kruskal-Wallis $\chi^2 = 86.951$, df = 1, $p < 0.001$), but not significantly different in 2007 (Kruskal-Wallis $\chi^2 = 0.2757$, df = 1, $p = 0.5996$) and 2009 (Kruskal-Wallis $\chi^2 = 0.0796$, df = 1, $p = 0.7778$). As sample sizes in terms of sampled populations were low for *A. polystachyus* in 2008 (Tab. 2.5), we did not take this year into account for comparison of nectar concentration within years. However, pooling nectar concentration values from all other years, we found it to be significantly higher for *A. polystachyus* (Kruskal-Wallis $\chi^2 = 15.73$, df = 1, $p < 0.001$).

A. eminens offered significantly more pollen (68.50 grains per sample) than *A. polystachyus* (26.85 grains per sample, anova, Tab. 2.7). Upper and lower anthers differed in pollen content, with between 10 and 20% more pollen in the upper anther in *A. eminens* and *A. polystachyus*, respectively. Pollen grain counts differed between populations.

Table 2.5: Average nectar content of flowers of *A. eminens* and *A. polystachyus* for all measured flowers, split by study years. Values including measure of variability are mean±sd.

A. eminens	n[a]	Empty[b] [%]	Amount [µl]	Conc. [g/l]	Sugar [mg]	Reward[c] [mg]
All[d]	372	21.2(49)	5.57±2.23	470±191	2.62±1.55	2.43±1.36
2003	92(8)	–	4.61±1.30	400±156	1.60±0.43	–
2007	75(7)	30.6(23)	3.79±2.36	237±96	1.05±1.07	0.86±1.01
2008	162(5)	7.4(15)	5.02±2.40	667±153	3.48±1.75	3.19±1.64
2009	43(4)	25.6(11)	8.82±4.70	576±97	4.34±2.06	3.23±2.34
A. polystachyus	n[a]	Empty[b] [%]	Amount [µl]	Conc. [g/l]	Sugar [mg]	Reward[c] [mg]
All[d]	244	45.5(84)	2.82±1.19	566±232	1.62±0.76	0.76±0.36
2003	85(8)	–	2.15±0.55	823±162	1.70±0.54	–
2007	66(5)	40.9(29)	2.16±1.14	280±95	0.63±0.48	0.36±0.27
2008[e]	*30(2)*	*40.0(14)*	*2.37*	*501*	*1.67*	*1.04*
2009	63(4)	55.6(41)	4.60±5.95	662±26	2.47±2.71	0.88±1.35

[a] Values in brackets reflect number of sampled populations

[b] Values in brackets indicate absolute number of nectarless flowers

[c] Average of mean values for each population, including zeroes for nectarless, unparasitized flowers. Values for 2003 not provided due to differences in sampling methodology

[d] Average calculated from pooled raw data from all years. Please note that data from 2003 was excluded from overall averages of rewardless flowers and average floral reward due to differences in sampling methodology

[e] Data collection was impaired by political unrest following the presidential election in Kenya in December 2007. sd not shown due to low sample size.

Table 2.6: Nested ANOVA for floral sugar content and amount of nectar. As values of nectar concentration did not show normal distribution, they were analyzed using nonparametric statistics (see text). Values were log-transformed to optimize for normality.

Amount of nectar	df	Sum Sq	Mean Sq	F value	Pr(>F)	
Species	1	51.4	51.4	72.189	<0.001	***
Year[a]	6	12.7	2.1	2.983	0.007	**
Population[b]	35	91.8	2.6	3.684	<0.001	***
Residuals	440	313.1	0.7			

Floral sugar content	df	Sum Sq	Mean Sq	F value	Pr(>F)	
Species	1	29.7	29.7	40.834	<0.001	***
Year[a]	6	145.7	24.3	33.327	<0.001	***
Population[b]	35	140.3	4.0	5.501	<0.001	***
Residuals	440	320.5	0.7			

[a] nested in Species

[b] nested in Year and Species

Table 2.7: Nested ANOVA for pollen grain counts, testing differences in pollen counts from upper and lower anthers nested in populations nested in species.

	df	Sum Sq	Mean Sq	F value	Pr(>F)	
Species	1	134,317	134,317	512.436	<0.001	***
Population[a]	14	1,137	1,137	4.337	<0.001	***
Anther[b]	16	663	663	2.530	0.001	**
Residuals	278	262	262			

[a] nested in Species

[b] nested in Population and Species

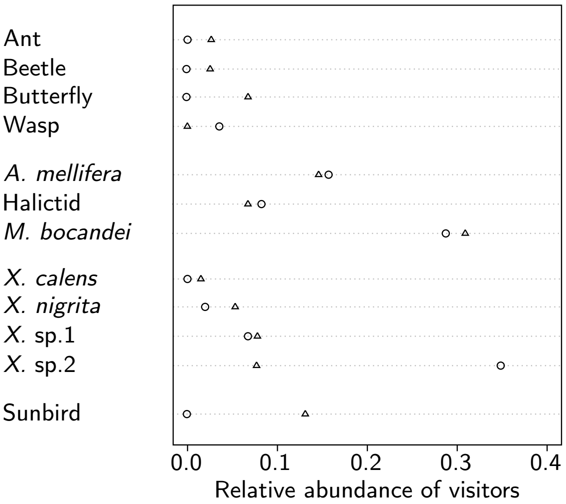

Figure 2.4: Relative abundance of floral visitors, based on population means of 75 standardized observation units on *A. eminens* (○, 40 units in four populations) and *A. polystachyus* (△, 35 units in three populations). The lower five species (*Xylocopa* and sunbirds) are likely pollinators, while the upper two species groups (split into various species (top group) and small bees (second group) are presumed to represent nectar and pollen robbers.

VISITORS

We were not able to complete visitor observations and were limited to populations in the north of the main forest fragment due to restricted mobility in the wake of political unrest following the presidential election in late 2007. Still, we were able to obtain observation data from four populations of *A. eminens* and three populations of *A. polystachyus*, observing the species for 20 and 17.5 hours, respectively. The abundances of honeybees, halictids, the stingless bee *M. bocandei* and three species of carpenter bees, *X. calens*, *X. nigrita* and *X.* sp.1, were nearly identical in either species (Fig. 2.4). The strongest differences were displayed in sunbirds, which have not been seen visiting flowers of *A. eminens* during observations, and in the carpenter bee *X.* sp.2, the latter being almost five times more abundant on flowers of *A. eminens* than on those of *A. polystachyus*. *A. eminens* and *A. polystachyus* shared 66% of flower visitors (Sœrensen similarity between relative abundances).

DISCUSSION

Although they are clearly distinguishable by their colour, flowers of *Acanthus eminens* and *A. polystachyus* show many similarities. Though *A. eminens* has significantly larger flowers than *A. polystachyus*, the range of floral trait sizes strongly overlaps, and is subject to strong variations both between populations and years. Our findings are in accordance with our first hypothesis that flowers of *A. eminens* and *A. polystachyus* differ in size, but the observed similarity warrants an analysis whether this difference is likely to have an impact on floral isolation between species, and on the species' pollination ecology.

FLORAL ISOLATION IN *ACANTHUS*

Flowers of species with partially overlapping ranges have been found to display stronger morphological divergence in sympatry, which has been interpreted as the result of selection for divergence due to interspecific competition (Grant 1994). In *Acanthus*, there is no evidence for divergence of floral morphology due to the proximity of the congener in either species. Flowers of *A. polystachyus* may be broader in populations which are largely surrounded by forest in comparison with more open populations, but generally, none of the factors included in our analysis was found to explain variations in flower size.

Selection on floral traits may be dependent on complex interactions with other flowers when they are part of larger interaction networks (reviewed by Strauss and Irwin 2004), but we do not find evidence that individual *Xylocopa* bees observed on flowers of *Acanthus* forage on other flowers with high frequencies during the flowering period of the congeners. Both stigmatic pollen loads of *Acanthus* and visitor pollen loads consist mainly of pollen of the congeners (N. Hasenbein, unpublished). However, previous studies have found the *Xylocopa* bees observed in this study to interact with flowers of several other plant species (Gikungu 2006; Hagen 2008).

Variability of flower size may indicate pollinator-mediated selection (Maad 2000), driven by reproductive advantages of specialization to subsets of available pollinator species (Conner *et al.* 1996; Armbruster and Baldwin 1998; Kay and Sargent 2009; Schluter 2009; Alonso *et al.* 2010). However, temporal and spatial variation in flower morphology have been described in several species (Maad 2000; Herrera 2005; Alarcón *et al.* 2008), and may be caused by factors not related to interspecific competition.

Selection for floral isolation between the congeners may be restricted by genetic constraints on flower morphology, or may be too slow to detect after recent habitat change as found in our study area (Schaab *et al.* 2010). Though Galen (1996) demonstrated a rapid evolutionary response to changes in pollinator regimes, evolutionary responses of long-living shrub species may only be measurable after several decades. Also, this study does not include morphological data from populations of either species outside the natural range of the respective congener, and a comparison with flower material from such populations may provide additional information whether the species influence each other's flower morphology.

Divergent floral morphologies due to adaption to divergent pollinator communities in different populations of *Acanthus* may be related or unrelated to the proximity to and density of the respective congener, depending on whether the abundance of *Acanthus* influences pollinator abundance. In a study on spatiotemporal variation of flower-visitor networks in Kakamega Forest, Hagen and Kraemer (2010) found comparatively larger bee species inside the forest habitat, suggesting in accordance with Gathmann *et al.* (1994), Herrera (1997) and Kato (2005) that these species might be better adapted to greater foraging ranges, scattered and low resources, and foraging under low-light conditions, than smaller bees. Hence, larger flowers may be adapted to these pollinators (Herrera 2005). The larger flowers of *A. polystachyus* found in more forested populations may indicate an adaption to larger pollinators, but the evidence is weak, and the adaptive nature of this finding highly speculative. However, this line of inquiry may be of interest for future studies.

On the other hand, due to the habitat requirements of *A. eminens* and *A. polystachyus*, spatial distance between the plant species may also represent an environmental gradient and it may be difficult to discriminate between character displacement caused by ecological divergence and selection for reproductive isolation (Grant 1994).

Variation in pollinator abundance, both temporal and spatial, may cause differences in pollination success, and consequently influence selection on floral traits (Galen *et al.* 1987; Eckhart 1991, 1992; Conner and Neumeier 1995; Fishbein and Venable 1996; Emms and Arnold 2000; Herrera 2000). Different pollinators may exert selection divergent in both strength and direction (Nilsson 1988; Young and Stanton 1990; Campbell *et al.* 1991), and selection on flower morphology has been found to vary across years and sites in other studies (Campbell 1989; Schemske and Horvitz 1989; Campbell *et al.* 1991; Johnston 1991, reviewed by Harder and Johnson 2009). Nectar and pollen thieves, parasites and herbivores may influence selection on flower morphology in either species, modifying the outcome of selection mediated by pollinators (e.g. Brody 1992; Galen 1999; Galen and Cuba 2001; Fenner *et al.* 2002; Ehrlén *et al.* 2002). Though variability in fitness due to variable selection may prevent directional selection for or against certain traits, selection may be consistent even when subsequent seasons vary in pollinator abundance or climatic conditions (Conner *et al.* 1996).

However, considering the strong variation in flower morphology between flowering seasons, directional selection on floral traits seems unlikely in *Acanthus*. The observed variation in flower morphology may reflect year-to-year changes in environmental conditions and cause differences in pollinator faunas between years (Maad 2000). We do not find the same pattern of morphological similarity between populations in separate flowering periods of either species, and different populations displayed the largest or the smallest flowers in either species and year. Hence, putative genetic differentiation of flower sizes between populations is masked by other factors which are subject to strong temporal variation. It is likely that several factors influence selection simultaneously, differently, and with different strength during separate flowering periods. Long-term studies and experimental manipulation of floral traits and pollination are needed to reveal the strength and direction of potential selection on floral traits in *Acanthus*, and to clarify which factors shape floral morphology of either species.

PARTITIONING OF POLLINATORS

In either species, flowers on an inflorescence decrease in size from the largest flower on the base to the smallest at the tip. We measured medium-sized flowers from the middle of inflorescences, which we found to be highly similar between species. Though it is likely that the range of flower sizes is not identical in *A. eminens* and *A. polystachyus*, we consider the flower architecture of the congeners not to be a mechanism for partitioning of pollinators between the species.

There is no indication for functional differences, e.g. placement of pollen on different pollinator body parts through divergent sexual architecture, and we conclude that flowers of both species of *Acanthus* are functionally identical. As no trait of floral architecture is distinctly different from the corresponding trait of the respective congener, both flower types seem to allow access to the same floral visitors. In addition, pollen placement on pollinators is likely identical in either species, which increases the likelihood of interspecific pollen transfer and pollen removal on heterospecific flowers (Mitchell *et al.* 2009).

Though the data on relative visitor abundance included in this study is limited, we found a strikingly similar relative abundance of many species on flowers of both species of *Acanthus*, and the shrubs share about two thirds of their pollinators. Still, visitor faunas may be separated by divergent abundance of pollinators in different populations of the species, or by pollinator behaviour, e.g. preference of visitors for either one of the species. As our data on visitation was sampled from a rather small subset of populations, it is likely that local abundance of visitors strongly influences our observations. We were limited to the north of the forest, which is under protection by KWS, and less disturbed than the forest areas in the south, and more detailed observations will have to include populations in all parts of the forest.

Pollinator behaviour may be more important in determining pollen deposition on and pollen removal from pollinator bodies than the fit between pollinators and flowers, even in flowers seemingly specialized to certain pollinators (Wilson 1995). Also, foraging behaviour has been found to determine pollination effectiveness in a study by Young *et al.* (2007). However, pollinator abundance has been shown to vary both spatially and temporally (Alarcón *et al.* 2008), and a detailed study of pollinator abundance on flowers of *Acanthus*, including populations with divergent habitat traits, is needed to clarify the congruence in pollinator faunas between the species. In addition, the spatio-temporal abundance of pollinator species in Kakamega is of interest when trying to predict whether future changes in habitat composition due to forest fragmentation may have any influence on populations of pollinators and the pollination of *Acanthus*.

Though we cannot claim to know the real contributions of each animal species to the pollination of *A. eminens* and *A. polystachyus*, the similarity of abundance of many visitor species corroborates that the plants share visitors and pollinators, and the pronounced differences in some groups indicates that pollinator faunas may be partitioned to some degree, which may be one of several, cumulative barriers preventing gene flow between *A. eminens* and *A. polystachyus* (Marques *et al.* 2007). On the other hand, partitioning of pollinators through local pollinator abundance does not provide an explanation for the observation that heterospecific populations display the highest reproductive success in either species. In these populations, pollinator behaviour may reduce pollen transfer between species, and pollinator movements may be influenced by a divergence in floral rewards.

FLORAL REWARDS

Both *A. eminens* and *A. polystachyus* produce nectar at the base of the ovary, which is sealed off by a ring of hairlike structures around the tube at the flower's base, preventing access by short-tongued animals. Flowers of *A. eminens* are more likely to contain any nectar, and offer more nectar than those of *A. polystachyus*. As a result, flowers of *A. eminens* on average contain three times more sugar than those of *A. polystachyus*, though the latter species offers higher sugar concentrations.

The average amount of sugar offered per flower varies between flowering periods and populations in both species. However, relative differences between the congeners are largely maintained between all years. For example, both *A. eminens* and *A. polystachyus* offer similar amounts of nectar when comparing flowering periods 2008 and 2009, but in both species nectar production was reduced in 2007. It is likely that climatic conditions during flowering provides an explanation for this co-variation in floral nectar content.

The higher floral sugar content of *A. eminens* may be an adaption to attract pollinators over large distances. It may also be an adaption to the isolation of populations, as high and constant nectar rewards may constrain pollinator movements, and cause a single pollinator to visit numerous flowers in a population (Keasar *et al.* 1996). Pollinators of flowers containing low amounts of floral rewards have to switch between flowers more frequently, which increases visitation frequency (Heinrich 1975). The lower amount of floral rewards in flowers of *A. polystachyus* may be an adaption to increase the likelihood of pollinator movements between flowers of different individuals. On the other hand, as plants of *A. polystachyus* are on average roughly eight (2008) to eleven (2009) times larger than those of *A. eminens*, they offer more nectar per plant, and higher resource density and total amount of resources per population, which may increase their attractiveness for pollinators relative to individuals and populations of *A. eminens*.

Inside the forest, *A. eminens* may be an important source of energy in the understorey plant community. The increased assurance of nectar rewards in *A. eminens* as well as the higher amount of sugar produced by its flowers points towards an adaption to large pollinator species crossing the comparatively large distances between populations (Heinrich and Raven 1972).

Large species with higher foraging range have been found to cross large distances between populations more frequently (Gathmann *et al.* 1994). On the other hand, pollinators have been found to leave populations earlier when they frequently encounter empty flowers or low amounts of floral rewards (Hodges 1985), and an increased likelihood of flowers offering nectar may also be an adaption to facilitate higher pollination rates within remote populations.

Flowers of *A. eminens* offer three to four times more pollen grains than flowers of *A. polystachyus*. As flower number and density are lower and distances between populations are greater in *A. eminens*, this may be an adaption facilitating greater pollen loads of pollinators and increased long-distance dispersal between populations (compare Groom 1998; Schulke and Waser 2001). Pollen of either species are accessible to and collected by a range of species including large *Xylocopa* spp., honeybees and similar-sized bees like *Meliponula* sp., and minute animals like halictids. In either shrub species, anthers are clustered around the style, preventing mechanical pollen losses. Pollen robbers are not excluded by flower morphology, and we observed stingless bees, especially *Meliponula bocandei*, frequently feeding on pollen in both species of *Acanthus*.

INTERSPECIFIC POLLEN TRANSFER AND HYBRIDIZATION

Even though there have been indications of fruit set from interspecific pollination (Dietzsch 2004), and other *Acanthus* species have been found to hybridize (McDade *et al.* 2005), we do not find any indication for hybridization or introgression between the two species. This may be due to the similarity of the analyzed morphological traits. Even if there were hybrid flowers of intermediate floral architecture, they would likely be indistinguishable from one of the paternal species (Anderson 1948; Neuffer *et al.* 1999). Intermediate colours may be an identifier of hybrid individuals (e.g. in *Ipomopsis*, Melendez-Ackerman 1997), but in *Acanthus*, no flowers of intermediate colour were found.

Successful hybridization is a rare event even in compatible species (Emms and Arnold 2000), and in a previous study, hand-pollination treatments with pollen of the congener have been found to decrease reproduction in either species of *Acanthus*. Pure heterospecific pollen was found to dramatically reduce fruit and seed set, but fruit development from interspecific pollen transfer was observed (Dietzsch 2004). The finding that fruit set in either species is not reduced due to the presence of the respective congener (Chapter 1) lead to the assumption that floral isolation is strong. But in this study, we do not find evidence that pollen flow between species is reduced by floral architecture.

Floral isolation may provide a strong barrier to hybridization which largely breaks down as soon as a F1 hybrid is formed (Emms and Arnold 2000), and we cannot rule out completely that increased interaction between the species due to anthropogenic habitat change will at some point cause the emergence of a hybrid plant, which may facilitate a breakdown of species barriers. Still, we conclude that there are no hybrids of *A. eminens* and *A. polystachyus* growing naturally in our study area. Even if there were inconspicuous hybrid or introgressed individuals, it seems reasonable to assume that they do not play an ecological role in Kakamega Forest due to their rarity.

One factor potentially limiting interspecific pollen transfer and, consequently, hybridization is a prevalence of geitonogamy. The simultaneous presentation of numerous flowers in large individuals may facilitate self-pollination, as pollinators likely visit several flowers of the same plant (Klinkhamer and Jong 1993; Harder *et al.* 2004). Both species are capable of selfing (Dietzsch 2004), and though flowers of *Acanthus* are protandrous, in plants with numerous inflorescences both sexual functions are displayed simultaneously.

Self-compatibility may be an adaption to rapid spreading in isolated habitats (Baker 1955), and Dietzsch (2004) found that selfing may cause higher seed set in *A. eminens* than in *A. polystachyus*. On the other hand, protandry may be an efficient barrier to selfing in founder populations, as co-occurrence of both sexual flower phases on plants carrying few flowers is less likely than individuals with large floral displays. Reduced seed set due to protandry may increase the founder population's genetic diversity through an increase in the ratio of seeds resulting from cross-pollination. Both an adaption to fast reproduction and to increase genetic diversity may play an important role in *A. eminens* due to the spatial isolation of its populations in recent clearings in densely forested areas.

Attraction of pollinators through large floral displays might incur fitness costs due to reduced reproduction through losses of genetic variability (Harder and Barrett 1995). Harder *et al.* (2004) have argued that, consequently, selection on display size should be balanced between pollinator attraction and costs of geitonogamy in selfing species (see also Jong *et al.* 1992; Harder and Barrett 1995). This tradeoff might play a major role in populations of *A. eminens*, as they form distinct patches which may consist of just a few genets, and depend on long-distance pollinator movements for genetic exchange. In *A. polystachyus*, variability of nectar content through 'empty' flowers could be an adaption to reduce geitonogamy (Biernaskie *et al.* 2002), inducing more frequent pollinator movements between individuals and populations (Hodges 1985).

A previous study found indications for pollen limitation in either species of *Acanthus* (Chapter 1). As both species display numerous hermaphroditic flowers, pollen transfer efficiency seems low, which may mitigate male fitness (Maad 2000; Ashman and Morgan 2004): Fruit set in *A. eminens* and *A. polystachyus* may be limited by pollen receipt, and consequently, pollinator-mediated selection through female reproductive success should favor traits which facilitate higher stigmatic pollen loads (Ashman and Morgan 2004).

CONCLUSIONS

The analysis of floral traits of *A. eminens* and *A. polystachyus* does reveal differences between species, but does not explain the apparent lack of reproductive interference between species demonstrated in a previous study. Our finding that the species share two thirds of their floral visitors indicates potential for partitioning of pollinators, and we conclude that pollinator-mediated interaction between *A. eminens* and *A. polystachyus* is very likely, especially in heterospecific populations. Since inter-population distances and densities of *A. eminens* and *A. polystachyus* are supposedly affected by forest fragmentation, it seems reasonable to expect that it causes changes in pollination of both *A. eminens* and *A. polystachyus*. A more detailed analysis of fruit and seed set, as well as of the role of potential pollen limitation in either species, may reveal whether restricted pollinator movement between species and populations causes or contributes to reproductive isolation and provide additional insight into the pollination ecology of the congeners.

CONTRIBUTIONS

Nectar, pollen and morphology field data from the reproductive period in 2002/2003 ('2003') were collected and analyzed by Kristina Gebhardt, and presented as part of her master thesis ('Staatsexamensarbeit') at the University of Bonn in 2004 (Gebhardt 2004). Nils Hasenbein collected morphology, nectar, and spectrometry data during flowering periods 2007, 2008 and 2009, (re-)analyzed all data, and wrote this paper.

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APPENDIX

Table 2.A: Correlation between morphological traits for *A. eminens*, based on data from flowering period 2003. Correlation coefficients (r) based on Pearson's Product-Moment-Correlation (above diagonal, *italics* indicate significance) and corresponding p-values (below diagonal). p-values corrected by Holm-Bonferroni procedure. Stars indicate p < 0.001.

	Flower		Gyn.	Tube			Lower Stamen			Upper Stamen		
	Length	Width		Length	Diameter 1	Diameter 2	Filament	Anther	Complete	Filament	Anther	Complete
Flower												
Length		*0.830*	*0.717*	*0.559*	*0.389*	*0.652*	*0.632*	*0.402*	*0.669*	*0.493*	*0.467*	*0.609*
Width	***		*0.615*	*0.423*	*0.440*	*0.628*	*0.517*	*0.381*	*0.520*	*0.428*	*0.421*	*0.502*
Gynoecium	***	***		*0.451*	*0.227*	*0.579*	*0.594*	*0.282*	*0.649*	*0.517*	*0.371*	*0.541*
Tube												
Length	***	***	***		0.186	*0.470*	*0.390*	*0.320*	*0.399*	*0.348*	*0.359*	*0.374*
Dia. 1	***	***	0.030	0.136		*0.442*	0.089	0.174	0.119	0.018	*0.249*	0.112
Dia. 2	***	***	***	***	***		*0.432*	*0.297*	*0.489*	*0.346*	*0.330*	*0.428*
Lower Stamen												
Filament	***	***	***	***	0.761	***		*0.311*	*0.865*	*0.817*	*0.373*	*0.749*
Anther	***	***	0.003	***	0.176	0.001	0.001		*0.450*	*0.289*	*0.770*	*0.412*
Complete	***	***	***	***	0.761	***	***	***		*0.748*	*0.517*	*0.827*
Upper Stamen												
Filament	***	***	***	***	0.814	***	***	0.002	***		*0.401*	*0.808*
Anther	***	***	***	***	0.013	***	***	***	***	***		*0.529*
Complete	***	***	***	***	0.761	***	***	***	***	***	***	

Table 2.B: Correlation between morphological traits for *A. polystachyus*, based on data from flowering period 2003. Correlation coefficients (r) based on Pearson's Product-Moment-Correlation (above diagonal, *italics* indicate significance) and corresponding p-values (below diagonal). p-values corrected by Holm-Bonferroni procedure. Stars indicate p < 0.001.

	Flower		Gyn.	Tube			Lower Stamen			Upper Stamen		
	Length	Width		Length	Diameter 1	Diameter 2	Filament	Anther	Complete	Filament	Anther	Complete
Flower												
Length		*0.675*	*0.475*	*0.485*	*0.218*	*0.319*	*0.611*	*0.556*	*0.649*	*0.543*	*0.484*	*0.608*
Width	***		*0.191*	*0.218*	*0.353*	*0.444*	*0.443*	*0.420*	*0.504*	*0.368*	*0.358*	*0.420*
Gynoecium	***	0.042		*0.375*	0.044	*0.232*	*0.495*	*0.305*	*0.466*	*0.448*	*0.314*	*0.442*
Tube												
Length	***	0.020	***		0.098	*0.266*	*0.397*	*0.430*	*0.409*	*0.412*	*0.405*	*0.380*
Dia. 1	0.020	***	0.556	0.378		*0.384*	*0.358*	*0.236*	*0.304*	*0.254*	*0.128*	*0.247*
Dia. 2	***	***	0.012	0.004	***		*0.448*	*0.298*	*0.426*	*0.414*	*0.362*	*0.401*
Lower Stamen												
Filament	***	***	***	***	***	***		*0.545*	*0.897*	*0.800*	*0.537*	*0.798*
Anther	***	***	0.001	***	0.011	0.001	***		*0.587*	*0.429*	*0.774*	*0.529*
Complete	***	***	***	***	0.001	***	***	***		*0.773*	*0.580*	*0.844*
Upper Stamen												
Filament	***	***	***	***	0.006	***	***	***	***		*0.488*	*0.869*
Anther	***	***	***	***	0.261	***	***	***	***	***		*0.574*
Complete	***	***	***	***	0.008	***	***	***	***	***	***	

Table 2.C: Correlation between morphological traits for *A. eminens*, based on data from flowering period 2007. Correlation coefficients (r) based on Pearson's Product-Moment-Correlation (above diagonal, *italics* indicate significance) and corresponding p-values (below diagonal). p-values corrected by Holm-Bonferroni procedure. Stars indicate $p < 0.001$.

	Flower		Gyn.			Lower Stamen	Upper Stamen
	Length	Width	Style	Ovary	Complete	Complete	Complete
Flower							
Length		*0.287*	*0.421*	*0.263*	*0.472*	*0.475*	*0.466*
Width	***		*0.228*	0.123	*0.278*	*0.281*	*0.374*
Gynoecium							
Style	***	0.004		*-0.220*	*0.977*	<0.001	<0.001
Ovary	0.001	0.214	0.005		−0.011	0.214	0.466
Complete	***	***	***	0.876		<0.001	<0.001
Lower Stamen	***	***	0.396	0.130	0.367		<0.001
Upper Stamen	***	***	0.389	0.081	0.317	0.782	

Table 2.D: Correlation between morphological traits for *A. polystachyus*, based on data from flowering period 2007. Correlation coefficients (r) based on Pearson's Product-Moment-Correlation (above diagonal) and corresponding p-values (below diagonal). p-values corrected by Holm-Bonferroni procedure. Stars indicate $p < 0.001$.

	Flower		Gyn.			Lower Stamen	Upper Stamen
	Length	Width	Style	Ovary	Complete	Complete	Complete
Flower							
Length		*0.512*	*0.452*	*0.264*	*0.501*	*0.485*	*0.426*
Width	***		*0.235*	0.148	*0.262*	*0.269*	*0.309*
Gynoecium							
Style	***	0.002		-0.023	*0.977*	<0.001	<0.001
Ovary	***	0.076	0.728		*0.191*	0.123	0.004
Complete	***	***	***	0.015		<0.001	<0.001
Lower Stamen	***	***	0.518	0.124	0.535		<0.001
Upper Stamen	***	***	0.354	0.220	0.395	0.718	

Table 2.E: Hierarchical partitioning table for potential factors influencing flower length and flower width as floral traits representing overall flower variation. Z-Scores based on 5,000 randomizations. n-values reflect the number of populations, bracketed values indicate the total number of measured flowers. Stars indicate significance.

A. eminens	Flower Length		Flower Width	
	% Var.	Z-Score	% Var.	Z-Score
2003, n = 8 (145)				
Canopy	16.67	−0.53	13.98	−0.72
Distance	21.10	−0.24	30.49	−0.27
Forest Cover [a]	46.50	1.28	29.38	0.22
Lat. Gradient	15.79	−0.59	26.14	0.00
2007, n = 9 (206)				
Canopy	8.97	−1.02	19.55	−0.44
Distance	28.28	−0.47	55.97	1.24
Forest Cover [a]	5.70	−1.15	10.78	−0.86
Lat. Gradient	57.06	0.44	13.70	0.70

A. polystachyus	Flower Length		Flower Width	
	% Var.	Z-Score	% Var.	Z-Score
2003, n = 7 (139)				
Distance	28.17	−0.90	5.97	−1.54
Forest Cover [a]	6.81	−1.40	−1.31	−1.61
Lat. Gradient	65.02	0.34	95.34	0.25
2007, n = 7 (232)				
Distance	55.37	−0.53	5.78	−0.78
Forest Cover [a]	32.76	−0.75	90.12	2.39 *
Lat. Gradient	11.87	−0.90	4.11	−0.85

[a] Forest cover in 500 m radius around population center

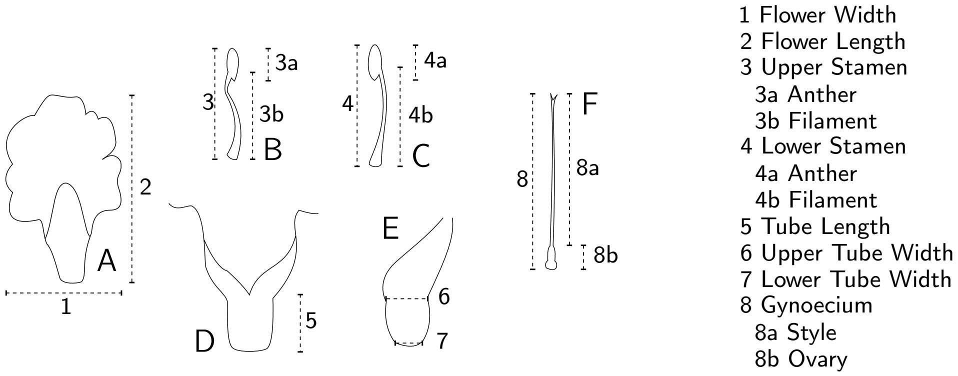

Figure 2.A: Diagram of *Acanthus* flower parts demonstrating the floral traits measured in *A. eminens* and *A. polystachyus*. A: Flower lip, seen from above; B: Upper stamen; C: Lower Stamen; D: Base of flower, from above; E: Base of flower, from side; F: Ovary and style, unbent. Adapted from drawings by Kristina Gebhardt (Gebhardt 2004).

3 SPATIOTEMPORAL VARIATION IN VISITATION FREQUENCIES AND VISITOR ABUNDANCE OF TWO SYMPATRIC SPECIES OF *ACANTHUS* L.

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ABSTRACT

Acanthus eminens and *A. polystachyus* display a striking similarity in flowering phenology and flower morphology, and are visited and pollinated by the same group of animals, carpenter bees of the genus *Xylocopa*. Partitioning of pollinator faunas has been proposed as a mechanism of isolation between the sympatric species, as there is no indication of reproductive interference. By analyzing spatiotemporal patterns of visitor and pollinator abundance, taking into account environmental factors which influence pollinator visitation rates and abundance, this study aims to contribute to both the understanding of reproductive isolation of congeners and the effects of anthropogenic habitat change on plant-pollinator systems. We demonstrate a moderate to high level of overlap between pollinator faunas of *A. eminens* and *A. polystachyus*, with each species mainly being pollinated by a different species of *Xylocopa*. In both species, habitat availability for pollinators is likely to be the main determinant of pollinator species composition, and high levels of geitonogamous pollen transfer may provide a barrier for reproductive interference.

INTRODUCTION

Apart from habitat preference (Waser 1978b; Kephart 1983; Armbruster and Herzig 1984; Ackermann *et al.* 2008) and separation of flowering times (Baker 1961; Stiles 1977; Botes *et al.* 2008), partitioning of pollinators is one of the most effective barriers to reproductive interference between plant species (Levin 1971; Armbruster and Herzig 1984; Stone *et al.* 1998, for a review of mechanisms of floral isolation, see Grant 1994). As most plants are not pollinated exclusively by a single animal species (Waser *et al.* 1996; Bosch *et al.* 1997, see references in Richardson *et al.* 2000), many sympatric and co-flowering plants share some proportion of their pollinators, the consequences of which have been scrutinized by several studies (e.g. Schemske 1981; Campbell and Motten 1985; Herrera 1996; Gómez and Zamora 1999; Cozzolino and Scopece 2008).

Pollinator fauna similarity between any given species pair and its outcome is shaped by the traits of the animal and plant species involved and consequently depends on complex interactions of many factors. Optimal foraging strategy predicts that pollinators will maximize their resource gain while reducing the cost of foraging, e.g. flight and handling time, to a minimum (Heinrich and Raven 1972; Heinrich 1975). Consequently, flower morphology (Stout *et al.* 1998; Hegland and Totland 2005; Stang *et al.* 2006, 2009), floral rewards (Pleasants 1981; Makino and Sakai 2007) and plant population density (Inouye 1978; Sowig 1989; Karron *et al.* 1995; Kunin 1997; Bosch and Waser 2001; Feldman 2008) have been found to influence pollinator abundance and behaviour, which may also be influenced by pollinator density (Fontaine *et al.* 2008). In addition, variance in the pollinator fauna of a certain species is commonly caused by spatial and temporal variations of pollinator abundance (Herrera 1988; Horvitz and Schemske 1990; Ashman and Stanton 1991; Kwak and Jennersten 1991; Gómez and Zamora 1999; Fenster and Dudash 2001; Herrera 2005; Price *et al.* 2005, see Cane *et al.* 2005)

Several studies have described that co-flowering plants may benefit from increased reproductive success through facilitation of pollinator visitation (Laverty 1992; Johnson *et al.* 2003; Feldman *et al.* 2004; Ghazoul 2006, see also Toräng *et al.* 2006). However, neutral or negative effects of co-flowering and competition for pollinators have been described more often (e.g. Levin and Anderson 1970; Rathcke 1988; Armbruster and McGuire 1991; McGuire 1993; Brown *et al.* 2002; Bell *et al.* 2005; Matsumoto *et al.* 2011).

When pollinators are shared, pollen may be transferred between species (e.g. Campbell 1985; Waser and Fugate 1986; Bosch *et al.* 1997; Bell *et al.* 2005; Theiss *et al.* 2007). Recently, the importance of interspecific pollen transfer (IPT) as factor of reproductive interference has been questioned, as several studies failed to demonstrate any effect of heterospecific pollen deposition (Campbell and Motten 1985; Kohn and Waser 1985; Kwak and Jennersten 1986; Stout *et al.* 1998; Nielsen *et al.* 2008, but see Waser 1978b; Kanchan and Chandra 1980; Thomson *et al.* 1982; Waser and Fugate 1986). Morales and Traveset (2008) found the loss of pollen due to grooming of pollinators and during foraging flights to and visits on heterospecific flowers to be of greater importance than IPT when co-flowering mitigates reproductive success.

IPT may play a greater role when species are compatible (Brown and Mitchell 2001; Matsumoto *et al.* 2009), and lead to hybridization and subsequent introgression in congeneric species (Anderson 1948; Brown and Mitchell 2001; Ackermann *et al.* 2008). Studies on reproductive isolation have often focussed related species, deepening our understanding of both speciation and competition in plant species, and, more recently, of alien plant invasions (e.g. Anderson and Hubricht 1938; Sinclair 1968; Arnold *et al.* 1993; Hodges and Arnold 1994; Ramsey *et al.* 2003; Kay 2006; Botes *et al.* 2008; Matsumoto *et al.* 2009).

Negative effects of co-flowering strongly depend on relative plant density (Levin and Anderson 1970; Campbell and Motten 1985; Stout *et al.* 1998; Takakura *et al.* 2008). As pollinator foraging behaviour is subject to physiological and energetic constraints (Heinrich and Raven 1972; Heinrich 1975), spatial distance between flowers and populations is an important factor of floral and, consequently, reproductive isolation.

Ecosystem fragmentation and anthropogenic habitat change may have dramatic effects on species abundance and spatial barriers between species, changing patterns of floral competition and interactions between plants and pollinators. For example, habitat change has been described as an important reason for increased rates of hybridization and reproductive interference between plant species naturally growing in spatially close, but distinct habitats (Anderson 1948; Levin *et al.* 1996; Arnold 1997; Wolf *et al.* 2001). More recently, studies have demonstrated how habitat change and change of flowering context, e.g. through species invasion, influence reproductive species traits and may cause, modify or disrupt links between species (Lamont *et al.* 2003; Ashworth *et al.* 2004; Hersch and Roy 2007; Junker *et al.* 2010).

Anthropogenic habitat loss has also been identified as a driver of biodiversity loss, and cascading effects may lead to a dramatic decrease of biodiversity (see Fahrig 2003 and references therein). Though generalized pollination networks have been shown to be resilient to disturbance, individual species may differ strongly in their response to habitat fragmentation (Memmott *et al.* 2004; Kaiser-Bunbury *et al.* 2010). Pollinator mediated interactions between species are highly complex, and detailed knowledge of species and processes in threatened ecosystems is required to reliably predict consequences of shifts in flower abundance due to anthropogenic habitat change (Lázaro *et al.* 2009). However, studies from many of the planet's biodiversity hotspots are few, and limit our knowledge of global evolutionary and ecological processes, especially in pollination ecology (Vamosi *et al.* 2006).

In this study, we assess the flower visitor fauna overlap between two species of *Acanthus* in Kakamega Forest, Western Kenya. *Acanthus eminens* and *A. polystachyus* exhibit a striking synchrony in flowering phenology (Chapter 1), and highly similar flowers and floral rewards (Chapter 2). In a previous study, based on a limited set of observations on flower visitor abundance, they have been found to share 66% of their pollinators (see Chapter 2), which another study in a single, heterospecific population found to be carpenter bees of the genus *Xylocopa* (Dietzsch 2004). Therefore, reproductive interaction between the species seems likely, either increasing fruit set of both species by facilitation of pollination events, or decreasing fruit set of at least one of the species through competition for pollinators. However, neither a positive nor a negative effect of distance to and habitat availability of the congener on fruit set could be demonstrated in either species (Chapter 1).

In this study, we quantify the overlap between pollinator faunas of *A. eminens* and *A. polystachyus* through detailed observations in several populations of each species. Pollinator faunas likely differ between habitats, as movement of pollinators between two separated habitat patches has been shown to depend on the matrix surrounding them (Bartomeus and Winfree 2011; Lander *et al.* 2011), and foraging ranges of solitary bees may be small (Gathmann and Tscharntke 2002). We propose that pollinator faunas of *A. eminens* and *A. polystachyus* are more similar in spatially close populations, both 1) within and 2) between species. Also, we hypothesize that 3) partitioning of pollinators is an important factor of reproductive isolation between *A. eminens* and *A. polystachyus*, decreasing potential for reproductive interaction and competition for pollinators.

Materials and Methods

Study area

Our study was conducted in Kakamega Forest in Western Kenya (0"170N, 34"540E). Kakamega Forest is considered the easternmost remnant of the lowland Guineo-Congolian rain forest belt, and forest fragmentation has led to the formation of 5 fragments (130 to 1,400 ha) and a remnant main forest (8500 ha, Schaab *et al.* 2010), comprising a total of 12,000 ha of rainforest vegetation at an altitude between 1,500 to 1,700 m above sea level. It is managed by the Kenyan Wildlife Service (KWS) in the north and the Kenyan Forest Service (KFS, former Forest Department) in the south. Mean monthly temperatures range from 11°C to 29°C, with an average temperature of 22°C. Rainfall averages 2,000 mm per year, with two distinct rainy seasons between March and May ('long rainy season') and September and November ('short rainy season') and two distinct dry seasons between December to February ('dry season') and from June to August ('cold dry season'). The forest is under significant pressure due to illegal logging, firewood collection, grazing, and hunt for bushmeat, and large parts are highly disturbed (Schaab *et al.* 2010). The forest is surrounded by densely populated (336 to 746 inhabitants per km^2, Mitchell 2004), highly diverse and structured farmland with 0.2 to 0.7 ha of land per household (Greiner 1991; Kenyan Ministry of Agriculture 2006). 62% of all households generate their income from agriculture, and the district's poverty rate is about 52% (Dose 2007).

Plant species

Acanthus eminens Clarke is a shrub growing in clearings in primary and old secondary rainforest between 1,500 and 2,800 m above sea level in Kenya. It is also found on the Ugandan side of Mt Elgon, and in the Imatong Mts in Sudan, very likely spreading to north Uganda. It forms distinct populations of between 65 and 2574 plants in Kakamega Forest, as well as relatively continuous but less dense stands along riversides. The species grows up to five meters in height, carries spiny, robust leaves and flowers in decussate spikes up to to 50 flowers. Small individuals carry one, large individuals up to over 100 inflorescences. *A. eminens* shares shape and functionality of its zygomorphic flowers and of its fruit capsules with *Acanthus polystachyus* var. *polystachyus* Delile (referred to as *Acanthus polystachyus*). The latter grows as a common shrub in hedgerows, on the outer edges of forests and around grasslands inside forests between 1,600 and 1,800 m above sea level, and reaches its easternmost range in Kenya's Western Province. It is missing on the plains around lake Victoria. Flowers of *A. polystachyus* are slightly smaller than those of *A. eminens*, while plants are larger and may carry up to several hundred inflorescences. Both the violet-blue flowers of *A. eminens* and the magenta/pink flowers of *A. polystachyus* are visited mainly by carpenter bees, as well as, occasionally, by sunbirds (Bergsdorf 2006). Both offer nectar in a compartment at the base of the flower, containing the ovary, which is enclosed by a short tube at the base of the flower, partially sealed off to visitors by a ring of hair-like structures. The style protrudes several cm from the ovary. Anthers are clustered around style in the last third of its length, and release pollen only when forced apart by visitors. Though *Acanthus* species are known to hybridize readily in cultivation (McDade *et al.* 2005), and at least two of the other species of this genus have been suggested to be of hybrid origin (Furness 1996), to our knowledge there is no report of hybridization between *A. eminens* and *A. polystachyus*.

In this study, we analyzed data from 4 main forest and 5 forest fragment populations of *A. eminens*, and four main forest and three fragment populations of *A. polystachyus*. Two fragment populations were heterospecific stands with less than 10 m distance between individuals of both *Acanthus* species, and distances between other populations ranged between 220 and 4,000 m. Data was collected during the flowering period between November 2008 and February 2009.

Flower visitors

We observed flowers of both species during standardized observation units, simultaneously observing ten, in one case five, flowers on different inflorescences for 30 minutes, followed by ten minutes of insect catching on all flowers of the population. Observations were started on the hour between 9 a.m. and 2 p.m., which was established as the time of highest visitation rates by prior observation. Species accumulation curves indicate that not all floral visitors were observed in either species (see Appendix, Fig. 3.A). However, curve shapes confirm that we have likely observed all potential pollinators of our main focus plant *A. eminens*. For *A. polystachyus*, there may be more pollinator species than we observed.

Reference insect specimens were killed, pinned and stored. Unknown species were identified by Dr. M. Gikungu, National Museum of Kenya, Nairobi. Unidentifiable individuals, e.g. those observed but not caught, were grouped into morphospecies, including a group of completely 'unknown' individuals. Two species of *Xylocopa*, belonging to the sub-genus *Xylomellisa*, were not identifiable to species level. Hence, they are referred to as *X.* sp.1 and *X.* sp.2. Based on pollinator efficiency measurements on both species of *Acanthus* (Dietzsch 2004), *Xylocopa* bees were assumed to be capable of transferring pollen in both species, while all other, significantly smaller animals, were considered non-pollinators (see Discussion). We refer to the groups as pollinators (all *Xylocopa*), non-pollinating visitors (all non-*Xylocopa*), and visitors (all observed animals).

We calculated the abundance of each visitor species in each population by dividing the sum of individuals of a single species by the sum of all individuals regardless of species during all non-empty observation units in each population.

Relative abundance of each visitor species on either species of *Acanthus* was calculated by averaging these population relative abundances for each animal species for all populations of each shrub.

Abundance values were normalized by dividing mean relative abundance of each visitor species by the sum of means for each plant species. Thus, these values were calculated to represent, as a ratio ranging from zero to one, the likelihood of any visitor from the respective sample (population of a species or species) belonging to a certain (morpho-)species.

Flower visitation rates

For flower visitation rates, we calculated relative importance of visitor species and visitation rates based on the number of visits to observed flowers. Relative importance was calculated as demonstrated for flower visitors above, representing the likelihood of any given visitation event on the respective *Acanthus* species to be caused by a member of one of the observed animal groups.

Visitation rates for each animal species are presented as visits per hour and flower during all observation units in a population. Average visitation rates on each plant species are average values from all populations of the respective species.

Forest cover and Inter-Population distances

Forest cover and inter-population distances were kindly provided by the team of BIOTA E02 (Prof. Dr. G. Schaab, University of Applied Sciences, Karlsruhe), based on GPS measurements inside populations. Note that we re-calculated models including forest cover for all available scales (radius of 100, 500, 1,000 and 2,000 m around population centers) separately for both species, and used only the scale which provided most explanatory power.

Canopy cover and population size

Automatic, continuous measurements of microclimatic data were impossible due to the high intensity of human disturbance inside the forest. To reflect climatic conditions within the forest, we used two measures of canopy cover, which is linked to many abiotic factors, especially to humidity and exposure to sunlight. First, we recorded canopy cover during transect walks. In each population, we used 2 perpendicular transects of 100 m, subjectively measuring canopy cover from 0 (no cover) to 8 (very dense cover) every 10 m. In addition, we validated our measurements in a subset of populations using digital image analysis, using the Software GapLightAnalyzer (Institute of Ecosystem Studies, Simon Fraser University, Burnaby, Canada), corrected for the GPS location of Kakamega Forest. For each population, (10-)25 images were taken with a digital camera (Coolpix 5400, Nikon, Tokyo, Japan) equipped with a fisheye lens (FCE9 with UR-E10 adapter, focal length 5.8 mm, Nikon, Tokyo, Japan), mounted on a leveled tripod. All images were taken in the late afternoon to avoid overexposure, and north-aligned with a compass. Distance between individual images was >10 m, which reduced overlap between image area. We found our subjective measurements confirmed by comparison with canopy openness [%] as calculated by the software (Linear regression, $F_{1,7} = 27.06$, $r^2 = 0.795$, $p = 0.0013$). Analyses presented here are based on transect walks, as not all remote populations could be accessed in time for canopy imaging.

Population size was assessed during the same transect walks as canopy cover, counting all flowering and non-flowering plants of *A. eminens*. Population size was estimated by multiplying the mean number of flowering plants per square meter by the area covered by the population, which was calculated from GPS measurements.

Statistics

For data analysis and visualization, we used R (R Development Core Team 2011) Version 2.12.2 and newer. For tests of normality, we used package 'car' (Fox and Weisberg 2011). Most of the more detailed analyses were based on population means, which sufficed conditions for parametric analysis. Non-parametric statistics were used where required.

Similarities between visitor and pollinator faunas were calculated using the method described by Morisita (1959), modified by Horn (1966). For calculation, we excluded values for unknown species of *Xylocopa* and for unknown other visitor species. For presentation, similarities ranging from zero (no similarity) to one (identity) were converted into percentage values, which we consider to be more intuitive.

Sources of variation in visitation rates were partitioned using a permutational multivariate analysis of variance using distance matrices (ADONIS, command 'adonis', package 'vegan', Oksanen *et al.* 2011). The same package was used for calculations of trait dissimilarities.

For comparison of population visitor faunas, we correlated dissimilarities and spatial distance, using an ordinary PROCRUSTES analysis (Gower 1971; Jackson 1995). PROCRUSTES superimposes two matrices through translation, scaling and rotation, minimizing their shape differences. Statistical significance is calculated through repeated PROCRUSTES scaling, calculating a correlation-like coefficient from the sum of squares (ss) between matrices ($r = \sqrt{1 - ss}$). PROCRUSTES has been found to be equally or more powerful than the familiar Mantel test procedure, which is commonly used for this type of analysis (Peres-Neto and Jackson 2001). All our analyses used 10,000 permutations.

To assess which factors contribute to variance of visitation rates between populations of the species, we used hierarchical partitioning (HP, package 'hier.part', Walsh and Mac Nally 2008) to quantify the contribution of each measured variable to the variance of the observed data (for a detailed description of HP, and a comparison with other methods of factor selection, see Mac Nally 1996, 2000, 2002; Quinn and Keough 2002). HP partitions variance between predictors included into the model by calculating the independent contribution of each factor alone, and the relative contribution of each predictor to variance of the response variable across all models based on all potential combinations of the factors, weighed by the explanatory power of each model. Statistical significance is based on Z-Scores derived from randomization of the original data, with $Z \geq 1.65$ representing an upper 0.95 confidence limit equivalent to $p \leq 0.05$. All our HP analyses were based on r-square measures of goodness of fit, and 5,000 randomizations. Note that HP partitions the variance explained by the factors included in the model, highlighting the importance of each factor relative to the other factors included, but does not provide an absolute measure of factor importance.

We calculated standard linear regressions for models including the factors found to have significant contribution to data variance based on HP, in order to quantify the percentages of variance explained by each factor in a way compatible with standard r-squared calculations.

We present standard deviation as estimator of sample variability. When displaying ratios (e.g. the ratio of pollinated stigmas in a sample based on presence/absence), we present standard errors as a measure of uncertainty of the estimate of the mean, as standard deviation does not provide meaningful information about ratios of presence and absence. When plotting two measured variables against each other, we used standard errors in both if necessary for either one. Error bars are constrained to potential data range in plots, e.g. when found to indicate flower visitation rates less than zero.

RESULTS

In total, we observed 1,795 flowers of *A. eminens* in nine populations during a total of 180 observation units (90 hours of observation), recording 255 visitors in 790 interactions with the observed flowers. In *A. polystachyus*, we observed 1,340 flowers in seven populations during 134 observation units (67 hours of observation), recording 195 visitors in 642 interactions with the observed flowers. Flower visitation rates were found to be similar over the course of day in both *A. eminens* (Kruskal-Wallis rank sum test, $\chi^2 = 3.43$, df = 5, p = 0.635) and *A. polystachyus* ($\chi^2 = 3.98$, df = 5, p = 0.552), and over the timespan of observations between 12/10/2008 and 01/28/2009 (Kruskal-Wallis rank sum test, $\chi^2 = 35.00$, df = 35, p = 0.468).

Pollinator visits made up about 68.8% of all flower visits on *A. eminens* (Fig. 3.1, see Appendix Tab. 3.A and 3.B). Based on flower visitation, the principal pollinators of this species were *X.* sp.1 and *X.* sp.2, which contribute 55.0% and 20.6% of pollinator visitation events, respectively. In *A. polystachyus*, *X.* sp.2 was the most common pollinator, followed by *X.* sp.1 and *X. nigrita*, with 45.1%, 20.0% and 15.7% of pollinator visitation events being caused by theses species, respectively. In *A. polystachyus*, 54.7% of visitors were pollinators. On *A. eminens*, the non-pollinating visitor species were dominated by unidentified small insect species (21.7%), followed by *Lasioglossum* sp.1 (23.8%), honeybees (18.8%), and the stingless bee *M. bocandei* (15.7%). The latter species accounted for more than half of non-pollinating visitation events on *A. polystachyus* (53.4%), followed by *A. mellifera* (27.3%).

X. sp.1 was not only the most important pollinator species in *A. eminens* based on visitation rates, but also the most abundant pollinator, as 68.7% of pollinator individuals were found to belong to this species (Fig. 3.2, see Appendix Tab. 3.C). The species was also the most common and most frequent visitor when taking all

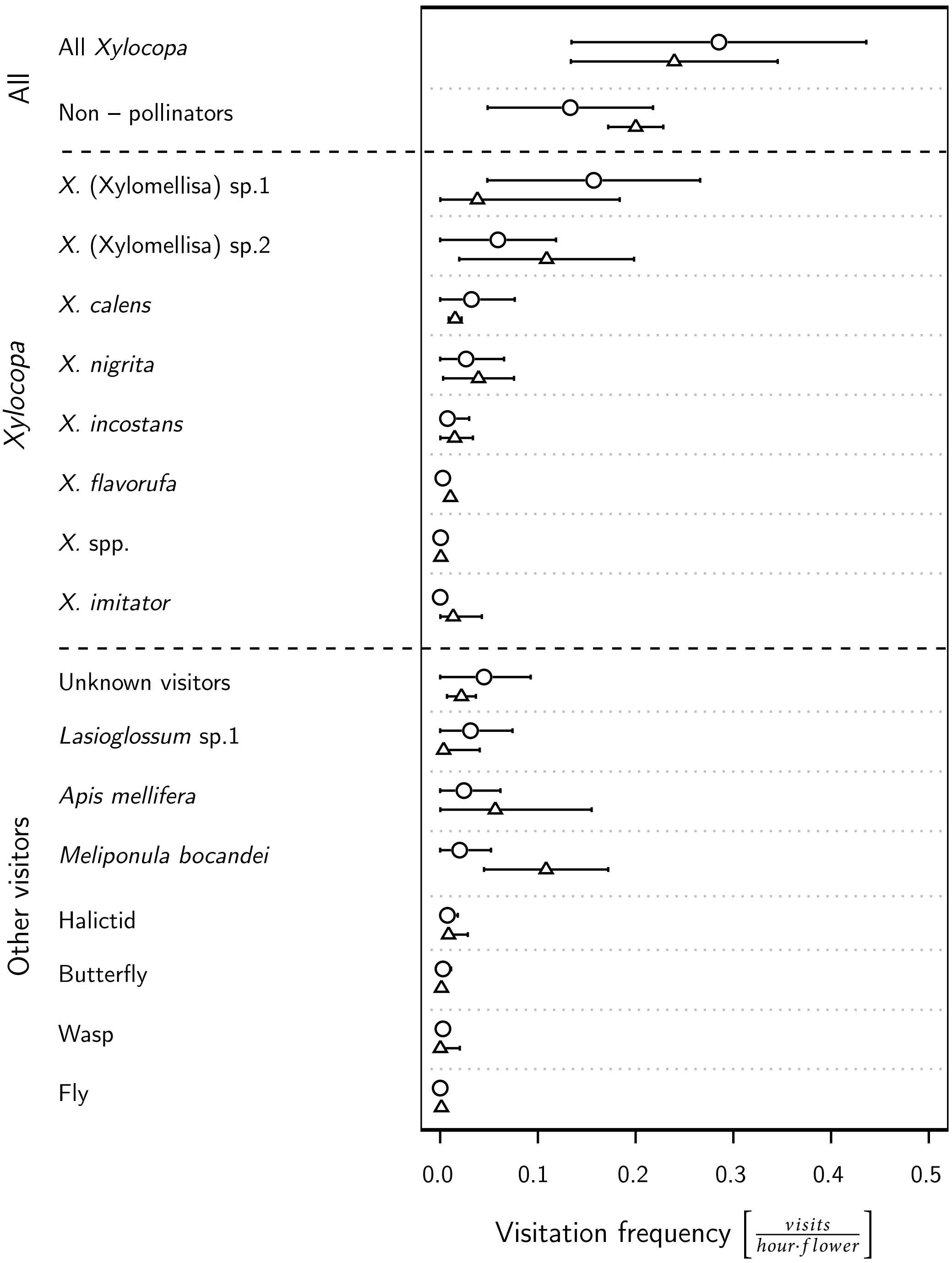

Figure 3.1: Rates of flower visitation on *A. eminens* (○, in n = 9 populations, 180 observation units) and *A. polystachyus* (△, n = 7 populations, 134 observation units), sorted from top to bottom by overall visitation, visitation of likely pollinators (*Xylocopa*) and visitation of likely non-pollinators. Values in groups sorted by decreasing importance for *A. eminens*. mean±sd of population mean values.

floral visitors into account, and makes up over one third (36.2%) of *A. eminens*' visitor fauna. In *A. polystachyus* the most frequent pollinator, *X.* sp.2, was also the most abundant, with 28.1% of pollinator individuals belonging to this species. It also made up one fifth (20.1%) of total flower visitors. However, the most important visitor was the non-pollinator *M. bocandei*, consituting 22.8% of *A. polystachus*' visitor fauna.

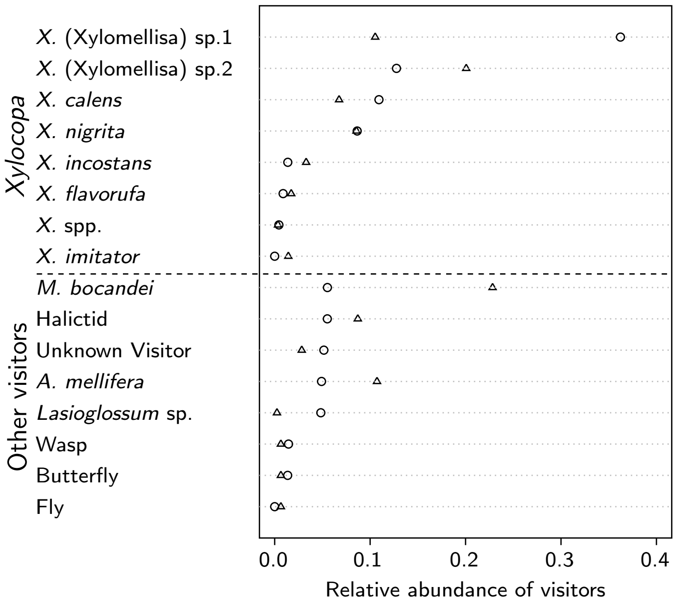

Figure 3.2: Mean relative abundance of individuals of pollinating and non-pollinating visitors (upper and lower section, respectively) in populations of *A. eminens* (○, in n = 9 populations, 180 observation units) and *A. polystachyus* (△, n = 7 populations, 134 observation units). Values in groups are sorted by decreasing importance for *A. eminens*. As values represent overall means of relative abundance in each population ranging between 0 and 1, no confidence interval is provided.

Similarity between floral visitor faunas was high, both when considering visitation by all visitors (61.4%) and pollinators (70.1%). Similarity was slightly higher based on visitor (64.7%) and pollinator abundance (74.9%). We conclude that the species share between two thirds and three quarters of their pollinators.

EFFECTS OF SPATIAL DISTANCE BETWEEN POPULATIONS

We tested whether spatial distance influences flower visitor faunas, based on dissimilarities of both visitation frequencies and abundance of individuals (Tab. 3.1, for full dissimilarity tables, see Appendix Tab. 3.D–3.G). Though we found no effect for all visitors or all pollinators in *A. eminens*, pollinator faunas of *A. polystachyus* were found to be more similar in spatially close populations when based on visitation frequencies. Based on visitor abundance, this correlation was marginally significant.

As we were interested whether *A. eminens* and *A. polystachyus* share more of their pollinators when growing closely together, we repeated these analyses for spatially close heterospecific population pairs. Correlation of faunistic and spatial dissimilarities did not

Table 3.1: PROCRUSTES test results for matrix correlation between visitor and pollinator fauna dissimilarities (upper and lower section, respectively) and spatial distance for *A. eminens* and *A. polystachyus*. Statistics based on 10,000 permutations of fauna dissimilarities. Stars indicate significance.

Visitation frequencies			
	Procrustes r	p-value	
All Visitors			
A. eminens	0.463	0.670	
A. polystachyus	0.610	0.250	
Pollinators			
A. eminens	0.519	0.288	
A. polystachyus	0.696	0.049	*
Visitor abundance			
	Mantel r	p-value	
All Visitors			
A. eminens	0.394	0.771	
A. polystachyus	0.522	0.400	
Pollinators			
A. eminens	0.362	0.542	
A. polystachyus	0.671	0.079	.

provide evidence for any link between the matrices (Procrustes analysis for visitation rates of visitors: $r = 0.510$, $p = 0.259$, for visitation rates of pollinators: $r = 0.521$, $p = 0.264$; for abundance of visitors: $r = 0.380$, $p = 0.640$, for abundance of pollinators: $r = 0.354$, $p = 0.576$).

Factors contributing to visitor and pollinator fauna dissimilarity

Population species identity, distance to the next heterospecific population, forest cover around the population center and flower density of the populations explained 53.0% of variance in dissimilarity values based on visitation rates, and 56.9% of variance based on visitor abundance (Tab. 3.2). In both analyses, species identity was the strongest predictor, with the other three factors displaying similar influence on the data.

Table 3.2: Factors influencing flower visitor faunas of *A. eminens* and *A. polystachyus*, based on flower visitation rates and visitor abundance. Variance was partitioned by species, distance to the next heterospecific population, forest cover in a radius of 1,000 m around population centers. Model statistics based on 10,000 randomizations in a non-parametric analysis of variance ('adonis'). Randomizations were restricted within species. Interaction terms were not significant, and consequently removed from the model.

Visitor frequency				
Predictor	r^2	F-Stat. $F_{1,15}$	p-value	
Species	0.189	4.419	<0.001	***
Dist. het.	0.130	3.033	0.005	**
Forest cover	0.100	2.329	0.040	*
Flower density	0.111	2.590	0.018	*
Visitor abundance				
Predictor	r^2	F-Stat. $F_{1,15}$	p-value	
Species	0.205	5.245	<0.001	***
Dist. het.	0.128	3.281	0.004	**
Forest cover	0.100	2.546	0.009	**
Flower density	0.136	3.461	0.003	**
Xylocopa frequency				
Predictor	r^2	F-Stat. $F_{1,15}$	p-value	
Species	0.202	4.782	0.004	***
Dist. het.	0.129	3.052	0.019	*
Forest cover	0.093	2.193	0.082	.
Flower density	0.110	2.600	0.044	*
Xylocopa abundance				
Predictor	r^2	F-Stat. $F_{1,15}$	p-value	
Species	0.271	7.585	0.003	**
Dist. het.	0.147	4.126	0.002	**
Forest cover	0.062	1.743	0.152	
Flower density	0.127	3.547	0.006	**

Factors influencing visitation rates and abundances of most important pollinators

For HP of variance in pollinator visitation rates and abundance, we included spatial distance, forest cover in 1,000 m radius around population centers, flower density and, for *A. eminens*, canopy cover and population size as potential predictors. HP revealed canopy cover to be the most influential factor affecting visitation rates of *X.* sp.1 in populations of *A. eminens* (For full HP tables, see Appendix Tab. 3.H). The link is shown to be to be positive in linear regression ($r^2 = 0.667$, $F_{1,7} = 14.03$, $p = 0.007$). The surrounding forest cover was the main factor influencing visitation rates of *X.* sp.2 in populations of *A. eminens*, with populations in open areas being visited significantly more often by the species compared with those in densely forested areas ($r^2 = 0.619$, $F_{1,7} = 11.36$, $p = 0.012$). In *A. polystachyus*, *X.* sp.1 was strongly influenced by flower density inside the population, with decreasing visitation rates in more dense populations ($r^2 = 0.638$, $F_{1,5} = 8.79$, $p = 0.031$). Visitation rates of *X.* sp.2 on *A. polystachyus* are shown to be influenced by the distance to the next heterospecific population in HP. However, we could not corroborate this finding in a linear model ($r^2 = 0.394$, $F_{1,5} = 3.25$, $p = 0.131$).

HP found only one significant predictor of abundance of *X.* sp.1 in *A. eminens* (see Appendix Tab. 3.I), which was positively linked to the distance to the next heterospecific population ($r^2 = 0.854$, $F_{1,7} = 41.09$, $p < 0.001$). No predictor was significantly linked to visitation rates of *X.* sp.2 in *A. eminens*, and no parameter was having any influence on the abundance of the main pollinators of *A. polystachyus*.

DISCUSSION

VISITOR AND POLLINATOR FAUNA OF *ACANTHUS*

Carpenter bees are the principal pollinators of *Acanthus eminens* and *A. polystachyus*, and flowers of either shrub are visited by the same six species of carpenter bees, with frequencies varying between plant species and populations. Another species of *Xylocopa*, *X. imitator*, infrequently visits flowers of *A. polystachyus*. Two distinct but as of yet unnamed species of carpenter bees, *X.* sp.1 and *X.* sp.2, are regular visitors on flowers of both shrubs, with *X.* sp.1 being the most frequent and the most abundant *Xylocopa* visiting *A. eminens*, and *X.* sp.2 being the most frequent and most abundant *Xylocopa* visiting *A. polystachyus*. Together, these species account for over half of all flower visitation events in *A. eminens*, and about a third of flower visits in *A. polystachyus*.

All *Xylocopa* brush reproductive structures of flowers of *Acanthus* with their thorax during foraging, and are classified as pollinators in accordance with a study on pollinator efficiency by Dietzsch (2004). Based on this assessment, the congeners share nearly three quarters of their pollinators.

We cannot rule out completely that specific physiological or ethological traits influence pollinator effectiveness, and the observed carpenter bees may differ in their pollination efficiency (Stout *et al.* 1998; Sahli and Conner 2007; Theiss *et al.* 2007). It has been demonstrated that the divergent ability of floral visitors to transfer pollen may reduce their importance as pollinators, which may be overestimated when based on visitation only (Alarcón 2010). However, as the pollinators of *Acanthus* belong to the same genus and are of similar size, we consider putative differences in effectiveness to be small (but see e.g. Thøstesen and Olesen 1996). Due to the pronounced differences in species abundance, it seems likely that the most abundant pollinators of *Acanthus* also are the most important (Vázquez *et al.* 2005; Sahli and Conner 2007). All other visitors are much smaller in body size, and collect pollen from anthers without touching stigmas during our observations.

Sunbirds, which we observed to visit flowers of both species in some populations (compare Chapter 2), are likely capable of pollinating either species. However, they were not observed during our standardized observation units, and consequently their ecological role as pollinators of *Acanthus*, if any, is neglectable. Still, birds may travel larger distances than bees, and could be an important vector for long-distance pollen transfer even when this is a rare event (Stiles 1978).

On average, *A. eminens* and *A. polystachyus* share about two thirds of flower visitors. Considering the biodiversity-rich surroundings, the number of species visiting flowers of *Acanthus* is low. In comparison, Hagen and Kraemer (2010) found 40 and 59 visitor species on flowers of *Justicia flava* (Acanthaceae) in the forest understorey and at the forest edge, respectively. Compared with *A. eminens*, the visitor fauna of *A. polystachyus* shows a higher ratio of non-pollinators, which may be due to greater bee species diversity and abundance in the structurally rich and comparatively dry farmland surrounding its habitat (Hagen and Kraemer 2010). Greater landscape diversity may facilitate small-scale structuring in comparison to the relatively homogenous forest habitat, and provide an explanation for the link found between forest cover of the surrounding landscape to variance in visitor dissimilarity (Steffan-Dewenter and Tscharntke 2001; Steffan-Dewenter *et al.* 2002).

FACTORS INFLUENCING POLLINATOR ABUNDANCE

The two principal pollinators of *A. eminens* and *A. polystachyus* differ in abundance between flowers of the congeners and between habitats, which is likely caused by differences in habitat-specific abundance. For example, we find *X.* sp.1 to be more frequent and abundant in forest habitats and populations with dense canopy cover. Hence, it is less common at forest edges, where *A. polystachyus* displays its highest flower density. This constellation of fewer bee individuals and increased density of flowers provides an explanation for the decrease in visitation frequency of *X.* sp.1 found in dense populations of *A. polystachyus* (compare Grindeland *et al.* 2005). *X.* sp.2 shows higher visitation rates in more open populations of *A. eminens*. This and its greater abundance on flowers of *A. polystachyus* may be explained by a preference of the species for more open habitats. However, no other population trait can be linked to visitation frequency or abundance of *X.* sp.2.

In accordance with our first hypothesis, spatial distance and pollinator fauna similarity in *A. polystachyus* are linked, which may also be attributed to the differences in species abundance between habitats as described above. However, as all observed populations were in close vicinity of the forest, additional data on floral visitation of *A. polystachyus* in farmland-dominated countrysides may provide further insights as to which *Xylocopa* are dependent on extensive forest habitats, and which are ubiquitous. In *A. eminens*, pollinator fauna composition of populations is not linked with spatial distance, corroborating that forest areas may provide a largely homogenous habitat for pollinator species. Hence, we reject the first hypothesis for *A. eminens*.

Kunin and Iwasa (1996) have shown that pollinators foraging on highly abundant flowers will specialize on the most adequate source of food, which may provide an effective barrier to interspecific pollen transfer. *A. eminens* and *A. polystachyus* display highly similar flower morphologies, but offer different amounts of floral rewards (Chapter 2), which may influence foraging behaviour (Pleasants 1981, see Stout *et al.* 1998). Flowers of *A. eminens* offer more sugar per flower, and consequently a single flower of *A. eminens* should attract more visitors than a single flower of *A. polystachyus* (Chittka and Schurkens 2001). However, the lower sugar content is more than compensated by the abundance of flowers of *A. polystachyus* (Chapter 2). Hence, its flowers may be more attractive for pollinators due to higher resource density.

Floral display size has been found to influence pollinator abundance, and larger displays may increase, decrease, or have a neutral effect on flower visitation rates (Robertson and MacNair 1995; Grindeland *et al.* 2005; Kudo and Harder 2005). Kunin (1997) demonstrated that flower density may play a greater role for pollinator behaviour and seed set than population size. We do not find a significant increase or decrease in pollinator visitation rates linked with population size or population density, and this neutral effect indicates that increased numbers of pollinator individuals compensate the higher number of flowers in denser and larger populations (Feldman *et al.* 2004; Grindeland *et al.* 2005).

Partitioning of pollinators

We do not find any evidence for a link between distance between heterospecific populations and visitor and pollinator similarity, as close heterospecific population pairs are as similar in their pollinator faunas as more distant pairs. In addition, the adaption of both species to the same genus of pollinators, the species of which are similar in size and likely of similar pollinator efficiency, limits the potential for partitioning of pollinators between the species. Consequently, we reject both our second and third hypothesis, concluding that spatial distance does not contribute to floral isolation through partitioning of pollinators. Hence, pollinator partitioning does not provide a clear barrier to interspecific pollen flow between *A. eminens* and *A. polystachyus*. However, though the congeners share about three quarters of their pollinators, pollinator partitioning may contribute to reproductive isolation as one of several other, cumulative mechanisms acting as barrier to interspecific pollen flow (compare Macior 1977; Kay 2006; Marques *et al.* 2007).

The similarity of pollinator faunas found in *A. eminens* and *A. polystachyus* may be caused by low overall resource availability for pollinators. Dry seasons may be low in flowers (Murali and Sukumar 1994), and individuals may forage on any available flowers especially when they are as similar as those found in our system (Chapter 2).

Specific pollinator visitation rates and their relative importance are probably subject to variations both within and between flowering seasons of *Acanthus* (see e.g. Herrera 1988; Fleming *et al.* 2001; Alarcón *et al.* 2008). Even in more specialized plant-pollinator systems, relative abundance of pollinators may be subject to large variations (Fenster and Dudash 2001). However, even if partitioning of pollinators is an important factor of reproductive isolation during some flowering periods of *Acanthus*, directional selection favoring floral adaption of either species to a subset of *Xylocopa* species is highly unlikely when there is strong variation in seasonal importance of pollinators (Schemske and Horvitz 1989; Pettersson 1991).

On the other hand, the amount of pollinator sharing warrants an analysis whether competition for pollinators influences reproduction of the congeners (Waser 1978a).

Interspecific pollen transfer

In a previous study on a heterospecific population of *A. eminens* and *A. polystachyus*, *Xylocopa* bees have been found visiting both flower types indiscriminately (Dietzsch 2004). In the same study, fruit set resulting from congeneric mixed pollen treatments was reduced, indicating detrimental effects of heterospecific pollen transfer on reproductive success. These two findings, as well as the observations on pollinator abundance presented here, led to the assumption that fruit set is mitigated by IPT in mixed populations of *A. eminens* and *A. polystachyus*. But *A. eminens* has been found to display its highest fruit set in spacially close and heterospecific populations during flowering season 2007/2008, and no effect of habitat availability or spatial distance to the congener on fruit set of either species could be demonstrated (Chapter 1).

Considering that the species share their pollinators, we propose either pollen transfer between species itself or its effects to be overestimated, superimposed by other factors, or both.

Pollen transfer between species may be limited because individual pollinators do not forage in spatially distinct populations (Austerlitz *et al.* 2004), and solitary bee foraging ranges have been found to be generally small in a temperate system (Gathmann and Tscharntke 2002). However, this does not provide an explanation for the lack of reproductive interference in heterospecific populations of *Acanthus*.

In these populations, we observed individuals to indiscriminately switch between species (in accordance with Dietzsch 2004). Generally, individuals often forage on spatially close inflorescences, and were observed to commonly visit several, and often all, open flowers of an inflorescence. Hence, pollen transfer between flowers of the same or spatially close individuals seems very likely, as has been demonstrated in similar cases (Snow *et al.* 1996; Rademaker *et al.* 1997; Karron *et al.* 2003). Consequently, only the very first flower after a switch between species may receive substantial proportions and amounts of heterospecific pollen, and most of it may be lost during this visit (Flanagan *et al.* 2009, in contrast to the findings of Kohn and Waser 1985), reducing pollen flow between congeners.

Therefore, we believe that it is very likely that most pollen grains reaching stigmas are conspecific, though pollen grains of the congeners are indiscriminable using methods within the limits of this study (Furness 1996). Hand-pollination with mixtures of pollen from both species have been found to reduce seed set in either species, and almost no fruits were set in flowers pollinated exclusively with heterospecific pollen (Dietzsch 2004). However, it seems unlikely that the ratio between *A. eminens* and *A. polystachyus*-pollen on stigmas is a strong factor limiting reproduction, as these effects should cause a decrease of reproduction in heterospecific populations, which could not be demonstrated in our study area (Chapter 1). On the other hand, we cannot rule out that pollen transfer may have strong effects on the development of seeds and their ability to germinate.

There are indications for other, strong limitations of fruit set which may mask the effects of IPT. Fruit set is generally low, with only about one third of flowers developing into fruits in *Acanthus*. A previous study has demonstrated climatic constraints on reproduction of either species, and found stigmatic pollen loads to be low in *Acanthus*, which may indicate potential pollen limitation (Chapter 1). Even though carpenter bees have been found to be the most abundant pollinators, it is not clear how much pollen they actually transfer. Large proportions of pollen may be lost either in flight (Campbell and Motten 1985; Campbell 1985; Inouye *et al.* 1994; Rademaker *et al.* 1997; Johnson *et al.* 2005), during interactions with other flowers (Murcia and Feinsinger 1996), or through grooming of bees (Harder and Wilson 1998; Johnson *et al.* 2005; Flanagan *et al.* 2009). In a recent study, loss of conspecific pollen during foraging has been found to be more detrimental for reproduction than heterospecific pollen deposition on stigmas (Morales and Traveset 2008). But in the case of carpenter bees on *Acanthus*, commonly foraging on several flowers within the same inflorescence and on numerous flowers within a radius of a few meters, pollen loss during flight may be low. Based on the high purity of pollen loads (N. Hasenbein, unpublished), *Xylocopa* bees seem to forage mainly on *Acanthus* flowers, and consequently, loss of pollen during visits on heterospecific flowers seems unlikely.

However, reproduction only is pollen-limited when pollen quantity and quality are the strongest constraints on fruit and seed set. Though this seems highly likely considering the low ratio of pollinated stigmas described in Chapter 1, only experimental manipulation of stigmatic pollen loads and observation of the resulting fruit and seed set will reveal whether pollen limitation is prevalent in *Acanthus*.

In addition, the high probability of short-ranged pollen transfer which we have described to limit pollen transfer between species also renders geitonogamous pollination highly likely. Most of the pollen of a single flower may be deposited on flowers of the same plant when the number of open flowers of an individual is high (Hessing 1988; Karron *et al.* 2003), which commonly is the case in *Acanthus*. This geitonogamous selfing may limit male reproductive success, as pollen is not available for outcrossing (Jong *et al.* 1993; Harder and Barrett 1995). In self-compatible species, however, geitonomy may be considered as a mechanism of reproductive assurance. It may also be an adaption of the congeners to rapidly spread into available habitats, which in our case are only temporary or likely subject to constant change.

Though flowers of *Acanthus* are protandric, the numerous flowers in asynchronous sexual phases on a single plant are not likely to reduce geitonogamous reproduction in large plants of *Acanthus* (Klinkhamer and Jong 1993). However, protandry may well play a role in very small and ‘founder’ populations. It may facilitate outcrossing in initial populations of *A. eminens*, and selfing and vegetative reproduction may help the plants to spread rapidly within a few reproductive cycles.

Considering the high likelihood of geitonogamous pollen transfer, stigmatic pollen loads have been found to be surprisingly low in both species. Geitonogamy increases the risk of inbreeding. Though no effect of spatial distance or habitat availability of each species on reproduction of the congener could be demonstrated

(Chapter 1), Bell *et al.* (2005) have demonstrated decreased outcrossing rates for plants competing for pollinators. As *Acanthus* plants within a population are likely to be closely related, and either species is capable of selfing (Dietzsch 2004) and vegetative reproduction, additional studies are needed to assess whether low genetic diversity affects reproduction of *Acanthus* in Kakamega Forest. A detailed study on the effects of pollinator visitation on pollination success in terms of stigmatic pollen loads, as well as on seed set and seed viability is needed to gain further insight into the interlocking pollination processes of *A. eminens* and *A. polystachyus*.

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APPENDIX

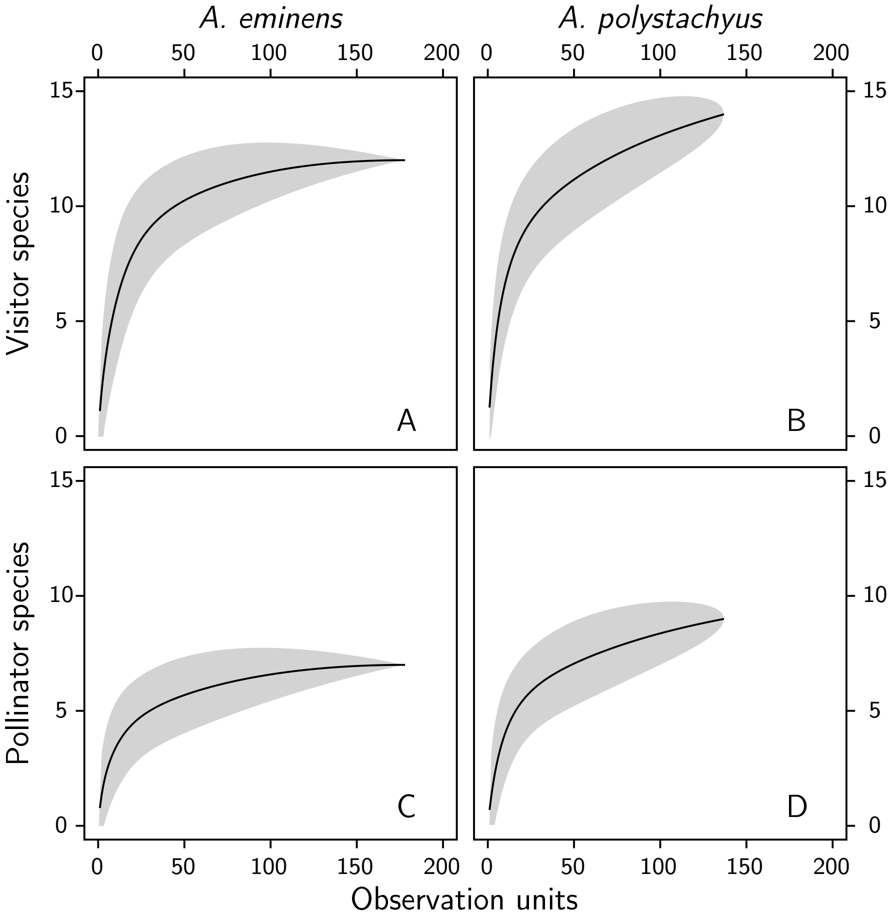

Figure 3.A: Species accumulation curves for flower observations of A: *A. eminens* and B: *A. polystachyus*, split for data on all floral visitors (A+B) and pollinators (C+D, *Xylocopa* bees and Sunbirds). Shaded area represents estimate of standard deviation. Graphs and values calculated using method 'exact' as specified in function 'specaccum', package 'vegan' (Oksanen *et al.* 2011), in R (R Development Core Team 2011).

Table 3.A: Flower visitation rates of flowers of *A. eminens* [$\frac{visits}{flower \cdot h}$]. n-values reflect observation units per population. mean±sd.

A. eminens	Buzambuli	Colobus	Ikuywa	Kisere		Rondo	Salazar		Yala
				A	B		1	2	
	n = 23	n = 22	n = 26	n = 15	n = 15	n = 15	n = 16	n = 25	n = 23
A. mellifera	0.00±0.02	0.01±0.04	0.02±0.07	0	0.05±0.14	0.12±0.18	0	0.02±0.07	0
Lasioglossum sp.1	0.07±0.17	0	0	0	0.12±0.23	0.05±0.16	0	0.03±0.11	0
M. bocandei	0	0	0.02±0.09	0	0	0	0	0.11±0.16	0.05±0.11
Halictid	0	0.02±0.07	0	0	0.06±0.12	0.09±0.25	0	0	0.01±0.06
Butterfly	0.01±0.04	0	0	0	0	0	0.01±0.05	0	0.00±0.02
Unknown	0	0.04±0.10	0	0	0.07±0.17	0.09±0.12	0	0.02±0.10	0.03±0.09
Wasp	0	0	0	0	0	0	0.03±0.07	0	0
Fly	0	0	0	0	0	0	0	0	0
X. calens	0.01±0.03	0	0.05±0.22	0.05±0.13	0.14±0.17	0.04±0.07	0	0	0
X. imitator	0	0	0	0	0	0	0	0	0
X. incostans	0.00±0.02	0.03±0.13	0.02±0.09	0	0.02±0.08	0	0	0	0
X. nigrita	0.05±0.19	0	0.02±0.07	0.14±0.36	0	0.01±0.05	0	0.02±0.07	0.00±0.02
X. Xylomellisa sp.1	0.13±0.21	0.25±0.27	0.10±0.20	0.04±0.12	0.28±0.20	0.06±0.15	0.15±0.19	0.21±0.27	0.19±0.35
X. Xylomellisa sp.2	0.01±0.03	0.12±0.20	0.06±0.16	0.11±0.30	0.16±0.28	0.03±0.10	0.02±0.08	0	0.02±0.07
X. flavorufa	0	0	0.02±0.07	0	0	0	0	0	0
X. sp.	0	0	0.00±0.02	0	0	0	0	0	0
Non-pollinators	0.09±0.17	0.06±0.14	0.04±0.11	0	0.29±0.22	0.35±0.28	0.04±0.08	0.18±0.24	0.10±0.18
Total *Xylocopa*	0.20±0.26	0.40±0.41	0.27±0.30	0.35±0.59	0.59±0.39	0.14±0.20	0.17±0.21	0.23±0.28	0.21±0.34

Table 3.B: Flower visitation rates of flowers of *A. polystachyus* [$\frac{visits}{flower \cdot h}$]. n-values reflect observation units per population. mean±sd.

A. polystachyus	Buzambuli	Colobus	Ikuywa	Kisere	Isecheno	Salazar	Yala
	n = 33	n = 14	n = 9	n = 12	n = 13	n = 30	n = 23
A. mellifera	0.09±0.21	0.17±0.24	0	0	0.02±0.08	0.02±0.07	0.09±0.24
Lasioglossum sp.1	0.01±0.03	0	0	0	0	0	0.02±0.06
M. bocandei	0.24±0.31	0.06±0.16	0	0.04±0.10	0.24±0.28	0.05±0.11	0.14±0.29
Halictid	0	0	0.03±0.08	0.05±0.12	0.03±0.05	0	0.01±0.04
Butterfly	0	0	0	0	0.01±0.03	0	0
Unknown	0.01±0.05	0	0	0	0	0	0.10±0.22
Wasp	0	0	0	0	0.01±0.03	0	0
Fly	0	0	0	0	0.01±0.03	0	0
X. calens	0.04±0.10	0.01±0.05	0.03±0.06	0	0.02±0.06	0.01±0.04	0
X. imitator	0	0.08±0.21	0	0	0	0	0
X. incostans	0.04±0.13	0	0	0	0	0.02±0.05	0.04±0.14
X. nigrita	0.07±0.25	0.01±0.05	0	0.02±0.06	0.02±0.08	0.10±0.18	0.04±0.13
X. Xylomellisa sp.1	0.04±0.13	0.01±0.05	0.08±0.29	0.08±0.21	0.06±0.16	0.03±0.10	0.04±0.10
X. Xylomellisa sp.2	0.14±0.27	0.11±0.16	0.08±0.16	0.28±0.36	0.02±0.06	0.16±0.27	0.01±0.06
X. flavorufa	0	0	0	0	0	0	0.05±0.16
X. sp.	0	0	0	0	0	0.00±0.02	0
Non-pollinators	0.34±0.36	0.24±0.26	0.03±0.08	0.09±0.14	0.32±0.30	0.07±0.12	0.36±0.54
Total *Xylocopa*	0.32±0.40	0.23±0.20	0.19±0.30	0.38±0.50	0.12±0.21	0.32±0.43	0.20±0.23

Table 3.C: Relative abundance of floral visitors in populations of *A. eminens* and *A. polystachyus*.

A. eminens	Buzambuli	Colobus	Ikuywa	Kisere		Rondo	Salazar		Yala
				A	B		1	2	
A. mellifera	0.04	0.06	0.09	0.00	0.05	0.18	0.00	0.06	0.00
Butterfly	0.04	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.03
Fly	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Halictid	0.00	0.13	0.00	0.00	0.08	0.29	0.00	0.00	0.00
Lasioglossum	0.14	0.00	0.00	0.00	0.28	0.03	0.00	0.03	0.05
M. bocandei	0.00	0.00	0.09	0.00	0.00	0.00	0.00	0.32	0.14
Unknown	0.00	0.09	0.00	0.00	0.03	0.18	0.00	0.10	0.11
Wasp	0.00	0.00	0.00	0.00	0.00	0.00	0.14	0.00	0.00
X. calens	0.07	0.00	0.17	0.50	0.20	0.13	0.00	0.00	0.00
X. flavorufa	0.00	0.00	0.09	0.00	0.00	0.00	0.00	0.00	0.00
X. imitator	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
X. incostans	0.04	0.03	0.04	0.00	0.03	0.00	0.00	0.00	0.00
X. nigrita	0.07	0.00	0.13	0.50	0.00	0.03	0.00	0.10	0.03
X. spp.	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00
X. Xylomellisa sp.1	0.54	0.44	0.26	0.29	0.25	0.11	0.71	0.39	0.59
X. Xylomellisa sp.2	0.07	0.25	0.09	0.57	0.10	0.05	0.07	0.00	0.05

A. polystachyus	Buzambuli	Colobus	Ikuywa	Kisere	Isecheno	Salazar	Yala
A. mellifera	0.15	0.30	0.00	0.00	0.05	0.07	0.18
Butterfly	0.00	0.00	0.00	0.00	0.05	0.00	0.00
Fly	0.00	0.00	0.00	0.00	0.05	0.00	0.00
Halictid	0.00	0.00	0.14	0.30	0.14	0.00	0.03
Lasioglossum	0.02	0.00	0.00	0.00	0.00	0.00	0.00
M. bocandei	0.42	0.15	0.00	0.20	0.45	0.20	0.18
Unknown	0.02	0.00	0.00	0.00	0.00	0.00	0.18
Wasp	0.00	0.00	0.00	0.00	0.05	0.00	0.00
X. calens	0.07	0.05	0.29	0.00	0.05	0.02	0.00
X. flavorufa	0.00	0.00	0.00	0.00	0.00	0.00	0.12
X. imitator	0.00	0.10	0.00	0.00	0.00	0.00	0.00
X. incostans	0.07	0.00	0.00	0.00	0.00	0.07	0.09
X. nigrita	0.10	0.05	0.00	0.10	0.05	0.24	0.06
X. spp.	0.00	0.00	0.00	0.00	0.00	0.02	0.00
X. Xylomellisa sp.1	0.03	0.10	0.14	0.20	0.09	0.05	0.12
X. Xylomellisa sp.2	0.13	0.25	0.43	0.20	0.05	0.32	0.03

Table 3.D: (Morisita-Horn) Dissimilarities between flower visitor faunas of populations of *A. eminens* and *A. polystachyus*, ranging from 0 (identity) to 1 (complete dissimilarity). Based on visitation frequencies of all morphospecies, excluding unidentified *Xylocopa* and non-pollinators which could not be assigned to any systematic group.

		A. eminens									*A. polystachyus*					
		Buzambuli	Colobus	Ikuywa	Kisere		Rondo	Salazar		Yala	Buzambuli	Colobus	Ikuywa	Isecheno	Kisere	Salazar
					A	B		1	2							
A. eminens																
Colobus		0.589														
Ikuywa		0.528	0.506													
Kisere	A	0.661	0.598	0.480												
	B	0.583	0.323	0.563	0.646											
Rondo		0.564	0.710	0.551	0.684	0.529										
Salazar	1	0.401	0.471	0.553	0.778	0.671	0.727									
	2	0.438	0.473	0.556	0.820	0.571	0.678	0.499								
Yala		0.482	0.377	0.510	0.782	0.595	0.699	0.294	0.276							
A. polystachyus																
Buzambuli		0.752	0.639	0.563	0.481	0.614	0.609	0.871	0.627	0.755						
Colobus		0.866	0.709	0.628	0.632	0.711	0.569	0.901	0.736	0.753	0.458					
Ikuywa		0.607	0.437	0.368	0.368	0.569	0.517	0.527	0.730	0.536	0.670	0.675				
Isecheno		0.682	0.758	0.588	0.588	0.766	0.622	0.710	0.483	0.578	0.354	0.675	0.625			
Kisere		0.712	0.501	0.528	0.528	0.549	0.643	0.697	0.670	0.563	0.587	0.619	0.422	0.630		
Salazar		0.704	0.560	0.504	0.504	0.619	0.759	0.845	0.696	0.701	0.362	0.505	0.614	0.658	0.429	
Yala		0.673	0.771	0.601	0.601	0.763	0.569	0.829	0.498	0.671	0.333	0.572	0.809	0.426	0.732	0.594

Table 3.E: (Morisita-Horn) Dissimilarities between pollinator faunas of populations of *A. eminens* and *A. polystachyus*, ranging from 0 (identity) to 1 (complete dissimilarity). Based on visitation frequencies, including only data for individuals which could be identified to species level.

		A. eminens Buzambuli	Colobus	Ikuywa	Kisere		Rondo	Salazar		Yala	*A. polystachyus* Buzambuli	Colobus	Ikuywa	Isecheno	Kisere	Salazar
					A	B		1	2							
A. eminens																
Colobus		0.525														
Ikuywa		0.416	0.483													
Kisere	A	0.607	0.583	0.446												
	B	0.617	0.207	0.470	0.561											
Rondo		0.450	0.655	0.303	0.514	0.648										
Salazar	1	0.293	0.436	0.501	0.773	0.571	0.502									
	2	0.285	0.346	0.541	0.770	0.496	0.590	0.295								
Yala		0.307	0.321	0.491	0.756	0.480	0.489	0.173	0.137							
A. polystachyus																
Buzambuli		0.594	0.486	0.425	0.226	0.502	0.512	0.786	0.782	0.767						
Colobus		0.786	0.616	0.586	0.481	0.670	0.623	0.844	0.876	0.818	0.454					
Ikuywa		0.486	0.441	0.265	0.442	0.512	0.284	0.470	0.607	0.482	0.436	0.467				
Isecheno		0.383	0.713	0.440	0.598	0.745	0.235	0.479	0.534	0.529	0.601	0.676	0.427			
Kisere		0.622	0.471	0.502	0.521	0.505	0.590	0.641	0.671	0.629	0.456	0.550	0.412	0.630		
Salazar		0.633	0.528	0.554	0.243	0.541	0.672	0.821	0.814	0.800	0.173	0.475	0.538	0.677	0.419	
Yala		0.495	0.721	0.516	0.636	0.809	0.595	0.710	0.684	0.703	0.488	0.804	0.708	0.512	0.743	0.595

Table 3.F: Morisita-Horn dissimilarities between flower visitor faunas of populations of *A. eminens* and *A. polystachyus*, ranging from 0 (identity) to 1 (complete dissimilarity). Based on visitor abundance, including only data for individuals which could be identified to species level.

		A. eminens									*A. polystachyus*					
		Buzambuli	Colobus	Ikuywa	Kisere		Rondo	Salazar		Yala	Buzambuli	Colobus	Ikuywa	Isecheno	Kisere	Salazar
					A	B		1	2							
A. eminens																
Colobus		0.146														
Ikuywa		0.261	0.335													
Kisere	A	0.480	0.380	0.295												
	B	0.241	0.382	0.335	0.495											
Rondo		0.637	0.483	0.507	0.659	0.422										
Salazar	1	0.100	0.167	0.426	0.617	0.491	0.748									
	2	0.253	0.361	0.305	0.644	0.539	0.726	0.296								
Yala		0.092	0.144	0.356	0.596	0.468	0.680	0.051	0.148							
A. polystachyus																
Buzambuli		0.823	0.769	0.488	0.629	0.774	0.734	0.913	0.341	0.727						
Colobus		0.661	0.484	0.408	0.442	0.662	0.500	0.759	0.534	0.686	0.299					
Ikuywa		0.597	0.354	0.444	0.192	0.415	0.463	0.687	0.807	0.668	0.700	0.464				
Isecheno		0.785	0.731	0.566	0.794	0.780	0.653	0.802	0.274	0.605	0.117	0.523	0.766			
Kisere		0.577	0.274	0.485	0.315	0.566	0.418	0.632	0.505	0.511	0.457	0.408	0.254	0.405		
Salazar		0.730	0.553	0.428	0.193	0.729	0.728	0.848	0.561	0.748	0.254	0.248	0.406	0.479	0.254	
Yala		0.595	0.574	0.305	0.722	0.682	0.552	0.692	0.323	0.565	0.244	0.296	0.820	0.345	0.552	0.436

Table 3.G: Morisita-Horn dissimilarities between pollinator faunas of populations of *A. eminens* and *A. polystachyus*, ranging from 0 (identity) to 1 (complete dissimilarity). Based on pollinator abundance, including only data for individuals which could be identified to species level.

		A. eminens									*A. polystachyus*					
		Buzambuli	Colobus	Ikuywa	Kisere		Rondo	Salazar		Yala	Buzambuli	Colobus	Ikuywa	Isecheno	Kisere	Salazar
					A	B		1	2							
A. eminens																
Colobus		0.089														
Ikuywa		0.222	0.312													
Kisere	A	0.481	0.381	0.235												
	B	0.171	0.217	0.126	0.331											
Rondo		0.290	0.358	0.118	0.258	0.036										
Salazar	1	0.053	0.119	0.404	0.642	0.301	0.449									
	2	0.038	0.170	0.330	0.580	0.319	0.433	0.040								
Yala		0.041	0.117	0.381	0.621	0.295	0.437	0.002	0.026							
A. polystachyus																
Buzambuli		0.636	0.523	0.324	0.077	0.449	0.375	0.802	0.745	0.784						
Colobus		0.534	0.313	0.420	0.147	0.399	0.403	0.632	0.662	0.627	0.221					
Ikuywa		0.570	0.376	0.388	0.176	0.247	0.216	0.678	0.751	0.681	0.244	0.151				
Isecheno		0.152	0.190	0.059	0.145	0.105	0.110	0.315	0.250	0.292	0.290	0.297	0.302			
Kisere		0.436	0.192	0.434	0.148	0.420	0.459	0.507	0.536	0.500	0.248	0.095	0.184	0.274		
Salazar		0.699	0.505	0.491	0.097	0.633	0.573	0.825	0.766	0.806	0.081	0.187	0.303	0.381	0.138	
Yala		0.383	0.428	0.243	0.539	0.494	0.570	0.502	0.438	0.483	0.494	0.628	0.733	0.389	0.569	0.581

Table 3.H: Results of hierarchical partitioning of variance of pollinator visitation. Predictors: distance to next heterospecific population, forest cover in 1,000 m radius around population center, flower density, and, for *A. eminens*, canopy cover and population size. Z-Statistics based of 5,000 randomizations, with a Z-score threshold of 1.65 and a confidence interval of 0.95. * marks significant results.

A. eminens	% Variance	Z-Score	
X. Xylomellisa sp.1			
Canopy	41.0	2.13	*
Flower density	30.6	1.34	
Population size	11.5	−0.13	
Distance	8.5	−0.36	
Forest Cover	8.4	−0.34	
X. Xylomellisa sp.2			
Canopy	11.6	−0.12	
Flower density	3.3	−0.78	
Population size	6.6	−0.53	
Distance	14.9	0.12	
Forest Cover	63.5	3.83	*

A. polystachyus	% Variance	Z-Score	
X. Xylomellisa sp.1			
Flower density	68.6	1.79	*
Distance	16.3	−0.36	
Forest Cover	15.1	−0.33	
X. Xylomellisa sp.2			
Flower density	17.0	−0.21	
Distance	58.1	1.74	*
Forest Cover	24.9	0.16	

Table 3.I: Results of hierarchical partitioning of variance of abundance of the two most important pollinator species of *Acanthus*. Predictors: distance to next heterospecific population, forest cover in 1,000 m radius around population center, flower density, and, for *A. eminens*, canopy cover and population size. Z-Statistics based of 5,000 randomizations, with a Z-score threshold of 1.65 and a confidence interval of 0.95. * marks significant results.

A. eminens	% Variance	Z-Score
X. Xylomellisa sp.1		
Distance	49.5	2.90 *
Forest Cover	20.0	0.66
Flower density	9.8	−0.23
Canopy	9.2	−0.29
Population size	10.7	−0.16
X. Xylomellisa sp.2		
Distance	24.0	−0.45
Forest Cover	13.9	−0.66
Flower density	12.1	−0.75
Canopy	14.6	−0.73
Population size	35.4	−0.23

A. polystachyus	% Variance	Z-Score
X. Xylomellisa sp.1		
Distance	36.0	0.87
Forest Cover	35.8	1.00
Flower density	28.2	0.61
X. Xylomellisa sp.2		
Distance	38.3	0.59
Forest Cover	12.1	−0.51
Flower density	49.6	1.10

4 FACTORS DETERMINING REPRODUCTIVE SUCCESS OF TWO SPECIES OF *ACANTHUS* IN KAKAMEGA FOREST, KENYA

NILS HASENBEIN AND MANFRED KRAEMER

ABSTRACT

The two sympatric species *Acanthus eminens* CLARKE and *A. polystachyus* var. *polystachyus* DELILE flower simultaneously, display a highly similar pollinator morphology, and are both pollinated by carpenter bees (*Xylocopa*). Contrary to expectations, previous studies have demonstrated that fruit set of neither species is influenced by the respective congener, indicating that strong barriers mitigate reproductive interference. On the other hand, these studies found a high variability in fruit set between populations of either species. Here, we provide a detailed analysis of fruit set, seed set and seed viability of *A. eminens* and *A. polystachyus*, and present evidence that reproductive success is more closely linked to environmental factors than to stigmatic pollen loads or pollinator visitation rates.

INTRODUCTION

Synchronously flowering plants may compete for pollinators, reducing their reproductive success (Levin and Anderson 1970; Brown and Mitchell 2001). Apart from reduced visitation rates, co-flowering and pollinator sharing may also incur fitness costs due to incompatible pollen transfer (Waser 1978a; Campbell 1985; Bosch *et al.* 1997; Theiss *et al.* 2007) or hybridization (Anderson 1948; Anttila *et al.* 1998; Wilsdon and Richards 2009). In addition, conspecific pollen discounting during visits on heterospecific flowers has been identified as a factor strongly reducing male reproductive success (Campbell and Motten 1985; Stout *et al.* 1998; Morales and Traveset 2008).

However, plants flowering simultaneously may benefit through increased pollinator attraction in heterospecific floral displays (Thomson 1978; Laverty 1992; Moeller 2004; Ghazoul 2006). On the other hand, and quite commonly, plant reproduction may not be influenced by the presence of co-flowering species at all (Armbruster and McGuire 1991; McGuire and Armbruster 1991; McGuire 1993).

Reproductive isolation of plant species may be caused by several cumulative barriers (Marques *et al.* 2007), which have been repeatedly focussed by scientists interested in mechanisms of speciation and reproductive interference between species, as well as in the ecological consequences of the latter (e.g. Anderson and Hubricht 1938; Sinclair 1968; Barone *et al.* 1992; Chari and Wilson 2001; Ramsey *et al.* 2003; Kay 2006; Yang *et al.* 2007; Bänziger *et al.* 2008; Botes *et al.* 2008; Wu and Zhang 2010). From these studies, it has been concluded that prezygotic isolation may be more common and stronger than postzygotic mechanisms (Widmer *et al.* 2008, but see Wendt *et al.* 2008).

Pre-empting reproductive interference completely, temporal isolation through a complete divergence of flowering time and spatial isolation through divergent habitat preferences may be the most effective mechanisms of floral isolation (Stiles 1975, 1977; Waser 1978a). Anthropogenic habitat change has long been known to reduce distances between previously isolated species and to change species abundance, causing reproductive interference (Anderson 1948; Rathcke and Lacey 1985). Commonly, its effects are stronger on less abundant and rare species (Levin and Anderson 1970; Burgess *et al.* 2005, 2008; Morales and Traveset 2008). The likelihood of heterospecific pollen transfer may increase with the number of competing pollen donors (Levin and Anderson 1970; Ghazoul 2002). Some of the most drastic effects of interspecific pollination with significant ecological impact have been demonstrated in various cases of plant species invasions, which may be seen as a special case of anthropogenic breakdown of spatial isolation between species (e.g. Matsumoto *et al.* 2009, 2011).

Partitioning of pollinators may reduce reproductive interference in plants flowering both simultaneously and in close vicinity. For example, flowers may selectively allow access only to a subset of the local fauna (Grant 1949). Floral morphology may facilitate specific placement and removal of pollen on and from pollinator bodies, assuring a high proportion of conspecific pollen on stigmas (Botes *et al.* 2008). Alternatively, ethological traits of pollinators such as selective foraging may reduce pollen flow between species (Grant 1949). The latter may be dependent on floral abundance, as pollinators may prefer the most abundant flower type (Hopper and Burbidge 1978). Abundant flowers receive a high proportion of conspecific pollen when pollinators do not preferentially visit a certain flower type (Levin and Anderson 1970; Inouye 1978; Feinsinger and Tiebout 1991).

The transfer of heterospecific pollen may reduce reproductive success, e.g. through mechanical clogging (Waser 1978b; Kanchan and Chandra 1980; Thomson *et al.* 1982; Waser and Fugate 1986; Palmer *et al.* 1989; Scribailo and Barrett 1994). On the other hand, several studies have failed to demonstrate an effect of pollen deposition on incompatible stigmas (Campbell and Motten 1985; Kohn and Waser 1985; Kwak and Jennersten 1986; Stout *et al.* 1998; Nielsen *et al.* 2008). In closely related species, heterospecific pollen is more likely to germinate on stigmas (Brown and Mitchell 2001; Matsumoto *et al.* 2009), and though pollen tube growth is often reduced in these cases (Williams *et al.* 1982; Brown and Mitchell 2001, but see Carney *et al.* 1996), the development of fertile hybrid seeds has been described for many congeneric species (Levin 1971; Neuffer *et al.* 1999; Vilà *et al.* 2000; Emms and Arnold 2000; Lexer *et al.* 2003; Matsumoto *et al.* 2009). Through repeated hybridization and introgression between plants in disturbed habitats, one of the involved species may outcompete the other (Levin *et al.* 1996; Rhymer and Simberloff 1996; Anttila *et al.* 1998; Mallet 2005), and in some cases, species have been shown to merge into new 'hybrid species' (Mallet 2007). More rarely, stable hybrid zones may be formed in transitional habitats between those of the parental species (e.g. Anderson 1948; Lamont *et al.* 2003).

Consequently, co-flowering in close vicinity is likely to affect reproduction in species sharing pollinators, especially when these species are highly similar and closely related. The effects mentioned above may endanger rare species and those found in fragments of increasingly rare habitats when their relative abundance is reduced, either by a reduction of flowers of the species itself or by an increase in the abundance of the competitor.

Acanthus eminens and *A. polystachyus* grow sympatrically in Kakamega Forest in Western Province, Kenya. The forest supposedly is the last Kenyan fragment remaining from the original Guineo-Kongolian rainforest belt. During the last 100 years, it lost nearly two thirds of forest cover, and several forest areas once connected are now forming separate fragments (Schaab *et al.* 2010).

As *A. polystachyus* grows at forest edges and in hedgerows, and *A. eminens* in clearings and along rivers inside the forest, habitat change has reduced the distance between the species. In previous studies, they were found to display a striking synchrony in flowering phenology (Chapter 1), as well as a strong similarity in floral morphology (Chapter 2) and pollinator faunas (Chapter 3), indicating strong potential for pollinator-mediated reproductive interference. However, none of the previous studies has analyzed in detail which factors influence fruit set, and to date, no data on seed set and seed quality of *A. eminens* and *A. polystachyus* are available.

Though it seems highly likely that the species are subject to interspecific pollen transfer, no effect on fruit set due to the distance to and habitat availability of the respective congener could be demonstrated (Chapter 1). Following up on this analysis, we hypothesize that 1) there is no effect of the respective congener on seed set and seed viability.

A comparison of pollinator abundance and visitation rates between populations found indications that abundance of pollinators depends more on habitat than on selective pollinator preference for either plant species (Chapter 3). Bergsdorf (2006) demonstrated higher fruit set and visitation rates in fragment populations of *A. eminens*. Here, we propose that higher pollinator visitation rates increase 2) the likelihood of stigmas to receive pollen, and 3) reproductive success in terms of fruit set, seed set, and seed viability.

Increased humidity (Chapter 1) and geitonogamous self-pollination have been suggested as factors limiting reproduction in *Acanthus* (Chapter 2, 3). We hypothesize that 4) microclimate, represented by canopy and forest over, influences reproduction in *A. eminens* and *A. polystachyus*, and that reduced reproduction rates are found in more humid habitats.

MATERIALS AND METHODS

STUDY AREA

Our study was conducted in Kakamega Forest in Western Kenya (0"170N, 34"540E). Kakamega Forest is considered the easternmost remnant of the lowland Guineo-Congolian rain forest belt, and forest fragmentation has led to the formation of five fragments (130 to 1,400 ha) and a remnant main forest (8500 ha, Schaab *et al.* 2010) comprising 12,000 ha of rainforest vegetation at an altitude between 1,500 to 1,700 m above sea level. It is managed by the Kenyan Wildlife Service (KWS) in the north and the Kenyan Forest Service (KFS, former Forest Department) in the south. Mean monthly temperatures range from 11°C to 29°C, with an average temperature of 22°C. Rainfall averages 2,000 mm per year, with two distinct rainy seasons between March and May ('long rainy season') and September and November ('short rainy season') and two distinct dry seasons between December to February ('dry season') and from June to August ('cold dry season'). The forest is under significant pressure due to illegal logging, firewood collection, grazing, and hunt for bushmeat, and large parts are highly disturbed (Schaab *et al.* 2010). The forest is surrounded by densely populated (336 to 746 inhabitants per km^2, Mitchell 2004), highly diverse and structured farmland with 0.2 to 0.7 ha of land per household (Greiner 1991; Kenyan Ministry of Agriculture 2006). 62% of all households generate their income from agriculture, and the district's poverty rate is about 52% (Dose 2007).

Plant species

We studied two species of *Acanthus* L.. *Acanthus eminens* Clarke is a shrub growing in clearings in primary and old secondary mountain rainforest between 1,500 and 2,800 m above sea level in Kenya (Beentje 1994). It is also found on the Ugandan side of Mt Elgon (pers. obs.), as well as in Ethiopia and in the Imatong Mts in Sudan, very likely spreading to north Uganda (Vollesen 2007). Though not a rare species in Kenya, it is limited to highland and mountaineous forest areas, which have suffered heavily from deforestation in more recent history. *A. eminens* grows up to five meters in height, carries spiny, robust leaves and flowers in decussate spikes. Plants may flower at relatively small size, carrying only one inflorescence, but most carry several, and up to over 100 inflorescences.

Flowering starts at the end of rainy season between october and november, and continues up to the end of dry season in mid-february. Fruits of *A. eminens* are dehiscent capsules with up to four seeds which are explosively expelled from the fruit on dehiscence by a woody central column in the fruit. Fruits and seeds do not show any sign of adaption to fruit dispersion by animals (e.g. clinging hairs, pulp, elaiososomes), and do not germinate after being damaged (pers. obs.). *A. eminens* is capable of vegetative reproduction, and forms distinct populations of between 65 and 2,574 (median: 142) plants in Kakamega forest area, as well as relatively continuous, but less dense, stands along riversides.

Acanthus polystachyus var. *polystachyus* Delile (referred to as *A. polystachyus*) is a related species (McDade *et al.* 2005) growing along forest edges and grasslands inside forests between 1,100 and 2,500 m above sea level in Ethiopia, Eastern Sudan, Eastern Uganda and Tanzania. In Kenya, it occurs only in Western Province and is missing on the plains around lake Victoria (Vollesen 2007). In Kakamega area, it is often found invading grasslands and is part of natural forest regeneration.

A. polystachyus does not form distinct populations as it grows in more or less loose clusters in small copses and hedgerows, also forming dense, continuous stands along forest edges. Morphologically similar to *A. eminens*, *A. polystachyus* has softer, hairy leaves and is generally larger than its congener. Its inflorescences are slightly larger than those of *A. eminens*, and a single plant carries up to several hundred of them.

Flowers of either species are zygomorphic, and of similar size and functionality, but different colour (Chapter 2). In the study area, the species are pollinated by the same group of bee species, carpenter bees of the genus *Xylocopa* (Chapters 2, 3).

In this study, we analyze data from five main forest and four forest fragment populations of *A. eminens*, and from five main forest and two fragment populations of *A. polystachyus*. Two fragment populations were heterospecific stands with less than 10 m distance between individuals of both *Acanthus* species. Nearest distances between heterospecific populations ranged between 220 and 4,000 m. This study includes data from two reproductive periods, between start of November and end of February 2007/2008 and 2008/2009 (referred to as 2008 and 2009, respectively). Sample sizes, especially from *A. polystachyus*, are reduced for 2008 due to constrained mobility in the wake of the political unrest which followed presidential elections in december 2007.

Fruit set

As reproductive success may vary with plant density as well as between flowers in different positions within an inflorescence, we analyzed the influence of these two factors on fruit and seed set. To assess whether flowers in different positions on an inflorescence show different levels of fruit and seed set, we marked a single flower in each third along the length on an inflorescence and monitored fruit set until maturity. Relative position (RP) of flowers was determined by dividing the position of each flower in order of flowering (zero to n), by the total number n of flowers of an inflorescence. Flowers were grouped into low (RP 0.245±0.057) middle (RP 0.539±0.059) and upper (RP 0.847±0.029) flowers. Fruits were collected and treated as described below.

At the end of both flowering seasons, we collected fully mature fruits shortly before dehiscence from up to five inflorescences of 16 to 40 and (4-)19 to 30 individual plants in nine and seven populations of *A. emi-*

nens and *A. polystachyus*, respectively. We only sampled inflorescences in which all flowers had been spent.

We noted the number of fruit capsules and total potential flowers on each inflorescence. Fruits were stored and treated as described in section 'seed set' below.

We calculated fruit set of each inflorescence by dividing the number of fruits on an inflorescence by the size of the inflorescence measured in potential flowers. Average fruit set per plant was calculated and used for the final calculation of population average fruit set.

Seed set

Fruits were individually stored in paper bags and dried until release of seeds. Seeds of each fruit were counted and assigned to one of three categories (underdeveloped, fully developed and healthy, fully developed and damaged). In the latter category, we also noted the kind of damage (predation marks, mould etc.). Each seed category for each plant was stored individually.

For each fruit and category of seed, we calculated the ratio of seeds in relation to the maximum of four potential seeds. The averages of these values per plant were used as measure of individual seed set. Mean values of individual seed set are used as measure of population seed set.

Seed viability

Preliminary tests with seeds from the flowering period 2006/2007 showed that only seeds classified as healthy and fully developed are capable of growing into seedlings. Consequently, only these seeds were used in further analyses.

Seeds were weighed and sown into labeled wells of 54-well trays at the greenhouse facilities of Bielefeld University, Germany. Seeds from both species, all populations and all collections were randomly assigned to wells, and trays were rotated weekly to reduce potential environmental effects on development. Trays were watered to field capacity every two days. Germination and seedling development were recorded twice per week during the first four weeks, and once per week thereafter.

Calculation of reproductive success

We calculated reproductive success as the product of the ratio of fruits found per flower, the ratio of fully developed, healthy seeds in each fruit capsule, and germination rate of these seeds. Hence, the resulting value for reproductive success represents the number of potential seedlings produced by a single flower in each population, theoretically ranging from zero (no seedling) to one (four seedlings).

Seed health was calculated by dividing the number of mature, undamaged seeds by the sum of all mature seeds per fruit.

Pollination success

We used stigmatic pollen loads as measure for primary pollination success (PPS).

Styles were collected from flowers of both species which did not show any sign of wilting or physical damage to their stigmas after they had dropped their corolla. The opposing flower on the inflorescence, albeit not collected, was checked for damage or infection to further ensure sample quality. No sampled flower showed presence of nectar, or was observed to be visited by potential pollinators. We therefore assume that these flowers had received all, if any, potential pollinator visits possible during their lifetime. Styles were broken off at their base using forceps, and stored individually in labeled tubes of 70% alcohol. For analysis, stigmas were cut off using a razor blade, and embedded in fuchsine jelly cubes (based on Kearns and Inouye 1993 p. 289, protocol modified for Agarose by U. Zumkier). The cubes were molten on glass slides, squashed under a glass cover, and pollen grains were counted using a standard light microscope.

Flowers of *Acanthus* have two locules with two ovules each. As reviewed by Cruden 2000, flowers have to receive more pollen than they have ovules for optimal pollination success, with most studies suggesting four to six times more pollen than ovules. McDade (1983) found that *Trichanthera gigantea*, another Acanthaceae, only sets fruit when pollinated with one pollen grain per ovule. We chose to consider all stigmas with more than four pollen grains of any kind to have been visited by pollinators, assuming this number to be sufficient for fruit set. This also facilitates comparability with an earlier study using the same set of data (Chapter 1), in which the percentage of pollinated stigmas was found to be constant during the course of the observed flowering periods. The results presented here are based on average values of all measurements in each population.

Pollinator visitation rates

We observed flowers of both species during standardized observation units, simultaneously observing ten, in one case five, flowers on different inflorescences for 30 minutes, followed by ten minutes of insect catching on all flowers of the population. Observations were started on the hour between 9 a.m. and 2 p.m., which was established as time of highest visitation rates by prior observation.

Reference insect specimens were killed, pinned and stored. Unknown species were identified by Dr. M. Gikungu, National Museum of Kenya, Nairobi. Unidentifiable individuals, e.g. those observed but not caught, were grouped into morphospecies, including a group of completely 'unknown' individuals. Two species of *Xylocopa*, belonging to the sub-genus *Xylomellisa*, were not identifiable as known species. Hence, they are referred to as *X.* sp.1 and *X.* sp.2. Based on pollinator efficiency measurements on both species of *Acanthus* (Dietzsch 2004), *Xylocopa* bees were assumed to be capable of transferring pollen in both species, and only these species are included in the analysis presented here. For more details on floral visitors of either species in our study system, please refer to Chapter 3.

Plant and flower density

For each fruit collected in 2009, we counted neighbouring conspecific inflorescences in a two and five meter radius around each inflorescence from which we collected fruits and seeds. We discriminated between inflorescences of the same individual, inflorescences from the same plant species, and inflorescences of the respective congener. Preliminary testing revealed stronger influence of the two meter scale on reproductive traits of *A. eminens*, and of the five meter scale on *A. polystachyus*. As these two counts are not independent, we used only the factor with greater predictive power for analyses. We used a generalized linear mixed model to analyze whether ratios of fruit set, seed set, and seed health are dependent on flower density, which was treated as fixed effect in the model. To account for replications, plant identity, nested in population identity, was included as random effect.

Forest cover and Inter-Population distances

Forest cover and inter-population distances were kindly provided by the team of BIOTA E02 (Prof. Dr. G. Schaab, University of Applied Sciences, Karlsruhe), based on GPS measurements inside populations. Note that we re-calculated models including forest cover for all available scales (radius of 100, 500, 1,000 and 2,000 m around population centers) separately for both species, and used only the scale which provided most explanatory power.

Canopy cover and population size

Continuous measurements of microclimatic data were not possible due to the high intensity of human disturbance inside the forest. To reflect climatic conditions within the forest, we used two measures of canopy cover, which is linked to many abiotic factors, especially to humidity and exposure to sunlight. First, we recorded canopy cover during transect walks. In each population, we used two perpendicular transects of 100 m, subjectively measuring canopy cover from 0 (no cover) to 8 (very dense cover) every 10 m. In addition, we validated our measurements in a subset of populations using digital image analysis, using the Software GapLightAnalyzer (Simon Fraser University, Institute of Ecosystem Studies), corrected for the GPS location of Kakamega Forest. For each population, (10-)25 images were taken with a digital camera (Coolpix 5400, Nikon, Tokyo, Japan) equipped with a fisheye lens (FCE9 with UR-E10 adapter, focal length 5.8 mm, Nikon, Tokyo, Japan), mounted on a leveled tripod. All images were taken in the late afternoon to avoid overexposure, and north-aligned with a compass. Distance between individual images was as large as possible for a minimum of ten images ((10–)15 m), in order to minimize overlap between image area. We found our subjective measurements confirmed by comparison with canopy openness [%] as calculated by the software (Linear regression, $F_{1,7} = 27.06$, $r^2 = 0.795$, $p = 0.0013$). Analyses presented here are based on transect walks, as not all remote populations could be accessed in time for canopy imaging.

Population size of *A. eminens* in terms of number of flowering plants was assessed during the same transect walks as canopy cover, and calculated by multiplying the mean number of flowering plants per square meter by the area covered by the population, which was calculated from GPS measurements.

Results

In total, we collected 1,973 fruits in nine populations of *A. eminens* and 1,403 fruits in seven populations of *A. polystachyus*, yielding 820 and 1,099 undamaged, fully developed seeds for germination experiments, respectively.

There were no significant differences between fruit set and seed set of flowers in different positions on inflorescences in either species (Tab. 4.1), and consequently, RP of flowers was excluded from further analyses.

We found low levels of fruit set in both species of *Acanthus*. In *A. eminens*, only 28.8% (2008) and

Table 4.1: Nested anova of square-root transformed values of population means of fruit and seed set in three groups of positions on inflorescences of *A. eminens* and *A. polystachyus*.

	df	Mean Sq	F	p-value
Fruit set				
Species	1	0.016	0.228	0.636
Position[a]	4	0.037	0.527	0.717
Residuals	42	0.071		
Fully developed seeds				
Species	1	0.149	1.720	0.205
Position[a]	2	0.012	0.143	0.868
Residuals	19	0.087		
Healthy, fully developed seeds[b]				
Species	1	0.026	0.403	0.533
Position[a]	2	0.038	0.576	0.572
Residuals	19	0.066		

[a] nested in Species

[b] Note that over 90% of seeds in this category were found to be viable

23.6% (2009) of flowers developed into mature fruits (For population values of reproduction, see Appendix, Tab. 4.A and 4.B). In *A. polystachyus*, 19.5% and 24.6% of flowers matured into fruit capsules. Within fruits, the ratio of seeds reaching full size was high, with 74.9% and 60.4% maturing in *A. eminens* and 78.4% and 66.7% maturing in *A. polystachyus* in 2008 and 2009, respectively. Hence, most fruits contained two to three (out of four) fully developed seeds. We found 22.1% and 5.7% of healthy, fully developed seeds per capsule in *A. eminens* and 29.9% and 28.9% in *A. polystachyus* in 2008 and 2009, respectively, indicating that most fruits in either species contain only a single seed with potential for germination, while in 2009, only one in twenty fruits contained a healthy seed in *A. eminens*. We found low percentages of viable seeds in both species of *Acanthus*, with *A. eminens* producing only 2.1% and 0.2% of the potential four viable seeds in 2008 and 2009, respectively. With 3.1% and 0.6% of four potential viable seeds found in *A. polystachyus*, the reproductive success of latter species was slightly higher. Taking the high viability of between 50 and 88.9% and the high number of fully developed seeds between 60 and 78.4% per fruit capsule into account, the two factors contributing most to the low overall reproductive success in either species were low fruit set, which resulted in a 70-80% loss of potential seedlings, and damage to fully developed seeds, which caused a loss between 60 and 90%.

Reproductive interference

Fruit set was not found to be linked with distance to the next heterospecific population in either species (Fig. 4.1, Tab. 4.2).

Table 4.2: Statistics for linear regressions of fruit set dependent on the distance to the next heterospecific population of the congeners (Fig. 4.1).

Species	Year	r^2	F-Stat.	p-value
AE	2008	0.190	$F_{1,6} = 1.41$	p = 0.280
	2009	0.001	$F_{1,7} = 0.01$	p = 0.945
AP	2008	0.141	$F_{1,3} = 0.49$	p = 0.533
	2009	0.192	$F_{1,5} = 1.19$	p = 0.325

Seed set of fully developed seeds was not found be dependent on distance to the next heterospecific population in *A. eminens* in 2009, but a negative link was found in 2008. In the same species, the ratio of damaged fully developed seeds was not influenced by distance to the next heterospecific population. In *A. polystachyus*, seed set of fully developed seeds was not affected in either year, whereas the ratio of damaged to all fully developed seed decreased with distance to the next heterospecific population in 2009, while it was unaffected in 2008 (Fig. 4.1, Tab. 4.3).

Finally, germination was not linked with distance to the next heterospecific population in either species and year. However, there was a marginal positive link in *A. polystachyus* in 2009, with a higher seed viability in populations farther away from a population of the congener.

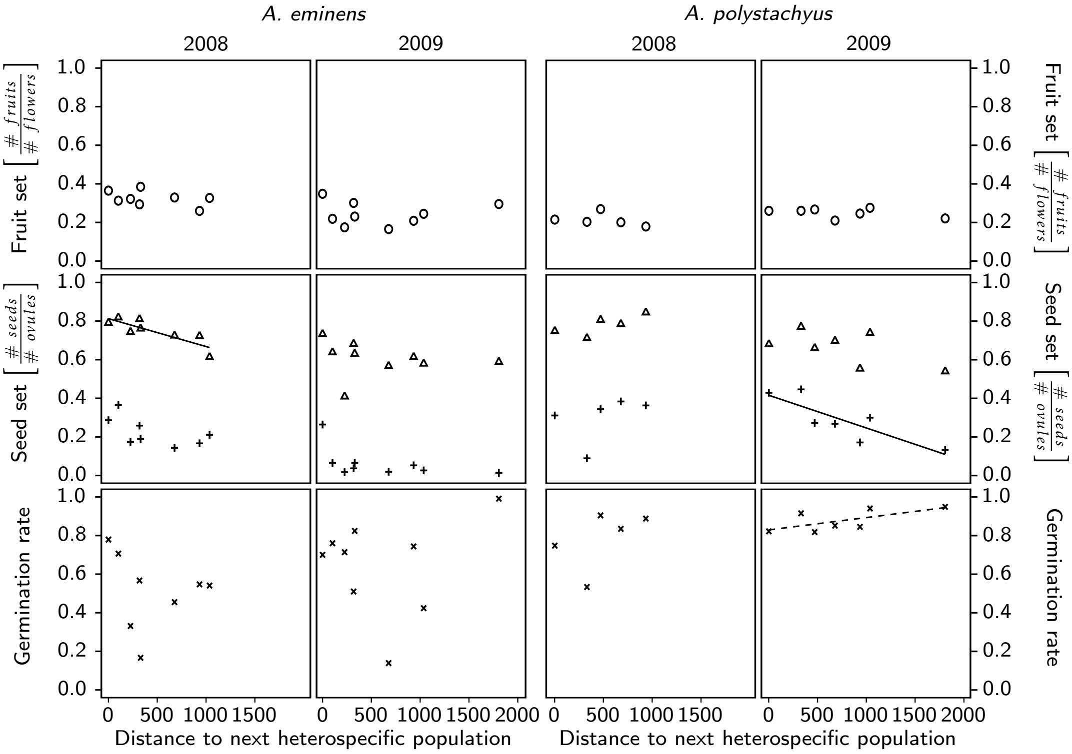

Figure 4.1: From top to bottom: Fruit set (○), seed set of fully developed seeds per four ovules/per fruit (△), ratio of undamaged to all fully developed seeds (+), and germination rate of undamaged fully developed seeds (×) during reproductive periods 2008 and 2009 plotted against distance to the next heterospecific population of *A. eminens* (left) and *A. polystachyus* (right). Lines represent model predictions over the range of data for significant (dashed lines: marginally significant) linear regressions.

POLLINATION SUCCESS

We did not find the ratio of pollinated stigmas to be dependent on combined pollinator visitation rates (Fig. 4.2, Tab. 4.4), and also not on the visitation rate of the most frequent pollinator of the same (*X.* sp.1 on *A. eminens*, *X.* sp.2 on *A. polystachyus*) and the respective other species (*X.* sp.2 on *A. eminens*, *X.* sp.1 on *A. polystachyus*). In addition, there was no link between fruit set or seed set and the ratio of stigmas found with four or more adherent congeneric pollen grains (Tab. 4.5).

Table 4.3: Statistics for linear regressions of seed set of fully developed seeds, the ratio of damaged fully developed seeds and seed germination rate dependent on the distance to the next heterospecific population of the congeners (Fig. 4.1). Stars mark significant results.

Fully developed seeds

Species	Year	r^2	F-Stat.	p-value
AE	2008	0.714	$F_{1,6} = 14.95$	p = 0.008**
	2009	0.036	$F_{1,7} = 0.26$	p = 0.623
AP	2008	0.583	$F_{1,3} = 4.19$	p = 0.133
	2009	0.376	$F_{1,5} = 2.31$	p = 0.143

Ratio of damaged fully developed seeds

Species	Year	r^2	F-Stat.	p-value
AE	2008	0.166	$F_{1,6} = 1.20$	p = 0.316
	2009	0.267	$F_{1,7} = 2.54$	p = 0.155
AP	2008	0.103	$F_{1,3} = 0.34$	p = 0.600
	2009	0.786	$F_{1,5} = 18.41$	p = 0.008**

Germination

Species	Year	r^2	F-Stat.	p-value
AE	2008	0.030	$F_{1,6} = 0.19$	p = 0.680
	2009	0.026	$F_{1,7} = 0.19$	p = 0.677
AP	2008	0.269	$F_{1,3} = 1.10$	p = 0.371
	2009	0.448	$F_{1,5} = 4.06$	p = 0.100 .

Table 4.4: Statistics for linear regressions of the ratio of stigmas found with four or more adherent congeneric pollen grains dependent on pollinator visitation rates (Fig. 4.2).

Species	Pollinator	r^2	F-Stat.	p-value
AE	All	0.150	$F_{1,7} = 1.23$	$p = 0.304$
	X. sp.1	0.074	$F_{1,7} = 0.56$	$p = 0.479$
	X. sp.2	0.249	$F_{1,7} = 2.32$	$p = 0.172$
AP	All	0.007	$F_{1,4} = 0.15$	$p = 0.445$
	X. sp.1	0.169	$F_{1,4} = 0.82$	$p = 0.418$
	X. sp.2	0.076	$F_{1,4} = 0.33$	$p = 0.597$

Table 4.5: Statistics for linear regressions of fruit set and seed set dependent on PPS.

Fruit set Species	Year	r^2	F-Stat.	p-value
AE	2008	0.000	$F_{1,6} = 0.00$	$p = 0.968$
	2009	0.077	$F_{1,7} = 0.08$	$p = 0.790$
AP	2008	0.096	$F_{1,3} = 0.32$	$p = 0.612$
	2009	0.394	$F_{1,5} = 3.25$	$p = 0.131$

Seed set Species	Year	r^2	F-Stat.	p-value
AE	2008	0.024	$F_{1,6} = 0.15$	$p = 0.713$
	2009	0.019	$F_{1,7} = 0.13$	$p = 0.725$
AP	2008	0.092	$F_{1,3} = 0.30$	$p = 0.621$
	2009	0.003	$F_{1,5} = 0.02$	$p = 0.900$

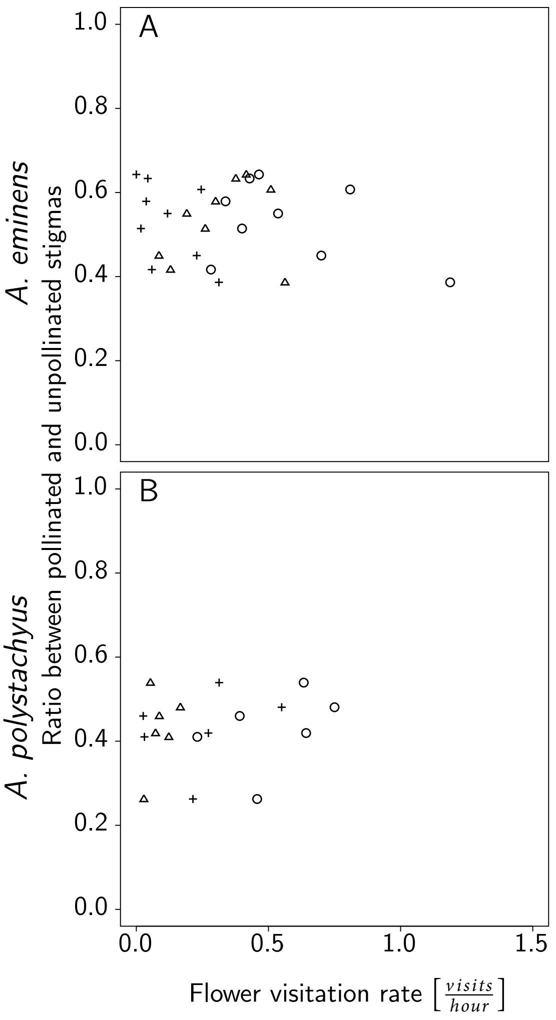

Figure 4.2: Ratio of stigmas found with four or more adherent pollen grains plotted against visitation rates of all putative pollinators (○), *X.* sp.1 (△) and *X.* sp.2 (+), for populations of *A. eminens* (A) and *A. polystachyus* (B). For sample sizes, see Appendix, Tab. 4.C.

VISITORS AND REPRODUCTION

We found a marginally significant negative link between fruit set and *X.* sp.1 visitation rate in *A. eminens* (Fig. 4.3, Tab. 4.6), and a marginally significant link between the ratio of fully developed seeds and visitation rates of all pollinating species. In addition, we found a significant negative link between the ratio of fully developed seeds and the visitation rate of *A. eminens*' most common pollinator, *X.* sp.2. In *A. polystachyus*, we did not find any link between visitation rates and fruit and seed set.

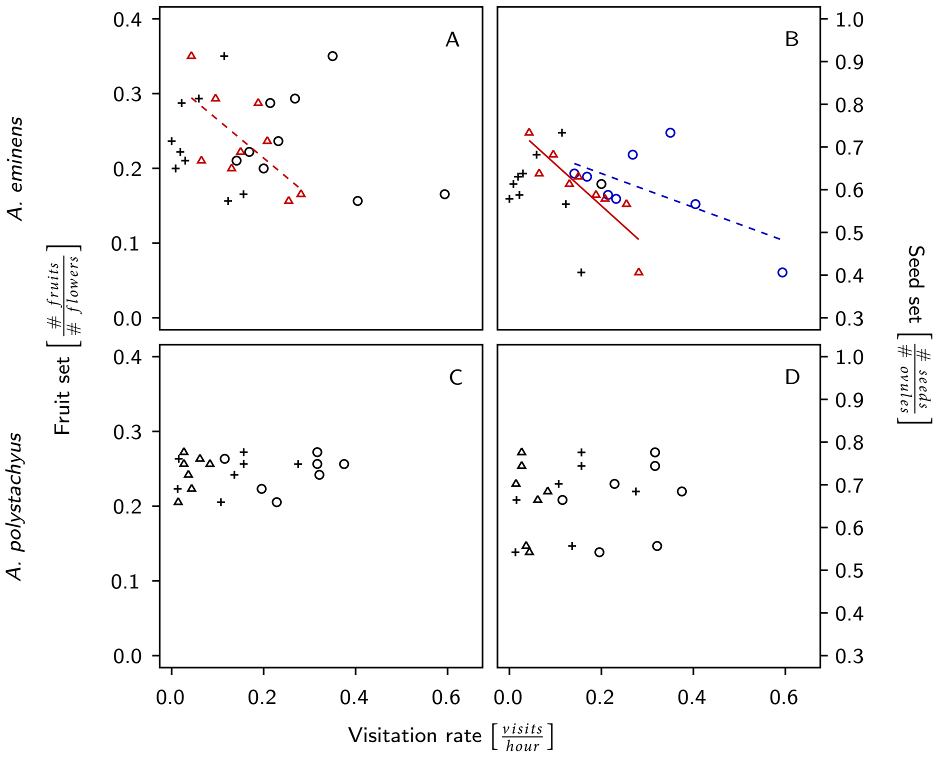

Figure 4.3: Ratio of fruit set (A+C) and ratio of seed set (B+D, fully developed seeds per four ovules) of *A. eminens* (A+B) and *A. polystachyus* (C+D) against visitation rates of all putative pollinators (○), *X.* sp.1 (△) and *X.* sp.2 (+). Lines represent model predictions over the range of data for significant (dashed lines: marginally significant) linear regressions.

Table 4.6: Statistics for linear regressions of fruit set and seed set of fully developed seeds dependent on pollinator visitation rates (Fig. 4.3). Stars mark significant results.

Fruit set	Species	r^2	F-Stat.	p-value
AE	All	0.086	$F_{1,7} = 0.658$	p = 0.444
	X. sp.1	0.439	$F_{1,7} = 5.468$	p = 0.052 .
	X. sp.2	0.030	$F_{1,7} = 0.216$	p = 0.656
AP	All	0.077	$F_{1,5} = 0.417$	p = 0.547
	X. sp.1	0.136	$F_{1,5} = 0.788$	p = 0.415
	X. sp.2	0.123	$F_{1,5} = 0.703$	p = 0.440
Seed set	**Species**	**r^2**	**F-Stat.**	**p-value**
AE	All	0.387	$F_{1,7} = 4.423$	p = 0.074 .
	X. sp.1	0.782	$F_{1,7} = 25.14$	p = 0.002**
	X. sp.2	0.127	$F_{1,7} = 1.017$	p = 0.347
AP	All	0.074	$F_{1,5} = 0.400$	p = 0.555
	X. sp.1	0.047	$F_{1,5} = 0.245$	p = 0.642
	X. sp.2	0.187	$F_{1,5} = 1.151$	p = 0.332

Reproduction and flower density

Using generalized linear mixed model analysis (fixed effect: flower density; random effect: plant identity, nested in population identity), we found only the health of fully developed seeds to be marginally linked with inflorescence density in *A. eminens* (n = 9(516), z = -1.898, p = 0.058, number in brackets reflects total number of plants, n reflects number of sampled populations), which had no effect on fruit set (z = 1.610, p = 0.107) and seed set of fully developed seeds (z = -0.276, p = 0.782). In *A. polystachyus*, fruit set (n = 7(640), z = 0.713, p = 0.476) and seed health (n = 7(681), z = 0.159, p = 0.874) were not significantly linked to flower density, while we found a marginal positive link between inflorescence density and seed set of fully developed seeds (n = 7(681), z = 1.729, p = 0.084).

Reproduction and climate

Seed set and seed health of *A. eminens* were reduced in populations with denser canopy in 2008 (Tab. 4.7), Fig. 4.4). The same trend for seed set, albeit marginally significant, was found in 2009. Fruit set was unaffected by canopy cover, while there was a marginally signif-

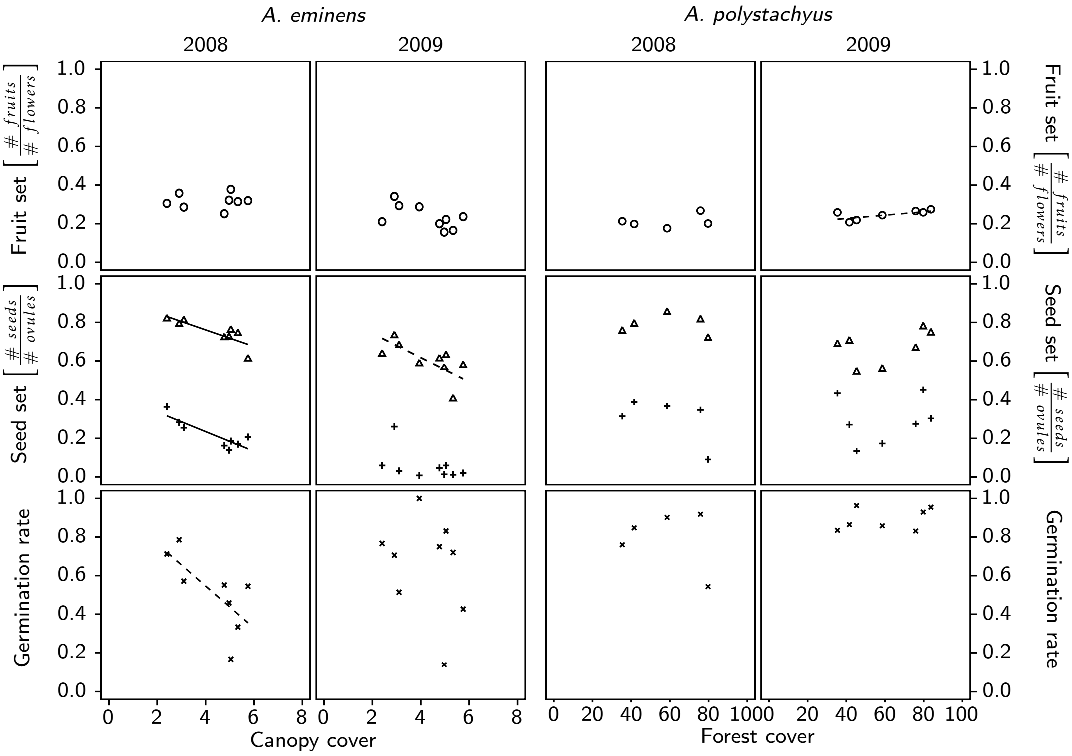

Figure 4.4: From top to bottom: Fruit set (○), seed set of fully developed seeds per four ovules/per fruit (△), ratio of undamaged to all fully developed seeds (+), and germination rate of undamaged fully developed seeds (×) during reproductive periods 2008 and 2009 plotted against canopy cover of populations of *A. eminens* (left) and against forest cover in a 2,000 m radius around populations of *A. polystachyus* (right). Lines represent model predictions over the range of data for significant (dashed lines: marginally significant) linear regressions.

icant trend of reduced germination rates of seeds of *A. eminens* from populations with dense canopy in 2008. However, this effect could not be demonstrated in 2009.

In *A. polystachyus*, we only found a slight increase in fruit set in populations surrounded my more forested areas in 2009, while seed set, seed health and germination rates were independent of forest cover.

Table 4.7: Results for linear regression analyses of fruit set, seed set of fully developed seeds, ratio of undamaged to all fully developed seeds and germination rate dependent on canopy cover in populations of *A. eminens* and percentage forest cover of the area within a 2,000 m radius around populations of *A. polystachyus*. (Fig. 4.4). Stars mark significant results.

Fruit set				
Species	Year	r^2	F-Stat.	p-value
AE	2008	0.004	$F_{1,6} = 0.02$	p = 0.889
	2009	0.328	$F_{1,7} = 3.42$	p = 0.107
AP	2008	0.120	$F_{1,3} = 0.41$	p = 0.567
	2009	0.471	$F_{1,5} = 2.31$	p = 0.089.

Seed set				
Species	Year	r^2	F-Stat.	p-value
AE	2008	0.687	$F_{1,6} = 13.19$	p = 0.011**
	2009	0.435	$F_{1,7} = 5.38$	p = 0.053.
AP	2008	0.007	$F_{1,3} = 0.02$	p = 0.894
	2009	0.212	$F_{1,5} = 1.34$	p = 0.299

Ratio of damaged fully developed seeds				
Species	Year	r^2	F-Stat.	p-value
AE	2008	0.747	$F_{1,6} = 17.72$	p = 0.006**
	2009	0.242	$F_{1,7} = 2.23$	p = 0.179
AP	2008	0.322	$F_{1,3} = 1.43$	p = 0.318
	2009	0.032	$F_{1,5} = 0.16$	p = 0.702

Germination				
Species	Year	r^2	F-Stat.	p-value
AE	2008	0.494	$F_{1,6} = 5.85$	p = 0.052.
	2009	0.077	$F_{1,7} = 0.58$	p = 0.471
AP	2008	0.073	$F_{1,3} = 0.24$	p = 0.660
	2009	0.104	$F_{1,5} = 0.58$	p = 0.481

DISCUSSION

In animal-pollinated plants, fruit set, seed set and seed viability are dependent on quantity and quality of pollinator visitation (Herrera 1987, 1989). Both components may be altered by competition between species when flowers receive fewer visits (e.g. Brown *et al.* 2002; Ghazoul 2004), pollen is lost due to foraging on heterospecific flowers, or incompatible pollen is placed on stigmas (Campbell and Motten 1985; Waser and Fugate 1986; Takakura *et al.* 2008). In this study, we do not find evidence for reproductive interference between the sympatric species *Acanthus eminens* and *A. polystachyus*, which is in accordance with the findings described in Chapter 1. In fact, seed set is higher in proximity to *A. polystachyus* in populations of *A. eminens* in 2008, and seed health is greater in proximity to *A. eminens* in populations of *A. polystachyus* in 2009, though facilitation of pollination in our system could already be ruled out based on pollinator observations (Chapter 3).

We conclude that there is no reproductive interference between the species, as has already been indicated in Chapter 1. As floral morphology and rewards are similar (Chapter 2), pollinators are shared to a at least moderate extent (Chapter 3), and heterospecific pollen transfer is detrimental to reproduction in either species (Dietzsch 2004), post-pollination effects may play a (partial) role in reproductive isolation of the species.

The likelihood of each ovule to produce a seed capable of developing into a seedling may be cumulatively reduced by the failure of each consecutive step between anthesis and seed germination. In *Acanthus*, only few viable seeds per ovule are finally released, and while both the number of fully developed seeds per fruit and the germination rates of fully developed, healthy seeds are reasonably high, most of the reproductive potential in either species is lost due to low fruit set and high proportions of damage to fully developed seeds.

Low fruit set may result from low pollen deposition on stigmas (Bierzychudek 1981; Burd 1994), low pollen quality (McDade 1983) and low resource availability, and commonly from a combination of these factors (Campbell and Halama 1993, see Haig and Westoby 1988).

In *A. polystachyus*, reproduction in terms of fruit set, seed set and seed viability does not vary with varying frequencies of pollinator visitation. In *A. eminens*, however, we find evidence that seed set is actually reduced in populations where its most common pollinator visits flowers with high frequency during one flowering season, which is in contrast to the findings of Bergsdorf (2006), who attributed increased fruit set in *Acanthus* to higher floral visitation rates.

A weak link between pollinator visitation rates and fruit and seed set has long been demonstrated in several species (Bertin 1982; Motten 1983; Silander and Primack 1978; Snow 1982). McDade and Davidar (1984) have demonstrated low fruit set even when resource limitation and limited pollen quantity can be ruled out, which they interpreted as indicator of low or strongly varying visit quality, with pollinators either failing to deposit pollen, or deposited pollen being of low quality. Compared to flower visitation rates, conspecific stigmatic pollen loads may be a better predictor for fruit and seed set (but see Mulcahy and Mulcahy 1983), but we do not find any link between pollinator visitation frequency and stigmatic pollen loads in *Acanthus*, and conclude that pollinator visitation rates do not limit reproduction in either species.

We considered stigmas to be pollinated when they received at least one pollen grain per ovule, and fruit set has been found at even lower pollination intensity (McDade 1983). On the other hand, more than one pollen grain per ovule may be necessary for seed set (Silander and Primack 1978; McDade 1983). However, we found less than two thirds of stigmas of either species with more than four adherent pollen grains in either year, rendering pollen limitation to be very likely in either of our study species. This is in accordance with the findings of Vamosi *et al.* (2006), who have demonstrated that pollen limitation is relatively common in ecosystems with high biodiversity.

A straightforward explanation for this finding would be low pollinator abundance, but pollinator visitation rates indicate a high likelihood for each flower to be visited at least once during its female phase (Chapter 3). Therefore, the low pollination efficiency of *Xylocopa* bees seems not to be caused by visitation frequency, but by low visit quality (see Herrera 1987). In other words, carpenter bees are ineffective pollinators of *Acanthus*, visiting with high frequency, but transferring little pollen. Another member of the genus, *X. virginica*, has been found to show low pollination effectiveness during legitimate visits on flowers by Adler and Irwin (2006). Ineffective pollinators may contribute to fruit and seed set of species when they are abundant, but have also been found to mitigate reproductive success in the presence of more efficient pollinators (Lau and Galloway 2004). However, there was no indication that either shrub is visited by a potentially more effective pollinator (Chapter 3). On the other hand, *Xylocopa* bees frequently groom after retreating from flowers, and removal of pollen from the thorax may play an important role in determining their pollinator effectiveness (Flanagan *et al.* 2009).

Another possible explanation for low pollen transfer which recently has been explored in detail is loss of conspecific pollen during visits on heterospecific flowers (Murcia and Feinsinger 1996) and during foraging flights (Inouye *et al.* 1994; Rademaker *et al.* 1997; Johnson *et al.* 2005), which may actually be more detrimental to reproduction than interspecific pollen transer (Morales and Traveset 2008; Flanagan *et al.* 2009). Though stigmatic pollen loads of *Acanthus* are almost completely congeneric, we cannot completely rule out the possibility that pollinators visit other flowers in between visits to *Acanthus*. However, pollinators frequently switch between flowers of the same and spatially close congeneric plants (Chapter 3), in which case a reduction of conspecific pollen loads during flight is ruled out as the distance between conspecific flowers is as low as it can be.

In addition, we found pollen loads of bees to consist of *Acanthus*-pollen only (pers. obs.). Hence, it is likely that pollinators forage predominantly on flowers of *Acanthus*, and a loss of pollen my be largely attributed to foraging movements between flowers and populations of *Acanthus*. Pollen grains of *A. eminens* and *A. polystachyus* are morphologically similar (Furness 1996) and were indiscriminable in our samples. Hence, we cannot rule out that differences in congeneric pollen composition affect reproduction in our study (Dietzsch 2004). However, our results indicate that putative effects of heterospecific pollen transfer likely are neglectable in *Acanthus*, as they may be completely masked by other factors (see Chapter 1).

Low pollen transfer effectiveness may also provide an explanation for the lack of reproductive interference demonstrated in *Acanthus* (Fishman and Wyatt 1999). When large amounts of pollen, regardless of which of the two species, are lost during even short flights, most pollen reaching stigmas may be geitonogamous self pollen, as pollinators have been found to commonly visit all flowers of an inflorescence and several inflorescences per plant (Chapter 3). It has been shown that only the first flower visited on an individual may receive high proportions of pollen from another plant, with consequent visits to other flowers of the same individual largely transferring self-pollen (Flanagan *et al.* 2009, in contrast to the findings of Kohn and Waser 1985).

Fruit set commonly varies with floral display size. In large floral displays, pollinators may visit more flowers, but the proportion of visited flowers is commonly reduced or remains constant (see Snow *et al.* 1996; Ohashi and Yahara 2002; Harder *et al.* 2004). Individual flowers may be visited with similar frequency in displays of different sizes when attraction of pollinators counters the increase in flowers (Karron *et al.* 2003; Harder *et al.* 2004), which seems to be the case in *Acanthus*, as floral density does not affect reproduction in any way.

When plants are limited in their fruit set, they may selectively abort development of fruits of low quality, e.g. those containing few seeds or seeds of low quality (Stephenson 1981). In *Acanthus*, this may provide an additional explanation for the lack of reproductive interference between the congeners. When flowers receiving large proportions of heterospecific pollen are aborted, and sufficient ovules remain which are fertilized by conspecific pollen, fruit set may be constant in surroundings with different densities of the congener.

Dietzsch (2004) found that fruit set from a congeneric mixture of *Acanthus* pollen depends on the ratio between conspecific and heterospecific pollen: The more pollen is transferred between species, the lower is the likelihood of the receiving flower developing into a mature fruit, and this may reflect the abortion of flowers receiving low-quality (heterospecific) pollen.

An additional indicator for a proportion of pollinated flowers being aborted is the rate of pollination compared to fruit set, as, with just few exceptions, we find a higher ratio of pollinated stigmas than of flowers developing into fruits. The potential to abort fruits and seeds of low quality in self-compatible plants allows both for selection of outcross progeny when sufficient outcross pollen is deposited on stigmas, as well as for reproduction via selfing when the availability of outcross pollen is low (Becerra and Lloyd 1992; Vaughton and Carthew 1993), which may be part of a mechanism of reproductive assurance in *A. eminens* (Kalisz and Vogler 2003). For the annual *Clarkia unguiculata* Lindl. (Onagraceae), Bowman (1987) argued that a preference for outcross pollination while allowing for autogamy may be an adaption to a frequent, potentially annual need to replenish the seed bank of the species. The strategy of *Acanthus* may be similar, as seeds germinate rapidly after watering, and random strong rain events during late dry season may result in the loss of large parts of the seed crop when seedlings dry out before continuous rains set in. Generally, seeds are short-lived, and likely do not remain viable until the next flowering period.

Reproductive success in either species was similar in hand-pollination experiments using self- and cross-pollen (Dietzsch 2004), but it is possible that self-pollen is less likely to set seed when both pollen types compete on a stigma (Bateman 1956; Bowman 1987). However, other limitations on fruit set unrelated to pollination may cause independence of fruit set from stigmatic pollen loads. For example, pollination may not play a role in determining fruit set when exceedingly more flowers are pollinated than may develop into fruits due to resource limitations.

Early-developing fruits as well as fruits resulting from outcrossing and those containing more seeds of high quality may have a higher likelihood of developing under resource limitation in multi-flowered plants (Stephenson 1981). In many species, late-flowering flowers have a reduced chance of maturing into seeds when early flowers are successfully pollinated. These later 'surplus flowers' may be interpreted as 'reserve ovaries' which mature only when earlier fruits are lost (Ehrlén 1991), and will not develop otherwise due to resource limitations. It is also possible that a proportion of flowers acts as 'decoy' for parasites, abortion of infected flowers reducing parasitism rates of developing fruits and seeds (Ghazoul and Satake 2009). In *Acanthus* we find few fruits and seeds to be damaged by predation, but uncommon, atypical rains during dry season may frequently occur, and have have been suggested to mitigate fruit development (Chapter 1).

The position of flowers opening one after another on an inflorescence may influence fruit set when it is resource limited (Emms 1996). A previous study has demonstrated temporal variability in fruit set based on inflorescence average (Chapter 1). In *Acanthus*, however, we do not find evidence for any variation in fruit or seed set in flowers in different positions within an inflorescence, and the ratio of pollinated stigmas does not vary during the reproductive period (Chapter 1). Hence, a divergent capability of flowers in different positions to develop into fruits and seeds may largely be ruled out. In addition, fruit set increases during the course of the reproductive period (Chapter 1), which is contrary to expectations when assuming a 'reserve ovary' system under unchanging pollination regimes, or resource limitation, which should increase as more fruits are developed over the course of the flowering season (compare Kwak and Jennersten 1991).

In *A. eminens*, on average three, in *A. polystachyus*, two or three out of four potential seeds are fully developed. Hence, we consider pollen quality of conspecific pollen grains on stigmas of flowers which develop into mature fruits to be moderate to high. This is in accordance with a putative abortion of fruits resulting from low-quality pollination as described above, as well as with generally high pollen quality.

A previous study of pollinator behaviour has indicated that pollen transfer between spatially close, and likely closely related individuals may cause low pollen quality in *Acanthus* (Chapter 3). This effect may be increased during flowering peaks (Melampy 1987; Elzinga *et al.* 2007) and in large individuals (Hessing 1988; Karron *et al.* 2003), as more flowers are available within short range of the pollen donor.

Both species are capable of selfing with high fruit and seed set (Dietzsch 2004), and geitonogamy may have no effect on fruit set or seed set in either species. However, it may affect population genetic diversity (Harder and Wilson 1998), and geitonogamous pollen transfer may be a factor strongly limiting outcrossing (Jong *et al.* 1993; Harder and Barrett 1995). Also, female reproductive success may be limited when seed quality, e.g. seed viability and seedling survival, is reduced through selfing or other effects (Rademaker *et al.* 1997; Campbell 2000). The putative benefits of reproductive assurance due to outcrossing can be mitigated by low quality resulting from autogamy (Vaughton *et al.* 2008), and in this case, seed production may be an inadequate predictor for reproductive success (Campbell 2000).

We find high germination rates for seeds classified as fully developed and healthy from all observed populations in either species, and consequently, seed quality does not seem to be an important factor limiting reproduction in *Acanthus*. A detailed survey of natural seedling performance and survival was beyond the scope of this study, but generally, only few seedlings are found in populations of either species (pers.obs.). However, the multitude of factors involved renders a prediction of future population development difficult (Price *et al.* 2008), and only long-term observations may reveal whether constraints on genetic diversity, pollination and reproduction are of major importance in *A. eminens* and *A. polystachyus*.

Many seeds are damaged before being released. Though a large proportion of seeds in either species is fully developed, a high proportion is infected by mould or rotten, with only some seeds being damaged by predators. We conclude that seed survival in *A. eminens* and *A. polystachyus* is mainly reduced through abiotic influences. This is in accordance with the findings of a previous study in which decreasing humidity due to the progress of dry season was found to increase fruit set in either species. Fruit capsules of *Acanthus* are dehiscent when dry, but with the onset of rainy season, unopened capsules and the seeds in them will invariably succumb to mildew (Chapter 1).

Apart from being influenced by selection on flowers and during flowering, early stages of reproductive phenology like budding and anthesis have been found to be adapted to optimized fruiting phenology, ensuring that ripe seeds are released under optimal conditions to facilitate germination and seedling survival (Rathcke and Lacey 1985; Schaik *et al.* 1993). In either species of *Acanthus*, seeds germinate within a few days after watering (pers. obs.). Seedlings are susceptible to drought, and consequently, release of seeds in *Acanthus* is optimally timed at the end of dry season, when conditions are ideal for the dehiscence of fruit capsules and the beginning rainy season ensures that seeds receive enough water during their establishment.

In *A. eminens*, an increase in fruit set for populations close to *A. polystachyus* has been described (Chapter 1). As explanation, it has been proposed that, for *A. eminens*, populations close to the congener provide better climatic conditions for reproduction than those deeper within the forest habitat. As fruit set is not linked to stigmatic pollen loads, which in turn could not be linked to pollinator visitation rates, a possible explanation for the negative link found between *X.* sp.1 visitation rates and seed set in *A. eminens* also is provided by the susceptibility of fruit set to humidity in *Acanthus*: *X.* sp.1 is more common in populations with dense canopy (3), which in turn are likely more humid than those in more open forests, and consequently display lower fruit set.

In addition, there is evidence that humidity may also play a role in determining seed set and seed health in *A. eminens*, which are reduced in populations under dense canopies. We do not find evidence for this effect in *A. polystachyus*, but we consider it likely that the species shows the same susceptibility to humidity as *A. eminens*, but is less affected by it as it grows in more open, drier habitat.

CONCLUSIONS

Fruit and seet set in *A. eminens* and *A. polystachyus* are mitigated by increased humidity. Though *A. eminens* seems to be more susceptible to moisture, we consider it likely that this is due to its more humid habitat. Pollinators seem to be of low effectiveness, and reproduction in both species may be limited by pollen deposition as pollen quantities on stigmas are low. In addition, the species are self-compatible and, consequently, they may be subject to inbreeding depression. This effect may be more pronounced in the spatially isolated populations of *A. eminens*. As spatial isolation may be increased due to anthropogenic habitat fragmentation, it is of interest for conservation to assess whether inbreeding affects reproduction in *A. eminens* and *A. polystachyus*. However, low fruit and seed set may also be caused by resource limitation, and additional studies on the effect of pollen quantity and quality on the reproduction of *A. eminens* and *A. polystachyus* are needed to determine whether the pollination of either congener is affected by habitat change.

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Appendix

Table 4.A: Reproductive success of flowers in different populations of *A. eminens* and *A. polystachyus* in 2008.

A. eminens		n[a]	Fruit set	Seed set[b]		Germination	Cumulative[b]
				Mature	Undamaged		
Buzambuli		38/13	0.252±0.134	0.723±0.184	0.162±0.241	0.551	0.012
Colobus		40/22	0.322±0.164	0.725±0.148	0.138±0.180	0.458	0.010
Ikuywa		30/14	0.286±0.184	0.811±0.136	0.255±0.325	0.571	0.027
Kisere	A	37/35	0.358±0.127	0.792±0.147	0.283±0.248	0.786	0.028
	B	21/4	0.314±0.173	0.744±0.175	0.170±0.302	0.333	0.010
Rondo		30/27	0.305±0.193	0.820±0.103	0.363±0.273	0.712	0.050
Salazar	1	20/4	0.378±0.142	0.762±0.158	0.186±0.278	0.167	0.004
	2	23/13	0.319±0.220	0.613±0.250	0.207±0.244	0.544	0.025
Mean			0.317±0.039	0.749±0.066	0.221±0.075	0.515±0.198	0.021±0.015
A. polystachyus							
Buzambuli		26/22	0.174±0.128	0.851±0.098	0.365±0.199	0.900	0.042
Colobus		29/26	0.196±0.131	0.790±0.140	0.386±0.189	0.846	0.043
Isecheno		30/26	0.265±0.109	0.812±0.156	0.345±0.223	0.917	0.035
Kisere		27/20	0.210±0.122	0.754±0.121	0.312±0.206	0.758	0.029
Salazar		19/4	0.199±0.112	0.716±0.171	0.088±0.170	0.542	0.005
Mean			0.209±0.031	0.784±0.052	0.299±0.121	0.793±0.153	0.031±0.015

[a] Number of plants sampled for fruits/number of plants in germination experiment

[b] Ratio based on four potential seeds per flower

Table 4.B: Reproductive success of flowers in different populations of *A. eminens* and *A. polystachyus* in 2009.

A. eminens							
		n[a]	Fruit set	Seed set[b]		Germination	Cumulative[b]
				Mature	Undamaged		
Buzambuli		16/5	0.200±0.086	0.613±0.276	0.047±0.111	0.900	0.002
Colobus		19/9	0.156±0.093	0.566±0.283	0.013±0.057	0.139	0.000
Ikuywa		30/13	0.293±0.120	0.682±0.195	0.031±0.050	0.514	0.002
Kisere	A	36/30	0.350±0.135	0.733±0.171	0.261±0.143	0.706	0.011
	B	28/5	0.165±0.073	0.406±0.300	0.011±0.048	0.720	0.000
Rondo		28/17	0.210±0.126	0.638±0.265	0.059±0.124	0.767	0.003
Salazar	1	24/11	0.222±0.102	0.630±0.211	0.059±0.133	0.831	0.002
	2	22/7	0.236±0.106	0.579±0.204	0.020±0.068	0.427	0.001
Yala		21/2	0.287±0.133	0.588±0.221	0.008±0.025	1.000	0.000
Mean			0.236±0.064	0.604±0.091	0.057±0.079	0.650±0.254	0.002±0.003
A. polystachyus							
Buzambuli		30/27	0.242±0.085	0.557±0.181	0.171±0.137	0.857	0.003
Colobus		30/21	0.205±0.075	0.702±0.124	0.269±0.185	0.863	0.005
Isecheno		30/29	0.263±0.073	0.664±0.165	0.273±0.180	0.830	0.005
Kisere		9/9	0.256±0.107	0.684±0.209	0.431±0.334	0.833	0.016
Salazar	1	25/25	0.256±0.072	0.776±0.111	0.449±0.164	0.928	0.007
	2	29/27	0.272±0.072	0.744±0.144	0.301±0.171	0.953	0.002
Yala		28/21	0.223±0.094	0.542±0.201	0.132±0.114	0.961	0.001
Mean			0.246±0.024	0.667±0.089	0.289±0.119	0.889±0.057	0.006±0.006

[a] Number of plants sampled for fruits/number of plants in germination experiment

[b] Ratio based on four potential seeds per flower

Table 4.C: Ratio of stigmas found with four or more *Acanthus* pollen grains and flower visitation rates in $\frac{visits}{flower \cdot h}$ of *X.* sp.1, *X.* sp.2 and all *Xylocopa* bees in populations of *A. eminens* and *A. polystachyus*. Flowering season 2009. mean±se.

A. eminens				
	X. sp1	*X.* sp2	All *Xylocopa*	Ratio of pollinated stigmas
Buzambuli	0.26±0.09	0.02±0.01	0.40±0.11	0.51±0.09
Colobus	0.51±0.12	0.25±0.08	0.81±0.18	0.61±0.09
Ikuywa	0.19±0.08	0.12±0.07	0.54±0.13	0.55±0.11
Kisere A	0.09±0.06	0.23±0.16	0.70±0.31	0.45±0.08
Kisere B	0.56±0.10	0.31±0.14	1.19±0.19	0.39±0.07
Rondo	0.13±0.07	0.06±0.05	0.28±0.10	0.42±0.07
Salazar 1	0.30±0.09	0.04±0.04	0.34±0.10	0.58±0.12
Salazar 2	0.42±0.11	0	0.46±0.11	0.64±0.06
Yala	0.38±0.15	0.04±0.03	0.43±0.14	0.63±0.09
A. polystachyus				
Buzambuli	0.07±0.05	0.27±0.09	0.64±0.14	0.42±0.09
Colobus	0.03±0.03	0.21±0.09	0.46±0.11	0.26±0.05
Isecheno	0.12±0.09	0.03±0.03	0.23±0.12	0.41±0.08
Kisere	0.17±0.12	0.55±0.21	0.75±0.29	0.48±0.08
Salazar	0.05±0.04	0.31±0.10	0.63±0.16	0.54±0.05
Yala	0.09±0.04	0.03±0.03	0.39±0.10	0.46±0.07

5 EXPERIMENTAL ASSESSMENT OF POLLEN LIMITATION IN TWO SPECIES OF *ACANTHUS* L. IN KAKAMEGA FOREST, KENYA

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ABSTRACT

In the sympatric, co-flowering species *Acanthus eminens* CLARKE and *A. polystachyus* var. *polystachyus* DELILE, previous studies have found less than two thirds of stigmas to be pollinated with more than one pollen grain per ovule. Low fruit set per flower with moderate to high seed set per fruit has lead to the conclusion that reproduction in either species may be pollen-limited. In addition, it has been suggested that low genetic diversity may affect reproduction of the species. In this study, we compared reproductive success of naturally pollinated flowers and supplementary hand-pollinated flowers receiving pollen from different sources. We find strong evidence for pollen quantity limitation in either species, as both treatments significantly increase fruit set, but not seed set and germination rates, in either species as compared to control flowers. However, there are no significant differences between the effects of pollen from different sources, indicating that pollen quality does not limit reproduction in either species of *Acanthus* in our study area.

INTRODUCTION

In flowering plants, it is a common phenomenon that a large proportion of flowers does not set seeds, and that less seeds per ovule than possible are produced under natural conditions (Willson 1979; Sutherland 1986; Horvitz and Schemske 1988). The ultimate reason for the development of these surplus flowers may not be their own fertilization and seed set. For example, they may serve as reserve flowers in case fertilized ovules are lost (Ehrlén 1991), increase reproduction under comparatively rare favourable conditions (Udovic and Aker 1981; Aker 1982), or act as decoys for parasites (Ghazoul and Satake 2009) or as pollen donors in diocious plants (Willson 1979; Horvitz and Schemske 1988). In addition, they may be sterile attractants for pollinators, e.g. in Asteraceae, or even serve as reward for pollinators as found in *Ficus*.

On the other hand, fruit set is often limited by resource limitation, pollen quantity or quality, or by a combination of these factors (Lloyd 1980; Stephenson 1981; Haig and Westoby 1988; Campbell and Halama 1993). Pollen limitation (PL) of reproductive success has been demonstrated to be common, with 62% of 258 species in studies reviewed by Burd (1994), and 73% of 85 species in studies reviewed by Ashman *et al.* (2004) being pollen limited. Insufficient pollen transfer to stigmas may be caused by low pollinator visitation rates due to low pollinator abundance (Motten 1986) and diversity (Ghazoul 2005). In addition, pollinators may be ineffective, depositing only few pollen grains per visit (Chapter 4).

Low fruit set may be prevalent even when low pollen quantity and resource limitations can be ruled out (McDade and Davidar 1984), and generally is a common phenomenon in plants (Lloyd 1980; Sutherland 1986). One possible explanation for this finding is limited pollen quality (Ashman *et al.* 2004; Aizen and Harder 2007). Whether pollen is of low pollen quality may depend on the degree of relatedness between parent individuals. In plants with low selfing rates, success of self-pollination by autogamy or by transfer of pollen within the same individual is limited and the same effect may extend to closely related plants, especially when populations suffer from inbreeding depression. For example, Vaughton and Ramsey (2010) have demonstrated that autonomous self-pollination is responsible for a significant reduction of seed set in *Bulbine bulbosa*.

As pollinator abundance and behaviour may strongly depend on the local floral community, e.g. through plants competing for pollinators or facilitating each other's visitation (Levin and Anderson 1970; Waser 1978; Laverty 1992; Ghazoul 2006), both the quantity and quality component of pollen limitation may be habitat dependent. Recently, PL has been demonstrated to be more common in ecosystems with higher biodiversity (Vamosi *et al.* 2006), which may be attributed to greater competition for pollinators or to the greater likelihood of habitat degradation and its effects (Hegland and Totland 2008). Species generally experiencing low visitation rates are more likely to experience PL, and anthropogenic habitat change may have an effect on its extent when species colonize new habitats where their pollinators are rare, or habitat fragmentation disrupts natural pollination processes (Aizen and Feinsinger 1994; Steffan-Dewenter and Tscharntke 1999; Ashman *et al.* 2004).

In habitats with high flower diversity, pollen quality may be mitigated when outcrossing rates are reduced due to competition for pollinators (Bell *et al.* 2005). Though the presence of heterospecific pollen may reduce the ability of conspecific pollen to germinate and develop (Kanchan and Chandra 1980), Morales and Traveset (2008) have demonstrated that the loss of pollen during visits on heterospecific flowers (Murcia and Feinsinger 1996) and during foraging flights (Inouye *et al.* 1994; Rademaker *et al.* 1997; Johnson *et al.* 2005) are more important in limiting plant reproduction than actual pollen transfer between heterospecifics. This effect might be of great importance in isolated populations which may be harder to locate for or avoided by pollinators (Groom 1998). In addition, bees flying shorter distances are likely to deliver more pollen than those crossing greater distances (Herrera 1987).

Reproduction fails in small populations when their viability decreases disproportionally with their size (Allee *et al.* 1949). In plants, this effect may be attributed to a lower attractiveness of these populations to pollinators (Schemske 1980; Mehrhoff 1983; Sowig 1989; Jennersten 1988; Klinkhamer and Jong 1990), and to lower pollen quality on stigmas due to close relatedness of parent individuals (Lamont *et al.* 1993; Groom 1998).

Several problems have been identified which complicate experimental assessments of pollen limitation. For example, seed set may not be a reliable predictor for reproductive success when seed quality, e.g. in terms of seed weight and seed viability, varies. Both are rarely measured together with fruit and seed set (reviewed by Ashman *et al.* 2004; Knight *et al.* 2005, but see Hegland and Totland 2008). Seed quality and seedling performance is commonly higher when seeds result from cross-pollination (Price and Waser 1979; Schoen 1983; Waser and Price 1983; Waller 1984; Mitchell-Olds and Waller 1985). In self-compatible flowers, discrimination against pollen tubes of self-pollen in the presence of cross-pollen is considered to be a strategy to increase offspring quality when cross pollination is frequent, simultaneously assuring reproductive success through self-pollination when it is not (Bowman 1987).

There may be tradeoffs between seed number and quality (e.g. Primack 1987; Ågren 1989), and surplus pollen on stigmas may allow for higher seed quality through increased male competition (Snow 1986) or female choice (Marshall and Ellstrand 1988, see also Hegland and Totland 2008). Potential mechanisms responsible for this effect are selective abortion of low-quality progeny (Janzen 1977; Lee 1984), and differences in pollen tube growth between self- and cross-pollen (Stout 1920; Arasu 1968; Weller and Ornduff 1989; Manasse and Pinney 1991; Montalvo 1992), both of which may increase average offspring quality.

In *Acanthus eminens* and *A. polystachyus*, afrotropical shrubs growing in sympatry in the area of Kakamega Forest in Western Province, Kenya, previous studies have found strong indications for pollen limitation. In Chapter 1 and Chapter 4, we found less than two thirds of stigmas of either species with more than four adherent pollen grains, and on average, less than four out of ten flowers of either species to develop into fruits. However, seed set and germination rates in either species were moderate to high. Though this suggests that pollen quality is high, this assumption has not been tested. In Chapter 3, we demonstrated that pollen transfer in *Acanthus* may be largely geitonogamous, and though plants were found to be highly capable of selfing (Dietzsch 2004), it is not clear whether pollen transfer within the same population reduces seed viability.

High fruit and seed set after self-pollination in treatments excluding pollinators suggest that self-pollen is not detrimental to fruit and seed set in *Acanthus* (Dietzsch 2004).

In a previous study, we found pollinators (carpenter bees, genus *Xylocopa*), to visit flowers of *Acanthus* with moderate frequency (Chapter 3), but transferring little pollen (Chapter 4). Hence, though bees commonly have been found to carry more pollen grains than other taxonomic groups (see Herrera 1987 and citations therein), *Xylocopa* either carry little pollen or few pollen grains are transferred from bees to stigmas. However, a higher ratio of stigmas were found to be pollinated with congeneric pollen than flowers were found to develop into fruit. This indicates that either some proportion of these pollen was heterospecific, and hence, incompatible. Alternatively, some pollen may have been of low quality, either not germinating at all or causing abortion of the flower during pollen tube or seed development.

Habitat loss and habitat change may cause isolation between populations of *A. eminens*, which in turn may decrease pollination success (Jennersten 1988; Lamont *et al.* 1993; Rathcke and Jules 1993). In addition, its spatially distinct populations, surrounded by a matrix which may be less suitable for bees than the surrounding farmland, may suffer from inbreeding depression, reducing the vigour of offspring from self-pollination or crosses with near relatives. This may be due to the expression of recessive deleterious genes, which is possible at any point during an individual's life-cycle (Lande and Schemske 1985; Charlesworth and Charlesworth 1987; Husband and Schemske 1996).

A common method to assess pollen limitation in plants is by comparing reproduction of flowers receiving supplementary cross-pollen with naturally pollinated controls (Ashman *et al.* 2004; Knight *et al.* 2006; Aizen and Harder 2007). However, this alters both the quantity of pollen as well as the quality of pollen (Ashman *et al.* 2004; Aizen and Harder 2007; Vaughton and Ramsey 2010). Vaughton and Ramsey (2010) have proposed an experimental protocol using emasculated flowers, excluding self-pollination. However, it is likely that geitonomamous pollen transfer is prevalent in either species of *Acanthus* (Chapter 3); and as an emasculation of all flowers of an individual was impossible, and since populations may largely consist of clonal and closely related individuals, we would have had to emasculate all flowers of a population to completely exclude the possibility of self-pollen transfer.

It has also been proposed that for an experimental assessment of pollen limitation, all flowers of a single plant should receive the same pollination treatment, as otherwise an increase in reproductive success of treated flowers could be caused by resource allocation to these flowers, whereas the overall seasonal or lifetime reproductive success of the individual could remain constant (Bawa and Webb 1984; Zimmerman and Aide 1989). However, this is not feasible in large individuals which flower over long periods. Still, resource allocation may be different even within an inflorescence (Wesselingh 2007), and therefore, flowers assigned to treatment and control have to be selected with care.

In this study, we experimentally test whether pollen quantity and pollen quality influence fruit set, seed set and seed viability of *A. eminens* and *A. polystachyus*. To reduce the effects of divergent flower position as much as possible, we compare the reproductive success of hand-pollinated flowers receiving conspecific pollen from within the same or from another population with the reproductive success of the opposing, naturally pollinated flower of the same decussate inflorescence. We hypothesize that 1) reproduction is limited by pollen quantity, and that supplementary hand-pollination increases fruit set. In accordance with the findings presented in Chapter 4, we further propose that 2) pollen quality does not influence reproductive success, and that fruit set, seed set and seed viability are similar in supplemental pollen treatments, regardless of pollen source.

MATERIALS AND METHODS

STUDY AREA

Our study was conducted in Kakamega Forest in Western Kenya (0"17ON, 34"54OE). Kakamega Forest is considered the easternmost remnant of the lowland Guineo-Congolian rain forest belt, and forest fragmentation has led to the formation of five fragments (130 to 1,400 ha) and a remnant main forest (8,500 ha, Schaab *et al.* 2010) comprising a total of 12,000 ha of rainforest vegetation at an altitude between 1,500 to 1,700 m above sea level. It is managed by the Kenyan Wildlife Service (KWS) in the north and the Kenyan Forest Service (KFS, former Forest Department) in the south. Mean monthly temperatures range from 11°C to 29°C, with an average temperature of 22°C. Rainfall averages 2,000 mm per year, with two distinct rainy seasons between March and May ('long rainy season') and September and November ('short rainy season') and two distinct dry seasons between December to February ('dry season') and from June to August ('cold dry season'). The forest is under significant pressure due to illegal logging, firewood collection, grazing, and hunt for bushmeat, and large parts are highly disturbed (Schaab *et al.* 2010). The forest is surrounded by densely populated (336 to 746 inhabitants per km^2, Mitchell 2004), highly diverse and structured farmland with 0.2 to 0.7 ha of land per household (Greiner 1991; Kenyan Ministry of Agriculture 2006). 62% of all households generate their income from agriculture, and the district's poverty rate is about 52% (Dose 2007).

PLANT SPECIES

We studied two species of *Acanthus* L.. *Acanthus eminens* CLARKE is a shrub growing in clearings in primary and old secondary mountain rainforest between 1,500 and 2,800 m above sea level in Kenya (Beentje 1994). It is also found on the Ugandan side of Mt Elgon (pers. obs.), as well as in Ethiopia, and in the Imatong Mts in Sudan, very likely spreading to north Uganda (Vollesen 2007). Although not a rare species in Kenya, it is limited to highland and mountaineous forest areas, which have suffered heavily from deforestation in more recent history. *A. eminens* grows up to five meters in height, carries spiny, robust leaves and flowers in decussate spikes. Plants may flower at relatively small size, carrying only one inflorescence, but most carry several, and up to over 100 inflorescences. The species is capable of vegetative reproduction, and forms distinct populations of between 65 and 2,574 (median: 142) plants in the Kakamega Forest area, as well as relatively continuous, but less dense, stands along riversides.

Acanthus polystachyus var. *polystachyus* Delile (referred to as *A. polystachyus*) grows along forest edges and grasslands inside forests between 1,100 and 2,500 m above sea level in Ethiopia, Eastern Sudan, Eastern Uganda and Tanzania. In Kenya, it occurs only in Western Province and is missing on the plains around lake Victoria (Vollesen 2007). In the Kakamega area, it is often found invading grasslands and is part of natural forest regeneration. *A. polystachyus* does not form distinct populations as it grows in more or less loose clusters in small copses and hedgerows, also forming dense, continuous stands along forest edges. Morphologically similar to *A. eminens*, *A. polystachyus* has softer, hairy leaves and is generally larger than *A. eminens*. Its inflorescences are slightly larger than those of its congener, and a single plant carries up to several hundred of them.

Flowers of either species are zygomorphic, and of similar size and functionality, but different colour (Chapter 2). Both species display a distinct peak of flowering during winter dry season between October and February. *A. polystachyus* also flowers sporadically throughout the year. Both species display protandry, with female receptiveness being indicated by the bending of the previously straight stigma one to two days after anther dehiscence. Both species are self-compatible (Dietzsch 2004). In the study area, the species are pollinated by the same group of bee species, carpenter bees of the genus *Xylocopa* (Dietzsch (2004), Chapter 2, 3).

Supplementary pollination

In the beginning of both species' flowering season in 2008/2009, we randomly marked two flowers of up to 30 plants of nine and five populations of *A. eminens* and *A. polystachyus*, respectively. Flowers were colour-coded on their bracts with acrylic paint, assigning them to one of two treatments, 'local' and 'remote' pollination. Flowers in the latter treatment received a mixture of pollen from five different conspecific plants of another than the focal population. Pollen was collected from a randomly chosen population, extracted and mixed with toothpicks in a 1.5 ml reaction tube, and applied as soon as possible. Application was standardized, with three strokes of a pollen-covered toothpick over the stigma. For the 'local' treatment, flowers were prepared using similar methodology, with pollen samples collected from five conspecific plants of the same population as the focal plant. Pollen was stored for thirty minutes before application to exclude potential effects of differences in storing time to 'remote' treatment. Toothpicks were discarded between treatments. In each population, both treatments were applied on the same day. All treatments were applied by the same experimenter (N.H.). As control, we observed and sampled the corresponding opposite untreated flower on the same decussate inflorescence.

Fruits were collected shortly before dehiscence and individually stored in paper bags. On collection, we noted the overall number of flowers of each inflorescence, as well as its total fruit set. Each fruit was dried until release of seeds. Seeds of each fruit were counted and each assigned to one of three categories (underdeveloped, fully developed and healthy, fully developed and damaged). In the latter category, we also noted the kind of damage (predation marks, mould etc.). Each seed category for each flower was stored individually.

Seeds classified as both fully developed and healthy were weighed and sown into labeled wells of 54-well trays at the greenhouse facilities of Bielefeld University, Germany. Seeds from both species, all populations and all treatments and controls were randomly assigned to wells to avoid environmental effects. Trays were rotated weekly to further reduce environmental effects on development, and watered to field capacity every two days. Germination and seedling development were observed twice per week during the first four weeks after first watering. No seeds germinated after more than seven days after the beginning of observations.

Calculation of reproductive success

We calculated reproductive success as the product of the ratio of fruits found per flower, the ratio of fully developed, healthy seeds in each fruit capsule, and germination rate of these seeds. Hence, the resulting value for reproductive success represents the number of potential seedlings produced by a single flower in each population, theoretically ranging from zero (no seedling) to one (four seedlings).

Average fruit set, seed set, and seed viability values were calculated for each treatment (local, local-control, remote, remote-control) and population. Comparisons are based on population means.

Statistics

All data sufficed conditions for parametric analysis. Though an Anova was possible, we were not interested in all potential pairwise comparisons (e.g., we were not interested in comparisons between species, or between results from treated 'local' and control 'remote' flowers). Hence, we used pairwise t-tests for comparison of success of treated and untreated ('control') flowers for each treatment. Results were corrected by Bonferroni-Holm procedure for multiple comparisons, as implemented in R (R Development Core Team 2011).

We tested whether there is a difference between treatment effects in fruit set and seed set. For each treatment and population, we calculated the relative increase or decrease in fruit set or seed set by dividing the number of fruits/seeds resulting from supplementary pollination by the respective value from controls. Differences between these values were assessed by pairwise t-tests.

In *A. polystachyus*, treated flowers were destroyed in several populations due to trimming of hedgerows, likely by firewood collectors, reducing sample sizes. In addition, sample size is partly reduced in each consecutive step from fruit set to germination when fruits did contain few seeds, or when all seeds were damaged and not available for germination experiments. t-test statistics were only calculated when both groups contain three or more samples. Due to low sample sizes, a comparison between treatments was only possible for fruit set in both species, and seed set in *A. eminens*.

RESULTS

Untreated flowers displayed an average fruit set of 0.23±0.16 fruits per flower in *A. eminens*, and 0.24±0.11 fruits per flower in *A. polystachyus* (for population values, see Appendix, Tab. 5.A). This is in accordance with previous finding on fruit set of *Acanthus* in another survey during the same flowering period (Chapter 4), as is the ratio of fully developed seeds (*A. eminens*: 0.69±0.16, *A. polystachyus*: 0.59±0.10, for population values, see Appendix, Tab. 5.B). Less than five percent of fruits stopped to develop before reaching maturity, and the ratio of these aborted fruits did not differ between treatments ($p > 0.05$).

In *A. eminens*, fruit set of untreated flowers was significantly lower than in flowers additionally receiving local ($t = 6.402$, $df = 8$, $p < 0.001$) and remote pollen ($t = 4.117$, $df = 8$, $p = 0.003$), and fruit set in *A. eminens* is generally pollen limited (Fig. 5.1). In *A. polystachyus*, fruit set is increased by hand pollination with remote pollen ($t = 4.626$, $df = 2$, $p = 0.044$), the effect being only marginally significant when flowers were treated with local pollen ($t = 2.490$, $df = 3$, $p = 0.088$). However, sample sizes are small, and our results indicate a similar degree of pollen limitation as for *A. eminens*.

Both in *A. eminens* and *A. polystachyus*, there were no significant differences between values for seed health in either treatment (Fig. 5.2. *A. eminens* – Local treatment: $t = -0.628$, $df = 8$, $p = 0.548$; Remote treatment: $t = -0.628$, $df = 8$, $p = 0.548$. *A. polystachyus* – Local treatment: $t = -0.851$, $df = 2$, $p = 0.485$; Remote treatment: sample size too small ($n = 2$)).

There were no differences in the effects of the separate treatments in either species (Fruit set: *A. eminens* – $t = -0.637$, $df = 8$, $p = 0.542$, *A. polystachyus* – $t = -0.335$, $df = 2$, $p = 0.770$. Seed set: *A. eminens* – $t = -0.743$, $df = 4$, $p = 0.499$, *A. polystachyus* – $t = -0.921$, $df = 1$, $p = 0.526$. For population-wise ratios, see Appendix Tab. 5.C).

As both quantity and quality of pollen may depend on isolation of habitats, we tested whether the difference in reproductive success between treatments differs between habitats of either species, using data on forest cover in a radius of up to 2,000 m around each population (see Chapter 1). However, linear models did not reveal any link between the ratio of fruit set or seed set between treatments and forest cover at all spatial scales ($p > 0.05$), and consequently, details are not included in this study.

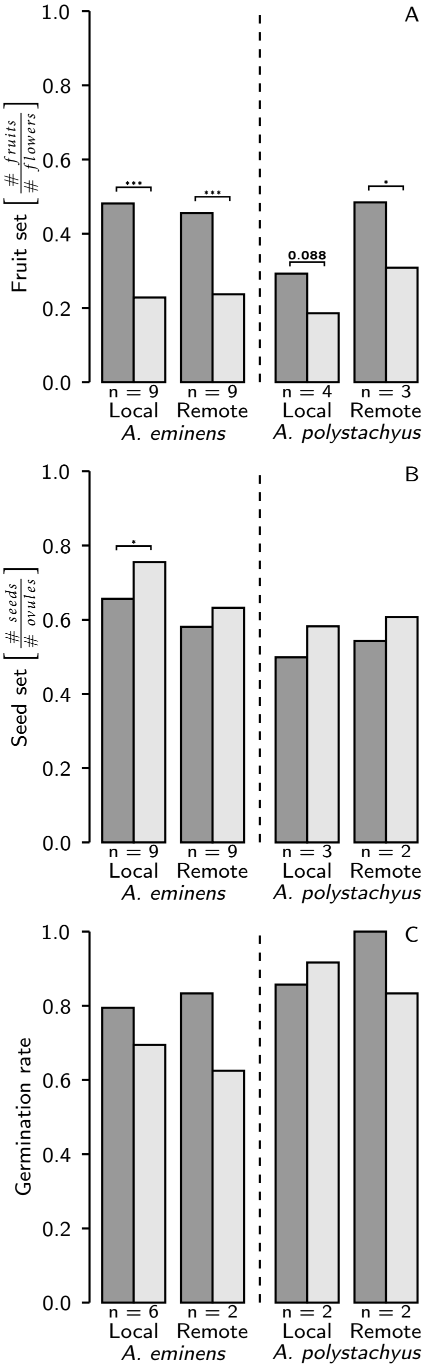

Figure 5.1: Ratio of fruit set per flower (A), seed set of fully developed seeds per fruit (B), and germination of fully developed, healthy seeds (C) from treatments (dark grey bars) and controls (light grey bars) in populations of *A. eminens* and *A. polystachyus*. Flowers received either 'local' (conspecific pollen from five different plants from the same population as the treated flower) or 'remote' (likewise, but from another population) pollen. Stars indicate significance (*: p ≤ 0.05, **: p < 0.01, ***: p < 0.001). Numeric p-values provided for marginally significant values (0.05 < p ≤0.1).

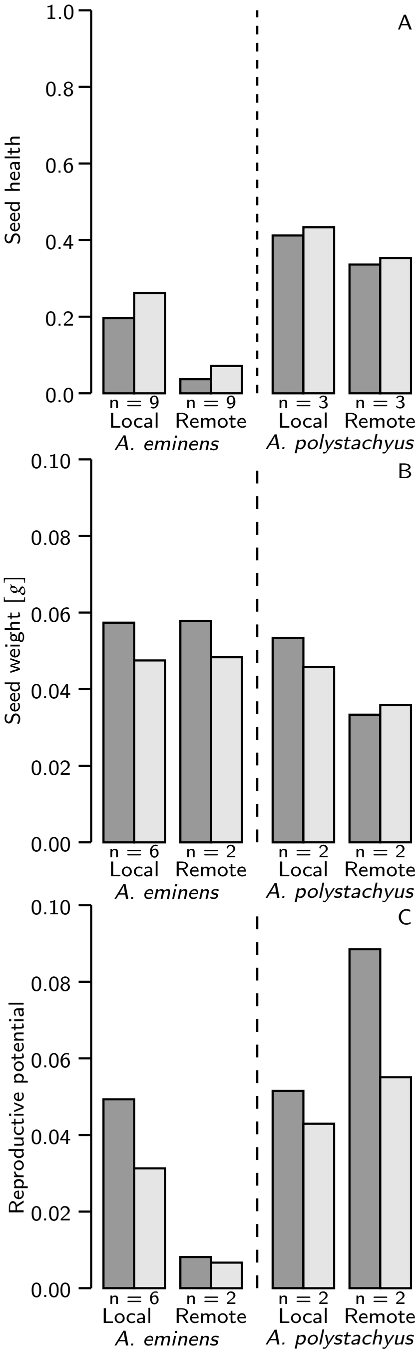

Figure 5.2: Seed health (A), seed weight (B) and reproductive potential (C) from treatments (dark grey bars) and controls (light grey bars) in populations of *A. eminens* and *A. polystachyus*. Flowers received either 'local' (conspecific pollen from five different plants from the same population as the treated flower) or 'remote' (likewise, but from another population) pollen. Seed health is expressed as ratio between viable fully developed seeds and fully developed seeds per flower. Reproductive potential is expressed as the average number of seedlings resulting from single flowers (four ovules). All comparisons (calculated for n > 2 only) were not significant.

Discussion

Fertilization is one of the most crucial steps in plant reproduction, and depends on the transfer of sufficient pollen quantities and the quality of pollen deposited on on receptive stigmas (Herrera 1987, 1989). In the congeners *Acanthus eminens* and *A. polystachyus*, both have been proposed as factors limiting fruit set, which along with seed health is the strongest factor limiting reproduction in either species (Chapter 4).

In this study, we find fruit set strongly limited by pollen quantity, with supplementary hand-pollination of otherwise untreated flowers nearly doubling the likelihood of flowers developing into fruits. In contrast, we do not find conclusive effects on seed set or germination rates of seeds from treated and control flowers.

Stephenson (1981) has argued that a higher likelihood of hand-pollinated flowers to set fruit does not necessarily indicate pollen limitation on fruit set. For example, the resources of an individual may be cumulatively depleted by each flower setting fruit, reducing the likelihood of fruit and seed set in subsequent flowers. In this case, the total reproductive success of an individual during a flowering season does not change through supplementary pollination. In addition, 'surplus' flowers may allow for high seed set when plants, by chance, receive high amounts of compatible, high-quality pollen (Haig and Westoby 1988; Burd 1994), and may represent an adaption of flowers to variability in pollination success.

However, as we used opposing flowers within the same inflorescence for our treatments, we believe any putative temporal effect of resource allocation to be neglectable, as there were several cases in which both flowers developed. Also, progressing resource limitation should render fruit set more likely in both earlier flowering inflorescences and earlier flowers, for both of which there was no indication in previous studies. On the contrary, fruit set increased over the course of the flowering season and did not vary between flowers in different positions on an inflorescence, while the ratio of stigmatic pollen loads did not vary over time (Chapter 1 and 4, respectively). Hence, on population level, pollen limitation does not seem to vary over time in *Acanthus*, even though this effect has been demonstrated in other species (Zimmerman 1980; Ramsey 1995).

It is also possible that levels of pollen limitation vary between flowering seasons, though a majority of studies conducted over more than one year reviewed by Ashman and Morgan (2004) found pollen limitation in all study years. The described temporal patterns of fruit set and stigmatic pollen loads were identical for two consecutive flowering seasons of *A. eminens* and *A. polystachyus* (Chapter 1). Though reproductive success may not be independent between seasons, as plants spending many resources during one reproductive period may suffer from resource limitation in the next (Janzen *et al.* 1980), there is no indication for such an effect in *Acanthus*. Our results corroborate that fruit set in *Acanthus* is strongly limited by the number of conspecific pollen grains on stigmas.

However, pollen and resource limitation are not mutually exclusive, and only half of hand-pollinated flowers in both *A. eminens* and *A. polystachyus* develop into fruits, indicating that both pollen and resource availability likely are limiting reproduction in *Acanthus*. Low fruit set per flower may represent the difficulty of assuring sufficient pollen transfer (Pleasants and Chaplin 1983; Southwick 1984, reviewed in Burd 1994). On the other hand, 'surplus' flowers which do not develop into fruits may represent an adaption to decrease pollen limitation, as a greater number of flowers may cause greater pollen grain availability (Conner *et al.* 1996; Totland *et al.* 1998; Maad 2000; Ehlers *et al.* 2002; Ehrlén 1993; Lawrence 1993; Sandring and Ågren 2009), and attract more pollinators.

Humidity has been shown to have a strong negative influence on fruit set in *Acanthus*, and additional flowers may also serve as reserve ovaries in case of occasional rain events. Fruits experiencing high levels of humidity will rot (Chapter 1), and irregular, infrequent rains are possible throughout the three-month peak flowering period of the congeners. Under these conditions, staggered flowering may be an adaption to the likelihood of fruits from earlier flowers being lost, at the same time allowing for high fruit set under very favourable conditions. As a way to increase pollination by their ineffective pollinators (Chapter 4), both species of *Acanthus* may display large numbers of flowers to attract more visitors over great distances and to serve as pollen donors, increasing the likelihood of successful pollen transfer (Willson 1979).

It is likely that several mechanisms, acting on different temporal and spatial scales, influence and determine the reproductive success and system of *Acanthus*. It may be advantageous for each species to develop large, attractive displays, at the same time producing much pollen and providing numerous flowers both as insurance against losses during early flowering and to allow for high reproductive success under optimal conditions.

Supplementary pollination more often increases fruit than seed set, which may be caused by an advantage of developing 'packages' of propagules due to resource usage efficiency (Burd 1994). Flowers of *Acanthus* contain four ovules, and consequently, a single visit from a pollinator transferring as little as four grains of pollen of adequate quality could in theory result in all ovules being fertilized. However, *Xylocopa* bees are ineffective pollinators, and stigmatic pollen loads in either species of *Acanthus* are low even though visitation frequencies are not (Chapter 3, 4).

As there is no difference between the two treatments, we conclude that quantity of pollen is limiting fruit set, while its quality is not. Though we were not able to collect enough fruits yielding fully developed, undamaged seeds to allow for statistical analyses of our germination experiment, we found a slight decrease in seed set in flowers of *A. eminens* receiving additional 'local' pollen. Similarly, Flanagan *et al.* (2009) found reduced seed set in flowers of *Mimulus ringens* L. (Phrymaceae) receiving hand-applied conspecific pollen compared to flowers visited by suitable pollinators. However, they attributed this finding to potential differences in pollen placement, which we consider no applicable explanation for a reduction in seed set in *Acanthus* due to our hand-pollination protocol.

Germination rates of seeds classified as fully developed and healthy were found to be generally high both in this study as well as in a more extensive germination experiment described in Chapter 4. It seems likely that seed quality and viability in *Acanthus* are generally high. This may be due to the abortion of low-quality offspring, which was already found to be likely due to the difference between the ratio of pollinated stigmas and the ratio of flowers setting seed described in Chapter 4. Following this line of thought, we cannot rule out natural transfer of outcross pollen from other populations to flowers receiving the 'local' treatment, and especially in the case of abortion of low-quality offspring, some proportion of seeds in the 'local' treatment may result from outcross pollination (Becerra and Lloyd 1992). However, this is unlikely as pollen transfer in *Acanthus* is generally infrequent (Chapter 4). Most pollen transfer in *Acanthus* probably is geitonogamous or happens between spatially close and closely related individuals (Chapter 3), but the number of fruits aborted while developing was low. If abortion is a factor responsible for a large reduction of fruit and seed set in *Acanthus*, it acts at a very early stage of fruit development, or even before fertilization (Becerra and Lloyd 1992; Vaughton 1995).

Due to the likelihood of populations of either species to consist of closely related individuals, as well as the probability of geitonogamous pollen transfer, populations of *Acanthus* may be inbred. Anthropogenic habitat fragmentation as found in Kakamega Forest may isolate populations, e.g. by reducing gene flow between populations, causing inbreeding and increasing its effects through a simultaneous reduction of habitat quality and pollinator abundance (Segal *et al.* 2006). This can affect seedling performance in natural populations, as inbreeding may reduce the ability of plants to adapt to unfavorable habitat conditions (Johnston and Schoen 1996; Ramsey and Vaughton 1998), and its effects may be more pronounced when habitat conditions change (Schemske 1983; Johnston 1992; Wolfe *et al.* 1998). Also, reproduction in small, isolated populations fails when small floral displays do not attract sufficient pollinators or when isolation deteriorates pollen quality (Groom 1998).

Small differences between the development and viability of fruits and seeds resulting from cross-pollination and pollen transfer from closely related individuals as found in *Acanthus* may be caused by rigorous purging of the genetic load of populations through previous inbreeding (Vaughton 1995) or selfing (Barrett and Charlesworth 1991) due to high amounts of selfing and pollen transfer between closely related plants (Levin 1984; Charlesworth 1989; Burbidge and James 1991). The colonization of new habitats by few individuals as found in *A. eminens* may result in close relatedness of all individuals of the species in the study area, with no or little genetic differentiation between populations and, consequently, between outcross- and self-pollen. For example, Vaughton (1995) found low levels of inbreeding depression in *Grevillea barklyana* F. MUELL. (Proteaceae), which she attributes to frequent purging of genetic loads. Alternatively, she considers that the species could chronically lack genetic diversity as it forms small, isolated population in disturbed areas resulting from a small amount of founder individuals, which is a highly similar strategy as found in *A. eminens*.

Changes in abundance, identity and diversity of pollinators, as well as their behaviour, influence pollen limitation (Gómez *et al.* 2010), and habitat change and forest fragmentation could affect all of these factors in *Acanthus* (Chapter 3).

Previous studies have speculated that geitonogamous pollen transfer could play a major role in the pollination of *Acanthus*. Whether plants benefit from self-pollination strongly depends on their ecological context (Darwin 1877; Holsinger 1991; Lloyd 1992; Barrett *et al.* 1996; Dudash *et al.* 1997; Fishman and Wyatt 1999; Eckert 2000; Fausto *et al.* 2001; Goodwillie 2001; Herlihy and Eckert 2002; Kalisz and Vogler 2003). Also, the frequency of outcross pollen transfer, itself dependent on various factors, strongly determines whether outcross- or self-pollination is prevalent in a population (Levin and Berube 1972; Jain 1976; Lloyd 1979; Schoen *et al.* 1996; Vogler and Stephenson 2001). When outcross pollen is rare or its transfer unreliable, autogamous seed set provides reproductive assurance (Darwin 1877; Baker 1955; Lloyd 1979). Consequently, self-compatibility may be an adaption to rapid spreading into isolated habitats after initial colonization in *Acanthus* (Baker 1955). Protandry may be an efficient barrier to selfing in founder populations, as a co-occurrence of both sexual flower phases is less likely in plants carrying few flowers than in individuals with large floral displays. Consequently, protandry may be an adaption to increase a founder population's genetic diversity through an increase in the ratio of seeds resulting from cross-pollination.

To have an effect on populations of either species of *Acanthus*, pollen limitation must limit e.g. population growth, population vigour, or even on the ability to spread into new habitats (Ashman and Morgan 2004; Horvitz *et al.* 2010). It is difficult to predict whether anthropogenic habitat change affect the reproduction and vigour of *A. eminens* in our study area, as to date there have been no studies on the dispersal and performance of *Acanthus* seeds in natural populations. Given the seemingly limited range of seed dispersal and the low likelihood of pollen transfer, populations in different habitat fragments could be isolated. However, the same applies for populations embedded in natural forest. A detailed analysis of genetic relatedness between populations and of seedling paternity, as well as studies on the movement of pollinators, e.g. through radio-tracking, may reveal whether anthropogenic disturbance affects genetic diversity and gene flow in *Acanthus*, and may help to decide whether low genetic diversity is an important factor influencing reproduction of the congeners.

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APPENDIX

Table 5.A: Fruit set per flower (mean±se) in hand-pollinated (T) and ‘control’ (C) flowers of *A. eminens* and *A. polystachyus*.

‘Local’ Treatment

	Fruit set		Ratio of aborted fruits		
	T	C	T	C	n
A. eminens					
Buzambuli	0.33±0.14	0.08±0.08	0.08±0.08	0	12
Colobus	0.36±0.09	0.04±0.04	0.04±0.04	0.04±0.04	28
Ikuywa	0.59±0.10	0.41±0.10	0	0	27
Kisere.A	0.33±0.07	0.17±0.06	0.10±0.05	0.03±0.03	29
Kisere.B	0.64±0.15	0.27±0.14	0.09±0.09	0	11
Rondo	0.74±0.09	0.39±0.10	0	0.04±0.04	23
Salazar.1	0.58±0.15	0.17±0.11	0	0	12
Salazar.2	0.40±0.11	0.35±0.11	0.15±0.08	0.15±0.08	20
Yala	0.40±0.16	0.20±0.13	0	0	10
A. polystachyus					
Buzambuli	0.23±0.12	0.08±0.08	0	0	13
Colobus	0.38±0.10	0.22±0.08	0.15±0.07	0	26
Salazar.1	0.15±0.10	0.15±0.10	0.08±0.08	0	13
Salazar.2	0.38±0.08	0.26±0.08	0.20±0.06	0.06±0.03	25

‘Remote’ Treatment

	Fruit set		Ratio of aborted fruits		
	T	C	T	C	n
A. eminens					
Buzambuli	0.25±0.10	0.10±0.07	0.15±0.08	0	20
Colobus	0.36±0.09	0.04±0.04	0	0.04±0.04	28
Ikuywa	0.58±0.12	0.47±0.12	0	0	19
Kisere.A	0.27±0.09	0.08±0.05	0.27±0.09	0.04±0.04	26
Kisere.B	0.65±0.12	0.06±0.06	0.12±0.08	0.06±0.06	17
Rondo	0.70±0.10	0.52±0.11	0	0	23
Salazar.1	0.37±0.09	0.22±0.08	0.07±0.05	0.07±0.05	27
Salazar.2	0.43±0.11	0.39±0.10	0.22±0.09	0.09±0.06	23
Yala	0.50±0.19	0.25±0.16	0	0	8
A. polystachyus					
Buzambuli	0.52±0.11	0.30±0.10	0	0	23
Colobus[a]	-	-	-	-	-
Salazar.1	0.30±.015	0.20±0.13	0	0	10
Salazar.2	0.63±0.11	0.42±0.12	0	0	19

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[a] *A. polystachyus* plants were destroyed in Colobus

Table 5.B: Seed set per flower (mean±se), seed weight (mean±sd) and ratio of germinated seeds (mean±se) in hand-pollinated (T) and 'control' (C) flowers of *A. eminens* and *A. polystachyus*.

'Local' Treatment

	Seed set per flower				Seed Weight[a] [g]		Germination			
	T	n	C	n	T	C	T	n	C	n
A. eminens										
Buzambuli	2.75±0.75	4	4	1	0.06±0.02	0.04	0.50±0.50	2	1.00	1
Colobus	2.70±0.33	10	2.50±1.50	2	0.05±0.01	0.03	0.50±0.50	2	0.00	1
Ikuywa	1.80±0.29	10	2.17±0.31	6	0.07±0.01	0.06±0.01	1.00±0.00	4	0.67±0.33	3
Kisere A	2.69±0.40	13	3.00±0.63	6	0.06±0.00	0.06±0.01	0.86±0.14	7	0.50±0.50	2
Kisere B	2.67±0.71	6	3	1	0.06±0.01	–	1.00±0.00	2	–	–
Rondo	2.65±0.26	17	2.80±0.39	10	0.07±0.02	0.06±0.01	1.00±0.00	2	1.00±0.00	2
Salazar 1	2.86±0.51	7	4	1	0.07±0.01	–	1.00±0.00	3	–	–
Salazar 2	2.78±0.32	9	2.71±0.61	7	0.03±0.00	0.05±0.02	0.50±0.50	2	1.00±0.00	4
Yala	2.75±0.48	4	3.00±1.00	2	–	–	–	–	–	–
A. polystachyus										
Buzambuli	1.33±1.33	3	2	1	–	–	–	–	–	–
Colobus	1.89±0.51	9	2.60±0.60	5	0.07±0.01	0.04±0.01	1.00±0.00	4	1.00±0.00	3
Salazar 1	–	–	–	–	–	–	–	–	–	–
Salazar 2	2.25±0.31	8	2.29±0.42	7	0.04±0.02	0.05±0.01	0.71±0.18	7	0.83±0.17	6

'Remote' Treatment

	Seed set per flower				Seed Weight[a] [g]		Germination			
	T	n	C	n	T	C	T	n	C	n
A. eminens										
Buzambuli	2.67±0.49	6	3.00±0.00	2	–	0.06	–	–	0	1
Colobus	2.29±0.52	7	1.50±1.50	2	–	–	–	–	–	–
Ikuywa	1.75±0.43	12	2.13±0.30	8	0.08	–	1	1	–	–
Kisere A	1.77±0.41	13	3	1	–	–	–	–	–	–
Kisere B	3.11±0.31	9	2.00±1.00	2	–	–	–	–	–	–
Rondo	2.75±0.37	16	2.75±0.35	12	0.06±0.02	0.07±0.01	1.00±0.00	3	1.00±0.00	3
Salazar 1	2.27±0.41	11	2.14±.059	7	–	–	–	–	–	–
Salazar 2	1.92±0.47	12	2.75±0.31	8	0.03±0.00	0.03±0.00	0.50±0.50	2	0.50±0.50	2
Yala	2.40±0.40	5	3.50±0.50	2	–	0.03	–	–	1	1
A. polystachyus										
Buzambuli	1.85±0.45	13	2.86±0.51	7	0.03±0.01	0.04±0.01	1.00±0.00	2	0.67±0.33	3
Salazar 1	–	–	–	–	–	–	–	–	–	–
Salazar 2	2.50±0.50	2	2.00±1.15	3	0.04±0.01	0.04±0.01	1.00±0.00	3	1.00±0.00	2

[a] for n-values, see Germination

Table 5.C: Fruit and seed set ratio between treatments. Values >1 indicate higher reproduction when flowers receive pollen from remote populations, values <1 indicate higher reproduction when flowers are supplementary pollinated with pollen from the same population.

	Relative change	
	Fruit set	Seed set
A. eminens		
Buzambuli	0.63	1.29
Colobus	1.00	1.41
Ikuywa	0.84	0.99
Kisere A	1.75	0.66
Kisere B	4.71	1.75
Rondo	0.71	1.06
Salazar 1	0.48	1.48
Salazar 2	0.97	0.68
Yala	1.00	0.75
A. polystachyus		
Buzambuli	0.57	0.97
Colobus	0.00	0.00
Salazar 1	1.50	–
Salazar 2	1.18	1.51

General Discussion

The pollination ecology of *Acanthus*

Acanthus eminens and *A. polystachyus* are highly similar in their flowering phenology. During *A. polystachyus*' main and *A. eminens*' only flowering period, starting with the onset of winter dry season in October and ending with the onset of the new year's rains in February, flowering progresses in synchrony (Chapter 1).

As Stiles (1975) pointed out, it is unlikely that two species which flower simultaneously coexist unless there are other mechanisms reducing competition for pollinators or reproductive interference in compatible species (Arnold *et al.* 1993; Chari and Wilson 2001; Kay 2006; Marques *et al.* 2007). In numerous plant species flowering at the same time, divergent flower morphologies or floral rewards contribute to a partitioning of pollinators, thereby limiting or preventing pollen exchange (Stang *et al.* 2006; Botes *et al.* 2008).

However, the flowers of *A. eminens* and *A. polystachyus* are highly similar, and though those of *A. polystachyus* are slightly smaller, each species displays a range of flower sizes which renders the discrimination of specific flowers by size impossible (Chapter 2). In addition, there is significant spatiotemporal variation in flower sizes in either of the congeners, and a partitioning of pollinators through flower morphology, e.g. by allowing access only to certain animals (Stang *et al.* 2006; Alarcón *et al.* 2008) or by different handling costs (Waser and Price 1983; Stout *et al.* 1998) is highly unlikely.

Even though flowers of *A. eminens* are more reliable sources of nectar, offer a greater amount of sugar and contain more pollen (Chapter 2), this is more than compensated by the greater resource density found in *A. polystachyus*, as populations and individual plants are much larger. This high resource density may render stands of *A. polystachyus* highly attractive for visitors able to access the floral rewards (Schemske 1980; Conner *et al.* 1996; Ågren 1996). Consequently, shifts in the relative abundance of the species may cause the ratio of flower visits to each of the congeners to shift, which likely increases heterospecific pollen transfer to the less abundant species (Hopper and Burbidge 1978).

Habitat fragmentation not only causes shifts in the relative abundance of *A. eminens* and *A. polystachyus* as the size of their respective habitats changes. It also affects the distance between populations of the species, which shrinks as forest fragments get smaller. I suspected that spatial distance as one of several putative barriers to reproductive interference may be mitigated by anthropogenic influences (see Anderson 1948). Pollen transfer may be more likely between populations which are spatially close, but only if pollinators are shared.

I found flowers of both species to be visited most frequently by carpenter bees (*Xylocopa*), which have been identified as pollinators of *Acanthus* by Dietzsch (2004) (Chapter 3). Though the abundance of specific *Xylocopa* differs between plant species and populations, this is likely caused by pollinator abundance and not by a preference of the bees for a certain flower type (Chapter 3). Consequently, most species are found on flowers of both *A. eminens* and *A. polystachyus*, which therefore are likely to interact through their pollinators. I suspected that the congeners may compete for or facilitate each other's pollinator visits (Waser *et al.* 1996; Ghazoul 2006). In addition, *Acanthus* species are known to hybridize in cultivation (McDade *et al.* 2005), and at least two of the other species of this genus (*A. leucostachyus* Wallich and *A. longipetiolatus* Kurz) have been suggested to be of hybrid origin (Furness 1996). Hence, I considered that *A. eminens* and *A. polystachyus* may affect each other's reproduction or even hybridize when found in close vicinity.

However, I did not find evidence for reproductive interference (Chapter 1, 4) or hybridization (Chapter 2) between the species, though both are more likely in related species (Levin 1971). Reproduction is increased in heterospecific populations (Chapter 1, 4), which is not attributable to hybrid seed set as the potential for hybridization is limited (Dietzsch 2004).

Both species currently coexist in heterospecific populations, which indicates that there may be no intermediate habitat for hybrids, which has been shown to be one of the most important requirements for the establishment of hybrid swarms and zones (Anderson 1948; Lamont *et al.* 2003). With both parent species occupying disturbed sites associated with the forest, it is hard to imagine a habitat where putative hybrids would not be outcompeted by one or the other parent. As there is no evidence for the existence of hybrid individuals in the wild (Chapter 2), the relevance of hybridization is likely neglectable even if, occasionally, a hybrid plant occurs.

From this observation as well as from the patterns of reproduction described above, I conclude that there is no reproductive interference between *A. eminens* and *A. polystachyus*, or that it is masked by stronger effects.

Both species of *Acanthus* likely are important sources of nectar and pollen for their pollinators during dry season in Kakamega Forest. Even though pollinator visitation rates render it likely that all flowers are visited at least once (Chapter 3), stigmatic pollen loads are low (Chapter 1, 4), and not all flowers are pollinated. Reproduction of either species is pollen-limited (Chapter 5), which has recently been described to be more likely in biodiversity-rich habitats (Vamosi *et al.* 2006; see also Knight *et al.* 2006).

The low pollen transfer effectiveness implied by low stigmatic pollen loads of *Acanthus* is surprising when considering the high likelihood of short-distance pollen transfer, and the apparent paradox that flowers are pollen limited despite being frequently visited by legitimate pollinators indicates that *Xylocopa* bees are ineffective pollinators. Morales and Traveset (2008) have demonstrated that losses of conspecific pollen between visits are more likely to affect reproduction than interspecific pollen transfer, but *Xylocopa* fail to reliably transfer pollen grains even though they often visit spatially close flowers, which should reduce the potential for pollen discounting during longer flights (Chapter 3).

Whether fragmentation isolates populations depends on whether pollinators travel between fragments, which in turn depends on the distance and habitat type between them as well as on the flight range and behaviour of pollinators. Pollinators may cross large distances between isolated populations (Schulke and Waser 2001), but in *Acanthus*, the low level of pollen transfer renders this effect to be negligible. On the other hand, given the low range of seed dispersal in both species, with seeds being expelled from fruit capsules and not dispersed further by other vectors, pollen flow likely is the most important mechanism for gene flow between populations.

Though both species are protandric, releasing their pollen before their stigmas become receptive, most individuals are functional hermaphrodites, as there are nearly always flowers of both sexual stages open simultaneously. The striking phenological synchronicity between populations of each species may represent an adaption to allow for pollen transfer between populations (Rathcke and Lacey 1985), but pollinators frequently forage on all flowers of an inflorescence, several inflorescences of the same plant, and several plants within a population (Chapter 3). Consequently, the probability of geitonogamy, either within the same plant or between neighbouring clones or close relatives, is high. Especially during the peak of flowering in dense thickets of *Acanthus*, outbreeding rates may be low and close to neglectable (Melampy 1987; Elzinga *et al.* 2007). Hence, long-distance pollen transfer may play a role in founder populations with few flowers, or when bees visit the first flowers within a population. However, it will not significantly contribute to seed production.

On the other hand, the quality of pollen transferred in either species of *Acanthus* is high, as fruits generally contain many seeds and most undamaged seeds are viable (Chapter 4). This is corroborated by my finding that supplementary pollination increases fruit set, while seed set remains unaffected (Chapter 5). This also indicates that reproduction of neither species is mitigated by inbreeding, as this increase is independent of pollen source (compare Segal *et al.* 2006). Given that both species are likely adapted to colonize habitats by rapidly increasing population size from just a few founder individuals, this may indicate that both species regularly purge their genetic loads (Barrett and Charlesworth 1991; Vaughton 1995).

These effects may also constitute another barrier to reproductive interference between the species, as low pollen transfer efficiency also limits pollen flow from flowers of *A. eminens* to those of *A. polystachyus* and vice versa, except in heterospecific populations. In these, reproduction is comparatively high, indicating that other factors than pollinator-mediated interaction between the congeners are stronger determinants of fruit and seed set in *Acanthus*.

I found high levels of seed damage as well as reduced fruit set due to environmental conditions in either species (Chapter 1, 4, 5). Plants in more humid habitats as well as those flowering at the beginning of dry season show reduced reproduction, while it is highest in dry habitats and at the end of dry season. This also provides an explanation for the conundrum why there is a negative link between the reproductive success of *A. eminens*, and the abundance of its most common pollinator, *Xylocopa* sp1 (Chapter 4): The bees are most common in forest habitats, which are more humid and hence less suitable for the reproduction of *A. eminens.* In the dries habitats at the forest edge, where the bees are rarer, reproduction of *A. eminens* is high due to favourable climatic conditions.

HABITAT ADAPTIONS

Though *A. eminens* and *A. polystachyus* occupy different habitats, a closer look reveals their strategies to be highly similar. Both species are more or less associated with the forest, and form dense populations consisting of many-flowered individuals in disturbed and quickly changing, successional habitats. *A. polystachyus* grows at forest edges, in fallows, hedges and copses, and spreads into more open habitats. *A. eminens* on the other hand colonizes gaps inside the forest as well as riversides, both of which are subject to constant change which the species compensates by rapid and at least partly clonal growth. The potential for clonal reproduction also indicates that populations may consist of less genets than meets the eye, with consequences for genetic diversity and the likelihood of inbreeding effects.

The observed prevalence of geitonogamous selfing described above may be an adaption to fast reproduction, further increasing the ability of both species to spread into available habitats. The lack of inbreeding depression indicates that low genetic diversity simply does not affect reproduction, or that selfing purges the genetic load of *Acanthus*. Protandry of flowers may increase the likelihood of genetic exchange with other individuals when populations are small, or during early flowering.

In larger populations, either species offers an abundance of floral rewards. *A. eminens* produces more nectar and more pollen, which likely is an adaption to increase attractiveness for pollinators in its resource-poor environment. *A. polystachyus*, on the other hand, may be a highly attractive resource through its sheer mass, and consequently offers less nectar and pollen, and even some nectarless flowers.

Most fruits of either species are released just before the onset of rains in February, and the striking synchrony of flowering displayed by *Acanthus* likely is a mutual adaption to optimize fruit development, seed release and germination, and seedling survival (compare Rathcke and Lacey 1985; Wheelwright 1985; Schaik *et al.* 1993; Murali and Sukumar 1994). In both species, the likelihood of flowers developing into fruits increases with progressing dry season, and I found fruits to contain fewer seeds and the seeds more likely to be damaged in populations of *A. eminens* with a denser canopy.

Both species grow quickly from seed. As *A. eminens*' seeds are likely to germinate immediately when watered after being dispersed, there probably is no soil seed bank of the species. Instead, it is the seedlings which slowly grow on the dim forest floor, spreading into available habitats when opportunity arises. These small, vegetative plants of *A. eminens* are found in many parts of the forest. When they increase in size, they start flowering with one or two inflorescences. These may receive a high proportion of outcross pollen, this effect being bolstered by flower protandry. As plants increase in size, so does the likelihood of geitonogamy. But geitonogamy also is a mechanism of reproductive assurance, and the species is able to spread rapidly into available habitats, forming dense stands with numerous flowers which release many seeds. Permanent habitats of *A. eminens*, e.g. at riversides, also provide a source for seeds to be dispersed along the river and into the surrounding forest. I believe that it is from these two population types that seeds are spread in the surrounding forest, leading to the establishment of new seedlings, waiting for a chance to colonize newly available habitats and starting the cycle all over again.

Populations of *A. polystachyus* on the other hand permanently line the forest edges, forming dense stands with many flowers. The species spreads aggressively into the surrounding open habitat, while it is overgrown and succeeded by larger forest species. There are few small plants and seedlings of *A. polystachyus*, but all observed populations were mature and well-established, and the large shrubs may spread mainly through vegetative growth. In more recently colonized habitats, or in areas suffering from extreme disturbances like fire or landslides, seedlings may be more prevalent, and sexual reproduction may be of greater importance.

ACANTHUS AND CONSERVATION

Apart from its effects on pollination processes, forest fragmentation reduces habitat for *A. eminens*. Fragmentation rarely leaves behind enclaves of intact forest: Most fragments are highly frequented by firewood collectors and hunters for bushmeat, and subject to selective logging and collection of medicinal plants. At the end of this deterioration, small copses of common trees of little economic importance remain, with a network of small footpaths reducing vegetation cover and altering microclimate of the herb and shrub layer. Though *A. eminens* thrives at forest edges, it does not spread into the farmland, and seems absent from heavily degraded forest. For example, the species has vanished during the last five years in one heavily forested and degraded forest fragment (Kaimosi), likely through a combination of selective removal and reduced habitat quality. Consequently, the abundance of *A. eminens* in Kakamega Forest may dwindle if disturbance intensifies.

There are no indications that *A. polystachyus* will suffer reduced population sizes or reduced vigour in my study area. Unless agriculture shifts towards large-scale, industrial farming, clearing landscapes and removing hedgerows and copses to give way to large fields spanning several hectares, the species will remain common. Even if the loss of forest were complete, it would still be found at roadsides and in less arable fallows, even though it would likely not form the continuous belts now lining the rainforest.

Rhymer and Simberloff (1996) have argued that a loss of traits resulting from gene flow between genetically divergent plants of the same species may lower the genetic diversity of a species (see Ackermann *et al.* 2008). Hence, it represents a cryptic loss of biodiversity which may reduce the ability of populations to adapt to changing habitat conditions. It was far beyond the scope of this study to analyze these effects, but as habitat change changes plant and pollinator abundance, it is quite possible that patterns of gene flow also change. Due to the costs and effort of long-term analyses of trends in gene flow and genetic diversity between and in populations of species inhabiting increasingly fragmented landscapes, this will likely remain a neglected area of pollination and plant ecology for some time.

Shifts in relative abundance of plant species may have a strong impact on pollination processes and populations (Kunin 1993). In the light of this effect, it is unsurprising that pollination processes have often been found vulnerable to habitat change, which involves the reduced abundance of species in the original habitat and an increase in abundance of those species found in the emerging landscape.

It is important to keep in mind that *A. eminens* and *A. polystachyus* may be important resources for pollinators. Though only a survey of the visitor fauna of all plants flowering simultaneously with the congeners would begin to reveal the dependence of *Xylocopa* bees on *Acanthus*, its importance for carpenter bees is strongly indicated. It is known that *Xylocopa* visit several different plant species in Kakamega Forest and species have been found foraging year-round (Gikungu 2006), but detailed data on the mutual dependence of the forest's plants and pollinators are lacking. However, caught on flowers of *Acanthus*, carpenter bees only carry pollen of *Acanthus*, and the common shrub species may help to sustain the populations of *Xylocopa* at a high level, thereby increasing pollination success for other plant species flowering later (Waser and Real 1979). Consequently, the loss of such common species may result in resource limitation of pollinator population size, and reduce reproductive success of later-flowering species (Saavedra *et al.* 2003). *Xylocopa* bees are pollinators of some crop species, like beans and passion fruit, and it might be of interest to investigate the link between the abundance of *Acanthus* flowering in dry season and the abundance of *Xylocopa* during crop flowering in the following rainy season.

Mutualistic interactions and conservation

Generally, mutualistic interactions between plants and animals are commonly negatively affected by anthropogenic change (Tylianakis *et al.* 2008). Recently, several studies have analyzed the impact of invasive species on plant-pollinator networks, and have repeatedly found negative effects on native plants (e.g. Lopezaraiza–Mikel *et al.* 2007; Aizen *et al.* 2008; Bartomeus *et al.* 2008).

At the community scale, plant-pollinator networks are often quite robust in the face of anthropogenic change even when single species are lost (Memmott *et al.* 2004; Bascompte *et al.* 2006). However, interaction strength may still be modified by habitat change (Lopezaraiza–Mikel *et al.* 2007; Memmott *et al.* 2007). Network resilience strongly depends on whether core or keystone species, which link most parts of the network as interaction 'hubs', are affected by change (Jordano *et al.* 2003). Changes in abundance of these species may strongly affect all other species in a network, increasing their importance for conservation.

The benefits of natural pollinators acting as one of several potential 'ecosystem services' have repeatedly been used to argue for conservation of natural plant-pollinator communities (e.g. Klein *et al.* 2003; Steffan-Dewenter *et al.* 2005; Klein *et al.* 2007). The increase in crop production caused by pollinators provides a tremendous opportunity to convince stakeholders and policy makers of the economic value of natural ecosystems. However, the functionality of pollination as an ecosystem service does not necessarily depend on each and every species a given habitat contains in a pristine state. Hence, the conservation of a functional pollinator community which allows for an increase in agricultural yield or continued pollination of several indigenous plant species does not necessarily need to consider rare natural species. The finding that networks are robust to species extinction (Memmott *et al.* 2004) may indicate that many ecosystem functions may persists even when some species are lost. Hence, network functionality will likely persist even when rare species vanish.

On the other hand, pollinator-rich, semi-natural habitats may provide a means to reconcile agriculture and conservation even if some rare natural species are lost. Depending on their management, even agricultural ecosystems may sustain many species (Tscharntke *et al.* 2002; Holzschuh *et al.* 2007). In fact, conservation-friendly management may be the only way to conserve a part of nature's riches in areas under pressure from growing population und economic needs (Vandermeer and Perfecto 1997). However, conservationists have to be aware that arguing solely with the economic value of nature does not necessarily facilitate conservation, as costs and benefits will be compared and nature may, even if only because of short-sighted planning, lose to industrialized agriculture (Ghazoul 2007).

In 100 years, will there be *Acanthus* in Kakamega forest?

Like most other recent research in Kakamega Forest, my study aims to provide a scientific background for conservation policy in Kenya. Each species of *Acanthus* is a characteristic floral element in its respective habitat, and may serve as an indicator for the effects of anthropogenic habitat change. In addition, both species are abundant enough to collect large numbers of samples, and the congeneric species offered the opportunity to analyze the effects of habitat change on highly similar species.

Pollination of neither species seems to be affected by recent habitat change, and proves to be resilient to shifts in pollinator species composition and abundance. Fruit and seed set are more closely tied to climatic conditions than to pollinator visitation, and consequently, it seems that shifts in local climate due to deforestation or in global climate due to anthropogenic climate change are more likely to pose a threat to the reproduction of *A. eminens* or *A. polystachyus* than forest fragmentation.

Several climate change models predict a general, in some models dramatic, increase of rainfall for my study area, which may be bolstered by extreme rain events during *Acanthus*' flowering period caused by El Niño/Southern Oscillation (ENSO) events (Hulme *et al.* 2001), disrupting patterns of rainy and dry seasons. Apart from obvious implications for agriculture, my study indicates that a change in patterns of rainfall may have severe effects on the few remaining natural ecosystems in East Africa, even in habitats like rainforests, which normally are expected either to be adapted to strong rainfall or to benefit from an increase in humidity. Aseasonal rains may reduce reproductive success in species of these habitats, changing their population dynamics (Chapman *et al.* 2005).

The effects of habitat and climate change may be slow, and this lag makes it difficult to predict the consequences landscape modifications may have after more than a few decades (Ewers and Didham 2006; Gardner *et al.* 2009). After recent fragmentation, perennial species may not immediately show reduced vigour. But the details of plant-pollinator interactions may help to predict the outcome of anthropogenic habitat change on long-lived plant species (Rathcke and Jules 1993).

Species like *A. eminens* and *A. polystachyus*, which are not endangered themselves, may be important floral resources for rare large bee species during their time of flowering. Shifts in their abundance patterns may have cascading effects, affecting population sizes of their pollinators and, in turn, reproductive success of other plant species both in endangered natural habitats and crop plantations (Pauw 2007). Long-term monitoring of pollinators and keystone plant species is urgently needed to provide fundamental information for policy makers, to allow deeper insights into impacts of climate change on tropical ecosystems, and to deepen our understanding of ecological and evolutionary processes shaping reproductive phenology of plants. In addition, these data will help to assess whether processes which do not immediately respond to habitat change slowly deteriorate, which is of importance for both natural and agricultural systems.

Whether the protected areas of Kakamega Forest will persist as partly or fully functional forest systems strongly depends on the population of the surrounding areas (Wittemyer *et al.* 2008). Even the most remote areas of Kakamega Forest may not be primary forest, as hunting and selective logging may have already altered species abundance and composition (Schaab *et al.* 2010; see Gardner *et al.* 2009, and citations therein). Degraded forests offer opportunity for conservation, as they may not harbour rare taxa, but still the greater part of species of the original ecosystem (Tscharntke *et al.* 2002; Holzschuh *et al.* 2007). Even though conservation of pristine areas takes priority, protecting near-pristine, and even secondary and disturbed habitats may help to conserve large parts of an area's original biodiversity. The preservation of these modified ecosystems may remain the only option when anthropogenic change has already advanced to the point where no primary habitats remain. It has to remain a priority of conservationists to continue and increase the efforts made to ensure the collaboration of people living near natural habitats in conserving these ecosystems for future generations.

The future of Kakamega Forest depends on whether the growing needs of the increasing number of people living around the forest can be reconciled with conservation of the forest. Sadly, the open question is not whether there will still be *A. eminens* in Western Kenya, but whether there will still be a Kakamega Forest one hundred years from now.

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Hiermit versichere ich, dass ich diese Arbeit selbständig verfasst, keine anderen Quellen und Hilfsmittel als die angegebenen benutzt und die Stellen, die anderen Werken dem Wortlaut oder Sinn nach entnommen sind, kenntlich gemacht habe. Diese Arbeit hat in dieser oder ähnlicher Form keiner anderen Prüfungsbehörde vorgelegen.

Bielefeld, den 2. April 2012

Nils Hasenbein