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Role of sunbirds in Afromontane pollination systems

Ph.D. Thesis

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ANNOTATION

Bird pollination is a widespread phenomenon that has evolved in ten phylogenetically independent bird families to the point that birds have become significant pollinators for at least some flowers. Among them, hummingbirds (Trochilidae) from the New World tropics are both the best known and the most explored group. Meanwhile, the second most important and abundant family, the Old World sunbirds (Nectariniidae), is relatively underappreciated in the literature. Much of the research on plant-sunbird relationships which has been so far done is from South Africa, in particular from the highly specific Cape region. Very little work has been conducted in tropical Africa, the centre of sunbirds diversity.

The thesis presents five case studies investigating the roles of local sunbird species in pollination systems of several co-flowering plant species in tropical montane areas in Cameroon. The achieved results importantly contribute to understanding of the processes on pollinator and plant community level.

DECLARATION [IN CZECH]

Prohlašuji, že svoji disertační práci jsem vypracovala samostatně pouze s použitím pramenů a literatury uvedených v seznamu citované literatury. Prohlašuji, že v souladu s § 47b zákona č. 111/1998 Sb. v platném znění souhlasím se zveřejněním své disertační práce, a to v úpravě vzniklé vypuštěním vyznačených částí archivovaných Přírodovědeckou fakultou elektronickou cestou ve veřejně přístupné části databáze STAG provozované Jihočeskou univerzitou v Českých Budějovicích na jejích internetových stránkách, a to se zachováním mého autorského práva k odevzdanému textu této kvalifikační práce. Souhlasím dále s tím, aby toutéž elektronickou cestou byly v souladu s uvedeným ustanovením zákona č. 111/1998 Sb. zveřejněny posudky školitele a oponentů práce i záznam o průběhu a výsledku obhajoby kvalifikační práce. Rovněž souhlasím s porovnáním textu mé kvalifikační práce s databází kvalifikačních prací Theses.cz provozovanou Národním registrem vysokoškolských kvalifikačních prací a systémem na odhalování plagiátů.

České Budějovice, 16.9.2014

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LIST OF PAPERS AND AUTHOR’S CONTRIBUTION

The thesis is based on the following papers:

Food selection by avian floral visitors: an important aspect of plant–flower visitor interactions in West Africa.

Janeček Š., Riegert J., Sedláček O., Bartoš M., Hořák D., Reif J., **Padyšáková E.**, Fainová D., Antczak M., Pešata M., Mikeš V., Patáčová E., Altman J., Kantorová J., Hrázský Z., Brom J. & Doležal J. (2012): Biological Journal of the Linnean Society 107: 355–367 (IF= 2.413).

Eliška Padyšaková participated in experiment preparation and data collection in the field.

Hovering sunbirds in the Old World: occasional behaviour or evolutionary trend?

Janeček Š., Patáčová E., Bartoš M., **Padyšáková E.**, Spitzer L. & Tropek R. (2011): Oikos 120: 178–183 (IF=3.329).

Eliška Padyšaková participated in experiment preparation and data collection in the field, following samples processing and revision of the manuscript.

Generalization versus specialization in pollination systems: visitors, thieves, and pollinators of *Hypoestes aristata* (Acanthaceae).

Padyšáková E., Bartoš M., Tropek R. & Janeček Š. (2013): PLoS ONE 8(4): e59299 (IF= 3.730).

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Interference competition between sunbirds and carpenter bees for the nectar of *Hypoestes aristata*.

Tropek R., Bartoš M., **Padyšáková E.** & Janeček Š. (2013). African Zoology 48(2): 392-394 (IF=0.848).

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Asymmetric competition for nectar between large nectar thief and small pollinator: an energetic point of view.

Padyšáková E., Brown M. Bartoš M. & Janeček Š. manuscript

Eliška Padyšaková participated in experiment preparation and data collection in the field, and is responsible for data assembly, partially for statistical analysis and for writing the manuscript.

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CHAPTER I

General introduction

Pollination of flowering plants represents a vital ecosystem service of great value for the sustainable health of the planet and food security. Efficient pollination of plants is crucial to ensure food supplies for animals, as plants are the foundation of all food chains on the planet. In view of the fact that 74 – 98% of all flowering plants are animal pollinated (Ollerton et al. 2011), pollinators are key components of global biodiversity also they are usually associated with more rapid speciation of plants (Dodd et al. 1999, Kay et al. 2006). Besides its practical significance, the plant-animal relationship has been a focus for scientists for many decades inquiring into some of the fundamental aspects of biology, from evolution and ecology to behaviour and reproduction (Willmer 2011).

~ · The origin of animal-mediated pollination · ~

Since plants are immobile organisms, they usually rely on external agents to transport their pollen among conspecific flowers. The enormous diversity in modes of pollination among living plants can be broadly divided into pollination involving either animals (biotic) or wind/water (abiotic). Since the interpretation of pollination in extinct plants faces significant difficulties and only rarely is there direct evidence of flower-pollinator interactions it is therefore difficult to establish the ancestral pollination mode for angiosperms. Evidence from the pre-angiosperm fossil record indicates that although wind pollination was almost certainly widespread among earliest Cretaceous seed plants, different kinds of insect pollination were already established in the Late Triassic, Jurassic and early Cretaceous (Friis et al. 2011). However, adaptive shifts from insect to wind pollination and vice versa are well known (Stebbins 1970). Gradually, the majority of all plants formed tightly co-evolved mutualism with animals, especially insects, representing reciprocal exploitation with an underlying evolutionary conflict.

~ · Pollination mutualism · ~

Mutualism such as animal-mediated pollination is, in fact, neither symmetrical nor cooperative. Indeed, pollination derives inadvertently from fully antagonistic pollinivory by beetles and sawflies in early seed plants. Since the goals of plants and pollinators remain distinct there is still a potential conflict of interest between mutualists (Kearns et al. 1998, Bronstein 2001, Richardson 2004). Floral traits that increased chances of pollination and encouraged visits by the most effective pollinators, ideally without providing any reward, were favoured by selection on one side, and flower-visiting animal traits that ensure exploitation of available food resources as effective as possible on the other. Hence, conflicts of interest between mutualists seem unavoidable, because the commodity experienced by one partner as a cost is often experienced as a benefit by the other. For example, nectar that serves as a floral reward in most pollination mutualisms is one of the costs to plants but one of the benefits to animals (Rathcke 1992). Such a long-lasting conflict, for instance, over the optimal amount of nectar that should be deployed per flower – with pollinators preferring a higher amount and plants a lower amount – dictates that natural selection will act in divergent ways on plants and pollinators.

The plant is under selection to maximize its attractiveness for potential mutualists at the lowest cost possible while minimizing the detrimental effects of antagonists. In other words, floral traits are likely to evolve as much defence against some animals as adaptations increasing the effectiveness of others (Janeček et al. 2011). These traits should all be produced for minimum costs and adjusted in suitable extent, especially floral rewards (nectar, pollen, oil), to keep visitors close to their limits of getting a profit but ensure that the animal moves around as many conspecific flowers as possible.

Flower visitors are usually thought to follow those rewards offered by plant as best they can. Evolved animal traits should facilitate foraging profit – in relation to how much energy is extracted from visited flowers – and minimize the effort in extracting it and in moving between plants to get it. A well-adapted foraging animal selects those plants where the energy gained from the flowers visited does not exceed its energy expenditure.

~ · Specialization versus generalization · ~

Plant-pollinator interactions have commonly been regarded as tightly coevolved, with general evolutionary trend toward increased specialization (Waser and Ollerton 2006). The paradigm that the adaptive value of floral traits is related to particular visitors began at around 250 years ago (Kölreuter 1761, Sprengel 1793) with descriptions of how floral features promote established interactions between plants and visitors. This idea was later elaborated by many studies (Darwin 1862, Müller and Delpino 1869, Müller 1883, Baker 1963, Grant and Grant 1965, Stebbins 1970, Faegri and van der Pijl 1979, Johnson and Steiner 2000) with the view that plants can control specific pollinator types to achieve cross-pollination by evolving corresponding floral-trait combinations. If some floral visitors are more effective in pollen transfer among conspecific plants (Muchhala et al. 2009); selection should favour traits promoting these effective pollinators (Aigner 2001, Whittall and Hodges 2007, Brunet 2009, Schlumpberger et al. 2009). Stebbins (1970) highlighted several evolutional principals involved in the process of adaptive radiation for pollen vectors, especially the well known "the most effective pollinator principle" among others. The suggested principle underlines the predominant influence of those pollinators that visit the plant most frequently and effectively in shaping the characteristics of flowers. It does not mean that the predominant and most effective vectors are constantly the exclusive plant pollinators but rather stress their essential role in the origin of particular forms of flowers. Such selection favouring specialization is assumed to promote pollinator-mediated speciation, floral divergence, and reproductive isolation, thereby explaining the current diversity of flowering plants (Waser 1998, Levin 2000).

On the other hand, if several diverse flower visitors provide similar pollination service, and if plant costs of interactions are comparable, plants are not forced to specialize on attracting a particular group of visitors. Recent studies working at the level of entire communities liven up the notion that the flowers of many plant species are visited by a diverse assemblage of pollinators (Herrera 1988, Horvitz and Schemske 1990, Gomez et al. 1996, Gomez and Zamora 1999, Dilley et al. 2000, Lippok et al. 2000, Thompson 2001), and pollination systems are more generalized and dynamic than previously suggested (Waser et al. 1996, Memmott 1999). The problem is that highly diversified communities make it difficult to assess the totality of their biotic interactions and quantify generality at the community level (Waser et al. 1996, Johnson and Steiner 2000). Nevertheless, new approaches initiated in the mid-1990s, which borrowed the tools from the theory of food webs and interaction networks, have revealed several novel patterns that bear impact on the generalization-specialization topic (Memmott 1999, Olesen et al. 2007, Stang et al. 2009, Vazquez et al. 2009). For example, pollination networks show asymmetry in the degree of specialization between pairs of interacting species: many specialist plants tend to have generalist visitors, and many specialist visitors tend to visit generalist plant species. This finding instigated reanalyzing the distinction between the priorities of plants and pollinators as well as the terms generalization and specialization itself in the pollination ecology community. Plant-pollinator networks also display nested structure, in which the links of specialists are subsets of the links of more generalized species (Bascompte et al. 2003). Nestedness has important implications for the conservation of pollination interactions and adds robustness to a network (Memmott et al. 2004, Genini et al. 2010). Robustness to the loss of plants or pollinators can, however, ultimately differ among the species of different roles and positions within the network (Jordano et al. 2006).

There is a long-term debate on the apparent contradiction between the ecological observation that generalization is quite common (Waser et al. 1996, but see Johnson and Steiner 2000) and the general perception that much of floral evolution has been toward specialization of a functional group of pollinators (Ollerton 1996, Johnson and Steiner 2000, Aigner 2001). This stems from the confusion between the terms "ecological specialization"-the state of being specialized- and "evolutionary specialization"-the process of evolving toward greater specialization. The opinion that some plants are associated with generalist pollination systems is mostly based on lists of flower visitors while detailed analysis of pollinator performance on these plants show some, at least, ecological specialization (Lindsey 1984, Zych 2007). Additionally, empirical and theoretical statements about the prevailing generalization in pollination systems have sometimes ignored the finding that not all flower visitors are effective pollinators (Memmott 1999, Armbruster et al. 2000, Padyšáková et al. 2013). Moreover, pollinating visitors are likely to vary in pollination efficiency, and only a subset of these pollinators generates selection (Armbruster et al. 2000). As the composition of pollinator communities is highly variable in time and space (Herrera 1988, Eckhart 1992, Cane and Payne 1993, Herrera 1995), specialization may have occurred even if the resulting population appears not to be very specialized at first glance.

To characterize variability in the nature and strength of interactions in pollination system it is necessary to clearly distinguish effective pollen vectors from non-pollinating visitors, and to quantify the percentage of pollen removal and fertilization caused by each pollinator (Kay and Schemske 2004). While pollination efficiency of all flower visitors is not yet estimated in most studies (Olesen and Jordano 2002), it has been successfully calculated for the insects visiting individual plant species (Schemske and Horvitz 1984, Fishbein and Venable 1996, Bingham and Ort 1998). Using the same techniques to species-rich communities, broad network surveys have to be aware of an important likelihood -not observing an interaction that actually occurs (Kay and Schemske 2004). In fact, constructing a comprehensive pollination web remains a heady goal. Until now, it seems that neither extreme ecological specialization nor generalization in pollination interactions is the prevailing rule, while most of the plant-pollinator systems lie along the specialization-generalization continuum (Waser and Ollerton 2006).

~ · Pollination syndromes · ~

Flowers of most angiosperms reflect specialized adaptations to one or another pollinator type, because they are exploiting the abilities and preferences of particular kinds of visitor while preventing access to the other (Willmer 2011). These broad flower types converged on certain morphologies and reward patterns that are formally described in "pollination syndromes" concept (Faegri and van der Pijl 1979). They are responses to common selection pressures exerted by shared unrelated pollinators, which generate correlations among traits. These traits include flower shape, size, colour, odour, reward type and amount, nectar composition, timing of flowering, etc. For example, tubular red odourless flowers with copious nectar often attract birds; foul smelling flowers with nectar easily accessible attract carrion flies or beetles. Moth-and-bat-pollinated flowers tend to be white, night-opening, large and showy with tubular corollas and a strong, sweet scent produced in the evening, night or early morning. Fly-pollinated plants more often do not emit a strong scent, are typically purple, violet, blue, and white, and have open dishes or tubes; and bee-pollinated flowers can be very variable in their size, shape and colouration, often with nectar guides, scent and sucrose-dominated nectar (Willmer 2011).

Whilst it is clear that plants are often recognized as being clustered in phenotype-space around some of the classic "syndromes", there has been much debate amongst scientists as to how frequent they are and to what extent we can use the classical syndromes to classify plant-pollinator interactions (Ollerton 1998). Contradictory observations that flowers are often visited by wide range of potential pollinators do not fit the traditional "syndromes". The common notion that syndromes represent a serious oversimplification have led to a critical re-evaluation of the syndromes, the evolution of adaptations and ecology of species' interactions in general (Fenster et al. 2004, Wilson et al. 2004, Armbruster and Muchhala 2009, Ollerton et al. 2009a, Ollerton et al. 2009b). It should be noted that the main authors of the syndrome idea were fully aware of that pollination syndromes are intended to be statistical rather than diagnostic constructs and their formalization could be misconstrued. It is well-known that pollination guilds vary from a single species to large suites of very different species, guilds may differ within species across geographical ranges and over time, and visitor groups such as "flies", "beetles" and "bees" are simplified constructs that contain much internal variation in foraging patterns.

Despite these certain limitations, the "syndrome" concept, if not fixedly defined, has utility and can serve to make an educated guess of the most likely pollinators as well as test assumptions of pollinator-mediated selection on specific traits (Armbruster et al. 2000, Pellmyr 2002, Willmer 2011). For this purpose, the concept implies that pollinators are organized into functional rather than taxonomical groups according to presumed similarities in the selection pressure they exert, for example long-tongues flies, large bees, etc. (Fenster et al. 2004). If we apply these more broad functional definitions of pollination agents, plants with "unspecialized" floral morphology can be regarded as functionally specialized (Ollerton et al. 2007, Niemirski and Zych 2011). Although it is hard to determine how plants adapted to one functional group of pollinators could shift to a different group, it is known that shifts among syndromes occur, in some lineages repeatedly (Perret et al. 2003, Kay et al. 2005, Thomson and Wilson 2008).

~ · Costs and benefits of animal pollination · ~

Using animal pollination for pollen dispersal provides several benefits for immobile plants (Pellmyr 2002). The process is more direct because animals can actively seek out isolated flowers, and thus a pollen grain collected on one flower may travel further than it would by wind. Moreover, the pollen dispersal on an animal can happen in all possible directions and abiotic characters, such as water flow or actual wind blow, do not drive it. Similarly, animal-mediated pollination can take place in habitats with very little or unreliable wind environments, such as closed-canopy rain forests. At the same time, plants have to deal with novel costs that came with animal pollination. Plants need to produce visual and olfactory cues as well as rewards such as nectar and additional pollen to attract, feed and maintain flower pollinators. Considering pollen has a higher probability of reaching and fertilizing an ovule of different plant when travelling on a pollinator body, animal- pollinated plants may invest fewer resources to pollen production than wind-pollinated plants.

It is important to note that not all animal pollinated plants are reliant solely on animals to seed set at all times (Ollerton et al. 2011). Many species have a mixed mating system using self-pollination without the assistance of animals. In a long-term species perspective, however, plants with mixed mating systems require animal pollination to maintain out-crossing among individuals of the same species.

~ · Animals involved in pollination mutualisms · ~

At least 130 000 species of animals, and probably up to 300 000, are regular flower visitors and potential pollinators (Buchmann and Nabhan 1996, Kearns et al. 1998). A very large proportion of all regular flower visitors involve three extant groups of animals that have evolved flight – insects, birds and bats (Herrera and Pellmyr 2002, Willmer 2011).

Among the insects, flower visiting species are particularly frequent within the large orders Hymenoptera (bees and wasps), Lepidoptera (moths and butterflies), Diptera (flies) and Coleoptera (beetles). Bees predominate in many habitats across world and almost uniquely use both nectar and pollen as foods. Since they are highly variable we need to consider several different types of "bee syndromes" to make sense of their interactions with flowers (e.g. solitary bees, carpenter bees, euglossine bees, bumblebees, stingless bees, honey bees) Lepidopterans are reasonably effective pollinators, especially large and partially endothermic sphingid species. Compared to butterflies that must land and settle to feed, hawkmoths feed mainly while hovering. Sphingids' both physical and behavioural attributes enhance their effectiveness as pollinators of dusk or night flowering plants. They became especially important in regions where dusk temperatures were higher (Mediterranean-type and savannah habitats), in moist tropics as well as on the large and isolated island of Madagascar. Flies are important flower visitors in some tropical and semiarid zones, but their importance increases especially in regions where other visitor groups are uncommon, such as some islands and high-altitude habitats. From a worldwide perspective, most flies could best be included within the generalist syndrome although three groups – long-tongued flies, carrion flies, and hoverflies – represent syndromes in their own right. Beetles are often overlooked although quite an important cohort of pollinators, especially in the southern hemisphere and in arid or tropical climates. Beetles are less mobile than bees or flies, and are rarely sufficiently specific in their visits to the generalist flower types. Many flower-visiting beetles are destructive feeders and may damage or consume whole flowers, including petals and ovule tissues.

Among birds, ten phylogenetically independent groups have diversified as flower-visitors and pollinators. Bird families involved in pollination include the American Trochilidae (hummingbirds and hermits), Palaeotropical and Pacific Nectariniidae (sunbirds, flowerpeckers), south African Promeropidae (sugarbirds), Australian Meliphagidae (honeyeaters, spinebills and bellbirds), southern hemisphere Zosteropidae (white-eyes), Australian Psittacidae (brushtongued parrots), New World and Hawaiian honeycreepers (Fringilidae and Thraupidae), Australian and southern Asian Dicaeidae (flower-peckers), and New World Icteridae (orioles and caciques). They are important pollinators especially in tropical and subtropical areas where plants can provide enough floral resources throughout the year. Since they are generally of large size and homoeothermic, nectarivorous birds must forage throughout the day to obtain enough energy. Birds are thus more dependable pollinators where cold or/and rainy conditions are frequent, such as high mountain or Mediterranean-climate winters. Hummingbirds and some sunbirds can hover efficiently in front of the flowers while others must perch to feed. These two categories of nectarivorous birds imply two somewhat different floral syndromes. Many of the flower-visiting birds are only occasional visitors and do more damage to flowers. On the other hand, even birds that are not specialized for flower visiting (such as tits, warblers, blackcaps, finches, starlings, thrushes or drongos) can contribute to pollination success of some plant species. As an exception to tropical and subtropical distribution, migratory hummingbirds in North America breed in temperate to alpine areas and pollinate many plant species there.

Among bats, megachiropterous fruit bats are important flower visitors and pollinators in tropical regions on all continents, while microchiropterous Phyllostomidae occur only in the New World. Bats' range of occurrence in tropics includes humid zones as well as hot arid areas where they pollinate many desert plants, or high altitudes where they encounter cool and even freezing temperatures at night. Bats are primarily nocturnal and as large hovering endothermic mammals have extremely high energy requirements. Hence it is no surprise that bat-pollinated plants show a rather specific bat pollination syndrome.

Other, non-flying groups of vertebrates, able to access and pollinate flowers either by climbing and gliding among trees or by seeking pendant flowers close to the ground, include lizards and mammals. Lizards do visit flowers and can sometimes be important as pollinators, especially on islands where they achieve higher densities due to lower predation risk. The group of non-flying mammals regularly visited and pollinated flowers is mainly composed of marsupials, monkeys, lemurs and rodents, plus some occasional pollinators such as small carnivores, musk shrews or elephant shrews. We can find this type of plant-mammal relationships in different habitats across different continents, particularly tropical rain forests and higher-altitude cloud forest in South America, fynbos flora in Africa or Mediterranean-type bushes in Australia. There are no known specific adaptations involved in pollination by non-flying mammals, and hence these systems are considered to be a relict that has survived from ancient times in certain areas (Sussman and Raven 1978).

In addition, new types of plant-pollinator interactions, involving "unusual" pollinating animals are regularly being discovered, such as specialized pollination by spider hunting wasps (Pompilidae) and fruit chafers (Cetoniidae) in the eastern grasslands of South Africa (Ollerton et al. 2003).

Examples from different habitats have reported regionally different patterns of animal flower visitors. In general, hymenopterans predominate among pollinators in central Europe while in tropical Central America with bird and bat pollination entering the picture and fewer fly visitors. Flies tend to be more dominant in high-altitude habitats where the vertebrates are absent (Warren et al. 1988, McCall and Primack 1992, Hingston and Mc Quillan 2000, Willmer 2011).

~ · Animals' adaptations related to flower-visiting · ~

Numerous animals that primarily visit and pollinate flowers have evolved extremely varied adaptations for efficient nectar gathering as well as pollen transfer. There are three principal techniques employed by nectar feeders: active suction, capillary suction and viscous dipping (Kim et al. 2011). Surprisingly, results of the latest study indicate that preferred nectar viscosity (i.e. optimal sugar concentration) depends exclusively on the feeding mechanism (tongue length, mouthpart morphology, aspects of behaviour while feeding) but not on body size, quantity of intake, or species (Kim et al. 2011, Willmer 2011).

The feeding habits of flies are highly variable, their mouthparts are essentially suctorial but different taxa are able to suck, lap, chew or bite. Flies from some families (e.g. Bombyliidae) are endowed with elongate proboscis and a powerful suctorial mechanism with a tongue that can penetrate and suck fluid from quite deep corolla tubes. Lepidopterans are liquid feeders, sucking up fluids using a long, coiled, and elastic proboscis along which a pressure gradient is generated by cibarial muscles (Kingsolver and Daniel 1979, Pivnick and McNeil 1985). Pollen can stick to lepidopteran's hairy body, especially their tongues and faces, while they are visiting flowers. Pollen stuck on the proboscis, nevertheless, tends to get redistributed when the tongue is coiled up after feeding. Hence only a proportion remains available for deposition on stigmas. Pollen carryover by some groups of butterflies (e.g. Papilionidae) is more effective when they flutter their wings and contact anthers/stigmas continuously while visiting a flower (Cruden and Hermannparker 1979). Most bees ingest nectar by dipping their lapping capillary tongue tip into, then extracting it from, the viscous nectar. They use a suction pump provided by a muscular chamber in the head that applies negative pressure to the channel within the tongue. The bee tongues vary greatly in overall length and are in close relation to flower choice. Nectar-feeding birds were believed to employ capillary suction, with tongue function like a pair of tiny, static tubes drawing up floral nectar. Instead, recent study demonstrated that the hummingbird's tongue tip is a highly efficient device that dynamically traps nectar within the lamellae while the tip leaves the fluid (Rico-Guevara and Rubega 2011). Moreover, the process purely results from the tongue structure itself, not requiring special energy to drive it. Hence, the whole nectar-feeding process in other nectarivorous birds needs to be re-evaluated. In general, birds have very varied beak length, width and curvature, and often show a close match to the corollas that they visit. Some nectar-feeding bird beaks have markedly serrated edges, which is probably related to insect catching (pers.com.). Pollen transfer takes place on a bird's body in a relatively specific place, such as forehead, chest, bill or solely feet (Johnson and Brown 2004, Willmer 2011). Nectarivorous bats have mainly pronounced snouts and a long slender tongue with a tip brush of fine bristles or narrow scales, often pointing slightly backward, and allowing nectar uptake by capillary action. Since nectar is only part of their diet, small non-flying mammals that can pollinate flowers show very little or no specialist adaptation to the flowers that they utilize. Most of them regularly take seeds, leaves, fruits, or insects as well as nectar, plus some pollen and flower tissues. Australian mammals with extended narrow snout and long brush-tipped tongue comprise the only exception to this lack of specialism.

~ · Nectar larceny · ~

The plant-pollinator relationship is considered a mutualism because the pollinator obtains a required food commodity such as nectar in exchange for its pollination service (Maloof and Inouye 2000). Both offered services and commodities, however, provide remarkable opportunity for exploitation by non-mutualistic species that can obtain them while providing nothing in return. Traditionally, all animals visiting flowers were included in pollination networks and considered pollinators (Niemirski and Zych 2011, Willmer 2011). But not all the flower visitors actually serve as pollinators and rather remove nectar without contacting plant reproductive organs and thus providing the expected service (pollination). The outcomes from illegitimate floral visits might have strong positive, negative, or neutral effects on plant reproductive success depending on the manner of larceny (Maloof and Inouye 2000). Nectar thieves enter flowers via floral opening and probably do not have much effect on the floral tissue itself. They are usually animals differing significantly in size or nectar feeding fashion from mutualistic pollinators with which the flower seems to have evolved. The mismatch of the morphologies enables them to creep into the flowers and remove the nectar. On the contrary, nectar robbers can make a hole in corolla tube to obtain nectar directly from nectaries (primary robbing). Other nectar-seeking animals may then exploit robber-made holes (secondary robbing). Nectar larcenists are expected to be universally detrimental cheaters in plant-pollinator mutualism (Maloof and Inouye 2000). The existing literature, however, shows that larcenists are as likely to be beneficial to the flowers they visit as they are to be detrimental. Robbers as well as thieves may alter the direct relationship between plants and pollinators via reducing nectar volume (Irwin and Brody 1998, Maloof 2001). Negative effects are typically seen in pollinator avoidance of stolen flowers with reduced nectar rewards (Irwin and Brody 1999, 2000, Navarro 2001). Nectar robbers might, moreover, reduce the plant's reproductive success directly, i.e. via damage of reproductive organs (McDade and Kinsman 1980, Traveset et al. 1998). On the contrary, close examination of robber behaviour often revealed that they can positively contribute to pollination success by moving pollen onto the stigma in the process of collecting nectar or pollen (Waser 1979, Higashi et al. 1988, Navarro 2000). Besides these direct positive effects of the "robber-like pollinators", reducing nectar volume by larcenists resulting in changing pollinator's behaviour may indirectly increase both female and male plant's reproductive success (Richardson 2004). Pollinators, encountering low-rewarding flowers, are forced to fly further and visit more flowers to maintain their daily energy budget (Maloof 2001). Due to longer distances between subsequent plants, pollinators move the pollen further from close relatives of the plants. This behaviour can decrease inbreeding (Inouye 1983, Fenster 1991), increase pollen flow distances and thereby increase outcrossing rate (Barrett and Harder 1996).

Floral larceny is likely ubiquitous in most plant-animal pollination systems where plants have tubular flowers or flowers with nectar spurs (Irwin and Maloof 2002). Despite the fact that nectar robbers may act as cheaters, the plant-pollinator system persists stably and is robust to robbers' invasion without pollinators being driven to extinction (Wang 2013). Moreover, when considering network topology of host plants cheaters seem to be important to overall stability and integration of natural systems (Genini et al. 2010). An increase in cheating in visitation networks may destroy nestedness and enforce modularity by which the structure would slow down the spread of disturbances (Olesen et al. 2007, Genini et al. 2010).

~ · Geographical variation in pollination systems on a global scale · ~

From the current debates arises the question about how enormous variation of terrestrial plant communities in their structural complexity and taxonomic diversity affect large-scale biogeography and levels of specificity of plant-animal interactions (Waser and Ollerton 2006). It is well documented that tropical rainforest communities show high levels of alpha and beta diversity and multilayer configuration of plants compared to other communities that either contain few species of plants and are structurally very simple (e.g. Arctic tundra) or are somewhere in between these two extremes (tropical and subtropical grasslands, temperate woodland). A similar latitudinal trend can be seen in geographical variation in diversity and specificity of pollination systems at the community level although the relationship between latitude and number of pollination systems does not appear to be simply linear. Disproportionately higher diversity of pollination systems in the tropics compared to equals in other latitudinal zones indicates a step change rather than smooth increase.

Community analyses suggest a number of reasons why this step increase can exist. 1) Tropical communities show greater species richness and diversity at most taxonomic levels than any other ecosystems (Ashton 1969, Appanah 1981, Bawa et al. 1985, Hillebrand 2004). There is a significantly greater number of animal pollinated plant species in tropical regions (Ollerton et al. 2011). 2) Lowland rainforest, where most of the tropical studies were conducted, are mostly structurally complex habitat which in itself is believed to have an effect on the number of pollination systems. Apparent distinction between complex habitat structures and those with simpler structure might be, however, confounded with latitudinal effect in available studies. 3) More opportunities for the evolution of functionally specialized systems may happen with new groups of pollinating animals appearing or becoming more important in tropics, such as birds, bats, or euglossine bees. Although there are some good examples of the one-to-one relationship type of specialization they seem to be an exception rather than the norm in the tropics (Feisinger 1983). Nonetheless, the majority of plant species are specifically pollinated by one species or a few species belonging to the same taxonomic or functional group. Several studies recently have addressed this question and have come to precisely opposite conclusions. While Olesen and Jordano (2002) concluded that tropical communities are more ecologically specialized at pollination level, others critically stated their results and interpretation as not well supported, mainly due to their network approach inappropriately applied to the issue of specialization (Ollerton and Cranmer 2002, Kay and Schemske 2004). 4) More diverse and specialized pollination systems can better evolve in long-term climatically stable environments (i.e. tropics) where the pollinators are better predictable in time and space.

All the observed disproportions might be, however, highly influenced by our inadequate knowledge caused by uneven coverage of altitudes and habitats among the performed studies (Ollerton and Cranmer 2002). Although we have particularly good data about the patterns in structurally complex lowland tropical forest some other regions, such as in Southern Hemisphere and including full range of altitudinal variations and habitat complexities, need to be surveyed to confirm the observed differences.

~ · Pantropical comparisons of bird pollination systems · ~

Although the vast majority of animal-pollinated plants relies on insect, substantial number of tropical and subtropical plants of various growth habits (trees, shrubs, herbs, epiphytes, and vines) are pollinated by birds (Fleming and Muchhala 2008). Specific interactions with these plants led to a substantial adaptive radiation of nectar-feeding birds, independently in three major tropical regions: the Neotropics, sub-Saharan Africa and seasonal Asia, and aseasonal Southeast Asia and Australia (Fleming and Muchhala 2008). New World birds are generally smaller and show a greater degree of feeding specialization than their Old World counterparts (Fleming 2005, Fleming and Muchhala 2008). The Old World tropics and subtropics show a substantial regional variation in the degree of evolutionary specialization. Pretty diverse large, mostly non-hovering sunbirds in sub-Saharan Africa exhibit specific relationships with certain flowers, whereas sunbirds in Southeast Asia and large, non-hovering lorikeets and honeyeaters in

Australia tend to have non-specific relationships with their food plants (Fleming and Muchhala 2008). New World hummingbirds' small size and specialized flight morphology enable them to effectively hover while visiting flowers, whereas most of the Old World bird nectar-feeders preferentially perch on flowers, or branches while feeding. A similar difference characterizes flower or inflorescence size and their nectar rewards (Pellmyr 2002). A flower adapted to hovering pollinators can be small and delicate, and can evolve in the Neotropics insect-pollinated plant lineages more easily than large sturdy flower needed to hold relatively heavy perching birds. This factor might explain the limited evolution of some palaeotropical bird pollination systems that contain less species and smaller morphological diversity in nectar-feeding birds (Fleming and Muchhala 2008).

~ · Objectives and content of the thesis · ~

Much of pollination biology over the past few centuries was logically best investigated on the continents where it originated, Europe and North America, whereas pollination relationships and breeding systems from other regions, particularly Africa's developing countries and much Asia and Latin America, are less well researched. In the literature on African pollination biology, the biggest part of the research is still focused on the nature of the relationships and breeding systems, indicating the general lack of this information from Africa.

In awareness of the need of pollination studies from poorly explored mountain tropical areas as well as the unique opportunity to explore one of the important hotspots of biodiversity, my colleagues and I have conducted studies of fundamental characteristics of target species and their interactions in pollination systems in Bamenda Highlands, Cameroon. During the ten years of research at the locality we aimed to look in detail at the plant-animal communities from many different points of view, and to apply different field techniques to provide the useful references for some of the aspects of pollination biology.

Here, I present 5 case studies dealing with the role of sunbirds in pollination systems of several co-flowering plant species with different pollination syndromes and degree of specialization. To date, most of the pollination systems involving sunbirds were studied in the highly specific Cape region in South Africa while very little work has been done in tropical Africa where sunbirds reach a peak of species diversity (Gill and Wolf 1978, Evans 1996, Rodger et al. 2004). To help fill this gap of knowledge we have surveyed in our studies: (i) how relative resource abundance influences plant selectivity by sunbirds and how this selectivity is related to phenotypic trait matching; (ii) what roles do individual visitors play in pollination systems of selected plants with different syndromes and how good predictors of effective pollinators are these syndromes; (iii) what interactions are between two distantly related visitors utilizing the same nectar source, and what the economics of foraging mechanisms and outcomes of such competition are.

Chapter II examines the nectar abundance effect and phenotypic complementarity between five plants with different flower morphologies and three sunbird visitors with different bill lengths.

Chapter III describes a highly specialized pollination system including frequent hovering bird pollination and discusses the possible selective pressures of sunbirds on the evolution of plant traits.

Chapter IV presents a test of validity for the concept of pollination syndromes on plants showing bee-pollination syndrome but being visited by a diverse animal assemblage and demonstrates the advantages of applied field methods.

Chapter V shows the interference competition between sunbirds and carpenter bees feeding on the same nectar plant.

Chapter VI compares the energetic requirements of different-sized nectar feeders and discusses potentially important selective pressures of their foraging activities on plant populations.

Chapter VII summarises the main results of this thesis.

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CHAPTER II

Food selection by avian floral visitors: an important aspect of plant–flower visitor interactions in West Africa

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Abstract

Community-level studies have shown that plant–pollinator interactions are much more generalized than previously expected. Consequently, many authors have questioned the significance of phenotypic complementarity between plants and pollinators and abundance effects in pollination interactions. Here, we compare the behaviour of three sunbird species feeding on the nectar of five plant species in afromontane vegetation. We studied the feeding behaviour with and without consideration of plant abundance (i.e. diet selectivity and diet composition, respectively). The aims of the study were to estimate: (1) how relative resource abundance influences flower selectivity; (2) the degree of phenotypic matching; and (3) whether different plant resource assessment methods give different answers to this question. The results showed that, although sunbirds frequently feed on both morphologically adapted and nonadapted plants, food selectivity data are consistent with the hypothesis of phenotypic complementarity. Moreover, we found that the type of plant abundance measurement can change conclusions in some cases, as individual plants differ in their growth habits and nectar production. This effect was most obvious for the assessment of selectivity of the northern double-collared sunbird (*Cinnyris reichenowi*) and for *Hypoestes aristata*, a plant producing inflorescences composed of a large number of small flowers possessing small amounts of nectar per flower (a high abundance of flowers, but a low abundance of nectar relative to the remaining plant community).

Introduction

An increase in ecological and subsequent phenotypic specialization because of natural selection is one of the fundamental principles of Darwin's evolutionary theory (Darwin, 1859). One of the most famous examples of specialization during a coevolutionary process is the highly specific pairwise interaction between Darwin's long-spurred orchid, *Angraecum sesquipedale*, and the subsequently discovered long-proboscis moth, *Xanthopan morganii* (Darwin, 1862). Instead, more recently, researchers have documented a much higher degree of ecological generalization in both mutualistic (Ollerton, 1996; Waser et al., 1996) and trophic (Binning et al., 2009) interactions than previously expected.

In studies focused on plant–pollinator interactions, the role of morphological thresholds has been highlighted. A flower tube of a particular length and width excludes floral visitors with mouthparts shorter and/or wider, whereas visitors with longer and narrower mouthparts are able to visit a wide spectrum of flower morphologies. Borrell (2005) showed that long-tubed flowers are specialized for pollination by long-tongued euglossine bees, but the long tongues of euglossine bees did not prevent them from feeding at short flowers. The long-proboscid flies (Nemestrinidae) feed not only on flowers with long tubes, but also on flowers with short tubes (Potgieter et al., 1999), or even on nontubular generalized flowers (Devoto and Medan, 2006). However, some studies have reported a lower efficiency of longtongued bees at open flowers (Inouye, 1980). Similar patterns have also been documented for nectarivorous birds. For example, the diets of Australian nectarivorous birds reflect habitat preferences rather than flower morphology, and they visit both tubular and more open flowers (Franklin and Noske, 2000). Similarly, the bill morphology of nectarivorous birds is not related to floral morphology in New Guinean rainforests (Brown and Hopkins, 1995). The highest degree of matching between the morphological traits of nectarivorous birds and flowers is demonstrated mainly by the New World long-billed hermit hummingbirds (Phaethornithinae) (Snow and Snow, 1972). Small, short-billed hummingbirds, however, visit a wide spectrum of floral morphotypes (Snow and Snow, 1972; Dalsgaard et al., 2009). The interactions between sunbirds (Nectariniidae) and plants seem to include signs of both ecological specialization and generalization. Although Fleming and Muchhala (2008) concluded that sunbirds visit mainly specialized (tubular) flowers, other authors observed that sunbirds frequently feed on a wide spectrum of unspecialized plant species (Cheke et al., 2001). The evolution of specialized long mouthparts in birds can also be seen as a specialization for ecological generalization, which enables floral visitors to enlarge the spectrum of visited flowers (Stang et al., 2007).

In addition to trait matching, the abundance of plants and visitors has been suggested as a necessary prerequisite for a proper understanding of plant–pollinator interactions (Vázquez and Aizen, 2004; Stang et al., 2006; Vázquez et al., 2007, 2009a). It has been shown that the abundance of individual species in a community explains the high proportion of variability in the attributes of plant–pollinator networks, including network nestedness, connectance, interaction evenness and interaction asymmetry (Vázquez and Aizen, 2004; Vázquez et al., 2007, 2009b; for an explanation of individual terms, see Bascompte and Jordano, 2007 or Vázquez et al., 2009a). Although it has been suggested that employing different measurements of plant resource abundance (e.g. individuals, flowers, pollen or nectar) may influence significantly the results (Vázquez et al., 2009a, b), studies considering different levels of plant resource abundance are rare. This issue is more pronounced when we consider pollination from the pollinator's point of view. For instance, energetic values of individual flowers can be more important than the number of flowers or individuals (Vázquez et al., 2005; Stang et al., 2009). Given that growth and floral display habits often vary within a community, the method of assessment of plant resource abundance may have a large effect on how different species are prioritized, e.g. plants with rich inflorescences of small nectar-poor flowers will have high abundance in terms of flower number, but relatively low abundance in terms of nectar production.

In this study, we focused on the plant abundance effect and on the phenotypic complementarity between plants and flower visitors in a community consisting of three sunbirds of different bill lengths and five plants with different flower morphologies in the Bamenda Highlands, Cameroon. We aimed to describe the feeding behaviour of sunbirds both without considering plant abundance (diet composition in terms of resource use) and with relative plant abundance taken into account (food selectivity as a function of both resource use and resource availability). Specifically, we questioned: (1) whether the diet selectivity approach would expose trait matching between bill and flower morphology; and (2) whether three types of plant abundance measurements (nectar production, number of flowers or number of plants) would affect the assessment of sunbird selectivity.

Methods

Study area

The study site was situated in the Mendongbuo area, within the Elba Ranch, in the Bamenda-Banso Highlands above Big Babanki village (Cameroon, 6°5'N, 10°18'E, 2200 m a.s.l.). It included approximately 0.5 ha of stream mantel vegetation growing around a small stream. The plant community was dominated by *Gnidia glauca* (Thymelaeaceae), *Croton macrostachyus* (Euphorbiaceae) and *Pittosporum viridiflorum* (Pittosporaceae) in the tree layer, and *Phyllanthus mannianus* (Euphorbiaceae), *Psychotria peduncularis* (Rubiaceae), *Hypericum revolutum* and *Hypericum roeperianum* (Hypericaceae) in the shrub layer. Pastures and *Pteridium aquilinum* growth surround this vegetation.

Plant species

Our study focused on five plant species on which sunbirds fed during our unpublished pilot observations: *Pycnostachys eminii* Gürke (Lamiaceae), *Lobelia columnaris* Hook. f. (Campanulaceae), *Hypoestes aristata* (Vahl) Roem & Schult (Acanthaceae), *Hypericum revolutum* Vahl (Hypericaceae) and *Impatiens sakeriana* Hook. f. (Balsaminaceae). According to pollination syndromes (Faegri and van der Pijl, 1979), *I. sakeriana* can be considered as a typical bird-pollinated species (see also Janeček et al., 2011 and Bartoš et al., 2012), whereas *Hypericum revolutum* and *P. eminii* are typical insect-pollinated species (Janeček et al., 2007; Bartoš et al., 2012). The other two species (*L. columnaris* and *Hypoestes aristata*) have some traits associated with insect pollination and others with bird pollination. We used corolla length as a factor limiting the accessibility of the nectar resources (Stang et al., 2007; Dalsgaard et al., 2009). For *L. columnaris*, calyx length was measured as it is the main nectar barrier for sunbirds. Measurements of corolla length were taken from 30 individual plants of each species, randomly chosen within the study area (Table 1, Fig. 1).

Table 1. Characteristics of plant species studied in 2003 and 2007 in the Bamenda Highlands, Cameroon. Corolla length (ANOVA, d.f. = 3, F = 635, P < 0.01), amount of sugar per flower (ANOVA, d.f. = 4, F = 85.49, P < 0.01), nectar concentration (ANOVA, d.f. = 4, F = 66.13, P < 0.01) and nectar volume (ANOVA, d.f. = 4, F = 90.74, P < 0.01). The same superscripts indicate nonsignificant differences between individual plant species [*post-hoc* Tukey's honestly significant difference (HSD) test]. For more information on nectar traits of target plant species, see Bartoš et al. (2012)

Plant species	Flower colour	Flower shape	Corolla length (mm)	Amount of sugar per flower (mg)	Nectar concentration (w/w)	Nectar volume per flower (µL)
Impatiens sakeriana	Red	zyg., spur.	23.2 ± 2.1^{A}	14.02 ± 10.93^{A}	30.88 ± 5.97^{A}	38.42 ± 28.41^{A}
Lobelia columnaris	Pale blue	zyg., tub.	19.0 ± 1.9^{C}†	11.34 ± 10.90^{A}	42.33 ± 14.93^{B}	24.46 ± 26.44^{B}
Hypoestes aristata	Mauve	zyg., tub.	10.2 ± 0.6^{B}	0.98 ± 1.13^{B}	62.23 ± 23.43^{C}	1.27 ± 1.52^{C}
Pycnostachys eminii	Pale blue	zyg., tub.	8.7 ± 0.7^{D}	0.24 ± 0.22^{B}	61.77 ± 23.82^{C}	0.29 ± 0.22^{C}
Hypericum revolutum	Yellow	act., flat	–	6.73 ± 7.25^{A}	42.93 ± 30.87^{B}	19.46 ± 22.84^{B}

Values are means ± standard deviation.

†For*L. columnaris*, calyx length was measured as it represents the nectar barrier (see Fig. 2).

act., actinomorphic; spur., spurred; tub., tubular; zyg., zygomorphic.

Measurements of nectar production

Nectar production of individual plants was measured using sets of bagged flowers. The number of sets was species specific to cover the whole flower lifetime, and 16 flowers per set were analysed. Each set was used for one harvest and individual harvests represented different flower age classes. The harvests were performed at the same time during the day – 06.30 and 16.30 h for the long-flowering species (*I. sakeriana* and *L. columnaris*) and 06.30, 11.30 and 16.30 h for the short-flowering species (*Hypericum revolutum*, *Hypoestes aristata* and *P. eminii*). Nectar was extracted from flowers using 5-, 10- or 25-*µ*L microcapillaries or Hamilton syringes based on flower size and nectar volume. Sugar content was measured with a pocket refractometer (ATAGO PAL-1; USA); small amounts of nectar (which were usually highly concentrated and highly viscous) were diluted with distilled water before measurement, with the original sample sugar concentration calculated on the basis of this dilution. The total amount of sugar per flower was calculated using sugar concentration per unit volume (mg μL^{-1}) and sugar volume (Bolten et al., 1979). To calculate the sugar amount per microlitre from the w/w concentration (the concentration measured using a refractometer), we used an exponential regression equation (Galetto and Bernardello, 2005). Because the quantity of nectar in one flower of *P. eminii* was too small for nectar analyses, we collected nectar from several flowers in one inflorescence, determined the nectar volume based on the combined sample and calculated the nectar volume per flower. For the purposes of this study, we determined nectar abundance on each observed plant as a mean total amount of sugar content per flower (Table 1) multiplied by the number of flowers on the plant. More details on nectar production in the target plant species studied are given in Bartoš et al. (2012).

Sunbirds

We focused on the feeding behaviour of all three sunbird species (Table 2, Fig. 1) that occur in the study area: (1) the northern double-collared sunbird *Cinnyris reichenowi*, which is the most abundant species in open woodlands, forest clearings and ecotones; (2) the orange-tufted sunbird *Cinnyris bouvieri*, an abundant species in open habitats; and (3) the Cameroon sunbird *Cyanomitra oritis*, a species inhabiting the dense vegetation in forest clearings and shrubby patches (Reif et al., 2006, 2007). The last species is endemic to the Cameroon Mountains; the other two species are distributed throughout West-Central and East Africa (Borrow and Demey, 2001). Sunbirds are territorial, but often exhibit off-territory forays for nectar. To obtain morphological measurements, we trapped sunbirds with mist nets during November and December in both 2003 and 2007. Two morphological traits were measured for each trapped individual: body weight and bill length. The bill length was measured as the length of the exposed culmen (from the bill tip to where the feathers end on the upper bill). In total, 246 individuals were measured. Bill lengths of the three sunbird species decrease in the sequence *Cyanomitra oritis* > *Cinnyris bouvieri* > *Cinnyris reichenowi*, and bill length was positively related to body mass Table 2). The trapped sunbirds were colour ringed to avoid pseudoreplication in the dataset.

The study was carried out during the breeding season at the start of the dry period when pair formation, courtship and incubation occur. The observations finished when the incubation and chick-rearing periods started because the young are fed mainly on insects (Cheke et al., 2001; Procházka et al., 2010).

Observations of sunbird foraging behaviour

We established observation points to record sunbird behaviour. Each observation point (a space observed by one researcher) comprised all individuals of the five plant species visited by sunbirds and growing in an approximately 10-m-long segment of stream mantel vegetation (approximately 16 plants per observation point). Individual points were observed during 30-min sessions, with a minimum total of 40 h spent at each observation point. Because pollination behaviour may vary between years (Alarcón et al., 2008; Petanidou et al., 2008), we repeated the observations at the beginning of two dry seasons:between 19 November 2003 and 9 December 2003 and between 29 November 2007 and 19 December 2007. We observed 20 points and gathered data on 363 individual plants in 2003, and we observed 16 points and gathered data on 231 individual plants in 2007. We estimated the number of flowers for all plant individuals of the target plant species during consecutive 5-day periods to eliminate changes caused by the phenological shift of individual plants (see Supporting information, Table S1).

Observations were evenly distributed during the study periods in both the years (50% of the observations occurred from 06.00 to 11.30 h and 50% between 12.30 and 16.00 h). A voice recorder was used to record the observations of feeding sunbirds. For each individual bird on each individual plant, we recorded the plant species, sunbird species and length of time the bird spent on feeding nectar. As most of the studied plants are clonal, individual plants were often considered as ‘plant clusters’, which probably originated by clonal spread.

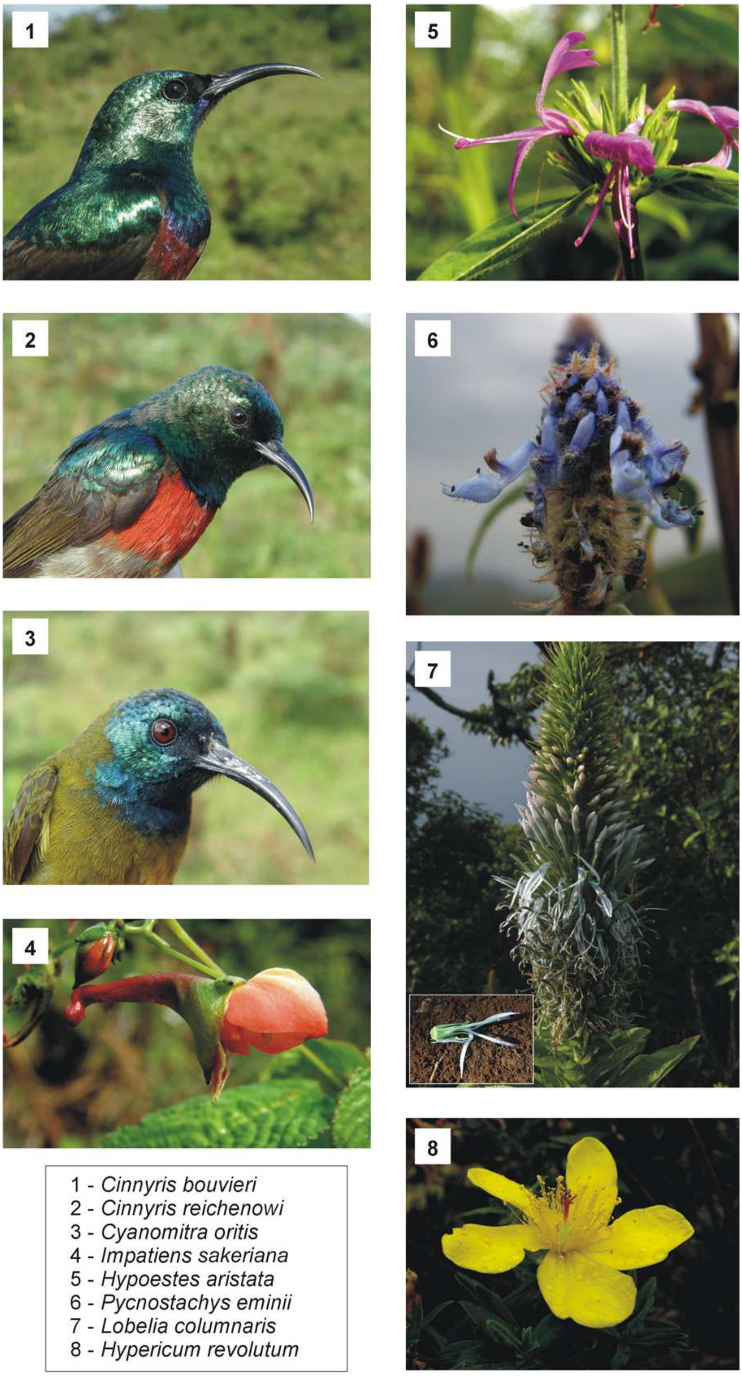


Fig. 1. Target plant and sunbird species (photographs 1–3 were taken by Pavla Blažková and photographs 4–8 by Jan Riegert).

Table 2. Characteristics of sunbird species studied in 2003 and 2007 in the Bamenda Highlands, Cameroon. Bill length (ANOVA, d.f. = 2, $F = 983$, $P < 0.01$) and body weight (ANOVA, d.f. = 2, $F = 203$, $P < 0.01$). The same superscripts indicate nonsignificant differences between individual sunbird species [*post-hoc* Tukey's honestly significant difference (HSD) test]

Sunbird species	Bill length (mm)*	Body weight (g)*	Number of individuals measured
Cinnyris reichenowi	16.0 ± 1.2^{A}	8.1 ± 1.2^{A}	144
Cinnyris bouvieri	17.3 ± 1.1^{B}	8.3 ± 0.8^{A}	22
Cyanomitra oritis	25.5 ± 1.6^{C}	11.9 ± 1.8^{B}	80

*Values are means ± standard deviation.

Sunbird selectivity and statistical analyses

The analysis of variance (ANOVA) in the program STATISTICA version 10 was used to compare plant (Table 1) and sunbird (Table 2) traits.

We used Jacobs' selectivity index, $D_i = (r_i - p_i)/(r_i + p_i - 2r_ip_i)$ (Jacobs, 1974), to determine the feeding selectivity of sunbird species. This index contains the resource use ratio (r_i), the ratio of resource i used to the amount of all used resources, and the resource availability ratio (p_i), the ratio of the resource i in the system with respect to the amount of all available resources. The index D_i varies from 1 (positive selection) to -1 (negative selection). We approximated the resource use ratio as the ratio between feeding durations on an individual plant and total feeding duration on all plants including inter-flower flights (for advantages and disadvantages of this approach, see Discussion). For statistical analyses, we calculated the selectivity indices of individual sunbird species for each plant individual in three ways, each differing in plant resource abundance assessment. As a measurement of plant resource abundance, we considered: (1) the number of plants (i.e. total number of plants present); (2) the number of flowers (number of flowers on the individual/total number of flowers present); and (3) nectar production. The plant resource availability ratio calculated in terms of nectar production is the amount of sugar produced by a plant individual (the number of flowers of an individual plant multiplied by the mean sugar amount per flower for a given species) divided by the total sugar produced by all the observed plant individuals in the community. It should be noted that the resource use ratio (r_i) was the same for all three types of selectivity index.

The selectivity indices of each sunbird species were calculated for each of the 594 observed plants. These selectivity indices thereafter represent the response variables. When we target on sunbird selectivity (Table 3); each plant individual (coded as plant identification) was used as the random factor, and sunbird species, plant species and year were employed as fixed factors. As the data distributions do not fulfil the assumptions of traditional ANOVA (e.g. most observed plants were unvisited and had a selectivity index of -1), we used analogical permutation tests in the program PERMANOVA+ for PRIMER (Anderson et al., 2008). Using permutation tests, the pseudo-*F* ratio was calculated in a similar manner to the *F* ratio in traditional methods, but does not correspond to Fisher's *F* distribution, and the appropriate distribution under a true null hypothesis is obtained by the permutation procedure (Anderson et al., 2008).

PERMANOVA was also used to test the differences between individual methods of plant abundance estimation on assessment of individual sunbird species preferences (Table 4). In these analyses, the plant individual (plant identification) represents a random factor and method type, year and plant affiliation to species represent the fixed factors.

To determine whether the methods differed for individual sunbirds in individual years and for individual species, we performed the PERMANOVA tests in the same way, but separately, for each sunbird species–plant species–year interaction (Table 5). In consequence, two factors were only included in these analyses: plant individual as random factor and method as fixed factor. When the permutation ANOVA was statistically significant, we performed permutation pairwise comparisons, which corresponded to parametric *t*-tests (Anderson et al., 2008).

Results

Sugar amounts and nectar volumes per flower were much larger for *L. columnaris*, *Hypericum revolutum* and *I. sakeriana* than for *P. eminii* or *Hypoestes aristata* (Table 1). The sugar concentration was highest in the nectar of

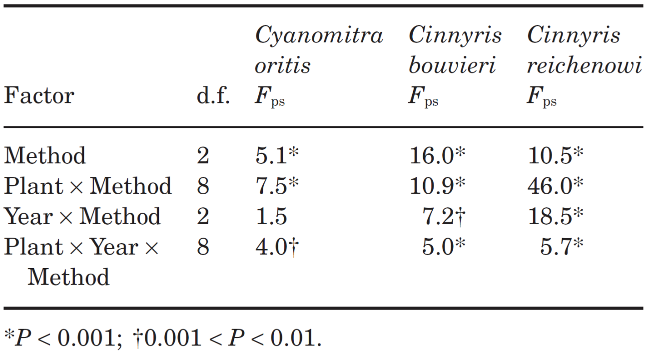
Hypoestes aristata and *P. eminii* and lowest in the nectar of *I. sakeriana* (Table 1). At the community level, *L. columnaris* and *Hypericum revolutum* were the largest nectar sources in the observed area (Fig. 2A). The contribution of *L. columnaris* to the total nectar available in the community was greater in 2007 than in 2003 (Fig. 2A), because the peak of *L. columnaris* flowering occurs late in the dry season, and the observation period was later in 2007 than in 2003. Considering the number of flowers and number of plants as measures of plant abundance, the highest number of flowers was recorded for *Hypoestes aristata* and the highest number of plants was recorded for *Hypericum revolutum* for both years (Fig. 2B, C). The longest handling times per plant were recorded for *I. sakeriana* in the year 2003 and the shortest for *P. eminii* (for data on handling times per plant, see Table S1). The handling times did not differ between sunbird species at individual plants (results not shown). In 2003 and, to a lesser degree, in 2007, all sunbirds spent a high proportion of their feeding time (*r*) on *Hypericum revolutum* (Fig. 2D–F). The time spent on other plant species differed depending on the sunbird species. There were no records of *Cinnyris bouvieri* feeding on *I. sakeriana* or of *Cyanomitra oritis* feeding on *P. eminii*. Sunbirds fed more on *L. columnaris* in 2007 than in 2003 (Fig. 2D–F), when the highest abundances of *L. columnaris* were recorded. Sunbird species selected different plant species, and the pattern of selectivity was the same for both years; this was true for all selectivity measurements considering different measures of plant abundance (Table 3, General test). Birds also differed in selectivity for all target plant species with only one exception (selectivity for *P. eminii*, considering the number of flowers as a measure of plant abundance) and different bird selectivity between years was detected only for *Hypericum revolutum* (Table 3, part A). Individual sunbird species differently selected individual plant species, regardless of whether considering nectar production, number of flowers or number of plants (Table 3, part B).

Table 3. Selectivity of individual sunbird species: D_n, Jacobs' selectivity index, where nectar production represents plant resource abundance; D_f, Jacobs' selectivity index, where the number of flowers represents plant resource abundance; D_p, Jacobs' selectivity index, where the number of plants represents plant resource abundance. Pseudo-F values (F_{ps}) are shown. Year, plant species (Plant) and bird species (Bird) were considered to be fixed factors. Always significant results for the random factor plant identification, which was included in the General test and tests for individual plant species, are not shown

		D_n	D_f	D_p
	d.f.	F_{ps}	F_{ps}	F_{ps}
General test				
Year	1	0.0	0.0	0.2
Bird	2	83.7*	69.4*	80.7*
Plant	4	14.1*	18.7*	24.0*
Year × Plant	4	0.7	0.5	1.9
Year × Bird	2	19.2*	19.9*	7.2*
Bird × Plant	8	23.1*	19.3*	27.2*
Year × Bird × Plant	8	1.5	1.7	1.7
Part A – selectivity for individual plant species				
Impatiens sakeriana				
Year	1	1.4	0.9	0.6
Bird	2	24.4*	29.6*	28.6*
Year × Bird	2	1.6	1.2	1.7
Lobelia columnaris				
Year	1	0.2	0.0	1.3
Bird	2	24.0*	24.5*	25.5*
Year × Bird	2	0.2	0.4	1.3
Hypoestes aristata				
Year	1	0.0	0.1	3.5
Bird	2	57.2*	25.1*	46.0*
Year × Bird	2	2.3	1.5	0.0
Pycnostachys eminii				
Year	1	0.0	0.0	0.7
Bird	2	3.4‡	1.8	4.4†
Year × Bird	2	3.0‡	2.0	1.2
Hypericum revolutum				
Year	1	0.0	0.0	0.2
Bird	2	46.5*	46.7*	44.9*
Year × Bird	2	21.6*	25.2*	11.6*
Part B – selectivity of sunbirds				
Cyanomitra oritis				
Year	1	4.0	5.0‡	4.1
Plant	4	11.9*	13.5*	14.6*
Year × Plant	4	3.0‡	2.7‡	2.7‡
Cinnyris bouvieri				
Year	1	6.5‡	6.2‡	2.5
Plant	4	24.6*	31.5*	33.9*
Year × Plant	4	0.3	0.2	1.6
Cinnyris reichenowi				
Year	1	17.9*	18.4*	4.7‡
Plant	4	19.5*	12.8*	27.4*
Year × Plant	4	0.7	0.9	1.3

*$P < 0.001$; †$0.001 > P < 0.01$; ‡$0.01 > P < 0.05$.

Table 4. The effect of using different abundance approaches (Method) on Jacobs' selectivity index. Pseudo-*F* values (F_{ps}) are shown. The plant individual identification was used as a random factor (results for this always significant factor are not shown), and method, plant species and year as fixed factors. For more information, see Material and methods



Factor	d.f.	*Cyanomitra oritis* F_{ps}	*Cinnyris bouvieri* F_{ps}	*Cinnyris reichenowi* F_{ps}
Method	2	5.1*	16.0*	10.5*
Plant × Method	8	7.5*	10.9*	46.0*
Year × Method	2	1.5	7.2†	18.5*
Plant × Year × Method	8	4.0†	5.0*	5.7*

*$P < 0.001$; †$0.001 < P < 0.01$.

The method of plant abundance measurement had a significant effect on the selectivity of individual sunbirds, and the method used affected the selectivity differently for individual plant species in individual years (Tables 4 and 5). It should be noted that Table 5 shows the preference on an individual level and, in consequence, the preference is indicated by a less negative preference value rather than by a positive value. *Cyanomitra oritis* mostly selected for plants of *I. sakeriana* and *Cinnyris bouvieri* for plants of L. columnaris without regard to the method of plant abundance assessment. *Cinnyris reichenowi* selected mostly for *Hypoestes aristata* when considering nectar production as the plant resource abundance measurement, but for *I. sakeriana* when considering the number of flowers or number of plants as the measure of plant resource abundance (Table 5). A similar pattern was observed when the selectivity indices were calculated on the plant specimen level (selectivity indices calculated for each of the 594 plants, Table 5) instead of on the plant species level (selectivity index calculated for each plant species, Fig. 3).

Discussion

When considering phenotypic specialization and dietary niche breadth of pollinators, our results are in agreement with those of previous studies showing that phenotypically specialized birds are able to feed on a wider spectrum of plants than predicted by their specialized traits (Snow and Snow, 1972; Woodell, 1979; Brown and Hopkins, 1995; Franklin and Noske, 2000; Fleming and Muchhala, 2008; Dalsgaard et al., 2009). For the sunbirds studied, we therefore argue that there is no clear trade-off between the evolution of phenotypic specialization and feeding on plants with easily accessible nectar (e.g. *Hypericum revolutum*). In other words, even though sunbirds have phenotypically specialized bills, they readily feed on nonspecialized flowers and can be considered to be ecologically generalized. Nevertheless, our study demonstrated the strong effect of plant abundance on feeding behaviour, similar to that observed in studies on plant–flower visitor networks (Vázquez, 2005; Vázquez and Aizen, 2006; Vázquez et al., 2007). When plant abundance was considered, we detected a clear pattern of selectivity for specialized long tubular flowers, as well as trophic niche partitioning, among the sunbirds studied.

The sunbird with the longest bill, *Cyanomitra oritis*, selected *I. sakeriana*, which was the plant with longest flowers. The sunbird with a bill length that was somewhat shorter than that of *Cyanomitra oritis*, i.e. *Cinnyris bouvieri*, selected *L. columnaris*, whose calyx (which is a nectar barrier in this species) was somewhat shorter than the corolla of *I. sakeriana.* This general pattern was clear regardless of which approach to the measurement of plant abundance was used (nectar production, number of plants or number of flowers). *Cinnyris reichenowi* selected *Hypoestes aristata* only if nectar production or the number of plant individuals was considered as the measure of plant abundance, and *Hypoestes aristata* was the most selected species during both years only if nectar production was used as the measure of abundance.

Table 5. Mean selectivity of sunbirds for one individual plant. D_n, Jacobs' selectivity index, where nectar production represents the plant resource abundance; D_f, Jacobs' selectivity index, where the number of flowers represents the plant resource abundance; D_p, Jacobs' selectivity index, where the number of plants represents the plant resource abundance; F_{ps}, pseudo-F value calculated by individual permutation ANOVAs comparing differences between methods of plant abundance approach. The same letters indicate nonsignificant differences between plant abundance approaches. Note that the ANOVA test was not calculated for plants which were not visited a single time ('–'), and pairwise comparisons were calculated only when the ANOVA test was significant. The plant individual identification was used as a random factor. For more information, see Material and methods.

		Cyanomitra oritis				*Cinnyris bouvieri*				*Cinnyris reichenowi*			
Year	Plant	D_n	D_f	D_p	F_{ps}	D_n	D_f	D_p	F_{ps}	D_n	D_f	D_p	F_{ps}
2003	*I. sak.*	-0.493^A	-0.386^B	-0.424^{AB}	3.8‡	−1.000	−1.000	−1.000	–	-0.097^A	0.169^B	0.092^{AB}	6.1*
	L. col	−0.873	−0.816	−0.850	2.3	-0.231^A	-0.119^B	-0.346^C	10.9*	-0.555^A	-0.438^B	-0.609^A	3.7‡
	H. ari.	-0.814^A	-0.894^B	-0.738^A	5.3†	-0.692^A	-0.781^B	-0.684^A	5.1*	-0.097^A	-0.516^B	-0.001^A	36.0*
	P. emi.	−1.000	−1.000	−1.000	–	-0.740^A	-0.911^B	-0.866^C	6.4*	−0.953	−0.996	−0.982	1.6
	H. rev.	-0.798^A	-0.770^B	-0.821^C	14.4*	-0.813^A	-0.773^B	-0.848^C	19.9*	-0.666^A	-0.631^B	-0.689^A	10.7*
2007	*I. sak.*	-0.071^A	0.013^B	-0.029^A	3.3‡	−1.000	−1.000	−1.000	–	-0.019^A	0.208^B	0.034^A	13.0*
	L. col	−0.937	−0.930	−0.925	1.2	-0.312^A	-0.171^B	-0.192^B	13.1*	-0.521^A	-0.357^B	-0.396^B	7.8*
	H. ari.	−0.896	−0.926	−0.911	1.3	−0.873	−0.919	−0.886	2.4	0.086^A	-0.417^B	-0.147^C	34.2*
	P. emi.	−1.000	−1.000	−1.000	–	−1.000	−1.000	−1.000	–	−0.694	−0.883	−0.978	1.0
	H. rev.	−0.932	−0.927	−0.930	1.4	-0.919^A	-0.912^B	-0.936^{AB}	3.2‡	-0.397^A	-0.324^B	-0.545^C	47.7*

H. ari., *Hypoestes aristata*; *H. rev.*, *Hypericum revolutum*; *I. sak.*, *Impatiens sakeriana*; *L. col.*, *Lobelia columnaris*; *P. emi.*, *Pycnostachys eminii*.

*$P < 0.001$; †$0.001 < P < 0.01$; ‡$0.01 < P < 0.05$.

On the basis of selectivity analyses, we can conclude that patterns of food preference observed in our study area are in accordance with the suggestion that complementary phenotypes are important determinants of plant–flower visitor interactions (Inouye, 1980; Armbruster and Guinn, 1989; Stang et al., 2006, 2007, 2009). Our results indicating that sunbirds select plants with a corolla length similar to the length of their bills, and that this selectivity decreases for plants with both longer and shorter corollas, are in accordance with the findings of Stang et al. (2009), who studied a plant–pollinator interaction network in the Mediterranean community, including insect pollinators, and with the findings of the theoretical models of Santamaría and Rodríguez-Gironés (2007). Stang et al. (2009) showed that the interactions between plants with openly accessible nectar and pollinators with long proboscises are less frequent than interactions between plants and pollinators with related or complementary morphologies. Our results, however, provide one exception to this scenario, as *Cinnyris reichenowi* (a short-billed sunbird) selected the long-spurred *I. sakeriana* for feeding, regardless of which measure of plant resource abundance was used. This finding seems to contradict the concept of phenotypic complementarity. We suggest, however, that this discrepancy might be explained by our observations of *Cinnyris reichenowi* 'stealing' nectar by piercing the flower spur of *I. sakeriana* in more than 50% of visits (Janeček et al., 2011). In other words, *Cinnyris reichenowi* evades the plant size threshold defined by the spur.

Unfortunately, our methods of observation did not allow us to collect data on the number of visited flowers, the feeding time per flower or nectar extraction efficiency, as performed in other studies using different types of observational approach (Wolf et al., 1972; Gill and Wolf, 1978; Montgomerie, 1984). The advantage of our method is that more plants can be observed from greater distances, and this method can be used for plants for which the assessment of the number of visited flowers is difficult (e.g. the small flower heads of *P. eminii* in our study). We also did not consider possible differences in extraction efficiency among sunbirds. Therefore, phenotypic complementarity might be underestimated, as it has been shown that nectarivorous birds explore phenotypically related flowers more efficiently (Wolf et al., 1972; Gill and Wolf, 1978; Montgomerie, 1984).

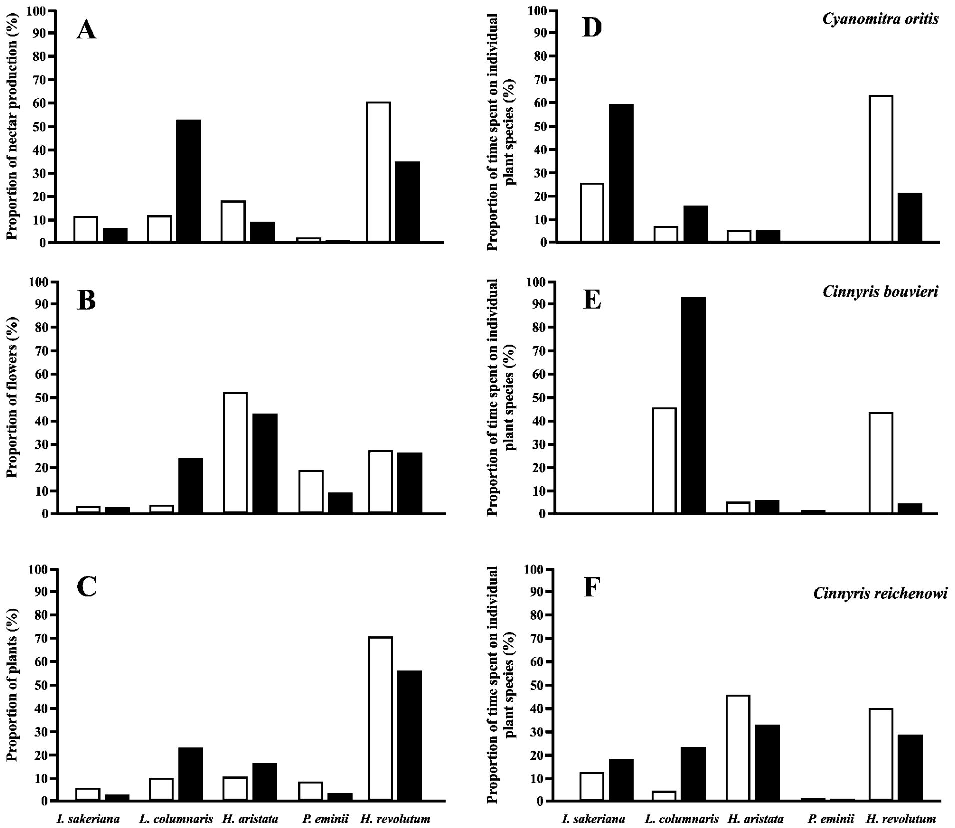


Fig. 2. Proportion of observed resources, considering nectar production (A), number of flowers (B) and number of plants (C), and proportion of time spent by individual sunbird species on target plants (D–F). Open bars, 2003; filled bars, 2007. It should be noted that the abundance from which the proportions were calculated were weighted by minutes of observation on each individual plant (abundance/observation time); this is why they differ slightly from the proportions calculated from the data in Supporting information (Table S1), which show the total number of flowers and plants at the study site. The plants on the *x* axis are arranged in the order of corolla length (*Impatiens sakeriana* > *Hypericum revolutum*).

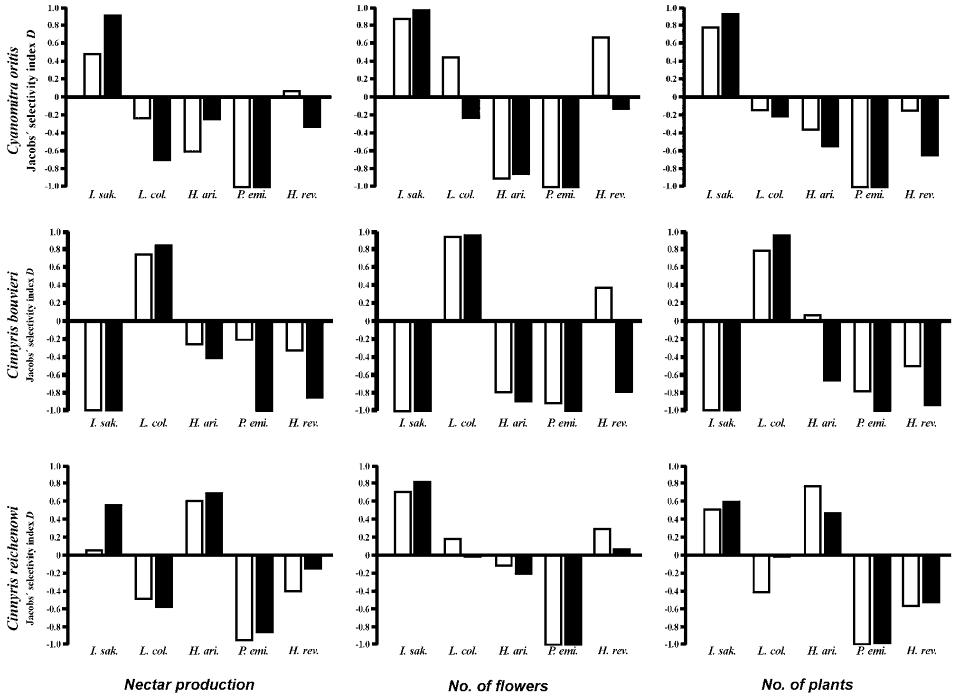


Fig. 3. Jacobs' selectivity indices (*D*) of individual sunbird species for target plant species, considering different assessments of plant abundance measures (nectar production, number of flowers and number of plants). Open bars, 2003; filled bars, 2007. The plants on the *x* axis are arranged in the order of corolla length (*Impatiens sakeriana* > *Hypericum revolutum*). The bill lengths decrease from the top in the sequence: *Cyanomitra oritis* > *Cinnyris bouvieri* > *Cinnyris reichenowi*. *H. ari.*, *Hypoestes aristata*; *H. rev.*, *Hypericum revolutum*; *I. sak.*, *Impatiens sakeriana*; *L. col.*, *Lobelia columnaris*; *P. emi.*, *Pycnostachys eminii.*

Nevertheless, we believe that our approach was sufficient to support the phenotypic complementarity hypothesis. Subsequent studies in our system should also target on mechanisms that can modify the bird–plant interactions, including sunbird competition, aggression and territoriality (e.g. Feinsinger, 1976; Frost and Frost, 1980).

Although, we only investigated the bird's point of view in this study, it is possible to compare our current results with those of our previous studies that focused on the pollination systems of two plant species visited by sunbirds (Janeček et al., 2007, 2011). In the study on *Hypericum revolutum* (Janeček et al., 2007), we showed that the fitness of this plant species is independent of sunbird visits. Therefore, we infer that there is no clear selection pressure driving floral adaptations and that sunbirds can be seen as robbers that utilize a relatively small proportion of the *Hypericum revolutum* rewards (as they have negative selectivity for *Hypericum revolutum*). In contrast, in a study that included a plant with morphologically specialized flowers (*I. sakeriana*), we showed that the sunbirds *Cyanomitra oritis* and *Cinnyris reichenowi*, which prefer this species, are exclusive pollinators of *I. sakeriana* (Janeček et al., 2011); this study also showed that *Cyanomitra oritis* is a much more effective pollinator than *Cinnyris reichenowi*. On the basis of these findings, we can conclude that both reciprocal ecological specialization and phenotypic complementarity between *Cyanomitra oritis* and *I. sakeriana* support the hypothesis that these two species have coevolved.

In this study, we have demonstrated that the food selectivity approach provides important insights into plant–visitor interactions and, when used with other approaches (as with studies on plant–pollination systems of individual plant species, e.g. Janeček et al., 2007, 2011), can be useful for constructing hypotheses concerning coevolution, for explaining the evolution of specialized adaptations and for demonstrating trophic niche separation between individual actors. Moreover, we show that the type of plant abundance measurement can change the conclusions in some cases. Future studies should include experimental manipulation, and should be focused on the exploration of the mechanisms underlying these observed patterns, mainly on the factors affecting food selectivity, such as the effect of competition (e.g. Rodríguez-Gironés and Santamaría, 2010), differences in foraging strategies and energetics (e.g. Gill and Wolf, 1978) and differences in the preferences for nectar quality (e.g. Johnson and Nicolson, 2008).

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Supporting information

Table S1. Number of plant individuals at the study site, number of flowers at the study site (mean across counts performed every 5 days), number of visits of individual sunbird species (*N*) and mean handling time (*Ht*) per plant in seconds.

Plant species	**Year**	**No. of plants**	**No. of flowers**	***C. oritis***		***C. bouvieri***		***C. reichenowi***	
				N	***Ht***	***N***	***Ht***	***N***	***Ht***
Impatiens sakeriana	2003	15	202	35	62	0	-	96	32
	2007	12	95	18	23	0	-	115	17
Lobelia columnaris	2003	33	419	14	34	207	19	64	14
	2007	51	1018	5	29	196	16	135	21
Hypoestes aristata	2003	35	4224	9	13	12	14	188	31
	2007	35	1989	3	9	4	28	180	21
Pycnostachys eminii	2003	27	3293	0	-	6	15	4	5
	2007	6	384	0	-	0	-	4	7
Hypericum revolutum	2003	253	2143	85	23	79	15	235	18
	2007	127	1210	7	19	14	15	221	21

CHAPTER III

Hovering sunbirds in the Old World: occasional behaviour or evolutionary trend?

Janeček Š, Patáčová E, Bartoš M, Padyšáková E, Spitzer L and Tropek R (2011)

Oikos 120: 178–183

Abstract

The nectarivory of sunbirds in the Old World and hummingbirds in the New World evolved independently. While both groups are specialised in their feeding apparatuses, hummingbirds are moreover famous for their adaptations to sustained hovering flight. Recently, an example of a pollination system of the invasive plant *Nicotiana glauca* has been used to show that less adapted sunbirds also are frequently able to hover. Nevertheless, the question has remained why plants adapted to bird hovering pollination do not occur outside the New World. In this paper we show that the long-peduncle Cameroonian *Impatiens sakeriana* is not capable of autonomous selfing and can be pollinated only by two often hovering sunbirds, the Cameroon sunbird *Cyanomitra oritis* and the northern double-collared sunbird *Cinnyris reichenowi*. Our study revealed that this plant is highly specialised for pollination by *C. oritis*. *Cinnyris reichenowi* hovers less frequently and often thieves nectar by piercing the flower spur when perching. This study shows that pollination systems occurring in the Old World follow similar evolutionary trends as systems including hovering hummingbirds in the New World.

Introduction

Convergence and divergence of an organism's character during natural selection is among the fundamental principles of Darwin's evolutionary theory (Darwin, 1859). One of the most famous examples of convergent adaptation studied since Darwin's time is comprised of the morphological and behavioural adaptations of nectarivorous birds in relation to their feeding activity. Moreover, recent molecular studies have showed that the two largest groups of nectarivorous birds (Old World sunbirds and New World hummingbirds) are members of two superordinal phylogenetic clades and represent morphologically convergent forms of nectarivorous birds (Fain and Houde, 2004). While the feeding apparatus adaptations of the two groups appear to be very similar, they evidently differ in their adaptations for hovering flight (Schuchmann, 1999; Altshuler and Dudley, 2002). Sunbirds, which are not capable of sustained hovering flight as are hummingbirds, are considered to be mainly perching birds and observations of hovering sunbirds during nectar feeding are rare (Ley and Classen-Bockhoff, 2009). However, Westerkamp (1990) suggested that attention should be directed to the actual functioning of flowers rather than to systematic affiliation of birds. He also supposed some plants in the Old World (including *Impatiens sakeriana,* which is the focus of our study) have blossoms oriented into free space which could be adapted to hovering birds. Nevertheless, there have been no detailed studies on their pollination ecology, and after the discovery of fossil hummingbirds in the Old World it was hypothesised that these plants co-evolved with hummingbirds' ancestors (Mayr, 2004).

The view of sunbirds as perching birds was also supported by many plant adaptations which have been studied mainly in southern Africa. Some ornithophilous species (e.g. *Erica* spp. (Siegfried et al., 1985), *Satyrium* spp. (Johnson, 1996) and many *Iridaceae* species (Goldblatt and Manning, 2006)) have thick stems to support avian pollinators in perching. *Syncolostemon densiflorus* (Lamiaceae) has a compact terminal inflorescence that enables feeding from a single perching position (Ford and Johnson, 2008). *Strelitzia nicolai* (Musaceae) creates a perch from anther-sheath and stigma (Frost and Frost, 1981) and *Babiana ringens* (Iridaceae) facilitates perching by an unusual sterile inflorescence axis (Anderson et al., 2005). In other African regions pollination studies have been less frequent, but have also showed pollination systems including the perching behaviour of sunbirds - e.g. in East Africa, *Nectarinia johnstoni* perch on *Lobelia telekii* (Evans, 1996) and several sunbird species perch on *Leonotis nepetifolia* (Gill and Wolf, 1978).

More recently, an example of a pollination system of the invasive plant *Nicotiana glauca* has been used to show that less adapted sunbirds also are frequently able to hover. Nevertheless, the question has remained why plants adapted to bird hovering pollination do not occur outside the New World (Geerts and Pauw, 2009a).

In our study, we focused on the pollination ecology of *Impatiens sakeriana*. This plant has a bird-pollination syndrome including red flowers, a spur up to 25 mm long (Grey-Wilson, 1980), and produces a high volume (38 *µ*l per flower) of dilute nectar (31% of sugar w/w); (Bartoš et al., unpubl.). Moreover, *I. sakeriana* have flowers on long peduncles oriented into free space (Grey-Wilson, 1980) and so seem to be pollinated by hovering birds (Westerkamp, 1990; Mayr, 2004). While the pollination system of this plant has not so far been studied, it has been the object of evolutionary hypotheses, which we tested: 1) *Impatiens sakeriana* represents a plant which is adapted to sunbird hovering pollination (Westerkamp, 1990). 2) Floral traits of *I. sakeriana* have evolved in the past in co-evolution with extinct hovering hummingbirds and, nowadays, it is pollinated by insects (Mayr, 2004) or is dependent on autonomous selfing. The first hypothesis can be supported by the occurrence of indications that this plant could specialise for the long-billed (25 – 33 mm) sunbird *Cyanomitra oritis* (Cheke et al., 2001). *Impatiens sakeriana* and *C. oritis* have an identical distribution area (occurring only in the Cameroonian mountains and on Fernando Po), they occupy the same habitats at higher altitudes and *I. sakeriana* is a predictable nectar resource, as it flowers continuously throughout the year (Grey-Wilson, 1980; Cheke et al., 2001). The second hypothesis can be supported by the fact that no African pollination system including frequent hovering bird pollination has so far been described.

Methods

Study site

The study was carried out in the Mendong Buo area (6°5’ N, 10°18’ E; 2100 – 2200 m a.s.l.); Bamenda Highlands, NorthWest Province, Cameroon. The vegetation of this area is a mosaic of high *Hypparhenia* grasslands, pastures dominated by *Sporobolus africanus* and *Pennisetum cladestianum*, *Gnidia glauca* woodlands, often burned forest clearings dominated by *Pteridium aquilinum,* and remnants of species-rich montane tropical forests dominated mainly by *Schefflera abysinica*, *S. manii*, *Bersama abyssinica*, *Syzigium staudtii*, *Carapa grandiflora* and *Ixora foliosa* . Only in these montane forest areas does *Impatiens sakeriana* occur.

Insect observation

Insect visitors were recorded from November to December 2007 in 8 transects (ca 10 m long) of stream mantel vegetation with common occurrence of *I. sakeriana.* Five minutes were spent on each transect during one visit. (in total, 7 h, evenly distributed in daytime during the whole study period). The recording was limited from 9 a.m. to 5 p.m., when the insect activity was the most intensive, and to suitable weather (at least half the day with no clouds). Occasionally, *I. sakeriana* was also observed at night.

Reproductive system of I. sakeriana

In our first experiment, we studied the reproductive system of *I. sakeriana* to assess the importance of sunbirds on its pollination and to better understand the pollination ecology of this species. The experiment was realised from November

2008 to January 2009 and was performed on eight populations of *I. sakeriana* . In this experiment we established five treatments: parthenogenesis: flowers bagged and emasculated; autogamy: flowers bagged; geitonogamy: flowers bagged and hand pollinated from flowers of the same plant; outcrossing: flowers bagged and hand-pollinated by the pollen of a distant population;

control: natural pollination. These treatments were performed in seven replicates in each population. Emasculation was performed after flower opening before thecae dehiscence. One replicate of treatments was performed on one plant if possible, but as we were often unable to find enough numbers of plants with sufficient numbers of flowers we used for one replicate more plants growing close together.

Sunbird effectiveness

In our second experiment, which was realised simultaneously with the first one in the same *I. sakeriana* populations, we tested the pollination effectiveness of both sunbird species with respect to their behaviour. Randomly selected flowers were bagged during their flowering, except to be exposed for a single sunbird visit. In this way, we tested the effect on both male (pollen removal) and female (seed yield) plant functions. To estimate sunbird effectiveness on male function, the flowers in the male period, when the pollen is exposed, were uncovered and after one sunbird visit the rest of the pollen was collected into Eppendorf tubes. In parallel, we collected pollen from seven unvisited (bagged) flowers in each population to estimate pollen production. The pollen removal was then determined as the difference between mean pollen production in the population and the pollen left remaining by the sunbird in this population. Pollen grains were counted in a laboratory with a microscope using a haemocytometer (Roulston, 2005). To assess effectiveness of sunbirds on female function, the flowers were uncovered in the female period, when the stigma is exposed. After visitation, the flowers were again bagged and left until fruit maturation. Sunbird behaviour was noted in both the female and male parts of the experiment. As it is impossible to achieve well-balanced numbers of visited flowers in each bird species-population combination, we aimed to have an equal number of pollen removals (n = 8) and pollinated flowers (n = 12) by *Cyanomitra oritis* as the 'species of higher interest' in each population. The total observation time was 358 h (147 h pollen removal and 211 h sunbird pollination). For each observed flower the visitation rate (no. of visits per flower per hour) was estimated as the duration from observation start to visit multiplied by 2 (as we suppose that the observer starts to observe in the middle of two visits).

Seeds from both experiments were counted, weighed, and germinated in a greenhouse in pots 10 cm in diameter and 5 cm deep with a soil-sand mixture. Seedlings were counted after two months when seeds stop germinating.

Statistical analyses

For statistical analyses we used software STATISTICA 8.0 (StatSoft, Inc. 2008; <www.statsoft.com>). Because pseudo-replications in individual populations could occur (e.g. individual sunbirds could visit more target flowers and individual plant could be of the same genotype thanks to clonal spreading of *I. sakeriana*), we considered population as a random factor in the analyses. Data on seed numbers was log transformed to improve normality. Data on visitation rates were highly positive skewed thanks to many zero values, because of this we used the nonparametric Mann–Whitney test to test differences between visitation rates of both sunbird species.

Results

During our observation of insect visitors on *Impatiens sakeriana* just one sighting of *Apis mellifera* was recorded. Moreover, we observed no insect on *I. sakeriana* flowers during our further studies. Thus, *I. sakeriana* could not be effectively pollinated by insects.

By contrast, during our pilot bird observations on *I. sakeriana* we noted two sunbird species visiting *I. sakeriana* flowers – the aforementioned *Cyanomitra oritis* and *Cinnyris reichenowi.*

Our first experiment on the reproductive system of *I. sakeriana* shows that *I. sakeriana* is unable to produce seeds by either parthenogenesis (54 fruits aborted, n = 56) or autogamy (53 fruits aborted, n = 56). In contrast, both geitonogamy (10 fruits aborted, n = 55) and outcrossing (11 fruits aborted, n = 55) pollination were successful. The mean number of seeds per fruit and proportion of germinated seeds was higher (although non-significantly) after outcrossing versus geitonogamous pollination. Seeds which developed after outcrossing pollination were significantly heavier (Fig. 1). *Impatiens sakeriana* was not pollen limited and control flowers which were accessible to sunbirds (*n* = 51) produced even somewhat higher number of seeds than artificially pollinated ones. The seeds of control flowers had mean weight falling between those of the other two treatments and a somewhat poorer germinating ability than did outcrossed seeds (Fig. 1). Hovering was a common behaviour for both sunbird species. We observed a higher proportion of hovering for *C. oritis* relative to *C. reichenowi* (Fig. 2, 3). *Impatiens sakeriana* produced a high number of pollen grains (mean = 489 996, mean SD in population = 176 953). *Cyanomitra oritis* removed more than twice the pollen grains per visit (mean = 191 350, SD = 113 147, *n* = 80) as did *C. reichenowi* (mean = 89 776, SD = 148 441, *n* = 35) (Table 1). *Cyanomitra oritis* was also the more effective sunbird from the plant's female function point of view (Fig. 4, Table 1). Nevertheless, the effectiveness of both its hovering and perching behaviour was similar. In contrast, the perching behaviour of *C. reichenowi* (the prevailing behaviour of this species) resulted in low pollination effectiveness and more than 50% of these visits were illegitimate, as they resulted in piercing the flower spur. The hovering of *C. reichenowi* was much more effective than its perching, and no nectar robbing was observed. Nevertheless, the hovering pollination of *C. reichenowi* was still less effective than that of *C. oritis* (Fig. 4). We detected no effect of either bird species or of their behaviour on weight per seed and seed germination ability (Table 1). *Cyanomitra oritis* visited target flowers approximately two times more frequently (mean 0.66 visits per flower per hour, SD = 1.13) than did *C. reichenowi* (mean 0.31 visits per flower per hour, SD = 0.88); (Mann-Whitney-test, $U = 36\ 533$, $Z = 8.110$, $p < 0.000$).

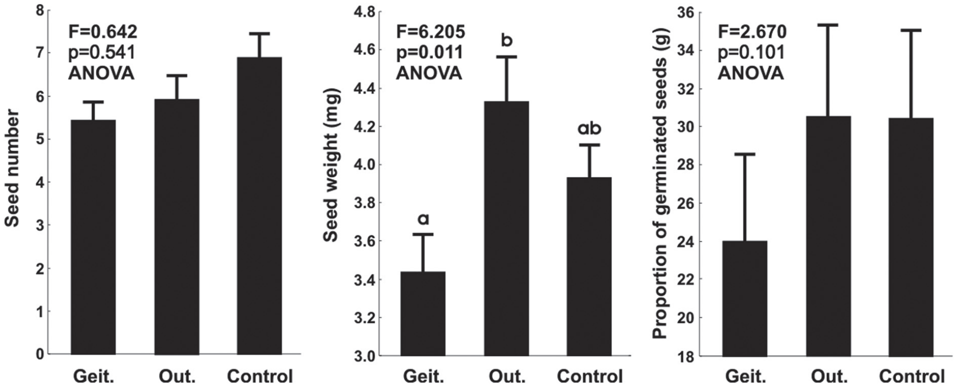

Fig. 1. Mean seed number per fruit, weight and proportion of germinated seeds of *Impatiens sakeriana* in three treatments where fruits developed: Geit. – geitonogamy, Out. – outcrossing and control. Differing letters above the boxes indicate significant differences between treatments (post-hoc test – unequal n HSD test). Error bars represent SE.

Fig. 2. Hovering *Cyanomitra oritis* during feeding on *Impatiens sakeriana*.

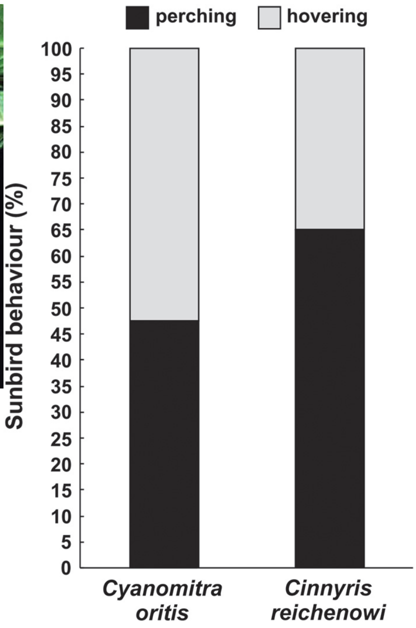

Fig. 3. Behaviour of *Cyanomitra oritis* (n = 181) and *Cinnyris reichenowi* (n = 64) during feeding on *I. sakeriana*.

Table 1. Sunbirds' effectiveness. ANOVA-mixed effect model with population as a random factor. Seed number was log transformed to improve normality. *p*-values < 0.05 are in bold.

	Pollen removal		Seed number		Seed weight		Proportion of germinated seeds	
	F	*p*	*F*	*p*	*F*	*p*	*F*	*p*
Species (SP)	7.872	**0.006**	39.66	**0.000**	0.086	0.770	1.662	0.201
Behaviour (BE)	0.108	0.743	3.72	0.056	1.117	0.294	3.07	0.083
SP × BE	0.063	0.802	5.94	**0.016**	0.448	0.505	3.322	0.072

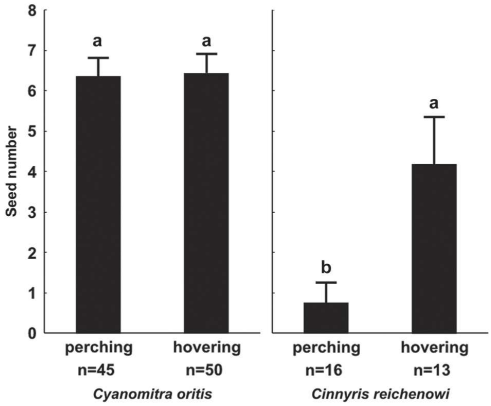

Fig. 4. Sunbirds pollination effectiveness. Differing letters above the boxes indicate significant differences between sunbirds' behaviour (post-hoc test – unequal n HSD test). Error bars represent SE.

Discussion

The experiment on the reproductive system of *Impatiens sakeriana* shows that this plant is not able to be autonomously self-pollinated and is fully dependent on sunbird pollination. Sunbirds deposit enough pollen on stigmas, and *I. sakeriana* is not pollen limited. Outcrossing seems to be more advantageous than geitonogamy but both result in high seed numbers. During flower longevity anthers are exposed first, and after their dehiscence the stigma is exposed. This scheme together with our pollination tests confirmed protandry, which was also shown for other species of *Impatiens* genus (Tian et al., 2004; Caris et al., 2006; Sreekala et al., 2008). No exceptions from this protandrous reproductive system, such as cleistogamy reported for *I. pallida* and *I. capensis* (= *I. biflora*), were observed (Schemske, 1978; Waller, 1980).

Cyanomitra oritis was the more important pollinator from both effectiveness and visitation rate point of view. Both perching and hovering pollination were observed. The finding that the perching behaviour of *C. oritis* has the same pollination effectiveness as its hovering behaviour cannot explain the evolution of *I. sakeriana*'s characteristics: mainly long peduncles, which are typical for hovering flight (Westerkamp, 1990). The logical explanation as to which selection pressures caused the evolution of these characters could be found in the behaviour and effectiveness of the occasional nectar robber *C. reichenowi*. The perching and thieving of nectar by this species decrease fitness (seed production) of *I. sakeriana* . We therefore hypothesise that the adaptations of *I. sakeriana,* which seems to be adaptations to hovering flight (Westerkamp 1990), evolved rather as a defence against *C. reichenowi* than as adaptations increasing the effectiveness of *C. oritis* . This idea can be also supported by the biogeographical distribution of *C. reichenowi* in West Africa, which includes the areas of both *C. oritis* and *I. sakeriana* (Cheke et al., 2001). Our hypothesis agrees with those suggesting nectar robbers have not just a simple negative impact but the effect of nectar robbing can be more complex and can affect co-evolution between plant and pollinators (Maloof and Inouye, 2000). Another reason why the *C. reichenowii* thieves the nectar despite lower hovering ability can be the fact that it has a bit lower maximal bill length (22 mm; Cheke et al., 2001) than is the maximal spur length of *I. sakeriana* (25 mm; Grey-Wilson, 1980). Similar nectar robbing by short billed sunbirds on long tube flowers was shown by Geerts and Pauw (2009b) in South Africa. Nevertheless, we must point out that these hypotheses arise from the current reality and we have no information on which other interactions formed this pollination system in the past.

Compared to the pollination system of the invasive plant *Nicotiana glauca* in South Africa, (Geerts and Pauw, 2009a) proportions of hovering of *C. oritis* (50%) and *C. reichenowi* (30%) are higher than that of *Cinnyris chalybea* (less than 10%), comparable with *Cinnyris fuscus* (about 40%), but lower than that of *Nectarinia famosa* (almost 80%). Our study shows that the 'dogma' that hummingbirds hover whereas sunbirds perch should be abandoned and we need to return to the suggestion that it is neither systematic affiliation of birds nor their traits but rather plant traits that determine sunbird behaviour (Westerkamp, 1990; Geerts and Pauw, 2009a).

A persisting question is whether the studied system that includes frequent hovering pollination is unique in the Old World, or if it is an overlooked and more widely distributed phenomenon. We expect that the ideas on pollination systems including sunbirds and their evolution are misrepresented due to an unbalanced knowledge between individual African regions. Whereas we have a relatively large number of studies from marginal areas of sunbird distribution (mainly South Africa), the pollination systems in tropical Africa, the centre of sunbirds' diversity, remain mainly unrecognised.

For example, the species of genus *Impatiens* could play an important role in sunbird radiation processes in the African mountains. About 15 African sunbird species are endemic to restricted mountain areas (like *C. oritis*), with the highest diversity in East Africa (Cheke et al., 2001). The same biogeographical pattern can be seen for about 27 species of African *Impatiens* bearing bird-pollination syndrome and which have been suggested to be pollinated by birds (Grey-Wilson, 1980). Nearly all of them are, like *I. sakeriana,* endemic to small mountain areas and have their greatest diversity in the East African mountains (Grey-Wilson, 1980). About 16 species with bird-pollinating syndromes have sums of peduncle and pedicel lengths exceeding 50 mm and which can force sunbirds to hover. Nevertheless, except for this study there are neither detailed studies on African nor on other bird-pollinated Old World *Impatiens* species.

In our study we have presented the highly specialised pollination system of *I. sakeriana*, and have shown the first well-documented pollination system including frequent hovering of sunbirds in the Old World. Nevertheless, we are just at the start of acquiring knowledge on the functioning and evolution of bird-pollination systems in tropical Africa. Without more intensive study of these systems we will be not able to evaluate properly convergence processes between sunbirds and hummingbirds as their New World counterparts. Further, the determination of intensity and directionality of selective pressures of individual sunbirds on plant traits, which seems to be related with bird hovering (e.g. peduncle length) should be performed.

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CHAPTER IV

Generalization versus specialization in pollination systems: visitors, thieves, and pollinators of *Hypoestes aristata* (Acanthaceae)

Padyšáková E, Bartoš M, Tropek R and Janeček Š (2013)

PLoS ONE 8(4): e59299

Abstract

Many recent studies have suggested that the majority of animal-pollinated plants have a higher diversity of pollinators than that expected according to their pollination syndrome. This broad generalization, often based on pollination web data, has been challenged by the fact that some floral visitors recorded in pollination webs are ineffective pollinators. To contribute to this debate, and to obtain a contrast between visitors and pollinators, we studied insect and bird visitors to virgin flowers of *Hypoestes aristata* in the Bamenda Highlands, Cameroon. We observed the flowers and their visitors for 2-h periods and measured the seed production as a metric of reproductive success. We determined the effects of individual visitors using 2 statistical models, single-visit data that were gathered for more frequent visitor species, and frequency data. This approach enabled us to determine the positive as well as neutral or negative impact of visitors on *H. aristata*'s reproductive success. We found that (i) this plant is not generalized but rather specialized; although we recorded 15 morphotaxa of visitors, only 3 large bee species seemed to be important pollinators; (ii) the carpenter bee *Xylocopa* cf. *inconstans* was both the most frequent and the most effective pollinator; (iii) the honey bee *Apis mellifera* acted as a nectar thief with apparent negative effects on the plant reproduction; and (iv) the close relationship between *H. aristata* and carpenter bees was in agreement with the large-bee pollination syndrome of this plant. Our results highlight the need for studies detecting the roles of individual visitors. We showed that such an approach is necessary to evaluate the pollination syndrome hypothesis and create relevant evolutionary and ecological hypotheses.

Introduction

Debates about the generalization or specialization of pollination systems have been a prevailing theme in pollination ecology for many years. During that time, the view has been that pollination systems permanently balanced on the specialization–generalization continuum (Willmer, 2011). The original idea that coevolution often resulted in the specialization of plants and their pollinators came firstly up with Darwin's evolutionary theory (Darwin, 1859) and then was extended in later works (Stebbins, 1970). The specialization has been discussed over a long period and is closely related to the concept of pollination syndromes (Faegri and van der Pijl, 1979; Renner and Feil, 1993; Hodges and Arnold, 1994; Manning and Goldblatt, 1997), which are defined as a set of traits that convergently evolved as adaptations to similar pollinators. Simultaneously, the pollination syndrome concept has been opposed by some pollination biologists who noted that the links between floral traits and observed visitors are much weaker than predicted (Waser et al., 1996; Ollerton et al., 2009) and that co-evolution is often diffuse (Strauss et al., 2005). Whereas the existence of generalized pollination systems was firstly manifested only for some plant species (Beattie, 1971; Primack, 1979; Bullock et al., 1989), the more recent community-wide studies have shown that flowers of most plants are visited by a relatively high diversity of visitors and that generalization is much more common than was previously expected (Memmott, 1999; Memmott and Waser, 2002; Olesen and Jordano, 2002; Jordano et al., 2003; Olesen et al., 2007).

Nevertheless, this broad generalization hypothesis has been criticized by other researchers (Kishore et al., 2011; Niemirski and Zych, 2011; Wilmer, 2011) who argue that some floral visitors that are usually considered in pollination webs are actually ineffective pollinators. In fact, a broad spectrum of diverse floral visitors with positive, neutral, and even negative effects on plant reproductive success can be found (Inouye, 1980; Roubik, 1982; Maloof and Inouye, 2000; Janeček et al., 2011). Several different techniques can be used to test the effects of particular pollinators. Indirect techniques, such as estimating visitor frequency rates (Herrera, 1987, 1989; Stone, 1996) or direct measuring the total amount of pollen grains brought onto the stigma during a single visit of a particular visitor (Herrera, 1987, 1989; Inouye et al., 1994; Mayfield et al., 2001; Adler and Irwin, 2006; Young et al., 2007), may not sufficiently consider the real contribution of particular visitors to the plant's reproduction (Ne´eman et al., 2010). One possible way to detect the visitor's actual contribution directly is by using estimates from single visits to virgin flowers (Olsen, 1997; Sahli and Conner, 2007; Robertson and Leavitt, 2011). However, the single-visit approach has several weaknesses. Although it allows positive contributions to plant reproduction (i.e. the contribution of effective pollinators) to be quantified, it is not possible to reveal any negative effects of other visitors, so those visitors are simply classified as ineffective pollinators. Since many studies have shown negative effects of floral visitors (Lara and Ornelas, 2001; Dedej and Delaplane, 2004; Castro et al., 2008), these should be considered whenever hypotheses on floral evolution are developed (Lara and Ornelas, 2001).

Here, we focus on the pollination system of a broadly distributed Afrotropical plant species, *Hypoestes aristata*. This species shows the pollination syndrome (van der Pijl, 1960; Faegri and van der Pijl, 1979) associated with bee pollination. Typically, its flowers have nectar-guide markings and produce a small amount of highly concentrated nectar. However, according to previous studies it is visited by a much broader spectrum of potential pollinators, including long-proboscid flies in South Africa (Potgieter and Edwards, 2005; Larsen et al., 2008) and various sunbirds, bees, flies, butterflies, and moths in our study area in the Bamenda Highlands, Cameroon (Riegert et al., 2011; Bartoš et al., 2012). In this area, *H. aristata* is the most favoured food plant of the sunbird *Cinnyris reichenowi* (Janeček et al., 2012). Although the *H. aristata* morphology suggests pollinator specialization, it is apparently visited by a variety of birds and insects. Thus, *H. aristata* is an ideal model plant species for testing the validity of the concept of pollination syndromes. Simultaneously, examining its pollination system can contribute to the current debate about the proportion of generalization and specialization in pollination biology. The aim of our study was to answer the following main questions: (1) What is the spectrum of floral visitors of *H. aristata*? (2) Which visitors are effective pollinators? (3) Which visitors have neutral or negative effects on the reproduction of *H. aristata*? (4) Is the pollination system of *H. aristata* rather generalized, as suggested by previous studies on its floral visitors, or more specialized, as predicted by its floral traits? and (5) Is the bee pollination syndrome a good predictor of effective pollinators?

Methods

Study site

The study site was situated in the Mendong Buo area (6°5´26´´N 10°18´9´´E; 2100–2200 m a.s.l.), ca. 5 km southeast from Big Babanki (Kedjom-Keku community), in the Bamenda Highlands, North-West Province, Cameroon. This area is a mosaic of extensive pastures, frequently burned forest clearings dominated by *Pteridium aquilinum*, shrubby vegetation along streams, and remnants of species-rich tropical montane forests with a frequent occurrence of *Schefflera abyssinica*, *Schefflera manii*, *Bersama abyssinica*, *Syzygium staudtii*, *Carapa procera*, and *Ixora foliosa*. There is a single wet season from March to November, with annual precipitation ranging from 1780 to 2290 mm/year (For more details see: Cheek et al., 2000; Reif et al., 2007; Tropek and Konvicka, 2010).

Our research was permitted by the Ministry of Scientific Research and Innovations of the Republic of Cameroon (permit no. 93/MINRESI/B00/C00/C10/C12) and the Ministry of Forestry and Wildlife of the Republic of Cameroon (permit no. 2306/PRBS/MINFOF/SG/DFAP/SDVEF/SC). Voucher insects were exported with the permission of the Ministry of Agriculture and Rural Development of the Republic of Cameroon (permit no. 15347/A/PPP/LBE). Our research was also permitted by Benjamin Vubangsi, the local chief of the Kedjom-Keku community, which owns the study area. The study was not conducted in any of the protected areas or on any protected species.

Plant species

Our target plant species, *Hypoestes aristata* (Vahl) Sol. ex Roem. & Schult var. *aristata* (family Acanthaceae), is a clonal herb that grows up to 1.5 m high and is native to tropical sub-Saharan Africa (Hepper, 1963; Balkwill and

Norris, 1985). The plant has hermaphroditic, zygomorphic flowers that are crowded into verticillate inflorescences. Dark purple blossoms with white nectar-guide markings on the upper lip have a pistil and 2 stamens long exerted from the corolla (Fig. 1). *H. aristata* produces a low volume (1.27 μl per flower) of hexose-dominant nectar of highly variable concentration (62.21% ± 24.13; mean concentration ± [SD] w/w; i.e. sucrose equivalent mass/total mass; (Bartoš et al., 2012). Nectar is accumulated in its 1-cm-long, narrow corolla-tube. Individual flowers last for about 5 days and can be found throughout the dry season. After pollination, a flower turns into a dehiscent capsule with up to 4 seeds (pers. obs.). *H. aristata* forms dense clumps, with several shoots flowering more or less simultaneously, which increases its local attractiveness for visitors. Usually, the plant dominates locally in disturbed montane forests, at their edges, in shrubby vegetation along streams, and in successionally older clearings. Experimental hand-pollinations during a preliminary study showed that *H. aristata* cannot effectively reproduce via autonomous selfing or parthenogenesis, and thus, is fully dependent on its pollinators (File S1; Fig. A in File S1; Table A in File S1).

Flower visitors and pollination effectiveness

The flower visitors were studied from November to December 2010, when the plants of *H. aristata* are in full bloom. Ten shoots in 10 patches of flowering *H. aristata* were chosen within the whole study area. Shoots with several target flower buds were bagged individually with a fine mesh and the buds were marked. The bags were large enough to allow the flowers to completely open inside the netting. The following day, all open marked flowers on a shoot (5.3 ± 1.29 per shoot; mean ± standard deviation [SD]) were observed simultaneously for a 2-hour session (i.e. one shoot with several open flowers was observed in one session) and all flowers were bagged again immediately after the observation. During each observation session, all animals that visited the marked flowers were recorded and identified to morphotaxa (Table 1, Movie S1). Observations of individual shoots were equally distributed throughout the day (between 0700 and 1800) to include all possible diurnal visitors and were limited to suitable weather conditions (sunny or partly cloudy). Fruits were harvested after maturation and their seeds were counted and weighed.

Statistical analyses

Due to many zero values, the data on seed production were not normally distributed. We thus analysed the effects of particular flower visitors on seed production using non-parametric permutation models. Seed numbers produced by individual flowers served as a dependent variable and visits of individual visitors as explanatory variables (i.e. each visitor represents one explanatory variable in each analysis). These explanatory variables contained either abundance data (i.e. numbers of visits to individual flowers during 2-hour observations – see Model 1 below) or presence-absence data (i.e. the information if the visitor at least once visited or did not visit the flower – see Model 2 below). Note that we also considered the value of zero at the unvisited flowers for abundance data in Model 1. To avoid the variability in seed production that can be explained by having more than one visitor to a flower during the 2-hour session we used the Type II sums of squares approach for a given explanatory variable (Littell et al., 2002; Quinn and Keough, 2002; Eberly, 2007). In this way, the sum of squares for each visitor (explanatory variable) was calculated as the increase of the model sum of squares (and equivalently the decrease in the error sum of squares) due to adding this visitor into a model that already contained all of the other visitors (Littell et al., 2002). Thus, only the variability that could not be explained by other than just the tested visitor was considered. Two models with different biological predictions were established. Model 1 assumed that the number of developed seeds increases or decreases with visitation frequency (e.g. visitors continuously saturate the stigma with pollen grains or continuously consume nectar from the flower and decrease the attraction of the flower by this way). Model 2 assumed that the most important is whether the visitor visit the flower or not (e.g. flower receives enough pollen to produce the maximum number of seeds after a single visit from each pollinator or the nectar is completely depleted during the single visit). Following these approaches, the log (x+1) transformed numbers of visits by individual visitors to each flower were used in the first model as an explanatory variable, whereas binary coded visits (i.e. at least one visit = 1, no visit = 0) to each flower were analysed in the second model. In both the models, the factors (visitors) with high *p*-values and negligible contribution to total variation in seed set among flowers indicated by negative estimates of the component of variation were stepwise excluded from the model (Searle et al., 1992; Quinn and Keough, 2002; Anderson et al., 2008). After exclusion of the term with the lowest negative value of the component of variation, the models were recalculated. Consequently, only visitors with positive values of components of variance remained in the models (Anderson et al., 2008). The spatial autocorrelation effect (i.e. the term 'shoot') was considered in the models as a random variable. This term was always significant (i.e. individual shoots differed), and we have not shown the results for this term in Table 1. Except for the above described whole models, where all visitors were considered, we calculated marginal tests for each of the visitors. These tests demonstrate how visits of each visitor are related to seed production when each visitor is taken alone, ignoring others. Permutation tests were run with PERMANOVA+ for PRIMER (Anderson et al., 2008).

Results

During the observations of 539 flowers, 1979 flower visits, involving fifteen visitor morphotaxa, were recorded (Table 1). On average, 198 (±68.52) visits per patch and 3.67 (±2.61) visits per flower were detected. Although more than 95% of the flowers were visited at least once, less than 15% of the visited flowers produced fruit with viable seeds.

The total visitor community was highly dominated by two carpenter bees: *Xylocopa* cf. *inconstans* (Fig. 1A; including *X. inconstans* and *X. caffra*, which are hardly recognisable from each other in the field) and *Xylocopa lugubris* (Fig. 1B); followed by the honeybee *Apis mellifera* (Fig. 1F) and the northern double-collared sunbird *Cinnyris reichenowi* (Fig. 1C; Fig. 2). Nevertheless, the visitors' abundances and community composition differed considerably among patches (Figure S1).

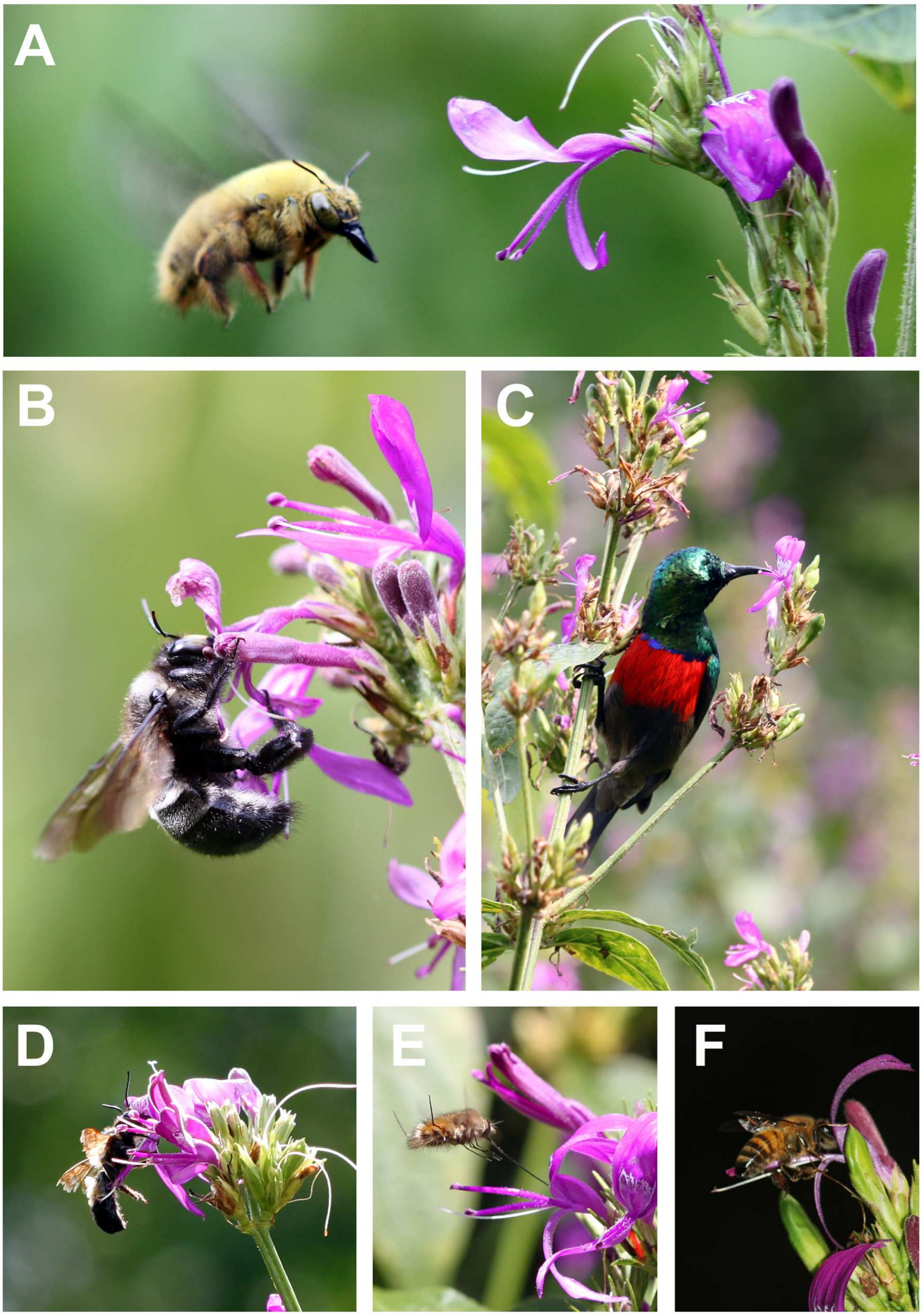

Fig. 1. The visitors of *Hypoestes aristata*: (A) *Xylocopa* cf. *inconstans*; (B) *Xylocopa lugubris*; (C) *Cinnyris reichenowi*; (D) *Megachile* sp.; (E) Bombyliidae; (F) *Apis mellifera*. Photos (A)–(E) by R. Tropek, (F) by Š . Janeček.

All the studied patches had a similar pattern of visitor distribution, with one or a few highly abundant taxa, while most other visitors were rarely observed. *X. lugubris* was the only visitor taxon observed at all studied patches.

Although 5 visitor taxa significantly affected seed production, if the other visitors were not considered (marginal tests for models 1 and 2, Table 1), only three visitor taxa were able to explain the variability in the reproductive success of *H. aristata* when the variability which could be explained by more visitors was eliminated (whole models 1 and 2, Table 1). Both the whole models indicated that the carpenter bee *X.* cf. *inconstans* and the leafcutter bee *Megachile* sp. (Fig. 1D) increased plant reproductive success, whereas the honeybee *A. mellifera* was related to fruit abortion (Table 1). According to the estimated values in the first model, *X.* cf. *inconstans* is three times more important pollinator than *Megachile* sp. Most of the variability in the second model was explained by the visits of *A. mellifera*.

Table 1. The effect of individual flower visitors on seed production in *H. aristata*.

			MODEL 1				**MODEL 2**				
			Marginal tests		**Whole model**		**Marginal tests**			**Whole model**	
Order	**Family**	**Species**	**r**	**F**	**Es.**	**F**	**Pr.**	**Ab.**	**F**	**Es.**	**F**
Passeriformes	Nectariniidae	*Cinnyris bouvieri*	0.030	0.49	–	–	0.75	0.36	1.24	0.003	1.78
		Cyanomitra oritis	0.044	1.06	0.011	1.91	0.80	0.36	3.03	–	–
		Cinnyris reichenowi	−0.093	**4.73***	–	–	0.19	0.41	3.91	–	–
Diptera	Bombyliidae		−0.021	0.23	–	–	0.26	0.37	0.23	–	–
	Syrphidae		0.045	1.07	–	–	0.58	0.35	1.22	–	–
	Other dipterans		0.091	**4.51***	0.002	1.82	0.36	1.14	**4.51***	0.001	1.71
Lepidoptera			−0.033	0.58	–	–	0.00	0.37	0.58	–	–
Hymenoptera	Apidae	*Apis mellifera*	−0.084	3.77	0.010	3.22	0.21	0.42	**4.73***	0.035	**8.58****
		Anthophora sp.	0.036	0.70	–	–	0.52	0.36	0.79	–	–
	Megachilidae	*Megachile* sp.	0.089	**4.71***	0.011	**6.41***	1.00	0.36	**4.31***	0.010	**6.01***
	Other wild bees		−0.027	0.40	–	–	0.21	0.37	0.36	–	–
	Apidae	*Xylocopacf.inconstans*	0.176	**17.2****	0.040	**10.64****	0.50	0.22	**10.78****	0.010	**4.00***
		Xylocopa nigrita	−0.028	0.41	–	–	0.00	0.37	0.43	–	–
		Xylocopa erythrina	−0.028	0.41	0.000	1.116	0.00	0.37	0.43	0.002	1.60
		Xylocopa lugubris	0.025	0.33	0.006	2.2228	0.39	0.35	0.21	–	–

Permutation models: ***Model 1*** assumed that visitors continuously saturate stigmas with pollen grains, i.e. the number of visits by individual visitors represented the explanatory variables. Marginal tests for this model represent individual regressions. ***Model 2*** is based on the idea that the flower received sufficient pollen to produce the maximum of seeds after one visit from a pollinator (i.e. visitor presence/absence data were used). Marginal tests represent the individual permutation ANOVAs. Abbreviations:**r,**Pearson correlation coefficient; **F,** F ratio;**Es.,**unbiased estimate of the components of variation, which shows the relative importance of individual terms in the model in relation to overall variation;**Pr.,**mean number of seeds which developed from flowers visited at least once by the visitor; and **Ab.,**mean number of seeds which developed from flowers not visited by the visitor. Significant differences (*$0.01<p<0.05$;**$p<0.01$) are in bold. The results for the random term 'shoot', which were always significant, were included in the whole models but are not presented. For more details, see Methods.

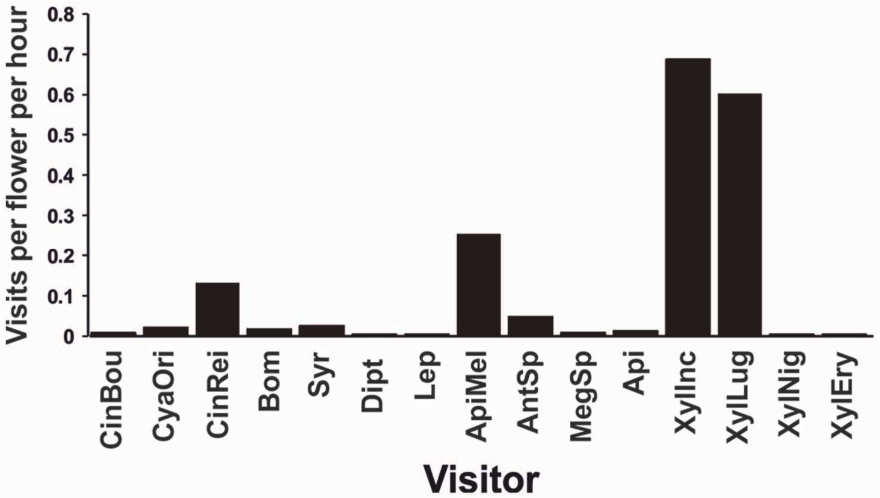

Fig. 2. Total visitation frequencies. Abbreviations: CinBou = *Cinnyris bouvieri*, CyaOri = *Cyanomitra oritis*, CynRei = *Cinnyris reichenowi*, Bom= Bombyliidae, Syr = Syrphidae, Dipt = other dipterans, Lep = Lepidoptera, ApiMel = *Apis mellifera*, AntSp = *Anthophora* sp., MegSp= *Megachile* sp., Api = other bees, XylInc = *Xylocopa* cf. *inconstans*, XylLug = *Xylocopa lugubris*, XylNig = *Xylocopa nigrita*, XylEry = *Xylocopa erythrina.*

The majority of the flowers were visited repeatedly during our observations, usually by more than one visitor taxon, but 79 observed flowers were visited just once. These single visits were made by the four most frequent visitors, but flowers produced seeds only after a single visit of either *X.* cf. *inconstans* or *X. lugubris*, not of *A. mellifera* or *C. reichenowi* (Table 2). Although the flowers visited once by these four visitors did not significantly differ in seed production (permutation ANOVA; d.f. = 3; F = 1.98; p = 0.114), *Xylocopa* spp. differed from *A. mellifera* and *C. reichenowi* which were indicated by the models (Table 1) as visitors with rather negative influence on the seed production (permutation ANOVA; d.f. = 2; F = 5.07; p = 0.039). Although a honeybee might receive a pollen load from the anthers, it rarely deposits the pollen because it is too small to touch the stigma when inserting its head into the flower to forage on nectar (see Fig. 1F). Similarly, sunbirds, while visiting, introduced their bills partially or totally into the floral tube in a space between the upper lip and both sexual organs.

Table 2. List of visitors with more than 5 single-visits, and the mean number of seeds ± standard deviation (SD) for each flower.

Visitor	**Number of single-visits**	**Seeds/visit**
Xylocopa* cf. *inconstans	22	0.455±1.06
Xylocopa lugubris	21	0.238±0.70
Apis mellifera	14	0±0
Cinnyris reichenowi	13	0±0

Summarizing all the analyses performed, the carpenter bee *X.* cf. *inconstans* seemed to be the main pollinator of the plant in the study area. The importance of the other carpenter bee, *X. lugubris*, followed from its total high frequency of visits. *X. lugubris* equally visited the successfully and unsuccessfully pollinated flowers, which means that, in total, it contributed to pollination of the flowers only occasionally. Its high frequentness, however, guarantees a relatively bigger contribution to seed production than the less frequent visitors. The leafcutter bee *Megachile* sp. positively affected seed production of *H. aristata* (Models I and II in Table 1), nevertheless its visitation rate was too low (Fig. 2) to be crucial to *H. aristata*'s reproduction in the study area.

Discussion

We have described the reproductive and pollination system of *H. aristata*, and have shown that the apparently generalized pollination system is actually highly specialized in the study area and that the effective pollinators are in agreement with the pollination syndrome of this plant.

Due to our experimental approach, we were able to determine not only the pollinator effectiveness but also the negative impact of visitors on the studied plant's reproduction. Interestingly, single visits from 2 frequent visitors, the honeybee *A. mellifera* and the sunbird *C. reichenowi*, did not result in any seed production, and visits of *A. mellifera* even decreased the reproduction success of *H. aristata*.

The effectiveness of both the above mentioned carpenter bees in the *H. aristata* pollination system is in accordance with statements of other researchers, showing the *Xylocopa* species as extremely important pollinators in various tropical systems (Renner, 1989; Raju and Rao, 2006; Janeček et al., 2007). The honey bee *A. mellifera* is commonly considered to be a generalist forager, visiting many plant species (Traveset and Richardson, 2006). Although it usually visits flowers more frequently than other flower visitors (Silva-Montellano and Eguiarte, 2003; Escaravage and Wagner, 2004; Fumero-Caban and Melendez-Ackerman, 2007), its effectiveness as a pollinator is likely to differ, depending upon its foraging behaviour (Hansen et al., 2002; Escaravage and Wagner, 2004) and the morphology of the flowers (Silva-Montellano and Eguiarte, 2003). Our finding that *A. mellifera* had a negative impact on *H. aristata* seed production might be because of a combination of both of the above-mentioned factors. We assume that, as has been shown by other studies (Hansen et al., 2002; Celebrezze and Paton, 2004), *A. mellifera* acted as a floral thief, removing a substantial part of the available nectar or pollen and thus making the flower unattractive for other visitors.

Among the three sunbird species visiting *H. aristata*, *C. reichenowi* was the most frequent visitor (Riegert et al., 2011; Janeček et al., 2012), but it did not effectively pollinate the flowers. Its ineffectiveness could be related to the relatively small and specialized flowers of *H. aristata* that do not fit the birds' heads (Fig. 1C). Thus, the anthers and stigma contacted the lower part of the bird's bill, which seems to be inappropriate for pollen transfer. In bird-pollinated flowers, pollen grains typically attach firmly to a bird's crown when the bird inserts its bill into the perianth to extract nectar (Castellanos et al., 2003; Nassar and Ramirez, 2004). On the basis of our results, we consider *C. reichenowi* to be a nectar thief, although there was no obvious negative effect on *H. aristata* reproduction, in contrast to that by *A. mellifera.* In accordance with our observations (Fig. 1), we agree that 'trait-matching' between flowers and their visitors plays an important role in pollination interactions (Stang et al., 2006, 2007, 2009; Janeček et al., 2011, 2012).

A limitation of our study is the relatively small study area size and short time in which the study was performed. It has been shown that diversity, abundance, and the importance of individual visitors may differ depending on the time and place (Herrera 1988, 1995; Eckhart, 1992; Cane and Payne, 1993). Conversely, *H. aristata* in South Africa is also visited by carpenter bees (Potgieter and Edwards, 2005); thus, there is a high possibility that they are the main pollinators in that region. Moreover, our findings are in accordance with the expectations from 'trait-matching'; i.e. the honeybee *A. mellifera* rarely reaches the stigma to deposit pollen and the sunbird carries pollen on its lower bill. Therefore, neither of these species should be an effective pollinator. Nevertheless, similar studies conducted in different African regions would substantially contribute to this debate.

Choosing the right field technique for measuring the pollination or plant reproductive success is important since there are several possible methods with various weaknesses and benefits (Ne´eman et al., 2010). Because of the shortcomings of using the single-visit method to estimate pollination effectiveness (Olsen, 1997; Robertson and Leavitt, 2011), we chose the approach based on 2-hour observation periods. Basing observations on time-defined periods is more suitable to detect the potential effects of the whole spectrum of floral visitors, including occasional visitors; and to reveal both positive and negative effects of individual visitors. This method is, moreover, less laborious than bagging flowers after each single visit. If the length of the observation period is well chosen the dataset can also include single-visit data, at least for the more frequent pollinators. A drawback of this method follows the fact that the seed set is usually formed after multiple visits from the same or different visitors.

The analyses of the pollination system of *H. aristata* show different roles for individual visitors. Our finding that the two carpenter bees were the only important pollinators among the wide spectrum of floral visitors is in accordance with the bee pollination syndrome of *H. aristata* and with the concept of pollination syndrome (van der Pijl, 1960; Faegri and van der Pijl, 1979). Nevertheless, as much as successful pollination is highly dependent on 'trait-matching' between flowers and their visitors (Stang et al., 2006, 2007, 2009; Janeček et al., 2011, 2012), we agree that the visitor's body size plays an important role in the assessment of the pollination syndrome validity. The large bees were effective pollinators whereas the relatively smaller bee *A. mellifera* had a negative effect on *H. aristata* reproduction. This assumption supports the idea that the bee pollination syndrome should be divided further into large-bee and small-bee syndromes (Heithaus, 1974; Grant and Grant, 1979). Our results are also in accordance with the most effective pollinator principle (Stebbins, 1970), supposing that the plant traits evolved as a response to the most effective pollinators. In contrast to the expected generalization of this system, we found a high degree of specialization. This would be even more apparent if we followed the ideas of Fenster et al. (2004) and considered the similarly large bees *Xylocopa* spp. and *Megachile* sp. as one functional group exerting similar selection pressures. Moreover, we also observed visitors with negative or potentially negative effects on plant reproductive success. As shown in other studies (Lara and Ornelas, 2001), these visitors can create different selection pressures on various floral traits. If they are overlooked or even considered as pollinators, then our understanding could lead to a total misinterpretation of the pollination systems. Our conclusions would be completely different if we considered all visitors as pollinators as is typical in plant-pollinator web studies (Fig. 3). It also clearly demonstrates why pollination networks frequently show flowers to be phenotypically specialized but ecologically generalized (Ollerton, 1996).

Although we assume that the progress from studies on simple pollination systems (often including just one pollinator and one plant species) to community level studies is the right direction for pollination biology, we must urge, together with other researchers (Schemske and Horvitz, 1984; Wilmer, 2011), that without any knowledge of the roles of individual visitors, we cannot confirm the validity of the pollination syndrome hypothesis, determine the degree of generalization, nor create a relevant evolutionary hypothesis.

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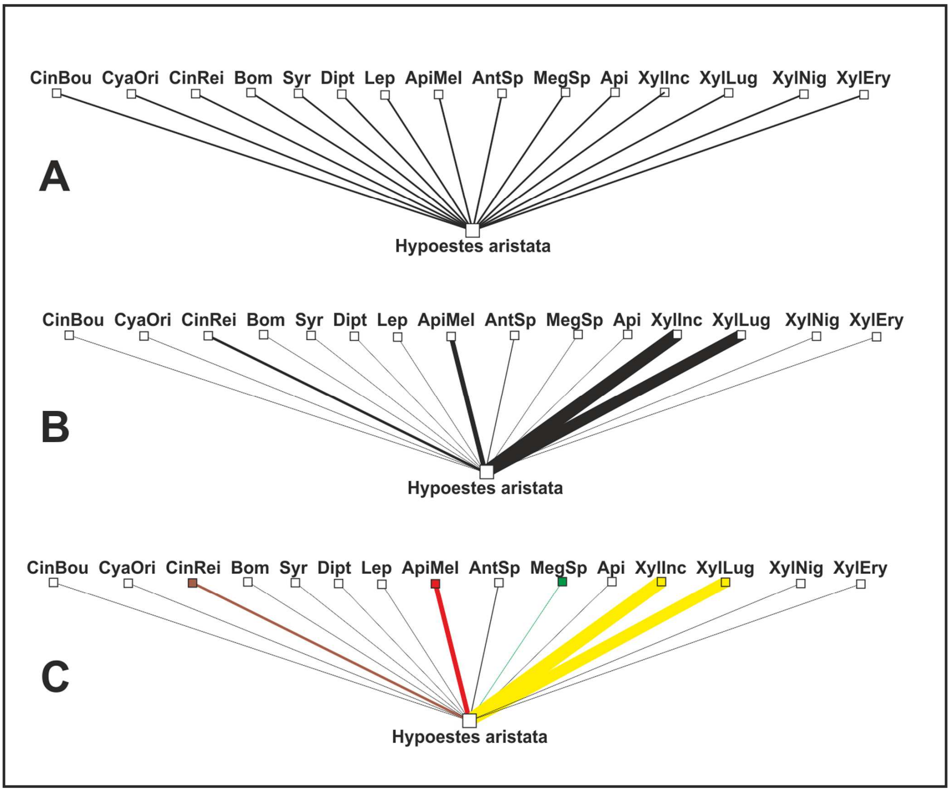

Fig. 3. Interactions between *H. aristata* and its visitors. (A) Binary interactions showing just the visitor-plant interaction - the approach commonly used in pollination networks. (B) Quantitative interactions showing the frequencies of visits by individual visitors - the less frequently used approach in pollination networks. (C) Quantitative interactions indicating the role of individual visitors: yellow = important effective pollinators, green = pollinators with a marginal effect on *H. aristata* reproduction, red = nectar thieves with a negative impact on *H. aristata* reproduction; brown = nectar thieves with a potential negative effect on *H. aristata* reproduction; and black, visitors with no effect on *H. aristata* reproduction. Abbreviations: CinBou = *Cinnyris bouvieri*, CyaOri = *Cyanomitra oritis*, CynRei = *Cinnyris reichenowi*, Bom= Bombyliidae, Syr=Syrphidae, Dipt = other dipterans, Lep =Lepidoptera, ApiMel = *Apis mellifera*, AntSp = *Anthophora* sp., MegSp = *Megachile* sp., Api = other bees, XylInc = *Xylocopa* cf. *inconstans*, XylLug = *Xylocopa lugubris*, XylNig = *Xylocopa nigrita*, XylEry = *Xylocopa erythrina*.

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Supporting information

Additional Supporting Information may be found in the online version of this article: **http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0059299**

Fig. S1 Figure of the visitation frequencies, given separately for each studied patch.
(DOC)
File S1 Preliminary study on the breeding system of *Hypoestes aristata*. The breeding system was studied by emasculation and pollen supplementation in five experimental treatments. The results showed that the experimental treatments differed in the reproductive success of *H. aristata*; i.e. in the number and total weight of seeds per fruit. Table A, Results of the handpollination experiment done by permutation mixed models. Fig. A, Seed number per flower (Means and Standard Errors) of *Hypoestes aristata* in five experimental treatments.
(DOC)

Movie S1 The video file attached shows the representative visitors of *Hypoestes aristata* while foraging for the nectar. Shots were taken at the study site by the small hand camcorder during the field studies in 2010 and 2012. Some of the presented shots were intentionally slowed to better show the visitors behaviour. High definition of the video file was converted to fit the size limit given by the journal.
(ZIP)

CHAPTER V

Interference competition between sunbirds and carpenter bees for the nectar of *Hypoestes aristata*

Tropek R, Bartoš M, Padyšáková E and Janeček Š (2013)

African Zoology 48(2): 392-394

Abstract

Interference competition for nectar sources has been repeatedly described between hummingbirds and various insects, but rarely recorded in other nectarivorous birds. We observed aggressive behaviour by African sunbirds (*Cinnyris reichenowi* and *Cinnyris bouvieri*) defending the nectar plant *Hypoestes aristata* against carpenter bees (*Xylocopa caffra* and *Xylocopa inconstans*) in the Bamenda Highlands, Cameroon. During 200 hours of observation, we recorded 38 cases of the sunbirds attacking carpenter bees; all these intrusions occurred only in the flower-richest patches of the plant. We predict that similar aggressive interactions will occur between other Old World nectarivorous birds and insects. Such interference competition between distantly related taxa could have an important impact on the evolution of pollination systems.

Introduction

Interference competition between birds and insects is rather rare. So far, it has been repeatedly described only in hummingbirds, which compete with various insects for nectar sources. Aggressive foraging interactions were usually recorded between hummingbirds and large bees or wasps (Hymenoptera) (Stoaks 2000; Galindo-Gonzales & Ornelas 2002; Jacobi & Antonini 2008), and butterflies or moths (Lepidoptera) (Primack & Howe 1975; Carpenter 1979; Thomas et al. 1986; Jacobi & Antonini 2008).

Reports of interspecific competition between nectarivorous birds and insects in the Old World are rare, we know of only three published cases (Akinpelu 1989; Ollerton & Nuttman in press). In Nigeria, Western Olive Sunbirds (*Cyanomitra obscura*) were attacked by honey bees (*Apis melifera*) in defence of nectar of *Tecoma stans* (Akinpelu 1989). Eastern Olive Sunbirds (*Cyanomitra olivacea*) aggressively displaced carpenter bees *Xylocopa nigrita* from flowers of *Lagenaria sphaerica* in Tanzania (Ollerton & Nuttman in press). Nuttman (Ollerton & Nuttman in press) also observed Palestine Sunbirds (*Nectarinia osea*) defending flowers of *Erythrina* sp. against *Xylocopa pubescens*. In these cases, there is apparent competition between similarly-sized pollinators for the available nectar sources. Sunbirds are known to establish territories around nectar-rich sources and defend these against other nectarivorous birds (e.g., Evans & Hatchwell 1992; Riegert et al. 2007; Ollerton & Nuttman in press). Although such competition between birds and insects occurs relatively commonly at least in the New World (e.g., Boyden 1978; Kodric-Brown & Brown 1979), its effect on the co-evolution of plants and their pollinators is little studied. Here we describe a case of interference competition between sunbirds and carpenter bees for the nectar of *Hypoestes aristata* in Bamenda Highlands, Cameroon.

Methods

Our study was conducted in the Mendong Buo area (6°5´26´´N, 10°18´9´´E; 2100–2200 m a.s.l.), ca 5 km southeast from the Big Babanki village, Bamenda Highlands, NW Cameroon. The study area experiences a single wet season from March⁄April to mid-November (annual precipitation 1780-2290 mm; Cheek et al. 2000) and comprises a mosaic of mountain forest remnants, forest clearings dominated by *Pteridium aquilinum* and regenerating shrubs, submontane grasslands maintained by occasional grazing, and species-rich shrubby vegetation along streams with several plant species visited by sunbirds and/or carpenter bees (see Bartoš et al. 2012; Janeček et al. 2012).

During the study of the pollination system of *Hypoestes aristata* (Acanthaceae), an up to 1.5 m clonal herb with purple zygomorphic flowers crowded into verticillate inflorescences, we targeted ten patches of the flowering plants (Padyšáková et al. 2013). This locally relatively abundant plant produces a low volume of hexose-dominant nectar of highly variable concentration (Bartoš et al. 2012). From November to December 2010, each of the patches was observed for ten 2-hour sessions (i.e. 20 hours per patch) from an observational tent eliminating disturbance of floral visitors. All sessions were equally distributed within the day (between 07:00 and 18:00) and limited to suitable weather (sunny or partly cloudy). During the sessions all interactions between sunbirds and carpenter bees were recorded, including species and sex of both attacker and victim. Additionally, we counted the number of currently flowering plants in each study patch for a rough estimate of the nectar availability (Table 1).

Results and Discussion

In total, we recorded 38 cases of sunbirds attacking carpenter bees (Table 1). The observed chases were very rapid and intensive, often accompanied by aggressive pecking (50 % of the recorded interactions). The sundirds were observed to start an attack when feeding, flying around or sitting on the near twig. They chased carpenter bees not only when feeding on flowers but also when the bees were only flying close to the nectar source. The aggresive behaviour was interrupted when an attacked bee was chased away from the nectar source. On the other hand, this behaviour was quite rare, as most feeding carpenter bees were ignored by sunbirds (see Padyšáková et al. 2013 that *X.* cf. *inconstans* was still the most common visitor of *H. aristata*), however we do not have any data to quantify it.

Table 1. Numbers of flowering *Hypoestes aristata* plants and aggressive interactions between sunbirds and carpenter bees in particular study sites.

Site	Nr. of plants	Intrusions
1	518	19
2	37	0
3	77	1
4	66	0
5	46	0
6	97	0
7	202	16
8	182	2
9	58	0
10	42	0

The major aggressor was the northern double-collared sunbird *Cinnyris reichenowi* (23 males / 1 female) while attacks from the orange-tufted sunbird *Cinnyris bouvieri* were less frequently observed (14 males / no female). The only carpenter bee attacked was *Xylocopa* cf. *inconstans* (including *X. inconstans* and *X. caffra*, hardly distinguishable from each other in the field). All these species were previously recorded as relatively common visitors to *H. aristata* flowers (Padyšáková et al. 2013). We never observed any attacks on a smaller carpenter bees species, *X. lugubris*, which was even more often in some of the studied patches (Padyšáková et al. 2013) nor on other less common visiting insects. Whereas *X.* cf. *inconstans* constitutes one of the most important pollinators (together with *X. lugubris*) of the plant in the study area, the sunbirds steal nectar without any effect on pollination (Padyšáková et al. 2013), and especially for *C. reichenowi* this plant is the favourite nectar source in the study area (Janeček et al. 2012). Thus these aggressive interactions can potentially influence the plant's reproductive success. Some previous studies (Hansen et al. 2002 on Mauritius; Geerts & Paws 2011 in South Africa) have reported a negative impact of honeybees on the frequency of visits by nectarivorous birds to flowers. These studies, however, did not mention any aggressive interactions and suggested depletion of nectar supplies. Earlier, Prendergast (1983) speculated that sunbirds and butterflies might compete for nectar based on observations in Côte d'Ivoire, but provided no direct evidence. On the other hand, we do not know any study showing changes in visiting frequencies of any insect pollinators caused by competition with nectarivorous birds. Further study, including experimental exclusion of the competing visitors, will be needed.

In the study area, male carpenter bees were attacked more frequently than females (29 vs. 9 attacks). The carpenter bee females have stings and are able to sting also in flight (P. Bogusch & J. Straka, pers. comm.), and have aposematic coloration, which could inhibit attack by the birds. On the other hand, without data on visitor frequency, we cannot exclude the simple explanation that male and female carpenter bees differed in abundance. While several authors reported that large bees and wasps might attack competing birds (e.g., Akinpelu 1989; Stoaks 2000; Galindo-Gonzales & Ornelas 2002), we did not record this during our study.

Boyden (1978) and Thomas et al. (1986) reported a positive correlation between the amount of defended nectar and intensity of energetically expensive intrusions of insects, but this relationship still has not been broadly studied. In the two Cameroonian sunbirds, we recorded most interference in notably flower-rich patches, suggesting that sunbirds defend only the energetically valuable resources, similarly to hummingbirds. On the other hand nectar-richer patches might be included in the territories of more aggressive sunbirds. The richer nectar sources could be also visited by more sunbirds and carpenter bees, with more frequent attacks as a consequence of higher encounter rates between these two groups of visitors. However, Ollerton & Nuttman (in press) quantified numbers of flower visits and did not find any relationship with the attack frequencies. With no measures of the abundance of the carpenter bees and sunbirds we cannot resolve this question.

Our preliminary results provide evidence of highly sophisticated pollination systems including sunbirds, and we anticipate that such interspecific defence of nectar sources may be also found in other species and areas of the Old World. Clearly more detailed research into the evolutionary effects of interference competition by such distantly related pollinators is needed.

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CHAPTER VI

Asymmetric competition for nectar between large nectar thief and small pollinator: an energetic point of view

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manuscript

Abstract

There are two alternative hypotheses related to body size and competition for restricted food sources. The first one supposes that larger animals are superior because of their increased feeding abilities whereas the second one assumes superiority of smaller animals because of their lower food requirements. We examined the relationship between two unrelated species of different size, drinking technique, energy requirements and roles in pollination system to reveal the features of their competitive interaction and mechanisms enabling their co-existence while utilizing the same nectar source. We observed diurnal feeding behaviour of main pollinator, carpenter bee *Xylocopa caffra* and nectar thief, northern double-collared sunbird *Cinnyris reichenowi* on 19 clumps of *Hypoestes aristata* (Acanthaceae) in Bamenda Highlands, Cameroon. We recorded times of visits, numbers of visited flowers and handling times. Moreover we measured diurnal nectar characteristics and consumption. For comparative purpose, we used approximate values of resting metabolic rates. We revealed the energetic gain-expenditure balance of the studied visitor species in relation to diurnal changes in nectar quality and quantity. In general, smaller energy requirements and related ability to utilize smaller resources made the carpenter bee competitively superior to the larger sunbird. Nevertheless, sunbirds are endowed with several mechanisms to reduce asymmetry in exploitative competition, such as use of nectar resources in times of the day when rivals are inactive, aggressive attacks on carpenter bees while defending the nectar plants, and higher speed of nectar consumption. From plant point of view, competition for nectar between nectar thieves and pollinators might negatively impact the reproduction success of the plant. Although next studies are needed, we suppose that it is not the case of the studied system and the nectar consumption of sunbird might rather increase number of flowers visited and pollinated by carpenter bees. We moreover hypothesize that excessive production of nectar do not evolved to support just pollinators but rather to enable coexistence of both pollinator and thief.

Introduction

Nectar is an important resource of energy and nutrients for a large spectrum of flower visitors including bacteria, yeasts, mites, and diverse orders of insects, birds, reptiles and mammals. Nectar might, however, be temporally and spatially limited and in consequence interspecific competition will occur whenever the foraging of two or more organisms overlap in time and space (Brown et al. 1981, Ramalho et al. 1991, Galen and Geib 2007, Tiple et al. 2009). Such interactions are common in nature and play an important role in the organization of guilds of nectar-feeding animals and the co-evolution of plant-pollinator relationships (Ferriere et al. 2007).

Competition for nectar raises the question of sympatric coexistence of nectarivorous organisms, mainly in situations when competitive interactions occur among distantly related taxa where competition is expected to be highly asymmetric (Persson 1985, Barnes 2003). In general, competitive advantage in exploitative competition is related to foraging economy i.e. costs and benefits of foraging (Kodric-Brown and Brown 1979). It is believed that animals of small body size and low energetic requirement might forage profitably even when the quantity of nectar per flower is insufficient for larger nectar feeders, and thus exclude larger competitors, especially if they are outnumbered (Heinrich and Raven 1972, Heinrich 1975). There are, however, some ways for larger competitors to eliminate and even reverse this asymmetry. (1) Larger animals may obviously benefit from direct size effects in having lower costs of active defence of nectar resources (Persson 1985). Aggressive behaviour during the defence makes sense only in situations when the saved nectar outweighs the cost in time and energy related to this behaviour (Kodric-Brown and Brown 1979). Based on this, it is not surprising that aggressive defending of nectar resources is mostly documented among closely related species of the similar size (Colwell 1973, Johnson and Hubbell 1974, Kodric-Brown and Brown 1978). In contrast, only a few studies have reported this behaviour among unrelated nectarivores (Jacobi and Antonini 2008, Ollerton and Nuttman 2013, Tropek et al. 2013). This interference competition mostly happens between small birds and large insects (Lyon and Chadek 1971, Primack and Howe 1975, Boyden 1978, Tropek et al. 2013). (2) Large visitors might have better adaptations, improving foraging economy, such as longer feeding apparatus enabling consumption of larger nectar amounts from tubular flowers, or better movement ability among flowers, decreasing handling time and increasing nectar intake speed (Inouye 1980, Temeles and Roberts 1993). Moreover, larger nectarivores usually have better thermoregulation which brings higher energetic requirements on one hand, but allows them to be active in conditions of low ambient temperatures and thus harvest nectar when smaller competitors are inactive. This advantage is particularly evident when large animals are foraging on plants which accumulate nectar during the night and/or increase production rates in the morning (Brown et al. 1981, Bartoš et al. 2012).

These competitive interactions are not only important from the visitors' perspective, but also from the plants point of view. Individual nectarivore species play diverse roles in host plant reproductive systems. Only some visitors are effective pollinators (King et al. 2013, Padyšáková et al. 2013), whereas others either steal the nectar without pollen pick up or deposition, or illegitimately rob nectar by destruction of floral tissues (Inouye 1980). This pattern leads to the question of what would happen if the ineffective visitor was competitively superior. In this case, the plant-pollinator system seems to be ecologically and evolutionary fragile and selection pressure on plant traits which will eliminate this superiority should occur (Jones et al. 2012). Irwin et al. (2008) highlighted that one of the important mechanisms can be the plant tolerance by increasing nectar production to decrease the competition and satisfy both pollinators and larcenists. In the systems where both larcenists and pollinators occur, detailed experimental and observational studies have shown that the effect of nectar robbing on pollinated hosts might be also positive (Irwin and Brody 1998). Considering this, it is rather advantageous for plants to maintain the equilibrium in competition between pollinators and larcenists. The dynamics of plant-pollinator-robber systems thus pose important selection pressures on the evolution of floral traits.

In this paper we assess competition for nectar between two unrelated visitors of the herb *Hypoestes aristata*. Our research to date indicates that although *H. aristata* is visited by many insect functional groups and also by sunbirds, especially *Cinnyris reichenowii*, this plant is in fact specialized to be pollinated by large bees (Janeček et al. 2012, Padyšáková et al. 2013). The carpenter bee *Xylocopa caffra* is both the most frequent and the most effective pollinator while the northern double-collared sunbird *C. reichenowi* does not affect the seed production of visited flowers (Padyšáková et al. 2013). Moreover, this sunbird species protects its favourable nectar sources by aggressively driving the carpenter bee out of *H. aristata* patches (Tropek et al. 2013).

Based on previous results, we tested following hypotheses: 1) The smaller visitor and pollinator *X. caffra* will profit more from feeding on *H. aristata* because of lower energetic requirements and ability to use smaller resources (i.e. plants with smaller number of flowers) 2) The sunbird will balance this asymmetrical competition by aggressive behaviour, harvesting nectar in times when *X. caffra* is inactive, extracting higher amounts of nectar from individual flowers, and by higher speed of nectar intake (i.e. shorter handling time).

Methods

Study site

Our studies were conducted in the vicinity of Big Babanki, North-West Province, Cameroon (6°5´26´´N 10°18´9´´E) at high elevation in the central Bamenda Highlands (2100–2200 m a.s.l.). There is a single wet season from March to November, with annual precipitation ranging from 1 780 to 2 290 mm/year (For more details see: Cheek et al. 2000, Tropek and Konvicka 2010). The area is mostly open second growth of extensive pastures, frequently burned forest clearings dominated by *Pteridium aquilinum*, shrubby vegetation along streams, and remnants of species-rich tropical montane forests with a frequent occurrence of *Schefflera abyssinica*, *Schefflera manii*, *Bersama abyssinica*, *Syzygium staudtii*, *Carapa procera*, and *Ixora foliosa*.

Study species

Our target plant species, *Hypoestes aristata* (Vahl) Sol. ex Roem. & Schult var. *aristata* (Acanthaceae), is a clonal herb that grows up to 1.5 m high and is native to tropical sub-Saharan Africa (Hepper 1963, Balkwill and Norris 1985). The flowers of *H. aristata* cumulate nectar in 1-cm-long, narrow corolla-tubes. Nectar is hexose-dominant and the concentration is highly variable (30-45% w/w). The higest amounts of nectar can be found early in the morning but the highest nectar concentration is at midday (Bartoš et al. 2012). For more details about the plant see Padyšáková et al. (2013) and Bartoš et al. (2012). We studied *H. aristata* near the peak of its flowering season.

The northern double-collared sunbird *Cinnyris reichenowi* is distributed throughout West-Central and East Africa (Borrow and Demey 2001). This is the smallest of the local sunbirds (mean weight = 8.6 g) and is the most abundant species in open woodlands, forest clearings and ecotones where a variety of flowering plants provide an energy supply in the form of nectar (Reif et al. 2006, Reif et al. 2007). Sunbirds are territorial but often exhibit off-territory forays for nectar (Riegert et al. 2014). Mismatch between small flowers of *H. aristata* and sunbird's head apparently cause ineffective pollination service (Padyšáková et al. 2013).

The medium-sized carpenter bee *Xylocopa caffra* (mean weight = 0.768 g) is a wide-spread African species (Eardley 1983). This species together with some other less abundant large bees is the main pollinator of *H. aristata* (Padyšáková et al. 2013) as well as of the other plant species in the target area (Janeček et al. 2007). We never observed carpenter bees gathering pollen while visiting the flowers.

For comparative purpose, we got available values of resting metabolic rates (RMR) of closely related species having similar weight and foraging behaviour as surrogates from literature. We used the whole-body resting metabolic rate at 25°C of *Nectarinia veroxii* for *C.reichenowi* (Q = 725.175 J/hr; Prinzinger et al. 1989) and *Bombus terrestris* for *X. caffra* (Q = 2.958 J/hr; Makarieva et al. 2008).

Field observations and measurements

Nineteen clumps at 6 different study sites were surveyed from November to December 2012 when the plants of *H. aristata* were in full bloom. Clumps of different size (number of flowers) were chosen to test the effect of resource size on visitation rates. Three clumps (four in one case) were observed simultaneously in 2-hour sessions equally throughout four days (between 0600 and 1800) making 24 hours per clump in total. Before each observation session, we counted the number of open flowers on studied clumps to know the availability of food supply. Since the number of flowers for individual clumps did not differ much during the study we used the mean number of flowers for a given clump as a measure of clump size. While observing we focused only on visitations done by all individuals of the carpenter bee *X. caffra* and northern double-collared sunbirds *C. reichenowi*. The total time measured included the time the species spent probing flowers and removing nectar as the major component, and the time among flowers within an inflorescence and some brief transits among inflorescences as a minor fraction.

To estimate the amount and concentration of nectar a carpenter bee or sunbird removed from a flower we chased it from a clump with more than 10 open flowers after it had probed minimally 3 flowers (mean 6 flowers). We then measured the nectar volume and concentration in the same number of visited and unvisited flowers using 5-μL microcapillaries and a pocket refractometer (ATAGO PAL-1; USA). We calculated the difference (A-B) between (A) the nectar volume per unvisited flower in particular time of day and (B) the volume per visited flower in particular time of day. When the nectar volume was too low to measure the concentration we assigned the mean value appropriate for particular time of day. To calculate the sugar amount (in mg) per microlitre from w/w concentration (the concentration measured using a refractometer), we used an exponential regression equation (Galetto and Bernardello 2005). We repeated this procedure again from November to December 2013 to detect potential variability in nectar consumption and characteristics between years.

Statistical analyses

Counting data (number of visited flowers per plant, Number of visits per plant, Number of flowers per plant) were log transformed to improve normality. Most analyses were done in STATISTICA 10 (StatSoft, Inc. 2013). The data on nectar remnants (nectar volume left after visitation) with many zeroes did not meet normality assumption even after transformation and in this case we used the non-parametric permutation ANOVA in the program PERMANOVA+ for PRIMER (Anderson et al. 2008).

Results

In total, we recorded 365 visits of sunbirds *C. reichenowi* and 185 visits of carpenter bees *X. caffra*. In terms of flowers, *C. reichenowi* visited 14 192 and *X. caffra* 3 517 flowers. Data gathered in individual years nevertheless did not significantly differ so we pooled them in subsequent analysis. We found no consistent sexual differences in nectar removal so we combined our data for both sexes of the sunbird species. 99% of carpenter bee visits were done by females. The amount of nectar left after one visit did not differ between sunbirds and carpenter bees (PERANOVA, Pseudo-$F_{(1,137)}=0.4$; $p=0.525$). Similarly, both species left on average 15% of the nectar offered. Compared to *X. caffra*, *C. reichenowi* visited significantly more flowers per plant (ANOVA, $F_{(1,545)}=83.1$; $p<0.01$, Fig. 1a) as well as more flowers per second (ANOVA, $F_{(1,545)}=164.2$; $p<0.01$, Fig. 1b). Both *C. reichenowi* and *X. caffra* highly preferred plants with bigger number of flowers, with a marginally significant higher tendency in the case of sunbirds (GLM, Number of flowers: $F_{(1,34)}=33.6$; $p<0.01$; Number of flowers*Species: $F_{(1,34)}=3.2$; $p=0.08$; Fig. 2).

First foraging carpenter bees were occasionally spotted no sooner than 07h00 probably due to low ambient temperature and high condensed moisture caused by big difference between day and night temperatures typical for tropical mountain environments (Fig. S1); their feeding activity peaked around midday. Conversely, sunbirds started to regularly forage with dawn and fed more or less evenly during the day with slight decrease of activity over midday when the energetic intake per flower was the lowest (Fig. 3).

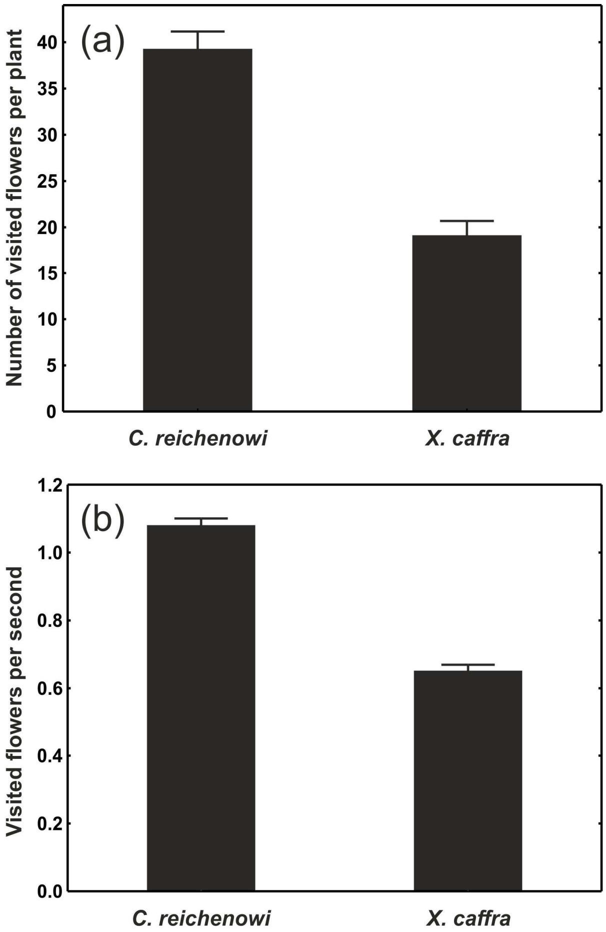

Fig. 1 The differences between *C. reichenowi* and *X. caffra* in numbers of visited flowers per plant (a) and visitation speed (b). Error bars represent standard error.

A theoretical relation between absolute energetic gains and nectar-feeders metabolism provision is illustrated in Fig. 4 where energetic gain is considered to be the percent of the covered 24-h MR in the case that nectar quality of that particular time of day remains the same for the rest of day, and a forager continuously feeds on such nectar continuously for 12 daylight hours. Based on their resting metabolic rates, the energy provision by nectar feeding was significantly different between the surveyed species (t test for dependent samples, t=-12.6, p<0.01; Fig. 4). The energy intake of *X. caffra* greatly exceeded the resting metabolic requirements, no matter the time of day, whereas metabolism of *C. reichenowi* was covered and surpassed only when feeding on morning and late afternoon nectar (Fig. 4).

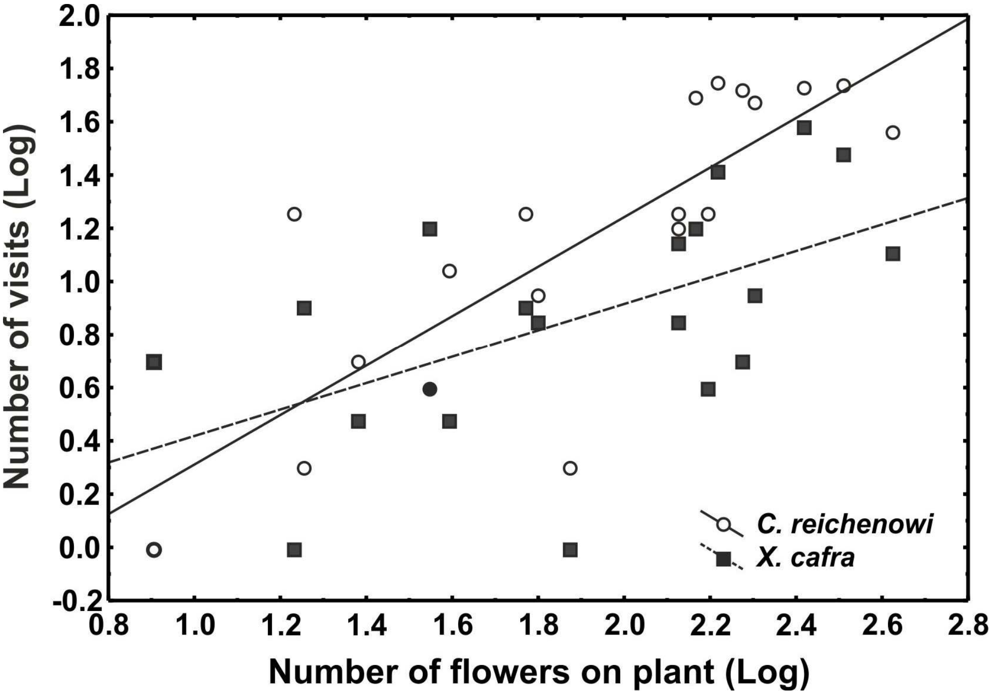

Fig.2 Effect of Number of flowers on plants of *H. aristata* on number of visits on the plants.

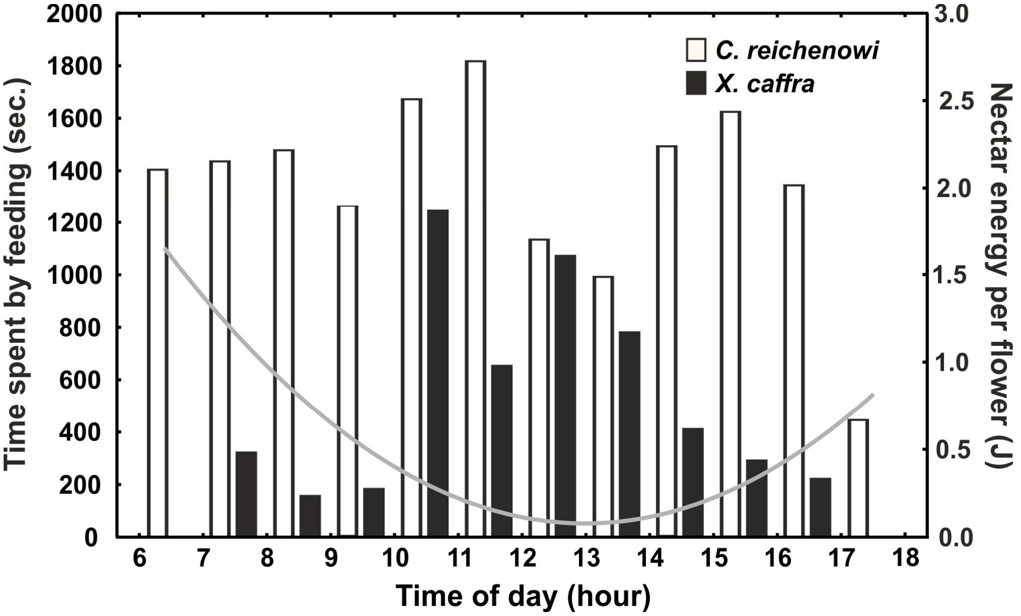

Fig. 3 Feeding activity of *C. reichenowi* and *X. caffra* and diurnal changes in standing crop (grey line –polynomial function)

Discussion

In our study, we reveal mechanisms underlying competition for nectar between the bigger nectar thief (sunbird) and smaller pollinator (carpenter bee). Our energetic approach shows that this competition is highly asymmetric in favour of the smaller pollinator and as a consequence we support the hypothesis that smaller visitors are, thanks to smaller energy requirements and related ability to explore smaller resources, competitively superior. Nevertheless, we also show many mechanisms that enable the larger competitor to reduce this superiority and avoid exclusion from the use of the resources. These mechanisms included interference competition (Tropek et al. 2013), use of resources in times when the smaller competitor is inactive, higher speed of nectar consumption and higher preference for bigger plants.

While visiting clumps of *H. aristata*, sunbirds move quickly around the rings of flowers and probe consecutively into adjacent flowers to look for nectar. They fly or jump quickly among the shoots within a dense clump and rarely skip flowers. The sunbirds usually perch on the stalk and insert their curved bills in all directions from the actual sitting position often resulting in entering the flowers from above and behind. To our opinion, this action contributes to ineffective pollination service by sunbirds. On the contrary, carpenter bees fly among flowers, lands individually on flowers and takes longer than the sunbirds to probe a flower because it always enters the flowers from the front.

Both empirical data and theoretical models show that body size has a predominant influence on an animal's energetic requirements, its potential for resource exploitation, and its susceptibility to natural enemies (Hamrin and Persson 1986, Werner 1994, Ballance et al. 1997, Bystrom and Garcia-Berthou 1999). As a contrast to some previous studies (Schoener 1983), ours and others experiments indicate that in exploitation competition directly based on food depletion, smaller species have greater net gain and are less affected by a decline in resources (Persson 1985, Werner 1994, Ballance et al. 1997). The smaller animal’s advantage is best regarded as a combination of feeding ability as well as utilization of limited resource availability (Persson 1985). In our case, when considering that a sunbird (weight 8.6 g) expends much more energy than carpenter bee (weight 0.768 g) just to cover its resting metabolism, and the mean energy load gained by each given species per one flower visit is pretty similar (0.0819 J for sunbird and 0.0738 J for carpenter bee, respectively), our study clearly reveals the smaller harvester to be advantaged.

To counterbalance the exploitative losses, large dominant species often take advantage of a direct size effect and aggressively outcompetes the smaller subordinate species in interference competition (Persson 1985). From this point of view interference competition is viewed as consequence of asymmetrical exploitative competition (Persson 1985). The aggressive chasing of *X. caffra* by *C. reichenowii* in the studied system, firstly reported by (Tropek et al. 2013) as well as observed eight times during actual study, supports mentioned idea. This behaviour, nevertheless, in comparison with 185 observed visits of *X. caffra* can be seen as rather rare. Since intensive defending is a very costly strategy (Gill and Wolf 1975), an aggressor should optimally defend areas of highest productivity and tolerate smaller subordinate species to forage in less profitable areas (Gill and Wolf 1975, Pimm et al. 1985, Ballance et al. 1997, Riegert et al. 2011). In the study area, Riegert et al. (2014) described feeding niche partitioning between two local sunbird species mostly driven by interference competition. The submissive sunbird *C. reichenowi* was forced by chasing from *Cinnyris bouvieri* to feed on plant species with relatively lower nectar productivity. Similarly, a previous study on sunbird-carpenter bee competition detected sunbirds' aggressive defending attacks to carpenter bees exclusively in high density patches (Tropek et al. 2013). Our actual results complement the observed responses by showing sunbirds' stronger affinity to plants with more flowers (Fig. 2). As shown in many studies, differences in body size in terms of metabolic costs affect the higher preference of larger species to utilize and defend the richest food sources, maximizing the foraging efficiency (Gill and Wolf 1975, Ballance et al. 1997, Palmer et al. 2003, Riegert et al. 2011, Tropek et al. 2013). This pattern might explain why chasing of insect by relatively large sunbirds (Ollerton and Nuttman 2013, Tropek et al. 2013) is reported much less often then chasing of insect by small hummingbirds (Primack and Howe 1975, Stoaks 2000, Jacobi and Antonini 2008).

Patterns of effective utilization of variable nectar volumes are correlated with morphological differences of feeders' mouthparts and drinking technique employed. Our observation showing carpenter bees feed even at midday on the remaining highly concentrated nectar left in flowers after sunbird visits finds its explanation in the bee feeding technique. Most bees actually ingest nectar by dipping their tongue into, then extracting it from, the viscous nectar (Kim et al. 2011). On the contrary, nectar-feeding birds, which employ capillary suction, optimally utilize higher volume of less concentrated nectar. Considering drinking style, we guess that feeding sunbirds may leave a small amount of untapped nectar in the flowers, especially on the corolla walls. Simply based on physical and physiological constraints, these nectar leftovers are out of reach to sunbirds' bills as well as to our microcapilaries which we used for measuring the nectar volume. Viscous dipping carpenter bees, however, may take advantage of it and feed even on flowers previously visited by sunbirds. The nectar dipping technique, nevertheless, could result in longer handling times particularly for flowers containing small volumes (Harder 1986).

Both species consumed and depleted food resource that was not later available for sympatric nectar-feeding rivals. One might argue that increased activity in the morning and again in the afternoon with cessation at midday by sunbirds reflects a normal pattern of bimodal daily bird activity (Bednekoff and Houston 1994) rather than exclusion by rival nectar feeders due to low nectar availability. There is no doubt that sunbirds heavy feeding activity in the morning might get the quantity of their own food supply to less profitable levels, but later activity of carpenter bees does contribute substantially to nectar depletion. Field and laboratory studies, however, testing hummingbirds feeding throughout the day by recording their visits to artificial nectar feeders providing constant nectar source clearly showed that birds fed continually from early morning to late afternoon (Wolf and Hainsworth 1977, Brown et al. 1981). Conversely, many nectarivorous birds show a pattern of steady decline in feeding intensity through the day (Bednekoff and Houston 1994). A recent study testing Baker's proposition (Baker 1975) on sunbird species strongly supported the hypothesis that the most efficient energy intake occurs at sugar concentrations that represent a compromise between low energy content and high viscosity (Koehler et al.

2010). The finding that nectarivorous birds should favour lower nectar concentration would correspond with observed values of *H. aristata* nectar in the morning and late afternoon (Bartoš et al. 2012). It seems to us that sunbirds midday cessation was a response to high nectar concentration and low volume, partly caused by carpenter bees' visits, which made them switch to other nectar sources.

When considering low profit from exclusive feeding on *H. aristata* nectar (Fig. 4), sunbirds must visit other nectar plants producing higher caloric reward to cover its daily energy demands. Among them, *Hypericum lanceolatum* and *Lobelia columnaris* are typical components of the floral community within the sunbird territory and one of the often visited plant species (Janeček et al. 2012; pers.obs.) providing multiple larger nectar standing crop (Bartoš et al. 2012). This poses an interesting question: why does the sunbird clearly prefer (Janeček et al. 2012), actively defend (Tropek et al. 2013) and spend so much time of day feeding on a nectar source plant that does not meet its energy demands? Since most of actually studied sunbird individuals were coloured ringed we could detected that particular plants are visited regularly and almost exclusively by particular individuals and their mates. It implies that *H. aristata* nectar depletion is part of sunbird‘s territorial defence. Moreover, Riegert et al. (2011), studying ordinary daily activities in northern double-collared sunbirds, observed a substantial percentage of time spent by active insect feeding, reaching 15.4% in females. Using stable isotope analyses to detect diet and niche segregation among sympatric species in their study area, Procházka et al. (2010) revealed these sunbirds derive most of their nitrogen from invertebrates. We can hypothesize that this combination of sources provide sufficient energetic intake to cover sunbird overall metabolic expenditures as well as defending less profitable resource, such as *H. aristata*, forced to do so by being excluded from other plants by larger sunbirds (Riegert et al. 2014).

From the plant perspective, the optimal rate of nectar production should be the rate that yields the greatest difference between the costs and benefits (Zimmerman 1988). In our studied system, the plant species apparently produces much higher amounts of nectar per plant than would be necessary to satisfy energetic requirements of its mutualistic pollinator, the carpenter bee and thus attracts thieving sunbirds as well. Excessive nectar production has most likely something to do with an established plant-pollinator-thief system as was suggested by other authors (Barrows 1976, Roubik et al. 1985, Morris 1996, Maloof and Inouye 2000). Although previous study has shown C. reichenowi as directly ineffective pollinator its presence in the visitor community might have some indirect effect on reproductive success of *H. aristata*. Recent studies have brought innovative evidence showing nectar robbers and thieves to not always have negative effects on seed set (Irwin and Brody 1999), but sometime neutral or even positive outcomes via required increased pollen flow distances by changing the behaviour of the pollinators (Maloof and Inouye 2000, Maloof 2001, Richardson 2004). This view partly modifies the suggestion made elsewhere (Heinrich 1975) that natural selection would tend to produce enough food reward for optimal pollinator, and at the same time, do not provide too great food quantity to force a pollinator make flower to flower and plant to plant movements. Natural selection for sufficiently low production of nectar expected in mutualistic relationships can be substituted, in case of plant-pollinator-thief system, rather by regulation of thieving member in the system. Although we do not have direct evidence we consider that this would also be the case in our system. We hypothesize that the accurate amount of food reward is maintained by sunbirds and in consequence carpenter bees move more often between plants to match their daily energy budgets. This hypothesis should, nevertheless, be tested in next experimental studies.

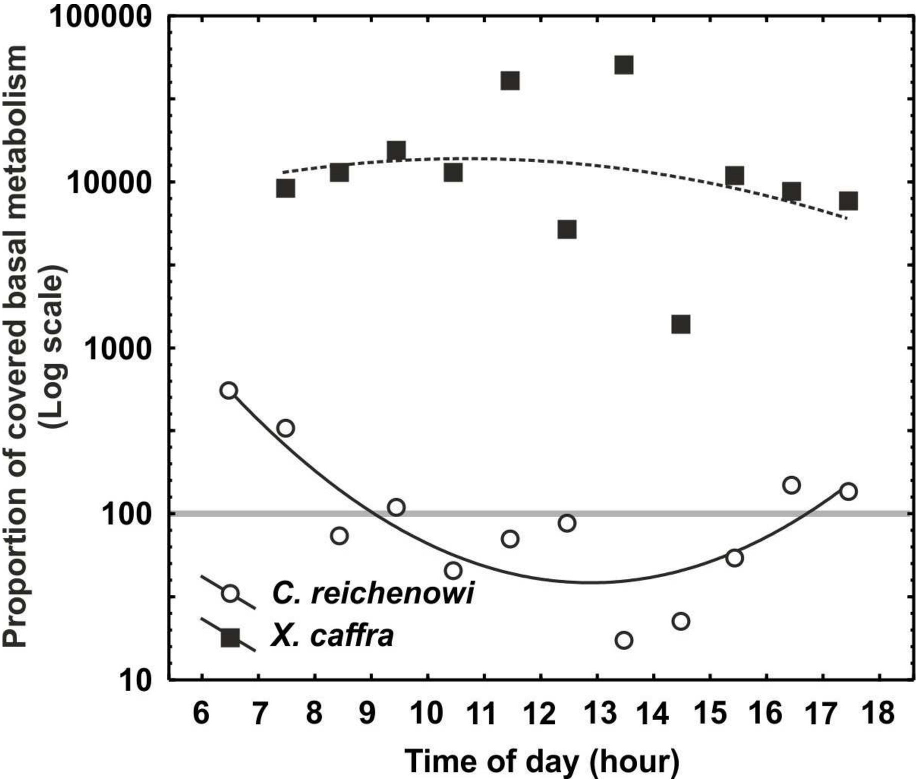

Fig. 4 Metabolism energy provision by nectar feeding. Each point shows percentage of 24-h metabolism coverage assuming that the visitor will take nectar continuously during the day (06h00 to 18h00) from the flowers with nectar specific properties relevant at that time of day. Approximation done by polynomial function. Grey line indicates 100% coverage.

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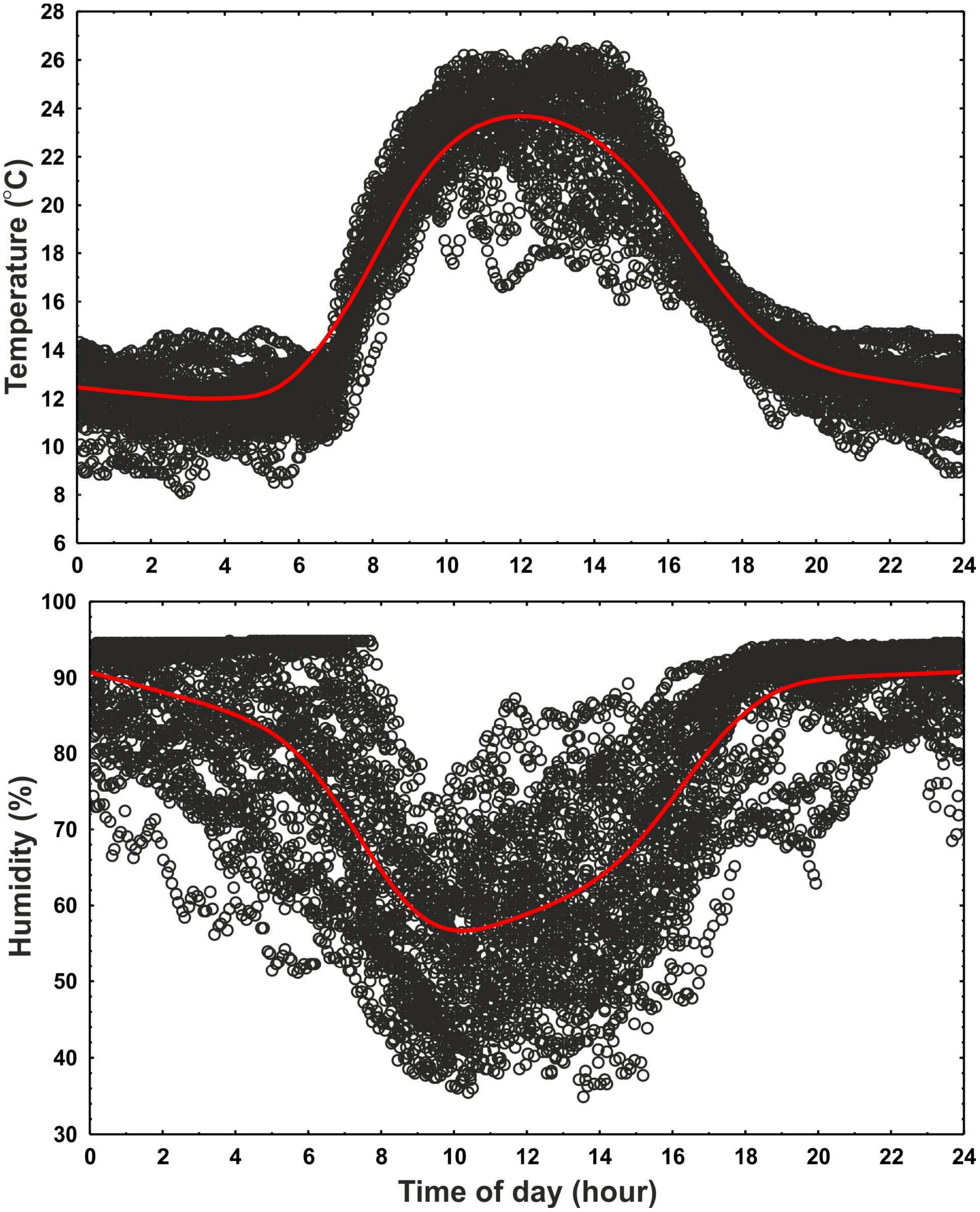

Fig. S1 Diurnal changes in temperature (°C) and air humidity (%) at the study area in the time of the fieldwork. Red line indicates mean values.

CHAPTER VII

Summary of results

~ · Summary of results · ~

Various hypotheses have been tested to help explain the role of sunbirds in the function and evolution of pollination systems in tropical mountains of West Africa. Sunbirds comprise a substantial part of local pollinator assemblage, and their foraging activities exert potentially important selective pressures on plant populations.

Although sunbirds are able to feed on flowers bearing traits related to various pollination syndromes (Chapter II, IV and VI), we detected a clear pattern of selectivity for specialized long tubular flowers when plant abundance was considered (Chapter II). Different sunbirds, however, are not equally effective as pollinators and some take an advantage of the mutualistic relationships between plants and their pollinators by stealing available nectar from flowers without pollinating them (Chapter III and IV). Amazingly, the same sunbird species occasionally acts as nectar robber which steals the rich nectar rewards from specialized, bird pollinated flowers by cutting through tube in one pollination system (Chapter III). It can also act as a nectar thief which steals the low-volume nectar from specialized, carpenter bee pollinated flowers by entering the flowers without contacting the reproductive organs due to a mismatch of morphologies (Chapter IV and VI). Employing either strategy, these larcenists not only compete directly with legitimate pollinators while aggressively defending their nectar sources (Chapter V), but in so doing they also reduce the amount of nectar available (Chapter III, IV and VI), and are likely to change pollinator behaviour and consequently affect pollen transfer (Chapter VI). Plants are hence likely to evolve in response to competition among flower foragers by increases in characteristics which exclude ineffective visitors and force the pollinators to hover at the flowers to reach nectar (Chapter III), or by nectar overproduction to maintain both thief and pollinator whose co-existence is necessary for the indirect positive fitness effect of increased outcross pollination due to longer flight distances (Chapter IV and VI). Although this can be inferred from our results, much additional research is required to clarify the ecological mechanisms and evolutionary consequences of these interactions.

In another aspect of the thesis, applied field techniques enabled us to try various approaches in practise and compare their weaknesses and advantages with other methods used in pollination studies. Hence, we highlight the idea of our colleagues who propose to move away from the broadly used network approach categorizing an interaction as either present or absent. It is necessary to move toward more direct measurements of the nature and strength of interactions, ideally by estimating the pollination effectiveness of individual flower visitors and determining their roles in pollination systems. To construct relevant hypotheses concerning plant-visitor co-evolution and explain the evolution of specialized adaptations, future studies have to especially focus on intensive sampling of underrepresented communities across diverse habitats and latitudinal as well as altitudinal zones.

Overall, our studies help to compensate the enormous lack of pollination studies conducted in West Africa, particularly in mountain tropical regions. The presented results have brought significant practical evidence confirming the validity of some concepts or statements made in other areas: these include phenotypic complementarity as important determinant of plant-visitor interaction (Chapter II); asymmetric specialization in plant-pollinator relationships (Chapter II); pollination-syndrome concept (Chapter III and IV); and larger animals tendency to be superior to smaller ones in interference competition (Chapter V), but not in exploitative competition (Chapter VI). Additionally, our surveys have revealed novel phenomena observed on the African continent, such as the first well-documented native-African-plant pollination system including frequent hovering of sunbirds (Chapter III), or interference competition for nectar sources between African sunbirds and carpenter bees (Chapter V). Without further work in Africa we cannot find more differences between this continent and the rest of the world, and thus better elaborate the overall conceptualization of the amazingly diverse pollination systems in different ecosystems.

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EDUCATION

2004 – up to now University of South Bohemia, Faculty of Science
Ceske Budejovice, Czech Republic
RNDr., Mgr.

- Bc. study programme: *The impact of predation on duck breeding success*
- Mgr. study programme: *The effects of local and landscape-scale habitat characteristics and prey availability on corridor use by carnivores*
- Dr. study programme: *The role of vertebrates in Afromontane pollination systems*

SHORT RESEARCH AND STUDY STAYS

2007 – 2013 (5x) Bamenda Highlands, Cameroon

- field work
- data gathering

2013 (six weeks) University of KwaZulu-Natal, South Africa
The Tsitsikamma Fynbos Research Program

- research fellowship
- setting up and running a plant-bird pollination project

PUBLICATIONS

Šálek, M., Červinka, J., **Padyšáková, E.** & Kreisinger, J. (2014). Does spatial co-occurrence of carnivores in a Central European agricultural landscape follow the null model? *European Journal of Wildlife Research* 60(1), 99-107.

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Bartoš, M., Tropek, R., Spitzer, L., **Padyšáková, E.**, Janšta, P., Straka, J., Tkoč, M. & Janeček, Š. Specialization on pollination systems of two co-flowering phenotypically generalized *Hypericum* species (Hypericaceae) in Cameroon. *in prep.*

INTERNATIONAL CONFERENCES AND ORAL PRESENTATIONS

2013 Bird Club meeting, Plettenberg Bay, South Africa

"The outcomes of the long-term pollination ecologist work in Cameroon"

- oral talk

2014 Tropical Ecosystems – Between Protection and Production, Munich, Germany

"Importance of plant-pollinator relationships in a changing afromontane landscape"

- oral talk

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Ph.D. Thesis

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