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Sunbird foraging behaviour on flowers of *Impatiens sakeriana*

Potravní chování strdimilů na květech *Imptaiens sakeriana*

Master's thesis

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Prague, 2019

Statement:

I hereby state that I have completed this thesis by myself and that I have properly cited all literature and other information sources I have used. Neither this thesis nor its parts have been submitted to achieve any other academic title(s).

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Abstract

Although the Old World sunbirds are generally considered to be an ecological analogy of the New World hummingbirds, until recently it was believed that in contrast to hummingbirds, sunbirds perch while feeding. This opinion was largely supported by several studies, mostly from South Africa, describing adaptations of plants facilitating this behaviour. However, recent studies have shown that the Old World nectarivores hover while feeding in front of flowers more frequently than previously thought.

We focused on a specialised West African pollination system of *Impatiens sakeriana* and the foraging behaviour of its two major pollinators, the Northern Double-collared Sunbird (*Cinnyris reichenowi*) and the Cameroon Sunbird (*Cyanomitra oritis*). Based on continuous monitoring in their natural habitat via camera systems, we evaluated factors influencing bird foraging behaviour on a flower, i.e. bird's decision whether to perch or to hover. Our results indicate that sunbird foraging behaviour choice depends on plant architecture, namely on the length of peduncles and pedicels. Surprisingly, weather affects pollinator's behaviour just slightly. The data also indicate that feeding and moving among flowers require less time if the bird hovers and therefore this behaviour is associated with higher flower visitation rate.

Additionally, we studied in detail hovering flight of both sunbird species. It has been hypothesized that passerines, unlike hummingbirds, are not able to sustainably hover for longer periods and that the majority of them exhibit intermittent flight with regular interruptions in flapping. Our findings show that even though actual frequency of flapping slightly decreases in time, both studied sunbird species are able to hover steadily without any interruptions in flapping for several seconds with wingbeat frequency averaging 20 Hz.

Key words: *Impatiens sakeriana*, *Cynniris reichenowi*, *Cyanomitra oritis*, foraging behaviour, hovering, bird pollination

Abstrakt

Ačkoliv jsou strdimilové nového světa učebnicovou ekologickou a evoluční paralelu novosvětských kolibříků, do nedávné doby byli považováni za ptáky, kteří na rozdíl od kolibříků při konzumaci nektaru sedí. V řadě studií, převážně z jižní Afriky, byl tento rozdíl demonstrován na adaptacích rostlin, které jim toto chování usnadňují. Současné studie však ukazují, že krmení za letu je u strdimilů častější, než se dříve předpokládalo.

Zaměřili jsme se na specializovaný polinační systém netykavky druhu *Impatiens sakeriana* rostoucí v horách západní Afriky a potravní chování jejich dvou ptačích opylovačů, strdimilů *Cinnyris reichenowi* a *Cyanomitra oritis.* Na základě souvislého monitoringu několika divoce rostoucích jedinců rostliny a jejich návštěvníků jsme se pokusili vyhodnotit vliv faktorů ovlivňujících chování ptačích opylovačů u květu (tedy zda pták během krmení sedí, či se krmí za letu). Naše výsledky ukazují, že ptačí chování u květu je výrazně ovlivněno architekturou rostliny, konkrétně délkou květní stopky a osy květenství. Překvapivě, intenzita deště ovlivňuje ptačí chování pouze mírně. Naše data také ukazují, že krmení za letu je spojeno jak s kratšími návštěvami květu, tak s rychlejšími přesuny mezi květy a umožňuje tak ptákům navštívit více květů za jednotku času.

Zvláštní pozornost pak byla věnována třepotavému letu (letu na místě), který bývá u květů strdimily často praktikován. Na základě několika studií se má za to, že pěvci nejsou na rozdíl od kolibříků déle trvajícího souvislého letu na místě schopni a pokud třepotají, přerušují opakovaně mávání křídel vždy po několika cyklech. Z analýzy našich nahrávek je však patrné, že i přes mírný pokles v aktuální frekvenci úderů křídle v čase, oba studované druhy jsou schopny nepřerušovaného třepotavého letu na místě po dobu několika sekund. Frekvence úderů křídel se u obou druhů pohybuje okolo 20 Hz.

Klíčová slova: *Impatiens sakeriana*, *Cynniris reichenowi*, *Cyanomitra oritis*, potravní chování, třepotání, opylení ptáky

Contents

Chapter I:

Introduction

General introduction

Around 85 % of flowering plants (over 300 000 species) rely on animals for pollen transfer (Ollerton et al. 2011). Most of them are pollinated by insects. Based on the insect-pollination precursor, numerous independent evolutionary processes led to the origin and development of bird-plant pollination systems. Nowadays, plants primarily pollinated by birds embody a remarkable fraction of all vascular plants, comprising members of ca. 500 genera from over 65 families (Cronk and Ojeda 2008). Among others, these are for example Fabaceae, Rubiaceae or Lamiaceae.

Pollination by birds

Bird-pollination is generally widespread but reaches major importance and highest degree of specialization in tropical regions. Birds (and other endotherms) are less constrained by rainfall and cold weather than invertebrate pollinators, thus their role is relatively weightier in mountain regions and during rainy seasons (González et al. 2009). Furthermore, thanks to their high mobility, limited ability for pollen grooming (especially it is if distributed around bill) and frequent interactions among individuals, the bird-pollination results in two times higher paternal plant diversity than the insect pollination (Krauss et al. 2017). This makes the pollination by birds a highly advantageous strategy and explains why various plants across continents and taxa exhibit several adaptations to increase bird visitation, avoid visitation of less effective pollinators such as insects, and maximize the amount of pollen deposited on bird's body.

Most obvious is the consistence in floral characteristics, sometimes denoted as syndrome of ornithophily. The absence of odour or vivid coloration belong among the most apparent signs. Ornithophilous flowers are often red, this colour is known as well visible for birds and at the same time difficult to register for many insect species (Altshuler 2003). The tendency for maximalization of pollen load on bird's body is reflected in tubular, gullet or brush-like floral morphology (Faegri and van der Pijl 1979). Furthermore, absence of insect-landing platforms and orientation toward free space evoke the intention of visitor filtration. However, some plants provide perches for birds to facilitate access to flowers (Stiles 1981; Westerkamp 1990; Cronk and Ojeda 2008;). Visitor filtration is additionally enhanced by nectar properties. Bird-pollinated flowers usually produce large amount of relatively dilute nectar (20-25 %) with only diminutive content of amino acids (Baker and Baker 1983; Nicolson 2002).

Nectarivorous birds across continents

Nectar serves as a reward and forms a crucial diet component for more than 900 bird species (Krauss et al. 2017). In contrast to relatively high taxonomical scattering of bird-pollinated plants, specialised nectarivorous birds are restricted to only few lineages with the absolute majority belonging to three families: hummingbirds (Trochilidae), sunbirds, spiderhunters (Nectariniidae) and honeyeaters (Meliphagidae).

Hummingbirds with over 330 species represent the largest and most specialized nectarivorous clade and, as they form part of the order Apodiformes, one of only two groups of non-passerine specialised bird pollinators. Members of this family occur exclusively in the New World, ranging from Alaska to southern South America. In contrast, the vast majority of Old World nectar-feeding birds are from the order Passeriformes. Around 130 species of sunbirds and spiderhunters are distributed across warmer parts of Africa and

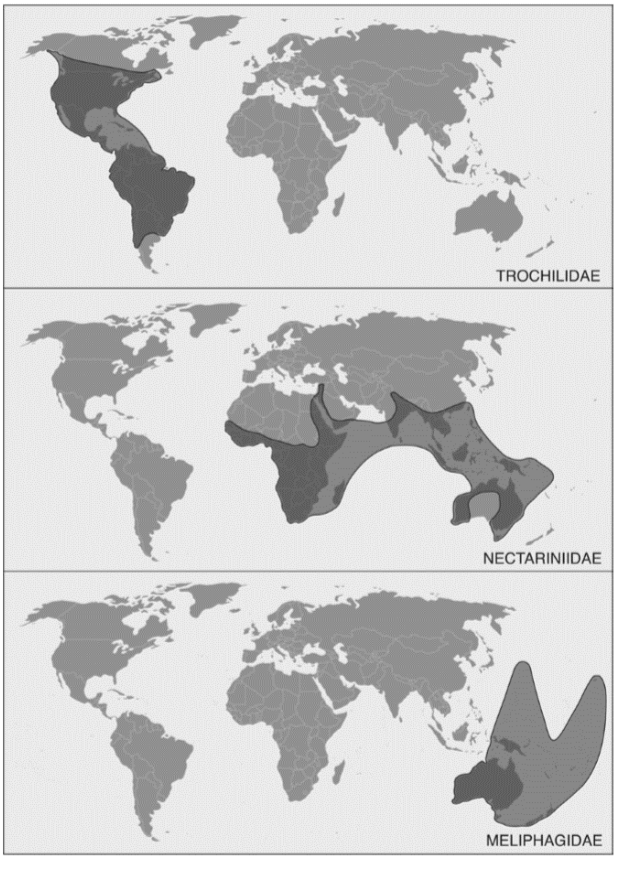


Figure 1
Distribution of the tree major nectraivorous families: hummingbirds (Trochilidae), sunbirds (Nectarinidae) and honeyeaters (Meliphagidae). Picture from Cronk and Ojeda 2008.

Asia with few species inhabiting Australia. Close to 180 species of honeyeaters are restricted to Australia and Indonesia (Stiles 1981). In consequence of their phylogenetical severance and minimal overlap in distribution, these birds legitimately often serve as an example of convergent evolution.

Even though, I will limit further discussion only to the three major families which have been already mentioned, I believe it is desirable to present also other predominantly nectar-feeding birds. These are the white-eyes (Zosteropidae), flower-peckers (Dicaeidae), lorries and lorikeets (Lorini, Psittasidae), some species of sugarbirds (Promeropidae), tanagers (Thraupidae), Hawaian honeycreepers (Fringillidae: Carduelinae) and American Orioles (Icteridae).

Interestingly, analogous disproportion as seen on a higher taxonomical level may be found also on species level, since many plants are effectively pollinated only by few bird species, while most birds exploit a large range of plant resources (Krauss et al. 2017).

Convergent adaptations of nectar-feeding bird taxa

Complementary to plant adaptations for bird-pollination, nectar-feeding birds exhibit several physiological and morphological adaptations in reaction to the specificity of their diet and associated lifestyle (Stiles 1981).

Nectar is basically a dilute sugar solution with a negligible portion of other components, indeed nectarivorous birds must deal with an extremely high daily water intake. Together with a low concentration of salt presented in the diet, this has led to changes in renal morphology that allows the production of unexceptionally dilute urine (Casotti and Richardson 1992; Casotti et al. 1998; Lotz and Nicolson 1999). Consequently, they have developed an ability to regulate renal activity according to actual water consumption in order to avoid excessive water loss (Bakken 2004; McWhorter 2004; Purchaseet al. 2013). High percentage of sugars together with high metabolism rate requires very fast and effective transport of consumed sugars across the intestinal epithelium (Lotz and Nicolson 1996; McWhorter et al. 2006; Napier et al. 2008). Furthermore, these birds are tolerant to an exceptionally low nitrogen intake, still all nectar feeding bird at least partly supplement protein deficit by consummation of insects (Paton 1982; Roxburgh and Pinshow 2000; Lopez-Calleja 2003; Riegert et al. 2011). For plants, nectar is costly to product thus is not offered in large quantities. Moreover, it is often not easily accessible. For that reason, birds show many corresponding morphological features. The most obvious is probably their small body size. Some nectar-feeding birds belong among the smallest living endotherms

(Amethyst Woodstar, *Calliphlox amethystina* only 2.3-2.5 g; Züchner and Kirwan 2019), but generally, body mass of the most specialised ones rarely exceeds 20 g (Stiles 1981). Accordingly, their metabolism is unusually fast, and they use torpor or hypothermia to survive periods of starving (Collins et al. 1980; Prinzinger et al. 1992; Downs and Brown 2002).

Nectar-feeding birds generally have long and curved bills which perfectly match in deep floral corollas (Stiles 1981) and long tubular tongues that allow enhanced nectar extraction using capillarity in addition to active sucking and licking (Fleming and Muchhala 2008).

Occurrence and evolution of hovering flight

On the other hand, some traits are unique to a particular bird family. The most famous example is the insect-like hovering (i.e. whirring according to Westerkamp 1990) flight typically performed by hummingbirds while feeding on flowers. It is realisable due to an extraordinary rotation in wrist together with reduction in the relative size of the proximal skeletal wing elements, large pectoral muscles and some other morphological and physiological alterations (Warricket al. 2005; Hedrick et al. 2012).

For a decades it has been thought that thanks to these adaptations, only hummingbirds are capable of sustainable hovering flight and that this fact is reflected in evolution of bird-pollinated plants across continents. This concept was supported by many studies, mainly from South Africa, showing Old World plants facilitating perching of birds during flower visits (Fleming and Muchhala 2008). The premise formulated by Miller (1985) and promoted by Westerkamp (1990), that behaviour of bird pollinators is ultimately determined by plant architecture as this obligates hummingbirds to hover, had been almost forgotten. Westerkamp noted that some examples of plants with flowers orientated toward free space (a typical trait displayed by hummingbird-pollinated plants) can be found even in Africa or Asia. Mayr (2005) hypothesised that these plants might be relicts after hummingbird ancestors, which were possibly (with respect to discovery of hummingbird-like fossils in Europe) distributed also in the Old World, nowadays pollinated by insects.

New insights into this topic were brought by two relatively recent studies. First of them is the study Geerts and Pauw (2009) describing behaviour of three sunbird species during visits of South American tree tobacco (*Nicotiana glauca*) invasive to South Africa. All observed sunbird species hovered while feeding, the Malachite Sunbird (*Nectariniia famosa)* even in 80 % of all visits. This study shows that regardless isolated evolution, sunbirds are able to hover for feeding. The second crucial work is the observation of

Janeček et al. (2011) demonstrating a native African species, *Impatiens sakeriana,* to be pollinated by regularly hovering sunbirds, the Cameroon Sunbird (*Cyanomitra oritis*) and Northern Double-collared Sunbird (*Cinnyris reichenowi*). It may well be, that Old-World bird-plant pollinating systems have been evolving in similar direction as in the New World. Furthermore, Wester (2013) compiled available information concerning sunbird and honeyeater foraging behaviour, which points out that 46 species of sunbirds and 15 species of honeyeater have been observed hovering for feeding.

According to the newest observations, some sunbirds prefer to hover while feeding even if an adequate perch is available (Padyšáková and Janeček 2016). This finding is in concordance with an alternative explanation for hovering performance proposed by Pyke (1981), who claims that behaviour of pollinators may be explained simply by rules known from Optimal Foraging Theory (MacArthur and Pianka 1996). In his opinion, there are situations in which hovering is beneficial for birds, as it allows visiting more flowers and thus increase the energy intake in time. Moreover, based on observation of Eastern Spinebill (*Acanthorhyncus tenuirostris),* he assumes motivation for hovering should be higher if flowers are situated further from each other as the advantage connected with hovering is relatively greater in these cases. His suggestion is supported by the finding that hovering flower visits are generally shorter than perching ones (Padyšáková and Janeček 2016).

Flying in the rain

Besides plant characteristics and anatomical (or physiological) constrains of its visitor, plant-pollinator (in our case bird) interactions are also affected by climatic condition (possibly throughout bird physiology). One of the major climatic factors is the intensity of precipitation (González et al. 2009). Due to their high metabolism rate (Lasiewski 1963; Prinzinger et al. 1989), in order to stay alive, nectarivorous birds must keep feeding on flowers also under moderate or even heavy rain.

Naturally, flying in the rain represents a big challenge, mainly for simple mechanical reasons. Water loads remaining on bird's body increases its effective body mass elevating energy expenditure related to maintenance in the air. At the same time, drops hitting wing area complicate wing movement and their uneven distribution may negatively affect manoeuvrability. Furthermore, acting forces of falling raindrops are directly proportional to their size and second power of their velocity, thus the negative effect of rain rapidly increases with its intensity (Angulo-Martínez et al. 2016). Negative consequences of striking droplets and plumage wetting are disproportionally higher for smaller flying animals. If we consider the high metabolism rate typical for small animals and extreme costs of hovering flight (Lasiewski 1963; Evans and Thomas 1992), energetics costs associated with hovering in the rain must be enormous.

To my knowledge, there are few studies focusing on the effect of rain on flight performance in relation to migrations (as could be expected, numbers of migrating birds, especially passerines, are lower under moderate rain then decrease to zero under heavy rain (Nisbet et al. 1968) and only two studies describing how hummingbirds deal with intense rain. Ortega-Jimenez and Dudley (2012a) noticed, that Anna's Hummingbird (*Calypte anna*) performs aerial and perched shaking to remove accumulated water from their bodies. Additionally, their studies of hovering performance of *C. anna* under increasing intensity of precipitation, demonstrate hummingbird's capability to hover even under heavy rain (22.4 mm·h^{-1}; Ortega-Jimenez and Dudley 2012b).

Studies on hovering passerines (a brief comparison with hummingbirds)

Despite many evidences of hovering passerines, there is no doubt hovering flight proper to hummingbirds is exceptional among birds. Their wings are fully extended throughout the entire wingbeat cycle and can be extensively supinated so that also upstroke is aerodynamically active (similarly as in hovering butterflies) and contributes by 25 % to full weight support (Warrick et al. 2005; Hedrick et al. 2012). Furthermore, they are capable of immediate and extremely fast oxidation of absorbed sugars in enlarged pectoral muscles (Suarez et al. 1990; Welch and Suarez 2007). In contrast, weight support provided by wings of flying passerines is at any speed (including hovering) exhibited ultimately during downstroke. While being elevated, wings are bounded to bird's body (Tobalske 2010).

Due to the dissimilarities mentioned above, passerines (unlike hummingbirds) are thought to not be able to perform sustainable hovering, but to regularly interrupt flapping and hold their wings either bounded to body or extended for period corresponding to one or more wingbeat cycles (Zimmer 1943; Tobalske 2010). However, little passerines, such as zebra finches, tend to reduce the length and frequency of pauses in flapping with decreasing flight speed. It has been suggested that wing anatomy, namely low wing aspect ratio (i.e. ratio of length and width of wing, in *Taenopygia guttata* equalling to 4.5) rather than muscle physiology constrains zebra finch to use intermittent flight instead continuous flapping (Tobalske et al. 1999). Latest studies show that passerine birds whose lifestyle requires slow or hovering flight display several mechanisms to compensate for nearly inactive upstroke. Muijres et al. (2012a) claim, that regardless practically unused wings, upstroke does not necessarily need to be otiose. For example, Pied Flycatcher (*Ficedula hypoleuca*) generates lift also during upstroke by changes in body-tail configuration. This lift is higher at lower speed and may provide close to 23 % of weight support. In addition, leading-edge vortices produced by its wings during downstroke contributes up to 49 % of weight support (Muijres et al. 2012b), which is three times more than reported for hummingbirds and even slightly exceeds the contribution known for drosophila (Tobalske 2010). And it is not the only species reported to actively use tail during hovering (Su et al. 2012). Additionally, lift production may be enhanced by ventral wing clapping (Chang et al. 2011).

It is important to mention that above cited findings are results of only few flight analyses. Out of them a single study was focused on a specialized nectar-feeding bird, the Scarlet-chested Sunbird (*Chalcomitra senegalensis*; Zimmer 1943); therefore, almost nothing is known about hovering performance of nectarivorous passerines.

Target species

As I have already noted, in reaction to the established dogma of hovering New World and perching Old Word nectar-feeding birds a paper written by Westerkamp was published in 1990. In this paper he claims that to understand bird-plant interactions “the actual functioning of flowers must be in focus, and not the geographic distribution nor the systematic affiliation of their visitors”. To manifest the irrelevance of bird phylogeny and distribution in this relationship, he arguments by the presence of plants with flowers adapted to hovering birds also in the Old World. He reveals some examples of such plants: *Canarina canariensis*, *C. eminii* (Campanulaceae), Himalayan *Agapetes* spp. (Ericaceae) and *Impatiens sakeriana* (Balsaminaceae).

Impatiens sakeriana

I. sakeriana Hook. f. (Balsaminaceae) is a perennial shrub reaching up to 3-4 meters in height, but often lower. It is native to tropical western Africa, endemic to mountains of western Cameroon and Bioko, where it grows in moist shade places of mountain forests, at their edges and in shrubby vegetation along streams at the altitude ranging from 900 to 3000 m a.s.l. (Grey-Wilson 1980).

Flowers, present throughout the entire year, are characteristically red with long spurs (up to 2.5 cm), organized in inflorescence of two. As correctly pointed out by Westerkamp (1990), thanks to long peduncles and pedicels (3.7-11.7 cm and 1-2.6 cm, respectively) they are oriented toward free space which evokes an adaptation to hovering pollinator (Grey-Wilson 1980). They generally produce a high volume (38 µl) of sucrose dominant and relatively dilute nectar with concentration around 30 %. (Bartoš et al. 2012). Flowers are protandrous, the first 3–4 days being in male phase, then converting for 3-4 days into female phase. Stigma and anthers are within the mouth of the corolla and enable perfect pollen placement on birds' heads (Grey-Wilson 1980).

I. sakeriana can be effectively pollinated only by two species of sunbirds, the Northern Double-collared Sunbird (*Cinnyris reichenowi*) and Cameroon Sunbird (*Cyanomitra oritis*), who both often hover while feeding on its flowers (Janeček et al. 2015).

Figure 2
The Inflorescence of *Impatiens sakeriana*

Cinnyris reichenowi

C. reichenowi is a medium size (BM=5.2-8.0 g) sunbird, common in mountain forests, their edges, clearing and gardens of west-central and north-eastern Africa at the elevation from 1 200 to 2 800 m a.s.l. Sometimes, two subspecies are recognised, *C. r. preussi* inhabiting highlands of Nigeria, Cameroon and Bioko and *C. r. reichenowi* distributed across mountains of Democratic Republic of Congo, Rwanda, Burundi, Uganda, Kenya and southern Sudan (Cheke et al. 2001).

Males of this species (as seen on figure 3) are brightly coloured, with head, mantle and back metallic green and red breast with narrow blue or purple band bellow the throat, in contrast females are inconspicuous, olive green with slightly paler underparts.

Both sexes have relatively short bill (around 1.6-2.2 cm) and are known to feed on nectar from a variety of plants, including *Lobelia* sp., *Salvia* sp. or *Psychotria* sp., plus insects and spiders.

Individuals of this species are territorial, aggressively defending food sources (Riegert et al. 2014) and are considered as altitudinal migrants, appearing in lower elevations that the breading range in wet seasons. Time of breading differs according to the distribution, but generally is linked to dryer climatic conditions (Cheke et al. 2001)

This species belongs among the most abundant at higher elevated areas of western Cameroon (Reif et al. 2006; Sedláček et al. 2015).

Figure 3
A male of *Cinnyris reichenowi*

Cyanomitra oritis

C. oritis is medium sized sunbird, markedly bigger than *C. reichenowi* (BM=9.8-13.8 g), endemic to mountain forests (1 200-2 100 m a.s.l) of eastern Nigeria, Cameroon and Bioko, where it inhabits dense vegetation in forest clearings and shrubby patches (Reif et al. 2007). Sometimes three subspecies are recognised, *C. o. bansoensis* occupying most of the Cameroonian highlands and east Nigeria, *C. o. oritis* restricted to Mt. Cameroon and *C. o. poensis* occurring exclusively on Bioko (Cheke et al. 2001).

Both sexes of this species are similar, olive green with head, throat and upper breast metallic steel-blue and yellow pectoral tufts (can be seen on figure 4). They have long (2.5-2.9 cm) moderately curved bill and often feed on several *Impatiens* species (Janeček et al. 2015). According to Cheke et al. (2001), on Mt. Cameroon they may move to lower altitudes during rainy season. Females have been reported to lay eggs, besides dry season, also in March, April and July (Cheke et al. 2001).

This species has been recognised as the most effective pollinator of *I. sakeriana* in Bamenda Highlands (Janeček et al. 2011).

Figure 4
An individual of *Cyanomitra oritis*

Although sunbirds are considered to be the most specialized nectar feeding clade of the Old World (Stiles 1981) and they are often demonstrated as an example of convergent evolution and compared to the New World's hummingbirds, the number of studies conducted on particular families is largely unbalanced. Considering the lack of studies on behaviour and morphological and physiological adaptations to nectarivory of sunbirds, especially form sub-Saharan Africa, where they reach their highest diversity (Cheke et al. 2001) and reflecting the reference made by Westerkamp (1990), we decided to take the advantage of background provided by previous studies and focus again and more in details on the relatively well framed (one plant-two pollinators) pollination system of *I. sakeriana* described above.

Aims of thesis

For decades, there have been a discussion on why, in contrast to hummingbirds, nectarivorous birds of the Old World perch while eating. Traditionally, this discrepancy has been explained by bird phylogeny and absence of crucial adaptations for hovering flight in passerines. Nowadays, we know Old world nectarivores also hover for nectar and this behaviour is much more common than expected (Wester 2013). I believe it is time to move on a bit and look in detail on hovering performance of these birds.

Foraging behaviour of sunbirds

If we slightly modify the original question, we can ask: "Why nectarivorous bird (in our case sunbirds) hover and why do they perch?" To answer, in chapter I, I aim to evaluate possible factors affecting hovering / perching decision (herein often referred as foraging behaviour) of *C. oritis* and *C. reichenowi* on flowers of *I. sakeriana*.

According to Westerkamp (1990) plant architecture is a crucial factor affecting pollinator's behaviour. Janeček et al. (2011) showed that the orientation of flowers towards free space influences bird's hovering/perching decision and Pyke (1981) noted that occasional hovering enables honeyeater to visit more flowers per unit of time, especially if they are at larger distances from each other. With respect to previous studies, we attempt to evaluate the effect of plant architecture on bird foraging behaviour, choosing the sum of length of pedicel and peduncle (PedPed) and distance between flowers as a relevant characteristic.

Distribution of our target species overlaps with one of the rainiest areas in the world (Tye 1991), hence most probably even feeding sunbirds are exposed to precipitation of various intensity. Regarding challenges which moving in rain represents especially to small volatile animals (Ortega-Jimenez and Dudley 2012b), we aim to evaluate the effect of rain intensity on the probability of hovering.

Pyke (1981) hypothesised, for bird hovering could represent an evolutionary stable strategy how to increase the energy intake in time. His suggestion is partly supported also by Padyšáková and Janeček (2016) who found hovering flower visits to be shorter than perching ones. Furthermore, they assume, this could be also advantageous for the plants as higher visitation rate means enhanced pollination speed. We try to evaluate whether there is a correlation between the flower visitation rate and the ratio of hovering performed and whether this is caused by shorter flower visits of faster movement between flowers.

Hovering flight of sunbirds

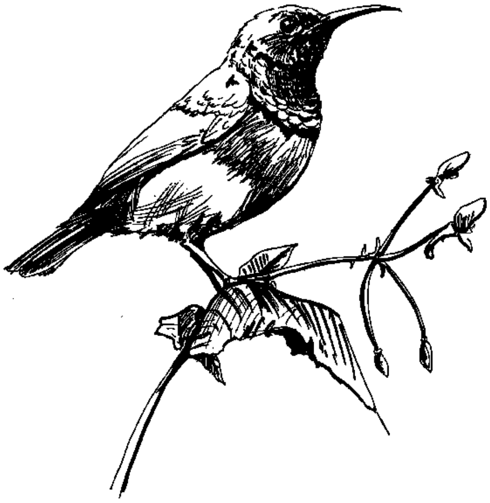
Even though, several sunbirds have been reported to hover (Wester 2013), in general, there is a lack of studies on their hovering kinematics. The only such a study is from 1943 (Zimmer 1943). Based on this and few other surveys of slow flight of passerines, sunbirds are thought not to be able to hover continuously and repeatedly interrupt flapping for one or more wingbeat cycles. It has been hypothesised that hovering ability could be constrained by relatively rounded wings (Tobalske et al. 1999). On the other hand, it has been suggested that often hovering passerines may exhibit several adaptations for this type of flight (Muijres et al. 2011).

In chapter III, I look in detail on hovering performance of *C. oritis* and *C. reichenowi*. My original plan was to describe all the basic kinematic characteristics, including wing amplitude, wingtip trajectory, body angle, tail movement and eye stability in 3-dimensional space, with the ambition of testing whether these sunbirds exhibit any adaptation for hovering formerly noted in other passerines. Unfortunately, due to several technical problems, I was not able to fully analyse the recordings until now. Therefore, here I present only the results of wingbeat frequency as a function of time spend in the air.

I aim to estimate the proportion and average length of pauses in flapping performed by both species while hovering. Furthermore, I intend to relate my findings to wing anatomy and compare them with previous findings on hovering passerines.

Chapter II:

Foraging behaviour of sunbirds



Methods

Data collection

The study took place in Mount Cameroon National Park, Southwest Region, Cameroon, at the elevation of 2000 m a.s.l. (4° 8' N, 9° 7' E) in August, the middle of the rainy season, 2017.

We randomly selected ten flowering individuals of *Imatiens sakeriana* and recorded all their visitors in four days. Recording was performed with VIVOTEK IB8367-T security cameras during 12-h periods (6:00-18:00; unless the recording failed) from 21st to 24th of August.

The number of open flowers changed in days, nevertheless with only two exceptions, there was more than one open flower on every monitored plant each day. I used an inch-tape to measure linear distances between each two opened flowers (i.e. apertures of their corollas) and vernier caliper to measure the lengths of straightened peduncles and pedicels (further referred as PedPed length, see figure 5), both with the accuracy of 0.1 cm.

The actual precipitation was measured by two rain gauges placed in two different forest clearings within the area of selected plants, a photo of level of water in each of them was taken every ten minutes.

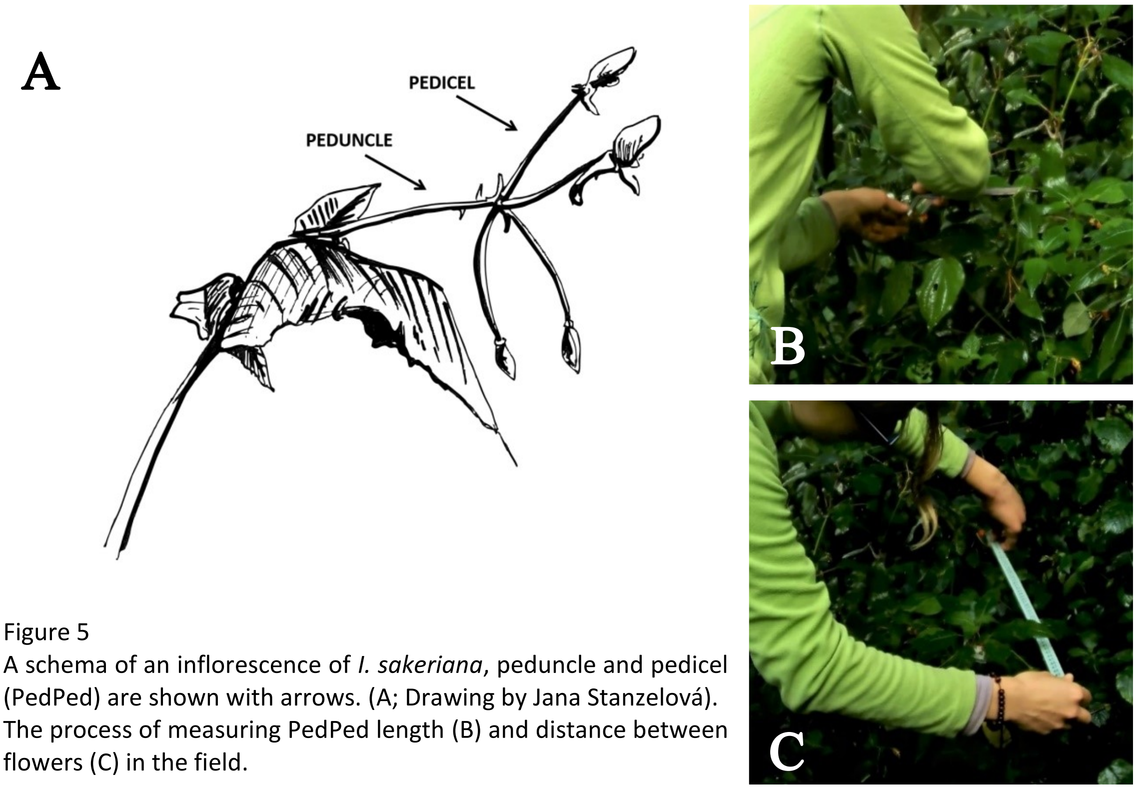


Figure 5
A schema of an inflorescence of *I. sakeriana*, peduncle and pedicel (PedPed) are shown with arrows. (A; Drawing by Jana Stanzelová). The process of measuring PedPed length (B) and distance between flowers (C) in the field.

Recording was preceded by 6 days of mist netting in the closest neighbourhood of chosen plants. Captured sunbirds were marked with metal rings and a unique combination of coloured rings for a better approximation of the total number of individuals arriving to the plants.

Video processing

To detect visitor arrivals an open software MotionMeerkat (Weinstein 2015) was used. Non-bird visitors and birds that apparently did not drink from at least one flower were ignored. We determined the species, sex and according to colour ring combination an individual ID, if possible. We noted the type of behaviour (hovering, perching or a combination of both) on the flower (from the insertion of the bill into a floral corolla to its extrusion), while moving among flowers (from the extrusion of the bill from one floral corolla to its insertion to another floral corolla) and the exact time (in frames, i.e. $\frac{1}{24}$ s) spent by each activity.

Statistical analysis

For analysis of the effects of PedPed length and the effect of precipitation on type of sunbird behaviour, I used binomial generalized linear mixed model (GLMM) with an assumed Bernoulli distribution, setting flower identifier nested within an individual plant as random factor. Each variable was tested in a separate model.

Only two types of behaviour were included in the analysis (hovering and perching), because the third type (a combination of both) was not comparably frequent. For analysis of the effect of PedPed length, perching on surrounding vegetation was excluded to restrict the test only to the effect of architecture of a proper *Impatiens* plant.

Average precipitation was logarithmically (log+1) transformed to reduce the skewness towards larger value, because we obtained many hours of recordings and visit events during modular rain but only few in heavy rain (see table 1) and I wanted to include in the analysis also observation under no rain.

Unfortunately, we were not able to obtain an adequate number of observations for most of the flowers visited by *C. oritis*, therefore we decided to test the effect of PedPed length on sunbird behaviour considering only the most frequent type of behaviour on a particular flower and use solely plant identifier as a random factor.

For evaluation of the effect of behaviour and precipitation on time spent on the flower and on transfer time among flowers I used nested ANOVA with plant identifier as an error variable. For the same reason as mentioned above, precipitation was logarithmically (log+1) transformed. Transfers and visit duration were also logarithmically transformed to achieve their normal distribution.

Another nested ANOVA with plant identifier as an error variable was performed to analyse the effect of sunbird behaviour on flower visitation rate. Also, here the explained variable was logarithmically transformed to normalize its distribution. I indicated the sum of distance between visited flowers as first explanatory variable to filter out its effect.

Considering the huge difference in sizes and character of obtained datasets of *C. reichenowi* and *C. oritis*, we decided not to compare the results among species.

All statistical analyses were performed in R (Ihaka and Gentleman 1996).

Results

Visitation of *Impatiens sakeriana*

In total we analysed 405.4 hours of videos, i.e. 40.54 hour per plant on average, even though there were slight differences in time recorded in various days and plants. Due to some technical problems, some of the recordings failed resulting in four plants being recorded only for three days (on figure 6 these are: Imp3, Imp5, Imp7, Imp9) and one plant being recorded only for two days (Imp8). Altogether, we observed 86 flowers located on 10 plants of *I. sakeriana*.

The most common visitors were males of *C. reichenowi* with 1326 flower visits within 360 individual arrivals to plant, i.e. the average frequency of arrivals to plant per hour f = 0.861 ± 0.269 (mean ± s.d.). In contrast, not a single female of this specie was seen on any recording. Visits of *C. oritis* were considerably less frequent, only 181 flower visits within 58 arrivals to plant, i.e. the average frequency of arrivals to the plant per hour f = 0.161 ± 0.099 (mean ± s.d.). No other visitor besides individuals of target species feeding on any flower was noticed.

During mist netting we managed to capture and mark 12 males and 7 females of *C. reichenowi* and 14 individuals of *C. oritis* with colour rings. We decided not to distinguish sexes of *C. oritis,* because it was off the breeding season and the characteristics for male determination of this species (yellow pectoral tufts; according to Cheke et al. 2001) seems to be present also on females to some extent. Anyway, only one ringed visitor of *C. oritis* was recorded twice arriving to plant, which corresponds to 3.4 % of all arrivals of this species. Five different ringed individuals of *C. reichenowi* appeared on the video, but still their arrivals represented only 37.2 % of all arrivals of the species (see figure 6).

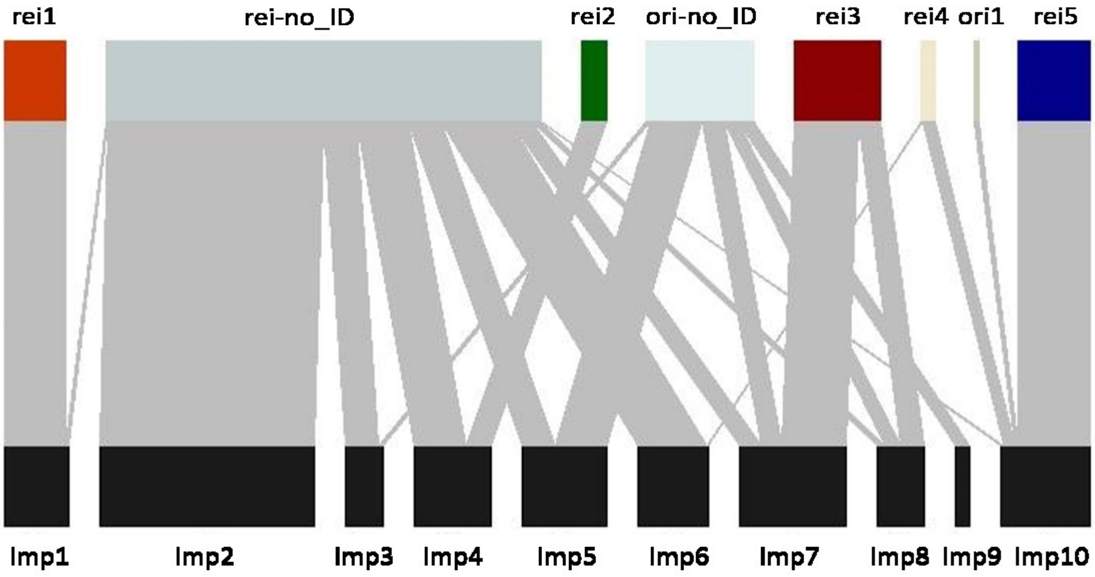
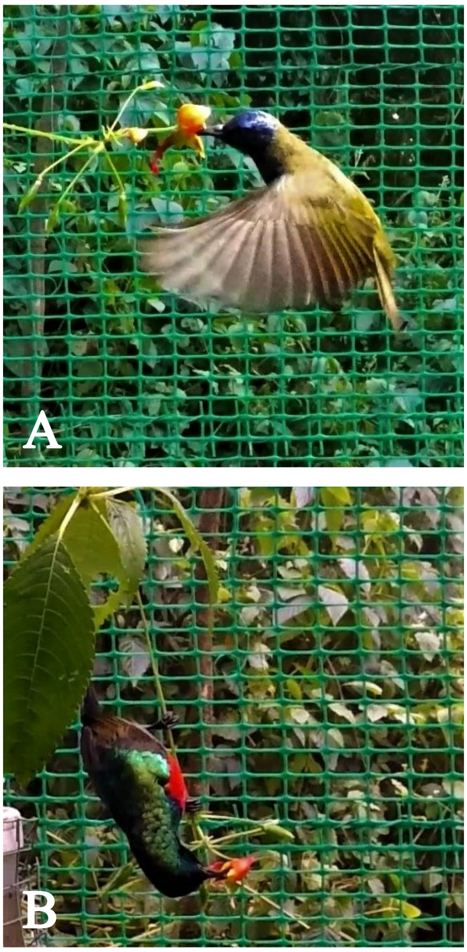


Figure 6
Observed visitation network
Ringed individuals of *C. reichenowi* (rei1-rei5) and *C. oritis* (ori1) and not ringed individuals of both species (rei-no_ID or ori-no_ID) are displayed up, individuals of visited plants (Imp1-Imp10) are displayed down. Note that each of ringed birds is mainly visiting one maximally two plants. In total, we observed 418 individual arrivals to plant, for more information see results.

Foraging behaviour of sunbirds

Three types of behaviour on plants were observed (see figure 7). In most of the cases, birds of both species hovered in front of the flower while feeding on it. Hovering visits represented 59.9 % (n = 758) and 75.7 % (n = 128) of all visits by *C. reichenowi* and *C. oritis*, respectively. The second most frequent type of behaviour was perching, representing 36.5 % (n = 456) and 21.9 % (n = 37) of all visits. Birds perched on the plant that they were feeding from (212 and 20 visits for *C. reichenowi* and *C. oritis*, respectively), on surrounding vegetation (192 and 5 visits for *C. reichenowi* and *C. oritis*, respectively), but in many cases proper perch was not well distinguishable (164 and 24 visits for *C. reichenowi* and *C. oritis*, respectively). Rarely a combination of hovering and perching of the same individual during a visit on the flower was observed. These visits represented only 3.7 % and 2.4 % (46 and 4 visits for *C. reichenowi* and *C. oritis*, respectively) and were not involved in statistical analysis.



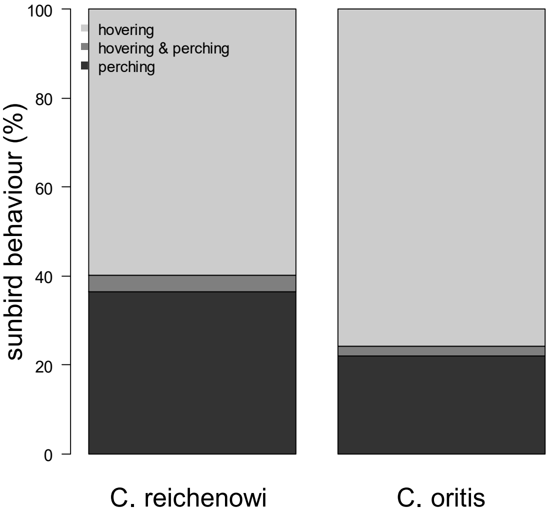


Figure 7
The occurrence in percentage of the types of behaviour observed on flower (upper figure). Hovering (59.9 %, 75.7 %) in light grey, perching (36.5 % and 21.9 %) in dark grey. The less frequent type, combination of hovering and perching (3.7 %, 2.4 %) in grey was excluded from further analysis. (Percentage and barplots for *C. reichenowi* and *C. oritis*, respectively.)
The two most frequently practised types, hovering (A; here performed by *C. oritis*) and perching (B; here on peduncle of host plant, performed by *C. reichenowi*) can be seen on the left. Pictures were taken during experiment described in chapter III.

Table 1
Behaviour while moving between two flowers in context of combination of behaviour performed on those flowers.

X	***C. reichenowi*** **769 observed switches**			***C. oritis*** **76 observed switches**		
Behaviour between and on flowers	**flying directly**	**resting**	**hopping**	**flying directly**	**resting**	**hopping**
perch-perch	3	14	151	1	0	11
perch- hover	32	107	130	0	2	11
hover-hover	114	156	62	17	34	0
sum	149	277	343	18	36	22

Individual rows represent combination of behaviour on two flowers. Individual columns represent transfer behaviour between flowers; flying directly = direct flight between flowers, resting = one stop on a flight from the first to the second flower, hopping= bird did not fly at all and was hopping toward the second flower or did not fly directly and stopped several times. Note “resting” is most frequent between two hovering events and “hopping” between two perching events or perching and hovering event.

While moving between flowers, birds often did not fly directly, but stopped and perched few seconds. These stops were performed especially between two hovering flower visits. In contrast, between two perching visits birds often did not fly, but hopped instead (see table 1).

Rain intensity

Over the four days of recording (48.5 hours) we measured actual intensity of precipitation. Only in 40 % (equals to 19.2 hours, 518 flower visits) it was not rainy. During the remaining 60 % (29.3 hours, 964 flower visits) actual intensity of precipitation ranged between 0.3 and 63 mm·h^{-1}, for more details see table 2.

Table 2
Time recorded, number of flower visits by *C. reichenowi* and *C. oritis* at several intensities of precipitation.

Rain intensity (mm·h^{-1})	**Total time (hours)**	**Flower visits by *C. reichenowi***	**Flower visits by *C. oritis***
0	19.2	467	51
0.1-10	25.9	773	96
10.1-20	1.5	21	18
20.1-30	1.2	38	10
30.1-40	0.2	0	0
40.1-50	0.3	7	0
More than 50	0.2	0	0
Not measured	X	20	6

Note, that some individuals of both species were recorded feeding under heavy rain (over 20 mm·h^{-1}). Unfortunately, not all the minutes of camera recordings of plant were covered by rain gauging.

Factors affecting the probability of hovering

The effect of plant architecture and the effect of rain

As shown on figure 8, longer peduncles and pedicels of a flower significantly increased the probability of hovering if visited by *C. reichenowi* ($N = 923$; $\chi^2 = 7.326$; $df = 1$; $p = 0.007$). Almost identical pattern was observed for *C. oritis,* but it was not significant ($N = 25$; $\chi^2 = 2.326$; $df = 1$; $p = 0.205$). The distance between flowers had no impact on bird behaviour. There was a slightly negative (but significant, $N = 1185$; $\chi^2 = 5.049$; $df = 1$; $p = 0.025$) effect of precipitation on behaviour of *C. reichenowi*. Again, the relationship was not significant in case of *C. oritis* ($N = 157$; $\chi^2 = 0.012$; $df = 1$; $p = 0.913$). Probability of hovering was not affected by the distance among visited flowers ($N_{rei} = 760$, $N_{ori} = 81$; $\chi^2_{rei} = 0.788$, $\chi^2_{ori} = 1.59$; $df = 1$ for both species, $p_{rei} = 0.375$, $p_{ori} = 0.208$).

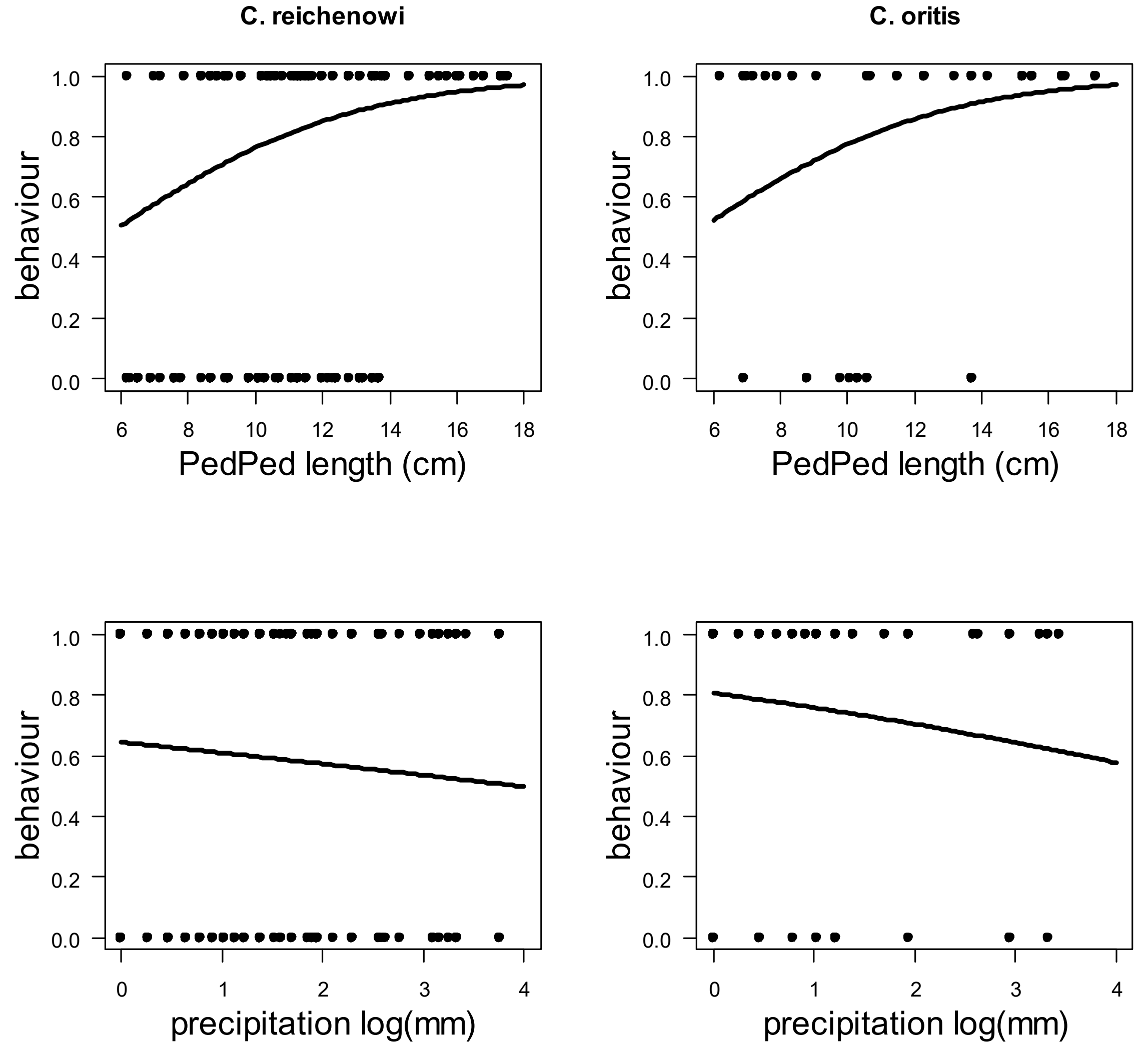


Figure 8
The influence of external factors on behaviour of *C. reichenowi* and *C. oritis*.
0 represents perching events, 1 represents hovering events, regression line indicates the probability of hovering on a flower. First two plots show the effect of pedicels and peduncles (PedPed) length (in cm) on sunbird behaviour, last two plots show the effect of precipitation (in mm per hour) on sunbirds behaviour. Both factors affect significantly hovering probability of *C. reichenowi*, but not behaviour of *C. oritis*.

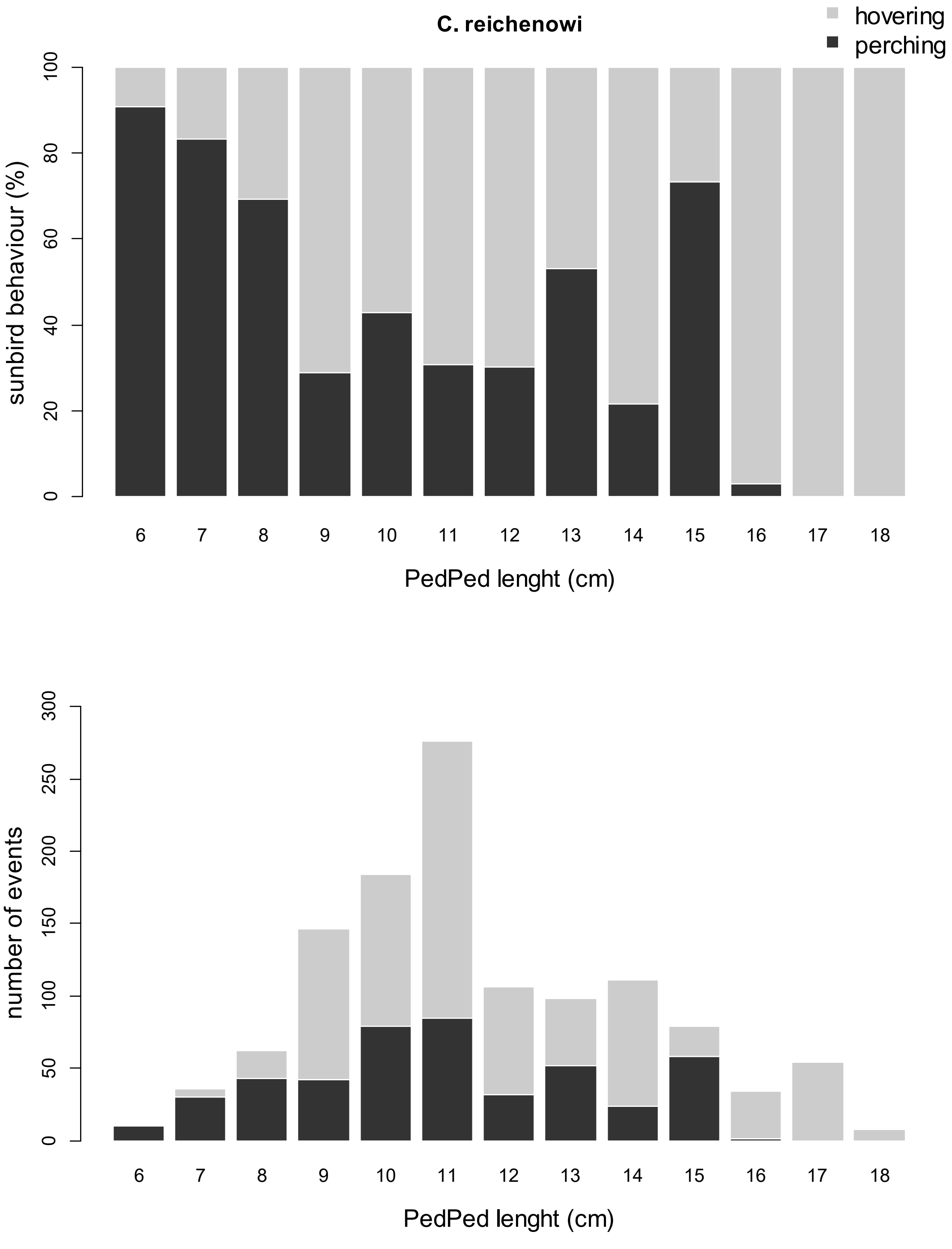


Figure 9
The percentage and the absolute number of hovering and perching events performed by *C. reichenowi* on flowers of various PedPed length.
Perching events are represented in dark grey, hovering events are represented in light grey.

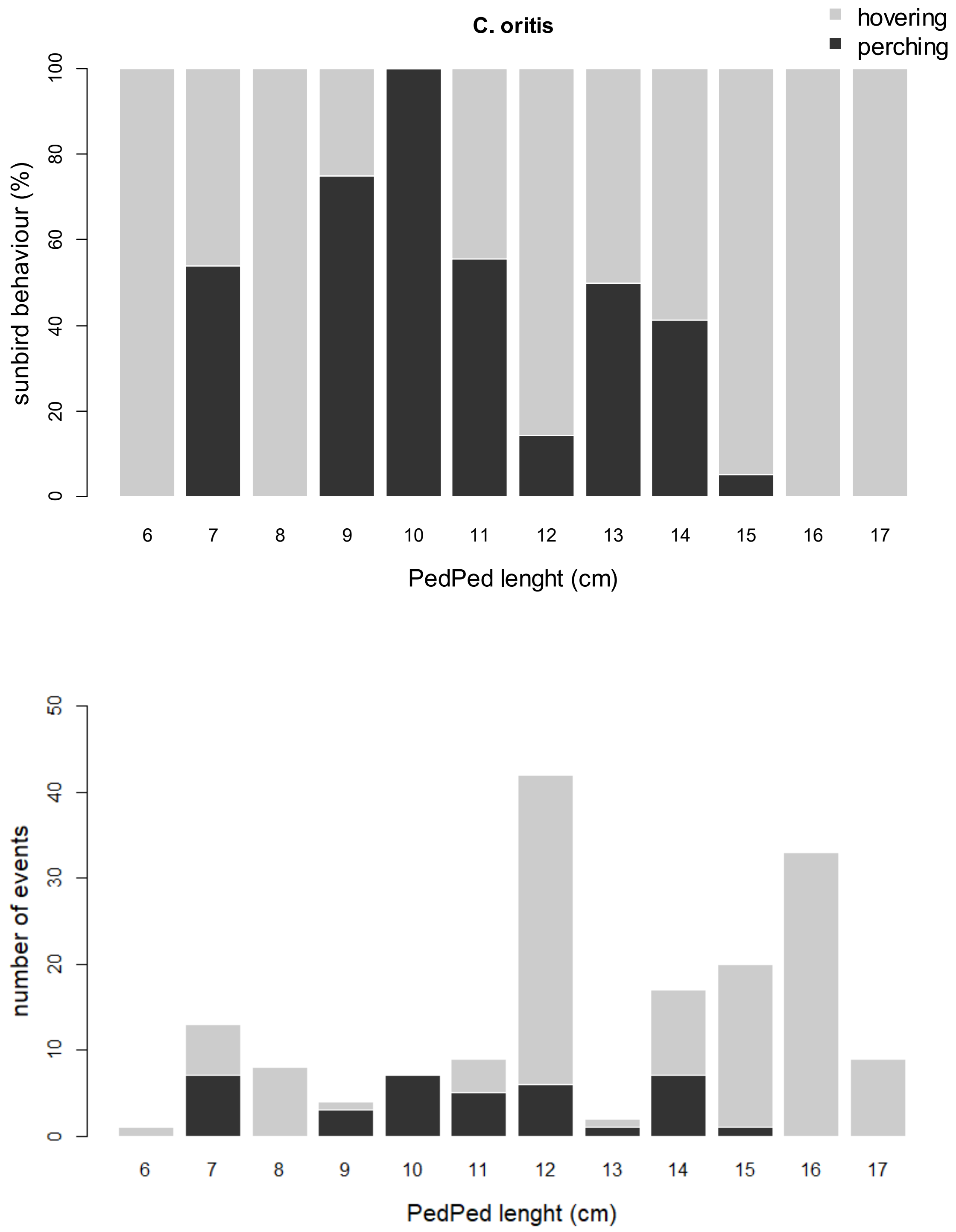


Figure 10
The percentage and the absolute number of hovering and perching events performed by *C. oritis* on flowers of various PedPed length.
Perching events are represented in dark grey, hovering events are represented in light grey.

Hovering and flower visitation rate

In general, hovering enabled birds of both species to increase the number of flowers visited per unit of time ($N_{rei} = 276$, $N_{ori} = 36$; $F_{rei} = 111.6$, $F_{ori} = 12.7$; $df = 1$ for both species; $p_{rei} < 0.001$, $p_{ori} < 0.01$), see figure 11. This was thanks to faster switching between flowers ($N_{rei} = 701$, $N_{ori} = 78$; $F_{rei} = 4.87$, $F_{ori} = 9.59$; $df = 2$ for both species; $p_{rei} = 0.008$, $p_{ori} < 0.001$) and at the same time, hovering was associated with significantly shorter time spent on flower ($N_{rei} = 1079$, $N_{ori} = 150$; $F_{rei} = 227.16$, $F_{ori} = 20.93$; $df = 1$ for both species; $p < 0.001$ for both species), figures 12 and 13, respectively. The duration of flower visit by *C. reichenowi* was affected negatively by precipitation ($N = 1079$; $F = 22.15$; $df = 1$; $p < 0.001$), perching birds were affected more strongly ($F = 4.34$; $df = 1$; $p = 0.04$), figure 12. No relationship between the intensity of precipitation and duration of transfer between flowers was detected.

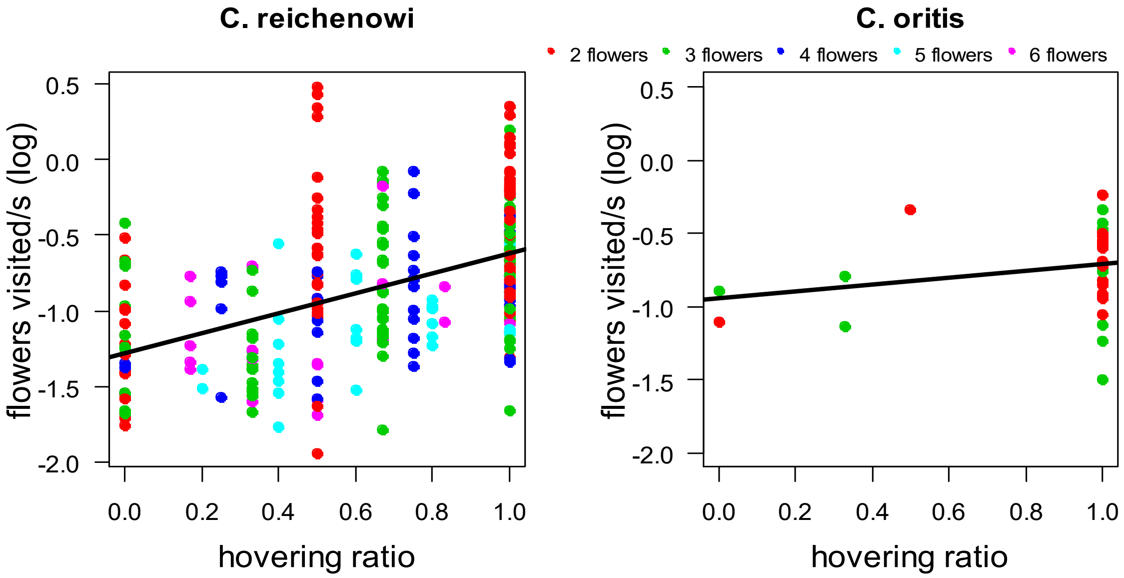


Figure 11
The link between ratio of hovering performed and the rate of flower visitation
Hovering allows sunbirds to visit more flowers per unit of time.
0 = perching only, 1 = hovering only; different colours represent distinct total number of flowers visited during one plant visit, this creates relatively higher variability in the data than real. Flower visitation rate is shown in logarithmical scale. Graphs for *C. reichenowi* and *C. oritis*, respectively.

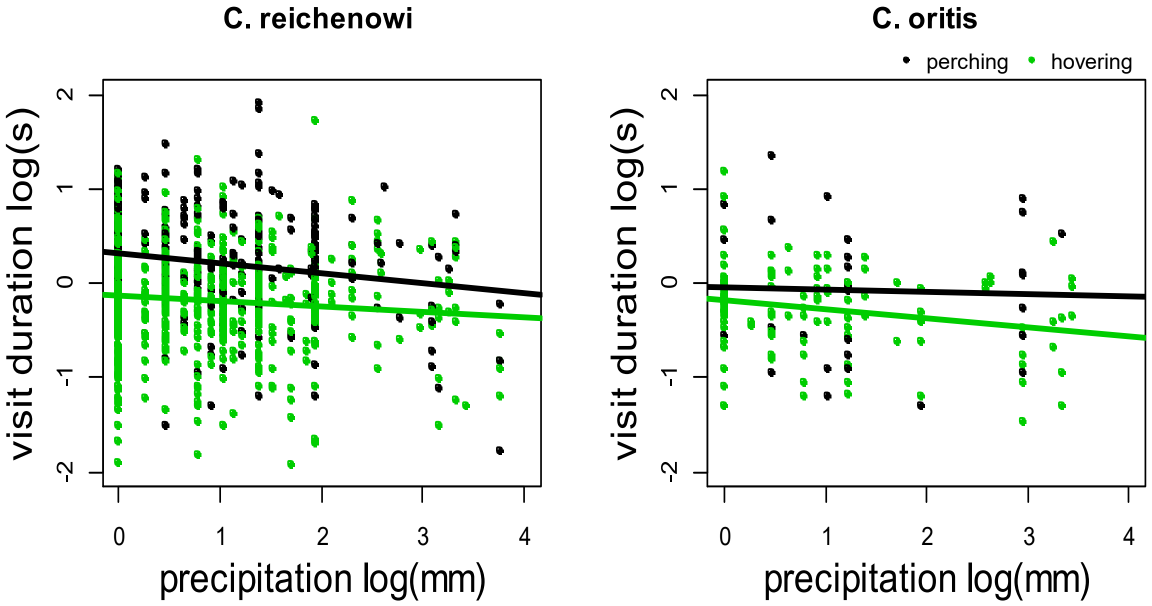


Figure 12
The relationship between duration of flower visit and precipitation (in mm per hour).
There is significant effect of precipitation only on visit duration by *C. reichenowi*. Behaviour influenced significantly the duration of visit of both sunbird species.
Perching events are represented in black, hovering events are represented in green. Both variables are in logarithmical scale.

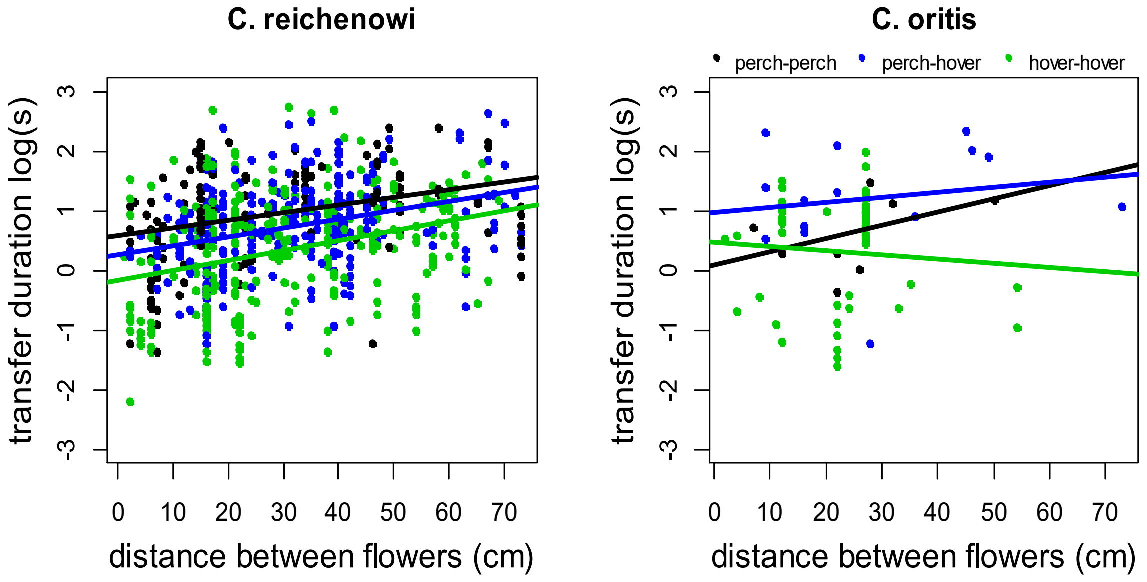


Figure 13
The relationship of duration of transfer between flowers and the distance among them.
Transfer duration between flowers is affected by bird 's behaviour (hovering/perching) on both visited flowers. However, there was no interaction amongst behaviour and distance between flowers. Suspicious negative slope visible in the second plot for transfer between two hovering events is probably due to absence of stops between two distant flowers.
Duration of transfer is shown in logarithmical scale. Transfers between two perching events are represented in black, transfers between two hovering events are represented in green and transfers between one hovering one perching event are represented in blue.

Discussion

Visitation of *Impatiens sakeriana*

Even though, the number of observed individuals was relatively small, the study gives us a reliable picture of sunbird foraging in tropical mountain forest in the central rainy season. Despite our expectations, the total number of visits differed among the sunbird species in an order of magnitude in favour of *C. recihenowi* (1326 visits vs. 181 by *C. oritis*). This finding is in contrast with observation of Janeček et al. (2011, 2012) who found *C. oritis* to be two times more frequent visitor of *I. Sakeriana*. A possible explanation for this discrepancy could be simply the fact that the local abundancies of both species between Mt. Cameroon (our study site) and Bamenda Highlands (the site of a prior study) differ. It is true that *C. reichenowi* is locally more abundant in the study area (Sedláček et al. 2015) and also the total number of captured specimens of these species during mist netting foregoing plant monitoring was slightly higher if compared to specimens of *C. oritis*, but the difference was not that high (19 of *C. reichenowi* vs. 14 of *C. oritis*). And at the same time, according to (Reif et al. 2007), *C. reichenowi* is locally more common also in Bamenda Highlands where the study of Janeček et al. was conducted. More probable reason is the fact, that data collections took place in different seasons. The observations of Janeček et al. (2011, 2012) were performed in the wet-dry transition and dry seasons (November to January) whether our study was conducted in August, the central rainy season. At the time our study was performed, there were only few flowers of *Hypericum revolutum* and no flowering *Lobelia columnaris* neither *Hypoestes aristata,* on Mt. Cameroon, i.e. plant species which were often visited by sunbirds in previous studies from Bamenda Highlands. Talking about frequencies of visits, it is important to consider the high seasonality of this area and do not generalize the results. An identical system may exhibit different patterns if studied a month later or earlier. None of the target sunbird species relies explicitly on one single species of plants (Janeček et al. 2012), flowering species vary over the year and so does nectar supply, bird visitation follows (van Schaik et al. 1993).

It is conceivable that in this year period *C. oritis* exploits alternative food resources. Some nectarivorous birds change the proportion of nectar in their diet compared to other food sources (mainly insect) through seasons (Paton 1982; Symes and Woodborne 2011). However, I would expect relative decrease of nectar consumption to occur in the shortage of nectar supply or in time of higher protein requirements, mainly during breeding season (even though in this case it would be actually caused by increasing of protein intake) which is for *C. oritis*, similarly to most of tropical mountain bird species, mainly the dry season and additionally from March to July (Serle 1954; Tye 1991; Cheke et al. 2001)

As we know, during the rainy period this species is common in lower elevations, therefore I assume, it could be an example of altitudinal migrant with relatively low number of individuals persisting in the highest altitudes year-round. There is an evidence of migration across elevational gradient for several bird species including sunbirds. For example, a relatively widespread Olive Sunbird (*Cyanomitra olivaceae*) or Banded Green Sunbird (*Anthreptes rubritongues*) endemic to Eastern Arc Mountains in mountain forests (over 1200 m a.s.l.) in East Africa are known to move to lower altitudes in the beginning of the cold rainy season and return to higher altitudes again in the beginning of the warm rainy season (Burgess and Mlingwa 2000). In Neotropics, nectarivorous birds (together with frugivores) are the prevailing migrating food guilt (Barçante et al. 2017). Even Cheke et al. (2001) admits that migration of *C. oritis* across elevational gradient of Mt. Cameroon may occur.

Traditionally, altitudinal movements have been explained by seasonal resource food availability variation (Barçante et al. 2017). One of many alternative hypotheses, which seems to be reliable in this particular case, is migration driven by harsh environmental conditions in higher altitudes during rainy seasons (Boyle 2011; Boyleet al. 2010). Boyle et al. (2010) found that a negative correlation between variation in rainfall intensity and intensity of migration to lowlands was stronger for smaller bird species, frugivores and nectarivores. Furthermore, she proposed that for above mentioned physiological challenges, smaller males of White-ruffed Manakins (*Corapipo altera;* a neotropical mountain species, of body mass slightly higher than our target species) are more likely to migrate to lowlands, leaving more resources for larger females (Boyle 2008). Reversely, if we consider that based on our long-term data from several elevations on Mt. Cameroon, females of *C. recihenowi* are in average by almost 1 g lighter than males ($BM_{female} = 8.07 \pm 0.577$ g, $n_{female} = 89$; $BM_{male} = 8.92 \pm 0.717$ g, $n_{male} = 206$; mean ± s.d.), similar process as proposed by Boyle could stand behind our finding about the absolute absence of females of *C. reichenowi.*

Regarding *C. oritis*, I am not able to comment on any gender dissimilarities in visitation frequency neither behaviour, as due to their lesser sexual dimorphism, it is not possible to distinguish the sexes from videos and only one ringed individual (of undetermined sex) was recorded.

Actually, the negligible number of ringed individuals of *C. oritis* recorded on flowers despite the foregoing ringing effort (one ringed individual arriving twice to one plant in comparison to 5 ringed individuals arriving repeatedly to several plants; see figure 6) may well be a result of diverse foraging strategy displayed by the two sunbird species, which has been already reported. At least during the dry season, *C. reichenowi* is known to be actively defending food resources (Riegert et al. 2014), whereas behaviour *C. oritis* more reminds trap-line behaviour characteristics for highly specialized hermit hummingbirds (Padyšáková and Janeček 2016). Trap-lining is related to extended pollen transmission and might possibly represent outcome of another evolutionary pressure leading to fitness maximalization of involved plant (Krauss et al. 2017). Unfortunately, our data are largely insufficient to make any conclusion on this topic.

Factors affecting the probability of hovering

The effect of pedicel and peduncle length

For both sunbird species, hovering was generally more frequent than previously recorded (Padyšáková and Janeček 2016). From figures 8, 9 and 10, it is obvious that the probability of hovering rapidly increased as peduncle and pedicel got larger. This pattern is in perfect accordance to what was described by Padyšáková and Janeček (2016), although there is an interesting disagreement in the maximum PedPed length bird still perched. Whereas in above mentioned study, there was no perching event on a flower with PedPed larger than 10 cm, we recorded several perching events on flowers with PedPed length up to 13.8 cm. This could be a methodological artefact as the analysis of Padyšáková and Janeček contained only events when the birds perched on peduncle or pedicel of visited flower whilst in our analysis, regarding relatively small total number of perching events, we decided to include perching on any part of visited plant.

On the other hand, it may be nicely illustrating intraspecific variation across separated populations of *I. sakeriana* and its pollinators. This hypothesis could be supported by the fact that out of 106 perching events of *C. reichenowi* feeding on a flower with PedPed length above 10 cm, 34 times the perch was a peduncle, which is not a negligible quantity. As I have already mentioned, hovering was relatively more frequent in our study if compared to previous studies on the same system, but this trend corelates with bigger average PedPed length of *I. sakeriana* found on Mt. Cameroon (T-test; N = 115; t = -3.28; df = 31.465; p = 0.003; see figure 14).

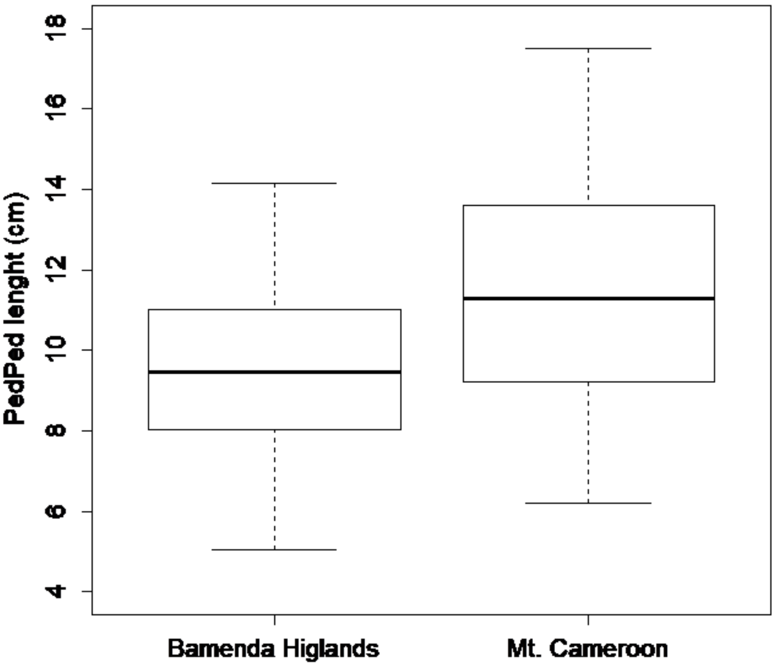


Figure 14
Lengths of pedicels and peduncles from Bamenda Highlands (data from Padyšáková and Janeček 2016) compared to those from our study on Mt. Cameroon.

The effect of rain

Values for the intensity of precipitation must be taken only as an approximation. The exact rain intensity in lower strata of tropical mountain forest where *I. sakeriana* grows varies according to the vegetation densities of strata above. Unfortunately, we were not able to measure the actual precipitation at the location of every recorded plant. We used two rain gauges, placing them in two relatively open areas in between locations of all recorded plants. Results obtained from both gauges differ to some extent, but I believe, for this proposes and especially if taken in logarithmical scale, they can serve sufficiently.

The number of visits under moderate and heavy rain (see table 2) supports the premise of Stiles (1978) that vertebrates should gain higher importance as pollinators in wet and cold conditions. As expected, with increasing precipitation bird tented to perch slightly more, but were able to hover even under heavy rain. At the intensity over 40 $mm \cdot h^{-1}$ there were four hovering events on a flower by *C. recihenowi* (compared to three perching events). Both the effect of PedPed length and the effect of precipitation were not significant in case of *C. oritis*, but similar patterns as for *C. reichenowi* are visible. I strongly believe, at least the effect of PedPed length would be significant also for *C. oritis* if we managed to collect larger and better-balanced dataset.

The effect of distance between flowers

Despite our expectations, we did not find any significant relationship between hovering probability and the distance from the last visited flower, therefore we did not confirm hypothesis of Pyke (1981) on a link between plant architecture, optimal foraging theory and pollinator's behaviour. As I already mentioned in the introduction, according to his scenario hovering may permit bird higher flower visitation rate and consequently higher energy intake in time. This benefit is supposed to grow with an increasing distance between visited flowers. However, very often, after leaving a flower bird stopped and perched for several seconds sometimes even minutes before visiting following one. In both sunbird species, this rest was most often observed between two hovering events and rapidly slowed down average speed of movement (see table 1). I think, this is a phenomenon, Pyke did not count on in his theory and it may explain why proposed relationship was not found.

Hovering and flower visitation rate

Regardless frequent stops between flowers, hovering frequency was still correlated with higher flower visitation rate as proposed by Pyke (1981). This correlation also support the idea of Padyšáková and Janeček (2016) that frequent hovering could enhance pollination speed.

Higher flower visitation rate was permitted partly thanks to faster switching between flowers as expected and based on Pyke's hypothesis (1981). The visitation rate – distance relationship of *C. oritis* even shows almost the same pattern as proposed originally by Pyke, still the interaction of distance and behaviour was not significant. At the same time, similarly to finding of Padyšáková and Janeček (2016), hovering was associated with shorter flower visits.

Theoretically, shorter flower visits could be caused by relatively low maximum time for which bird is able to hover. In that case it would not be necessarily connected with higher energetic intake, as substantial amount of nectar could remain in a flower. However, birds rarely returned to just visited flower which would be logical if there was still nectar left and they we noted them to often hover for considerably longer time. For that reasons, I suggest that hovering drinking speed is higher if compared to perching drinking speed.

There is a relatively high variance in the data for each hovering ratio (see figure 11). It is a methodological artefact caused by missing arrival duration to first flower visited together with different total number of flowers visited. Even thought, if we separate the data according to number of flowers visited, the slope of the relationships remains almost the same, the absolute values reported should be treated with caution as they are clearly overestimated.

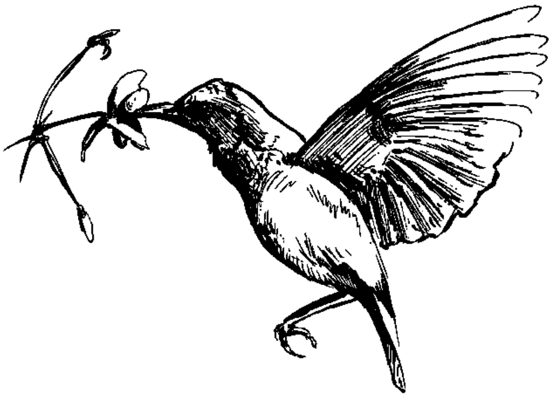
Conclusion

Our data show markedly different pattern in flower visitation frequencies on *I. sakeriana* by *C. oritis* if compared to previous studies of this species. In our study, *C. reichenowi* was the most common visitor, with the total number of visits exceeding the one of *C. oritis* in an order of magnitude. Also, regarding visitation frequencies, our observations point out an interesting discrepancy between sexes of *C. reichenowi*.

At least for *C. reichenowi*, our data favours Miller's (1985) and Weserkamp's (1990) hypothesis, that pollinators behaviour on a flower is determined by plant architecture, namely by the length of peduncles and pedicels. We did not prove hovering decision to be determined by the distance between flowers (another characteristic of plant architecture tested) as proposed by Pyke (1981). Still, our results support his assumption that frequency of hovering is positively correlated with flower visitation rate (corresponding to rate of energy intake). As expected, precipitation negatively affected hovering frequency, but this relationship was significant only for *C. reichenowi.*

Chapter III:

Hovering flight of sunbirds



Methods

Data collection

The study was conducted in August 2018, on the south-eastern side of Mount Cameroon (Southwest Region, Cameroon) at the elevation of 2000 m a.s.l. (4° 10' N, 9° 11' E).

Hovering flight of ten individuals of each of the two target species was analysed. Unfortunately, due to shifts in species distribution during the rainy season which have been already discussed in chapter II, we did not manage to obtain a sufficient number of representatives of both sexes of each species, therefore we analysed hovering flight of ten males of *C. reichenowi*, six females and four males of *C. oritis*.

All recorded specimens were captured by mist nets in the forest or grassland close to the study site. None of individuals was moulting. They were weighted using 20 g Pesola spring scale with the accuracy of 0.25 g and placed in an experimental cage of the dimensions 1 m × 1 m × 1.7 m situated in the middle of forest glade (for limitation of wind), where recording took place. There was a tarpaulin situated above the cage which served as a protection against rainfall (see figure 15). The air temperature during recording was approximately 16° C.

In the middle of the cage, at the height of approximately 120-130 cm, we fixed a flower of *Impatiens sakeriana*. Before placing a bird inside the cage, I used Hamilton syringe (20 µl) to refill the flower with 50 µl of 30 % (w/w) sucrose solution. Even thought, sugar concentration was comparable to natural nectar concentration of this species, the total volume fairly exceeded daily production of one flower (38 µl; Bartoš et al. 2012). So large volume was used to motivate birds to hover for as long time as possible.

To each bird I showed the flower and enabled it to taste the artificial nectar inside. Then, I released the specimen inside the cage. Often very soon after being released, the bird visited the flower. All the flower visits were recorded on four GoPro HERO 5 Black Edition video cameras with a resolution of 720p and at frame rate corresponding to 240 frames per second. Each camera was placed facing expected bird's position from frontal, lateral, posterior and dorsal view. Two artificial lights, LED Yongnuo YN1410, were used to improve natural light condition and consequently image sharpness.

The birds were released from the cage after the first floral visit (or several ones in case that more visits followed immediately). Those birds, which did not visit the flower were liberated after 45 minutes.

Figure 15
Experimental cage
A view at the experimental cage situated in the middle of forest glade (A) and the position of the flower of *I. sakeriana,* cameras and lights inside it (B).

Wingbeat frequency analysis

Recorded videos were analysed using Argus software (Jackson et al. 2016). For analysis of wingbeat frequency, I used only recordings from the camera situated laterally to bird's body.

Since the beginning and the end of drinking is often not clearly distinguishable, the total time of visit was established as the time from the first touch of flower by bird's bill to the end of last upstroke with the bill still inside the flower. Only the longest visit of each individual was analysed.

To estimate actual wingbeat frequency, every beginning of downstroke (i.e. when the wing was most up and steady; see figure 16), was manually marked. Average frequency was counted as the number of wingbeats (n-1) in time between the first and the last beginning of downstroke (in $\frac{1}{240}$).

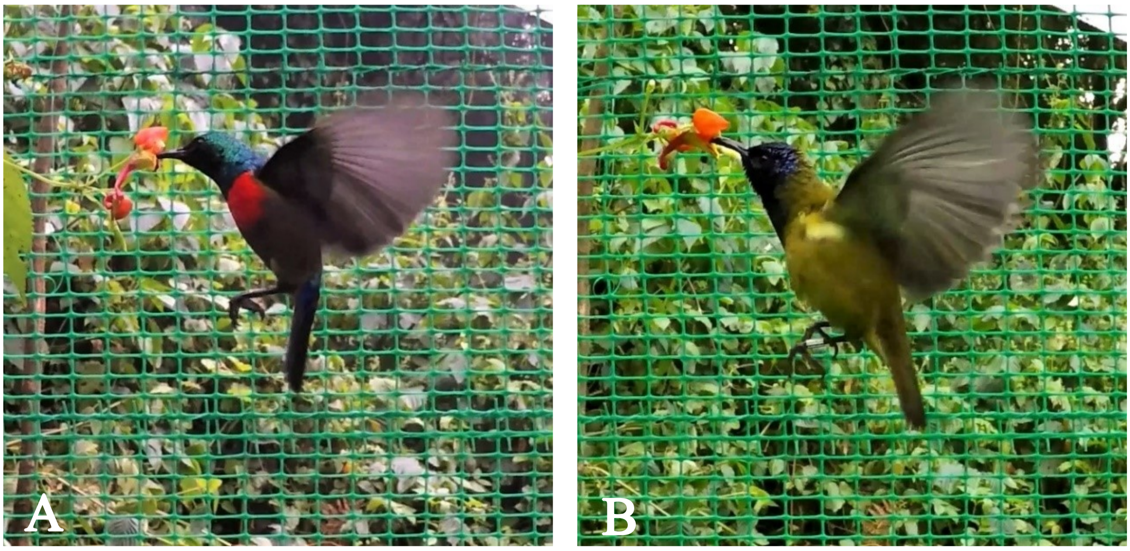


Figure 16
Beginning of downstroke, a view from lateral camera
The position of wing marked for counting of actual wingbeat frequency. Pictures of *C. reichenowi* (A) and *C. oritis* (B).

Wing aspect ratio estimation

Unfortunately, due to complicated management related to recording process, we were not able to obtain pictures for estimating wing aspect ratio of those individuals which were recorded while hovering. Therefore, for at least approximate estimation of the wing aspect ratio of the studied species, photos of an extended wing of 5 randomly selected individuals of each species on a square graph plate were taken.

An area of one wing ($\frac{1}{2}$ S) and a half of wing span ($\frac{1}{2}$ B) were measured in ImageJ software (Abràmoff et al. 2004), see figure 17. A wing aspect ratio (R_a) was calculated as $R_a = \frac{B^2}{S}$, where B is the wing span (i.e. the distance from one wing tip to the other one) and S is the wing area of both wings including the part of the body between the wings (Pennycuick 2008).

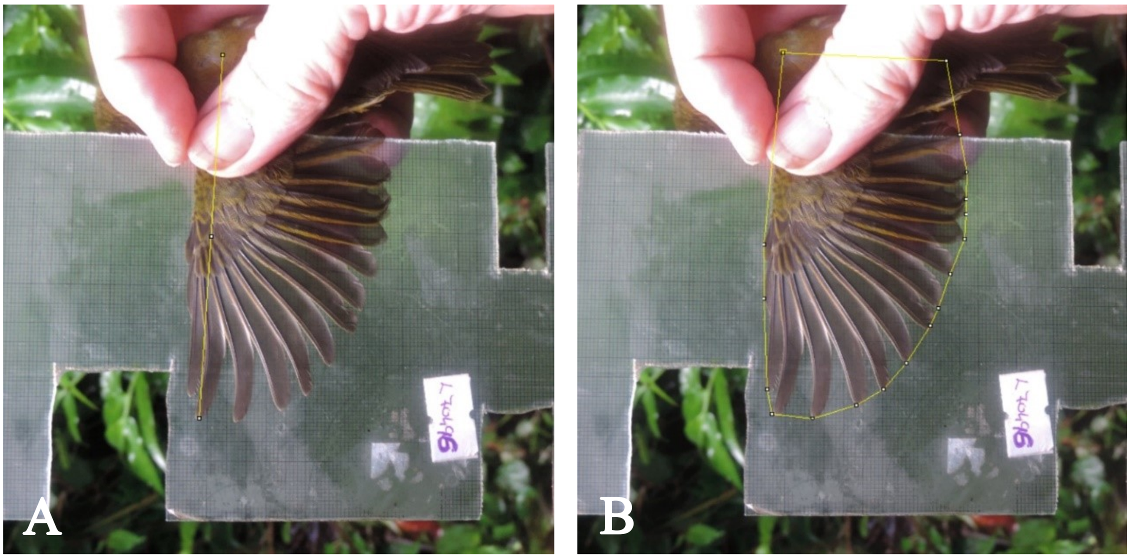


Figure 17
Measurement of wing
Wing aspect ratio was estimated based on the mid-wingspan ($\frac{1}{2}$ B; picture A) and one wing area ($\frac{1}{2}$ S; picture B).

Statistical analysis

I used nested ANOVA with a bird ID as an error variable to test the change of actual wingbeat frequency in time (two separated models) and to test the interaction between time and species (the third model).

Average wingbeat frequency, total time of the visit and the average wing aspect ratio were compared between species using T-test.

Statistical analyses were performed in R (Ihaka and Gentleman 1996).

Results

The time of hovering performance and the average wingbeat frequency

The average time of hovering (i.e. time of a flower visit) performed by *C. reichenowi* was T = 2.31 ± 1.24 s, recorded hovering of *C. oritis* was on average little longer:

T = 2.85 ± 1.59 s. The wingbeat frequency was generally around 20 Hz, slightly lower in case of *C. reichenowi*: f = 19.85 ± 1.52 Hz, than in case of *C. oritis*: f = 20.15 ± 0.65 Hz (mean ± s.d.), see table 3. None of the characteristics differed significantly between species ($t_{time} = 0.843$; $df_{time} = 16.97$; $p_{time} = 0.411$ and $t_{freq} = 0.58$; $df_{freq} = 12.23$; $p_{freq} = 0.572$).

Table 3
List of birds recorded for analysis of hovering flight

Ring number	Species	Sex	Body mass (g)	Hovering Time (s)	Average wingbeat frequency (Hz)
L55993	C. oritis	female	11.25	2.75	21.16
L56212	C. oritis	female	12.75	4.04	19.59
L56219	C. oritis	male	12.0	3.86	20.30
L56250	C. oritis	female	9.75	1.26	20.66
L56272	C. oritis	male	13.25	0.77	20.58
L70032	C. oritis	male	11.5	3.30	20.05
L70145	C. oritis	female	11.25	3.89	19.40
L70146	C. oritis	female	9.5	2.43	19.79
L70151	C. oritis	female	12.75	0.65	20.80
L70157	C. oritis	male	12.75	5.54	19.19
L55988	C. reichenowi	male	9.5	2.99	18.54
L56284	C. reichenowi	male	9.0	3.89	18.54
L56299	C. reichenowi	male	10.25	0.48	23.30
L70001	C. reichenowi	male	9.25	3.65	20.55
L70006	C. reichenowi	male	9.5	2.73	20.40
L70154	C. reichenowi	male	9.0	1.24	19.17
L70155	C. reichenowi	male	9.75	1.84	18.38
L70156	C. reichenowi	male	9.75	2.94	19.10
L70161	C. reichenowi	male	9.75	0.46	20.97
L70170	C. reichenowi	male	9.5	2.90	19.54

Actual wingbeat frequency

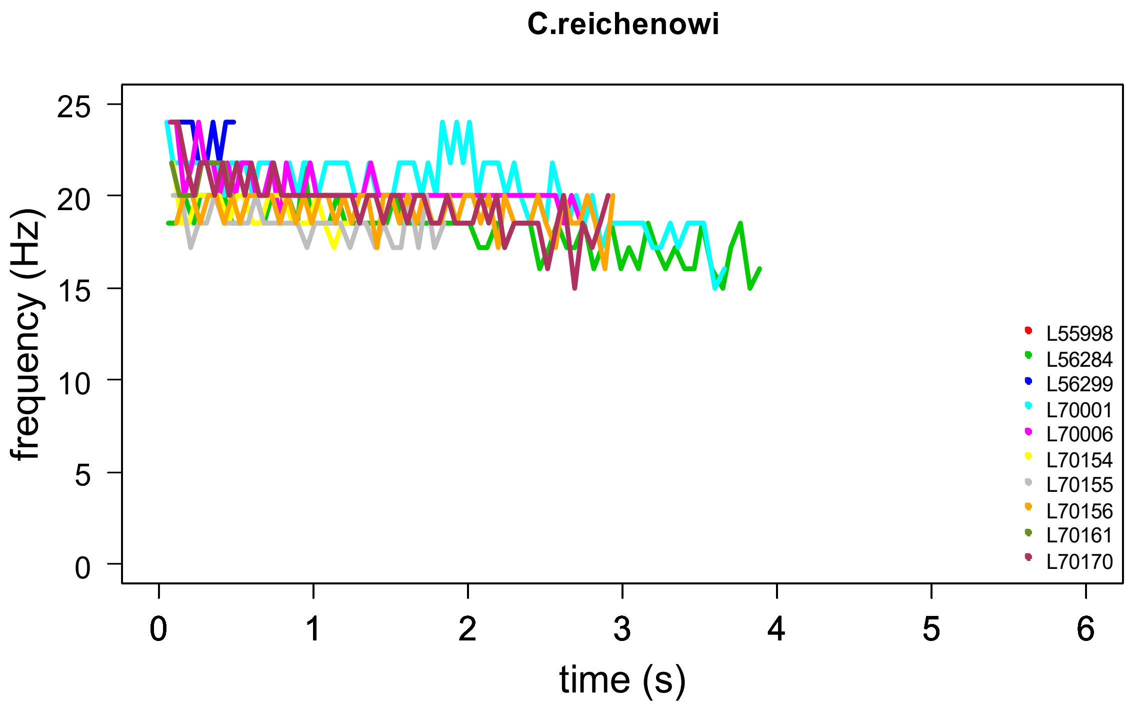
In both species, the beginning of feeding was associated with relatively higher wingbeat frequency (up to 24 Hz) at time when the bird was still not completely stable. The actual wingbeat frequency then decreased with time spent in the air ($F_{rei} = 248.8$, $F_{ori} = 135.3$; $df = 1$ for both species; $p < 0.001$ for both species). The decrease was more obvious in case of *C. reichenowi,* where the wingbeat frequency of majority of individuals which hovered longer than 2.5 seconds was then irregularly falling to only 15 - 16 Hz. This decrease equals to almost 25 % of the mean wingbeat frequency (19.85 Hz) of this species. In contrast, the actual wingbeat frequency of *C. oritis* was decreasing constantly and rather slower ($F = 54.68$; $df = 1$; $p < 0.001$) with its minimal value equalling to 17.14 Hz, see figure 18.

Wing aspect ratio

Wing aspect ratio of both species was rather low, $AR_{rei} = 4.54 \pm 0.01$, $AR_{ori} = 4.61 \pm 0.34$ (mean ± s.d.) and did not differ between species ($t = 0.457$; $df = 4.69$; $p = 0.668$).

Table 4
List of birds used for estimation of wing aspect ratio of each species

Ring number	**Species**	**Sex**	**Body mass (g)**	**Wing area (mm^2)**	**Wing span (mm)**	**Wing aspect ratio**
L55913	C. oritis	not determined	13.5	8916	206	4.76
L55919	C. oritis	not determined	12.0	9326	200	4.29
L55929	C. oritis	not determined	11.0	8778	206	4.83
L70493	C. oritis	not determined	12.0	8072	200	4.96
L70496	C. oritis	not determined	12.0	8756	192	4.21
L55916	C. reichenowi	male	9.5	7218	184	4.69
L55921	C. reichenowi	male	8.8	7746	186	4.47
L55927	C. reichenowi	male	10.0	8262	192	4.46
L70497	C. reichenowi	male	9.5	7698	188	4.59
L70500	C. reichenowi	male	8.5	8392	194	4.48



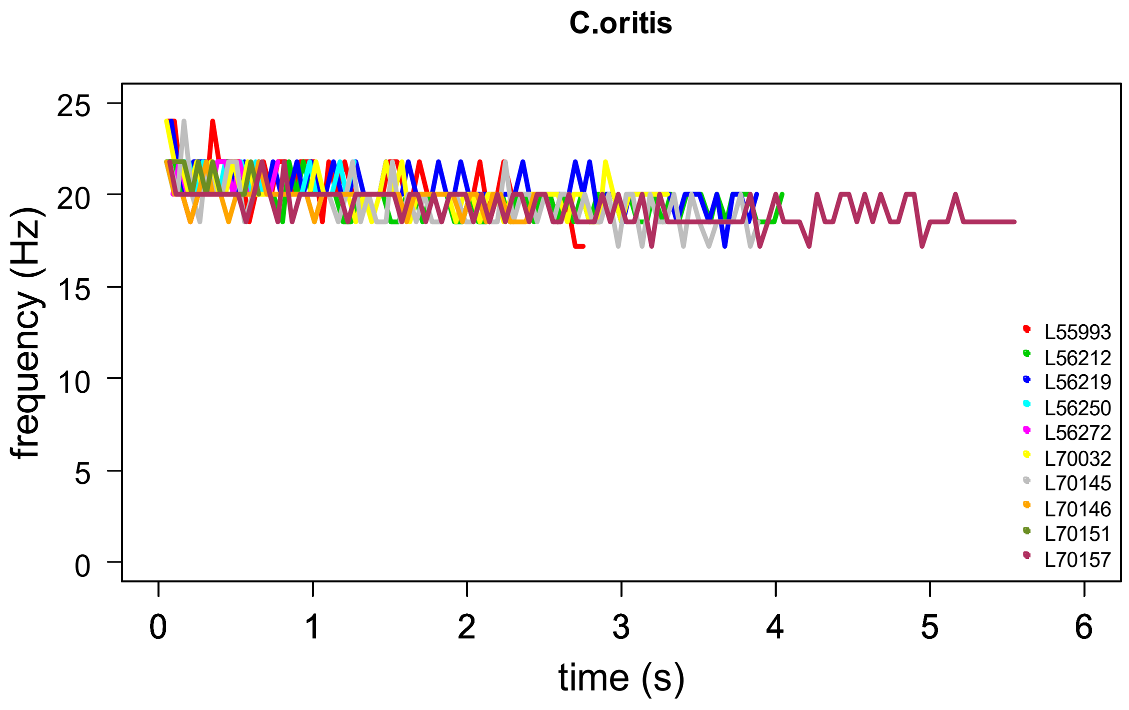


Figure 18

Actual wingbeat frequency during hovering flower visit.

Individuals are shown by distinct colours. Graphs for *C. reichenowi* and *C. oritis.* Note the relatively higher uniformity and slower decrease of wingbeat frequency in performance of *C. oritis*.

Discussion

The time of hovering performance and the average wingbeat frequency

Representatives of both species were able to hover for few seconds, the mean time of recorded hovering by *C. reichenowi* corresponds to 2.3 s and by *C. oritis* to 2.9 s. This is fairly less than several minutes of hovering known at some hummingbirds (Lasiewski 1963). However, the hovering time of each individual reported in our study should be treated with caution. Almost surely it does not correspond with the maximal possible time of its hovering performance, because none of the birds had been habituated to the cage before. The fact of being closed in a cage (often for the first time) and the foregoing manipulation is most probably for a wild bird very stressful and energy demanding. Moreover, they often hovered in the cage for some time even before visiting the flower.

The average reported wingbeat frequency during hovering was almost identical in both species and equalled to 20 Hz. This finding could seem surprising if we consider the facts that a wingbeat frequency is known to decrease with body size (Tobalske 2001; Altshuler and Dudley 2003) and body mass of recorded individuals significantly differs among species: $BM_{rei} = 9.52 \pm 0.38$ g versus $BM_{ori} = 11.67 \pm 1.28$ g (mean ± s.d., T-test; $t = 5.07$, $df = 10.58$, $p < 0.001$). However, the differences in body mass are minimal and the flight ability is also affected by other morphological features (Pennycuick 1990). Compared to other species, the wingbeat frequency of our target sunbirds is twice as high as that known for the only previously analysed hovering sunbirds, *Chalcomitra senegalensis* ($f = 10$ Hz; Zimmer 1943), of slightly higher body mass ($BM = 11.1\text{-}17.2$ g according to Cheke et. al. 2001). Nevertheless, it is comparable to the wingbeat frequency of other passerines of similar size (Tobalske et al. 1999; Chang et al. 2011) and also to the wingbeat frequency of the largest hummingbird, the Giant Hummingbird, *Patagona gigas* with its body mass $BM = 24$ g (Altshuler and Dudley 2003).

Actual wingbeat frequency

Actual wingbeat frequency of all individuals shows a periodical pattern, I think, largely it is a methodological artefact caused by relatively low framerate (240 fps) of recordings. Because the wingbeat frequency was not precisely 20 Hz, the wing was captured only 12 times per a wing cycle and its position slightly differed in every picture, thus I was forced to mark the beginning of downstroke sometimes a frame earlier or later. Nevertheless, it does not have any influence on the pattern found.

There is a slight decrease in the wingbeat frequency of individuals of both species in time from the beginning of hovering, but I did not notice any interruption in flapping that would last for at least one wingbeat cycle as would be expected base on the definition of intermittent flight and prediction made by (Tobalske 2001). Our finding also does not correspond to finding of Zimmer (1943), who observed an individual of *Chalcomitra senegalensis* (also a representative of specialised nectar-feeding birds) regularly interrupting flapping for one or more wingbeat cycles during hovering. Of course, technological equipment used in his study cannot be compared with the one used in our study.

There were few obvious irregularities in the wingbeat frequency of those individuals of *C. reichenowi,* hovering for more than 2.5 s. After 40-50 cycles of continuous flapping, the actual wingbeat frequency of each of them repeatedly dropped to 15-16 Hz. These prominent changes could correspond to pauses in flapping for half of the wingbeat cycle. However so far, I am not able to say whether this pattern is really a result of flapping interruption or general slowdown during the entire wingbeat cycle. At the same time, there were no such irregularities in the wingbeat frequency during entire hovering performances by individuals of *C. oritis*, which seems to be able to hover without any interruption in flapping for more than 100 wingbeat cycles.

Here, our results contrast with results of Tobalske at al. (1999), who observed zebra finches *Taenopygia guttata* (m=13 g) to interrupt flapping during hovering on average after seven wingbeat cycles, and therefore indicate that both target species, similarly to other species whose life style requires hovering or slow flying (Muijres et al. 2011), could be better adapted for hovering than an average small passerine.

Besides body mass, also wing morphology, especially a wing span and a wing area, are known to affect bird flying abilities and wingbeat frequency (Pennycuick 1990). It has been hypothesised that a low wing aspect ratio may constrain small passerines to use intermittent instead of continuous flapping also during hovering (Tobalske et al. 1999). Our findings does not support this assumption, as measured wing aspect ratio of both species, *C. reichenowi* and *C. oritis* was relatively low, approximately 4.5. This value is almost similar to the aspect ratio of *T. guttata* (AR=4.2), that is not capable of continuous flapping as discussed above (Tobalske et al. 1999)

Conclusion

Our results show, that despite their relatively low wing aspect ratio, individuals of both species *C. reichenowi* and *C. oritis* are capable of hovering lasting several seconds without any interruption in flapping. The actual wingbeat frequency of all individuals slowly decreases in time his decrease is more obvious in case of *C. reichenowi*, where after 2.5 s of constant flapping its wingbeat frequency irregularly drops to only 75 % of its mean value. However, before making a general conclusion, more effort needs to be dedicated to the flight performance of these birds in order to understand better their possible adaptations to hovering flight.

Conclusions of the thesis

For decades, there have been discussions on why, in contrast to hummingbirds, nectarivorous birds of the Old World perch while eating. Nowadays, we know they also often hover for nectar. We looked in detail on foraging behaviour of two sunbird species, *Cinnyris reichenowi* and *Cyanomitra oritis* and evaluated possible factors affecting their decision whether to perch or to hover. Furthermore, we analysed their wingbeat frequency during hovering in front of a flower to bring at least basic information on their capacity for this kind of flight.

Foraging behaviour of sunbirds

Our results reported in chapter II support Miller's (1985) and Westerkamp's (1990) opinion, that the plant architecture is a crucial factor affecting birds foraging behaviour. The probability of hovering increased with the length of peduncles and pedicels. We found this pattern to be significant only for *C. reichenowi*. Almost identical trend was visible for *C. oritis*, however it should be treated with caution. The dataset for this species was insufficient and largely unbalanced and the result was not significant.

Our findings do not correspond to the assumption of Pyke (1981), that hovering is preferred especially on those flowers, which are situated at bigger distances from each other. I believe, that largely is this caused by rests often performed between two hovering events, which were not included in Pyke's theories.

Sunbirds were observed feeding at rain intensities up to 50 $mm \cdot h^{-1}$. As expected, the probability of hovering was negatively affected by the rain intensity. Still there were several birds hovering even under heavy rain. Again, this behaviour was significant only for *C. reichenowi.*

As hypothesized by Pyke (1981), for both species we found hovering associated with higher flower visitation rate. This correlation was caused by shorter flower visits and, despite the rests often performed between two hovering events, also by faster transfer between flowers. Such correlation may have interesting consequences on bird energetics or on pollination speed, but still more research on this topic is required.

Hovering flight of sunbirds

From our detail analysis of the wingbeat frequency of both studied species reported in chapter III, it seems that despite relatively low wing aspect ratio, sunbirds are not limited to intermittent flapping during hovering as proposed by Tobalske (2010) but are able to hover steadily for several seconds. It indicates that these birds could be better adapted for hovering flight than an average small passerine.

Recorded average wingbeat frequency is similar for both species and equals to 20 Hz. Actual wingbeat frequency shows slow decrease in time from the beginning of hovering. This decrease is more prominent in case of *C. reichenowi*, where the wingbeat frequency after 2.5 s often irregularly drops to only 15 Hz. So far, I am not able to say whether this is caused by the interruptions in flapping for half of a wingbeat cycle or whether it a consequence of a general slowdown during the entire wingbeat cycle.

To know if sunbirds exhibit adaptation for hovering similarly to other often hovering or slow flying passerines as demonstrated by Muijres et al. (2011), more detailed analysis of their flight is needed.

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