Research

**Oikos 120: 178–183, 2011**
doi: 10.1111/j.1600-0706.2010.18612.x

Subject Editor: Jan van Gils. Accepted 28 May 2010

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

**Štěpán Janeček, Eliška Patáčová, Michael Bartoš, Eliška Padyšáková, Lukáš Spitzer and Robert Tropek**

*Š. Janeček (janecek@butbn.cas.cz), E. Patáčová, M. Bartoš and E. Padyšáková, Inst. of Botany, Academy of Sciences of the Czech Republic, Dukelská 135, CZ–379 82 Třeboň, Czech Republic. – EPat, MB, EPad, L. Spitzer and R. Tropek, Faculty of Science, Univ. of South Bohemia, Branišovská 31, CZ–370 05 České Budějovice, Czech Republic. – LP and RT also at: Inst. of Entomology, Biology Centre, Academy of Sciences of the Czech Republic, Branišovská 31, CZ–370 05 České Budějovice, Czech Republic.*

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.

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 μl 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 melifera* 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 *Cyanomoitra 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).

## 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

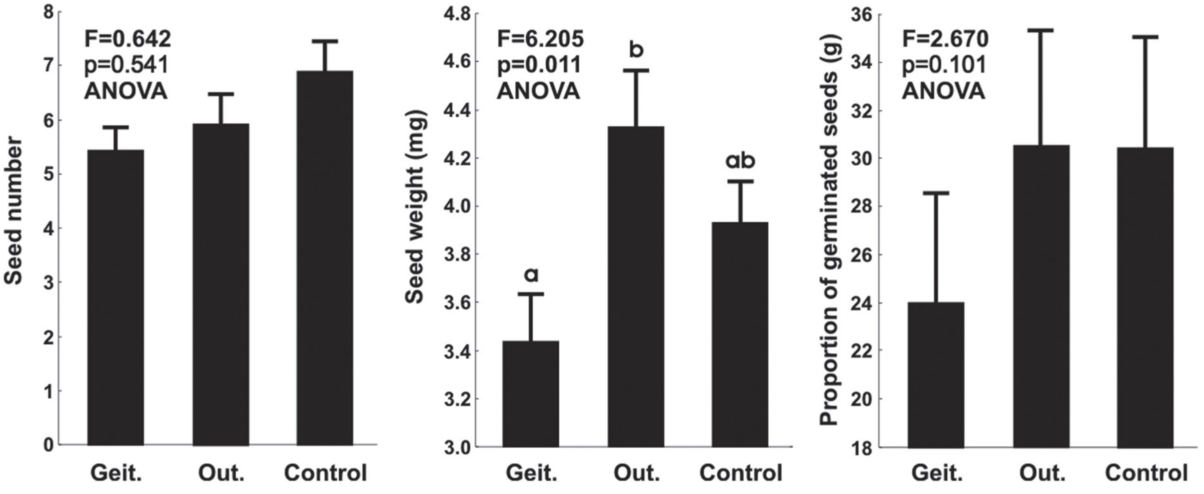

Figure 1. Mean seed number per fruit, weight and proportion of germinated seeds of *Impatiens sakeriana* in three treatments where fruits developed: Geit. – geitonogamy, Out. – outcrossing and control. Differing letters above the boxes indicate significant differences between treatments (post-hoc test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

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

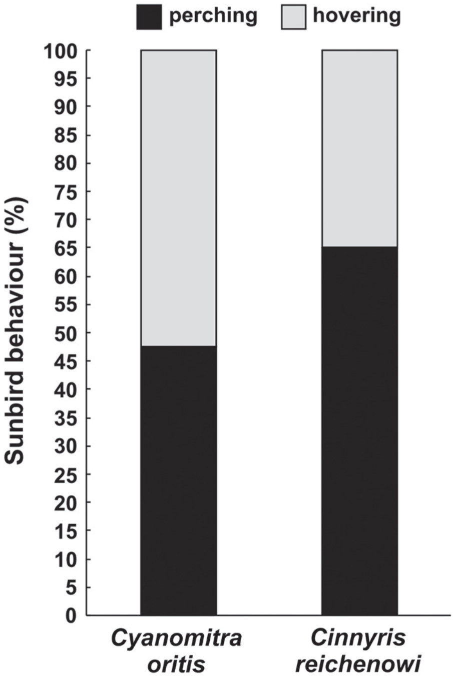

Figure 3. Behaviour of *Cyanomitra oritis* (n = 181) and *Cinnyris reichenowi* (n = 64) during feeding on *I. sakeriana.*

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 (Tiang 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 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).

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 |

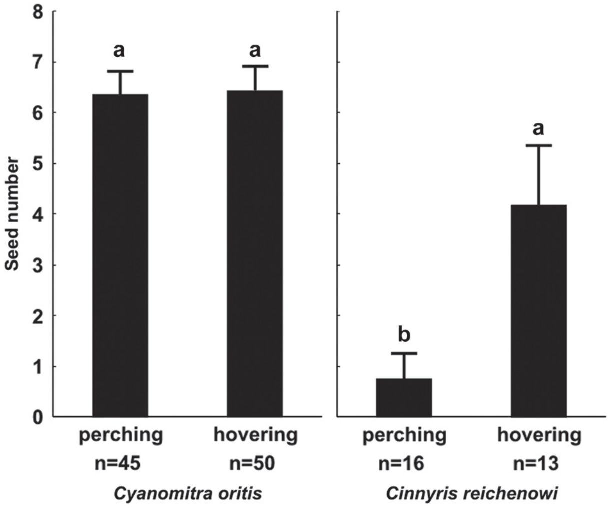

Figure 4. Sunbirds pollination effectiveness. Differing letters above the boxes indicate significant differences between sunbirds' behaviour (post-hoc test – unequal n HSD test). Error bars represent SE.

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.

*Acknowledgements* – We would like to thank Michal Pešata for help during pilot studies; David Hořák, Ondřej Sedláček and Jitka Klimešová for comments on the manuscript; Ernest Vunan for technical support, and Matthew Sweney for English proofreading. This work was supported by the Grant Agency of the Czech Academy of Sciences (IAA601410709 and B601110703), Czech Science Foundation (206/08/H044), GAJU 136/2010/P and by project AV0Z60050516.

## References

Altshuler, D. L. and Dudley, R. 2002. The ecological and evolutionary interface of hummingbird flight physiology. – J. Exp. Biol. 205: 2325–2336.

Anderson, B. et al. 2005. Specialized bird perch aids cross-pollination. – Nature 435: 41–42.

Caris, P. L. et al. 2006. Floral development in three species of *Impatiens* (Balsaminaceae). – Am. J. Bot. 93: 1–14.

Cheke, R. A. et al. 2001. Sunbirds: a guide to the sunbirds, flowerpeckers, spiderhunters and sugarbirds of the World. – Christopher Helm, London.

Darwin, C. 1859. On the origin of species. – Murray, J., London.

Evans, M. R. 1996. Nectar and flower production of *Lobelia telekii* inflorescences, and their influence on territorial behaviour of the scarlet-tufted malachite sunbird (*Nectarinia johnstoni*). – Biol. J. Linn. Soc. 57: 89–105.

Fain, M. G. and Houde, P. 2004. Parallel radiations in the primary clades of birds. – Evolution 58: 2558–2573.

Ford, C. M. and Johnson, S. D. 2008. Floral traits, pollinators and breeding systems in *Syncolostemon* (Lamiaceae). – Plant Syst. Evol. 275: 257–264.

Frost, S. K. and Frost, P. G. H. 1981. Sunbird pollination of *Strelitzia nicolai*. – Oecologia 49: 379–384.

Geerts, S. and Pauw, A. 2009a. African sunbirds hover to pollinate an invasive hummingbird-pollinated plant. – Oikos 118: 573–579.

Geerts, S. and Pauw, A. 2009b. Hyper-specialization for long-billed bird pollination in a guild of South African plants: the malachite sunbird pollination syndrome. – S. Afr. J. Bot. 75: 699–706.

Gill, F. B. and Wolf, L. L. 1978. Comparative foraging efficiencies of some montane sunbirds in Kenya. – Condor 80: 391–400.

Goldblatt, P. and Manning, J. C. 2006. Radiation of pollination systems in the *Iridaceae* of sub-Saharan Africa. – Ann. Bot. 97: 317–344.

Grey-Wilson, C. 1980. *Impatiens* of Africa. – A.A. Balkema, Rotterdam.

Johnson, S. D. 1996. Bird pollination in South African species of *Satyrium* (Orchidaceae). – Plant Syst. Evol. 203: 91–98.

Ley, A. C. and Classen-Bockhoff, R. 2009. Pollination syndromes in African Marantaceae. – Ann. Bot. 104: 41–56.

Maloof, J. E. and Inouye, D. W. 2000. Are nectar robbers cheaters or mutualists? – Ecology 81: 2651–2661.

Mayr, G. 2004. Old world fossil record of modern-type hummingbirds. – Science 304: 861–864.

Roulston, T. 2005. Pollen as a reward. – In: Dafni, A. et al. (eds), Practical pollination biology. Enviroque, Cambridge, pp. 236–260.

Schemske, D. W. 1978. Evolution of reproductive characteristics in *Impatiens* (Balsaminaceae): the significance of cleistogamy and chasmogamy. – Ecology 59: 596–613.

Schuchmann, K. L. 1999. Family Trochilidae (hummingbirds). – In: Hoyo, del J. et al. (eds), Handbook of the birds of the World. Vol. 5. Lynx Editions, pp. 468–680.

Siegfried, W. R. et al. 1985. Stem thickness of *Erica* plants in relation to avian pollination. – Oikos 45: 153–155.

Sreekala, A. K. et al. 2008. Reproductive biology of *Impatiens coelotropis* Fischer, a critically endangered balsam from the southern Western Ghats. – Curr. Sci. 95: 386–388.

Tiang, J. et al. 2004. Pollination ecology and pollination system of *Impatiens reptans* (Balsaminaceae) endemic to China. – Ann. Bot. 93: 167–175.

Waller, D. M. 1980. Environmental determinants of outcrossing in *Impatiens capensis* (Balsaminaceae). – Evolution 34: 747–761.

Westerkamp, C. 1990. Bird–flowers: hovering versus perching exploitation. – Bot. Acta 103: 366–371.