



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

ŠTĚPÁN JANEČEK^{1*}, JAN RIEGERT², ONDŘEJ SEDLÁČEK³, MICHAEL BARTOŠ^{1,2}, DAVID HOŘÁK³, JIŘÍ REIF^{4,5}, ELIŠKA PADYŠÁKOVÁ^{1,2}, DRAHOMÍRA FAINOVÁ², MARCIN ANTCZAK⁶, MICHAL PEŠATA¹, VÁCLAV MIKEŠ², ELIŠKA PATÁČOVÁ¹, JAN ALTMAN^{1,2}, JANA KANTOROVÁ¹, ZÁBOJ HRÁZSKÝ^{1,2}, JAKUB BROM⁷ and JIŘÍ DOLEŽAL^{1,2}

¹*Institute of Botany, Academy of Sciences of the Czech Republic, Dukelská 135, CZ-379 82 Třeboň, Czech Republic*

²*Faculty of Science, University of South Bohemia, Branišovská 31, CZ-370 05 České Budějovice, Czech Republic*

³*Department of Ecology, Faculty of Science, Charles University, Viničná 7, CZ-128 44 Praha, Czech Republic*

⁴*Institute for Environmental Studies, Charles University in Prague, Benátská 2, 128 01 Praha 2, Czech Republic*

⁵*Department of Zoology and Laboratory of Ornithology, Faculty of Science, Palacky University, tr. Svobody 26, 77146 Olomouc, Czech Republic*

⁶*Department of Behavioural Ecology, Adam Mickiewicz University, Faculty of Biology, Umultowska 89, 61-614 Poznań, Poland*

⁷*Department of Landscape Management, Faculty of Agriculture, University of South Bohemia, Studentská 13, CZ-370 05 České Budějovice, Czech Republic*

Received 30 January 2012; revised 3 April 2012; accepted for publication 4 April 2012

Community-level studies have shown that plant–pollinator interactions are much more generalized than previously expected. Consequently, many authors have questioned the significance of phenotypic complementarity between plants and pollinators and abundance effects in pollination interactions. Here, we compare the behaviour of three sunbird species feeding on the nectar of five plant species in afro-montane vegetation. We studied the feeding behaviour with and without consideration of plant abundance (i.e. diet selectivity and diet composition, respectively). The aims of the study were to estimate: (1) how relative resource abundance influences flower selectivity; (2) the degree of phenotypic matching; and (3) whether different plant resource assessment methods give different answers to this question. The results showed that, although sunbirds frequently feed on both morphologically adapted and nonadapted plants, food selectivity data are consistent with the hypothesis of phenotypic complementarity. Moreover, we found that the type of plant abundance measurement can change conclusions in some cases, as individual plants differ in their growth habits and nectar production. This effect was most obvious for the assessment of selectivity of the northern double-collared sunbird (*Cinnyris reichenowi*) and for *Hypoestes aristata*, a plant producing inflorescences composed of a large number of small flowers possessing small amounts of nectar per flower (a high abundance of flowers, but a low abundance of nectar relative to the remaining plant community). © 2012 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2012, ●●, ●●–●●.

ADDITIONAL KEYWORDS: Cameroon – diet composition – food selectivity – pollination – specialization – sunbird.

*Corresponding author. E-mail: janecek@butbn.cas.cz

INTRODUCTION

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

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

The evolution of specialized long mouthparts in birds can also be seen as a specialization for ecological generalization, which enables floral visitors to enlarge the spectrum of visited flowers (Stang, Klinkhamer & van der Meijden, 2007).

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

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

MATERIAL AND METHODS

STUDY AREA

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

PLANT SPECIES

Our study focused on five plant species on which sunbirds fed during our unpublished pilot observations: *Pycnostachys eminii* Gürke (Lamiaceae), *Lobelia columnaris* Hook. f. (Campanulaceae), *Hypoestes aristata* (Vahl) Roem & Schult (Acanthaceae), *Hypericum revolutum* Vahl (Hypericaceae) and *Impatiens sakeriana* Hook. f. (Balsaminaceae). According to pollination syndromes (Faegri & van der Pijl, 1979), *I. sakeriana* can be considered as a typical bird-pollinated species (see also Janeček *et al.*, 2011 and Bartoš *et al.*, 2012), whereas *Hypericum revolutum* and *P. eminii* are typical insect-pollinated species (Janeček *et al.*, 2007; Bartoš *et al.*, 2012). The other two species (*L. columnaris* and *Hypoestes aristata*) have some traits associated with insect pollination and others with bird pollination. We used corolla length as a factor limiting the accessibility of the

nectar resources (Stang *et al.*, 2007; Dalsgaard *et al.*, 2009). For *L. columnaris*, calyx length was measured as it is the main nectar barrier for sunbirds. Measurements of corolla length were taken from 30 individual plants of each species, randomly chosen within the study area (Table 1, Fig. 1).

MEASUREMENTS OF NECTAR PRODUCTION

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

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

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

*Values are means \pm standard deviation.

†For *L. columnaris*, calyx length was measured as it represents the nectar barrier (see Fig. 2). act., actinomorphic; spur., spurred; tub., tubular; zyg., zygomorphic.

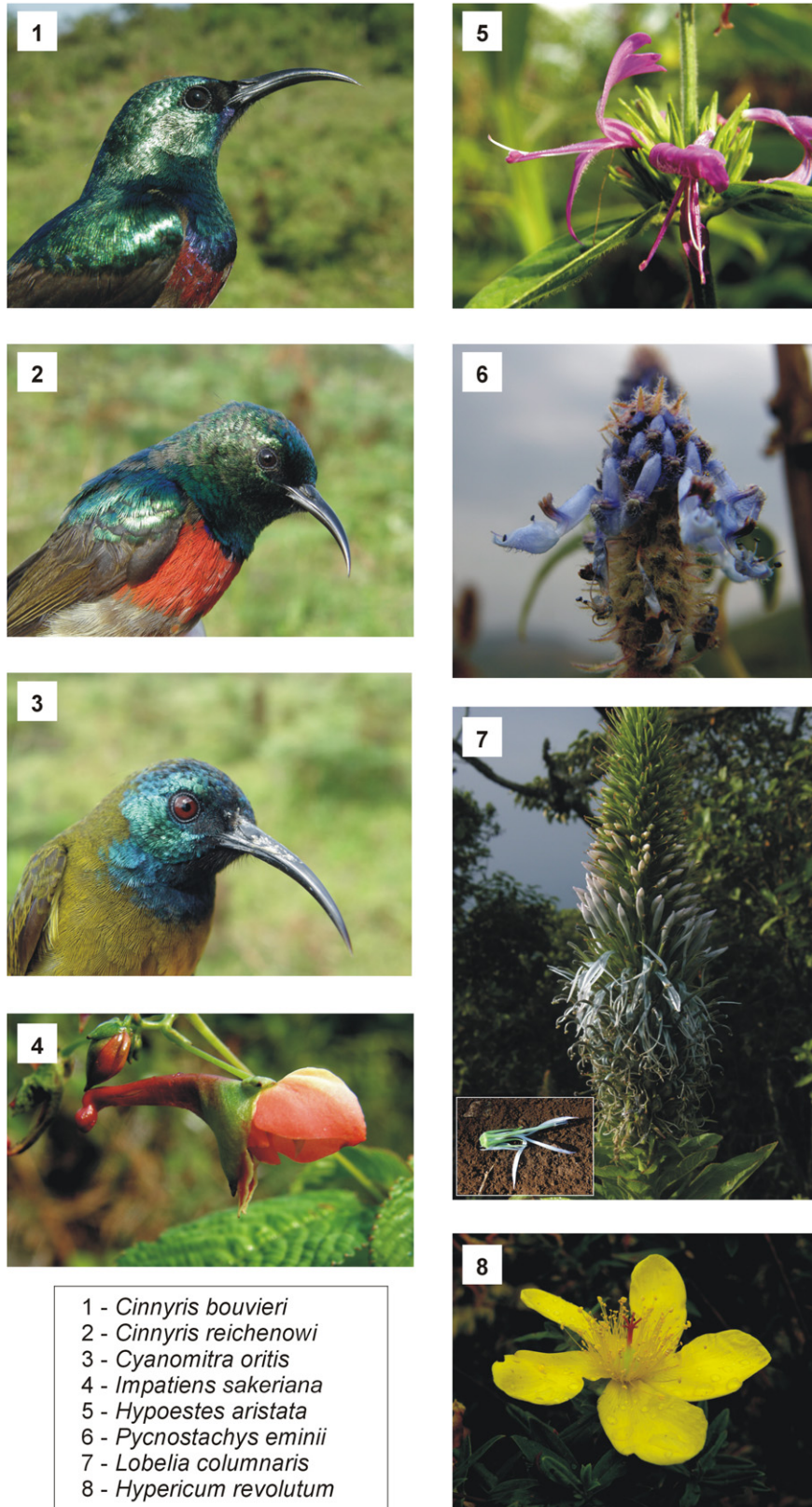


Figure 1. Target plant and sunbird species (photographs 1–3 were taken by Pavla Blažková and photographs 4–8 by Jan Riegert).

small for nectar analyses, we collected nectar from several flowers in one inflorescence, determined the nectar volume based on the combined sample and calculated the nectar volume per flower. For the purposes of this study, we determined nectar abundance on each observed plant as a mean total amount of sugar content per flower (Table 1) multiplied by the number of flowers on the plant. More details on nectar production in the target plant species studied are given in Bartoš *et al.* (2012).

SUNBIRDS

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

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

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

*Values are means ± standard deviation.

(Table 2). The trapped sunbirds were colour ringed to avoid pseudoreplication in the dataset.

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

OBSERVATIONS OF SUNBIRD FORAGING BEHAVIOUR

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

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

SUNBIRD SELECTIVITY AND STATISTICAL ANALYSES

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

We used Jacobs' selectivity index, $D_i = (r_i - p_i) / (r_i + p_i - 2r_i p_i)$ (Jacobs, 1974), to determine the feeding selectivity of sunbird species. This index contains the resource use ratio (r_i), the ratio of resource i used to the amount of all used resources, and the resource availability ratio (p_i), the ratio of the resource i in the

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

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

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

To determine whether the methods differed for individual sunbirds in individual years and for individual species, we performed the PERMANOVA tests in the same way, but separately, for each sunbird species–plant species–year interaction (Table 5). In consequence, two factors were only included in these

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

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

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

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

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

* $P < 0.001$; † $0.001 < P < 0.01$.

analyses: plant individual as random factor and method as fixed factor. When the permutation ANOVA was statistically significant, we performed permutation pairwise comparisons, which corresponded to parametric *t*-tests (Anderson *et al.*, 2008).

RESULTS

Sugar amounts and nectar volumes per flower were much larger for *L. columnaris*, *Hypericum revolutum* and *I. sakeriana* than for *P. eminii* or *Hypoestes aristata* (Table 1). The sugar concentration was highest in the nectar of *Hypoestes aristata* and *P. eminii* and lowest in the nectar of *I. sakeriana* (Table 1). At the community level, *L. columnaris* and *Hypericum revolutum* were the largest nectar sources in the observed area (Fig. 2A). The contribution of *L. columnaris* to the total nectar available in the community was greater in 2007 than in 2003 (Fig. 2A), because the peak of *L. columnaris* flowering occurs late in the dry season, and the observation period was later in 2007 than in 2003. Considering the number of flowers and number of plants as measures of plant abundance, the highest number of flowers was recorded for *Hypoestes aristata* and the highest number of plants was recorded for *Hypericum revolutum* for both years (Fig. 2B, C). The longest handling times per plant were recorded for *I. sakeriana* in the year 2003 and the shortest for *P. eminii* (for data on handling times per plant, see Table S1). The handling times did not differ between sunbird species at individual plants (results not shown).

In 2003 and, to a lesser degree, in 2007, all sunbirds spent a high proportion of their feeding time (*r*) on *Hypericum revolutum* (Fig. 2D–F). The time spent

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

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

H. ari., *Hypoestes aristata*; *H. rev.*, *Hypericum revolutum*; *I. sak.*, *Impatiens sakeriana*; *L. col.*, *Lobelia columnaris*; *P. emi.*, *Pycnostachys eminii*. * $P < 0.001$; † $0.001 < P < 0.01$; ‡ $0.01 < P < 0.05$.

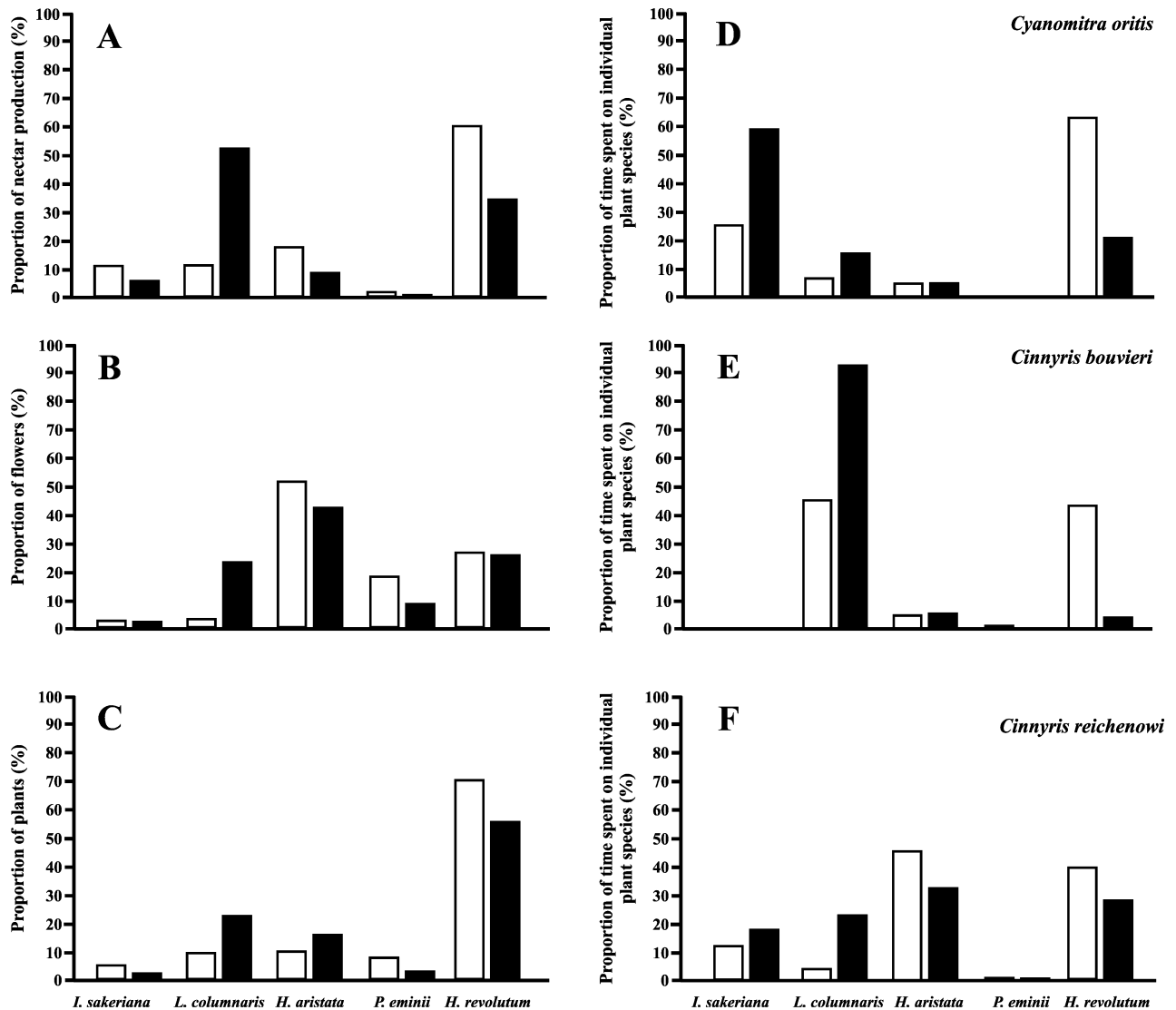


Figure 2. Proportion of observed resources, considering nectar production (A), number of flowers (B) and number of plants (C), and proportion of time spent by individual sunbird species on target plants (D–F). Open bars, 2003; filled bars, 2007. It should be noted that the abundance from which the proportions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

on other plant species differed depending on the sunbird species. There were no records of *Cinnyris bouvieri* feeding on *I. sakeriana* or of *Cyanomitra oritis* feeding on *P. eminii*. Sunbirds fed more on *L. columnaris* in 2007 than in 2003 (Fig. 2D–F), when the highest abundances of *L. columnaris* were recorded.

Sunbird species selected different plant species, and the pattern of selectivity was the same for both years; this was true for all selectivity measurements considering different measures of plant abundance (Table 3,

General test). Birds also differed in selectivity for all target plant species with only one exception (selectivity for *P. eminii*, considering the number of flowers as a measure of plant abundance) and different bird selectivity between years was detected only for *Hypericum revolutum* (Table 3, part A). Individual sunbird species differently selected individual plant species, regardless of whether considering nectar production, number of flowers or number of plants (Table 3, part B).

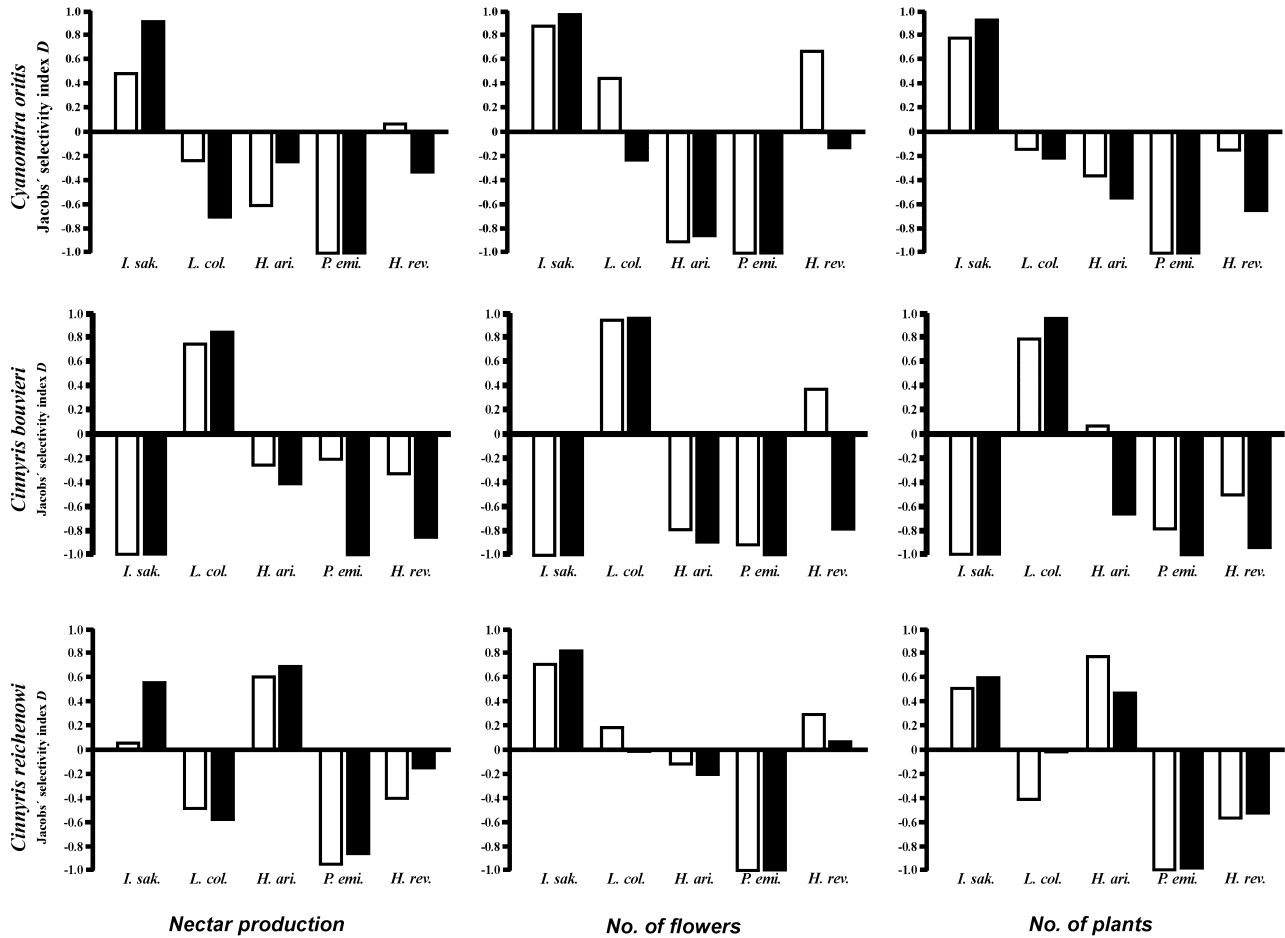


Figure 3. Jacobs' selectivity indices (*D*) of individual sunbird species for target plant species, considering different assessments of plant abundance measures (nectar production, number of flowers and number of plants). Open bars, 2003; filled bars, 2007. The plants on the *x* axis are arranged in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

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

pattern was observed when the selectivity indices were calculated on the plant specimen level (selectivity indices calculated for each of the 594 plants, Table 5) instead of on the plant species level (selectivity index calculated for each plant species, Fig. 3).

DISCUSSION

When considering phenotypic specialization and dietary niche breadth of pollinators, our results are in agreement with those of previous studies showing that phenotypically specialized birds are able to feed on a wider spectrum of plants than predicted by their specialized traits (Snow & Snow, 1972; Woodell, 1979; Brown & Hopkins, 1995; Franklin & Noske, 2000; Fleming & Muchhala, 2008; Dalsgaard *et al.*, 2009). For the sunbirds studied, we therefore argue that

there is no clear trade-off between the evolution of phenotypic specialization and feeding on plants with easily accessible nectar (e.g. *Hypericum revolutum*). In other words, even though sunbirds have phenotypically specialized bills, they readily feed on non-specialized flowers and can be considered to be ecologically generalized.

Nevertheless, our study demonstrated the strong effect of plant abundance on feeding behaviour, similar to that observed in studies on plant–flower visitor networks (Vázquez, 2005; Vázquez & Aizen, 2006; Vázquez *et al.*, 2007). When plant abundance was considered, we detected a clear pattern of selectivity for specialized long tubular flowers, as well as trophic niche partitioning, among the sunbirds studied. The sunbird with the longest bill, *Cyanomitra oritis*, selected *I. sakeriana*, which was the plant with longest flowers. The sunbird with a bill length that was somewhat shorter than that of *Cyanomitra oritis*, i.e. *Cinnyris bouvieri*, selected *L. columnaris*, whose calyx (which is a nectar barrier in this species) was somewhat shorter than the corolla of *I. sakeriana*. This general pattern was clear regardless of which approach to the measurement of plant abundance was used (nectar production, number of plants or number of flowers). *Cinnyris reichenowi* selected *Hypoestes aristata* only if nectar production or the number of plant individuals was considered as the measure of plant abundance, and *Hypoestes aristata* was the most selected species during both years only if nectar production was used as the measure of abundance.

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

mentarity. We suggest, however, that this discrepancy might be explained by our observations of *Cinnyris reichenowi* ‘stealing’ nectar by piercing the flower spur of *I. sakeriana* in more than 50% of visits (Janeček *et al.*, 2011). In other words, *Cinnyris reichenowi* evades the plant size threshold defined by the spur.

Unfortunately, our methods of observation did not allow us to collect data on the number of visited flowers, the feeding time per flower or nectar extraction efficiency, as performed in other studies using different types of observational approach (Wolf, Hainsworth & Stiles, 1972; Gill & Wolf, 1978; Montgomerie, 1984). The advantage of our method is that more plants can be observed from greater distances, and this method can be used for plants for which the assessment of the number of visited flowers is difficult (e.g. the small flower heads of *P. eminii* in our study). We also did not consider possible differences in extraction efficiency among sunbirds. Therefore, phenotypic complementarity might be underestimated, as it has been shown that nectarivorous birds explore phenotypically related flowers more efficiently (Wolf *et al.*, 1972; Gill & Wolf, 1978; Montgomerie, 1984). Nevertheless, we believe that our approach was sufficient to support the phenotypic complementarity hypothesis. Subsequent studies in our system should also target on mechanisms that can modify the bird–plant interactions, including sunbird competition, aggression and territoriality (e.g. Feinsinger, 1976; Frost & Frost, 1980).

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

In this study, we have demonstrated that the food selectivity approach provides important insights into

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

ACKNOWLEDGEMENTS

We are grateful to the Bamenda Highlands and Kilum-Ijim Forest Projects and, especially, to Dr Michael Boboh Vabi, Dr Guillaume Dzikouk and Gonwouo Nono Legrand (Cameroon Biodiversity Conservation Society, Yaoundé, Cameroon) for enabling us to perform our research in the Bamenda Highlands. The project was authorized by the Ministry of Research and Innovation, Yaoundé, Cameroon under Research Permits nos. 023 and 118. The study was performed with the kind permission of the neighbouring Ndawara-Belo ranch. We thank the entire Kedjom-Keku community and, particularly, Ernest Vunan Amohlon and Devin Chikelen from non-governmental organization Sustainable Agricultural Technicians for their kind reception in the Big Babanki village; Dagmar Bystrická for help in the field; and Jitka Klimešová for helpful comments on an earlier version of the manuscript. We also thank the three anonymous reviewers and Martina Stang for their helpful comments. The study was supported by The Grant Agency of the Academy of Sciences of the Czech Republic (IAA601410709, KJB601110703), Ministry of Education, Youth and Sports (6007665801, 6007665806, 0021620828 and 6198959212), The Grant Agency of the University of South Bohemia (136/2010/P, 138/2010/P), a long-term research development project no. RVO 67985939 and by The Grant Agency of the Czech Republic (P505/11/1617).

REFERENCES

Alarcón R, Waser NM, Ollerton J. 2008. Year-to-year variation in the topology of a plant–pollinator interaction network. *Oikos* **117**: 1796–1807.

- Anderson MJ, Gorley RN, Clarke KR. 2008.** *PERMANOVA+ for PRIMER: guide to software and statistical methods*. Plymouth: PRIMER-E.
- Armbruster WS, Guinn DA. 1989.** The solitary bee fauna (Hymenoptera: Apoidea) of interior and arctic Alaska: flower associations, habitat use, and phenology. *Journal of the Kansas Entomological Society* **62**: 468–483.
- Bartoš M, Janeček Š, Padyšáková E, Patáčová E, Altman J, Pešata M, Kantorová J, Tropek R. 2012.** Nectar properties of the sunbird-pollinated plant *Impatiens sakeriana*: a comparison with six other co-flowering species. *South African Journal of Botany* **78**: 63–74.
- Bascompte J, Jordano P. 2007.** Plant–animal mutualistic networks: the architecture of biodiversity. *Annual Review of Ecology and Systematics* **38**: 567–593.
- Binning SA, Chapman LJ, Cosandey-Godin A. 2009.** Specialized morphology for a generalist diet: evidence for Liem's paradox in a cichlid fish. *Journal of Fish Biology* **75**: 1683–1699.
- Bolten ABS, Feinsinger P, Baker HG, Baker I. 1979.** On the calculation of sugar concentration in flower nectar. *Oecologia* **41**: 301–304.
- Borrell BJ. 2005.** Long tongues and loose niches: evolution of euglossine bees and their nectar flowers. *Biotropica* **37**: 664–669.
- Borrow N, Demey R. 2001.** *Birds of Western Africa*. London: Christopher Helm Publishers.
- Brown ED, Hopkins MJG. 1995.** A test of pollinator specificity and morphological convergence between nectarivorous birds and rainforest tree flowers in New Guinea. *Oecologia* **103**: 89–100.
- Cheke RA, Mann CF, Allen R. 2001.** *Sunbirds: a guide to the sunbirds, flowerpeckers, spiderhunters and sugarbirds of the world*. London: Christopher Helm Publishers.
- Dalsgaard B, Gonzáles AMM, Olesen JM, Ollerton J, Timmermann A, Andersen LH, Tossas AG. 2009.** Plant–hummingbird interactions in the West Indies: floral specialization gradients associated with environment and hummingbird size. *Oecologia* **159**: 757–766.
- Darwin C. 1859.** *On the origin of species*. London: J. Murray.
- Darwin C. 1862.** *On the various contrivances by which British and foreign orchids are fertilised by insects, and on the good effect of intercrossing*. London: J. Murray.
- Devoto M, Medan D. 2006.** Diversity, distribution and floral specificity of tangle-veined flies (Diptera: Nemeletridae) in North West Patagonia, Argentina. *Revista Chilena de Historia Natural* **79**: 29–40.
- Faegri K, van der Pijl L. 1979.** *The principles of pollination ecology*, 3rd revised edn. Oxford: Pergamon Press.
- Feinsinger P. 1976.** Organization of a tropical guild of nectarivorous birds. *Ecological Monographs* **46**: 257–291.
- Fleming TH, Muchhala N. 2008.** Nectar-feeding bird and bat niches in two worlds: pantropical comparisons of vertebrate pollination systems. *Journal of Biogeography* **35**: 764–780.
- Franklin DC, Noske RA. 2000.** Nectar sources used by birds in monsoonal north-western Australia: a regional survey. *Australian Journal of Botany* **48**: 461–474.

- Frost SK, Frost PGH. 1980.** Territoriality and changes in resource use by sunbirds at *Leontotis leonurus* (Labiatae). *Oecologia* **45**: 109–116.
- Galetto L, Bernardello G. 2005.** Nectar. In: Dafni A, Kevan PG, Husband BC, eds. *Practical pollination biology*. Cambridge: Enviroquest Ltd., 261–313.
- Gill FB, Wolf LL. 1978.** Comparative foraging efficiencies of some montane sunbirds in Kenya. *Condor* **80**: 391–400.
- Inouye DW. 1980.** The effect of proboscis and corolla tube lengths on patterns and rates of flower visitation by bumblebees. *Oecologia* **45**: 197–201.
- Jacobs J. 1974.** Quantitative measurement of food selection. *Oecologia* **14**: 413–417.
- Janeček Š, Hrázský Z, Bartoš M, Brom J, Reif J, Hořák D, Bystrická D, Riegert J, Sedláček O, Pešata M. 2007.** Importance of big pollinators for the reproduction of two *Hypericum* species in Cameroon, West Africa. *African Journal of Ecology* **45**: 607–613.
- Janeček Š, Patáčová E, Bartoš M, Padyšáková E, Spitzer S, Tropek R. 2011.** Hovering sunbirds in the Old World: occasional behaviour or evolutionary trend? *Oikos* **120**: 178–183.
- Johnson SD, Nicolson SW. 2008.** Evolutionary associations between nectar properties and specificity in bird pollination systems. *Biology Letters* **4**: 49–52.
- Montgomerie RD. 1984.** Nectar extraction by hummingbirds: response to different floral characters. *Oecologia* **63**: 229–236.
- Ollerton J. 1996.** Reconciling ecological processes with phylogenetic patterns: the apparent paradox of plant–pollinator systems. *Journal of Ecology* **84**: 767–769.
- Petanidou T, Kallimanis S, Tzanopoulos J, Sgardelis SP, Pantis JD. 2008.** Long-term observation of a pollination network: fluctuation in species and interactions, relative invariance of network structure and implications for estimates of specialization. *Ecology Letters* **11**: 564–575.
- Potgieter CJ, Edwards TJ, Miller RM, Van Staden J. 1999.** Pollination of seven *Plectranthus* spp. (Lamiaceae) in southern Natal, South Africa. *Plant Systematics and Evolution* **218**: 99–112.
- Procházka P, Reif J, Hořák D, Klvana P, Lee RW, Yohannes E. 2010.** Using stable isotopes to trace resource acquisition and trophic position in four Afrotropical birds with different dietary requirements. *Ostrich* **81**: 273–275.
- Reif J, Hořák D, Sedláček O, Riegert J, Pešata M, Hrázský Z, Janeček Š, Storch D. 2006.** Unusual abundance–range size relationship in an Afrotropical bird community: the effect of geographical isolation? *Journal of Biogeography* **33**: 1959–1968.
- Reif J, Sedláček O, Hořák D, Riegert J, Pešata M, Hrázský Z, Janeček Š. 2007.** Habitat preferences of birds in a montane forest mosaic in the Bamenda Highlands, Cameroon. *Ostrich* **78**: 31–36.
- Rodríguez-Gironés MA, Santamaría L. 2010.** How foraging behaviour and resource partitioning can drive the evolution of flowers and the structure of pollination networks. *The Open Ecology Journal* **3**: 1–11.
- Santamaría L, Rodríguez-Gironés MA. 2007.** Linkage rules for plant–pollinator networks: trait complementarity or exploitation barriers? *PLoS Biology* **5**: 354–362.
- Snow BK, Snow DW. 1972.** Feeding niches of hummingbirds in a Trinidad valley. *Journal of Animal Ecology* **41**: 471–485.
- Stang M, Klinkhamer PGL, van der Meijden E. 2006.** Size constraints and flower abundance determine the number of interactions in plant–flower visitor web. *Oikos* **112**: 111–121.
- Stang M, Klinkhamer PGL, van der Meijden E. 2007.** Asymmetric specialization and extinction risk in plant–flower visitor webs: a matter of morphology or abundance? *Oecologia* **151**: 442–453.
- Stang M, Klinkhamer PGL, Waser NM, Stang I, van der Meijden E. 2009.** Size-specific interaction patterns and size matching in a plant–pollinator interaction web. *Annals of Botany* **103**: 1459–1469.
- Vázquez DP. 2005.** Degree distribution in plant–animal mutualistic networks: forbidden links or random interactions? *Oikos* **108**: 421–426.
- Vázquez DP, Aizen AM. 2004.** Asymmetric specialization: a pervasive feature of plant–pollinator interactions. *Ecology* **85**: 1251–1257.
- Vázquez DP, Aizen MA. 2006.** Community-wide patterns of specialization in plant–pollinator interactions revealed by null models. In: Waser NM, Ollerton J, eds. *Plant–pollinator interactions: from specialization to generalization*. Chicago, IL: The University of Chicago Press, 200–209.
- Vázquez DP, Blüthgen N, Cagnolo L, Chacoff NP. 2009a.** Uniting pattern and process in plant–animal mutualistic networks: a review. *Annals of Botany* **103**: 1445–1457.
- Vázquez DP, Chacoff NP, Cagnolo L. 2009b.** Evaluating multiple determinants of the structure of plant–animal mutualistic networks. *Ecology* **90**: 2039–2046.
- Vázquez DP, Melián CJ, Williams NM, Blüthgen N, Krasnov BR, Poulin R. 2007.** Species abundance and asymmetric interaction strength in ecological networks. *Oikos* **116**: 1120–1127.
- Vázquez DP, Morris WF, Jordano P. 2005.** Interaction frequency as a surrogate for the total effect of animal mutualists on plants. *Ecology Letters* **8**: 1088–1094.
- Waser NM, Chittka L, Price MW, Williams NM, Ollerton J. 1996.** Generalization in pollination systems, and why it matters. *Ecology* **77**: 1043–1060.
- Wolf LL, Hainsworth FR, Stiles FG. 1972.** Energetics of foraging: rate and efficiency of nectar extraction by hummingbirds. *Science* **176**: 1351–1352.
- Woodell SRJ. 1979.** The role of unspecialized pollinators in the reproductive success of Aldabran plants. *Philosophical Transactions of the Royal Society B: Biological Sciences* **286**: 99–108.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

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

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.