

Fly pollination in *Ceropegia* (Apocynaceae: Asclepiadoideae): biogeographic and phylogenetic perspectives

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- **Background and Aims** *Ceropegia* (Apocynaceae subfamily Asclepiadoideae) is a large, Old World genus of >180 species, all of which possess distinctive flask-shaped flowers that temporarily trap pollinators. The taxonomic diversity of pollinators, biogeographic and phylogenetic patterns of pollinator exploitation, and the level of specificity of interactions were assessed in order to begin to understand the role of pollinators in promoting diversification within the genus.
- **Methods** Flower visitor and pollinator data for approx. 60 *Ceropegia* taxa were analysed with reference to the main centres of diversity of the genus and to a cpDNA–nrDNA molecular phylogeny of the genus.
- **Key Results** *Ceropegia* spp. interact with flower-visiting Diptera from at least 26 genera in 20 families, of which 11 genera and 11 families are pollinators. Size range of flies was 0.5–4.0 mm and approx. 94 % were females. *Ceropegia* from particular regions do not use specific fly genera or families, though Arabian Peninsula species are pollinated by a wider range of Diptera families than those in other regions. The basal-most clade interacts with the highest diversity of Diptera families and genera, largely due to one hyper-generalist taxon, *C. aristolochioides* subsp. *deffersiana*. Species in the more-derived clades interact with a smaller diversity of Diptera. Approximately 60 % of taxa are so far recorded as interacting with only a single genus of pollinators, the remaining 40 % being less conservative in their interactions. *Ceropegia* spp. can therefore be ecological specialists or generalists.
- **Conclusions** The genus *Ceropegia* has largely radiated without evolutionary shifts in pollinator functional specialization, maintaining its interactions with small Diptera. Intriguing biogeographic and phylogenetic patterns may reflect processes of regional dispersal, diversification and subsequent specialization onto a narrower range of pollinators, though some of the findings may be caused by inconsistent sampling. Comparisons are made with other plant genera in the Aristolochiaceae and Araceae that have evolved flask-shaped flowers that trap female flies seeking oviposition sites.

Key words: Apocynaceae, Asclepiadoideae, *Brachystelma*, *Ceropegia*, Diptera, flower evolution, generalization, mutualism, pollination, *Riocreuxia*, specialization, Stapeliinae.

INTRODUCTION

Species-rich genera of biotically pollinated flowering plants are often considered to have diversified via shifts in pollination niche, brought about by reproductive isolation from sister taxa (e.g. Kay and Schemske, 2008) following adaptation to novel pollinators (Grant, 1949; Stebbins, 1970; Waser, 1998). Several models can account for this (Armbruster, 2009) and reproductive isolation without pollinator shifts (i.e. in relation to the abiotic environment) is also a common mechanism (Stebbins, 1970; Johnson, 1996). Plant–pollinator interactions are therefore an important component of the biodiversity of terrestrial communities. However, the diversity of pollination systems has been studied in relatively few large genera (with >100 species) principally because of the logistical difficulties of observing pollinators for any but a small fraction of the species in a genus. Rarer still are studies that have employed a molecular phylogenetic approach to

understanding floral diversification (e.g. Armbruster, 1993; Bruneau, 1996, 1997; Goldblatt *et al.*, 2002; Wilson *et al.*, 2004; Ronsted *et al.*, 2005, 2007; Marussich and Machado, 2007).

The scarcity of phylogenetic studies of the pollination biology of large genera over broad geographical scales is a significant, if understandable, limitation of the literature on plant–species diversification. Significant because it constrains our ability to understand species radiations and the diversification of floral traits in relation to shifts in pollination systems; and understandable because observing plant–pollinator interactions for many species over a wide range is time consuming; e.g. Armbruster’s ground breaking, pantropical research on *Dalechampia* spans three decades and is still ongoing.

The present study focuses on the genus *Ceropegia* (Apocynaceae: Asclepiadoideae, Ceropegieae), a large taxon of over 180 accepted species, with new species being regularly discovered (e.g. Dold, 2006; Malpure *et al.*, 2006). The tribe Ceropegieae is restricted to the Old World, and *Ceropegia*

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TABLE 1. *The centres of diversity for Ceropegia, their terminal taxon richness (including species, subspecies and natural varieties) and the sample sizes for this study*

	No. of described taxa in the region	No. of <i>Ceropegia</i> taxa with pollinator/flower visitor data	Percentage of described taxa	No. of accessions* for those taxa
East Africa	62	30	48	67
Asia	60	3	5	5
Southern Africa	52	16	31	25
West Africa	25	11	44	14
Madagascar	20	4	20	5
Arabian Peninsula	13	8	62	24

Pollinator/flower visitor totals exclude data from cultivated plants. In the second column, 20 taxa have been counted more than once because they occur in more than one biogeographic region. Regions are ranked in order of their *Ceropegia* taxon richness.

*'Accessions' refers to records of *Ceropegia* flowers at a particular locality that have been found to contain insects.

has a distribution from the Canary Islands, across most of sub-Saharan Africa, Madagascar, the Arabian Peninsula, south-east Asia (including the Indian Subcontinent, Laos, Thailand and China), to the south-western Pacific Region (including Indonesia, Philippines, Papua New Guinea and north-east Australia). Within this range there are six recognized centres of diversity: East Africa, West Africa, southern Africa, the Arabian Peninsula, the Indian Subcontinent and Madagascar (Table 1). The genus is keenly collected by plant growers, and the functional details and floral Bauplan of the trap-flower 'Kesselfallen-Blüten' have been known for some time (Knuth, 1909; Vogel, 1961, 1993; Endress, 1996; Masinde, 2004). However, there are relatively few published data on the flower visitors and pollinators of *Ceropegia* proportional to the size of the genus (Vogel, 1961; Bhatnagar, 1986; Sabrosky, 1987; van der Meutter, 1988; Ollerton and Forster, 1995; Ollerton, 1999; Masinde, 2004).

The present study analyses data on flower visitors and pollinators for 59 species, subspecies and natural varieties of *Ceropegia* (approx. one-third of known terminal taxa in the genus; Table S1 in Supplementary data available online) across the whole distribution of the genus and for all major clades (Meve and Liede-Schumann, 2007). It has been possible to assemble this large data set because of two unusual features of the pollination biology of these plants. First, being asclepiads, *Ceropegia* species present pollen as discrete masses (pollinia) with mechanical clips that attach to the pollinator; identifying pollinators amongst all of the flower visitors is therefore straightforward (Wyatt and Broyles, 1994; Ollerton and Liede, 1997). Secondly, as far as is known, all species of *Ceropegia* temporarily trap their pollinators, releasing them after a period of about 24 h, during which time pollinaria have often been picked up and/or deposited by the pollinators. Botanists who collect *Ceropegia* specimens therefore often also collect the flower occupants and, in spirit-preserved material, these can be removed with minimum damage to the flowers (Ollerton and Forster, 1995). The

pollinaria are mechanically persistent and therefore the status of flower visitors as pollinators can be confirmed. These two biological factors provide an opportunity to accumulate plant–pollinator interaction data much more rapidly than would normally be the case for such a large genus. The Kew Herbarium holds some 650 spirit specimens of *Ceropegia* and these have been systematically examined for the presence of insects. In addition, published and unpublished field data collected by ourselves and others have been used to assemble a data set of 136 records in which *Ceropegia* flowers at a particular locality have been found to contain insects. These records are hereafter collectively referred to as 'accessions'. This data set has been used to provide a first assessment of the diversity of pollination systems within the genus *Ceropegia*, across its whole range, and in both biogeographic and phylogenetic contexts. The present study addressed the following questions. (1) How diverse are pollinator–plant interactions within *Ceropegia*, given that all described species of *Ceropegia* have an identical floral Bauplan (*sensu* Endress, 1996), though flowers are highly variable among taxa (Fig. 1)? (2) *Ceropegia* has several regional centres of diversity; are there distinct biogeographic patterns of pollinator exploitation within the genus? (3) Do the different clades of *Ceropegia* exploit particular pollinator taxa, or are the same groups of pollinators used throughout the genus? (4) Within the context of a strict Bauplan but variable floral detail (question 1), how specific are the pollination systems of *Ceropegia* at the levels of insect order, family and genus?

Addressing these questions should inform our understanding of the biogeography and phylogeny of plant–pollinator interactions in a moderately large genus of mainly tropical plants across most of its native range.

MATERIALS AND METHODS

Insects removed from the Kew Herbarium accessions were predominantly Diptera (see Results) and were initially identified to family or genus by one of us (A.W.); especially critical families were sent to experts for further determination (see Acknowledgements). Using a dissecting or stage microscope, the body length of all insects was measured and each was examined for the presence of persistent pollinaria, which was taken as confirmation that these fly taxa were legitimate pollinators; in asclepiads, removal of pollinaria is spatially and behaviourally correlated with pollinia insertion and therefore insects that are large enough, and in the correct position and orientation on the flower, are likely to both remove and deposit pollen.

Samples of *Ceropegia* flowers collected in the field by four of us (J.O., U.M., M.P., S.M.) and colleagues were similarly dissected and flies measured, assessed and sent to A.W. for initial determination. Field-collected South African material was identified by B. Stuckenberg (Natal Museum, South Africa). In addition, all of the available data on *Ceropegia* pollinators from the published literature were collated. The resulting data of *Ceropegia* pollinators and flower visitors were included in the ASCLEPOL database and is available online at http://www.uni-bayreuth.de/departments/planta2/research/pollina/as_pol_t.html.

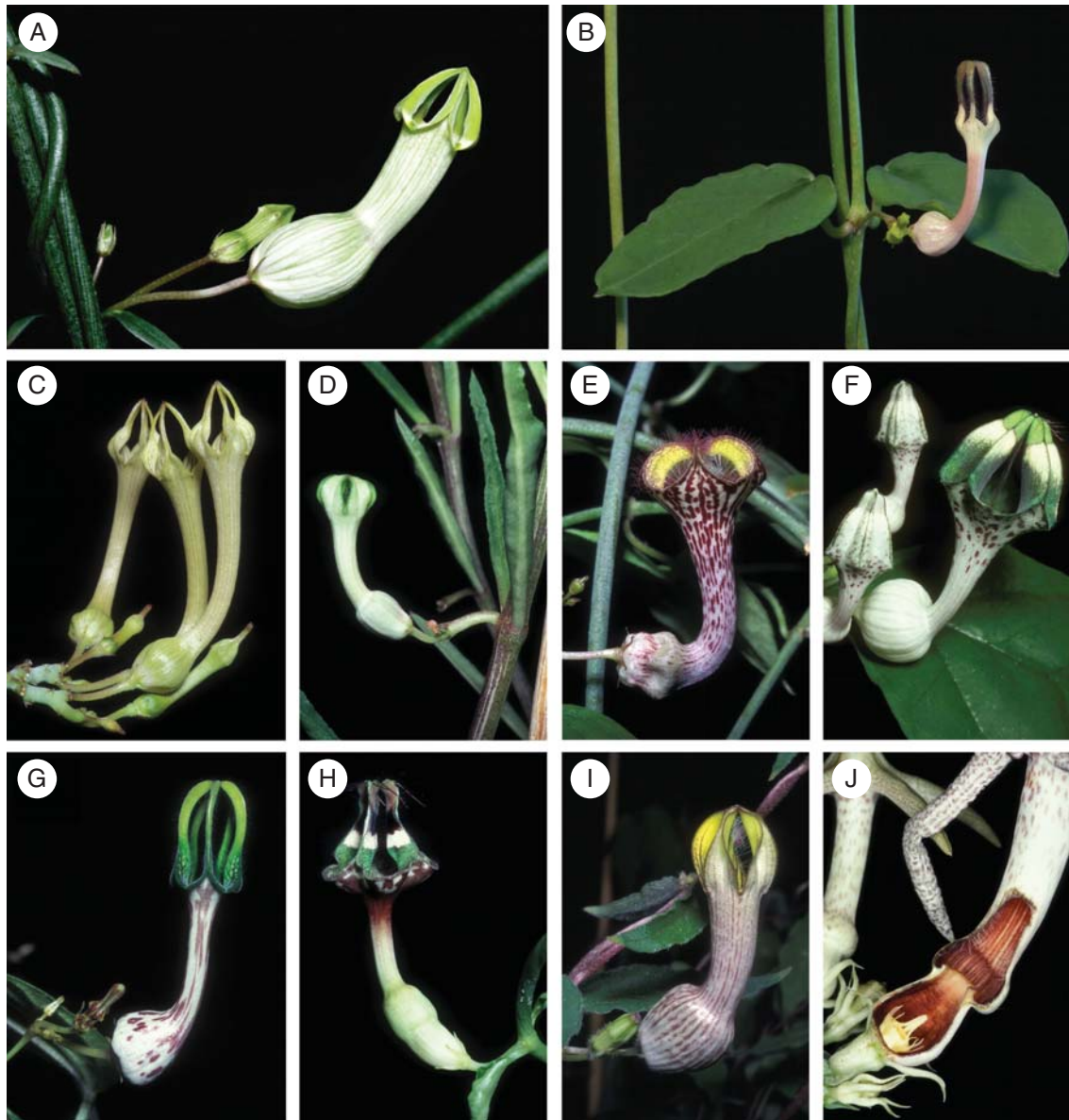


FIG. 1. Representative taxa of *Ceropegia* illustrating floral diversity in the genus: (A) *C. ampliata*; (B) *C. imbricata*; (C) *C. aristolochioides* subsp. *aristolochioides*; (D) *C. crassifolia* var. *copleyae*; (E) *C. affinis*; (F) *C. sankuruensis*; (G) *C. meyeri-johannis*; (H) *C. denticulata*; (I) *C. cufodontii*; and (J) *C. variegata* (basal inflation opened, exposing corona). Photographs: U. Meve.

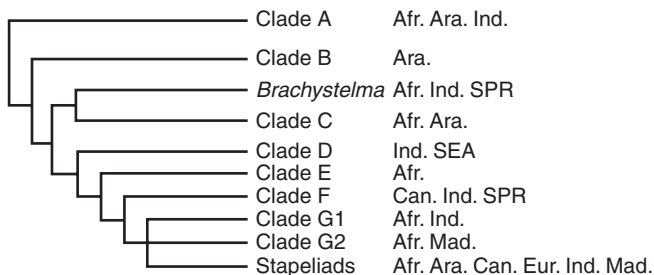


FIG. 2. Summary phylogeny of *Ceropegia*, showing the major clades and their relationship to the genus *Brachystelma* and the stapeliads, and the distribution of those clades (adapted from Meve and Liede-Schumann, 2007). Distribution abbreviations: Afr. = Africa; Ara. = Arabian Peninsula; Can. = Canary Islands; Eur. = Europe; Ind. = Indian Subcontinent; Mad. = Madagascar; SEA = south-east Asia; SPR = south-western Pacific Region.

Data analysis

Some of the flies in the published literature and from the authors' own collections were identified to species level. However, the taxonomy of tropical small-sized Diptera is so poorly studied that it was decided to limit the analyses to the level of genus and above. The data on Diptera–*Ceropegia* interactions were therefore classified into four levels of taxonomic–functional resolution:

- (i) Flies that have been identified to family level and which may or may not have been carrying pollinaria (i.e. all flower visitors).
- (ii) Flies that have been identified to family level and which carried pollinaria (i.e. pollinators only).

- (iii) Flies that have been identified to genus level and which may or may not have been carrying pollinaria (i.e. all flower visitors).
- (iv) Flies that have been identified to genus level and which carried pollinaria (i.e. pollinators only).

The largest of these four categories is I, encompassing virtually all of the records that we possess, because most flies could be determined to family level at least. The most restrictive category is IV – relatively few of the flies determined to genus level carried pollinaria. Whilst it is important to work with as large a sample size as possible, it is categories II and IV that are of most interest from the perspective of pollination biology. Nonetheless, flies that did not carry pollinaria also represent species interactions, between *Ceropegia* and flies that may be legitimate pollinators, but which had not yet picked up pollinaria; or flies which may be parasitic on *Ceropegia*, e.g. gall-forming Cecidomyiidae (see Results). An important determinant of whether or not a fly acts as a pollinator of *Ceropegia* is likely to be its size: the fly must be small enough to manoeuvre down the narrowed corolla tube and within the flower chamber, but also large (and strong) enough to be able to remove pollinaria (see Results). The results are therefore presented with appropriate qualifiers as to the status of the Diptera as flower visitors or confirmed pollinators.

Phylogenetic analysis

Ceropegia, with some 180 species, represents the largest taxon within the tribe Ceropegieae, followed by *Brachystelma* R.Br. (approx. 100 species). The tribe consists of about 770 species altogether, distributed over four subtribes and 46 genera (Meve and Liede, 2004; Endress *et al.*, 2007). The largest subtribe, the Stapeliinae (the stem-succulent stapeliads) corresponds to the terminal clade in the tribal phylogeny, with all genera separated by rather short genetic distances (Meve and Liede, 2004). In a recent molecular analysis of *Ceropegia* by Meve and Liede-Schumann (2007), based on non-coding markers of cpDNA (trnT-L and trnL-F spacers and the trnL intron) and nrDNA (ITS), the taxa investigated scatter over a grade of seven clades (labelled A–G, Fig. 2). The taxa that group together on these clades often lack morphological similarity, and show different distribution areas (e.g. Africa and Asia), pointing to a complex biogeographical history of the whole genus. On the contrary, of the 13 investigated *Ceropegia* taxa of East Africa, ten belong to clade C, and all the species endemic to Madagascar belong to clade G2 (Fig. 2). Asian taxa, however, are underrepresented in this study. The phylogenetic analysis suggests that *Ceropegia*, despite its morphological conformity, is twice paraphyletic. One of the seven clades retrieved is shared by some *Ceropegia* and all *Brachystelma* species investigated (Fig. 2), making *Ceropegia* without *Brachystelma* paraphyletic. Furthermore, all endemic Madagascan *Ceropegia* taxa investigated, plus the East African *C. robynsiana* and a subclade comprising *C. bulbosa* and *C. konasita*, share a terminal (but in most analyses unresolved) clade with the stapeliads (Fig. 2). Thus again, *Ceropegia* without the stapeliads is paraphyletic; both *Brachystelma* and the stapeliad group emerged from within *Ceropegia* as traditionally circumscribed. In the absence of adequate morphological characters supporting

taxonomical reclassification of the groups involved into monophyletic units, Meve and Liede-Schumann (2007) proposed to hold on to the current taxonomy, since the genus *Ceropegia* is convincingly characterized by its pitfall flowers. Consequently, the phylogeny of *Ceropegia* and the Stapeliinae remains not as fully understood and more complex than that of other tribes of Asclepiadoideae (cf. Liede-Schumann *et al.*, 2005; Rapini *et al.*, 2006). In this study, for the sake of brevity, those clades which split earliest from the phylogeny (i.e. clades A, B and C in Fig. 2) are referred to as ‘basal’ or ‘basal-most’ and the remaining clades as ‘derived’, with the understanding that this makes no value judgement on their ‘primitiveness’ or otherwise.

RESULTS

Flower visitors and pollinators of Ceropegia

The vast majority of visitors to *Ceropegia* flowers, and all of the confirmed pollinators, are small species of Diptera (Table S1 in Supplementary data available online; ASCLEPOL database). Those Diptera for which the gender could be determined were overwhelmingly female (550 of 584 insects = 94.2%). Of the flies that carried pollinaria, 81 of 83 specimens (97.6%) carried them on their mouthparts; the remaining two flies carried them on a foreleg and the prothorax. The non-dipteran flower occupants included sap-sucking Aphididae and Cixiidae (Homoptera), Cicadellidae (Heteroptera), Collembola, herbivorous micro-Lepidoptera, and various families of parasitoidal Hymenoptera, including Chalcidoidea and Mymaridae (the latter frequently laying their eggs on the eggs of Homoptera; Noyes, 2003). Ants (Hymenoptera: Formicidae) were also occasionally found (Ollerton, 1999).

The size range of the Diptera was 0.5–4.0 mm in length; however, on average, the flies that were found with pollinaria attached were significantly larger than flies without pollinaria [mean \pm s.d. (n) = 1.8 \pm 0.6 mm (67) versus 1.6 \pm 0.6 mm (153); z -test: z = 29.8, $P \ll 0.01$]. This suggests that some of the smallest Diptera (those less than approx. 0.8 mm, the lower range of pollinaria-carrying flies) are unlikely to be large enough to remove pollinaria. There was a significant positive correlation between fly size and the size of the pollinia being carried (Spearman's r = 0.46, n = 30, P = 0.01; Fig. 3) and between fly size and the length of the basal inflation of the corolla tube (Spearman's r = 0.39, n = 48, $P \ll 0.006$; Fig. 4), supporting the idea that the function of flies as pollinators is constrained by their size, as both basal inflation and pollinia size are correlated with overall flower size (Spearman Rank Correlations: flower length vs. size of basal inflation: r = 0.71, n = 47, $P \ll 0.0001$; flower length vs. pollinia length \times width: r = 0.35, n = 25, P = 0.08). Thus flies that are too small cannot remove pollinaria, whereas flies that are too large would not be able to manoeuvre within the basal inflation.

Taxonomic diversity and functional ecology of Ceropegia pollinators

Based on the data available so far, *Ceropegia* taxa are known to interact with at least 26 genera of Diptera belonging

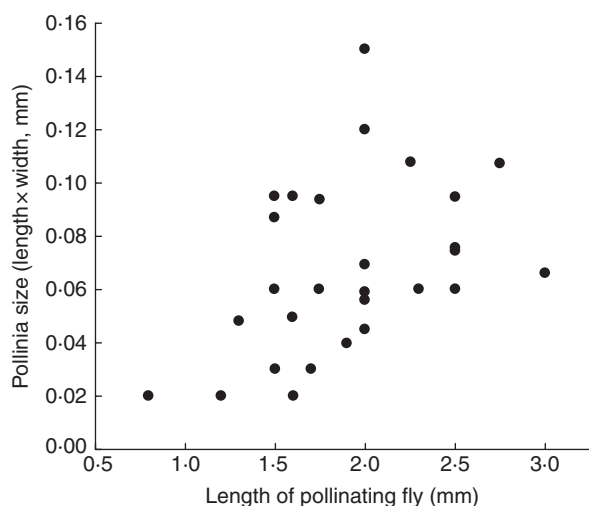


FIG. 3. Relationship between fly size (length) and size of the pollinia carried (pollinia length \times width) for Diptera pollinating *Ceropegia* spp.

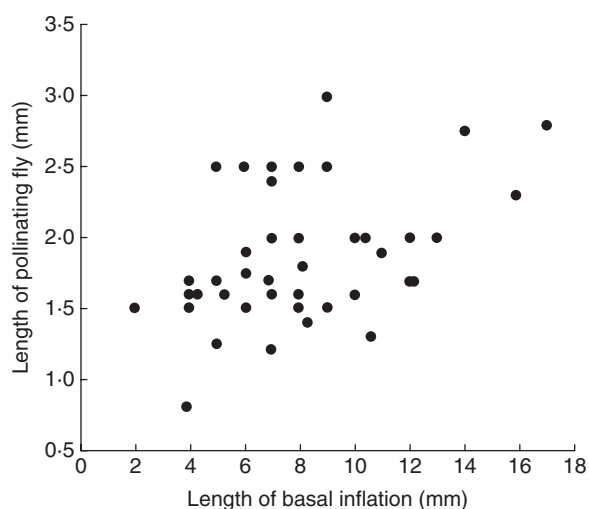


FIG. 4. Relationship between the size of the basal inflation of *Ceropegia* flowers and the size of the flies trapped within them.

to 20 families (Table S1 in Supplementary data available online; Table 2). Of these flower visitors, 11 genera and 11 families are known to act as pollinators. This high taxonomic diversity of pollinators will undoubtedly increase as more data become available for *Ceropegia* spp. included in this study (confirming flower visitors as pollinators) and for the remaining species of *Ceropegia* that have not been studied. Some of the species removed from the Kew specimens are new to science (Disney, 2009).

Of the non-pollinating Diptera in Table 2, some families are repeatedly found in *Ceropegia* flowers but never carry pollinaria and may never be part of the pollinating fauna of these taxa. For example, Cecidomyiidae (found in six taxa) may be visiting flowers to lay eggs because their larvae are gall-making parasites, or predators of mites or of other insects (see Ollerton, 1996).

Given the wide phylogenetic range of Diptera families it is no surprise that the functional ecologies of these flies are similarly diverse and range from taxa that feed on living plant and fungus material, to carrion, dung and rotting vegetation feeders, to commensals and parasitoids of other insects (Table S2 in Supplementary data available online). However, the larvae of most taxa feed on decaying organic material of some description.

The biogeographic perspective

The broadest diversity of flower-visiting and pollinating families and genera of Diptera is found in the Arabian Peninsula region, where *Ceropegia* taxa are known to interact with 16 families (eight known to pollinate) and 16 genera (seven known to pollinate) of flies (Table 2). However, a significant amount of that diversity is attributable to a single taxon, *Ceropegia aristolochioides* ssp. *deflersiana*, that interacts with at least 15 families of flies in this region (Table S1 in Supplementary data available online). A closely related taxon, *C. aristolochioides* ssp. *aristolochioides* similarly interacts with six families in East Africa, so this may be a feature of this particular species (see also ‘The phylogenetic perspective’ below), which correlates with the unusually large variability of flower morphology present in both subspecies of *C. aristolochioides* (Meve *et al.*, 2001). Within the ranges of the two subspecies there exist numerous distinct variants which apparently freely interbreed and for which there is no clear geographical pattern of distribution (Masinde, 2006).

East Africa and southern Africa have similar levels of diversity of Diptera that interact with *Ceropegia* flowers, while the remaining regions (West Africa, Madagascar and Asia) are all relatively depauperate in terms of the diversity of *Ceropegia*–Diptera interactions (Table 2 – though see caveats regarding sampling effort below). Some Diptera families and genera repeatedly interact with *Ceropegia* as pollinators across all or most regions; e.g. Ceratopogonidae (*Forcipomyia*), Milichiidae (*Desmometopa*), the hyper-diverse genus *Megaselia* (Phoridae), and Chloropidae. Other families and genera are restricted to only one or two regions. Apart from the high diversity in the Arabian Peninsula discussed above, however, there is no distinct pattern of biogeographic occurrence of *Ceropegia* taxa interacting with particular families or genera in different biogeographic regions (Table 2).

The phylogenetic perspective

The basal-most clade A of *Ceropegia* (Fig. 2; Meve and Liede-Schumann, 2007) interacts with the highest diversity of Diptera families (at least 16) and genera (at least 24) as flower visitors (Table 3). Fewer taxa are confirmed pollinators (seven families and six genera) but this diversity is nonetheless greater than for any of the other ‘*Ceropegia*’ clades labelled in Fig. 2. Uneven sampling between clades (see below) means that comparisons must be tentatively made; however, comparing clade A with the more abundantly sampled, but more-derived clade C suggests that the higher diversity found in the basal-most clade is not an artefact of sampling (Table 3). The two most-derived clades (G1 and G2; Fig. 2) differ in

TABLE 2. Records of Diptera families and genera as flower visitors and pollinators of *Ceropegia* spp. in the main regions of diversity of the genus

Region	Genera*		Families*	
	Flower visitors	Pollinators	Flower visitors	Pollinators
Arabian Peninsula	<i>Allotrichoma</i> <i>Amauromyza</i> <i>Asteia</i> <i>Atrichopodon</i> <i>Chaetosciara</i> (?) <i>Chlororhinia</i> (?) <i>Desmometopa</i> <i>Diclasioipa</i> <i>Forcipomyia</i> <i>Goniurella</i> <i>Hecamedoides</i> <i>Leptomotopa</i> <i>Lygistorrhina</i> <i>Megaselia</i> <i>Meoneura</i> <i>Phyllomyza</i> <i>Phytomyptera</i> <i>n</i> = 6 (11) [†]	<i>Allotrichoma</i> <i>Atrichopodon</i> <i>Chlororhinia</i> (?) <i>Desmometopa</i> <i>Forcipomyia</i> <i>Leptomotopa</i> <i>Phytomyptera</i> <i>n</i> = 6 (9)	Agromyzidae Asteiidae Calliphoridae Carnidae Cecidomyiidae Ceratopogonidae Chironomidae Chloropidae Drosophilidae Ephydriidae Lygistorrhinidae Milichiidae Phoridae Sciaridae Tachinidae Tephritidae	Calliphoridae Ceratopogonidae Chloropidae Drosophilidae Ephydriidae Milichiidae Sciaridae Tachinidae
East Africa	<i>Asteia</i> <i>Bradysia</i> (?) <i>Dasyhelea</i> <i>Desmometopa</i> <i>Forcipomyia</i> <i>Leptomotopa</i> <i>Megaselia</i> <i>Milichiella</i> <i>Phyllomyza</i> <i>Rhexoza</i> <i>Stomosis</i> <i>n</i> = 20 (42)	<i>Desmometopa</i> <i>Forcipomyia</i> <i>Leptomotopa</i> <i>Megaselia</i> <i>n</i> = 6 (8)	<i>n</i> = 8 (22) Asteiidae Cecidomyiidae Ceratopogonidae Chloropidae Drosophilidae Milichiidae Psychodidae Phoridae Scatopsidae Sciaridae	<i>n</i> = 7 (13) Ceratopogonidae Chloropidae Drosophilidae Milichiidae Sciaridae
Asia	<i>Desmometopa</i> <i>Forcipomyia</i> <i>n</i> = 2 (3)	<i>Desmometopa</i> <i>Forcipomyia</i> <i>n</i> = 2 (3)	<i>n</i> = 26 (67) Ceratopogonidae Chloropidae Milichiidae <i>n</i> = 3 (4)	<i>n</i> = 13 (19) Ceratopogonidae Milichiidae
Madagascar	<i>Leptomotopa</i> <i>Stomosis</i> <i>Desmometopa</i> <i>n</i> = 2 (3)	<i>Desmometopa</i> <i>Leptomotopa</i> <i>Stomosis</i> <i>n</i> = 2 (3)	<i>n</i> = 3 (4) Cecidomyiidae Chloropidae Milichiidae <i>n</i> = 4 (6)	<i>n</i> = 2 (3) Milichiidae
Southern Africa	<i>Brachypogon</i> <i>Desmometopa</i> <i>Drapetis</i> <i>Forcipomyia</i> <i>Leptomotopa</i> <i>Megaselia</i> <i>Neophyllomyza</i> <i>Rhexoza</i> <i>Swammerdamella</i>	<i>Desmometopa</i> <i>Forcipomyia</i> <i>Megaselia</i> <i>Rhexoza</i> <i>Swammerdamella</i>	Cecidomyiidae Ceratopogonidae Chironomidae Chloropidae Drosophilidae Hybotidae Milichiidae Phoridae Scatopsidae Sciaridae <i>n</i> = 16 (29)	<i>n</i> = 2 (3) Ceratopogonidae Chloropidae Drosophilidae Milichiidae Scatopsidae
West Africa	<i>n</i> = 5 (8) <i>Desmometopa</i> <i>Forcipomyia</i> <i>Leptomotopa</i> <i>Megaselia</i> <i>n</i> = 4 (5)	<i>n</i> = 5 (8) <i>Desmometopa</i> <i>Forcipomyia</i> <i>Leptomotopa</i> <i>Megaselia</i> <i>n</i> = 4 (5)	<i>n</i> = 16 (29) Ceratopogonidae Chloropidae Milichiidae Mycetophilidae Phoridae Sciaridae <i>n</i> = 11 (14)	<i>n</i> = 7 (9) Ceratopogonidae Chloropidae Milichiidae Phoridae

*Families and genera were considered pollinators if pollinaria were found attached to the flies. The names are arranged in alphabetical order within cells.

[†]*n* is the number of *Ceropegia* taxa contributing to that cell; the number following it in parenthesis is the number of accessions of those taxa, i.e. records of *Ceropegia* flowers at a particular locality that have been found to contain insects.

their patterns of Diptera diversity; despite very similar sample sizes, the largely Madagascan clade G2 interacts with a diversity of flower-visiting families and genera, whereas all of the

African and Indian Subcontinent clade G1 species so far sampled are pollinated by flies of the genus *Forcipomyia* (Ceratopogonidae; Table 3).

TABLE 3. Records of Diptera families and genera as flower visitors to the major clades of *Ceropegia*

Clade	Families*	Genera*
A	Agromyzidae	<i>Amauromyza</i>
	Asteiidae	<i>Asteia</i>
	Calliphoridae	<i>Chlororhinia?</i>
	Carnidae	<i>Meoneura</i>
	Cecidomyiidae	Undetermined genus
	Ceratopogonidae	<i>Atrichopodon</i>, <i>Forcipomyia</i>
	Chironomidae	Undetermined genus
	Chloropidae	Undetermined – possibly >1 genus
	Drosophilidae	Undetermined – possibly >1 genus
	Ephyridae	<i>Hecamedoides</i>, <i>Diclasiope</i>, <i>Allotrichoma</i>
	Lygistorrhinidae	<i>Lygistorrhina</i>
	Milichiidae	<i>Phyllomyza</i>, <i>Stomosis</i>, undetermined genus
	Phoridae	<i>Megaselia</i>
	Sciaridae	<i>Chaetosciara?</i>, undetermined genus
Tachinidae	<i>Phytomyptera</i>	
Tephritidae	<i>Goniurella</i>	
	(7/22) [†]	
B [‡]	(0/0)	
C	Cecidomyiidae	Undetermined genus
	Ceratopogonidae	<i>Forcipomyia</i>, <i>Dasyhelea</i>, <i>Brachypogon</i>
	Chironomidae	Undetermined genus
	Chloropidae	Undetermined – possibly >1 genus
	Drosophilidae	Undetermined genus
	Hybotidae	<i>Drapetis</i>
	Milichiidae	<i>Desmometopa</i>, <i>Milichiella</i>, <i>Leptometopa</i>, <i>Neophyllomyza</i>
	Phoridae	<i>Megaselia</i>
	Scatopsidae	<i>Rhexozia</i>, <i>Swammerdamella</i>
	Sciaridae	Undetermined genus
	(23/60)	
D	Milichiidae	<i>Desmometopa</i>
	(1/1)	
E	Psychodidae	Undetermined genus
	Phoridae	<i>Megaselia</i>
	(1/2)	
F	Chloropidae	Undetermined genus
	Mycetophilidae	Undetermined genus
	Sciaridae	Undetermined genus
	(3/4)	
G1	Ceratopogonidae	<i>Forcipomyia</i>
	(4/9)	
G2	Cecidomyiidae	Undetermined genus
	Chloropidae	Undetermined – possibly >1 genus
	Milichiidae	<i>Desmometopa</i>, <i>Leptometopa</i>, <i>Stomosis</i>
	Sciaridae	<i>Bradysia?</i> , undetermined – possibly >1 genus
	(6/12)	

*Families and genera which are confirmed as pollinators are presented in bold. The names are arranged in alphabetical order by family within cells.

[†]The numbers in parentheses are sample sizes: number of taxa of *Ceropegia* (species and subspecies) followed by number of accessions (see text for details). Only taxa that could be firmly assigned to clades have been included.

[‡]No flower visitor data are available for clade B which is included only for completeness.

Specificity of *Ceropegia*–pollinator interactions

The specificity of interactions between *Ceropegia* and its flower visitors was examined by using three approaches: (1) *Ceropegia* taxa that had multiple samples were assessed to look at specificity within and between regions (Table 4); (2) the frequency with which more than one Diptera family or genus was found in an individual *Ceropegia* flower was analysed to look at specificity at

particular sites on specific dates (Fig. 5); and (3) the few cases where pollinators had been found in species sampled from the wild and in cultivation were compared (Table 5).

From Table 4, the proportion of the 17 terminal taxa (counting the *C. linearis* complex as a single taxon) that show evidence of conservatism in their interactions with particular fly genera as flower visitors or pollinators is approx. 60% (10 of 17 taxa). For example, *Ceropegia nilotica* is so far known to interact only with flies of the genus *Desmometopa* (Milichiidae) across multiple samples from West Africa and East Africa, whilst *C. bulbosa* interacts with flies only in the genus *Forcipomyia* (Ceratopogonidae) in Asia and the Arabian Peninsula (Table 4). The remaining 40% (7 of 17 taxa) appear to be rather less conservative in their interactions, such as *C. arabica* var. *powysii*, which interacts with flies belonging to four genera within samples from East Africa, and *C. aristolochioides* ssp. *deflersiana* which is known to be highly variable in its interactions in the Arabian Peninsula (Table 4).

From a sub-sample of 46 *Ceropegia* flowers from the Kew collection, over half (54%) contained only a single individual fly. The frequency distribution was strongly right skewed with over one-third (37%) of flowers containing two to six flies and only single examples of flowers that when opened contained 9, 10, 11 and 21 flies. Of the 135 *Ceropegia* accessions, 25 (18.5%) contained more than one genus of fly, either in the same flower or from a different flower in that collection. Therefore most species blooming at a particular locality in a given season interacted with and were pollinated by only a single genus of Diptera (Fig. 5).

Ceropegia is a genus that is actively collected and grown by horticultural enthusiasts and botanical gardens. In the ASCLEPOL database, there are data on flies trapped in 20 *Ceropegia* taxa in cultivation from 26 accessions. For two of these taxa (the *C. linearis* complex and varieties of *C. stapeliiformis*) there are enough data to compare patterns of interaction with Diptera within their native range of southern Africa and in cultivation in Europe (Table 5). Taxa within the *C. linearis* complex are remarkably conservative in their exploitation of pollinators and are known only to interact with Diptera of the genus *Forcipomyia* (Ceratopogonidae), reinforcing the results in Table 4. In contrast, varieties of *C. stapeliiformis* in the wild and in cultivation in southern Africa are pollinated by flies belonging to *Leptometopa* (Milichiidae), whereas in Europe they are known to be pollinated by at least three genera in the families Chloropidae and Milichiidae (Table 5; Vogel, 1961). The limited evidence from these cultivated examples strengthens the conclusion that some taxa of *Ceropegia* are strict specialists, and conservative in their use of pollinators at least at the genus level, whereas other taxa are much less conservative and attract a range of different genera and families of Diptera that serve as pollinators.

The effect of sampling effort on the results

The number of Diptera families and genera known to be flower visitors and pollinators of *Ceropegia* taxa in different regions is strongly affected by sampling effort (i.e. the number of *Ceropegia* taxa for which there are data and number of accessions per taxon). For example, across the

TABLE 4. Comparisons of *Ceropegia* flower visitors and pollinators (Diptera genera) for taxa with three or more accessions, within and between biogeographic regions

Clade	<i>Ceropegia</i> taxon	Accession*	Region	Genera [†]
A	<i>C. aristolochioides</i> ssp. <i>deffersiana</i>	K44108	AP	Chlororhinia? , Atrichopodon , Allotrichoma , Amauromyza , Chaetosciara? , Hecamedoides , Diclasioipa , Meoneura
		K53946	AP	Asteia , Phyllomyza
		K45745	AP	Phytomyptera , Diclasioipa
		K39277	AP	Lygistorrhina , Megaselia
		K44111	AP	Goniurella
		K58573	EA	Stomosis
C	<i>C. arabica</i> var. <i>abbreviata</i>	F604	EA	Forcipomyia
		K47780	AP	Leptometopa
	<i>C. arabica</i> var. <i>superba</i>	K49831	AP	Leptometopa , Desmometopa
		K47779	AP	Desmometopa
	<i>C. arabica</i> var. <i>powysii</i>	K49406	AP	Desmometopa
		K47779	AP	Desmometopa
	<i>C. nilotica</i>	SM686	EA	Forcipomyia
		SM828	EA	Leptometopa , Milichiela
		F727	EA	Desmometopa
		K40459	EA	Desmometopa
		K1930	EA	Desmometopa
		K3989	EA	Desmometopa
		K56450	EA	Desmometopa
		K51019	EA	Desmometopa
		K22867	WA	Desmometopa
		K23284	WA	Desmometopa
	<i>C. denticulata</i>	K50488	SA	Desmometopa , Neophyllomyza , Forcipomyia
		K58558	EA	Desmometopa
		K5303	EA	Desmometopa
	<i>C. stenantha</i>	K58533	EA	Desmometopa
		K47158	EA	Desmometopa
		K1920	SA	Desmometopa , Swammerdamella , Drapetis
		K1921	SA	Swammerdamella
		K3983	SA	Rhexoza , Brachypogon
		K3982	SA	Rhexoza
		K1045	EA	Rhexoza
	<i>C. linearis</i> ssp. <i>tenuis</i>	K53328	EA	Dasyhelea
		K40179	EA	Dasyhelea
	<i>C. linearis</i> ssp. <i>woodii</i>	K49196	SA	Forcipomyia
		K49209	SA	Forcipomyia
	<i>C. linearis</i>	SM389/2	EA	Forcipomyia
		K58544	EA	Forcipomyia
	<i>C. meyeri-johannis</i>	K6715	EA	Forcipomyia
		K3975	EA	Forcipomyia
		M&L3368	EA	Forcipomyia
		B872	EA	Forcipomyia
G1	<i>C. bulbosa</i>	Bhatnagar (1986)	A	Forcipomyia
		Ali (1994)	A	Forcipomyia
	<i>C. imbricata</i>	K54293	AP	Forcipomyia
		K54293	EA	Forcipomyia
G2	<i>C. albisepta</i>	A404	EA	Forcipomyia
		Sabrosky (1987)	M	Leptometopa
	<i>C. ballyana</i>	RM31	M	Stomosis
		K47775	EA	Leptometopa
	<i>C. papillata</i>	K58598	EA	Leptometopa
		K29852	EA	Bradysia?
?	<i>C. papillata</i>	K1037	EA	Forcipomyia
		K3975	EA	Forcipomyia
		K3403	EA	Forcipomyia
		K1039	EA	Forcipomyia
		K3405	EA	Leptometopa
		K1922	SA	Forcipomyia
		K3985	SA	Forcipomyia

Continued

TABLE 4. *Continued*

Clade	<i>Ceropegia</i> taxon	Accession*	Region	Genera [†]
	<i>C. filipendula</i>	K3400	EA	<i>Phyllomyza</i>
		K3401	EA	<i>Leptomelopa</i>
	<i>C. claviloba</i>	K1040	EA	<i>Forcipomyia</i>
		K1033	SA	<i>Forcipomyia</i>

*Accessions from the Kew Herbarium are prefixed K, other prefixes refer to collectors, and references refer to previously published data.

[†]Confirmed pollinators are shown in bold. Species which cannot presently be assigned to a clade are indicated by a ‘?’.

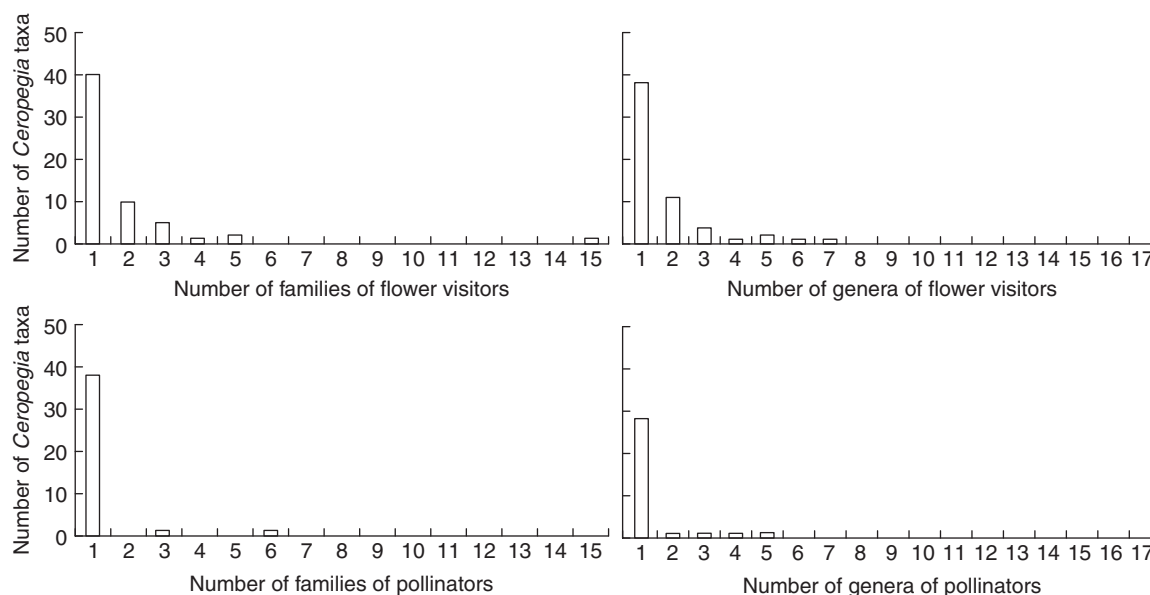


FIG. 5. Frequency distributions of the number of genera (right-hand graphs) and families (left-hand graphs) of Diptera known to interact as flower visitors (top graphs) and confirmed as pollinators (bottom graphs) of *Ceropegia* species, subspecies and natural varieties ('taxa' on the y-axes).

biogeographic zones, the number of pollinating genera per region is marginally significantly correlated with number of *Ceropegia* taxa studied from that region (Spearman Rank Correlation: $r = 0.77$, $n = 6$, $P = 0.07$) and significantly correlated with the number of accessions from each region ($r = 0.84$, $n = 6$, $P = 0.04$). Similar results were found for number of genera as flower visitors and for numbers of families of visitors and pollinators (results not shown). In short, variation in the number of taxa or accessions in these analyses makes it difficult to compare between taxa and regions, a long-standing problem in ecological sampling generally and studies of pollination ecology specifically (Ollerton and Cranmer, 2002; Herrera, 2005). This limits our ability to say definitively whether, for example, *Ceropegia* taxa in the Arabian Peninsula really do interact with a wider diversity of fly families than *Ceropegia* taxa in East Africa (Table 2) and whether basal clades interact with a broader range of Diptera than more-derived clades (Table 3), or, alternatively, if these patterns are due to the larger number of taxa and accessions of Arabian Peninsula and basal-most *Ceropegia* in the present data set. One way to control for sampling artefacts is to compare pairs of *Ceropegia* taxa that have a similar number of accessions in

the Arabian Peninsula with other regions. Similarly, one can compare *Ceropegia* taxa from different clades that have similar levels of sampling. These comparisons should give an indication of whether the observed patterns of relative diversity of pollinators have a real biological basis or are an artefact of different sampling efforts in different regions and across clades (Tables 6 and 7).

For the biogeographic comparison, the results show that the higher diversity of Arabian Peninsula *Ceropegia* pollinators is largely an artefact, not of sampling *per se*, but of the influence on the data set of the hyper-generalist taxon *Ceropegia aristolochioides* ssp. *deflersiana* (Table 6). On a per-accession basis, other taxa have very similar diversities of interacting Diptera families and genera in the Arabian Peninsula compared with other regions.

For the clade contrasts, given a similar level of sampling effort, taxa in derived clades can be as specialized or generalist in their interactions with Diptera as the basal clades (Table 7). In all cases, the sampling effort in these examples is three or four accessions per taxon, except for *C. albisepta* that is included as an example of a member of a derived clade (G2) that interacts with four genera of Diptera despite low sampling effort ($n = 2$ accessions).

TABLE 5. Examples of *Ceropegia* taxa interacting with pollinating Diptera within compared with outside (in cultivation) their native ranges

<i>Ceropegia</i> taxon	Region	Families of pollinators	Genera of pollinators
<i>C. linearis</i>	East Africa	Ceratopogonidae	<i>Forcipomyia</i>
<i>C. linearis</i>	East Africa	Ceratopogonidae	<i>Forcipomyia</i>
<i>C. linearis</i>	East Africa	Ceratopogonidae	<i>Forcipomyia</i>
<i>C. linearis</i>	East Africa	Ceratopogonidae	<i>Forcipomyia</i>
<i>C. linearis</i> ssp. <i>tenuis</i>	Southern Africa	Ceratopogonidae	<i>Forcipomyia</i>
<i>C. linearis</i> ssp. <i>woodii</i>	Southern Africa	Ceratopogonidae	<i>Forcipomyia</i>
<i>C. linearis</i>	Europe	Ceratopogonidae	<i>Forcipomyia</i>
<i>C. linearis</i> ssp. <i>woodii</i>	Europe	Ceratopogonidae	<i>Forcipomyia</i>
<i>C. stapeliiformis</i> var. <i>serpentina</i>	Southern Africa	Milichiidae	<i>Leptometopa</i>
<i>C. stapeliiformis</i> var. <i>serpentina</i>	Southern Africa	Milichiidae	<i>Leptometopa</i>
<i>C. stapeliiformis</i> var. <i>serpentina</i>	Southern Africa (cultivation)	Milichiidae	<i>Leptometopa</i>
<i>C. stapeliiformis</i>	Europe	Chloropidae	<i>Conioscinella</i>
<i>C. stapeliiformis</i>	Europe	Milichiidae	<i>Madiza</i> , <i>Tricimba</i>

Details of the references and accessions are to be found in the ASCLEPOL database.

TABLE 6. Flower visitor diversity (Diptera families and genera) of *Ceropegia* taxa in the Arabian Peninsula contrasted with other biogeographic regions

<i>Ceropegia</i> taxa	Region	No. of accessions	No. of families	No. of genera
<i>C. aristolochioides</i> ssp. <i>deflersiana</i>	Arabian Peninsula	9	15	18
<i>C. aristolochioides</i> ssp. <i>aristolochioides</i>	East Africa	7	6	6
<i>C. arabica</i> var. <i>abbreviata</i>	Arabian Peninsula	2	1	1
<i>C. bulbosa</i>	Asia	2	1	1
<i>C. arabica</i> var. <i>arabica</i>	Arabian Peninsula	3	2	2
<i>C. arabica</i> var. <i>superba</i>	Arabian Peninsula	3	1	1
<i>C. arabica</i> var. <i>powysii</i>	East Africa	4	3	5
<i>C. variegata</i>	Arabian Peninsula	1	1	1
<i>C. variegata</i>	East Africa	1	1	1

DISCUSSION

TABLE 7. Comparison of the effect of sampling effort (number of accessions) on the diversity of Diptera families and genera recorded for relatively basal and more-derived clades of *Ceropegia* (see Fig. 2)

<i>Ceropegia</i> taxa	Clades	No. of accessions	No. of families	No. of genera
Basal clades				
<i>C. denticulata</i>	C	4	1	1
<i>C. meyeri-johannis</i>	C	3	1	1
<i>C. arabica</i> var. <i>arabica</i>	C	3	2	≥2
<i>C. arabica</i> var. <i>superba</i>	C	3	1	1
<i>C. arabica</i> var. <i>powysii</i>	C	4	3	5
<i>C. variegata</i>	C	3	3	≥3
<i>C. linearis</i>	C	3	1	1
<i>C. denticulata</i>	C	4	1	1
Derived clades				
<i>C. imbricata</i>	G1	3	1	1
<i>C. bulbosa</i>	G1	3	1	1
<i>C. ballyana</i>	G2	3	3	≥3
<i>C. robynsiana</i>	G2	4	2	≥2
<i>C. albisepta</i>	G2	2	3	4

The floral Bauplan of *Ceropegia* comprises a fused corolla that is more or less tubular, often partially closed at the mouth to form a cage, and frequently with a basally inflated region. However, there is enormous variation on this theme, with species and subspecies varying considerably in size, colour, shape, odour and ornamentation (Fig. 1). Despite this variation, as far as is known the large majority of flower visitors and all confirmed pollinators are small Diptera, usually female, and typically <3 mm in length. This confirms earlier work on pollination within a much smaller subset of the genus (Vogel, 1961; Bhatnagar, 1986; Sabrosky, 1987; van der Meutter, 1988; Ollerton and Forster, 1995; Masinde, 2004) but does not preclude discovering pollination by other insect orders (e.g. Coleoptera) in *Ceropegia* spp. that have not yet been studied. Insects such as aphids (Hemiptera) and small wasps (Hymenoptera) that are occasionally found in *Ceropegia* flowers probably feed on floral tissues or developing seeds. Other infrequent flower occupants are ants (Hymenoptera: Formicidae) whose scavenging, omnivorous habits suggests that their presence in *Ceropegia* flowers may be to steal nectar or to scout possible food sources (indeed one individual was preserved in the act of consuming a trapped fly; see Ollerton, 1999).

Ceropegia is therefore an example of a large genus that has diversified despite an apparently functionally specialized (*sensu* Fenster *et al.*, 2004; Ollerton *et al.*, 2007), conservative pollination system which exploits only small Diptera. However, the Diptera that are known to interact with *Ceropegia* spp. have a taxonomic span of at least 26 genera belonging to 20 families, with a large range of life histories and ecologies (Tables S1 and S2 in Supplementary data available online). The overwhelming majority of these flies were female and it seems reasonable to infer that *Ceropegia* pollination systems are essentially brood site deception systems in which the flowers mimic substrates for egg laying, as is found in other ‘trap flower’ genera such as *Arisaema* (Vogel and Martens, 2000). Identifying the models that different *Ceropegia* taxa are mimicking is not straightforward because human sensory modes differ from those of Diptera. Larval feeding substrates of some *Ceropegia*-associated fly families includes carrion and dung (Calliphoridae), and rotting plant and fungus material (Ceratopogonidae, Drosophilidae, Milichiidae, Scatopsidae, Sciaridae). The occurrence of the predominantly aquatic Ephydriidae and parasitoidal Tachinidae as pollinators of *C. aristolochioides* subsp. *defflersiana* deserves mention and may be due to the uncharacteristically ecologically generalized (*sensu* Fenster *et al.*, 2004; Ollerton *et al.*, 2007) nature of this taxon’s interactions with its pollinators (Table 4 and Table S2 in Supplementary data available online). Why this should be the case is not known and deserves further study, given its variable flower morphology (Meve *et al.*, 2001).

Pollinaria placement was in almost all cases on the mouthparts of the flies, a phylogenetically conservative trait within *Ceropegia* (e.g. Vogel, 1961; Masinde, 2004), and the Ceropegieae as a whole (Meve and Liede, 1994; Ollerton and Liede, 1997; Ollerton *et al.*, 2003) although it does occur in other clades (e.g. Ollerton and Liede, 2003; Ollerton *et al.*, 2003; Yamashiro *et al.*, 2008).

Floral scent has been suggested to be the most important cue for distant attraction of the pollinators in *Ceropegia* (Vogel, 1961) and is produced by specialized epithelia (‘osmophores’) located on the upper side of the tips of the corolla lobes. In some species of *Ceropegia*, flower scents can be detected by the human nose, while other species are apparently unscented. Ongoing analyses of floral scent in *Ceropegia* species should improve our understanding of the role of scent in the pollination biology of the genus (S. Dötterl, A. Jürgens and U. Meve, unpubl. res.). For example, flower morphology and colour varies much between *Ceropegia* species visited by the same fly species (J. Ollerton *et al.*, unpubl. res.), and it may be that scent is the main mode of attraction for at least some members of this genus. In which case, how have these complex flower (non-scent) phenotypes evolved? What is the role of specific corolla colours, vibratile hairs, and so forth? Are they effective attractive cues in *Ceropegia*? Or are they just ‘accessory attractants’ for short-distance attraction as Vogel (1961) presumed?

The proportion of *Ceropegia* taxa for which there are flower visitor or pollinator data varies considerably from region to region (Table 1). The African regions and the Arabian Peninsula are strongly represented in terms of proportion of described *Ceropegia* taxa. In contrast there are fewer data available for Asia and Madagascar. Therefore any

biogeographic conclusions that are drawn regarding the use of particular genera and families as pollinators of *Ceropegia* must perforce be preliminary. In fact, across the whole of the distributional range of *Ceropegia*, a rather similar suite of flies is exploited as pollinators and several fly genera and families re-occur as *Ceropegia* pollinators from region to region (Table 2). This suggests some level of evolutionary conservatism in the use of a phylogenetically varied group of Diptera as pollinators, and may be due to the fact that these fly genera are typically widespread and common, mainly occurring as larvae on decaying plant and animal material (Table S2 in Supplementary Data, available online). At the moment there is no strong evidence that *Ceropegia* in particular regions have specialized on particular fly genera or families. The one exception is that Arabian Peninsula *Ceropegia* exploit a much wider range of Diptera families and genera than those in other regions (Table 2). However, this result is due largely to the influence of a single taxon, *C. aristolochioides* spp. *defflersiana* that interacts with and is pollinated by a wider range of Diptera families than any other taxon (Table S1 and Table 5). A closely related subspecies, *C. aristolochioides* spp. *aristolochioides* occurs in East Africa and is not visited by such a range of families, despite a similar level of sampling effort (Table S1); this is unlikely to be due to biogeographic differences in fly faunas as East Africa is adjacent to the Arabian Peninsula and none of these Diptera families (and genera where known) are endemic to the regions. The most likely explanation is therefore that *C. aristolochioides* spp. *defflersiana* has locally adapted to being pollinated by a diverse range of Diptera, possibly by losing the ‘private signals’ to specific pollinators that may characterize other *Ceropegia* (see below).

The two most basal clades for which there are data (clades A and C) interact with a broader range of Diptera families and genera than do the more-derived clades D to G2 (Table 3). If confirmed, this would be an intriguing finding as it suggests that the evolution of the more-derived groups of *Ceropegia* has been driven in part by specialization to particular fly families or genera. However, there are three caveats to that suggestion. First of all, if the basal-most clades are older than the more-derived clades and they could possibly occupy a wider range of ecological communities (though it is noted that basal clades are not more widely distributed than derived clades; see Fig. 2). Secondly, for some clades the diversity of pollinators is either unknown (clade B) or under-sampled (e.g. clades D and E), making any conclusions regarding phylogenetic shifts in specialization preliminary. Finally, sampling effort (numbers of taxa and accessions with pollinator data) is significantly greater in the basal-most clades compared with the more-derived clades (Table 1) and that has almost certainly affected the present findings. In fact, detailed consideration of these results in relation to sampling effort implies that species in the derived clades are no more specialized in their interactions, on average, than the basal clades (Table 7). On the other hand, the basal clades contain both very specialized and hyper-generalized *Ceropegia* taxa, and so it is possible that the variance in diversity of pollinators per taxon may be greater in the basal clades compared with the derived clades (Table S1 in Supplementary Data, available online).

It is clear from the molecular phylogenetic evidence that trap flower pollination has evolved and been lost several times within the tribe Ceropegieae. The genus *Riocreuxia*, once synonymized with *Ceropegia* but now known to be a member of subtribe Anisotominae and therefore only distantly related (Meve, 1998; Meve and Liede, 2004; Masinde, 2005) also possesses trap flowers that are very similar to those of *Ceropegia*; small Diptera, as yet unidentified, have been recovered from flowers (Masinde, 2005; J. Ollerton, unpubl. res.). In addition, the paraphyletic relationship of *Ceropegia* to *Brachystelma* and the stapeliads (Meve and Liede-Schumann, 2007) suggests that trap flowers have been lost at least twice. However, within *Brachystelma* and the stapeliads, there are species that possess at least some of the features of trap flowers such as a tubular corolla that is longer than wide or corolla tips fused into a cage, e.g. *Brachystelma stenophyllum* amongst others, and stapeliads such as some *Echidnopsis* spp. and *Stapeliopsis urniflora*. Trap-flower pollination therefore appears to be an evolutionarily labile strategy. The transition from Diptera pollination of trap flowers to open flowers has evidently been accompanied by a shift in pollinator identity. Although not as yet evaluated using gas chromatographic analyses, few if any *Ceropegia* species produce the fetid floral odours produced by many of the stapeliads (Jurgens *et al.*, 2006) and some *Brachystelma* species. This correlates with the utilization primarily of carrion- and faeces-breeding Calliphoridae and Muscidae by the stapeliads (Meve and Liede, 1994) compared with the more varied and generally smaller Diptera employed by *Ceropegia* taxa. Interestingly there are no examples of New World fly-pollinated asclepiads with trap flowers (e.g. within the Gonolobinae; Ollerton and Liede, 1997), making this an example of convergent evolution that is both phylogenetically and biogeographically constrained within the Apocynaceae.

Pollination systems similar to that of *Ceropegia*, which utilize temporarily trapped Diptera as pollen vectors, have evolved several times in unrelated plant families such as Araceae and Aristolochiaceae. These taxa apparently have

highly convergent pollination systems, with flowers or functional floral units which share traits such as flask-shaped corollas (or their analogues in the Araceae) that retain the Diptera, 'light windows' that affect the behaviour of the flies, dull or sombre colouration, complex corolline appendages or hairs, and odours that are often fetid or fermented. Although there is compelling evidence that these pollination systems are examples of convergent evolution, in fact the Diptera that are used as pollen vectors differ significantly across some of these genera (Table 8). In the Araceae, *Arisaema* mainly exploits fungus-breeding flies (Vogel and Martens, 2000) whilst *Arum* species mimic a range of larval food sites including dung and rotting fruit, with some species using beetles (Coleoptera) as pollen vectors (Gibernau *et al.*, 2004). It is the genus *Aristolochia* (Aristolochiaceae) that is most similar to *Ceropegia* in the fly families it uses as pollen vectors (Table 8); there is an extraordinary similarity in floral morphology and behaviour between some members of these genera that strongly suggests convergent evolution to attract similar flies.

Specializing on small flies as pollinators might be considered to be a risky and possibly ineffectual strategy for a plant. However, in temperate communities at least, small dipteran pollinators can be very abundant and active for the entire flowering season (e.g. Ollerton and Diaz, 1999). In contrast much less is known about tropical dipteran pollinators, largely due to the tendency for entomologists and insect ecologists to neglect such small, cryptic taxa (Kearns, 2001; Larson *et al.*, 2001). Concerns about pollinator extinctions have for the most part focussed on large, obvious species such as birds, bees and moths (Kearns, 2001). However, the role of small Diptera as pollinators for some significantly diverse clades of flowering plants underscores the conservation value of understanding these flies and their interactions with genera such as *Ceropegia*, which is far from being an exceptional case. Within other major clades of the asclepiads, flies belonging to diverse families and genera are important pollinators (Meve and Liede, 1994; Ollerton and Liede, 1997) and

TABLE 8. Comparison of the most commonly encountered pollen-carrying Diptera families of *Ceropegia* with those of other well studied trap flower genera (in addition to the major pollen vectors, other fly families are less frequently encountered in all plant genera)

Family	Genus	Major pollen vectors	References
Apocynaceae	<i>Ceropegia</i>	Ceratopogonidae Chloropidae Milichiidae	This study
Araceae	<i>Arisaema</i>	Mycetophilidae Sciaridae	Vogel and Martens (2000)
	<i>Arum</i>	Ceratopogonidae Drosophilidae Psychodidae Sphaeroceridae	Gibernau <i>et al.</i> (2004)
Aristolochiaceae	<i>Aristolochia</i>	Calliphoridae Ceratopogonidae Chloropidae Drosophilidae Milichiidae Phoridae	Wolda and Sabrosky (1986), Hall and Brown (1993), Trujillo and Sersic (2006), Sakai (2002), Burgess <i>et al.</i> (2004), Razzak <i>et al.</i> (1992), Bänziger and Disney (2006), J. Ollerton (unpubl. res.)

recent studies have reinforced these findings. For example, Diptera are significant pollinators within the *Tylophora–Vincetoxicum* complex in Japan and appear to be responsible for some speciation within the group (Yamashiro *et al.*, 2008), whilst Medeiros *et al.* (2008) have identified disease-carrying black flies (Simuliidae) as pollinators of *Tassadia* spp. in Brazil. The asclepiads are not unique in this respect and research continually identifies ‘unusual’ Diptera as pollinators (and agents of selection on floral phenotype) of diverse genera; for example, fungus gnat (Mycetophilidae)-pollinated *Mitella* spp. (Saxifragaceae) (Okuyama *et al.*, 2008). The biodiversity of plant–pollinator interactions continues to surprise us in its breadth and novelty.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxfordjournals.org/ and consist of the following. Table S1: *Ceropegia* taxa and their Diptera flower visitors. Table S2: Synopsis of the higher-level phylogenetic positions of Diptera families known to be flower visitors to *Ceropegia*, together with their associated genera, and information about the feeding ecology of these flies (where known).

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LITERATURE CITED

- Ali T. 1994. *Pollination ecology of some asclepiads (Asclepiadaceae) from Pakistan*. PhD Thesis, University of Karachi.
- Armbruster WS. 1993. Evolution of plant pollination systems – hypotheses and tests with the neotropical vine *Dalechampia*. *Evolution* **47**: 1480–1505.
- Armbruster WS. 2009. Associations between floral specialization and species diversity: cause, effect, or correlation? *Evolutionary Ecology* **23**: 159–179.
- Bänziger H, Disney RHL. 2006. Scuttle flies (Diptera: Phoridae) imprisoned by *Aristolochia baenzigeri* (Aristolochiaceae) in Thailand. *Bulletin de la Société Entomologique Suisse* **79**: 29–61.
- Bhatnagar S. 1986. On insect adaptations for pollination in some asclepiads of Central India. In: Kapil RP, ed. *Pollination biology – an analysis*. New Delhi: Inter-India Publications, 37–57.
- Bruneau A. 1996. Phylogenetic and biogeographical patterns in *Erythrina* (Leguminosae: Phaseoleae) as inferred from morphological and chloroplast DNA characters. *Systematic Botany* **21**: 587–605.
- Bruneau A. 1997. Evolution and homology of bird pollination syndromes in *Erythrina* (Leguminosae). *American Journal of Botany* **84**: 54–71.
- Burgess KS, Singfield J, Melendez V, Kevan PG. 2004. Pollination biology of *Aristolochia grandiflora* (Aristolochiaceae) in Veracruz, Mexico. *Annals of the Missouri Botanical Garden* **91**: 346–356.
- Disney RHL. 2009. Insects of Arabia scuttle flies (Diptera: Phoridae) Part II: the genus *Megaselia*. *Fauna of Arabia* (in press).
- Dold AP. 2006. *Ceropegia macmasteri* (Apocynaceae-Asclepiadoideae-Ceropegieae), a new species from Eastern Cape, South Africa. *South African Journal of Botany* **72**: 144–146.
- Endress P. 1996. *Diversity and evolutionary biology of tropical flowers*. Cambridge: Cambridge University Press.
- Endress ME, Liede-Schumann S, Meve U. 2007. Advances in Apocynaceae: the enlightenment, an introduction. *Annals of the Missouri Botanical Garden* **94**: 259–267.
- Fenster CB, Armbruster WS, Wilson P, Dudash MR, Thomson JD. 2004. Pollination syndromes and floral specialization. *Annual Review of Ecology, Evolution and Systematics* **35**: 375–403.
- Gibernau M, Macquart D, Przetak G. 2004. Pollination in the genus *Arum* – a review. *Aroideana* **27**: 148–166.
- Goldblatt P, Savolainen V, Porteous O, *et al.* 2002. Radiation in the Cape flora and the phylogeny of peacock irises *Moraea* (Iridaceae) based on four plastid DNA regions. *Molecular Phylogenetics and Evolution* **25**: 341–360.
- Grant V. 1949. Pollination systems as isolating mechanisms in angiosperms. *Evolution* **3**: 82–97.
- Hall DW, Brown BV. 1993. Pollination of *Aristolochia littoralis* (Aristolochiales) by males of *Megaselia* spp. (Diptera, Phoridae). *Annals of the Entomological Society of America* **86**: 609–613.
- Herrera CM. 2005. Plant generalization on pollinators: species property or local phenomenon? *American Journal of Botany* **92**: 13–20.
- Johnson SD. 1996. Pollination, adaptation and speciation models in the Cape Flora of South Africa. *Taxon* **45**: 59–66.
- Jurgens A, Dötterl S, Meve U. 2006. The chemical nature of fetid floral odours in stapeliads (Apocynaceae-Asclepiadoideae-Ceropegieae). *New Phytologist* **172**: 452–468.
- Kay KM, Schemske DW. 2008. Natural selection reinforces speciation in a radiation of Neotropical rainforest plants. *Evolution* **62**: 2628–2642.
- Kearns CA. 2001. North American dipteran pollinators: assessing their value and conservation status. *Conservation Ecology* **5**: 5 (URL: <http://www.consecol.org/vol5/iss1/art5/>).
- Knuth P. 1909. *Handbook of flower pollination*, Vol. 3. Oxford: Clarendon Press.
- Larson BMH, Kevan PG, Inouye DW. 2001. Flies and flowers: taxonomic diversity of anthophiles and pollinators. *The Canadian Entomologist* **133**: 439–465.
- Liede-Schumann S, Rapini A, Goyder DJ, Chase MW. 2005. Phylogenetics of the New World subtribes of Asclepiadeae (Apocynaceae-Asclepiadoideae): Metastelmatinae, Oxypetalinae, and Gonolobinae. *Systematic Botany* **30**: 184–195.
- Malpure NV, Kamble MY, Yadav SR. 2006. A new species of *Ceropegia* L. (Asclepiadaceae) from the Western Ghats of India with a note on series *Attenuatae* Huber. *Current Science* **91**: 1140–1142.
- Marussich WA, Machado CA. 2007. Host-specificity and coevolution among pollinating and nonpollinating New World fig wasps. *Molecular Ecology* **16**: 1925–1946.
- Masinde PS. 2004. Trap-flower fly pollination in East African *Ceropegia* L. (Apocynaceae). *International Journal of Tropical Insect Science* **24**: 55–72.
- Masinde PS. 2005. A revision of the African genus *Riocreuxia* Decne. (Apocynaceae: Asclepiadoideae-Ceropegieae). *Kew Bulletin* **60**: 401–434.
- Masinde PS. 2006. Species concepts in *Ceropegia* L. (Apocynaceae-Ceropegieae): a case example of *Ceropegia aristolochioides* Decne. In: Ghazanfarand S, Beentje HJ, eds. *Taxonomy and ecology of African plants, their conservation and sustainable use*. London: Royal Botanic Gardens, Kew, 583–589.
- Medeiros JF, Rapini A, Barbosa UC, Py-Daniel V, Braga PIS. 2008. First record of Simuliidae (Diptera) with pollinaria of Asclepiadoideae (Apocynaceae) attached. *Neotropical Entomology* **37**: 338–341.

- van der Meutter L. 1988. Notes and photographs: fly visitors to flowers – possible pollinators in cultivation. *Asklepios* **44**: 19–25.
- Meve U. 1998. *Emplectanthus* N.E.Br.: a close relative of *Riocreuxia* Decne. in the Asclepiadaceae–Stapeliaceae. *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie* **120**: 123–130.
- Meve U, Liede S. 1994. Floral biology and pollination in stapeliads – new results and a literature review. *Plant Systematics and Evolution* **192**: 99–116.
- Meve U, Liede S. 2004. Subtribal division of Ceropegieae (Apocynaceae–Asclepiadoideae). *Taxon* **53**: 61–72.
- Meve U, Liede-Schumann S. 2007. *Ceropegia* (Apocynaceae, Ceropegieae, Stapeliinae): paraphyletic but still taxonomically sound. *Annals of the Missouri Botanical Garden* **94**: 392–406.
- Meve U, Masinde PS, Sentner U, Liede S. 2001. RAPD analysis and taxonomic reconsideration of the *Ceropegia aristolochioides* complex (Apocynaceae–Ceropegieae). *Plant Biology* **3**: 622–628.
- Noyes JS. 2003. Universal Chalcidoidea Database, British Museum of Natural History [http://www.nhm.ac.uk/research-curation/projects/chalcidoids] accessed 11 March 2007.
- Okuyama Y, Pellmyr O, Kato M. 2008. Parallel floral adaptations to pollination by fungus gnats within the genus *Mitella* (Saxifragaceae). *Molecular Phylogenetics and Evolution* **46**: 560–575.
- Ollerton J. 1996. Interactions between gall midges (Diptera: Cecidomyiidae) and inflorescences of *Piper novae-hollandiae* (Piperaceae) in Australia. *The Entomologist* **115**: 181–184.
- Ollerton J. 1999. Fly trapping in *Ceropegia* flowers – evidence of ant predation of pollinators. *Asklepios* **77**: 31–32.
- Ollerton J, Cranmer L. 2002. Latitudinal trends in plant–pollinator interactions: are tropical plants more specialised? *Oikos* **98**: 340–350.
- Ollerton J, Diaz A. 1999. Evidence for stabilising selection acting on flowering time in *Arum maculatum* (Araceae): the influence of phylogeny on adaptation. *Oecologia* **119**: 340–348.
- Ollerton J, Forster P. 1995. Diptera associated with flowers of *Ceropegia cumingiana* in Australia. *Asklepios* **66**: 21–22.
- Ollerton J, Liede S. 1997. Pollination systems in the Asclepiadaceae: a survey and preliminary analysis. *Biological Journal of the Linnean Society* **62**: 593–610.
- Ollerton J, Liede S. 2003. Corona structure in *Cynanchum*: linking morphology to function. *Ecotropica* **9**: 107–112.
- Ollerton J, Johnson SD, Cranmer L, Kellie S. 2003. The pollination ecology of an assemblage of grassland asclepiads in South Africa. *Annals of Botany* **92**: 807–834.
- Ollerton J, Killick A, Lamborn E, Watts S, Whiston M. 2007. Multiple meanings and modes: on the many ways to be a generalist flower. *Taxon* **56**: 717–728.
- Rapini A, Chase MW, Konno TUP. 2006. Phylogenetics of South American Asclepiadoideae (Apocynaceae). *Taxon* **55**: 119–124.
- Razzak MA, Ali T, Ali SI. 1992. The pollination biology of *Aristolochia bracteolata* Lamk. (Aristolochiaceae). *Pakistan Journal of Botany* **24**: 79–87.
- Ronsted N, Weiblen GD, Cook JM, Salamin N, Machado CA, Savolainen V. 2005. 60 million years of co-divergence in the fig-wasp symbiosis. *Proceedings of the Royal Society series B–Biological Sciences* **272**: 2593–2599.
- Ronsted N, Salvo G, Savolainen V. 2007. Biogeographical and phylogenetic origins of African fig species (*Ficus* section *Galoglychia*). *Molecular Phylogenetics and Evolution* **43**: 190–201.
- Sabrosky CW. 1987. A new species of *Leptometopa* (Diptera, Milichiidae) from Madagascar pollinating *Ceropegia* (Asclepiadaceae). *Proceedings of the Entomological Society of Washington* **89**: 242–243.
- Sakai S. 2002. *Aristolochia* spp. (Aristolochiaceae) pollinated by flies breeding on decomposing flowers in Panama. *American Journal of Botany* **89**: 527–534.
- Stebbins GL. 1970. Adaptive radiation of reproductive characteristics in Angiosperms. 1. Pollination mechanisms. *Annual Review of Ecology and Systematics* **1**: 307–326.
- Trujillo CG, Sersic AN. 2006. Floral biology of *Aristolochia argentina* (Aristolochiaceae). *Flora* **201**: 374–382.
- Vogel S. 1961. Die Bestäubung der Kesselfallen-Blüten von *Ceropegia*. *Beiträge zur Biologie der Pflanzen* **36**: 159–237.
- Vogel S. 1993. Betrug bei Pflanzen: Die Täuschblumen. *Akademie der Wissenschaften und der Literatur, Mainz. Abhandlungen der mathematisch-naturwissenschaftlichen Klasse* 1993(1): 5–48.
- Vogel S, Martens J. 2000. A survey of the function of the lethal kettle traps of *Arisaema* (Araceae), with records of pollinating fungus gnats from Nepal. *Botanical Journal of the Linnean Society* **133**: 61–100.
- Waser NM. 1998. Pollination, angiosperm speciation, and the nature of species boundaries. *Oikos* **82**: 198–201.
- Wilson P, Castellanos MC, Hogue JN, Thomson JD, Armbruster WS. 2004. A multivariate search for pollination syndromes among penstemons. *Oikos* **104**: 345–361.
- Wolda H, Sabrosky CW. 1986. Insect visitors to 2 forms of *Aristolochia pilosa* in Las Cumbres, Panama. *Biotropica* **18**: 295–299.
- Wyatt R, Broyles SB. 1994. Ecology and evolution of reproduction in milkweeds. *Annual Review of Ecology and Systematics* **25**: 423–441.
- Yamashiro T, Yamashiro A, Yokoyama J, Maki M. 2008. Morphological aspects and phylogenetic analyses of pollination systems in the *Tylophora-Vincetoxicum* complex (Apocynaceae–Asclepiadoideae) in Japan. *Biological Journal of the Linnean Society* **93**: 325–341.