SPECIAL INVITED PAPER—EVOLUTION OF PLANT MATING SYSTEMS

POLLINATION AND MATING SYSTEMS OF APODANTHACEAE
AND THE DISTRIBUTION OF REPRODUCTIVE TRAITS
IN PARASITIC ANGIOSPERMS

SIDONIE BELLOT2 AND SUSANNE S. RENNER2

Systematic Botany and Mycology, University of Munich (LMU), Menzinger Str. 67 80638 Munich, Germany

• Premise of the study: The most recent reviews of the reproductive biology and sexual systems of parasitic angiosperms were published 17 yr ago and reported that dioecy might be associated with parasitism. We use current knowledge on parasitic lineages and their sister groups, and data on the reproductive biology and sexual systems of Apodanthaceae, to readdress the question of possible trends in the reproductive biology of parasitic angiosperms.

• Methods: Fieldwork in Zimbabwe and Iran produced data on the pollinators and sexual morph frequencies in two species of Apodanthaceae. Data on pollinators, dispersers, and sexual systems in parasites and their sister groups were compiled from the literature.

• Key results: With the possible exception of some Viscaceae, most of the ca. 4500 parasitic angiosperms are animal-pollinated, and ca. 10% of parasites are dioecious, but the gain and loss of dioecy across angiosperms is too poorly known to infer a statistical correlation. The studied Apodanthaceae are dioecious and pollinated by nectar- or pollen-foraging Calliphoridae and other flies.

• Conclusions: Sister group comparisons so far do not reveal any reproductive traits that evolved (or were lost) concomitant with a parasitic life style, but the lack of wind pollination suggests that this pollen vector may be maladaptive in parasites, perhaps because of host foliage or flowers borne close to the ground.

Key words: Apodanthaceae; dioecy; field observations; fly pollination; phylogeny; sexual system.

A ground-breaking study published 5 years ago revealed that among flowering plants, parasitism evolved at least 13 times (Barkman et al., 2007), namely in the Apodanthaceae, Balanophoroaceae, Cassytha (Lauraceae), Cuscuta (Convolvulaceae), Cynomoriaceae, Cytinus (Cytinaceae), Hydnoraceae, Krameria (Krameriaceae), Lennoaceae, Mitrastemon (Mitrastemonaceae), Rafflesiaceae, higher Santalales, and Orobanchaceae (excluding the first-branching genus Lindenbergia; Fig. 1). Together, these clades may comprise some 4500 species, 2040 of them in the Orobanchaceae and another 2100 in the Santalales (Table 1 provides species numbers and references). The phylogenetic placements of 12 of the 13 parasite lineages found by Barkman et al. (2007) have been upheld in studies with denser gene or taxon sampling (Nickrent, 2007; Vidal-Russell and Nickrent, 2008a, b; Filipowicz and Renner, 2010). Only the placement of Cynomoriaceae, which was unsupported in the preferred topology of Barkman et al., remains unclear. The single genus of this family has been placed in Myrtales, Rosales, Santalales, Saxifragales, and Sapindales (Nickrent et al., 2005; Jian et al., 2008; Qiu et al., 2010; Zhang et al., 2009), but the situation is not yet settled (Zhang et al., 2011).

With the independent parasitic lineages of flowering plants now clear, it is timely to return to the question of traits that may be associated with the evolution of parasitism. This question was last addressed in 1995 in a review of the reproductive biology of parasitic angiosperms that found no discernible trends unique to parasitic plants (Molau, 1995). The review followed the classification most accepted at the time (Cronquist, 1988), which lumped several parasite groups since revealed to be independent, such as Apodanthaceae and Rafflesiaceae, and Balanophoroaceae and Cynomoriaceae, while separating others since revealed to have a single ancestor, such as Eremolepidaceae and Santalaceae. The focus of the review also was not evolutionary since relevant sister group relationships were not yet known.

An obvious parallel evolutionary trend in parasitic angiosperms is the reduction of the capacity to photosynthesize. The loss of photosynthetic capacity is complex because genes coding for photosynthesis are partly located in the chloroplast genome, partly in the nucleus, and plastids are involved in other metabolic pathways than those directly related to photosynthesis (Neuhaus and Emes, 2000; Krause, 2012). All holoparasites investigated so far probably therefore still have a plastid genome (Table 1; column 4). Other life history or morphological traits that could have undergone parallel evolution in parasites concern the timing of reproduction (especially in seasonal
dioecious species, only 1% contained parasitic species (Renner and Ricklefs, 1995). A seeming correlation between dioecy and a parasitic life style was also noted in the flora of the southeastern United States: ca. 17% of Carolina heterotrophs and mycoheterotrophs are dioecious (Conn et al., 1980). More recently, an analysis of diversification rates in parasitic plants (Hardy and Cook, 2012) revealed that a parasitic life style may contribute to high extinction risks (for hosts and parasites alike), and this risk might be compounded by a dioecious sexual system in which species may suffer a competitive disadvantage because only half of the individuals in a dioecious population are seed bearing (Heilbuth et al., 2001; Vamosi and Vamosi, 2004). It would therefore be surprising if dioecy were indeed overrepresented among parasitic angiosperms.

Here we use today’s data on the placement of the 13 parasite families and their reproductive biology to readdress the question of pollination. For example, animal pollination might be more reliable than wind pollination if parasite flowers are often located near the ground or under host foliage.

Because most parasitic lineages can only be studied in natural conditions, not in the laboratory or botanical gardens, knowledge of their reproductive biology is patchy. Pollinators of Cynomoriumaceae and Lennoaceae have never been observed, and the first data on the pollination of Apodanthaceae are reported in the present study. Parallel or convergent evolution in the reproductive modes of parasitic angiosperms is thus an open question. In terms of sexual systems, a study published 17 yr ago suggested an overrepresentation of dioecy among parasitic angiosperm genera. This was based on the finding that of 959 angiosperm genera with dioecious species, at least 43 (4%) also contained parasitic species. Of the angiosperm genera lacking dioecious species, only 1% contained parasitic species (Renner and Ricklefs, 1995). A seeming correlation between dioecy and a parasitic life style was also noted in the flora of the southeastern United States: ca. 17% of Carolina heterotrophs and mycoheterotrophs are dioecious (Conn et al., 1980). More recently, an analysis of diversification rates in parasitic plants (Hardy and Cook, 2012) revealed that a parasitic life style may contribute to high extinction risks (for hosts and parasites alike), and this risk might be compounded by a dioecious sexual system in which species may suffer a competitive disadvantage because only half of the individuals in a dioecious population are seed bearing (Heilbuth et al., 2001; Vamosi and Vamosi, 2004). It would therefore be surprising if dioecy were indeed overrepresented among parasitic angiosperms.

Here we use today’s data on the placement of the 13 parasite families and their reproductive biology to readdress the question of pollination. For example, animal pollination might be more reliable than wind pollination if parasite flowers are often located near the ground or under host foliage.
<table>
<thead>
<tr>
<th>Parasitic clades: No. of genera/No. of species</th>
<th>Flower phenotype</th>
<th>Population sexual system, Minimum no. of dioecious species</th>
<th>Plastid genome investigated</th>
<th>Pollination</th>
<th>Fruit or seed dispersal</th>
<th>Longevity</th>
<th>Sister group and no. of genera/ no. of species</th>
<th>Flower phenotype in sister group</th>
<th>Population sexual system in sister group</th>
<th>Pollination in sister group</th>
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</thead>
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<tr>
<td>Apodanthaceae: 2/18</td>
<td>Unisexual</td>
<td>Monocious or dioecious 5</td>
<td>Ongoing: <em>Pilostyles aethiopica</em> and <em>P. hamiltonii</em> (this study)</td>
<td>Birds or other animals eating small fruits</td>
<td>Rodents, rain wash 6</td>
<td>Unknown</td>
<td>Cucurbitales (all or some) up to 2600 species in 109 genera</td>
<td>Mostly unisexual</td>
<td>Mostly monocious</td>
<td>Biotic or abiotic</td>
</tr>
<tr>
<td>Balknophoraceae: 16/42</td>
<td>Unisexual</td>
<td>Monocious or dioecious 4, 21</td>
<td>None</td>
<td>Flies, wasps, ants, moths, beetles 65; bees 66; bats 65</td>
<td></td>
<td></td>
<td>Other Santalales 106–138/1552–3304 parasites + 11–1344–83 autotrophs</td>
<td>Bisexual or unisexual 4</td>
<td>Hermaphrodite, monocious or dioecious (or other sexual systems) 8</td>
<td>Nectar-foraging birds, moths, bees, wasps, flies 48</td>
</tr>
<tr>
<td>Casytha (Lauraceae): 1/21</td>
<td>Bisexual 12</td>
<td>Hermaphrodite 12</td>
<td>None</td>
<td>Nectar-foraging animals 12</td>
<td>Perennial 14</td>
<td>Lauraceae without Cryptocaryae ca. 40/1730 57</td>
<td>Unisexual or bisexual 15</td>
<td>Hermaphrodite, monocious or dioecious 15</td>
<td>Nectar-foraging short-tongued insects 43</td>
<td></td>
</tr>
<tr>
<td>Cuscuta (Convolvulaceae): 1/200</td>
<td>Bisexual 42</td>
<td>Hermaphrodite 42</td>
<td>Published: <em>Cuscuta reflexa</em>, <em>C. exaltata</em>, <em>C. obtusiflora</em>, <em>C. gronovii</em> 65, 54</td>
<td>Birds 61, 58, water 50</td>
<td>Mostly annual 12, 43</td>
<td>Somewhere in Convolvulaceae 52</td>
<td>Mostly bisexual but also unisexual 7</td>
<td>Hermaphrodite, some dioecious 7</td>
<td>Nectar-foraging animals</td>
<td></td>
</tr>
<tr>
<td>Cynonorium (= Cynonoriaceae): 1/1</td>
<td>Unisexual and bisexual 16</td>
<td>Monocious or dioecious 16, 6</td>
<td>None</td>
<td>Flies feeding on pollen?</td>
<td>Possibly rain 58</td>
<td>Perennial 17</td>
<td>Some Saxifragales lineage?</td>
<td>?</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Cytophanteae: 2/10</td>
<td>Unisexual or bisexual 18</td>
<td>Monocious, polygamomonoecious 50, or dioecious 13, 6</td>
<td>None</td>
<td>Rodents and ants 19, beetles 65</td>
<td>Mechanical 68</td>
<td>Perennial 8</td>
<td>Muntingiaceae 3/35</td>
<td>Bisexual 20</td>
<td>Hermaphrodite 20</td>
<td>Bees 21</td>
</tr>
<tr>
<td>Hydroraceae: 2/9</td>
<td>Bisexual 7</td>
<td>Hermaphrodite 7</td>
<td>None</td>
<td>Based on morphology and scent, prob. flies 60 or beetles 22, 65</td>
<td>Mammals 52</td>
<td>Perennial 13</td>
<td>Aristolochiaceae 5–8/480 57</td>
<td>Bisexual 25, 7</td>
<td>Hermaphrodite 25, 7</td>
<td>Typically flies 80</td>
</tr>
<tr>
<td>Krameria (= Krameriaceae): 1/18</td>
<td>Bisexual 27</td>
<td>Hermaphrodite 27</td>
<td>None</td>
<td>Oil-collecting bees 27</td>
<td>Nuts barbed with spines 27</td>
<td>Perennial 7</td>
<td>Zygophyllaceae 22/285 57</td>
<td>Mostly bisexual 7</td>
<td>Hermaphrodite or dioecious 7</td>
<td>Insects 7</td>
</tr>
<tr>
<td>Lennoaceae 2/4</td>
<td>Bisexual 27</td>
<td>Hermaphrodite 27</td>
<td>None</td>
<td>Probably Lepidoptera 62</td>
<td>Wind, rain 62, 63</td>
<td>Perennial and annual 17, 53</td>
<td>Ehretiaceae 8/170 57</td>
<td>Bisexual or unisexual 18</td>
<td>Hermaphrodite or dioecious 11</td>
<td>Insects, maybe sometimes abiotic 30</td>
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<td>Mitrastemon (= Mitrastemonaceae): 1/2</td>
<td>Bisexual 12</td>
<td>Hermaphrodite 12</td>
<td>None</td>
<td>Meliphagidae 1</td>
<td>Birds, rain 12</td>
<td>Perennial 8</td>
<td>Ericales excluding Balsaminaceae, Maregraviaceae, and Tetrameristaceae 9</td>
<td>Hermaphrodite, monocious or dioecious 34</td>
<td>Mostly animal-pollinated 49</td>
<td></td>
</tr>
<tr>
<td>Orobancheae (excluding Lindenbergia): 9/6/2040 33</td>
<td>Bisexual 12</td>
<td>Hermaphrodite 12</td>
<td>Published: <em>Epifagus virginianus</em> 60, ongoing: 7 other species 69</td>
<td>Nectar-foraging bees 58</td>
<td>Grazing mammals 18, rain 12</td>
<td>Perennial and annual 12</td>
<td>Lindenbergia (Orobanchaceae) 3–30</td>
<td>Bisexual</td>
<td>Hermaphrodite</td>
<td>Nectar-foraging bees</td>
</tr>
<tr>
<td>Parasitic clades:</td>
<td>Flower phenotype</td>
<td>Population sexual system, Minimum no. of dioecious species</td>
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<td>Pollination in sister group</td>
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<tr>
<td>Rafflesiaceae:</td>
<td>Bisexual or unisexual</td>
<td>None</td>
<td>Ovipositing flies</td>
<td>Mammals</td>
<td>Perennial</td>
<td>Euphorbiaceae 218/5735</td>
<td>Unisexual</td>
<td>Monocious or dioecious</td>
<td>Mostly biotic</td>
<td></td>
</tr>
<tr>
<td>Santalales (Amphorogynaceae, Aiptanidae, Cervantesiaceae, Comandraeaceae, Loranthaceae, Misodendraceae, Nanodaceae, Octoknemaceae, Olacaceae, Opiliaceae, Santalaceae, Schoepfiaceae, Thesiaceae, Viscaceae and Ximeniaceae, excluding Balanophoraceae and the three basal-branching lineages Coulaceae, Strobiosiaceae, Erythroplacaceae):</td>
<td>Bisexual or unisexual</td>
<td>None</td>
<td>Nocturnal-foraging birds, moths, bees, wasps, flies, two fly-pollinated species of Arceuthobium</td>
<td>Birds, wind, explosion</td>
<td>Perennial, some annual in Thesium</td>
<td>Coulaceae 2–3/2–4</td>
<td>Bisexual</td>
<td>Hermaphroditic</td>
<td>?</td>
<td></td>
</tr>
</tbody>
</table>

whether parasitic angiosperm lineages exhibit shared traits in their pollination biology, dispersal modes, or sexual systems. We also describe the pollination and mating system of two species of Apodanthaceae. Apodanthaceae comprise 18 species in two genera, Apodanthes and Pilostyles (a third genus name, Berlinianche, has never been validly published). They occur in North and South America, Africa, Iran, and Australia. Like most other Cucurbitales, Apodanthaceae have unisexual flowers. The flowers and fruits measure 4–10 mm in diameter (Blarer et al., 2004; present paper) and appear on the hosts’ stems and branches once a year (Fig. 2). Insect pollination had been inferred from flower morphology and color, and occasional observations of visitors in Africa, Australia, and Brazil (Dell and Burbidge, 1981; Blarer et al., 2004; Brasil, 2010). Blarer et al. (2004) detected no flower odor in the African Pilostyles (Berlinianche) aethiopica Welwitsch, while Blassingame (1968) perceived an indoloid odor in the Californian P. thurberi Gray.

Questions about reproductive trait correlations across angiosperms are difficult to answer because of insufficient data; however, any strong trend, such as lack of wind pollination or preponderance of bisexual flowers, can be picked up even from incomplete data.

MATERIALS AND METHODS

Fieldwork on Pilostyles aethiopica and Pilostyles haussknechtii—Pilostyles aethiopica Welwitsch was studied between 16 and 29 February 2012 in the Mukuvisi woodlands, at 17°50’44.16”S and 31°05’24.12”E in Harare, Zimbabwe. These woodlands are protected. The parasite flowers were borne on the branches of 1.5 to 4 m tall trees of Julbernardia globiflora (Benth.) Troupin (Fabaceae). Pilostyles haussknechtii Boissier was studied between 28 May and 11 June 2012 in two populations in Iran, one north of Teheran in the province of Alborz (35°55’14.09”N and 51°03’12.78”E), the other in the province of Isfahan (32°55’11.3”N and 50°41’23.1”E). The local vegetation type was steppe (Fig. 2), and the hosts were 0.20 to 1 m tall shrubs of Astragalus floccosus Boissier near Teheran and of A. verus Olivier in Isfahan. Herbarium vouchers of the parasites and their hosts have been deposited in the herbarium of Munich, M (S. Bellot 5 to S. Bellot 56).

Fig. 2. Habits and floral morphology of two species of Pilostyles. (A) Pilostyles haussknechtii on Astragalus floccosus near Isfahan, Iran. (B) Pilostyles aethiopica on Julbernardia globiflora in the Mukuvisi woodlands, Harare, Zimbabwe. (C) Possible monoecy, with male (left) and female (right) flowers of P. haussknechtii on a stem of A. floccosus. (D) Male flower of P. aethiopica. (E) Female flower of P. aethiopica (courtesy of D. Plowes).
At the Harare site, 20 host trees carrying numerous parasite flowers were selected. Male *Pilosyles aethiopica* flowers have a shiny, white hair crown at the top of their central column, whereas female flowers do not (Fig. 2), making it possible to sex flowers by eye. At the Isfahan site, 27 infected shrubs were studied, and at the Teheran site, 26 infected and 123 uninfected shrubs. To sex the flowers of *P. haussknechtii* required close observation or prying open flowers, and it was therefore only possible to sex a subset of the flowers on each host. At the Teheran site, we also studied whether host flowering was more or less abundant in infected vs. noninfected hosts.

In Harare, parasite flowers in five host tree patches were observed during 10-min periods between 0900 and 1700 hours on several days for a total of 36 observation periods. Notes were taken on flower sex, scent, number of insect visitor, and visitor behavior. Visitors were also photographed, and captured visitors have been deposited in the zoological collections of Munich (Zoologische Staatsammlung München; vouchers S. Bellot 1 to 6). Photographs and specimens were studied and identified by the fly and ant specialists listed in the acknowledgments. For the six most common visitors to both flower sexes, visitation frequency was quantified. The scent of male and female flowers was assayed by letting it accumulate in closed small glass vials. Nectar presence was tested with diabetes testing strips that were inserted into flowers.

**Assessment of reproductive traits in parasitic angiosperms and their sister groups**—All angiosperm genera recorded as parasitic were databased; following previous reviews, we included Santalales lineages thought to be parasitic based on their phylogenetic position but lacking information on haustoria formation. Most important sources were D. L. Nickrent’s Parasitic Plant Connection website (http://www.parasiticplants.si.edu/UsingPPC.html, last accessed on 28 October 2012) and R. Walker’s Parasitic plants database (http://www.omnistrerra.com/bot/pp_home.cgi, last accessed 28 October 2012). For each genus, species names were downloaded from Tropicos (http://www.tropicos.org), and synonyms or illegitimate names were deleted. The Plant List (http://www.theplantlist.org/) and the Angiosperm Phylogeny Website (Stevens, 2001) were also consulted. This resulted in a table of 5058 accepted species names (Appendix S1; see Supplemental Data with the online version of this article); for the largest groups, Cuscuta, Orobanchaceae, and Santalales, we accepted species numbers of 200 (Digital Atlas of *Cuscuta*, Costea [2007], 2040 [Stevens, 2001]), and 2111 (Nickrent et al., 2010), slightly higher (*Cuscuta*) or lower than those obtained from other sources.

Traits scored for parasitic species (Appendix S1) were flower morphology (whether unisexual or bisexual), population sexual system(s), longevity (annual, perennial), pollinator(s), and seed and fruit dispersal (partly assessed only from fruit types). Data sources (Appendix S1) included online floras, websites, articles in journals, and books of parasitic plants, most importantly Kuijt (1969) and Heide-Jorgensen (2008). The scored data were used to construct a summary table at the parasitic family level or other relevant taxonomic levels (Results).

**RESULTS**

**Sexual systems and flowering of Apodanthaceae**—Table 2 summarizes the results of the distribution of flower morphs in the Zimbabwean and Iranian populations. In Zimbabwe, 12 hosts carried only male parasite flowers, seven only female flowers, and one flowers of both sexes. In the two Iranian populations, 19 or 17 host shrubs carried only male parasite flowers, while six or 10 carried only female parasite flowers. One shrub carried parasite flowers of both sexes (Fig. 2C). At the Teheran site, of 123 uninfected hosts almost all flowered, while of the 26 infected hosts, only seven flowered. When the parasite was flowering on a flowering host, flowers of host and parasite never occurred on the same host stem.

**Flowers visitors and floral scent**—At the Harare site, four fly species (Fig. 3A–D) and two ant species (Fig. 3E, F) were observed on both flower sexes and during more than one observation period. The ant species *Camponotus flavomarginatus* Mayr, 1862 and *Lepisiota capensis* Mayr, 1862 (both Formicinae; vouchers S. Bellot 4, 5, 6) are unlikely pollinators because they return to the nest between forays to different food sources. In Harare, the most common flies were *Chrysomya chloropyga* Wiedemann, 1818 (voucher S. Bellot 1; Fig. 3B) and *Stomorhina* sp. (not captured; Fig. 3A), both belonging to the Calliphoridae. In Iran, the fly *Timia* (*Empyrocerca*) spec. aff. *abstersa* (*Ulidiiidae*; voucher S. Bellot 7; Fig. 3G) was common on flowers of both sexes. At both sites, flies alighted on branches near flowers (which were too small for them to land on), approached the flowers, and then probed the stamens or the base of the floral column with their proboscis, apparently looking for pollen grains or liquid. Their entire heads, including the upper part of the proboscis, came in contact with the anther ring or stigma. In Harare, floral scent could be perceived throughout the day, with no apparent change in intensity; female flowers produced scent before they opened. In Iran, flower scent was not always perceptible and was weak, even after accumulation in glass vials (done at ca. 1000 and 1500 hours). No difference in scent between male and female flowers could be perceived in either species. Nectar presence could not be verified with the diabetes testing strips because nectar quantities were too minute. No insect eggs or larvae were found in any flower.

**Reproductive traits of parasitic angiosperms and their sister groups**—Of a total of 4510 parasitic species, about 10% are dioecious (Table 1 and Appendix S1). Dioecy is found in Apodanthaceae, Balanophoraceae, higher Santalales, Cytinaceae, and Raflesiacaeae, and in the sister groups of three of them (Table 1). Of the eight parasitic lineages lacking dioecious species, five have sister groups that include dioecious species (Table 1). Table 1 also shows that all parasitic lineages contain perennial species, including trees (Fig. 4E), while five contain perennial as well as annual species. Only *Cuscuta* is predominantly annual. All dioecious parasite species appear to be perennial.

With the exception of the Viscaceae genus *Arceuthobium*, in which nectar-offering and fly-pollinated species may sometimes receive wind-carried conspecific pollen (Player, 1979), parasitic species are animal-pollinated, with all pollinator categories from vertebrates to insects represented (Table 1). Figure 4 shows some of the pollination syndromes represented (Fig. 4C: bee/bumblebee pollination, Fig. 4D: animal pollination with trapping). Among parasites’ sister groups, several contain wind-pollinated species (Table 1). Fruit or seed dispersal by animals predominates in the parasite lineages (Fig. 4A, B), except for Cynomoriaceae, Lenoaeeae, and some Orobanchaceae, the

<table>
<thead>
<tr>
<th>Species</th>
<th>Population</th>
<th>Infected hosts</th>
<th>Hosts with only male parasite flowers</th>
<th>Hosts with only female parasite flowers</th>
<th>Hosts with male and female parasite flowers</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. aethiopica</em></td>
<td>Mukuvisi woodlands</td>
<td>20</td>
<td>12</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td><em>P. haussknechtii</em></td>
<td>Teheran</td>
<td>26</td>
<td>19</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Isfahan</td>
<td>27</td>
<td>17</td>
<td>10</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 2. Host distribution of male and female flowers of *Pilosyles aethiopica* and *P. haussknechtii*
A study of the Brazilian species *P. ingae* (Karsten) Hooker recently reported that 1.3% of the male flowers had small pistils, but were functionally male (Brasil, 2010: The numbers of dissected flowers were not given.). In the species studied here, *P. aethiopica* and *P. haussknechtii*, dioecy appears to be the normal sexual system (Table 1). Among the remaining Apodanthaceae, three or four are monoecious, namely the Australian *P. collina* B. Dell and *P. coccoidea* K.R. Thiele (Thiele et al., 2008), the Brazilian *Apodanthes minorum* Vattimo (de Vattimo, 1973), and possibly the Brazilian *P. stawiarskii* de Vatt. (de Vattimo, 1950). Most remaining species seeds of which are dispersed by rain splash or wind (cf. references in Table 1).

**DISCUSSION**

Sexual systems and reproductive biology of Apodanthaceae—Flowers of *Pilostyles aethiopica* and *P. haussknechtii* were strictly unisexual, without traces of aborted organs of the other sex. Unisexual flowers are the ancestral condition in the Cucurbitales, apparently combined with a dioecious mating system (Zhang et al., 2006). A study of the Brazilian species *P. ingae* (Karsten) Hooker recently reported that 1.3% of the male flowers had small pistils, but were functionally male (Brasil, 2010: The numbers of dissected flowers were not given.). In the species studied here, *P. aethiopica* and *P. haussknechtii*, dioecy appears to be the normal sexual system (Table 1). Among the remaining Apodanthaceae, three or four are monoecious, namely the Australian *P. collina* B. Dell and *P. coccoidea* K.R. Thiele (Thiele et al., 2008), the Brazilian *Apodanthes minorum* Vattimo (de Vattimo, 1973), and possibly the Brazilian *P. stawiarskii* de Vatt. (de Vattimo, 1950). Most remaining species...
Fig. 4. Examples of reproductive traits of parasitic angiosperms. (A) Seedlings of the hemiparasite *Krameria* sp. (Krameriacae) at the foot of a host (Cactaceae) with barbed seeds (inset) adapted to exozoochory. (B) Bisexual flowers and fruits of the bird-pollinated and dispersed *Tristerix longibracteatus* (Loranthaceae). (C) Herb habit of the root parasite *Orobanche lavandulacea* (Orobanchaceae) and close-up of its bee-pollinated inflorescence (inset). (D) Bisexual flower of the root holoparasite *Prosopanche americana* (Hydnoraceae) in male phase (courtesy of B. Schlumpberger). (E) Tree habit and inflorescence (inset) of the hemiparasite *Gaiadendron* (Loranthaceae). Images A–C, E: courtesy of G. Gerlach.
are dioecious \([P. \text{ ingae} \text{ and } P. \text{ thurberi}] \) (Brasil, 2010); the Australian \(P. \text{ hamiltonii} \) (Thiele et al., 2008), the American \(P. \text{ mexicana} \) (Brandegee) Rose, \(P. \text{ blanchetii} \) (Gardner) Brown, \(P. \text{ calliandrae} \) (Gardner) Brown (de Vattimo, 1971), \(A. \text{ tribracteata} \) Rusby (Rusby, 1920), and \(A. \text{ caseariae} \) Poiteau (Poiteau, 1824)]. The sexual system of the American species \(P. \text{ galactiae} \) Ule, \(P. \text{ guyanensis} \) Ule, and \(A. \text{ berteroi} \) (Gill.) Gardner, and of the African \(P. \text{ holzzii} \) Engler is unknown, the later species being known only from a type destroyed during World War II.

The endoparasitic lifestyle of Apodanthaceae makes it difficult to decide whether cases of monoecy in fact represent multiple infections with two or more parasite individuals, each representing one sex. Next-generation sequencing data that will facilitate the design of highly variable genetic markers have now been generated for \(P. \text{ hamiltonii} \) and \(P. \text{ aethiopica} \) and may resolve this question (S. Bellot and S. S. Renner, unpublished data). Nothing is known about germination and initial haustorium formation, but animal dispersal is certain because the fruits are small red, orange, or yellow berries with numerous tiny \((0.5 \text{ mm})\) seeds, embedded in a fleshy sticky pulp (Bouman and Meijer, 1994; S. Bellot, personal observation). Birds have been observed eating the fruits of \(P. \text{ stylos} \) \(P. \text{ ingae} \) in Brazil (Brasil, 2010).

Apodanthaceae flowers are sessile, minute, and dark-red or yellow (depending on the species), with nectar offered in minute quantities (this study) on a shallow nectary cushion at the base of the flowers. Pollination by short-tongued nectar-foragers was therefore expected, and as demonstrated here, at least \(P. \text{ aethiopica} \) is indeed pollinated by short-tongued flies. An earlier study of this species also reported flies as flower visitors, namely \(Drosophila, \) Sciaridae, Psychidae, and Cecidomyiidae (Blárár et al., 2004), but provided neither details on their foraging behavior on the flowers nor whether they visited both sexes.

The flower scent likely consists of ethanol and related compounds in addition to fruity compounds as known from other fly-pollinated species (Ibanez et al., 2004, 2006; Brasil, 2010), with the Panicoideae of grasses, for example, known to have fruity scents (e.g., \(A. \text{ tribracteata} \) Rusby, 1920; Brasil, 2010). But \(P. \text{ blanchetii} \) Pilostyles and \(P. \text{ aethiopica} \) have been slower to accumulate than molecular ones. All animal pollinators are represented among parasitic angiosperms, ranging from ants, via birds, to bees, butterflies, beetles, and flies, to shrews (Figs. 3, 4; Table 1 and its references). Animal pollination is overrepresented in parasitic angiosperms—As shown in Table 1, parasitic angiosperms are animal-pollinated, while among the remaining angiosperms at least 24,000 species are wind-pollinated, with wind pollination having evolved at least 65 times (Linder, 1998). Ecological reasons for the absence of wind pollination among parasites may be their occurrence at ground level (root parasites), on the stems of trees (e.g., Apodanthaceae), or within leafy tree crowns (e.g., mistletoes), where pollen export and import by wind might be relatively difficult. All animal pollinators are represented among parasites, ranging from ants, via birds, to bees, butterflies, beetles, and flies, to shrews (Figs. 3, 4; Table 1 and its references). Among seed dispersal modes, dispersal by animals predominates, although not as pervasively as animal pollination.

Conclusions—In conclusion, the absence of wind pollination is clearly a feature uniting parasitic angiosperms (not noted by Molau, 1995). Dioecy appears to be more common among parasites than it is among other flowering plants, but phylogenetic resolution so far is insufficient to infer shifts to and from dioecy. The African and Iranian species of Apodanthaceae studied here are dioecious and pollinated by flies. Resolving whether a combination of animal pollination, perennial life history, and dioecy facilitated the evolution of parasitism or evolved subsequent to the evolution of parasitism will require ancestral trait reconstructions in several of the 13 lineages. At the moment, we are far from understanding the ecological and evolutionary interactions among hosts, parasites, their pollinators and dispersers even in the best-studied systems, \(Cuscuta, \) \(Viscum, \) or the economically important pests in Orobanchaceae, and compared to earlier reviews (Kuijt, 1969; Molau, 1995), ecological data have been slower to accumulate than molecular ones.

LITERATURE CITED


Bellot and Renner—Reproductive Biology of Parasitic Angiosperms


