Charles L. Argue

The Pollination Biology of North American Orchids: Volume 2

North of Florida and Mexico

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Preface

This is the second of two volumes treating the pollination biology of all native and introduced orchid species occurring north of Mexico and Florida. Both volumes provide an up-to-date collation of a vast literature previously scattered in numerous, often obscure, journals and books. Like Volume 1, Volume 2 supplies detailed information on genetic compatibility, breeding systems, pollinators, pollination mechanisms, fruiting success, and limiting factors for each species. Distribution, habitat, and floral morphology are also summarized. In addition, original, detailed line drawings emphasize orchid reproductive organs and their adaptation to known pollinators. All drawings are by the author, sometimes based on the published work of others, as indicated.

Volume 1 furnished a brief introduction to the general morphology of the orchid flower and the terminology used to describe orchid breeding systems and reproductive strategies. It treated the lady's-slippers of genus *Cypripedium*, subfamily Cypripedioideae, and introduced nine genera of the subfamily Orchidoideae, including the diverse rein orchids of genus *Platanthera*.

Volume 2 continues the treatment of the Orchidoideae with nine North American genera of tribe Cranichideae. These include the rattlesnake plantains of genus *Goodyera*, often recognized by their clusters of variegated leaves, and the ladies' tresses of genus *Spiranthes* and their relatives, well known for the often spiral arrangement of their flowers in spicate inflorescences.

Seven North American tribes of the large subfamily Epidendroideae are considered next. Tribe Neottieae, with three genera, includes the twayblades of genus *Listera* (*Neottia*) with their long lips and paired stem leaves. Also in this group are the helleborines, including the strange, ghostly white phantom orchid of genus *Cephalanthera* and the native stream orchid and broad-leaved helleborine, both of genus *Epipactis*. The tribe Triphoreae comprises a single North American genus, *Triphora*, the three-birds orchid with an asymmetrical perianth. The tribe Malaxideae includes the diminutive and easily overlooked adder's mouth orchid of genus *Malaxis* and a second genus, *Liparis*, which shares the common name twayblade with *Listera*, but differs in having only basal leaves. The tribe Calypsoeae comprises four genera native to our flora. The fairy slipper orchid of genus *Calypso*, considered by some as the most beautiful terrestrial orchid in North America; the crane-fly orchid of genus *Tipularia*, with straggly long-spurred flowers that suggest a crane-fly in flight; the coral-roots of genus Corallorhiza, mycoparasitic herbs of varying color; and the puttyroot or adam-and-eve orchid of genus *Aplectrum*, with a distinctive pleated, white-ribbed basal leaf. Tribe Cymbideae includes a single southeastern North American genus, *Eulophia*. Tribe Epidendreae embraces a coral root look-alike, *Hexalectris*, and the green fly orchid of genus *Epidendrum*, the only representative of this very large genus in our flora and the only epiphytic orchid found naturally north of Florida. The dragon's mouth (genus *Arethusa*) and grass-pink (genus *Calopogon*) are members of tribe Arethuseae, which along with the rose pogonia (genus *Pogonia*) of subfamily Vanilloideae, share the development of ultra-violet absorbing false stamens on their lips. Other North American members of subfamily Vanilloideae include the large and small whorled pogonias of genus *Isotria* and the spreading pogonias of genus *Cleistesiopsis*.

Although great progress has been made over the last several decades, many aspects of orchid reproduction are not fully understood or have been studied in only a few populations. Areas where information is limited are clearly indicated, spotlighting particular needs for further research.

The Pollination Biology of North American Orchids will be of interest to both regional and international audiences including:

- Researchers and students in this field of study who are currently required to search through the scattered literature to obtain the information gathered here.
- Researchers and students in related fields with an interest in the coevolution of plants and insects.
- Conservation specialists who need to understand both the details of orchid reproduction and the identity of primary pollinators in order to properly manage the land for both.
- Orchid breeders who require accurate and current information on orchid breeding systems. The artificial cultivation and breeding of native orchids is an important conservation measure aimed at reducing and hopefully eliminating the collection and sale of wild orchids.
- General readers with an interest in orchid biology. Technical terminology is kept to a minimum, and an extensive glossary is provided for the nonspecialist reader.

Saint Paul, MN Charles L. Argue

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Part I
Subfamily Orchidoideae (Part Two)

Tribe Cranichideae

The Cranichideae with seven subtribes, 93 genera, and approximately 600 species, is widely distributed in the tropics and subtropics. Subtribes in our flora include the Goodyerinae, Cranichidinae, and Spiranthinae.

Chapter 1

Subtribes Goodyerinae and Cranichidinae

Abstract Bumblebees are the most important pollinators of North American species of *Goodyera*, but pollinator recruitment appears to be limiting. Autogamy and agamospermy are unknown in our flora. Factors contributing to reproductive success are summarized. The flowers of *Zeuxine* are chiefly autogamous or apomictic. The pollination of *Platythelys* has yet to be studied, but flower morphology suggests insect pollination. *Ponthieva* is self-compatible but not autogamous. Possible pollinators include small halictid bees or oil collecting anthophorid bees.

Keywords *Goodyera* • *Zeuxine* • *Platythelys* • *Ponthieva* • Autogamy • Apomixes • Bumblebees • Small flies • Small halictid and anthophorid bees

Goodyerinae

The Goodyerinae include 34 genera found in both the Old and New Worlds, especially in the tropics and subtropics (Pridgeon et al. 2003). Three genera, two native and one introduced, occur in our flora.

Goodyera R. Brown (Rattlesnake Plantains)

Goodyera is a genus of about 80–100 species with a worldwide distribution (Pridgeon et al. 2003). Four are present in North America north of Mexico. The pollination biology of *G. oblongifolia* Raf. (Menzie's or great rattlesnake plantain), *G. repens* (L.) Br. Fernald (lesser rattlesnake plantain), and *G. tesselata* Lodd. (tessellated rattlesnake plantain) is well documented. Limited data on pollination are also available for *G. pubescens* (Willd.) R.Br. (downy rattlesnake plantain).

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Habitat and Distribution

Goodyera oblongifolia is found in moist to dry coniferous or hardwood forests and on the margins of wooded bogs and swamps (Luer 1975; Kallunki 1976, 2002; Case 1987). In the east, it is locally common in the Great Lakes region and near the mouth of the St. Lawrence. In the west, it occurs from southeastern Alaska to central California, Idaho, and Wyoming, especially in the mountains and along the Pacific Coast. Scattered populations also extend south to New Mexico and Arizona. Southern members often occur in spruce-fir forests at high elevations.

Goodyera repens is found in cool, acidic, nutrient deficient soils under a full or partial canopy. It is common in the shaded, mossy grounds of bogs and coniferous forests over much of its range but is also present in dry to mesic, mixed coniferous forest. In subarctic forests, it occupies upland sites and a wide variety of habitats (Case 1987; Smith 1993; Kallunki 2002). Populations range from Alaska to Newfoundland south through British Columbia, the Prairie Provinces, and the Great Lakes to New Jersey (Kallunki 1976). It is also present in the Appalachians to Georgia and scattered in the Rockies to New Mexico and Arizona (Catling 1990; Kallunki 2002).

Goodyera tesselata is probably an allotetraploid ($2n=60$) derived by hybridization between the diploids *G. oblongifolia* and *G. repens* (Kallunki 1976, 1981). It can occur in areas where the diploids are absent, supporting its treatment as a legitimate species. This orchid not only shows a preference for the dry soils of upland coniferous or mixed forest, but also occurs in white cedar swamps or on the edges of spruce-tamarack bogs (Kallunki 2002). It ranges from northeastern Minnesota and southeastern Manitoba through the Great Lakes to New Jersey and Newfoundland.

Goodyera pubescens ($2n=26$) differs morphologically and chemically from the other species in our flora (Kallunki 1981). It is highly flexible in its requirements and occurs in a variety of frequently woody habitats on dry or wet, usually acidic soils from Minnesota, southern Ontario, Quebec and Maine to Tennessee and Georgia. Scattered populations also occur in Nova Scotia, Florida, Missouri, Arkansas, and Oklahoma (Kallunki 2002). According to Kallunki (1981), it is seldom included in the mixed species populations of *Goodyera* that are common in northern Michigan.

Floral Morphology

A variable number of small, whitish, resupinate flowers are arranged in a spicate inflorescence (Fig. 1.1a) (Table 1.1). The dorsal sepal and lateral petals converge in all four species to form a hood above the lip and column (Figs. 1.1 and 1.2). Lateral sepals are spreading or reflexed. The lip is fleshy with a ligulate or pointed apex and a concave or saccate base. Except in *G. pubescens*, its inner surface bears two to four rows or ridges of glandular papillae (Kallunki 1981). The column is short with a pointed to blunt rostellum separating the anther and stigma (Fig. 1.2) (Luer 1975;

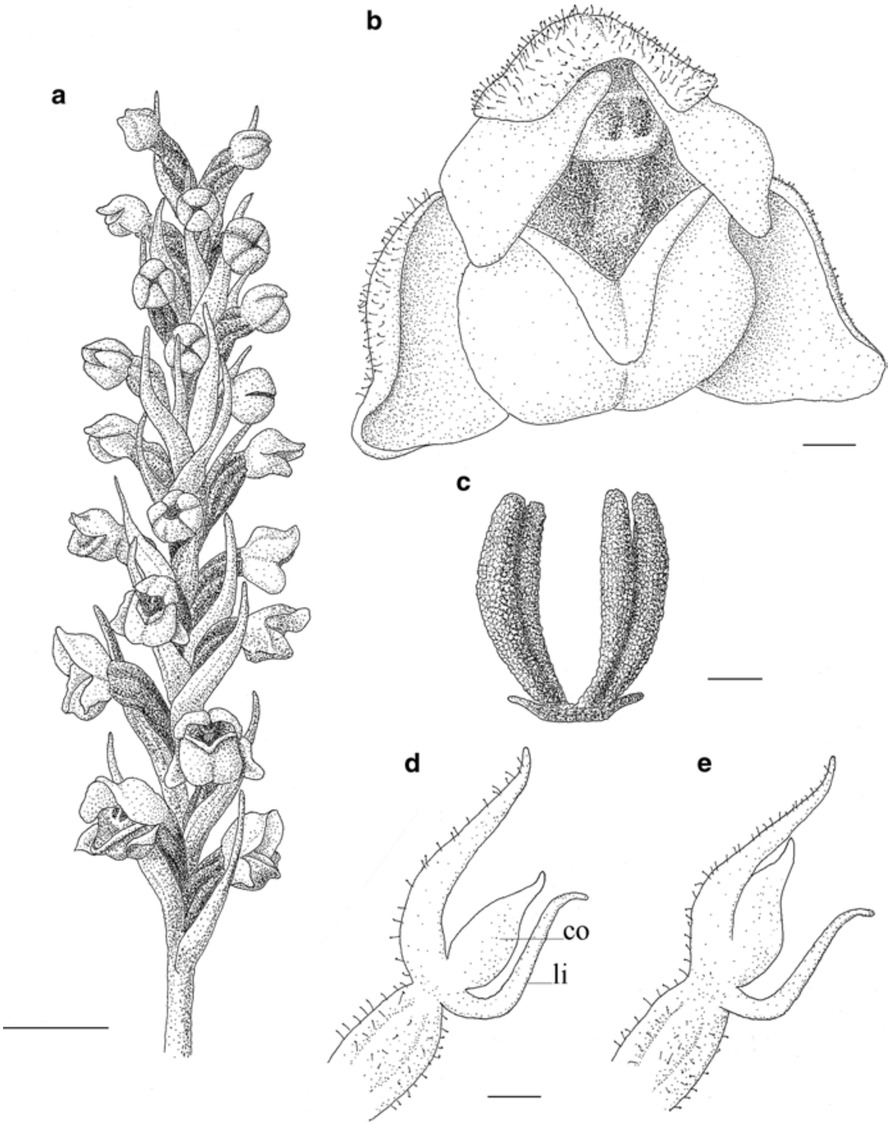

Fig. 1.1 *Goodyera pubescens*. (**a**) Inflorescence, scale bar = 1 cm; (**b**) Flower, front view, scale bar = 0.5 mm; (**c**) Pollinarium, scale bar = 0.02 mm; (**d**) Sagittal section of a flower showing early (male) stage; (**e**) Sagittal section of older flower showing separation of lip and column, scale bar = 1 mm. *co* column, *li* lip

Case 1987). The anther is bilocular, erect, dorsal, and positioned at the distal end of the column. Each anther bears a pair of sometimes cleft, large, sectile pollinia comprise tetrads (e.g. Fig. 1.1c) (Zee et al. 1987; Dressler 1993). The pollinia are

Table 1.1 Data on *Goodyera* (Kallunki 2002)

Character	*G. oblongifolia*	*G. pubescence*	*G. repens*	*G. tesselata*
Plant height (cm)[a]	9.2–27.2	to 50[b]	6.2–15.3	6.4–22.8
Inflorescence (cm)	Spiral or secund	Cylindric	Secund or infrequently spiral	Spiral or second
Flower number	10–48	10–57	7–36	5–72
Dorsal sepal (mm)[a]	6.7–9.3	4–5.5[b]	3.0–5.2	3.9–7.1
Lateral sepals (mm)	5.7–7.8	3.1–5.3	3.0–5.2	3.8–6.0
Hood/Helmet (mm)	5–10	3.6–5.7	3.0–5.5	3.9–7.1
Lip (mm)	4.9–7.9 × 1.3–3.2	2.5–4.2 × 2.2–3.5	1.8–4.8 × 1.4–3.2	3.0–5.5 × 1.2–3.1

[a] Kallunki (1976)
[b] Luer (1975)

attached at their apices to a single, shared viscidium held by a forked or notched rostellar beak at the tip of the column (Fig. 1.2) (Kipping 1971; Johnson and Edwards 2000; Kallunki 2002). The stigma lobes are connate and positioned under the base of the rostellum (Pridgeon et al. 2003).

Average flower size differs slightly (Table 1.1), ranging from about 3 to 5 mm long in *G. repens* and *G. pubescens* through *G. tesselata* (about 4–7 mm long) to *G. oblongifolia* (about 5–10 mm long) (Luer 1975; Kallunki 1976, 1981; Smith 1993). Kallunki (1981) described the perianth in *G. repens* as distinctly whiter than in *G. oblongifolia* and *G. tesselata*, where the sepals are frequently tinged with green. The reflectance pattern also varies under ultraviolet light, where the labella of *G. oblongifolia* and *G. tesselata* appear bright yellow–green and that of *G. repens* does not fluoresce at all. Ultraviolet patterns can play a role in the orientation of hymenopterous pollinators (Jones and Buchmann 1974) and may, in addition, attract specific vectors and function as an effective pre-pollination isolating mechanism (Kevan 1972; Guldberg and Atsatt 1975; Jones 1978).

Floral odors perceptible to the human observer are present in *G. oblongifolia* and *G. tesselata*, but not in *G. repens*; they are stronger during the day than at night (Kallunki 1981). Nectar is present in some flowers around the clock but is relatively much less abundant in *G. repens* than in the other two (Kallunki 1981).

Compatibility and Breeding System

Hagerup (1952) noted a lack of pollinia coherence and bud autogamy, but no agamospermy, in some populations of *G. repens* from Denmark, and Pridgeon et al. (2003) reported autogamy in *G. inmeghema* Ormerod from Vanuatu in the South Pacific. Occasional reports have also suggested the possible occurrence of autogamy in our flora. Catling (1983), for example, found a few large colonies of *G. tesselata* and *G. pubescens* in Canada, where all the ovaries in all the inflorescences produced ripe capsules. He noted that the rostellum in these species was smaller than in *G. oblongifolia*.

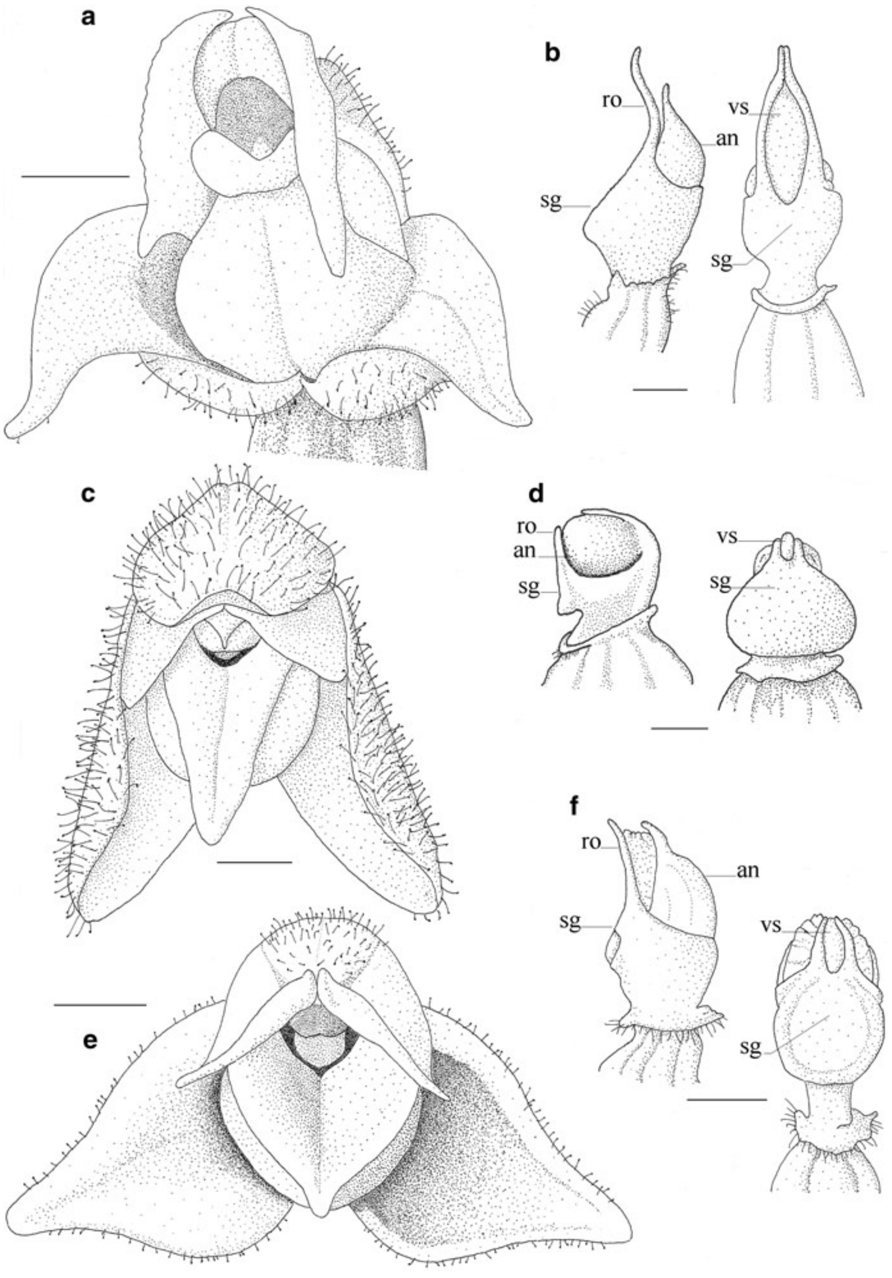

Fig. 1.2 (**a**, **b**) *Goodyera oblongifolia*; (**c**, **d**) *G. repens*; (**e**, **f**) *G. tesselata.* Flower, front view (*left*); column side view (*center*), front view (*right*), scale bars = 1 mm. *an* anther, *po* pollinium, *ro* rostellum, *sg* stigmatic area, *vs* viscidium. **b**, **d**, and **f** modified from Kallunki (1976) with permission

Table 1.2 Results for open pollinated [mean (range)] and experimental treatments on four species of *Goodyera* enclosed in nylon net (percentage capsule set/percentage fertile seed [median (range)]) (Kallunki 1981)

Variables	*G. oblongifolia*	*G. pubescens*	*G. repens*	*G. tesselata*
Source of plants	Michigan	Wisconsin, Rhode Island	Michigan	Michigan
Open pollinated[a]				
Flowers visited (%)	49.8 (3–84)		86	50.5 (3–97)
Flowers pollinated (%)	44.2 (3–66)		79	48.8 (3–96)
Fruit set (%)	49.8 (8–70)		49 (76)[b]	41.0 (2–84)
Unmanipulated, caged	0	0	0	0
Pollinarium removed, caged	0	0	0	0
Selfed	100/40 (2–87)	90/77 (12–94)	88/36 (6–88)	86/88 (38–94)
Cross-pollinated	92/60 (0–93)	94/64.5 (8–92)	100/63.5 (0–92)	100/79 (40–97)

[a] Means and range of means for *G. oblongifolia* and *G. tesselata* and the means of one population for *G. repens*

[b] Kallunki (1976)

Agamospermy has also been suggested. Leavitt (1901) reported polyembryonic seeds in *G. tesselata* and *G. pubescens*. Ackerman (1975) also found a few polyembryonic seeds in some highly fertile capsules of *G. oblongifolia*, but embryological studies are needed to determine whether these were produced by cleavage of the fertilized zygote or were adventitious and asexual in origin. The former interpretation is favored by the fact that there were never more than two embryos, and these were always partially fused.

Kallunki (1981) has conducted systematic experimental pollinations on caged plants of all four species collected from the wild. Each was capable of producing seed with embryos when artificially self- or cross-pollinated, but no autogamy or agamospermy was found (Table 1.2). Kipping (1971), Stevenson (1973), Ackerman (1975), and Kallunki (1976) also observed no conclusive evidence for either autogamy or agamospermy in our species.

In Kallunki's (1981) experiments, outcrossed and selfed plants often differed in capsule and seed production (Table 1.2), but none of the differences were statistically significant. In addition, although Ackerman (1975) reported average seed fertility in selfed plants to be somewhat lower than that in naturally pollinated plants, fertility remained high. There has, therefore, been little to no selection for self-incompatibility barriers in these species. Mechanisms for the promotion of outcrossing are nevertheless present (see below), and *Goodyera* may be said to process a clonal outbreeding system. For a summary of the advantages and disadvantages of this system, see the discussion in the section on *Isotria verticillata* (Chap. 9) and (Mehrhoff 1983, p. 1451).

Experimental crosses between any two of the four species of *Goodyera* also produced fertile seed (Table 1.3) (Kallunki 1981), but intraspecific crosses usually produced a higher median percentage of fertile seeds than interspecific crosses (Kallunki 1981). This suggests that some degree of incompatibility may be present, but interspecific fertility levels remained high in all cases (Table 1.3). *Goodyera*

Table 1.3 Results for hybridization experiments on four species of *Goodyera* enclosed in nylon net (percentage capsule set/percentage fertile seed [median (range)]) (Kallunki 1981)

Variables	*G. oblongifolia*	*G. pubescens*	*G. repens*	*G. tesselata*
Source of plants	Michigan	Wisconsin, Rhode Island	Michigan	Michigan
Hybridized				
G. pubescens X	71/57.5 (1–96)			
G. repens X	96/34 (0–80)	82/46 (3–76)		
G. tesselata X	78/42 (0–87)	90/68 (20–98)	74/73.5 (24–96)	
Chromosomes ($2n$)	30	26	30	60 (45)

tesselata, intermediate in size and other characters between *G. oblongifolia* and *G. repens*, is apparently not mechanically or ecologically isolated from either species. It probably hybridizes freely with one or both of the diploids in areas, where they are sympatric. Triploids ($2n=45$) were found only in areas, where *G. tesselata* and *G. oblongifolia* or both diploids were also present (Kallunki 1981). These plants had irregular meiosis and resembled *G. tesselata* in morphology. The presence of occasional parthenocarpic plants (Ackerman 1975; Kallunki 1981) might also be related to triploidy. The 95% confidence limits on the percentage of plants in mixed populations that looked like *G. tesselata* but were actually triploids ranged from 33 to 79% (Kallunki 1981).

Given the occurrence of mixed populations and the absence of significant chromosomal sterility barriers it might be asked what, if anything, restricts the level of hybridization. Seedling establishment can occur within existing colonies of all four species (Ames 1921; Reddoch and Reddoch 2007), but rhizome growth gives rise to extensive clones, and the clonal population structure plus the absence of substantial self-incompatibility barriers probably results in much inbreeding (Ackerman 1975; Kallunki 1981). Self-compatibility would be expected to augment seed production in a clonal species (Estes and Brown 1973; Estes and Thorpe 1974) and in combination with vegetative reproduction, contribute to the maintenance of species integrity in hybridizing, mixed populations (Ackerman 1975; Kallunki 1981). Clonal structure also leads to a clustering of inflorescences which could enhance the orchid's visual and olfactory appeal to pollinators and provide a concentrated food source (Ackerman 1975), factors that might further reduce hybridization by restricting pollen transport and gene flow, especially in years when genets produce large numbers of flowers (Kallunki 1981).

A certain amount of isolation would therefore result from self-compatibility and a perennial, clonal growth habit. However, experiments with florescent pigment particles revealed that vectors frequently moved between and cross-pollinated the flowers of *G. tesselata* and *G. oblongifolia*, and hybrids resulting from crosses between *G. tesselata* and either diploid could be present in any mixed-species population (Kallunki 1981). There are, as yet, no reports of diploid hybrids between *G. repens* and *G. oblongifolia* despite a median of 34% fertile seed obtained in experimental crosses (Table 1.3). Apparently, cross-pollination between these species occurs infrequently under natural conditions or there is a problem with the establishment of the hybrid seeds. It may be significant that *G. repens* and *G. oblongifolia* are among the most dissimilar members of the genus in our flora

(Kallunki 1981). Their different, though overlapping, flowering periods; partial mechanical isolation based on size differences of the flowers; and partial ethological isolation related to differences in ultraviolet reflectance, odor, and nectar reward may affect the frequency of hybridization. On the other hand, since the flowers of *G. tesselata* resemble those of *G. oblongifolia* in ultraviolet reflectance and odor production and are intermediate between those of *G. repens* and *G. oblongifolia* in size, bees may have greater difficulty distinguishing them from flowers of the other two species. The presence of *G. tesselata* may therefore increase the probability of hybridization whenever it occurs in mixed populations with one or both of the diploids (Kallunki 1981).

Goodyera pubescens, with a different base chromosome number, is also interfertile with the other three species, producing a median of 46–68% fertile seed in hand pollinations (Table 1.3). Natural hybrids might therefore occur in mixed populations, but again, none have yet been reported. Kallunki (1981) did not study this species in detail, and information on ultraviolet reflectance, floral odor, and many other details are not available. However, differences in lip shape and ornamentation might contribute to its apparent reproductive isolation. It has been reported to attract different pollinators than the other three *Goodyera* species (see below), but further study is needed.

Phenological separation might play an important, perhaps a primary role in the reduction of hybridization between *G. tesselata* and the two diploids in some areas. Although flowering periods overlap, the median blooming dates in mixed populations for all pairwise comparisons of the three species differ significantly in northern Michigan (Kallunki 1981). *Goodyera tesselata* blooms first followed by *G. repens* and then *G. oblongifolia* (Kallunki 1976, 1981). Backcrosses between triploid offspring and their diploid as well as probably their tetraploid parents may be largely sterile. If so, selection for a prezygotic isolating mechanism, such as phenological separation to reduce the number of wasted gametes, might be expected. Levin and Kerster (1967) have demonstrated that such selection can occur in perennial as well as in short-lived annual species.

Brown (1985), in a 20-year study, confirmed the order of flowering reported from Michigan in mixed populations on the Bruce Peninsula in Ontario. However, Barclay-Estrup et al. (1991) found a different sequence of overlapping flowering periods in the Thunder Bay District. Here, *G. repens* apparently blooms earlier than *G. tessselata* with *G. oblongifolia* again blooming last. The significance of these differences remains to be determined.

Finally, in plants with a clonal outbreeding system each intraspecific pollination event results in the production of a large number of seeds. Both geitonogamy and cross-pollination, therefore, also counter the detrimental effects of hybridization.

Pollinators and Pollination Mechanisms

Bumblebees, attracted by nectar at the base of the saccate lip, are the most important pollinators of *G. oblongifolia*, *G. repens*, and *G. tesselata* in North America

(Ackerman 1975; Kallunki 1981). They are able to access the recessed nectar source, likely to be beyond the reach of smaller, short-tongued bees, and are strong enough to inadvertently rupture the rostellum (Kipping 1971).

Kipping (1971) recorded pollen-bearing workers of *Bombus vosnesenskii* Radoszkowski visiting flowers of *G. oblongifolia* in Nevada County, California. Another visitor, *B. mixtus* Cresson, carried no pollinaria (Kipping 1971). Ackerman (1975) observed and captured pollen-bearing queens of *B. occidentalis* Greene on this orchid in Humboldt County. The bumblebees here were few in number, were seen mostly on sunny days, and quickly examined several flowers on each inflorescence before departing.

Kallunki (1976, 1981) also described bumblebees systematically visiting and removing pollinaria from the flowers of *Goodyera* in Michigan. She observed *Bombus perplexus* on *G. repens* with pollinaria on their proboscises, captured specimens of *B. vagans* on plants of *G. oblongifolia*, and noted but did not capture or identify other bumblebees visiting the flowers of *G. tesselata* in the same area. She also reported halictid bees and syrphid flies on the flowers of *G. repens*, *G. tesselata*, or *G. oblongifolia*, but none carried any pollinaria. Stevenson (1973), however, observed the halictid, *Augochlora pura* (Say), removing pollinaria from *G. pubescens* in North Carolina, and Homoya (1993) recorded *Augochlorella aurata* (Smith) visiting flowers of this species in Indiana.

Ackerman (1975) described the pollination mechanism in *G. oblongifolia*, and according to Kallunki (1981), the process is the same in *G. tesselata* and *G. repens*. The flowers are slightly protandrous. The column in young flowers lies close to and parallel with the lip, obstructing access to the stigma (Fig. 1.1d). At this stage, the elongate rostellum and viscidium in combination with the central groove of the labellum form a narrow tube. This tube is large enough to admit the proboscis of a visiting bee so long as it does not bear any pollinaria. The position of the column thus prevents the bee from depositing pollen on the stigma of a young flower. A bee with a naked proboscis probing for nectar at this stage ruptures the rostellum, contacts the sticky viscidium with its proboscis (galea), and removes the pollinaria as it withdraws from the flower (Fig. 1.3). In older flowers, the column and lip separate (Ackerman 1975; Luer 1975), exposing the stigma (Fig. 1.1e); the viscidium, if it has not been removed, dries up (Ackerman 1975). Visiting bees carrying pollinaria can now easily deposit packets of pollen or massulae on the exposed stigma. As noted elsewhere, bees usually move upward on the inflorescence (e.g., Ackerman 1975; Corbet et al. 1981), and this behavior, in combination with the slight protandry of the flowers, promotes outcrossing, although it does not, of course, exclude geitonogamy among inflorescences in a clone or even among flowers within an inflorescence (see *Spiranthes* for a full discussion) (Kallunki 1981). Outcrossing is also favored by sectile pollinia, which allow massulae from a single pollinium to be deposited on a number of successively visited stigmas. Since all genes from a pollen parent are present in each massula, pollen genotypes are more widely dispersed, and the chances of a variety of genotypes being contributed to a single capsule may also be increased (Freudenstein and Rasmussen 1997).

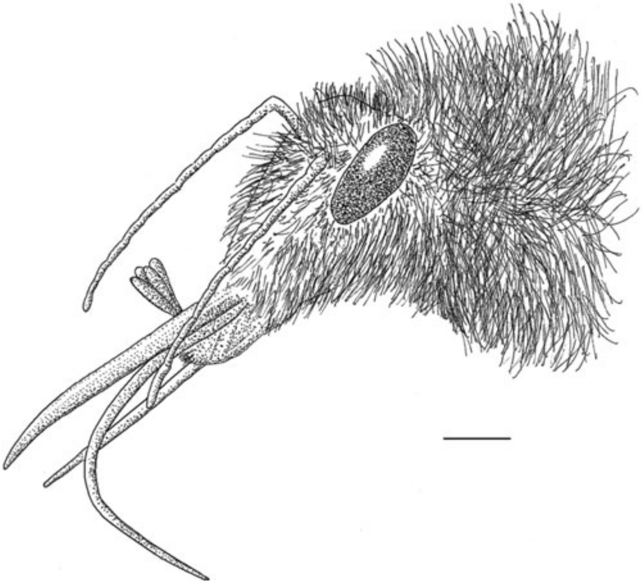

Fig. 1.3 Head of bumblebee with pollinarium of *Goodyera* attached to the proboscis, scale bar = 2 mm

The question of whether the presence of other simultaneously blooming, rewarding plant species increases the rate of pollination by attracting larger numbers of pollinators or reduces it by increasing competition for pollinator service has been much discussed (e.g., Levin 1972; Neiland and Wilcock 2000; Johnson et al. 2003). Kallunki (1981), for her part, considers that pollination rates would vary positively with the abundance of *Goodyera* species and inversely with the presence and number of competing species. The flowers of *G. tesselata*, *G. oblongifolia*, and *G. repens* all attract the same type of pollinators, elicit the same type of pollinator behavior, and despite the differences noted above, are similar morphologically (Kallunki 1981). Macior (1971), Mosquin (1971), Straw (1972), Heinrich (1975), and others believe such similarities to be significant in attracting pollinators and reinforcing pollinator visits when, as in the case of *Goodyera*, individuals of each species are not abundant and flower late in the season when there are few other plants in bloom. If similarities in floral morphology and overlapping flowering periods increase bumblebee visitation and lead to an augmentation of seed production, restrictions on the development of both ethological and phenological isolation along with some continuing level of interspecific mating might be expected.

Fruiting Success and Limiting Factors

In a 30-year study in southwestern Quebec, Reddoch and Reddoch (2007) reported that juvenile plants of *G. pubescens* produced from seed developed more slowly and took longer to flower than clonal ramets. Nevertheless, a variable but usually high number of capsules were usually produced (Correll 1978; Brackley 1985; Homoya 1993; Reddoch and Reddoch 1997). The percentage of flowering rhizomes in each patch usually ranged from 0 to 8%. However, at 2–4 year intervals the percentage increased to over 8% and up to 30%. Although rhizomes were no longer connected to one another, the intermittent large flowerings were synchronized within patches, between patches, and between populations. Reddoch and Reddoch (2007) correlated this synchrony with the occurrence of an extended warm, dry period in May of the preceding year.

Kallunki (1981) found wide intraspecific variation in fruit set in four mixed, open-pollinated populations of *G. tesselata* and *G. oblongifolia* from Michigan (Table 1.2). A fruit set of 90% reported by Kipping (1971) for a small population of *G. oblongifolia* in California extends the range even further. Kallunki attributed the variation she observed to differences in the abundance and visibility of *Goodyera* species, the presence or absence of competing species, and/or differential exposure to strong winds and human activity. In any case, mean fruit set values in naturally pollinated plants of *G. tesselata*, *G. repens*, and *G. oblongifolia* were markedly lower than in hand-pollinated plants (Table 1.2), suggesting that pollinator recruitment might be a limiting factor. Flowering plants also produced a higher mean number of new rhizomatous growths than nonflowering plants (Ackerman 1975), implying an absence of resource limitation. Long-term studies are needed, however, to sort out possible initial differences in plant vigor.

In Ackerman's (1975) study of *G. oblongifolia* in California, pollinaria were removed from 45% of open-pollinated flowers and deposited on 59% of exposed stigmas. Mature capsules developed in 46% of the flowers. Ackerman considered the mortality rate, taken as the difference between the number of flowers pollinated and the number of capsules that matured, to be significant. Intervals between pollination and fertilization and between fertilization and maturation of the capsules were short. Seeds were sometimes liberated within a few weeks of flowering (Catling 1990). Although Swamy (1949) thought such short intervals represented a primitive condition, Ackerman (1975) believed they were related to mortality rates, representing a secondary adaptation to environmental conditions.

Wind distribution of the seeds and adaptation to common and widely occurring pollinators indicate a potential for wide dispersal (Muesebeck et al. 1951; Ackerman 1975). Ackerman (1975) suggests that the disjunct population structure of *G. oblongifolia* may reflect this potential. However, Case (1987) believes that, at least in the Great Lakes region, disjunct populations may represent remnants of a once more widespread distribution fragmented by glaciation.

A number of factors, therefore, appear to contribute to the reproductive success of one or more species of *Goodyera* (Ackerman 1975). Seed production is increased by self-compatibility, clonal growth, aggregation of flowers, nectar production, and adaptation to common pollinators; maintenance of genetic variability results from occasional outcrossing promoted by protandry, and hybridization is restricted to an extent that permits individual species to maintain their identities; capsule mortality may be minimized by short maturation time; and the establishment of new populations is facilitated by adaptation to wide-spread pollinators, wind dispersal of the seeds, and vegetative reproduction.

Goodyera populations are known to be sensitive to disturbance. Logging practices and habitat destruction have led to local extirpations in areas of the Pacific Northwest and are a threat at most other locations (St. Hilaire 2002). For example, populations of *G. pubescens*, perhaps the most threatened species of *Goodyera*, have been much reduced due to reduction of habitat in northeastern North America (Light 2000). The loss of a mature canopy is thought to expose the rhizomes of this species to damaging climatic fluctuations (Reddoch and Reddoch 2007). Other threats include predation, especially by deer, invasive exotic plants, low genetic diversity, acid rain, climate change, and collection for horticultural purposes (e.g., St. Hilaire 2002 and references therein).

Other Goodyerinae

Zeuxine Lindley

Zeuxine is a genus of about 30 (Ackerman 2002a) to 70 (Pridgeon et al. 2003) widely distributed species. *Zeuxine strateumatica* (L.) Schltr. (soldier's orchid) (Fig. 1.4a), native to Arabia, Asia, Australia, and the Pacific Islands, was first introduced to North America in Florida and is now also found in Georgia, Mississippi, Louisiana, and Texas (Ames 1938; Thieret 1972; Correll 1978; Ackerman 2002a). Luer (1975) observed small flies visiting the colorful and highly fragrant flowers, but based on its prolific seed production, rapid spread, and free reproduction in hot houses, he and Ackerman (2002a) concluded that it is almost certainly autogamous

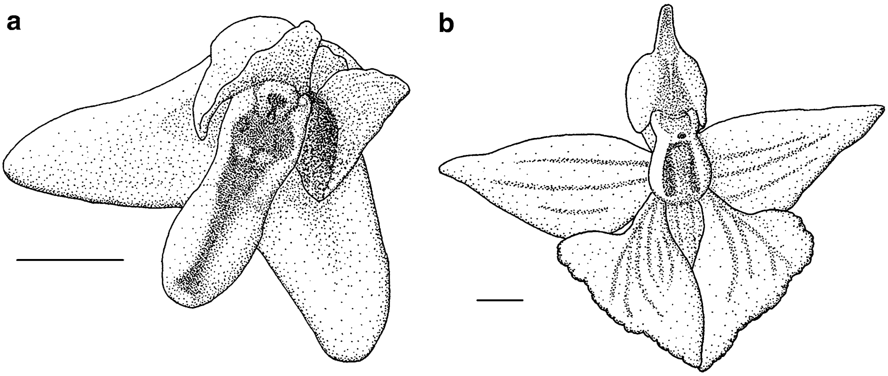

Fig. 1.4 (**a**) *Zeuxine strateumatica*, flower, front view; (**b**) *Ponthieva racemosa*, nonresupinate flower, front view, scale bars = 2 mm

or apomictic. Sun and Wong (2001) found that populations near Hong Kong are apomictic and occur only as colonizers of recently disturbed habitats. Adventitious embryony was, in fact, documented earlier for *Z. sulcata* (Roxb.) Lindl. ex Wight (Sesshagiriah 1941; Swamy 1946), now recognized as synonymous with *Z. strateumatica* (World Checklist of Monocotyledons 2008). Such a breeding system would be consistent with reported low levels of genetic variation within populations and high levels of population differentiation (Sun and Wong 2001). Of five identified cytotypes all but the $2n=20$ cytotype have abnormal meiosis (Vij and Vohra 1974; Ackerman 2002a).

Platythelys Garay

Platythelys is a genus of about 10 species found in the southern United States, Mexico, Central and South America, and the Caribbean (Pridgeon et al. 2003). A single species, *P. querceticola* (Lindl.) Garay (low erythrodes, jug orchid) (*Erythrodes querceticola* (Lindley) Ames), occurs in Florida and Louisiana. No information is available on its pollination, but the presence of a rostellum, viscidium, and saccate nectar spur (Ackerman 2002b) implies insect vectors.

Cranichidinae

The Cranichidinae include about 17 genera largely restricted to South and Central America, Mexico, and the Caribbean (Pridgeon et al. 2003). A single genus is present in our flora.

Ponthieva R. Brown

Ponthieva is a genus of about 30 widely distributed, tropical and subtropical, Western Hemisphere species (Pridgeon et al. 2003). One, *Ponthieva racemosa* (Walter) C. Mohr (shadow witch), is present in our flora. It has a nonresupinate flower (Fig. 1.4b) and occurs in the Atlantic and Gulf Coast states from Virginia to east Texas (Luer 1972; Ackerman 2002c). Information is limited, but Ackerman (1975) found Florida populations to be self-compatible and not autogamous. He reported natural fruit set in 35% of the flowers at one site in northern Florida. Luer (1972) observed very small halictid bees visiting the flowers in central Florida, but Dressler (1993) reported that the lip secretes oil, not nectar, and suggested the pollinators might be anthophorid bees that collect the oil to feed to their larvae.

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Chapter 2
Subtribe Spiranthinae

Abstract Breeding systems in *Spiranthes* are diverse. Taxa or populations within taxa can produce seed sexually or asexually or by a combination of sexual and asexual means. Mechanisms include insect pollination, autogamy, and agamospermy. A partial correlation is evident between ploidy level, mode of reproduction, and seed type. The primary pollinators of most sexual or facultatively sexual plants are medium sized to comparatively large, long-tongued bees. Smaller halictine bees are adapted to the pollination of *S. lucida.* Protandry, acropetaly, and pollinator foraging habits favor cross-pollination over geitonogamy in young flowers. Available data on the pollination of *Dichromanthus*, *Deiregyne*, *Schiedeella*, and *Microthelys* are discussed.

Keywords *Spiranthes* • *Dichromanthus* • *Deiregyne* • *Schiedeella* • *Microthelys* • Protandry and acropetaly • Sexual and asexual species • Polyembryonic seed • Long- to short-tongued bees and hummingbirds

The subtribe Spiranthinae with about 40 genera is mostly confined to the Neotropics (Pridgeon et al. 2003). Five genera, all native, are represented in our flora.

Spiranthes Richard (Ladies'-Tresses)

Spiranthes is a nearly cosmopolitan genus with approximately 45 species worldwide. About 23 are present in North America north of Mexico and Florida. All produce an upright stem bearing a terminal spike of resupinate flowers routinely coiled in a tight to loose spiral of several more or less vertical ranks (Fig. 2.1) (Luer 1975). The flowers are nodding to ascending, tubular or urn shaped (urceolate) to gaping (ringent), and variable in size (Tables 2.1 and 2.2) (Luer 1975; Sheviak and

C.L. Argue, *The Pollination Biology of North American Orchids: Volume 2: North of Florida and Mexico*, DOI 10.1007/978-1-4614-0622-8_2,

Fig. 2.1 *Spiranthes romanzoffiana*. (**a**) Inflorescence, scale bar = 2 mm; (**b**) habit, scale bar = 1 cm; (**c**) flower, exploded view, scale bar = 2 mm

Brown 2002). In our species, they range in color from white or greenish white to cream colored or yellow. The lateral petals are adherent to the dorsal sepal to form a hood over the column (Figs. 2.1 and 2.2). The lateral sepals, with straight to slightly reflexed or upturned tips, may be either spreading or confluent with the lip and lateral petals to produce a floral tube (Catling 1980b). Except for its tip, the lip is often concealed by the other members of the perianth (Fig. 2.1, see below). It is clawed and varies in shape from lanceolate to ovate or sometimes pandurate (violin shaped) and is frequently recurved with a crenulate to lacerate and often crisped apical margin (Figs. 2.1 and 2.2). Paired callosities or nectar glands are present at its base. The dorsal surface of the usually short column bears an erect, bilocular anther containing a pair of deeply cleft, soft, mealy, sectile pollinia (Figs. 2.3 and 2.4).

Table 2.1 Data on asexual/facultatively sexual species of *Spiranthes* (Sheviak and Brown 2002)

Character	*S. casei*	*S. cernua*	*S. magnicamporum*	*S. ochroleuca*	*S. odorata*
Plant height (cm)	4–77	10–50	7–60	10–55	10–100+
Dorsal sepal (mm)	5.2–8	6–12	5–14	6–14	4–18
Lateral sepals (mm)	5.2–8	6–12	5–14	6–14	4–18
Lateral petals (mm)	3.9–7.6	6–12	4.9–13		4–18
Lip (mm)	4.1–8 × 3.2–5.1	6–10.5 × 2–7.5	4.9–12 × 3.3–7	7–12 × 4–7	4–16 × (4–) 7–9.5
Viscidia		Linear to linear-lanceolate	Linear	Linear	Linear to linear-lanceolate

Table 2.2 Data on uniformly sexual species of *Spiranthes* (Sheviak and Brown 2002)

Character	*S. laciniata*	*S. vernalis*	*S. lacera*	*S. romanzoffiana*	*S. tuberosa*	*S. lucida*	*S. diluvialis*	*S. ovalis*
Plant height (cm)	20–95 (–100)	20–65	15–65	8–55	5–30	4–37	20–62	5–40
Dorsal sepal (mm)	6–10×2–3	6–10×2–3	5×1	5.3–12.5	5×1	4.5×5.5	7.5–15	3.5–6.1 to 1–1.5
Lateral sepals (mm)	6–10×2–3	6–10×2–3	5×1	5.3–12.5	5×1	5–6×1–2	7.5–15	3.5–6.1 to 1–1.5
Lateral petals (mm)	7–9×2	6–9×2	5×1	No data	5×1	5–6×2–4	No data	6×1
Lip (mm)	6–9×4	5–8×4	5×2.5	4.8–10×1.6–6.8	5×2.5	5–6×2–4	7–12×2.5–6.8	5×3

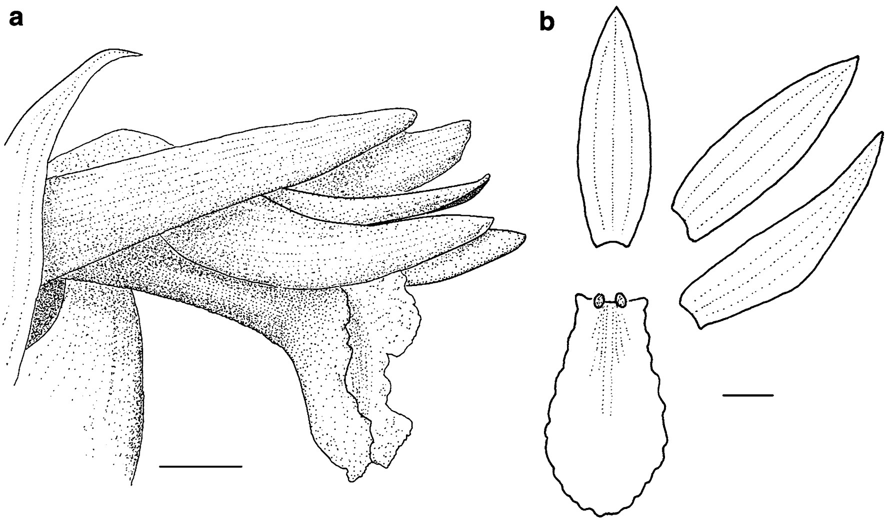

Fig. 2.2 *Spiranthes magnicamporum*. (**a**) Flower, oblique lateral view; (**b**) flower, exploded view, scale bars = 2 mm

These are attached by their apices to a single, usually long viscidium at the distal end of the rostellum, which, when removed, leaves a V-shaped notch (Fig. 2.3) (Luer 1975; Dressler 1993; Johnson and Edwards 2000). A wide, flat, stigmatic surface is positioned on the ventral side of the column behind the rostellum (e.g., Sipes and Tepedino 1995).

Breeding systems are diverse. Taxa or populations within taxa can produce seed sexually or asexually or by a combination of sexual and asexual means. Mechanisms include insect pollination (e.g., Darwin 1862; Godfery 1922, 1933; Catling 1980b, 1982; Sipes and Tepedino 1995), autogamy (Ridley 1888; Hagerup 1952; Catling 1980a, b, 1982), and agamospermy (e.g., Leavitt 1900, 1901; Schnarf 1929; Swamy 1948; Catling 1979, 1980b, 1982; Schmidt 1987; Schmidt and Antlfinger 1992).

Asexual Species or Populations

Wholly or partially agamospermous taxa include *S. cernua* (L.) L. C. Richard (nodding ladies'-tresses), *S. ochroleuca* (Rydberg) Rydberg (yellow nodding ladies'-tresses), *S. magnicamporum* Sheviak (Great Plains ladies'-tresses), *S. odorata* (Nuttall) Lindley (fragrant ladies'-tresses), *S. casei* Catling and Cruise var. *casei* (Case's ladies'-tresses), and *S. casei* Catling and Cruise var. *novaescotiae* Catling (Case's ladies'-tresses) (Table 2.1).

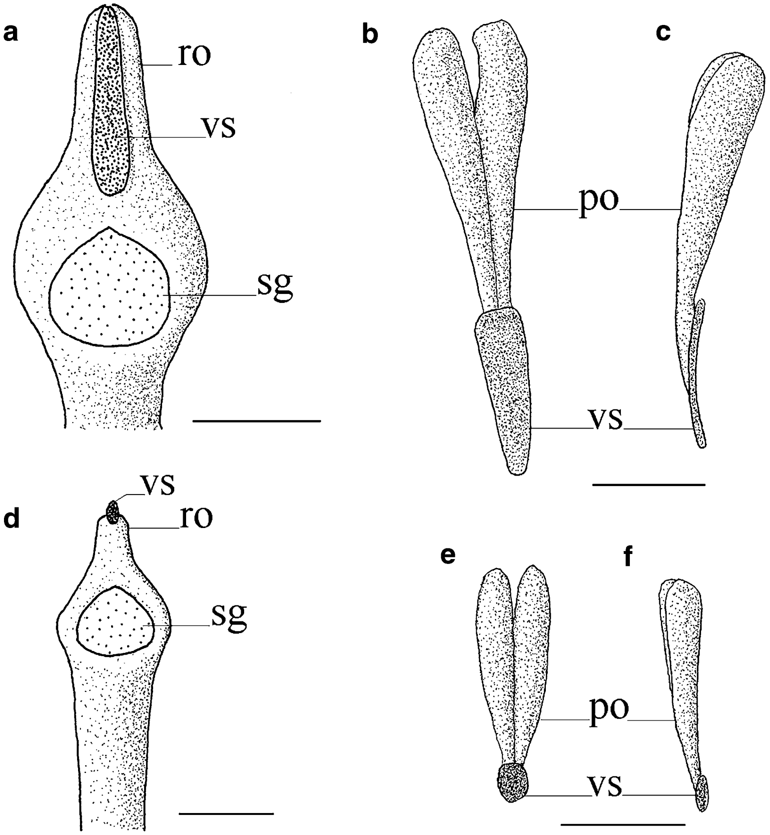

Fig. 2.3 Columns and pollinia. (**a**)–(**c**) *Spiranthes ochroleuca*; (**d**)–(**f**) *S. lucida*. Columns viewed from below (*left*); pollinia, ventral view (*center*), lateral view (*right*), scale bars = 1 mm. *po* pollinia, *ro* rostellum, *sg* stigma, *vs* viscidium

Distribution and Habitat

S. cernua commonly forms colonies in usually acidic, sunny areas of marshes, meadows, and woodland openings. It is found from Nova Scotia to Georgia and west to southern Ontario, Minnesota, and Texas (Luer 1975; Catling 1980b; Sheviak and Brown 2002). *S. ochroleuca* prefers dryer and shadier sites than *S. cernua* and occurs in developing woodland or meadows and barrens. It ranges from Nova Scotia to Tennessee and South Carolina west to Michigan and Indiana (Sheviak and Brown 2002). *S. magnicamporum* may be present in wet prairies and fens but is frequently found on dry, grassy uplands in calcareous soil, where it is largely isolated ecologically from *S. cernua* (Catling and Brown 1983; Catling 1990; Sheviak and Brown 2002). It ranges continuously from western Nebraska to Indiana, north to southern Manitoba, and south to Texas, with scattered populations occurring east and west of this region (Luer 1975; Sheviak and Brown 2002; Johnson 2006).

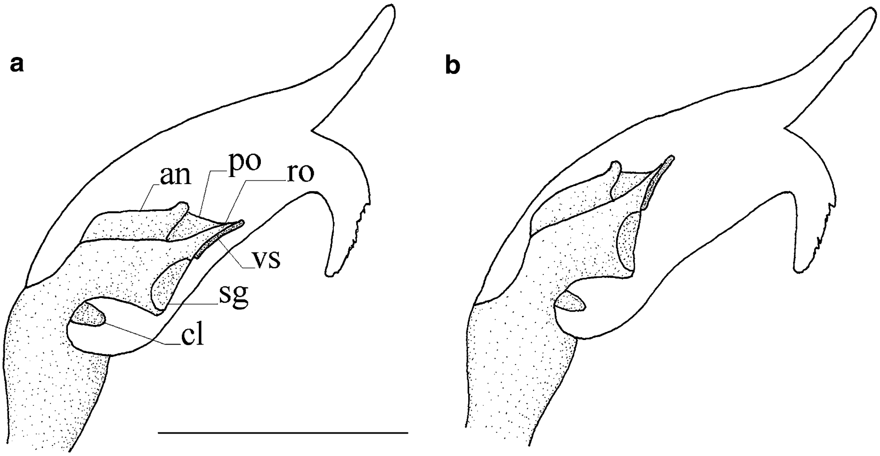

Fig. 2.4 *Spiranthes vernalis* showing the position of column in newly opened flowers (**a**) and 2–4-day-old flowers (**b**), scale bar = 5 mm. *an* anther, *cl* callus, *po* pollinia, *ro* rostellum, *sg* stigma, *vs* viscidium

S. odorata is semiaquatic to aquatic, occurring in cypress and hardwood swamps, marshes, and prairies (Sheviak and Brown 2002). According to Luer (1975), two forms occur. One prefers heavily shaded, wet woods and rich, acid humus, the other, full sun in alkaline marshes and wet prairies. It is distributed along the Atlantic and Gulf Coasts from New Jersey to Texas with disjunct populations in Kentucky and Tennessee (Catling 1982; Sheviak and Brown 2002).

S. casei var. *casei* grows on mesic to dry, open sites in periodically disturbed, coarse, sandy soils, frequently on barren ridge tops (Catling and Cruise 1974). It is most common from Nova Scotia and northern New England through northern Pennsylvania, southern Quebec, and southern Ontario to Wisconsin (Catling 1990; Sheviak and Brown 2002). *S. casei* var. *novaescotiae* is found on acidic soils on barrens and dry roadsides in southern Nova Scotia (Catling 1981).

Floral Morphology

The lip is white or ivory colored and may have a darker or yellow center. Flowers of *S. ochroleuca* are variously reported to be strongly fragrant (Sheviak 1973) or to have a very weak and musty odor (Catling and Brown 1983) while those of *S. odorata* are said to have a scent similar to that of coumarin, vanilla, or jasmine (Luer 1975). Flowers of *S. cernua* are either odorless or emit a faint scent resembling that of fresh *Cypripedium* roots (Sheviak 1973; Catling and Brown 1983). *S. magnicamporum*, on the other hand, has flowers that produce a strong, sweet, and coumarin-like fragrance, distinct from that of *S. cernua.* Flowers of agamospermous taxa bloom for 3–10 days and initiate capsule dehiscence within 2 weeks of blooming (Catling 1982).

Compatibility and Breeding System

Catling (1982) tested taxa from northeastern North America for agamospermy, autogamy, intrafloral selfing, geitonogamy, and xenogamy. He found autonomous agamospermy (i.e., agamospermy without pollination) in widely dispersed populations of *S. casei* var. *casei*, *S. casei* var. *novaescotiae*, and *S. cernua*. Emasculated specimens from these populations enclosed in insect-proof cages developed enlarged ovaries containing abundant seed. Schmidt (1987) obtained similar results for *S. cernua*, and Sheviak (1982) demonstrated normal capsule and seed development in specimens of this species with excised columns.

Autonomous agamospermy was also observed in isolated populations of the otherwise sexual species *S. magnicamporum* and *S. ochroleuca* located near the northeastern limits of their ranges. Agamospermous races of *S. magnicamporum* occurred in extreme southwestern Ontario, eastern Michigan, northern Ohio, and part of Indiana and Illinois, agamospermous races of *S. ochroleuca* in southern Nova Scotia and western Prince Edward Island (Catling 1982). *S. odorata* (*S. cernua* var. *odorata*) was also agamospermic at 12 localities near the northeastern limit of its distribution in New Jersey, Delaware, and northern Virginia (Catling 1982).

The preceding six taxa are members of the *S. cernua* complex (e.g., Catling 1980b). All are similar morphologically, bloom in the autumn, and were formerly included in *S. cernua* (Correll 1978; Catling 1982). *S. odorata*, *S. ochroleuca*, and *S. magnicamporum* are diploid ($2n=30$) (Sheviak 1982). *S. cernua* is tetraploid; a few triploids and aneuploids have also been found (Sheviak 1982). Counts of chromosome numbers for *S. casei* vary between 60 and 75 (Catling 1980b).

Sheviak (1982) established a partial correlation between ploidy level, mode of reproduction, and seed type in this complex. Diploids are sexual, undergo regular meiosis with perfect bivalent formation, and produce monoembryonic seed. Polyploids show variable levels of meiotic irregularity, as well as different levels of agamospermy and polyembryonic seed production, extruded embryos, free embryos, and ruptured embryo sacs.

Contrary to Swamy (1948), Sheviak (1982), among others, reported a high percentage of monoembryonic seed in the agamospermic allotetraploids of this complex. Single embryos were usually present in 40–60% of mature seeds, but their incidence ranged from 20 to 85% (Catling 1980b, 1982). On the other hand, polyembryony is extremely rare or absent in obligately sexual individuals (Sheviak 1982; Schmidt and Antlfinger 1992). Based on these correlations and an examination of some of Catling's material, Sheviak (1982) concluded that the specimens of *S. magnicamporum, S. ochroleuca,* and *S. odorata* examined by Catling from near their northeastern range limits were polyploid. However, Catling's (1980b) report of a diploid, agamospermic specimen of *S. magnicamporum*, acknowledged by Sheviak (1982), indicates that the correspondence is not perfect.

Based on direct anatomical studies of *S. casei* var. *casei, S. casei* var. *novaescotiae*, *S. cernua*, and an agamospermous race of *S. magnicamporum*, Catling (1982)

Table 2.3 Pollinators of *Spiranthes casei* and *S. cernua*

Species	Pollinator/state or province	References
S. casei	Apidae	
	Bombus terricola Kirby/NY	Sheviak (1982)
	Halictidae	
	Dialictus versans (Lov.)/ON	Catling (1980b, 1983c)
S. cernua	Apidae	
	B. fervidus (Fabricios)/NH	Sheviak (1982)
	NY	Sheviak (1982)
	VT	Sheviak (1982)
	NS	Catling (1980b, 1983c)
	NH	Luer (1975), p. 120
	B. impatiens Cresson/MA	Sheviak (1982)
	B. terricola Kirby/NY, KS	Sheviak (1982)
	B. sp./NY, NE	Sheviak (1982)
	NC	Stevenson (1973)
	Halictidae	
	D. sp./NC	Stevenson (1973)

confirmed earlier reports (Leavitt 1900, 1901; Swamy 1948) that agamospermy in the *S. cernua* complex occurs by adventitious embryony. While the flower is still in bud and at about the time the megasporocyte undergoes its first division, one or two cell layers of the inner integument, usually in an area near the micropylar end of the ovule, start to enlarge and divide (Swamy 1948; Catling 1982). By anthesis or several days thereafter, proliferation in this area has produced 10–30 sometimes greatly enlarged cells, and degeneration of the nucellus has occurred (Catling 1982). Embryo sac development usually fails to proceed beyond the 4-nucleate stage (Swamy 1948; Catling 1980b, 1982; Lakshmanan and Ambegaokar 1984). The enlarged cells ultimately give rise to one or multiple embryos.

Unlike Leavitt (1900), Swamy (1948) found that in a few collections of "*S. cernua*" individual capsules sometimes contained both seeds with adventitious embryos and seeds with embryos derived from the fertilization of normally developed embryo sacs. Although in asexual individuals adventive embryos developed very early, in these so-called "intermediate" individuals their development was delayed until after the normally developed ovules had been fertilized.

The northeastern species of *Spiranthes* studied by Catling (1980b, 1982) differ from many plants with adventitious embryony in not needing pollination to stimulate development of the embryo (pseudogamy), but this does not mean that agamospermy is obligate (Leavitt 1901; Stebbins 1941; Maheshwari 1952; Catling 1982). None of the taxa with adventitious embryony have lost the morphological features required for pollination (Catling 1981, 1982; Sheviak 1982). The flowers remain in anthesis 3–10 days, secrete nectar, are functionally capable of pollen production and reception, may emit a characteristic fragrance (Catling 1980b, 1982), and are frequently pollinated by insects (Tables 2.3–2.9).

Table 2.4 Pollinators of *Spiranthes magnicamporum*, *S. ochroleuca*, and *S. odorata*

Species	Pollinator/state or province	References
S. magnicamporum	Apidae	
	Bombus fervidus (Fabricus)/ND	Sheviak (1982)
	B. nevadensis var. *americanum*/WI	Hapeman (1996)
	B. sp./ON, IL	Catling (1980b, 1983c)[a]
S. ochroleuca	Apidae	
	B. impatiens Cresson/MA	Sheviak (1982)
	B. pennsylvanica (DeGeer)/ne U.S.	Ames (1921), p. 81
	B. vagans/VT	Sheviak (1982)
	B. sp./PA, MA, ON	Catling (1983c)[a]
S. odorata	Apidae	
	B. pensylvanicus (DeGeer)/FL	Dodson in Luer (1975, p. 120) [as *B. americanorum* (Fabr.)]
	B. pennsylvanicus/FL	Sheviak (1982)
	B. fervidus/NH	Luer (1975)
	B. impatiens Cresson/MA	Sheviak (1982)
	B. nevadensis Cresson/NC	Stevenson (1973)

[a]Unobserved; likely based on floral morphology and/or flowering pattern

Table 2.5 Pollinators of *Spiranthes lacera*

	Pollinator/state or province	References
Andrenidae	*Calliopsis andreniformis* Smith/IL	Robertson (1893, 1929)
Apidae	*Bombus americanum* (Fabricus)/IL	Robertson (1893, 1929)
	B. perplexus Cresson/ON	Catling (1980b, 1983c)
	B. terricola Kirby/ON	Catling (1980b, 1983c)
	B. vagans Smith/ON	Catling (1980b, 1983c)
	B. sp./NJ	Catling (1983c)[a]
Halictidae	*Dialictus immitatus* (Lov.)/ON	Catling (1980b, 1983c)
Megachilidae	*Anthidium notatum* Latreille/FL	Robertson (1893)
	Hoplitis truncata Cresson/ON	Catling (1980b, 1983c)
	Megachile brevis Say/FL	Robertson (1893, 1929)
	M. inermis Prov./ON	Catling (1980b, 1983c)

[a]Unobserved; likely based on floral morphology or flowering pattern

Studies examining population variability and seed morphology are consistent with the occurrence of differing levels of sexuality in agamospermous taxa. In a multivariate analysis of morphological data based on plants from southwestern Ontario, Catling and Brown (1983) found that populations of *S. magnicamporum*, although clearly agamospermous based on their frequent development of polyembryonic seed, were also distinctly more variable than those of *S. cernua*. It is likely that the differences in variability reflect differences in levels of sexuality and that agamospermy is facultative in populations of *S. magnicamporum* at this site (Swamy 1948; Catling 1982; Catling and Brown 1983).

Based on breeding experiments and levels of polyembryonic seed production, Schmidt (1987) and Schmidt and Antlfinger (1992) concluded that agamospermy is also the predominant mode of reproduction for *S. cernua* near the edge of its range in eastern Nebraska. However, experimental treatments testing for geitonogamy and xenogamy produced a significantly higher proportion of nonpolyembryonic seeds than tests for agamospermy (Schmidt and Antlfinger 1992). They considered that agamospermy was most likely facultative on the assumption that some of the additional nonpolyembryonic seeds were produced sexually. Based on seed morphology in natural populations, the maximum estimates of sexual seed production per capsule ranged from about 20 to 34% in successive years. This would provide sufficient genetic diversity to account for the amount of allozyme variation observed in this population (Schmidt and Antlfinger 1992).

S. parksii Correll, described as a facultatively agamospermous tetraploid species endemic to postoak savannas in East Central Texas (Catling and McIntosh 1979; Catling 1990; Sheviak and Brown 2002), is not distinct from *S. cernua* (Dueck 2008; Dueck and Cameron 2008).

Fruiting Success and Limiting Factors

Schmidt (1987) suggested that the high proportion of agamospermic seeds produced in eastern Nebraska populations of *S. cernua* might be due to pollinator limitation (discussed below under sexual populations) or to the precocious development of adventitious embryos prior to anthesis. Indeed, Sheviak (1982) reported that capsules developed prior to anthesis in some plants of this species, and as noted earlier, Catling (1982) described the initiation of adventitious embryos while the flower was still in the bud stage. Precocious agamospermic reproduction might increase the proportion of asexual embryos by reducing the resources available for the later development of meiotic embryos (e.g., Nogler 1984). If so, any factors that suppress or delay the initiation of asexual reproduction might be expected to permit the maturation of more megagametophytes and hence to increase the level of sexual reproduction (Catling 1982), but such factors have yet to be identified (Schmidt 1987).

Schmidt (1987) and Schmidt and Antlfinger (1992) observed significant yearly fluctuations in the proportion of polyembryonic seeds in unmanipulated plants of *S. cernua* at their study site. Although such fluctuations might be attributed to differences in pollinator availability or to genetic diversity among the plants observed, they might also reflect environmental influences other than those affecting pollinator abundance. Clausen (1954) and Marshall and Brown (1981) considered the level of agamospermy to mirror an interplay of environmental and genetic factors, and Knox (1967), for example, demonstrated that differences in photoperiod at different latitudes correlated with the level of agamospermy in grasses. However, a more complete understanding of interrelationships between asexual and sexual seed production in *S. cernua* must await the results of additional genetic and developmental studies.

Facultative agamospermy is more common and versatile in angiosperms than obligate agamospermy and does not represent the evolutionary blind alley often associated with the latter (Clausen 1954; Nygren 1966; DeWet and Stalker 1974; Asker 1979; Catling 1981, 1982). When compared with autogamy, agamospermy requires less extreme morphological adaptation (Ornduff 1969) and produces no inbreeding depression (Charlesworth and Charlesworth 1979). At the same time, fertility is assured; the potential for rapid colonization is retained; heterozygosity is maintained; and the fixation of particular patterns of variability (new adaptive gene combinations) may be facilitated (Baker 1955; Antonovics 1968; Jain 1976; Solbrig 1976; Marshall and Weir 1979; Catling 1980b, 1982; Lloyd 1988). Short-term fitness may, thus, be combined with the advantages of genetic recombination (Catling 1982). The latter includes, for example, the ability to recombine new, advantageous mutants, permitting evolutionary responses to changing environmental conditions; the ability of the best-adapted genotypes to escape the accumulation of linked, disadvantageous but nonlethal mutants ("Muller's Ratchet"); and the ability to fill the maximum number of environmental niches through a high level of genetic variation among individuals.

Agamospermy is also thought to confer advantages over sexual reproduction in certain areas, where it may reflect adaptation to isolation or to a reduction in the availability or activity of pollinators (Lloyd 1980, 1988; Manning 1981; Catling 1982; Schmidt and Antlfinger 1992). Such sites include wide expanses of recently glaciated territory, edges of a species range, recently established vegetation zones, or more or less isolated bioclimatic zones (e.g., Bayer and Stebbins 1980; Catling 1982; Schmidt and Antlfinger 1992). For example, the three species, *S. magnicamporum, S. ochroleuca*, and *S. odorata*, which appear to be sexual over most of their distributions, reveal an apparent association between agamospermy and range limits, particularly northeastern range limits (Catling 1982). *S. casei* is distributed within formerly glaciated territory (Prest 1970; Ives et al. 1975; Catling 1990), and *S. casei* var. *novascotiae* lies in the relatively isolated bioclimatic zone of southern Nova Scotia (Fernald 1921; Roland and Smith 1962–1969; Catling 1981, 1982, 1990). The association of agamospermy with northeastern range limits, glaciated territory, and isolated bioclimatic zones, its augmentation of other isolating mechanisms, and its presence in a complex of closely related taxa suggest that it has been a significant factor in the production of new species (Catling 1982, 1983a).

Sexual Species or Populations

This section examines sexual reproduction in uniformly sexual species of *Spiranthes* and the facultatively agamospermous populations of the *S. cernua* complex whose asexual reproduction was discussed above. Uniformly sexual species include: *S. lacera* (Rafinesque) Rafinesque var. *lacera* (northern slender ladies'-tresses), *S. lacera* var. *gracilis* (Bigelow) Luer (southern slender ladies'-tresses), *S. tuberosa* Rafinesque (little ladies'-tresses), *S. vernalis* Engelmann and Gray (spring ladies'-tresses), *S. laciniata* (Small) Ames (lace-lip ladies'-tresses), *S. romanzoffiana* Chamisso (hooded ladies'-tresses), *S. diluvialis* Sheviak (Ute ladies'-tresses), *S. lucida* (H. H. Eaton)

Ames (wide-leaved ladies'-tresses), *S. ovalis* Lindley var. *erostellata* Catling (oval ladies'-tresses), and *S. ovalis* var. *ovalis* (oval ladies'-tresses) (Table 2.2).

Distribution and Habitat

Most species are variously distributed in the eastern USA from the Atlantic to Saskatchewan, Nebraska, Kansas, and Texas. The range of *S. romanzoffiana*, however, extends across Canada from Newfoundland to the Aleutian Islands and at elevation to New Mexico, Arizona, Nevada, and California. *S. diluvialis* is an amphiploid ($2n = 74$) resulting from hybridization of *S. romanzoffiana* ($2n = 44$) and *S. magnicamporum* ($2n = 30$), probably during the last glacial period (Sheviak 1984; Arft and Ranker 1998). A rare riparian species, it is confined to mesic or wet meadows and marshes near permanent drainage systems, springs, or large lakes scattered from Nebraska and Montana to Nevada and Washington (Sipes et al. 1993; Sheviak and Brown 2002; Bjork et al. 2008). *S. lucida*, *S. romanzoffiana*, and *S. laciniata* are also frequently found in wet to mesic sites from calcareous fens and shorelines to meadows (Luer 1975; Catling 1980b; Sheviak and Brown 2002). The remaining species occur in a wide range of situations, including open forests, grassy meadows, old fields, and roadside ditches (Luer 1975; Sheviak and Brown 2002).

Floral Morphology

The lip varies from white, often with a yellowish tinge or a yellowish or greenish center, to rich saffron yellow with green tints in *S. lucida*. The column lacks a rostellum and viscidium in *S. ovalis* var. *erostellata* (Catling 1983b).

The flowers of *S. diluvialis* have a faint, coumarin-like scent while those of *S. romanzoffiana* are said to produce a weak and musty-sweet or vanilla-like odor (Sheviak 1984; Larson and Larson 1990). Flowers of sexual species bloom for 10–40 days and initiate capsule dehiscence 14 (7–21) days after pollination (Catling 1982). Flowers over 30 days old are no longer functional. *S. lucida* blooms much earlier than the other sexual species and is further distinguished by a number of floral features, including the shape of its viscidium, the location of its nectar, and the orientation of its stigma (see below) (Catling 1983c, 1990).

Compatibility and Breeding System

Catling (1982) found that there was no expansion of the ovary or development of seed in caged, unmanipulated flowers of *S. lacera* var. *lacera*, *S. lucida*, *S. romanzoffiana*, *S. vernalis*, or populations of *S. ochroleuca* from Pennsylvania and New York or *S. magnicamporum* from western Illinois. Observations of other species of *Spiranthes* maintained in insect-proof cages indicated that *S. laciniata*, *S. lacera* var *gracilis*, and *S. tuberosa* were also uniformly sexual (Catling 1980b, 1982).

Experimental self-pollination, geitonogamous pollination, and cross-pollination in examined species all usually led to full ovary expansion. However, when compared to self- and geitonogamous pollinations, cross-pollinations produced a higher percentage of ovaries with seeds (Catling 1980b, 1982). Catling attributed the difference to partial self-incompatibility or postzygotic embryo abortion (e.g., Brink and Cooper 1939, 1947).

Embryo development followed a similar pattern. Although highly variable, the percentage of seeds with well-developed embryos was reduced in cases of self-pollination and geitonogamous pollination when compared to cross-pollination (Catling 1980b, 1982). Catling (1980b, 1982) suggested that this might be related to the rate of pollen-tube growth and/or a reduced rate of pollen germination in intraplant pollinations (e.g., Weller and Ornduff 1977). Seed development and capsule dehiscence occurred up to 5 days earlier in cross-pollinated as compared to self-pollinated individuals (Catling 1980b). In addition, seed development only occurred in the upper portion of the ovary in cases of geitonogamous and self-pollination, especially in populations of *S. lacera* var. *lacera* and *S. lucida* (Catling 1980b, 1982). Thus, it appears that some interference with the development of the pollen tubes occurred in these treatments. Inbreeding depression in *S. lucida* was also suggested by a reduction in the size of the embryo in many seeds resulting from self- or geitonogamous pollinations, often to one-quarter or less of the usual size (Catling 1982). No small embryos were produced in cross-pollinated ovaries: all seed coats contained either large embryos or no embryos at all (Catling 1980b, 1982).

Unlike *S. ovalis* var. *ovalis* (an outcrossing or geitonogamous plant), the absence of a rostellum in *S. ovalis* var. *erostellata* permits the pollinia to develop in direct contact with the stigmatic surface (Catling 1983b). The seeds are monoembryonic, and routine experimental tests suggest that the flowers are autogamous, although pseudogamy cannot be entirely excluded (Catling 1980b, 1983b). In addition, the flowers are partly open, and a limited amount of outcrossing might be possible (Catling 1983b; Sheviak and Brown 2002).

Autogamy can confer advantages pertinent to plants colonizing disturbed or successional sites, and *S. ovalis* var. *erostellata* has recently become more common in old-field and second growth woodland habitats in some northern parts of its distribution (Sheviak 1974). Like agamospermy, the level of autogamy can be responsive to both genetic and environmental factors (Uphof 1938; Jain 1976; Frankel and Galun 1977) and is, therefore, subject to selection (Catling 1983a). Mixed mating systems may be highly adaptive to characteristics of the local environment, such as the availability of pollinators. As in facultative agamospermy, protracted adjustment of autogamy and outcrossing may reach equilibrium, balancing short-term fitness with the advantages of genetic recombination (Catling 1982). In addition, distinct modifications in floral morphology, associated with restricted gene flow, accompany autogamy (see Ornduff 1969) and may be related to the development or augmentation of ethological isolating mechanisms (Levin 1971).

The absence of a rostellum and the occurrence of autogamy are unknown among other North American species of *Spiranthes* (Catling 1980b, 1983b). However, among European and Asiatic members, autogamy is present in northern Danish populations of *S. spiralis* (L.) Chev. (Hagerup 1952), at least some Australian populations of *S. sinensis* (Pers.) Ames (Darwin 1862; Fritzgerald 1876; Ridley 1888) and Hong Kong populations of *S. hongkongensis* Hu and Barr (Hu 1977). The column also lacks a rostellum in some populations of *S. sinensis* (Ridley 1888) and a viscidium in *S. hongkongensis* (Hu 1977; Sun 1996), and the pollinia come into direct contact with the stigma in both.

Another type of mixed mating system based on varying levels of selfing and outcrossing is found in the rare orchid *S. diluvialis* at sites in Colorado and Utah (Sipes and Tepedino 1995). Experimental self-pollination, geitonogamous pollination, and cross-pollination produced no statistically significant differences in fruit and seed set (Sipes 1995; Sipes and Tepedino 1995). Thus, no indication of self-incompatibility or inbreeding depression is present in this species, and although outcrossing is favored by protandry, acropetally, and pollinator foraging habits (see below), male and female phases overlap and facilitated selfing and geitonogamy occur. Tests for autogamy and agamospermy led to no fruit production, and no polyembryonic seeds were found in any of the populations examined (Sheviak 1984; Sipes and Tepedino 1995). As in other nonautogamous members of *Spiranthes* (Catling 1982), autogamy is precluded by the structure of the column which prevents contact between the pollinium and stigma in the absence of a pollinator (Fig. 2.4) (Sipes and Tepedino 1995).

Various factors contribute to the maintenance of species integrity in taxa with overlapping distributions (Sheviak and Brown 2002). Phenology is highly variable, but may be of some significance in the isolation of particular species. For example, in southwestern Ontario, *S. ochroleuca* and *S. magnicamporum* bloom simultaneously after *S. cernua* has passed its peak (Catling and Brown 1983). Both can cross with *S. cernua* but, like most members of the *S. cernua* complex, are more or less genetically isolated from one another (Sheviak 1982). They tend to be spatially isolated by soil pH as well, *S. ochroleuca* preferring acidic conditions and *S. magnicamporum* alkaline (Catling and Brown 1983). In addition to a degree of phenological isolation, *S. cernua* is also partly isolated from *S. magnicamporum* and *S. ochroleuca* by a preference for relatively moist sites (Catling and Brown 1983). Differences in ploidy level may also restrict introgressive hybridization between *S. cernua* with $2n=60$ chromosomes and *S. ochroleuca* or *S. magnicamporum* with $2n=30$ (Sheviak 1976; Catling 1980b; Sheviak and Catling 1980). Occasional crosses do occur, however, and it is likely that hybridization between *S. cernua* and related diploids accounts for a large part of the variability observed in this species (Sheviak 1982; Catling 1990; Johnson 2006).

Sheviak (1973, 1982) suggested that pollinator discrimination might further isolate *S. cernua* and *S. magnicamporum*. Although this hypothesis has not been corroborated by subsequent studies (Sheviak 1976, 1982; Catling 1983c) and both species share at least one common pollinator in eastern North America (Tables 2.3 and 2.4), the flowers and inflorescences of these plants differ in appearance, and as already noted the flowers of *S. magnicamporum* have a strong and distinctive fragrance (Sheviak 1973, 1976, 1982). Catling and Brown (1983) think that pollinators in mixed populations of these species may very well respond to these differences. At the same time, a general similarity in floral morphology may serve to attract and reinforce the behavior of pollen vectors as in *Goodyera* (Kallunki 1981; Catling and Brown 1983).

Pollinators and Pollination Mechanisms

The primary pollinators of this genus are medium sized (ca. 9-mm long) to comparatively large (15–20-mm long), long-tongued bees, particularly species of *Bombus* and to lesser extent members of the Megachilidae (Tables 2.3–2.9) (Darwin 1862; Robertson 1893, 1929; Ames 1921; Godfery 1931, 1933; Catling 1980b, 1983c; Sheviak 1982). Smaller halictine bees (species of *Lasioglossum* (*Dialictus* Robertson) and *Augochlorella*) are adapted to the pollination of *S. lucida* (Table 2.7); visits of these bees to other species are often erratic and associated with inefficient pollen transfer (see below) (Catling 1980b, 1983c). Among the few remaining tabulated insects, the importance of the andrenid *Calliopsis andreniformis* as a pollinator of *S. lacera* (Table 2.5) remains to be determined. The same may be said of the long-tongued bees of the genus *Anthophora* and the typhiid wasp, *Myzinum*, reported as pollinators of *S. diluvialis* (Table 2.8). Sipes and Tepedino (1995) only rarely observed them bearing any pollinaria, and they have not been implicated in the pollination of other species of *Spiranthes* (Catling 1983c).

In most *Spiranthes*, the nectar glands (calli) secrete their nectar into the base of the floral tube (Fig. 2.5a) (Ames 1921; Correll 1978; Catling 1982, 1983c). The relatively long, curved galea and projecting tongue of *Bombus* species and Megachilidae are well-adapted to reach this nectar source. The galea is hinged to the Stipes and when extended it reaches well forward of the head. As the insect inserts its head into the flower to obtain the nectar, it brings the dorsal surface of the flat-topped galea into contact with the viscidium (Fig. 2.5b) (Catling 1983c). The viscidium is elongated and rigid in all northeastern species of *Spiranthes*, except *S. lucida* (Fig. 2.3) (see below), and attaches readily to the stiff, flat-topped galea (Fig. 2.5c) (Catling 1980b, 1983c; Catling and Catling 1991). It is oriented parallel to the long axis of the proboscis and adheres to the middle or proximal upper surface in bees with a short galea and to the distal upper surface in bees with a long galea. A similar mode of attachment was reported for *Bombus* pollinators of *S. romanzoffiana* and *S. diluvialis* (see below) in western North America (Tables 2.6 and 2.8) (Larson and Larson 1987; Sipes and Tepedino 1995) and *S. spiralis* in England (Darwin 1862; Godfery 1933).

The flowers open first near the base of the spike, and blooming proceeds upward in sequence (e.g., Darwin 1862; Catling 1983c; Sipes and Tepedino 1995). Individual flowers are protandrous. In newly opened flowers, the lip and column are close together and the stigma hidden (Fig. 2.4a). At this male stage, incoming pollinia

Table 2.6 Pollinators of *Spiranthes romanzoffiana*

	Pollinator/state or province	References
Apidae	*Apis melifera* L./BC	Larson and Larson (1987)
	Bombus bifarius Cresson/BC	Larson and Larson (1987, 1990)
	B. borealis Kirby/ON	Catling (1980b, 1983c)
	B. flavidus (Fabricius)/ON	Catling (1980b, 1983c)
	B. flavifrons Cresson/BC	Larson and Larson (1987, 1990)
	B. cf. *insularis* (Smith)/NL	Catling (1980b, 1983c)
	B. perplexus Cresson/ON	Catling (1980b, 1983c)
	B. vagans Smith/QC	Godfery (1933), p. 96
	/ON, QC	Catling (1980b, 1983c)
	B. vagans ssp. *bolsteri* Franklin/NL	Catling (1980b, 1983c)
	B. terricola Kirby/NL, ON	Catling (1980b, 1983c)
	B. terricola occidentalis Greene/BC	Larson and Larson (1987, 1990)
	B. ashtoni (Cresson)/NL	Catling (1980b, 1983c)
	B. insularis (Smith)/BC	Larson and Larson (1987)
Halictidae	"*Chloryalictus smilacini*" presents identity unknown/QC	Godfery (1933), p. 96
	Halictus confuses Smith (as *H. provancheri* D.T.)/QC	Godfery (1933), p. 96
	Lasioglossum zonulum (Smith)/NL	Catling (1980b, 1983c)
Megachilidae	*Megachile melanophea* Smith/NL	Catling (1980b, 1983c)

Table 2.7 Pollinators of *Spiranthes laciniata*, *S. lucida*, *S. ovalis* var. *ovalis*, and *S. parksii* (= *S. cernua*)

Species	Pollinator/state or province	References
S. laciniata	Apidae	
	Bombus sp./FL	Catling (1980b)[a]
S. lucida	Halictidae	
	Augochlorella aurata (Smith)/ON	Catling (1980b, 1983c)
	Dialictis immitatus (Smith)/ON	Catling (1983c)
S. ovalis var. *ovalis*	Apidae	
	B. sp./?	Catling (1980b)[a]
	Halictidae /?	Catling (1980b)[b]
S. parksii (= *S. cernua*)	Unknown/TX	Catling and McIntosh (1979), Catling 1990; Sheviak and Brown (2002)

[a] Unobserved; likely based on floral morphology and/or flowering pattern
[b] Confirmation needed
[?] Study sites were not given, and therefore no data are available

Table 2.8 Pollinators of *Spiranthes diluvialis*

	Pollinator/state or province	References
Anthophoridae	*Anthophora terminalis* Cresson/CO	Sipes and Tepedino (1995)
	Anthophora Latreille sp./UT	Sipes and Tepedino (1995)
Apidae	*Bombus fervidus*/UT	Sipes and Tepedino (1995)
	B. morrisoni Cresson/UT	Sipes and Tepedino (1995)
Tiphiidae	*Myzinum* Latreille sp./UT	Sipes and Tepedino (1995)

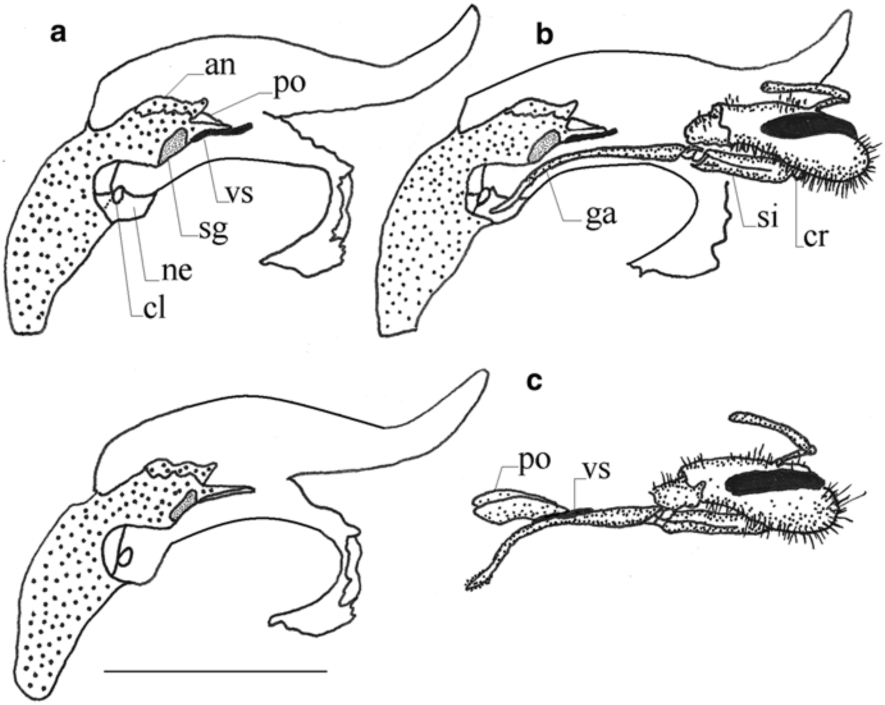

Fig. 2.5 *Spiranthes romanzoffiana* and *Bombus vagans*. (**a**) Lateral view of flower with part of the perianth removed; (**b**) bee removing nectar, showing the position of head and mouthparts; (**c**) lateral view of flower following removal of the pollinarium and the head of *B. repens* with the pollinarium attached to its galea, scale bar = 5 mm. *an* anther, *cl* callus, *cr* cardo, *ga* galea, *ne* nectar, *po* pollinia, *sg* stigma, *si* stipes, *vs* viscidium. After Catling 1983c, ©2008 of NCR Canada. Reproduced with permission

attached to the upper surface of a bumblebee's proboscis cannot readily reach the stigmatic surface but are instead diverted above and around the column by the downward pointing rostellum at its tip (Fig. 2.4a) (Catling 1980b, 1983c). However, the galea of probing bees can easily contact the exposed viscidia at the tip of the rostellum and extract the pollinia (Fig. 2.5) (Catling 1980b, 1983c). After 2–4 days, the column and lip separate and the stigma is exposed so that pollinia are easily deposited on its surface (Fig. 2.4b). In some species (e.g., *S. lacera* var. *lacera* from Ontario, *S. vernalis* from New Jersey), the stigmatic surface becomes stickier after the column separates from the lip (Catling 1983c).

Because of the bumblebee's tendency to move from the base toward the top of the inflorescence, in full anthesis the older, functionally female flowers at the base of the inflorescence are visited first and receive pollen from other plants (e.g., Darwin 1862; Gray 1862a; Catling 1980b, 1983c; Corbet et al. 1981; Willems and Lahtinen 1997). The younger flowers near the top are functionally male and donate pollen prior to the maturation and exposure of their stigmas (Catling 1983c).

The bumblebees that arrive as pollen donors, thus, depart as pollen recipients, and selfing and geitonogamous pollination are reduced while cross-pollination is promoted (Catling 1983c). The effectiveness of protandry has now been demonstrated experimentally (Jersakova and Johnson 2007).

Darwin (1862) first drew attention to protandry and sequential flowering as factors promoting cross-pollination in *S. spiralis*. This flowering pattern has now been confirmed for *S. spiralis* (Willems and Lahtinen 1997) and reported for other species, including *S. cernua* and *S. lacera* var. *gracilis* (Gray 1862b), *S. romanzoffiana* (Godfery 1933; Summerhayes 1951; Larson and Larson 1987, 1990), *S. sinensis* (Coleman 1933), and *S. diluvialis* (Sipes and Tepedino 1995).

In addition, Catling (1980b, 1982, 1983c) confirmed the association of this flowering pattern with pollination by *Bombus*, a few other Apidae, and some Megachilidae. The relationship is documented for North American species of *S. cernua* var. *cernua* from southeastern Ontario, southwestern Quebec, and New England; *S. lacera* var. *lacera* from Ontario, Quebec, Nova Scotia, and Pennsylvania; *S. romanzoffiana* from Ontario, New Brunswick, Newfoundland, and Quebec; *S. vernalis* from New Jersey, Florida, and Georgia; and, based on more limited data, *S. odorata* from the southern Coastal Plain. Floral morphology, acropetal flowering, and protandry imply that bumblebees are also the primary pollinators of northeastern North American species of *S. lacera* var. *gracilis* in New Jersey; *S. laciniata* in central Florida; *S. magnicamporum* in southwestern Ontario (facultatively agamospermic population) and western Illinois (sexual population); *S. ochroleuca* in Pennsylvania, Massachusetts, and southwestern Ontario; and *S. tuberosa* in New Jersey (Catling 1980b, 1982, 1983c).

The upward movement of bumblebees on the inflorescence of *Spiranthes* has been verified in North America for *Bombus terricola* Kirby, *B. vagans* ssp. *vagans* Smith, *B. vagans* ssp. *bolsteri* (Franklin) (= *B. vagans* Smith), *B. flavifrons* Cresson, *B. bifarius* Cresson, *B. morrisoni* Cresson, *B. fervidus* (Fabricius), and 15 other unidentified *Bombus* species (Catling 1983c; Larson and Larson 1987, 1990; Sipes et al. 1993; Sipes and Tepedino 1995). Consistent movement in one direction may benefit the forager by reducing its chances of revisiting newly emptied flowers (e.g., Pyke 1978; Heinrich and Waddington 1979). Corbet et al. (1981) found that bees moved upward when flowers of the inflorescence were visited head up and downward when they were visited head down. Floral morphology in *Spiranthes* requires that bees of the Apidae and Megachilidae visit the flower in an upright position to extract nectar (Catling 1983c). In addition, it is probably more difficult for large bees to crawl down a spike than up (Catling 1983c). Although Corbet et al. (1981) found bee movements to be independent of the vertical pattern of reward, Catling (1983c) maintained that the presence of more nectar in older flowers at the base of the inflorescence makes it more energy efficient for bees to visit these flowers first.

Changes in the perianth may sometimes permit bees to identify the flowers at the base of the inflorescence that contain the largest amounts of nectar. Thus, for example, two characteristic brown spots appear on the lips of old or pollinated flowers of *S. vernalis* (Luer 1975; Catling 1980b). In most species, pollination simply leads to a halt in nectar secretion followed by a wilting and fading of flower color within 1–3 days. In *S. ochroleuca*, however, 30–40-day-old, unpollinated flowers with a dry stigma, nonfunctioning viscidium, and dead ovules still contain nectar and remain fresh looking (Catling 1983c). Catling (1980b, 1983c) suggested that these flowers might improve the chances of pollination in other nearby plants of *S. ochroleuca* by rewarding and, thus, reinforcing pollinator visitations. Selection for this condition might occur within a genet or in populations of closely related plants.

Depending on the species and the weather, all flowers in a spike become functionally female after 5–30 days. However, a functional viscidium can still be present (e.g., Catling 1983c). Thus, although outcrossing is initially favored by protandry, acropetally, and the predominantly upward movements of bumblebees on the inflorescences, circumstances change if the pollinia are still in place a number of days after the beginning of anthesis. Flowers then contain exposed, receptive stigmas and fully viable pollen, and pollinators may effect self-pollination (Sipes and Tepedino 1995). The level of selfing may consequently be dependent on the frequency of pollinator visits (Sipes 1995; Sipes and Tepedino 1995). For example, when visitation rates were low in populations of *S. diluvialis* from Colorado and Utah, more flowers reached the female stage with a functional viscidium, and a higher percentage of pollinaria (>80%) were removed during this hermaphroditic stage. When rates were high, only 16% of pollinaria were removed during this stage (Sipes and Tepedino 1995). Of course, the number of pollinations per visit would be higher in an inflorescence with functionally hermaphroditic flowers than in one with flowers in separate male and female stages (Sipes 1995). As compared to obligate outcrossing, this circumstance could provide a selective advantage in species with low levels of self-incompatibility and few pollinator visitors (Sipes and Tepedino 1995).

Unlike the other examined taxa of *Spiranthes*, *S. lucida* is specifically adapted to pollination by halictine bees (Table 2.7) (Catling 1983c). The elongated viscidium and protandry associated with bumblebee and megachilid pollination are absent: the viscidium is oval (Fig. 2.3), and the inflorescence is relatively short and not protandrous or only weakly so (Catling 1980b, 1983c). The stigma is glutinous and more upright and accessible in newly opened flowers, and pollinaria are easily removed at this stage (Catling 1983c).

Due to the relatively greater length of its column and the short claw of its lip, the calli in *S. lucida* secrete nectar onto the ventral surface of the column rather than into the base of the floral tube (Fig. 2.6) (Catling 1983c). It is here more easily reached by a short-tongued bee. In direct contrast with the Megachilidae and Apidae, the basal parts of the proboscis in halictines are relatively long with a well-developed cardo, prementum, and stipes (Fig. 2.6) (Catling 1980b, 1983c). At the same time, the terminal parts, including the galea, palpus, and glossa, are small (Catling 1980b, 1983c). This morphology appears to be well-adapted to reach the nectar on the ventral surface of the column behind the stigma. As the bee inserts its head into the relatively open flower, the clypeus, between the eyes and below the antennae, contacts the oval viscidium (Fig. 2.6b), and the pollinia are extracted as the bee withdraws (Fig. 2.6c) (Catling 1983c).

Catling (1980b, 1983c) observed *Augochlorella aurata* [as *A. stricta* (Prov.)] and *Lasioglossum imitatum* (Smith) [= *Dialictus immitatus* (Smith)] pollinating this species in Ontario. Species of *Bombus* were present in the study area, but none

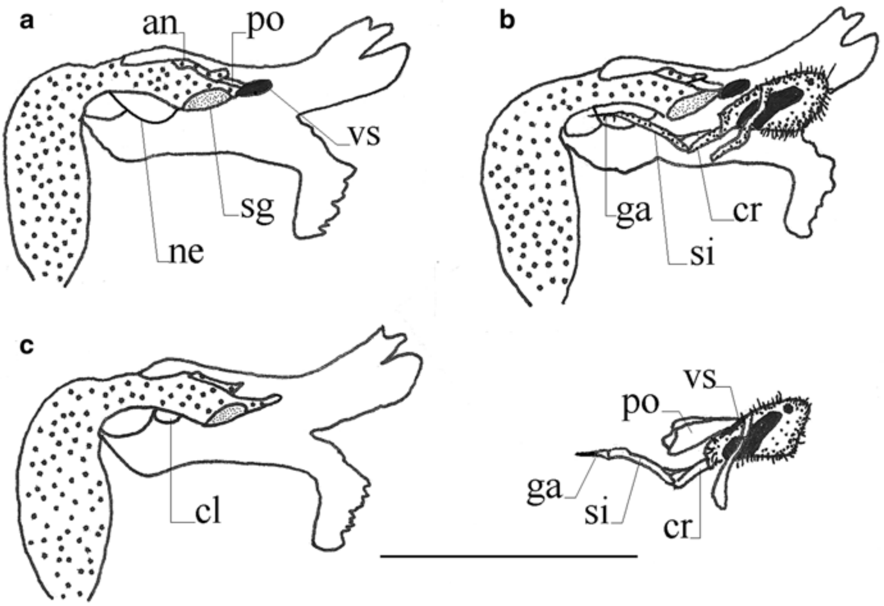

Fig. 2.6 *Spiranthes lucida* and *Augochlorella aurata*. (**a**) Lateral view of flower with part of perianth removed. (**b**) Bee removing nectar, showing the position of head and mouthparts. (**c**) Lateral view of flower after removal of the pollinarium and head of *A. aurata* with pollinarium attached to the clypeus (forehead), scale bar = 5 mm. *an* anther, *cl* callus, *cr* cardo, *ga* galea, *ne* nectar, *po* pollinia, *sg* stigma, *si* stipes, *vs* viscidium. After Catling (1983c), ©2008 of NCR Canada. Reproduced with permission

carried pollinaria of *S. lucida*. *A. aurata* usually visited only a single flower in each inflorescence, even though the spikes normally bore a number of fully open blooms. When visits to a second adjacent flower occurred, they always involved flight. In no case did the bee begin its exploration on the lowermost open flower. Compared to *Bombus*, halictine visits to a spike were less systematic and involved much less crawling and fewer flower visits.

Other species of *Spiranthes*, particularly taxa having relatively small, amply-distended flowers, often with small viscidia and pollinia, are also sometimes pollinated by halictines (e.g., *S. casii* var. *casii*, *S. lacera* var. *lacera*, *S. lacera* var. *gracilis*, *S. tuberosa*) (Tables 2.3, 2.5, 2.9) (Stevenson 1973; Catling 1980b, 1983c). The pollinia of *S. casii* var. *casii* are occasionally found attached between the compound eyes of *Lasioglossum versans* (Lovell) [= *Dialictus versans* (Lovell)] (Catling 1983c). However, this is probably abnormal, and except for *S. lucida* it is likely that halictines usually enter the flowers of *Spiranthes* upside down in order to reach the nectar at the base of the floral tube (Catling 1980b, 1983c). The viscidium is then attached to the underside of the prementum or to the ventral edges of the stipes. Catling (1980b, 1983c) observed a *L. imitatum* (= *Dialictus immitatus*) pollinating *S. lacera* var. *lacera* in this manner, but considered it a rare event.

Table 2.9 Pollinators of *Spiranthes tuberosa* and *S. vernalis*

Species	Pollinator/state or province	References
S. tuberosa	Apidae	
	Bombus sp. /?	Catling (1980b)[a]
	Halictidae	
	Augochlorella pura (Say)/NC	Stevenson (1973)
	Unknown bee, possibly Halictidae /?	Catling (1980b)
S. vernalis	Apidae	
	Apis melifera L./GA	Catling (1980b, 1983c)
	B. impatiens/NC	Stevenson (1973)
	B. pennsylvanicus/NC	Stevenson (1973)
	B. sp. /?	Catling (1980b)

[a] Unobserved; likely based on floral morphology and/or flowering pattern
[?] Study sites were not given, and therefore no data are available

The available evidence indicates a lower level of pollinator specificity among most northeastern species of *Spiranthes* (Catling 1983c). Sheviak (1982), for example, noted that in greenhouse-grown specimens, *B. impatiens* moved between *S. cernua* and *S. odorata* and between *S. cernua* and *S. ochroleuca* effecting pollination. A similar lack of specificity in natural populations is clearly evident from the tabulation of *Spiranthes* species and their known pollinators (Table 2.3–2.9). Thus, for example, Catling (1980b, 1983c) identified *B. perplexus* Cresson, *B. terricola*, and *B. vagans* as pollinators of *S. lacera* var. *lacera* (Table 2.5) and *S. romanzoffiana* (Table 2.6) even though these orchids differ in the size and structure of their flowers (Catling 1983c). Hybrids are known and the resulting nothospecies is recognized as *S.* × *simpsonii* Catling and Sheviak (Simpson and Catling 1978; Catling 1980b; Catling and Sheviak 1993). In general, the granular pollinia in *Spiranthes* permit the deposition of pollen from a single pollinium on several successively visited flowers, augmenting any potential for hybridization (Catling 1983c).

Larson and Larson (1987, 1990) studied the foraging behavior of bumblebees on *S. romamzoffiana* at three sites on Vancouver Island. Although they also found low levels of pollinator specificity, visitation rates were usually high, possibly in response to a continuous and/or abundant nectar reward. Within a 5 × 12-m study plot of a dozen sparsely distributed plants, the pollinators, principally *B. bifarious*, zigzagged along a clearly directional flight path and were attracted to conspicuous, tall plants with long inflorescences more frequently than to short plants with smaller inflorescences, which they often bypassed. An association between larger inflorescences and higher pollinator visitation rates is not uncommon (e.g., Schmid-Hempel and Speiser 1988). In the case of *S. romanzoffiana*, a foray by a single bee did not usually involve repeat visits to any one plant; however, in a series of forays, bees revisited the four most "conspicuous" plants at frequencies that may have exceeded the optimal foraging behavior wanted to maximize nectar rewards (Larson and Larson 1990).

Visits were also correlated at a lower level with nearest neighbor distance and the number of open flowers per inflorescence (Larson and Larson 1990). Other studies have also shown increased pollinator visits in populations with closely spaced inflorescences (e.g., Rathcke 1983). However, when the inflorescences are nonclonal, competition between plants could also be higher and the number of flowers visited per plant might decrease (Klinkhamer and De Jong 1990). Duffy and Stout (2008), in a study in western Ireland, found that intraspecific competition among inflorescences of *S. romanzoffiana* increased and visitation rates per inflorescence decreased at high densities. At the same time, they reported a positive relationship between the number of pollinator visits to *S. romanzoffiana* and total floral density in mixed patches of this and other rewarding plants, the so-called magnet species effect.

Many *Spiranthes* species congregate in patches, and outcrossing may be restricted because pollen transport is usually undirected and occurs chiefly among densely spaced plants that are likely to be closely related (Ackerman 1975; Larson and Larson 1987). However, Larson and Larson (1987, 1990) found that when bees finished foraging on *S. romanzoffiana* at their study site, they consistently moved on to other scattered patches of this orchid in the adjacent forest. This, along with their clearly directional flight path within plots, suggests that the bees may trapline through the forest, effecting some level of gene flow between patches (Heinrich 1976).

Some parallels between *S. cernua* in northeastern North America and *G. tessellata* are noteworthy. Both are polyploid (apparently of hybrid origin) and sympatric with two closely related diploid species. Like *G. tessellata*, *S. cernua* blooms before the diploids, and this phenological separation may be a statistically significant obstacle to hybridization (Catling and Brown 1983). Northeastern North American species of *Spiranthes* (14 of 15 taxa) also resemble *Goodyera* in column structure as well as mode of viscidia attachment, and both genera are protandrous with sequential acropetal flowering (Darwin 1862; Ames 1921; Hagerup 1952; Kipping 1971; Ackerman 1975; Catling 1980b, 1983c).

Fruiting Success and Limiting Factors

Extensive studies are available on the reproduction of Nebraska populations of *S. cernua*. According to Schmidt (1987), artificially crossed plants produced a significantly higher proportion of monoembryonic seeds than unmanipulated (control) plants. This suggests that pollinator limitation may influence the proportion of agamospermic seeds produced. Schmidt (1987) also found that the frequency of polyembryonic and monoembryonic seeds did not differ significantly for emasculated, bagged, and unbagged plants. Presumably, if there was no pollinator limitation, a higher proportion of monoembryonic seeds would be expected in the unbagged treatment.

Sheviak (1982), Catling (1980a, 1983a, c), and Schmidt and Antlfinger (1992) saw few pollinators visiting the flowers, and few pollinaria were removed. Factors that might limit pollinator visits have yet to be identified. This species might simply be less attractive to pollinators than other fall-flowering taxa (Anderson and Schelfhout 1980; Kaul and Rolfsmeier 1987) or fragmentation of the prairie may have decreased pollinator populations (Schmidt 1987). In either case, the mating system of *S. cernua* might provide independence from pollinators, permitting it to colonize and survive in small numbers on isolated remnants of its prairie habitat (Schmidt and Antlfinger 1992).

Although a large percentage of the stored reserves in Nebraska populations of *S. cernua* was allocated to reproduction, the demographic data suggested that the effects on subsequent flowering and growth, at least in larger individuals, were lower than in some other orchid genera with similar reproductive effort (Whigham 1984; Schmidt 1987; Snow and Whigham 1989; Primack and Hall 1990; Calvo 1993; Primack et al. 1994; Antlfinger and Wendel 1997). Less than half of the Nebraska population produced inflorescences in a given season (Antlfinger 1991), but of the plants that flowered two-thirds were reproductive for at least 2 years in a row and one-fourth of these for 3 or more years in a row (Antlfinger and Wendel 1997). In fact, as compared to nonflowering plants, those that flowered in 1 year were usually more likely to produce flowers again the next year (Antlfinger and Wendel 1997). Similar results have been reported for *S. delitescens* Sheviak (McClaran and Sundt 1992).

Plant size was a significant factor. Seed production, flower number, and inflorescence size were positively correlated with plant size as estimated by dry weight and leaf area (Antlfinger and Wendel 1997). Plant size was also correlated between years. The production of inflorescences had relatively little effect on subsequent reproductive effort in large plants producing 31 or more flowers per year. A cost of reproduction was not observed in these plants for several years, and it remains unclear if reproductive effort or below-average rainfall during the study period contributed most significantly to a progressive decrease in leaf size, stored photosynthate, and eventual transition to a vegetative state (Antlfinger and Wendel 1997). Smaller plants producing 16–30 flowers showed a smaller increase or a reduction in size a year after flowering compared to those that did not flower and a more frequent reversion to a vegetative state for a year or more after flowering. The reproductive cost was highest in the smallest plants, those producing 1–15 flowers. Although highly variable, this group was the one most likely to remain vegetative or to be absent following a year of flowering, presumably because they had fewer stored reserves than larger plants (Antlfinger and Wendel 1997). If larger plants consistently produce more flowers and capsules, the effective size of the population is reduced. Schmidt (1987) believes that this could lead to inbreeding and genetic drift with changes in the population's gene pool.

Antlfinger (1991) considers that frequent flowering in *S. cernua* may be worth the cost. Formation of the inflorescence in a facultative apomict assures the production of a large number of seeds even where sexual reproduction may be pollinator limited (Schmidt and Antlfinger 1992; Antlfinger and Wendel 1997). Schmidt (1987), for example, found that 92% of surviving flowers produced capsules at his study site in Nebraska. In addition, the inflorescence in *S. cernua*, a species with often fugaceous leaves, is photosynthetically active and plays a role in carbon assimilation (an estimated 8.4% of the season total) that may contribute significantly to a reduction in the cost of reproduction (Antlfinger and Wendel 1997). This carbon assimilation may, in fact, account for nearly half of the requirement for inflorescence production and maintenance, the remainder presumably coming from stored carbohydrates.

Many species of *Spiranthes* may persist as mycorrhizomes and be dependent on fungal associates for nourishment for many years before leaves differentiate and photosynthesis begins (Summerhayes 1951; Wells 1981). However, *S. cernua* is capable of initiating photosynthesis shortly after germination (Stoutamire 1974; Schmidt 1987). Flowering may follow within 2 or 3 years (Ames 1921; Stoutamire 1974). Thousands of seeds are dispersed by the wind in October and November in Nebraska (Schmidt 1987), and although further studies are needed, success in the establishment of new populations might benefit from an association between agamospermy and frequent flowering if it leads to a disproportionate representation of these genotypes in the seed bank and in colonizing individuals (Antlfinger and Wendel 1997).

Some species of *Spiranthes* appear to differ from *S. cernua* in reproductive investment and resource allocation. For example, Willems and Dorland (2000) conducted demographic studies on *S. spiralis*, a sexual, Old World species totally dependent on insects for its reproduction (Willems and Lahtinen 1997). Here, more than 80% of flowering plants failed to flower again the next year, and this result was independent of the age structure of the population (cf. Wells 1967). Flowering had a negative effect on the size of the rosette the following year, but it also reduced leaf size in the year of flowering due to allocation of limited underground resources to both the developing inflorescence and the rosette (Willems and Dorland 2000). Thus, the cost of reproduction exceeded the resources available in a given flowering year. Most plants required only 1 year to accumulate the reserves needed to flower again, but plants that flowered for 2 or more successive years required longer recovery times (Willems and Dorland 2000). Longevity is unknown for *S. cernua*, but *S. spiralis* may live several decades (Wells 1967; Tamm 1972), and life span must be factored into the balance between current reproductive investment and future fitness with long-lived species spreading their reproductive output more or less evenly over the course of their lifetime.

Other species appear to differ from *S. cernua* in pollinator recruitment. Sipes et al. (1993) found no reduction in the mean number of fertile seeds produced in open-pollinated compared to hand-pollinated flowers in *S. diluvialis*. They concluded that seed production is not limited by pollinator visitation rates. Natural fruit set may, however, be affected by the position of the flower on the inflorescence. Fruits were produced by a higher percentage of flowers (85%) from the bottom half of the inflorescence than from the top half (50%) (Sipes and Tepedino 1995). This result might be expected based on protandry and the acropetal movement of bumblebees. Schmidt (1987) also reported the occurrence of larger capsules at the bottom of the inflorescence in *S. cernua*, but this occurred in artificially pollinated plants and was related to factors other than the frequency of pollination.

Pollinator limitation was also not apparent in *S. romanzoffiana* from British Columbia. Bees visited each inflorescence an average of about once every 4 min (Larson and Larson 1987, 1990), an unusual rate, particularly for an orchid (Ackerman 1975, 1981; Heinrich 1976, 1979a, b; Pyke 1978; Hodges and Miller 1981; Zimmerman 1982; Catling 1983c). Over 75% of the flowers produced seed, and all the plants examined bore capsules (10–45/plant) containing seed. Larson and Larson (1987, 1990) suggested that the level of visitation at their study site might be in response to a continuous or rich nectar supply. This was apparently not the case for this orchid in western Ireland, where Duffy and Stout (2008) could establish no correlation between nectar reward and visitation rates.

Additional factors influencing reproductive success in *Spiranthes* include fire, which may stimulate flower production (Sheviak 1982) and predation, which can be significant in some areas. Schmidt (1987) found that in Nebraska populations of *S. cernua*, predators destroyed 55% of the plants with flower buds prior to anthesis. Antlfinger and Wendel (1997) also found an overall loss of 22–39% of the inflorescences of this species to herbivores, probably to voles. Other possible causes of mortality in eastern Nebraska included interspecific competition and the loss of associated fungi.

Additional Species of *Spiranthes*

Ten other *Spiranthes* taxa occur in North America north of Florida and Mexico: *S. porrifolia* Lindley (creamy ladies'-tresses), *S. stellata* P. M. Brown, Dueck, and Cameron (no common name), *S. infernalis* Sheviak (ash meadows ladies'-tresses), *S. delitescens* Sheviak (reclusive ladies'-tresses), *S. longilabris* Lindley (giant spiral ladies'-tresses), *S. brevilabris* Lindley (texas ladies'-tresses), *S. floridana* (Wherry) Cory (florida ladies'-tresses), *S. eatonii* Ames ex P. M. Brown (eaton's ladies'-tresses), *S. praecox* (Walter) S. Watson (greenvein ladies'-tresses), and *S. sylvatica* P. M. Brown (woodland ladies'-tresses), although the latter may not be distinct from *S. praecox* (Dueck and Cameron 2008). Nothing is yet known of their pollination biology. However, the presence of a narrow, elongated viscidium in *S. praecox* suggested to Catling (1980b) that it is protandrous and pollinated by *Bombus*. In addition, *S. infernalis* is very similar to *S. porrifolia*, and Sheviak (1989) believes that it may have evolved from it. *S. porrifolia*, in turn, was formerly recognized as a variety of *S. romanzoffiana*, an otherwise uniformly sexual species. Finally, Sheviak and Brown (2002) and Brown et al. (2008) reported that these taxa produce exclusively monoembryonic seeds, implying that the breeding system is sexual in the populations they examined.

Other Spiranthinae

Dichromanthus Garay

Dichromanthus includes three species distributed from the southern USA through Mexico to Honduras. Two are present in our flora.

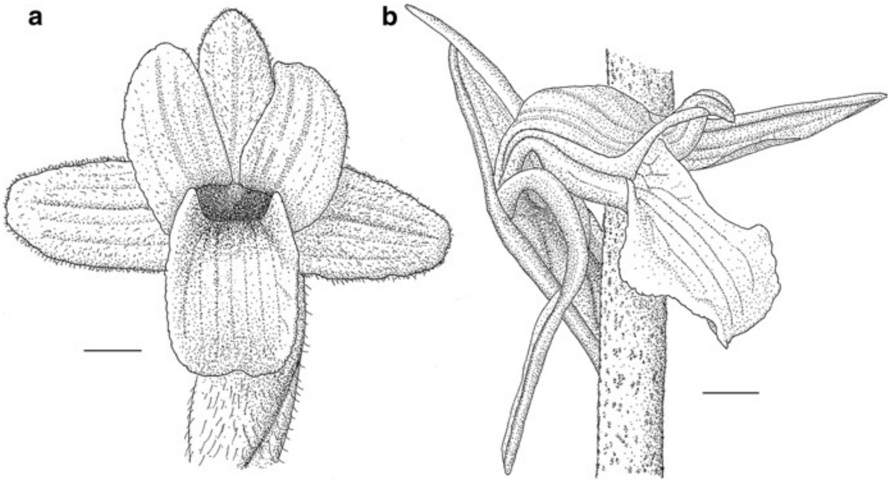

Fig. 2.7 (**a**) *Dichromanthus michuacanus*, flower, front view; (**b**) *Deiregyne confusa*, flower, oblique view, scale bars = 3 mm

Dichromanthus cinnabarinus (Llave and Lexarza) Garay (= *S. cinnabarina* (LaLave and Lexarza) Hemsl.) (cinnabar ladies'-tresses) is found from southwest Texas to Guatemala (Brown 1998) and *D. michuacanus* (LaLave and Lexarza) Salazar and Soto Arenas (= *Stenorrhynchos michuacanum* (LaLave and Lexarza) Lindley or *Spiranthes michuacana* (LaLave and Lexarza) Hemsl.) (michoacan ladies'-tresses) from southeastern Arizona and southwest Texas to Mexico (World Checklist of Monocotyledons 2008). Balogh and Greenwood (1982) originally suggested without elaboration that *D. cinnabarinus* might be pollinated by bees. However, the flowers are odorless and along with the entire spike are bright red to yellow–orange, features which suggest hummingbird pollination (Luer 1975; Brown 2002a; Pridgeon et al. 2003). According to Light (1998), the perianth is very hard and may curb the efforts of nectar-robbing insects and birds to puncture the flower base. Pollination rates are low. Coleman et al. (2006) found only three maturing capsules in one population from Brewster County, Texas.

D. michuacanus (Fig. 2.7a), on the other hand, usually has white, cream-colored, or pale green flowers with dark green stripes (nectar guides) and during the day produces a strong lemony and faintly urine-like scent (e.g., Luer 1975; Catling and Brown 2002). An exception is forma *armeniacus* R. A. Coleman, which has flowers with a rich, apricot-yellow background color and a stronger, sweet odor (Coleman 2009). Regardless of color, the plants are large with robust flowers and may be pollinated by bumblebees or carpenter bees. Coleman (2005) monitored four sites in Arizona over a period of 9 years. He found that about 60% of plants initiating inflorescences over this period failed to reach anthesis, mostly as a result of herbivory. About 52% of plants that attempted to bloom in 1 year also attempted to bloom again the following year. Relatively, few plants attempted to bloom more than 2 years in a row. Plants not flowering again a year after a blooming event were often either stunted or failed to emerge, sometimes for 1 or 2 years, usually reappearing, if at all, as small plants. The availability of stored energy reserves may, therefore, play a role in limiting the reproductive success of this orchid.

Deiregyne Schlechter

Deiregyne is a genus of about 15–20 species largely confined to the mountains of Mexico and Guatemala. One species *D. confusa* Garay (confusing ladies'-tresses) (Fig. 2.7b), however, extends into Texas in the Chisos Mountains of Brewster County. Its flowers produce nectar and a diurnal fragrance, and their size, shape, and color suggest pollination by bumblebees (Pridgeon et al. 2003).

In a study in the state or Durango, Mexico, Luer (1975) noted bumblebee pollination of a plant identified as *S. durangensis* Ames and Schweinfurth (= *Deiregyne durangensis* (Ames and Schweinfurth) Garay = *Schiedeella saltensis* Schlechter). Although Kew's World Checklist of Monocotyledons places this taxon in south Texas, Garay (1982), in his study of Spirantheae, referred all specimens of *S. durangensis* that he examined, including those from Texas, to *D. confusa.* Brown (2002c) also recognizes this taxon as *D. confusa* in the Flora of North America. (Although Garay and Brown are followed here, the World Checklist of Monocotyledons (2008) recognizes the species as *Schiedeella confusa* (Garay) Espejo and Lopez-Ferr.) Luer described the bumblebee as a huge, yellow and black queen that moved slowly upward in the spicate inflorescence, grasping the lateral sepals and thrusting its head far down into each flower in search of nectar. In the process, its head contacted the viscidia, and pollinaria were extracted.

Schiedeella Schlechter

Schiedeella, a genus of nine (Brown 2002b) to 15 (Pridgeon et al. 2003) species is found from the southern USA to Panama (except Belize) and the Caribbean (Cuba, Hispaniola). One species, *S. arizonica* P. M. Brown (red-spot ladies'-tresses), occurs in the USA from Arizona to Texas (Brown 2002b). There are no published observations on the pollination of this orchid. Among related species, Szlachetko (1993) reported autogamy in *S. romeroana* Szlach. from Mexico. A rostellum is lacking or poorly developed in this species, and the pollinia come to lie in direct contact with the stigma at anthesis. However, the pollinia in *Schiedeella* are usually attached dorsally to a well-developed, sheathing, ventral viscidium. Thus, for example, *S. llaveana* (Lindl.) Schltr. and *S. trilineata* (Lindl.) Burns-Bal., from Mexico and Central America, are xenogamous and thought to be pollinated by foraging bees, possibly bumblebees, based on the size, color, and odor of the flowers (Pridgeon et al. 2003).

Microthelys Garay

Microthelys is a genus of about ten species once thought to be restricted to scattered montane habitats in Mexico, Central America, and Ecuador (Pridgeon et al. 2003). However, *M. rubrocallosa* (Robins and Greenm.) Garay (green medusa orchid) was recently discovered in the Sacramento Mountains in New Mexico (R. A. Coleman and Baker 2006). No detailed studies have been published on its pollination, but some plants or populations are apparently autogamous. The putatively self-pollinating individuals include forms with both poorly developed and normal-appearing rostella. The pollinia lie in direct contact with the rim of the stigma in both (Pridgeon et al. 2003).

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Part II
Subfamily Epidendroideae

The subfamily Epidendroideae, with 650 genera and about 18,000 species, is the largest subfamily in the Orchidaceae. Although present in temperate regions, this group is best represented in the tropics of both the Eastern and Western Hemispheres. Members of seven tribes occur in North America north of Mexico and Florida.

Chapter 3
Tribe Neottieae

Abstract *Listera cordata* is self-compatible, but outcrossing improves seed production and is promoted by protandry. Autogamy is absent. Fungus gnats are common pollinators. *Listera ovata* is self-compatible, but selfing reduces embryo number and size. Autogamy is prevented or reduced by column structure. Floral morphology in other *Listera* suggests particular pollinator characters or behaviors. *Cephalanthera austinae* is facultatively autogamous and usually employs a strategy of deceit to attract pollinators. *Epipactis gigantea* is a self-compatible, clonal orchid. Reproduction routinely involves a single colonization event followed by asexual propagation and selfing. Syrphid flies are primary pollinators. *Epipactis helleborine* is self-compatible and usually allogamous. Wasps are the primary pollen vectors.

Keywords *Listera* • *Cephalanthera* • *Epipactis* • Protandry • Male and female function • Fruit production and inflorescence size and plant density • Deliberate pollen collection • Syrphid flies and wasps and prey mimicry • Fungus gnats

The Neottieae are distributed in the Old World from Europe to tropical Africa and east to Sri Lanka, Southeast Asia, China, Japan, New Guinea, and Australia. In the Western Hemisphere, representatives are found in the western USA, Central America, and northern South America (Pridgeon et al. 2005). Three genera are present in our flora.

Listera R. Brown

Listera is reduced to synonymy under *Neottia* Guett. in the World Checklist of Monocotyledons (2008), but Magrath and Coleman (2002) recognized it as distinct in the Flora of North America and their treatment is followed here. The genus includes 25 species distributed in cool temperate regions of the Northern and Southern

C.L. Argue, *The Pollination Biology of North American Orchids: Volume 2: North of Florida and Mexico*, DOI 10.1007/978-1-4614-0622-8_3,

Hemisphere. Eight are found in our flora, but detailed accounts of pollination biology are available only for *L. cordata* and the Eurasian species, *L. ovata* (L.) R. Br. The latter is now also found in Ontario (Magrath and Coleman 2002), but its reproduction in North America has not been studied.

Listera cordata (L.) R. Brown (Heart-Leaved Twayblade)

Distribution and Habitat

L. cordata is a common but inconspicuous and often overlooked orchid of moist sphagnum hummocks in northern woods and cold balsam–cedar–spruce bogs, but is also found in thick, rather dry moss mats on headlands and in the humus or needle-duff of deciduous and coniferous forests (Case 1987). It is circumboreal in distribution. In North America, it ranges from Greenland and Labrador south to Virginia and west to Alaska and northern California. Southern extensions occur in the Rocky Mountains to New Mexico and in the Appalachians to North Carolina (Luer 1975; Magrath and Coleman 2002).

Floral Morphology

Variable numbers of small, light green to purple resupinate flowers are borne in a slender, terminal raceme (Fig. 3.1a; Table 3.1) (Ackerman and Mesler 1979; Magrath and Coleman 2002). Individual flowers persist for 10–25 days (Ackerman and Mesler 1979). The linear-oblong lip, flattened and bent sharply downward from a point near its insertion, is deeply cleft at its apex into two linear-lanceolate lobes (Fig. 3.1b, c). A short, erect to arcuate column is positioned in the center of the flower. The anther is suberect and located near the apex of the column behind a large, thin, leaf-like rostellum. It dehisces in the bud and two soft and mealy, club-shaped pollinia containing numerous tetrads are released (Dressler 1993). A viscidium and a caudicle are absent, and the pollinia are held unattached and enfolded by the incurved margins of the rostellum (Fig. 3.1d) (Ackerman and Mesler 1979). The stigma is entire and located directly beneath the rostellum (Fig. 3.1f). A narrow nectary or nectar groove extends down the center of the labellum for most of its length (Fig. 3.1c); a second nectary is present on a basal disk immediately below the column (Ackerman and Mesler 1979).

Compatibility and Breeding System

A number of authors have reported autogamy or apogamy in *Listera* (e.g., Hooker 1854; von Kirchner 1922; Hagerup 1952; Kugler 1970; Prochazka and Velisek 1983), and indeed Lojtnant and Jacobsen (1976) found the fruiting to be so abundant and widespread in Greenland populations of *L. cordata* that they supposed this

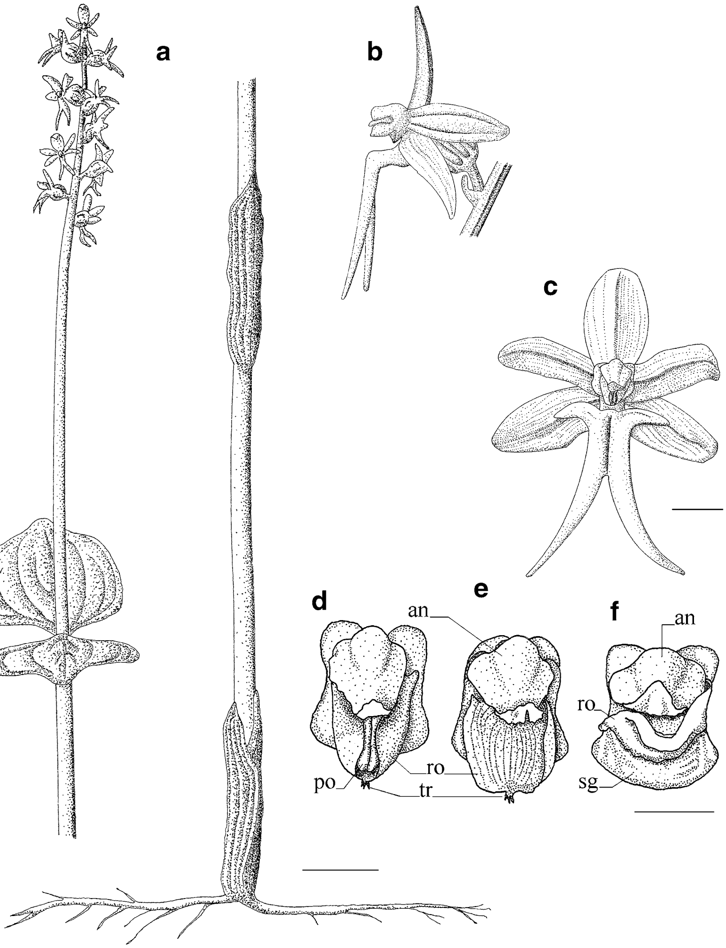

Fig. 3.1 *Listera cordata*. (**a**) Habit, scale bar = 1 cm; (**b**) flower, side view; (**c**) flower, front view, scale bars (**b**, **c**) = 1 mm; (**d**)–(**f**) front view of column; (**d**) rostellum intact; (**e**) column after triggering of rostellum and release of the pollinia; (**f**) column with an elevated rostellum and exposed stigma, scale bar = 0.3 mm. *an* anther, *po* pollinia, *ro* rostellum, *sg* stigma, *tr* trigger hairs. (**d**)–(**f**) after Ackerman and Mesler (1979) with permission

species might also have such a breeding system. However, the tetrads of *L. cordata* are more coherent, less likely to fragment, and less likely to autopollinate than those of other *Listera* while cross-pollination is common and appears to be favored.

Melendez-Ackerman and Ackerman (2001), for example, found that subalpine populations in Gunnison County, Colorado, although also self-compatible, produced a significantly higher percentage of fertilized ovules and more seeds per capsule when cross-pollinated. Similarly, Ackerman and Mesler (1979), in a 3-year study in Humboldt County, California, found that geitonogamy occurred but outcrossing was promoted by protandry, and Mesler et al. (1980) estimated the relative frequency of geitonogamous versus xenogamous pollination in northern California

Table 3.1 Data on *Listera* (Magrath and Coleman 2002)

Character	*L. cordata*	*L. ovata*
Plant height (cm)	5–33	20–30
Inflorescence length (cm)	2–10	5–20
Flower number	5–25	10–100
Dorsal sepal (mm)	2–3 × 1	5–6 × 2–3
Lateral sepals (mm)	2–3 × 0.5–1.5	4 × 2–3
Lateral petals (mm)	1.5–2.5 × 0.5–1	4 × 1
Lip (mm)	3–4 × 1–1.5	8–10 × 4
Column (mm)	0.5 × 0.5	2 × 1.5
Chromosomes (2*n*)	36, 38, 40, 42	

(by comparing percentage fruit set of emasculated plants with controls) and found the movement of pollen between plants to be common. In addition, the California populations reproduced chiefly by sexual means rather than vegetatively as reported elsewhere (e.g., Ziegenspeck 1936).

Pollinators and Pollination Mechanisms

Ackerman and Mesler (1979) have described the pollination in *L. cordata* in detail. The downward pointing labellum serves as a landing platform for visiting insects. A minute amount of nectar is secreted into a superficial groove, the aforementioned nectar groove, which runs down the center of the labellum (Fig. 3.1c). The insect, feeding on this nectar, crawls slowly up the labellum. It is usually oriented more or less randomly at this stage, but when it reaches the base, it may begin to feed on nectar secreted from the nectar disk beneath the rostellum. If so, it usually positions its head toward the column. If the head touches any of the three small trigger hairs on the base of the rostellum (Fig. 3.1d, e), an adhesive, held under pressure, is forcibly ejected onto it, and the rostellum immediately reflexes, releasing the pollinia which fall onto the drop of rostellar glue (Fig. 3.1e) (Ramsey 1950; Ackerman and Mesler 1979). The glue dries in several seconds, and the pollinia are firmly attached. Ejection of the rostellar glue and deposition of the pollinia are so nearly simultaneous that Ackerman and Mesler (1979) were usually unable to touch the trigger hairs with a needle and remove it quickly enough to avoid taking the pollinia.

About a day following discharge of the pollinia, the rostellum folds upward freeing the passage to the receptive stigma (Fig. 3.1f), which has now become very sticky (Ackerman and Mesler 1979). Nectar is resecreted into the nectar groove, and if another insect that has visited a younger flower and has pollinia cemented to its head makes its way up the labellum as before, the pollinia may contact the stigma. Since only fragments of the soft, mealy pollen mass detach, a single pollinium might pollinate several flowers. If the pollinia are not removed from the flower within about 4 days, the triggering device is inactivated and the rostellum folds upward to expose the stigma.

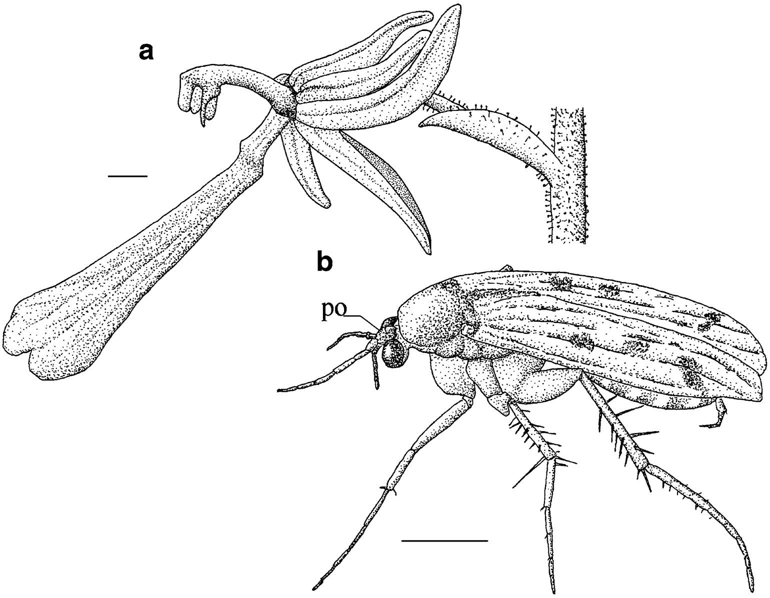

Fig. 3.2 (**a**) *Listera convallarioides*, flower, oblique view; (**b**) fungus gnat (*Mycetophila* sp.) with pollen mass of *L. cordata* attached to its head, scale bars = 1 mm. *po* pollen mass

The most commonly reported pollinators of *L. cordata* in the coastal redwood forests of northern California were fungus gnats (Fig. 3.2b) in the families Mycetophylidae (*Mycetophila* Meigen sp.) and Sciaridae (*Sciaria* Meigen sp.) (Ackerman and Mesler 1979; Mesler et al. 1980). Hapeman (1996) also reported fungus gnats as pollinators in Door County, Wisconsin. These insects did not always orient themselves properly with respect to the column and did not always contact the trigger hairs of the rostellum or deposit pollinia on the stigma (Mesler et al. 1980). The probability of pollination per flower visit was low, but this was compensated by an abundance of gnats and a large number of visits.

The flowers of *L. cordata* show no evident adaptation to a specific pollinator. The nectar is well-exposed, and the pollination mechanism is simple and can probably be operated by many small insects with short mouthparts. Members of other insect groups reported carrying pollinia in northern California included hymenoptera in families Ichneumonidae and Braconidae (*Microgaster* Latreille sp.). Although interaction with these insects was sporadic and accounted for less than 0.1% of the flower visits, Ackerman and Mesler (1979) and Mesler et al. (1980) thought that the dominant role of fungus gnats at their site might be a regional phenomenon related to the local abundance of these insects and that the primary pollinator might differ at other sites, where fungus gnats are less common (Ackerman and Mesler 1979). Other pollinators have, in fact, been observed elsewhere. For example, *Tipula subnodicornis* Zetterstedt (Tipulidae) and a species of *Microgaster* (Braconidae) were recorded in Finland (Silen 1906) and a species of *Limnophila* Macquart (Tipulidae) in Great Britain (Willis and Burkhill 1908). Keenan (1998) mentioned that mosquitoes might also be involved, but even though present in immoderate numbers, they played no apparent role in the pollination of this orchid at a site in Quebec.

Although Ackerman and Mesler (1979) consider nectar to be the primary attractant, a fetid odor is also mentioned, implying adaptation to the attraction of sapromyophilous or mycetophilous insects. A similar scent is found in some other species of *Listera* (Ramsey 1950; Schremmer 1961; van der Pijl and Dodson 1966; Ackerman and Mesler 1979). Nevertheless, the flowers of *L. cordata*, at least, do not appear to be pollinated by egg-laying insects normally attracted by the smell of feces or decaying plant or animal bodies nor do they chemically mimic fungi to attract ovipositing fungus gnats (Faegri and van der Pijl 1971; Ackerman and Mesler 1979). Ackerman and Mesler (1979) reported no evidence of oviposition or of eggs or larvae on the flowers. Moreover, they found that both male and female fungus gnats were attracted and observed no evidence of sexual activity, which might suggest that the males were attracted only by the presence of females.

Fruiting Success and Limiting Factors

Floral rewards are widely scattered in the cool, damp coastal redwood forests. Efficient pollinators, such as bumblebees, require ample and relatively concentrated energy sources and are not common in this habitat (Moldenke 1976). Plants, such as *L. cordata*, may of necessity depend on small, less-efficient pollinators that require less-abundant nectar supplies (Stebbins 1974). The behavior of most flies and wasps is often considered erratic (Sprengel 1793; Kunth 1898–1905; van der Pijl and Dodson 1966; Proctor and Yeo 1972; Moldenke 1976; Leppik 1977), yet 61–78% of the flowers monitored in three northern California populations of *L. cordata* produced capsules (Ackerman and Mesler 1979). This percentage is higher than that recorded for some temperate, terrestrial, nectariferous orchids pollinated by butterflies, bumblebees, moths, and syrphids (Ackerman 1975; Smith and Snow 1976; Ivri and Dafni 1977; Ackerman and Mesler 1979). Although Mesler et al. (1980) attributed this rate of success to the large number of gnats present at their study site rather than to their efficiency as pollinators, they found that the gnats were effective vectors not only in terms of overall fruit set, but also in their ability to bring about cross-pollination. The potential of a single pollinium to pollinate several flowers may also compensate for pollinator inefficiency (Mesler et al. 1980).

Listera, thought by Darwin (1862) to have one of the most refined flower types among the orchids, provides an example of a genus that has evolved specialized flowers but includes members which are, nevertheless, effectively pollinated by relatively unspecialized insects. The significant feature here is the exposed nectar, which presumably allows the utilization of the flower by a wide variety of insects with primitive mouthparts. Although trends leading to a reduction in the number of pollinators is thought to result in more efficient pollination and a lowering of the cost of pollen transfer (e.g., Tremblay 1992), other factors, such as climate, pollinator availability, and reliability, need to be considered, and the success of *L. cordata*, as reflected in its circumboreal distribution, may be related to a broad pollinator spectrum.

Additional Species of *Listera*

Some workers have reported that *L. ovata* is apomictic (Prochazka and Velisek 1983) or autogamous with incoherent pollen falling directly onto the surface of the stigma (e.g., Hooker 1854; von Kirchner 1922; Kugler 1970). Others assert that pollen in the latter case is dislodged by the activity of thrips and that the process should, therefore, not be considered autogamy but a form of entomogamy (Darwin 1862, 1869). Nilsson (1981a, b), on the other hand, found no evidence for either autogamy or thrip-mediated entomogamy in extensive and detailed studies of *L. ovata* on Oland and in Uppland, Sweden, and bagging experiments have now demonstrated that a vector is needed for pollination (Brys et al. 2008).

Artificial self-pollination produced fruit, and since *L. ovata* is clonal some level of inbreeding probably occurs. However, selfing led to a reduction in embryo number and size. Like *L. cordata*, cross-pollination is promoted by the positioning of the rostellum.

Natural fruit set varied from up to 80% in one population from Gotland to 13–70% (mean value, 37%) on Oland. Marked differences were evident among populations and years and also among individual plants within populations.

In a study in eastern Belgium, Brys et al. (2008) examined the effects of population size, local plant density, and floral display on pollination success and reproduction. They found that pollination efficiency, pollen removal, and fruit set were positively correlated with population size up to a threshold value of 30–40 plants. Beyond this number, pollination efficiency and reproductive output decreased. Pollinia removal and fruit set values were higher in plants with larger floral displays. Fruit production was also positively correlated with local plant density, and this relationship was equally valid in emasculated plants, implying that female function was unaffected by geitonogamous pollination in this population. These results differ from Melendez-Ackerman and Ackerman's (2001) observations on *L. cordata* in Colorado, where reproductive success was found to be independent of plant density, except in highly dispersed plants.

Pollinators of *L. ovata* in North America are unknown, but the flowers are larger than those of *L. cordata* (Table 3.1) and the pollinators are probably also larger. Nilsson (1981a, b) considers that the flowers are adapted for strong, 8–12-mm long insects with nearly hairless heads and short, fairly small mouthparts. In studies conducted in Europe, sawflies (Hymenoptera, Symphyta) and more than 70 species of ichneumons (Hymenoptera, Ichneumonidae) were attracted to the flowers in large numbers (Darwin 1862; Kunth 1898–1905; Porsch 1958; Nilsson 1981b). Also attracted were beetles, primitive flies, and other hymenoptera (Sprengel 1793; Muller 1883; Kunth 1898–1905; Heimans and Thijsse 1907; Godfery 1931; Porsch 1958; Schremmer 1961; van der Pijl and Dodson 1966; Nilsson 1981b), including wasps of the genus *Argogorytes*, otherwise well-known as the exclusive, pseudocopulatory pollinators of the fly orchid, *Ophrys insectifera* L. (Nilsson 1981b). *Tipula* (crane fly) also visited along with some predatory insects (e.g., *Chloroperla torrentium* Pict.) usually destructive to flowers and generally associated with primitive blossom types (Darwin 1862; Muller 1873; Silen 1906; Heimans and Thijsse 1907; Faegri and van der Pijl 1971).

Where pollinia were removed, they attached to the head of legitimate pollinators (Nilsson 1981b). The pollination mechanism is essentially the same as in *L. cordata* (Nilsson 1981b), except that, according to Darwin (1862), ejection of the rostellar glue and release of the pollinia do not occur as highly synchronized, independent events. Rather, the glue contacts both the tips of the pollinia and the insect as it is ejected, cementing the two together. Also extrafloral nectaries are present in *L. ovata* (Proctor and Yeo 1972), and these may prolong foraging of some insects until the floral nectar is located.

Little information is available on the pollination biology of other North American species of *Listera*, but certain generalizations have been extrapolated from similarities and differences in floral morphology. Apparently, all species of *Listera* secrete nectar and have a touch-sensitive column (Melchior and Werdermann 1954–1964; Dressler and Dodson 1960; Dressler 1993), but only *L. cordata* and *L. ovata* share a strongly bent labellum, a feature Nilsson (1981a) believes to be adaptive for pollinators that crawl between flowers in a raceme. Other North American species, such as *L. convallarioides* (Swartz) Nuttall (Fig. 3.2a), *L. borealis* Morong, *L. auriculata* Wiegand, *L. caurina* Piper (= *L. banksiana* Lindley), *L. australis* Lindley, and *L. smallii* Wiegand, lack such a bend and may be serviced by other pollinators which fly, rather than crawl, between the flowers (Nilsson 1981a). Similarly, *L. cordata* and *L. ovata* both have a very short column adapted to insects with short mouthparts. The long, overarching columns in North American species, such as *L. auriculata*, *L. borealis*, and *L. convallarioides* (compare Figs. 3.1b and 3.2a), suggest adaptation to other groups of pollinators with long mouthparts or long legs (Nilsson 1981a).

Hapeman (1996) photographed a small Dipteran, possibly a fungus gnat, on the flower of *L. auriculata* in Wisconsin, and Ambs (2009) stated, without reference or further detail, that this orchid might be pollinated by mosquitoes. It must share at least one common pollinator with *L. convallarioides* as a naturally occurring hybrid of these species (*L.* × *veltmanii* Case) is known (Case 1964). Although he observed no pollinators, Kipping (1971) found that 50–70% of the flowers of *L. convallarioides* set fruit over a 2-year period in El Dorado County, California. Like *L cordata*, the orchid is protandrous (Ramsey 1950), and despite the differences in floral morphology noted above, Kipping believes that nectar secretion, pollinator movements, and pollinia removal may be similar in the two species.

Other Neottieae

Two additional genera of this tribe, *Cephalanthera* L. C. Richard and *Epipactis* Zinn. occur in North America. These genera are similar in general appearance and labellar morphology, but differ in column structure (Dressler 1993). In addition, flowers of *Cephalanthera* are sessile and the ovary is spirally twisted, whereas those of *Epipactis* are stalked and the pedicels are long enough to include most of the twist of resupination (Luer 1975). The common name "Helleborine" is still often applied to both, and natural intergeneric hybrids occur (Dressler 1993).

Cephalanthera Richard

Cephalanthera is a genus of about 15 species found in North Africa, temperate Eurasia, and western North America (Pridgeon et al. 2005). A single species is represented in our flora.

Cephalanthera austinae (A. Gray) A. Heller (Phantom Orchid, Snow Orchid)

Habitat and Distribution

C. austinae is a perennial, achlorophyllous plant totally dependent on mycorrhizal fungi for survival. It is found in coniferous forests on dry to moist mineral soils from southern British Columbia and northern Idaho to California (Sheviak and Catling 2002).

Floral Morphology

Variable numbers of resupinate flowers are borne in a loose, terminal raceme (Table 3.2). Like the rest of the plant, they are essentially pure white, except for a yellow mark on the lip (Kipping 1971; Luer 1975). The lateral sepals are spreading while the petals are partially connivent with the hood-like dorsal sepal (Fig. 3.3a). The lip is constricted in the center, giving rise to distal and basal sections (Fig. 3.3b). The basal section (hypochile) is concave with triangular lateral lobes that curve upward to flank the column. The distal section (epichile) is deflexed and cordate with a rounded apex and a yellow center. Its dorsal surface bears three to five, fine longitudinal ridges (Fig. 3.3b); these are ciliated, yellow or orange in color, and thought by some to represent pseudopollen (Kipping 1971; Luer 1975; van der Cingle 2001; Sheviak and Catling 2002). The flowers produce neither scent nor nectar. The column is slender, upright, and nearly round in cross section. The anther is dorsal, and two pairs of soft, elongate pollinia are present under a hinged anther cap at the end of the column (Fig. 3.3c, d). Each pollinium comprises two granular strands of white, mealy pollen. The stigma, made up of three functional, confluent lobes, is positioned immediately below the anther (Fig. 3.3c, d) (Kipping 1971; Luer 1975; Sheviak and Catling 2002; Pridgeon et al. 2005). The pollinia have no caudicle and are commonly said to also lack a viscidium and rostellum

Table 3.2 Data on *Cephalanthera* (Sheviak and Catling 2002)

Character	*Cephalanthera austinae*
Plant height (cm)	19–65
Inflorescence length	5–15[a]
Flower number	8–30[a]
Dorsal sepal (mm)	12–20 × 4–7
Lateral sepals (mm)	12–20 × 4–7
Lateral petals (mm)	10–17 × 3–6
Lip (mm)	8–12 × 9–14
Column (mm)	10 × 2[b]

[a] Kipping (1971)
[b] Luer (1975)

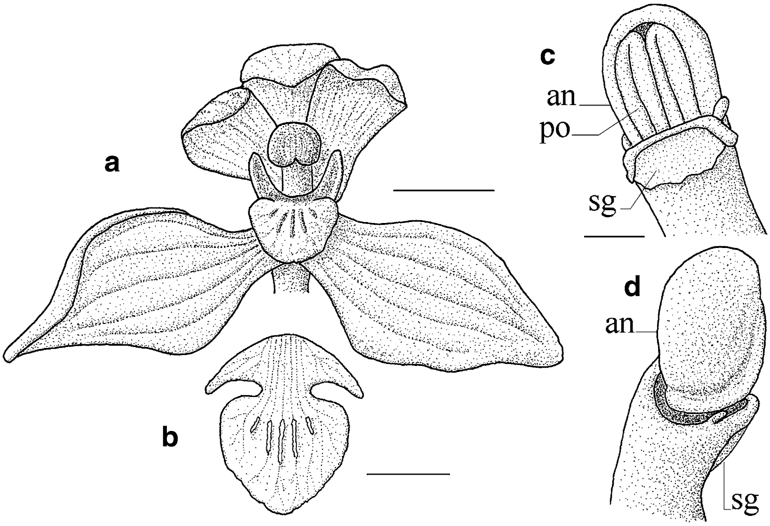

Fig. 3.3 *Cephalanthera austinae*. (**a**) Flower, front view, scale bar = 4 mm; (**b**) lip, flattened, scale bar = 5 mm; (**c**) top of column, ventral view showing anther with anther cap; (**d**) top of column, side view, scale bar (**c**, **d**) = 1 mm. *an* anther, *po* pollen mass, *sg* stigma

(Kipping 1971; Luer 1975). Dressler (1993), however, noted that the median stigma lobe, although reduced in some autogamous forms, normally projects well beyond the axis of the column (Fig. 3.3c, d) and bears a deposit of glue on its margin that very probably plays some role in pollination (Rasmussen 1982). For further discussion, see Burns-Balogh et al. (1987).

Compatibility and Breeding System

Kipping (1971), in a 2-year study in El Dorado County, California, enclosed young plants with unmanipulated, unopened buds in screen cages. About 14–30% of the enclosed flowers produced fruit. He also observed cross-pollination, and similar reports are available for a number of Old World species of *Cephalanthera*, where hybridization, autogamy, and apomixis have also been recorded (Darwin 1862; Godfery 1933; Evans 1934; Hagerup 1952; Dafni and Ivri 1981; Nilsson 1983; Prochazka and Velisek 1983; Scacchi et al. 1991).

Pollination Mechanism and Pollinators

Kipping (1971) reported that autogamy was frequent in older flowers following the withering and dehiscence of the anther caps. The basal section of the labellum also pulled away from the column as the flowers aged, and the pillars of mealy pollen, left unsupported, often fell over, sometimes landing on the viscid stigma.

Kipping also reported insect pollination. During the first year of his study, he captured three, small halictid bees, *Lasioglossum pullilabre* (Vachal) [cited as *Evylaeus pullilabris* (Vachal)], on the flowers of *C. austiniae*. Two carried the white pollinia of this orchid glued to their thoraxes. The third carried no pollinia, but the flower from which it had just emerged bore two strands of white pollen on its stigma. The bees, after hovering momentarily before a flower, landed on the distal section of the lip. Each, then, entered the narrow opening between the column and concave basal section of the lip (Fig. 3.3a), where it remained for 10 or 15 s. While backing out, the bee's thorax contacted the stigma and acquired a deposit of viscid material. As it passed under the anther, some of the pollen adhered to this deposit and was extracted. A second species, *Lasioglossum nigrescens* (*Craeford*) [as *Evylaeus nigrescens* (Crawford)], also captured on a flower of *C. austinae*, carried no pollinia.

Based on these observations, *C. austinae* would appear to be a nonrewarding, facultatively autogamous orchid employing a strategy of deceit to attract pollinators. Although floral mimicry is involved in the attraction of insects to some nonrewarding, Old World species of *Cephalanthera* (e.g., Dafni and Ivri 1981; Nilsson 1983; Nazarov and Ivanov 1991), there is no evidence for or against mimicry in *C. austinae.* Moreover, the flowers may not always be unrewarding. Kipping (1971) observed a distinctly different behavior during the second year of his study when another species of halictid bee, *Lasioglossum nevadense* (Crawford) (as *Dialictus nevadensis* Crawford), visited the flowers in large numbers. After landing on the lip, this bee immediately crawled into its concave basal section, ascended the column to the anther, removed pollen with its mandibles and front legs, and transferred the grains to its hind legs and the ventral surface of its abdomen. In the course of its visit, the bee often crawled randomly over the surface of the column, and subsequent examination revealed the presence of white pollen on several stigmas (Kipping 1971). The levels of self-pollination versus cross-pollination were not determined, but either way this appears to be a case where orchid pollen is deliberately collected by an insect and pollen transfer is not achieved by deceit. Such behavior has been reported elsewhere in North America only in *Cleistesiopsis*, possibly *Calopogon* (Gregg 1991, see below) and *Cypripedium* (Light 2005).

Fruiting Success and Limiting Factors

Kipping (1971) found that 15–27% of open-pollinated flowers produced fruit over the course of his 2-year study in northern California. Data on specific factors that may affect fruit set in this orchid are not yet available, but the percentages observed in unmanipulated caged (14–30%) versus open-pollinated flowers imply that autogamy is frequent in this population.

Epipactis Zinn

The genus *Epipactis* includes 16 (Pridgeon et al. 2005) to 30 (Thornhill 1996) temperate Eurasian and African species. One is native to North America and Asia. Two others are introduced to our flora.

Epipactis gigantea Douglas ex Hooker (Giant Helleborine, Stream Orchid)

Habitat and Distribution

This native, North American orchid requires a continuous water supply for its roots and is often found along shores on wet gravely substrates and sand bars or seepage banks (Coleman 1995). It also occurs around springs and in riparian woodlands or chaparral. It is distributed from British Columbia to southern California west to Montana and Texas with isolated populations in Oklahoma and southwestern South Dakota (Coleman 1995; Brown and Argus 2002).

Floral Morphology

Two to thirty-two rather showy, resupinate flowers are borne in an often one-sided, lax raceme (Table 3.3) (Coleman 1995; Brown and Argus 2002). The sepals are often greenish-yellow with purple veins. The laterals are widely spreading (Fig. 3.4a). The petals extend forward over the column and are usually pale pink to reddish or orange with darker red or purple veins. As in *Cephalanthera,* a central constriction divides the lip transversely into two parts (Fig. 3.4a). The basal part (hypochile) is concave with centrally located, small, warty calli and subtriangular lateral lobes. The calli are red to yellow, and the lobes are yellowish with purple to brown veins. The distal part (epichile), hinged and mobile, is oblanceolate and grooved to a pink apex. Two erect, wing-like, yellow–orange calli are again present near the base. The column is erect. The stigma and rostellum are positioned between a pair of lateral processes below the anther cap (Fig. 3.4b, c) (Luer 1975). The anther

Table 3.3 Data on *Epipactis* (Brown and Argus 2002)

Character	*Epipactis gigantea*	*Epipactis helleborine*
Plant height (cm)	To 140	25–80(–100)
Flower number	2–32	15–30
Dorsal sepal (mm)	15–20 × 6–8[a]	
Lateral sepals (mm)	16–24 × 8–9	10–13 × 5–6
Lateral petals (mm)	13–17 × 6–8	9–11 × 4–6
Lip (mm)	14–20	14–17 × 13
Column (mm)	5–10 × 3	3–6
Chromosomes (2*n*)	40	38, 40[b]

[a] Luer (1975)

[b] Hollingsworth and Dickson (1997)

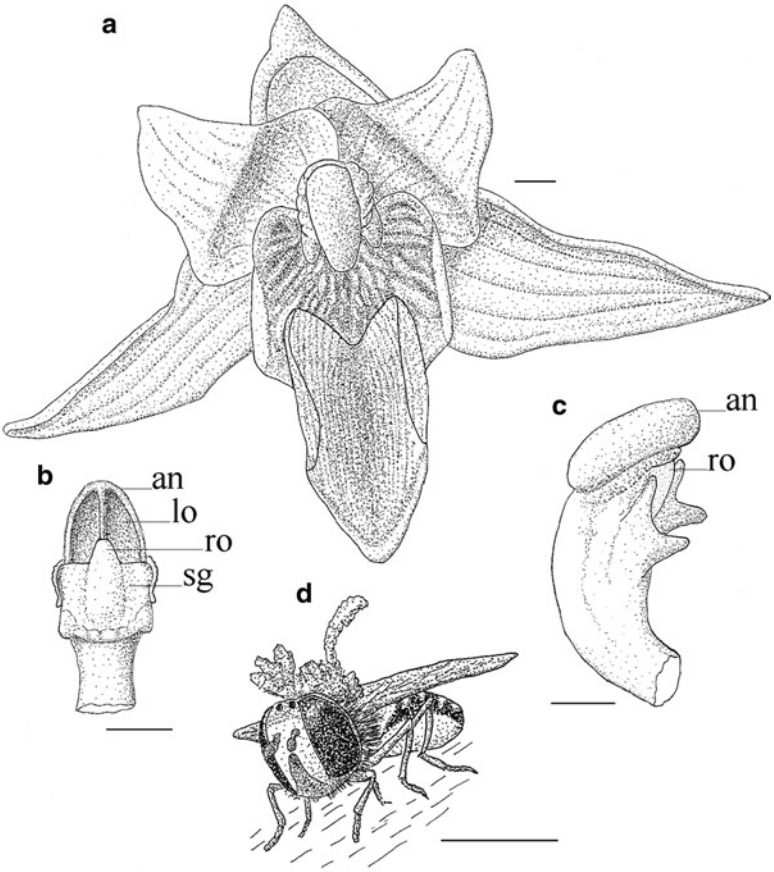

Fig. 3.4 *Epipactis gigantea*. (**a**) Flower, front view; (**b**) column, ventral view with anther cap lifted; (**c**) column, side view, scale bars (**a**–**c**) = 2 mm; (**d**) syrphid fly with pollinia attached to dorsal thorax, scale bar = 1 mm. *an* anther, *lo* locule, *ro* rostellum, *sg* stigma

is dorsal, two-chambered, and contains two pairs of soft, mealy pollinia comprised of pollen tetrads. Rostellar glue is located at the top of the rostellum and covered by a fragile membrane, but caudicles are absent and the pollinia are probably not attached to the rostellar glue prior to the intervention of a pollinator (Dressler 1993).

Compatibility and Breeding System

Based on hand pollinations, Wilson (2009) found that 59% ($n = 101$) of self-pollinated flowers, 72% ($n = 85$) of outcrossed flowers (using donor pollen from plants at least 10 m distant), and 63% ($n = 67$) of flowers receiving pollen from other flowers in the same raceme (geitonogamy) set fruit. Contrary to Brunton (1986) and Thornhill (1996), tests for autogamy and agamospermy were also positive. Thirty-eight percent ($n = 679$) of unmanipulated, bagged flowers and 11% ($n = 62$) of emasculated, bagged flowers set fruit, although some level of experimental error was suspected in the latter case (Wilson 2009).

The establishment of self-compatibility is of interest as this orchid is known to form vigorous clonal colonies (Allen 1982; Mantas 1993; Coleman 1995; Thornhill 1996). Thornhill [personal communication in Wilson (2009)], in a study of 12 populations in California, Oregon, Idaho, and New Mexico, concluded that dispersal routinely involves a single colonization event followed by asexual reproduction and selfing. Subsequent drift can lead to high levels of differentiation among colonies, suggesting a very low level of pollen or seed exchange among populations. However, despite low levels of intrapopulation heterozygosity, extensive examination of enzyme loci indicates that colonies are not entirely clonal and that the occasional influx of individuals from other populations may contribute to differentiation from the original founder.

Pollinators and Pollination Mechanism

E. gigantea is pollinated by syrphid flies, but provides no compensation in the way of nectar or other reward (e.g., Wiefelsputz 1970; Correll 1978; Ross 1988; Wilson 2009). Long-distance attraction is visual and, according to Ross (1988), is based on the orchid's yellow lip, a color particularly visible to insects. At close range, the flies are apparently induced to enter the flowers by deceit. The floral fragrance mimics the smell of honeydew, the excess sweet sap excreted by aphids during feeding. Adult syrphids eat honeydew, and the females lay their eggs among masses of aphids, the normal food supply for syrphid larvae. Coleman (2002) suggested that the warty calli on the lip might be mimetic, the syrphids mistaking them for aphids.

Wilson (2009), in a study conducted over 2 years at three elevations in the Rocky Mountains of Colorado, identified six species of syrphid flies in four genera as pollen vectors: *Dasysyrphus creper* (Snow), *Eupeodes americanus*, *E. luniger* (Wiedemann), *E. volucris* Sacken, *Platycheirus immarginatus* (Zetterstedt), and most frequently, *Sphaerophoria philanthus* Meigen. A closely related, nonsyrphid pollinator, *Copestylum satur* (Sacken), was also captured. Wasps were occasionally trapped, but none carried any pollen. However, Mantas (1993) identified yellow jackets (*Vespula vulgaris*) as pollinators at sites in northwestern Montana ranging in altitude from 945 to 990 m.

Ross (1988) also saw syrphid flies depositing eggs in flowers of *E. gigantea* at widespread sites from Del Norte County, California, to Zion Canyon, Utah. He reported many flies entering or leaving the flowers and many hovering in the vicinity with one or two yellow pollinia attached to their dorsal thoraxes (Fig. 3.4d). After landing on the epichile, the fly crawled under the column, its thorax brushed against the stigma, and picked up a deposit of rostellar glue. When it later backed out of the flower, the sticky deposit contacted and extracted pollinia from the anther. Pollination occurred during the entry of subsequently visited flowers.

Wilson (2009) observed similar behavior at her Colorado sites. The pollinators revisited many flowers but usually moved from the bottom of the inflorescence upward, spending 1–3 s on each open flower. If, as Wilson reported, the plants are not protandrous, the direction of pollinator movement would not affect the levels of geitonogamy or outcrossing. The pollinia were received on the thorax with some syrphids carrying two or more sets. Flies occasionally deposited eggs on the perianth. However, like Ross (1988), Wilson noted that aphids or aphid eggs were usually not present on the flower or other parts of the plant, and the hatched syrphid larvae would therefore not survive.

Ivri and Dafni (1977) and Sugiura (1996) reported a similar strategy in the nectar-producing orchids, *E. helleborine* (L.) Crantz subsp. *helleborine* in Israel and *E. thunbergii* A. Gray in Japan. In addition to male syrphids that sometimes fed at and pollinated the flowers, females laid eggs on the labellum. The hypochile, as in *E. gigantea*, bears warts or markings on its surface that may mimic the shape and color of aphids, and these, along with the nectar, are thought to attract the females. Feeding behavior, before or after egg laying, may lead to pollination of the flower. The presence of a mimetic system in a rewarding orchid might perhaps be thought of as a supplementary pollinator attractant, effecting a net increase in pollination and reproductive success. Attraction by deceit in rewarding orchids is known elsewhere as, for example, in *Epidendrum amphistomum* A. Rich., where male moths are lured by production of a pheromone mimic but pollination is accomplished during nectar feeding not pseudocopulation (Adams and Goss 1976).

In some species of *Epipactis*, insect-induced movements of the distal labellar segment are thought to play a role in pollination, changing the position or affecting the balance or exit trajectory of the pollinator and thus bringing it into contact with the column (e.g., Darwin 1862; Sugiura 1996). This subject needs additional study, but Nilsson (1981a) called attention to an apparent correlation between pollinator species and the development of the labellar hinge mechanism. *E. gigantea* has a well-developed labellar hinge, and its rotation could play a role in effecting contact between the thorax of the visiting syrphid fly and the column.

Fruiting Success and Limiting Factors

Thornhill (1996) reported fruit set values of 40–60% in natural populations and suggested that *E. gigantea* might be pollinator limited. This possibility is supported by Wilson's (2009) observation of a statistically significant difference in fruit set between open-pollinated control plants (62%) and hand-pollinated outcrossed plants (72%). She tracked individual flowers and found that 100% of those visited by syrphids produced fruit and seed. She believes that pollinator limitation may in this case be compensated by facultative autogamy, probably as a backup in older, unvisited flowers.

According to Coleman (1986), almost all the flowers in some California populations set fruit, whereas other nearby populations remained unpollinated. The reason for the difference is not known, but where fruiting is successful seedling recruitment can be high.

Mantas (1993), in a 2-year study in northwestern Montana, found that the percentage of ramets producing flowers was very low, from 3.2 to 4.8%. Each flowering ramet produced 4.2 (1–9) flowers. At the same time, the yearly percentage of flowers producing mature fruits varied widely from 6.8 to 63.5%. Mantas attributed the variation to high levels of rainfall during the blooming period of 1 year, reducing pollinator visitation rates. Wilson (2009) also observed variation in fruit set among sites and years and speculated that the differences might in part reflect differential supplemental pollination by other insects, such as ants and aphids.

Mantas (1993) also reported that seed production was higher in open stands, which routinely produced taller ramets and more flowers than dense stands. The latter were more likely to reproduce vegetatively. Arditti et al. (1981) found that germination rates averaged 20% under laboratory conditions, that a shady environment was needed for germination, and that seeds from immature capsules germinated better than those from ripe fruit. Although the latter could imply that seeds decrease in viability during maturation, it also suggests that mature seeds may pass through a period of dormancy prior to germination, a phase often absent in orchids (Arditti et al. 1981 and references therein). The seeds are able to tolerate a range of variation in soil acidity, retaining a constant rate of germination (20%) over a pH range of 5–7.5. Apparently, however, the plants must survive a fairly long period of development before reproducing. Myers and Ascher (1982) report that the interval from seed to flowering is 39 months.

Variation occurs in the genetic diversity of populations and may reflect differences in the relative importance of clonal versus sexual reproduction. Nevertheless, clonal growth appears to be the most frequent overall reproductive strategy in *E. gigantea* (Thornhill 1996). At the same time, the production of numerous small seeds permits long-distance dispersal and self-compatibility, plus genetic variation among populations allows colonization of a wide variety of geographically isolated habitats.

Little is known about the effects of predation and disease on this orchid. Mantas (1993) found that browsing by slugs, insects, and ungulates occurred at one site in northwestern Montana, but that the effects were not significant. Also, anthracnose occasionally destroyed some flowering buds; although the long-term effects are unknown, very few ramets were infected during the study period. In some areas, serious degeneration of the habitat has resulted from the invasion of exotic species and such human activity as hot springs development, stream flow reduction caused by extensive groundwater pumping, construction of hydroelectric dams, housing development, recreation, timber harvest, grazing, and expanding agriculture (Brunton 1986; Coleman 2002).

Epipactis helleborine (L.) Crantz (Broad-Leaved Helleborine)

Habitat and Distribution

Naturalized in North America since 1879 (Day 1882; Correll 1978), this orchid is found on disturbed sites or in cedar swamps, shaded deciduous, or mixed forest and along wooded streams, frequently on rocky substrates; it often grows as individual stems among young trees, where few other herbs are present (Light and MacConaill 1991; Boesse 2000; Brown and Argus 2002; Pridgeon et al. 2005). Many isolated populations are scattered throughout the USA and southern Canada (Brown and Argus 2002). A more or less continuous distribution is present from New Brunswick to New Jersey west to Wisconsin. Several extended ranges are also found in California and the Washington–British Columbia area.

Floral Morphology

A number of small, resupinate flowers are borne in a loose to moderately dense, often one-sided raceme (Table 3.3) (Judd 1972; Brown and Argus 2002). The sepals are spreading and greenish with tinges of purple. The petals are pale green to pink, purple, or yellowish and, along with the dorsal sepal, extend forward over the column (Brown and Argus 2002). The lip is divided by a transverse constriction into a distal and basal section, but unlike *E. gigantea* the hinge mechanism is poorly developed. The basal section (hypochile) is purplish to brownish and lacks papillae; deeply concave, it secretes and holds nectar (Fig. 3.5a) (Luer 1975). The distal section (epichile) is triangular, recurved, and white, green, or purplish with a pair of fleshy calli near the base. A short column (Table 3.3) bears lateral processes (staminodia) (Luer 1975) and a dorsal anther containing a pair of sessile, cleft, mealy pollinia comprised of tetrads (Fig. 3.5b, d) (Luer 1975; Burns-Balogh et al. 1987). The three-lobed stigma is wide, positioned below the anther, and separated from it by a prominent, rounded rostellum (Luer 1975). A fragile membrane again covers the top of the rostellum and bursts on contact, emitting a sticky liquid (Young and Renz 1958).

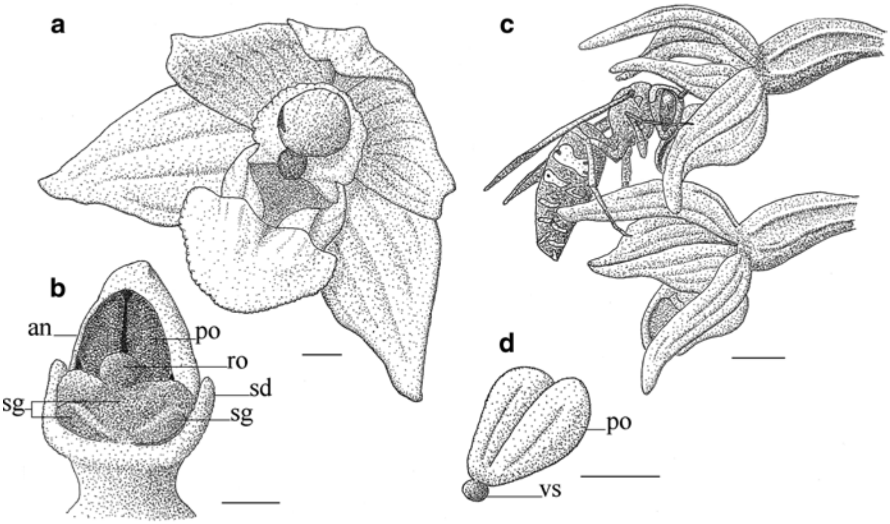

Fig. 3.5 *Epipactis helleborine*. (**a**) Flower, front view, scale bar = 3 mm; (**b**) column, slightly oblique side view, scale bar = 1 mm; (**c**) wasp entering flower, scale bar = 3 mm; (**d**) polliniarum, scale bar = 1 mm. *an* anther, *po* pollinia, *ro* rostellum–viscidium, *sd* staminodium, *sg* stigma, *vs* viscidium

Compatibility and Breeding System

Although the flowers are self-compatible, Muller (1988) concluded that autogamy is absent, and Ehlers et al. (2002) found no evidence for it in bagging experiments conducted on 13 populations from 3 geographic regions along a latitudinal gradient of about 1,000 km from northern to southern Sweden. Similarly, Talalaj and Brzosko (2008) found allogamy to predominate in a 5-year study of three populations from northern Poland. Autogamy is usually prevented by the positioning of the large rostellum and stigma (Fig. 3.5b) (Mousley 1927; Ehlers et al. 2002; Talalaj and Brzosko 2008). In addition, insect visitation with removal of the pollinia often occurs very quickly after the flowers open.

Although van der Cingle (2001) attributed reports of autogamy to taxonomic confusion, namely, the misidentification of distinct, but closely related autogamous taxa as *E. helleborine* s.s., other workers have reported that following elevation of the anther cap, the pollinia sometimes pivot at their points of attachment on the rostellum and fall onto the stigmatic surface (Martens 1926; Mousley 1927; Light in Squirrell et al. 2001). Also, in older, unvisited flowers, the pollinia may expand and become friable, and an elevated anther cap then allows pollen fragments to break away, some reaching the stigma (Mousley 1927; Talalaj and Brzosko 2008). Hagerup (1952) described a similar process in Denmark, where tetrads were routinely liberated from the pollinia prior to or just after opening of the flower. Based on these reports, occasional autogamy may result either from rotation of the entire pollinium onto the stigma or from a lack of pollen coherence in older flowers (Catling 1983). Talalaj and Brzosko (2008), however, note that pollen viability is reduced in aging flowers.

Other European taxa of this genus are clearly facultatively to obligately autogamous or even cleistogamous (e.g., Hagerup 1952; Young and Renz 1958; Sundermann 1975; Richards 1986; Claessens and Kleynen 1996; Mered'a and Potucek 1998). Robatsch (1983), in fact, claimed that 60% of all species are autogamous. Most are reported to produce very little nectar or floral scent and to share a breakdown of the rostellum along with powdery pollen that falls directly onto the stigma. Some autogamous species are thought to have evolved independently within local populations of *E. helleborine* (Richards 1982; Harris and Abbott 1997; Ehlers and Pedersen 2000; Johnson and Edwards 2000; Pedersen and Ehlers 2000; Squirrell et al. 2001, 2002; Talalaj and Brzosko 2008). Facultative and obligate subspecies and varieties are associated with dry habitats, such as coastal sand dunes of the Netherlands and Denmark. Based on the levels of pollen removal, pollinator visitation may be lower in dry than in mesic habitats (Ehlers et al. 2002) and could lead to selection for autogamy (e.g., Baker 1955; Wyatt 1988).

Since the plants of *E. helleborine* may be multistemmed (e.g., Richards 1982; Burns-Balogh et al. 1987; Muller 1988; Light and MacConaill 1998), between spike as well as within spike geitonogamy is possible. Based on some reports, geitonogamy is the most probable mode of pollination and may be promoted by pollinator behavior (Light 1994; Light and MacConaill 1998; Talalaj and Brzosko 2008). At the same time, pollinators often carry the pollinia for extended periods, depositing pollen slowly (Richards 1986), increasing pollen carryover and the potential for cross-pollination. The extraction or deposition of pollinia does not affect the overall attractiveness of an inflorescence (Piper and Waite 1988). Vectors bearing pollinia may, therefore, visit previously pollinated flowers, and data on pollen germination suggest the possibility of pollen-tube competition (Light 1994).

Both cross-pollination and geitonogamy are known to occur in Quebec (Mousley 1927; Light and MacConaill 1998), and cross-pollination is routinely reported in European populations (e.g., Sundermann 1975; Richards 1982; Talalaj and Brzosko 2008). Piper and Waite (1988) inferred a high frequency of cross-pollination in England based on a marked temporal separation of pollen export and import. A genetic structure consistent with Hardy–Weinberg expectations and random mating also suggests that outcrossing is predominant and selfing is rare or subject to inbreeding depression within local populations in both North America and the Old World (Hollingsworth and Dickson 1997; Ehlers and Pedersen 2000; Squirrell et al. 2001). At the same time, significant differentiation is present among local populations, implying that pollen flow is largely restricted to flowers within populations (Richards 1982; Hollingsworth and Dickson 1997; Ehlers and Pedersen 2000; Squirrell et al. 2001).

Pollinators and Pollination Mechanisms

E. helleborine is pollinated by wasps (Fig. 3.5c). These insects are visually oriented and often attracted to brown, yellow–green, and dull red colors (Wiefelsputz 1970). In addition, Brodmann et al. (2008) recently demonstrated that *E. helleborine* produces compounds that mimic green-leaf volatiles, chemicals released when herbivores, such as caterpillars, feed on leaves (mostly six-carbon aldehydes, alcohols, and acetates). Caterpillars, such as those of *Pieris rapae* (L.) (cabbage white), are known prey of social wasps, and *E. helleborine* evidently mimics green-leaf volatiles in order to attract wasps for pollination.

Judd (1972) found wasps to be the exclusive floral visitors in southern Ontario. *Vespula arenaria* (Fabricius) [= *Dolichovespula arenaria* (Fabricius)] (sandhills hornet), *V. consobrina* (Saussure) (blackjacket), and *V. vidua* (Saussure) (ground hornet) carried up to three pairs of pollinia attached to the hairs on their lower frons and clypeus, indicating multiple visits. They were mostly female workers, although some males were also observed. Voth (1982) reported that female wasp pollinators in Europe were older workers that were no longer feeding larvae or seeking animal prey. A fourth species, *Polistes fuscatus* (Fabricius) (paper wasp), visited the flowers in Ontario but carried no pollinia (Judd 1972). In addition, Mousley (1927) reported two other wasps, *Vespula germanica* (Fabricus) (german yellow jacket) and, to a lesser extent, *V. maculata* L. (black wasp, bald-faced hornet) as the most common pollinators in Quebec, and Dowden (in Luer 1975) observed the common yellowjacket, *V. vulgaris* (L.) as a pollinator in New England. Catling (1983), Mantas (1993), and Light and MacConaill (1998) reported unspecified wasps as pollinators in Montana, Ontario, and Quebec, respectively. Honeybees are frequent visitors in New England, but their long proboscises often allow them to reach the nectar without removing the pollinia. Wasps, on the other hand, have short proboscises and are forced to penetrate deeply into the flower to reach the nectar. As a result, their heads almost always contact the pollinia.

Studies in the Old World indicate that the legitimate pollinators of outcrossing *Epipactis* species are also predominantly wasps of the family Vespidae (e.g., Darwin 1862; Kunth 1898–1905; Meeuse 1961; Judd 1972; Nilsson 1981a; Richards 1982, 1986; Ehlers and Olesen 1997; Ehlers et al. 2002). However, it has already been mentioned that syrphid flies are the principal pollinators of *E. consimilis* (= *E. helleborine* subsp. *helleborine*) in Israel and *E. thunbergii* in Japan (Ivri and Dafni 1977; Sugiura 1996). Although Kew's World Checklist of Monocotyledons lists *E. consimilis* as a synonym of *E. helleborine* var. *helleborine*, details of its pollination, especially the role of the hinge mechanism as described by Ivri and Dafni (1977), differ from that reported elsewhere for *E. helleborine*. Other Old World species are pollinated by various flies, beetles, bees, and other hymenoptera, including ants (e.g., Darwin 1862; Nilsson 1978, 1981a; Brantjes 1981; Richards 1986). Ants also supplement syrphid flies as pollinators of *E. thunbergii* in Japan (Sugiura 1996; Sugiura et al. 2006). Of particular interest, honeybees, the most important pollinators of *Epipactis* species in the Netherlands based on their abundance and mobility, collected pollen as a larval food (Brantjes 1981). In apparent contradiction of the reports from New England, the pollinia attached to the clypeus and distal fragments were groomed in flight to the corbiculae. The basal parts of the pollinia remained on the head and were available for pollination.

American authors agree, in general, with their European counterparts on the usual sequence of events leading to pollination. The wasp grasps the distal part of the lip and inserts its head under the column to feed on nectar contained in the deeply concave basal section (Fig. 3.5c). While doing so, pollinia borne from earlier flower visits are brushed against the stigma, positioned just above the basal lip section (hypochile). The amount of pollen deposited varies from a few hundred tetrads to a whole pollinium. Additional feeding movements bring the wasp's head into contact with the sticky rostellar fluid, which is extracted along with the adherent pollinia as the wasp withdraws from the flower. Even though a caudicle is absent, the pair of pollinia bend forward from the vertical as they dry, better positioning them to contact the stigma of the next flower visited (e.g., Darwin 1862; Meeuse 1961; Judd 1972; Nilsson 1981a; Richards 1986).

Wasps having fed on the nectar of this orchid sometimes become very lethargic (Lojtnant 1974; Burns-Balogh et al. 1987; Muller 1988). Muller (1988) detected small amounts of ethanol in the nectar, and Ehlers and Olesen (1997) have now found ethanol-producing microorganisms present. These might be airborne or transported to the flower from various ripe fruits by wasps. Lojtnant (1974) observed that "intoxication" of the wasps reduced the amount of pollen they groomed from their bodies. He speculated that this might increase the quantity transferred to other plants and that selection for the production of compounds with antimicrobial activity, found in the nectar of some plants (e.g., Gilliam et al. 1983), might be absent in *E. helleborine* (Ehlers and Olesen 1997).

More recently, Jakubska et al. (2005) detected the presence of other nectar components with potential narcotic effects. These included indole, morphinan, and phenol derivatives. They proposed that pollinators are first attracted to the flowers by volatile nectar components, such as vanillin, furfural, ethanol, eugenol, and their derivatives. Following nectar consumption, the narcotic effect of constituents, such as morphinan and indole derivatives, may extend the time the pollinators spend on the inflorescence and improve the chance of pollinating more flowers.

Jakubska et al. (2005) further suggested that because temperatures at their study site (often exceeding 28°C or 82°F) would result in rapid evaporation of ethanol, the level of ethanol production by microorganisms would have to be extraordinary to produce its purported effect on pollinators. Moreover, a number of nectar components, such as furfural, syringol, indol derivatives, eugenol, and methyleugenol, present in the nectar of *E. helleborine*, have known bactericidal and fungicidal properties. The species of *Cladosporium* and *Candida* found by Ehlers and Olesen (1997) are apparently susceptible to these compounds. The contribution of these microbes to the "intoxication" of pollinators may, therefore, be less significant than these authors suggest. Ethanol could, however, derive from the decomposition of some nectar components and might therefore still contribute to the lethargic behavior of pollinators.

Fruiting Success and Limiting Factors

Although precise details are lacking, pollination is often frequent, and Mousley (1927) reported finding three or four sets of pollinia on a single stigma of *E. helleborine* in southern Quebec. However, in Israel, where syrphid flies are the primary vectors, only 15.5% of 724 flowers on 24 plants were pollinated naturally (Ivri and Dafni 1977). In Europe, Richards (1982) found some capsules of *E. helleborine* containing nearly 100% viable seed and others with embryos present in only 1–15% of the seeds. He assumed the former to result from wasp visits and the latter to be a product of less-efficient autogamy, where the amount of pollen transferred to the stigma was inadequate to fertilize 1,000 or more ovules present in each ovary.

At a study site in Gatineau Park, Quebec, Light and MacConaill (1990, 1991, 1998) found that about 25% of the plants that emerged each year flowered. All pollinated flowers set fruit, and 4–79% of flowering plants developed capsules. The number of seeds produced per capsule decreased from the bottom toward the top of the inflorescence. However, because the decrease in seed number was abrupt and the capsules toward the top of the inflorescence were oddly shaped, they did not believe this decrease to be a result of resource limitation.

As in *Spiranthes spiralis* (Wells 1967), *Isotria medeoides* (Mehrhoff 1989), *Tipularia discolor* (Snow and Whigham 1989), *Cypripedium acaule* (Cochran 1986), and many other orchids, Light and MacConaill (1990, 1991, 2006) found that plants sometimes failed to appear above ground for 1 or more years, even though about half of the nonemergent plants still had living rhizomes. Three plants reappeared following an absence of 3 years and one of these flowered. This species may be able to obtain sufficient nourishment from its mycorrhizal fungus to remain underground and forego photosynthesis for several seasons before emerging to bloom. Most (62%) emerged only once over a 20-year period, and 27 plants reemerged after an absence of 7–18 years. The probability of any plant reappearing was highly variable from year to year, but was not related to whether or not it had bloomed in the previous season. According to Light and MacConaill (1990), the perennating bud, containing the next year's floral primordia, develops prior to flowering and fruiting. They believe that there is little evidence to indicate that the costs of flower and fruit production affect differentiation or development of this bud, although they have not yet ruled it out.

Kindlmann (1999), on the other hand, considered that the most likely explanation for yearly transitions between flowering and sterility in a European species of *Epipactis*, *E. albensis* Novakova and Rydlo, was based on the costs of sexual reproduction and leaf herbivory, as reported in a number of other orchids (Snow and Whigham 1989; Primack and Hall 1990; Whigham 1990; Whigham and O'Neill 1991; Zimmerman and Whigham 1992; Calvo 1993; Primack et al. 1994; Gill 1996). Additional work based on demographic modeling and costs associated with fruit rather than flower production in this species is needed to further test this idea (Kindlmann 1999; Ehlers et al. 2002).

In a study in Sussex, England, Piper and Waite (1988) found that rates of pollen import and export in *E. helleborine* were positively correlated with two secondary floral characters, the number and density (number of flowers/inflorescence length) of open flowers per inflorescence. For inflorescences of equal size, the number of pollinia exported (male function) always exceeded the number imported with a loss of about 58% of the pollinia from the population. Pollen import by itself is considered an unreliable measure of female function, which is better based on seed production (Stephenson 1981; Bell 1985). The latter was not examined in this study, but according to Bell (1985), seed production in xenogamous, hermaphroditic flowering plants is not closely related to secondary floral characters. Accordingly, Piper and Waite (1988) believe that the evolution of secondary floral characters in *E. helleborine* has been a product of selection for higher rates of pollen export. A more recent study on *Epipactis* appears to support this conclusion. Ehlers et al. (2002) found that an increase in flower number enhanced pollinia removal more than fruit set and also considered the evolution of large inflorescences in *Epipactis* to likely be more closely related to selection on male function than female function (e.g., Chaplin and Walker 1982; Vaughton and Ramsey 1988; Emms et al. 1997; Pickering 2000). Firmage and Cole (1988) reported a similar relationship for *Calopogon tuberosus*. However, Snow and Whigham (1989) found no differential effect of inflorescence size on male and female reproductive success in *T. discolor*, and according to Tremblay et al. (2005), a similar parallel response may be true of many other orchids as well.

Although Light and MacConaill (1990) reported that fruit set in *E. helleborine* was generally high, they found that herbivory, disease, and weather conditions could significantly reduce the number of capsules that reached dehiscence. These authors specifically mentioned small animal herbivores as a problem in Quebec, and Mousley (1927), based on studies in the same province, noted that larvae of *Spilosoma virginica* (Fabricus) (yellow, wooly-bear moth) were significant predators on the leaves of this species.

Additional Species of *Epipactis*

Epipactis atrorubens (Hoffman ex Bernhardi) Besser, a native of Europe and Asia with vanilla scented, reddish-purple flowers, is represented in North America by a small, naturalized population in northern Vermont (Mousley 1927; Brown and Argus 2002). Its survival here and elsewhere may be heavily dependent on connections to tree ectomycorrhizae (Gebauer and Meyer 2003).

All studies on its pollination have been conducted in Europe. Contrary to East (1940) and Tremblay et al. (2005), *E. atrorubens* is clearly self-compatible. Hagerup (1952) reported a predominance of bud autogamy in Denmark, but Sundermann (1975), Richards (1982), and Talalaj and Brzosko (2008) found it to be chiefly allogamous elsewhere. Nectar is produced on the hypochile and is accessible to almost any insect. Wiefelsputz (1970) and Robatsch (1983) suggested that the floral coloration and scent are likely to attract bumblebees and honeybees, and Talalaj and Brzosko (2008) found bumblebees (*Bombus* sp.) to be the primary visitors. Voth (1982, 1988) and I. Muller (1988) reported bumblebees, wasps, and honeybees as pollinators.

According to Darwin (1862) and Godfery (1933), English plants are usually pollinated by species of wasps in the genus *Vespa* L. Nilsson (1981a), however, observed only one species of vespid wasp visiting the flowers in Sweden, and it was not a pollen vector. He found solitary eumenid wasps of genus *Odynerus* s. l. Latreille to be the legitimate pollinators of this species in his study area, even though other pollinators, including bees (Apidae) and beetles, visited the flowers more frequently. Flowering of *E. atrorubens* coincides with the emergence of eumenid populations in Sweden and occurs distinctly earlier than the flowering of plants adapted to vespid wasps. Eumenids and vespids are usually polyphagous, often carnivorous, and visit flowers less frequently than other anthophiles, such as honeybees and bumblebees. The latter two, however, often have longer proboscises and are less well-adapted to the floral morphology of *E. atrorubens*, often taking nectar without extracting pollinia. As a result, Nilsson (1981a) believes that honeybees and bumblebees, in particular, have a negative effect on the pollination of this species, despite the fact that they are often cited as primary pollinators in the literature (e.g., Godfery 1933; Dressler 1993).

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Chapter 4
Tribe Triphoreae

Abstract The flowers of *Triphora trianthophora* are self-compatible but not autogamous. Predation and pollen limitation vary among populations, and vegetative reproduction can be significant in some seasons at some sites. However, sexual reproduction and outcrossing appear to predominate and may be facilitated by synchronous blooming. Fruit set appears related to population size at some locations. Pollination mechanisms are discussed. The primary pollinators are small, short-tongued bees of the family Halictidae.

Keywords *Triphora* • Outcrossing and synchronous blooming • Predation and pollen limitation • Vegetative reproduction • Fruit set and population size • Short-tongued halictid bees • Andrenid bees

The tribe Triphoreae comprises 4 genera and about 28–30 species (Pridgeon et al. 2005). Representatives are found in North-, Central-, and South America with one species restricted to tropical West Africa. A single genus is present in our flora.

Triphora Nuttall

Triphora is a genus of about 19 species found in North America, the West Indies, and through Middle America to central South America. Five species occur in North America north of Mexico; all but one are restricted to Florida.

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Table 4.1 Data on *Triphora* (Medley 2002)

Character	*Triphora trianthophora*
Plant height (cm)	7–20 (–30)
Flower number/raceme	(1–) 3–6 (–20)
Dorsal sepal (mm)	11–15 × 3–4
Lateral sepals (mm)	11–15 × 3–4
Lateral petals (mm)	11–14 × 3–4
Lip (mm)	8–20 × 6–10
Column (mm)	10 × 2
Chromosomes (2n)	18[a], 44[b]

[a] Brackley (1985)

[b] Dressler (1981) for tribe Triphoreae

Triphora trianthophora (Swartz) Rydberg (Three Birds Orchid, Nodding Pogonia)

Habitat and Distribution

T. trianthophora is distributed throughout eastern North America from southwest Maine to southern Ontario and Iowa south to central Florida and east Texas (Luer 1975). It is apparently found in a wide variety of usually shady habitats, including rich or sandy mesophytic or dry woodlands, dune forests, sandy flatwoods, floodplain forests, and tamarack swamps (Medley 1979, 2002; Keenan 1986; Ramstetter 2001).

Floral Morphology

Generally, six or fewer resupinate flowers are borne singly in the upper leaf axils (Table 4.1) (Medley 1979). They are gaping, nodding to nearly erect, and white, often tinged with pale pink (Luer 1975; Medley 2002). The dorsal sepal is erect, the laterals spreading (Fig. 4.1a). The lip is obovate, narrowly clawed, and three-lobed (Fig. 4.1c). The lateral lobes are ovate to ovate-triangular and entire. Along with the closely overarching lateral petals, they form a floral tube enclosing the column (Fig. 4.1a, b). The middle lobe is ovate to orbicular with a sinuous to denticulate margin. Three parallel, bright green, papillose crests extend from the claw to about half way down the length of the central lobe (Fig. 4.1a, c). The green crests along with a narrow strip extending from the crests to the distal end of the labellum fluoresce in ultraviolet and serve as nectar guides (Medley 1979). The column is white and bears a pale green to white, two-chambered anther with magenta margins (Fig. 4.1d, e). The anther is rigid, terminal, and incumbent and contains two reddish-purple, soft, and mealy pollinia comprised of tetrads and held together by viscin (Lownes 1920; Medley 1979, 2002). A drop of rostellar glue is covered by a fragile membrane (Dressler 1993); caudicles and true viscidia are not present (Freudenstein 1994). The stigma, positioned beneath the column just proximal to the anther, is 1–1.5-mm long with a flat, granular, viscid surface (Fig. 4.1d, e). The flower

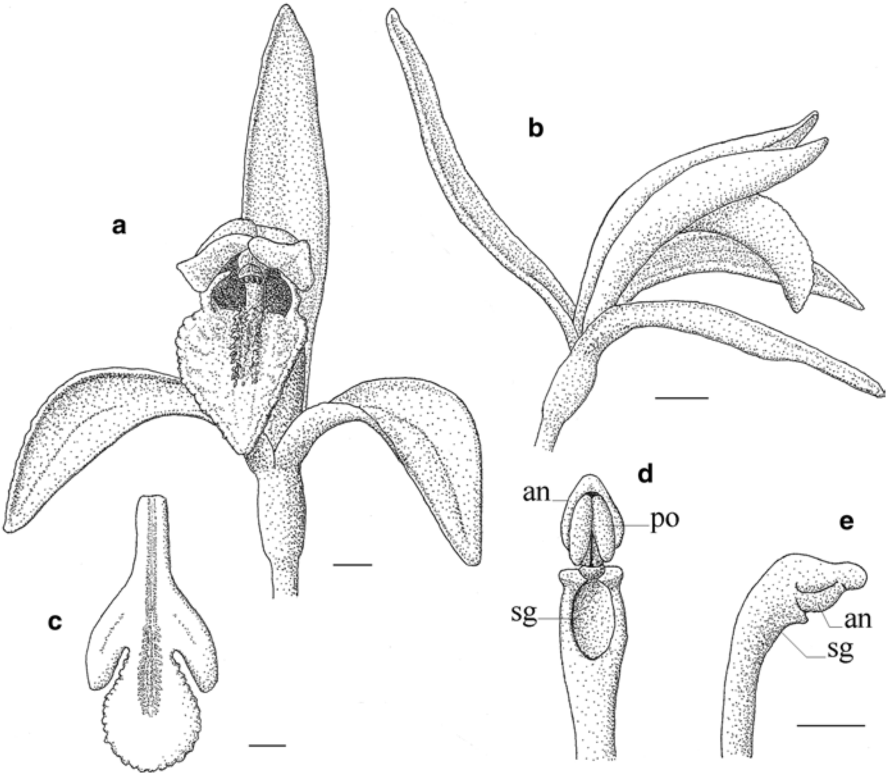

Fig. 4.1 *Triphora trianthophora*. (**a**) Flower, front view; (**b**) flower, side view; (**c**) lip, flattened; (**d**) column, ventral view with raised anther; (**e**) column, side view, scale bars = 2 mm. *an* anther, *po* pollinia, *sg* stigma

produces a faint odor variously described as both sweet and somewhat musty–fetid (Medley 1979). Small droplets of what Medley (1979) presumed to be nectar are present at the base of the lateral sepals and labellum.

Flowering of all plants in a particular area is largely synchronous, and the flowers often open in mid-morning and close by late afternoon on only 1 or occasionally 2–4 successive days (Lownes 1920; Medley 1979, 2002). According to Keenan (1986), flowers more than 1 day old are no longer in prime condition. Successive intervals of bud maturation and episodes of synchronous blooming may be repeated at intervals of several days from three to seven times in a single season and the synchrony may extend over a wide geographic area (Medley 1979; Keenan 1986, 1992). Each plant often produces from one to six buds (Table 4.1), but usually only one or two, occasionally three, flowers are open at the same time (Keenan 1986, 1992; Willems 1994). Blooming may be associated with late summer rains and frequently, but not always, occurs about 48 h after a drop in nighttime temperature. It is restricted to an interval from summer to late fall when relatively few sympatric flowers are in bloom, a circumstance that may reduce competition for pollinators (Sheviak 1974; Brackley 1981; Keenan 1986, 1988; Medley 2002). Extended periods of underground dormancy are known (e.g., Willems 1994), and Porcher (1977) claims that reemergence may be delayed for as long as 125 years.

Compatibility and Breeding System

Based on experimental pollinations, Medley (1979) found that the flowers are self-compatible but possess no mechanism for spontaneous selfing. He reported some revisitation of plants and flowers, indicating that selfing may occur under natural conditions, but outcrossing among individuals of the same population was usual. Synchronous blooming, with many plants displaying their flowers to pollinators at the same time, may facilitate outcrossing (e.g., Willems 1994). A number of other *Triphora* species, including *T. gentionoides* found in Florida, are known to be autogamous (Pridgeon et al. 2005).

Pollination Mechanisms and Pollinators

According to Medley (1979), the pollinator, invariably a bee, lands on the lip and following the ultraviolet nectar guides (Fig. 4.1a, c), enters the floral tube to reach the nectar at the base of the labellum. In the process, it crawls under the column bearing the anther and stigma. As it backs out of the flower, it presumably ruptures the rostellar membrane, and the pollinaria are glued to the rigid, backward pointing hairs on its dorsal thorax (Fig. 4.2). Pollination occurs when and if a bee bearing pollinia enters another flower and deposits pollen on the stigma. The bee might extract additional pollinia as it moves among previously unvisited flowers, and Medley (1979) reported some carrying up to four sets on their thorax.

The primary pollinators of *T. trianthophora* are small, short-tongued bees of the family Halictidae, attracted by the synchronous flowering of this orchid (Catling 1984). Lownes (1920) reported pollination by *Halictus quadrimaculatus* Robertson [= *Lasioglossum macoupinense* (Robertson)] at a site near Squam Lake in central New Hampshire. The most important pollinator in St. Joseph County, Indiana, and Berrien County, Michigan, was *Augochlora pura* (Fig. 4.2) with three or four species of *Lasioglossum* (*Dialictus*) (including *L. versans*, *L. obscurum* (Robertson), and *L. cressoni* (Robertson)) and an unknown species of *Sphecodes* and *Ceratina dupla* playing subsidiary roles (Medley 1979). In Cass County, Michigan, two other halictids, *L.* (*Evylaeus*) *quebecense* (Crawford) and *L. illinoense* (Robertson) functioned as primary pollinators even though *A. pura* was present at the site (Medley 1979). Keenan (1996) also collected several insects visiting *Triphora* flowers in New Hampshire, including an unidentified species of *Andrena* bee with purple pollinia on its back.

Medley (1979) listed a large number of additional, nonpollinating flower visitors. For example, bumblebees (*Bombus*) visited the flowers occasionally but were ineffective pollen vectors. The plants were unable to support their weight, and the bees departed the collapsing stems without removing any pollen or nectar. Willems (1994), however, reported that an unidentified, small species of bumblebee (*Bombus*) removed pollinia from a flower in western Massachusetts. Occasionally, late in the

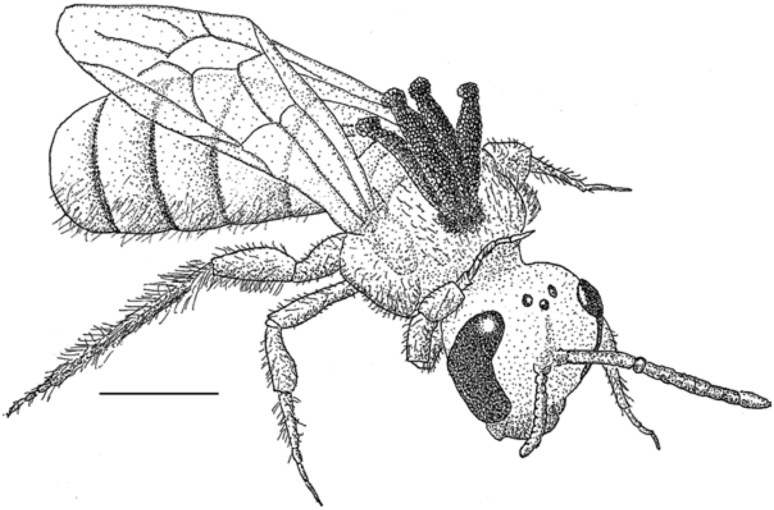

Fig. 4.2 *Augochlora pura* with pollinia of *Triphora trianthophora* attached to its thorax, scale bar = 1 mm

afternoon, *A. pura* and unidentified species of *Lasioglossum* may resort to thievery, robbing nectar at the base of the sepals. Medley (1979) thought that this activity was prompted by an absence of nectar at this time of day, and in his opinion it had no effect on pollination efficiency.

Fruiting Success and Limiting Factors

Medley (1979) found that capsule enlargement at his site in Berrien County, Michigan, approached 100% if the weather was favorable for bee activity and plants were grouped so that three or more flowers were available. Pollination was uncommon, however, in isolated groups of only one or two plants.

Willems (1994), on the other hand, reported very low levels of pollination and capsule set in a western Massachusetts population of this orchid over a 6-year period. Ninety percent or more of all emergent stems flowered, but less than 10% of the flower buds produced mature capsules. As only 5% of developing capsules were destroyed by predators, she attributed low fruit production to an abbreviated blooming period combined with a shortage of pollinators and considered that reproduction was primarily vegetative. Similarly, Lownes (1920) found that pollination rarely occurred at his study site in central New Hampshire and that seeds usually failed to ripen. This was true despite the fact that 20–40 plants were clustered within an area of a square foot. Like Willems, he thought that reproduction was chiefly vegetative.

In a more recent 11-year investigation at the site where Lownes conducted his 1920 study, Keenan (1996) found that slightly less than 50% of the flowers formed capsules, a level lower than that reported by Medley but far higher than that reported by Willems and Lownes. Although further work is needed, the available data indicate that sexual reproduction may at least sometimes play a significant role in the propagation of this orchid.

Reproductive success of *T. trianthophora* may be limited by predation. Keenan (1996) reported that an unspecified number of flowers and developing capsules were routinely lost to herbivores in New Hampshire. Willems (1994) found chipmunks and most particularly slugs to be serious predators of young plants in Massachusetts. Rapid seed maturation, usually occurring within 7 days after fertilization (Pridgeon et al. 2005), may reduce the loss to predators. In addition, development, logging, and other alterations of the habitat pose a threat. The small size of the plants and many of the populations also render them susceptible to stochastic events and inadvertent disturbances (Ramstetter 2001).

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Chapter 5
Tribe Malaxideae

Abstract Fungus gnats are the primary pollinators of *Malaxis paludosa* with the pollinia attaching on the ventral–anterior part of the thorax behind the mouthparts. Fruit set is substantial. *Liparis liliifolia* is self-incompatible, and fruit set is significantly higher when widely separated individuals are crossed. The pollinators remain unknown. *L. loeselii* is self-compatible and autogamous but not agamospermous. Autogamy is facilitated by rain, which on striking the anther cap propels the pollinia onto the surface of the stigma. Populations are short lived, expanding or disappearing with changing conditions, and are maintained primarily by seedling recruitment.

Keywords *Malaxis* • *Liparis* • Rain-assisted autogamy • Ventral thoracic viscidia attachment • Self-incompatibility • Seedling recruitment • Fungus and gall gnats • Cecidomyiid gall midge

The tribe Malaxideae includes 13 genera with a broad distribution in both hemispheres, especially in the tropics and subtropics (Pridgeon et al. 2005). Two genera occur in North America north of Mexico.

Malaxis Solander ex Swartz

Malaxis is a genus of about 300 primarily tropical and subtropical species found in both the Old and New World. A few temperate species are distributed in Europe and Asia as well as the Americas. Eight to ten species occur in North America north of Mexico, but the pollination biology of only one has been examined in any detail.

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Table 5.1 Data on *Malaxis* (Catling and Magrath 2002)

Character	*Malaxis paludosa*
Plant height (cm)	3–23
Raceme length (cm)	0.5–9
Flower number	2–55
Dorsal sepal (mm)	(1.6)2–2.5 × 1–1.6
Lateral sepals (mm)	(1.6)2–2.5 × 1–1.6
Lateral petals (mm)	1.4–1.9 × 0.5–1
Lip (mm)	1.2–1.8 × 0.7–1
Column (mm)	(0.3)0.5–0.7 × (0.3)0.5–0.7
Chromosomes (2*n*)	28

Malaxis paludosa (L.) Swartz (Bog Adder's-Mouth) (= Hammarbya paludosa (L.) Kuntze)

Habitat and Range

M. paludosa is usually found in open, sunlit black spruce (*Picea mariana* (Mill.) Britton, Stems, and Poggenburg) bogs, or swamps on hummocks of *Sphagnum* L. or occasionally *Mnium* L. moss (W. R. Smith 1993). Reeves and Reeves (1984) consider it an epiphyte on mosses. Circumboreal in distribution, this small and inconspicuous plant has been collected at a few North American sites in Alaska, western Canada, Ontario, Manitoba, and Minnesota, but probably occurs undiscovered in numerous bogs in between (Catling and Magrath 2002).

Floral Morphology

A highly variable number of pinhead-sized, greenish-yellow flowers are evenly spaced in a terminal raceme (Table 5.1) (Luer 1975; Smith 1993). The nearly microscopic yellowish lip is striped with green and stands erect due to a 360° twist of the pedicel and ovary (Fig. 5.1a) (Luer 1975). It is more or less ovate with an acute or sometimes constricted apex giving rise to a small, acuminate tip (Luer 1975; Smith 1993). The lower margins clasp the column resulting in a tubular entrance to the flower (Fig. 5.1b). The lateral petals are recurved and about as long as the lip (Luer 1975). The column is short and erect (Table 5.1) (Luer 1975; Dressler 1993). Two pairs of waxy, leaf-like pollinia are produced in a terminal, two-celled anther (Fig. 5.1c, e). The anther opens while the flower is still in bud, and the anther membrane contracts toward the base of the column, exposing the pollinia (Darwin 1862). The pollinia remain cradled in the cup-like clinandrum and shriveled anther membrane at their base. The attenuate upper ends project slightly beyond the top of the rostellum, and as the bud opens they contact the posterior surface of a small drop of viscid matter or rostellar glue which projects slightly above the rostellum (Fig. 5.1d). The pollinia are bound together by the glue, which shrinks and becomes more viscid

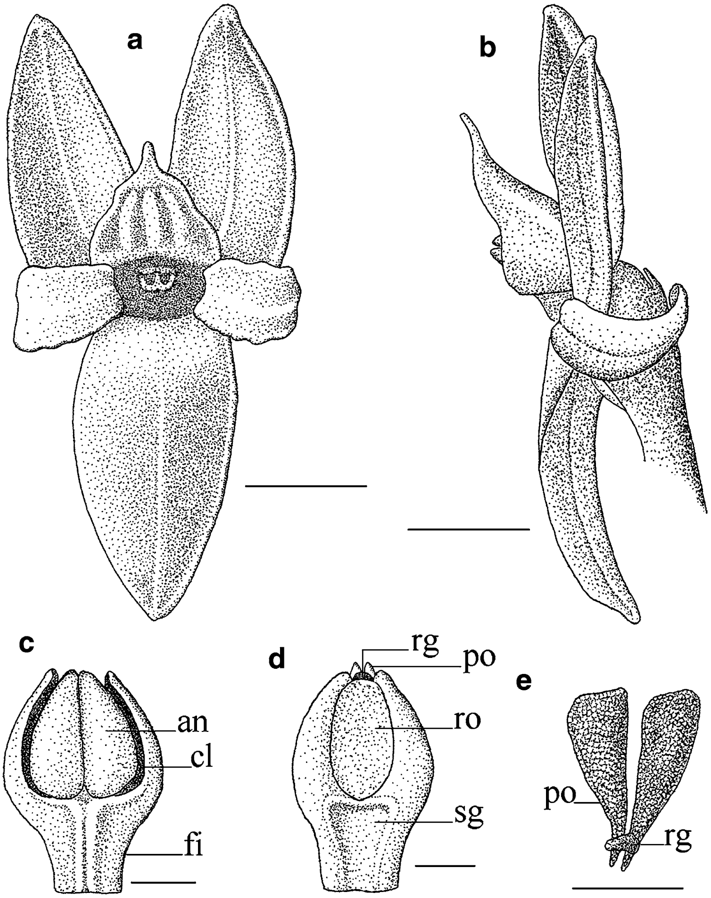

Fig. 5.1 *Malaxis paludosa*. (**a**) Flower, front view, scale bar = 1 mm; (**b**) flower, side view, scale bar = 1 mm; (**c**) column, back view, scale bar = 0.2 mm; (**d**) column, front view, scale bar = 0.2 mm; (**e**) pollinia (two shown) held together by drop of rostellar glue, scale bar = 0.2 mm. (**c**)–(**e**) Modified from Darwin (1862). *an* anther, *cl* clinandrum, *fi* filament, *po* pollinia, *ro* rostellum, *rg* rostellar glue, *sg* stigma

over time (Fig. 5.1e). Caudicles are absent. The stigma, located on the front half of the column at the base of the rostellum (Fig. 5.1d), is covered by a film of viscous fluid (Darwin 1862).

Flowering begins at the bottom of the inflorescence, and buds are produced from the apex throughout the flowering period. The first flowers to open remain fresh for the length of the blooming period, about 4–5 weeks, whereas late flowers may bloom for only a few days (Reeves and Reeves 1984). Only the lip wilts; the sepals and other petals on both the unfertilized flowers and developing fruit remain fresh and green into late August. The leaves of this tiny orchid are smaller than in other American species of *Malaxis*, and Reeves and Reeves (1984) suggest that the photosynthetic contribution of the enduring green perianth may be significant. Flowers of *M. unifolia* Michaux and *M. monophyllos* (L.) Swartz are not similarly persistent. A minute film of nectar, insufficient for analysis, is produced in an area at the base of the lip and column. The floral odor is sweet and cucumber-like and most detectable about 15 cm away from the plant. It is produced throughout the blooming period, although it is more noticeable early on. Foliar embryos may sprout from the margins and tips of the leaves to supplement seed reproduction and creeping and rooting annual growth (Taylor 1967).

Compatibility and Breeding System

Autogamy is known in a handful of *Malaxis* species scattered from the Western Hemisphere to Asia and the South Pacific (Ridley 1888; King and Pantling 1898; Schlechter 1911–1914; Smith 1928). However, in a study in Beltrami County, Minnesota, Reeves and Reeves (1984) reported that open pollinated plants of *M. paludosa* produced fruit, but plants bagged to exclude pollinators did not. The orchid, therefore, is probably neither autogamous nor apomictic. Catling (1983) also found no evidence for autogamy in a plant from the Queen Charlotte Islands. Rostellar development here was sufficient to effectively separate the pollinia and the stigma. In any case, the self-compatibility of this orchid has yet to be established. Self-incompatibility is known in at least one *Malaxis*, *M. massonii* (Ridl.) Kuntze, from Puerto Rico (Aragon and Ackerman 2001).

Pollinators and Pollination Mechanisms

Reeves and Reeves (1984) found pollinia attached to a single, 2.5-mm long, male fungus gnat, *Phronia digitata* Hackman (Diptera, Mycetophilidae). Attachment was at the ventral–anterior part of the thorax behind the mouthparts (Fig. 5.2). The insect, therefore, probably perched on the lowermost sepal and reached over the column to extract nectar from near the base of the lip and column with its proboscis. In the process, the area behind its mouthparts contacted the viscid droplet on the pollinia. If the column had been approached from the lip, the pollinia would have been attached elsewhere. In older flowers, the lip bends downward and the lowermost sepal upward, leaving entry from the lowermost sepal the only one possible (Reeves and Reeves 1984). When another flower is visited, the pollinia are likely to contact and adhere to the viscous fluid on the surface of the stigma. Reeves and Reeves (1984) observed pollinia in this position with emerging pollen tubes penetrating the stigmatic tissue. The pollinia were not inserted into a stigmatic pocket as reported by Darwin (1862).

Reeves and Reeves (1984) also observed a mosquito, *Aedes* sp. (Diptera, Culicidae), possibly probing for nectar near the base of the lip and column. No pollinia were found on mosquitoes, however, and these authors believe that they are too large to either contact the pollinia or bring about pollination. Five other species of dipterans and two hymenopterans were observed or collected on the plants but

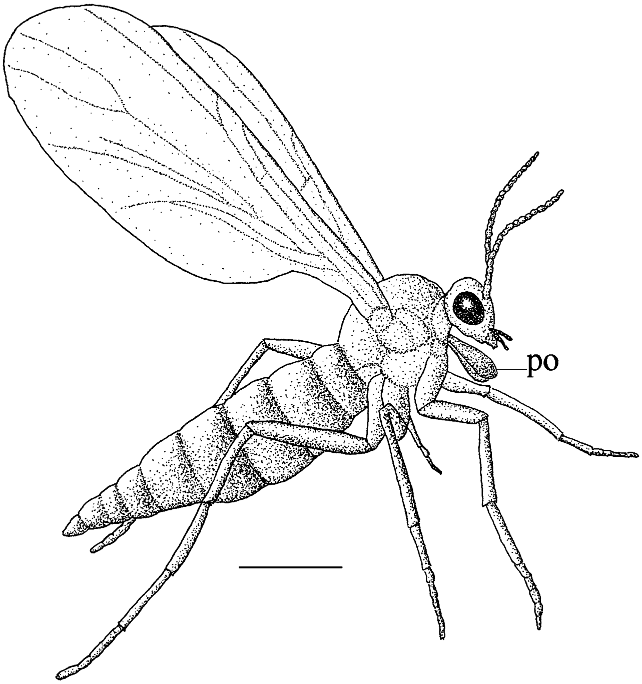

Fig. 5.2 Fungus gnat with pollinia of *Malaxis paludosa* attached to the anterior ventral thorax, scale bar = 0.3 mm. *po* pollinia

none carried pollinia. The smaller European version of *M. paludosa* is apparently pollinated by tiny bog flies (Davies et al. 1988).

Fruiting Success and Limiting Factors

Even though pollen vectors were only rarely observed, both Darwin (1862) and Reeves and Reeves (1984) reported that pollinia were removed from most flowers. Reeves and Reeves (1984) found that 20.5% of the flowers in their study area produced fruit in 1983 with a mean of 3.64 fruits per plant and a maximum of 7. In 1984, this average dropped to 2.17. The modal number for the 2 years was 4 and this number was produced by about 28% of the plants. In 1983, 64% of the plants produced 4–7 fruits compared to 22% in 1984 with the variation provisionally attributed to differences in rainfall. Although the sample was very limited, Darwin (1862) reported an even higher level of natural fruit set with 13 of 21 flowers (62%) on one spike producing capsules. The level of fruit production can therefore be substantial, although seed viability and seedling recruitment remain to be examined. By way of comparison, fruit set in a deceptive, obligately outcrossing, and reportedly pollinator limited, Puerto Rican species, *M. massonii* (Ridl.) Kuntze, was only 1.8% (Aragon and Ackerman 2001).

Predation, probably by rodents and insects, accounted for fruit losses ranging from about 34–39% over the 2 years of the Minnesota study (Reeves and Reeves 1984).

Additional Species of *Malaxis*

M. unifolia Michaux is sympatric with *M. paludosa* in Minnesota, Ontario, and Manitoba, and its resupinate flowers bloom at about the same time. In Minnesota, Reeves (in Christensen 1994) observed a cecidomyiid gall midge with a pollinium attached to its head crawling upward on the labellum toward the column of *M. uniflora*. The two species may, therefore, be pollinated by different insects carrying pollinia on different parts of their bodies. Other visitors to *M. unifolia* included species of *Bradysia* Winnertz (Sciaridae), *Aedes* (Culicidae), and *Trioxys* Haliday (Braconidae).

Based on flower size and phenology, Reeves (in Christensen 1994) believes that fungus and gall gnats may pollinate *M. soulei* L. O. Williams (now *M. macrostachya* (Lex.) Kuntz), *M. corymbosa* (S. Watson) Kuntze (now *M. brachystachys* (Rchb. f.) Kuntz), *M. ehrenbergii* (Rchb. f.) Kuntze, and *M. tenuis* (S. Watson) Ames (now *M. abieticola* Salazar and Soto Arenas) in southern Arizona. Similarly, Hapeman (1996) thinks that flower size and color as well as habitat indicate that fungus gnats probably pollinate the North American *M. monophyllos* var. *brachypoda* (Gray) Morris and Eames in Wisconsin. In an abstract, Reeves and Reeves (1985) noted the presence of insect pollinators as well as isolating mechanisms for this variety in Minnesota, but failed to identify the pollinators or provide any additional detail. Light (1998) reported that it produced 5-mm long, seed bearing capsules at an unspecified site in Canada. Tests in Ontario showed no evidence for autogamy in this orchid or in *M. unifolia*; both have well-developed rostella separating the stigma and pollinia (Catling 1983). However, the largely Old World *M. monophyllos* var. *monophyllos* (= var. *diphyllos* (Cham.) Luer), also found in Alaska and British Columbia, is reported to be mostly autogamous in Europe, although tiny flies may sometimes act as pollinators (Davies et al. 1988). So far as I am aware, nothing has been published on pollination of the three remaining North American species of *Malaxis*, *M. wendtii* Salazar, *M. bayardii* Fernald, and *M. spicata* Swartz, although again flower size and color imply the involvement of small flies, such as fungus gnats.

Liparis Richard

Liparis is a cosmopolitan genus embracing about 250 (Magrath 2002) to 320 (Pridgeon et al. 2005) species. Three occur in North America north of Mexico. One, *L. nervosa* (Thunberg ex Murray) Lindley is restricted to Florida. The other two, with very different breeding systems, are considered here.

Table 5.2 Data on *Liparis* (Magrath 2002a)

Character	*Liparis lilifolia*	*Liparis loeselii*
Plant height (cm)	9–25	6–26
Raceme length (cm)	4–15	
Flower number	5–31	2–15(19)
Dorsal sepal (mm)	8–11.5 × 1.2–2	4.5–6 × 1–2
Lateral sepals (mm)	8–11.5 × 1.2–2	4.5–5.5 × 1–2.2
Lateral petals (mm)	8.5–12 × 0.2–0.3	4.5–5.5 × 0.5–1
Lip (mm)	8–12 × 6–10	4–5.5 × 2.2–3.5
Column (mm)	3–4 × 1–1.5	2–3 × 0.5–1
Chromosomes (2*n*)		32, 36

Liparis liliifolia (L.) Richard ex Lindley (Lily-Leaved Twayblade or Large Twayblade)

Habitat and Distribution

L. liliifolia grows in mesic to moist open woods, often along streams and gullies. Frequently common in early to mid-successional stages or after logging and other disturbances, it is reportedly rare in the shade of mature forests (Curtis and Greene 1953; Sheviak 1974; Niemann 1986). It is distributed from southeastern Minnesota, southern Ontario, New Hampshire, and Massachusetts to northern Arkansas and the Carolinas with a disjunct population in southeastern Oklahoma (Magrath 2002).

Floral Morphology

Up to 31 small, resupinate flowers are borne in a loose raceme (Table 5.2) (Luer 1975). The sepals are light green; the dorsal is erect while the laterals are generally positioned beneath the lip (Fig. 5.3a) (Luer 1975; Magrath 2002). The petals are purple and pendant. Both petals and sepals have strongly revolute or involute margins (Fig. 5.3b). The lip is relatively wide (Table 5.2), recurved, and oblate with minutely erose margins. It is mauve and more or less translucent with darker reddish-purple veining (Luer 1975). The column is curved, slightly winged apically, and bears a pair of blunt tubercles on its inner surface near the base (Fig. 5.3a). Two pairs of waxy, yellow pollinia are contained within a two-celled, terminal anther (Magrath 2002). There are no caudicles (Pridgeon et al. 2005), and true viscidia are probably also absent. Nectar spurs are lacking, and although Whigham and O'Neill (1991) observed no nectar, they reported ants foraging on the lip, perhaps indicating that some nectar may be present here as in *Listera cordata* (Ackerman and Mesler 1979). Although purple flowers sometimes indicate a form of mimicry evolved to attract insects that feed on or deposit eggs in carrion, the flowers of *L. liliifolia* are reportedly odorless (Whigham and O'Neill 1991).

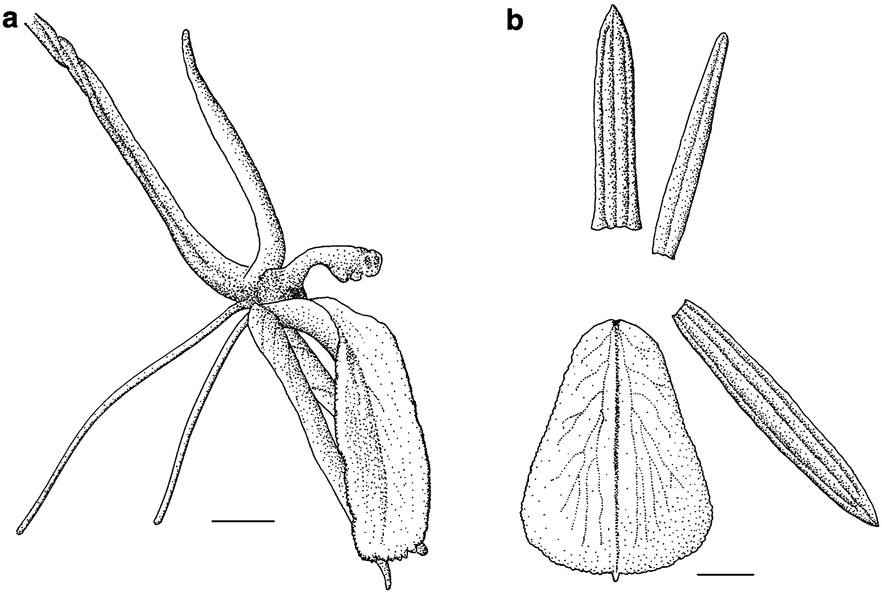

Fig. 5.3 *Liparis liliifolia.* (**a**) Flower, oblique view; (**b**) flower, exploded view with the sepals and petals flattened, scale bar = 2 mm

Compatibility and Breeding System

L. liliifolia is self-incompatible (Catling 1983; Gregg 1989). Moreover, fruiting success is positively related to the distance between parent plants. In a 5-year study at the Smithsonian Environmental Research Center, near Annapolis, Maryland, Whigham and O'Neill (1991) found that random hand pollination among caged plants growing within an area of about 400 m^2 produced fruit set in 29.4% of the flowers pollinated. Similar hand pollination between plants separated by a distance of about 1 km resulted in a fruit set of 62.8%. No fruit was set when pollen was exchanged between nearest neighbors presumably because of incompatibility among asexually generated individuals.

Pollinators and Pollination Mechanisms

The populations of *L. liliifolia* are small and scattered and might be pollinated casually by a number of unspecialized insects. However, pollination mechanisms have yet to be described and the pollinators remain unknown. Catling (1984) suggested that the long, arching column in this species (Fig. 5.3a) could imply a thoracic attachment of the pollinia or a pollinator with relatively long legs or mouthparts. Robertson (1929) reported species of *Hylemya* Robineau-Desvoidy (Anthomyiidae; Diptera) and *Lopidea* Uhler (*Lomatopleura* Reuter) (Mixidae; Hemiptera) as visitors but not as pollinators.

The pollination mechanism and a number of pollinators have been reported for other species. For example, Wallace (1974) studied the pollination of *L. reflexa* (RBr.) Lindley in southern Australia. The pollinator here was an unidentified dipteran in family Sarcophagidae. Attracted by an odor reminiscent of stale egg yolk, it landed on the labellum and crawled toward the base in search of nectar. A space between the two-winged column and curved labellum just accommodated the fly. As it withdrew from the flower, the top of its thorax contacted the rostellum and received a deposit of glutinous fluid. Continuing its withdrawal, it contacted and lifted the anther cap, and pollinia were discharged onto the deposit of sticky fluid. Franzen (1990) speculated that a similar pollinator might visit *L. furcata* (Hook f.) Ridley in West Malaysia. The latter has purplish flowers and a repulsive odor that might also attract members of the Sarcophagidae, which, as the name suggests, often deposit their eggs on decaying plant or animal material.

Different insect groups are probably involved in the pollination of other members of the genus, as already implied by the reported absence of a floral odor in *L. liliifolia.* Christensen (1994), for example, considered small gnats as potential pollinators of some *Liparis* species with long racemes and unpleasant smelling, tiny greenish flowers, and Peterson [in Christensen (1994)] found that *Culex* mosquitoes pollinated *L. viridiflora* (Blume) Lindley grown in a window at Aarhus University in Denmark. Darwin (1862) thought that the wings on either side of the entrance to the stigma in this and some other species might serve as a guide for the insertion of pollinia, but he observed no pollinators.

Reproductive Success and Limiting Factors

Once they reached a critical, minimal size, most plants at Whigham and O'Neill's (1991) Maryland site flowered regularly each year producing a more or less constant number of flowers per inflorescence. However, only 0.0–7.3% of open-pollinated flowers produced fruit. They attributed this very low level of fruit production to the combined effects of pollinator limitation; small, dispersed populations; and self-incompatibility. Asexual reproduction also occurs. Corms, loosely embedded at or near the surface of the litter layer, are able to reproduce by the production of offsets.

In addition to low levels of fruit production, limiting factors of particular significance in the northeastern USA, where this species is becoming scarce, include forest succession with increased shading; predation by herbivores, particularly deer; and competition from invasive plant species (e.g., Sheviak 1974).

Liparis loeselii (L.) Richard (Loesel's Twayblade or Fen Orchid)

Habitat and Distribution

Also found in Europe, *L. loeselii* is distributed throughout northeastern North America from eastern Nova Scotia to Saskatchewan and south to Iowa, Tennesse, and North Carolina. A number of more westerly and southerly disjunct populations are spread from Alabama to Washington and the Northwest Territories (Scoggan 1978; McMaster 2001; Magrath 2002). It is most commonly found in alkaline or circumneutral substrates in a wide range of habitats (Luer 1975; Catling 1980). These can include moist ravines, bogs, fens, wet meadows, forested wetlands, seeps, and dune slacks as well as dryer upland sites. Like the preceding species, it often shows a preference for early successional stages and disturbed areas (McLain 1968; Catling 1980; Thompson and MacGregor 1986).

Floral Morphology

Up to 19 very small, white to greenish, resupinate flowers are borne in a single loose raceme (Table 5.2) (McMaster 2001 and references therein). The sepals are spreading or directed forward, and the petals are pendant to spreading; both have revolute margins (Fig. 5.4a) (Luer 1975; Magrath 2002). The lip is obovate or suborbiculate to oblong and arcuate recurved with an obtuse to apiculate apex and crenulate-wavy margins. It is translucent to opaque, green to yellowish, and thickened down the center (Luer 1975; Magrath 2002). An incurved column bears wing-like projections on its upper part (Fig. 5.4e, f). Two pairs of waxy, yellow pollinia are contained within a two-celled terminal anther (Fig. 5.4f) (von Kirchner 1922; Luer 1975). A well-developed caudicle and viscidium are absent. The flowers produce no nectar and lack a perceptible odor (von Kirchner 1922).

Compatibility and Breeding System

L. loeselii is self-compatible and autogamous (von Kirchner 1922; Hagerup 1941; Catling 1980). In a study of plants from York County, Ontario, Catling (1980) established seven treatment groups. All were held in a large insect-proof enclosure in the greenhouse while in flower. Flowers in the first group were maintained as a control. In the second, they were emasculated, in the third they were vigorously agitated by "wind" from a strong fan, and in the fourth they were sprayed for 5 min with water from a watering can (fine spray) and hose (heavy spray) once every 2 days over the course of the 3-week flowering period. In the fifth, sixth, and seventh groups, the flowers were artificially fertilized by self-pollination, geitonogamous pollination, and cross-pollination, respectively.

No seed developed in emasculated flowers suggesting the absence of agamospermy. Artificial self-, geitonogamous-, and cross-pollinations produced seed in 94–100% of the ovaries tested compared to 17% in undisturbed (control) plants. Flowers agitated by "wind" did not differ from undisturbed plants in their levels of autogamous pollination. However, flowers receiving the simulated rain treatment (group 4) had quadruple the level of autogamy (viz. 70%) found in undisturbed plants watered from below (Catling 1980).

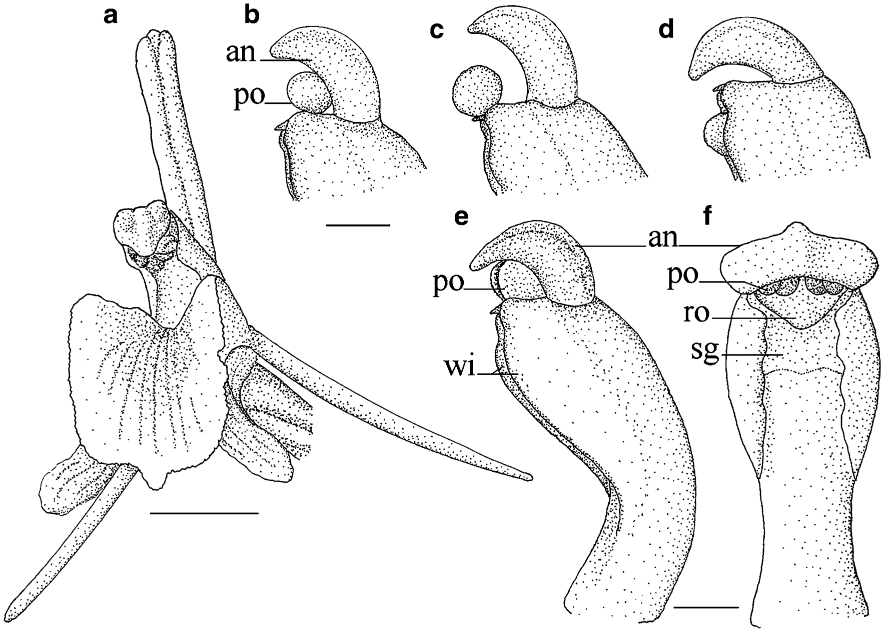

Fig. 5.4 *Liparis loeselii.* (**a**) Flower, slightly oblique view, scale bar = 2 mm; (**b**)–(**d**) movements leading to autogamy; (**b**) elevation of the anther cap (cf. "**e**"); (**c**) pollen masses (represented diagrammatically as a single unit) rotate out of the anther to lower margin of the rostellum; (**d**) quick downward movement of the anther cap pushes pollinia onto the stigmatic surface, scale bar = 0.5 mm; (**e**) column, side view; (**f**) column, front view, scale bar = 0.5 mm. *an* anther, *po* pollinium, *ro* rostellum, *sg* stigma, *wi* wing

Pollination Mechanism and Pollinators

Catling (1980) saw no insects visiting the flowers in York County despite 30 h of observation during the period of peak flowering. One to four days after anthesis the anther cap began to disintegrate and turn brown. Browning was accompanied by an elevation of the cap at its point of attachment on the top of the column probably due to a contraction of dead tissue (Fig. 5.4b). Its elevation released the pollen masses, permitting them to rotate or slide out of the anther compartments to the lower margin of the rostellum, where a small ridge separated the upper surface of the column from the stigma (Fig. 5.4c). A small deposit of adhesive material usually held the pollinia in this position, but in 17% of the flowers rotation onto the stigmatic surface occurred directly. If water droplets from an eyedropper were made to fall on the anther cap, the cap was forced quickly downward, pushing the pollinia around the edge of the margin onto the stigmatic surface (Fig. 5.4d). The pollen masses were also pulled onto the stigma by cohesive forces as the water droplets were slowly reduced in size by evaporation or as they ran rapidly over the flower surface.

In most cases, however, the rapid depression of the anther cap was sufficient to explain the movement of the pollinia onto the stigmatic surface.

Catling (1980) observed a similar mechanism in additional plants from York and Simcoe Counties, Ontario, and from Cattaraugus County, New York, and Adams County, Wisconsin. Here again, elevation of the anther cap led to a descent and rotation of the pollen masses. Placed in insect-proof cages and watered from below, these orchids showed levels of autogamy varying from 15 to 50%. Higher levels were once more observed in a small sample treated with a simulated rain spray.

Catling's (1980) results are consistent with earlier observations by von Kirchner (1922) on European plants of *L. loeselii*. Kirchner noted that the pollen masses slipped out of the anther onto the column and the stigmatic surface without the participation of a pollinator, but failed to note the role played by the elevation of the hinged anther cap. Hagerup (1941), on the other hand, reported that the downward movement of the anther cap expelled the pollen directly onto the stigmatic surface, but considered that this movement occurred without outside influence. Neither von Kirchner (1922) nor Hagerup (1941) associated the movement of the anther cap or pollen masses with rain.

In other European studies, Reinhard et al. (1991) considered autogamy to be obligatory, but Zeigenspeck and Kirchner (in Fuller 1966) mentioned mosquito pollination as a possible supplement to regular self-fertilization.

Reproductive Success and Limiting Factors

McMaster (2001), in Franklin County, Massachusetts; Wheeler et al. (1998), in East Anglia (UK); and Jones (1998), in South Wales, reported high mortality rates in populations of *L. loeselii*. The populations were short lived, expanding or disappearing with changing conditions, and were maintained primarily by seedling recruitment.

The level of seed production may, therefore, be critical. McMaster found that 51–77% of the flowers at his study site produced capsules over 7 years. These numbers correspond well with von Kirchner's (1922) report of self-pollination and capsule development in 72 of 102 (ca. 71%) flowers on 20 plants. Seed count per capsule averaged 4,270 and ranged from 1,601 to 11,748 for five capsules examined during 1 year at McMaster's site. Extrapolation to the 551 capsules counted that year gives a total estimate of over 2 million seeds produced in a 7 × 3-m area. Ramsey (in McMaster 2001) determined that about 81% of the seeds were viable in one natural population in England. Provided, then, that suitable conditions are available for the establishment and development of new individuals, prolific seed production, abundantly augmented by rain-assisted autogamy, may account for the continued survival of *L. loeselii* populations despite short life spans for individual plants.

McMaster (2001) reported that well-watered, fertilized, and cultivated transplants in partially shaded beds were significantly larger and produced many more flowers than the wild populations, suggesting that drought, temperature, herbivory, and/or resources might be limiting under natural conditions. Resources, however, were apparently not critical. In a 6-year study of wild populations, he found that fruit-bearing plants were more likely to survive, bear flowers, and produce fruit the following year than nonfruiting plants.

The quantity of stored resources needed for flowering might, in fact, be reduced in orchids such as *L. loeselii* that produce green, long-lived flowers potentially capable of making a photosynthetic contribution to the plant (McMaster 2001). Moreover, the small, nectarless flowers are probably relatively inexpensive to produce (Hagerup 1952; Neiland and Wilcock 1998).

McMaster (2001) recorded herbivore damage on 10% to nearly 60% of the plants at his Massachusetts study site over 6 years. Beyond the immediate effects of predation, grazed plants showed significant reductions in survival, flowering, and fruiting in subsequent years, suggesting that the long-term energy budget of *L. loeselii* is more significantly affected by herbivory than by flowering and fruiting. Similarly, Wheeler et al. (1998) reported that only 3% of plants in eastern England damaged by grazing produced flowers the following year compared to 22% for the entire population.

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Chapter 6
Tribe Calypsoeae

Abstract *Calypso bulbosa* is self-compatible but not autogamous. Outcrossing is predominant, and bumblebees are the primary pollinators. The flowers provide no reward and are pollinated by deceit. Fruit production over the lifetime of the plant is pollen limited. Natural populations of *Tipularia discolor* are maintained primarily by vegetative reproduction, but experimental work supports the occurrence of cross-pollination. The pollinators are nocturnal or crepuscular noctuid moths. Fruit set is limited by pollinator service. A variety of breeding systems are present in *Corallorhiza*, including cleistogamy, autogamy, outcrossing, and facultative autogamy. Known pollinators include species of *Empis*, *Andrena*, and *Pimpla pedalis*. Seed set in outcrossing taxa appears to be pollinator limited.

Keywords *Calypso* • *Tipularia* • *Corallorhiza* • *Aplectrum* • Variable breeding systems • Interpopulation gene flow • Deceit pollination and pollen limitation • Nocturnal/crepuscular moths • Dance flies and hymenoptera

The tribe Calypsoeae includes 12 genera widely distributed in Europe, northern Asia, North America, Central America, the Caribbean, and South America (Pridgeon et al. 2005). Four genera occur in our flora.

Calypso Salisbury

Calypso is a monotypic, circumboreal species.

C.L. Argue, *The Pollination Biology of North American Orchids: Volume 2: North of Florida and Mexico*, DOI 10.1007/978-1-4614-0622-8_6,

Table 6.1 Data on *Calypso* (Sheviak and Catling 2002)

Character	*Calypso bulbosa*
Plant height (cm)	4.5–22
Flower number	Usually 1
Dorsal sepal (mm)	10–24 × 1.5–5
Lateral sepals (mm)	10–24 × 1.5–5
Lateral petals (mm)	10–24 × 1.5–5
Lip (mm)	13–23 × 4–13
Chromosomes (2*n*)	28

Calypso bulbosa (L.) Oakes (Calypso, Fairy Slipper)

Habitat and Distribution

Two North American varieties of this wintergreen orchid are known. Variety *americana* (R. Brown) Luer occurs in wet coniferous forests, mixed forests, and bogs from Alaska to Newfoundland and south to Arizona, New Mexico, Minnesota, Michigan, and New Hampshire (Sheviak and Catling 2002). Variety *occidentalis* (Holzinger) B. Boivin is found in moist to dry, shady coniferous forests from northwestern British Colombia to California and western Montana.

Floral Morphology

A usually single, slightly pendant to horizontal, resupinate flower is borne terminally on a stalk of varying length (Table 6.1) (Luer 1975; Sheviak and Catling 2002). Sepals and petals are ascending to erect and loosely twisted (Fig. 6.1a). Both are usually colored pink or magenta. The lip is deeply saccate, producing a slipper with a basal orifice, a rounded heel, and a two-spurred toe or apex (Fig. 6.1a) (Luer 1975; Sheviak and Catling 2002). The margin of the lip is everted and dilated distally to form a broad, apron-like lamella. In variety a*mericana*, the lamella is white or washed with pink and bears a conspicuous cluster of contrasting yellow bristles at its base. In variety *occidentalis*, it is a dull white with reddish mottling, and the basal bristles are sparse, whitish, and inconspicuous (Luer 1975; Sheviak and Catling 2002). The inside of the slipper in both is marked by fine streaks and spots of rich purple leading down into the two horn-like spurs under the lip apex (Kipping 1971; Stoutamire 1971; Luer 1975). Contrary to many reports, Kipping (1971) did not say nectar was present in variety *occidentalis*, and Krell (1977) and Ackerman (1981) state specifically that it is absent. Variety *americana* is also nectarless according to Mosquin (1970), Stoutamire (1971), and Boyden (1982), and the double nectar spurs in both varieties are deceptive devices.

The column is rose pink, broadly winged, nearly circular, and convex, forming a petaloid hood over the lip just above the entrance to the flower (Fig. 6.1a, d) (Mosquin 1970; Ackerman 1981; Boyden 1982). An operculate, subterminal anther is positioned on the lower surface and contains two pairs of flattened and superposed,

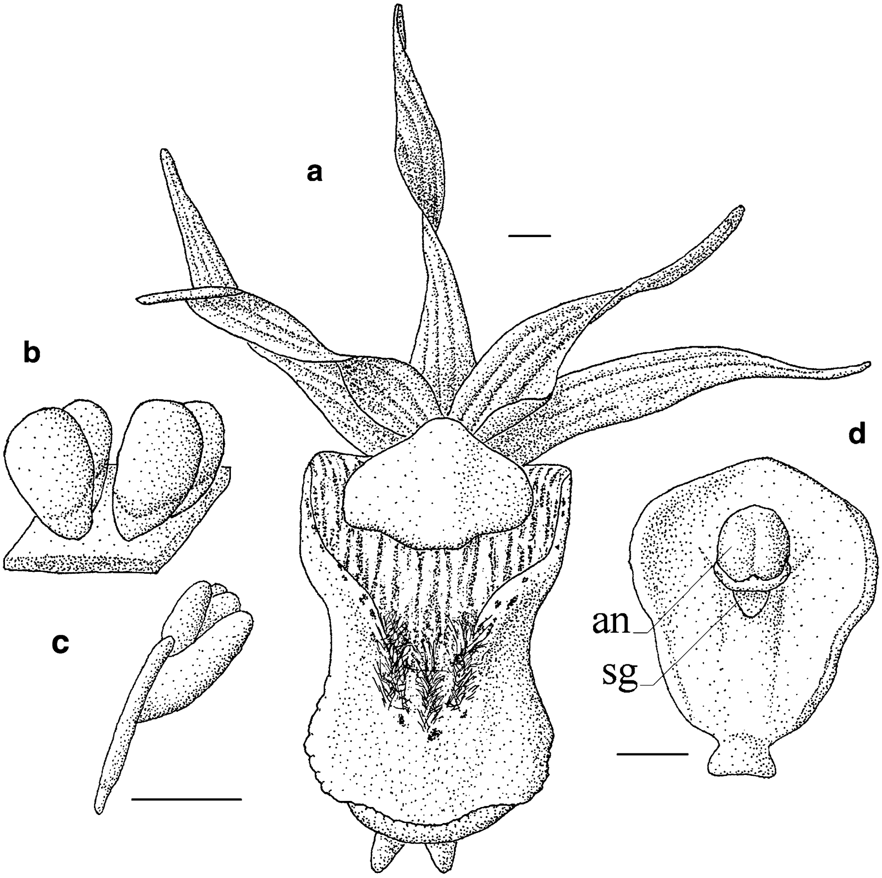

Fig. 6.1 *Calypso bulbosa*. (**a**) Flower, front view, scale bar = 2 mm; (**b**) Pollinarium, oblique view; (**c**) Pollinarium, side view, scale bar (**b**, **c**) = 1 mm; (**d**) Column, ventral view, scale bar = 2 mm. *an* anther, *sg* stigmatic area

waxy pollinia borne on short tegula in a single pollinarium (Fig. 6.1b, c) (Luer 1975; Freudenstein 1994a; Sheviak and Catling 2002; Pridgeon et al. 2005). The viscidium is large and quadrangular (Sheviak and Catling 2002). A concave stigma is located just behind the anther and is separated from it by a small ridge (Fig. 6.1d) (Mosquin 1970; Boyden 1982).

Unpollinated flowers of variety *americana* in Alberta (Mosquin 1970; Proctor and Harder 1995), Idaho (Lehmberg 2002), and Maine (Nylander 1922, 1935) may remain open for 1–3 weeks. Flowers of variety *occidentalis* in California persist in good condition for up to 3 or 4 weeks (Kipping 1971; Ackerman, personal communication in Krell 1977). Senescence involves a change in flower color and orientation as well as a change in the relative position of the column, sepals, and petals.

Krell (1977), studying variety *occidentalis*, and Boyden (1982), variety *americana*, found floral odor faded or even changed in character as the flowers aged. This may explain conflicting reports in the literature, where the odor of both varieties has been variously described as faint or strong, sweet and rose-like (variety *occidentalis*) or not rose-like (both varieties) or completely absent (Bradshaw 1919; Mousley 1924; Kipping 1971; Stoutamire 1971; Luer 1975; Krell 1977; Boyden 1982).

Vegetative reproduction and clonal growth are far more common in variety a*mericana* than in variety *occidentalis* (Mousley 1924, 1925; Krell 1977). Krell (1977) found that patches of variety *occidentalis* in northwestern Idaho usually contained three to nine plants in an area of one square meter or less. Such patches only occurred in densely shaded areas, where there was little interspecific competition. In areas that received more light, groups consisted of no more than two or three plants, and these were much more widely spaced. This contrasts with reports of large, dense clones of variety a*mericana* (Mousley 1925; Mosquin 1970). Terrill (in Mousley 1925), for example, reported up to 50 plants in a 1 m^2 area.

Compatibility and Breeding System

Both varieties are self-compatible but not autogamous. Ackerman (1981) examined the breeding system of variety *occidentalis* in Humboldt County, California. He obtained 100% fruit set and 81.6% viable seed in artificially self-pollinated, caged plants. Unmanipulated, caged plants failed to set any fruit. Artificially self-pollinated flowers in Marin County, California (Kipping 1971), and northwestern Idaho (Krell 1977) also developed fruit. In a study of variety *americana* at Banff National Park, Alberta, Mosquin (1970) found that self-pollinated plants, bagged to exclude insects, produced fruit and abundant seed, while unmanipulated plants, so treated, did not. Although the pollinaria are easily removed, in the absence of a visitor they remain in position until the flower withers; the relative positions of the anther and stigmatic surface are thought to minimize the chances of self-pollination.

Pollinators and Pollination Mechanisms

Insects captured bearing pollinaria or viscidia of *C. bulbosa* in North America are listed in Table 6.2 along with references and collection sites. All were bumblebees, mostly *Bombus* (including *Psithyrus*) queens, and the flowering of *Calypso* is coincident with their emergence (e.g., Boyden 1982). The flowers usually bloom before the workers appear in large numbers, and in many cases only queens are large enough to act as effective pollinators (Mosquin 1970; Ackerman 1981; Boyden 1982). However, due to variation in the size of the lip opening, larger workers sometimes transfer pollen, particularly in variety *americana* (Table 6.2) (Mosquin 1970, Thorp, personal communication in Ackerman 1981; Boyden 1982). The varieties are not isolated from one another by specialized or exclusive pollinators, and although purportedly allopatric, it is likely that some populations overlap in northwestern North America (Sheviak and Catling 2002). Varietal intercompatibility is likely but has apparently not yet been tested experimentally.

Table 6.2 Species of bumblebees with attached pollinaria of *Calypso bulbosa*

Pollinators	Caste[a]/variety[b]	Study site	Source
Bombus bifarius Cresson	Q/a, u	?	Krell (1977)
B. bifarius nearcticus Handlirsch	Q (W)/a	AB, NWT, BC, WA	Mosquin (1970)
	Q/o	ID	Krell (1977)
	Q/a	AB	Boyden (1982)
B. californicus F. Smith	Q/a	AB	Mosquin (1970)
B. caliginosus (Frison)	Q/o	CA	Ackerman (1981)
B. centralis Cresson	Q/u	BC, WA	Mosquin (1970)
	Q/o	?	Ackerman (1981)
B. frigidus Smith[c]	Q/u	QB	Mosquin (1970)
B. melanopygus Nylander[d]	Q/o	CA	Ackerman (1981)
B. flavifrons Cresson	Q/o	ID	Krell (1977)
	Q/a	?	Krell (1977)
B. frigidus Smith	Q, W/a	BC, QB, AB	Mosquin (1970)
B. lucorum moderatus Cresson	Q/u	AB	Mosquin (1970)
	Q/a	?	Krell (1977)
B. melanopygus Nylander	Q/o	AB	Ackerman (1981)
	Q/a	AB	Boyden (1982)
B. mixtus Cresson	Q, W/a	BC, AB	Mosquin (1970)
	Q/a	CA	Ackermann (1981)
B. occidentalis Greene	Q/a	AB	Mosquin (1970)
	Q/a	AB	Boyden (1982)
B. pleuralis Nylander	Q (W)/a	NWT, BC, WA, AB	Mosquin (1970)
	Q/o	?	Krell (1977)
B. rufocinctus Cresson	Q/o	?	Ackerman (1981)
B. sitkensis Nylander	Q/u	?	Krell (1977)
B. ternarias Say	Q/a	?	Krell (1977)
	Q/u	?	Krell (1977)
B. vagans Smith	Q/a	?	Krell (1977)
B. vosnesenskii Radoszkowski	Q/o	CA	Kipping (1971)
B. insularis (Smith)[e]	F/o	CA	Ackerman (1981)
B. fernaldae (Franklin)[f]	Q/a	?	Krell (1977)
B. insularis (Smith)[g]	F/a	BC, AB	Mosquin (1970)
	Q/a Q/u	?	Krell (1977)

[a] Caste of bumblebee: *Q* queen, *W* worker, *F* designated simply as "female," caste unidentified but probably workers rather than queens. Parentheses = less common

[b] Variety of *C. bulbosa*: a = *americana*, o = *occidentalis*, u = unknown

[c] As *Bombus couperi* Cresson

[d] As *Bombus edwardsii* Cresson

[e] As *Psithyrus crawfordii* Franklin

[f] As *Psithyrus fernaldae* (Franklin)

[g] As *Psithyrus insularis* (Smith)

C. bulbosa produces no floral rewards and is pollinated by deceit. It is a generalized food–flower mimic with floral characteristics that Heinrich (1979) described as typical of the bumblebee-pollinated food–flower syndrome. Pollen transport is apparently dependent on exploratory sampling visits of newly emerged, naïve, queen bumblebees, and pollination rates are positively correlated with bumblebee abundance (Mosquin 1970; Stoutamire 1971; Luer 1975; Wollin 1975; Krell 1977; Ackerman 1981). Boyden (1982) in a study near Banff, Alberta, found that 57% of 168 captured bumblebees carried at least one pollinarium or viscidium of variety *americana* while 43% carried none. Thus, over half of newly emerged bees apparently made an exploratory visit to the flowers of *Calypso*. They visited flowers more or less indiscriminately on their first foraging trips, but as indicated by a low pollination rate (see below), they soon learned, usually by the second to sixth trips, to select only species with the most rewarding flowers.

Boyden's data support Mosquin (1970), Stoutamire (1971), and Ackerman (1981) in their view that naïve bumblebee queens quickly learn to avoid the flowers of *Calypso* after visiting them only a few times. Bumblebee queens in temperate climates live only 1 year (Heinrich 1979), and selection may favor pollinators that quickly learn to recognize unrewarding flowers. Although pollination levels are low, a compensatory effect in this and other unrewarding orchids, possibly related to the evolution of deception, is the production of an enormous number of seeds in those few plants that are successfully pollinated.

Mimicry in various forms has been invoked as a possible strategy for luring pollinators to unrewarding flowers. Vogel (1978) suggested a role for deceptive structures that simulate the presence of pollen. In *Calopogon*, *Pogonia*, *Arethusa*, and other genera yellow hairs are present on the labella that appear to imitate the appearance of anthers and pollen (e.g., Stoutamire 1971; Thien and Marcks 1972; see below). The yellow hairs on the labellum of variety *americana*, also present in the Old World variety *bulbosa* but absent in variety *occidentalis*, may likewise function as pollen dummies to attract pollinators by deception (Boyden 1982). Comparative studies of UV absorption or visitation rates for the flowers of varieties *americana* and *occidentalis* are not yet available.

Mosquin (1970), in his study of variety *americana* in Alberta, suggested that the flowers might mimic those of *Dodecatheon radicatum* Greene, a plant with similarly colored flowers offering a rich pollen reward. He captured bees carrying pollinaria of *C. bulbosa* var. *americana* on the flowers of this plant. However, since he found flowers of *C. bulbosa* to be very plentiful and those of *D. radicatum* to be relatively uncommon, the latter would seem to be a poor model for a mimic based on relative abundance alone. Moreover, Boyden (1982), studying plants in the same area, found no significant difference in the number of pollinaria or viscidia of *C. bulbosa* var. *americana* on bumblebees visiting flowers of *D. radicatum* versus those captured on flowers of other species or flying free. Boyden's observations therefore fail to support Mosquin's (1970) mimicry hypothesis. The bees appear to be well capable of distinguishing between the flowers of these two species. In addition to a difference in floral odor, pollen is extracted from *Dodecatheon* flowers by buzz pollination, an entirely different type of bee behavior than exhibited by bees during visits to *Calypso* (Boyden 1982). In like manner, Ackerman (1981) found no evidence that variety *occidentalis* mimics other spring-blooming plants in northern California.

Some investigators have hypothesized that the number of pollinator visits to unrewarding plant species should be negatively correlated with population size (Stoutamire 1971) and positively correlated with the local frequency of rewarding plant species (Laverty and Plowright 1988; Laverty 1992). Thus, for example, the frequency of encounters between bees and flowers might be relatively high in large, dense populations resulting in a reduction in the time required for the bees to learn that the flowers offer no reward. At the same time, a local abundance of food plants might serve to attract pollinators, some of which might visit unrewarding flowers by mistake, the so-called magnet species effect (e.g., Dafni 1983).

Data on the influence of population parameters and the magnet species effect in *Calypso* are inconsistent and often contradictory. Alexandersson and Agren (1996), for example, found the hypothetical relationship to be true only in 1 year of a 3-year study on variety *bulbosa* in northern Sweden. In the other 2 years, pollen removal was neither significantly related to population size nor the local frequency of simultaneously flowering plants with richly rewarding flowers. In addition, the proportion of plants setting fruit was not significantly correlated with either variable in any year of the study. Similarly, Ackerman (1981) found that percent fruit set was largely independent of population size in northern California populations of variety *occidentalis*, even though larger populations produced more fruits, and some reduction of percent fruit set was observed in very small or very large populations.

Boyden (1982) found no relationship between population density and pollination rates in variety *americana* near Banff, where large, dense clones and thousands of flowers were spread over a wide area (Mosquin 1970). Krell (1977), on the other hand, observed that flowers of variety *occidentalis* were pollinated less frequently in northwestern Idaho when they occurred in dense patches than when they were more widespread.

Mosquin (1971) reported that flowering of variety *americana* in the Banff area extended from late May through the third week of June. Relatively few entomophilous plant species were in bloom in May, and insects were forced to compete for the relatively scarce nectar and pollen resources available. Later, plants, such as *Salix* and *Taraxicum officinale* L. that offered a nearly unlimited amount of nectar and pollen, came into bloom, and plants were then competing for pollinators. Under these circumstances, any magnet species effect resulting from the attraction of additional pollinators would almost surely be outweighed by the increased competition for their services.

Ackerman (1981) reported one to four pollinia present on each pollinated stigma of variety *occidentalis*. Thus, the four pollinia removed as a unit (Fig. 6.1b) are capable of separating. Although capsule set was unaffected, experimental crosses revealed a significant difference in seed production in flowers pollinated by one-half of a pollinium compared to those that received two pollinia. Proctor and Harder (1994) believe that two pollinia of variety *americana* are sufficient to fertilize most of the ovules contained in a single flower. They suggest, therefore, that the size of the pollinia rather than the size of the pollinarium was selected to correspond to the number of ovules present in each ovary and that the full complement of pollinia in each pollinarium is utilized in the complete pollination of several flowers.

In a study in the Rocky Mountain foothills of Alberta, Proctor and Harder (1995) noted that flowers receiving any amount of pollen senesced within 4 days, whereas those that were not pollinated or only had pollinaria removed remained in good condition for 8–11 days. The authors hypothesized that selection favors the onset of senescence following completion of the female function because it is the less easily satisfied. If removal of the pollinarium is significantly more likely to occur than its deposition on the stigma, senescence after deposition will probably have been preceded by at least some pollen removal. They reasoned that in deceptive flowers fulfillment of the male function was more likely to occur because it only requires fooling the pollinator one time, whereas satisfaction of the female function requires that the pollinator be fooled twice. Thus, senescence of the flower is triggered by completion of the function less likely to succeed.

The fact that the onset of senescence in *Calypso* is independent of pollen load may have further implications. We shall see below that the rate of flower senescence in *Cleistesiopsis bifaria* is associated with the amount of pollen received (Gregg 1989, 1991). Proctor and Harder (1994) believe that this relates to the fact that pollen dumped on the pollinator of *Cleistesiopsis* is granular, and loads could be received that contained far fewer tetrads than needed to fertilize the large number of ovules in the ovary. In *Calypso*, on the other hand, a minimum of one pollinium is deposited, and each pollinium is capable of fertilizing about 11,000 ovules (Proctor and Harder 1994). If the deposition of one pollinium produces a satisfactory number of seeds, selection for a more precise correlation between pollen load and floral senescence might be relatively low.

Kipping (1971), Stoutamire (1971), and Gumprecht (1977) agree generally on the pollination process. The foraging bee lands on the labellum and forces its head and thorax beneath the column (Fig. 6.1a). Discovering that no nectar is available in the lip, it backs out of the flower. As it withdraws, the pollinarium is attached to the hairless area at the rear of the dorsal thorax (Fig. 6.2). The placement is such that the bee has difficulty removing it, and it may remain in place for many days (Ackerman 1981; Boyden 1982). According to Ackerman (1981), attachment here requires that the bee penetrate deeply into the flower and back out with its body arched to bring the edge of the scutellum into contact with the viscidium. Moreover, the size of the bee is important; it must be able to enter beneath the winged column, and the latter must fit closely over the thorax. The stigma, viscid when receptive, is proximal to the anther (Fig. 6.1d). The withdrawing bee is, therefore, likely to deposit pollen previously attached to its thorax before it contacts and removes the pollinarium.

The mechanics of pollination clearly identify outcrossing as the predominant mode of pollen transfer. Besides, the plants produce only one flower each year, and bees do not usually reenter the same flower (Ackerman 1981). Kipping (1971) also reported that the extracted pollinarium retains its anther cap for several minutes, a feature that might prevent self-pollination should reentry occur. Geitonogamous pollination is unlikely in variety *occidentalis* because, as already noted, vegetative reproduction here is evidently rare. Ackerman (1981), for example, reported that it did not occur at all at his study sites in Humboldt County, California. Geitonogamy

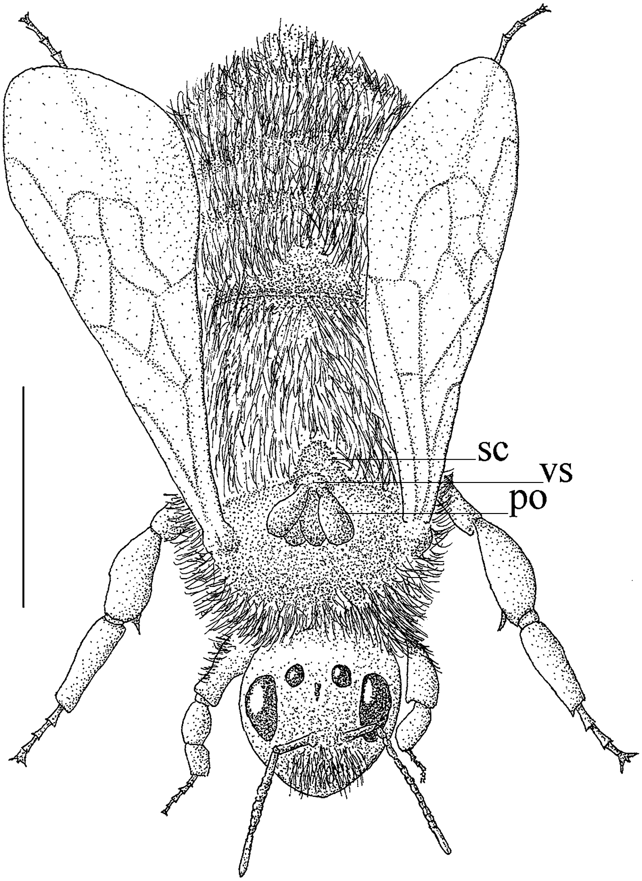

Fig. 6.2 Bumblebee with pollinarium of *Calypso bulbosa* attached to the hairless area at the rear of its dorsal thorax, scale bar = 5 mm. *po* pollinium, *sc* scutellum, *vs* viscidium

is more likely in some populations of variety a*mericana* in conjunction with the higher frequency of clonal growth noted for this taxon (Mousley 1925; Mosquin 1970). Clonal growth is also common in the Eurasian variety *bulbosa* (Wollin 1975), where Alexandersson and Agren (2000) reported high rates of self-pollination in addition to important levels of pollen flow among populations.

Fruiting Success and Limiting Factors

The ability of the bee to recognize flowers of *Calypso* as unrewarding is reflected in the levels of pollinator visitation. These levels can be estimated by the rates of pollinaria removal or displacement, pollinia deposition, and fruit set. Boyden (1982) found that only 101 (12%) of 843 examined flowers of variety *americana* in the

Banff area were visited, and only ten (1%) were pollinated. These results correspond closely to the levels that Mosquin (1970) reported for the same area. He found that 200 (12%) of 1,654 examined flowers had pollinaria missing, disturbed, or deposited on the stigma. Proctor and Harder (1995) obtained somewhat different results in a study of this variety from the Rocky Mountain foothills of Alberta. Here, 212 (41%) of 520 examined flowers were visited, 72 (14%) had only pollinaria removed, 11 (2%) had only pollinia deposited, and 129 (25%) had pollinia both removed and deposited. In Idaho, Lehmberg (2002) found pollinia removed from 14 (56%) of 25 plants. In variety *occidentalis*, Ackerman (1981) reported that 671 (53%) of 1,273 flowers at one site in Humboldt County were visited and 141 (11%) were pollinated, and Krell (1977) observed that 117 (41%) of 287 flowers were visited in northwestern Idaho and 63 (22%) were pollinated. At the latter site, 9% of the total number of flowers produced fruits as compared to 11–34% over five sites in Humboldt County (Ackerman 1981) and 34% in Marin County, California (Kipping 1971). Curiously, there is very little comparable fruiting data for variety *americana*, although Mousley (1924) reported that 6% or 3 of 50 plants developed capsules near Hartley, Quebec, and Lehmberg (2002) found enlarged ovaries in 8% or 2 of 25 plants in Idaho. Average fruit set in variety *bulbosa* from northern Sweden ranged from 21 to 48%, relatively high compared to the North American varieties, a difference that Alexandersson and Agren (1996) believe may be at least partly related to differences in when and how the data were collected.

In addition to quickly acquired ability of the bee to recognize unrewarding flowers, the levels of male and female success are reduced because pollinaria are not always removed from the flower or transported pollinia deposited on the stigma when bees do visit the flowers (Ackerman 1981). This is true despite the adaptation of the flower for a specific type of pollinator. Boyden (1982), for example, found that only 2 out of 60 bees that carried something other than just one pollinarium in the Banff area carried one pollinarium and one viscidium, a ratio that would reflect the possibility of a highly efficient pollen transfer mechanism. Forty-four bees bore only viscida. If it is assumed that the pollinia, once acquired, were removed during ensuing flower visits, all 44 of these bees deposited pollinia without removing a second pollinarium.

Moreover, as already suggested by the ratio of pollinaria removed to pollinia deposited, Boyden (1982) found that once acquired the transfer of pollinia from bee to flower is not highly efficient. A dozen bees bore two intact pollinaria indicating visits to at least one flower with no pollinia deposition. Two bees bore three intact pollinaria. Mosquin (1970), Ackerman (in Krell 1977), and Krell (1977) also observed bees with more than one pollinarium attached to their thorax. Kipping (1971) captured specimens of *Bombus vosnesenskii* with attached pollinaria at his study site in Marin County and placed them in a terrarium with newly opened flowers of variety *occidentalis*. The bees successfully extracted new pollinaria, but the original pollinia were not removed by the stigmas. He suggested that the stigmas might not be very receptive prior to the removal of the pollinaria, a mechanism that might prevent self-pollination. Krell (1977), however, found no evidence in support of protandry in this variety.

Ackerman (1981) noted that the size of the pollinator was variable, and the size of the throat gap, measured from the rim of the lip to the top of the column in variety *occidentalis* ranged from five to ten millimeters. He thought that the lack of precision in pollen transfer might be based on this variation, and Boyden (1982) suggested that evolution of a more precise fit is unlikely because size in both the bumblebee (Heinrich 1979) and flower are dependent on variable nutrient and growth factors.

Nevertheless, Proctor and Harder (1994) reported that naturally pollinated stigmas of variety *americana* in their Alberta study usually bore at least two pollinia (mean 2.53 ± 0.34, $n = 30$), the number considered sufficient to fertilize most of the ovules contained in a single flower. Just the same, the low frequency of pollination in both varieties very likely represents a limiting factor in the reproduction of these orchids even if a sufficient amount of pollen is transferred when pollination does occur.

Alexandersson and Agren (1996) found that although the effects of pollinator limitation in variety *bulbosa* varied considerably from year to year, fruit production over the lifetime of the plant was probably determined by the level of pollen transfer. Over half of the plants that failed to produce fruit did so because they were never pollinated, and hand-pollinated plants consistently produced more fruits than open-pollinated plants, although the difference was statistically significant in only 1 year of their 3-year study. At the same time, increased pollination intensity, based on supplemental hand pollination for 5 years in one population, resulted in the cumulative production of 1.8 times as many fruits as produced by the control plants, with no reduction in flowering, growth, or survival. There is, therefore, no experimental evidence indicating that fruit production in this orchid is resource limited.

Within season pollinator limitation is also suggested by a reported close correlation of pollinator availability and pollination levels in a northern California population of variety *occidentalis.* Ackerman (1981) observed two pollination peaks corresponding with two episodes of bumblebee emergence. The first pollination peak occurred in concert with the emergence of the first group of bumblebees, but the bees soon shifted to other resources. There followed a drop in pollination in the middle of the period of maximum flowering. The second pollination peak then corresponded with the emergence of the second group of bees and the process was repeated.

Fruit production was also limited by bad weather and herbivory. In northern Sweden, the amount of damage resulting from frost and drought was negatively correlated with tree cover, and animals sometimes consumed both the flowers and corms (Alexandersson and Agren 1996; Pridgeon et al. 2005).

Tipularia Nuttall

Tipularia includes three temperate zone species: one in East Asia, one in the Himalayas, and one in North America (Pridgeon et al. 2005).

Table 6.3 Data on *Tipularia* (Catling and Sheviak 2002)

Character	*Tipularia discolor*
Plant height (cm)	10–65
Raceme length (cm)	8–28
Flower number	(5–) 10–55
Dorsal sepal (mm)	5–8 × 1.5–2.8
Lateral sepals (mm)	5–8 × 1.5–2.8
Lateral petals (mm)	4–7 × 1–1.8
Lip (mm)	5–8 × 2.5–3
Spur length (mm)	10–23
Column (mm)	2.5–4

Tipularia discolor (Pursh) Nuttall (Crane-Fly Orchid)

Distribution and Habitat

T. discolor is a common wintergreen orchid in deciduous and coniferous forests throughout most of eastern North America. It is found from southern Missouri east to Massachusetts and south to Texas and Florida (Whigham and McWethy 1980; Brown 1998; Catling and Sheviak 2002).

Floral Morphology

A highly variable number of small, greenish-yellow to greenish-purple, resupinate flowers are loosely arranged on a slender raceme (Table 6.3) (Luer 1975). Flower symmetry is unusual with the column directed either to the right or to the left of the nectary opening. Both left- and right-handed flowers occur on each raceme (Stoutamire 1978). The sepals and petals also have an altered and irregular orientation, with one or more often asymmetrically positioned in the perianth (Fig. 6.3a, b) (Luer 1975; Stoutamire 1978). It is the only North American orchid that shows such modified symmetry. Both Asian species have bilaterally symmetrical flowers, and Stoutamire (1978) considered this the ancestral condition. The lip is trilobed with small, rounded, lateral lobes and a narrow central lobe, slightly spreading at the tip (Fig. 6.3b) (Luer 1975). It is extended basally into a long, narrow nectar spur (Fig. 6.3a; Table 6.3) (Luer 1975). A slightly curved, 2.5–4 mm long column bears one terminal, incumbent anther containing two pairs of hard, superposed, yellow pollinia (Fig. 6.3c, d). All four pollinia are attached to a single 1 mm long, elastic stalk with a basal viscidium, the latter enclosed in a bifid flap of the rostellum (Luer 1975; Catling and Catling 1991; Dressler 1993; Catling and Sheviak 2002). The stalk in *Tipularia* is a unique type of unrecurved stipe derived through elongation and detachment of the rostellum (Freudenstein 1994a). The stigma is sticky, entire, and located behind the anther (Whigham and McWethy 1980). The flowers produce a perceptible, nocturnal fragrance (Stoutamire 1978), which Schnell (1997, p. 438) described as “a very faint

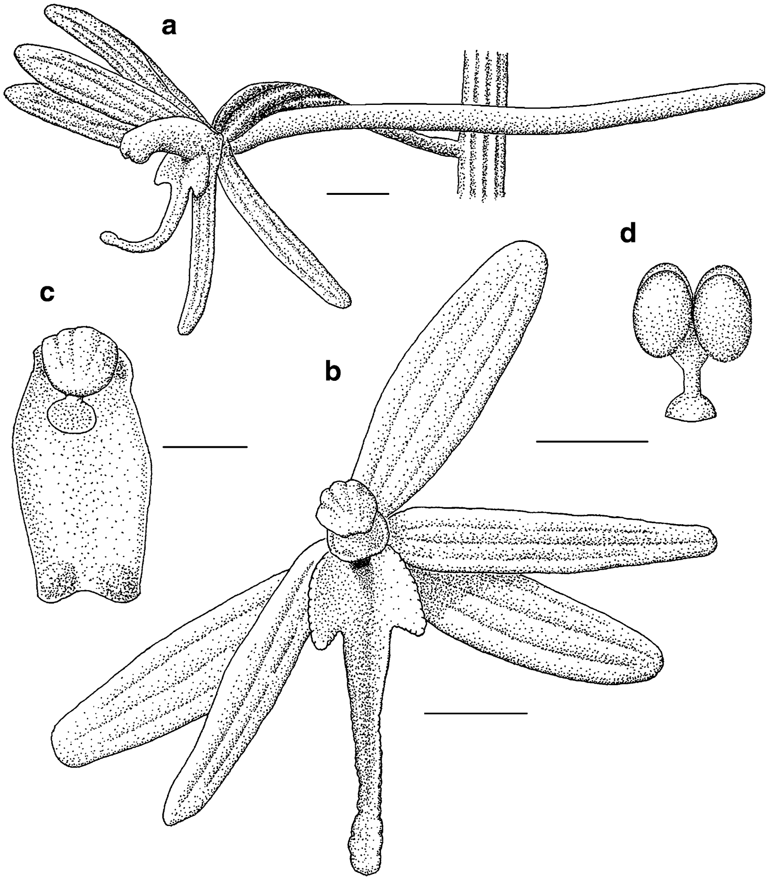

Fig. 6.3 *Tipularia discolor*. (**a**) Flower, side view, scale bar = 2 mm; (**b**) Flower, front view, scale bar = 2 mm; (**c**) Column, ventral view, scale bar = 1 mm; (**d**) Pollinarium following loss of anther cap with four superposed pollinia, scale bar = 1 mm

or citronella odor." Flowering occurs in mid-to late summer when relatively few other forest herbs are in bloom (Taylor 1974; Whigham and McWethy 1980).

Compatibility and Breeding System

Whigham and McWethy (1980) examined the reproductive biology of this orchid in a 2-year study at the Smithsonian's Chesapeake Bay Center for Environmental Studies near Annapolis, Maryland. They found that natural populations were maintained primarily by vegetative reproduction. Nevertheless, experimental crosses indicated that pollinator-mediated intrafloral selfing, geitonogamy, and xenogamy all have the potential for seed production, and a study of genetic markers supports the occurrence of gene flow among populations (Smith et al. 2002). In the Maryland study, artificial self-pollination resulted in seed set in 91% of the flowers. Intrainflorescence and interinflorescence crosses produced seed in 84% and 69% of the flowers, respectively. The lower percentages in outcrossed flowers resulted from abortion of developing fruit for unknown reasons during 1 year of the study. Examination of enclosed, unemasculated and emasculated flowers indicated that autogamy and apomixis are probably absent. In a later 2-year study at the same site, Snow and Whigham (1989) reported a similar level of fruit set with 47–89% of artificially cross-pollinated flowers developing capsules.

Pollinators and Pollination Mechanisms

Whigham and McWethy (1980) found a large, night-flying, noctuid moth, *Pseudaletia unipuncta* (Haworth) (Fig. 6.4), to be the sole pollinator of *T. discolor* at their Maryland study site. Two smaller geometrid moths [*Protoboarmia porcelaria* (Guenee) and *Xanthorhoe ferrugata* (Clerck)] were occasionally seen visiting the flowers, but neither deposited nor removed any pollinaria nor extracted any nectar.

Stoutamire (1978) also identified three species of noctuid moths as pollinators in North Carolina: *Plusia oxygramma* Geyer, *P. precationis* Guenee, and *Cucullia convexipennis* Grote and Robinson. The moths began their explorations just before complete darkness and continued to visit for about 45 min. Pollinators in both Maryland and North Carolina were presumably attracted to the inconspicuous, nocturnally fragrant flowers by their scent. According to Whigham and McWethy (1980) the random flight pattern of *Pseudaletia* was altered as individuals ventured within 3–5 m of a *Tipularia* inflorescence, when they moved directly to the flowers.

The moths observed by Stoutamire (1978) had 15 mm long proboscises which were used to extract nectar from 18 to 20 mm deep nectar tubes. The distance between the opening to the nectar spur and the column tip (1.5–2 mm) in *T. discolor* corresponded to the distance between the base of the proboscis and compound eyes of the noctuids. In all cases, the moths passed quickly from flower to flower, grasping the sepals and petals and maintaining a continual fluttering motion of their wings (Stoutamire 1978; Whigham and McWethy 1980). Stoutamire (1978) reported that movement proceeded from the base toward the top of the raceme. According to Whigham and McWethy (1980), only a few flowers on each inflorescence were visited, and although nectar was taken, not all the visited flowers had pollinaria removed. They considered that the chances of a compound eye contacting the viscidium increased with decreasing nectar volume, forcing the moths to insert their proboscises deeper into the nectar spurs. The moths oriented their bodies at right angles to the ground rather than in the plane of the column (Schnell 1997). As they withdrew from the flower they extracted a pollinarium on either their left or right eye, depending on the orientation of the column (Stoutamire 1978). A moth carrying a pollinarium on its right eye could only pollinate another right-handed flower and vice versa. Stoutamire (1978) speculated that asymmetry here might represent an adaptation to lateral, as opposed to mid-line, attachment of the viscidium in an orchid having only a single pollinarium.

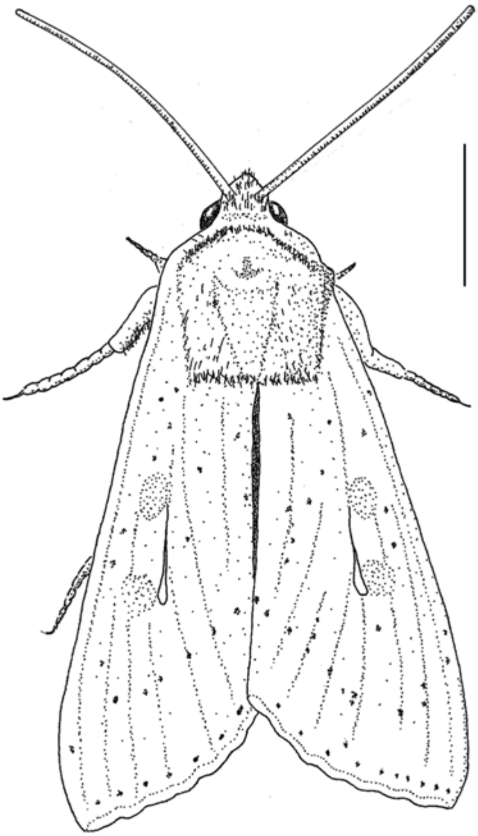

Fig. 6.4 *Pseudaletia unipuncta* (army worm), a pollinator of *Tipularia discolor*, dorsal view, scale bar = 5 mm

Whigham and McWethy (1980) suggested that self-pollination might occur when, following attachment of the viscidium to the moth's eye, the insect forces its head deeper into the flower. This would bring the pollinia into contact with the sticky stigmatic surface, positioned just posterior to the anther sac. However, because the surface of the viscidium is turned inward and faces the center of the flower, it probably is affixed to the back of the compound eye as the moth withdraws (Catling and Catling 1991). Even if the viscidium is attached as the moth enters the flower, the anther cap, which surrounds the four pollen masses on the end of the pollinarium (Fig. 6.3c), is retained for 8–40, usually 15–20 min following extraction, and pollinia are not generally deposited on a stigma until after the anther cap falls off (Snow and Whigham 1989; Catling and Catling 1991). Since the moths quickly visit only a few flowers on any given inflorescence before moving on to another plant, the period of anther cap retention provides a mechanical barrier during this interval that should prevent or greatly reduce the chances of selfing and promote outcrossing (Stoutamire 1978; Whigham and McWethy 1980; Snow and Whigham 1989; Catling and Catling 1991). No data are available on possible return visits to the same flower. Following loss of the anther cap (Fig. 6.3d), one or two of the four transported pollinia may attach to and remain on any contacted stigmatic surface (Catling and Catling 1991). Each pollinarium is therefore able to contribute pollen to 2–4 flowers.

Pollinator activity was related to the amount of nectar present. Whigham and McWethy (1980) found that moth visits at their study site began 4–5 days after anthesis and continued for 13–15 days, peaking on the 11th to 13th day. By about the 16th day when nectar had dropped to approximately 20% of its maximum volume, very few pollinators visited the flowers, even though they remained open with some nectar content for an additional 3–15 days. Apparently, *P. unipuncta* was sensitive to the total amount of nectar available and broke-off visits when this amount fell below some undetermined, minimum level. Willson and Bertin (1979) recorded similar behavior of this moth on *Asclepias* where it functioned as a common pollinator for only 1 week of an extended flowering period.

Whigham and McWethy (1980) found the pollinators not only responded to the overall cycle of nectar production, but also were able to concentrate their attention on the portion of the inflorescence that produced the most nectar. Both nectar production and anthesis occurred acropetally. Pollinator visits were initially restricted to the basal portion of the inflorescence. After about 5 days, nectar production was equally dispersed along the length of the inflorescence, and pollinator visits were also equally dispersed. After 10 days, nectar production was largely limited to the upper flowers, and pollinator visits were then concentrated in this area. All inflorescences were visited during the blooming period with a maximum of 25–45% of available flowers visited daily.

Fruiting Success and Limiting Factors

In their Maryland study, Snow and Whigham (1989) reported that naturally pollinated plants each produced an average of 6–8 fruits per year, with 18–25% of the flowers setting fruit. Eleven percent of the plants produced no fruit, 68% produced 1–10 fruits, and 6% produced over 15 fruits.

In their earlier study at the same site, Whigham and McWethy (1980) found that once pollen was successfully transferred to the stigmatic surface, flowers produced fruits an average of 70.7–93.5% of the time. However, pollinators were scarce during the mid-summer flowering period, and fruits were set in only 24% of unbagged, emasculated flowers. This result lies within the range of variation obtained for open-pollinated plants in the 1989 study, and since it excluded fertilizations resulting from facilitated self-pollinations, it is compatible with the postulated prevalence of cross-pollination in this species.

Both studies imply that fruit set in open-pollinated plants is limited by pollinator service. However, Snow and Whigham (1989) found that the potential advantage of increased pollination in this species, as in others we have discussed, might not translate into an increase in lifetime fecundity. Chances of sexual reproduction were correlated with corm size and leaf area, and fruit and flower development reduced the stored reserves available for corm and leaf growth (Snow and Whigham 1989; Whigham 1990; Efird 1987). Plants that produced 1–10 fruits, as in most naturally pollinated individuals, were more likely to flower the following year than those with many fruits: 23% did so compared to only 3% of those with over 10 fruits (Snow and Whigham 1989). Plants that produced less than 10 fruits also showed a reduction in leaf area and corm size the following season compared to nonfruiting plants, and naturally pollinated plants flowered on average only every 2.5 years. The percentage of plants blooming varied from 8.9% to 23% (Whigham and McWethy 1980), but the number of flowers produced per inflorescence remained more or less constant (Whigham and O'Neill 1991). Further studies of seasonal allocation patterns and photosynthetic characteristics of *Tipularia* have verified the importance of carbohydrate storage in the corm for future growth and reproduction (Zimmerman and Whigham 1992; Tissue et al. 1995).

Other researchers have reported that in addition to plant size, environmental conditions, such as variation in the availability of soil water or protective snow cover, affect flowering (Firmage and Cole 1988; Wells and Cox 1989). Whigham and O'Neill (1991), however, found no clear association between flowering and any climatological factor. They believe that differences in flowering and fruiting are related chiefly to costs associated with sexual reproduction. Recovery from reproductive costs may, however, have been prolonged by other factors (Snow and Whigham 1989; Whigham 1990).

Reproductive success was, for example, affected by herbivory. Whigham and O'Neill (1988) reported that white-tailed deer (*Odocoileus virginianus* Zimmerman) are important predators in Maryland and commonly remove the whole leaf. Most plants usually had their single leaf eaten once every 2 or 3 years. Although maintenance of a large part of the nutrient stores below ground may allow the plants to withstand relatively high levels of herbivory, complete and partial experimental defoliations resulted in a reduction in future growth and sexual reproduction; even higher costs were associated with the combined effects of simulated herbivory and fruit production (Whigham 1990,).

Immediate reproductive gains in species that are subject to heavy predation like *Tipularia* may be more important than future fitness (Whigham and O'Neill 1988 and references therein). The production of 6–8 fruits per year probably represents a compromise between the chances of immediate and future reproductive success. However, additional data on survivorship and age-specific fecundity of both parents and clonal descendents are needed to evaluate the possible advantages of reproduction early in the life cycle and the trade off in fitness between the numbers of fruits produced per season and the number of reproductive seasons (Cole 1954; Schaffer and Gadgil 1975; Snow and Whigham 1989).

In addition to sexual reproduction, fruit set had an effect on asexual reproduction, which, in turn, could affect fitness by attracting pollinators to neighboring inflorescences of the same genet (e.g., Firmage and Cole 1988) and by dispersing the risk of mortality over a larger area (e.g., Cook 1979). Plants frequently generated one leaf each year. Those that branched produced two leaves with the subsequent degeneration of connecting corms (Snow and Whigham 1989). Branching was correlated with the supply of available resources and was observed in 75% of the plants with no fruit, 50% of those with less than 10 fruits, and 34% of those with higher fruit set (Snow and Whigham 1989). Since branching occurred in about half the plants that exhibited natural levels of fruit production, development of a second leaf was common and could potentially compensate for the negative effects of flowering on leaf size (Whigham and O'Neill 1991). This, in combination with the comparatively low fruit set under natural conditions, might help to keep the costs of reproduction in *Tipularia* to a minimum.

Corallorhiza Gagnebin (Coral Roots)

Corallorhiza is a genus of 11 mostly temperate North American species (Magrath and Freudenstein 2002). One is circumboreal, and several extend as far south as Honduras and Nicaragua (Freudenstein 1997). All lack laminate leaves and are essentially mycoparasitic (Campbell 1970; Freudenstein 1994b; Zimmer and Gebauer 2008; Cameron et al. 2009). Seven species occur in North America north of Mexico. Literature is available on the pollination of *Corallorrhiza maculata* Rafinesque (spotted coral-root), *Corallorhiza striata* Lindley var. *striata* (striped coral-root), *Corallorhiza odontorhiza* (Willdenow) Poiret var. *odontorhiza* and var. *pringlei* (Greenman) Freudenstein (autumn coral-root), *Corallorhiza bentleyi* Freudenstein (Bently's coral-root), and *Corallorhiza trifida* Chatelain (early or northern coral-root).

Habitat and Distribution

C. maculata and *C. striata* var. *striata* are widely scattered across the USA and southern Canada while *C. odontorhiza* is largely restricted to the eastern half of this range. All three are often found in moist to dry deciduous, coniferous, or mixed woods with *C. striata* var. *striata* also occurring in coniferous swamps and on lakeshores. *C. trifida* is circumboreal and extends continuously across nearly all of Canada and the northern states to New Mexico in the Cordillera. Northern populations occupy habitats similar to the above as well as tundra and muskeg, while southern populations are usually found on stream banks or in cold, often coniferous, swamps, wet deciduous woods, and bogs (Freudenstein 1997; Magrath and Freudenstein 2002). *C. bentleyi* was originally found on an abandoned railroad grade in West Virginia, but has since been located at several adjacent, somewhat disturbed sites in the Appalachian mountains of Virginia and West Virginia (Freudenstein 1999; Horwitz 2006). Recent analysis based on plastid DNA and morphological characters supports its recognition as a separate species and indicates a close relationship with *C. striata* var. *involuta* (Greenm.) Freudenstein (Barrett and Freudenstein 2009).

Floral Morphology

The flowers are resupinate and vary in size and number (Table 6.4). They can be inconspicuous or showy and loosely to densely arranged in short to long racemose inflorescences (Freudenstein 1997, 1999; Magrath and Freudenstein 2002). *C. odontorhiza* produces both chasmogamous and cleistogamous flowers (Fig. 6.5). These occur on separate plants, sometimes in a single population, with cleistogamous individuals more common over the range of the species (Magrath and Freudenstein 2002). The flowers of *C. bentleyi* are also cleistogamous with red lips at some sites and chasmogamous with yellow lips at others (Fig. 6.6) (Freudenstein 1999; Horwitz 2006).

The sepals and lateral petals may be spreading, curved, or directed forward, with the petals almost clasping the column in *C. trifida* (Fig. 6.7a) (Freudenstein 1997). In *C. odontorhiza*, *C. trifida*, and *C. maculata* the lateral sepals unite with the base of the labellum and column at the top of the ovary forming a mentum or small spur, more prominent in the latter than in the other two species (Fig. 6.8b) (Freudenstein 1997). The dorsal sepal, spreading to sometimes arching over the column, is connivent with the lateral petals in chasmogamous members of *C. odontorhiza* to form a hood over the flowers (Fig. 6.5a, b).

The labellum varies in shape and may be lobed or unlobed (Figs. 6.5a, b, 6.6b, 6.7a, 6.8a, and 6.9a, b) (Freudenstein 1997, 1999). It is narrowed basally to a short claw, 0.4–1.0 mm wide. Basal lamellae or ridges are present and vary from 1.5 to 4 mm in length. In *C. bentleyi* and *C. striata*, they are thickened and fused into a callus, and the labellar margins are involute (Figs. 6.6b and 6.9a).

The column varies in size (Table 6.4) and is straight to strongly curved toward the labellum It bears two pairs of hard, superposed, unequal pollinia at its apex in a fully encumbent, operculate anther (Figs. 6.5–6.9) (Freudenstein 1997). The pollinia have a common stalk as part of a single pollinarium. The stalk comprises a stipe with an adhesive base and a distal end that connects to the pollinia via elastic caudicles, visible as soon as the anther opens (Claessens and Kleynen 1998). The stipe in many members of the Calypsoeae, including *Aplectrum* and *Corallorhiza*, is termed a hamulus (Freudenstein 1994a). It is derived from an upwardly curved extension of the rostellar apex that attaches to the caudicles (Freudenstein 1994a, b). Because of its placement at the apex of the rostellum, rotation at its base can effect a transfer of the pollinia from their usual position on the dorsal side of the rostellum to the ventral side, where the stigma is located (e.g., Fig. 6.8e, f) (Freudenstein 1994a, 1997). The hamulus remains attached to the pollinia following contact with the stigma, permitting identification of those flowers that have self-pollinated (Catling 1983). It and other components of the rostellum are very reduced or absent in cleistogamous plants of *C. odontorhiza* and are poorly developed in *C. trifida* and *Aplectrum* (compare Fig. 6.5c, d and 6.5e, f). In addition, a pair of adaxial auricles at the base of the column, which guides the pollinator's proboscis, is much less well developed in cleistogamous plants. The stigmatic surface is concave, viscid, and variously shaped, usually with an orientation perpendicular to the long axis of the column (Table 6.4).

Table 6.4 Data on *Corallorhiza* (Freudenstein 1997; Magrath and Freudenstein 2002)

			C. odontorhiza			
Treatment	*C. bentlyi*	*C. maculata*	var. *odontorhiza*	var. *pringlei*	*C. striata*	*C. trifida*
Plant height (cm)	To 20	10–68	9–33	9–46	10–67	8–35
Raceme (cm)	3.0–10.5 × 1.0–2.5	2–22 × 1–4.0	1.0–9.0 × 1.0–2.6	1.4–11.5 × 1.1–2.5	1.0–23 × 1.0–4.5	1.5–8.5 × 0.9–2.6
Flower Number	2–20	6–41	2–22	5–30	2–35	3–18
Dorsal sepal (mm)	5.9–6.1 × 2.1–2.5	4.7–15	3.2–4.5 × 0.8–1.5	3.2–4.5 × 0.8–1.5	5–18	3.7–7
Lateral sepals (mm)	5.5–6.1 × 2.1–2.5	4.7–15	3–4.5	3–4.5	5–18	3.5–7
Lateral petals (mm)	5–5.8 × 2.2–3	4.5–11.5	2.5–3.8	2.5–3.8	5–18	3–5.5
Lip (mm)	4.9–5.7 × 3.1–3.5	4–9 × 1.5–6	2.6–3.8 × 1.7–2.3 (–3)	2.7–4.6 × 2–3.7	2–16.5 × 1.5–8.5	2.5–4 × 1.5–3
Column (mm)	3–3.8 × 0.8–1	3.3–7.8 × 0.7–1.3	1.9–2.5 × 0.8–1	1.8–2.4 × 0.8–1.7	2.0–6.0 × 0.8–2.0	2–3.3 × 0.5–1.6
Stigmatic surface (mm)		0.7–1.3	0.2–0.4 × 0.3–0.5	0.3–0.5 × 0.7–1	1.1 × 0.7	0.4 × 0.8

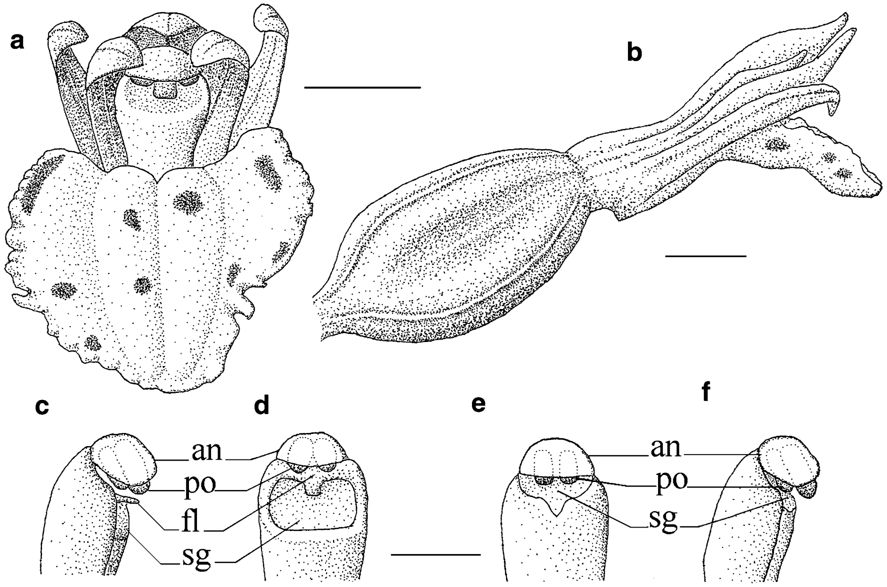

Fig. 6.5 *Corallorhiza odontorhiza* var. *pringleii* (**a**–**d**) with chasmogamous flowers and var. *odontorhiza* (**e**, **f**) with cleistogamous flowers showing contact of pollinia with stigmatic surface. (**a**) Flower, front view; (**b**) Flower, side view; (**c**) Column apex, side view; (**d**) Column apex, ventral view; (**e**) Column apex, ventral view; (**f**) Column apex, side view, scale bars = 1 mm. *an* anther, *fl* flap of rostellar tissue separating pollinia and surface of stigma, *po* pollinia, *sg* stigma

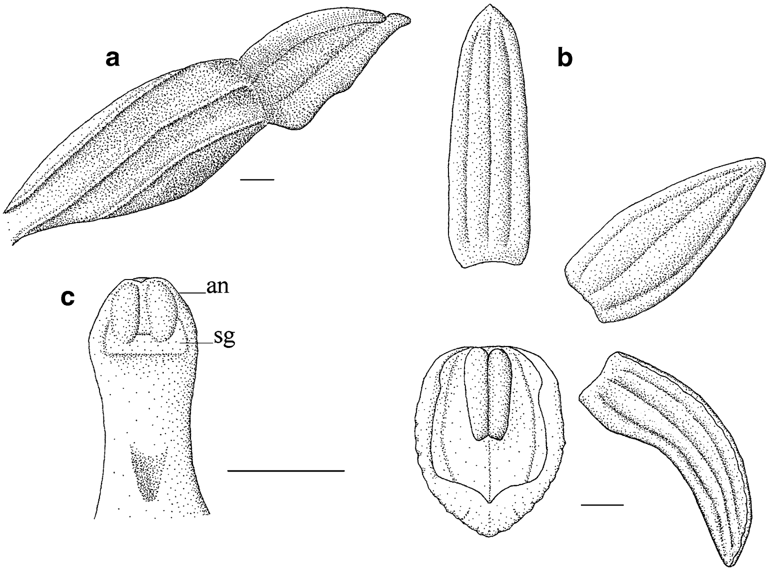

Fig. 6.6 *Corallorhiza bentleyi*. (**a**) Cleistogamous flower, side view; (**b**) Flower, exploded view; (**c**) Column, ventral view showing the absence of separation between the pollinia and the stigmatic surface, scale bars = 1 mm. *an* anther, *sg* stigma

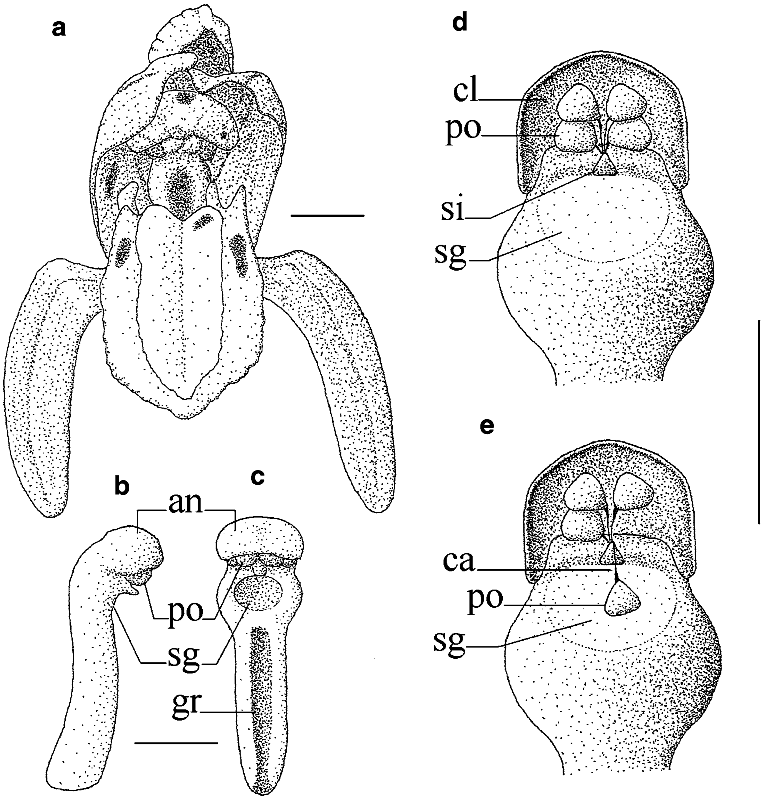

Fig. 6.7 *Corallorhiza trifida.* (**a**) Flower, front view; (**b**) Column, side view; (**c**) Column, ventral view; (**d**, **e**) Column apex, ventral view following loss of anther cap with pollinia in place (**d**) and with one pollinium on the stigmatic surface (**e**), scale bars = 1 mm. *an* anther, *ca* caudicle, *cl* clinandrum, *gr* groove, *po* pollinia, *sg* stigmatic area, *si* stipe

Extensive intraspecific variation occurs in flower color (Freudenstein 1997). The sepals and lateral petals are often whitish or greenish, sometimes suffused with red, purple, or brown, especially near their tips, but in *C. striata* they are basically salmon red with distinct purple stripes. The lip is often white, and all perianth parts may be spotted with purple.

Compatibility and Breeding System

Catling (1983) reported that individual chasmogamous and cleistogamous plants of *C. odontorhiza* from southern Ontario remain constant year after year and that their flower type is probably genetically determined. He conducted pollination experiments on both types. Chasmogamous plants produced 100% seed set in self-, geitonogamous-, and cross-pollination tests but no seeds in tests for autogamy or

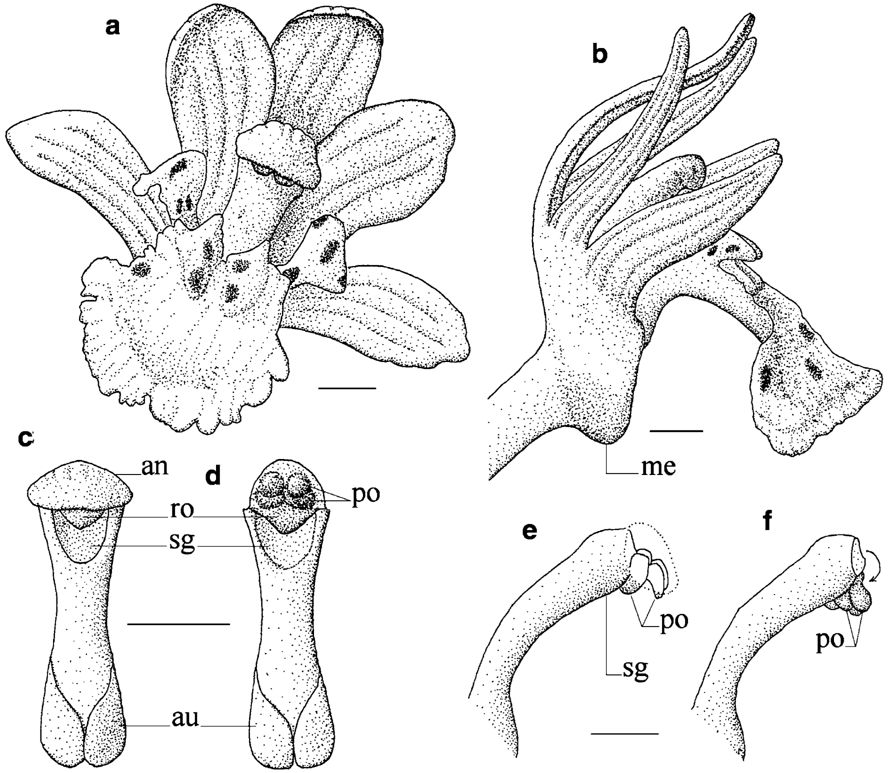

Fig. 6.8 *Corallorhiza maculata*. (**a**) Flower, front view; (**b**) Flower, side view; (**c**) Column, viewed from below, anther in place; (**d**) Column, viewed from below, anther dehisced; (**e**) Column, side view showing position of pollinia prior to anther dehiscence; (**f**) Column, side view following anther dehiscence and rotation of pollinia onto the stigma, scale bars = 1 mm. *an* anther, *au* auricle, *me* mentum, *po* pollinia, *ro* rostellum, *sg* stigma

apomixis. Cleistogamous plants achieved 100% seed set in tests for autogamy and 0% in tests for apomixis. The experimental transfer of pollen from cleistogamous to chasmogamous plants also resulted in 100% seed set. Freudenstein (1997) referred chasmogamous plants of *C. odontorhiza* to variety *pringlei* and cleistogamous plants to variety *odontorhiza*. Cleistogamous flowers of *C. bentleyi* are also known to be autogamous (Freudenstein 1999).

Catling (1983) also found 50–100% self-pollination in individual, unmanipulated plants of *C. trifida* maintained in insect proof cages in a greenhouse, supporting earlier reports of autogamy in this species (von Kirchner 1922a, b). Summerhayes (1951) observed similar levels of self-pollination with seed set occurring naturally in 85–100% of the flowers. Freudenstein (1997) also reported a high level of autogamy in a population in central New York. In this study, stipe rotation resulted in autogamy in 50% of the open flowers examined, no stigmas bore any pollinia that were not attached to the rostellum, and pollinia were removed from only 6.8% of open flowers.

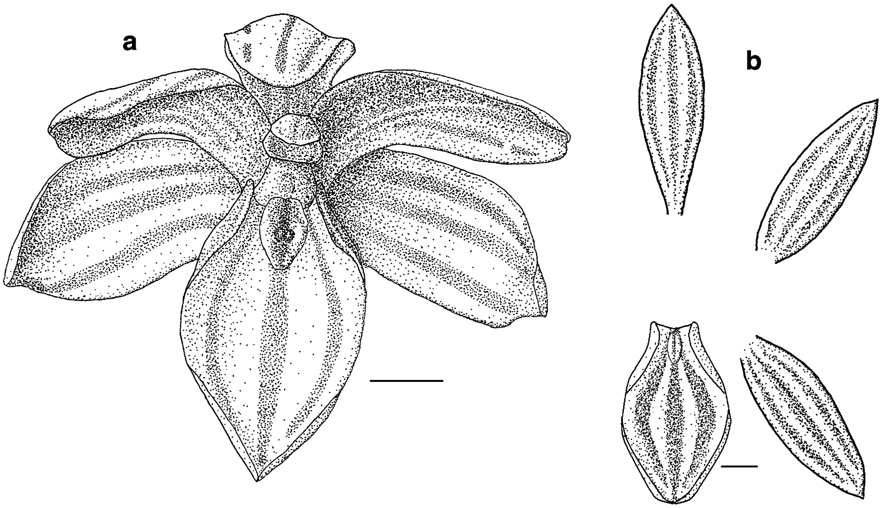

Fig. 6.9 *Corallorhiza striata*. (**a**) Flower, front view; (**b**) Flower, exploded view, scale bars = 2 mm

Autogamy is also present in southern Ontario populations of *C. maculata*. Based on examination of stipe rotation, Catling (1983) reported that self-pollination occurred in 5–50% of the flowers on each plant with variation evident both within and among populations. Kipping found fruit set ranging from 51 to 64% in flowers of *C. maculata* enclosed in screen cages in El Dorado and Marin Counties, California, respectively. Insect pollination and probable outcrossing is also recorded for this species (Kipping 1971; Luer 1975) and for *C. striata* var *striata* (Catling 1983; Freudenstein 1997; see below).

The possible development of autogamy within *C. odontorhiza*, differences in the mechanism of self-pollination (see below), and a hypothesis of relationships among the species (Freudenstein 1994b; Freudenstein and Doyle 1994; Senyo and Freudenstein 2000) all suggest that autogamy has originated independently in *C. bentleyi*, *C. trifida*, cleistogamous *C. odontorhiza*, and *C. maculata*.

A variety of breeding systems are therefore present in *Corallorhiza*, including cleistogamy and autogamy in *C. odontorhiza* var. *odontorhiza* (Catling 1983) and *C. bentleyi* (Freudenstein 1999), outcrossing in *C. striata* (Freudenstein 1997) and *C. odontorhiza* var. *pringlei* (Catling 1983), and facultative autogamy in *C. maculata* and possibly *C. trifida* (Catling 1983).

Pollinators and Pollination Mechanisms

Autogamy was long suspected in *C. odontorhiza* based on the connivent perianth parts in the majority of plants and the high levels of ovary expansion observed in natural populations (e.g., Luer 1975; Catling 1983; Case 1987). In his Ontario study, Catling (1983) found that cleistogamous flowers of *C. odontorhiza* essentially lack a hamulus and that autogamy occurs as a result of the direct growing together of the pollinia and the stigmatic surface rather than by a rotation of the hamulus as in many other species of *Corallorhiza*. More specifically, a small flap of the rostellum that separates the pollinia and stigma in chasmogamous flowers (Fig. 6.5c, d) is absent or poorly developed in cleistogamous flowers (Fig. 6.5e, f). This allows the pollen mass to contact the viscid secretion of the stigmatic surface leading to pollen expansion and germination of the pollen tubes. In *C. bentleyi*, a hamulus is present, but as the flowers remain closed it is not used. The pollinia germinate in place, and the pollen tubes grow down to the stigma (Fig. 6.6c) (Freudenstein 1999).

In *C. maculata* and *C. trifida*, on the other hand, Kipping (1971), Catling (1983), and Freudenstein (1994b, 1997) consider autogamy to occur through rotation of the pollinia onto the stigmatic surface. The pollinia in both are positioned near the apex of the column (Figs. 6.7b, c and 6.8c, e), where they are retained until the anther cap degenerates and falls away (Catling 1983). They are then free to rotate forward and downward on the hamulus through an angle of as much as 270°, bringing them into contact with the stigmatic surface on the underside of the column (Fig. 6.8f).

Claessens and Kleynen (1998) described a somewhat different process in *C. trifida*. Following opening of the anther cap and connection of the stipes with the caudicles and pollinia, the elevated anther cap dries out and falls off. The pollinia rest unattached in the clinandrum (Fig. 6.7d), and in response to the slightest vibration fall onto the stigmatic surface below (Fig. 6.7e). The caudicles are of the proper length and elasticity to assure that the pollinia land on the stigma. The stipes (hamuli) remain in place.

Since self-pollination does not occur until after the flower has opened and the anther cap has fallen away, exclusive insect pollination is possible during the interval separating these events. The anther cap may be retained for up to 48 h after the flower opens in *C. maculata* (Catling 1983), whereas, according to Freudenstein (1997), it is fugacious in *C. trifida*. The pollination mechanism could therefore be considered more truly facultative in *C. maculata* than *C. trifida.* At the same time, only half of each pollinium often touches the stigma and swells, leaving the other half free (Catling 1983). Insects are therefore able to remove fragments of the pollinia after, as well as before, self-pollination has occurred (Catling 1983).

In his study of coastal populations in Marin County, California, Kipping (1971) captured an unidentified species of *Empis* L. (dance flies, both sexes; Emperidae; Diptera) (Fig. 6.10a) carrying pollinia of *C. maculata* on its dorsal thorax (nota). Subsequent observation in a terrarium revealed that after landing on the labellum, *Empis* advanced slowly toward its base and probed the nectar opening with its proboscis. The proboscis was guided to the entrance of the nectary by the two ridges, about 3 mm long, on the surface of the labellum (Fig. 6.8a, b). These movements resulted in contact of the fly's back with the column. After feeding for about 3 min, it withdrew with a pollinarium attached to its dorsal thorax and canted slightly forward. In one case, Kipping (1971) observed transport and deposition of pollinia on the stigma of a second flower. Species of *Andrena* (Andrenidae; Hymenoptera) are also capable of removing the pollinaria of this orchid as illustrated by Luer (1975).

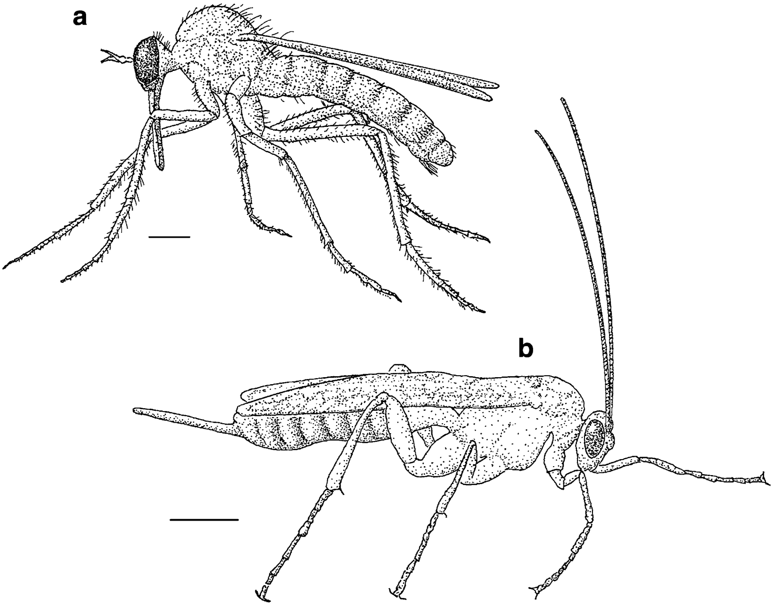

Fig. 6.10 (**a**) *Empis* sp., a pollinator of *Corallorhiza maculata*; (**b**) *Pimpla pedalis*, a pollinator of *Corallorhiza striata*, scale bars = 1 mm

Kipping (1971) captured several other flower visitors at his study site in the Sierra Nevada Mountains of El Dorado County. These included three *Lasioglossum* (*Evylaeus*) *ovaliceps* Cockerell (Halictidae); two different bombyliid flies; and an undescribed species of a small-headed fly, *Eulonchus* Gerstaecker (Acroceratidae). However, none bore pollinaria.

A number of insect visitors have also been reported for *C. trifida*. For example, Dungflies (Scalophaga), syrphid flies (Syrphidae) small hymenoptera, dance flies (*Empis*), and coleoptera have been recorded in Europe (Muller 1881; Kunth 1898–1905; Silen 1906b; Evans 1919; Godfery 1933; Summerhayes 1951; Danesch and Danesch 1962; Fuller 1980; Lang 1989). However, none were confirmed as pollinators. Silen (1906a, b) found that a sticky disk was lacking in *C. trifida* and that the pollinaria failed to adhere to either visiting insects or to a pencil tip inserted into the flower. Claessens and Kleynen (1998) maintain that a viscidium is present but that it loses its adhesive power in open flowers.

C. striata, on the other hand, with showy inflorescences and brightly colored, striped flowers, appears well adapted to insect pollination (Fig. 6.9). Freudenstein (1997) observed a parasitic wasp, *Pimpla pedalis* (Cresson) [as *Coccygomimus pedalis* (Cresson)] (Ichneumonidae; Hymenoptera) (Fig. 6.10b) removing pollinaria in Emmet County, Michigan. The possible role of this wasp as a major pollinator is consistent with a distribution largely coincident with that of the orchid (Townes and Townes 1960). According to Freudenstein (1997), rotation of the stipe and self-pollination occurred in only 4.7% of the flowers. In another 34.3%, the pollinia on the stigma were not attached to the rostellum, and the flowers were considered insect pollinated. These observations in conjunction with the morphology of the flower imply that this species is chiefly adapted to reproduction by outcrossing.

No pollinators have been identified for chasmogamous plants of *C. odontorhiza*. However, the absence of seed production in bagging experiments on both unmanipulated and emasculated flowers indicates that pollinators are necessary.

Fruiting Success and Limiting Factors

Reported high levels of natural capsule production in populations of *C. trifida* and cleistogamous *C. odontorhiza* correlate with the establishment of nearly obligate to obligate autogamy in these taxa (von Kirchner 1922a, b; Summerhayes 1951; Luer 1975; Catling 1983; Freudenstein 1997).

Although levels of self-compatibility in *C. maculata* have not been directly examined experimentally, Catling (1983) reported that seed set was abundant among naturally self-pollinating flowers in southern Ontario. Kipping (1971) found natural fruit set in 50.5% of the flowers in El Dorado County and 59% in Marin County. This is very close to his experimental results with unmanipulated, caged plants noted above (51% and 64%).

Although chasmogamous plants of *C. odontorhiza* are fully self-compatible, it is not unusual to find individuals in natural populations in which 75% of the capsules remain undeveloped (Catling 1983). Consistent with an absence of autogamy, this result, when compared with the 100% seed set obtained in experimental crosses, also suggests that pollinator service may be a limiting factor in the reproduction of this orchid.

Experimental crosses were not performed, but Freudenstein's (1997) report of natural insect pollination in about 34% of the flowers in a Michigan population of *C. striata* also suggests the possibility of pollinator limitation. Additional research is needed to further estimate levels of fruit set and seed viability in all *Corallorhiza* species and to evaluate the principal factors affecting these levels.

Additional Species and Varieties of *Corallorhiza*

No data are available on the reproductive biology of *C. striata* var. *vreelandii* (Rydberg) L. O. Williams, a taxon scattered from the Dakotas and New Mexico to Washington and California. However, the perianth segments, with the exception of the lip, tend to be connivent suggesting either adaptation to a specific pollinator or autogamy (Freudenstein 1997).

There are also no published accounts of pollination for *C. wisteriana* S. W. Conrad and *C. mertensiana* Bongard. The former is distributed in the east from Nebraska and Texas to Pennsylvania and Florida and in the west from Montana to New Mexico and Arizona, the latter, from southeastern Alaska and northern

California to Montana and Wyoming. The flowers are open in both with a brightly colored perianth in *C. mertensiana* and a well-developed stipe in *C. wisteriana*. Freudenstein (1997) examined 253 open flowers of *C. wisteriana* in a group of freshly cut racemes received by mail. Rotation of the stipe and autogamy had occurred in only 2.8% of the flowers. However, the data were ambiguous as no stigmas had received pollinia from other flowers, and only 15% had pollinaria removed. The floral characters in both species are nevertheless consistent with insect pollination and outcrossing.

Aplectrum Nuttall

Aplectrum is a monospecific genus found in the eastern United States and Canada (Pridgeon et al. 2005).

Aplectrum hyemale (Muhlenberg ex Willdenow) Nuttall (Adam and Eve Orchid, Putty-Root)

Habitat and Distribution

A. hyemale, a wintergreen orchid, is often densely aggregated in the rich soils of mesic to wet-mesic, deciduous forest (Sheviak 1974; Case 1987). It ranges from Minnesota, southern Quebec, and Massachusetts to Oklahoma and Georgia with occasional disjunct populations outside this range (Auclair 1972; Magrath 2002).

Floral Morphology

A relatively constant number of medium-sized, resupinate flowers are borne in a loose raceme (Table 6.5) (Luer 1975). Sepals and lateral petals vary in color from yellow to green, tinged with magenta or purple–brown (Luer 1975; Smith 1993). The sepals are spreading whereas the petals parallel the column (Fig. 6.11a). The labellum is obovate and 3-lobed (Fig. 6.11b). The central lobe, whitish with purple markings, is large and orbicular with an undulate margin and three fleshy lamellae on its lower half (Fig. 6.11a, b). Small, ovate lateral lobes are present on either side toward the middle and ascend to flank the column (Fig. 6.11a) (Luer 1975; Smith 1993; Magrath 2002). The column is compressed, elongate, 7 mm long, and pale green with purple spots. As in the preceding members of the Calypsoeae, it bears a terminal, incumbent anther and one pollinarium comprised of four hard, superposed, yellow pollinia attached to a viscidium by a short stipe (hamulus). The stigma is distinctly concave (Magrath 2002). The plants bloom after the spring ephemerals, and the flowers last about 4 or 5 days (Hogan 1983). Nectar is apparently absent (Hogan 1983).

Table 6.5 Data on *Aplectrum* (Magrath 2002)

Character	*Aplectrum hyemale*
Plant height (cm)	18–50
Raceme length (cm)	3–7 × 1.5
Flower number	6–10[a]
Dorsal sepal (mm)	10–15 × 1.8–4
Lateral sepals (mm)	10–15 × 1.8–4
Lateral petals (mm)	9–13 × 1.8–3.5
Lip (mm)	9–12 × 7–9
Column (mm)	7 × 2

[a] Luer (1975)

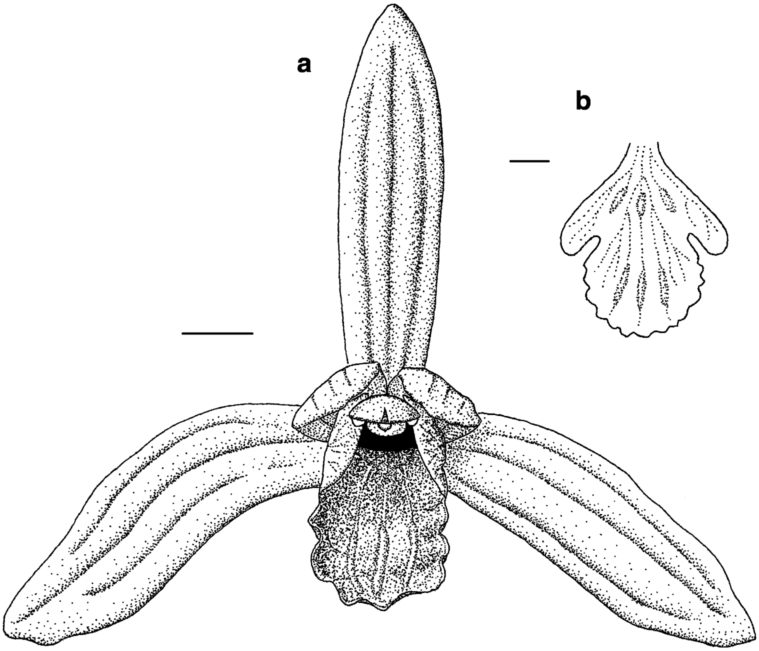

Fig. 6.11 *Aplectrum hyemale*. (**a**) Flower, front view; (**b**) Lip, flattened, scale bars = 2.5 mm

Compatibility and Breeding System

Hogan (1983) conducted a 2-year study of *A. hyemale* in Trelease Woods and Brownfield Woods northeast of Urbana in east central Illinois. He found that artificial geitonogamous pollination resulted in 100% fruit set, indicating full self-compatibility at the level of fruit production. Moreover, 71% of enclosed, unmanipulated flowers and 47% of enclosed, emasculated flowers set fruit, indicating the occurrence of autogamy and possibly, agamospermy. Although present elsewhere in our flora (e.g., *Spiranthes*), agamospermy is unusual in the orchid family (van der Pijl and Dodson

1966), and Hogan (1983) did not rule-out the possibility that fruit set in emasculated plants might reflect autogamy that occurred prior to emasculation. On the other hand, Leavitt (1901) reported polyembryony in *A. hyemale*, a trait often associated with agamospermy.

Artificial cross-pollination in the Illinois population resulted in 87% fruit set, but calculations based on Hogan's (1983) field observations indicated that insects visited only about 1 out of 50 flowers during the 5-day flowering period. Thus, although gene flow through pollen may occur, the available data do not confirm any actual transfer of pollen between plants (see below), and in Hogan's (1983) opinion, *A. hyemale* is routinely autogamous and perhaps, agamospermous. K. P. Kevin (in Catling 1983) and Catling (1984) also reported autogamy in Illinois and Canadian populations, respectively, although the plants in one population from Simcoe County, Ontario, were apparently self-incompatible (Cating 1982).

Pollinators and Pollination Mechanisms

The usual sequence of events leading to pollination in *A. hyemale* was the same in bagged and unbagged inflorescences (Hogan 1983). The pollinia were covered by the anther-cap in newly opened flowers (Fig. 6.11a). On the first or second day, the cap dropped off, laying bare the pollinia. On the 3rd or 4th day, the pollinia were displaced and fleshy, white lobes covered the stigma. According to Hogan (1983), the generation of these lobes was the result of contact between the stigmatic surface and the displaced pollinia. Catling (1984), indeed, described a 270° rotation of the pollinia onto the stigma following degeneration of the anther cap.

Freshly placed pollinia from another flower were never observed on the stigma. In fact, 26 h of observation over 4 days revealed only about 12 individuals of the short-tongued bee, *Lasioglossum oblongum* (Lovell) [as *Dialictus oblongus* (Lovell)] (Hymenoptera: Halictidae) visiting the flowers (Hogan 1983). All were seen on a single day between noon and 1:30 p.m. Most merely landed on the inflorescences, remaining for up to one-half minute. Only five entered flowers. Each entered only one and remained about 5 s. None were observed bearing pollinaria either before or after the flower visit. In one case, a bee dislodged the anther-cap, and although no pollinia were removed, two were observed to be in contact with the stigma following the bee's departure. Thus, insect facilitated self-pollination could be a factor.

Fruiting Success and Limiting Factors

Comparisons of leaf measurements suggest that plants must attain some minimum size before flowering (Hogan 1983), and only one or two individuals from aggregates of 100 or more plants may flower in a given year (Case 1987; Smith 1993). About 82% of flowering, open-pollinated plants set fruit (Hogan 1983). On average, each fruit produced 31.1 mg of seed, and 26.0% of the seeds were viable. Seed weight and the level of viability did not differ significantly among open-pollinated, unmanipulated and bagged, or artificial geitonogamous or xenogamous treatments, and there was, therefore, no evidence of inbreeding depression.

Hogan (1983) recorded 0.00012 insect visits per flower per 10 min. This is about 1/4,000th the frequency observed for several species of earlier blooming spring ephemerals at the same site (Schemske et al. 1978). The difference cannot be accounted for by the lack of nectar in *Aplectrum* because the visitation rates among nectarless spring ephemerals were 0.03 to 0.71 per flower per 10 min (Schemske et al. 1978). Instead, Hogan (1983) suggested the reduced visitation rate might be due to a change in pollinator availability between the blooming period for *A. hyemale* and the ephemerals: pollinators might have become less common or *Aplectrum* might have faced increased competition from other, nectar producing plants (Schemske et al 1978; Nilsson 1980; Motten 1982; Hogan 1983).

A limited number of pollinators and/or a high level of competition for their services during the blooming period could have led to selection for autogamy. A change in phenology might have provided an alternative (Hogan 1983); however, the blooming time in this wintergreen orchid may be set by the period available for high carbohydrate production and accumulation in spring prior to flowering. This period is restricted to the interval between snowmelt or spring warming and closure of the canopy (Stevens and Dill 1942; Adams 1970).

According to Hogan (1983) the patches of *A. hyemale* are probably clonal. The genetic variability resulting from autogamy (or agamospermy) would then approximately equal that resulting from pollen transfer within populations, and fruit set could be increased with no loss in genetic diversity. A breeding system based on clonal growth and autogamy and/or agamospermy is consistent with the limited genetic variability in this species observed by Adams (1970) and Auclair (1972). Restricted variability, in turn, may be reflected in the absence of diversification in this monospecific genus (Stebbins 1957; Hogan 1983).

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Chapter 7
Tribes Cymbidieae and Epidendreae

Abstract *Eulophia alta* and *E. ecristata* occur in North America north of Florida and Mexico, but their pollination has not been studied here. Although autogamy has been reported elsewhere, a recent Florida study of *E. alta* found that autogamy was rare and produced seeds that developed more slowly than seed produced by other treatments. Morphology of the flower of *Epidendrum magnoliae*, along with its production of an intense nocturnal fragrance, suggests pollination by nocturnal moths. *Hexalectris revoluta* var. *colemanii* is an obligate outbreeder, whereas *H. nitida*, *H. spicata* var. *arizonica*, and *H. revoluta* var. *revoluta* are autogamous. *H. revoluta* and *H. nitida* appear to be resource limited.

Keywords *Eulophia* • *Epidendrum* • *Hexalectris* • Autogamy and retarded seed development • Intrageneric variation in breeding systems • Resource limitation • Nocturnal moths

Cymbidieae

The Cymbidieae includes 28 widespread genera (Stern and Judd 2002). If *Pteroglossaspis* Reichenbach f. is treated as a synonym of *Eulophia* in accordance with the World Checklist of Monocotyledons (2008), only one genus occurs in our flora.

Eulophia R. Brown ex Lindley

Two of two hundred and fifteen to two hundred and twenty-five species belonging to this large, pantropical but primarily African and Asian genus occur in the southeastern USA north of Florida: *Eulophia alta* (L.) Fawcett and Rendle (Fig. 7.1a, b) in southeastern Georgia and *E. ecristata* (Fernald) Ames (= *Pteroglossaspis ecristata* (Fernald) Rolfe) in coastal South and North Carolina, Alabama, and Louisiana (Romero-Gonzalez 2002a, b).

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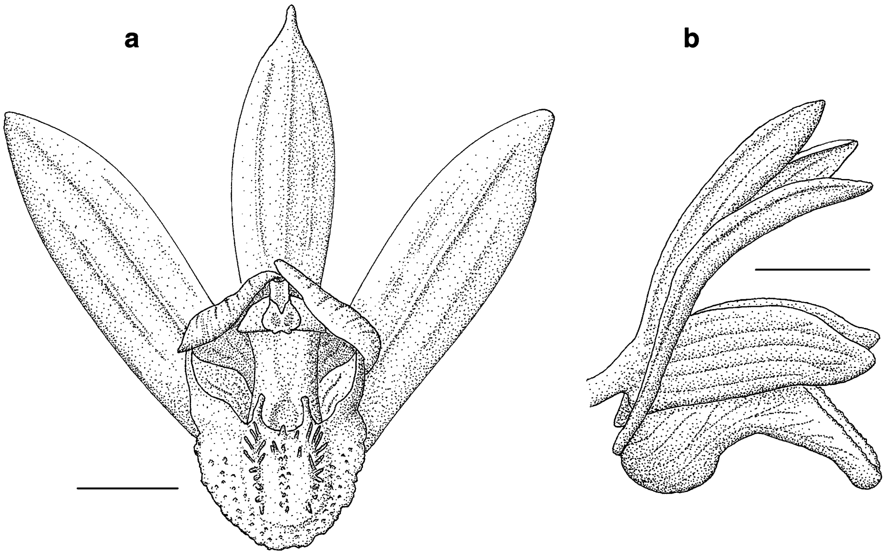

Fig. 7.1 *Eulophia alta*. (**a**) Flower, front view; (**b**) flower, side view, scale bars = 5 mm

Little pollination data are available for either species in North America. Williamson (1984) reported autogamy in *E. alta* and a number of other *Eulophia* species in Zambia, and autogamy has also been reported in populations of *E. alta* from Mexico, South Africa, Florida, and Puerto Rico (Goss 1973; Catling 1990; van der Cingle 2001). However, a recent study at a site in south Florida (Johnson et al. 2009) found spontaneous autogamy to be rare, with only 7.1% of the observed flowers developing capsules. Moreover, autogamy produced seeds that developed more slowly than seeds produced by other treatments. Hand pollinations significantly increased capsule formation. Capsule set resulting from induced autogamy was 46.4%; geitonogamy, 64.3%; xenogamy with pollen from plants 10–100-m distant, 42.9%; xenogamy with pollen from plants greater than 10-km distant, 67.9%. Johnson et al (2009) concluded that autogamy is uncommon in *E. alta* at their study site and that the large numbers of capsules found in natural populations are probably the product of unobserved cross-pollination events. Outcrossing is, in fact, a common mode of sexual reproduction elsewhere in the genus (e.g., Lock and Profita 1975; Singer and Cocucci 1997; Sun 1997; van der Cingle 2001; Peter and Johnson 2006; Jurgens et al. 2009).

Epidendreae

The Epidendreae includes 86 genera found in the Caribbean and North, Central, and South America. Only two are present in North America north of Mexico and Florida (Pridgeon et al. 2005).

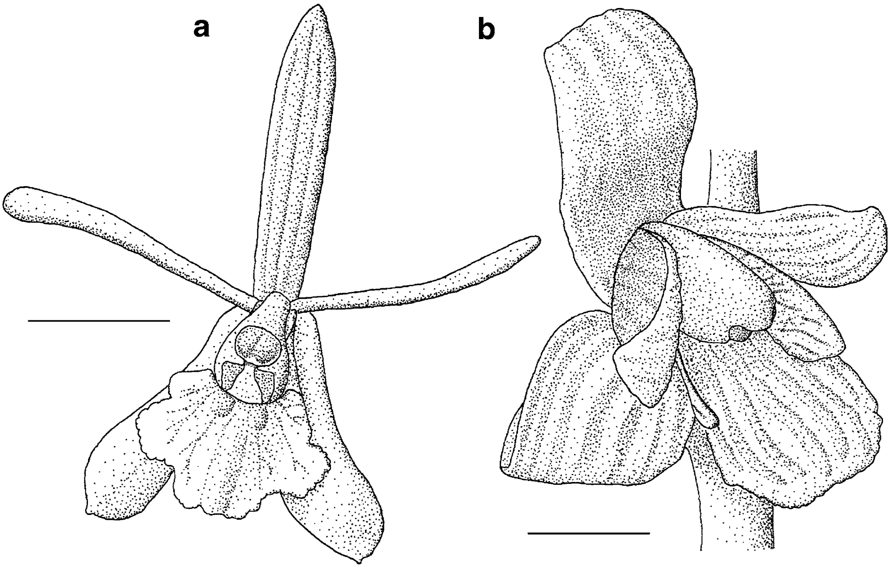

Fig. 7.2 (**a**) *Epidendrum magnoliae*, flower, front view; (**b**) *Hexalectris spicata*, flower, oblique view, scale bars = 5 mm

Epidendrum L.

Over 1,000 tropical and subtropical species of *Epidendrum* have been described (Hagsater 2002). Seven are present in North America north of Mexico. Among these, *E. magnoliae* Muhlenberg (green-fly orchid) (Fig. 7.2a) is the only representative of the genus and the only epiphytic orchid found naturally north of Florida (Luer 1972; Correll 1978). It occurs in swamps, hammocks, and moist hardwood forests along the Atlantic and Gulf Coasts from North Carolina into Florida and west to Louisiana (Luer 1972; Hagsater 2002).

Although much information is available on tropical and subtropical species of the genus, no studies have been published which specifically treat the reproductive biology of *E. magnoliae*. Bush and Kutz (2006) detected genetic drift within and high levels of gene flow between populations, a finding they attributed to pollinator movement as well as seed dispersal. The flowers are said to be intensely fragrant at night (Subrahmanyam 2004), and the odor is described as honey-like. This, along with the morphology of the flower (van der Pijl and Dodson 1966; Luer 1972), suggests pollination by nocturnal moths. Adams and Goss (1976) and Goss (1977) described such a syndrome for two other species, *E. amphistomum* A. Richard (as *E. anceps* Jacquin) and *E. floridense* Hagsater (as *E. difforme* Jacquin) in the Big Cypress Swamp of southern Florida (synonymy based on Hagsater 2002). Both are self-incompatible and therefore obligately xenogamous. Although nectar is provided, the initial attraction in these species may be pheromone-like since the flowers are pollinated almost exclusively by male moths.

Hexalectris Rafinesque

Kennedy and Watson (2010) recently revised the genus *Hexalectris* and proposed several significant changes in nomenclature and distribution. However, pending further study, the present discussion follows the treatments of Goldman et al. (2002) and Catling (2004).

Hexalectris is a mostly Mexican genus of seven mycoheterotrophic species often confused with *Corallorhiza*, but differing, among other things, in having a number of raised crests running down the center of the lip (Fig. 7.2b) (Coleman 2002). Two species are found only in Mexico or Mexico and Guatemala, but five others extend into the USA. All, except the widespread *H. spicata* (Walter) Barnhart var. *spicata* (crested coralroot), are restricted to scattered sites in Texas, New Mexico, and Arizona (Goldman et al. 2002). Limited information on pollination biology is available for *H. nitida* L. O. Williams (Shining or Chisos or Glass Mountain coralroot), *H. spicata* (Walter) Barnhart variety *arizonica* (S. Watson) Catling and V. S. Engel (Arizona crested coralroot), *H. revoluta* Correll variety *revoluta* (Curly corralroot, Correll's cock's-comb), and variety *colemanii* Catling (Coleman's coralroot).

H. nitida includes forms having open flowers with revolute sepals and petals (Luer 1975) and others with predominantly closed flowers (Engel 1987). Catling (1990, 2004) reported autogamy for this species: the holotype has no rostellum, and the pollinia develop in direct contact with the stigmatic surface.

H. spicata var. *arizonica* is also autogamous, routinely lacks a rostellum, and usually has a connivent or only a slightly spreading perianth (Catling 1990, 2004: Catling and Engel 1993). Open flowers (Fig. 7.2b) have only been reported in a single, predominantly cleistogamous population near Dallas. The tips of the anthers in open flowers are dehydrated, pointed, and reddish, whereas in closed flowers they are enlarged, green, and fleshy. The distended anther tips in closed flowers along with a terminally expanded midvein on the lip may contribute to squeezing the pollinia onto the stigmatic surface as the column stretches and buckles during the aging process (Catling and Engel 1993). Coleman (2002), however, reported that the pollinia slide from under the anther cap early on and are present on the stigma in freshly opened and in young cleistogamous flowers.

Autogamy also occurs in *H. revoluta* Correll var. *revoluta* (Catling 2004). In this case, the evidence suggests that the pollen masses rotate onto the stigmatic surface following elevation of the anther cap. Catling (2004) observed a pollinarium with the viscidium still attached to the edge of the rostellum but with four pollinia resting on the stigmatic surface and firmly attached there by developing pollen tubes. The second variety, *H. revoluta* var. *colemanii*, is an obligately outbreeding taxon with a distinct rostellum (Catling 2004).

Insects may also cross-pollinate the flowers of *H. revoluta* var. *revoluta* and open flowers of *H. nitida* or *H. spicata* var. *arizonica*, but this has yet to be demonstrated (Catling and Engel 1993). Recent observations in *H. nitida* suggest that autogamy here might be obligate (Catling 2004). Even if outcrossing occurs, hybridization between the varieties of *H. spicata* would likely be restricted by differences in blooming dates, variety *arizonica* blooming later than variety *spicata* in at least some areas where they are sympatric (Coleman 2002; Goldman et al. 2002).

Coleman (2005) found that summer rains (June–September) were fairly constant during a 9-year period in Arizona, but that variations in winter rains (October–May) correlated with the number of blooming plants in *H. revoluta*, var. *colemanii*, and Engel (1987) reported a correlation between blooming and ample late spring rains over a period of 7 years for *H. nitida* in Texas. The process of blooming and presumably fruiting apparently requires the expenditure of a significant fraction of the available stored reserves. Coleman (2002, 2005) reported that *H. revoluta* and *H. spicata* did not reemerge for one or more years after blooming. However, additional research is needed on the causes of dormancy in these orchids.

Reproduction in *H. spicata* var. *arizonica* was also limited by browsing and by the unexplained abortion of 10–80% of the developing spikes and buds. In some years, only one or two plants in a colony of 50 or 60 that began development survived to produce flowers (Coleman 2002).

No reports have been published on the pollination biology of the remaining American taxa, *H. spicata* var. *spicata*, *H. warnockii* Ames and Correll (purple-spike coralroot), and *H. grandiflora* (A. Richard and Galeotti) L. O. Williams (Greenman's coralroot).

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Chapter 8
Tribe Arethuseae (*Calopogon* R. Brown and *Arethusa* L.) and Subfamily Vanilloideae (Part One) (*Pogonia* Jussieu)

Abstract *Arethusa bulbosa*, *Calopogon tuberosus*, and *Pognia ophioglossoides* are self-compatible but adapted to outcrossing. The flowers produce little or no reward and are probably pollinated primarily by deceit. Fruit production is pollen limited. Pollinators are bees apparently attracted to the large, colorful perianths and ultraviolet absorbing, anther-like brushes on the lips. Larger flowers of *C. tuberosus* discriminate among pollinators and prevent hybridization with three smaller, sympatric species. Reproductive isolation among the smaller flowered taxa is maintained primarily by differences in phenology, habitat, and pollinator preference. The flowers of *C. oklahomensis* are similar in size to those of *C. tuberosus*, but this species is hexaploid and blooms earlier.

Keywords *Arethusa* • *Calopogon* • *Pogonia* • Deceit pollination • Ultraviolet absorbing pseudostamens • Reproductive isolation • Pollen-limited fruit set • Clump and inflorescence size effects • Bumblebees

The tribe Arethuseae comprises 26 genera native to eastern Asia, New Guinea, the southwest Pacific Islands, eastern North America, and the northern Caribbean (Pridgeon et al. 2005). Two genera, *Arethusa* and *Calopogon*, are found in our flora. *Arethusa* includes one species from North America and one from Japan while *Calopogon*, with five species, is restricted to North America. Most information on its pollination biology is based on a single species.

Pogonia, with one North American and three or four East Asian species, is also considered here. It is assigned to subfamily Vanilloideae (see below) (Pridgeon et al. 2003) and is not closely related to *Calopogon* and *Arethusa* (e.g., Dressler 1981, 1993; Freudenstein and Rasmussen 1997; Cameron and Case 1999; Goldman et al. 2001; Panasarin and Barros 2008). However, the flowers of all three are similar in size and color (Luer 1975), and the lip in each is similarly structured and shares a cluster of stamen-like, yellow–white protuberances or brushes which absorb ultraviolet light and are thought to function in the attraction and orientation

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of pollinating insects (Thien 1971; Thien and Marcks 1972). Some insects visit all three species, and it is expedient to consider them together in order to address questions concerning their interfertility, pollination mechanisms, and reproductive isolation.

Arethusa bulbosa L. (Dragon's-Mouth), *Pogonia ophioglossoides* (L.) Ker Gawler (Rose Pogonia), and *Calopogon tuberosus* (L.) Britton, Sterns, and Poggenburg (Grass-Pink)

Distribution and Habitat

A. bulbosa is distributed from Manitoba and Minnesota to Newfoundland and through the Great Lakes to New Jersey with isolated populations in North Carolina and Saskatchewan (Sheviak and Catling 2002a). *C. tuberosus* and *P. ophioglossoides* overlap *A. bulbosa* in the north, extending further south through Florida to eastern Texas, again with a few disjunct populations outside this range (Luer 1975; Goldman et al. 2002a; Sheviak and Catling 2002b, c). The three species are often found together and may occur in bogs, fens, swamps, wet woods, savannas, meadows, and prairies (Thien and Marcks 1972; Goldman et al. 2002b; Sheviak and Catling 2002a, b, c). Where sympatric, *P. ophioglossoides* tends to prefer the wetter, *C. tuberosus* and *Arethusa* the drier sites, with *C. tuberosus* exhibiting wider tolerances than *Arethusa* (Boland and Scott 1992). According to Yannetti (in Pridgeon et al. 2005), *Arethusa* is short lived and may be more dependent on frequent seed propagation than the other two taxa.

Floral Morphology

P. ophioglossoides bears a single terminal or two racemic, resupinate, and gaping flowers on stems of varying length (Table 8.1) (Thien and Marcks 1972; Sheviak and Catling 2002b). Flower color is pink to occasionally white, but according to Heinrich (1975) is usually fairly constant. The lip is spatulate with dark red, involute margins, lacerate and deeply fringed toward the apex (Fig. 8.1a). Its ventral surface is ornamented with purple veins and bears three rows of ultraviolet absorbing, yellowish bristles, which become long, red processes toward the apex (Luer 1975; Smith 1993). The column is curved and pink with an incumbent, terminal anther housing a pair of soft and mealy, ovoid, yellow pollen masses (Fig. 8.1b, c) (Luer 1975; Dressler 1993). True pollinia are absent (Pridgeon et al. 2003). Pollen is loose and dispersed in clumps made up of binucleate monads (Gregg 1991). The stigma is flat (Sheviak and Catling 2002b). A viscidium is absent (e.g., Stoutamire 1971).

Table 8.1 Data on *Arethusa*, C*alopogon*, and *Pogonia*

Character	*A. bulbosa*[a]	*C. tuberosus*[b]	*P. ophioglossoides*[c]
Plant height (cm)	2–40	4–110(135)	4–70
Flower number	1(2)	1–15(25)	1–2
Dorsal sepal (mm)	20–55 × 3–9	15–31 × 5–18	14–23 × 3–7
Lateral sepals (mm)	20–55 × 3–9	13–26 × 5–16	14–23 × 3–7
Lateral petals (mm)	23–49 × 4–10	15–28 × 4–14	13–25 × 3–11
Lip (mm)	19–35 × 10–19	11–23 × 9–21	12–25 × 4–10
Column (mm)	ca. 23[d]	12–25 × 1–2	
Width distal end (mm)	ca. 10[d]	6–10	
Chromosomes (2*n*)	40, 44[e]	26, 40, 42	18

[a] Sheviak and Catling (2002c)
[b] Goldman and Orzell (2000)
[c] Sheviak and Catling (2002d)
[d] Correll (1978)
[e] Thien and Marcks (1972)

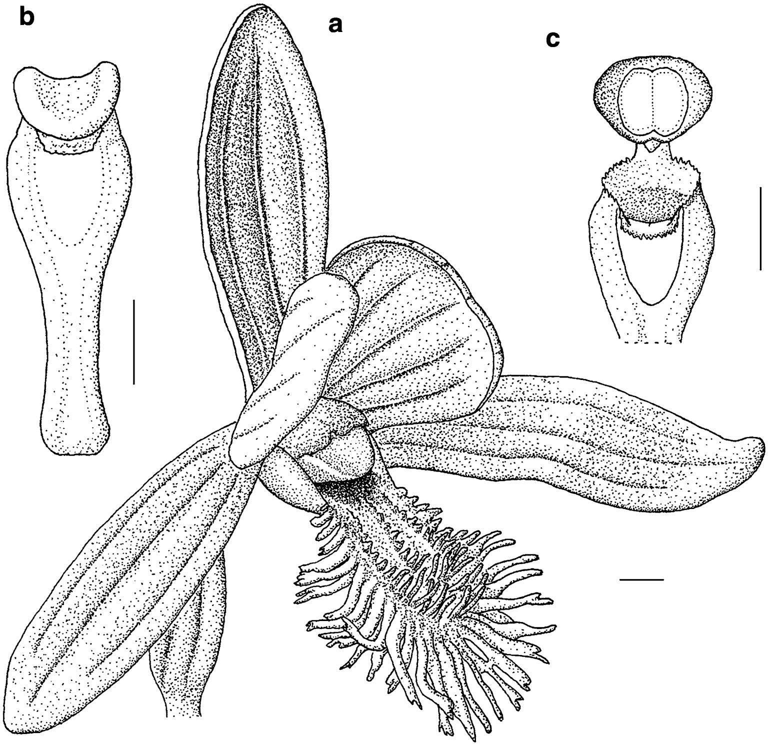

Fig. 8.1 *Pogonia ophioglossoides*. (**a**) Flower, oblique view; (**b**) Column, ventral view; (**c**) Column, ventral view with anther turned backward, scale bars = 2 mm

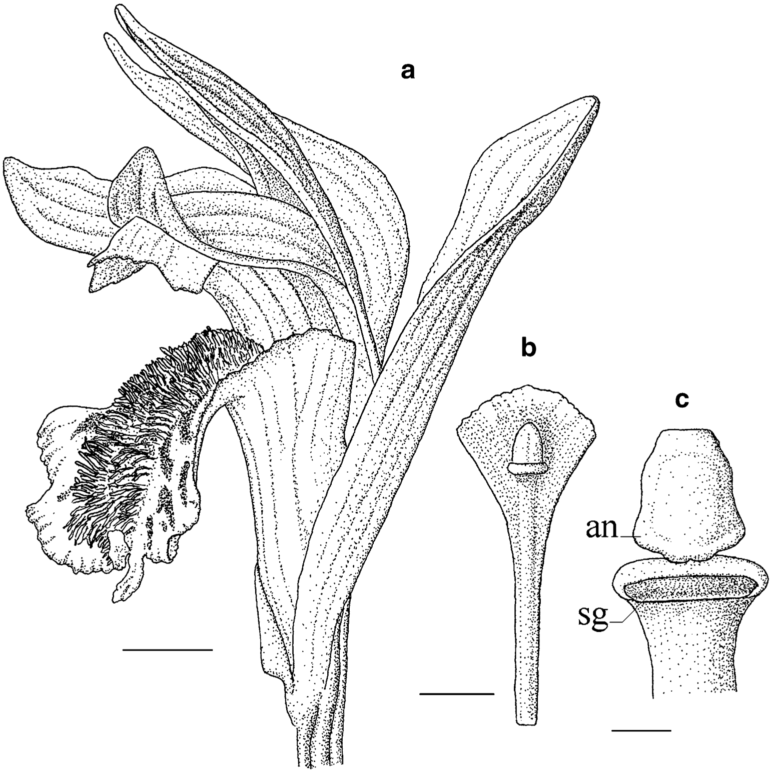

Fig. 8.2 *Arethusa bulbosa*. (**a**) Flower, oblique view, scale bar = 5 mm; (**b**) Column, ventral view, scale bar = 5 mm; (**c**) Close up of anther (an) and stigma (sg), scale bar = 1 mm

The flowers of *A. bulbosa* are relatively large and usually solitary and terminal on scapes of variable length (Table 8.1). Commonly rose–purple, they range from magenta to white (Luer 1975; Case 1987; Yannetti 2003). All three sepals are erect, whereas the petals are positioned with the lip to form a loose tube around the column (Fig. 8.2a). The lip is obovate to oblong with two indistinct lateral lobes and a large, downcurved middle lobe. The latter has a notched apex, crenulate to erose lateral margins, and deep purple veining or blotching on an otherwise white or pinkish-white ventral surface. This surface also bears ultraviolet absorbing crests of yellow lamellae, which become fleshy processes toward the apex (Luer 1975). The column is pink, flattened, and arched with lateral wings and a distally erose margin (Fig. 8.2a, b) (Luer 1975; Case 1987). Positioned below the apex, the incumbent anther (Fig. 8.2b, c) produces two pairs of soft and mealy, weakly sectile, yellow–green pollinia (Luer 1975; Hesse et al. 1989; Dressler 1993). The massulae are irregularly shaped and comprised of tetrads (Hesse et al. 1989). The stigma is emergent and proximal to the anther (Fig. 8.2b, c) (Sheviak and Catling 2002a). A viscidium is absent (Stoutamire 1971).

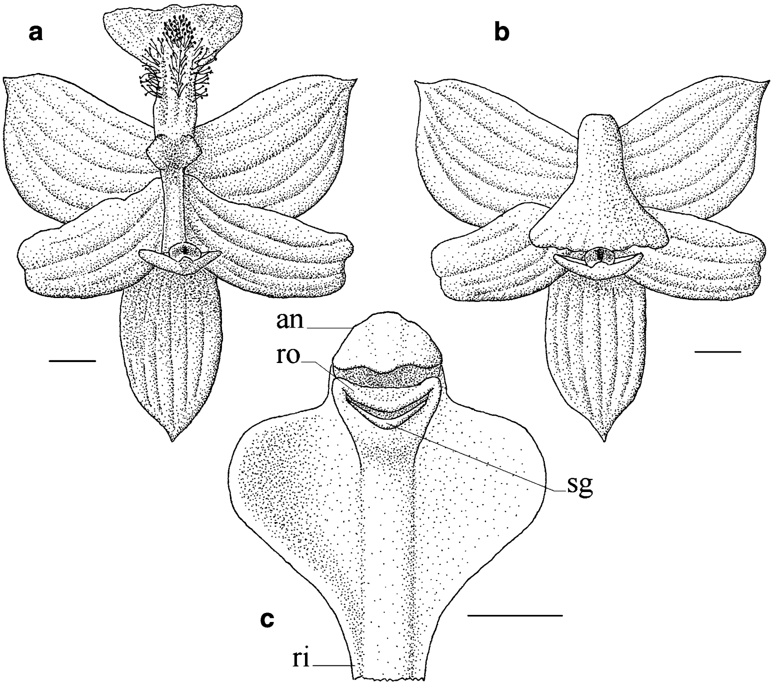

Fig. 8.3 *Calopogon tuberosus*. (**a**) Flower, front view with lip erect, scale bar = 5 mm; (**b**) Flower, front view with lip depressed, scale bar = 5 mm; (**c**) Apex of column, scale bar = 2.5 mm. *an* anther, *ri* ridge, *ro* rostellum, *sg* stigma

C. tuberosus develops a variable number of non-resupinate flowers in 1 (–3) loose, terminal, scapose raceme(s) (Table 8.1). Flower color is highly inconstant and ranges from pale pink or occasionally white to deep magenta (Heinrich 1975; Luer 1975; Case 1987; Boland and Scott 1991; Smith 1993). Firmage and Cole (1988) report fewer flowers per inflorescence for populations in Maine, the lower numbers purportedly a response to a cooler climate. The lateral sepals are reflexed distally. The lip is erect and includes two small and obscure, basal lateral lobes and a linear middle lobe with a broadly winged, obtuse apex (Fig. 8.3a) (Luer 1975). Its ventral surface is densely bearded with short, ultraviolet absorbing, filiform to clavate bristles arranged in rows (Case 1987; Smith 1993). The column is incurved and widely winged at its tip (Fig. 8.3c). An incumbant, terminal anther produces masses of tetrads in two pairs of soft pollinia. The stigma is perpendicular to the column and proximal to the anther; a rostellum is usually present, but a viscidium is absent (Fig. 8.3c) (Stoutamire 1971; Sheviak and Catling 2002c). The absence of a viscidium and the occurrence of loosely formed, friable pollen masses in all three orchids may not represent a primitive condition but may, as in *Cypripedium*, be an adaptation to pollination by hairy insects (Stoutamire 1971).

Although, according to Goldman et al. (2004), *C. barbatus* is the only *Calopogon* that completely lacks floral fragrance, the flowers of *C. tuberosus* are also often described as odorless or faintly scented; they produce no nectar and the pollen is not usually collected for food (Stoutamire 1971; Thien and Marcks 1972; Dressler 1993; Goldman et al. 2002b). The flowers of *Pogonia* emit a mild, sweet odor and may produce very small amounts of nectar in a small tube-like nectary formed by the convergence of the column and lip (Guignard 1879; Heinrich 1975; Gregg 1991). According to Thien and Marcks (1972), *Arethusa* also produces a floral odor and a very small amount of nectar. Yannetti (1996) reported the presence of fragrance in 15–20% of the plants at a site in the Pine Barrens of southern New Jersey and described a well-developed nectary, lined with papillose cells, concealed within the ovary. He speculated that the direction of evolution proceeded from a system of reward to one of deceit, but that some fragrant plants retained the capacity to produce a little nectar. Stoutamire (1971) was neither able to detect any odor nor find any liquid in the flower and concluded that nectar was either absent or the pollinators obtained it by piercing the tissues. Dressler (1993) also characterized the Arethusinae as nectarless.

Compatibility and Breeding System

The flowers of *C. tuberosus*, once thought to be capable only of outcrossing (e.g., Thien and Marcks 1972), are now known to be self-compatible (Table 8.2). In a study in northern Florida, Thien (1973) found that 67% of artificially self-pollinated flowers produced fruit. Similarly, Firmage and Cole (1988) reported that 87% of artificially self-pollinated flowers and 61% of geitonogamous pollinations in south central Maine produced fruit compared to 83% of cross-pollinated plants. However, insects are required for pollination: no fruit was set in enclosed, unmanipulated plants (Firmage and Cole 1988). Under natural conditions, the pollination mechanism (see below) probably reduces selfing in individual flowers while the timing of flower opening reduces transfer of pollen among flowers on a single plant (Thien and Marcks 1972; Thien 1973; Firmage and Cole 1988).

Pogonia and *Arethusa* are similarly self-compatible (Table 8.2). Artificial self-pollination produced fruit sets of 89–95% in *P. ophioglossoides* and about 95% in *A. bulbosa* (Thien and Marcks 1972; Thien 1973). However, flower structure and number indicate both are also adapted to outcrossing (see below) (Robertson 1887; Guignard 1879; Thien and Marcks 1972).

Evaluations of intergeneric crosses in the three orchids, based on the development of F1 seeds with embryos, indicate that *A. bulbosa* and *C. tuberosus* are interfertile (Thien and Marcks 1972), and a naturally occurring hybrid has been reported in Newfoundland (Pinkepank 1993). Plastid DNA sequences support a close relationship between *Calopogon* and *Arethusa* (Goldman 2000; Goldman et al. 2001). Crosses of either of these species with *Pogonia* are usually sterile; occasional high seed production with *Pogonia* as the female parent is attributed to apomixis (Thien

Table 8.2 Summary of data on percentage fruit set resulting from hand pollination and open pollination in *Calopogon tuberosus*, *Pogonia ophioglossoides*, and *Arethusa bulbosa*

Taxon	Study site	Selfing	Geitonogamy	Outcrossing	Open	Authors
C. tuberosus	FL	67				Thien (1973)
	ME	87	61	83	26 (12–40)	Firmage and Cole (1988)
	NL				19 (17–22)	Boland and Scott (1991)
	WI				16	Thien and Marcks (1972)
P. ophioglossoides	NL				30 (29–33)	Boland and Scott (1991)
	WI	89–95			10–100	Thien and Marcks (1972), Thien (1973)
A. bulbosa	NL				16 (13–20)	Boland and Scott (1991)
	WI	95			5	Thien and Marcks (1972)

and Marcks 1972). The chromosomes of *Pogonia* (2*n* = 18) average 18 µm in length versus 3.8 µm in *Arethusa* (2*n* = 40, 44) and 4 µm in *C. tuberosus* (2*n* = 26, 40 or 42) (Baldwin and Speese 1957; Thien and Marcks 1972; Love and Love 1981; Goldman et al. 2002b; Sheviak and Catling 2002a, b). The number and size differences between the chromosomes of *Pogonia* and the other two species apparently inhibit hybridization.

Pollinators and Pollination Mechanisms

Since the flowers of all three orchids produce little or no reward, it is likely that they are pollinated primarily by deceit. The pollinators are apparently attracted to the large, colorful perianths and the ultraviolet absorbing, anther-like brushes on their lips (Stoutamire 1971; Thien and Marcks 1972). Thien and Marcks (1972) studied the pollination of all three species in a bog in Vilas County, Wisconsin. Visitors were infrequent, but all were bee pollinated (see below).

In *C. tuberosus*, the base of the upright lip is narrow and functions as a hinge (Fig. 8.3a). A pollen-seeking bee of the proper size and weight landing on the ultraviolet absorbing tuft of anther-like hairs causes the lip to fall forward and down (Fig. 8.3b), and the pollinator is deposited on the upper surface of the column. According to Thien and Marcks (1972), it lands on its back and skids downward along the column, where it first contacts the stigma and then the anther (Fig. 8.3c). The stigma is viscid, and a sticky secretion is deposited on the bee's dorsal abdomen. As it slides past the anther, pollinia may adhere to this secretion and be extracted from the anther.

The process sounds untidy, but the column, shaped like a slide, has ridges along both margins that constrain and position the body of the pollinator. As a result, an effective pollen vector, a bee of the proper size, usually receives the pollinia on the dorsal surface of its first abdominal segment (Fig. 8.4b). The lip snaps back to the vertical position when released by the bee, and the flower is ready for another visitor. The entire process is completed in less than 4 s. If the bee, now carrying pollen, visits another flower and the process is repeated, pollen may be deposited on the stigma during the slide down the column.

In *Arethusa*, the pollinator lands on the anther-like cluster of ultraviolet absorbing hairs and crawls toward the base of the lip searching for nectar (Fig. 8.2a) (Thien and Marcks 1972). It follows the ridges of the crested lip beneath the over-arching winged column, thrusting its head and thorax deep into the flower (Stoutamire 1971). As the bee backs out, a downward curvature near the center of the lip (Fig. 8.2a) forces its thorax against the column. It first brushes against the stigma (Fig. 8.2b, c), which deposits a sticky secretion on the hairless rear edge of the thorax and on adjacent hairs. Next, it contacts a small protrusion on the underside of the hinged anther case; the anther case is pulled open, and the pollinia are attached to the stigmatic secretion (Fig. 8.4a) (Stoutamire 1971; Thien and Marcks 1972). Once the insect has passed, the anther case snaps shut (Stoutamire 1971). Since pollinia

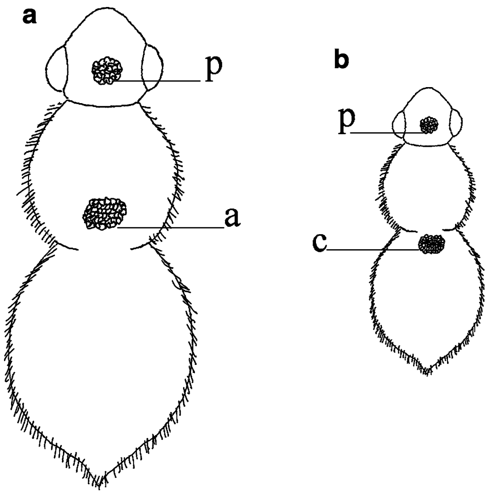

Fig. 8.4 Schematic dorsal views of two pollinators showing placement of pollen. (**a**) A large bee, such as a *Bombus* queen; (**b**) A small bee, such as a *Bombus* worker or *Megachile*. Position of pollen placement for *Arethusa bulbosa* (a), *Calopogon tuberosus* (c), and *Pogonia ophioglossoides* (p)

can be removed only as the insect is leaving the flower and it rarely, if ever, visits the same flower more than once, self-fertilization is usually avoided (Thien and Marcks 1972). On subsequent floral visits, pollen may be transferred when the thorax contacts the stigma; the head and abdomen of the insect usually fail to contact either the anther case or the stigma.

The process is similar in *Pogonia*. The pollinator again lands on the fleshy, ultraviolet absorbing hairs of the lip (Fig. 8.1a) and crawls toward its base, inserting its head deep into the flower (Guignard 1879; Thien and Marcks 1972). However, the flower of *Pogonia* is so constructed that pollen grains from the anther are attached to the head, rather than the thorax or abdomen, of the withdrawing bee (Fig. 8.4a, b). On subsequent floral visits, the head may deposit pollen on the stigma. The insect's thorax and abdomen usually fail to contact either the anther or the stigma.

The geographic ranges and flowering periods of all three species overlap in northern regions, and all have been reported to share a number of insect visitors, yet natural hybrids are rare (Thien and Marcks 1972; Pinkepank 1993). The differences in the positioning of pollinia on the body of the pollinator have often been considered to provide an explanation for intraspecific fidelity in pollen transfer, acting as a mechanical isolating mechanism and presumably permitting, in some cases, the simultaneous placement of pollen from two or even all three orchids on different parts of a common pollinator's body (Thien and Marcks 1972).

However, the intergeneric crossing experiments outlined above clearly imply that differences in the positioning of pollinia are redundant in the reproductive isolation of contemporary populations of *Pogonia* from *Arethusa* and *C. tuberosus*. The effect of pollinia placement on other factors, such as the avoidance of stigma contamination by foreign pollen, might yet prove to be important, however. Such contamination can significantly reduce male and female fitness by limiting the available space and relative numbers of compatible pollen grains on the stigma and

Table 8.3 Pollinators of *Arethusa* and *Pogonia*

Orchid Pollinators	Caste	Study Location	Authors
Arethusa bulbosa			
Bombus borealis Kirby or *B. sandersoni* Franklin	Q	NL	Boland and Scott (1991)
B. ternarius Say	Q	WI	Thien and Marcks (1972)
B. terricola Kirby	Q	WI	Thien and Marcks (1972)
Pogonia ophioglossoides			
Bombus borealis Kirby or *B. sandersoni* Franklin	Q, W	NL	Boland and Scott (1991)
B. fervidus (Fabricius)	X	ME	Heinrich (1975)
B. ternarius Say	X	ME	Heinrich (1975)
	Q, W	WI	Thien and Marcks (1972)
B. terricola Kirby	X	ME	Heinrich (1975)
	Q, W	WI	Thien and Marcks (1972)
B. vegans Smith	X	ME	Heinrich (1975)
	Q	WI	Thien and Marcks (1972)

Abbreviations: *Q* queen, *W* worker, *X* unspecified

by interfering with the developing pollen tubes from these grains; reduction in male fitness can also result from wastage of pollen that could otherwise have been transferred to conspecific plants (Holsinger et al. 1984; Lloyd and Webb 1986; Charlesworth and Charlesworth 1987; de Jong et al. 1992; Schoen and Lloyd 1992; Klinkhamer and de Jong 1993; Snow et al. 1996).

There is now reason to believe that pollinia placement also represents only one of several factors restricting hybridization between *A. bulbosa* and *C. tuberosus*. Seasonal isolation may be significant, at least in northern Wisconsin. The peak flowering period for *A. bulbosa* is usually relatively early and only partially overlaps that of *C. tuberosus* (Thien and Marcks 1972). In addition, there may be partial segregation based on pollinator size. The lip and anther cap are separated by a distance of about 5 mm in *A. bulbosa,* and only queens of *Bombus ternarius* Say and *B. terricola* were able to pollinate the flowers (Table 8.3). Workers of these and other bees were often too small to scrape the anther and remove the pollen. Thien and Marcks (1972) found pollen of *C. tuberosus* on workers of *B. terricola*, on both queens and workers of *B. ternarius*, and on queens of *Bombus vagans* and *Megachile melanophaea* (Table 8.4). They believe, however, that contrary to Dressler (1981), the smaller workers were most effective at removing the pollen. Their emergence coincided with anthesis in *C. tuberosus* when flowering in *Arethusa* was nearly finished (Stoutamire 1971; Thien and Marcks 1972). The queens were much larger and often too big to function as effective pollinators. Strong fliers, they were commonly able to prevent or delay the descent of the lip and to exit the flower before contacting the stigma. When they did occasionally touch the column, they usually had already begun their exit and only received pollen on their legs or on one side of their thorax. Because of their size, they also tended to enter the flowers from one side, and the pollinia were again inconsistently positioned. In either event,

Table 8.4 Pollinators of *Calopogon tuberosus*

Pollinators	Caste	Locality	Authors
Augochlora Smith sp.	X	ME	Heinrich (1979a)
Bombus sandersoni Franklin	W	NL	Boland and Scott (1991)
B. fervidus (Fabricius)	X	ME	Heinrich (1975)
	X	ME	Firmage and Cole (1988)
B. nevadensis auricomus (Robertson)	X	TX	Lehmberg (2002)
B. ternarius Say	X	ME	Heinrich (1975)
	Q, W	WI	Thien and Marcks (1972)
B. terricola Kirby	X	ME	Heinrich (1975)
	W	WI	Thien and Marcks (1972)
B. vegans Smith	X	ME	Firmage and Cole (1988)
	X	ME	Heinrich (1975)
	Q	WI	Thien and Marcks (1972)
Megachile melanophaea Smith	Q	WI	Thien and Marcks (1972)
Xylocopa micans Lepeletier	X	FL	van der Pijl and Dodson (1966)
Unidentified halictids	X	ME	Firmage and Cole (1988)

Abbreviations: *Q* queen, *W* worker, *X* unspecified

the chances for pollination were poor, and queens that served as pollinators of *A. bulbosa* (Table 8.3) were usually ineffective in transferring the pollen of *C. tuberosus* (Table 8.4) (Thien and Marcks 1972).

Firmage and Cole (1988) also observed several species of smaller bees in the family Halictidae as well as the bumblebees *B. vagans* and *B. fervidus* pollinating the flowers of *C. tuberosus* in Maine, confirming Heinrich's (1975) earlier observations here (Table 8.4). Heinrich (1975, 1979) found that species of *Augochlora* Smith and the *Bombus* species already identified as pollinators in Wisconsin to be also effective pollinators in Maine (Table 8.4). In addition, *Xylocopa micans* Lepeletier (Apidae, carpenter bees) (van der Pijl and Dodson 1966) and *Bombus nevadensis auricomus* (Lehmberg 2002) have been reported as pollinators of this orchid in Florida and Texas, respectively (Table 8.4). The caste of these bumblebees and most other pollinators was not provided.

Queens of *B. vagans* and both queens and workers of *B. ternarius* and *B. terricola* also pollinated flowers of the incompatible species *P. ophioglossoides* in northern Wisconsin (Thien and Marcks 1972), and the same species along with *B. fervidus* visited the flowers in Maine (Table 8.3) (Heinrich 1975). *Bombus borealis* or *B. sandersoni* Franklin visited the flowers of all three orchids in Newfoundland; again queens pollinated *Arethusa*; workers pollinated *Calopogon*, and both workers and queens pollinated *Pogonia* (Tables 8.3 and 8.4) (Boland and Scott 1991).

It has been suggested that the similarities in flower size, color, and lip structure, as well as the ultraviolet absorbing brushes in these orchids, are the result of floral convergence (Thien and Marcks 1972; Heinrich 1975). The assumption that simple, deceptive flowers may occasionally evolve mimicry is reasonable, as this could enhance their chances of successful sexual reproduction. However, the sometimes-cited examples of *P. ophioglossoides* (Heinrich 1975) or *A. bulbosa* (Thien and Marcks 1972) serving as models are problematic. Mimicry would make little sense here, where none of the plants provide any incentive in the form of a significant food source for pollinators, the size of bumblebees. Firmage and Cole (1988) found that the level of fruit set in *C. tuberosus* at their Maine study site, where none of the proposed orchid models occur, was greater than the level that Thien and Marcks (1972) reported for their Wisconsin population, where such a model was present (Table 8.2).

If mimicry is involved, it seems more likely that the orchids have converged upon other more common taxa in the same biotype that offer a more bountiful reward. Pollinators preconditioned to other pink flowers might visit the similar flowers of the orchids by mistake (Mosquin 1970). Seeking the possible identities of these other species in northern Wisconsin and Maine bogs, Thien and Marcks (1972), Heinrich (1975), and Firmage and Cole (1988) have found bumblebees actively pollinating many pink flowered ericaceous shrubs, as well as *Sarracenia purpurea* L., *Epilobium* L. species, and *Polygala paucifolia* Willd. The latter is thought to be a particularly good model as one of its pink–magenta petals also has a brush-like structure that absorbs light in the ultraviolet range, and it flowers when the three orchids are in bloom (Thien and Marcks 1972). Wal Upton (in van der Cingle 2001) reports that Douglas H. Goldman has also observed mimicry of *Rhexia virginica* L. in eastern Texas. Of course, unless the deception is very good, the presence of sympatric models that offer a reward might actually reduce rather than increase the frequency of pollinator visits to *C. tuberosus.* Moreover, the thing mimicked may not be another flower at all, but rather the pollen food source purportedly simulated by the anther-like brushes on the lip, the so-called pseudopollen of Dafni (1984).

On the other hand, it cannot be excluded that the flowers of *Pogonia* or *Arethusa* do contain sufficient nectar to serve as a basis for convergence (e.g., Thien and Marcks 1972; Heinrich 1975). Heinrich (1975) reported frequent movement of bumblebees among simultaneously blooming plants of *Calopogon* and *Pogonia* in Maine, and considered that this activity might be prolonged by food rewards collected from *Pogonia*. Given the effectiveness of the pollination mechanism, successive visits of a single pollinator to nonrewarding flowers could lead to the production of abundant seed (Heinrich and Raven 1972). However, such movement implies the chance transfer of pollen between *Pogonia* and *Calopogon* or, at other sites, between *Pogonia* and *Arethusa* with the possible production of sterile hybrids or seeds. Such hybridization would reduce the reproductive success of both species and would tend to remove one from mixed populations (Lewis 1961; Heinrich 1975) or lead to selection for character displacement or changes in phenology or habitat (Levin and Kerster 1967; Levin and Schaal 1970; Heinrich 1975).

Gregg's (1991) report of a carpenter bee (*Xylocopa virgincia* L.) possibly collecting pollen from *Calopogon pallidus* Chapman in North Carolina suggests a different basis for mimicry. Carpenter bees have not as yet been implicated in the pollination of either *Pogonia* or *Arethusa*; however, Thien and Marcks (1972) noted that some pollinators were probably overlooked and that those identified in Wisconsin are absent from the southern range of all three orchids.

Convincing evidence for mimicry remains to be established. The flowers, which, after all, possess a suite of characters usually associated with bumblebee pollination, may not need to mimic those of any other species, and pollen transfer may simply depend largely upon the early, tentative explorations of potential new food resources by recently emerged, inexperienced bees (Heinrich 1975, 1979; Boyden 1982; Little 1983). In *C. tuberosus* and perhaps *Arethusa*, the occurrence of odorless or faintly scented flowers may force the pollinator to rely heavily on flower shape or color, and the variation in the latter may extend the number of visits required for the bee to learn that the flowers have no nectar (Heinrich 1975; Ackerman 1981; Yannetti 2003).

Fruiting Success and Limiting Factors

The data on experimental crosses can be compared to data on natural pollination obtained from three widely separated locations (Table 8.2). In Maine, the percentage of plants producing fruit in *C. tuberosus* over a 7-year period ranged from 12 to 40% with a mean of 26% (Firmage and Cole 1988). In Wisconsin, the percentage of flowers producing capsules under natural conditions was 5% in *A. bulbosa*, 16% in *C. tuberosus*, and 10–100% in *P. ophioglossoides*, the high percentages in some populations of the latter apparently due to apomixes (Thien and Marcks 1972; Thien 1973). In Newfoundland, capsule set ranged from 16 (13–20)% in *A. bulbosa* and 19 (17–22)% in *C. tuberosus* to 30 (29–33)% in *P. ophioglossoides* (Boland and Scott 1991).

The relatively low level of capsule production in open compared to artificially pollinated plants (Table 8.2) suggests pollinator limitation (Thien and Marcks 1972), as might be expected if bees learn to discriminate between species offering abundant and poor rewards (Free and Butler 1959; Stoutamire 1971; Firmage and Cole 1988). Firmage and Cole (1988), for example, observed that in *C. tuberosus* only one visit every 3–5 days per plant resulted in any pollen removal or deposition on the stigma. However, low capsule set in these species may again be compensated by the production of very large numbers of seeds in those plants that are effectively pollinated (Stoutamire 1971; Firmage and Cole 1988).

Thien and Marcks (1972) found that bees in northern Wisconsin were most attracted to flowering plants of *C. tuberosus* and *A. bulbosa* that occurred in groups. Similarly, in Maine, Firmage and Cole (1988) found that in *C. tuberosus* the percentage of flowers setting fruit varied significantly with plant distribution. Flowers on plants occurring in clumps of 2–8 within a 1 m radius had a higher probability of setting fruit than flowers on either solitary plants or on plants in clumps of nine or more. Presumably, groups of 2–8 attracted more pollinators than solitary flowers, and larger groups were abandoned following a few unrewarding visits. In a study of *Arethusa* and *Pogonia* in Newfoundland, Boland and Scott (1991) reported an inverse relationship between fruit set and the percentage of plants producing flowers. In *A. bulbosa*, about 27% of the plants flowered, and as noted above, a mean of 16% of these set fruit. In *P. ophioglossoides*, only 3% flowered, but 30% set fruit. Bowland and Scott reasoned that a higher number and density of *A. bulbosa* flowers may have allowed pollinators to learn more quickly that they were a poor source of food.

In *C. tuberosus*, male and female reproductive success at the Maine site increased with increasing inflorescence size (Firmage and Cole 1988). The number of open flowers (typically 2–4) was independent of inflorescence size, and larger inflorescences did not attract more pollinators at any one time; however, the overall probability of pollinator visits was increased by a longer period of sequential flowering.

A larger inflorescence and longer display time might represent one response to a competition for scarce pollinators (Willson and Price 1977, 1980; Wyatt 1982; Firmage and Cole 1988). Yet, both the Maine and Wisconsin study sites were located in sphagnum bogs, an environment commonly considered to be low in nutrient resources. Nutrient limitation might be expected to produce the opposite trend in inflorescence evolution (Firmage and Cole 1988). Where both factors are significant, inflorescence size and display time should reflect some level of compromise between the need to attract scarce pollinators and the limited resources available to produce the inflorescence (Firmage and Cole 1988). In addition to a reduction in inflorescence size and display time, the savings realized by allocating limited resources to purposes other than a pollinator reward must likewise be balanced against a resulting reduction in the attractiveness of the flowers and a contingent decrease in annual reproductive success (Firmage and Cole 1988).

Additional Species of Calopogon

The four other species of *Calopogon* native to North America resemble *C. tuberosus* in flower morphology, color, and ultraviolet reflectance. *Calopogon multiflorus* Lindley, *C. barbatus* (Walter) Ames, and *C. pallidus* Chapman are found in pine savannas and grasslands of the southeastern United States (Thien 1971; Luer 1972; Goldman et al. 2002b). They have $2n = 40$ or 42 chromosomes, as does *C. tuberosus* in this area, and all are self-compatible and interfertile with each other and with *C. tuberosus* (Thien 1973; Goldman et al. 2002b, 2004). However, *C. tuberosus* has larger flowers, about 4 cm across compared with about 2.5–3 cm in the other three (Tables 8.1 and 8.5). According to van der Pijl and Dodson (1966) and Thien (1973), a resulting difference in "fit" discriminates among prospective pollinators and prevents gene flow between *C. tuberosus* and these species.

Flower size does not separate the more recently discovered midwestern species, *C. oklahomensis* G. H. Goldman, from *C. tuberosus* (Tables 8.1 and 8.5). However, *C. oklahomensis* is a hexaploid with 120 or 114 chromosomes and it blooms earlier than *C. tuberosus* over the principal part of their remaining sympatric range. It is not known to hybridize with either *C. tuberosus* or the other species of *Calopogon* (Goldman 1995; Goldman et al. 2004).

Thien (1973) considers reproductive isolation among the small-flowered species to be maintained primarily by differences in phenology and habitat. Although the ranges and flowering dates of *C. barbatus* and *C. multiflorus* often overlap, *C. multiflorus* is different in its ecological preferences from *C. barbatus* and *C. tuberosus* and is usually found in dryer situations (Goldman et al. 2004). The blooming seasons

Table 8.5 Data on other species of *Calopogon* (Goldman et al. 2002)

Character	*Calopogon barbatus*	*Calopogon multiflorus*	*Calopogon oklahomensis*	*Calopogon pallidus*
Plant height (cm)	11–34	17–33(50)	20–100+	15–50(67)
Flower number	1–12	2–15	1–20(25)	3–20
Dorsal sepal (mm)	12–20 × 4–9	9–17 × 5–8	18–25 × 5–10	12–20 × 4.5–6
Lateral sepals (mm)	11–16 × 5–9	10–13.5 × 6–9	15–20 × 7.5–13	10–15 × 5–9
Lateral petals (mm)	11–16 × 4–6	8–14 × 4–8	18–25 × 5–10	9–18 × 3–5.5
Lip (mm)	7–13.5 × 7–11	7–12 × 7–13	12–23 × 5.5–16	9–13 × 5–11
Column (mm)	6–9 × 1.5–2	5.5–7.5 × 1–2	15–25 × 1.5–2	7–11 × 1–1.5
Width distal end (mm)	5–8	5–7	4.5–8	6–9
Chromosomes (2*n*)	38[a], 40, 42	38[a], 40, 42	114[a], 120	38[a], 40, 42

[a] Goldman et al. (2004)

of *C. barbatus* and *C. pallidus* barely overlap, and little pollen transfer occurs between them (Thien 1973). In addition, the flowers of *C. multiflorus* produce a strong, pungent fragrance at peak anthesis, whereas those of *C. barbatus* and *C. pallidus*, respectively, have either no odor or only an occasional, weak fragrance (Goldman and Orzell 2000). Thus, pollinator attraction may differ. Heinrich (1975) and Luer (1975) reported species of *Bombus*, *Xylocopa virginica* and *Apis mellifera* as pollinators of *C. pallidus*. Robertson (1887) reported *Augochloropsis sumptuosa* (Smith) (as *Augochlora sumptuosa* (Smith)) and an unidentified species of *Augochlora* as pollinators of *C. barbatus.* Both were females. The pollination mechanism is the same as reported for *C. tuberosus* with pollen attaching dorsally to the first abdominal segment. *Bombus griseocollis*, *Augochlora pura pura* (Say), (as *A. festiva* Smith) and unidentified species of *Halictus* Latreille also occasionally visited the flowers but were ineffective pollen vectors (Robertson 1887, cf. Thien 1973). Rare natural hybridization events among these species are not, however, unknown (Goldman and Orzell 2000; Goldman et al. 2004).

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Part III
Subfamily Vanilloideae (Part Two)

Subfamily Vanilloideae includes two tribes and 13 genera with a worldwide distribution. A single tribe is found in North America.

Chapter 9
Tribe Pogonieae

Abstract *Isotria verticillata* is a clonal orchid with a xenogamous–geitonogamous breeding system. Autogamy and agamospermy are absent. *Isotria medeoloides* is autogamous but not agamospermous. *I. verticillata* is pollinated by short-tongued, wide-ranging, polylectic bees. Pollinator visitation may limit fruit production. *Cleistesiopsis bifaria* is a clonal, pollen-limited orchid with a geitonogamous–xenogamous breeding system, but selfing reduces the percentage of healthy embryos. Autogamy is absent. Bumblebees and leaf-cutter bees are primary pollinators. Anthers disperse pollen tetrads in successive doses of decreasing size. Flowers pollinated with small pollen doses fade more slowly and sometimes receive additional pollen to increase their seed output. Flowers also provide a pollen reward.

Keywords *Isotria* • *Cleistesiopsis* • Divergent breeding systems • Pollen-load partitioning • Strategies of deceit and reward • Short-tongued bees • Bumblebees • Leaf-cutter bees

The tribe Pogonieae is represented by five disjunct genera found on three continents (Pridgeon et al. 2003). Two, in addition to *Pogonia*, are present in our flora.

Isotria Rafinesque

Isotria, comprised of two species, is restricted to North America.

C.L. Argue, *The Pollination Biology of North American Orchids: Volume 2: North of Florida and Mexico*, DOI 10.1007/978-1-4614-0622-8_9,

Isotria verticillata **(Muhlenberg ex Willdenow) Rafinesque (Large Whorled Pogonia) and** *Isotria medeoloides* **(Pursh) Rafinesque (Small Whorled Pogonia)**

Distribution and Habitat

Both species are eastern woodland plants with a preference for acidic substrates. *I. verticillata* occurs in sphagnum bogs or dry to mesic, forested uplands from southern Maine and Michigan to Georgia and east Texas (Luer 1975). It can be abundant with a single genet producing as many as 300 closely spaced flowering shoots (ramets) (Mehrhoff 1983). *I. medeoloides*, considered one of the rarest orchids in temperate North America (Mehrhoff 1983; Vitt and Campbell 1997), occurs at scattered sites from Maine to Georgia and west to Michigan and Missouri (Luer 1975; Vitt and Campbell 1997; Mehrhoff and Homoya 2002). Each site usually has only 3–10 plants, although some northern stations include over 100 (Mehrhoff 1983; Brackley 1985; Vitt and Campbell 1997). Often occurring in disturbed areas, its preferred habitat appears to be relatively open, dry-mesic to mesic, second growth deciduous or deciduous–coniferous forest (e.g., Gregory 1988; Mehrhoff 1989a, b; Mehrhoff and Homoya 2002). Prolonged periods of dormancy have been reported (Brumback and Fyler 1988; Ware 1990; Vitt 1991).

Floral Morphology

Both species produce one, occasionally two, terminal, resupinate flowers on each shoot (Mehrhoff 1983; Vitt and Campbell 1997). The flowers of *I. verticillata* are multicolored and distinctly larger than the uniformly light green flowers of *I. medeoloides* (Table 9.1). The sepals are widely spreading, and the lateral petals converge over the column (Figs. 9.1 and 9.2) (Luer 1975; Correll 1978). The lip is obovate and apically 3-lobed with a rounded middle lobe. The middle lobe in *I. verticillata* is expanded and white with a broad, fleshy, green ridge along the middle of the disk and a revolute, undulate margin. The lateral lobes, with purple margins, are turned upward, forming a floral tube with the other two petals (Fig. 9.1c). The rounded, yellowish-green to greenish-white lip of *I. medeoloides* is less ornate with a slightly emarginated middle lobe and yellowish-green, keel-like projections extending from the base and expanded above into blunt, elongated, wart-like processes. The narrowly triangular lateral lobes are involute and again form a floral tube with the lateral petals (Fig. 9.2a) (Luer 1975; Mehrhoff 1983; Mehrhoff and Homoya 2002). The column is white and has a white, terminal, incumbent anther containing two, soft, mealy pollen masses with upwards of 2,000 tetrads in each (Figs. 9.1b and 9.2b) (Luer 1975; Mehrhoff 1983). True pollinia are absent (Pridgeon et al. 2003); granular pollen in this group is again thought to represent a reversion rather than a primitive condition. The stigmatic area is well defined and pubescent in both, but the rostellar flap, between the anther and the stigma, is reduced in *I. medeoloides* (Figs. 9.1b and 9.2b) (Mehrhoff 1983). Viscidia are absent (Dressler 1993). Young flowers of *I. verticillata* produce a scent (Andrews 1901;

Table 9.1 Data on *Isotria* (Mehrhoff and Homoya 2002)

Character	*Isotria verticillata*	*Isotria medeoloides*
Plant height (cm)	4–40	4–25
Flower number	1(2)	1(2)
Dorsal sepal (mm)	34–67 × 2–4	12–25 × 2–3
Lateral sepals (mm)	34–67 × 2–4	12–25 × 2–3
Lateral petals (mm)	15–25 × 3–7	12–18 × 2–4
Lip (mm)	15–25 × 4–5	11–16 × 4–5
Column (mm)	8–10	8–10
Chromosomes (2*n*)	18	18

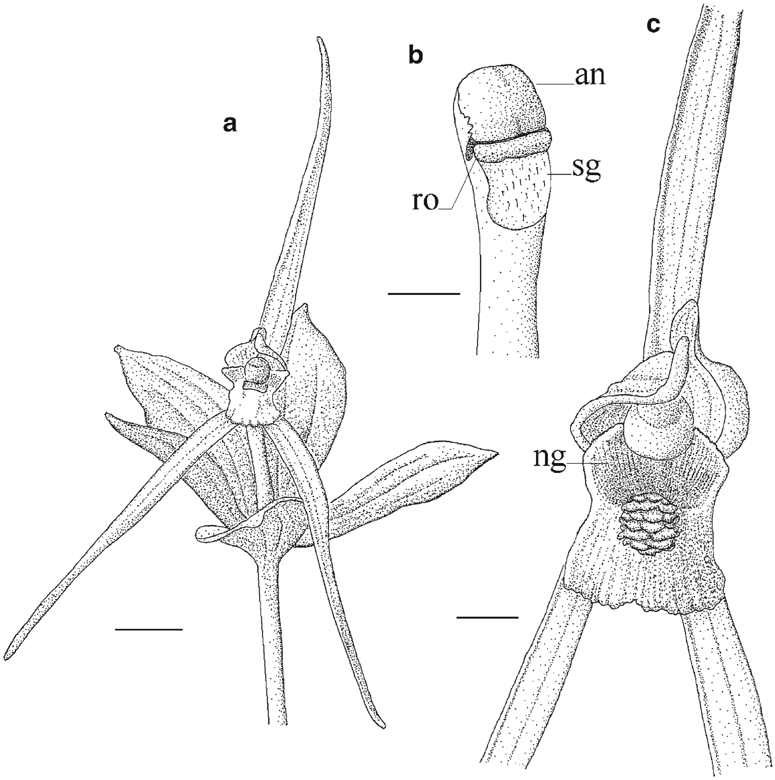

Fig. 9.1 *Isotria verticillata*. (**a**) Upper part of stem and flower, front view, scale bar = 10 mm; (**b**) Column, slightly oblique ventral view, scale bar = 2 mm. *an* anther, *ng* nectar guides, *ro* rostellum, *sg* stigma; (**c**) Close-up of flower, front view, scale bar = 2 mm

Homoya 1977; Mehrhoff 1980) while those of *I. medeoloides* do not (Homoya 1977; Mehrhoff 1983). Neither species makes nectar, but *I. verticillata* possesses apparent pollination (nectar) guides (Mehrhoff 1983). Individual flowers of *I. verticillata* remain open for about 4–7 days with the flowering period for the population lasting about 2 weeks in North Carolina (Mehrhoff 1983). Individual flowers of *I. medeoloides* bloom

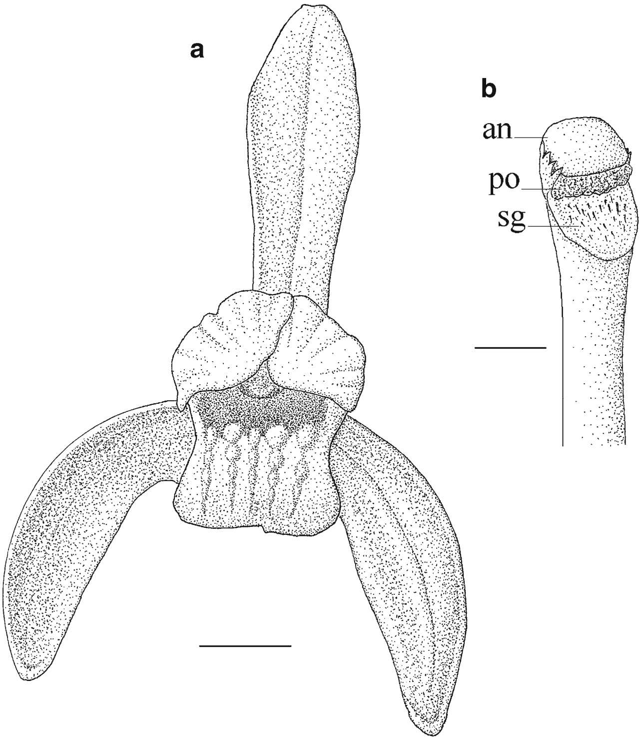

Fig. 9.2 *Isotria medeoloides*. (**a**) Flower, front view; (**b**) Column, oblique ventral view showing pollen mass in contact with the stigma, scale bars = 2 mm. *an* anther, *po* pollen mass, *sg* stigma

from 4 to 12 days in North Carolina, opening 1–2 weeks later than those of *I. verticillata*, where the species are sympatric (Mehrhoff 1983).

Compatibility and Breeding System

In a study of *I. verticillata* in the southeastern United States, Mehrhoff (1983) found that capsule development was initiated in 95% of flowers that were artificially self-pollinated or "cross-pollinated" within a clone, 92% of flowers that were artificially cross-pollinated between clones in the same population, 85% of flowers that were artificially cross-pollinated between populations, 0% of flowers that were enclosed in an insect-proof mesh bag, and 0% of flowers that were emasculated and similarly enclosed (Table 9.2). *I. verticillata* is therefore self-compatible, potentially allogamous, and shows no evidence of autogamy or agamospermy. It is a clonal orchid with a xenogamous–geitonogamous breeding system very similar to that found in *Goodyera* (q.v.) (Ackerman 1975; Kallunki 1981). This kind of breeding system may represent an effective adaptation to pollinator scarcity, but not pollinator absence (Ames 1922; Mehrhoff 1983).

Table 9.2 Percent of flowers forming capsules in *Isotria*

Treatment	*Isotria medeoloides*[a]	*Isotria verticillata*[b]
Open pollination	83 (89)[c]	21
Selfing/geitonogamy	82	95
Cross-pollination	82	85[d], 92[e]
Unmanipulated and bagged	67	0
Emasculated and bagged	0	0

[a] Vitt and Campbell (1997)
[b] Mehrhoff (1983)
[c] Vitt (1991)
[d] Between populations
[e] Between clones in same population

Table 9.3 Data on reproduction in *Isotria* (Mehrhoff 1983)

Variable	*I. verticillata*	*I. medeoloides*
Flower production		
Total number of ramets observed	1,706	70
Number of flowering ramets	470	16
Percentage of ramets that flowered	27.5	22.9
Percentage of flowers lost	7	29
Pollination		
Percentage of all flowers naturally pollinated	19.6	62.5
Percentage of all surviving flowers pollinated	21.0	83.3
Capsule production		
Percentage of all flowers producing mature capsules	5.5	57.1
Percentage of pollinated flowers (capsules initiated) producing mature capsules	43.3	80.0
Percentage of entire population (of ramets) producing mature capsules	1.45	11.40

Mehrhoff (1983) noted some potential advantages of this strategy. First, the genet produces a large number of closely spaced, flowering ramets, which may attract more pollinators. In the case of *I. verticillata*, the percentage of pollinated flowers was higher in larger clones. Second, the development of a number of flowering ramets augments the number of seeds produced per genet. Third, if clones are not isolated, the capacity for interclonal pollen flow and extensive outcrossing are retained. Fourth, the clonal growth habit may permit the survival of the genetic individual despite destruction of some of its ramets. Fifth, a single seed can provide for the founding and expansion of a colony. On the negative side, the massing of flowering ramets may be responsible for a reduction in outcrossing and an increase in predation of the plants and capsules (Mehrhoff 1983). More capsules were lost to predation in *I. verticillata* than in *I. medeoloides* (Table 9.3) (Mehrhoff 1983).

Mehrhoff (1983) chose not to conduct experimental manipulations on *I. medeoloides* because of potentially destructive effects for the small populations in his study area. Vitt and Campbell (1997), however, examined the breeding system in several, relatively large populations in southern Maine, each with over 100 individuals.

In a 2-year study, they observed no fruit production among emasculated and enclosed flowers, indicating an absence of agamospermy (Table 9.2). Fruit set did not differ among enclosed, emasculated plants that were artificially selfed or cross-pollinated (both 82%), indicating that selfing resulted in no decrease in fruit set. Moreover, the difference in fruit set between unmanipulated, enclosed (67%) and unenclosed, control plants (83%) was not statistically significant; autogamy was therefore sufficient to explain the level of fruit set observed in natural populations. Autogamy is also reflected in the development of relatively few, monocolored flowers per genet, small sepals and small, unembellished petals (Table 9.1), an absence of nectar guides and floral scent, a reduced rostellar flap, low levels of pollen and ovule production, and high levels of fruit production (Ornduff 1969; Mehrhoff 1983).

Mehrhoff (1983) attributes the development of autogamy in *I. medeoloides* chiefly to a scarcity of flowers within local populations and an associated reduction in the probability of effective pollen transfer. The median population of *I. medeoloides* produces only one or two widely separated flowers (Mehrhoff 1980). Under such circumstances, autogamy may provide the best chance for seed generation, insuring some seeds are formed even if only one flower is developed (Baker 1955). At the same time, the mechanism of self-pollination in this orchid might not exclude the possibility of insect visitation. Such visits could, however, prove disruptive. A vector that removed pollen and failed to transport it to another flower would seriously deplete or eliminate the reproductive output of a population with only one or two flowers. Selection might therefore operate to minimize such floral visits by reducing the visual appeal of the flowers (Mehrhoff 1983).

Pollinators and Pollination Mechanisms

I. verticillata is pollinated by solitary, short-tongued bees in the Andrenidae (*Andrena ceanothi* Viereck, *A. miranda* Smith, and *A. nasonii* Robertson), Apidae (*Nomada sayi* Robertson and *N.* sp.), and Halictidae (*Augochlora pura* (Fig. 4.2) and *Lasioglossum cressonii* (Robertson) [as *Dialictus creasonii* (Robertson)] (Mehrhoff 1983). Mehrhoff (1983) believes that three other morphologically similar species (*Andrena imitatrix* Cresson, *A. miserabilis* Cresson, and *Nomada superba* Cresson), seen on flowers but not observed to remove pollen, may also be pollinators of *I. verticillata*. All of the species identified as pollinators have a wide geographic range and are known to visit a large variety of spring and summer flowers (Mitchell 1960). Widespread pollinators in combination with vegetative reproduction may facilitate the establishment of new populations.

Mehrhoff (1983) described the mechanics of pollination. The pollinator, searching for nectar, lands on the labellum and enters the floral tube (Fig. 9.1a, c). It squeezes beneath the anther and rostellar flap to the stigma (Fig. 9.1b), which deposits a sticky secretion on the dorsal surface of its thorax. As it backs out under the rostellar flap the anther cap catches on the posterior part of its upper thorax, is forced open, and dumps a mass of mealy pollen onto the stigmatic secretion. If the pollen is transported to another flower and the process repeated, the pollen mass may be deposited on the stigma.

The initial pollinator removes only some of the pollen; the rest remains available for transport by later vectors. The consequences of partitioning the pollen load are discussed below for *Cleistesiopsis*. One result for flowers receiving repeat visits is an increased probability of at least some pollen reaching the stigma of another flower. An obvious disadvantage is that only one pollinator may visit the flower, a particular concern in a species like *I. verticillata* where pollination is uncommon. A second disadvantage is that the number of pollen grains transferred may be insufficient to provide maximum seed production. Naturally pollinated flowers of *I. verticillata* do, in fact, sometimes produce few or no seeds while flowers artificially pollinated with whole "pollinia" produce seeds in abundance (Mehrhoff 1983).

Pollinators visited newly but fully opened fragrant flowers most frequently (Mehrhoff 1983). The number of pollinator visits was influenced by illumination and air temperature; insect activity increased with high values for both factors. Time of day and relative humidity also affected insect activity but to a lesser extent. The percentage of flowers pollinated per clone was positively related to the number of flowers produced per clone, but the probability of pollination was unaffected by a flower's proximity to other flowers in the clone or by its height above the ground.

In *I. medeoloides*, autogamy is initiated shortly after flower opening with a decent of the anther out of the anther socket and an extrusion of pollen from the pollen sacs. In a day or so following anthesis, these combined movements result in contact of the pollen and stigma (Fig. 9.2b) (Mehrhoff 1983; Vitt and Campbell 1997). Mehrhoff (1983) considers that such a mechanism might be easily derived in an already self-compatible species by minor repositioning of the anther and reduction in the rostellar flap.

The flowers of *I. medeoloides* are open, and according to Catling (1984), occasional cross-pollination could be promoted by higher growth rates of pollen tubes from other conspecific plants. However, neither Mehrhoff (1983) nor Vitt and Campbell (1997) observed any insect visitors. A single season survey of all flowering individuals revealed pollinia missing from only four of 52 flowers (Vitt and Campbell 1997), and these might not have been removed by pollinators. According to Mehrhoff (1980), the pollen mass may occasionally break from the anther and fall onto the labellum without contacting the stigma.

Fruiting Success and Limiting Factors

In *I. verticillata*, 21.0% of the flowers that were not lost to predation, abortion, or other factors were pollinated and 43.3% of these produced mature capsules. In *I. medeoloides*, the corresponding numbers were 83.3% and 80%, respectively (Table 9.3). Although the pollination system in *I. medeoloides* is dramatically more efficient than that in *I. verticillata* on a flower-by-flower basis, *I. verticillata* is capable of producing a much larger number of seeds per plant if genets rather than flowers are compared (Mehrhoff 1983). A higher percentage of *I. medeoloides* capsules survive to maturity, but seedling recruitment may be limiting, possibly due to the encroachment of surrounding vegetation in a species apparently adapted to relatively open seral stages (Mehrhoff 1989a, b).

Fresh capsule weight and mean seed weight in *I. medeoloides* did not differ among the treatments of Vitt and Campbell's (1997) study, but overall seed weight was highly correlated with capsule dimensions and with plant size. Larger plants had larger capsules containing a greater seed bulk. In addition, Vitt et al. (1996), in a 10-year study in Maine, found that a successful reproductive effort in 1 year was significantly correlated with a reduction in reproductive status the following year. Such a correlation suggests the presence of resource limitation just as the difference in the percentage of capsule production in artificially pollinated and open-pollinated plants of *I. verticillata* suggests pollinator limitation (Table 9.2). On the other hand, Mehrhoff's (1989a) demographic data provide no evidence for a high cost of reproduction in *I. medeoloides*. He found that 75% of flowering plants in stable populations flowered the following year, whereas only 24% of nonflowering plants did so. In declining but still reproductive populations, the numbers were 59% and 10%, respectively.

I. verticillata produced many more pollen grains and ovules per flower than *I. medeoloides*, but the pollen to ovule ratios (P:O) for these species were 3.9:1 and 3.8:1, respectively, suggesting efficient breeding systems (Mehrhoff 1983). Cruden (1977) predicted lower P:O ratios for autogamous as compared to xenogamous species, a not surprising expectation if it is assumed that relatively more pollen grains need to be produced when their probability of reaching the stigma is less certain. The P:O ratio for *I. verticillata* is exceptionally low for a xenogamous species. Mehrhoff (1983) noted that pollination in *I. verticillata* is a relatively rare event, and the low P:O ratio of this orchid may reflect a heavy reliance on propagation by cloning.

Cleistesiopsis Pansarin and Barros

Cleistesiopsis includes two species, both restricted to North America (Pansarin and Barros 2008). Formerly included in the genus *Cleisteis*, the designation of a new genus is based on an analysis of both molecular and morphological characters (for details, see Cameron and Case 1999; Cameron et al. 1999; Pansarin and Barros 2008; Pansarin et al. 2008).

*Cleistesiopsis divaricata **(L.) Pansarin and Barros (Spreading Pogonia) and** Cleistesiopsis bifaria **(Fernald) Pansarin and Barros (Smaller Spreading Pogonia)***

Distribution and Habitat

C. bifaria is found in the Appalachian Mountains from northern Georgia to West Virginia and on the Coastal Plain from North Carolina to Louisiana (Catling and Gregg 1992). It occurs in pine savannas, open oak–pine woods, and meadows on mountain ridges and grades. Recent molecular data imply that West Virginia and

Table 9.4 Data on *Cleistesiopsis* (Gregg and Catling 2002)

Character	*Cleistesiopsis bifaria*	*C. divaricarpa*
Plant height (cm)	(12)15–46(64)	(15)31–76
Flower number	1–2(3)	1
Dorsal sepal (mm)	24–55 × 2–5	31–65 × 3–5.5
Lateral sepals (mm)	24–55 × 2–5	31–65 × 3–5.5
Lateral petals (mm)	21–36 × 6–12	27–53 × 5–19
Lip (mm)	21–38 × 13–16	26–55 × 18–24
Column (mm)	13–19	21–29
Chromosomes (2*n*)	18	18
Keel		
Relative thickness	Thick	Thin
Width (central lip) (mm)	2.4–2.6	3–4
Depth of groove (mm)	0.3	1.5

North Carolina populations do not comprise a single genetically cohesive group and may be separate species (Smith et al. 2004). *C. divaricata* is absent in West Virginia and the Appalachians but is present in pine savannas on the coastal plain from northern Florida to southern New Jersey (Catling and Gregg 1992).

Floral Morphology

Cleistesiopsis typically bears one or occasionally, in West Virginia populations of *C. bifaria*, two or three terminal, resupinate flowers (Gregg 1989, 1991b; Catling and Gregg 1992). Plants of *C. bifaria* are generally shorter with flowers that are less colorful and about half as large as those of *C. divaricata* (Table 9.4) (Luer 1975; Gregg and Catling 2002). Adjacent flowering and vegetative stems may represent branches of the same rhizome (ramicauls) (Gregg 1989). Both species have three green to reddish brown or purple sepals borne in the same plane. These are often positioned at approximately right angles to one another and perpendicular to the horizontal, tubular corolla (Figs. 9.3a and 9.4a) (Gregg 1989; Catling and Gregg 1992). The latter is formed by the overlapping of two lateral petals with the upturned basal margins of the labellum. The lateral petals have recurved tips and are usually pink but vary from pale pink or white to deep rose with darker magenta veining (Catling and Gregg 1992). The labellum shows similar variation in color with darker rose to purple veining and a fleshy, pollen-mimicking central yellow crest (Figs. 9.3a, c and 9.4a). It is indistinctly three lobed and oblanceolate with a crenulate margin. Nectaries are absent (Catling and Gregg 1992). The column (Figs. 9.3b and 9.4b) is free, included, and concealed by the tubular corolla. Slightly curved and ragged at its tip, it bears a terminal, incumbent anther with a hinged anther cap (Catling and Gregg 1992). The anther produces two yellow, soft, and mealy pollen masses with an estimated total of 5,000–20,000 loosely coherent tetrads. As in *Isotria*, true pollinia are absent, and the production of

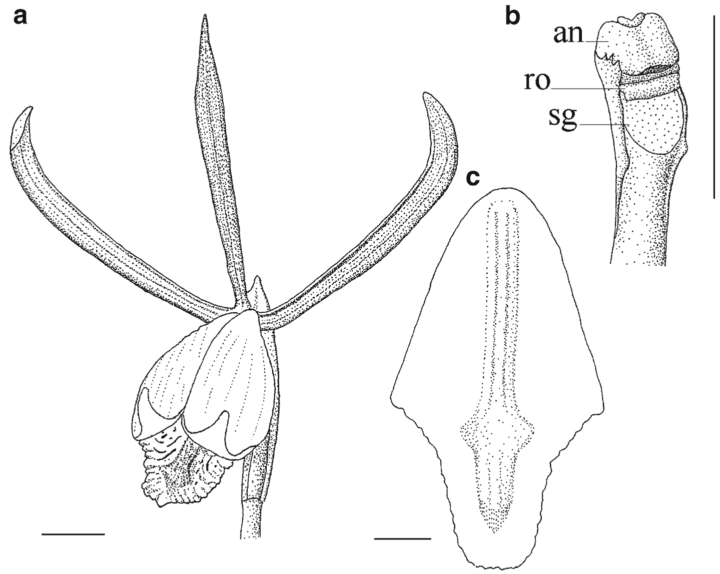

Fig. 9.3 *Cleistesiopsis*. (**a**) *C. divaricata*, flower, front view, scale bar = 10 mm; (**b**, **c**) Generalized figures for *C. divaricata* and *C. bifaria*; (**b**) Column, oblique ventral view with small protrusion on lower edge of anther cap, scale bar = 10 mm; (**c**) Lip, from above, flattened, showing position of keel, scale bar = 5 mm. *an* anther, *ro* rostellum, *sg* stigma

granular pollen is considered a secondary reversion (Gregg 1989; Pridgeon et al. 2003). The stigmatic area beneath the column is separated from the anther by a truncate flap of rostellar tissue (Figs. 9.3b and 9.4b) (Catling and Gregg 1992). Viscidia are absent (Dressler 1993). Receptive stigmas secrete a sticky, stigmatic fluid and remain receptive for a minimum of 8 days following anthesis in West Virginia populations of *C. bifaria* (Gregg 1989). Unpollinated flowers of this species usually remain open and attractive for about 10–12 days.

Compatibility and Breeding System

Gregg (1989, 1991a, b) studied the pollination biology of *C. bifaria* at an acidic mountain meadow site in northeastern Barbour County, West Virginia and *C. bifaria* and *C. divaricata* at Big Island Savanna in Brunswick County, North Carolina, where flowering specimens of the two species occurred in approximately equal numbers. Compatibility and breeding system data are available only for the *C. bifaria* population from West Virginia. Autogamy was absent, distant neighbor crosses (5–30 ft separation) resulted in 93–100% fruit set, and artificial intrafloral selfing and geitonogamy produced 89–100% fruit set. Average seed weight per capsule was not significantly different among the last three treatments, but intrafloral

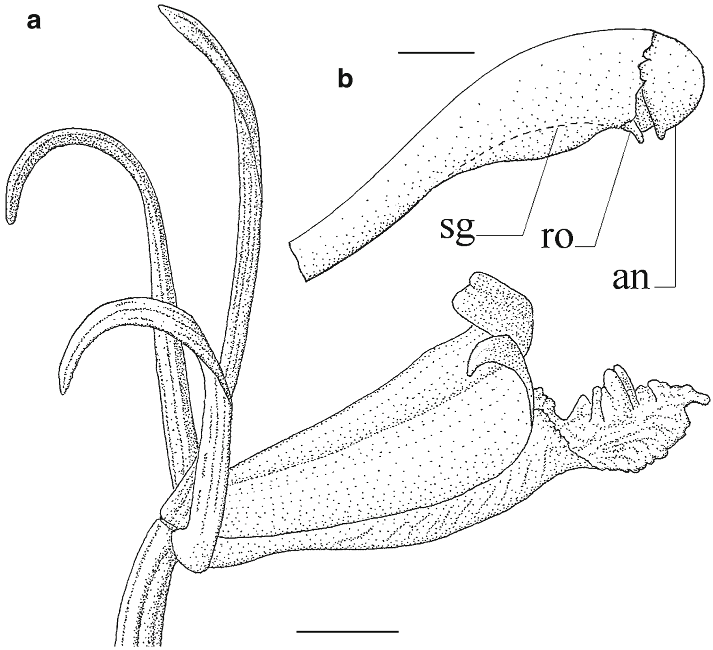

Fig. 9.4 *Cleistesiopsis*. (**a**) *C. bifaria*, flower, side view (sepal orientation variable); (**b**) Generalized column for *C. divaricata* and *C. bifaria*, side view with small protrusion on underside of anther cap; scale bars = 5 mm. *an* anther, *ro* rostellum, *sg* stigmatic area

selfing and geitonogamy reduced the percentage of healthy embryos. Vegetative propagation by root shoots is common (Gregg 1991c).

The two species of *Cleistesiopsis* are interfertile and artificial crosses between them did not result in lower seed production than crosses within the parental species (Catling and Gregg 1992). However, naturally occurring hybrids were not observed.

Pollinators and Pollination Mechanisms

Although insect visits were rare, bumblebees (*Bombus* spp.) in North Carolina and both bumblebees and leaf-cutter bees (*Megachile* spp.) in West Virginia are the primary pollinators of *Cleistesiopsis* (Gregg 1989, 1991b). The bee, usually a worker, first lands on the labellum and crawls toward its base. As it passes beneath the column, its head and dorsal thorax touch the stigma and are smeared with sticky, stigmatic fluid. On backing out of the flower its back contacts a small protrusion on the underside of the anther (Fig. 9.4b). The hinged anther cap is pulled open and clumps of loosely coherent tetrads are dumped onto the stigmatic fluid covering the bee's dorsal thorax and head. The anther cap then snaps back to its original position. On later floral visits, the dorsal thorax and head contact the stigma and pollen is transferred. The process is essentially identical to that found in *Pogonia ophioglossoides* and *I. verticillata* (Gibson 1905; Thien and Marcks 1972; Mehrhoff 1983), except that tetrads rather than monads are transferred (Gregg 1991b).

The pollination mechanism in *Cleistesiopsis* can dispense an average of 16 clumps of tetrads before the anther is emptied; thus, a number of successive pollinators can receive pollen from a single flower (Gregg 1991a, b). Gregg (1991a) examined the effect of successive pollen dumps on capsule set, capsule development, seed number, individual seed weight, and percentage of healthy embryos per fruit. She found that a flower's first three pollen dumps, each of which contained more tetrads than later dumps, produced heavier capsules with more seeds. Some first dump pollinations contained enough tetrads to produce maximum seed set, and a pollen to seed ratio of 4, very low for a nonautogamous breeding system, is probably related to the energy efficient production of pollen clumps large enough to fertilize most of the ovules in an ovary (Cruden 1977; Gregg 1989). Pollination with later, lower dosage pollen releases did not reduce percent capsule set, percent of set capsules achieving ripeness, individual seed weight, or the percentage of healthy embryos. Lower dosage dumps were, however, unexpectedly associated with increased energy expenditure in the amount of pericarp produced per seed.

Flowers which received high pollen dosages all faded within 5–7 days, while less than half of the flowers receiving low pollen dosages faded in this time period, and many persisted as long as unpollinated flowers (Gregg 1991a). More sparsely pollinated flowers thus remained attractive to pollinators and sometimes received additional pollen to increase their seed output.

The pollination mechanism in *Cleistesiopsis* probably also enhances paternal success. When a single flower releases pollen in more than one package, the number of pollinators is increased along with the chances of successful pollen transport should any one vector fail to deliver pollen to a receptive stigma. Pollen would also be distributed to a larger number of stigmas and variation in the sired offspring would be increased (Catling and Catling 1991; Gregg 1991a).

Harder and Thomson (1989) reported an analogous pollen dispensing relationship in *Erythronium grandiflorum* Pursh. Here, the amount of pollen removed from the flower was inversely related to the proportion deposited on subsequently visited stigmas. According to this model, individual plants would improve their total pollen dispersal when the number of pollen-removing visits increased and the amount of pollen removed by each pollinator was limited.

A disadvantage of this pollination strategy is that when pollinators are limiting the chance visitation of a single pollinator would result in only part of the pollen load being transported (Gregg 1991a). Moreover, although it might result in an increase in variability among the offspring (Ter-Avanesian 1978), the presence of relatively few pollen grains on a stigma receiving later, lower pollen dosages would likely reduce competition among grains for ovules along with the advantageous effect of such competition on fitness (Mulcahy et al. 1983; Gregg 1991a).

The flowers of *Cleistesiopsis*, with their pollen-mimicking, fleshy labellar crest, have often been assumed to function as bee-food mimics (Gregg 1989, 1991b). As such they would attract naïve bees and achieve pollination by deception much as in

Calopogon tuberosus, *Arethusa bulbosa*, *P. ophioglossoides*, and *Calypso bulbosa* (Heinrich 1970, 1975, 1979a; Thien and Marcks 1972; Ackerman 1981; Boyden 1982; Gregg 1989). However, in addition to *Cleistesiopsis* tetrads on the head and dorsal thorax, Gregg (1991b) found them in the corbicular loads (pollen baskets) of 22 worker bumblebees. Thousands of *Cleistesiopsis* tetrads representing one-third to nearly 100% of the corbicular masses were found on eight bumblebees from North Carolina and one from West Virginia. Several hundred were found in the corbiculae of four others from North Carolina and one from West Virginia. In all, 42% of the bees captured that were carrying pollen of *Cleistesiopsis* had deliberately stored significant amounts of it in their corbiculae. Pollen was removed from the frons and scutum and transferred to the hind tibial corbiculae by grooming. Although most retained some tetrads on their dorsal thorax, five of the bumblebees in North Carolina and one in West Virginia that carried tetrads in corbicular masses had none on their dorsal thorax or head. Apparently, all the pollen is therefore sometimes lost to collecting, but such loss may be offset by an increase in the number of visiting bees seeking a genuine reward with a consequent overall increase in capsule set.

The type of bees carrying and actively collecting pollen differed in North Carolina and West Virginia. In North Carolina, *Cleistesiopsis* pollen was found on the dorsal thorax of three species of *Bombus*, but only *B. pennsylvanicus* had large numbers of tetrads in its corbiculae. Of 21 bees captured carrying *Cleistesiopsis* pollen, 16 were *B. pennsylvanicus*; nine of these had just visited *C. divaricata* and four, *C. bifaria*. The remaining three had most recently entered other flowers, including those of *P. ophioglossoides* and *Calopogon pallidus*. Smaller numbers of *Cleistesiopsis* tetrads, one to 27, were also present in the corbiculae of *B. griseocollis*, *B. impatiens*, and four other *B. pennsylvanicus* and may have been accidentally transferred while grooming.

In West Virginia, *Cleistesiopsis* pollen was observed on the dorsal thorax in four species of *Bombus* and at least one *Megachile*, but only single individuals of *B. fervidus* and *B. vagans* carried large numbers of tetrads in their corbiculae. In contrast to North Carolina, only one of the five examined individuals of *B. pennsylvanicus* carried any *Cleistesiopsis* tetrads and none were present in its corbicular load.

If large corbicular loads consisting of hundreds or thousands of *Cleistesiopsis* tetrads are considered, the frequency of bees collecting pollen was significantly higher at the North Carolina than at the West Virginia study site. Following attraction by long-distance cues, bees were more likely to enter a flower at the North Carolina than at the West Virginia location. Inspected flowers of *C. bifaria* in West Virginia had no detectable odor, but flowers of both *C. bifaria* and *C. divaricata* produced scents at Big Island Savannah in North Carolina (Gregg 1991b; Catling and Gregg 1992). The odors apparently differ in the two species. Flowers of *C. bifaria* are said to smell like vanilla and those of *C. divaricata*, like Easter lilies or daffodils (Catling and Gregg 1992). There was, however, no difference in the pollinator response to the floral odors of these orchids at the savannah site in North Carolina (Gregg 1991b).

Floral fragrances may be associated with reward and probably provide a close range recognition signal that stimulates the bee to enter the flower (Heinrich 1975, 1979b; Nilsson 1979; Boyden 1982). Competition for pollinator service among a variety of pink flowered species at the North Carolina site, not present at the West

Virginia site, may have influenced selection for floral fragrance (Gregg 1991b). Bees learn to recognize scents more rapidly than colors or flower shapes: they are correctly recognized 97–100% of the time following only a single exposure (Kolterman 1969). Colors, on the other hand, are recognized at the 90% level only after three to five visits (Menzel 1967) and shapes require at least 20 visits (Wehner 1967). An absence of floral odor in the West Virginia population may explain why most associated bees have failed to discover the presence of a pollen reward (Gregg 1991b).

The pollination of *Cleistesiopsis*, then, is not entirely by deceit. Bumblebees regularly and actively collected its pollen, packing the grains into their hind tibial corbiculae. Since bees discard unwanted pollen (Heinrich, personal communication in Gregg 1991b), we may infer that *Cleistesiopsis* pollen is transported back to the nest as a food resource. However, the marked difference in the behavior of bees at the two sites implies that *Cleistesiopsis* depends largely on a strategy of deceit in West Virginia and more on a strategy of reward in North Carolina (Gregg 1991b).

One explanation for a difference in reproductive strategies may be morphological. West Virginia plants sometimes produced two flowers per stem or several simultaneously flowering stems from the same clone (Gregg 1991b). No instances of the former and very few of the latter were encountered in 7 years of observation in North Carolina. Thus, geitonogamy would be more likely to occur in the West Virginia population, and we have seen that artificial geitonogamous crosses reduce the percentage of healthy embryos (Gregg 1989, 1991b). Provision of a reward might be expected to increase geitonogamous pollination and reduce the overall fitness of the small West Virginia population, a population that produces only a few hundred, scattered flowers per year. Ackerman (1986) regarded a reduction in geitonogamy as one of the possible advantages of deceptive pollination.

Flower color, pattern, and sepal orientation can be highly variable in *C. bifaria*, and this lack of uniformity may represent an additional adaptation to deceit pollination at the West Virginia site (Gregg 1991b). As noted already, Heinrich (1975, 1979 a), Boyden (1982), and Little (1983) believe that such variation increases the time required for pollinating insects to learn to recognize the flowers. In North Carolina, pollinators may show a strong preference for scented flowers, and the similar variation in flower color and pattern observed here might be less important (Gregg 1991b).

The mechanism by which these two highly interfertile species are maintained has yet to be determined. A hypothesis that differences in column length (Table 9.4) play a role in reproductive isolation has not been substantiated by the pollinator data presently available (Catling and Gregg 1992). However, phenological differences may contribute. Gregg (1991b) noted a difference in peak flowering times at the North Carolina site: fresh flowers of *C. bifaria* were mostly gone by the time the flower buds of *C. divaricata* began to open.

Fruiting Success and Limiting Factors

In the West Virginia, population of *C. bifaria* about 50–74% of open-pollinated plants produced capsules over a 7-year period (Gregg 1989). This is a markedly lower level of fruit set than that observed in hand pollinated plants (89–100%) and suggests the possibility of pollinator limitation (Gregg 1989, 1991a). Other characteristics of *Cleistesiopsis* suggesting pollinator limitation include an extended period of female receptivity in both unpollinated flowers and those receiving low pollen dosages and the ripening of capsules containing relatively few seeds (Willson and Schemske 1980; Gregg 1991a). In addition, anthesis is timed to occur in mid to late June in the West Virginia population of *C. bifaria* when few other plants are in bloom (Gregg 1989).

Despite adaptations to avoid the deleterious effects of geitonogamy in *C. bifaria*, such crosses undoubtedly occur in a plant that produces more than one flower per stem and a number of simultaneously flowering ramets. However, as Mehrhoff (1983) noted for *Isotria,* the potentially disadvantageous effects of such a breeding system might be compensated, where pollinator visitation is a significant limiting factor because the aggregation of flowering stems increases the probability that pollinator visits will occur, however brief or uncommon (Gregg 1989).

Cleistesiopsis bears a maximum of about 20,000 seeds per capsule rather than the hundreds of thousands or millions produced by many other orchids (Gregg 1991a). Moderate seed yields may be beneficial in a plant like *Cleistesiopsis* with a small amount of storage tissue. Less drain occurs on the nutrient resources of the plant at a time when capsule maturation and the production of next year's primordia are proceeding simultaneously. An absence of within season resource limitation is suggested by low levels of capsule abortion regardless of pollen dosage (Willson and Schemske 1980; Gregg 1991a) and an absence of any second year reduction in flowering and fruiting in a large sample of plants that produced capsules the first year (Gregg 1989). Long-term studies are needed to examine the possibility of lifetime resource limitation.

Fruit set over 50% has been reported in other nonrewarding orchids (Steiner et al. 1994; Nazarov 1995), and further study of the pollination dynamics in such species may help to explain the frequency of seemingly inefficient deceptive pollination systems in this family (Lipow et al. 2002). Gregg (1991b) recommends further investigation of the relationships between insects and nectarless orchids with mealy or soft pollinia currently presumed to employ a deceit strategy to achieve pollination.

The limiting effect of herbivore damage was examined in West Virginia (Gregg 1989). The damage was minimal in 5 years of a 7-year study. In the two remaining years, grazing, most likely by deer, had a significant impact, destroying about 30% of the plants 1 year. Among flowers that set fruit that year about 85% produced mature capsules. Insect damage, deer predation, and fungal infection accounted for most of the losses.

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Glossary

Abdomen In insects, the posterior segment of the body.

Achlorophyllous Lacking chlorophyll and therefore non-photosynthetic and dependent on fungi for nourishment.

Acropetal A process that begins at the base and proceeds toward the apex.

Acuminate Tapering gradually into a slender, extended point.

Acute Margins (e.g., of leaves or flower parts) meeting at an angle of less than 90°, the tip distinct but not extended.

Adaptation The modification of an organism, a character, or a habit to fit the environment.

Adaxial The side of an organ (leaf, bract, or floral part) directed toward the main axis, normally the inner or upper surface.

Agamospermy A form of apomixes in which seeds and embryos are produced asexually from a diploid cell with no fertilization; cf. pseudogamy.

Allogamy Cross-fertilization or outbreeding.

Allozyme In our sense, the variants of an enzyme genetically coded by different alleles of a single gene as distinguished through electrophoretic analysis of enzyme phenotypes.

Aneuploidy (Aneuploid) The occurrence of one or more extra or missing chromosomes.

Angiosperms A class of vascular plants that characteristically produce seeds enclosed in an ovary, flowering plants.

Annual A plant that germinates from a seed, grows, flowers, produces seeds, and then dies within a single year; cf. perennial.

Anther The part of the stamen or male organ that produces pollen.

Anther cap A structure covering the pollinia in orchids.

Anthesis Specifically the opening of the stamens, but more generally, the stage when the flower bud opens and the period during which the flower is open and functional.

Apex (pl. apices, adj. apical) The tip or terminal point of a perianth part, leaf, bract, or stem.

C.L. Argue, *The Pollination Biology of North American Orchids: Volume 2: North of Florida and Mexico*, DOI 10.1007/978-1-4614-0622-8,

Apiculate Ending abruptly with a short point at the apex.

Apogamy Reproduction of the sporophyte directly from the female gametophyte without fertilization by male cells.

Apomixis (apomictic) As used here, the production of seeds without fertilization.

Arcuate Bent or curved.

Asexual Reproduction by vegetative means.

Attenuate Gradually tapering to a slender point; cf. acuminate.

Auricle Projecting lobes at the base of a leaf, bract, or petal; in orchids often a small, lateral outgrowth of the column, possibly part of a sterile anther.

Autogamy Intrafloral self fertilization without the aid of a pollen vector.

Auto-pollination Self pollination without the aid of a pollen vector.

Bifid Forked or cleft into two parts.

Bilaterally symmetrical Shaped so that only a cut through one plane will divide the object into matching halves.

Biotype Organisms of a population sharing a specified genotype or a particular characteristic in one environment that is not shared by other populations of the same species in other environments.

Bursicle (or bursicula) A sac-like or purse-like structure enclosing a viscidium in some Orchidaceae.

Buzz pollination A method of pollen collecting based on the vibration of flowers by bees; the vibrations are based on a “shivering” of the bee’s flight muscles and cause the pollen to drop out of the flower onto the body of the bee.

Calcareous Composed largely of calcium carbonate or growing on a substrate composed chiefly of calcium carbonate (limestone, chalk).

Callus A fleshy or other protuberance, for example, of the labellum.

Capsule A dry, dehiscent fruit opening along one or more sutures.

Caudicle A slender extension of the pollinium usually composed of pollen and viscin, connecting the pollinia to the stipe or, in the absence of a stipe, directly to the viscidium; derived from the anther.

Chasmogamous (chasmogamy) A plant with flowers that open to expose the reproductive organs.

Ciliate Fringed with fine hairs or cilia.

Classification The placement and delimitation of taxa within divisions of a hierarchical system.

Clavate Shaped like a club, thick above and tapering to the base.

Claw (clawed) The narrowed basal part of a sepal, lip, or other petal in some plants.

Cleistogamy Self-pollination (autogamy) in closed flowers.

Clinandrium The part of the column under or surrounding the anther; the anther bed.

Clone A population of genetically identical individuals derived by asexual reproduction.

Clypeus A shield-like plate on the front of an insect’s head.

Column A centrally positioned organ of the orchid flower formed by the partial or complete fusion of the male and female parts (= gynostemium).

Column-foot A ventral extension of the column base in some orchids that is attached to the labellum.

Column-wing Projections on both sides of the column in some orchids thought to possibly represent sterile anthers.

Competition Activity occurring when two or more individuals belonging to the same or different species vie with one another for some limited resource.

Connate United to a part or organ of the same kind as one petal to another; cf. adnate.

Connivent Parts or organs that are convergent and touching but not fused.

Conspecific Two or more organisms belonging to the same species.

Convergence Process involving the independent evolution of a similar character in two species or the occurrence of a similar character in a set of species that was not found in their common ancestor, a convergently evolved character.

Corbicula (pl. Corbiculae) A smooth area on each hind tibia of a bee that is edged with a fringe of stiff hairs and functions in the collection and transport of pollen, the pollen basket.

Cordate Heart-shaped, indented at the base.

Corm A short, enlarged fleshy base of a stem, usually underground or near the surface.

Corolla Collective term for all the petals of a flower comprising the inner whorl of the perianth when a calyx is present.

Cost of reproduction The energy invested in the production of offspring.

Crenulate A margin with very small, rounded teeth, diminutive or crenate.

Crest An elevated line or ridge, usually on some part of a flower.

Crisped (crispate) A margin that is irregular, wavy, or ruffled.

Cross-fertilization Union of an egg with sperm from a different plant following cross-pollination.

Cross-pollination Transfer of pollen from the anther of one plant to the stigma of another.

Deception Simulation of non-existent rewards to attract pollinators.

Deciduous Leaves or other plant parts shed naturally prior to winter or the dry season or at a prescribed developmental stage.

Deflexed Bent or turned sharply downward.

Dehiscence The spontaneous splitting open of certain plant organs (e.g., anthers, fruits) along prescribed lines to discharge their contents.

Demographic data Data on the dynamic balance of a population especially with regard to age structure, density, and capacity for expansion of decline.

Denticulate Diminutive of dentate, finely toothed.

Derived A relative term referring to a feature that has evolved from another.

Diploid Possessing two sets of chromosomes in each somatic cell nucleus, indicated as “2n.”

Distal The part of a structure farthest away from the structures point of attachment; cf. proximal.

DNA (Deoxyribonucleic acid) the stuff of which genes are made and the genetic code is written.

Dolabriform In the shape of an axe-head.

Dorsal The outer or underside of leaves, bracts, and floral parts, synonymous with abaxial.

Drift See genetic drift.

Emarginate Having a notch at an obtuse apex, usually in reference to leaves and floral parts, synonymous with retuse.

Embryo In plants a young individual in early stages of development following differentiation of the proembryo into suspensor and embryo.

Embryo sac The female gametophyte in angiosperms, containing the egg cell and a number of other haploid cells.

Endemic An organism with a restricted distribution native to a certain geographic area.

Entire A margin that is continuous, smooth, and undivided, lacking lobes or teeth, said of leaves and perianth parts.

Entomogamy, entomogomous The floral syndrome based on insect pollination.

Entomophilous Pollinated by insects; cf. entomogamy.

Ephemerals A plant or flower that is short-lived.

Epichile The distal (terminal) part of a labellum differentiated into a hypochile, (mesochile), and epichile.

Epiphytic The condition of a plant attached to and growing on another plant or object but not parasitic.

Erose A margin that is irregularly eroded, notched, or jagged, as if it had been chewed.

Ethological isolation Barrier to pollen exchange based on behavioral differences of the pollinators.

Evolution A process of genetic change in biological populations in response to environmental changes (see natural selection).

Extrafloral nectaries A sugar secreting gland outside the flower.

Fecundity The production or the capacity to produce offspring in abundance.

Female fitness The differential contribution of the female to the production of viable offspring; the relative reproductive success of genes contributed by ovules as compared to pollen.

Female function Seed production.

Fen An open plant community on flat land generally growing on alkaline or neutral wet peat.

Fidelity A constancy where a pollinator is particularly attracted to a narrowly adapted type of flower.

Filiform Thread-shaped.

Fitness A measure of differential reproductive success among members of the same species in their contribution to the gene pool of the next or to succeeding generations.

Food-flower mimic A usually unrewarding flower that mimics the appearance of flowers that provide a reward such as nectar and/or pollen.

Founder effect (Founder) The proposition that a small, pioneer community established in genetic isolation from the main population will possess only a small fraction of the genetic variation present in the parent population.

Frons A segment of an insect's cranium usually positioned between and below the antennae and above the clypeus; the anterior, uppermost part of the head of an insect, forehead.

Fugacious Early withering or dehiscence of a plant part.

Galea Part of an insect's maxilla, the outer lobes.

Gametes Sex cells, eggs and sperm.

Geitonogamy Fertilization of a flower with pollen from a different flower on the same plant or clone.

Gene flow In plants the movement of genes from one population to another conspecific population by cross-pollination.

Gene pool All the genes present in a breeding population or species at one time.

Genet A term describing a single plant comprised of a number of vegetatively produced, genetically identical stems (ramets), a clone.

Genetic drift Changes in gene frequency entirely as a result of chance rather than natural selection; most likely to occur in very small populations where the probability of non-random mating is high.

Genotype The genetic makeup of an individual or the shared genetic makeup of a group of individuals as contrasted with physical appearance (phenotype).

Gland An organ comprised of one or more cells that secretes specific chemical compounds.

Glossa A tonguelike mouthpart in an insect.

Gynostemium See column.

Hamulus A kind of stipe derived from an upwardly curved, distal extension of the rostellar apex.

Hand pollination The transfer of pollen to stigma by human hand, artificial as opposed to natural or open pollination.

Herbaceous Pertains to plants with little woody tissue, particularly if the above ground parts endure less than a year.

Hermaphroditic A flower having both male and female reproductive structures or a plant with only perfect flowers.

Heterotrophic An organism that is not able to synthesize food and receives its nourishment from the consumption or absorption of organic substances.

Hexaploid An organism having six sets of paired chromosomes in each nucleus, termed "6n."

Humus The dark organic matter in soil derived from the decomposition of plant or animal matter.

Hybrid An individual produced from genetically different parents; as used here from different species, genera, or other taxonomic groups.

Hybridization The production of hybrids by natural or artificial means.

Hypochile The basal part of a lip that is divided into two or three parts, the hypochile, (mesochile), and epichile.

Inbreeding Selfing or breeding with closely related individuals.

Inbreeding depression A decline in vigor in normally outcrossing species resulting from inbreeding and problems associated with homozygous recessive lethals and semilethals.

Incumbent Resting on or bending downwards, as the anthers of many orchids that bend downward during development.

Incurved Bent or curved toward the center of an organ.

Inflorescence A cluster of flowers or flowering branches that may include bracts but not foliage leaves.

Integument Protective cell layers enclosing the nucellus of the ovule, maturing to become the seed coat.

Intergeneric Between genera.

Introgression (Introgressive) The transfer of genes from one species to another through recurrent backcrossing of a fertile hybrid with one of its parents.

Involute Edges rolled inward or toward the upper surface; cf. revolute.

Labellum (1) The median petal in an orchid flower, usually differing in size, shape, and/or color from the others, also known as the lip. (2) a fleshy pad terminating a fly's proboscis.

Lamella (pl. lamellae) A thin layer, plate, or elevation.

Lanceolate Longer than wide with the maximum width toward the base and tapering toward the apex.

Larva An independent, immature feeding stage of an insect, usually in reference to one undergoing complete metamorphosis.

Lateral Pertaining to a structure positioned on either side of a medial line dissecting a flower into two halves; e.g., "lateral petal."

Lax Loosely arranged, not dense or crowded.

Ligulate Shaped like a tongue or strap.

Lip See labellum.

Magnet species The idea that species with flowers very attractive to pollinators can increase the local abundance of pollinators and thereby increase visitation to sympatric species having less attractive or non-rewarding flowers.

Male fitness The differential contribution of the male to the production of viable offspring; the relative reproductive success of genes contributed by pollen as compared to ovules.

Male function Pollen donation.

Massula (pl. Massulae) A mass or packet of pollen grains in orchids having sectile pollinia.

Maxilla (pl. Maxillae) A component of the insects mouthparts lying just behind the jaws. In bumblebees it forms a sheath surrounding the proboscis.

Medial Located in or near or pertaining to the middle of something.

Median In a series of recorded values that quantity having an equal number of observations on either side of it or an average of two middle values when the number of values recorded is an even number.

Megagametophyte The female gametophyte or haploid generation.

Megasporocyte A special cell of the megasporangium also known as the megaspore mother cell that undergoes reduction division or meiosis to produce four haploid megaspores within the ovule.

Mentum A chin-like extension at the base of some orchid flowers resulting from a fusion of the lateral petals with the base of the lip or column (column-foot).

Mesic Related to or adapted to a moderately moist environment.

Mimicry A resemblance between two unrelated species advantageous to one (Batesian mimicry) or both (Mullerian mimicry) and therefore favored by natural selection.

Monad(s) A single pollen grain, not attached to other grains as in tetrads.

Monocotyledons A presumably monophyletic subclass of the angiosperms, sometimes placed in class Liliopsida, having embryos with a single cotyledon (absent in most orchids), narrow parallel-veined leaves, flower parts usually inserted in threes, a fibrous root system with adventitious roots, a stele with scattered vascular bundles, and phloem plastids with deltoid protein inclusions.

Morphology The study of form in organisms, especially external features.

Mycetophilous Fungus loving.

Mycoparasites (Mycoparasitic) Parasites that derive their nutrition from fungi rather than dead material.

Mycorrhiza An association between the roots of higher plants and an infecting fungus, often regarded as symbiotic.

Nectar guide Structures or contrasting colors, sometimes in the ultraviolet range, on the lip or other petals that indicate to a flower visitor where to search for the nectar.

Nectar tube A variously shaped but often more or less cylindrical tube derived from the lip and/or other perianth parts that may or may not contain nectar.

Nectary A gland that secretes nectar, often part of the lip in orchids.

Nodding To droop or bend downward.

Non-resupinate Flower orientation in orchids with the lip uppermost.

Notum In an insect, the dorsal part of each thoracic segment.

Nucellus In seed plants, the diploid tissue lying between the embryo sac and the integuments.

Oblanceolate Longer than wide with the maximum width above the middle and tapering to the base and the tip; cf. lanceolate.

Oblong Longer than wide with nearly parallel sides.

Obovate Longer than wide with an outline like that of an egg, the broader end apical; cf. ovate.

Obtuse Blunt or rounded at the tip, the sides meeting at an angle of more than 90°.

Operculate Having a caplike or lid-like structure; in anthers sometimes said of the shriveled, often dehisced anther wall following anthesis.

Orbicular More or less roundish or circular.

Outcrossing As used here, a cross between two nonclonal individuals of the same species.

Ovary The basal part of the pistil which contains the ovules and develops into the fruit (in orchids a capsule).

Ovate Having an outline like that of an egg, the broader end basal (below the middle); cf. obovate.

Oviposition The laying or deposition of eggs especially by means of an ovipositor.

Ovoid A solid in the shape of an egg.

Ovule A sporangium containing the female gamete and other haploid nuclei in a central embryo sac, the surrounding nucellus or megasporangium, and one or two layers of protective integument; develops into a seed following fertilization.

Pandurate Constricted in the middle with rounded ends, violin-shaped.

Papilla (pl. papillae), papillose A small rounded projection or nipple-like structure, usually from an epidermal cell, often considered a type or trichome.

Parthenocarpy (parthenocarpic) The development of the ovary of a flower into a fruit without fertilization. Fruits that develop parthenocarpically are typically seedless.

Pedicel The stalks immediately beneath single flowers attaching them to the main axis (peduncle) of an inflorescence.

Peduncle The primary stalk of an inflorescence or of a solitary flower.

Perennial A plant that survives year after year.

Perianth The sepals and petals or tepals of a flower.

Pericarp The wall of the fruit derived from the wall of the matured ovary.

Petal An individual segment of the corolla generally positioned just inside the sepals when both are present, often colorful and showy.

pH A measure of acidity or alkalinity based on the hydrogen ion concentration; a reduction in pH represents a decrease in alkalinity and an increase in acidity.

Phenology The science studying the influence of seasonality on the recurrence of such annual phenomena of animal and plant life as bird migration, budding etc.

Phylogenetics/Phylogeny A reconstruction of the relationships and evolutionary history of organisms.

Pollen Initially one-celled microspores produced by microsporogenesis in anthers, subsequently giving rise to the male gametophytes.

Pollen-tube An outgrowth from the pollen grain that usually emerges through an aperture in the pollen wall and grows through the styler tissue toward an ovule where it enters and releases its gametes.

Pollinarium (pl. pollinaria) A functional unit of pollen transfer usually consisting of pollen packets (pollinia), a stalk, and a viscidium.

Pollination biology Study of the mechanisms and processes involved in the pollination of flowers.

Pollinator limitation The condition in which the number of pollinator visits limits the number of seeds or fruits produced.

Pollinium (pl. Pollinia) A coherent and more or less compact mass of pollen grains.

Prementum In insects, the basal component of the distal part of the labium (prelabium) which bears laterally a pair of segmented labial palpi.

Primitive An ancestral or plesiomorphic feature; the term is relative and the same character may be primitive in one group and derived or advanced in another.

Proboscis (pl. Proboscises) In insects, elongate, beaklike mouthparts adapted for sucking or piercing.

Protandrous (Protandry) Condition where the anthers dehisce before the pistil in the same flower reaches maturity.

Proximal Denoting the part of an organ situated nearest to the point of origin or attachment to the main body.

Pseudocopulation A type of mimicry in which flowers resemble female insects and the males, attracted by sexual instincts, attempt to copulate with them.

Pseudogamy Pollination stimulates agamospermy but no fertilization occurs.

Pseudopollen Pollen-imitating structures on the flower that attract pollinators by deception or nutritive, granular, pollen-like cells offered as a floral reward.

Pubescent Provided with short hairs, especially when soft and down-like.

Raceme A simple, indeterminate inflorescence with pedicelled flowers on a common, more or less elongate central axis.

Racemose In racemes or having a raceme-like inflorescence.

Ramet A stem and apparently individual plant belonging to a genet or clone.

Recombination A major source of variation that arises during meiosis through crossing over and the reassortment of entire chromosomes.

Recurved Curved backward or downward.

Reflexed Abruptly bent downward or backward.

Reproductive success The relative production of fertile offspring by a particular genome.

Resource limitation Limitation of the number of seeds or fruits produced as a result of insufficient nutrients, water, or light.

Resupinate Twisting or bending of the orchid pedicel or ovary so that the lip, which is uppermost in the bud, is positioned on the lower side when the flower is mature.

Revolute Edges rolled downwards or toward the lower surface.

Rhizome An indeterminate, prostrate or subterranean stem that in plants such as sympodial orchids is made up of the bases of successive aerial shoots.

Ringent Gaping or opening wide.

Rostellum A part of the median stigma lobe in orchids that separates the stigmatic surface from the anthers and that produces a glue that attaches the pollinia to the pollinator.

Saccate Deeply concave, pouch- or sac-shaped.

Sapromyophily (Sapromyophilous) The floral syndrome associated with pollination by carrion- and dung-flies attached by dark colors and putrid odors.

Scape A leafless flowering stalk (peduncle) arising from the ground and carrying a single flower or inflorescence.

Scapose Having a scape or scape-like structure.

Scutellum In insects, the posterior part of the second and third dorsal thoracic segments, the mesonotum and metanotum; usually used in reference to the mesonotum as it is much reduced on the metanotum in most insect groups.

Scutum In insects, the second and largest part of the upper surface of a thoracic segment. It is preceded by the prescutum and followed by the scutellum.

Sectile Referring to soft, granular pollinia that are subdivided into small packets (massulae) interconnected by elastic threads.

Secund Directed to one side only, as with flowers arranged in a row on one side of the stem.

Self-compatible Capable of producing seed by self-pollination.

Self-incompatible (Self-sterility) Incapable of producing seed by self-pollination.

Self-pollination The transport of pollen from the anthers to the stigma of the same flower, a flower on the same inflorescence, or a flower on the same genet.

Sepal An individual component of the outermost whorl of the perianth.

Sessile Attached without any kind of stalk.

Sinuous Wavy or curving in alternate directions.

Spatulate (Spathulate) Having a broad, rounded apex tapering to a narrow base; spatula-shaped.

Spike A type of simple, indeterminate inflorescence with sessile flowers borne on a more or less elongate common axis.

Spur A hollow tubular or sac-like extension of the labellum or other floral part that may or may not contain nectar.

Stamen The male or pollen producing organ of the flower typically comprised of an anther, filament, and connective, but variously modified in orchids.

Stigma The part of the carpel on which pollen lands and germinates.

Stipe, Stipes (pl. Stipites) (1) In orchids, a stalk of the pollinarium derived from the rostellum, not the anther, connecting the viscidium to the caudicle or pollinium. (2) in insects, a mouthpart, specifically, a stalk-like component of the maxilla distal to the cardo and bearing the maxillary palps.

Subspecies A subdivision in the taxonomic hierarchy with a rank subordinate to species.

Superposed Positioned on top of one another; said of pollinia that are flattened parallel to the long axis of the clinandrum or anther bed.

Sympatric Refers to populations or species distributed in the same or overlapping geographic areas; cf. allopatric.

Syndrome A group of functionally correlated characters reflecting adaptation to a particular combination of environmental or biotic conditions.

Taxon (pl. Taxa) Any named taxonomic group such as a species, genus, or family.

Tegula A kind of stipe or pollinium stalk derived from the dorsal epidermis of the rostellum; cf. hamulus.

Terrestrial In reference to plants, growing in soil on the ground; cf. epiphytic.

Tetrad A group of four cells formed by meiosis; as applied to pollen, four pollen grains that remain attached as a unit at maturity.

Tetraploid An organism or cell with four complete sets of chromosomes in each nucleus.

Thorax In insects, the middle subdivision of the body positioned between the head and abdomen and bearing the legs and wings (when present).

Throat The orifice in a gamopetalous corolla or calyx, positioned between the tube and the limb.

Tibia The fourth segment and lowermost long segment of an insect's leg.

Ultraviolet Electromagnetic radiation with wavelengths (between about 40 and 400 nm) that are invisible to humans but visible to many pollinators.

Undulate Having a wavy margin or surface; cf. sinuate.

Vegetative reproduction A type of asexual reproduction in which specialized organs such as rhizomes, tubers, corms, bulbs, psedobulbs, and gemmae generate new growth.

Ventral In plants, the inner or upper side of lateral organs such as leaves, bracts, or petals (adaxial).

Viscidium (pl. Viscidia) The sticky part of the rostellum, often connected to the pollinia and functioning in its attachment to a pollinator.

Viscin An elastic, more or less glutinous, often thread-like material which binds pollen together in the pollinia and caudicles.

Viscous A fluid with little tendency to flow; thick, glutinous, sticky, adhesive.

Xenogamy The transfer of pollen between different genets.

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