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The Pollination Biology of North American Orchids: Volume 2

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Charles L. Argue, Ph.D
University of Minnesota
College of Biological Sciences
Saint Paul, MN, USA
argue001@umn.edu

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

Contents

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).

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;
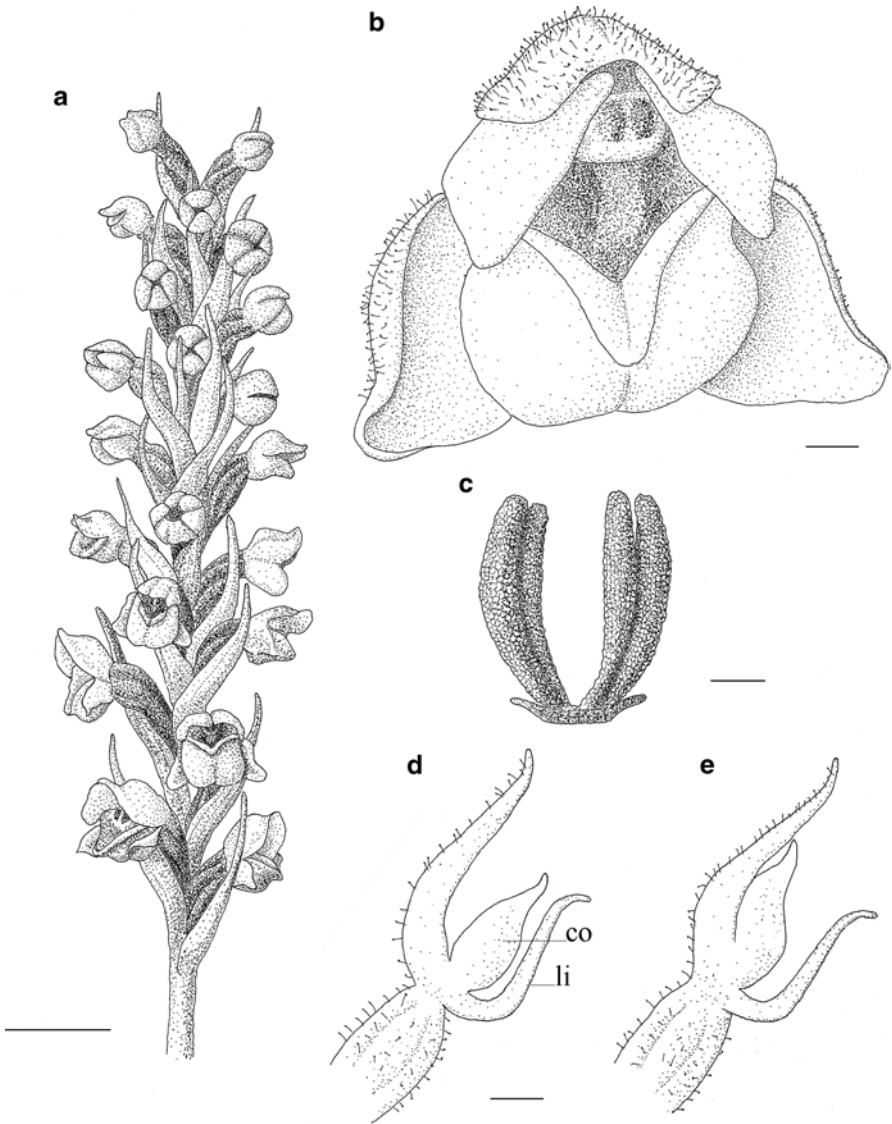
Fig. 1.1 *Goodyera pubescens*. (**a**) Inflorescence, scale bar = 1 cm; (**b**) Flower, front view, scale bar = 0.5 mm; (**c**) Pollinarium, scale bar = 0.02 mm; (**d**) Sagittal section of a flower showing early (male) stage; (**e**) Sagittal section of older flower showing separation of lip and column, 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 secund
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*.
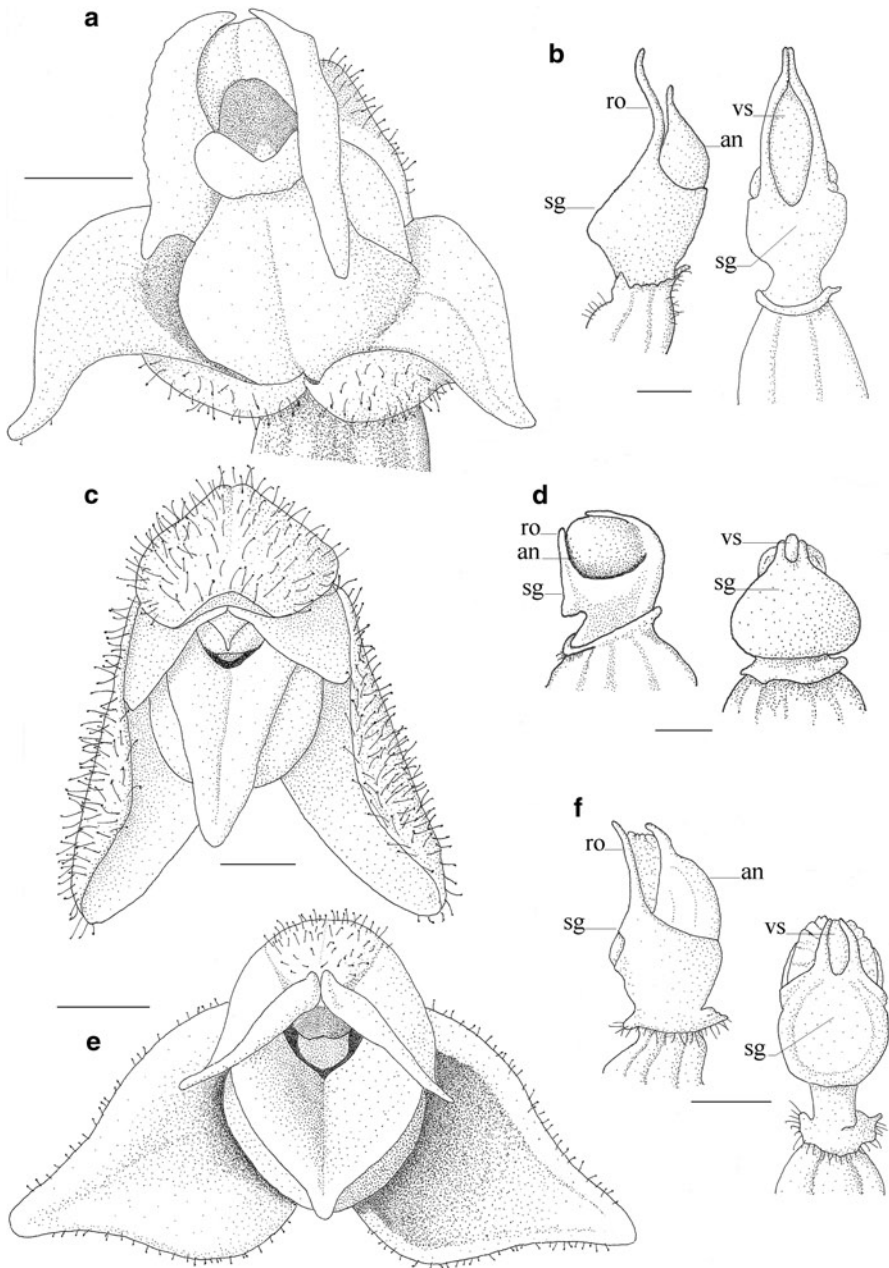
Fig. 1.2 (**a**, **b**) *Goodyera oblongifolia*; (**c**, **d**) *G. repens*; (**e**, **f**) *G. tesselata.* Flower, front view (*left*); column side view (*center*), front view (*right*), scale bars = 1 mm. *an* anther, *po* pollinium, *ro* rostellum, *sg* stigmatic area, *vs* viscidium. **b**, **d**, 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 (2*n*)	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).