

## 15 PLANT-POLLINATOR INTERACTIONS AND FLORAL RADIATION IN *PLATANThERA* (ORCHIDACEAE)

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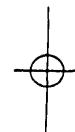
Jeffrey R. Hapeman and Ken Inoue

The Orchidaceae, with ca. 20,000 species (Dressler 1993), is perhaps the largest family of flowering plants. Given the spectacular floral diversity of the orchids, plant-pollinator interactions are thought to be one of the primary selective forces driving the remarkable diversification of this family (Benzing 1987; Dressler 1993). The significance of floral specialization in the radiation of the orchids was first recognized by Charles Darwin (1877) in his book *The Various Contrivances by which Orchids are Fertilized by Insects*. Darwin described the importance of orchid floral structure in attracting pollinators and the relation of floral structure to pollinator morphology, as well as the important role of the pollinators in orchid reproduction. Written at a time when many in the scientific community felt that floral structure had little or no relation to pollination, his book convincingly demonstrated the adaptive nature of orchid floral structure. In particular, Darwin perceptively described how floral morphology and pollinator morphology could interact, leading to the possibility of what would now be termed plant-pollinator coevolution.

The research of Darwin and those who followed him (including Dodson 1962; van der Pijl and Dodson 1966; Dressler 1981, 1993; Williams 1982; Nilsson 1992) has continued to demonstrate the importance of floral differentiation in orchid diversification. The spectacular variation in floral form and color, the production of ethereal scents to lure oil-collecting bees, and the tremendous variation in the placement of pollinia on pollinators have been studied and attempts have been made to relate them to orchid phylogeny. Yet much of this research has been plagued by one nagging problem: orchid phylogeny is based on a systematic scheme derived primarily from floral morphology, the same characters whose adaptive significance is typically under study (Chase and Palmer 1992). In order to avoid bias or circularity (Maddison and Maddison 1992), an independent source of characters is needed when studying orchid floral evolution (contra Brooks and McLennan 1994). Historically, this has been difficult to achieve using morphology, given that vegetative characters are fairly conserved, particularly at the generic level (Benzing 1987; Dressler 1993). The advent of molecular techniques of phylogenetic inference, with the possibility of a wealth of variable characters (Hillis 1987; Clegg 1993), has provided an excellent solution to the problem of character bias (Sytsma 1990; Givnish et al. 1995) and has opened up the possibility of investigating floral evolution in the orchids in an unbiased fashion (Chase and Palmer 1992).

We have begun a molecular systematic study of the genus *Platanthera* (Orchidaceae) and related orchidoid genera to investigate the role of floral divergence and pollinator diversification (and the interaction between the two) in orchid diversification. *Platanthera*, with ca. 85 species, is the largest genus of north temperate terrestrial orchids





**Table 15.1.** Distribution, floral syndrome, and pollination ecology of *Platanthera* species under study

Section/Group Taxon	Distribution	Color	Pollination time	Pollinia placement	Pollinator	References
<b>Blephariglottis*</b>						
<i>blephariglottis</i>	E U.S.	white	both	eye	moths, butterflies	Smith & Snow 1976
<i>ciliaris</i>	E U.S.	orange	diurnal	eye	<i>Papilio</i> spp.	Robertson & Wyatt 1990
<i>cristata</i>	SE U.S.	orange	diurnal	eye?	butterflies, bees?	Folsom 1979, Hapeman unpubl.
<i>integrilabia</i>	SE U.S.	white	both	eye	hawkmoths, butterflies	Zettler 1996
<i>grandiflora</i>	E U.S.	purple	diurnal	eye	<i>Hemaris</i> spp., butterflies	Stoutamire 1974, Moldenke 1949
<i>lacera</i>	E us.	green/white	both	proboscis	noctuid moths, <i>Hemaris</i> spp.	Duckett 1983, Stoutamire 1974
<i>leucophaea</i>	E U.S.	white	nocturnal	proboscis	hawkmoths	Sheviak & Bowles 1986
<i>peramoena</i>	E U.S.	purple	diurnal	eye	<i>Hemaris thysbe</i>	Hapeman 1997
<i>praeclara</i>	central U.S.	white	nocturnal	eye	hawkmoths	Cuthrell 1994
<i>psycodes</i>	E us.	purple	both	proboscis	<i>Hemaris</i> spp., butterflies, moths	Stoutamire 1974
<b>Gymnadeniopsis†</b>						
<i>clavellata</i>	E U.S.	green	selfing	proboscis	selfing	Catling 1991
<i>integra</i>	SE U.S.	yellow	diurnal	proboscis	bees, butterflies, selfing	Morong 1893, Luer 1975
<i>nivea</i>	SE U.S.	white	?	proboscis	butterflies?	
<b>Platanthera?</b>						
<i>bifolia</i>	palaearctic	green/white	nocturnal	proboscis	hawkmoth	Nilsson 1983
<i>metabifolia</i>	E palaearctic	white	nocturnal	proboscis	hawkmoth	Inoue 1983
<i>orbiculata</i>	nearctic	green/white	nocturnal	eye	noctuid moths	Stoutamire unpubl.
<i>okuboii</i>	Japan	green/white	nocturnal	proboscis	hawkmoths	Inoue 1983
<i>obtusata</i>	nearctic, Sweden	green/white	nocturnal	eye	mosquitoes, pyralid moths	Thien and Utech 1972, Voss and Riefner 1983
<i>hookeri</i>	E nearctic	green/yellow	nocturnal	eye	noctuid moths?	
<i>macrophylla</i>	E nearctic	green/white	nocturnal	eye	noctuid moths or hawkmoths?	
<b>Boninensis</b>						
<i>boninensis</i>	Bonin Isl.	cream	nocturnal	proboscis	noctuid moths	Inoue 1983

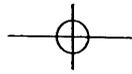


<b>Tipuloides*</b>						
<i>tipuloides</i> ssp. <i>nipponica</i>	Japan	green	nocturnal	proboscis	noctuid moths	Inoue 1983
<i>tipuloides</i> ssp. <i>sororia</i>	Japan	green	nocturnal	proboscis	hawkmoths, noctuid moths	Inoue 1983
<b>Sachalinensis</b>						
<i>sachalinensis</i>	E Asia	green	nocturnal	proboscis	noctuid moths	Inoue 1983
<b>Mandarinorum*</b>						
<i>mandarinorum</i> ssp. <i>ophrydioides</i>	Japan	green	nocturnal	eye	noctuid & geometid moths	Inoue 1983
<i>mandarinorum</i> ssp. <i>hachijoensis</i>	Japan	green	nocturnal	eye	hawkmoths	Inoue 1983
<i>amabilis</i>	Japan	green	nocturnal	proboscis	noctuid moths?	
<i>minor</i>	E Asia	green	xnocturnal	eye		Inoue 1983
<b>Limnorchis</b>						
<i>dilatata</i> var. <i>leucostachys</i>	NW U.S.	white	nocturnal	proboscis	noctuid moths	Ripping 1971
<i>hyperborea</i> var. <i>hyperborea</i>	nearctic	green	both	proboscis	bees, butterflies, noctuid moths	Catling & Catling 1989
<i>hyperborea</i> var. <i>viridiflora</i>	Japan, Aleutia	green	?	proboscis	noctuid and/or pyralid moths?	
<i>stricta</i>	NW. U.S.	green	both	proboscis	geometrid moths, bees, flies	Patt et al. 1989
<b>Japonica</b>						
<i>japonica</i>	E Asia	white	nocturnal	proboscis	hawkmoths?	
<b>Tulotis</b>						
<i>fuscescens</i>	E Asia, Aleutia	green	nocturnal	proboscis	noctuid moths?	
<i>sonoharae</i>	Ryukyu Isl.	green	nocturnal	proboscis	noctuid moths?	
<i>ussuriensis</i>	E Asia	green	nocturnal	proboscis	pyralid moths	Inoue 1983

\* polyphyletic

† paraphyletic

? not enough resolution to determine



(Ohwi 1965; Inoue 1983; Mabberley 1987; Wood et al. 1993). Species of *Platanthera* occur in North America, Asia, Europe, North Africa, as well as Borneo and Sarawak. Major centers of diversity are found in North America and East Asia. Compared to other genera in the tribe Orchideae, *Platanthera* has apparently undergone a tremendous radiation in floral form and pollination syndrome (Plate 3). Flowers range from green and white to bright purple or orange, and may have elaborate dissections of the **labellum** and petals, lending the flowers a “fringed” appearance (Plate 3). All species have a nectar spur on the lip, whose length may exceed 4 cm in some **taxa**. The majority of species are pollinated by noctuid and **pyralid** moths, but there are species pollinated by beetles, butterflies, hawkmoths, bumblebees, flies and even mosquitoes (see Table 15.1). The diversity of pollination syndromes found in *Platanthera* thus represents nearly all of the nondeceptive pollination syndromes found in the Orchidaceae (van der Pijl and Dodson 1966). As a consequence, *Platanthera* serves as a small-scale model for understanding the role of pollinators and floral **specialization** in the adaptive radiation of the Orchidaceae as a whole.

This paper will examine preliminary results from our research on floral evolution in *Platanthera*. Questions addressed include:

- What have been the pathways to different floral syndromes?
- How have important traits such as pollinia placement and flower color evolved? Is there evidence of repeated independent evolution (i.e., convergence) of similar floral morphology as a result of sharing similar pollinators?
- How useful is floral morphology for inferring phylogeny?

To address these questions, we overlay floral morphological traits and pollination data on the molecular phylogeny to determine the most parsimonious states for the origin and evolution of these traits (Kocher et al. 1993; Crisp 1994; Givnish et al. 1995).

### Systematic Considerations

*Platanthera* is a member of the tribe Orchideae, which contains approximately 1,300 species of terrestrial orchids in 57 genera. Relationships between the genera are poorly understood, and indeed, even the generic boundaries are unclear (Dressler 1993). Traditionally, American botanists have taken a broad taxonomic view by including all North American species of *Platanthera* in the genus *Habenaria* (Ames 1910; Correll 1950). More recently, however, specialists on the Orchidaceae have recognized *Platanthera* as distinct (Luer 1975; Case 1987; Smith 1993; Homoya 1994); Dressler (1993) goes so far as to place *Platanthera* and *Habenaria* in different subtribes of the Orchideae. As part of our search for appropriate outgroup **taxa**, we are investigating intergeneric relationships in the Orchideae (in collaboration with colleagues at the Royal Botanic Gardens, Kew). As we will report elsewhere, we have found that *Platanthera* is clearly monophyletic and rather distantly removed from *Habenaria* s.s. Thus, the current trend to recognize *Platanthera* as distinct appears to be well justified; however, a precise definition of synapomorphic morphological characters that unite the *various* species of *Platanthera* is still lacking. Currently, *Pla-*



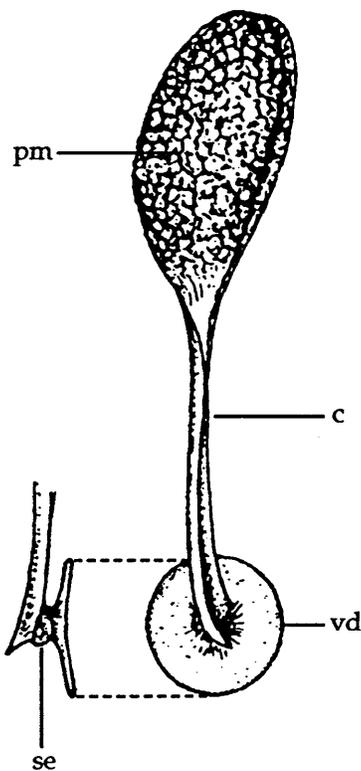
*tanthera* is defined by (i) the relatively broad anther; (ii) the presence of fusiform or elongate root-tuberoids (or tuberoids lacking), as opposed to spheroid or globose tuberoids; and (iii) a stigma that lacks processes and is united into one large receptive surface. The inadequacy of these characters in defining *Platanthera* is demonstrated by the fact that several species (*P. nivea*, *P. clavellata*, and *P. integra*) have stigmatic processes, although they are smaller than those typically found in species of *Habenaria* s.s.

### Ecology and Morphology of *Platanthera*

*Platanthera* grow in a variety of habitats ranging from acid, peaty savannas and bogs to circumneutral floodplains and prairies, and can also be found in both temperate and boreal woodlands. A few species grow in the tropical montane rain forests of Borneo (Luer 1975; Case 1987; Smith 1993; Wood et al. 1993; Homoya 1994).

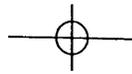
Vegetative characters shared by *Platanthera* and other taxa in the tribe Orchideae include soft, entire, and sometimes fleshy leaves. The leaves are **cauline** in most species, although some are characterized by basal leaves.

Flowers of *Platanthera* are typical of those of other orchidoid genera. The single fertile anther (a synapomorphy uniting all orchid taxa above the primitive apostasioids and cypripedioids) has been secondarily divided into two structures, variously called pollinia, pollinaria, or **hemipollinaria** (hereafter referred to as pollinia) (Dressler 1993). The pollinia are composed of pollen masses held together by elastic threads, and are drawn out at one end into a caudicle. The caudicle is capped at its apex by a sticky disk of tissue, the viscidium, which serves to attach the pollinium to the pollinator (Figure 15.1). Since most of the pollinators of *Platanthera* are lepidopterans and thus are hairy/scaly over most of their body, the only smooth surfaces where the sticky viscidia can effectively attach to the pollinators are the eyes and proboscis. Indeed, the pollinia attach to one of these two locations in the pollinators of *all species* of *Platanthera*. The **labelum** is well developed, resupinate, and bears a nectar spur which varies in length from about 2 mm in *P. stricta* to more than 40 mm in *P. praeclara* (Patt et al. 1989; Cuthrell 1994). The nectar spur varies in shape from short and saccate (*P. stricta*), to elongate (I? *praeclara*), to somewhat S-shaped



**Figure 15.1.** A pollinium of *Platanthera chlorantha*. pm=pollen mass, c=caudicle, vd=viscidium, se=stipe (from Nilsson 1978, used with permission).





(*P. peramoena*). Inoue (1983) has suggested that spur curvature may be functionally related to pollinator class. The petals and labellum are typically entire but are occasionally dentate or deeply divided, the latter giving the flowers a “fringed” appearance. The labellum may bear fleshy tubercles or projections. Flowers may be yellow, orange, purple, white or green, although most are green or whitish-green (see Plate 3). Some flowers (such as those of *P. leucophaea* and *P. chlorantha*) are strongly scented, while others (such as *P. peramoena*) are at most weakly scented. Scent production is often strongly periodic. In many species the scent is only released in the early hours of the evening when pollinators are most active, while at other times the flowers are virtually scentless (Nilsson 1978; Sheviak and Bowles 1986).

Apart from these general characteristics, many species express a variety of additional specializations. In a number of species, such as *Platanthera dilatata* and *P. hyperborea*, the labellum is “trapped” in an upright position for the first few days of anthesis, blocking access to the stigma and thus making the flowers functionally protandrous (Plate 3 J, M). A tubercle on the labellum in section *Tulotis* serves to deflect the proboscis of the pollinator, allowing removal of only one pollinium at a time (Plate 3 G) (Stoutamire 1971; Inoue 1983). The flowers of *P. hookeri* are strongly “hook-shaped” in profile which may force pollinators to approach the flower from one side at a time, serving a similar function to the tubercle in *Tulotis* (Catling and Catling 1991).

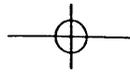
There is a tremendous diversity of pollination syndromes within *Platanthera*, involving apparent adaptations to attract and ensure fertilization mediated by specific groups of insects (Table 15.1). The majority of taxa are pollinated by noctuid and pyralid moths, and hawkmoths are also common pollinators. One or two taxa are pollinated by bumblebees, several by butterflies, and one by beetles (I? *chorisiana*, not included in this analysis). Several taxa are pollinated by both moths and mosquitoes (Thien and Utech 1970; Voss and Riefner 1983), and at least one species is pollinated by flies, bees, and moths (Patt et al. 1989). Some species, such as *P. hyperborea*, appear to be pollinated by several different types of pollinators. Detailed studies, however, usually reveal that one class of pollinator or one class of proboscis length predominates, although a number of insects may act as pollinators of a given species (Inoue 1983; Nilsson 1978, 1983; Patt et al. 1989). Catling and Catling (1991) have suggested that the number of pollinator species varies inversely with spur length in *Platanthera*.

Pollination data were primarily collected from the literature. The pollination biology of *Platanthera* has been well studied, primarily through the efforts of Warren Stoutamire in the United States, Ken Inoue in Japan and eastern Asia, and L. Anders Nilsson in Europe. The senior author has supplemented this with field observations of several critical taxa during the past two years.

### Molecular Systematics

Fresh or silica gel-dried plant material was collected for 19 taxa during numerous trips throughout the eastern United States by the senior author. Ken Inoue collected leaf material for all of the East Asian taxa included in the analysis, and Mark





Chase kindly provided DNA for additional U.S. taxa. Leaf material for a number of other taxa was obtained by the senior author through correspondence with various colleagues. Leaf material was obtained from all currently recognized sections of the genus, usually with more than one taxon representing each section. Voucher specimens for DNA samples are deposited at the University of Wisconsin Herbarium (WIS) and the Shinshu University Herbarium, Japan (SHIN).

#### DNA extraction

Total DNAs were extracted from fresh, silica gel-dried or -80° C frozen leaf tissue collected from individual plants using either a 2% CTAB (Doyle and Doyle 1987) or a modified 6% CTAB procedure (Smith et al. 1991). Leaf tissue was precooled in liquid nitrogen to maximize DNA yield. Some DNA samples were further purified on CsCl gradients.

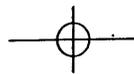
#### Amplification and sequencing

The Internal Transcribed Spacer (ITS) region of nuclear ribosomal DNA (nrDNA) was amplified from total genomic DNA via the polymerase chain reaction (PCR) (Oste 1988; Ehrlich 1989) using the primers "ITS4" and "ITS5" (White et al. 1990) and the protocol detailed in Baum et al. (1994). The ITS region has been shown to evolve rapidly, and is useful for resolving phylogenetic relationships at fairly low taxonomic levels (Baldwin 1992; Baum et al. 1994; Baldwin et al. 1995; Yuan and Küpfer 1995).

PCR-amplified DNA was cleaned with the GeneClean II™ kit (Bio 101) and then directly sequenced via the dideoxy method (Sanger et al. 1977), using the Sequenase™ 2.0 kit (US Biochemicals). Primers for sequencing included the two primers used for amplification, as well as "ITS2" and "ITS3B" (White et al. 1990). A modified double-stranded sequencing protocol described in Baum et al. (1994) was followed. Some taxa were sequenced with the AmpliTaq FS dye-terminator cycle sequencing kit (Perkin-Elmer) and analyzed on an ABI Model 377 automated sequencer (Applied Biosystems Inc.). PCR-amplified DNA for these taxa was cleaned using Qiaquick spin columns (Qiagen Inc.).

#### Sequence alignment and phylogenetic analysis

Sequences of ITS1 and ITS2 were manually aligned using SeqApp 1.9 (Gilbert 1993) and analyzed using Fitch parsimony in PAUP 3.1.1 (Swofford 1993). Alignment required the insertion of a number of gaps; however, most alignments were fairly unambiguous. When several alignment alternatives were found, the alignment that yielded the fewest informative characters was chosen. This is the most conservative approach and minimizes the a priori creation of informative characters (Baum et al. 1994). Due to the large size of the data set, the heuristic search strategy of PAUP was implemented. The random addition sequence and steepest descent options were utilized to increase the probability of finding all possible "islands" of trees (Maddison 1991). In addition to unweighted parsimony, character-state step-matrix weighting of transitions-transversions (Albert and Mishler 1992) and coding of informative indels (i.e., those shared by two or more taxa) were explored (for a discussion of gap



coding see Baldwin 1993; Baum et al. 1994). The phylogenetic "signal" of the sequences was assessed by the CI (Kluge and Farris 1969) and the  $g_1$  statistic (Hillis and Huelsenbeck 1992) (the latter by generating 10,000 random trees using the Random Trees option in PAUP).

Due to concerns about the monophyly of *Platanthera*, and in order to select the proper outgroups, 16 taxa representing 13 genera from the tribe Orchideae were included in the data set which was then analyzed using global parsimony (Maddison et al. 1984). To assess the monophyly of *Platanthera*, the taxa of *Platanthera* were not constrained to the ingroup. Based on a larger *rbcL* study of the Orchidaceae, two species of *Orchis* and *Ophrys* were chosen as super-outgroups (K. Cameron et al. unpubl. data). In an effort to gauge the strength of each clade in the strict consensus tree, both bootstrap (Felsenstein 1985) and decay analyses (Bremer 1988) were conducted. While bootstrapping and decay analyses may have their limitations (see Sanderson 1989, 1995), we feel they give at least some relative measure of internal support for the resulting clades.

#### Evolutionary analyses

Patterns of floral morphological evolution and the evolution of pollination syndromes were investigated by overlaying the appropriate morphological and ecological characters onto the molecular phylogeny with MacClade 3.05 (Maddison and Maddison 1992). Analyses in MacClade were conducted using the ACCTRAN option, a more conservative approach which minimizes convergence and parallelisms in character-states (Maddison and Maddison 1992). This procedure required that the *Platanthera ciliaris-blephariglottis-cristata* polytomy be resolved. All three possible branching patterns were investigated. The order of branching had no effect on character evolution, with the exception of floral color, where two arrangements agreed with each other but differed from the third. Consequently, the basal floral color for that group was coded as equivocal. The trees were redrawn with the polytomy present. Morphological and ecological characters were obtained both through the study of specimens (both live and herbarium) and through a detailed review of the literature on *Platanthera* and its relatives. The results of individual character analyses were compared to investigate the relations between phylogeny, morphology, and ecology.

## Results and Discussion

#### Sequence alignment and analysis

The lengths of both ITS regions were within the ranges reported for other angiosperms and were similar for all the species investigated; differences in length were primarily the result of small indels. Alignment required the insertion of 39 gaps, 18 in ITS1 and 21 in ITS2; the gaps ranged from one to 14 base pairs in length. Seven of the gaps were potentially informative. Using the method of Baum et al. (1993), these gaps were coded and added to the data set. There was a total of 447 sites, of which 254 were potentially informative.



Sequence divergence (treating gaps as missing) was fairly high, ranging from 0.3% to 38.0% for ITS1 and 0.0% to 37.7% for ITS2. While these high values may imply high levels of “noise,” comparison of the  $g_1$  statistic from our data with the published data of Hillis and Huelsenbeck (1992) indicated a highly significant ( $p < 0.01$ ) phylogenetic signal (i.e., a significant amount of non-random structure in the data set). In addition, the CI value of the resulting trees indicated a relatively low level of homoplasy given the number of taxa and characters under consideration (see Chapter 2).

### Phylogenetic analysis

Analysis of the data yielded two most parsimonious trees of 1123 steps (excluding uninformative characters, CI = 0.540); addition of the indels yielded the same two trees, and simply increased the length of several branches (1178 steps, CI = 0.546). Weighting the transversions over the transitions 2:1 yielded one shortest tree, which was identical to one of the two trees found in the unweighted analysis. Bootstrap and decay analyses were conducted on this tree, which was chosen as a working model for floral character evolution in *Platanthera* (Figure 15.2).

All analyses supported a monophyletic *Platanthera*, with *Galearis* as the sister-group. Contrary to the beliefs of many systematists, *Platanthera* was rather far removed from *Habenaria* s.s. In all analyses, five major clades could be recognized within *Platanthera*: *Blephariglottis*, *Lacera*, *Limnorchis*, *Platanthera*, and *Tulotis* (Figure 15.2). These informal clade names are derived from traditional sectional names where the clades overlap significantly with the sections. The *Lacera* clade represents a group of species not previously recognized at the sectional level, and is named for one species of the clade, *P. lacera*. While the clades overlap traditional sections to some degree, the monophyly of few sections of *Platanthera* was well supported (Table 15.1). It should be noted, however, that while relationships within the clades are rather well supported, the relationships between the clades are only weakly supported (at least by such measures as bootstrap support).

### Floral evolution in *Platanthera*

Any discussion of large-scale patterns of floral evolution in *Platanthera* must be accompanied by a cautionary note: Relationships between clades of *Platanthera* are only weakly supported by our ITS data, even though relationships within clades are generally well supported. **However**, given the lack of other suitable phylogenies with which to test our hypotheses, the models we present are currently the best estimates of floral evolution in the genus. While it may be possible to strengthen the relationships between the clades by adding more characters to the data set, it is likely that the major clades of *Platanthera* rapidly diversified; if this is the case, it is possible that no amount of sequencing will allow us to confidently resolve intersectional relationships in this genus (Sytsma 1990).

**POLLINATION SYNDROME**—The basal condition in *Platanthera* appears to involve generalized settling moth pollination (i.e., noctuids and pyralids) (Figures 15.3, 15.4). This corresponds with the assessment of previous workers (van der Pijl and Dodson 1966; Nilsson 1983; Dressler 1993), but is inconsistent with Inoue (1983) who sug-



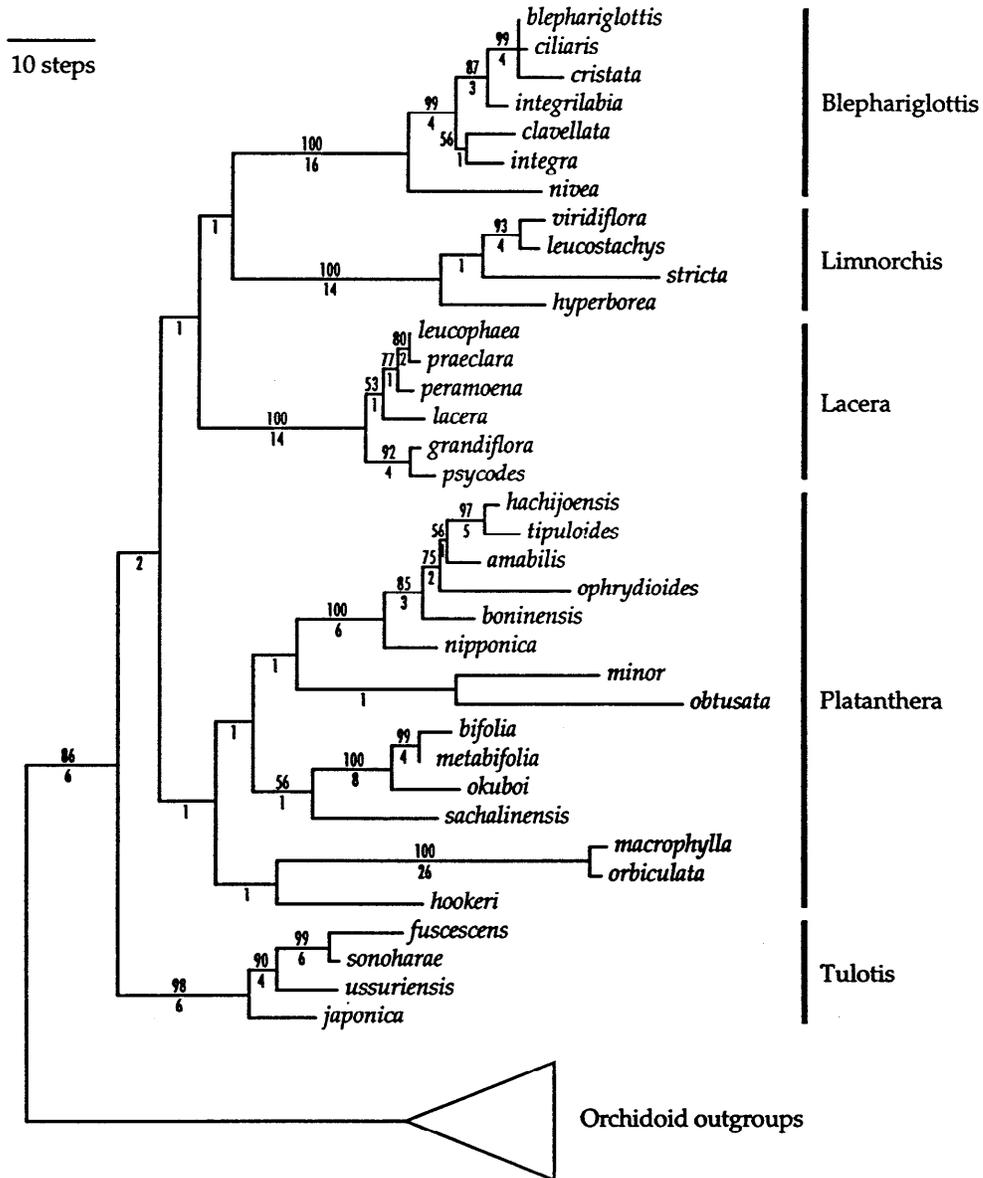
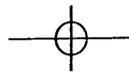
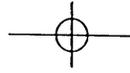
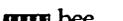


Figure 15.2. Phylogram of *Platanthera* based on a weighted parsimony analysis of nuclear ribosomal ITS sequence data. This is the only tree produced by the weighted analysis, and is identical to one of the two trees from the unweighted analysis. Bootstrap values greater than 50% are listed above the branches; decay values are below the branches. The outgroups are represented by a wedge; relationships among the outgroups will be discussed elsewhere.



Pollination Syndrome:

-  culicid
-  nocturnal settling moth
-  diurnal sphingid
-  nocturnal sphingid
-  bee
-  selfing
-  butterfly

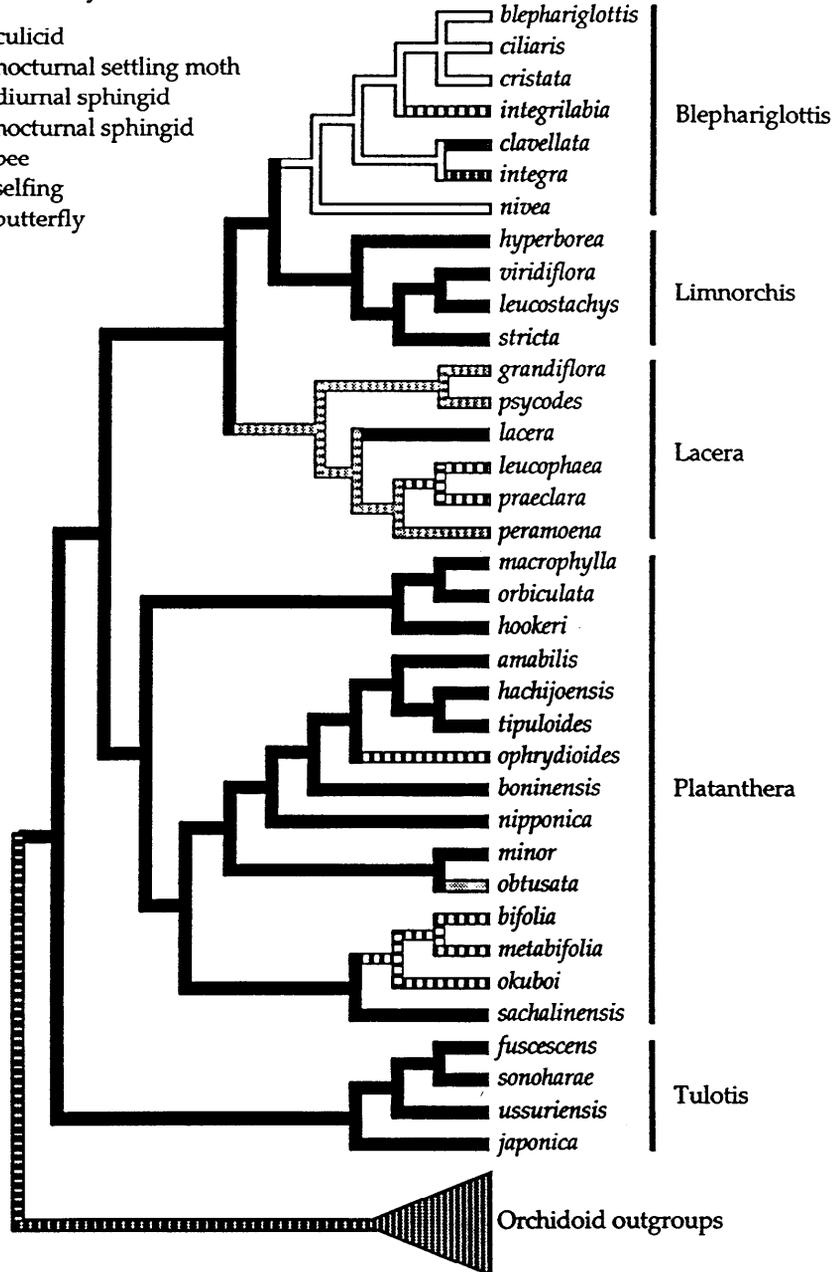


Figure 15.3. Cladogram illustrating pollination syndrome evolution in *Platanthera*, drawn from an ACC-TRAN-optimized analysis of the pollination data on the ITS-derived phylogeny using MacClade 3.05 (Maddison and Maddison 1992).

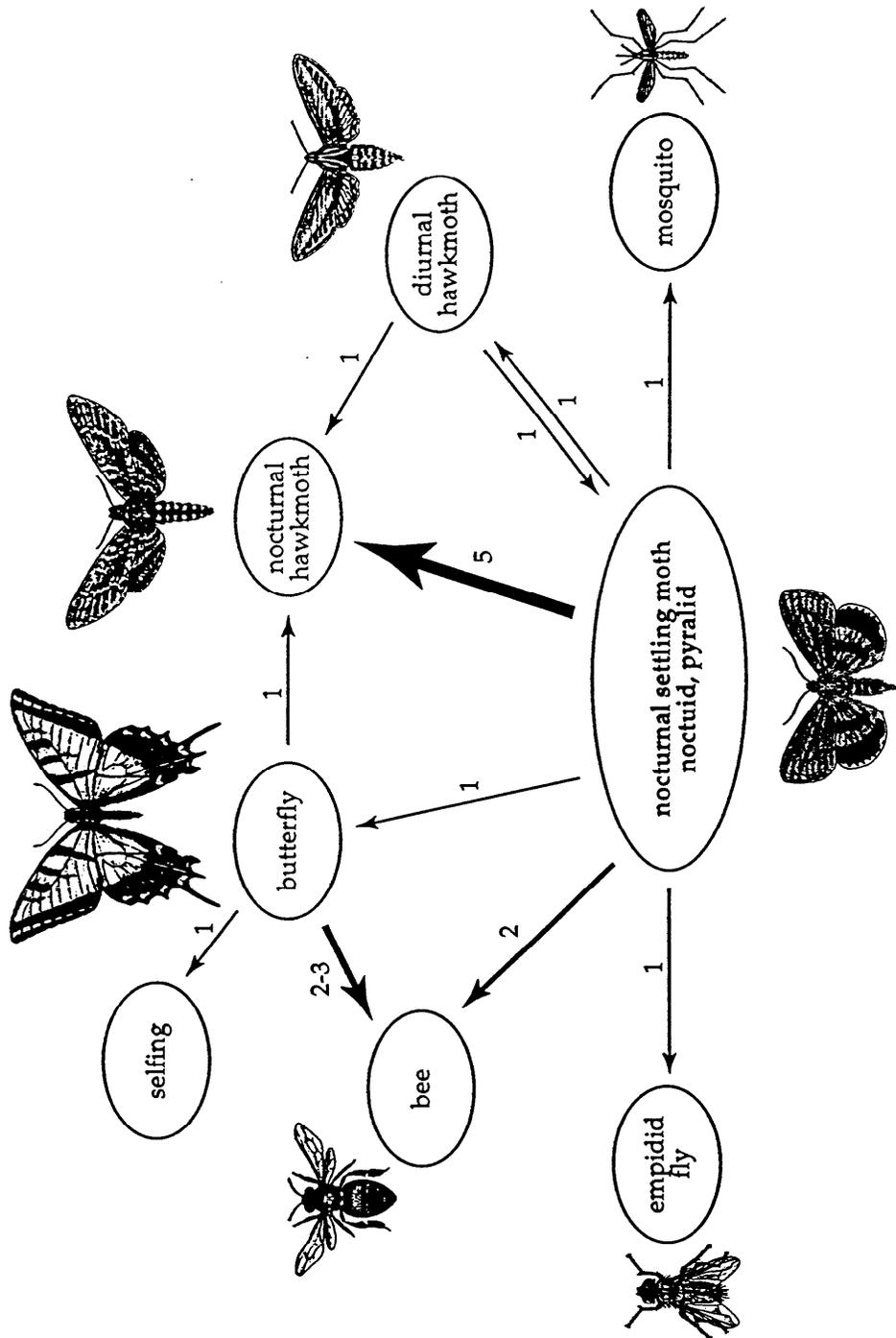
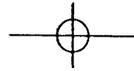
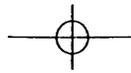


Figure 15.4. Diagram illustrating pollination syndrome evolution in *Platanthera*, redrawn from Figure 3 to demonstrate more clearly the evolutionary pathways to individual syndromes. The arrows indicate the direction of pollination syndrome evolution. The width of the arrows (and the numbers next to them) indicate the number of independent gains (i.e., >1 = convergence) of the given syndrome. (Figures of insects modified from Swann and Papp 1972, used with permission.)



gested that either bee or beetle pollination was the basal condition. Diurnal hawkmoth pollination has arisen only once, in the *Lacera* clade, and diurnal butterfly pollination has also arisen only once or at most twice. These syndromes apparently require a change in color (to purple and orange or yellow, respectively), in addition to numerous other morphological modifications to insure proper placement of the pollinia and the presence of the appropriate attractive stimuli. In contrast, hawkmoth pollination has arisen convergently five times, and requires only slight modifications from the generalized green to greenish-white floral syndrome of most species of *Platanthera*, such as increased scent production, a shift to whiter flowers and perhaps an increase in spur length. Thus, most species of *Platanthera* may be somewhat preadapted for hawkmoth pollination, facilitating the frequent shifts to this syndrome.

Dipteran (mosquito and/or fly) pollination has arisen two times, each time in a different clade of *Platanthera* (Figures 15.3, 15.4). Species pollinated by flies and mosquitoes are very similar morphologically to those pollinated by small moths, and apparently all species that are pollinated by mosquitoes and flies are pollinated by moths as well (Thien and Utech 1970; Voss and Riefner 1983; , Patt et al. 1989; Catling and Catling 1991). A switch from moths to dipterans may require only slight changes, perhaps in spur length and odor. As with hawkmoth pollination, the convergent shifts to dipteran pollination may also reflect some degree of preadaptation to this syndrome.

Finally, bumblebee pollination has arisen either two or three times, in two different clades (Table 15.1). The three species for which there are sufficient pollination records by bumblebees are apparently also pollinated by moths and/or butterflies. Therefore, the presence of a "bee pollination" syndrome in *Platanthera* is questionable at this point.

**FLORAL COLOR** – The primitive floral color is green (Figure 15.5). A number of species have shifted to greenish-white coloration, while pure white flowers have convergently arisen five times involving four sections. It should be noted that all hawkmoth-pollinated species have white flowers, but not all whit&lowered species are hawkmoth-pollinated. Thus, while hawkmoths might exert some selective pressure for the development of white flowers, other pollinators apparently do so as well (Table 15.1). Brightly colored flowers have arisen in two clades: yellow and orange in the *Blephariglottis* clade and purple in the *Lacera* clade (Figure 15.5).

The adaptive significance of floral color has been investigated by many pollination ecologists (e.g., Grant and Grant 1965; Faegri and van der Pijl 1966; Kevan 1983; Scogin 1983). The colors of flowers of *Platanthera* fit the classical syndromes: white flowers are typically pollinated by hawkmoths or noctuids; green and greenish-white flowers are pollinated by moths (noctuids and pyralids); yellow and orange species are primarily pollinated by butterflies (Table 15.1). The purple-flowered species are all pollinated, at least in part, by day-flying hawkmoths in the genus *Hemaris*. Observations on adult nectar hosts of *Hemaris thysbe* (Fleming 1970) indicate that this species has a distinct preference for purple to purplish-pink flowers. Thus, selection by these diurnal hawkmoths may have led to the evolution of purple flowers in *Platanthera*.

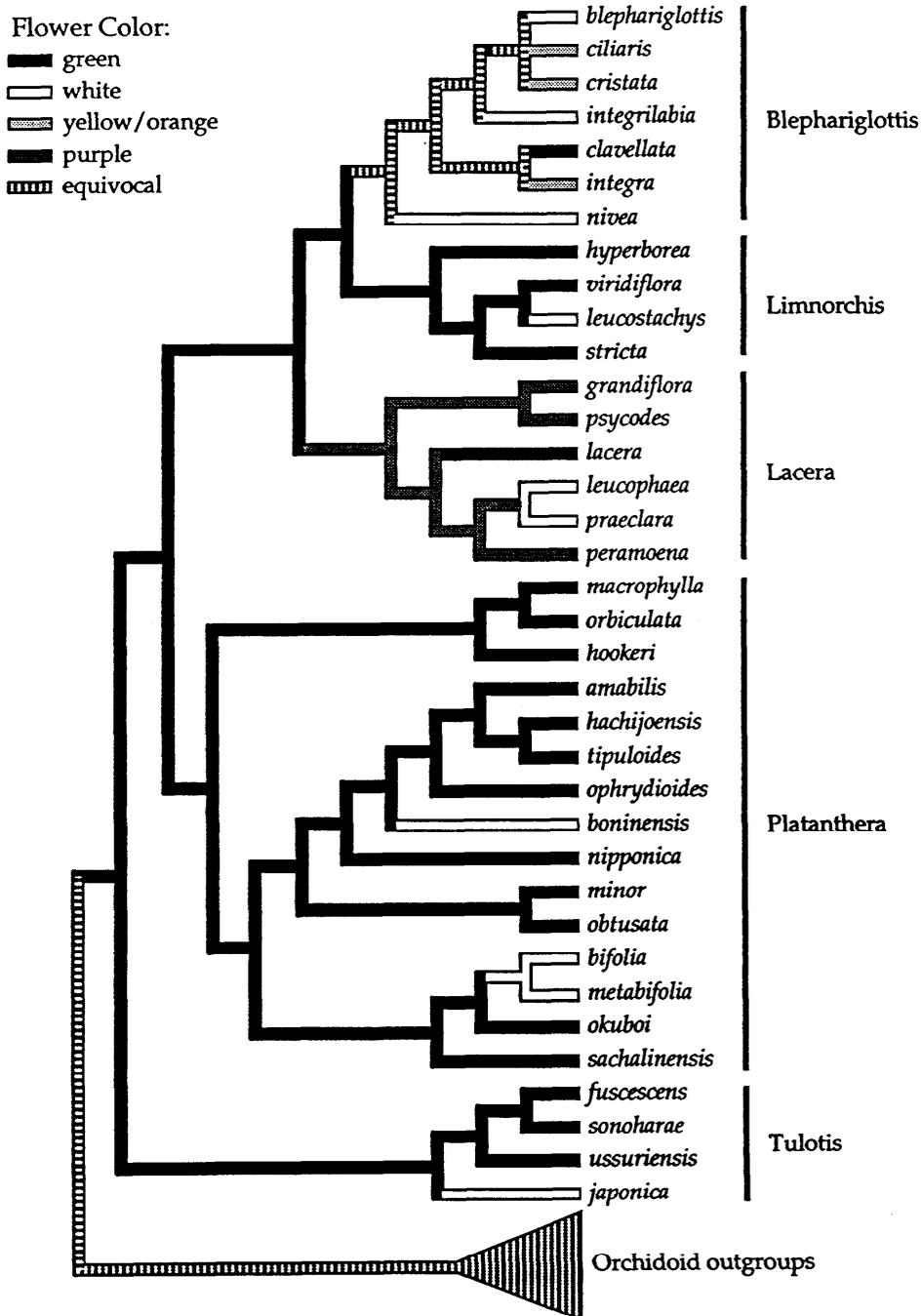
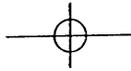


Figure 15.5. Cladogram illustrating floral color evolution in *Platanthera*, drawn from an ACCTRAN-optimized analysis of the pollination data on the ITS-derived phylogeny using MacClade 3.05 (Maddison and Maddison 1992).



Pollinia Placement:

▨ eye  
■ proboscis

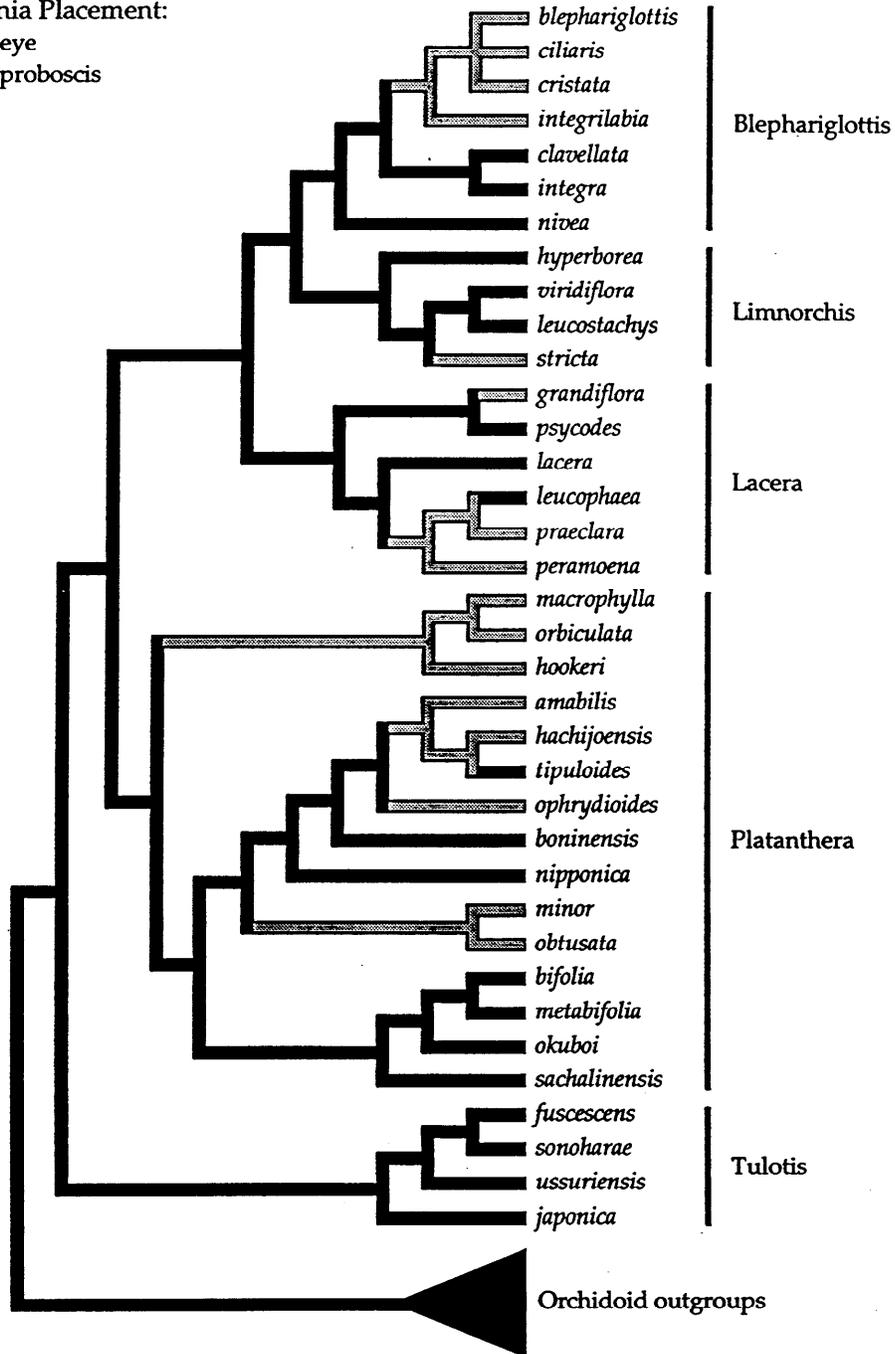
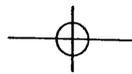


Figure 15.6. Cladogram illustrating the evolution of pollinia placement (and hence column morphology) in *Platanthera*, drawn from an ACCTRAN-optimized analysis of the pollination data on the IS-derived phylogeny using MacClade 3.05 (Maddison and Maddison 1992).



**POLLINATION TIME** – While only a few species are diurnally pollinated, diurnal pollination has arisen twice: once in the yellow, orange or white species of the *Blephariglottis* clade and once in the purple-flowered species of the *Lacera* clade. Comparisons of pollination time and color show that only the diurnally pollinated species are brightly colored; floral color and pollination time are clearly closely linked.

**POLLINIA PLACEMENT** – While the primitive condition is proboscis placement of the pollinia, eye placement has arisen independently at least seven times, involving all of the clades except *Tulotis* (Figure 15.6). Pollinia placement is a particularly homoplasious character; in the *Platanthera* clade alone, eye placement has convergently arisen at least three times. Placement of the pollinia on the eyes enforces a strong floral specificity, as both the distance between the viscidia and the length of the spur must match, respectively, the eye spacing and proboscis length of the pollinator. Figures 15.7 and 15.8 illustrate the functional constraints imposed by eye placement of the pollinia. With proboscis placement, eye spacing is irrelevant and the proboscis need only be long enough to reach the nectar (Nilsson 1978, 1983, 1988). Given this, it would seem that placement of pollinia on the eyes might result in fewer successful pollinations than placement on the proboscis. However, the frequent convergent gains of eye placement argue for some selective advantage to this trait. Further research on the pollination biology of *Platanthera* will be necessary to determine what may be the selective advantage(s) of this trait.

The switch from tongue to eye placement (or perhaps vice versa) of the pollinia has apparently been important in speciation in *Platanthera*. Three species-pairs (*P. psychodes*-*P. grandiflora*, *P. leucophaea*-*P. praeclara*, and *P. bifolia*-*P. chlorantha*) are differentiated primarily by placement of the pollinia. Two of these pairs are found in the *Lacera* clade. In each of these pairs, the orchids share at least one pollinator species and are sympatric in some areas of their respective ranges. The shift in column morphology allows the plants to effectively “partition” the pollinators. These pairs may have speciated **sympatrically** through **bi-directional** selection on column morphology from an ancestrally intermediate or polymorphic column type. Studies of the developmental morphology of the column may provide insight as to how this process might have **occurred**.

Pollinator partitioning via pollinia placement has been the subject of a great deal of research in the neotropical *Catasetinae* and *Gongorae* (Dressler 1968; Williams 1982). Apparently this study is the first to investigate pollinator partitioning via pollinia placement in an unbiased phylogenetic context, in part due to the previous difficulties in clearly determining the phylogenetic relationships of the species in question. In many cases, the differences in column structure, and thus pollinia placement, are used in separating species and in assessing their relationships. The homoplasious nature of column structure in *Platanthera* demonstrates that caution in character selection is necessary when studying floral evolution in groups, such as the orchids, where runaway sexual selection is expected.

**FRINGING** – Fringing of the **labellum** and petals has arisen only twice, in the *Blephariglottis* and *Lacera* clades. The convergent nature of this character is of particular interest, as fringing has previously been considered a synapomorphy uniting the species of section *Blephariglottis*. The degree of floral dissection has been shown to be



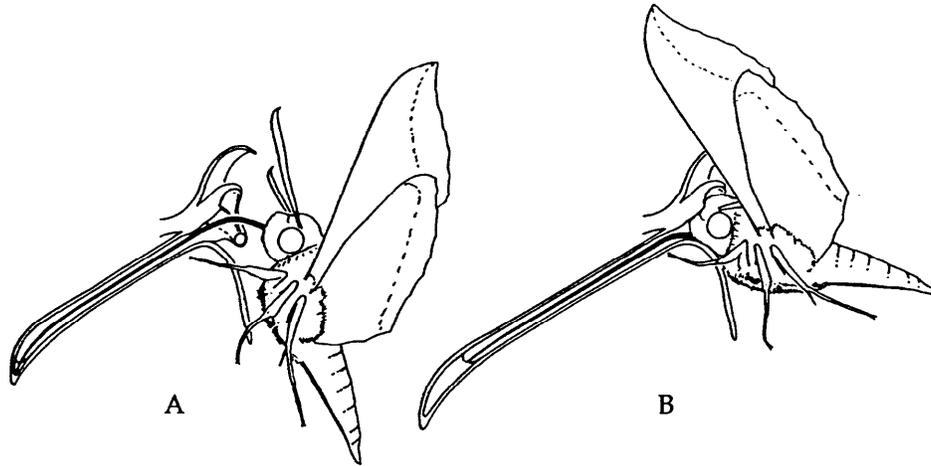
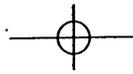


Figure 15.7. Constraints placed on spur length by eye placement of pollinia in *Platanthera*. In A, the spur is shorter than the distance from the tip of the moth's tongue to the base of its eyes; consequently, the eyes do not contact the viscidia, the pollinia are not removed, and the moth is effectively a nectar thief. In B, the spur is longer than the distance from the tip of the moth's tongue to the base of its eyes, and pollination is effected. (Taken from Nilsson 1978, used with permission.)

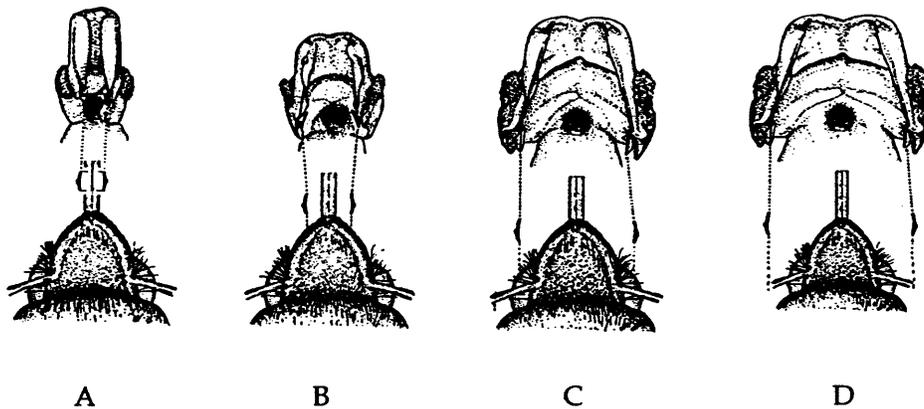
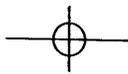


Figure 15.8. Column morphology and pollinia placement in *Platanthera*. A and C demonstrate proboscis and eye placement, respectively. In B, the moth's head is wider than the distance between the viscidia, which are also too widely placed to contact the moth's proboscis. Consequently, the viscidia will contact the scaly face of the moth, where they are unlikely to stick. D illustrates a moth that is too small to effect efficient pollination; the moth's head fits between the viscidia without contacting them. While this moth may occasionally serve as a pollinator by removing one pollinium at a time, it would not be as effective as the moth in C p=pollinium, v=viscidium. (Taken and adapted from Nilsson 1983, used with permission)



related to the frequency of pollination in the hawkmoth-pollinated *Viola cazorlensis* Gand. (Violaceae) (Herrera 1993). In fact, seven of the nine fringed species of *Platanthera* are pollinated at least in part by hawkmoths. If fringed flowers confer an advantage in pollination frequency, the selective advantage of fringing for *Platanthera* would be great, as several species of *Platanthera* are apparently pollinator-limited (Inoue 1983; Cuthrell 1994).

**NECTAR SPURS** – The general trend has been from shorter to longer nectar spurs. There have been several reversals to shorter spurs, however, in species pollinated by flies, mosquitoes and bees. All of these insects have shorter mouthparts than most lepidoptera. Very long spurs (>2.5 cm) appear to have evolved independently several times in response to hawkmoth and papilionid butterfly pollination.

**OTHER TRAITS** – A thickened callus of tissue, or tubercle, is found on the **labella** of all species of the Tulotis clade (with the exception of *P. japonica*) (Plate 3 G) and on the **labella** of *P. limosa* and *P. hologlottis* (not included in this study). The tubercle appears to function as a barrier preventing a forward approach to the flowers, forcing the pollinator to insert its proboscis from one side of the flower at a time. As a result, the pollinator can remove only one pollinium at a time. This may allow the plant to increase the odds of outcrossing, particularly if the pollinia are removed by different pollinators, as would be expected (Stoutamire 1971; Catling and Catling 1991). Because *P. limosa* and *P. hologlottis* are more closely related to the Limnorchis clade, the tubercle they share with the Tulotis clade is convergent (J. Hapeman, unpubl. data).

Other species also exhibit specializations that ensure that pollinia are removed individually. Several other species of the Limnorchis clade have the **labellum** “trapped” in an upright position initially, which **allows** removal of only one pollinium at a time (perhaps increasing outcrossing) and also apparently makes the flowers **functionally** protandrous by blocking access to the stigma (Plate 3 I, J, M). The **ice tong**-shaped flowers of *P. hookeri* also have been suggested to serve in manipulating pollinators into removing only one **pollinium** (Catling and Catling 1991).

## Conclusions

Several broad conclusions can be drawn from our results. First, molecular data provide an adequate and non-biased foundation for studying floral evolution. Sequence data supply a large number of homologous, variable characters which are independent of the floral characters. Without this independent data set, it **would** have been difficult (if not impossible) to accurately study floral evolution in this fascinating and diverse genus. *In Platanthera*, most of the taxonomy is based on floral morphology, as the plants are vegetatively fairly uniform. The poly- or **para**-phyletic nature of many **sections** of *Platanthera* is an indication of how convergent floral morphology has historically led to misinterpretation of phylogenetic relationships in this genus.

Second, given the frequency of convergence and reversals in floral morphology, it is clear that floral characters are unsuitable for inferring phylogeny *in Platanthera*. Much of floral morphology appears to be rather homoplasious; that is, it is related





more to ecology than to phylogeny. Shifts in column structure (= pollinia placement) as a result of pollinator partitioning is a particularly clear example of this. A second example would be the convergent evolution of "fringed" flowers in hawkmoth-pollinated lineages. While some features of floral morphology may be useful for inferring phylogeny, it is difficult or impossible to determine *a priori* which characters are suitable and which are homoplastic. This is similar to the findings of Chase and Palmer (1992) for the Oncidiinae (Orchidaceae). These two results taken together hold **potentially** large repercussions for much of orchid systematics, which historically has been based heavily on floral morphology.

Finally, pollinators have been important in shaping the evolution of *Platanthera*, affecting a diversity of changes in floral color and morphology. Hawkmoths have been involved in the evolution of long nectar spurs. Diurnal hawkmoths and butterflies have driven the evolution of brightly colored flowers. Partitioning of pollinators via differential pollinia placement has led to speciation through shifts in column morphology. *Thus, if Platanthera* is an adequate model for the Orchidaceae, then floral diversification as a result of pollinator-mediated selection has been an important driving force in the radiation of this fascinating and diverse family

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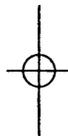
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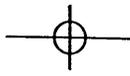
### References

- Albert, V. A., and Mishler, B. D. 1992. On the rationale and utility of weighting nucleotide sequence data. *Cladistics* 8:73-83.
- Ames, **0.1910**. *Illustrations and studies of the family Orchidaceae Volume IV: The genus Habenaria in North America*. Stanfordville, NY: Earl L. Coleman.
- Baldwin, B. G. 1992. Phylogenetic utility of the internal transcrii spacers of nuclear ribosomal DNA in plants: an example from the Compositae. *Molecular Phylogenetics and Evolution* 1:3-16.



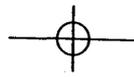
- Baldwin, B. G. 1993. Molecular phylogenetics of *Calycadenia* (Compositae) based on ITS sequences of nuclear ribosomal DNA in plants: cluomosomal and morphological evolution reexamined. *American Journal of Botany* 80:222-238.
- Baldwin, B. G., Sanderson, M., Porter, J. M., Wojciechowski, M. F., Campbell, C. S., and Donoghue, M. J. 1995. The ITS region of nuclear ribosomal DNA a valuable source of evidence on angiosperm phylogeny *Annals of the Missouri Botanical Garden* 82:247-277.
- Baum, D. A., Sytsma, K. J., and Hoch, P. C. 1994. The phylogeny of *Epilobium* L. (Onagraceae) based on nuclear ribosomal ITS sequences- *Systematic Botany* 19:363-388.
- Benzing, D. A. 1987. Major patterns and processes in orchid evolution: a critical synthesis Pp. 33-78 in *Orchid biology: reviews and perspectives, IV*, J. Arditti, ed. Ithaca, NY: Cornell University Press.
- Bremer, K. 1988. The limits of amino acid sequence data in angiosperm phylogenetic reconstruction *Evolution* 42:795-803.
- Brooks, D. R., and McLennan, D. A. 1994. Historical ecology as a research programme: scope, limitations and the future. Pp. 1-27 in *Phylogenetics and ecology*, P. Eggleton and R. I. Vane-Wright, eds. London, England: Academic Press.
- Case, F. W. 1987. *Orchids of the western Great Lakes region*. Bloomfield, MI: Cranbrook Institute of Science.
- Catling, P. M., and Catling V. R. 1989. Observations of the pollination of *Platanthera huronensis* in southwestern Colorado. *Lindleyana* 4:78-84.
- Catling, P. M., and Catling, V. R. 1991. A synopsis of breeding systems and pollination in North American orchids. *Lindleyana* 6:187-210.
- Chase, M. W., and Palmer, J. D. 1992. Floral morphology and chromosome number in subtribe Oncidiinae (Orchidaceae): evolutionary insights from a phylogenetic analysis of chloroplast DNA restriction site variation. Pp. 324-339 in *Molecular systematics of plants*, P. S. Soltis, D. E. Soltis, and J. J. Doyle, eds. New York, NY: Chapman and Hall.
- Chase, M. W., Soltis, D. E., Olmstead, R. G., Morgan, D., Les, D. H., Mishler, B. D., Duvall, M. R., Price, R. A., Hills, H. G., Qiu, Y., Kron, K. A., Rettig, J. I., Conti, E., Palmer, J. D., Manhart, J. R., Sykma, K. J., Michaels, H. J., Kress, W. J., Karol, K. G., Clark, W. D., Hedrén, M., Gaut, B. S., Jansen, R. K., Kim, K.-J., Wimpee, C. F., Smith, J. F., Fumier, G. R., Strauss, S. H., Xiang, Q.-Y., Plunkett, G. M., Soltis, P. S., Swensen, S. M., Williams, S. E., Gadek, P. A., Quinn, C. J., Eguiarte, L. E., Golenberg, E., Learn, G. H., Graham, S. W., Barrett, S. C. H., Dayanandan, S., and Albert, V. A. 1993. Phylogenetics of seed plants: an analysis of nucleotide sequences from the plastid gene *rbcl*. *Annals of the Missouri Botanical Garden* 80:528-580.
- Clegg, M. T. 1993. Chloroplast gene sequences and the study of plant evolution. *Proceedings of the National Academy of Science, USA* 90:363-367.
- Cole, F. R., and Firmage, D. H. 1984. The floral ecology of *Platanthera blephariglottis*. *American Journal of Botany* 71:700-710.
- Correll, D. S. 1950. *Native orchids of North America north of Mexico*. Stanford, CA: Stanford University Press.
- Crisp, M. D. 1994. Evolution of bird-pollination in some Australian legumes (Fabaceae). Pp. 1-27 in *Phylogenetics and Ecology*, P. Eggleton and R. I. Vane-Wright, eds. London, England: Academic Press.
- Cuthrell, D. L. 1994. Insects associated with the prairie fringed orchids, *Platanthera praeclara* Sheviak and Bowles and *P. leucophaea* (Nuttall) Lindley. M. S. thesis, North Dakota State University.
- Darwin, C. 1877. *The various contrivances by which orchids are fertilized by insects*. 2nd ed., revised. Chicago, IL: University of Chicago Press.
- Doyle, J. J., and Doyle, J. L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemistry Bulletin* 19:11-15.
- Dressler, R. L. 1993. *Phylogeny and classification of the orchid family*. Portland, OR: Dioscorides Press.
- Duckett, C. 1983. Pollination and seed production of the ragged fringed orchid, *Platanthera lacera* (Orchidaceae). Honor's thesis, Brown University, Providence, RI.
- Ehrlich, H. A. 1989. *PCR technology: principles and applications for DNA amplification*. New York, NY: Stockton Press.
- Faegri, K., and Pijl, van der L. 1966. *The principles of pollination ecology*. New York, NY: Pergamon Press.
- Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39:783-791.
- Fernald, M. L. 1950. *Gray's manual of botany*. 8th ed. New York, NY: D. Van Nostrand.
- Fleming, R. C. 1970. Food plants of some adult sphinx moths (Lepidoptera: Sphingidae). *The Michigan Entomologist* 3:17-23.





- Folsom, J. P. 1984. A reinterpretation of the status of relationships of taxa of the yellow-fringed orchid complex. *Orquidea* 9:320-345.
- Gilbert, D. 1993. SeqApp version 1.9, a multiple sequence editor for Macintosh computers. Published electronically on the Internet, available via anonymous ftp from ftp.bio.indiana.edu.
- Givnish, T. J., Sytsma, K. J., Smith, J. E., and Hahn, W. J. 1995. Molecular evolution, adaptive radiation, and geographic speciation in *Cyanea* (Campanulaceae). Pp. 288-337 in *Hawaiian biogeography: evolution on a hot spot archipelago*, W. L. Wagner and V. A. Funk, eds. Washington, DC Smithsonian Institution Press.
- Grant, V., and Grant, K. A. 1965. *Flower pollination in the phlox family*. New York, NY: Columbia University Press.
- Hapeman, J. R. 1997. Pollination and floral biology of *Platanthera peramoena* (A. Gray) A. Gray (Orchidaceae) Lmdleyana (in press).
- Herrera, C. M. 1993. Selection on complexity of corolla outline in a hawkmoth-pollinated violet. *Evolutionary Trends in Plants* 7:9-13.
- Hillis, D. M. 1987. Molecular versus morphological approaches to systematics. *Annual Review of Ecology and Systematics* 18:23-42.
- Hillis, D. M., and Huelsenbeck, J. P. 1992. Signal, noise, and reliability in molecular phylogenetic analyses. *Journal of Heredity* 83:189-195.
- Homoya, M. A. 1993. *Orchids of Indiana*. Bloomington, IN: Indiana University Press.
- Inoue, K. I. 1983. Systematics of the genus *Platanthera* (Orchidaceae) in Japan and adjacent regions with special reference to pollination. *Journal of the Faculty of Science, the University of Tokyo Section III*, 13: 285-374.
- Kevan, P. G. 1983. Floral colors through the insect eye: What they are and what they mean. Pp. 3-30 in *Handbook of experimental pollination biology*, C. E. Jones and R. J. Little, eds. New York, NY: Van Nostrand Reinhold.
- Kipping, J. L. 1971. *Pollination studies of native orchids*. M.S. Thesis, San Francisco State College.
- Kluge, A. G., and Farris, J. S. 1969. Quantitative phyletics and the evolution of anurans. *Systematic Zoology* 18:1-32.
- Kocher, T. D., Conroy, J. A., McKaye, K. R., and Stauffer, J. R. 1993. Similar morphologies of cichlid fishes in Lakes Tanganyika and Malawi are due to convergence. *Molecular Phylogenetics and Evolution* 2:158-165.
- Luer, C. A. 1975. *The native orchids of the United States and Canada*. New York, NY: New York Botanical Garden.
- Mabberley, D. J. 1987. *The plant book: a portable dictionary of the higher plants*. New York, NY: Cambridge University Press.
- Maddison, W. P., Donoghue, M. J., and Maddison, D. R. 1984. Outgroup analysis and parsimony. *Systematic Zoology* 33:103-103.
- Maddison, W. P., and Maddison, D. R. 1992. *MacClade: analysis of phylogeny and character evolution, version 3.05*. Sunderland, MA: Sinauer Associates.
- Moldenke, H. N. 1949. *American wildflowers*. New York, NY: Van Nostrand.
- Morong, T. 1893. A new species of *Listera*, with notes on other orchids. *Bulletin of the Torrey Botanical Club* 20:31-39.
- Nilsson, L. A. 1978. Pollination ecology and adaptation in *Platanthera chlorantha* (Orchidaceae). *Botaniska Notiser* 131:35-51.
- Nilsson, L. A. 1983. Processes of isolation and introgressive interplay between *Platanthera bifolia* (L.) Rich. and *P. chlorantha* (Custer) Reichb. (Orchidaceae). *Botanical Journal of the Linnean Society* 87:325-350.
- Nilsson, L. A. 1992. Orchid pollination biology. *Trends in Ecology and Evolution* 7:255-259.
- Ohwi, J. 1965. *Flora of Japan*. Washington, DC: Smithsonian Institution Press.
- Oste, C. 1988. Polymerase chain reaction. *Biotechniques* 6:162-167.
- Patt, J. M., Merchant, M. W., Williams, D. R. E., and Meeuse, B. J. D. 1989. Pollination biology of *Platanthera stricta* (Orchidaceae) in Olympic National Park, Washington. *American Journal of Botany* 76:1097-1106.
- Pijl, L. van der, and Dodson, C. H. 1966. *Orchid flowers: their pollination and evolution*. Miami, FL Fairchild Tropical Garden and the University of Miami Press.
- Robertson, J. L., and Wyatt, R. 1990. Evidence for pollination ecotypes in the yellow-fringed orchid, *Platanthera ciliaris*. *Evolution* 44:121-133.
- Sanderson, M. J. 1989. Confidence limits on phylogenies: the bootstrap revisited. *Cladistics* 5:113-129.
- Sanderson, M. J. 1995. Objections to bootstrapping phylogenies: a critique. *Systematic Biology* 44:299-320.





- Sanger, F., Nicklen, S., and Coulson, A. R. 1977. DNA sequencing with chain terminating inhibitors. *Proceedings of the National Academy of Science, USA* 74:5463.
- Scogin, R. 1983. Visible floral pigments and pollinators. Pp. 160-172 in *Handbook of experimental pollination biology*, C. E. Jones and R. J. Little, eds. New York, NY: Van Nostrand Reinhold.
- Sheviak, C. J., and Bowles, M. L. 1986. The prairie fringed orchids: a pollinator-isolated pair. *Rhodora* 88:267-290.
- Smith, G. W., and Snow, G. E. 1976. Pollination ecology of *Platanthera ciliaris* and *P. blephariglottis* (Orchidaceae). *Botanical Gazette (Crawfonsville)* 137:133-140.
- Smith, J. E., Sytsma, K. J., Shoemaker, J. S., and Smith, R. L. 1991. A qualitative comparison of total cellular DNA extraction protocols. *Phytochemical Bulletin* 23:2-9.
- Smith, W. R. 1993. *Orchids of Minnesota*. Minneapolis, MN: University of Minnesota Press.
- Stoutamire, W. P. 1971. Pollination in temperate American orchids. Pp. 233-243 in *Proc. 6<sup>th</sup> world orchid conference*, M. J. G. Corrigan, ed. Sydney, Australia: Halstead Press.
- Stoutamire, W. P. 1974. Relationships of the purple-fringed orchids *Platanthera psycodes* and *P. grandiflora*. *Brittonia* 26:42-58.
- Swofford, D. L. 1993. *PAUP: phylogenetic analysis using parsimony, version 3.1.1*. Champaign, IL: Illinois Natural History Survey
- Sytsma, K. J. 1990. DNA and morphology: inference of plant phylogeny *Trends in Ecology and Evolution* 5:104-110.
- Thien, L. B., and Utech, F. 1970. The mode of pollination in *Habenaria obtusata* (Orchidaceae). *American Journal of Botany* 57:1031-1035.
- Voss, E. G., and Riefner, R. E. 1983. A pyralid moth (Lepidoptera) as pollinator of blunt-leaf orchid. *Great Lakes Entomologist* 16:57-60.
- White, T. J., Bims, T., Lee, S., and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp. 315-322 in *PCR protocols: a guide to methods and applications*, M. Innis, D. Gelfand, J. Sninsky, and T. White, eds. San Diego, CA: Academic Press.
- Wood, J. J., Beaman, R. S., and Beaman, J. H. 1993. *The plants of Mount Kinabalu: 2. orchids*. London, England: Royal Botanic Gardens, Kew.
- Yuan, Y., and Küpfer, P. 1995. Molecular phylogenetics of the subtribe Genticinae (Gentianaceae) inferred from the sequences of internal transcribed spacers (ITS) of nuclear ribosomal DNA. *Plant Systematics and Evolution* 196:207-226.
- Zettler, L. W., Ahuja, N. S., and McInnis, T. M. 1996. Insect pollination of the endangered monkey-face orchid (*Platanthera integrilabia*) in McMinn County, Tennessee - one last glimpse of a once common spectacle. *Castanea* 61:14-24.

