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The Functional Significance of Staminodes, With Special Reference to *Penstemon* (Scrophulariaceae)

by

Jennifer Walker-Larsen

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ABSTRACT

Sterile stamens (staminodes) commonly originate from evolutionary stamen loss through progressive suppression of stamen development. They occur scattered throughout the angiosperms, but their functional significance remains poorly understood. A review of staminode occurrence in the context of Chase et al.'s (1993) DNA phylogeny of angiosperms indicates that most staminodes are transitional and likely non-functional. However, staminodes are occasionally modified to serve various floral roles. In some groups (e.g. Magnoliales/Laurales), staminodes form integral components of floral design. More commonly, functional staminodes characterize small lineages and serve incidental roles usually provided by alternate mechanisms. Such incidental staminode modification occurs in a few genera of the Scrophulariales. Staminode removal experiments involving four *Penstemon* (Scrophulariaceae) species indicate that the staminode facilitates contact between the pollinator and the sexual organs in bee-pollinated species. In contrast, the staminode of derived hummingbird-pollinated species appears functionless. Generally, staminode functioning seems limited to specific pollination systems.

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1. INTRODUCTION

1.1 Flower function

The flowers of hermaphroditic plants present their sexual organs in such a way as to promote reproductive success through both male and female function. Plants, being sessile, rely on the transport of pollen by vectors to gain access to mates. In animal-pollinated plants, floral design and display mediate pollinator attraction and facilitate male function through pollen export and female function through pollen receipt (Harder and Barrett, 1996). Animals typically visit flowers to gain rewards produced by flowers (reviewed by Neff and Simpson, 1983), most commonly nectar and/or pollen. Pollinators learn to associate these resources and their location with visual and olfactory cues such as the size of multi-flowered inflorescences (Klinkhamer and de Jong, 1993), flower colour (Kevan, 1983), flower size and shape (Schemske et al., 1996), and odour (Williams, 1983).

Pollen dispersal (and pollen receipt if resources do not limit seed production) is generally enhanced by increased pollinator visitation (thus increased attractiveness: Stanton et al., 1986) and floral mechanisms that mediate pollinator contact with the stigma and anthers (Harder and Barrett, 1996). Flowers direct pollinators to the location of rewards with colour patterns and odour (Kevan, 1983; Williams, 1983) and flower structure may, to a greater or lesser extent, manipulate pollinator movement to facilitate contact with the sexual organs (Campbell et al., 1994, 1996).

Floral evolution in angiosperm families has been characterized by trends towards reductions in male reproductive effort (pollen production) per flower (Stebbins, 1970; Proctor and Yeo, 1972). This reduction may relate to increased specialization in pollination. More ancestral animal-pollinated species tend to produce flowers that attract diverse pollinators (polyphilic) whereas more derived species tend to produce flowers specialized for pollination by a specific group of pollinators (oligophilic: Gilbert and Raven, 1975; Faegri and van der Pijl, 1979; Crepet 1983, 1984; but see Waser et al., 1996). Polyphilic flowers tend to be open and easily accessible and produce large

amounts of pollen which is liberally transferred to the bodies of insect visitors as they move about the flower (Richards, 1986). However, effective pollen transport to conspecific stigmas is unlikely due to the relatively low probability of a given pollinator consecutively visiting conspecific flowers (Herrera, 1987) and the pollinator's rather haphazard contact with floral organs. Oligophilic flowers, on the other hand, have shapes which tend to limit flower access to specific groups of obligate flower visitors which show greater floral constancy (Richards, 1986). These shapes also channel pollinator movement within the flower, allowing more precise pollen transfer between the flower and the pollinator (Richards, 1986). As a result, movement of pollen to conspecific flowers tends to be relatively high.

Increased precision in pollen transfer may have allowed an evolutionary decrease in pollen production per flower (Richards, 1986). Fitness of hermaphrodite plants includes fitness gains from both male function (realized through pollen donation) and female function (realized through seed production). Hence, flowers promote fitness to the extent that they allocate resources in a manner that optimizes both male and female function (Charnov, 1982; Brunet, 1992). Fitness gains for female function generally increase linearly with increased proportional allocation of floral resources when resource availability limits seed production (see Charnov, 1982, Fig 14.3). In contrast, fitness gains from male function (in animal-pollinated plants) likely decelerate with increased male effort (see Charnov, 1982, Fig. 14.3), due to pollinator saturation (Brunet, 1992), pollen layering on pollinators' bodies, and pollinator grooming (Harder and Wilson, 1994). As a result, the gains from increased allocation to male function likely diminish more rapidly with increased pollen transfer efficiency, thus favoring a shift of resources from male to female function.

Alternatively, reduced pollen production per flower could be related to the reallocation of floral resources from a small number of large flowers to a higher number of smaller flowers. In this situation, a constant proportional allocation of resources to

male function by a plant can be maintained by reducing absolute allocation to pollen production per flower.

1.2 The evolution and function of staminodes

Whether spurred by increased precision in pollen transfer or changes in flower size/number, reduction in male reproductive effort per flower largely involves loss of stamens (Stebbins, 1974). Stamen number may decrease through stamen fusion (Ronse Decraene and Smets, 1995), but more commonly involves progressive suppression of stamen development during morphogenesis (Tucker, 1988; Ronse Decraene and Smets, 1995). For example, among species of *Bauhinia* (Caesalpinaceae) a transition series is evident whereby fertile stamens cease pollen production, lose anthers, and filaments reduce progressively until the rudimentary organs themselves are lost (Endress, 1994). In this genus, stamen number varies among species from ten (the ancestral state) to one and 'missing' stamens are absent or represented by stamen remnants such as sterile stamens or filaments, termed staminodes (Tucker, 1988).

Within angiosperms, staminodes also result from at least two other processes. In spirally-arranged flowers, floral organs often transgress from one to another through intermediate structures. Transitional organs form between whorls of sepals and petals, petals and stamens, and stamens and carpels (Ronse Decraene and Smets, 1993). Non-functional organs between stamen and carpel, or stamen and petal whorls are referred to as staminodes. Staminodes may also result from a switch from hermaphrodite to unisexual flowers. Progressive suppression of male function in functionally female flowers is generally accompanied by transitions from fertile stamens to staminodes that produce sterile pollen (cryptic dioecy, Charlesworth, 1984) to stamen remnants and eventual stamen loss (dioecy). However, in this thesis I consider only staminodes in hermaphroditic flowers.

Despite the trend towards complete suppression of reduced stamens, evolutionary loss of staminodes is not inevitable. Selection may secondarily modify these remnant stamens to adopt other functions in the flower (Weberling, 1989). Commonly recognized

staminode functions include: pollinator attraction through visual conspicuousness and/or provision of attractants and rewards, as in species of the Eupomatiaceae and Lecythidaceae (Prance, 1976; Mori et al., 1978; Endress, 1984b); avoidance of self-pollination, as found in some Magnoliales (Endress, 1984a, 1984b, 1986, 1994); and facilitation of pollen removal and receipt through various trigger-mechanisms found in the Marantaceae (Kennedy, 1978; Rogers, 1984; Yeo, 1992) and Onagraceae (Eyde and Morgan, 1973; Plitmann et al., 1973; Heywood, 1985). Unfortunately, the basis for such assignments of function has been largely conjectoral or at best anecdotal, except for flowers exhibiting trigger-mechanisms. The conspicuous staminode of *Penstemon* (Scrophulariaceae) illustrates this trend, having generated much speculation as to its function without any experimental evidence (see Chapter 3).

The distribution of 'functional' staminodes in flowering plants is also unclear. Staminodes appear sporadically throughout the angiosperms (Stebbins, 1974), but their presence is often overlooked and seldom is the distinction made between 'functional' and 'remnant' staminodes. Highly modified staminodes may also be misinterpreted as derivations of other structures (Weberling, 1989).

1.3 Objectives

In this thesis I address two main objectives: 1) to review patterns of occurrence and postulated function of staminodes in angiosperms and 2) to identify the function of the staminode in *Penstemon* (Scrophulariaceae).

In Chapter 2, I discuss trends in floral design, stamen organization, staminode origin, and staminode function for each major angiosperm clade. I then extract common themes in floral design, staminode origin and staminode function, and discuss factors which influence staminode distribution and function. This analysis is conducted within the context of a recent DNA phylogeny of angiosperm families (Chase et al., 1993). To assess whether the coarse resolution of the Chase et al. (1993) phylogeny obscures evolutionary patterns, I also consider staminode evolution with a more fully resolved phylogeny of the Scrophulariales of the Asterid clade (compiled from Olmstead and

Reeves, 1995; A.D. Wolfe, pers. comm. 1997; Wolfe et al., 1997; and Spangler, pers. comm. 1998).

In Chapter 3, I assess the functional significance of the staminode in *Penstemon* (tribe Cheloneae: Scrophulariaceae) through staminode removal experiments. This study involves the determination of staminode function in both ancestral and derived pollination systems within the genus, with experimentation on two bee-pollinated (ancestral) and two bird-pollinated (derived) species. I assess the impacts of this removal on flower fitness by comparing components of male (pollen removal) and female (pollen receipt) function in flowers with and without staminodes. Staminode function is determined based on the impacts of its removal on pollen removal, pollen receipt, and on pollinator visit characteristics such as frequency and duration.

In Chapter 4, I summarize and synthesize the results and conclusions from Chapters 2 and 3. This overview illustrates the transitional and non-functional nature of most staminodes and the rare, opportunistic involvement of staminodes in floral function.

2 THE OCCURRENCE AND FUNCTIONAL SIGNIFICANCE OF STAMINODES IN ANGIOSPERMS

2.1 Introduction

Staminodes appear scattered throughout the angiosperms (Stebbins, 1974) and may be "non-functional", transitional stamen remnants, or "functional" stamen remnants, secondarily modified to take over other roles of the flower (Weberling, 1989). Historically, no distinction was made between functional and non-functional staminodes, and staminode presence in flowers was often overlooked (until recent reviews of androecium development in angiosperms by Ronse Decraene and Smets, 1992, 1993, 1995). These factors hinder assessment of the functional significance of staminodes in general and may have led to the premature dismissal of staminodes as unimportant structural components of angiosperm flowers.

In this chapter I review patterns of occurrence and functional significance of staminodes in the angiosperms, focusing on trends in floral evolution associated with staminode development, the separation of 'functional' versus 'remnant' staminodes, and trends in staminode functioning. I consider only staminodes in hermaphroditic flowers. Staminodes frequently occur in female flowers (e.g. in dioecious genera of the Asteraceae: Kuijt, 1987), resulting from incomplete suppression of male function. These staminodes may play important floral roles, as in *Saurauia* (Actinidiaceae) where staminodes provision pollinators with sterile pollen (Cane, 1993). However, as reduction of the entire androecium likely involves different genetic processes than partial reduction, these staminode types warrant separate investigation.

I conduct the analysis in the context of the Chase et al. (1993) phylogeny of the angiosperms based on chloroplast DNA *rbc*L sequences. Rice et al. (1997) caution against use of such large phylogenies due to significant limitations imposed by large data sets on parsimony analyses. However, a recent phylogeny of the angiosperms based on entire 18S ribosomal sequences by Soltis et al. (1997) shows general congruence with the Chase et al. (1993) analysis.

The Chase et al. (1993) phylogeny, along with staminode character states of each extant taxon in the analysis, allows me to examine a possible evolutionary history of staminodes within flowering plants. In this type of analysis, ancestral staminode character states are assigned to minimize the number of character state changes. According to Carlquist (1969), angiosperms should lose non-functional structures rapidly due to their plasticity and rapid evolution. Hence, if staminodes are largely non-functional and transitional, then they should only be evident in closely-related taxa at any given time in evolutionary history and should be lost quickly from the lineage. In this case, staminodes should appear primarily at the tips of phylogenies and larger taxa that include species with staminodes should be polymorphic. If, on the other hand, staminodes persist and are functional, then large lineages of taxa with staminodes should be apparent.

The level of resolution of staminode evolutionary history hinges on the resolution of the Chase et al. (1993) analysis, which is limited to the family level and above. Often, families are comprised of large numbers of species with different androecial arrangements, and the resulting polymorphic staminode states may obscure staminode evolution within a clade. To examine this possibility, I therefore present a more detailed DNA sequence analysis of the Scrophulariales (compiled from Olmstead, 1995; Wolfe, pers. comm. 1997; Wolfe et al., 1997; and R.E. Spangler, pers. comm. 1998).

2.2 Methods

Chase et al. (1993) constructed their phylogeny of the angiosperms from a parsimony analysis of DNA sequences for the chloroplast gene *rbc*L from 475 species representing all major taxonomic groups (subclasses and orders). This analysis resolves relationships within and above the order level, and in some cases, demonstrates paraphyly of families with several representatives (notably the Saxifragaceae and Grossulariaceae).

To examine the evolutionary history of staminodes, I determined androecial characteristics (number of stamen whorls and presence/absence of staminodes), floral design (symmetry, blossom type), mode of pollination, and staminode functionality (if present) for families considered in the Chase et al. (1993) analysis using a variety of

sources. In this analysis, "staminodes" refer to both "non-functional" and "functional" stamen remnants. Reviews of androecium development and evolution in the flowering plants by Ronse Decraene and Smets (1992, 1993, 1995) provided most androecial characteristics, whereas Cronquist (1981) and family synopses in various floras provided information on floral design. I determined pollination mechanisms and staminode functioning, when possible, from family synopses, reviews of pollination biology, and results of experimental pollination studies.

I coded staminode states for single-state families as 0-staminode(s) absent, 1-complete whorls of staminodes present, or 2-partial whorls of staminodes present. A partial whorl of staminodes may involve from one to many staminodes, but the number of staminodes is always less than the number of stamens in an intact whorl. To enhance resolution by limiting the number of polymorphic codings (following Maddison and Maddison, 1992), I coded polymorphic families as 01-states 0 and 1, 02-states 0 and 2, or 012-states 0, 1 and 2. I considered the gain of a single state as one step and the loss of a single state as one step, with no penalty for retaining polymorphisms. The character states were unordered, allowing state change in any direction.

I traced the staminode character onto the Chase et al. (1993) phylogeny using MacClade 3.0 (Maddison and Maddison, 1992). This software reconstructs character evolution by inferring ancestral character states that imply the fewest character state changes. Results are presented in the form of a cladogram. Cladogram branches are shaded with different patterns that represent hypothesized ancestral character states whereas boxes at the tip of each terminal branch display the current character states of extant taxa. Unlike extant taxa, ancestral branches cannot be assigned more than one character state. Occasionally, more than one ancestral character state is equally parsimonious and in these cases the branch is shaded with a pattern indicating its equivocal status.

I present the resulting phylogeny in five sections, corresponding to the major clades recognized by the Chase et al. (1993) analysis. A small, inset cladogram indicates

the position of the clade in the larger phylogeny. In some cases, paraphyletic families of the Chase et al. (1993) analysis divided along tribal boundaries. In these situations, I replaced the genus name with that of the representative tribe.

I also trace the evolution of staminodes within the Scrophulariales using MacClade (Maddison and Maddison, 1992). I use Olmstead and Reeve's (1995) *rbc*L and *ndh*F phylogeny of the Scrophulariales, with the addition of the Cheloneae (Scrophulariaceae) as determined by Wolfe et al.'s (1997) and A.D. Wolfe's (pers. comm. 1997) *rbc*L phylogeny. I also inset R.E. Spangler's (pers. comm. 1998) *rbc*L and *ndh*F phylogeny of the Bignoniaceae. I assigned unordered character states for extant taxa as 0 - stamen absent, 1 - stamen represented by small rudimentary staminode, 2 - stamen represented by large staminode, and 3 - stamen fertile. I considered each character state change as one step.

2.3 Results and Discussion

2.3.1 Incidence of stamen reduction and staminode formation

All major clades of flowering plants identified by Chase et al. (1993) exhibit reduction of entire stamen whorls (Table 2.1). In particular, 34.0% of Monocot, 46.2% of Hamamelid/Ranunculid, 67.7% of Rosid, and 88.7% of Asterid families include species with an ancestral stamen whorl that has been either incompletely or completely suppressed. Some species of the Magnoliales/Laurales clade also demonstrate reduction, but the prevalence of spirally arranged flowers confounds determination of stamen loss as discussed in section 2.3.2.1.2.

Interestingly, the incidence of whorls of staminodes does not parallel the loss of functional stamen whorls. Whorled staminodes are found in at least some species of 8.5% of Monocot, 23.1% of Hamamelid/Ranunculid, 25.0% of Rosid, and 7.0% of Asterid families (Table 2.1). Hence the clades with the lowest (Monocots) and the highest (Asterids) loss of stamen whorls equally have the lowest relative frequency of

staminodes at the family level. In the Magnoliales/Laurales, 63.6% of families include species with whorls of staminodes (Table 2.1).

Stamen reduction does not necessarily involve an entire ancestral stamen whorl. Often, individual stamens within a whorl are suppressed through partial reduction of a stamen whorl. The number of stamens involved vary from one to many, although the number of reduced stamens must be less than the number of fertile stamens in the ancestral whorl. Reduction of a partial stamen whorl occurs only in more derived clades, with 19.1% of Monocot, 15.4% of Hamamelid/Ranunculid, 34.4% of Rosid, and 23.9% of Asterid families having species with either an incompletely or completely reduced partial whorl(s) (Table 2.1). Partial whorl loss is strongly associated with zygomorphy (98.9% of 11,961 genera with a reduced partial whorl(s) are zygomorphic). Staminodes represent these partial whorls in 88.9% of Monocot, 100% of Hamamelid/Ranunculid, 36.4% of Rosid, and 52.9% of Asterid families that include species with a reduced partial whorl(s).

Most families, except most Asterids and those with few genera, demonstrate variable staminode states and androecial configurations. This suggests that the appropriate level to study staminode evolution is at the subtribe or genus level (see section 2.3.2.5.2). However, because floral trends differ in each major clade, analysis of androecial characters in the context of the Chase et al. (1993) phylogeny illustrates different general patterns of stamen loss, staminode formation and staminode function. Therefore, I first discuss floral trends, staminode origin, and staminode functions individually for each major clade individually and then consider common themes for angiosperms as a whole.

Table 2.1 Frequency of stamen reduction and associated staminode development in major angiosperm clades (sensu Cronquist 1981). A family is counted if at least one species exhibits the trait of interest rather than all species exhibit the trait.

	Magnoliales/ Laurales	Monocots	Hamamelids/ Ranunculids	Rosiidae	Asteriidae
# families with unambiguous[1] androecia	22	47	13	96	71
Loss of entire stamen whorl					
# families with a reduced ancestral whorl	undetermined[2]	16	6	65	63
# families with whorl of staminodes representing reduced whorl	14	4	3	24	5
Partial loss of stamen whorl(s)					
# families with a reduced part of an ancestral whorl(s)	0	9	2	33	17
loss associated with zygomorphy		9	2	27	17
# families with staminodes representing reduced partial whorl	0	8	2	12	9

[1]unambiguous androecia refers to stamen arrangements for which the developmental origins of each stamen are known

[2]staminodes may or may not be associated with reduction of fertile stamen whorls (see section 2.3.2.1.2)

2.3.2 Floral trends and staminode origin and function in major angiosperm clades

2.3.2.1 Magnoliales/Laurales

2.3.2.1.1 Floral trends

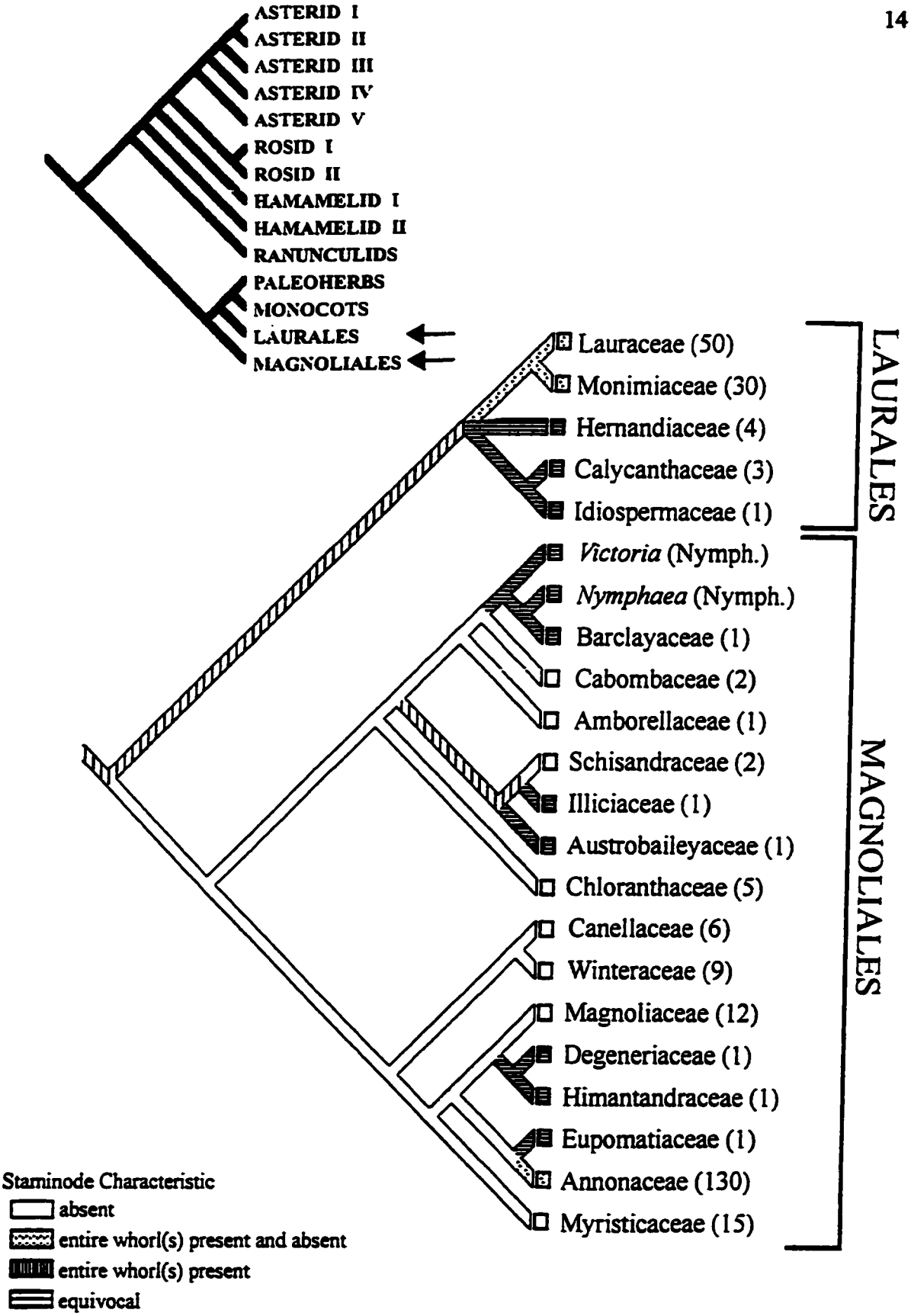
Two distinct floral types, both with or without staminodes, characterize the Magnoliales and Laurales: 1) short-lived, large, complex conspicuous flowers ('magnolids') and 2) longer-lived, small, simple inconspicuous flowers ('laurids'). Beetle-pollinated magnolid-type flowers are composed of numerous, spirally arranged parts, and often include an inner whorl of large, petaloid staminodes. These flowers typify the Idiospermaceae, Calycanthaceae, Nymphaeaceae, Barclayaceae, Austrobaileyaceae, Degeneriaceae, Himantandraceae, Eupomatiaceae, and Annonaceae. Laurid-type flowers are comprised of a reduced number of cyclically arranged parts, contain inner and outer whorls of small staminodes and are pollinated by small diverse insects. Laurid-type flowers characterize the Lauraceae, Monimiaceae, Hernandiaceae, Cabombaceae, Amborellaceae, Schisandraceae, Chloranthaceae, Canellaceae, and Myristicaceae. The Winteraceae contain flowers of both types.

2.3.2.1.2 Staminode origin

Staminodes arose independently at least five times within the Magnoliales/Laurales (Fig 2.1). Clades with staminodes are not restricted solely to branch tips of the cladogram in three cases, hence staminodes seem to persist and be maintained, indicating function.

The mechanism of staminode formation in magnolid-type flowers is unclear due to the absence of transition series within or between families. Staminodes may have resulted from stamen reduction by suppression of first- or last-formed stamen primordia (Ronse Decraene and Smets, 1993). Alternatively, staminodes may have originated as a consequence of the spiral arrangement of floral primordia. In spirally-arranged flowers, floral organs often transgress into each other through intermediate forms, forming carpellodes, staminodes, staminodial petals, etc. (Ronse Decraene and Smets, 1993).

Fig 2.1. Distribution of entire staminode whorls in the Magnoliales and Laurales clades based on the Chase et al. (1993) phylogeny. Positions of genera within the paraphyletic Nymphaeaceae (Nymph.) are included. Family names follow Cronquist (1981). Numbers in parentheses represent the number of genera comprising the family.


ASTERID I
ASTERID II
ASTERID III
ASTERID IV
ASTERID V
ROSID I
ROSID II
HAMAMELID I
HAMAMELID II
RANUNCULIDS
PALEOHERBS
MONOCOTS
LAURALES
MAGNOLIALES
Lauraceae (50)
Monimiaceae (30)
Hernandiaceae (4)
Calycanthaceae (3)
Idiospermaceae (1)
Victoria (Nymph.)
Nymphaea (Nymph.)
Barclayaceae (1)
Cabombaceae (2)
Amborellaceae (1)
Schisandraceae (2)
Illiciaceae (1)
Austrobaileyaceae (1)
Chloranthaceae (5)
Canellaceae (6)
Winteraceae (9)
Magnoliaceae (12)
Degeneriaceae (1)
Himantandraceae (1)
Eupomatiaceae (1)
Annonaceae (130)
Myristicaceae (15)
LAURALES
MAGNOLIALES
Staminode Characteristic
absent
entire whorl(s) present and absent
entire whorl(s) present
equivocal

In contrast, staminodes in cyclical laurid-type flowers arise in association with stamen-whorl reduction, as is evident in transition series in the Lauraceae. Flowers of ancestral members of this family likely produced four stamen whorls. However, in flowers of more derived taxa the innermost and sometimes two outermost stamen whorls range from fertile stamens (*Dodecadenia*, Allen, 1948) to staminodes (*Eusideroxylon*, Hutchinson, 1964) to absent (*Endiandra*, Hutchinson, 1964; *Misantheca*, Allen, 1948).

2.3.2.1.3 Staminode function

In magnolid-type flowers, staminodes function primarily to prevent self-pollination in concert with other floral mechanisms, such as synchronous floral anthesis within a plant and floral closure at night (Endress, 1984b). Petaloid inner staminodes between the carpels and stamens of these protogynous and self-compatible flowers bend inwards following female phase (Grant, 1950a; Faegri and van der Pijl, 1979; Miller, 1989; Endress, 1994), effectively shielding the receptive stigmas. This staminode movement occurs in all magnolid-type flowers with staminodes, except the modified trap flowers of the Nymphaeaceae (Schneider, 1976; Faegri and van der Pijl, 1979). Flowers without inner staminodes show alternate mechanisms, such as stigmas raised above the stamens (Magnoliaceae and Winteraceae: Friis and Endress, 1990) and stigma abscission (Annonaceae: Endress, 1994).

Secondary functions of inner staminodes in magnolid-type flowers include pollinator attraction through visual and olfactory signals and the provision of rewards. Contrasting coloured staminodes in the Calycanthaceae and Himantandraceae (Endress, 1984a,b) increase perianth conspicuousness, whereas the staminodes of the Austrobaileyaceae, Eupomatiaceae, and Degeneriaceae emit scent (Endress, 1994). Staminodes sometimes provide rewards, secreting food bodies in the Degeneriaceae (Endress, 1984b), Calycanthaceae (Grant, 1950a), and Eupomatiaceae (Endress, 1994).

In laurid-type flowers, staminodes attract pollinators through the provision of nectar (Woodson and Schery, 1948; Endress, 1984b, 1986). Although these flowers are

also protogynous and self-compatible, they lack obvious mechanisms to prevent autogamy.

2.3.2.2 Monocots

2.3.2.2.1 Floral trends

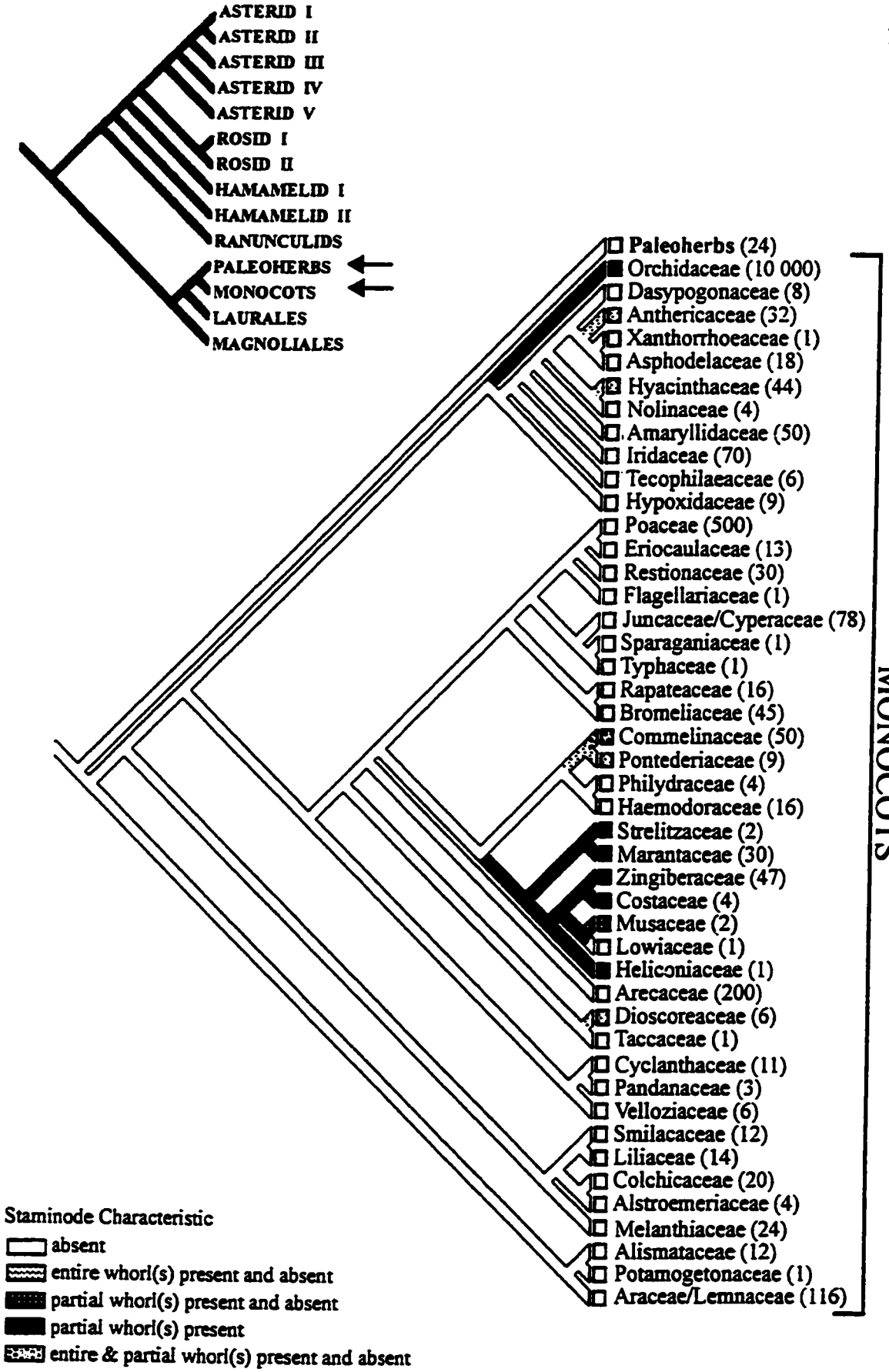
Three main floral types exist in the Monocots, depending on pollination syndrome. Animal-pollinated species have flowers either solitary or arranged in loose inflorescences (except for the dense spike-like panicles of some Pontederiaceae, Costaceae, and Xanthorroeaceae) and can be split into two groups based on flower shape. Regular flowers, pollinated by diverse, small insects, occur throughout animal-pollinated Monocots and likely represent the ancestral condition (Dahlgren and Clifford, 1982). Irregular flowers, with more specialized pollinators (typically bees and birds), evolved in the Commelinales-Zingiberales clade, the Orchidaceae, and the Iridaceae (Fig 2.2). Wind-pollinated plants, with small, reduced flowers generally crowded into dense spikes or heads, predominate in the Poaceae-Typhaceae clade and occur independently in the Potamogetonaceae, Pandanaceae and Arecaceae.

2.3.2.2.2 Staminode origin

Ancestrally, Monocot flowers produce two whorls of fertile stamens, though reduction of an entire stamen whorl occurs in 37.8% of Monocot families pollinated by polyphilic insects or wind. Stamen reduction involves inner and outer whorls with equal frequency and transition series occur in the Anthericaceae, Hycinthaceae, Tradescantieae (Commelinaceae) and insect-pollinated Pontederiaceae, in which the reduced whorl may be fertile, represented by small staminodes, or absent. Staminode whorls do not occur in wind-pollinated species.

At least four independent origins of whorled staminodes occur in the Monocots, with three origins restricted to single families (Fig. 2.2). Clades with whorled staminodes occur mainly at branch tips, indicating that staminode whorls are generally not maintained

Fig 2.2. Distribution of entire and partial staminode whorls in the Monocot clade based on the Chase et al. (1993) phylogeny. Family names follow Cronquist (1981). Numbers in parentheses represent the number of genera comprising the family. 'Paleoherbs' includes the Aristolochiaceae, Lactoridaceae, Saururaceae and Piperaceae.


ASTERID I
ASTERID II
ASTERID III
ASTERID IV
ASTERID V
ROSID I
ROSID II
HAMAMELID I
HAMAMELID II
RANUNCULIDS
PALEOHERBS
MONOCOTS
LAURALES
MAGNOLIALES
Paleoherbs (24)
Orchidaceae (10 000)
Dasypogonaceae (8)
Anthericaceae (32)
Xanthorrhoeaceae (1)
Asphodelaceae (18)
Hyacinthaceae (44)
Nolinaceae (4)
Amaryllidaceae (50)
Iridaceae (70)
Tecophilaeaceae (6)
Hypoxidaceae (9)
Poaceae (500)
Eriocaulaceae (13)
Restionaceae (30)
Flagellariaceae (1)
Juncaceae/Cyperaceae (78)
Sparaganiaceae (1)
Typhaceae (1)
Rapateaceae (16)
Bromeliaceae (45)
Commelinaceae (50)
Pontederiaceae (9)
Philydraceae (4)
Haemodoraceae (16)
Strelitzaceae (2)
Marantaceae (30)
Zingiberaceae (47)
Costaceae (4)
Musaceae (2)
Lowiaceae (1)
Heliconiaceae (1)
Arecaceae (200)
Dioscoreaceae (6)
Taccaceae (1)
Cyclanthaceae (11)
Pandanaceae (3)
Velloziaceae (6)
Smilacaceae (12)
Liliaceae (14)
Colchicaceae (20)
Alstroemeriaceae (4)
Melanthiaceae (24)
Alismataceae (12)
Potamogetonaceae (1)
Araceae/Lemnaceae (116)
MONOCOTS
Staminode Characteristic
absent
entire whorl(s) present and absent
partial whorl(s) present and absent
partial whorl(s) present
entire & partial whorl(s) present and absent

and do not persist. This pattern seems not to apply to the Commelinaceae-Pontederiaceae clade; an ancestral character state of whorled staminodes present and absent, as indicated for this clade, is unlikely. Most Pontederiaceae have two stamen whorls (Cronquist, 1981) and, given the seeming irreversibility of stamen whorl reduction in angiosperms (discussed in section 2.3.2.4.2), the ancestor must also have had two stamen whorls. This argues for independent staminode origins in both families. Hence whorled staminodes probably evolved five times within Monocots.

Reduction of partial stamen whorls occurs in taxa with specialized animal-pollinated flowers (37.8% of Monocot families). Loss of some stamens within a stamen whorl is strongly associated with zygomorphy (with the exception of the Iridaceae and some actinomorphic Pontederiaceae) and thus predominates in specialized insect-pollinated plants. In these groups, staminodes generally represent the reduced partial whorl either as small rudiments (Heliconiaceae, Musaceae, and Strelitzaceae) or as large, elaborate organs (Orchidaceae, Marantaceae, Zingiberaceae, and Costaceae). Partial staminode whorls originated independently twice in the Monocots (Fig. 2.2) and typify all species of the Zingiberales and Orchidaceae (except the Musaceae-Lowiaceae clade: Fig. 2.2). This retention of partial whorls of staminodes within lineages indicates that they are selectively maintained in these groups.

Reduction of stamens within whorls also occurs in some wind-pollinated plants with only one or two fertile stamens per flower (as in several members of the Poaceae, Pandanaceae, Eriocaulaceae, Cyperaceae, Sparaginaceae and Typhaceae). However, in contrast to animal-pollinated taxa, this reduction of the androecium resulted in complete stamen loss with no retention of staminodes.

Direct evolution of functional staminodes from functional stamens likely occurred in the Commelineae tribe of the Commelinaceae. Unlike a stamen reductive trend, the staminodes persist in all species and function as part of a pollen mimicry system (Vogel, 1978; Faden, 1992; but see Simpson et al., 1986). Within the tribe, a transition series is evident in which lower "fodder" stamens become larger and more conspicuous and upper

stamens become less conspicuous. Upper stamens remain fertile, whereas lower stamens produce smaller and fewer pollen grains (*Tinantia*: Simpson et al., 1986) until they become staminodial (*Commelina*). Staminodes may be rewarding (producing sterile pollen), or non-rewarding (Yeo, 1992). At all stages of this transition series, the lower stamens (or staminodes) remain functional, and hence likely evolved without an intervening non-functional stage.

2.3.2.2.3 Staminode function

Whorls of staminodes in Monocots generally appear to be non-functional and transitional in nature, as they are rarely elaborated to take over other roles except in the Xyridaceae (aligned with the Commelinales by Cronquist [1981] but not included in the Chase *et al.* [1993] analysis). *Xyris* flowers contain an outer whorl of large bifid staminodes tipped with tufts of moniliform hairs (Woodson and Schery, 1944; Kral, 1966). These staminodes may function as secondary pollen presenters (Yeo, 1992), but their similarity to staminodes of other pollen flowers such as *Commelina* (Commelinaceae) and *Sparmannia* (Tiliaceae) suggests an analogous function. In these flowers staminodes mimic larger amounts of pollen (Osche, 1983 per Endress, 1994) and may stimulate bees to carry out pollen-collecting movements (Vogel, 1978).

In contrast, partial whorls of staminodes often become an integral component of Monocot flower structure, as seen in the Orchidaceae and within the Zingiberales clade. Two lines have evolved from ancestral orchids, whose flowers included only the lateral two anthers of the inner whorl and one stamen of the outer whorl fertile and no staminodes (Burns-Balogh and Bernhardt, 1985). In monandrous orchids the remaining anthers of the inner whorl evolved into staminodes that range from small proturberances to flared appendages on the column (termed column wings) and function to direct and control pollinator movement (Burns-Balogh and Bernhardt, 1985). In diandrous orchids the remaining outer stamen became a staminode (Abraham and Vatsala, 1981) which forms a fully integrated component of the trap in the Cypripedioideae (Burns-Balogh and Bernhardt, 1985). This staminode, elaborated in a variety of ways (see Braem [1988] for

staminode diversity in *Paphipedilum*), generally mimics attractive structures such as brood sites (*Paphipedilum rothschildianum*: Atwood, 1985) and nectar (*Paphiopedilum villosum*: Banziger, 1996).

In the Zingiberales, staminodes function in pollinator attraction, trigger mechanisms that promote contact between the pollinator and the pollen-presenting style and stigma, and as nectar guides. Staminodes are large and elaborate in the Marantaceae, Zingiberaceae, Costaceae and Cannaceae (not included in the Chase *et al.* [1993] analysis, but commonly aligned with the Marantaceae [Cronquist, 1981; Kress, 1990]). In the complicated lip flowers of the Cannaceae, Costaceae, and Zingiberaceae, varying numbers of petaloid staminodes form the labellum (often the most conspicuous element of the flower: Faegri and van der Pijl, 1979; Hickey and King, 1988) and act with the perianth as advertising organs (Maas, 1972; Smith, 1987). A very different flower structure exists in the bee-pollinated Marantaceae, where staminodes are involved in a trigger mechanism for explosive pollination (see Kennedy, 1978; Rogers, 1984; Yeo, 1992). A petaloid (cucullate) staminode encloses the pollen-presenting style, whereas a hooded and lobed staminode orients the pollinator and braces the mechanism. When the bee touches a trigger appendage of the cucullate staminode, the style releases and contacts the pollinator, achieving both pollen removal and pollen release.

Such radical shifts in floral roles have not occurred in other Monocot families, such as the remainder of the Zingiberales and some Commelinaceae with partial staminode whorls. In the Musaceae, Heliconiaceae, Strelitzaceae and Lowiaceae (Zingiberales) pollinated by hummingbirds, bats, and flies (Wolf and Stiles, 1989; Heywood, 1985; Cronquist, 1981), only one stamen of the outer whorl has been reduced, usually represented by a small staminode (absent in the Lowiaceae). This staminode may direct pollinators' tongues towards the nectaries (Endress, 1994). The pollen flowers of the Commelineae tribe (Commelinaceae) exhibit a trend towards increased anther dimorphism as part of a pollen mimicry system (Vogel, 1978, Fader, 1992; but see Simpson et al., 1986). Lower "fodder" stamens/staminodes attract pollinators and provide (or mimic)

pollen, either fertile or sterile, whereas the inconspicuous upper stamens place fertile pollen onto the pollinator (Vogel, 1978; Yeo, 1992; but see Simpson et al., 1986)

2.3.2.3 Hamamelids/Ranunculids

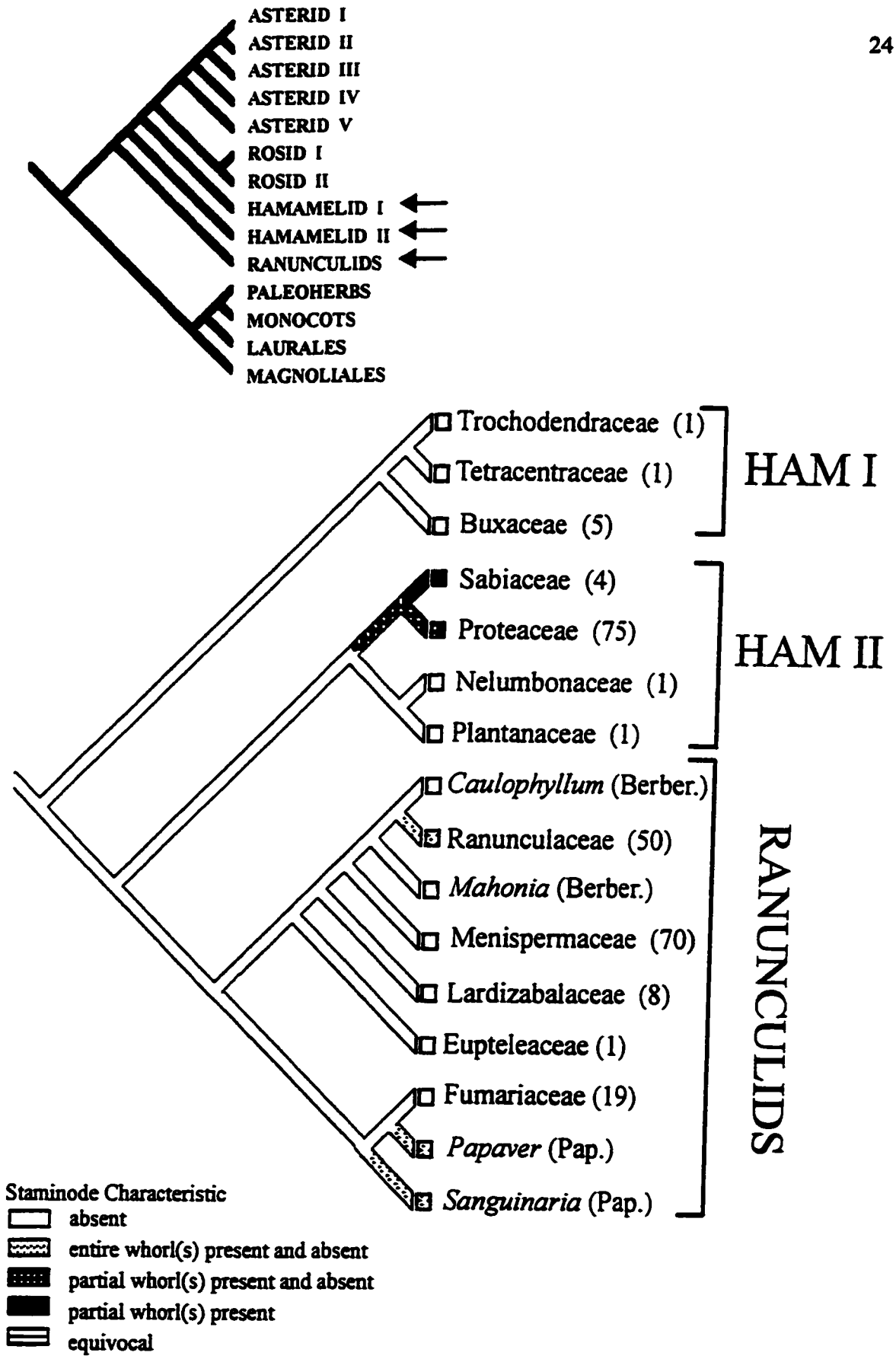
2.3.2.3.1 Floral trends

The Hamamelid and Ranunculid clades demonstrate a variety of pollination syndromes and floral trends. Flowers of the Ranunculid clade generally have floral parts arranged in whorls, with one to several whorls each of sepals, petals, and stamens (except the reduced wind-pollinated flowers of some Eupteleaceae and *Thalictrum* [Ranunculaceae]). The androecium is extremely variable, with flowers containing numerous spirally arranged stamens, numerous stamen whorls, two stamen whorls, or only one stamen whorl. Diverse insects pollinate most species, although species with spurred petals in the Fumariaceae and Ranunculaceae (*Aquilegia, Delphinium, Aconitium*) demonstrate more specialized pollinator relationships, with large-bodied bees and hummingbirds as principal pollinators. Flowers of the Hamamelid clades generally have one whorl each of perianth members (petals often lacking) and one whorl of stamens, although numerous, spirally-arranged stamens characterize the Trochendraceae and Nelumbonaceae. Wind-pollination occurs commonly in Hamamelid clades, characterizing all Hamamelid I members and the Platanaceae of the Hamamelid II clade (Fig. 2.3). Pollinators of the Sabiaceae and Proteaceae include insects, birds, and mammals. The enigmatic Nelumbonaceae share floral characteristics and pollination syndrome of "magnolid" Magnoliales and Laurales.

2.3.2.3.2 Staminode origin

In the Ranunculid clade, whorls of staminodes are associated with reduction of entire stamen whorls in three independent cases (Fig. 2.3). Members of the Ranunculaceae and Papaveraceae demonstrate reductive series from spiral polyandry and polycycly to only one whorl of fertile stamens. A small proportion of species in both families exhibit entire whorls of staminodes (Cronquist, 1981).

Fig 2.3. Distribution of entire and partial staminode whorls in the Hamamelid and Ranunculid clades based on the Chase et al. (1993) phylogeny. Positions of genera within the paraphyletic Berberidaceae (Berber.) and Papaveraceae (Pap.) are included. Family names follow Cronquist (1981). Numbers in parentheses represent the number of genera comprising the family.


ASTERID I
ASTERID II
ASTERID III
ASTERID IV
ASTERID V
ROSID I
ROSID II
HAMAMELID I
HAMAMELID II
RANUNCULIDS
PALEOHERBS
MONOCOTS
LAURALES
MAGNOLIALES
Trochodendraceae (1)
Tetracentraceae (1)
Buxaceae (5)
HAM I
Sabiaceae (4)
Proteaceae (75)
Nelumbonaceae (1)
Plantanaceae (1)
HAM II
Caulophyllum (Berber.)
Ranunculaceae (50)
Mahonia (Berber.)
Menispermaceae (70)
Lardizabalaceae (8)
Eupteleaceae (1)
Fumariaceae (19)
Papaver (Pap.)
Sanguinaria (Pap.)
RANUNCULIDS
Staminode Characteristic
absent
entire whorl(s) present and absent
partial whorl(s) present and absent
partial whorl(s) present
equivocal

Partial staminode whorls originated once in the Hamamelids and the Ranunculids (Fig. 2.3), associated with loss of a partial stamen whorl in the Sabiaceae and within the Proteaceae of the animal-pollinated Hamamelids (Fig. 2.3). Specialized zygomorphic flowers of each group (e.g. *Conospermum* [Proteaceae] and *Meliosma* [Sabiaceae]), have only two fertile stamens from an original whorl of four or five, with the remainder represented by staminodes (Cronquist, 1981).

2.3.2.3.3 Staminode function

Whorled staminodes are relatively rare, small, and most likely transitional in nature in the Ranunculids, with the exception of *Aquilegia* (Ranunculaceae). In *Aquilegia*, one or two inner whorls of broad, membranous staminodes form a sheath around the ovaries (Brayshaw, 1989). According to Brayshaw (1989), this sheath protects the ovaries against damage by pollinators.

In the Hamamelidaceae, partial staminode whorls form components of explosive pollination mechanisms. In *Conospermum* (Proteaceae), small staminodes at the base of the style hold the style back and act as triggers (Holm, 1978). When a pollinator probing for nectar touches the staminodes, the style is released and contacts the pollinator. Following style contact, the anthers of the two fertile stamens explode and dust the pollinator with pollen. A very different mechanism (see van Beusekom, 1971) exists in some *Meliosma* (Sabiaceae). A complex of two fertile stamens and three fleshy staminodes encircle the pistil of the open flowers with the top of the style protruding. Cavities in the staminodes enclose the anthers of the fertile stamens, which dehisce before flower anthesis. When a pollinator touches the flower, the stamen filaments release, explosively discharging pollen.

2.3.2.4 Rosidae

2.3.2.4.1 Floral trends

Most flowers in the Rosidae produce nectar as a reward, are open and radially symmetric, and are pollinated by diverse small insects. Trends towards more specialized

insect pollination occur within most families, with some members of a family typically developing irregular petals (such as *Lopezia* and *Gaura* of the Onagraceae) and becoming zygomorphic. Papillonaceous flowers, pollinated by bumble bees, characterize the Trigoniaceae, Fabaceae, and Polygalaceae. Tubular flowers occur rarely, formed by the extension and fusion of the hypanthium in Lythraceae, Punicaceae, and Combretaceae. Pollen flowers, producing only pollen as a reward and pollinated by female bees, occur throughout the Rosidae in the Molluginaceae-Aizoaceae clade (Fig. 2.4) and in families such as the Tiliaceae, Paeoniaceae, Ochnaceae, Rosaceae, Fabaceae, and Datiscaceae. Regular flowers specialized to large, animal pollinators occur within the Bombaceae-Dipterocarpaceae clade and the Onagraceae-Myrtaceae clade, including species pollinated by honeyeaters (some Malvaceae and Myrtaceae), bats (Bombaceae, Malvaceae, Tiliaceae) and small rodents (some Melastomataceae). Wind pollination characterizes families within most clades of the Rosid II group (Leitneriaceae, Bataceae, Haloragaceae) and occurs within the Viscaceae, Amaranthaceae, and Aceraceae. Wind pollination occurs less frequently in the derived Rosids, being common to only the Reduced Rosids I group.

2.3.2.4.2 Staminode origin

Reduction of one of two ancestral stamen whorls occurs commonly within animal-pollinated families of the Rosidae, with 72% of families having some species with only one whorl of fertile stamens. An early stage of stamen suppression is evident in some families, such as the Humiriaceae, where one of the two fertile whorls has smaller stamens (Ronse Decraene and Smets, 1993). Reduction seems to involve both inner and outer whorls of stamens, but such observations are confounded by positional shifts of the stamen primordia in the developing buds of some flowers, causing the two whorls to switch positions (Ronse Decraene and Smets, 1993).

Reduction of a stamen whorl in Rosids is never reversed. Most pollen flowers (except those with poricidal anthers as the Melastomataceae) and flowers specialized to large animal pollinators produce large amounts of pollen through a secondary increase of

stamen number. Stamen number increases through subdivision of fertile stamen primordia (Ronse Decraene and Smets, 1992), rather than re-initiation of suppressed stamen primordia. For example, in the Sterculiaceae flowers have one whorl of stamens and one whorl of staminodes present or absent. In *Dombeya* and *Guazuma* five stamen primordia of the fertile whorl have split to form 15 stamens, whereas the reduced whorl remains as five staminodes (Robyns, 1964; Young et al., 1984). Similar development of staminode whorls characterizes the Tiliaceae and some Myrtaceae.

Staminodes occasionally replace the reduced whorls. Staminode whorls evolved independently at least 14 times within the Rosidae (Figs 2.4 and 2.5), most commonly within individual families (indicated by character states with staminodes both present and absent). Staminodes typify only small and monotypic families (Pterostemonoideae and Moringaceae: Fig 2.4) and the small Greyiaceae-Francooideae clade (Fig. 2.5), indicating that staminodes seldom persist. This lack of persistence also characterizes the Celastraceae-Parnassiaceae clade (Fig. 2.5), despite the equivocal ancestral character state. Whorled staminodes seem an unlikely ancestral state, given the irreversible nature of stamen whorl loss and the presence of two fertile whorls in the Celastraceae. Therefore, the most parsimonious ancestral character (if the phylogeny is correct) is whorled staminodes present and absent, which does not indicate staminode persistence.

Reduction of stamens within stamen whorls is also a common trend in animal-pollinated Rosiidae, as 35.3% of families have species with partial whorl(s) of stamens. In these groups, stamen reduction mainly involves progressive suppression of stamen development (Ronse Decraene and Smets, 1995), but loss through stamen fusion occurs in some taxa (Cucurbitaceae: Ronse Decraene and Smets, 1995). Reduction of partial stamen whorls is strongly associated with zygomorphy (82% of species with partial stamen loss are zygomorphic compared to 0% of species with intact stamen whorls) and specialized entomophilous pollination syndromes. The Rosiidae demonstrate the complete spectrum of stamen reduction, from flowers with unstable androecia and variable numbers of fertile stamens and staminodes, as in *Pelargonium* (Geraniaceae), to flowers that have

Fig 2.4. Distribution of entire and partial staminode whorls in Rosid II clade based on the Chase et al. (1993) phylogeny. Positions of genera (or tribes) within the paraphyletic Capparaceae (Cap.), Grossulariaceae (Gross.), Saxifragaceae (Sax.), and Chenopodiaceae (Chen.) are included. Family names follow Cronquist (1981). Numbers in parentheses represent the number of genera comprising the family. 'Reduced Rosids II' include the Cercidiphyllaceae, Daphniphyllaceae and Hamamelidaceae, families with reduced flowers and ambiguous androecia.

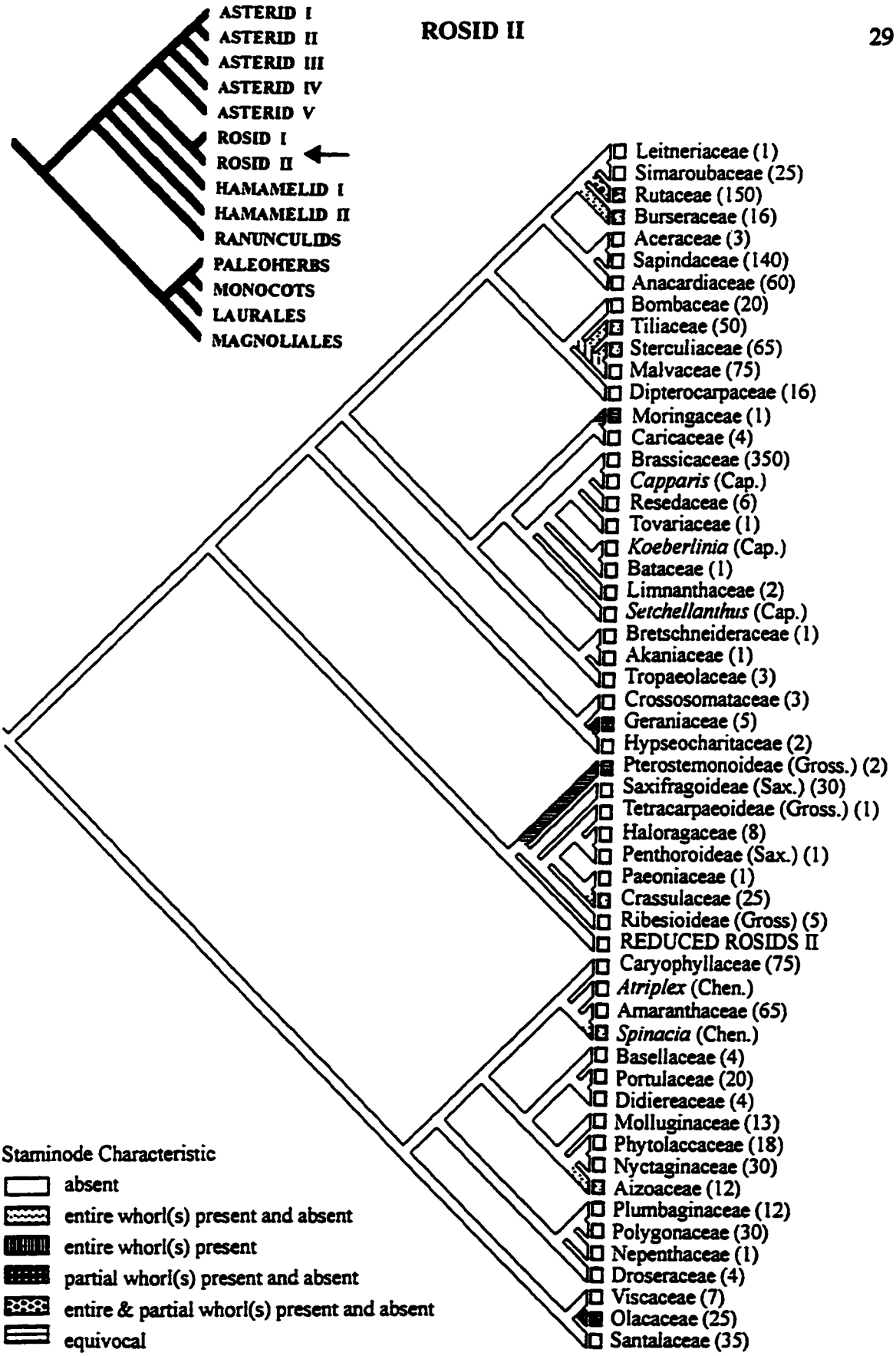
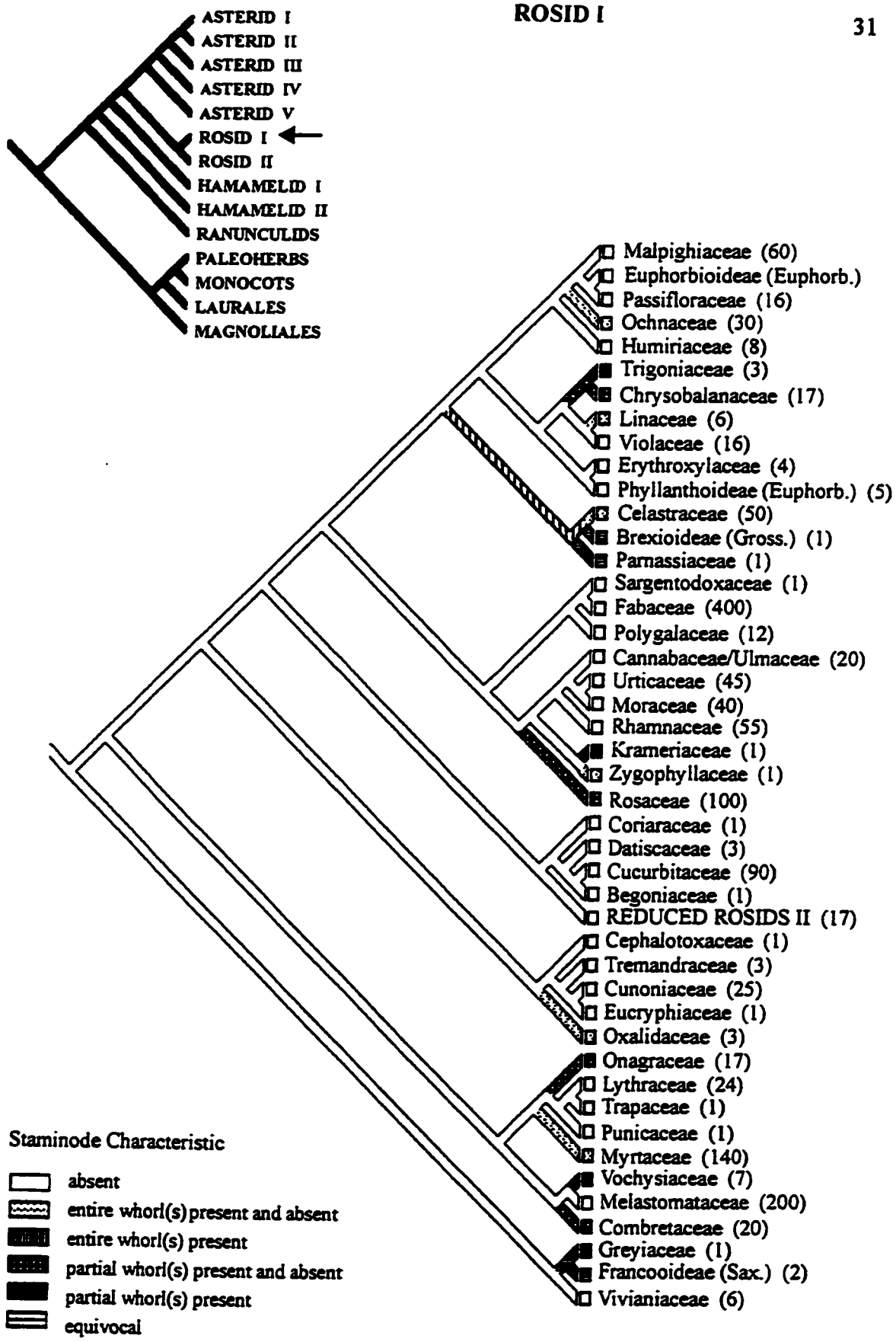

ASTERID I
ASTERID II
ASTERID III
ASTERID IV
ASTERID V
ROSID I
ROSID II
HAMAMELID I
HAMAMELID II
RANUNCULIDS
PALEOHERBS
MONOCOTS
LAURALES
MAGNOLIALES
Leitneriaceae (1)
Simaroubaceae (25)
Rutaceae (150)
Burseraceae (16)
Aceraceae (3)
Sapindaceae (140)
Anacardiaceae (60)
Bombaceae (20)
Tiliaceae (50)
Sterculiaceae (65)
Malvaceae (75)
Dipterocarpaceae (16)
Moringaceae (1)
Caricaceae (4)
Brassicaceae (350)
Capparis (Cap.)
Resedaceae (6)
Tovariaceae (1)
Koeberlinia (Cap.)
Bataceae (1)
Limnanthaceae (2)
Setchellanthus (Cap.)
Bretschneideraceae (1)
Akaniaceae (1)
Tropaeolaceae (3)
Crossosomataceae (3)
Geraniaceae (5)
Hypseocharitaceae (2)
Pterostemonoideae (Gross.) (2)
Saxifragoideae (Sax.) (30)
Tetracarpaeoideae (Gross.) (1)
Haloragaceae (8)
Penthoroideae (Sax.) (1)
Paeoniaceae (1)
Crassulaceae (25)
Ribesioideae (Gross) (5)
REDUCED ROSIDS II
Caryophyllaceae (75)
Atriplex (Chen.)
Amaranthaceae (65)
Spinacia (Chen.)
Basellaceae (4)
Portulaceae (20)
Didiereaceae (4)
Molluginaceae (13)
Phytolaccaceae (18)
Nyctaginaceae (30)
Aizoaceae (12)
Plumbaginaceae (12)
Polygonaceae (30)
Nepenthaceae (1)
Droseraceae (4)
Viscaceae (7)
Olacaceae (25)
Santalaceae (35)
Staminode Characteristic
absent
entire whorl(s) present and absent
entire whorl(s) present
partial whorl(s) present and absent
entire & partial whorl(s) present and absent
equivocal

Fig 2.5. Distribution of entire and partial staminode whorls in the Rosid I clade based on the Chase et al. (1993) phylogeny. Positions of tribes within the paraphyletic Euphorbiaceae (Euphorb.), Grossulariaceae (Gross.), and Saxifragaceae (Sax.) are included. Family names follow Cronquist (1981). Numbers in parentheses represent the number of genera comprising the family. 'Reduced Rosids I' include the Myricaceae, Betulaceae, Casuarinaceae and Juglandaceae, families with reduced flowers and ambiguous androecia.


ASTERID I
ASTERID II
ASTERID III
ASTERID IV
ASTERID V
ROSID I
ROSID II
HAMAMELID I
HAMAMELID II
RANUNCULIDS
PALEOHERBS
MONOCOTS
LAURALES
MAGNOLIALES
Malpighiaceae (60)
Euphorbioideae (Euphorb.)
Passifloraceae (16)
Ochnaceae (30)
Humiriaceae (8)
Trigoniaceae (3)
Chrysobalanaceae (17)
Linaceae (6)
Violaceae (16)
Erythroxylaceae (4)
Phyllanthoideae (Euphorb.) (5)
Celastraceae (50)
Brexioideae (Gross.) (1)
Parnassiaceae (1)
Sargentodoxaceae (1)
Fabaceae (400)
Polygalaceae (12)
Cannabaceae/Ulmaceae (20)
Urticaceae (45)
Moraceae (40)
Rhamnaceae (55)
Krameriaceae (1)
Zygophyllaceae (1)
Rosaceae (100)
Coriariaceae (1)
Datiscaceae (3)
Cucurbitaceae (90)
Begoniaceae (1)
REDUCED ROSIDS II (17)
Cephalotoxaceae (1)
Tremandraceae (3)
Cunoniaceae (25)
Eucryphiaceae (1)
Oxalidaceae (3)
Onagraceae (17)
Lythraceae (24)
Trapaceae (1)
Punicaceae (1)
Myrtaceae (140)
Vochysiaceae (7)
Melastomataceae (200)
Combretaceae (20)
Greyiaceae (1)
Francooideae (Sax.) (2)
Vivianiaceae (6)
Staminode Characteristic
absent
entire whorl(s) present and absent
entire whorl(s) present
partial whorl(s) present and absent
partial whorl(s) present
equivocal

completely lost reduced stamens (Bretschneideraceae-Tropaeolaceae clade and Fabaceae and Polygalaceae).

Partial staminode whorls evolved independently ten times within the Rosids (Figs 2.4 and 2.5), mostly within families. Staminodes only typify small families, such as the Trigoniaceae, Krameriaceae, and Vochysiaceae (Figs 2.4 and 2.5).

2.3.2.4.3 Staminode Function

Whorls of staminodes seem transitional in most Rosid groups, being small and unelaborated, but occasionally they are secondarily modified to take over other roles. Staminodes signal pollinators as part of the perianth, as in the large and petaloid staminodes of some Amaranthaceae or the contrasting coloured staminodes of several *Linum* species (Linaceae: Heywood, 1985). Staminodes also attract pollinators by mimicking rewarding structures. For example, in the pollen flowers of *Sparmannia* (Tiliaceae) an outer whorl of moniliform and bright yellow staminodes mimic large amounts of pollen (Vogel, 1978). Alternatively, in the Parnassiaceae the shiny, multifid and gland-tipped staminodes resemble nectaries (Muller, 1883; Knuth, 1908; Richards, 1986) and emit scent (Proctor and Yeo, 1972). Staminodes may also provide reward, forming a brightly coloured intrastaminal nectary disk in some members of the Ochnaceae (Cronquist, 1981). Staminodes may also present pollen secondarily, as in some beetle-pollinated *Verticordia* species, receiving pollen from the fertile stamens before flower anthesis (Holm, 1978; Yeo, 1992). Staminode movements in the slightly protandrous *Theobroma*, *Herrania* and *Dombeya* of the Sterculiaceae prevent selfing by mediating insect movement within the flower (Posnette, 1950; Cuatrecasas, 1964; Sampayan, 1966; and Entwistle, 1972). During male phase, staminodes closely surround the style (Young et al., 1987) and pollinating midges enter the flower from vertical petal ligules which act as a landing site (Young, 1984; Young et al., 1984) and exit without contacting the style. The staminodes flare outwards from the style during female phase and petal ligules reflex to a horizontal position (Young et al., 1987). Pollinators then land on the staminodes, crawl down between the staminodes and the style, and exit the flower through the petal

ligules (Young, 1984; Young et al., 1984). Staminode whorls may also enclose and protect the ovaries as in some Ochnaceae where an inner whorl of fused staminodes form a tube surrounding the ovaries (Cronquist, 1981). In many bird-pollinated, feather flowers (*Verticordia* and *Darwinia* of the Myrtaceae), staminodes prevent nectar robbing by bending inwards (sometimes along with fertile stamens) to cover the nectary disk (Yeo, 1992).

Partial staminode whorls are most often filaments or small rudiments with no ascribed functions, with the sole exception of the *Lopezieae* tribe of the Onagraceae. In this group two stamens of a four-stamen whorl have been lost, with only one fertile stamen and one staminode retained. The petaloid staminode encloses the fertile stamen and style at anthesis, releasing them explosively upon insect arrival (Eyde and Morgan, 1973; Plitmann et al., 1973; Heywood, 1985).

2.3.2.5 Asteridae

2.3.2.5.1 Floral trends

Animal pollination predominates in the Asteridae, except for a small wind-pollinated clade including Aucubaceae, Garryaceae, and Eucommiaceae in the Asterid I group (Figs 6 and 7) and isolated genera in other families (e.g. *Ambrosia* and *Artemisia*, Asteraceae). Three general flower types characterize the animal-pollinated Asteridae: 1) open, regular flowers similar to many Rosidae, 2) regular, tubular flowers and 3) bilabiate, tubular flowers. Open flowers typify the Asterid IV and Asterid V clades and occur in some groups of the Asterid II and Asterid III clades, such as the Cyrillaceae, Theaceae, Actinidiaceae, Clethraceae, Helwingiaceae-Aquifoliaceae clade and the Escallonioideae. In the Asterid IV clade these flowers are generally crowded into cymose inflorescences or heads (Nyssaceae), whereas the open flowers of the Asterid II and III clades tend to be solitary or in loose inflorescences. Regular tubular flowers characterize the remaining families of the Asterid II and III clades and some of the Asterid I clade (Solanaceae-Hydrophyllaceae clade, Gentianaceae-Loganiaceae clade, and the Byblidaceae and Oleaceae). In most cases, regular tubular flowers are solitary or in loose inflorescences,

but in the Asterid II group and basal members of the Asterid I group, flowers may be crowded into dense cymes (Asclepiadaceae, Loganiaceae, Dipsacales, Apiaceae, Pittosporaceae, and Goodeniaceae) or heads (Bruniaceae, Calyceraceae, Asteraceae). Bilabiate tubular flowers are restricted to the Scrophulariales clade (Callitrichaceae to Bignoniaceae) of the Asterid I group.

2.3.2.5.2 Staminode Origin

All flowers of the Asterid I and Asterid II clades (except for some Dipsacales) have only one whorl of stamens so reduction of an entire stamen whorl is restricted to the basal Asterids (III, IV, and V), among which 69.2% of families include species with only one whorl of fertile stamens. Whorls of staminodes occasionally replace reduced stamen whorls. Staminode whorls originated independently four times in the Asterids (Fig. 2.6), limited to single families. Staminodes occur in only a small proportion of species comprising the families (except Theophrastaceae). These staminodes are generally small and rudimentary but the corollas of *Sideroxylon* (Sapotaceae) and *Jacquinia* (Theophrastaceae) include large petaloid staminodes.

Reduction of a partial five-merous stamen whorl occurs only in the bilabiate flowers of the Scrophulariales in the Asterid I clade (Fig 2.7). In this group, the anthers of four stamens have been repositioned to the top of the corolla tube and the fifth stamen has been reduced to a staminode or lost completely. The staminode evolved at the base of the Scrophulariales (Bignoniaceae: Fig. 2.7) and re-appears in a few derived families (Scrophulariaceae and Acanthaceae). The coarse resolution of the Chase et al. (1993) phylogeny and variable staminode states within families, obscures the evolution and persistence of staminodes within the order. A more detailed phylogeny of the Scrophulariales reveals the varying representation of the fifth stamen throughout the group (Fig. 2.8). In most cases, the staminode is small and rudimentary but a large, elaborate staminode has evolved independently in flowers of the Cheloneae tribe (Scrophulariaceae) and *Jacaranda* (Bignoniaceae). Continued stamen loss occurs in many Lamiaceae and Scrophulariaceae, through reduction of one of the two stamen pairs (Cronquist, 1981) and

Fig 2.6. Distribution of entire staminode whorls in the Asterid III, IV, and V clades based on the Chase et al. (1993) phylogeny. Positions of genera within the paraphyletic Byblidaceae (Byblid.), Nyssaceae (Nyss.), and Araliaceae (Aral.) are included. Family names follow Cronquist (1981). Numbers in parentheses represent the number of genera comprising the family. The 'Ericoids' include the Ericaceae, Empetraceae, Epacridaceae, and Pyrolaceae.

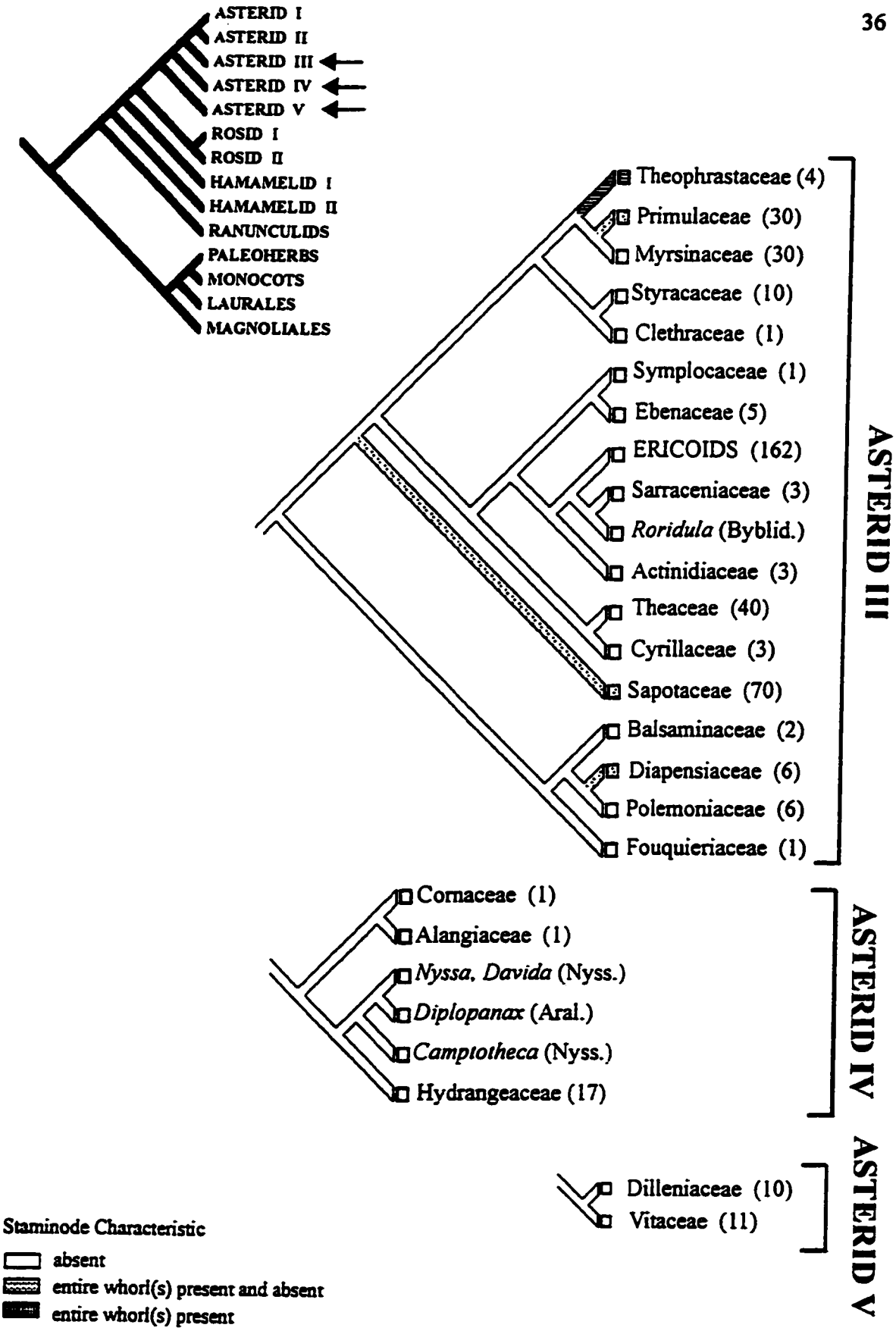

ASTERID I
ASTERID II
ASTERID III
ASTERID IV
ASTERID V
ROSID I
ROSID II
HAMAMELID I
HAMAMELID II
RANUNCULIDS
PALEOHERBS
MONOCOTS
LAURALES
MAGNOLIALES
Theophrastaceae (4)
Primulaceae (30)
Myrsinaceae (30)
Styracaceae (10)
Clethraceae (1)
Symplocaceae (1)
Ebenaceae (5)
ERICOIDS (162)
Sarraceniaceae (3)
Roridula (Byblid.)
Actinidiaceae (3)
Theaceae (40)
Cyrillaceae (3)
Sapotaceae (70)
Balsaminaceae (2)
Diapensiaceae (6)
Polemoniaceae (6)
Fouquieriaceae (1)
ASTERID III
Cornaceae (1)
Alangiaceae (1)
Nyssa, Davida (Nyss.)
Diplopanax (Aral.)
Camptotheca (Nyss.)
Hydrangeaceae (17)
ASTERID IV
Dilleniaceae (10)
Vitaceae (11)
ASTERID V
Staminode Characteristic
absent
entire whorl(s) present and absent
entire whorl(s) present

Fig 2.7. Distribution of partial staminode whorls in the Asterid I clade based on the Chase et al. (1993) phylogeny. Positions of genera (or tribes) within the paraphyletic families Araliaceae (Aral.) and Grossulariaceae (Gross.) are included. Family names follow Cronquist (1981). Numbers in parentheses represent the number of genera comprising the family.

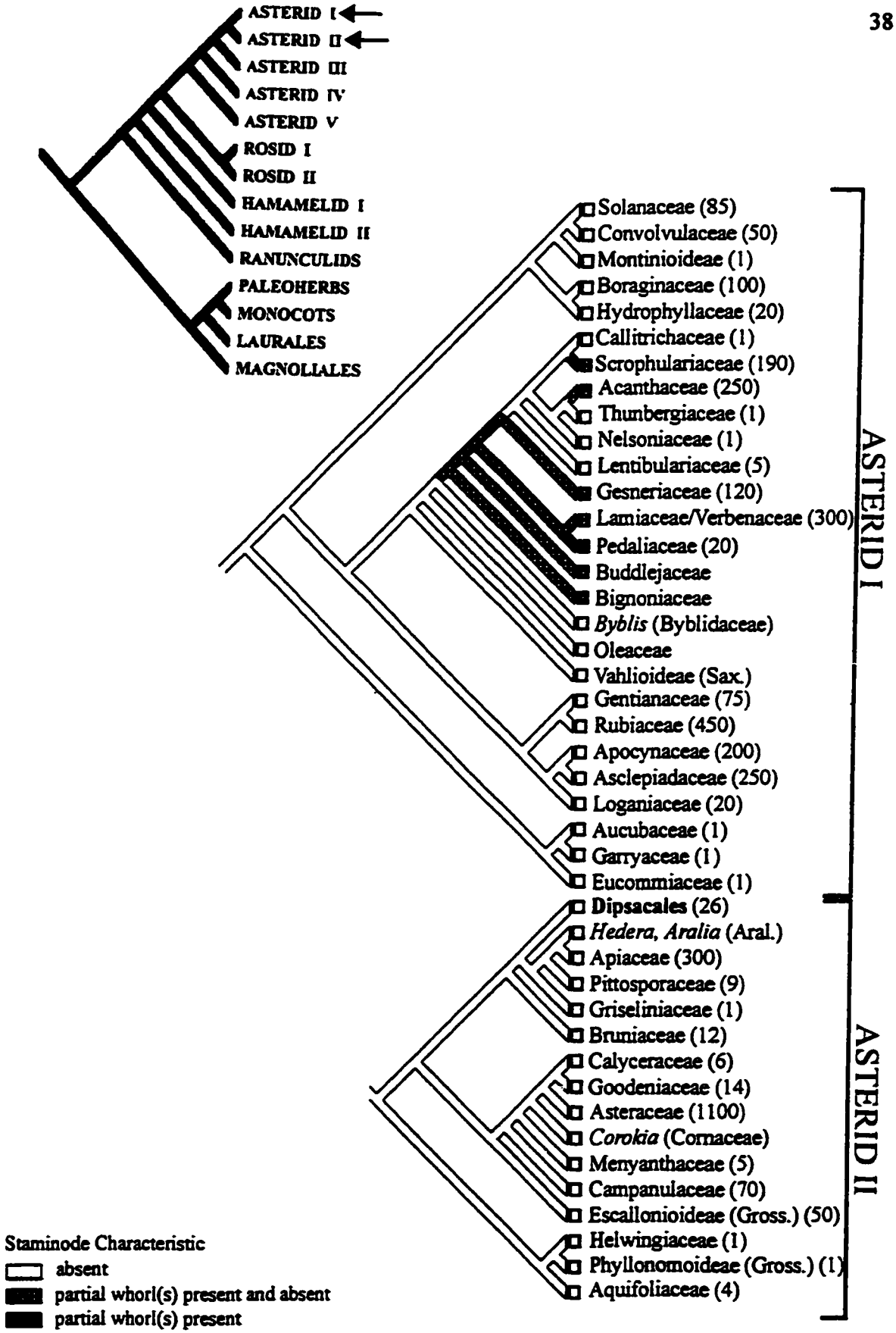
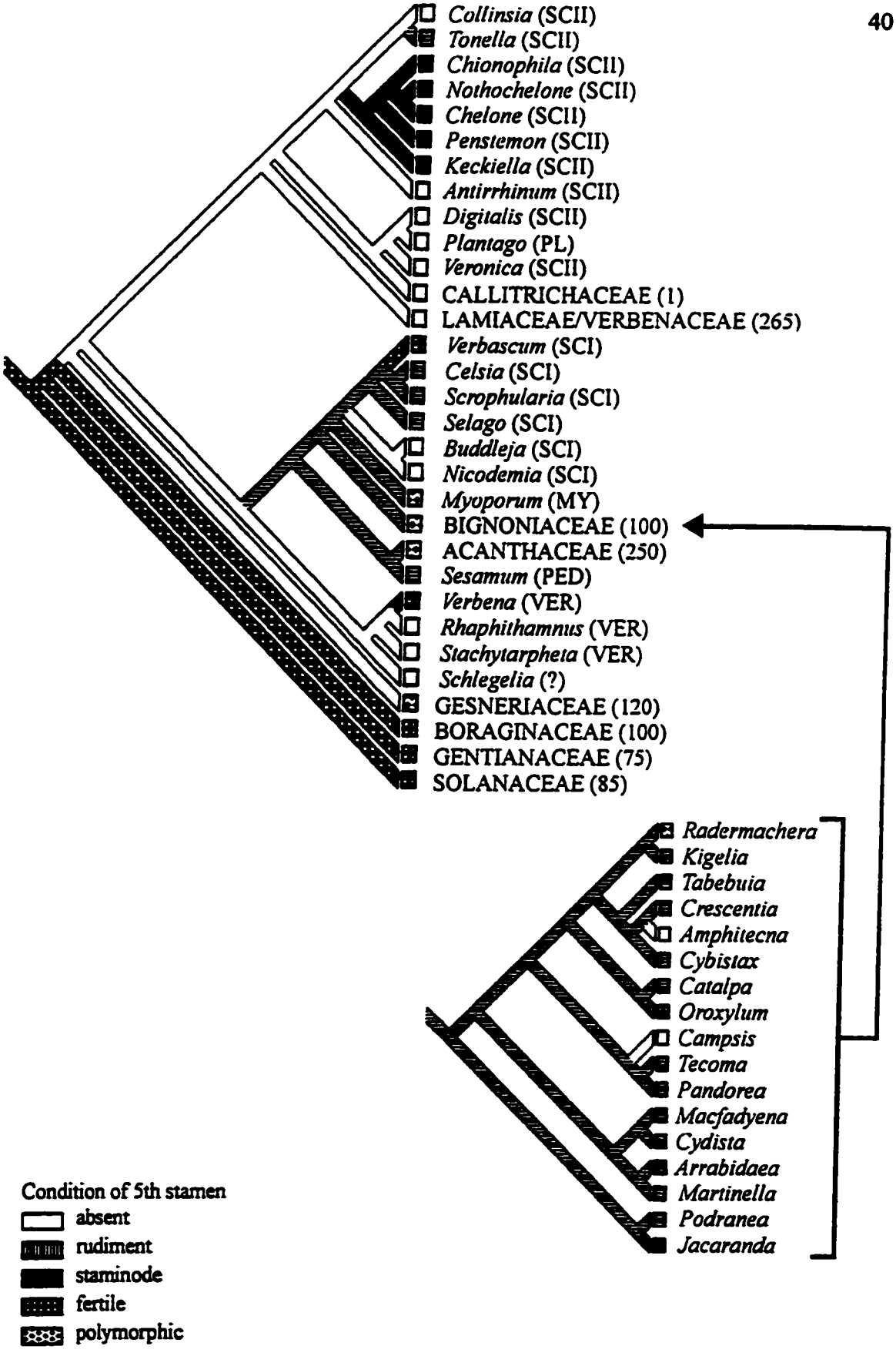

ASTERID I
ASTERID II
ASTERID III
ASTERID IV
ASTERID V
ROSID I
ROSID II
HAMAMELID I
HAMAMELID II
RANUNCULIDS
PALEOHERBS
MONOCOTS
LAURALES
MAGNOLIALES
Solanaceae (85)
Convolvulaceae (50)
Montinioideae (1)
Boraginaceae (100)
Hydrophyllaceae (20)
Callitrichaceae (1)
Scrophulariaceae (190)
Acanthaceae (250)
Thumbergiaceae (1)
Nelsoniaceae (1)
Lentibulariaceae (5)
Gesneriaceae (120)
Lamiaceae/Verbenaceae (300)
Pedaliaceae (20)
Buddlejaceae
Bignoniaceae
Byblis (Byblidaceae)
Oleaceae
Vahlioideae (Sax.)
Gentianaceae (75)
Rubiaceae (450)
Apocynaceae (200)
Asclepiadaceae (250)
Loganiaceae (20)
Aucubaceae (1)
Garryaceae (1)
Eucommiaceae (1)
Dipsacales (26)
Hedera, Aralia (Aral.)
Apiaceae (300)
Pittosporaceae (9)
Griseliniaceae (1)
Bruniaceae (12)
Calyceraceae (6)
Goodeniaceae (14)
Asteraceae (1100)
Corokia (Cornaceae)
Menyanthaceae (5)
Campanulaceae (70)
Escallonioideae (Gross.) (50)
Helwingiaceae (1)
Phyllonomoideae (Gross.) (1)
Aquifoliaceae (4)
ASTERID I
ASTERID II
Staminode Characteristic
absent
partial whorl(s) present and absent
partial whorl(s) present

Fig 2.8. Reduction of the posterior (5^{th}) stamen in flowers of the Scrophulariales based on Olmstead and Reeve's (1995) *rbc*L and *ndh*F phylogeny of the Scrophulariales, Wolfe et al.'s (1997) and Wolfe's (1998) *rbc*L and *ndh*F phylogeny of Cheloneae. Spangler's (1997) *rbc*L and *ndh*F phylogeny of the Bignoniaceae is inset. Numbers in parentheses represent the number of genera included in the family. The paraphyletic Scrophulariaceae is divided into two clades, SCII and SCII sensu Olmstead (1995). (PL=Plantaginaceae, MY=Myoporaceae, PED=Pedaliaceae, VER=Verbenaceae, ?=Family placement ambiguous)


Collinsia (SCII)
Tonella (SCII)
Chionophila (SCII)
Nothochelone (SCII)
Chelone (SCII)
Penstemon (SCII)
Keckiella (SCII)
Antirrhinum (SCII)
Digitalis (SCII)
Plantago (PL)
Veronica (SCII)
CALLITRICHACEAE (1)
LAMIACEAE/VERBENACEAE (265)
Verbascum (SCI)
Celsia (SCI)
Scrophularia (SCI)
Selago (SCI)
Buddleja (SCI)
Nicodemia (SCI)
Myoporum (MY)
BIGNONIACEAE (100)
ACANTHACEAE (250)
Sesamum (PED)
Verbena (VER)
Rhaphithamnus (VER)
Stachytarpheta (VER)
Schlegelia (?)
GESNERIACEAE (120)
BORAGINACEAE (100)
GENTIANACEAE (75)
SOLANACEAE (85)
Radermachera
Kigelia
Tabebuia
Crescentia
Amphitecna
Cybistax
Catalpa
Oroxylum
Campsis
Tecoma
Pandorea
Macfadyena
Cydista
Arrabidaea
Martinella
Podranea
Jacaranda
Condition of 5th stamen
absent
rudiment
staminode
fertile
polymorphic

in some species of Lamiaceae, further reduction of one theca of each anther (Cantino, 1992). In scattered genera throughout the Scrophulariales apparent reversion to actinomorphy has been accompanied by restoration of the suppressed stamen (see Fig. 2.8) such as *Verbascum* (Scrophulariaceae), *Oroxylum* (Bignoniaceae) and *Verbena* (Verbenaceae). These independent reversions, along with staminode re-appearance in derived taxa (Scrophulariaceae II, Fig. 2.8) from ancestors with complete loss of the 5th stamen, suggests that partial reduction of stamen whorls is reversible.

2.3.2.5.3 Staminode function

In most Asterid species with staminodes, staminode whorls and partial staminode whorls seem generally rudimentary and transitional, with no recognized function. Two rare exceptions are apparent to this nonfunctionality. Within the basal Asterids, the whorl of large petaloid staminodes in *Sideroxylon* and *Jacquinia* almost certainly attracts pollinators as part of the perianth. In contrast, the single staminode of bee-pollinated Cheloneae and *Jacaranda* has been ascribed diverse functions, including serving as a nectar guide (Delpino, 1868-1875), a lever mechanism that promotes pollen exchange with pollinators (Torchio, 1974; Chapter 3), a barrier mechanism to prevent nectar robbing (Straw, 1956), and a purchase for alighting pollinators (Pennell, 1948). Of these proposed mechanisms, experimental evidence favors the lever and barrier mechanisms, which both promote pollen exchange with pollinators (Chapter 3). The staminode may be functionless in bird-pollinated species of the Cheloneae (Chapter 3).

2.4 Common themes

2.4.1 Stamen reduction

Stamen reduction results from progressive suppression of stamen development (Tucker, 1988), except for a few cases of loss through stamen fusion as in the Cucurbitaceae (*Cyclanthera*: Takhtajan, 1991). In animal-pollinated plants, reduction of entire stamen whorls usually involves actinomorphic flowers with more than one whorl of

fertile stamens (Stebbins, 1974; Ronse Decraene and Smets, 1993, 1995), which are pollinated by diverse small insects. Reallocation of resources to more, smaller flowers and/or adaptations that increase efficiency of pollen dispersal likely prompt reduced pollen production per flower through stamen loss. These adaptations include pollen packaging and pollen dispensing mechanisms that limit pollen removal by individual pollinators (reviewed by Harder and Thomson, 1989) and more precise contact between pollinators and pollen presenters (including anthers) or pollinators and stigmas. Both adaptations result in higher proportions of pollen grains delivered to stigmas.

Reduction of partial stamen whorls accompanies the development of zygomorphy (Stebbins, 1974; Ronse Decraene and Smets, 1995) and specialization towards a particular type of pollinator. Zygomorphic flowers control pollinator entry, enhancing the precision of pollen placement (Richards, 1986) often through re-positioning of stamens (Stebbins, 1974). Loss of stamens during the evolution of zygomorphy likely reflects the increased efficiency of pollen transfer (Faegri and van der Pijl, 1979; Richards, 1986) and developmental constraints. In the Scrophulariales and Zingiberales, which have zygomorphic flowers with one whorl of stamens, anthers of fertile stamens have been re-positioned to the top of the corolla tube and loss of one stamen has resulted. In the Scrophulariales, reversion to radially symmetric flowers with an entire whorl of stamens occurs independently in several lines (*Verbena* [Verbenaceae], *Oroxylum* [Bignoniaceae], *Verbascum* [Scrophulariaceae]) and peloric mutants of usually zygomorphic species also lose stamen suppression (as in *Antirrhinum* mutants lacking *cyc* and *dich* genes: Coen, 1996; Luo et al., 1996). These results indicate close links in the genetic control of zygomorphy and suppression of partial stamen whorls.

Wind-pollinated plants do not exhibit any particular trend, demonstrating both reduction of entire and partial stamen whorls (usually to one fertile stamen in some Monocots, Rosids, and Asterids) and also increases in stamens (some Monocots, Asterids). Although wind-pollination is generally associated with an increase in stamen

number (Richards, 1986), flower aggregation may lead to stamen loss in individual flowers.

2.4.2 Staminode Formation

Staminodes generally seem to result incidentally from progressive suppression of stamens, as indicated by transition series from fertile stamens to staminodes to eventual stamen loss within groups such as the genus *Bauhinia* (Caesalpinaceae: Endress, 1994), Lauraceae (Allen, 1948; Hutchinson, 1964), and many rosid families (e.g. Sterculiaceae: Robyns, 1964; Crassulaceae: Cronquist, 1981; Zygophyllaceae: Ronse Decraene and Smets, 1995). Alternatively, functional staminodes may occasionally evolve directly from functional stamens. For example, in the Commelineae tribe (Commelinaceae) direct evolution of fertile stamens to sterile "fodder" stamens has occurred as part of a pollen mimicry system (Vogel, 1978). Direct evolution may similarly be involved in the magnolid-type flowers of the Magnoliales/Laurales, where transition series of a reductive process do not occur. However, it seems more likely that staminodes originated from the ancestral spiral arrangement of flower primordia. In spirally-arranged flowers, primordium whorls between whorls of fully formed petals and stamens and between stamens and carpels develop incompletely and produce staminodes (Ronse Decraene and Smets, 1993).

Whorled staminodes formed through stamen suppression are typically transitional but occasionally, usually in a few members of a family or all members of a small family, they take over other roles. Partial whorls of staminodes may similarly be transitional, incidentally becoming functional (as in the Scrophulariales), but in some groups they become integral components of flower structure within a group (e.g. Orchidaceae [Burns-Balogh and Bernhardt, 1985] and some Zingiberales [Faegri and van der Pijl, 1979; Hickey and King, 1988]).

The persistence of staminodes within a lineage likely relates to the number of functions they perform. Incidental staminodes typically adopt single functions. In such

cases, floral diversification could result in rapid staminode loss, should shifts in pollination pressures render such a staminode non-functional. In contrast, a multi-functional staminode would be less likely to become non-functional and lost. Indeed, multi-functional staminodes characterize groups such as the Magnoliales/Laurales, some Zingiberales, and Orchidaceae, where staminodes have become integral components of flower structure. In the Magnoliales/Laurales, staminodes prevent self-pollination and attract pollinators through visual conspicuousness, scent secretion, reward provisioning, and reward mimicking (Endress, 1984b, 1986, 1994; Miller, 1989). In some Zingiberales and the Orchidaceae, staminodes are incorporated with other floral organs (perianth of Cannaceae, Costaceae, and Zingiberaceae; column of Orchidaceae) and share the multiple roles of these structures.

Genetic control of the loss of entire versus partial stamen whorls differs. Reduction of stamen whorls seems irreversible, as secondary stamen increases (common to many Rosidae families) involve the division of fertile stamen primordia, not re-appearance of previously reduced whorls (Ronse Decraene and Smets, 1992). In contrast, reduction of partial stamen whorls appears plastic. For example, apparent reversion to actinomorphy has occurred in many taxa scattered throughout the Scrophulariales and is always accompanied by restoration of the suppressed stamen.

This difference in genetic control may explain why staminodes seem to occur more frequently from reduction of a partial stamen whorl than from reduction of an entire whorl. In particular, staminodes occur in 50.8% of families with evidence of reduction of a partial stamen whorl, but in only 14.5 % of families that exhibit some reduction of an entire stamen whorl. Following loss of an entire stamen whorl, limited opportunity for the modification of non-functional staminode whorls exists, given the permanence of stamen loss. In contrast, the plasticity of partial staminode whorls might allow repeated opportunistic involvement of staminodes in floral function. Certainly, this appears to be the case for the Scrophulariales, as taxa with staminodes (Cheloneae) have developed from taxa characterized by staminode loss (Fig. 2.8). Alternatively, the higher incidence

of partial staminode whorls may be related to the relatively recent evolutionary development of zygomorphy, resulting in a higher incidence of non-functional stamen remnants.

2.4.3 Staminode Function

Entire and partial whorls of staminodes fulfill many roles throughout the angiosperms, but some functions occur repeatedly in certain staminode types. Inner staminode whorls, positioned between the carpels and stamens, often protect the ovaries (Ranunculaceae: Brayshaw, 1989, Ochnaceae: Hickey and King, 1988) or prevent selfing through positional changes which either shield receptive stigmas (Magnoliales/Laurales: Grant, 1950a; Endress, 1984a, 1984b, 1986, 1994; Miller, 1989) or control pollinator movement on the flower (Sterculiaceae: Young, 1984; Young et al., 1984, 1987). Outer staminode whorls, or inner whorls when the two stamen whorls are fused, attract pollinators through visual conspicuousness (Magnoliales/Laurales: Endress, 1984b, 1986, 1994, Rosids: Heywood, 1985), provide rewards (Magnoliales/Laurales: Endress 1984b, 1986, 1994, and Rosidae: Cronquist, 1981), or mimic rewards (Rosidae: Muller, 1883; Knuth, 1908; Richards, 1986, Monocots: Endress, 1994). Incidental partial whorls of staminodes are commonly involved in mechanisms for explosive pollination (Monocots: Kennedy, 1978; Rogers, 1984; Yeo, 1992, Hamamelids, van Beusekom, 1971; Holm, 1978, Rosids: Eyde and Morgan, 1973; Plitmann et al., 1973; Heywood, 1985), whereas integral partial whorls (Orchidaceae: Burns-Balogh and Bernhardt, 1985, some Zingiberales: Maas, 1972; Smith, 1987) function in attraction and control of pollinator position.

Many of the functions adopted by staminodes are more commonly served by other floral mechanisms, including ovary protection, prevention of selfing, pollinator attraction through visual conspicuousness or provision of rewards, and control of pollinator position. Typical mechanisms to protect ovaries include their enclosure by other floral organs (epigyny: Grant, 1950b), and spatial separation of the ovary from rewards (e.g. nectar

spurs: Grant, 1950b; Hodges and Arnold, 1995). Common mechanisms preventing self-pollination involve the separation of pollen and stigma presentation, either in time (dichogamy: reviewed by Lloyd and Webb, 1986), or in space (herkogamy: reviewed by Webb and Lloyd, 1986) and unisexuality. The perianth typically attracts pollinators through visual display and positions them through flower shape. Floral rewards are most often provided by groups of secretory cells which produce nectar (less commonly oils, resins, and sexual attractants: Neff and Simpson, 1983), or tissues modified into food bodies (Neff and Simpson, 1983). When the androecium is involved in reward provisioning, as in pollen flowers, stamens typically provide fertile pollen as a reward, although staminodes provide sterile pollen in some heteranthic species (as in *Commelina*: Vogel, 1978).

In contrast, mechanisms for explosive pollination (although rare in flowering plants in general) often employ partial whorls of staminodes. In such cases, staminodes hold back the stamens and/or style in untripped flowers and act as triggers for their release, as in the explosive flowers of Proteaceae (*Conospermum*: Holm, 1978), Sabiaceae (*Meliosma*: van Beusekom, 1971), Onagraceae (Lopezieae: Eyde and Morgan, 1973; Plitmann et al., 1973; Heywood, 1985), and Marantaceae (Kennedy, 1978; Rogers, 1984; Yeo, 1992). In other examples of explosive pollination that do not employ staminodes, such as *Chamaepericlymenum* (Cornaceae: Mosquin, 1985), sunbird-pollinated Loranthaceae (Feehan, 1985), *Hyptis* (Lamiaceae: Brantjes and De Vos, 1981; Keller and Armbruster, 1981), *Kalmia* (Ericaceae: Henshaw, 1915) and some Fabaceae (*Medicago*, *Genista*, *Ulex*, and *Sarothammus*: Arroyo, 1981; Proctor and Yeo, 1992), the corolla encloses the stamens and/or style and acts as a trigger. Rarely, tension is provided by the exploding organ itself, as in *Stylidium* (Stylidiaceae: Erbar, 1992). In these flowers, the bent region of the column (comprising anthers and stigmas) is strongly reinforced by layers of thick-walled cells, which produce a rapid movement of the column after contact by a pollinator (Findlay and Findlay, 1975).

Reward mimicry also frequently involves partial or entire whorls of staminodes. Pollen flowers commonly display anther mimics (often staminodes: some Lecythidaceae, Tiliaceae, Commelinaceae [Vogel, 1978], Xyridaceae [Woodson and Schery, 1944; Kral, 1966]; but also hair tufts: *Bulbine, Narthecium, Anagallis* [Vogel, 1978]; and enlarged anther connectives: many Melastomataceae [Vogel, 1978]; or stamen filaments: *Dianella* [Vogel, 1978]) to enhance attractiveness. Staminodes occasionally mimic nectaries in nectar-producing flowers (Parnassiaceae: Muller, 1883; Knuth, 1908; Richards, 1986) but false nectar spots on petals occur more frequently (e.g. *Pelargonium* section Campylia: McDonald and Van der Walt, 1992). In the deceptive Cypripedioideae (Orchidaceae), the staminode commonly mimics nectaries (Little, 1983; Banziger, 1996) or other attractive structures such as brood sites (Atwood, 1985).

Although staminodes often represent non-functional, intermediate stages in stamen reduction, they have repeatedly provided the substrate for novel solutions to a variety of pollination problems. The diverse functioning of staminodes, even among closely related lineages such as the Sterculiaceae and Tiliaceae, suggests that staminodes can provide an evolutionary "quick fix" for functional problems specific to different pollination mechanisms. Presumably, modification of a non-functional structure would involve fewer constraints than the modification of a multi-functional floral organ. As a result, evolution of staminodes could occur more quickly and show greater flexibility than other floral structures. However, due to the transitional nature of staminodes, a narrow evolutionary window of opportunity for staminode modification exists. That modification of such transitional structures has regularly occurred reflects the rapid evolution of angiosperm flowers.

3 THE FUNCTIONAL SIGNIFICANCE OF THE STAMINODE IN *PENSTEMON* (SCROPHULARIACEAE)

3.1 Introduction

3.1.1 Stamen loss and staminode formation in the Scrophulariales

Floral morphology in the order Scrophulariales has undergone considerable evolutionary modification from the purported ancestral morphology which involved open, bowl-shaped flowers with five separate sepals, petals and stamens (Ronse Decraene and Smets, 1995). The flowers of most contemporary species in this order include fused and irregular petals, forming a two-lipped tubular flower with four of the original five stamens re-positioned to the top of the corolla tube (often further reduced to two stamens). This resulted in the loss of the fifth stamen, although it is represented by a rudimentary staminode in some groups. Notable exceptions include the five closely related genera comprising the tribe Cheloneae of the Scrophulariaceae and *Jacaranda* of the Bignoniaceae, which independently rederived a large and conspicuous staminode from an ancestrally reduced condition (Chapter 2, Fig 2.8). Staminodes of the Cheloneae vary considerably in length, thickness, apex shape, and extent of bearding. *Jacaranda* flowers demonstrate remarkable convergence with those of Cheloneae, sharing similar flower and staminode morphology.

This elaboration of the staminode suggests a functional role(s) that likely differs from that of the ancestral stamen filament from which the staminode evolved. In the Cheloneae and Bignoniaceae, the staminode has been proposed to play a variety of specialized roles in pollination (Endress, 1994), whether as a barrier to nectar robbers (Kerner, 1876; Errera, 1878; Straw, 1956; Kampny, 1995), a nectar guide (Delpino, 1868-1875; Endress, 1992), a purchase for alighting bees (Pennell, 1948), or a lever to bring the sexual organs into contact with pollinators (Torchio, 1974).

3.1.2 The staminode in *Penstemon*

Penstemon, the largest genus of flowering plants endemic to North America with almost 300 species (Holmgren, 1993), illustrates the variety of staminode form (and

presumably function) seen in the Cheloneae. Indeed, the genus name combines the Latin root '*paene*', nearly or almost, and the Greek '*stemon*', stamen, in reference to the characteristic prominent staminode (Keck, 1951). *Penstemon* flowers have lobed, tubular corollas held more or less horizontally on vertical inflorescences. Nectaries are located proximally in the flower at the filament bases of the upper pair of fertile stamens which surround the ovary. The filaments of the four fertile stamens curve around the inside of the corolla tube and position the anthers dorsally within the tube, one pair on either side of the stigma (see Fig. 3.1). The staminode originates from the dorsal upper portion of the corolla and descends in front of the ovary to lie along the lower surface of the corolla (see Fig. 3.1). Often the staminode is bearded with bright yellow hairs, hence the common name "beardtongue".

The remarkable variety of floral forms in *Penstemon* may be correlated with pollination system (Pennell, 1935; Straw, 1956); however, unclear phylogenetic relationships (including hybridization, Wolfe and Elisens, 1994) and a scarcity of pollinator records hamper such generalizations. Bee pollination is believed to be ancestral for *Penstemon*, but some species have since adapted to pollination by hummingbirds, masarid wasps, moths and flies (Scogin and Freeman, 1987). Of these derived systems, hummingbird- (about 15 species) and wasp-pollination (about 35 species) occur most frequently and seem to have evolved independently several times within the genus (Pennell, 1935; Cooper, 1952; Grant, 1994). Variation in staminode characteristics, such as length, colour and extent of bearding may be associated with different pollinators. Bearding demonstrates the most convincing pattern, with most bee-pollinated species possessing hairy staminodes, most hummingbird-pollinated flowers having glabrous staminodes, and bearded and glabrous staminodes occurring with equal frequency in wasp-pollinated species (Crosswhite, 1967).

3.1.3 Staminode function in *Penstemon*

The redevelopment of the posterior staminode in the Cheloneae tribe of the Scrophulariaceae, particularly *Penstemon* has long puzzled floral biologists. Ogle (1870)

believed the *Penstemon* staminode to be "perfectly useless" and considered its transverse position as merely the "next-best" evolutionary alternative to loss as an accommodation of the upper position of the style in evolving two-lipped flowers. In contrast, most other investigators attributed functional significance to the *Penstemon* staminode. Early European investigators developed hypotheses based on observations of the commonly garden-cultivated, bee-pollinated species, *P. gentianoides* and *P. hartwegii*. Delpino (1868-1875) believed the positioning of the staminode on the floor of the corolla helped guide visiting insects to the nectaries. In contrast, Kerner (1876) and Errera (1878) concluded that the transverse positioning of the staminode (along with the organization of the stamen filaments and style) created a barrier to nectar robbers, blocking the movement of these insects down the corolla tube. Merritt (1897) and Straw (1955, 1956) both endorsed this interpretation from their field observations of several Californian penstemons. Loew (1904) also suggested that the staminode excluded some pollinators, but he considered the hairiness and dilation of the bases of the staminode and anther filaments, not staminode positioning, to be important. Pennell (1948) asserted that the staminode, with its often enlarged or bearded apex, acts as a purchase for alighting bees. Pasquale (1893) suggested that the bearded staminode served pollination more directly by enabling autogamy in unvisited flowers, curling up with age and carrying pollen previously dropped from the anthers to the stigma. Finally Torchio (1974), from his observations of two wasp-pollinated species, conceded that the staminode (in wasp-pollinated species) may act secondarily as a nectar guide or a mechanical barrier, but proposed that it functioned primarily as part of a mechanical system that brings the sexual organs into contact with the pollinator. In particular, he observed wasps ramming the base of the staminode with their heads while accessing nectar and believed that this pressure was translocated to the top of the corolla tube, pushing the sexual organs down toward the pollinator.

Interestingly, similar theories of staminode function have not been proposed for hummingbird-pollinated species, even though the functions proposed for bee-pollinated

species seem inadequate in the context of bird pollination. The narrow corolla tubes of hummingbird-pollinated *Penstemon* tend to exclude potential nectar robbers and most plants lose nectar guides after an evolutionary switch to birds as pollen vectors (Grant and Grant, 1968). Indeed, Pennell (1935) viewed the maintenance of the staminode in this pollination system as a phylogenetic artefact. Obviously, to develop a clear understanding of the staminode in *Penstemon*, derived pollination systems must be considered.

3.1.4 Study design

I tested these diverse hypotheses regarding staminode function by examining its contribution to reproductive success for two bee-pollinated species, *P. ellipticus* Coult. & Fisher (subgenus Dasanthera) and *P. palmeri* A.Gray (subgenus Penstemon, section Peltanthera), and two hummingbird-pollinated species, *P. centranthifolius* Benth. (subgenus Penstemon, section Peltanthera) and *P. rostriflorus* Kellogg (subgenus Saccanthera, section Emersus). The pairs were chosen so as to minimize relatedness between members with similar pollinators. For each species I assessed whether the staminode is functional by assessing the impact of its removal on pollen removal and pollen receipt. To determine the mechanism responsible for altered pollen removal or receipt, I also assessed the impacts of staminode removal on pollinator visitation and visit characteristics.

3.2 Methods

3.2.1 Study sites

P. centranthifolius: A population of approximately 100 individuals was chosen growing in chaparral amongst *Ceanothus, Arctostaphylos, Adenostoma, Photinia, Rhus, Cerococarpus* and *Quercus* in bare sand (elev 1680 m) 1 km N of Lake Fulmore, adjacent to the University of California James Reserve in the San Bernardino National Forest, California (33°48'18"N, 116°46'36"W).

P. rostriflorus: A population of approximately 100 individuals was studied growing on a rocky floodplain along the South Fork Twin River, Toiyabe National Forest, Nevada (38°53'18"N, 117°14'39"W).

P. palmeri: A population of approximately 150 individuals was studied growing on the fill used to create Schroeder Dam amongst *Artemisia, Chrysothamnus,* and grasses in Beaver Dam State Park, Nevada (37°36'15"N, 114°04'11"W).

P. ellipticus: A population of approximately 200 individuals was studied growing on a southwest-facing talus slope of a bowl (elev 2100 m) on the south side of Mount Carthew along the Alderson-Carthew Lakes trail in Waterton Lakes National Park, Alberta (49°01'17"N, 114°00'49"W) amongst scattered *Pinus flexilis* James and *Juniperus horizontalis* Moench.

3.2.2 Treatment groups

All experiments contrasted three floral treatments: 1) unmanipulated flowers, 2) staminodeless flowers (staminode excised), and 3) control flowers (staminode incised but not removed). I manipulated all flowers while they were in bud to ensure against accidental pollen removal or deposition. To prevent disruption of the internal flower organization for staminodeless and control treatments, I made a small incision (< 3 mm) in the corolla tube next to the staminode insertion, through which I manipulated the staminode. I excised a staminode by pinching it off at its base with fine forceps and pulling it through the incision. For control flowers I removed only a strip of epidermal and outer tissue from the base of the staminode to simulate wounding effects caused by staminode removal.

3.2.3 Pollen receipt

To assess the effect of staminode removal on the amount of pollen received by a flower I tagged four flower buds on each of 25-30 plants, randomly assigned them to one of four treatments: staminodeless, unmanipulated, control, and bagged. Flowers receiving the first three treatments were manipulated as described above and bagged flowers were

enclosed in a mesh bag to exclude pollinators. I used bagged flowers to assess the incidence of autogamous self-pollination. I checked the flower buds hourly during daylight until bud break, when I removed the undehisced anthers of all treatment flowers (except bagged flowers) to prevent self-pollination. I collected the stigmas of all treatment flowers 48 h after bud break and stored them individually in small vials containing 70% ethanol. In the laboratory, I stained each stigma with 1% basic fuschin and counted the number of pollen grains deposited under a compound microscope (20X).

3.2.4 Pollen removal

Assessment of the effect of staminode removal on both the amount and rate of pollen removed from a flower involved seven flower buds on 25-30 plants. I randomly assigned two flowers to each of the staminodeless, unmanipulated and control treatments, manipulated and tagged these six buds, and collected the seventh bud. The anthers of this seventh bud provided a measure of pollen production by unvisited flowers for comparison with the pollen remaining in visited flowers. This bud was stored in 70% ethanol until the number of grains could be counted with a Particle Data Elzone 180XY particle counter (following Harder, 1990). I checked the remaining buds hourly during daylight for bud break and start of anther dehiscence (I used these data to determine timing of bud break and anther dehiscence at the population level). I collected the anthers from one flower for each treatment 3 h after the start of anther dehiscence (2 h for *P. palmeri*) and collected the anthers in the remaining flowers 6 h after the start of anther dehiscence (4 h for *P. palmeri*). Collection times were chosen based on observed pollinator activity and a corresponding estimate of the rate of pollen removal (from visual inspections of anthers at various times following dehiscence) for each species. Anthers were stored in 70% ethanol until the number of grains remaining could be counted with a Particle Data Elzone 180XY particle counter.

3.2.5 Pollinator visit characteristics

3.2.5.1 Hummingbird-pollinated species

P. centranthifolius - I assessed both the frequency and duration of hummingbird visits to different treatment flowers by observing and videotaping foraging bouts to the inflorescences of two plants that formed a small patch. This patch contained 12 inflorescences which were assigned randomly to 4 staminodeless, 4 control and 4 unmanipulated treatments. I manipulated all flowers on each inflorescence at the beginning of the experiment and checked the inflorescence twice daily to manipulate any recently opened flowers. The total number of flowers of each treatment was kept relatively equal throughout the observation periods. By videotaping foraging bouts I could measure visit durations accurately by counting the number of frames used to record each visit.

P. rostriflorus - Hummingbirds were observed feeding on the flowers, but visits were not characterized due to a small and reclusive bird population.

3.2.5.1 Bee-pollinated species

I assessed daily patterns of bee activity by counting the number of foraging bouts made by individuals of each pollinator group to a discrete, unmanipulated patch of plants for 10 min every hour throughout daylight for three days.

To determine pollinator visit characteristics, I randomly assigned all flowers in a discrete patch of plants (an individual plant in the case of the caespitose, spreading *P. ellipticus*) to either a staminodeless, control or unmanipulated treatment and manipulated them. I did not assign treatments to entire inflorescences as I had for the hummingbird-pollinated species because bees often visited only one or two inflorescences during a foraging bout and would not experience all flower treatments. Patches were checked twice daily for newly opened flowers, which were then randomly assigned a treatment and manipulated. Foraging bouts of individual bees to the patch were observed or videotaped to determine visit frequency per flower, visit duration, and the rejection rate

of treatment flowers (rejection occurred when a bee approached and inspected a flower but did not visit).

For *P. palmeri*, I assessed the pollination characteristics of primary pollinators on different floral treatments by presenting individual pollinators with virgin flowers in either male or female phase. I obtained virgin flowers by assigning an inflorescence to male or female function and bagging it to exclude pollinators. I randomly assigned all flower buds on the inflorescence to unmanipulated, staminodeless, and control treatments and manipulated them upon bud break. For male-phase inflorescences, one bud was also assigned to an unvisited treatment to assess pollen availability. For female-phase inflorescences I also removed the undehisced anthers of each flower to prevent self-pollen deposition on the stigma. I presented male-phase inflorescences to individual pollinators when all anthers of the treatment flowers had dehisced. Female-phase inflorescences were presented 24 hours after bud break. Immediately following a single visit from a pollinator (except flowers of unvisited treatment) I removed the stigma (if female phase) or the anthers (if male phase) and placed them in 70% ethanol. I later stained the stigmas with 1% basic fuschin and counted the number of pollen grains deposited under a microscope. I counted number of pollen grains remaining in the anthers with a Particle Data Elzone 180XY particle counter.

3.2.6 Nectar-robbing

For *P. palmeri*, the only species visited by appreciable numbers of nectar robbers, I assessed the incidence of nectar robbing for the three flower treatments. Inflorescences in a discrete patch were assigned to either the staminodeless, control or unmanipulated treatment, with equal numbers of inflorescences and flowers at any given time. I observed the patch for 32, 5-min periods separated by at least 30 min over two days and recorded both the number of flowers of each treatment visited by a nectar robber and the number of robbers that visited the patch.

3.2.7 Analysis

I analyzed pollen-receipt and pollen-removal data using Repeated-Measures Analysis of Variance. An Analysis of Variance was used for visit duration of pollinators to *P. centranthifolius* and *P. ellipticus* flowers, whereas a Repeated-Measures Analysis of Variance with between-bee factors and within-bee factors was conducted with Proc Mixed (Littel et al., 1996; SAS: SAS Institute Inc., 1997) for visit duration of *P. palmeri* pollinators. I performed G-tests for visit frequency and flower rejection rates. Values reported are mean ± se throughout.

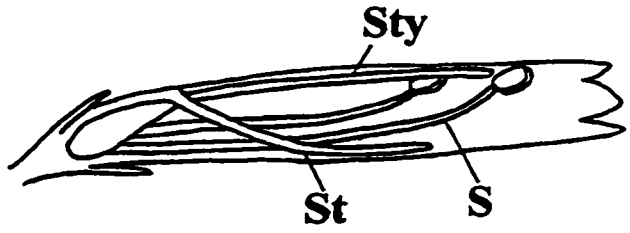
3.3 Results

3.3.1 Flower morphology

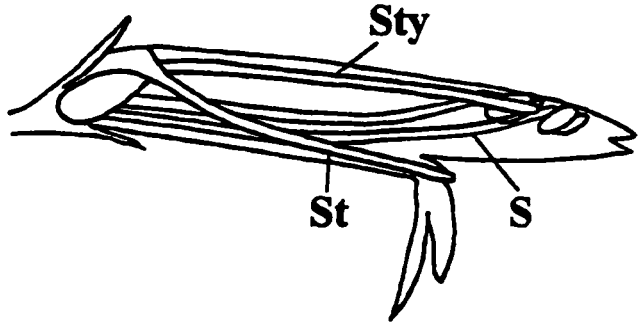
Penstemon species have a branching rootstock and display their flowers on several to many racemes. Most plants grow erect, with racemes from 3 to 14 dm high, although some alpine species (*P. ellipticus*) are low and spreading, forming dense mats. All *Penstemon* flowers have the same general organization, but flower structure varies considerably between species, even between those with similar pollinators (Fig 3.1). Flowers of the two hummingbird-pollinated species have similar colour, size, and corolla rigidity. However, the corolla of *P. rostriflorus* is deeply lobed with the lower lobe sharply reflexed compared to the shallow lobed and continuous-tubed *P. centranthifolius*. The two bee-pollinated species differ considerably. Flowers of *P. palmeri* have rigid, pouched corollas with dark nectar guides striping the lower lip and inside the corolla tube. The long, thick staminode protrudes from the corolla and bears profuse long, yellow hairs at its apex. Species in various sections of the subgenus *Penstemon* produce flowers similar to *P. palmeri*. Flowers of *P. ellipticus* have elongate corollas without obvious nectar guides. The thin corolla is pleated with prominent folds at the bottom and top of the corolla tube, which allow the flower to expand to accommodate larger bees. The anthers of the four stamens have woolly hair, which binds them together and prevents the filaments from springing apart as the bee pushes its way in, maintaining anther position.

Fig 3.1. Flowers of a) *P. centranthifolius*, b) *P. rostriflorus*, c) *P. palmeri*, and d) *P. ellipticus* in longitudinal section, revealing the style (Sty), stamens (S), and staminode (St). The scale bar represents 10 mm in the scale of the flower.

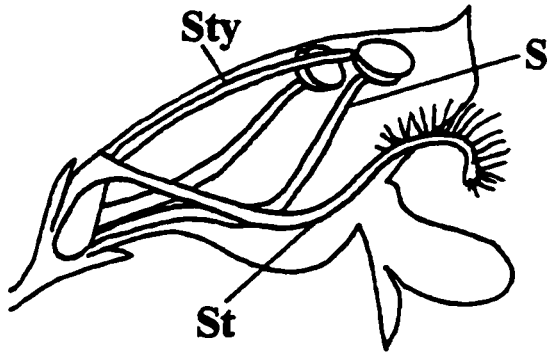
a)



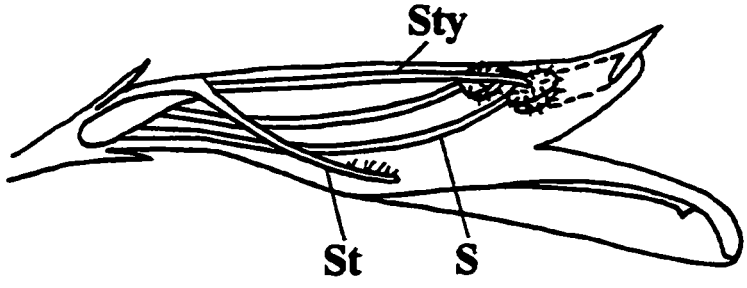
b)



c)



d)



This species has a short, thin staminode which is only sparsely hairy at its apex. The other species of subgenus *Dasanthera* are similar to *P. ellipticus* in terms of flower structure, although the extent of staminode bearding varies.

Penstemon flowers are generally protandrous. Male phase begins as the upper pair of anthers dehisce after bud break. The timing of upper anther dehiscence varies, with anthers of some flowers dehiscing immediately after bud break and others delaying for as long as 30 h (dependent on species). However, most flowers begin anther dehiscence within 6 h of bud break. The lower pair of anthers dehisce 2-3 h after the upper. The continuously-elongating style bends downwards when the stigma becomes receptive (usually 24 h after anthesis).

3.3.2 Hummingbird-pollinated species: *P. centranthifolius* and *P. rostriflorus*

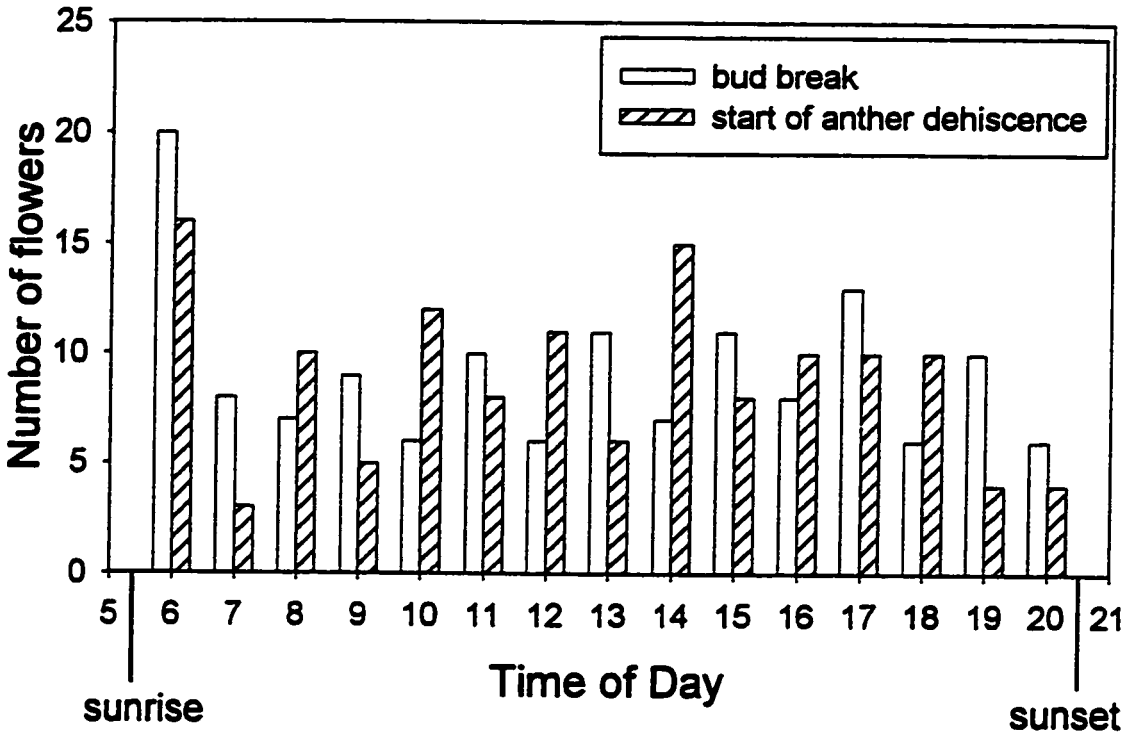
Flowers of both *P. centranthifolius* and *P. rostriflorus* opened at a relatively constant rate during the day (Fig. 3.2). Flowers opening throughout the night likely caused small peaks at 0600, the first observation time of the day, for both species.

3.3.2.1 Pollinator visit characteristics

Costa and Anna hummingbirds (*Calypte costae* Bourcier and *C. anna* Lesson) were numerous and visited *P. centranthifolius* very actively throughout the day at the San Bernardino site. One *P. centranthifolius* patch of 6 inflorescences (65 flowers) that I observed for 24 h received an average of 2.4 foraging bouts/h, with 14.4 ± 1.28 flowers visited/bout (n=58). Insects visited very rarely, with only a few nectar-robbing visits by *Hoplitis producta bernardina* Michener (4 observed) and *Bombus vosnesenskii* Rad. (2 observed) throughout the three-week study period. *Calypte anna* and *C. costae* visited unmanipulated, staminodeless and control *Penstemon centranthifolius* flowers similarly. During 52 foraging bouts (Table 3.1), Anna hummingbirds visited equal proportions of treatment flowers (ANOVA $F_{2, 52}$=0.83, P>0.25) and visited flowers for equal durations (ANOVA $F_{2, 230}$=0.45, P>0.50). Too few foraging bouts (n=6) by Costa hummingbirds

Fig 3.2. Diurnal patterns of bud break and start of anther dehiscence for flowers of a) *P. centranthifolius* (n=140) and b) *P. rostriflorus* (n=159).

a)


bud break
start of anther dehiscence
Number of flowers
0
5
10
15
20
25
5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Time of Day
sunrise
sunset

b)

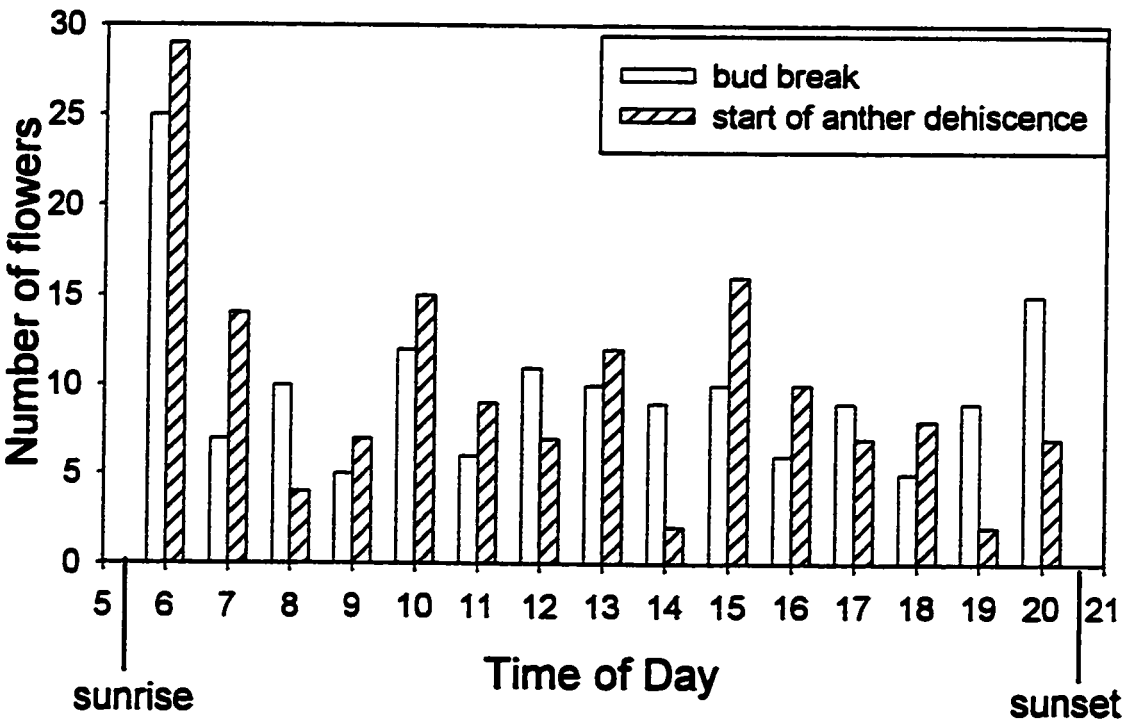

bud break
start of anther dehiscence
Number of flowers
0
5
10
15
20
25
30
5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Time of Day
sunrise
sunset

Table 3.1: Characteristics of Hummingbird (*Calypte anna* and *C. costae*) visits to *Penstemon centranthifolius* treatment flowers.

Characteristic	*Calypte anna*	*Calypte costae*
Mean proportion of flower visits/bout ± SE (*n*=52)		
Unmanipulated	0.23 ± 0.03	-----
Staminodeless	0.20 ± 0.03	-----
Control	0.24 ± 0.03	-----
Mean Visit Duration (s) ± SE		
Unmanipulated	0.62 ± 0.03 (*n*=93)	0.67 ± 0.03 (*n*=48)
Staminodeless	0. 59 ± 0.03 (*n*=60)	0.75 ± 0.04 (*n*=34)
Control	0.59 ± 0.03 (*n*=80)	0.75 ± 0.07 (*n*=43)

occurred to detect differences in proportions of flowers visited, but again, visit lengths to treatment flowers did not differ (ANOVA $F_{2,122}$=0.91, P<0.50).

In contrast to *P. centranthifolius*, *P. rostriflorus* received many fewer pollinator visits. During 24h of observation, broad-tailed hummingbirds (*Selasphorus platycercus* Swainson) visited a six-inflorescence patch (25 flowers) an average of 1.01 foraging bouts/h with 7.0 ± 0.95 flowers visited/bout (n=21). Pollen-collecting visits by small bees (*Lasioglossum egregium* Vachal, *Ceratina sp.*, *Halictus tripartitus* Cockerell, and *Evylaeus sp.*) were common, with the same six-inflorescence patch receiving, on average, 2.08 visits/h; however, these bees visited only 1.5 ± 0.12 flowers/bout (n=45).

3.3.2.2 Pollen receipt

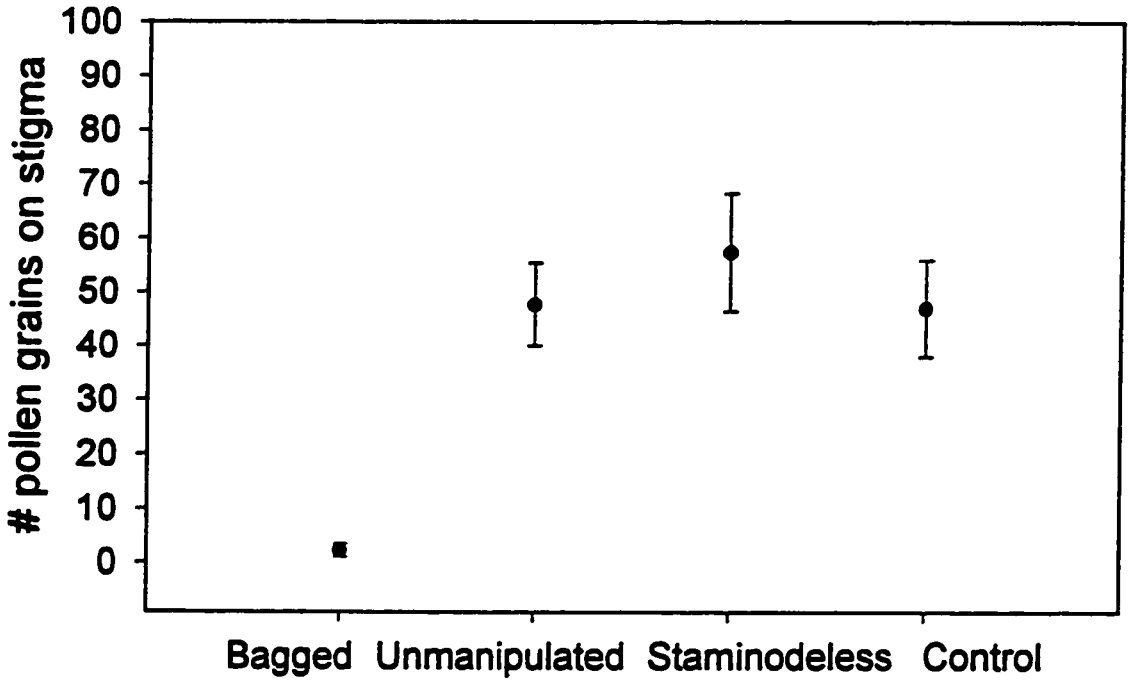
Given the number of ovules produced by flowers of *P. centranthifolius* (62.1 ± 2.5, n=20) and *P. rostriflorus* (105.8 ± 3.6, n=20) hummingbirds deposited relatively few pollen grains on their stigmas (Fig. 3.3). Bagged flowers received a few self-pollen grains in both cases, but these grains did not germinate. Flowers without staminodes tended to receive slightly more pollen grains than either unmanipulated or control flowers for both *P. centranthifolius* and *P. rostriflorus*, but this trend was not significant for either species (Repeated-Measures Analysis of Variance $F_{24,48}$=1.55, P>0.05; $F_{26,46}$=1.36, P>0.10).

3.3.2.3 Pollen removal

Flowers of *P. centranthifolius* and *P. rostriflorus* produced similar quantities of pollen (123,995 ± 1201 [n=10] and 130,948 ± 1700 [n=10] grains, respectively). Following anther dehiscence, the amount of pollen remaining in the anthers of both species decreased at a diminishing rate over time (Fig. 3.4). Unmanipulated, staminodeless and control flowers did not differ significantly with respect to either the amount of pollen removed (Repeated-Measures Analysis of Variance $F_{2,20}$=0.71, P>0.50 for *P. centranthifolius*; $F_{2,23}$= 0.27, P>0.75 for *P. rostriflorus*), or the rate of pollen removal

Fig. 3.3. Mean ± SE number of pollen grains received by flowers of each treatment group of hummingbird-pollinated a) *P. centranthifolius* (n=25) and b) *P. rostriflorus* (n=23).

a)


pollen grains on stigma
100
90
80
70
60
50
40
30
20
10
0
Bagged
Unmanipulated
Staminodeless
Control

b)

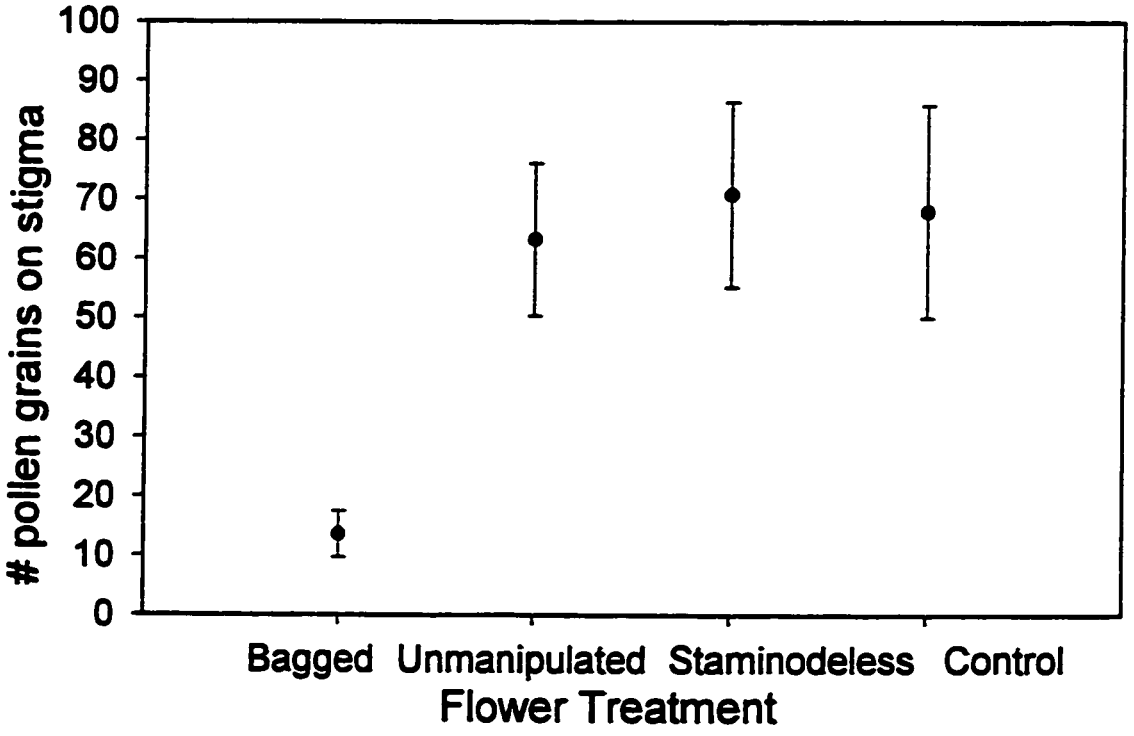
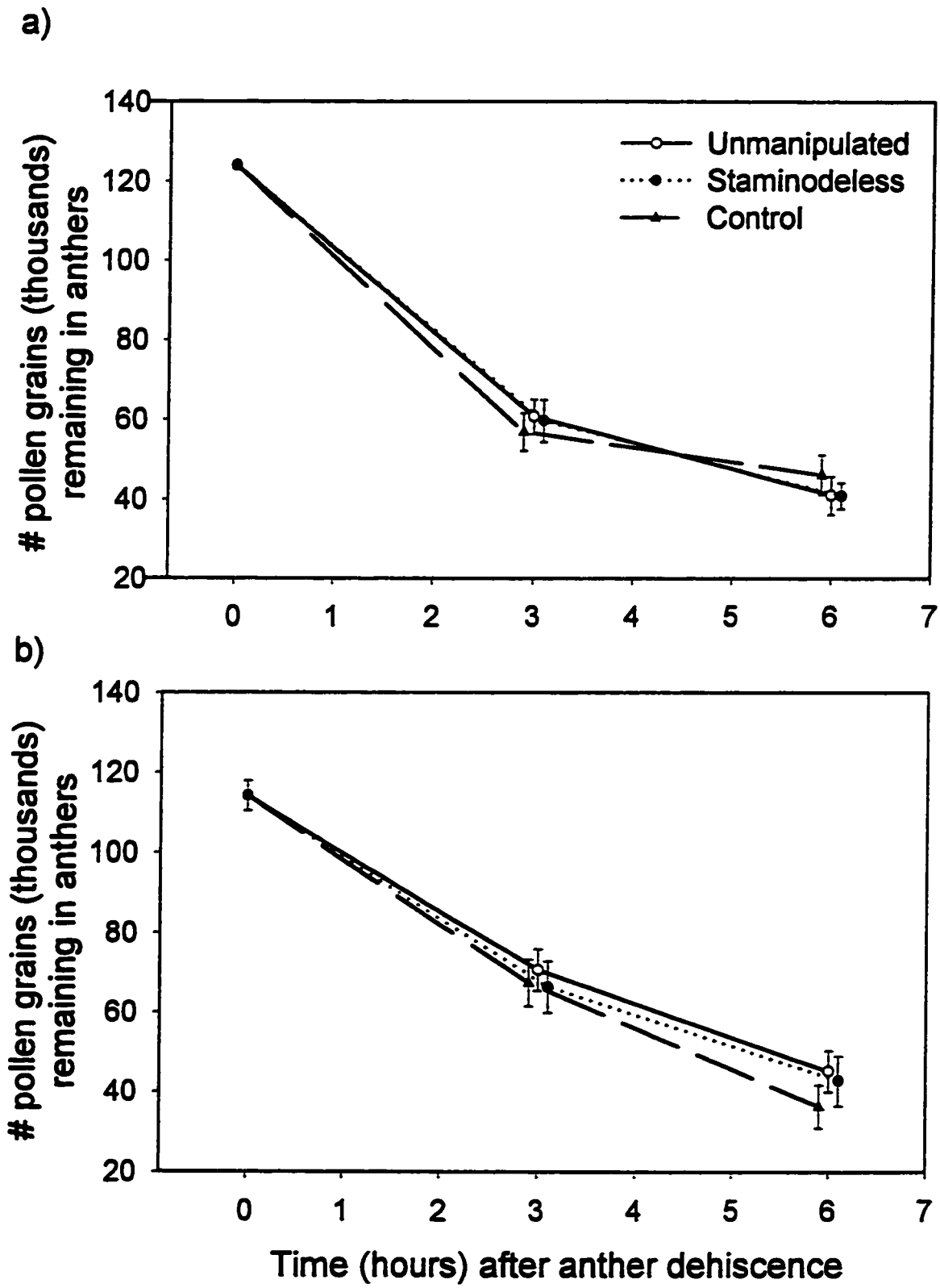

pollen grains on stigma
100
90
80
70
60
50
40
30
20
10
0
Bagged
Unmanipulated
Staminodeless
Control
Flower Treatment

Fig. 3.4. Mean ± SE number of pollen grains remaining in anthers of flowers of each treatment group of hummingbird-pollinated a) *P. centranthifolius* (n=23) and b) *P. rostriflorus* (n=25). The mean number of pollen grains at time 0 indicates mean total pollen production of flowers (n=10).


a)
b)
pollen grains (thousands) remaining in anthers
Unmanipulated
Staminodeless
Control
140
120
100
80
60
40
20
0
1
2
3
4
5
6
7
Time (hours) after anther dehiscence

(Repeated-Measures Analysis of Variance $F_{2, 42}$=0.75, P>0.25 for *P. centranthifolius*; $F_{2, 48}$=0.30, P>0.50 for *P. rostriflorus*) for either species.

3.3.3 Bee-pollinated species: *P. palmeri and P. ellipticus*

3.3.3.1 Pollinator visit characteristics

3.3.3.1.1 *Penstemon palmeri*

Flowers of *P. palmeri* opened primarily at dawn and dusk, with the start of anther dehiscence following a similar pattern (Fig 3.5). The six-inflorescence patch (35 flowers) observed for 10 h received intensive visitation from a variety of pollinators. *Callanthidium illustre* Cresson (66.2 foraging bouts/h with 2.45 ± 0.17 [n=127] flowers visited/bout) and *Xylocopa tabaniformis androleuca* Michener (17.3 foraging bouts/h with 5.01 ± 0.50 [n=72] flowers visited/bout) were the primary pollinators, but *Bombus morrisoni* Cresson (0.90 foraging bouts/h) and *Xylocopa californica arizonensis* Cresson (0.10 foraging bouts/h) visited infrequently. The small nectar robber, *Ashmeadiella australis* Cockerell, visited commonly (3.4 foraging bouts/h, with 2.38 ± 0.27 [n=34] flowers visited/bout). Other nectar robbers, such as *Agapostemon texanus* Cresson (0.2 foraging bouts/h), *Osmia clarescens* Cockerell (0.10 foraging bouts/h) and *Evylaeus sp.* (0.5 foraging bouts/h), rarely visited. Sphingid moths and hummingbirds also occasionally visited the flowers.

Callanthidium visited *P. palmeri* flowers for both pollen and nectar. During pollen-collecting visits bees landed on the lower lip of the flower and reached up and scrabbled at the anthers with their forelegs and mandibles, knocking pollen onto their bodies which they then packed into the scopa on the ventral side of their abdomen. To collect nectar, all large-bodied bees entered the flowers in an upright posture.

The timing of visits by bees followed a consistent daily pattern. *Callanthidium* was active throughout the day, with peaks in the early morning and evening. In contrast, *Xylocopa* visited only during early morning and evening (Fig 3.6). The absence of *Xylocopa* during most of the day seemed primarily related to aggressive behaviour by *Callanthidium*. Individual *Callanthidium* vigorously defended flower patches by ramming

and driving away other pollinator species (including sphingid moths and hummingbirds). This interaction would increase foraging costs disproportionately for the larger *Xylocopa*, likely making nectar collection from *P. palmeri* flowers unprofitable during midday when few flower buds open (Fig 3.5) and standing nectar crop diminishes. In contrast, daily fluctuations in visit frequency for *Callanthidium* may be related to an optimal temperature range. Peaks in activity occur at 0830 and 1800 (Fig 3.6c), when temperature at the site averaged approximately 29°C (Fig 3.7). Temperatures both higher and lower than 29°C resulted in lowered activity.

Visit duration to *P. palmeri* flowers differed between pollinator groups (Table 3.2). Male *Xylocopa* and *Callanthidium* visited flowers for equivalent durations (t=2.16, df=681, P>0.05) which were significantly shorter than visits by female *Xylocopa* (male *Xylocopa*, t=7.08, df=681, P<0.001; *Callanthidium*, t=5.62, df=681, P<0.001). However, all pollinator species visited flowers of different treatments for equivalent durations and rejected similar proportions of flowers regardless of treatment (Table 3.2). In contrast, visit frequency depended on species and sex of the pollinator (Table 3.2). Male *Xylocopa* found staminodeless flowers more attractive, visiting significantly more removed flowers than control or unmanipulated flowers, whereas female *Xylocopa* and *Callanthidium* showed no preference.

Nectar robbing did not differ between flower treatments of *P. palmeri*. Neither the mean number of robbers (ANOVA $F_{2,93}$=0.66, P>0.50) nor the number of flowers visited by each insect (ANOVA $F_{2,93}$=2.59, P>0.05) differed among flower treatments.

3.3.3.1.2 *Penstemon ellipticus*

Flowers of *P. ellipticus* opened primarily in late morning and early evening, whereas anther dehiscence occurred in early morning and late evening (Fig 3.8). Heavy morning dew at the high-elevation site likely delayed bud break until most of the moisture evaporated, but anther dehiscence was unaffected.

Only bumble bees visited *P. ellipticus*. *Bombus melanopygus* Cresson predominated, visiting an individual plant (75 flowers) observed for 5 h an average of 18.4

Fig. 3.5. Diurnal patterns of bud break and start of anther dehiscence for flowers of *P. palmeri* (n=155).

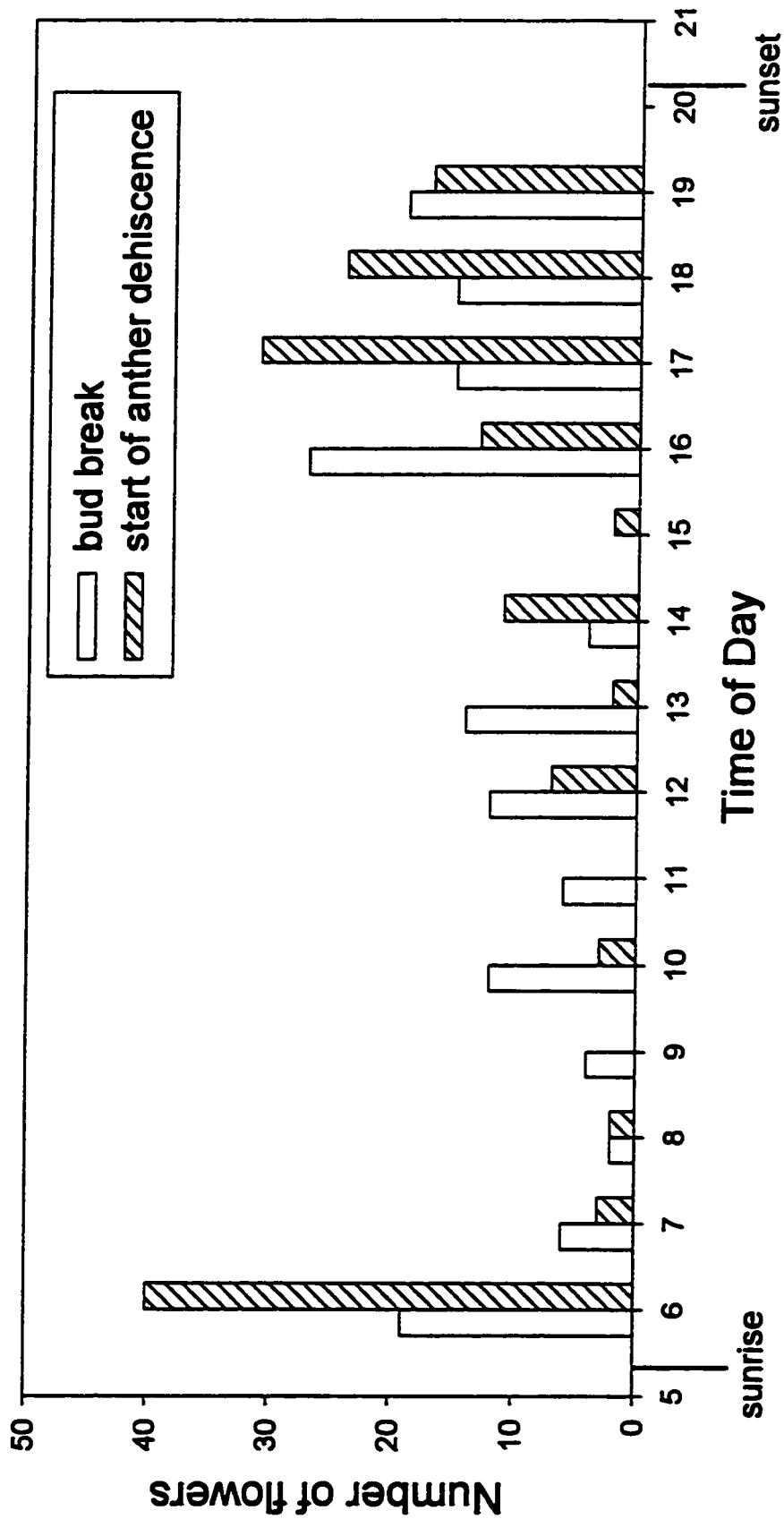

bud break
start of anther dehiscence
Number of flowers
0
10
20
30
40
50
Time of Day
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
sunrise
sunset

Fig. 3.6. Diurnal variation in *Xylocopa* and *Callanthidium* activity (mean number of foraging bouts to patch/10 min ± SE, n=3) on *P. palmeri* flowers.

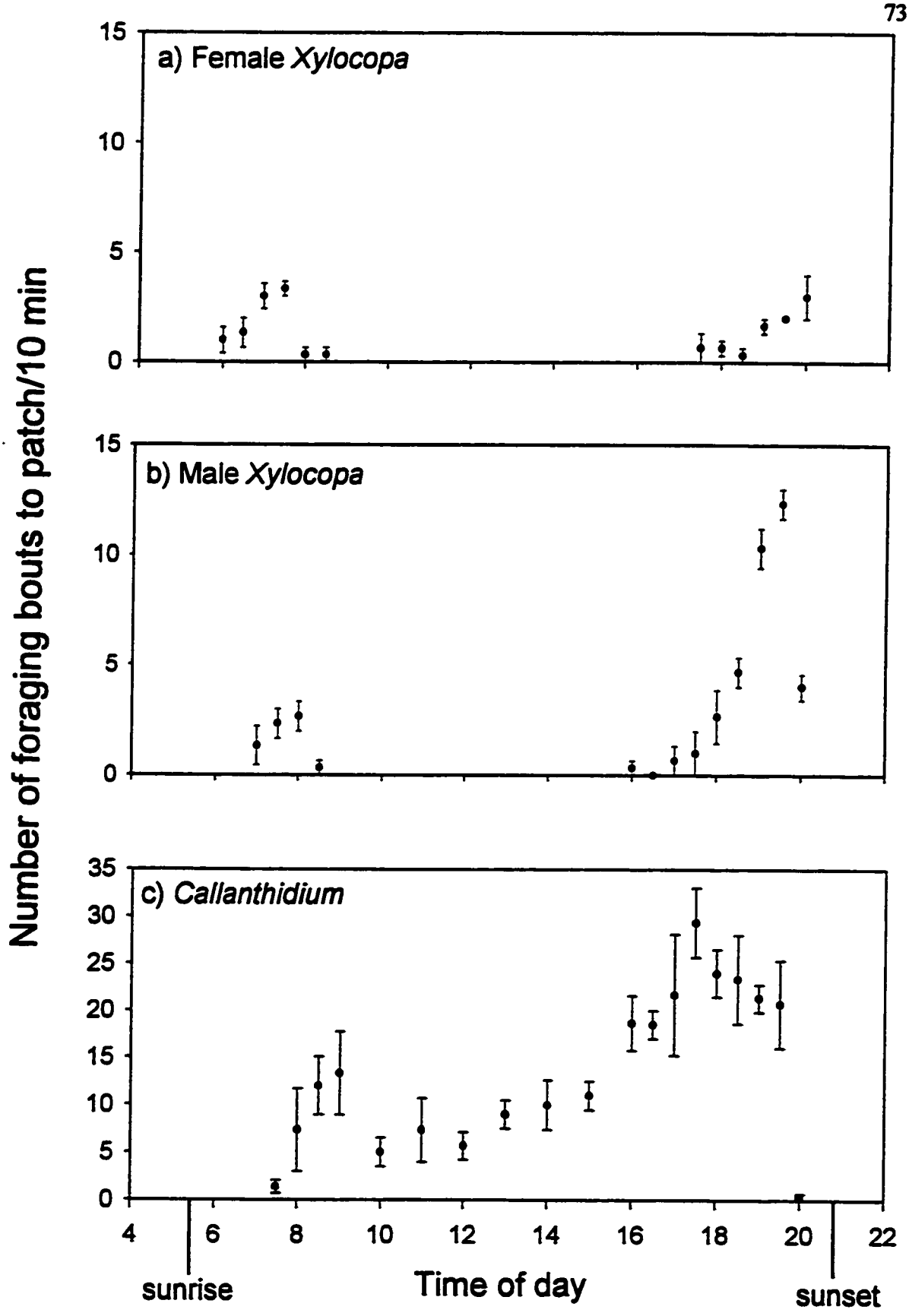
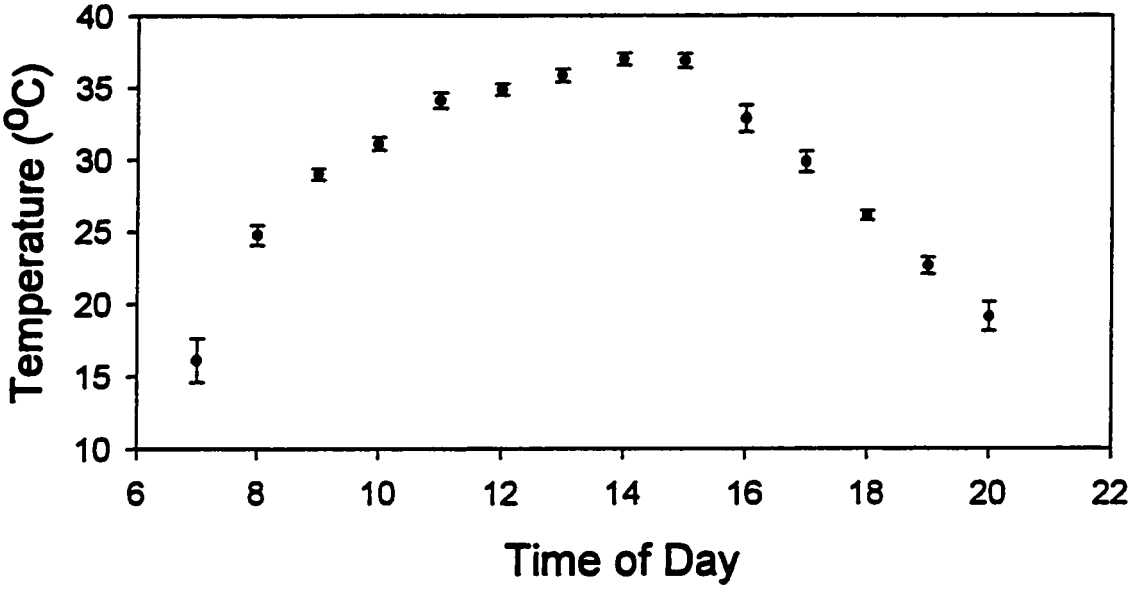

a) Female Xylocopa
b) Male Xylocopa
c) Callanthidium
Number of foraging bouts to patch/10 min
Time of day
sunrise
sunset
0
5
10
15
20
25
30
35
4
6
8
10
12
14
16
18
20
22

Fig. 3.7. Diurnal variation in ambient temperature (mean temperature ± SE, n=10) at *P. palmeri* site.


Temperature (°C)
40
35
30
25
20
15
10
6
8
10
12
14
16
18
20
22
Time of Day

foraging bouts/h with 5.7 ± 0.81 flowers visited/bout (n=71). Less frequent visitors included *B. mixtus* Cresson (1.9 visits/h with 8.7 ± 2.0 flowers visited/bout [n=7]) and *B. sylvicola* Kirby (0.94 visits/h with 7.5 ± 3 flowers visited/bout [n=4]). *Bombus melanopygus* activity at the site increased throughout the day until early evening, with a drop in activity during mid-afternoon (Fig 3.9a). Visit frequency seemed generally related to temperature (Fig 3.9b), except the mid-afternoon drop, which showed no clear relation to either temperature or bud break (hence standing nectar crop: Fig 3.9). However, periods of cloudcover occurred in the early afternoon on two of the observation days which may have decreased visitation.

All bees entered the *P. ellipticus* flowers in an upright posture. The flower closed after a bee entered, precluding observation of visit details, but the bee's movement could often be seen through the thin corolla when it was backlit. In this manner bees were observed to travel down the corolla until stopped by the transverse positioning of the staminode. They then extended their mouthparts as far as possible between the staminode filament and the corolla wall to access the nectary on that side. To access the opposite nectary, bees shifted their position and extended their mouthparts between the staminode and the opposite corolla wall.

Bombus melanopygus visited similar numbers of unmanipulated, staminodeless and control flowers of *P. ellipticus* and rejected equal numbers (Table 3.3). However, these bees visited staminodeless flowers significantly faster than unmanipulated and control flowers.

3.3.3.2 Pollen receipt

Given the number of ovules produced by flowers of *P. palmeri* (94.0 ± 2.6, n=20) and *P. ellipticus* (172.6 ± 6.8, n=20), bees at the two study sites delivered substantial pollen (Fig 3.10). As with the hummingbird-pollinated species, bagged flowers received very little pollen and those self-grains deposited did not germinate on the stigmas. Unmanipulated and control flowers received similar amounts of pollen (*P. palmeri*,

Table 3.2: Characteristics of pollinator visits to *Penstemon palmeri* treatment flowers.

a) Male *Xylocopa tabaniformis*

	Unmanipulated	Staminodeless	Control	Test statistic
# visits	83	129	87	$G_2 = 12.54$*
proportion of flowers rejected	0.12	0.07	0.05	$G_2 = 2.87$
Visit duration ± SE (s)	2.45 ± 0.11 (n=73)	2.36 ± 0.10 (n=120)	2.34 ± 0.13 (n=83)	$F_{2, 273} = 0.25$

b) Female *Xylocopa tabaniformis*

# visits	42	42	46	$G_2 = 0.24$
proportion of flowers rejected	0.07	0.07	0.09	$G_2 = 0.085$
Visit duration ± SE (s)	4.06 ± 0.40 (n=39)	3.26 ± 0.35 (n=39)	3.18 ± 0.25 (n=42)	$F_{2, 117} = 2.17$

c) *Callanthidium illustre*

# visits	151	152	140	$G_2 = 0.61$
proportion of flowers rejected	0.11	0.22	0.14	$G_2 = 5.30$
Pollen visit duration (s) ± SE	1.96 ± 0.20 (n=55)	1.94 ± 0.15 (n=46)	2.15 ± 0.25 (n=41)	$F_{2, 139} = 0.25$
Nectar visit duration (s) ± SE	2.95 ± 0.32 (n=30)	3.36 ± 0.33 (n=32)	2.99 ± 0.23 (n=34)	$F_{2, 93} = 0.60$

* 0.05>P>0.01, ** 0.01>P>0.001

Fig. 3.8. Diurnal patterns of bud break and start of anther dehiscence for flowers of *P. ellipticus* (n=166).

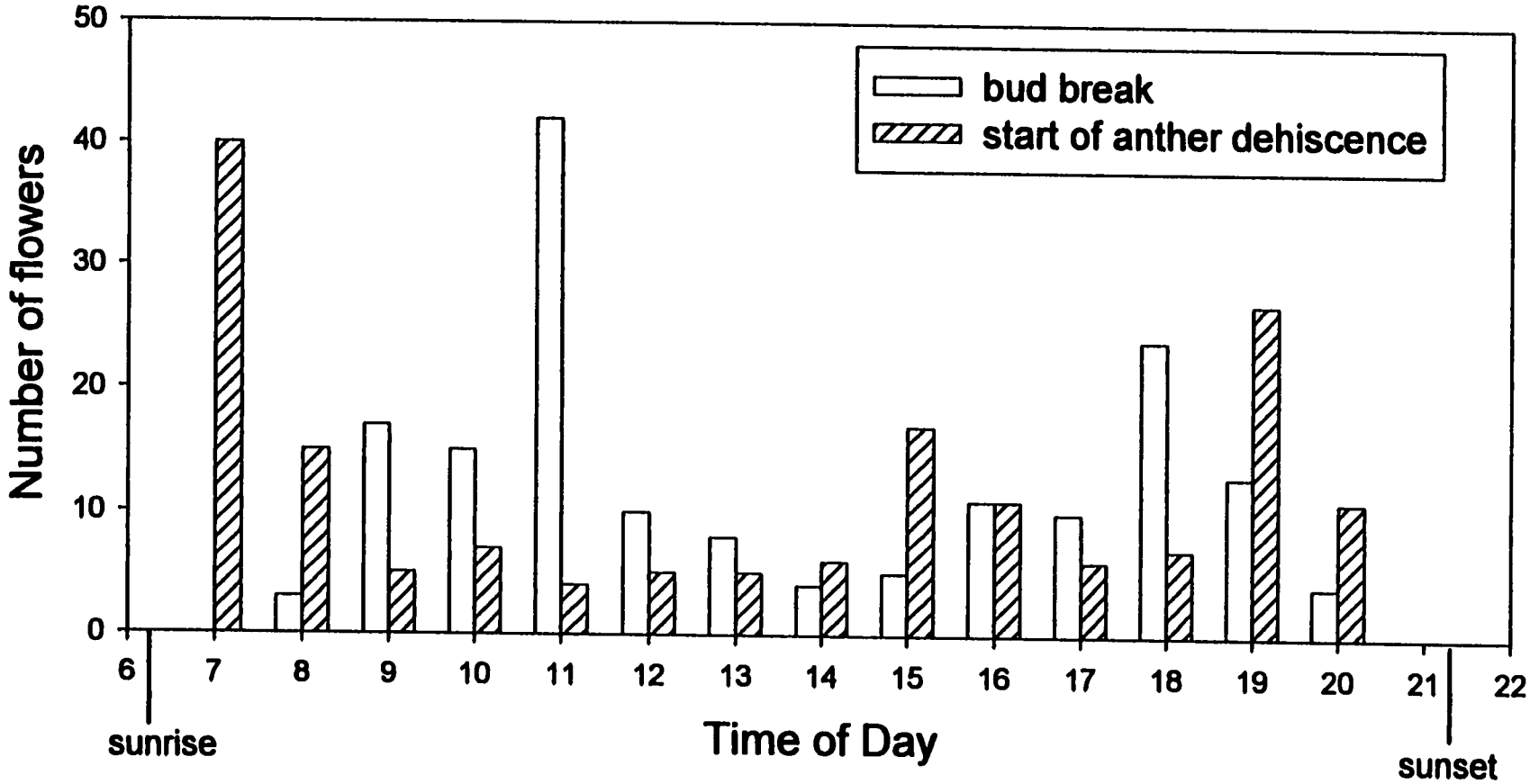
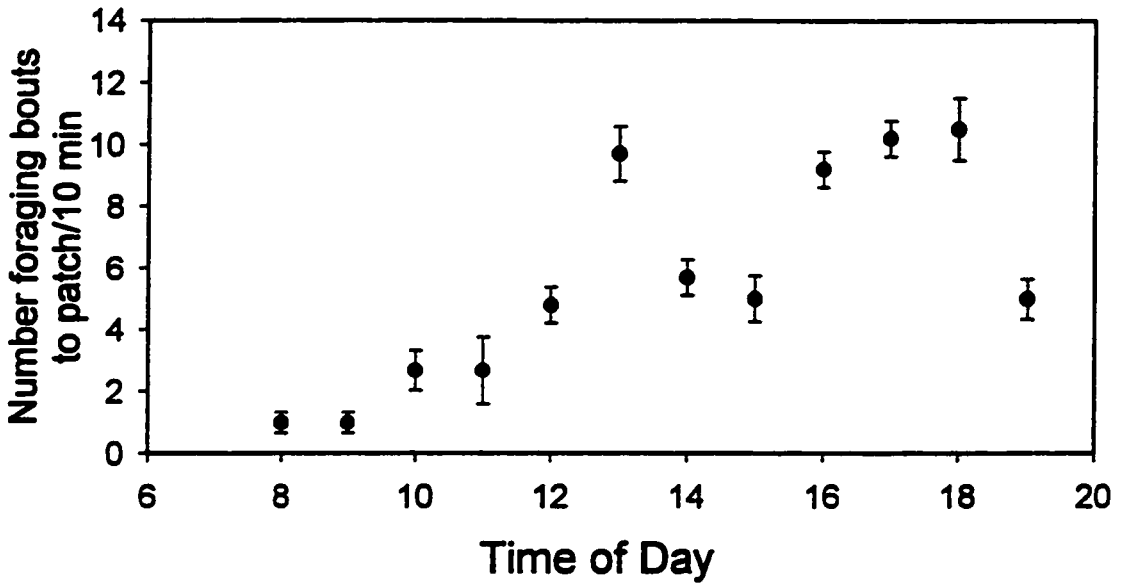
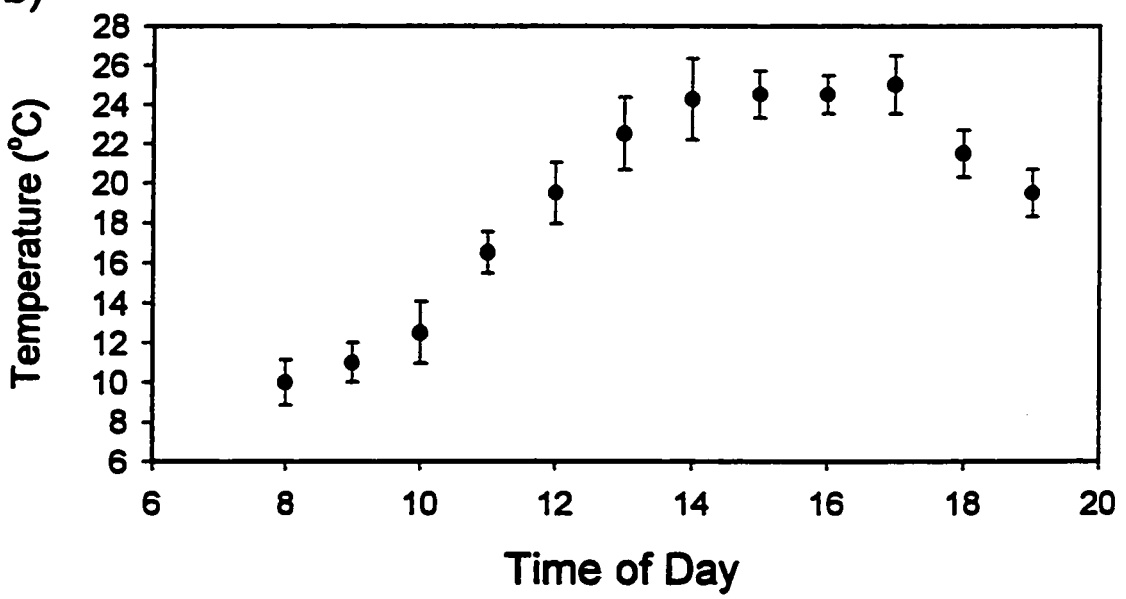

50
40
30
20
10
0
Number of flowers
bud break
start of anther dehiscence
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
sunrise
Time of Day
sunset

Fig. 3.9. Diurnal variation in a) *Bombus melanopygus* activity (mean number of foraging bouts to patch/10 min ± SE, n=3) on *P. ellipticus* flowers, and b) ambient temperature (mean temperature ± SE, n=10) at *P. ellipticus* site.

a)


Number foraging bouts to patch/10 min
0
2
4
6
8
10
12
14
6
8
10
12
14
16
18
20
Time of Day

b)


Temperature (°C)
6
8
10
12
14
16
18
20
22
24
26
28
6
8
10
12
14
16
18
20
Time of Day

t=1.48, df=3, P>0.75: *P. ellipticus*, t=1.17, df=3, P>0.10); however, staminodeless flowers received significantly fewer grains (*P. palmeri*, t=2.77, df=3, 0.01>P>0.001: *P. ellipticus*, t=3.22, df=3, 0.01>P>0.001). Single visits to virgin *P. palmeri* flowers by male *Xylocopa* indicate that these differences in pollen receipt arise from a pollinator's interactions with flowers during each visit (Fig. 3.11). In particular, as with the aggregate results, these bees deposited similar numbers of pollen grains on stigmas of unmanipulated and control flowers ($F_{1, 27}$=0.56,P>0.75), but significantly fewer grains on stigmas of staminodeless flowers ($F_{1, 41}$=8.74, 0.01>P>0.005). *Callanthidium* accepted too few of the presented flowers (n=5) to detect differences in pollen deposition between treatments.

3.3.3.4 Pollen removal

Flowers of *P. palmeri* and *P. ellipticus* produced large numbers of pollen grains (334,500 ± 1,177 and 146,033 ± 3,971 respectively). The total amount of pollen removed did not differ significantly between unmanipulated, staminodeless and control flowers for either *P. palmeri* ($F_{2, 21}$=2.21, P>0.10) or *P. ellipticus* ($F_{2, 25}$=1.46, P>0.25). The pollen remaining in the anthers of both species decreased at a diminishing rate over time (Fig. 3.12). Pollen removal rate did not differ significantly between treatments for *P. palmeri* ($F_{2, 21}$=0.44, P>0.50). In addition, *Xylocopa* males and *Callanthidium* removed similar amounts of pollen from *P. palmeri* during single visits (Fig 3.13: $F_{1, 61}$=0.35, P>0.90) and pollen removal did not differ between treatment flowers (*Xylocopa*, $F_{2, 40}$=0.37, P>0.75: *Callanthidium*, $F_{2, 17}$=0.60, P>0.75). In contrast, staminodeless flowers of *P. ellipticus* had a significantly lower rate of pollen removal than unmanipulated and control flowers ($F_{2, 25}$=4.79, 0.05>P>0.01).

3.4 Discussion

3.4.1 Impact of staminode removal on floral function

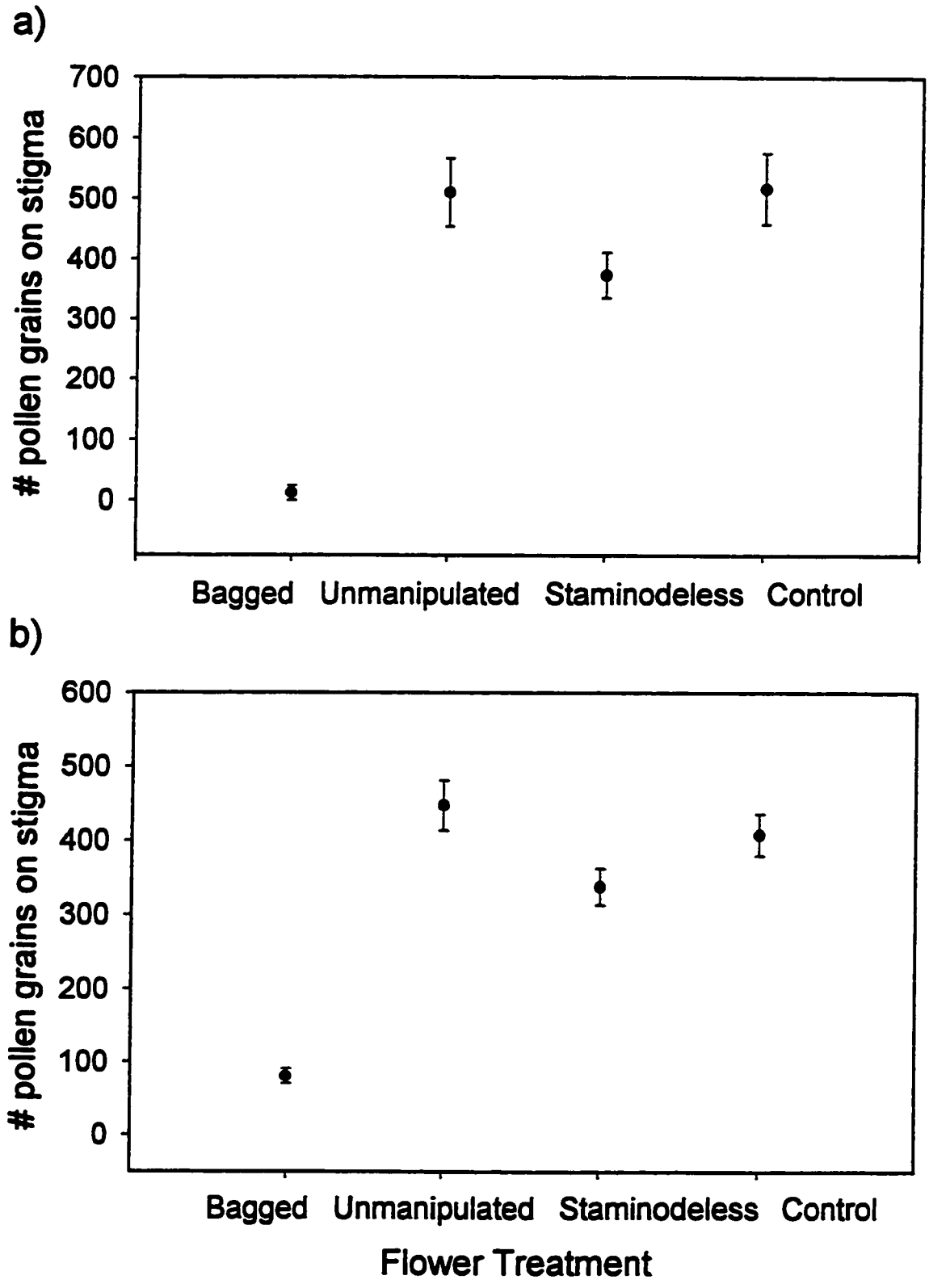
Based on my results, the staminode affects pollen dispersal by bee-pollinated *Penstemons*, but not by hummingbird-pollinated *Penstemons*. Staminode removal had no impact on either pollen receipt or pollen removal by hummingbird-pollinated

Table 3.3: Characteristics of *Bombus melanopygus* visits to *Penstemon ellipticus* treatment flowers. Analysis includes observed and videotaped data except for visit duration (videotaped data only).

	Unmanipulated	Staminodeless	Control	Test statistic
# visits	149	144	143	G = 0.162 (*df*=2)
Proportion of flowers rejected	0.34	0.31	0.32	G = 0.181 (*df*=2)
Visit length ± SE (s)	6.76 ± 0.35 (*n*=35)	5.38 ± 0.30 (*n*=41)	6.96 ± 0.37 (*n*=37)	$F_{2, 110}$= 6.59**

** 0.01>P>0.001

Fig. 3.10. Mean ± SE number of pollen grains received by flowers of each treatment group of bee-pollinated a) *P. palmeri* (n=29) and b) *P. ellipticus* (n=29).


a)
700
600
500
400
300
200
100
0
pollen grains on stigma
Bagged
Unmanipulated
Staminodeless
Control
b)
600
500
400
300
200
100
0
pollen grains on stigma
Bagged
Unmanipulated
Staminodeless
Control
Flower Treatment

P. centranthifolius or *P. rostriflorus*. In contrast, staminode removal from bee-pollinated species reduced pollen receipt by at least 20% (*P. palmeri*, 27%; *P. ellipticus*, 21%). Staminode removal also reduced the rate of pollen removal from *P. ellipticus*, but not for *P. palmeri*.

Staminode removal reduced pollen receipt by both *P. palmeri* and *P. ellipticus* flowers, but staminodeless flowers of both species received more pollen grains than the number of ovules they produced (nearly 4X for *P. palmeri* and 2X for *P. ellipticus*). Flowers typically require between 1.2 and 8 pollen grains per ovule for full seed set (Snow, 1982; McDade, 1983; Shore and Barrett, 1984; Bertin, 1990), so female function in staminodeless flowers could be compromised either through insufficient mating opportunities (fewer seeds produced) or through reduced mate choice (lower seed quality).

The lower rate of pollen removal observed for *P. ellipticus* could also hamper male function in flowers without staminodes. Although restricted pollen removal helps counteract the diminishing returns associated with animal pollination, less restrictive removal is advantageous when fertilization opportunities are not uniformly distributed through a flower's blooming period (Harder and Wilson, 1994, Fig. 5). Most *P. ellipticus* flowers open in late morning and start female phase approximately 24 hours later so that large numbers of flowers become receptive each morning. As a result, a male phase flower with a reduced rate of pollen removal might export fewer pollen grains to pistils with unfertilized ovules than flowers with more rapid removal. This would reduce siring success, limiting fitness gains through male function for these flowers.

3.4.2 Staminode function

The staminode of bee-pollinated *Penstemon* flowers could facilitate pollen receipt and removal either by increasing pollinator visitation or by enhancing contact by individual pollinators with a flower's sexual organs. Staminode removal did not reduce visit frequency to *P. ellipticus* or *P. palmeri* flowers, hence, the staminode seems generally not to act through visit frequency. That the staminode likely facilitates contact between

Fig. 3.11. Mean ± SE number of pollen grains received by *P. palmeri* flowers of each treatment group following a single visit by a male *Xylocopa*.

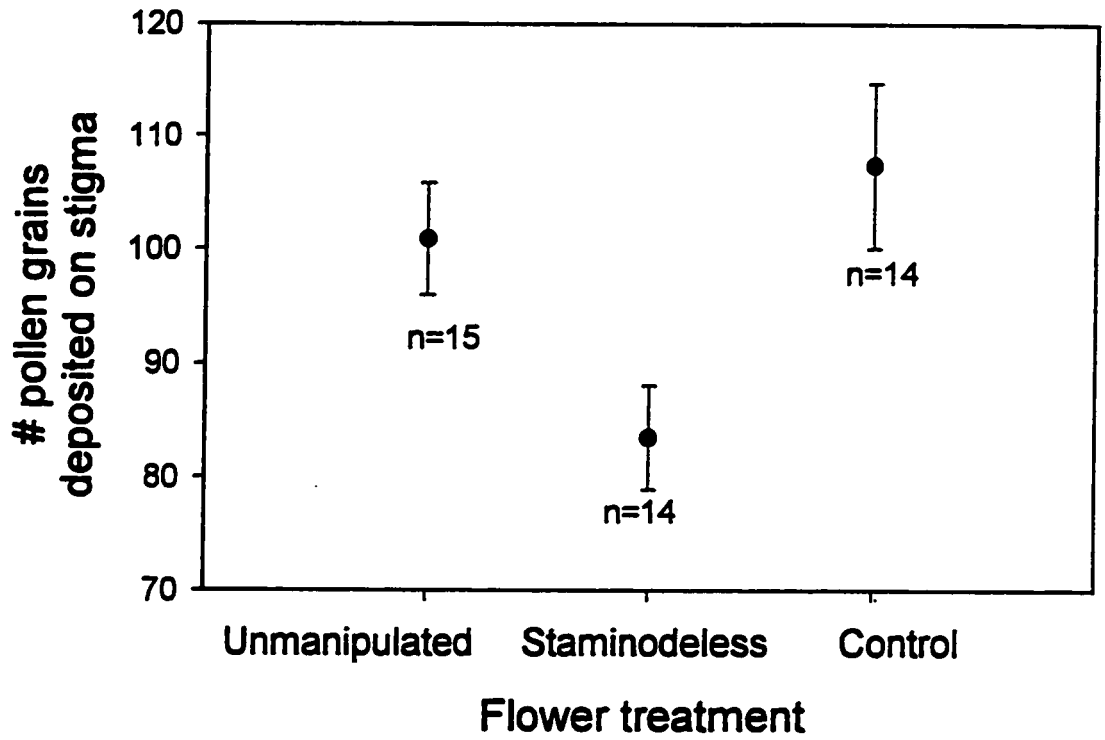
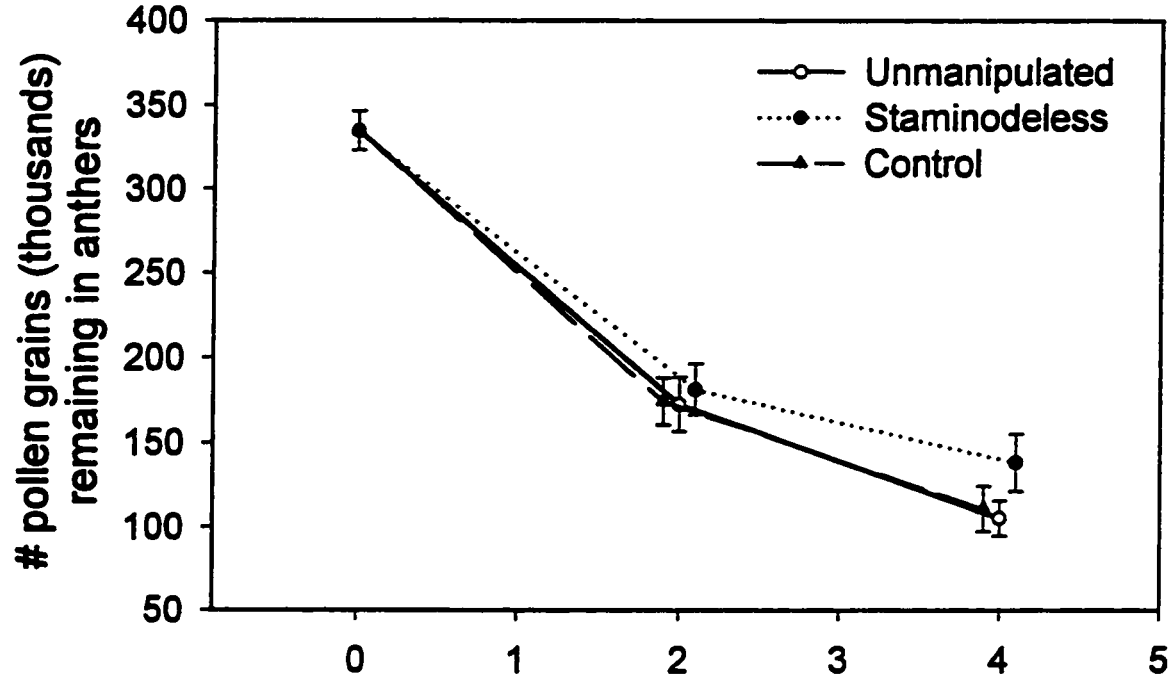

120
110
100
90
80
70
# pollen grains deposited on stigma
n=15
n=14
n=14
Unmanipulated
Staminodeless
Control
Flower treatment

Fig. 3.12. Mean ± SE number of pollen grains remaining in anthers of flowers of each treatment group of bee-pollinated a) *P. palmeri* (n=29) and b) *P. ellipticus* (n=25). The mean number of pollen grains at time 0 indicate mean total pollen production of flowers (n=10).

a)


400
350
300
250
200
150
100
50
pollen grains (thousands) remaining in anthers
Unmanipulated
Staminodeless
Control
0
1
2
3
4
5

b)

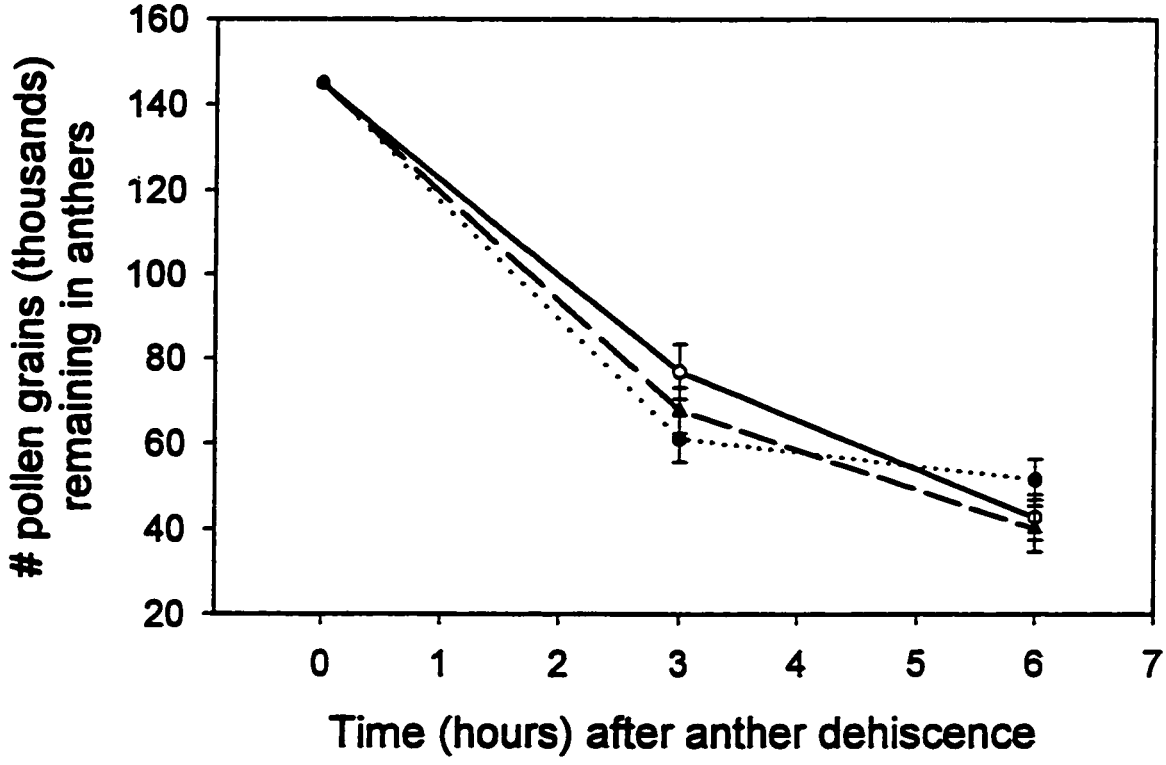
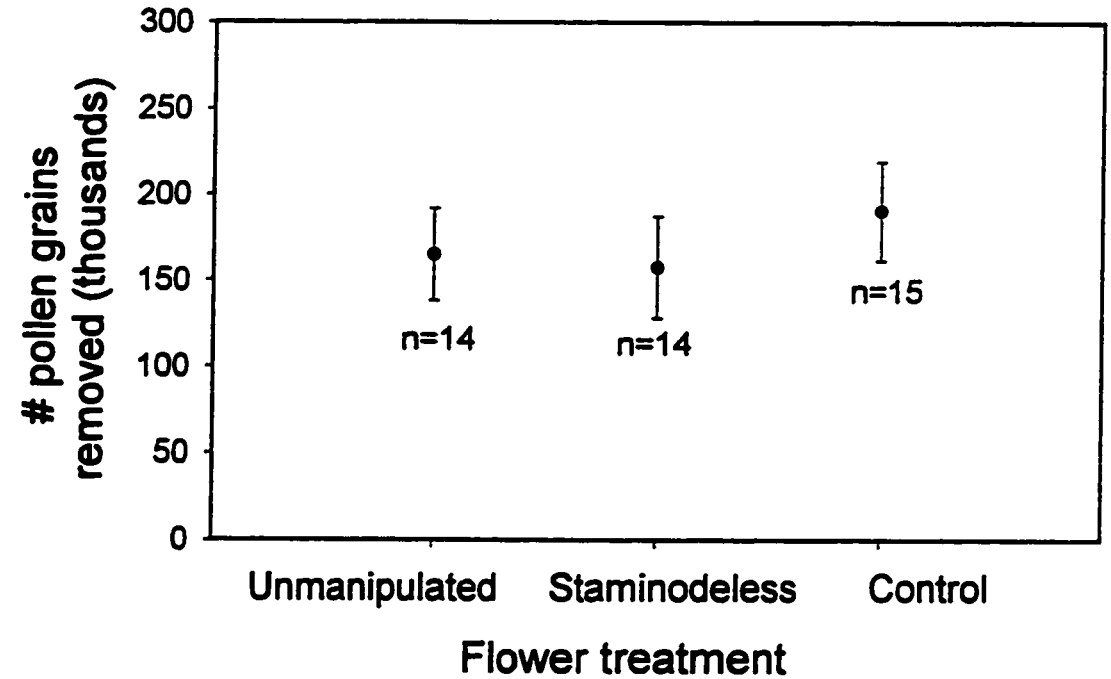
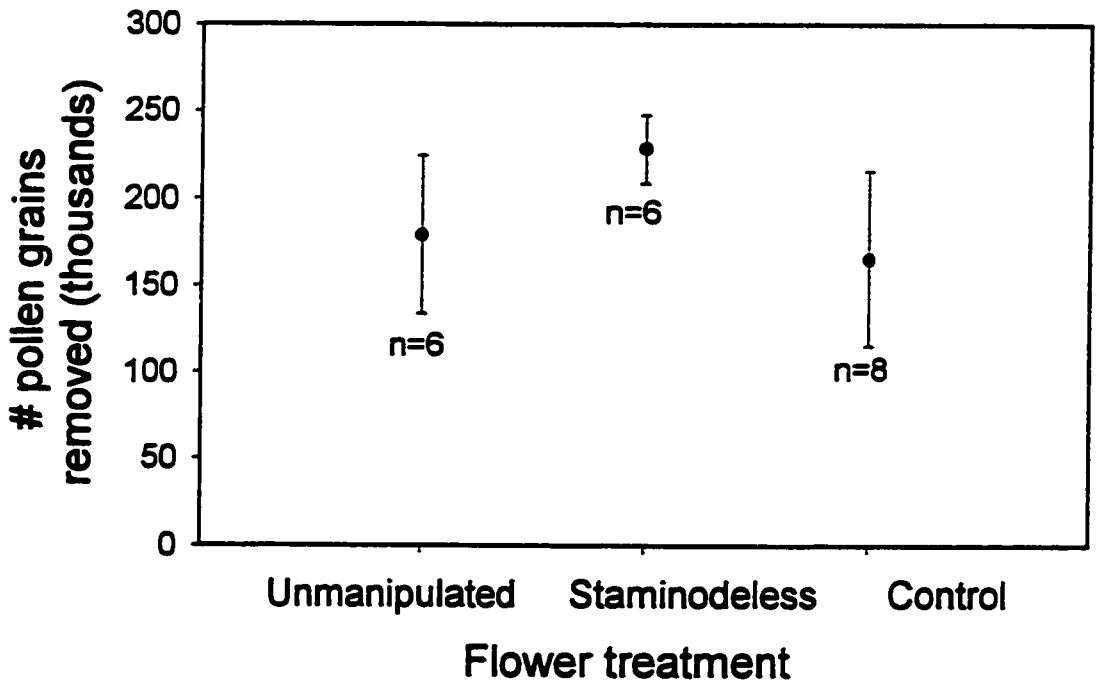

160
140
120
100
80
60
40
20
pollen grains (thousands) remaining in anthers
0
1
2
3
4
5
6
7
Time (hours) after anther dehiscence

Fig. 3.13. Mean ± SE number of pollen grains removed from anthers of *P. palmeri* flowers of each treatment group following a single visit from a) *Xylocopa*, or b) *Callanthidium*.

a)


300
250
200
150
100
50
0
# pollen grains removed (thousands)
n=14
n=14
n=15
Unmanipulated
Staminodeless
Control
Flower treatment

b)


300
250
200
150
100
50
0
# pollen grains removed (thousands)
n=6
n=6
n=8
Unmanipulated
Staminodeless
Control
Flower treatment

individual pollinators and a flower's sexual organs is supported by the reduced amount of pollen deposited during single visits by male *Xylocopa* to staminodeless *P. palmeri* flowers.

My data suggest that the mode of staminode action in governing a pollinator's contact with stamens and stigmas may differ between *Penstemon* species. Staminode removal did not affect visit duration by any of the main pollinators of *P. palmeri*, a species with relatively stiff corollas. This result is consistent with the lever mechanism that Torchio (1974) described for another species with relatively stiff corollas. Flower manipulations of *P. palmeri* demonstrated that pressure against the staminode caused the anthers and stigma to descend toward the pollinator. I have also observed this effect in the similar species, *P. eriantherus* Pursh (section Aurator, subgenus Penstemon).

Despite the observed lowering of the anthers, staminode removal did not significantly affect pollen removal from *P. palmeri* flowers. This is likely due to an independent mechanism that primarily facilitates contact between the pollinator and the stamens. In *P. palmeri*, and similar relatives, the rigid stamen filaments spread gradually outwards from the ovary, lying on the floor of the corolla, until roughly halfway down the corolla tube. The filaments then bend abruptly outwards and curve along the pouched corolla wall, positioning the anthers at the top of the corolla tube. The bend in the filaments provides a lever mechanism for the anthers. Flower manipulations of *P. palmeri* (and similar *P. eriantherus*) demonstrated that pressure against the inner surface of the filament at the bend causes the curved portion of the filament (plus anther) to swing down rapidly towards the pollinator. The downward movement of the anthers provided by the lever mechanism of the filaments was much greater than that provided by the lever mechanism of the staminode. Hence, in *P. palmeri* and similar relatives, the staminode probably acts primarily to facilitate pollen receipt.

For *P. ellipticus*, and similar relatives with flexible, pleated corollas, the staminode may facilitate contact with the sexual organs by creating a barrier that hinders access to the nectaries. As with Kerner's (1876) and Errera's (1878) barrier mechanism, the

transverse positioning of the staminode prevents further insect movement down the corolla tube towards the nectaries. However, the staminode does not function to prevent nectary access by nectar robbers (as proposed by Kerner [1876] and Errera [1878]), but instead hinders access to nectaries by pollinators in the same manner as a nectar spur. This increases visit duration, and hence contact between the pollinator and the stamens and stigma. Indeed, staminode removal from *P. ellipticus* flowers decreased the duration of pollinator visits by 20%. Once bees extend their mouthparts on one side of the staminode to access a nectary, they have to retract their tongue and approach from the other side of the staminode to access the opposite nectary. This time-consuming step would prolong a bee's contact with the anthers and stigma, resulting in greater pollen receipt and pollen removal from intact flowers than for flowers without staminodes. The preference of male *Xylocopa* bees for staminodeless flowers may reflect the shorter visit duration which would increase foraging efficiency.

This 'barrier' mechanism could work in concert with the lever mechanism; however, pressure against the staminode during flower manipulations of *P. ellipticus* (and similar *P. lyallii* Gray) did not change the position of the anthers or stigma. No lever mechanism for the anthers, as found in *P. palmeri* and relatives, operates in flowers of *P. ellipticus* and relatives.

Little evidence supported staminode functions other than the facilitation of pollinator contact with the anthers and stigma through Torchio's (1974) lever mechanism and the observed barrier mechanism. Staminode removal did not increase visit duration, indicating that the staminode does not function as a nectar guide. For *P. palmeri*, the only species visited by nectar robbers, removal of the staminode did not increase the frequency or duration of nectar-robber visits. Therefore it is unlikely that the staminode serves as a barrier to short-tongued nectar robbers. Pasquale's (1893) proposal that the staminode mediates autogamy can be excluded because bagged flowers received little pollen and *Penstemon* flowers are self-incompatible. The staminode may act secondarily as a purchase for some pollinators. *Callanthidium* bees often held onto the bearded staminode

of *P. palmeri* flowers as they alighted on the lower lip of the corolla. However, staminode removal did not visibly impede the ability of these insects to land or alter visit duration or flower preference. The need for the staminode to act as a purchase then, cannot be a driving selective force. This mechanism is not germane for *P. ellipticus* and related species with closed corollas, as the staminode is hidden and cannot affect pollinator landing.

A non-universal mechanism of staminode function for bee-pollinated species is perhaps an unsurprising result given the diversity in flower structure within the genus. To act both as a lever for the stigma (e.g. *P. palmeri*) and a barrier to hinder nectar access (e.g. *P. ellipticus*), only the transverse positioning of the staminode filament is essential. The elongation of the filament and various elaborations of the staminode tip in many *Penstemon* species may fulfill secondary roles more specific to various pollinating species. For example, bumble bees and carpenter bees commonly pollinate pouched flowers which have strongly exserted, bearded staminodes (e.g. *P. palmeri*, *P. eriantherus*: Pennell, 1948; Straw, 1956). The length and hairiness of the staminode may facilitate its lever action. A long staminode ensures that the staminode remains underneath the pollinator, whereas the hairiness may fix the position of the staminode, through friction with the insect's body, providing leverage.

The prevalence of the two mechanisms of staminode functioning within the genus as a whole awaits more extensive analysis. Given the consistency of flower form in the subgenus Dasanthera, the staminode likely serves as a barrier to hinder nectar access throughout the bee-pollinated species of that group. The barrier mechanism may also occur in the large number of bee-pollinated *Penstemons* of subgenus Penstemon with relatively short corollas that admit only the bee's head (e.g. subsection Proceri, *P. albertinus*). As for the lever mechanism, it was previously reported in wasp-pollinated species of subgenera Penstemon and Saccanthera (Torchio, 1974). I have also found it to operate in a bee-pollinated species of subgenus Penstemon. The barrier mechanism is a good candidate for the original function of the staminode in *Penstemon*. As *P. ellipticus*

illustrates, this function can be served by a short staminode. Other elaborations (increase in length, bearding) could follow, along with changes (or addition) to new functions. This is further supported by A.D. Wolfe's recent phylogenies of *Penstemon* based on chloroplast DNA sequences (personal communication, 1997), which place the subgenus Dasanthera basal to the rest of the genus.

Flowers of *Nothochelone*, *Pennellianthus*, and bee-pollinated *Keckiella* (Cheloneae) share comparable floral morphology to *Penstemon*, hence the staminode in these genera likely fulfills similar roles. However, differences in floral design for other bee-pollinated members of the Cheloneae, preclude similar staminode functioning. The partially closed flowers of *Chelone* can be manipulated only by large-bodied bees, which have sufficient mass to depress the lower lip and gain entrance. In this genus the short, thin staminode does not transverse the corolla tube, remaining on the dorsal surface. The short staminode of the small, narrow flowers of *Chionophila* shows similar positioning. Likely, the staminodes of these latter genera lack function.

In hummingbird-pollinated species, staminode removal did not impact flower success, suggesting that the staminode is non-functional in this pollination system. Indeed, short staminodes (not extending past the orifice) prevail in hummingbird-pollinated Penstemons (and Keckiellas), regardless of their ancestry, implying that the staminode is undergoing reduction in these species (Pennell, 1935).

The variation in elaboration and function of the staminode within the Scrophulariales is facilitated by the relatively simple genetic control of the fifth stamen in this order. In *Antirrhinum*, the action of only one gene (*cycloidea*) halts development of the fifth stamen at an early stage (Coen, 1996; Luo et al., 1996). Presumably, simple genetic alterations could modify this action. This flexibility would enable evolutionary convergence in staminode characteristics among distantly related species that experience similar pollination environments, as illustrated by *Jacaranda* (Bignoniaceae) and bee-pollinated *Penstemon* (Scrophulariaceae).

In bee-pollinated Scrophulariales, contact between different-sized bees and the sexual organs is facilitated by at least five floral mechanisms. First, in the Scrophulariaceae, flowers are often closed either partially (e.g. *Chelone*, *Mimulus*, *Chaenorrhinum*, some *Penstemon*) or fully (e.g. *Antirrhinum* and *Linaria*) by strongly arched corolla tubes (Kampny, 1995). A 'squeeze chute' mechanism results, whereby bees pushing their way into the tube are squeezed against the stigma and anthers. Second, in *Penstemons* of the subgenus Dasanthera, prominent folds at the top and bottom of the corolla tube adjust the size of the flower to the size of the visiting bee, ensuring contact with the anthers and stigma. Third, the barrier mechanism of staminode functioning facilitates contact by prolonging visit duration. Fourth, stigma contact may be enhanced by the lever mechanism of staminode action (*Penstemon*) which lowers the stigma. Fifth, various lever mechanisms for the anthers found in *P. palmeri* (and similar relatives) and *Salvia* (Lamiaceae: Knuth, 1908; Faegri and van der Pijl, 1979) facilitate anther contact. These mechanisms can be combined. The Dasanthera *Penstemons* have adjustable corollas and the barrier mechanism of staminode functioning, whereas *P. palmeri*, and similar relatives, have a "squeeze chute" corolla and the lever mechanism of staminode functioning.

The development of tubular, zygomorphic flowers in the Scrophulariales likely introduced a number of functional problems, such as exclusion of ineffective visitors, orientation of pollinators to rewards, and contact between the pollinators and the sexual organs. Selective pressure for more precise contact between the pollinators and sexual organs led to the re-positioning of stamens to the top of the corolla tube. This re-positioning involved only four of the original five stamens, the 5th stamen becoming non-functional. Suppression of the 5th stamen freed an alternate floral organ for modification to solve functional problems. In most cases, the 5th staminode was not modified and became lost. However, the staminode developed independently in two lineages to facilitate pollinator contact with the sexual organs. In *Penstemon* (Scrophulariaceae) and

Jacaranda, Bignoniaceae), the staminode has become a dynamic feature of floral morphology.

4. CONCLUSIONS

In angiosperms, staminodes occur largely as transitional stamen remnants. Taxa with staminodes are generally restricted to branch tips of the phylogenies and larger taxa that include species with staminodes are polymorphic. Staminodes appear functional in only a small proportion of species with staminodes, suggesting that staminodes rarely solve functional problems in flowers. Indeed, the diverse functions sometimes provided by staminodes are generally served by alternate mechanisms in other flowers.

Notable exceptions include several large lineages with staminodes, including the Magnoliales/Laurales clade and the Orchidaceae and Zingiberales. Such persistence and maintenance of staminodes indicate function and, indeed, staminodes form integral components of flower design in these groups. Staminodes provide a mechanism to prevent self-pollination in the Magnoliales/Laurales and work in concert with the perianth to attract pollinators and facilitate contact between pollinators and the anthers and stigmas in the Orchidaceae and Zingiberales. The persistence of staminodes within lineages may reflect the number of functions they perform. Multi-functional staminodes characterize groups with persistent staminodes, whereas single-function staminodes typify groups with incidental staminode presence.

The order Scrophulariales illustrates trends in stamen reduction and incidental secondary modification of staminodes. Flowers in this group have a partial whorl of fertile stamens due to the reduction and nearly universal absence of the 5th median stamen. Occasionally this stamen is represented as a rudimentary staminode, but species of the tribe Cheloneae (Scrophulariaceae) and *Jacaranda* (Bignoniaceae) possess a large and conspicuous staminode. In bee-pollinated flowers (the ancestral condition), the staminode facilitates contact between the sexual organs and the pollinator, increasing pollen receipt and sometimes pollen removal. This occurs in one of two ways depending on flower design: 1) as a lever mechanism for the style, or 2) as a barrier to hinder access to nectaries. Bee-pollinated flowers without developed staminodes in the Scrophulariales facilitate pollinator contact with anthers and stigma through alternate mechanisms,

commonly "squeeze chute" mechanisms (Kampny, 1995) and lever action for stamens. Species with staminodes often combine staminode function with these alternate mechanisms. In hummingbird-pollinated flowers (a derived condition), the staminode appears to be non-functional.

The opportunistic involvement of staminodes in floral function seen in some members of the Scrophulariales is characteristic of staminodes throughout the angiosperms. Specific selective pressures imposed by pollination may cause modification of the otherwise transitional floral structures to solve functional problems in flowers. That modification of such rapidly reduced structures occurs regularly illustrates the rapid and dynamic evolution of angiosperm flowers.

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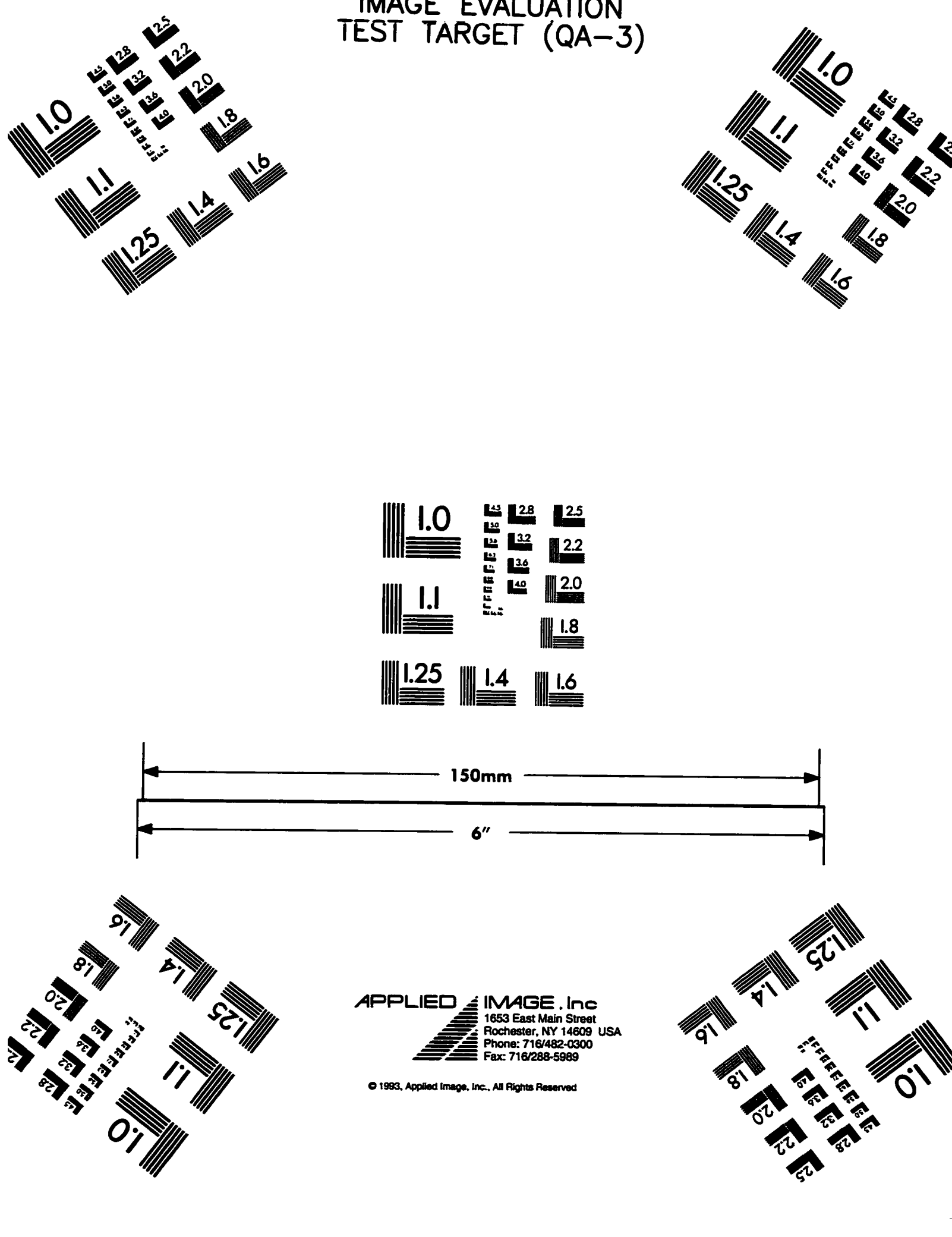
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