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FLORAL MORPHOMETRICS, DEVELOPMENT AND EVOLUTION OF HOMOSTYLY FROM DISTYLY IN *AMSINCKIA* (BORAGINACEAE)

by

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Submitted in partial fulfillment of the requirements
for the degree of Doctor of Philosophy

at

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ABSTRACT

The mechanisms that lead to the evolution of homostyly from distyly and the differentiation of two distylous floral morphs (pin and thrum) were studied by comparing floral morphometrics of homostylous and distylous groups within and among three evolutionary lineages in *Amsinckia*, in both mature and developing flowers. Twenty-six floral traits were included. In the two distylous flower morphs, stamen and pistil heights and many of the ancillary traits varied as expected from their close relationship to the definition of pins and thrums. In homostyles, traits related to anther height and pistil height were intermediate between pins and thrums in all lineages; for other traits homostyles generally had the smallest values. The functional anther-stigma distance and flower size were the two key characters discriminating homostyly from distyly. Stamen insertion height on the corolla tube was the major trait discriminating the three floral morphs (pin, thrum and homostyle) in *Amsinckia*, while style length was the major trait discriminating the four floral morphs (pin, thrum, large homostyle, and small homostyle) within *A. spectabilis*. Surprisingly, stigma thickness was the single most important trait discriminating the three evolutionary lineages.

Paedomorphosis through neoteny and progenesis was found to be the major developmental mechanism responsible for the evolution of homostyly from distyly within all three lineages. Nevertheless, multiple heterochronic processes were generally involved, and lineages differed in the developmental particulars, including the extent of paedomorphosis, developmental dissociation, changes of ontogenetic trajectories and involvement of some other developmental processes, such as peramorphosis by acceleration. Similar developmental mechanisms were found to cause the differentiation of pins from thrums in distyly independently in three lineages. The unique ontogenetic patterns in the large-flowered homostylous morph in the *A. spectabilis* lineage suggested that it may represent an intermediate morph in the evolution of homostyly from distyly.

Two additional major studies are included in this thesis. First, the concept and application of heterochrony, along with heterotopy and homeosis, in plant evolutionary studies have been thoroughly reviewed. Most heterochronic changes in plant evolution involve more than one of the six classic pure heterochronic processes. Neoteny, progenesis and acceleration were more common than hypermorphosis and predisplacement. Furthermore, the phenotypic effects of changes in the timing of onset or offset can be exaggerated, suppressed or reversed by changes in rate, and vice-versa.

In addition, for 36 species representing 13 angiosperm families, it was found that microsporocyte meiosis terminated at only three discrete relative times during flower development despite wide variations within and among species in absolute developmental durations. A single timing class characterized each species. The three timing classes were related to fractions based on the golden ratio. Timing class was not related to ploidy level, inflorescence architecture, pollination syndrome or mating system. These findings suggested that a single exogenous process may regulate the timing of premeiotic and postmeiotic floral development, or that one rate determines the other. They further implied that the underlying developmental processes have evolved in a limited number of ways among flowering plants.

LIST OF ABBREVIATIONS AND SYMBOLS

α	probability of rejecting null hypothesis (H_o) when H_o is true (Type I error)
τ	golden ratio
Λ	Wilks' lambda
#	number
%	percent
µm	micrometer
<	less than or smaller than
>	more than or larger than
$\approx$	similar to
AAFT	absolute age of a floral bud at microspore tetrad formation
ANOVA	analysis of variance
ASD	functional distance between anther and stigma
BUDL	flower length
BUDW	flower width
CDA	canonical discriminant analyses
CDF	canonical discriminant function
CFPL	fused petal length
CLBW	corolla lobe width
CPTL	petal length
cpDNA	chloroplast deoxyribonucleic acid
CTBL	corolla tube length
df	degrees of freedom
F	a test statistic; the ratio of two variances
FAA	formalin-acetic acid-ethanol
H	homostyle morph
KSL	sepal length
L1	lineage of *Amsinckia furcata* — *A. vernicosa*

L2	lineage of *A. douglasiana* — *A. tessellata gloriosa*
L3	lineage of *A. spectabilis*
LH	large homostyle morph
MANOVA	multivariate analysis of variance
mm	millimeter
N	sample size
P	pin morph
P	probability
PAPIL	stigma papilla length
PAPIW	stigma papilla width
PISL	pistil length (stigma height)
PMC	pollen mother cell
POLN	pollen number per flower
POLS	pollen size (diameter on long axis)
PSSL	style and stigma length
PSTA	stigma area
PSTH	stigma thickness
PSTL	stigma length
PSTW	stigma width
PSTYL	style length
R^2	coefficient of determination
RAFT	relative age of a floral bud with microspore tetrads
SANL	anther length
SANW	anther width
SE	standard error
SFIL	free filament length (portion not fused to petal)
SH	homostyle (small homostyle morph)
SINH	stamen insertion height
SSIL	stamen height (anther height)
STYLECA	style cross-sectional area

T	thrum morph
TRANSCA	style transmission tissue cross-sectional area

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CHAPTER 1

GENERAL INTRODUCTION

1.1. INTRODUCTION

Distyly and homostyly are two types of plant population with distinct floral morphology, mating patterns, incompatibility systems, and evolutionary standings. From a floral morphological point of view, the major difference between the two resides in the spatial arrangement of stigma and anthers in a flower. Distyly is a genetic floral polymorphism in which a plant population consists of two morphs that differ reciprocally in the heights of stigmas and anthers in flowers (Figs. 3.5-3.7). The pin morph has short stamens and a long style, while the thrum morph has the opposite arrangement. In homostylous flowers the anthers and stigma are positioned at approximately the same level (Figs. 3.5-3.7).

In most species distyly is associated with a genetic self-incompatibility system that also prevents matings between individuals of the same morph (Ganders, 1979a; Ganders et al., 1985). There are exceptions, including all distylous species of *Amsinckia* (Boraginaceae), which are self- and intra-morph compatible (Ray and Chisaki, 1957a; Ganders, 1975b, 1979a; Weller and Ornduff, 1977, 1989; Casper et al., 1988; Johnston and Schoen, 1996; Schoen et al., 1997). Thus, the mating pattern in most distylous species is legitimate pollination between reciprocal styles and stamens (Ornduff, 1971; Riveros et al., 1995). Homostylous plants in contrast are self-compatible and highly self-

pollinating (Ganders, 1979a; Piper et al., 1986; Boyd et al., 1990; Tremayne and Richards, 1993).

Distyly has been found in at least 28 angiosperm families (Barrett et al., 2000). It has usually been viewed as a floral device that promotes outcrossing (Darwin, 1892; Ganders, 1979a; Shore and Barrett, 1985; Nic Lughadha and Parnell, 1989; Barrett, 1990; Barrett et al., 2000; Lloyd and Webb, 1992a, 1992b; Richards and Koptur, 1993; Hermann et al., 1999). Homostyly, on the other hand, has been reported in at least eight families that contain heterostylous species (Dowrick, 1956). It is generally regarded as derived from the breakdown of distyly (Barrett, 1992b; Lloyd and Webb, 1992a).

In general, the dimorphic flower characters have been described in various species at different depths. No published study has examined more than a few traits. Therefore, a detailed understanding of the differences in floral morphology between distylous and homostylous flowers is still lacking. Many questions are still unanswered. For example, which portions or parts of the floral organs play the most important roles in the dimorphic characters of distyly or between distyly and homostyly? Do different distylous species have the same kinds of floral dimorphisms? Are the morphometric differences between distylous and homostylous flowers from different evolutionary lineages the same? In addition, there is almost no detailed ontogenetic information on distylous and homostylous flowers, which actually would be very important for a better understanding of how distyly breaks down to homostyly, and thus the way selfing evolves from outcrossing.

This thesis is unique in several respects.

First, I include three separate lineages within a genus.

Second, I measure the constituent parts of structures, thus enabling me to identify which of theses parts actually cause any differences between groups in the whole structure. Thus, this study includes far more traits than any other similar study.

Third, the development of all of these traits is quantified from early stages through flower opening to the final size. This complete picture of floral development enables me to pinpoint the time when morphs or lineages diverge. It also allows me to discover traits that have similar final size despite different developmental trajectories.

Fourth, This is the first study to describe the evolution of homostyly from distyly from the viewpoint of developmental processes such as heterochrony.

Finally, This is the first study to report both the consistency of microsporocyte meiosis timing within a species and the small number of such timing classes among species.

1.2. Research Objectives

Amsinckia (Boraginaceae) is a genus that has four evolutionary lineages (Ray and Chisaki, 1957a, 1957b, 1957c; Schoen et al., 1997). Each lineage consists of distylous and homostylous species or populations (see chapter three for detailed information). The main objectives of this study are to quantitatively compare 1) the floral morphometrics of distyly and homostyly across different species and evolutionary lineages in *Amsinckia*; 2) floral ontogenies of different floral morphs (pins, thrums and homostyles) with which to investigate the evolution of homostyly from distyly. In both cases, a major question is

whether the evolution of homostyly from distyly has proceeded in similar ways in the three evolutionary lineages studied here. This thesis has eight chapters. Below, I briefly outline the specific goals of each of the remaining seven chapters.

Chapter Two – I review what is currently known about heterochrony in plant evolution. The focus is on the application of the concept of heterochrony to plant evolutionary studies. I also discuss other developmental mechanisms, such as homeosis and heterotopy, which can also be responsible for morphological evolution.

Chapter Three – This chapter provides background information about the study species of Chapters Four, Five and Six. It includes information on inflorescence and floral morphology, mating systems, and phylogeny.

Chapters Four and Five – The goals of these two chapters are to assess the morphometrics of fully opened flowers of different floral morphs both within and among species and evolutionary lineages in *Amsinckia*. In Chapter Four, I focus on the quantitative comparisons of floral morphometrics mainly using univariate analyses. I not only examine the floral morphometrics between the two morphs (pin and thrum) of distyly both within and among distylous species and between the two styles (distyly and homostyly) both within and among the evolutionary lineages of *Amsinckia*, but also discuss the floral morphological characters associated with floral morphs and mating systems in conjunction with a mini-review of published studies in other distylous and homostylous plants. In Chapter Five, I use multivariate analyses to find major discriminative traits that separate floral morphs and evolutionary lineages among different groups (distyly vs. homostyly, pin vs. thrum vs. homostyle, pin vs. thrum vs.

large-flowered homostyle vs. homostyle in *Amsinckia spectabilis*). In addition, I also discuss all major discriminative traits in an evolutionary context.

Chapter Six – It is generally believed that homostyly is derived from distyly. In this chapter, I quantitatively study flower development, in terms of changes of floral morphometrics during flower ontogeny, in distylous and homostylous species, both within and among the evolutionary lineages of *Amsinckia*. I then use the concept of heterochrony, a mechanism linking development and evolution, to explain how homostyly has evolved from distyly, and therefore the evolution of selfing from outcrossing, in *Amsinckia*.

Chapter Seven – Starting from the accidental discovery that the relative pollen-mother-cell meiosis termination time during flower ontogeny was the same in all species, flower morphs and mating types in *Amsinckia*, I extend my study of meiosis time to 36 species from 13 angiosperm families. In this chapter, I report the discovery of the three discrete classes of meiosis termination time (RAFT: the time elapsed from flower primordium initiation to microspore tetrad formation as a proportion of the total time from the primordium initiation to flower opening). The study found that each species was characterized by only one of the three timing classes despite wide variations within and among species in absolute developmental durations. Further, this chapter discusses the astonishing mathematical relationships among the three numbers representing the three timing classes and explores their biological meanings. Special thanks to Dr. Mark Johnston who played critical roles in discovering and modeling the numerical relationships of these three timing classes.

Chapter Eight – Here I summarize the major points and conclusions of this study. I also provide some suggestions for possible future studies based on current knowledge in the subject of this thesis.

Please note that Chapters Two and Four to Seven have been written as self-contained research papers which are either published, submitted or will be submitted for publication. As a result there will inevitably be some repetition in the introductions, materials and methods, and discussions in some of the chapters. Chapters Two and Seven have been published in collaboration with Dr. Mark Johnston (Ch. 2 – Li, P. and M.O. Johnston, 2000. Heterochrony in plant evolutionary studies through the twentieth century. *The Botanical Review* 66: 57-88; Ch. 7 – Li, P. and M.O. Johnston, 1999. Evolution of meiosis timing during floral development. *Proceedings of the Royal Society: Biological Sciences* 266: 185-190). Chapter Four is in press in *Canadian Journal of Botany* [Li, P. and M.O. Johnston, 2001. Comparative floral morphometrics of distyly and homostyly in three evolutionary lineages of *Amsinckia* (Boraginaceae)].

CHAPTER 2

HETEROCHRONY IN PLANT EVOLUTIONARY STUDIES

2.1. ABSTRACT

The evolution of plant morphology is the result of changes in developmental processes. Heterochrony, the evolutionary change in developmental rate or timing, is a major cause of ontogenetic modification during evolution. It is responsible for both inter- and intraspecific morphological differences. Other causes include heterotopy, the change of structural position, and homeosis, the replacement of a structure by another. This paper discusses and reviews the role of heterochrony in plant evolution at the organismal, organ, tissue, cellular and molecular levels, as well as the relationships among heterochrony, heterotopy and homeosis. An attempt has been made to include all published studies through late 1999. It is likely that most heterochronic change involves more than one of the six classic pure heterochronic processes. Of these processes, I found neoteny (decreased developmental rate in descendant), progenesis (earlier offset) and acceleration (increased rate) to be more commonly reported than hypermorphosis (delayed offset) or predisplacement (earlier onset). I found no reports of postdisplacement (delayed onset). Therefore, while rate changes are common (both neoteny and acceleration), shifts in timing most commonly involve earlier termination in the descendant (progenesis). These relative frequencies may change as more kinds of

structures are analyzed. Phenotypic effects of evolutionary changes in onset or offset timing can be exaggerated, suppessed or reversed by changes in rate. Because not all developmental changes responsible for evolution, however, result from heterochrony, it is proposed that plant evolution be studied from a viewpoint that integrates these different developmental mechanisms.

2.2. INTRODUCTION

Heterochrony, a change in the relative timing and/or rate of developmental processes in a descendant relative to its ancestor, has become one of the most popular developmental and evolutionary topics in recent years. The symbol of this trend may be seen in recent book titles, such as *Heterochrony in Evolution* (McKinney, 1988b), *Heterochrony: the Evolution of Ontogeny* (McKinney and McNamara, 1991), *Evolutionary Change and Heterochrony* (McNamara, 1995), and reviews on heterochrony and development (Raff and Raff, 1987; Raff and Wray, 1989; Fink, 1988; Hall, 1990, 1992, 1998; Hall and Miyake, 1995; Carlson, 1991; Gould, 1992; Richardson, 1995; Hill, 1996; Klingenberg, 1996); heterochrony and evolution (Lord and Hill, 1987; Gould, 1988; McKinney, 1988c; McKinney and McNamara, 1991; Hill and Lord, 1990; Parichy et al., 1992; Mosbrugger, 1995; Alberch and Blanco, 1996; Zelditch and Fink, 1996); heterochrony and genetics (Atchley, 1987, 1990; Slatkin, 1987; Wiltshire et al., 1994); and some other perspectives on heterochrony (Guerrant, 1988; Klingenberg and Spence, 1993; Richardson, 1995; Fiorello and German, 1997; Reilly, 1997; Rice, 1997).

Heterochrony, as a term, has been defined and redefined many times since Haeckel (Haeckel, 1875, 1905) first formally used it. After a thorough review and analysis on the history and meaning of heterochrony proposed by previous authors, Gould (1977, p. 2) redefined heterochrony as "changes in the relative time of appearance and rate of development for characters already present in ancestors." Heterochrony is thus a "phyletic change in the timing of development, such that features of ancestors shift to earlier or later stages in the ontogeny of descendants" (Gould, 1992). Based on this concept, Alberch et al. (1979) and McKinney (1988a) further classified various heterochronic possibilities, which have become widely accepted (see Fig. 2.1). More recently, Reilly (1997) modified the current model of heterochrony, replacing some of the terminology with new nomenclature. Despite the recent attempts at clarification and consensus, controversy and confusion persist (McKinney, 1999). The debates will probably continue as data on more taxa accumulate and as heterochrony is further examined in relation to other developmental and evolutionary mechanisms.

Although predominantly studied in animals, heterochrony has been increasingly studied in plants during the past ten years. Here I briefly review some perspectives on heterochrony and its role in evolutionary changes of plant morphology. The main focus is on the evidence and progress that have been made in the study of heterochrony in plants, especially in the flower. Results are summarized in Appendix 1, which includes only those studies having adequate phylogenies and time-based developmental data (as well as some fossils). I will also discuss some of the limitations of heterochrony and suggest an integrative approach incorporating heterochrony, homeosis and heterotopy in plant ontogenetic and phylogenetic studies.

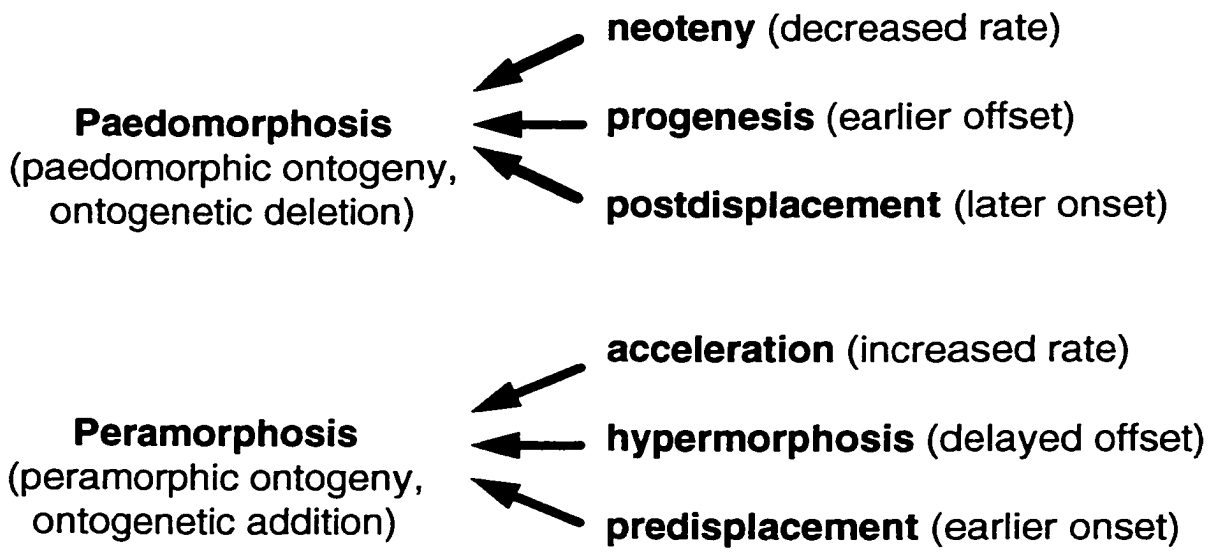


Figure 2.1. Two types of heterochrony and their developmental causes (after Alberch et al., 1979).

2.3. Heterochrony, Evolution and Development

Heterochrony has a special significance because it can produce dramatic novelties simply by changing the timing of developmental events and/or the rate of developmental processes. Heterochrony has both developmental and evolutionary components. Development is often studied by quantitative comparisons, which lead to the identification of particular developmental differences (timing, rate) that result in divergent phenotypes. The evolutionary component can be easily linked to the developmental results if one knows the probable phylogenetic relationships of the concerned groups. Thus, it is possible to draw a conclusion about the direction and type of developmental change associated with morphological evolution by integrating developmental information with phylogenetic hypotheses (Diggle, 1992).

"Ontogeny" usually refers to the sequence of events or stages occurring during development from a zygote to a sexually mature organism (Gould, 1977; Hall, 1992). In plants, especially in perennials, new leaves and flowers are produced on the mature plant body. Therefore, the development of a leaf or a flower starts from its primordium insertion on a mature plant to a fully expanded mature leaf or a fully opened flower and may be regarded as leaf or flower ontogeny. Ontogeny in any organism can also be the development of tissues or cells from their initiation to maturity (Gifford and Foster, 1989).

2.4. Types of Heterochrony

Heterochronic processes, or heterochronic changes of developmental processes, are the direct causes of morphological changes. The changes of development may involve onset time, offset time and rate (Alberch et al., 1979; Fink, 1982, 1988; Reilly, 1997). Based on the final effect of such perturbations, two basic heterochronic processes underlying organismal development can be identified: paedomorphosis and peramorphosis (Fig. 2.1). Paedomorphosis refers to a truncated developmental process which can result from a descendant having a shorter developmental duration or a lower developmental rate compared to its ancestor. Peramorphosis refers to an extended developmental process which can result from a longer developmental duration or a higher developmental rate (Alberch et al., 1979; Kluge, 1988; McKinney, 1988a; McKinney and McNamara, 1991). Paedomorphosis results in the descendant having an adult size and shape similar to the juvenile condition in the ancestor, while peramorphosis leads to the descendant having a larger adult size with a shape beyond that of the ancestor. The six heterochronic processes proposed by Alberch et al. (1979) were recently further illustrated by Wiltshire et al. (1994) in the garden pea, *Pisum sativum* (Leguminosae), using both real (mutant) and imagined developmental changes. Recently, Niklas (1994) proposed a third type of heterochronic process, akratomorphosis, which results in the descendant having an adult shape similar to that of its ancestor but with a difference in size, either larger ("gigas") or smaller ("dwarfism"). More recently, Reilly (1997) rejected the terms neoteny and progenesis, and proposed to use deceleration and hypomorphosis, instead, in an effort to reduce confusion about the actual meanings of these terms. For continuity and

standardization, however, I will use the original terms here. Based on the fact that not only the initiation or termination timing of developmental processes can be identical, earlier, or later, but also the developmental rate can be identical, faster, or slower, in descendants compared to those in ancestors, Niklas (1994, pp. 262-274) proposed a 3 x 3 x 3 matrix containing 27 possible heterochronic processes, and suggested that the same descendant phenotype can be achieved through different combinations of developmental processes (combinations of different onset timing, offset timing and growth rate).

Recently, Rice (1997) proposed a narrowed definition of heterochrony and stated that heterochrony is "a uniform change in the rate or timing of some ontogenetic process, with no change in the nature of the biological interactions going on within that process." In other words, heterochrony explains the developmental changes as a simple speed-up, slow-down or change of timing. Development, however, is a multi-dimensional process that is hardly uniform over time, and several studies have shown that both paedomorphosis and peramorphosis can be caused by either single or multiple developmental changes (Kluge, 1985; Klingenberg and Spence, 1993; Reilly, 1997). For example, the ontogenies of both the calyx and corolla lobes in *Veronica chamaedrys* (Scrophulariaceae), a species with putatively derived floral forms, show a slower early growth but an accelerated later development compared to *Veronicastrum virginicum* (Scrophulariaceae), having putatively ancestal floral forms (Kampny et al., 1993). In addition, the derivation of a number cleistogamous flower traits from the presumed ancestral chasmogamous flower in *Collomia grandiflora* (Polemoniaceae) results from two types of peramorphosis, namely, acceleration and predisplacement (Minter and Lord, 1983). It is probable that most observed morphological changes are the joint effect of

several types of heterochronic processes (Alberch et al., 1979; McNamara, 1993). In addition, some characters may have one kind of heterochrony, while other characters on the same organism may have no heterochrony or a different type. A good example is the derivation of the flowers of hummingbird-pollinated *Delphinium nudicaule* (Ranunculaceae) from those of bumblebee-pollinated *D. decorum* by a combination of paedomorphic and peramorphic ontogenies (Guerrant, 1982). Paedomorphic development (neoteny) of sepals and petals in *D. nudicaule* results in the mature flowers resembling the buds ("juveniles") of *D. decorum*, while peramorphic development (both acceleration and hypermorphosis) causes larger nectariferous petals in *D. nudicaule* than in *D. decorum*.

The evolution of any one character may sometimes also be the result of both paedomorphosis and peramorphosis. For example, the evolution of both male and female gametophytes in angiosperms from those of their gymnosperm ancestors results from both paedomorphosis (progenesis) and peramorphosis (acceleration; Takhtajan, 1976, 1991). The progenesis and acceleration of gametogenesis in flowering plants resulted in the loss of gametangia (antheridia and archegonia) on their gametophytes. The gametangium, in which the gametes are produced, is part of the sexual reproductive organ in most gymnosperms and all lower vascular plants. The loss of gametangia makes the gametophytes in flowering plants the most simplified among the higher plants. In general, reductions are regarded as an advanced features in evolution and probably usually result from paedomorphosis (Takhtajan, 1954, 1976, 1991; Stebbins, 1992). My studies (Chapter 6) on the development of various floral morphs in *Amsinckia spectabilis* (Boraginaceae) also indicate that both paedomorphic and peramorphic ontogenies are

involved in the derivation of small homostylous flowers from their putative ancestor, namely populations having large distylous flowers (see detail in section 2.9.2). Another example of both paedomorphosis and peramorphosis shaping the evolution of a single character is the derivation of larger sepals of *Veronica chamaedrys* by a slower development (neoteny) and a delayed offset (hypermorphosis) from the smaller sepals of *Veronicastrum virginicum* (Kampny et al., 1993).

Heterochrony may also cause intraspecific morphological differences in plants, such as variations in leaf morphology among individuals in *Begonia dregei* (Begoniaceae; McLellan, 1990, 1993; McLellan and Dengler, 1995). This is also found in my study (Chapter 6) on the evolution of small homostylous flowers in *A. spectabilis* in terms of changes in floral ontogenies. Heterochrony is usually responsible for shape and size variation of organs of the same type on a plant. It occurs in almost all plant organs, especially in leaves.

Just as different developmental changes can lead to different morphologies, the same or similar morphology can arise from a variety of developmental pathways. There are several examples of similar adult leaf morphology being produced by a variety of developmental patterns and processes (Kaplan, 1970, 1973b; Jones, 1988; McLellan, 1990). For example, the degree of incision of leaf margins varies among individuals in *B. dregei*; mature leaves from three least-incised varieties are very similar in shape (McLellan, 1990). Development of these varieties differs in size and shape of leaf primordium at initiation, in the timing of leaf incision and in growth rate. McLellan (1990) concluded that two different developmental pathways are involved in the formation of the similar leaf morphs among the three varieties. There are also floral

examples. Different developmental pathways have been found to result in similar mature carpels in *Persoonia falcata* and *Placospermun coriaceum* (Proteaceae; Douglas and Tucker, 1996); similarly long corolla tubes in *Pseudolysimachion* and *Veronicastrum* (Scrophulariaceae); and similarly long corolla lobes in *Pseudolysimachion* and *Veronica* (Scrophulariaceae; Kampny et al. 1994).

Caution must be taken while analyzing developmental and morphological changes in terms of heterochrony. The possible phenotypic effect caused by changes of developmental timing may be exaggerated or suppressed by changes of developmental rate, and *vice versa*. In other words, early onset (predisplacement) does not guarantee that the descendant final size or shape will be larger than or different from the ancestor because of a possible slower developmental rate (neoteny) and/or earlier offset (progenesis) in the descendant, in spite of the fact that it probably does happen in most situations. Similarly, delayed onset may not necessarily result in a smaller or different adult size or form.

Zygomorphic (bilaterally symmetrical) flowers are believed to be more specialized and advanced compared to the actinomorphic (radially symmetrical) flowers (Carlquist, 1969; Stebbins, 1992). The zygomorphic character in a flower may be initiated at earlier floral developmental stages (predisplacement; Tucker, 1987; Stebbins, 1992). The degree of zygomorphy, however, can be exaggerated or suppressed later in development. For example, flowers in *Cadia* and *Gleditsia* (Leguminosae) start to show their zygomorphic character at the sepal- and petal-initiation stages, but at anthesis they are no longer strongly zygomorphic because they were modified during later development by a lack of petal differentiation (Tucker, 1984, 1987), possibly caused by a

slower growth rate. A change in offset timing can enhance, reduce or eliminate the effects of a change in early developmental rate. This interaction between timing and rate is certainly important to morphogenesis, and seems often to be ignored in developmental and evolutionary studies, as well as in the discussion of the heterochronic models.

2.5. Problems and Solutions

2.5.1. Atomizing development

The use of heterochronic models such as the one proposed by Alberch et al. (1979), has as a shortcoming that the whole developmental process is conceptually divided into discrete stages. Sattler (1992, 1994) therefore advocated the use of process morphology, a dynamic approach to morphology based on the idea that structure is process. In his view, development is the combination of morphogenetic processes, and evolution occurs when these process combinations change. Process morphology gives a more integrated and more dynamic picture of development and evolution. Because process morphology uses process combinations that contain all kinds of parameters, however, it becomes more complicated and possibly difficult to use in analyzing developmental changes, compared to the heterochronic model. It may be difficult to use this outlook in practice, and it is probably not a very practical analytic tool for the study of development and evolution.

Heterochrony is seen as both a developmental process and an evolutionary pattern, causing confusion at times. Because of this, Alberch and Blanco (1996) recently

proposed that we "reduce the dependence of current thinking about heterochrony on the concept of 'timing' and instead focus on the organization of sequences of developmental events in ontogeny." Their new perspective on heterochrony searches for regularities in the developmental sequences, such as dissociation events (substitution/alteration of events in developmental sequence) and the nonterminal conservancy (insertion, addition or deletion of developmental events in the sequence), especially the terminal modification of developmental sequences. Examples of this type of study have shown its distinct value in understanding organismal morphological evolution (O'Grady, 1985; Alberch and Blanco, 1996).

2.5.2. Homology

While heterochrony is considered insufficient as a mechanism responsible for the integration between development and evolution (Raff and Kaufman, 1983; Raff, 1996; Gilbert et al., 1996), studying homology, including homologous genes and homologous developmental pathways, can help us understand the mechanisms underlying development and the relationships between development and evolution. Homology occurs at every level of organismal organization, development, and evolution. It is regarded as the hierarchical basis of comparative biology and the core concept in interpreting the logical relationships between ontogeny and phylogeny (Goodwin, 1989; Hall, 1994; Bolker and Raff, 1996). The role of homology in plant development and evolution is far less studied compared to that in animals (for reviews, see Kaplan, 1984; Donoghue and Sanderson, 1994; Sattler, 1994). After reevaluating the relationships between homology, developmental genetics and evolution, Gilbert et al. (1996) recently

reproposed the morphogenetic field, a discrete unit of embryonic development, as a major developmental unit. In such a view, genes and gene products create morphogenetic fields, and changes of these fields will modify organismal developmental pathways, and thus lead to evolutionary changes.

2.5.3. Developmental reference points

The most frequently used developmental termination reference in animals is sexual maturity. However, one must be cautious about using sexual maturity as an offset reference (Guerrant, 1982), because it is possible that some small changes of earlier developmental events may not be detected if sexual maturity occurs very late during development (Raff and Wray, 1989; Niklas, 1994). This is especially true in plants with indeterminate development. Different temporal references are often used in plant developmental studies. For example, the most-frequently used onset and offset points in floral studies are the initiation of primordia, meiosis, tetrad formation, anthesis, and fertilization.

2.5.4. Absolute versus relative timing

Consider the timing of two reference points R_1 and R_2 and that of the developmental event in the descendant, E_d. If the R_2 - R_1 period changes in the descendant, then heterochronies interpreted on relative scales can give different results from those on absolute scales. For example, if development of an organ commences earlier in the descendant (lower E_d), then evolution has occurred by predisplacerment. If, however, the total developmental time, R_2 - R_1, is also shorter in the descendant, then

heterochrony can be predisplacement, none or postdisplacement according to the proportional change in E_d compared to that in R_2 - R_1. The problem of absolute versus relative scales generally does not apply to neoteny or acceleration because these two rate-based heterochronies automatically incorporate the time separating the reference points R_2 - R_1. In short, the type of heterochrony can depend on whether absolute or relative scales are used when the proportional change in reference points differs from the proportional change in event timing (see also Raff and Wray, 1989).

2.5.5. Phylogenies

Heterochrony is often used in plant developmental and morphological studies even when phylogenetic information is absent. It is usually applied to explain the developmental differences between morphologically and/or functionally different organs. For example, the heteromorphic inflorescence in *Neptunia pubescens* (Leguminosae) produces three types of flowers. The perfect, male and neuter flowers are formed from the upper, middle, and basal sections of the inflorescence, respectively. Comparative developmental studies among the three types of flowers indicate that the most significant developmental divergence responsible for the flower type is the delay of floral organs' initiation in the male and neuter flowers, and thus, this was interpreted as heterochrony, a change of onset timing during development (Tucker, 1988). Because of the lack of phylogenetic information, it is difficult to know the direction of evolutionary change. Furthermore, strictly speaking, without a known phylogeny this is not a heterochrony. Therefore, it is a necessary challenge for biologists interested in heterochrony to obtain phylogenetic information or some knowledge of an organ's evolutionary history.

Ontogeny does not always provide a clear indication of phylogeny, and some organisms such as prokaryotes and single-celled eukaryotes may even lack ontogeny (Kluge, 1985). Therefore, heterochrony may not always be a responsible force in evolution, at least in some groups of organisms. In order to understand the phylogenetic relationships and evolution among different organisms, it will often be useful to employ other methods, such as comparisons with outgroups, multiple character congruence and parsimony (Kluge, 1985).

2.6. Allometry, a Tool Complementary to Heterochronic Study

Allometric study, "the study of size and its consequences" (Gould, 1966) or "the study of the consequences of size for shape" (Bookstein et al., 1985), can provide important developmental information even when age information is absent (McKinney, 1988a). It can further often illuminate the evolutionary adaptations of size or shape changes (Gould, 1966). Allometry has been extensively used by botanists (Niklas, 1994), for example in the comparative development of floral forms and size (Greyson, 1972; Lord, 1982; Minter and Lord, 1983; Mayers and Lord, 1983a; Kirchoff, 1983, 1988; Smith-Huerta, 1984; Kellogg, 1990; Jones, 1992).

During development, size, shape, developmental timing and rate are functionally interrelated. A change of these four variables may affect another, and such changes are subject to selection. Because a change in size or shape detected by allometry is not a function of time, allometry is not heterochrony. Results from allometric studies only

reveal the growth relationships between different parts of the organism or between a part of an organism in relation to the whole organism. To qualify as heterochrony, and for one to be able to distinguish the types of developmental processes and patterns, one must have developmental age information, and development must be studied over time. Unfortunately, there often is a practical difficulty with the identification of the types of heterochrony when organismal developmental data are examined over time instead of size. This is because developmental rate is often constantly changing over the time during organismal development. As Fiorello and German (1997) stated, "nonlinear growth data do not vary in simple factors like rate, timing, and starting size."

However, because allometry has its distinctive function in interpreting the relationships between size and shape, it is a useful tool in assisting heterochronic study (Blackstone, 1987; McKinney, 1988a). Klingenberg (1998) recently commented that "there are close connections between heterochrony and changes in allometric growth trajectories, although there is no one-to-one correspondence." Therefore, a complementary use of both size and time scales would give us a better understanding of the relationships between developmental process and evolution. To make use of allometry in developmental and evolutionary study, McKinney (1988a) proposed an allometry-heterochrony scheme, which is not only a useful tool for allometric analysis, but also distinguishes itself from heterochronic timing and/or rate effects.

2.7. Applicability of Heterochrony to Plant Studies

Heterochrony has been extensively studied as a source of animal variation and evolution. There are far fewer studies on the role of heterochrony in plant evolution. Most plant ontogenetic or morphogenetic studies focus on developmental processes, the sequence or description of the morphological changes of a plant or its organs during its development. Many studies lack data either on event timing or growth rate, mainly because of the difficulty in obtaining them, especially during the earliest developmental stages. This means that plant biologists are often unable to identify the heterochronic changes underlying plant or plant organ's development. Another main cause limiting the application of heterochrony in plant evolutionary study is indeterminate development. This is especially true for embryos and seedlings. Some plants or organs even have a period of dormancy during their normal development. The lack of distinction between the somatic "juvenile" phase and the sexually mature "adult" phase in many plants is certainly one of the reasons why heterochrony has not been well studied in this kingdom.

Some plant organs, such as flowers, fruits and leaves are determinate in their development. Their normal development, however, is easily affected by both their internal and external growth environments. For example, the final sizes and shapes of leaves can depend on the age of the plant and/or environmental conditions. A good example is heterophylly in aquatic plants, such as in *Ranunculus flabellaris* (Ranunculaceae; Young et al., 1995). Among organs with determinate development, the flower shows the least plasticity. For this reason most heterochronic studies in plants focus on flowers. Of course, from a paleobotanical point of view, heterochrony was also

involved in the evolution of land plants. There have been some discussions about heterochrony in relation to the evolution of plant life cycles, telome theory, stelar evolution and other aspects related to the evolution of land plants (Zimmermann, 1959; Takhtajan, 1991; Mosbrugger, 1995); these will not be discussed here.

2.8. Heterochrony in Fossil Plants

Heterochrony is believed to have played an important role in plant evolution, although fossils cannot provide direct evidence. For instance, the fossil crown-branched pseudoherb *Hizemodendron* is believed to be derived from a possibly crown-branched tree *Lepidodendron* by earlier cessation of stem elongation (progenesis; Bateman and DiMichele, 1991; Bateman, 1994). These two genera had very similar reproductive characters, but their vegetative architectures were very different. *Hizemodendron* was only about 0.1 - 0.5 m tall with simplicity in its anatomy, while *Lepidodendron* was about 30 m tall with relative complexity of anatomy. In another example, it is postulated that fossil *Chaloneria* (Isoetales) evolved from its putative ancestor *Sigillaria* (Lepidodendrales) by neoteny and progenesis (Bateman, 1994). The two genera differed not only in size but also in shape and the time of reproduction. *Sigillaria* was a tree about 15 m tall with both terminal and cauline lateral branches, and *Chaloneria* was a small-bodied shrub about 0.1 - 2 m tall with no branches. Bateman (1994) suggested that a reduced developmental rate caused the smaller size of descendant, and that terminal and nonterminal deletions during stem development resulted in the losses of all branches. The

paedomorphic development also shortened *Chaloneria*'s life history, and caused earlier reproduction.

As discussed earlier, a heterochronic approach is valid only when a developmental analysis is based on a time or age scale. Considering that it is almost impossible to reconstruct the timing of development in a fossil plant, the heterochrony-like analysis between putative ancestral and descendant fossil plants is meaningful only as a hypothesis.

2.9. Heterochrony in Flowering Plants

2.9.1. Heterochrony and timing of flowering

Most heterochronic studies in plants are focused on plant organs, and only a few heterochronic studies have been conducted at the whole-plant level for the reasons and difficulties mentioned earlier in this paper. One such study (Jones, 1992) was conducted on shoot development and flowering timing in two subspecies of *Cucurbita argyrosperma* (Cucurbitaceae), a cultivar (*C. argyrosperma* var. *argyrosperma*) and its wild progenitor (*C. argyrosperma*, subsp. *sororia*). Jones found that the nodal position, i.e., the timing, of flower production differed significantly in the two subspecies. In *C. sororia*, the earliest fertile male flower was produced at node 19, and the first fertile female flower was produced at node 39. In *C. argyrosperma*, however, the earliest fertile male and female flowers were produced at node 12 and 30, respectively. She concluded

that the shift to earlier flower production in the cultivar was a result of paedomorphic development by progenesis.

The phenomenon of heterochrony is most often seen when the timing of a developmental change is related to the onset of organismal sexual maturity, or to the time when the vegetative phase switches to the reproductive phase. The latter may occur when the shoot meristem or axillary bud, instead of producing leaves, starts to differentiate as a flower, a flower-producing branch or an inflorescence. The switch from vegetative to reproductive development is under both genetic and environmental controls. In several species, heterochronic mutations are known to change the phase length and/or the timing of the switch. For example, the *Tp2* mutation in maize increases leaf production, thus extending the vegetative phase and delaying the transition from vegetative to reproductive growth (Poethig, 1988). In contrast, the *leafy calyx* mutation in *Primula sinensis* (Primulaceae; Anderson and DeWinton, 1985) and *leafy* (*lfy*) mutation in *Arabidopsis thaliana* (Brassicaceae; Schultz and Haughn, 1991, 1993; Weigel et al., 1992; Weigel and Nilsson, 1995) can prolong the vegetative phase without delaying the onset of reproductive phase. In these mutations the flower (in *P. sinensis*) or inflorescence (in *A. thaliana*) is subtended by leaves or leaflike bracts, or even bract- or sepal-like floral organs (in *A. thaliana*).

It is also true that the vegetative growth phase often overlaps the reproductive growth phase in plants, which is evidenced by the production of new leaves and even new vegetative shoots while the plant is in the flowering phase. In such cases it would be difficult to conclude that the precocious flowering is a result of earlier offset of the vegetative growth phase.

By altering the onset of flowering, heterochrony can cause changes in life history (Zopfi, 1995; McKinney, 1999). Zopfi (1995) studied patterns of life history variation, morphology, ecology, and phylogeny in seven different habitat types of *Rhinanthus glacialis* (Scrophulariaceae). It was found that the onset of vegetative growth is about two weeks earlier in populations of subalpine hay meadows, postulated descendants, than in populations of alpine grassland, postulated ancestors. In addition, flowering time is about six to ten weeks later in populations of subalpine limestone grassland, postulated descendants, than in populations of alpine grassland, postulated ancestors, mainly due to later offset of vegetative growth of the main axis in plants. Plants from descendant populations have more internodes, taller stems, and more branches. Thus, it is suggested that populations of subalpine hay meadows are the peramorphic variants derived from populations of alpine grassland by predisplacement in vegetative growth, and that populations of subalpine limestone grassland are peramorphic variants derived from populations of alpine grassland by hypermorphosis in vegetative growth. Similarly, populations from grassland on rocks, the postulated descendants, have a later offset of vegetative growth compared to populations from dry continental meadows, the postulated ancestors, therefore, the former are proposed to have arisen from the latter through hypermorphosis in vegetative growth. In contrast, Zopfi (1995) also found that populations in litter meadows, postulated descendants, have earlier offset in vegetative growth than population form grassland on rocks, postulated ancestors. Plants from the postulated descendant populations have fewer internodes and branches as well as shorter stems compared to their ancestors. Thus, the populations in litter meadows are suggested to be the paedomorphic variants derived through progenesis.

2.9.2. Heterochrony and floral morphology

In general, the flower was derived from a primitive reproductive shoot of a seed fern, and most probably resulted from developmental deletion and subsequent modifications as well as specializations (Takhtajan, 1976). There are many examples demonstrating that not only the flower as a whole but also floral organs such as sepals, petals, stamens and carpels were all derived by progenesis and modified from some laminar structures (for details, see Takhtajan, 1976, 1991). Changes in the timing, rate and/or location of developmental events must have played important roles in the diversification and evolution of floral morphology. Kampny and Harris (1998) suggested that heterochrony is "the basis of floral shape evolution." Here my discussion on heterochrony will be mostly centered on the evolution of mating systems.

Within angiosperms, the evolution of the cleistogamous (CL) flower from the ancestral chasmogamous (CH) flower is generally believed to be the result of heterochrony (Lord and Hill, 1987; Gallardo et al., 1993). The mature CL flower looks like the young bud of the CH flower. Self-pollination occurs within the CL flower without opening. The mature CH flower is a typical open flower and there is a temporal difference in sexual maturity between stamen and pistil, causing some degree of outcrossing. CL flowers occur in many angiosperm species, usually on the same plant and often on the same inflorescence as CH flowers. The CL flower is often regarded as a progenetic dwarf derived from the CH flower (Lord and Hill, 1987; Gould, 1988; Guerrant, 1988), but various developmental pathways can result in the production of CL flowers. For example, in *Viola odorata* (Violaceae), the smaller size of the floral

primordium at its inception and the faster floral developmental rate (acceleration) caused earlier maturation, producing a CL flower (Mayers and Lord, 1983a, 1983b). The CL flower reached its sexual maturity 15 days earlier than the CH flower (Mayers and Lord, 1983a). In a study of flower development in *Lamium amplexicaule* (Labiatae), Lord (1979, 1982) found that the accelerated floral development after pollen-mother-cell meiosis resulted in the precocious maturation of the CL flower (about 10 days earlier compared to CH flower). A similar developmental pattern was also seen in *Astragalus cymbicarpos* (Fabaceae; Gallardo et al., 1993). In *Collomia grandiflora* (Polemoniaceae) accelerated development at early floral developmental stages (before pollen mother cell meiosis) and the earlier onset of pollen mother cell meiosis were responsible for the quicker development time of CL flowers (two days earlier than CH) (Minter and Lord, 1983). It was also reported that the small CL corolla form in *Salpiglossis sinuata* (Solanaceae) resulted from arrest of cell expansion (progenesis; Lee et al., 1979). From the examples above, it is clear that CL flower production involves not only progenesis, but also the acceleration of sexual maturity and therefore an increase in developmental rate. In other words, CL flowers can evolve through not only paedomorphic development by progenesis, but also peramorphic development by acceleration and/or predisplacement. It is probable that more than one type of heterochronic process is involved in the origin of cleistogamous flowers in most species.

Flowers of highly self-fertilizing species are often smaller than those of their outbreeding ancestors (Solbrig and Rollons, 1977; Wyatt, 1983; Guerrant, 1984, 1988, 1989; Diggle, 1992). Comparative floral developmental studies between *Limnanthes floccosa* (Limnanthaceae) and *L. alba* by Guerrant (1984, 1988) showed that *L. floccosa*

had its reproductive developmental stages (microsporocyte meiosis and tetrad formation) and maturity (anthesis) earlier than those in its putative ancestor, *L. alba*, although the two species had similar size-shape growth trajectories. *L. floccosa* produces small selfing flowers, while *L. alba* produces large outcrossing flowers. Early developmental offset (progenesis) was the primary cause of the precocious maturity of *L. floccosa* flower, although an increased floral developmental rate (acceleration) might also be involved (Guerrant, 1984, 1988).

Runions and Geber (1998) recently found that progenetic vegetative growth and accelerated sexual development lead to the derivation of self-pollinating *Clarkia xantiana ssp. parviflora* (Onagraceae) from cross-pollinating *C. xantiana ssp. xantiana*. The selfers of *C. xantiana* are smaller in plant size, flower earlier and produce smaller flowers. Runions and Geber's studies (1998) showed that the selfers possess shorter leaf and internode growth duration, flower 2.6 nodes earlier than that in the crossers, and have faster ovary elongation and ovule development rate compared to the crossers. It is reasonable to suggest that progenesis (early offset of vegetative growth lead to early flowering) and acceleration (relatively rapid maturation of ovaries and ovules) played an important role in the evolution of self-pollinating from cross-pollinating in *C. xantiana*.

I (Chapter 6) compared floral ontogenies between distylous and homostylous species in three separate evolutionary lineages of *Amsinckia* (Boraginaceae), and found that neoteny is primarily responsible for the derivation of highly self-fertilizing species from their outcrossing ancestors. The homostylous, small-flowered *A. vernicosa* evolved from distylous, larger-flowered *A. furcata*, and the tetraploid, smaller-flowered, homostylous *A. gloriosa* evolved from distylous, diploid, larger-flowered *A. douglasiana*

(Ray and Chisaki, 1957a, 1957b; Schoen et al., 1997). Individuals of distylous species bear either pin or thrum flowers. In pins, the stigma is exerted above the open corolla, while anthers are located at the lower portion of the corolla tube. In thrums, the stigma is positioned at the lower portion of the corolla tube while the anthers are at the entrance of the open corolla. In homostylous species the stigma and anthers in a flower are positioned almost at the same level. The larger distylous flowers are predominately outcross-pollinated, whereas homostylous flowers are smaller and predominately self-pollinated (Ganders, 1975b, 1976, 1979a; Johnston and Schoen, 1996; Schoen et al., 1997). My study finds that the developmental duration from the initiation of floral primordium to flower opening is the same between distylous and homostylous flowers in both lineages. The developmental rate for most floral traits such as floral bud length and width, pistil length, stamen filament length etc. in homostylous flower is highly significantly lower than that in distylous flowers (neoteny).

A change in developmental rate is not only responsible for floral evolution differentiating species, but also plays an important role in the derivation of different floral morphs and mating systems within a species. *Arenaria uniflora* (Caryophyllaceae) shows intraspecific variation in floral size and mating types. Plants in selfing populations produce small flowers, while those in outcrossing populations produce large flowers. Detailed morphological and growth-rate studies between the two types of flowers indicate that the selfing flowers have evolved from the outcrossing ones by a reduced developmental rate (neoteny) and longer growth duration (Hill et al., 1992). A similar result was also seen in my studies on the flower development and evolution of homostylous selfing flowers from distylous outcrossing ones within *Amsinckia*

spectabilis (Chapter 6). In this third evolutionary lineage of *Amsinckia* there are three types of population: distylous, large homostylous (sometimes including pins and thrums) and small homostylous. Outcrossing rates are approximately 50 - 70%, 25% and <1%, respectively (Johnston and Schoen, 1996; Schoen et al., 1997). The large homostylous flower is similar to the distylous flowers in floral developmental duration (18, 17 and 15 days for pin, thrum and large homostylous flowers, respectively), while the duration for the small homostylous flower is much longer (23 days). There is thus a later developmental offset (hypermorphosis) in small homostylous flowers. My study also shows that, compared to the two distylous morphs, the small homostylous flower has a significantly lower developmental rate (neoteny) and a later onset of pollen-mother-cell meiosis (postdisplacement). Therefore, a joint effect of hypermorphosis, neoteny and postdisplacement has resulted in the evolution of small homostylous flowers in *A. spectabilis*.

As discussed in section 2.5.4, different heterochronies can be obtained with relative and absolute time scales. In all three *Amsinckia* lineages studied, the timing of pollen-mother-cell meiosis shows no heterochrony when measured on a relative rather than absolute scale (Li and Johnston, 1999). On such a relative scale the period of flower development from primordium initiation to flower opening represents one unit. Thus, the fraction of floral development preceding (and following) pollen-mother-cell meiosis has remained invariant during extensive floral evolution.

It seems clear that the developmental processes responsible for the evolution of smaller, selfing flowers from larger, outcrossing progenitors vary among and within species. Early anther differentiation and precocious anther or floral maturation (all

examples of progenesis) are the major causes in many evolutionary processes, while changes of developmental rate (particularly neoteny) and growth duration are also involved in some cases.

Besides the organismal and organ levels, heterochrony can also be observed at smaller levels, such as floral parts, tissues and cells. Heterochrony has played a major role in the origin of the smaller size of anthers in self-pollinated flowers from the large anthers in outcross-pollinated flowers (Lord et al., 1989; Hill, 1996). The size and shape of stamen primordia for both types of flowers are almost the same, and the first noticeable difference during their development usually occurs at the archesporial cell stage (Lord, 1982; Minter and Lord, 1983; Hill and Lord, 1990). In *Collomia grandiflora* (Polemoniaceae), an earlier onset of CL anther differentiation (predisplacement; Lord et al., 1989; Hill and Lord, 1990), or a slower developmental rate (neoteny) and a shorter developmental duration (progenesis) between archesporial cell differentiation and microsporocyte meiosis in CL anthers (Minter and Lord, 1983; Lord et al., 1989), are responsible in CL flower for the precocious anther maturation and smaller mature anther size (about half the size of CH) with fewer pollen grains (only 1/10th the number of CH). A slower developmental rate (neoteny) and earlier anther dehiscence (progenesis) may be the causes of small anthers of CL flowers in *Bromus unioloides* (Gramineae; Langer and Wilson, 1965). The archesporial cells in the anthers of selfing flowers start to divide while the anthers are still small compared to the anthers of outcrossing flower in *Arenaria uniflora* (Caryophyllaceae). This causes the anthers in selfing flowers to reach maturity while still small (Hill and Lord, 1990; Hill, 1996) and indicates that the timing of archesporial cell division relative to the size of the developing anther has played a role in

shaping anther and floral morphologies. Because there was no time information available in the study of *A. uniflora*, however, we are unable to detect the type of heterochronic process responsible for the morphological changes. A short meiotic duration (progenesis) in CL anthers was reported to be responsible for the precocious maturation of anthers and flowers in *Bromus carinatus* (Gramineae; Harlan, 1945).

2.10. Heterochrony at the Cellular and Tissue Levels

The timing and pattern of cell division and differentiation in plants determine the type of organ, tissue or cell formed (Esau, 1977). For example, the timing, rate and duration of cell division, as well as differentiation during anther development, are believed to have a direct impact on anther final size and the amount of pollen produced (Minter and Lord, 1983; Hill, 1996). Changes of timing and rate of cell division during leaf development are the major developmental causes that lead to the formation of heteroblastic leaves on the same stem in some plants (Kaplan, 1973a, 1980; Richards, 1983; Dengler, 1992).

Heterochrony also exists in single-celled organisms, such as yeast. Mitosis in yeast is an indication of sexual maturity. Compared to the normal yeast, the heterochronic mutants of yeast undergo mitosis at an unusual time, either earlier (progenesis, mitosis occurs at smaller size) or later (hypermorphosis, mitosis occurs when it is over-size; Lee, 1988).

Heterochrony is not so well studied at cellular and tissue levels in plants. Nevertheless, heterochrony must exist at these levels because of the hierarchical nature of

development. For example, the type of leaf produced by the shoot apical meristem depends not only on whether and when leaflets are formed, but also relates to the timing of the offset of cell division or the onset of cell enlargement during leaf development (Dengler, 1984; Sinha et al., 1993). Comparative leaf developmental studies among three tomato genotypes showed that cell division precociously ceased and cells began to enlarge at a much earlier time in the development of simple entire leaf compared to those in half-compound and compound leaves (Dengler, 1984). Therefore, it can be inferred that the delayed offset of cell division and thus a later onset of cell enlargement during the expansion of leaf lamina were at least partially responsible for the formation of a large and/or compound leaf.

2.11. Heterochrony at the Molecular Level

Morphological evolution can arise not only from structural changes, but also from development-related gene regulation (Atchley, 1990). The composition, functional sequence and timing of activities of genes responsible for development determine both the duration of the developmental process and the timing of specific events. The underlying cause of developmental modification must include changes in temporal and/or spatial gene-expression patterns. Any morphological changes we observe, including the underlying changes in both rate and timing of physiological processes, are mostly caused by changes of gene combinations and/or their activities. From this point of view, any alteration of the temporal patterns of gene expression during development can be

regarded as a heterochronic change at the molecular level. If we follow Alberch and Blanco's (1996) recent idea that heterochrony should focus on the changes of sequences of developmental events, which is also supported by Raff (1996), then alteration of the gene-expression sequence during development is also a molecular heterochrony. In any case, comparative molecular data within and among taxa can provide insights into the variation of development, and thus the evolution of development.

It is clear that even a minor alteration of a plant developmental pathway could cause dramatic changes in phenotype (Wiltshire et al., 1994). A mutation that changed developmental rate or the timing of developmental events, such as meiosis, flower opening or the transition from vegetative growth to reproductive growth, is often termed a heterochronic mutant (Wiltshire et al., 1994). Examples include *Hairy-sheath-frayed1-O* (*Hsf1-O*) in maize (Bertrand-Garcia and Freeling, 1991; Freeling et al., 1992), and *early-flowering* (*elf*) in *Arabidopsis* (Zagotta et al., 1992). In *Pisum sativum* (Leguminosae) alone, nine heterochronic mutants have been found (Wiltshire et al., 1994). These mutants cause dramatic morphological changes by different types of heterochronic processes, including neoteny, progenesis, acceleration and hypermorphosis. For example, plants with the recessive mutant allele *sn*, under short-day conditions, begin to flower in the axil of first four-leaflet leaf and produce a total of only four leaves, all with four leaflets. Growth stops before the adult vegetative phase (no six-leaflet leaf is formed). Individuals with dominant allele *Sn*, on the other hand, begin flowering in the axil of first four-leaflet leaf, produce a total of seven four-leaflet leaves and grows until 17 six-leaflet leaves are formed. Thus, the earlier offset of vegetative

development, subsequent earlier flowering and earlier senescence in *sn* mutant are examples of progenesis (Wiltshire et al., 1994).

Recent experimental studies have found that over-expression of some genes can change flowering time. For example, under the control of the CaMV 35S promoter, over-expression of *LEAFY* can convert the inflorescence meristem into a flower meristem and cause early flowering in *Arabidopsis*. *LEAFY* can also induce transformed shoots to flower precociously in a hybrid aspen (*Populus tremulax* X *tremuloides*, Salicaceae), a plant that normally requires 8-20 years to flower (Weigel and Nilsson, 1995). It is also found that the over-expression of the *APETALA1* (*AP1*) gene alone can cause early flowering in *Arabidopsis*, by converting inflorescence shoot meristems into floral meristems, and thus dramatically reducing the time to flowering (Mandel and Yanofsky, 1995). Early flowering can be caused by additional heterochronic genes. For example, early flowering in *A. thaliana* can result from those genes mentioned earlier, as well as *terminal flower* 1 (*thl1*), *early-flowering* (*elf*) 1, 2, and 3 (Zagotta et al., 1992), *embryonic flower* (*emf*; Sung et al., 1992), and *early short days* (*esd*; Coupland et al., 1993). Mutations of some other genes can cause late flowering (Koornneef et al., 1991; Colasanti and Sundaresan, 1996). In *Arabidopsis* examples include mutants of *LD*, *FRI*, *CO* and *FCA* (Lee et al., 1994; Coupland, 1995; Colasanti and Sundaresan, 1996).

While changes in the expression of some genes can cause earlier or later flowering, mutations can also affect transitions between developmental stages, often leading to a retention of early developmental stages. It has been found that *Tp1*, *Tp2*, *Cg*, and *Hsf1-O* in maize can slow stage transitions during shoot development, and cause some juvenile stages to be prolonged, a result that could also be called paedomorphosis

(Bertrand-Garcia and Freeling, 1991; Freeling et al., 1992). It has also been found that *EMBRYONIC FLOWER (EMF), EARLY-FLOWERING (ELF), CONSTANS (CO)* and some other genes play important roles in controlling and regulating the transition time from vegetative to reproductive phase in *A. thaliana* (Haughn et al., 1995; Yang et al., 1995). The activity of the *EMF* genes gradually declines as vegetative growth proceeds during normal plant development. When *EMF* activity falls to a critical threshold, the plant or its shoot initiates a transition from vegetative to reproductive growth. The decline in *EMF* activity during vegetative growth in turn is regulated by *ELF* and *CO* genes, which can lead to promoting or delaying the transition time from vegetative to reproductive growth, and thus changing the offset time of vegetative growth or onset time of reproductive growth.

In addition to flowering time and floral morphology, inflorescence architecture may be changed by heterochronic genes as well. Coen et al. (1990, 1994) found that changes of *floricaula* (*flo*) gene expression timing or site will lead to a change of inflorescence types in *Antirrhinum majus* (Scrophulariaceae). For example, when activation of the *flo* gene was delayed, a compound cyme (thyrse) was produced instead of the normal single flower.

As Stebbins (1992) and Purugganan et al. (1995, 1996) have noted, any evolutionary change has a molecular basis, and in order to understand fully morphological evolution it is necessary to know the molecular basis of morphology. Molecular evolution of flower development has been the main focus in investigating plant evolution at the molecular level during recent years, and it has greatly advanced our

knowledge of genetic control of flower development. Most results have been from homeotic mutants, the importance of which to floral evolution remains unknown.

2.12. Homeosis

There is no doubt that heterochrony is one of the most important developmental mechanisms responsible for morphological evolution. Heterochrony, however, is not the only mechanism that can account for phenotypic evolution. Other developmental mechanisms include homeosis, heterotopy and homology.

Homeosis refers to a structure, "A," or part of "A," developing at the site of structure "B" (Sattler, 1988, 1994). In terms of "process morphology," homeosis occurs when "a process combination or process(es) of that combination are expressed at the site of another process combination (of the same organism)" (Sattler, 1992). According to this view, homeosis is the replacement of one developmental pathway by another, or of one part by another. A homeotic mutant then refers to a mutation that alters the normal developmental pattern and leads to organ "A" developing at the site of "B," and "B" could be partially or wholly replaced by "A."

Many homeotic mutants have been identified in plants, primarily in the flower (Bowman et al., 1989, 1992, 1993; Coen, 1991; Drews et al., 1991; Jack et al., 1992, 1993; Jordan and Anthony, 1993; Krol and Chua, 1993; Saedler and Huijser, 1993; Veit et al., 1993; An, 1994; Crone and Lord, 1994; Flanagan and Ma, 1994; Lord et al., 1994; Weigel and Meyerowitz, 1994) and leaf (Marx, 1987; Freeling et al., 1992; Murfet and

Reid, 1993; Schneeberger et al., 1995). The most well-known example of homeosis in plants is the replacement of one kind of floral organ by another. For example, both single- and double-flowered varieties exist in *Hibiscus rosa-sinensis* (Malvaceae). The single flower has about 60-70 stamens inside a pentamerous whorl of petals, while the double flower has many more modified petals and petalodia but fewer stamens. Floral developmental studies indicate that homeosis played a role in the replacement of stamens by petals or petalodia in the double flowers (MacIntyre and Lacroix, 1996).

A good example of partial homeosis is the development of male flowers on the heteromorphic inflorescences in *Neptunia pubescens* (Leguminosae). The flower usually produces petal-like stamens, called staminodia (Tucker, 1987, 1988). Staminodia develop from normal stamen primordia, but with altered developmental processes and patterns. Extended cell enlargement and large intercellular spaces lead to the formation of staminodial lamina. This is also different from the petal developmental process in which large amount of marginal meristem activities (cell divisions) are the main cause of petal lamina expansion (Tucker, 1987, 1988).

The term "serial homeosis" was proposed by Takahashi (1994) for a homeotic phenomenon occurring in flowers of *Trillium apetalon* (Liliaceae). *T. apetalon* is the only apetalous species in its genus. The whorl of three petals was replaced by three stamens, and this replacement triggered a serial floral-organ replacement in the inner whorls: the inner stamens replaced outer ones, and carpels replaced inner stamens.

Although most studies of homeosis in plants focus on its role in floral morphological evolutionary changes (e.g., Coen, 1991; Posluszny et al., 1990; Kirchoff, 1991; Lehmann and Sattler, 1996), homeosis in other plant organs has also been studied.

For example, Gerrath in a published discussion (Posluszny et al., 1990) used homeosis to explain the origin of tendrils in Vitaceae, *Pisum sativum* (Leguminosae) and *Passiflora guadrangularis* (Passifloraceae). Some of the pea (*P. sativum*) leaf mutants, such as *afila* (*af*) and *tendrilless* (*tl*), have been regarded as examples of homeosis in leaf ontogeny: the *af* mutant causes leaflets to be replaced by tendrils, and *tl* causes the opposite (Demason and Villani, 1998). Developmental study of double mutants and heterozygotes, however, shows that these genes interact to influence many aspects of leaf development, including timing, and that the conversion from one organ type to the other may actually be an example of heterochrony rather than homeosis (Demason and Villani, 1998).

In many cases, the developmental changes explained with heterochrony can also be interpreted by homeosis (Jordan and Anthony, 1993). The best examples in plants are the changes of floral morphogenesis caused by floral homeotic genes. Many homeotic genes have been identified and characterized, and most belong to the plant MADS-box regulatory gene family (Purugganan et al., 1995). Their expression can cause dramatic changes of flower morphology, and thus possibly result in the evolution of flower development. For example, both *apetala3* (*ap3*) in *Arabidopsis* and *deficiens* (*def*) in *Antirrhinum* can cause homeotic transformations from petals to sepals and from stamens to carpels (Bowman et al., 1989; Schwarz-Sommer et al., 1990; Jack et al., 1992, 1994; Weigel and Meyerowitz, 1993; Weigel, 1995). The developmental switch from petal to sepal possibly happens after the petal primordium is initiated (Hill and Lord, 1989). The expression of *Agamous* gene from *Arabidopsis* in tobacco flowers converts sepals to carpels and petals to stamens (Mandel et al., 1992; Martin, 1996). These facts demonstrate that a change at the gene level can lead to the production of a totally

different morphology, a replacement of parts in an organism. Therefore, homeotic genes may be responsible for at least some of morphological divergence during evolution.

2.13. HETEROTOPY

Heterotopy in plants usually refers to the formation of an organ at the "wrong place." A typical example might be epiphylly, the formation on angiosperm leaves of inflorescences, shoots, buds or leaves. For instance, flowers or inflorescences may form on the surface of leaf lamina, such as in *Callopsis volkensii* (Araceae; Dickinson, 1978), *Helwingia* (Cornaceae; personal observations), and *Tilia* (Tiliaceae; Dickinson, 1978), or in the sinus of leaf tips, such as in *Polycardia phyllanthoides* (Celastraceae; Perrier de la Bathie, 1946; Dickinson, 1978). In the genus *Begonia* (Begoniaceae), some species form inflorescences at the junction of petiole and leaf lamina (e.g., *B. paleacea* and *B. prolifera*), some species produce shoots/branches on the leaf lamina (e.g., *B. sinuata*), while others may form leaflike structures on the leaves (e.g., *B. manicata* and *B. phyllomaniaca*; Dickinson, 1978). In a well-known example of plant vegetative reproduction, the "maternity plant," *Kalanchoe daigremontaina* (Crassulaceae), produces many buds with roots ("plantlets") in the notches along its leaf margins.

Developmental studies of the epiphyllous inflorescences of *Phyllonoma integerrima* (Dulongiaceae; Dickinson and Sattler, 1974) and "hooded" barley (Gupta and Stebbins, 1969) have indicated that the inflorescence primordia are initiated on the leaf and bract primordia, rather than from the shoot apex. Similarly, epiphyllous leaflike structures are initiated from leaf primordia or young leaves in *Begonia hispida* var.

cucullifera (Lieu and Sattler, 1976; Maier and Sattler, 1977; Sattler and Maier, 1977), and epiphyllous branches/shoots are initiated from leaf primordia in *Chrysolidocarpus lutescens* (Fisher, 1973). The shifting of these developmental onset positions from their normal place on the stem constitutes heterotopy. The development of these epiphyllous structures may involve other developmental processes as well (for details, see Dickinson, 1978).

Besides on a larger scale, such as the occurrence of epiphylly, heterotopy also happens in a smaller scale in plant morphogenesis, for instance, the shifting of onset position of floral organ's primordia during flower development. The position of petal primordium inception is usually on the floral apex, in most species. The primordium, however, can also be initiated on the stamen primordia (Duchartre, 1844; Sattler, 1962), on the calyx tube (Cheung and Sattler, 1967), or on the common petal-stamen primordia (Sundberg, 1982).

In a broad sense, heterotopy is the positional displacement or translocation of an organ or structure. Thus, the homeotic replacement or transformation of floral organs, such as from petal to sepal, stamen to petal, petal to stamen, sepal to carpel, or stamen to carpel, might also be described as a displacement or translocation of organ's development, that is, heterotopy. Homeosis and heterotopy are therefore overlapping concepts; complete homeosis is simply heterotopy. Heterotopy is probably often involved in homeosis by initial changes to the developmental patterns.

Heterochrony changes developmental timing and/or rate, thereby altering only size and/or shape of an ancestral character. Heterotopy, in contrast, creates a character in a novel position by altering the ontogenetic trajectory. Therefore, the evolutionary effects

of heterotopy are more profound than those of heterochrony. Hall (1998, p. 388) stated that "heterochrony tinkers, but heterotopy creates." In actual morphological evolution, however, heterotopy may not be as common as heterochrony, because of the greater extent of developmental changes with heterotopy (Hall, 1998). On the other hand, heterotopy is little studied, especially in plants. In fact, the term "heterotopy" is usually not found in books dealing with botany or plant science. There is no doubt that both heterochrony and heterotopy play important roles in evolution. As Zelditch and Fink (1996) recently emphasized, "most ontogenies evolve by changes of spatiotemporal pattern." Heterochrony and heterotopy are probably two basic mechanisms underlying development and jointly responsible for evolution. It is time for developmental biologists to pay more attention to the role of heterotopy in evolution, and it is important to keep in mind that heterotopy has a distinct and complementary role to heterochrony in evolution. Heterochrony changes developmental timing and rate without changing the developmental trajectory, while heterotopy changes the trajectory but not the timing or rate. The simple quantitative changes involved in heterochrony may be more readily available in evolution than the more-qualitative changes involved in heteotopy.

2.14. Conclusions

Heterochrony leads to both inter- and intraspecific morphological changes in plants. Both paedomorphosis and peramorphosis can be caused by either single or multiple developmental changes. In fact, it seems likely that most heterochronic change involves

more than one of the six pure heterochronic processes defined by Alberch et al. (1979), so that an observed morphological change is often caused by the joint effect of several types of heterochronic processes representing paedomorphosis, peramorphosis or both. Heterochrony occurs at various organization levels within an organism and varies among organs or characters. Just as different developmental changes can lead to divergent morphologies, identical or similar morphologies can arise from different developmental pathways. The phenotypic effect caused by changes in developmental timing may be exaggerated or suppressed by changes in developmental rate, and vice versa. This timing and rate interaction determines final phenotype. To date, most studies simply list one type of heterochrony, probably from lack of information on the complete developmental trajectory rather than true lack of several types of heterochrony. Whether morphological evolution typically involves more than one of the six pure types will be resolved only with more time-based studies of complete developmental trajectories. This will often require measuring morphologies from the time of primordium initiation.

Heterochrony appears to be responsible for much morphological evolution, particularly in floral morphology. Heterochrony has clearly played an important role in the evolution of plant mating systems, where progenesis and neoteny are the major causes of the evolution of small selfing flowers from large outcrossing flowers. Heterochrony is also often responsible for changes of flowering time and the extent of vegetative-reproductive developmental overlap.

In addition to heterochrony, other development-related mechanisms such as homeosis, and heterotopy are important causes of evolutionary morphological change. The importance of heterochrony relative to other processes, and the levels at which it is

most commonly acts, are unresolved. It will be preferable to study plant evolution from an approach that integrates the different developmental mechanisms at various organizational levels.

Heterochrony has been the subject of much more discussion than actual quantification. The somewhat small number of studies found in the literature (Appendix 1) is almost certainly due to a lack of good phylogenic information at the species level. Of the six pure classic heterochronic processes, I found neoteny (decreased developmental rate in descendant), progenesis (earlier offset) and acceleration (increased rate) to be more commonly reported than hypermorphosis (delayed offset) or predisplacement (earlier onset, see Appendix 1). I found no reports of postdisplacement (delayed onset). Understanding the full importance of heterochrony to plant evolution requires additional studies employing sound phylogenies and time-based developmental trajectories. Only then will the true relative frequency of each process be known.

CHAPTER 3

STUDY SPECIES

3.1. INTRODUCTION AND SPECIES

Amsinckia (Boraginaceae), whose common name is fiddleneck, is primarily a western North American genus consisting of about 13 species of yellow- to orange-flowered annuals, of which five are distylous and the remaining are homostylous (Ray and Chisaki, 1957a, 1957b; Ganders, 1975b; Ganders et al., 1985; Johnston and Schoen, 1996; Schoen et al., 1997). In most cases, homostylous taxa in *Amsinckia* have smaller flowers compared with distylous taxa. Seven populations of five species in *Amsinckia* were studied in this research (Table 3.1). Of the seven populations, small-flowered homostylous *A. spectabilis* was collected from coastal area and the remainder were collected from inner regions in California (Table 3.1).

All *Amsinckia* plants studied are more or less bristly. Plants are about 1-2 feet tall. Leaves are simple, alternate, cauline and form basal rosette prior to flowering (Figs. 3.1-3.2). Plants flower between late March and early June in California.

3.2. INFLORESCENCE AND FLOWER MORPHOLOGY

The type of inflorescence in *Amsinckia* is variously termed a helicoid cyme, a coiled false spikes, or a coiled false raceme: a coiled determinate inflorescence whose flowers

Table 3.1. Summary of species, populations and floral morphs in *Amsinckia* studied in this research.

Species	Lineage	Floral morphs	Ploidy level (Chromosome no.)	Mating system (Selfing rate, *S*)	Population name and location
A. furcata	1	distylous	2n (14)	$S = 0.01$[a]	Griswold Hills,San Benito Co.
A. vernicosa	1	homostylous	2n (14)	$S > 0.99$[b]	Catway, Santa Barbara Co.
A. douglasiana	2	distylous	2n (12)	$S = 0.42$[c]	Bradley, Monterey Co.
A. t. gloriosa	2	homostylous	4n (24)	$S = 0.999$[c]	Lockwood, Monterey Co.
A. spectabilis	3	distylous	2n (10)	$S = 0.55$[c]	Nipomo, San Luis Obispo Co.
A. spectabilis	3	large homostylous	2n (10)	$S = 0.73$[c]	La Purisima, Santa Barbara Co.
A. spectabilis	3	small homostylous	2n (10)	$S = 0.998$[c]	Zmudowski St. Beach, Monterey Co.

[a] Schoen et al. (Schoen et al., 1997).

[b] Based on Johnston's unpublished study.

[c] Johnston and Schoen (Schoen et al., 1996).

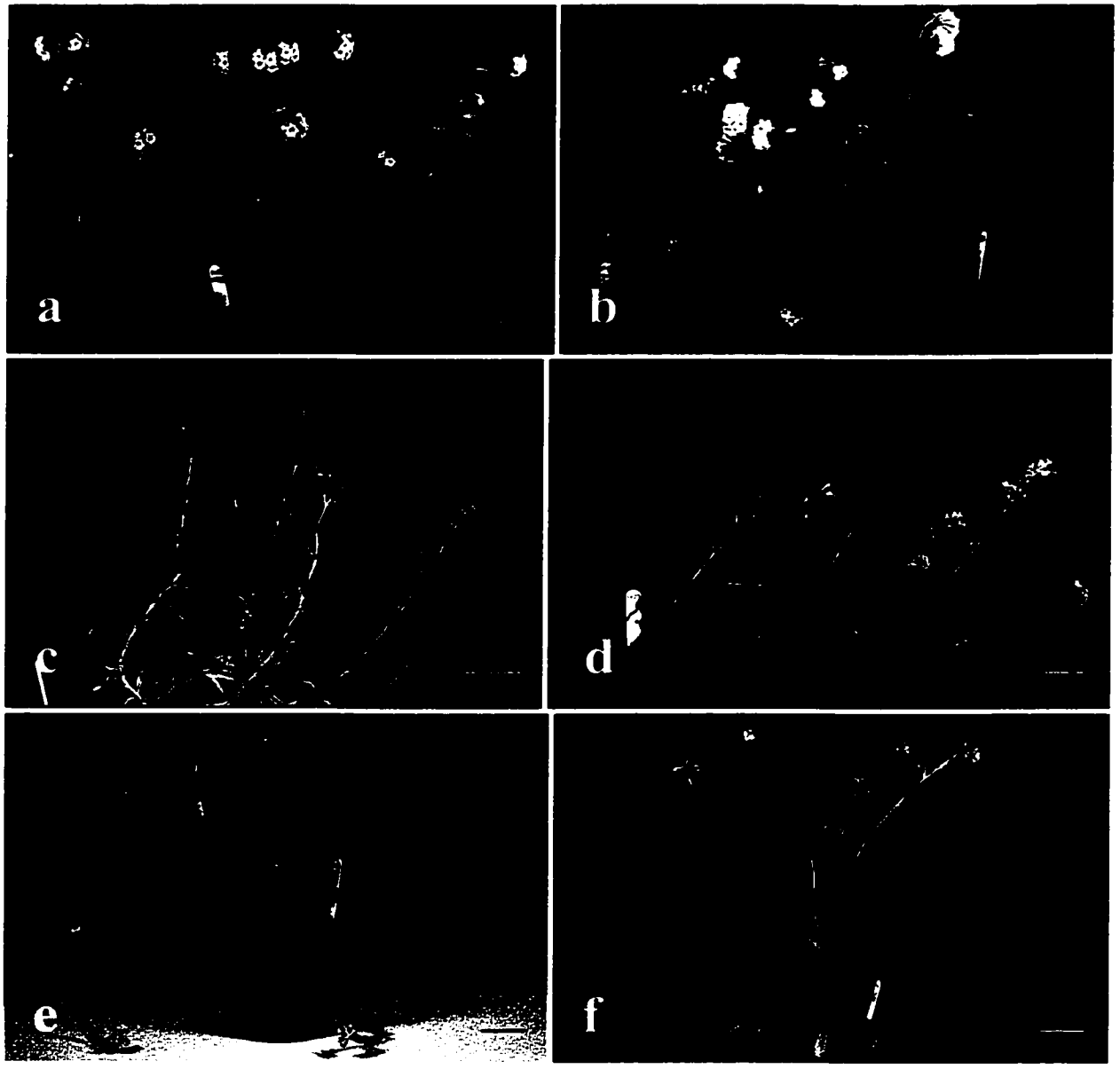


Figure 3.1. *Amsinckia* plants (part I). a. *A. furcata*, pin; b. *A. furcata*, thrum; c. *A. vernicosa*, homostyle; d. *A. douglasiana*, pin; e. *A. douglasiana*, thrum; f. *A. t. gloriosa*, homostyle. Note: Images were not scaled in size.

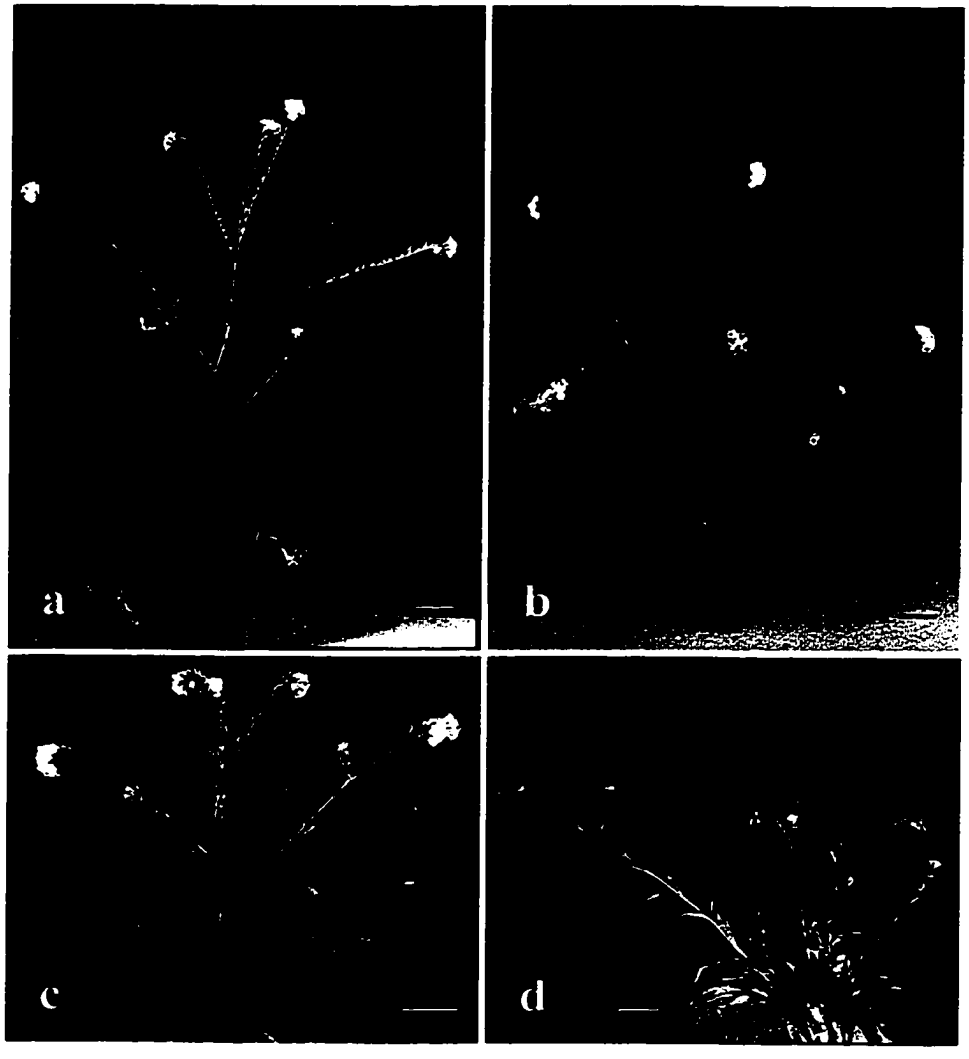


Figure 3.2. *Amsinckia* plants (part II). a. *A. spectabilis*, pin; b. *A. spectabilis*, thrum; c. *A. spectabilis*, large homostyle; d. *A. spectabilis*, small homostyle. Note: Images were not scaled in size.

develop from one side (outside) of the coiled axis in two rows (a zigzag pattern). The apical part of the inflorescence, containing unopened flower buds is coiled. As flowers open acropetally, the bottom part of the inflorescence containing opened flowers becomes uncoiled (Fig. 3.3).

Flowers of *Amsinckia* have five sepals that usually occur in the form of (2)+(2)+1 or (2)+(3). The five petals form a tube or funnel with the five lobes spreading at almost a right angles (a salver-form corolla). The five stamens are borne on the petals (epipetalous) and have anthers that dehisce by longitudinal slits. The superior, four-lobed ovary may form up to four one-seeded nutlets, which vary from smooth to roughened depending on species (Fig. 3.4).

In distylous species of *Amsinckia*, two floral morphs, pin and thrum, are produced by different individual plants. The two floral morphs differ reciprocally in style length and stamen height. In pin morph, epipetalous stamens are inserted and located at the lower part of the corolla, and the longer slender style positions the two-lobed stigma well above the anthers and often beyond the corolla (Fig. 3.5. a-b, g-h; Fig. 3.6. a-b). In contrast, epipetalous stamens are inserted and positioned at the top portion of the thrum corolla, while a shorter style positions the stigma at the middle to bottom part of the corolla (Fig. 3.5. c-d, i-j; Fig. 3.6. c-d).

Flowers of homostylous species or populations in *Amsinckia* are usually smaller than those of distylous ones. Exceptions exist in *A. spectabilis*, in which some populations consist of homostylous individuals with flowers nearly as large as in distylous populations. Both the stamens and stigma tend to be positioned near the middle portion of the corolla tube (Fig. 3.5. e-f, k-l; Fig. 3.6. g-h), except in flowers of large

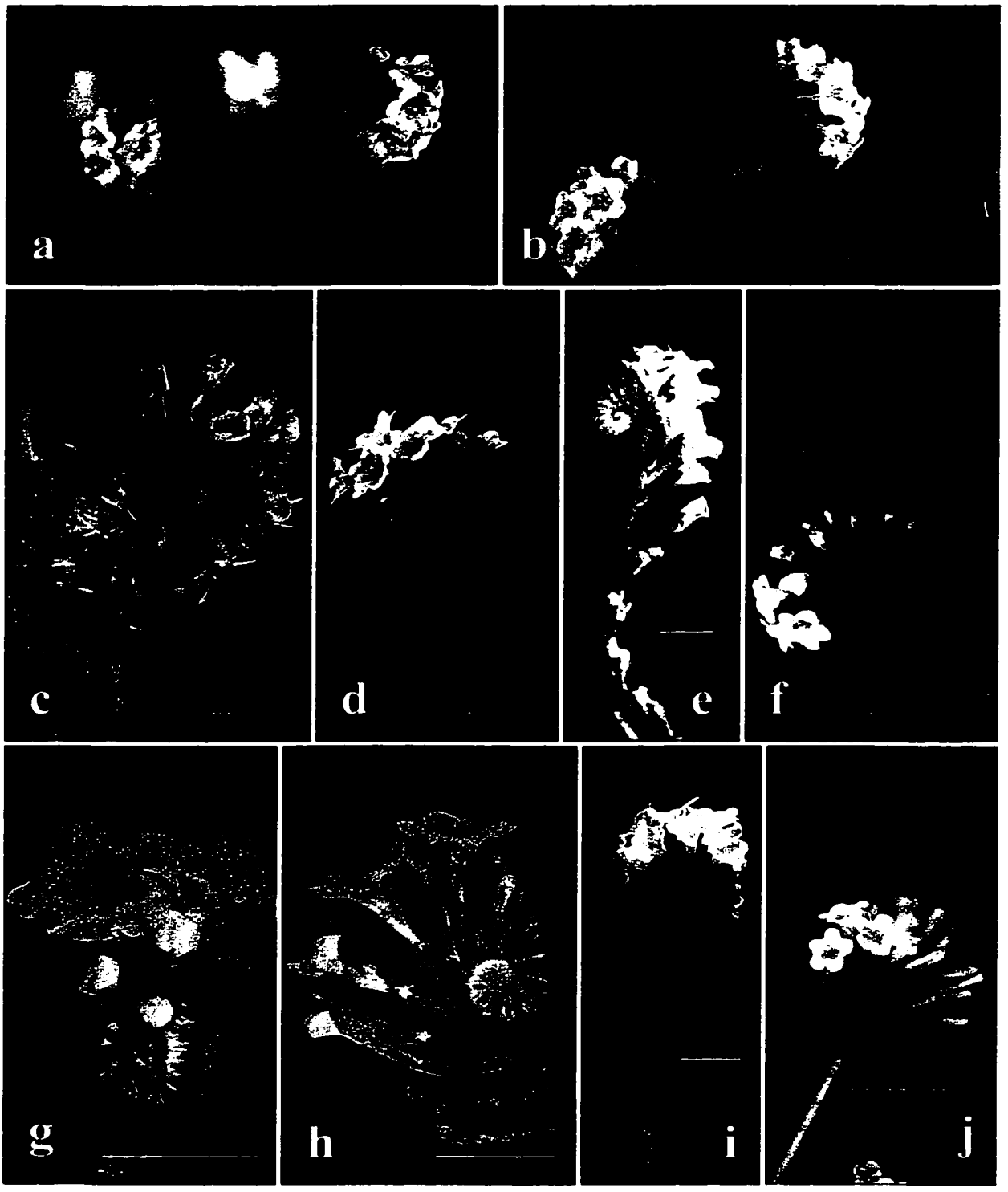


Figure 3.3. *Amsinckia* inflorescences. a. *A. furcata*, pin; b. *A. furcata*, thrum; c. *A. vernicosa*, homostyle; d. *A. douglasiana*, pin; e. *A. douglasiana*, thrum; f. *A. t. gloriosa* homostyle; g. *A. spectabilis*, pin; h. *A. spectabilis*, thrum; i. *A. spectabilis*, large homostyle; j. *A. spectabilis*, small homostyle. Note: Images were not scaled in size.

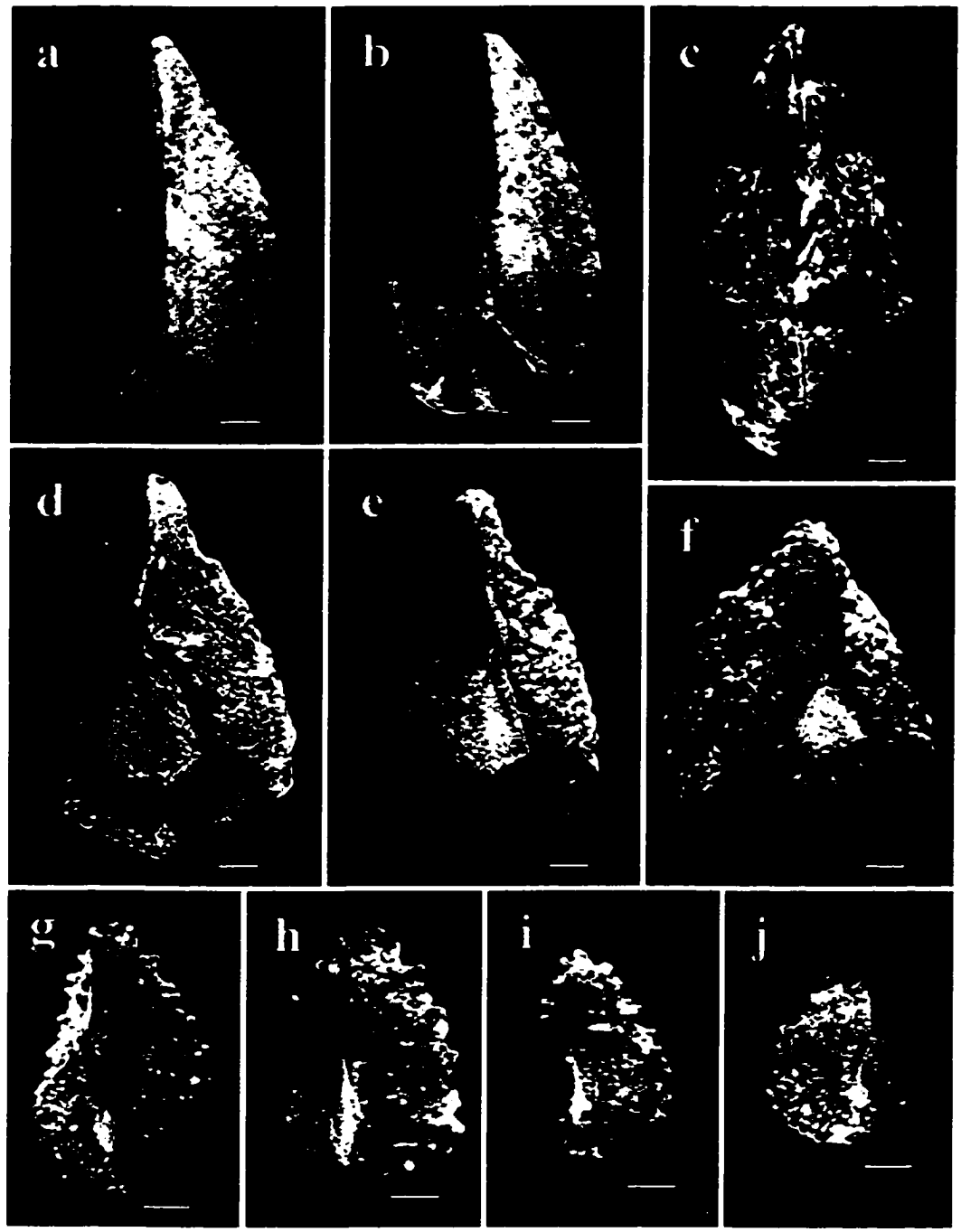


Figure 3.4. *Amsinckia* seeds. a. *A. furcata*, pin; b. *A. furcata*, thrum; c. *A. vernicosa*, homostyle; d. *A. douglasiana*, pin; e. *A. douglasiana*, thrum; f. *A. t. gloriosa* homostyle; g. *A. spectabilis*, pin; h. *A. spectabilis*, thrum; i. *A. spectabilis*, large homostyle; j. *A. spectabilis*, small homostyle. Scale bars = 400 μm.

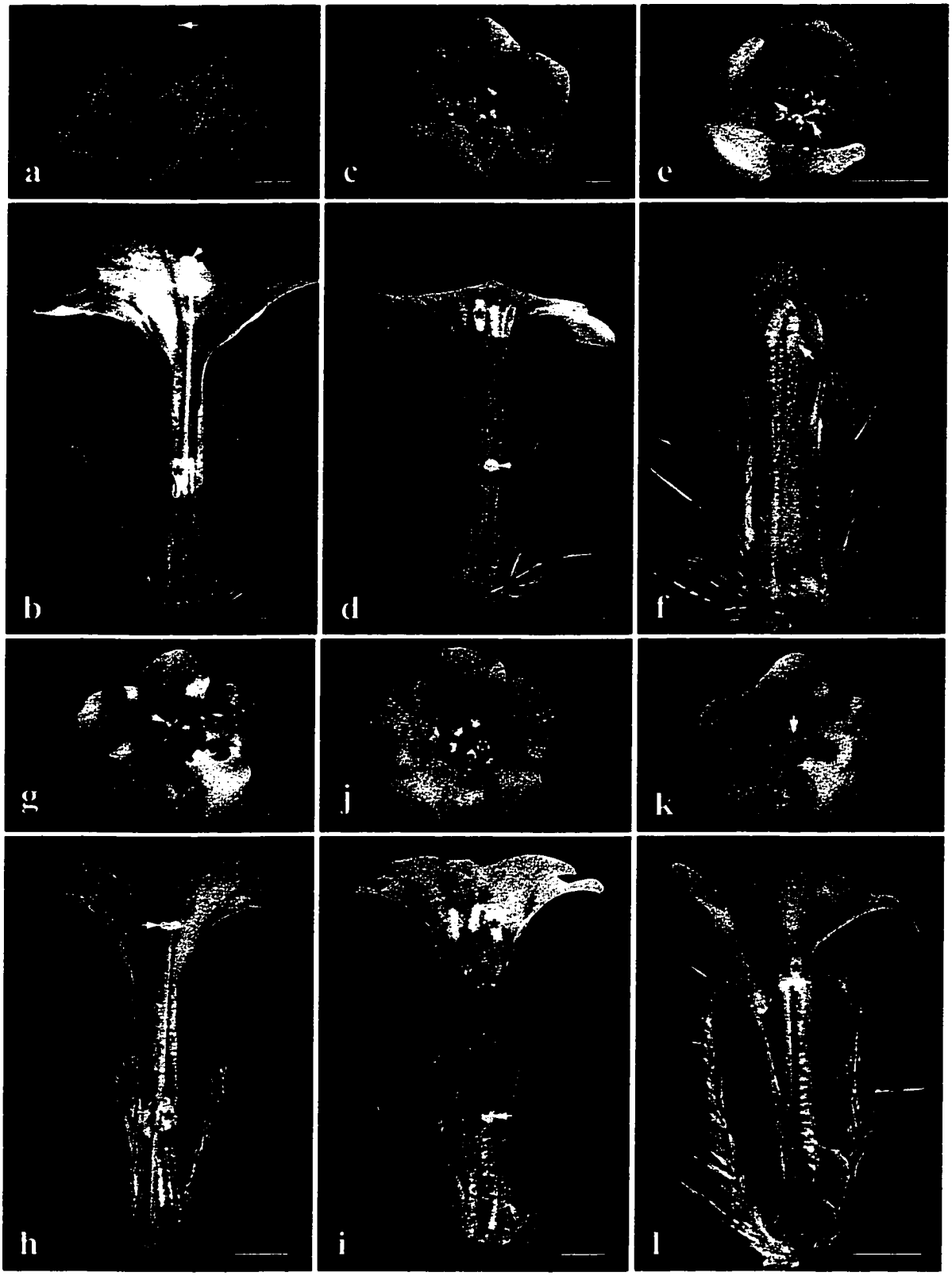


Figure 3.5. *Amsinckia* flowers (part I). a-b. *A. furcata*, pin; c-d. *A. furcata*, thrum; e-f. *A. vernicosa*, homostyle; g-h. *A. douglasiana*, pin; i-j. *A. douglasiana*, thrum; k-l. *A. t. gloriosa*, homostyle. Note: Images were not scaled in size.

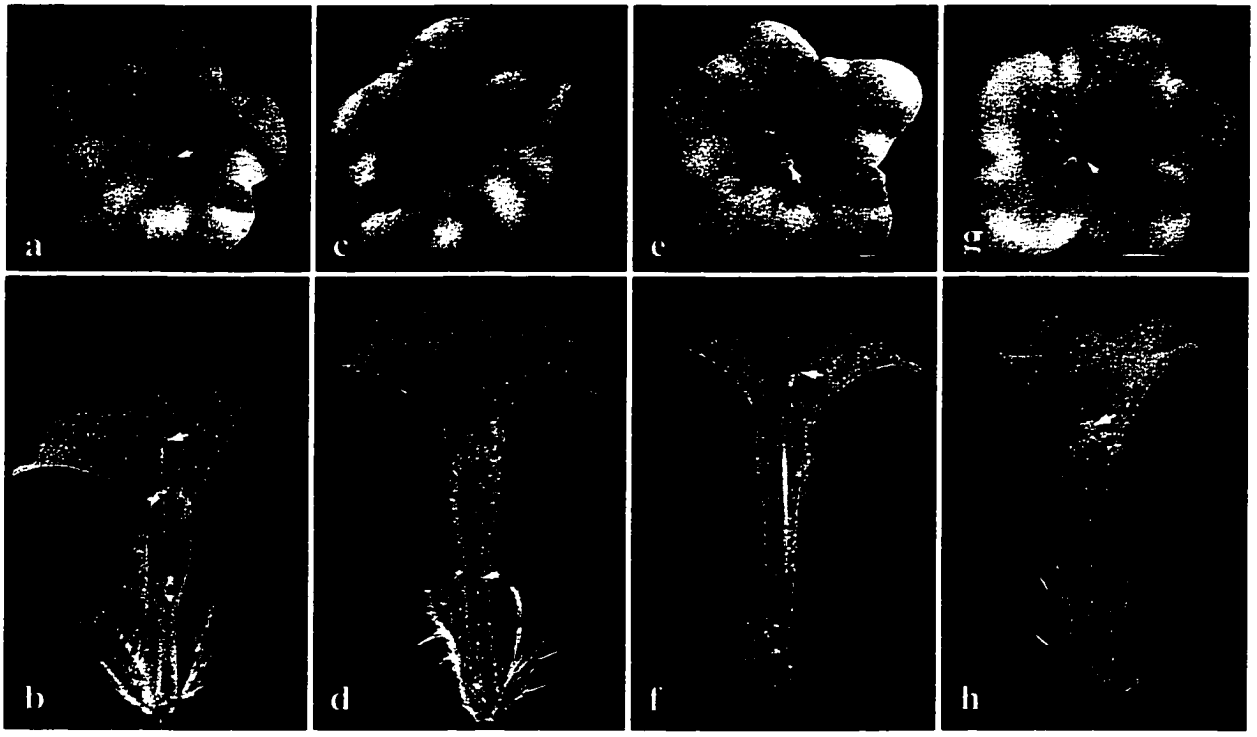


Figure 3.6. *Amsinckia* flowers (part II). a-b. *A. spectabilis*, pin; c-d. *A. spectabilis*, thrum; e-f. *A. spectabilis*, large homostyle; g-h. *A. spectabilis*, small homostyle. Note: Images were not scaled in size.

homostylous *A. spectabilis* in which both anthers and stigma are often positioned near the top portion of corolla tube (Fig. 3.6. e-f).

3.3. MATING SYSTEMS OF *AMSINCKIA*

The genus *Amsinckia* is a particularly appropriate group for the study of mating-system evolution. The genus exhibits a great diversity of mating systems, ranging from predominant cross-pollination, to intermediate cross-pollination to predominant self-pollination to nearly complete self-pollination (Table 3.1). Distylous species are pollinated mostly by butterflies and bees. Distylous outcrossing species are also self-compatible (Ray and Chisaki, 1957a, 1957b; Ganders, 1975b; Ganders et al., 1985; Weller and Ornduff, 1977; Johnston and Schoen, 1996; Schoen et al., 1997). Recent studies show that populations of distylous species have levels of selfing between 0 and 55%, while populations of homostylous species have selfing rates between 95 and 100% (Johnston and Schoen, 1996; Schoen et al., 1997).

Distylous species in *Amsinckia* do not possess the sporophytic incompatibility reactions typical of other distylous species, and are instead both self- and intramorph compatible (Ray and Chisaki, 1957a; Johnston and Schoen, 1996; Schoen et al., 1997). However, manipulated pollination studies have shown that they possess cryptic self-incompatibility, i.e., under mixed pollination circumstances the intermorph pollen usually succeeds in competition for fertilization over the self- and intramorph pollen grains (Weller and Ornduff, 1977; Casper et al., 1988). Differential pollen tube growth is

believed to be the cause of the cryptic self-incompatibility in *Amsinckia*, which is supported by the existence of more callose plugs and pollen tubes in the basal stylar region of intermorph cross-pollinated pistil than those of intramorph-pollinated pistil (Weller and Ornduff, 1989).

3.4. PHYLOGENY OF *AMSINCKIA*

On the basis of morphology and chromosome number studies, Ray and Chisaki (Ray and Chisaki, 1957b) proposed a phylogeny of *Amsinckia* which consists of four separate evolutionary transitions from predominant outcrossing to predominant selfing. Four of these separate evolutionary lineages are *A. furcata* to *A. vernicosa*; *A. douglasiana* to *A. tessellata gloriosa* (and *A. t. tessellata*); large-flowered, distylous *A. spectabilis* to large-flowered, homostylous *A. spectabilis* to small-flowered, homostylous *A. spectabilis*, and distylous *A. lunaris* to homostylous *A. lunaris* (Fig. 3.7). The first three of these four lineages were studied in this research. Evolution within *Amsinckia* appears to be related to a stepwise reduction in chromosome number from 2n = 14 in *A. furcata* to 2n = 8 in *A. lunaris*; the seed morphology changes from smooth with a groove to roughened with a scar; and the distylous *A. furcata* is the most primitive species while homostylous *A. lunaris* is a newly derived one in the genus (Ray and Chisaki, 1957b).

A recent phylogenetic study in *Amsinckia* using cpDNA data (restriction site variation in the chloroplast DNA; Schoen et al., 1997) has supported the phylogenetic tree proposed by Ray and Chisaki (1957b), and further suggested that the homostylous

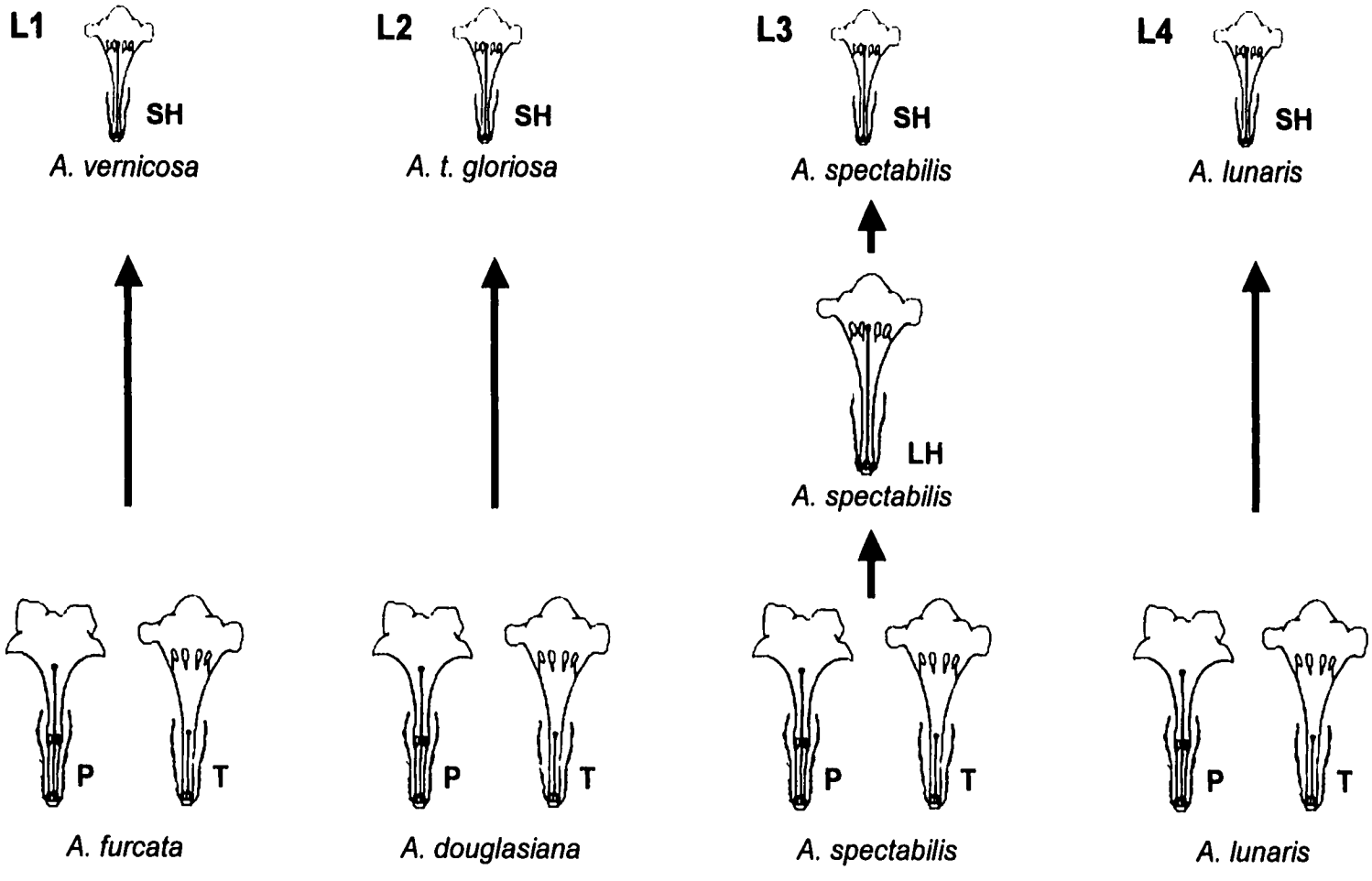


Figure 3.7. Diagram of four lineages of *Amsinckia* and their phylogenetic relationships (Ray and Chisaki, 1957b; Schoen et al., 1997). L1: Lineage 1; L2: Lineage 2; L3: Lineage 3; L4: Lineage 4; P: pin; T: thrum; LH: large homostylous flower; SH: small homostylous flower. Modified from Ray and Chisaki (1957b). The diagrams of flowers were modified from Ganders (1975b).

selfing taxa are recently derived from the distylous outcrossing ancestors, which occurred in each of the four lineages in *Amsinckia*, in comparison with length of branches separating the different outcrossing and selfing taxa. The molecular phylogenetic analysis indicates that the branches connecting two distylous species are about nine to 10 times longer than those connecting distylous species to their descendant homostylous species (Schoen et al., 1997). A more-recent phylogenetic analysis using both chloroplast and nuclear DNA sequences supports these conclusions (M.O. Johnston and W.J. Hahn, unpublished results).

Additionally, *A. spectabilis* contains large-flowered populations that are homostylous. Phenotypically, a flower of the large-flowered homostylous *A. spectabilis* has a long style that is similar to that in the pin morph and stamens whose anthers are positioned at the entrance of the corolla tube, as in a thrum. Therefore, it is not unreasonable to speculate that the flowers of large homostylous *A. spectabilis* may be a step in the evolutionary transition from distyly to a normal, smaller homostyly.

CHAPTER 4

COMPARATIVE FLORAL MORPHOMETRICS OF DISTYLY AND HOMOSTYLY IN THREE EVOLUTIONARY LINEAGES OF *AMSINCKIA* (BORAGINACEAE)

4.1. ABSTRACT

Twenty-six floral traits were measured in three evolutionary lineages of *Amsinckia* (Boraginaceae). Each lineage comprised a distylous ancestor and a homostylous descendant: (1) *A. furcata* / *A. vernicosa* ; (2) *A. douglasiana* / *A. tessellata gloriosa*; and (3) *A. spectabilis*. Comparisons were made between pins and thrums within the distylous groups, as well as among pins, thrums and homostyles. Differences among the morphs were also compared across the three lineages. The six traits directly related to stamen height or pistil height varied as expected from their close relationship to the definition of pins and thrums, with the stamen-height-related characters greater in thrums and the pistil-height-related characters greater in pins. Thrums made larger but fewer pollen grains in all lineages. Thrums also tended to have larger values for corolla size (six traits measured), stigma size (four traits), style cross-sectional area and style transmission tissue cross-sectional area. In two of three lineages, pins exceeded thrums in functional anther-stigma distance and in stigmatic papilla length and width. The size order of a trait in pins versus thrums was consistent in all lineages for 18 of 26 traits; in seven of the eight remaining traits *A. spectabilis* was the unusual lineage. In homostyles, traits related

to anther height and pistil height were intermediate between pins and thrums in all lineages; for other traits homostyles generally had the smallest values. For most traits lineages differed in the degree of differentiation among the three morphs.

4.2. INTRODUCTION

Distyly is a genetic polymorphism in which a population contains two floral morphs defined by the relative height of stigma and anthers. In pins the stigma is situated beyond the anthers, while thrums have the reverse arrangement. Distyly has arisen independently in at least 28 angiosperm families (Arroyo and Barrett, 2000; Barrett et al., 2000). Individuals in the majority of distylous populations are both self- and intramorph sterile. The reciprocal arrangement of male and female sexual organs therefore may reduce pollen wastage by increasing legitimate (i.e., intermorph) pollination (Darwin, 1877; Kohn and Barrett, 1992). In addition to natural selection for pollination proficiency, the persistence of distyly depends on tight linkage of the genes affecting anther height and stigma height (Lewis and Jones, 1992; Richards and Barrett, 1992).

Many genera or species with distylous members also contain other species or populations lacking distyly (Dowrick, 1956; Ganders, 1975a, 1979a; Barrett, 1989b). In most instances, such homostylous species or populations probably evolved from distylous ancestors (Ganders et al., 1985; Barrett, 1988, 1992b; Schoen et al., 1996, 1997), a process initiated by a cross-over or mutation in the distyly supergene (Lewis and Jones, 1992; Richards and Barrett, 1992). Homostylous populations are usually highly self-

fertilizing (Shore and Barrett, 1985; Piper et al., 1986; Boyd et al., 1990; Johnston and Schoen, 1996) because of the reduced or nonexistent anther-stigma separation and the loss of self-sterility caused by disruption of the supergene.

Pins, thrums and homostyles are defined by the relative positions of stigma and anthers. In addition to these primary, definitional traits, pins and thrums often differ in ancillary, nondefinitional traits, including pollen size, pollen number, style cross-sectional area and size of stigmatic papillae (Ganders, 1979a; Dulberger, 1992). The existence of differences in nondefinitional traits between pins and thrums indicates a correlation between definitional and nondefinitional traits. For example, in many distylous species, thrums produce larger pollen grains and smaller stigmatic papillae (Ganders, 1979a; Dulberger, 1992). In these species, anther height is therefore correlated positively with pollen size and negatively with papilla size; while stigma height is correlated negatively with pollen size and positively with papilla size. These phenotypic correlations between primary and ancillary traits could result from natural selection for proficient, legitimate pollen transfer as well as from pleiotropic effects of the genes affecting stigma and/or anther height. Unfortunately, most studies published to date examine a small number of ancillary traits, these studies differ in the ancillary traits examined and comparisons are limited to pins and thrums within distylous taxa. An understanding of changes in relationships among traits during evolution is best achieved by measuring traits in pins, thrums and homostyles within an evolutionary lineage.

Here I report measurements of approximately 26 floral traits in pins, thrums and related homostyles in three evolutionary lineages of *Amsinckia* (Boraginaceae). Within each lineage, homostyly is thought to have evolved from distyly (Ray and Chisaki,

1957b; Schoen et al., 1997). The goals of the study were, first, to identify differences in floral traits among the three morphs (pin, thrum, homostyle) and, second, to determine whether these differences are consistent among the three lineages. This study therefore examines differences, both within and among lineages, in a large number of single traits of mature flowers.

4.3. Material and Methods

4.3.1. Species and floral morphs

The species and populations studied are classified into three evolutionary lineages (Ray and Chisaki, 1957a, 1957b; Ganders et al., 1985; Schoen et al., 1997). These lineages are *Amsinckia furcata* — *A. vernicosa* (Lineage 1 or L1), *A. douglasiana* — *A. tessellata gloriosa* (Lineage 2 or L2) and *A. spectabilis* (Lineage 3 or L3; see Fig. 4.1). Each lineage consists of a distylous taxon, the presumed ancestor, and a homostylous taxon, the presumed descendant (Ray and Chisaki, 1957a, 1957b; Schoen et al., 1997). The *A. spectabilis* lineage additionally contains populations with an intermediate floral form characterized by large flowers that are not distinctly distylous. These populations are termed "mixed" (Ganders, 1975a) or "large-flowered homostylous" (see Fig. 4.1). This study therefore consisted of pins (P), thrums (T) and homostyles (H) in each of these lineages, plus the large-flowered homostyle in *A. spectabilis*, giving a total of 10 lineage-morph combinations. In this paper homostyly (H) of Lineage 3 (*A. spectabilis*) includes both large-flowered homostyly (LH) and small-flowered homostyly (SH). All study

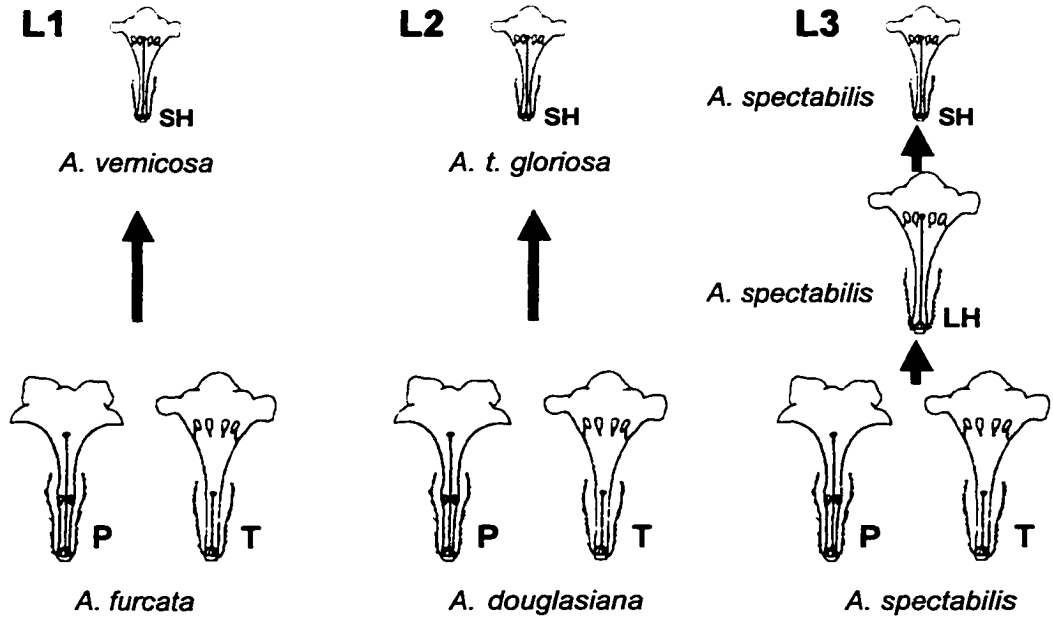


Figure 4.1. Diagram of three evolutionary lineages of *Amsinckia*. Figure Abbreviations: L1, Lineage 1; L2, Lineage 2; L3, Lineage 3; P, Pin; T: Thrum; LH, Large homostylous flower; SH, Small homostylous flower. Modified from Ray and Chisaki (1957b). The diagrams of flowers were modified from Ganders (1975b).

samples were collected from the field in California between 28 April and 6 May 1995. Eight to fifteen inflorescences were used for each floral morph of each species or population in the study. Each inflorescence was taken from a different individual plant. All inflorescences were fixed in formalin-acetic acid-ethanol (FAA) for about one week, and then stored in 70% ethanol for later studies. [Note on nomenclature: *A. tessellata gloriosa* (Ganders, 1993) is equivalent to *A. gloriosa* (Ray and Chisaki, 1957a; Schoen et al., 1997).]

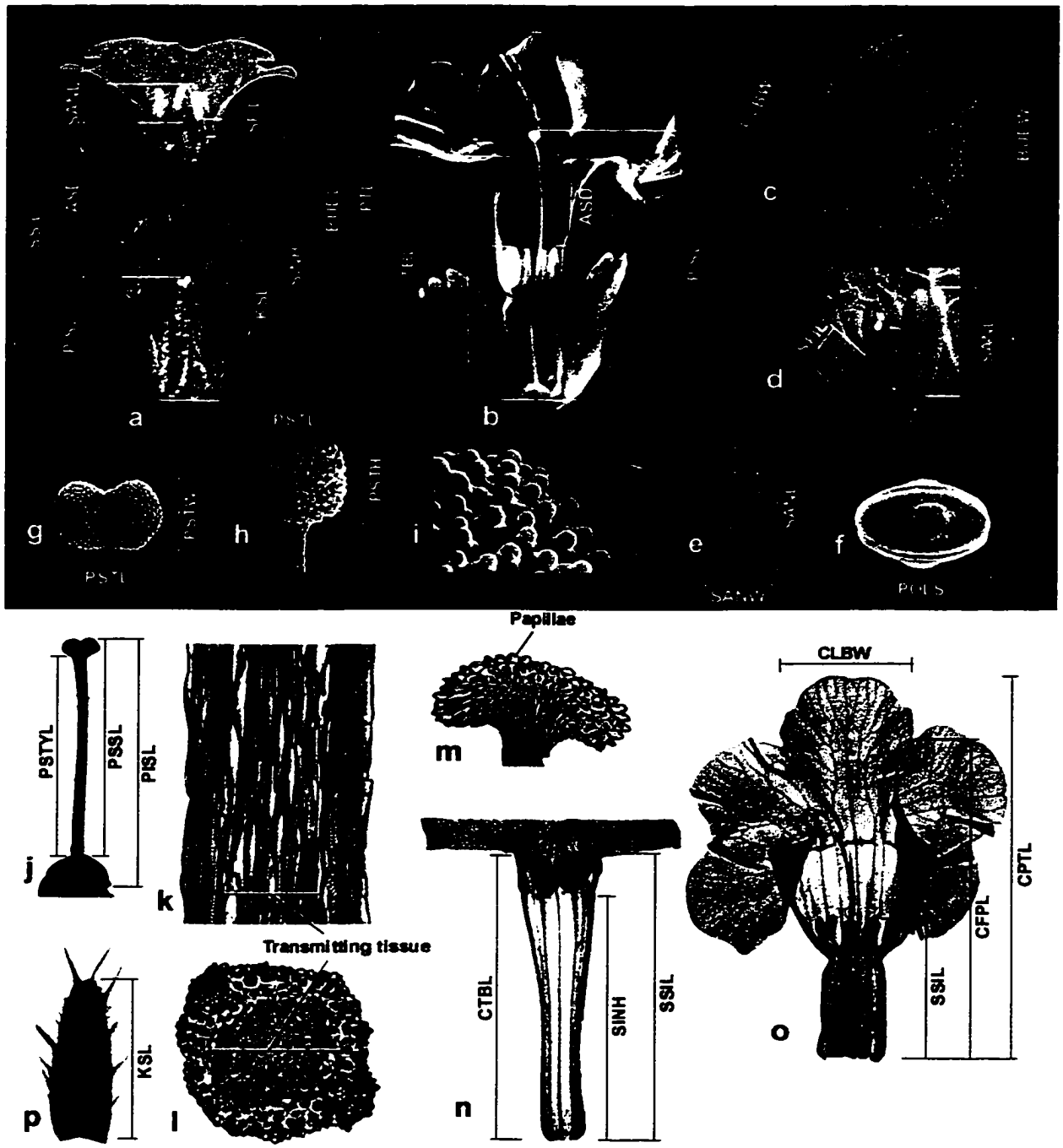
4.3.2. Measurements

In order from distal to proximal, the coiled *Amsinckia* inflorescence consists of unopened flowers (buds), fully opened flowers available for pollination, and senescing flowers. A typical inflorescence has two to eight fully opened flowers. For each inflorescence studied, at least three fully opened flowers were dissected and measured under an OLYMPUS SZH10 stereo microscope, which was connected to a video imaging system and computer. Measurements of floral traits were performed using the public domain NIH Image program (version 1.62, developed at the U.S. National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image) on images of dissected floral parts. The 26 quantitative traits used in the statistical analyses are listed in Table 4.1. All measurements or traits are defined or illustrated in Fig. 4.2. Most traits were named using a four-letter abbreviation with the first letter indicating the whorl: "K" for calyx, "C" for corolla, "S" for stamen and "P" for pistil. From among the flowers dissected and measured, the largest (named "maximum-sized" in the rest of the text) was used in the statistical analyses. Stigma papilla size (PAPIL and PAPIW), style cross-

Table 4.1. The 26 morphometric characters used in the ANOVAs. Morphometric measurements are illustrated in Fig. 4.2.

Abbreviation	Measurement scale	Whorl	Character
KSL	mm	Calyx	Sepal length
BUDL	mm	Corolla	Flower length
BUDW	mm	Corolla	Flower width
CFPL	mm	Corolla	Fused petal length
CLBW	mm	Corolla	Corolla lobe width
CPTL	mm	Corolla	Petal length
CTBL	mm	Corolla	Corolla tube length
POLN	# / flower	Stamen	Pollen number per flower
POLS	μm	Stamen	Pollen size (diameter on long axis)
SANL	mm	Stamen	Anther length
SANW	mm	Stamen	Anther width
SFIL	mm	Stamen	Free filament length (portion not fused to petal)
SINH	mm	Stamen	Stamen insertion height
SSIL	mm	Stamen	Stamen height (anther height)
PAPIL	mm	Pistil	Stigma papilla length
PAPIW	mm	Pistil	Stigma papilla width
PISL	mm	Pistil	Pistil length (stigma height)
PSSL	mm	Pistil	Style and stigma length
PSTYL	mm	Pistil	Style length
PSTH	mm	Pistil	Stigma thickness
PSTL	mm	Pistil	Stigma length
PSTW	mm	Pistil	Stigma width
PSTA	mm^2	Pistil	Stigma area
STYLECA	μm^2	Pistil	Style cross-sectional area
TRANSCA	μm^2	Pistil	Style transmission tissue cross-sectional area
ASD	mm	(Pistil/Stamen)	Functional anther - stigma distance

Figure 4.2 Dissected *Amsinckia* flowers, showing the morphometric characters and the measurement positions of various floral traits. All abbreviations in the figure are explained in Table 4.1. Magnifications vary among graphs. **a.** Longitudinal section of a live thrum flower with natural shape (3.8x). **b.** Longitudinal section of a flatted fixed pin flower (4.1x). **c.** Top view of a live homostylous flower (7.7x). **d.** Stamen attached to corolla tube (9x). **e.** Anther (11x). **f.** Pollen grain [scanning electron micrograph (SEM; 600x)]. **g.** Top view of a stigma (SEM; 25x). **h.** Stigma (SEM; 25). **i.** Stigma surface (SEM; 120x). **j.** Pistil (7x). **k.** Longitudinal section of a style (micrograph; 170x). **l.** Cross section of a style (micrograph; 170x). **m.** Longitudinal section of a stigma (micrograph; 46x). **n.** Longitudinal section of a flatted fixed corolla tube (3.5x). **o.** Dissected flatted pin corolla (4x). **p.** Sepal (3x).


a
b
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PSTL
ASD
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POLS
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PSSL
PISL
j
k
Transmitting tissue
CPTL
CFPL
SSIL
o
CTBL
SINH
SSIL
n
KSL
p
l

Figure 4.2.

sectional area (STYLECA) and style transmission tissue-cross sectional area (TRANSCA) were measured on sections made from paraffin-embedded stigmas and styles. STYLECA and TRANSCA were measured on cross-sections cut from the mid-portion of the styles. Stigma area (PSTA) was estimated as a five-sided box and was calculated as

$$PSTA = 2(PSTL \times PSTH) + 2(PSTW \times PSTH) + (PSTL \times PSTW)$$

Functional anther-stigma distance, ASD, measured the minimum distance separating the top of the stigma from the anthers. If the stigma top was within the anthers, that is, above the anther bottom and below the anther top (PISL > SSIL - SANL and PISL < SSIL), then ASD = 0. Otherwise, ASD was positive, and was calculated as follows for the two possible situations. If the stigma was below the anther bottom (PISL < SSIL - SANL), then ASD = SSIL - SANL - PISL. If the stigma was above the anther top (PISL > SSIL), then ASD = PISL - SSIL (see Fig. 4.2).

4.3.3. Statistical analysis

To quantitatively compare all floral traits among different flower morphs and among lineages, as well as the interactions between floral morphs and lineages, data were analyzed using ANOVA, followed by post hoc tests (Tukey Multiple Comparisons). Both lineage and morph were considered fixed factors. All ANOVAs were carried out with SYSTAT for the Macintosh (version 5.2, Evanston, Illinois, 1992). The sequential Bonferroni technique (Rice, 1989) was used to determine whether individual *P*-values were statistically significant at tablewide $\alpha = 0.05$.

4.4. RESULTS

4.4.1. The size of floral traits in different lineages and morphs

The mean size of a trait in a maximum-sized flower for the 10 lineage-morph combinations in five species of *Amsinckia* is presented in Table 4.2. The results of ANOVA followed by a post hoc test (Tukey Multiple Comparisons) for each trait among the different floral morphs within a lineage are also included in Table 4.2, and they are expressed using different letters in the superscript when the difference was significant ($P < 0.05$). Some traits showed the same kinds of difference between pins and thrums in all three lineages of *Amsinckia*. For example, thrums were always larger than pins in the mean value of corolla tube length (CTBL), stamen height (SSIL), stamen insertion height (SINH), stamen filament length (SFIL), style cross-sectional area (STYLECA), style transmission tissue cross-sectional area (TRANSCA), and pollen size (POLS; Table 4.2). On the other hand, pistil length (PISL), style and stigma length (PSSL), and style length (PSTYL) were significantly longer in pins than in thrums. Some traits, such as stigma papilla length (PAPIL) and papilla width (PAPIW), reversed their size order between pins and thrums depending on lineage. PAPIL and PAPIW in pin flowers were significantly larger than those in thrum flowers in both the lineages *A. furcata – A. vernicosa* (Lineage 1) and *A. douglasiana – A. t. gloriosa* (Lineage 2). The size order was reversed in *A. spectabilis* (Lineage 3).

Some traits were highly significantly different between pins and thrums in one or two lineages, but not in another. For instance, flower length (BUDL), petal length

Table 4.2. Mean ±SE of trait size in maximum-sized flowers and some basic statistical results. Means with different letters were significantly different ($P < 0.05$) among floral morphs within each lineage. Except for PSTH in Lineage 1, all the differences with a $P < 0.05$ were significant after tablewide correction ($\alpha = 0.05$) for multiple comparisons using the sequential Bonferroni technique (Rice, 1989) in the analyses across species and morphs within the lineage. Units: mm except PSTA (mm^2), PAPIL, PAPIW and POLS (μm), STYLECA and TRANSCA (μm^2). P = pin; T = thrum; H = homostyle; LH = large-flowered homostyle; SH = small-flowered homostyle.

Table 4.2.

Line-age	Species_morph & statistics	*N*	Trait					
			KSL	**BUDL**	**BUDW**	**CFPL**	**CLBW**	**CPTL**
L1	*A. furcata*_P	15	8.33 ± 0.22^{a}	16.66 ± 0.26^{a}	12.50 ± 0.23^{a}	14.25 ± 0.22^{a}	5.13 ± 0.16^{a}	16.77 ± 0.28^{a}
	*A. furcata*_T	8	8.41 ± 0.30^{a}	18.87 ± 0.39^{b}	13.23 ± 0.43^{a}	16.51 ± 0.32^{b}	5.23 ± 0.22^{a}	18.95 ± 0.39^{b}
	*A. vernicosa*_H	8	7.89 ± 0.30^{a}	9.61 ± 0.17^{c}	4.55 ± 0.16^{b}	8.61 ± 0.09^{c}	1.77 ± 0.06^{b}	9.60 ± 0.09^{c}
	F-ratio (2, 28)		0.93	220.31	245.19	227.45	118.99	216.69
	P-value		0.41	$<10^{-15}$	$<10^{-15}$	$<10^{-15}$	$<10^{-13}$	$<10^{-15}$
L2	*A. douglasiana*_P	8	7.58 ± 0.16^{ab}	14.93 ± 0.35^{a}	10.78 ± 0.30^{a}	13.63 ± 0.33^{a}	4.52 ± 0.17^{a}	15.26 ± 0.41^{a}
	*A. douglasiana*_T	11	7.91 ± 0.16^{a}	17.87 ± 0.22^{b}	11.53 ± 0.25^{a}	16.29 ± 0.28^{b}	4.95 ± 0.11^{a}	18.08 ± 0.26^{b}
	*A. t. gloriosa*_H	8	7.11 ± 0.17^{b}	12.12 ± 0.34^{c}	7.15 ± 0.35^{b}	10.62 ± 0.30^{c}	2.92 ± 0.13^{b}	12.18 ± 0.38^{c}
	F-ratio (2, 24)		6.12	100.33	60.96	93.50	59.49	78.30
	P-value		<0.01	$<10^{-11}$	$<10^{-9}$	$<10^{-11}$	$<10^{-9}$	$<10^{-10}$
L3	*A. spectabilis*_P	8	5.27 ± 0.10^{a}	13.97 ± 0.32^{ab}	10.67 ± 0.22^{ab}	12.29 ± 0.20^{a}	4.92 ± 0.10^{a}	14.14 ± 0.28^{a}
	*A. spectabilis*_T	8	4.73 ± 0.10^{b}	14.98 ± 0.54^{bc}	10.10 ± 0.46^{bc}	13.15 ± 0.51^{a}	4.60 ± 0.22^{ab}	15.00 ± 0.58^{a}
	*A. spectabilis*_LH	8	4.06 ± 0.10^{c}	12.52 ± 0.30^{a}	9.19 ± 0.29^{c}	10.81 ± 0.34^{b}	4.18 ± 0.10^{b}	12.61 ± 0.33^{b}
	*A. spectabilis*_SH	8	3.00 ± 0.10^{d}	8.84 ± 0.31^{d}	4.89 ± 0.15^{d}	7.62 ± 0.25^{c}	2.06 ± 0.05^{c}	8.90 ± 0.26^{c}
	F-ratio (3, 28)		93.59	49.75	74.52	49.12	89.75	49.11
	P-value		$<10^{-13}$	$<10^{-10}$	$<10^{-12}$	$<10^{-10}$	$<10^{-13}$	$<10^{-10}$

Table 4.2. Continued.

Line-age	Species_morph & statistics	*N*	Trait					
			CTBL	**POLN**	**POLS**	**SANL**	**SANW**	**SFIL**
L1	*A. furcata*_P	15	10.53 ±0.18[a]	19371 ±765[a]	32.27 ±0.31[a]	1.96 ±0.03[a]	0.66 ±0.01[a]	0.57 ±0.01[a]
	*A. furcata*_T	8	11.98 ±0.18[b]	16987 ±765[a]	41.70 ±0.31[b]	2.16 ±0.07[b]	0.80 ±0.02[b]	1.08 ±0.10[b]
	*A. vernicosa*_H	8	7.04 ±0.10[c]	6913 ±765[b]	35.50 ±0.31[c]	1.30 ±0.06[c]	0.59 ±0.02[c]	0.60 ±0.09[a]
	F-ratio (2, 28)		163.62	74.68	237.38	66.03	33.71	18.44
	P-value		$<10^{-15}$	$<10^{-15}$	$<10^{-15}$	$<10^{-10}$	$<10^{-7}$	<0.00001
L2	*A. douglasiana*_P	8	9.98 ±0.18[a]	22154 ±792[a]	25.99 ±0.28[a]	1.66 ±0.05[a]	0.61 ±0.01[a]	0.50 ±0.03[a]
	*A. douglasiana*_T	11	12.48 ±0.44[b]	18115 ±765[b]	36.41 ±0.28[b]	1.95 ±0.04[b]	0.77 ±0.01[b]	1.58 ±0.05[b]
	*A. t. gloriosa*_H	8	8.56 ±0.25[c]	9846 ±604[c]	35.18 ±0.31[c]	1.51 ±0.02[c]	0.61 ±0.01[a]	0.54 ±0.02[a]
	F-ratio (2, 24)		34.00	59.99	413.88	28.91	84.84	243.77
	P-value		$<10^{-7}$	$<10^{-15}$	$<10^{-15}$	$<10^{-6}$	$<10^{-10}$	$<10^{-15}$
L3	*A. spectabilis*_P	8	8.37 ±0.20[a]	12333 ±1025[a]	32.13 ±0.28[a]	1.48 ±0.04[a]	0.57 ±0.01[a]	0.63 ±0.02[a]
	*A. spectabilis*_T	8	10.12 ±0.38[b]	11163 ±677[a]	36.71 ±0.27[b]	1.47 ±0.05[a]	0.64 ±0.02[b]	0.88 ±0.04[b]
	*A. spectabilis*_LH	8	7.60 ±0.32[a]	7096 ±492[b]	34.77 ±0.23[c]	1.29 ±0.03[b]	0.60 ±0.01[ab]	1.01 ±0.06[b]
	*A. spectabilis*_SH	8	6.06 ±0.18[c]	4375 ±300[c]	30.86 ±0.26[d]	1.02 ±0.02[c]	0.48 ±0.01[c]	0.49 ±0.02[a]
	F-ratio (3, 28)		36.04	27.18	91.19	33.69	23.20	36.57
	P-value		$<10^{-9}$	$<10^{-14}$	$<10^{-15}$	$<10^{-8}$	$<10^{-7}$	$<10^{-9}$

Table 4.2. Continued.

Line-age	Species_Morph & Statistics	*N*	Trait					
			SINH	**SSIL**	**PAPIL**	**PAPIW**	**PISL**	**PSSL**
L1	*A. furcata*_P	15	5.26 ±0.12[a]	6.83 ±0.12[a]	55.89 ±1.65[a]	42.86 ±1.41[a]	14.14 ±0.21[a]	12.90 ±0.20[a]
	*A. furcata*_T	8	12.26 ±0.20[b]	14.24 ±0.18[b]	35.14 ±0.97[b]	27.12 ±0.99[b]	7.37 ±0.39[b]	5.28 ±0.14[b]
	*A. vernicosa*_H	8	7.00 ±0.10[c]	8.08 ±0.13[c]	31.57 ±0.63[c]	25.98 ±0.83[b]	8.36 ±0.08[c]	5.96 ±0.13[b]
	F-ratio (2, 28)		618.33	724.01	154.84	72.59	246.05	555.80
	P-value		$<10^{-15}$	$<10^{-15}$	$<10^{-15}$	$<10^{-15}$	$<10^{-15}$	$<10^{-15}$
L2	*A. douglasiana*_P	8	4.22 ±0.10[a]	5.40 ±0.14[a]	49.39 ±1.13[a]	34.45 ±1.14[a]	13.46 ±0.33[a]	12.21 ±0.36[a]
	*A. douglasiana*_T	11	11.74 ±0.25[b]	13.71 ±0.28[b]	33.97 ±0.64[b]	23.05 ±1.06[b]	5.85 ±0.12[b]	4.73 ±0.10[b]
	*A. t. gloriosa*_H	8	7.41 ±0.24[c]	8.44 ±0.22[c]	28.76 ±1.05[c]	28.60 ±1.48[c]	8.31 ±0.22[c]	6.94 ±0.18[c]
	F-ratio (2, 24)		295.01	323.10	108.55	13.60	317.31	313.99
	P-value		$<10^{-15}$	$<10^{-15}$	$<10^{-15}$	<0.00001	$<10^{-15}$	$<10^{-15}$
L3	*A. spectabilis*_P	8	4.66 ±0.11[a]	5.90 ±0.14[a]	30.63 ±0.73[a]	25.50 ±0.69[a]	9.76 ±0.17[a]	9.02 ±0.14[a]
	*A. spectabilis*_T	8	8.84 ±0.32[b]	10.38 ±0.32[b]	40.57 ±0.99[b]	29.58 ±0.87[b]	4.69 ±0.25[b]	3.77 ±0.18[b]
	*A. spectabilis*_LH	8	6.94 ±0.34[c]	8.35 ±0.36[c]	32.17 ±1.45[a]	24.44 ±1.14[a]	9.68 ±0.29[a]	8.98 ±0.26[a]
	*A. spectabilis*_SH	8	5.10 ±0.15[a]	5.97 ±0.19[a]	24.07 ±1.63[c]	18.72 ±0.97[c]	5.54 ±0.06[c]	4.73 ±0.09[c]
	F-ratio (3, 28)		57.55	62.54	34.23	15.97	160.60	235.32
	P-value		$<10^{-11}$	$<10^{-11}$	$<10^{-14}$	$<10^{-7}$	$<10^{-15}$	$<10^{-15}$

Table 4.2. Continued.

Line-age	Species_morph & statistics	*N*	Trait					
			PSTYL	**PSTH**	**PSTL**	**PSTW**	**PSTA**	**STYLECA**
L1	*A. furcata*_P	15	12.47 ±0.26^{a}	0.49 ±0.01^{a}	0.57 ±0.01^{a}	0.51 ±0.01^{a}	1.29 ±0.04^{a}	21302 ±659^{a}
	*A. furcata*_T	8	4.72 ±0.16^{b}	0.49 ±0.01^{ab}	0.65 ±0.02^{b}	0.56 ±0.01^{b}	1.51 ±0.08^{b}	24242 ±1134^{b}
	*A. vernicosa*_H	8	5.59 ±0.13^{b}	0.44 ±0.01^{b}	0.48 ±0.01^{c}	0.38 ±0.01^{c}	0.91 ±0.03^{c}	28537 ±1446^{c}
	F-ratio (2, 28)		354.48	3.40	27.12	67.23	23.79	11.45
	P-value		$<10^{-15}$	<0.05	$<10^{-6}$	$<10^{-10}$	$<10^{-6}$	<0.00005
L2	*A. douglasiana*_P	8	11.88 ±0.36^{a}	0.35 ±0.01^{a}	0.65 ±0.01^{a}	0.45 ±0.01^{a}	1.05 ±0.06^{a}	20743 ±767^{a}
	*A. douglasiana*_T	11	4.39 ±0.10^{b}	0.36 ±0.01^{a}	0.68 ±0.02^{a}	0.46 ±0.01^{a}	1.13 ±0.06^{a}	27878 ±1359^{b}
	*A. t. gloriosa*_H	8	6.71 ±0.18^{c}	0.25 ±0.01^{b}	0.47 ±0.02^{b}	0.47 ±0.01^{a}	0.69 ±0.01^{b}	19815 ±869^{a}
	F-ratio (2, 24)		316.85	26.77	27.72	0.02	9.07	13.35
	P-value		$<10^{-15}$	$<10^{-6}$	$<10^{-6}$	0.98	<0.001	<0.00001
L3	*A. spectabilis*_P	8	8.75 ±0.13^{a}	0.27 ±0.01^{a}	0.54 ±0.02^{ab}	0.41 ±0.01^{ab}	0.73 ±0.04^{a}	16732 ±404^{a}
	*A. spectabilis*_T	8	3.48 ±0.19^{b}	0.29 ±0.00^{a}	0.58 ±0.01^{a}	0.44 ±0.01^{a}	0.86 ±0.03^{a}	22931 ±1082^{b}
	*A. spectabilis*_LH	8	8.70 ±0.26^{a}	0.28 ±0.01^{a}	0.51 ±0.01^{b}	0.38 ±0.01^{b}	0.70 ±0.03^{a}	13731 ±1249^{c}
	*A. spectabilis*_SH	8	4.54 ±0.09^{c}	0.19 ±0.00^{b}	0.35 ±0.01^{c}	0.25 ±0.00^{c}	0.31 ±0.01^{b}	9527 ±315^{d}
	F-ratio (3, 28)		236.81	44.74	51.76	77.23	42.63	85.15
	P-value		$<10^{-15}$	$<10^{-10}$	$<10^{-10}$	$<10^{-12}$	$<10^{-9}$	$<10^{-15}$

Table 4.2. Continued.

Line-age	Species_morph & statistics	*N*	Trait	
			TRANSCA	**ASD**
L1	*A. furcata*_P	15	8767 ±245[a]	7.31 ±0.24[a]
	*A. furcata*_T	8	10861 ±698[b]	4.70 ±0.49[b]
	*A. vernicosa*_H	8	8407 ±443[a]	0.27 ±0.15[c]
	F-ratio (2, 28)		8.12	134.99
	P-value		<0.001	$<10^{-14}$
L2	*A. douglasiana*_P	8	4514 ±237[a]	8.05 ±0.27[a]
	*A. douglasiana*_T	11	8354 ±238[b]	5.91 ±0.33[b]
	*A. t. gloriosa*_H	8	7603 ±465[b]	0.12 ±0.30[c]
	F-ratio (2, 24)		68.78	185.15
	P-value		$<10^{-15}$	$<10^{-13}$
L3	*A. spectabilis*_P	8	7120 ±187[a]	3.86 ±0.13[a]
	*A. spectabilis*_T	8	13004 ±985[b]	4.23 ±0.24[a]
	*A. spectabilis*_LH	8	4570 ±549[c]	1.33 ±0.19[b]
	*A. spectabilis*_SH	8	3231 ±117[d]	0.43 ±0.16[c]
	F-ratio (3, 28)		134.49	141.54
	P-value		$<10^{-15}$	$<10^{-15}$

(CPTL), fused petal length (CFPL), and anther length (SANL) in thrums were significantly larger than those in pins in the first two lineages. The functional distance between anther and stigma (ASD) in pins was highly significantly larger than that in thrums in the first two lineages. These same traits, however, were not significantly different between pin and thrum morphs in the third lineage (Table 4.2). In addition, sepal length in pin flowers of the third lineage was significantly larger than that in thrum flowers. This difference was not statistically significant in the first two lineages (Table 4.2). Similarly, the number of pollen grains (POLN) produced in pin flowers was significantly greater than that in thrum flowers in the second lineage, but no significant difference was found in the other two lineages (Table 4.2).

Some of the traits did not differ between pins and thrums in any lineage, including flower width (BUDW), corolla tube width (CLBW), and stigma thickness (PSTH; Table 4.2).

Many of the traits in homostylous flowers (small homostylous in *A. spectabilis*) were significantly smaller than those in both pin and thrum flowers in all three lineages. They were BUDL, BUDW, CFPL, CLBW, CPTL, CTBL, SANL, POLN, PAPIL, and ASD (Table 4.2). These traits, except POLN, ASD and PAPIL, reflect overall flower size, which is smaller in homostylous flowers.

Other traits of homostylous flowers were intermediate in size compared with the same traits of pin and thrum. For example, the pistil length (PISL) of homostyles was smaller than that of pins but was larger than that of thrums in all lineages (Table 4.2). Conversely, pollen size (POLS) of homostyles was significantly larger than that of pins but was smaller than that of thrums (Table 4.2).

In all lineages stamen filament length (SFIL) of the homostylous flowers was similar to that in pin flowers (Table 4.2). The relative size of some homostylous floral traits, however, varied in different lineages in relation to the size of the same traits of pin and thrum. The size of a trait in a homostylous flower might be similar to the same trait in either a pin flower or a thrum flower (e.g., KSL of homostyle was similar to that of both pin and thrum in Lineage 1 but only similar to that of pin in Lineage 2; in Lineage 2, KSL, SANW and STYLECA of homostyle were similar to those of pin, whereas TRANSCA was similar to that of thrum). Furthermore, it might be similar to the same trait of a pin flower in one lineage but to the same trait of a thrum flower in another lineage (e.g., TRANSCA of homostyle was similar to that of pin in Lineage 1 but it was similar to that of thrum in Lineage 2; Table 4.2).

Within *A. spectabilis* all 26 traits were significantly larger in the large homostylous flower than in the small homostylous flower. Many (ten) of these traits were, in turn, significantly smaller than those of both pins and thrums, including KSL, CFPL, CPTL, SINH, SSIL, SANL, POLN, STYLECA, TRANSCA, and ASD. Comparison of the 26 traits in large homostylous flowers to those in pins and thrums showed that only three (SANW, PSTH, PSTA) were statistically indistinguishable from traits in both pins and thrums, three (BUDW, CLBW, SFIL) were different from pins but not from thrums, nine (BUDL, CTBL, PISL, PSSL, PSTL, PSTW, PSTYL, PAPIL, PAPIW) were different from thrums but not from pins, ten (KSL, CFPL, CPTL, SSIL, SINH, SANL, POLN, STYLECA, TRANSCA, ASD) were different (all smaller) than both pins and thrums, and one (POLS) was larger than pins but smaller than thrums.

4.4.2. A comparison of pin vs. thrum

All comparisons of floral traits among the morphs presented above were limited to within a lineage. In order to obtain more detailed information about the differences of traits among floral morphs and lineages, ANOVAs were carried out both within and among lineages (that is, containing both a morph and lineage factor). The remaining results are from such ANOVAs.

Twenty-two of 26 traits differed significantly in size between pin and thrum flowers as indicated by morph term in ANOVA (Table 4.3). The four traits showing no difference were flower width (BUDW), corolla lobe width (CLBW), sepal length (KSL), and stigma thickness (PSTH). In addition, the size of all 26 floral traits was significantly different among the lineages as indicated by lineage term in ANOVA (Table 4.3). More than half of the traits differed among all three lineages. Some other traits, however, showed no significant difference between two of the three lineages. For example, KSL, CFPL, CTBL, PSTYL, STYLECA, and POLN were not significantly different between Lineages 1 and 2; CLBW, PSTW, and PAPIW were not significantly different between Lineages 2 and 3; and SFIL, and TRANSCA were not significantly different between Lineages 1 and 3 (analysis not shown).

In about half of the studied traits, the differences between pin and thrum were lineage dependent, as indicated by the interaction term in ANOVAs. These lineage-dependent traits included those related to pistil length (PISL, PSSL, and PSTYL), stamen height (SSIL, SINH, and SFIL), functional anther-stigma distance (ASD), style transmission tissue cross-sectional area (TRANSCA), stigma papilla size (PAPIL and PAPIW), and pollen size (POLS; Table 4.3).

Table 4.3. Statistical comparisons of floral morphs. ANOVA results of the effect of lineage and morph (pin and thrum; distyly and homostyly; pin, thrum and homostyle) on maximum-sized traits in *Amsinckia*. In the *A. spectabilis* lineage, both small and large homostylous flowers were included.

Trait	Source	Pin vs. thrum			Distyly vs. homostyly			Pin vs. thrum vs. homostyle		
		F	*P*	R^2	*F*	*P*	R^2	*F*	*P*	R^2
KSL	Lineage	128.13	$<10^{-15}$*	0.84	292.85	$<10^{-15}$*	0.90	275.03	$<10^{-15}$*	0.91
	Morph	0.06	<0.80		32.94	$<10^{-6}$*		16.87	$<10^{-6}$*	
	Interaction	1.89	<0.16		5.02	<0.01		3.58	<0.01	
BUDL	Lineage	44.31	$<10^{-11}$*	0.73	9.15	<0.0003*	0.79	19.10	$<10^{-6}$*	0.86
	Morph	52.28	$<10^{-8}$*		232.43	$<10^{-15}$*		187.91	$<10^{-15}$*	
	Interaction	3.63	<0.05		12.48	<0.00002*		12.26	$<10^{-7}$*	
BUDW	Lineage	32.75	$<10^{-9}$*	0.57	1.17	<0.31	0.82	2.97	<0.06	0.83
	Morph	1.38	<0.24		304.17	$<10^{-15}$*		153.16	$<10^{-15}$*	
	Interaction	2.64	<0.08		25.81	$<10^{-8}$*		14.03	$<10^{-8}$*	
CFPL	Lineage	38.49	$<10^{-10}$*	0.73	12.95	<0.00002*	0.79	23.21	$<10^{-7}$*	0.87
	Morph	54.21	$<10^{-8}$*		228.13	$<10^{-15}$*		191.32	$<10^{-15}$*	
	Interaction	4.06	<0.05		7.44	<0.002*		8.45	<0.00002*	

Table 4.3. Continued.

Trait	Source	Pin vs. thrum			Distyly vs. homostyly			Pin vs. thrum vs. homostyle		
		F	*P*	R^2	*F*	*P*	R^2	*F*	*P*	R^2
	Lineage	4.49	<0.02	0.20	3.80	<0.03	0.76	0.48	<0.62	0.77
CLBW	Morph	0.23	<0.65		237.96	$<10^{-15}$*		117.08	$<10^{-15}$*	
	Interaction	2.20	<0.12		13.84	<0.00001*		7.71	<0.00003*	
	Lineage	39.32	$<10^{-10}$*	0.70	9.67	<0.0002*	0.79	18.71	$<10^{-6}$*	0.86
CPTL	Morph	41.45	$<10^{-7}$*		237.93	$<10^{-15}$*		181.27	$<10^{-15}$*	
	Interaction	3.36	<0.05		12.58	<0.00002*		11.67	$<10^{-6}$*	
	Lineage	28.42	$<10^{-8}$*	0.70	18.74	$<10^{-6}$*	0.72	35.24	$<10^{-11}$*	0.85
CTBL	Morph	61.88	$<10^{-9}$*		133.26	$<10^{-15}$*		146.39	$<10^{-15}$*	
	Interaction	1.76	<0.18		3.17	<0.05		5.02	<0.002*	
	Lineage	48.43	$<10^{-15}$*	0.26	53.54	$<10^{-15}$*	0.49	72.58	$<10^{-15}$*	0.51
POLN	Morph	13.71	<0.0005		314.67	$<10^{-15}$*		172.40	$<10^{-15}$*	
	Interaction	1.00	<0.37		10.88	<0.00005*		6.28	<0.0001*	
	Lineage	197.16	$<10^{-15}$*	0.74	37.68	$<10^{-15}$*		136.83	$<10^{-15}$*	0.63
POLS	Morph	1156.95	$<10^{-15}$*		0.93	<0.34		555.33	$<10^{-15}$*	
	Interaction	58.11	$<10^{-15}$*		36.58	$<10^{-15}$*	0.17	68.35	$<10^{-15}$*	

Table 4.3. Continued.

Trait	Source	Pin vs. thrum			Distyly vs. homostyly			Pin vs. thrum vs. homostyle		
		F	*P*	R^2	*F*	*P*	R^2	*F*	*P*	R^2
SANL	Lineage	73.72	$<10^{-14}$*	0.77	41.94	$<10^{-12}$*	0.80	71.73	$<10^{-15}$*	0.85
	Morph	16.50	<0.0002*		139.42	$<10^{-15}$*		99.09	$<10^{-15}$*	
	Interaction	4.80	<0.02		12.86	<0.00002*		11.84	$<10^{-6}$*	
SANW	Lineage	30.35	$<10^{-8}$*	0.76	9.77	<0.0002*	0.45	28.24	$<10^{-9}$*	0.74
	Morph	94.37	$<10^{-12}$*		28.74	$<10^{-6}$*		70.43	$<10^{-15}$*	
	Interaction	4.40	<0.02		0.83	<0.44		3.55	<0.02	
SFIL	Lineage	18.40	$<10^{-6}$*	0.89	1.21	<0.30	0.23	3.62	<0.04	0.77
	Morph	240.81	$<10^{-15}$*		9.66	<0.003*		84.44	$<10^{-15}$*	
	Interaction	37.02	$<10^{-9}$*		4.89	<0.01		17.27	$<10^{-9}$*	
SINH	Lineage	48.93	$<10^{-11}$*	0.97	2.36	<0.10	0.10	39.28	$<10^{-11}$*	0.94
	Morph	1424.50	$<10^{-15}$*		1.89	<0.17		496.38	$<10^{-15}$*	
	Interaction	36.74	$<10^{-9}$*		0.05	<0.95		14.16	$<10^{-8}$*	
SSIL	Lineage	62.09	$<10^{-13}$*	0.97	2.24	<0.11	0.1	38.71	$<10^{-11}$*	0.93
	Morph	1488.28	$<10^{-15}$*		3.99	<0.05		463.08	$<10^{-15}$*	
	Interaction	40.62	$<10^{-10}$*		0.12	<0.89		14.38	$<10^{-8}$*	

Table 4.3. Continued.

Trait	Source	Pin vs. thrum			Distyly vs. homostyly			Pin vs. thrum vs. homostyle		
		F	*P*	R^2	*F*	*P*	R^2	*F*	*P*	R^2
PAPIL	Lineage	44.75	$<10^{-15}$*	0.71	10.52	<0.00004*	0.35	35.20	$<10^{-13}$*	0.70
	Morph	97.38	$<10^{-15}$*		128.62	$<10^{-15}$*		168.44	$<10^{-15}$*	
	Interaction	125.00	$<10^{-15}$*		5.56	<0.005		64.23	$<10^{-15}$*	
PAPIW	Lineage	27.54	$<10^{-10}$*	0.57	11.25	<0.00003*	0.17	20.27	$<10^{-8}$*	0.42
	Morph	80.61	$<10^{-15}$*		30.27	$<10^{-7}$*		49.31	$<10^{-15}$*	
	Interaction	53.69	$<10^{-15}$*		6.12	<0.003*		24.87	$<10^{-15}$*	
PISL	Lineage	99.62	$<10^{-15}$*	0.96	5.64	<0.01	0.27	39.03	$<10^{-11}$*	0.89
	Morph	994.33	$<10^{-15}$*		3.56	<0.06		229.82	$<10^{-15}$*	
	Interaction	12.21	<0.00005*		2.97	<0.06		7.97	<0.00002*	
PSSL	Lineage	86.76	$<10^{-15}$*	0.98	1.64	<0.20	0.22	16.21	$<10^{-5}$*	0.90
	Morph	1573.43	$<10^{-15}$*		5.24	<0.03		272.33	$<10^{-15}$*	
	Interaction	18.95	$<10^{-7}$*		4.26	<0.02		12.57	$<10^{-7}$*	
PSTYL	Lineage	59.04	$<10^{-13}$*	0.97	1.28	<0.28	0.20	13.36	<0.00002*	0.90
	Morph	1252.62	$<10^{-15}$*		4.63	<0.04		267.23	$<10^{-15}$*	
	Interaction	15.59	$<10^{-5}$*		4.10	<0.03		12.06	$<10^{-7}$*	

Table 4.3. Continued.

Trait	Source	Pin vs. thrum			Distyly vs. homostyly			Pin vs. thrum vs. homostyle		
		F	*P*	R^2	*F*	*P*	R^2	*F*	*P*	R^2
	Lineage	129.70	$<10^{-15}$*	0.84	191.72	$<10^{-15}$*	0.87	202.22	$<10^{-15}$*	0.87
PSTH	Morph	0.94	<0.34		52.21	$<10^{-9}$*		25.34	$<10^{-8}$*	
	Interaction	0.63	<0.54		4.44	<0.02		2.40	<0.06	
	Lineage	14.22	<0.00002*	0.47	9.15	<0.0003*	0.64	13.06	<0.00002*	0.69
PSTL	Morph	11.01	<0.002*		100.45	$<10^{-15}$*		61.82	$<10^{-15}$*	
	Interaction	1.12	<0.34		2.35	<0.10		1.49	<0.21	
	Lineage	46.33	$<10^{-11}$*	0.65	7.49	<0.002*	0.37	6.84	<0.002*	0.38
PSTW	Morph	11.06	<0.002*		13.56	<0.0005*		7.59	<0.001*	
	Interaction	1.72	<0.19		4.26	<0.02		2.36	<0.06	
	Lineage	54.18	$<10^{-12}$*	0.68	42.65	$<10^{-12}$*	0.72	58.20	$<10^{-15}$*	0.75
PSTA	Morph	8.87	<0.005*		71.29	$<10^{-12}$*		43.19	$<10^{-13}$*	
	Interaction	0.87	<0.43		1.28	<0.28		1.20	<0.32	
	Lineage	10.08	<0.0001*	0.37	82.38	$<10^{-15}$*	0.54	54.99	$<10^{-15}$*	0.63
STYLECA	Morph	47.29	$<10^{-9}$*		4.05	<0.05		29.14	$<10^{-11}$*	
	Interaction	3.05	<0.05		28.05	$<10^{-11}$*		21.37	$<10^{-14}$*	

Table 4.3. Continued.

Trait	Source	Pin vs. thrum			Distyly vs. homostyly			Pin vs. thrum vs. homostyle		
		F	*P*	R^2	*F*	*P*	R^2	*F*	*P*	R^2
TRANSCA	Lineage	64.54	$<10^{-15}$*	0.60	24.99	$<10^{-9}$*	0.47	26.32	$<10^{-10}$*	0.71
	Morph	160.73	$<10^{-15}$*		12.91	<0.0005*		105.97	$<10^{-15}$*	
	Interaction	10.41	<0.00006*		20.53	$<10^{-8}$*		30.70	$<10^{-15}$*	
ASD	Lineage	43.86	$<10^{-11}$*	0.76	8.08	<0.001*	0.86	27.82	$<10^{-9}$*	0.82
	Morph	33.88	$<10^{-6}$*		403.53	$<10^{-15}$*		369.46	$<10^{-15}$*	
	Interaction	13.15	<0.00005*		15.01	$<10^{-5}$*		19.96	$<10^{-10}$*	

* Significant after tablewide correction ($\alpha = 0.05$) for multiple comparisons using the sequential Bonferroni procedure (Rice, 1989) in the analyses within the same category of comparison and source across traits.

df for Lineage, Morph and Interaction is 2, 1, 2 in analyses of pin vs. thrum and distyly vs. homostyly, and 2, 2, 4 in analysis of pin vs. thrum vs. homostyle, respectively.

df for the Error term ranges from 49 to 592 in analyses of pin vs. thrum, 80 to 988 in analyses of distyly vs. homostyly, and 77 to 985 in analysis of pin vs. thrum vs. homostyle.

4.4.3. A comparison of distyly vs. homostyly

Nineteen of 26 studied traits differed significantly between distylous (i.e., pin plus thrum) and homostylous flowers (following Bonferroni correction for multiple comparisons; Table 4.3). Distylous flowers tended to be larger overall (BUDL, BUDW, CFPL, CLBW, CPTL, and CTBL) and had larger sepals (KSL), anthers (SANL and SANW), stigmas (PSTH, PSTL, PSTW, and PSTA), functional anther-stigma distances (ASD), stigma papilla (PAPIL and PAPIW), style transmission tissue cross-sectional area (TRANSCA), and pollen production (POLN; Tables 4.2 and 4.3). In addition, distylous flowers exceeded homostylous flowers in filament length (SFIL; Tables 4.2 and 4.3).

Eighteen of 26 studied floral traits differed significantly between at least two of the three lineages (following Bonferroni adjustments for multi-comparisons; Table 4.3). Of the 18 traits, flower length (BUDL, CFPL, CPTL, and CTBL), sepal length (KSL), anther size (SANL and SANW), stigma size (PSTH, PSTL, PSTW, and PSTA), style cross-sectional size (STYLECA and TRANSCA), stigma papilla size (PAPIL and PAPIW), pollen size (POLS), and pollen number (POLN) were the most highly significantly different characters among lineages, while functional anther-stigma distance (ASD) was less so (Table 4.3). Among 26 studied floral traits, only PSTH, PSTA, STYLECA, and POLN differed significantly among all three lineages (analysis not shown).

Among those traits that showed significant difference between distylous and homostylous flowers, the sizes of BUDL, BUDW, CPTL, CLBW, SANL, POLS, POLN, STYLECA, TRANSCA, and ASD were highly significantly ($P < 0.00005$) lineage

dependent (Table 4.3). Some other traits, such as CFPL and PAPIW were also closely associated with the lineages ($P < 0.005$).

4.4.4. A comparison of pin vs. thrum vs. homostyle

All 26 traits differed significantly between at least two of the three floral morphs (pin, thrum, and homostyle), as indicated by the "morph" term in ANOVAs (following Bonferroni corrections; Table 4.3). Flower length (BUDL, CFPL, CPTL, and CTBL), pistil length (PISL, PSSL, and PSTYL), stamen height (SSIL and SINH), anther size (SANL and SANW), POLS, PSTL, PSTA, PAPIL, and ASD were highly significantly different among all three floral morphs (analysis not shown). The remainder of the traits, however, differed significantly between only two of the three morphs. For example, flower width (BUDW and CLBW), KSL, PSTH, PSTW, and POLN were not significantly different between pin and thrum morphs, and stamen filament length (SFIL) and the style cross-sectional size (STYLECA and TRANSCA) were similar between pins and homostyles. PAPIW was not statistically different between thrums and homostyles.

Almost all floral traits differed significantly among at least two of the three lineages (following sequential Bonferroni adjustments; Table 4.3). In particular, SANL, POLN, POLS, PISL, PSTH, PSTA, TRANSCA, PAPIL, and PAPIW were highly significantly different among all three lineages. Many other floral traits differed significantly between either Lineages 2 and 3, or Lineages 1 and 3, but not between Lineages 1 and 2 (analysis not shown). These traits were KSL, BUDL, CFPL, CPTL, CTBL, SSIL, SINH, SANW, PSSL, PSTYL, PSTL, PSTW, STYLECA, and ASD. On the other hand, SFIL was not different between Lineages 1 and 3.

Differences among the three floral morphs varied among lineages for 20 of 26 studied traits (following sequential Bonferroni adjustments; interaction term in Table 4.3), including flower size (BUDL, BUDW, CFPL, CPTL, CLBW, and CTBL), stamen height (SSIL, SINH, and SFIL), anther length (SANL), pollen size (POLS), pollen number (POLN), pistil length (PISL, PSSL, and PSTYL), style cross-sectional size (STYLECA and TRANSCA), stigma papilla size (PAPIL and PAPIW), and functional anther-stigma distance (ASD).

4.5. Discussion

4.5.1. Distyly vs. homostyly

In most taxa studied, the distylous flower is larger than the descendant homostylous flower (Ganders, 1979a), and this was found in all three lineages of *Amsinckia*. This significance included those traits related to corolla size, sepal length, anther size, stigma area and stigma papilla sizes, style cross-sectional size, and pollen production (Tables 4.2 and 4.3; Fig. 4.3). A few traits, however, including pistil length, stamen insertion height, and pollen size, were similar between distyly and homostyly within each lineage. The lack of difference for these traits between distyly and homostyly resulted from the fact that the size for distyly was averaged from pin and thrum, and because the trait size in homostylous flowers was often smaller than that in pins but larger than that in thrums, or vice versa. This averaging diminished or cancelled the actual differences between homostyly and the two floral morphs of distyly.

Figure 4.3. A summary of size-variations of 26 floral traits among floral morphs and three evolutionary lineages in *Amsinckia*. Note: The trait sizes are based on numerical order without regard to statistical significance. Figure Abbreviations: All abbreviations of traits are explained in Table 4.1; L1, Lineage 1; L2, Lineage 2; L3, Lineage 3; P, Pin; T: Thrum; H, homostyle. The large homostyle of *A. spectabilis* is omitted.

Trait / Lineage		P > T			T > P		
		Thrum smallest		Homostyle smallest		Pin smallest	
		P > H > T	H > P > T	P > T > H	T > P > H	T > H > P	H > T > P
KSL	L1 L2 L3			•	• •		
BUDL	L1 L2 L3				• • •		
BUDW	L1 L2 L3			•	• •		
CFPL	L1 L2 L3				• • •		
CLBW	L1 L2 L3			•	• •		
CPTL	L1 L2 L3				• • •		
CTBL	L1 L2 L3				• • •		
POLN	L1 L2 L3			• • •			
POLS	L1 L2 L3				•	• •	
SANL	L1 L2 L3			•	• •		
SANW	L1 L2 L3				• • •		
SFIL	L1 L2 L3				•	• •	
SINH	L1 L2 L3					• • •	
SSIL	L1 L2 L3					• • •	
PAPIL	L1 L2 L3			• •	•		
PAPIW	L1 L2 L3	•		•	•		
PISL	L1 L2 L3	• • •					
PSSL	L1 L2 L3	• • •					
PSTYL	L1 L2 L3	• • •					
PSTH	L1 L2 L3			•	• •		
PSTL	L1 L2 L3				• • •		
PSTW	L1 L2 L3				•		•
PSTA	L1 L2 L3				• • •		
STYLECA	L1 L2 L3				• •		•
TRANSCA	L1 L2 L3				• •	•	
ASD	L1 L2 L3			• •	•		

Fig. 4.3

Among those traits that were significantly larger in distyly than in homostyly, many of them differed in degree among the three lineages of *Amsinckia*. These traits were mostly those associated with flower size, anther length, functional anther-stigma distance, cross-sectional style structure size, pollen size and production. The literature contains few detailed floral comparisons between distyly and homostyly within the same evolutionary lineage, so it is difficult to compare the evolution of homostyly in *Amsinckia* to that in other species. Nevertheless, within *Amsinckia*, the existence of lineage-specific differences between homostyles and distyles suggests that each of these three lineages is at a different evolutionary stage in the evolution of homostyly, or that the genetic basis differs.

When data were analyzed without regard to lineage, distyly differed from homostyly in almost all studied floral traits, especially in traits related to flower size, sepal length, anther size, stigma and papilla size, anther-stigma distance, cross-sectional style structure, and pollen production. This is in general agreement with other descriptions of the differences between distyly and homostyly (Darwin, 1877; Bir Bahadur, 1970a, 1970b; Ganders, 1979a; Shore and Barrett, 1985; Hamilton, 1990).

4.5.2. Evolution of homostyly

The *Amsinckia* taxa with small, homostylous flowers are considered to be derived from distylous taxa (Ray and Chisaki, 1957b; Schoen et al., 1997). The similar anther and stigma heights within the small homostyles are not achieved by combining the ancestral thrum stigma position with the ancestral pin anther position. Instead, the small homostyles possessed values intermediate between pins and thrums for those traits

determining anther and stigma height. Specifically, this study included six such traits, three directly related to anther height (SFIL, SINH, SSIL) and three to stigma height (PISL, PSSL, PSTYL). Among these six traits, homostyles were intermediate between pins and thrums within a lineage in 17 of the 18 comparisons (Fig. 4.3). The single exception was free filament length (SFIL) in *A. spectabilis*. For the remaining 20 traits not directly related to anther or stigma height, small homostyles possessed smaller values than both pins and thrums in 54 of the 60 comparisons (Fig. 4.3).

The evolution of the homostylous flower can be compared among lineages both in kind and in degree. As described, homostylous *Amsinckia* flowers were intermediate between pins and thrums in absolute anther and stigma height but smaller in almost all other traits. In fact, the size order of traits in pins, thrums and homostyles was identical in 42 of the 78 comparisons (Fig. 4.3), indicating that homostyly has evolved in broadly similar ways in the three lineages, at least in Lineages 1 and 2. These similarities in kind among the three lineages existed despite the differences in degree found in the majority of traits (interaction term in Table 4.3).

4.5.3. Pin vs. thrum

Amsinckia furcata, *A. douglasiana*, and *A. spectabilis* exhibit the typical floral morphological syndrome of distylous species. Each of these species consists of two forms of individuals that reciprocally differ in both stigma and anther heights in the flowers. The pin flower has a relatively high stigma and low anthers, while the thrum flower has high anthers and a low stigma. The difference in pistil length or the height of the stigma between the two floral morphs is caused almost wholly by a difference in style length.

The other two components of the pistil length, the stigma thickness and the ovary height (latter not included in this paper), were not significantly different between the two morphs in this study, similar to results in other distylous species (Richards and Barrett, 1992). The differences of the two floral morphs in the three distylous species also include more than 20 other traits (Table 4.3; Fig. 4.3). The dimorphism of many traits was in agreement with what has been observed in other distylous species (Table 4.4; Bir Bahadur, 1968; Ornduff, 1971, 1976; Dulberger, 1973, 1974, 1992; Ganders, 1979a, 1979c; Philipp and Schou, 1981; Murray, 1990; Riveros et al., 1995). In particular, the stigma papilla size tended to be larger in pins than in thrums except in *A. spectabilis*, whereas style cross-sectional area, style transmitting-tissue area, stamen filament length, and pollen were larger in thrums.

4.5.3.1. *Relative reciprocity ratios*

Richards, Lloyd and Barrett (Richards and Koptur, 1993) have developed an index [(Anther height – Reciprocal stigma height)/(Anther height + Reciprocal stigma height)] to express the relative reciprocity ratio for pin organ level vs. thrum organ level for distylous species. The index is calculated separately for long and short organ levels; it quantifies reciprocal herkogamy and allows comparison of the reciprocity among heterostylous species. Reciprocal herkogamy is generally regarded as the defining feature of distyly (Arroyo and Barrett, 2000). If a species is perfectly reciprocal, the index should equal zero for both organ levels. The relative reciprocity indices for the long organ level vs. short organ level in three distylous species of *Amsinckia* are very close to zero (Fig. 4.4), especially in *A. furcata* (index = 0.004 and –0.038 for long and short organ levels,

Table 4.4. Floral trait size dimorphisms between pin (P) and thrum (T) flowers in three distylous species of *Amsinckia*. A general dimorphic status of floral traits in most other studied distylous species (listed as General) found in the literature are included for comparison. "≈" indicates lack of a statistically significant difference. For comparisons without regard to statistical significance, see Fig.4.3.

Traits	**General**	***A. furcata***	***A. douglasiana***	***A. spectabilis***
Sepal Length (KSL)	–	P ≈ T	P ≈ T	T > P
Flower length (BUDL)	–	T > P	T > P	P ≈ T
Flower width (BUDW)	–	P ≈ T	P ≈ T	P ≈ T
Fused petal length (CFPL)	–	T > P	T > P	P ≈ T
Corolla lobe width (CLBW)	–	P ≈ T	P ≈ T	P ≈ T
Petal length (CPTL)	–	T > P	T > P	P ≈ T
Corolla tube length (CTBL)	T > P	T > P	T > P	T > P
Pollen production (POLN)	P > T	P ≈ T	P > T	P ≈ T
Pollen size (POLS)	T > P*	T > P	T > P	T > P
Anther length (SANL)	T > P*	T > P	T > P	P ≈ T
Anther width (SANW)	–	T > P	T > P	T > P
Stamen filament length (SFIL)	T > P	T > P	T > P	T > P
Stamen insertion height (SINH)	–	T > P	T > P	T > P
Stamen height (SSIL)	T > P	T > P	T > P	T > P
Stigma papilla length (PAPIL)	P > T	P > T	P > T	T > P
Stigma papilla width (PAPIW)	P > T	P > T	P > T	T > P
Pistil length (PISL)	P > T	P > T	P > T	P > T
Style and stigma length (PSSL)	–	P > T	P > T	P > T
Style length (PSTYL)	–	P > T	P > T	P > T
Stigma thickness (PSTH)	–	P ≈ T	P ≈ T	P ≈ T
Stigma length (PSTL)	–	T > P	P ≈ T	P ≈ T
Stigma width (PSTW)	–	T > P	P ≈ T	P ≈ T
Stigma area (PSTA)	–	T > P	P ≈ T	P ≈ T

Table 4.4. Continued.

Traits	General	*A. furcata*	*A. douglasiana*	*A. spectabilis*
Style cross-sectional area (STYLECA)	T > P	T > P	T > P	T > P
Style transmitting-tissue area (TRANSCA)	T > P	T > P	T > P	T > P
Functional distance between anther and stigma (ASD)	P ≈ T*	P > T	P > T	P ≈ T

* Exceptions or opposite results exist in some cases.

– Either no common results or no information was found.

> Significantly larger ($P < 0.05$) than.

≈ Size difference is not significant at $P = 0.05$ level.

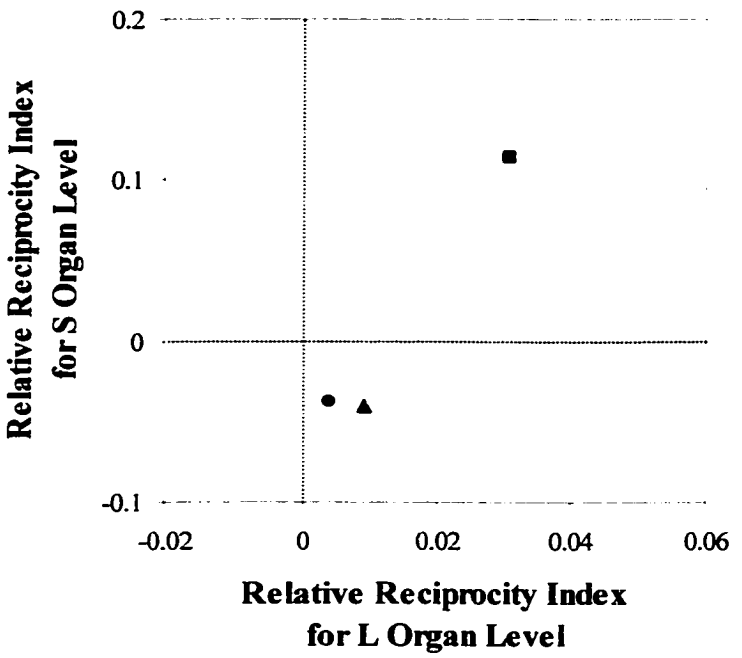

Figure 4.4. Relative reciprocity ratio for the long organ level vs. the short organ level for three distylous species of *Amsinckia*. Figure legends: ● *A. furcata*; ▲ *A. douglasiana*; ■ *A. spectabilis*.

respectively) and *A. douglasiana* (index = 0.009 and –0.040 for long and short organ levels, respectively). Although *A. spectabilis* has a higher relative reciprocity index (0.031 and 0.114 for long and short organ levels, respectively), it is still much lower than that in many other distylous species which can have indices close to ±0.15 or more (Richards and Koptur, 1993). *Amsinckia* is thus typical of distylous species and differs from another member of the Boraginaceae, *Anchusa officinalis* (Boraginaceae), which shows no evidence of reciprocal position of stigma and anthers in the two morphs, because the difference between the two morphs is much greater for the style length than for the anther height (Dulberger, 1970; Philipp and Schou, 1981). This species is considered to be stigma-height dimorphic rather than distylous (Baker, 2000).

Although *A. furcata* and *A. douglasiana* have typical reciprocity in their stamen and stigma positions in the two morphs, their pins have larger functional distance between stigma and anthers (ASD) than thrums do. This is similar to the situation in *Pentas lanceolata* (Rubiaceae; Bir Bahadur, 1970a) and *Jasminum fruticans* (Oleaceae; Thompson and Dommée, 2000), but is contradictory to what was found in *Quinchamalium chilense* (Santalaceae; Riveros et al., 1987), *Erythroxylum laurifolium*, *E. hypericifolium*, *E. sideroxyloides* (Erythroxylaceae; Pailler et al., 1998), and in two species of *Cordia* (Boraginaceae; Opler et al., 1975), in which the anther-stigma separation appears to be greater in thrums than in pins.. The significant difference in ASD between pins and thrums in *A. furcata* and *A. douglasiana* is related to the difference in anther length (SANL), which is significantly longer in thrums than in pins, and is probably also associated with the degree of bending of the style in the flowers. In *A.*

spectabilis, both ASD and SANL are not significantly different between pin and thrum flowers.

4.5.3.2. *Papillae, style and stigma*

The dimorphism of stigma papilla size (P > T in both papilla length and width) in *Amsinckia furcata and A. douglasiana* is similar to that in most other distylous species, such as *Anchusa hybrida* (Boraginaceae; Dulberger, 1970), *Linum pubescens*, *L. mucronatum* (Linaceae; Dulberger, 1967, 1973, 1974), *L. grandiflorum* (Dulberger, 1992), *Lythrum curtisii* (Lythraceae; Ornduff, 1978), *Menyanthes trifoliata* L. (Menyanthaceae; Nic Lughadha and Parnell, 1989), *Pentas lanceolata* (Bir Bahadur, 1970a), *Primula malacoides* (Pandey and Troughton, 1974), *P. obconica* (Dowrick, 1956), and *Pulmonaria obscura* (Boraginaceae; Olesen, 1979). In contrast, the stigma papilla size in *Amsinckia spectabilis* has an opposite dimorphism (T > P in both papilla length and width). Larger thrum papillae have also been reported in the distylous species *Amsinckia grandiflora* (Ornduff, 1976), *Luculia gratissima* (Rubiaceae; Murray, 1990), *Reinwardtia indica* (Linaceae; Bir Bahadur et al., 1984), and the stigma-height dimorphic species *Anchusa officinalis* (Schou and Philipp, 1984). The contrasting results found in *Amsinckia douglasiana* and *A. grandiflora* are interesting because of their very close phylogenetic relationship (Ray and Chisaki, 1957b; Schoen et al., 1997).

Because pin flowers in most studied distylous species have longer papillae, it has been suggested that the length of the stigma papilla is associated with the degree of style elongation, and that the elongation of both stigma papilla and style may have the same physiological basis (Dulberger, 1992). The existence, however, of a negative correlation

between stigma papilla length and style length found in *A. spectabilis* and the four other species mentioned above shows that the relationship between papilla and style differs among species. It thus appears doubtful that a common physiological basis underlies the elongation of papilla and style in all cases.

Similar to the relationship between stigma papilla size and style length, the relationship between stigma size (surface area) and style length also varies among distylous species. In all three of the distylous *Amsinckia* species studied here, thrums possessed larger stigmas than pins (Fig. 4.3), but the difference was statistically significant only in *A. furcata* (Table 4.2). The thrum stigma is larger in several other distylous species, including *A. grandiflora* (Ornduff, 1976), *Gelsemium sempervirens* (Loganiaceae; Bir Bahadur et al., 1984), *Hedyotis caerulea* (Ornduff, 1980), *Menyanthes trifoliata* (Nic Lughadha and Parnell, 1989), *Neanotis montholoni* (Rubiaceae; Bir Bahadur et al., 1984), *Primula malacoides* (Primulaceae; Pandey and Troughton, 1974), and *Palicourea lasiorrachis* (Rubiaceae; Feinsinger and Busby, 1987). In contrast, the pin stigma is larger in *Jepsonia heterandra* (Saxifragaceae; Ornduff, 1971), *Linum grandiflorum*, *L. mucronatum*, *L. pubescens*, *Plumbago capensis* (Plumbaginaceae; Dulberger, 1992), and many other distylous species (Dulberger, 1992; Richards and Barrett, 1992). The statement that "morph-specific differences in stigma size are closely linked to the size of the stigmatic papillae" (Hermann et al., 1999) is therefore not true of all species. The evidence from a variety of distylous species thus suggests that both papilla size and stigma surface area can be modified independently of style length. The degree to which natural selection shapes the relationships among these three characters is unknown.

In contrast to style length, the style cross-sectional area and the style transmitting-tissue area are significantly larger in thrums than in pins in all three distylous species of *Amsinckia*. Although information regarding the size of style structures in other distylous species is very limited, the results found in *Amsinckia* are the same as what has been observed in *Primula obconica* (Dowrick, 1956) and *Linum pubescens* (Dulberger, 1992). This could indicate the existence of opposite dimorphisms between the style length and diameter. The inverse correlation between the length and the cross-sectional size (area) of the style, especially the transmitting-tissue size, may have a physical effect on pollen tube growth, perhaps promoting or allowing more pollen-tube growth in thrums. Thus, it could be a factor associated with a lower seed set in pins of some distylous species, such as *Primula obconica* (Dowrick, 1956).

4.5.3.3. *Anther and filament*

As in most other heterostylous plants (Ganders, 1979a), *Amsinckia* flowers are sympetalous. The filaments of stamens are inserted on the corolla tube (Fig. 4.2d). Thus, filament length, corolla tube length, and stamen insertion height can contribute to the anther height in a flower. All three of these anther-height-related traits are highly significantly larger in thrum than pin flowers in all three distylous species of *Amsinckia*. This indicates that all three traits play important roles in the dimorphism of anther height in the three distylous species. The situation is similar to that in many other heterostylous species (Richards and Barrett, 1992), but is different from that in *Jepsonia heterandra* (Ornduff, 1971), *Erythroxylum coca* (Ganders, 1979b), *Cordia alliodora* and *C. trichotoma* (Gibbs and Taroda, 1983) in which the filament is the trait primarily

responsible for anther height. It also differs from that in *Hedyotis caerulea* (Ornduff, 1980), where stamen insertion height determines the anther height, and in *Cordia sebestena* (Percival, 1974), *Gaertnera vaginata* (Rubiaceae; Pailler and Thompson, 1997), *Bouvardia ternifolia* and *Psychotria chiapenis* (Rubiaceae; Faivre, 2000), where the anther height mainly depends on the corolla tube length. Collectively, these studies suggest that the major contributing traits to anther height in a flower differ among heterostylous species.

Anther length in *A. furcata* and *A. douglasiana* is dimorphic, being larger in thrum than pin flowers. Furthermore, anther width in all three studied distylous species of *Amsinckia* is larger in thrums than pins. This is similar to many other distylous species, such as *Hottonia palustris* (Primulaceae), *Nymphoides indica* (Menyanthaceae), *Pulmonaria angustifolia* (Darwin, 1877), and *Lithospermum* (Boraginaceae; Johnston, 1952). Ganders' (1979a) study on *A. furcata* also showed the same result. The anther length in *A. spectabilis* was not significantly different between the two morphs in my study. Ganders' (1979a) study of *A. spectabilis*, however, reported that the thrum anther was larger. This could indicate that anther size varies among populations within a distylous species, although larger pin anthers have not been reported in other species.

4.5.3.4. *Pollen*

Pollen is larger in thrums than in pins in all three distylous species of *Amsinckia*. Pollen size dimorphism occurs in most distylous plants and thrum pollen is usually larger than pin pollen (Ganders, 1979a; Dulberger, 1992; McKenna, 1992; Richards and Barrett, 1992; Guitian et al., 1998). The ratio of thrum to pin pollen size in most distylous species

varies from 1.06 to 1.80 (Dulberger, 1992). The ratios in *A. furcata*, *A. douglasiana*, and *A. spectabilis* are about 1.29, 1.40 and 1.14, respectively. The results in *A. furcata*, *A. douglasiana* are similar to what Ray and Chisaki (1957a) and Ganders (1976) found in the same species. Ornduff (1976) also observed similar pollen-size dimorphism in *A. grandiflora*. My result in *A. spectabilis*, however, differs from Ray and Chisaki's (1957a) observation, which showed no pollen-size dimorphism. It is known that exceptions to pollen-size dimorphism do exist. The absence of pollen-size dimorphism was reported in some distylous plants, such as in *Goniolimon tataricum* (Plumbaginaceae), *Limonium vulgare* (Plumbaginaceae; Weber, 1981), and *Linum pubescens* (Dulberger, 1973). It was also reported that thrum pollen was larger than pin pollen in one of the two studied populations of *Fauria crista-galli* (Menyanthaceae), but pin pollen was larger than thrum pollen in another nearby population of the same species (Ganders, 1979a). A significant difference in pollen size between populations of the same species was also observed in *A. furcata* (Ganders, 1976). Recent studies in tristylous species of the Lythraceae [*Decodon verticillatus* (Eckert and Barrett, 1994) and *Lythrum salicaria* (Mal and Hermann, 2000)] showed significant effects of populations on pollen size as well. Thus, the difference of the observations within *A. spectabilis* between this study and Ray and Chisaki (1957a) could be an indication of pollen-size variation among populations.

4.5.3.5. *Differences among lineages*

Most studied traits had similar dimorphisms between pins and thrums in all three lineages of *Amsinckia* (Tables 4.3 and 4.4; Fig. 4.3). There were, however, exceptions. Flower length (including petal length and fused petal length), anther length, and

functional anther-stigma distance were larger in thrum than pin flowers in *A. furcata* and *A. douglasiana*, but were not dimorphic in *A. spectabilis*. Differences in flower length were previously reported in *A. furcata*, *A. douglasiana* (Ganders, 1976), and *A. grandiflora* (Ornduff, 1976). Stigma length, width and area were dimorphic (T > P) only in *A. furcata*. The difference in pollen production between pin and thrum occurred only in *A. douglasiana*, in which pins produced more pollen than thrums. Pollen production in *A. furcata* and *A. spectabilis* was not significantly different between pin and thrum morphs in this study, though pins produced a little more pollen than thrums. Ganders (1975b), however, reported that thrum flowers produced more pollen in these two species. The reason for the contradictory results is not known. In most distylous species, pins produce more pollen than thrums (Ganders, 1979a; Dulberger, 1992). Sepal length was monomorphic in *A. furcata* and *A. douglasiana*, but it was longer in pin than in thrum in *A. spectabilis*, which is opposite to what Ganders (1979c) found in another member of the Boraginaceae, *Lithospermum cobrense* (Boraginaceae). The degree of dimorphism between pins and thrums differs among lineages for 12 out of 26 traits (Table 4.3).

The sizes of two flower-width traits (BUDW and CLBW) as well as stigma thickness (PSTH) showed no difference between pins and thrums in all three distylous species of *Amsinckia* included in this study. This result differs from some of the studies in other distylous species. For example, flower width (BUDW) was greater in thrums than in pins of *Luculia gratissima* (Murray, 1990) and *Lithospermum caroliniense* (Boraginaceae; Levin, 1968; Levin, 1972), but was larger in pins of *Anchusa officinalis* (Philipp and Schou, 1981). PSTH was not measured in most studies of dimorphism, but it was found to be larger in thrum than in pin of *Luculia gratissima* (Murray, 1990).

Overall, the size order of a trait in pins versus thrums was consistent in all lineages for 18 of 26 traits. In seven of the eight remaining traits *A. spectabilis* was the unusual lineage (Fig. 4.3). This pattern is consistent with phylogenies based on chloroplast DNA restriction-site divergence, in which the *A. furcata* and *A. douglasiana* lineages cluster in one clade, and the *A. spectabilis* lineage in another (Schoen et al., 1997).

CHAPTER 5

DISCRIMINANT ANALYSES OF DISTYLY AND HOMOSTYLY IN THREE EVOLUTIONARY LINEAGES OF *AMSINCKIA*

5.1. ABSTRACT

Using Canonical Discriminant Analyses (CDA) on 19 flower traits in *Amsinckia*, I searched for traits that best separate floral morphs and lineages among different groups: distyly vs. homostyly, pin vs. thrum vs. homostyle, pin vs. thrum vs. large-flowered homostyle vs. homostyle in *Amsinckia spectabilis*, and lineage *A. furcata – A. vernicosa* (L1) vs. lineage *A. douglasiana – A. tessellata gloriosa* (L2) vs. lineage *A. spectabilis* (L3) in *Amsinckia*. Functional anther-stigma distance and flower size are the key characters discriminating distyly from homostyly. Stamen height and especially its insertion height are the major traits discriminating the three floral morphs (pin, thrum and homostyle). Within *A. spectabilis*, pistil length, particularly the style length, is the major floral trait that discriminates the four floral morphs (pin, thrum, large homostyle, and small homostyle). One of the nondefinitional floral traits, stigma thickness, is the single most important trait discriminating the three evolutionary lineages. The overall size of stigma thickness among the three lineages is in the order of L1 > L2 > L3. I also performed CDA including only nondefinitional floral traits, in an attempt to find whether using nondefinitional traits alone can effectively discriminate different morphs and

lineages. The results not only varied in the degree of the discrimination, but also showed that the key discriminative traits differed depending on the groups being discriminated.

5.2. INTRODUCTION

Dimorphism is the typical floral morphological syndrome of distylous species, and it has been known for over a century (Darwin, 1877; Barrett, 1992a). The distinctive floral characters have been widely used as the key for identification of distylous and homostylous species, and the first time it was used as a key in the Boraginaceae was by Johnston in 1952. In the past two decades, several controversial models for the evolution of distyly have been proposed (Charlesworth and Charlesworth, 1979b; Lloyd and Webb, 1992a, 1992b; Richards, 1998). In related taxa containing both distylous and homostylous groups, it is generally believed that homostyly is derived from distyly (Ganders, 1975a, 1979a; Ganders et al., 1985; Barrett, 1989a). Distyly, as distinctive floral morphs of distylous species, occurs in at least 28 angiosperm families (Arroyo and Barrett, 2000; Barrett et al., 2000). Although homostyly usually characterizes a species, it can also occur as one of the floral forms within some heterostylous species (Bir Bahadur, 1968; Gibbs and Taroda, 1983). Variations of floral morphology in both distyly and homostyly from different taxa do exist (Ganders, 1979a; Dulberger, 1992; Richards and Barrett, 1992; Richards and Koptur, 1993). It is necessary to have quantitative morphological data and to thoroughly examine all the traits associated with either floral morphological

dimorphisms or monomorphism in order to understand the evolution and maintenance of the dimorphism and monomorphism, i.e., distyly and homostyly.

By minimizing the possible confounding effect of differences in evolutionary history among species, comparative studies among closely related taxa can achieve more convincing and "meaningful" conclusions (Harvey and Pagel, 1991). *Amsinckia* (Boraginaceae) is a genus consisting of both distylous and homostylous species, which exhibits a great diversity of mating systems, ranging from predominant cross-pollination, to intermediate cross-pollination to predominant self-pollination to nearly complete self-pollination (Ray and Chisaki, 1957a, 1957b; Ganders, 1975b; Ganders et al., 1985; Johnston and Schoen, 1995, 1996; Schoen et al., 1997). The evolution of homostyly from distyly has occurred at least four times independently in the genus (Ray and Chisaki, 1957b; Schoen et al., 1997). Three of these lineages are *A. furcata* to *A. vernicosa*; *A. douglasiana* to *A. tessellata gloriosa* (and *A. t. tessellata*); and large-flowered, distylous *A. spectabilis* to large-flowered, homostylous *A. spectabilis* to small-flowered, homostylous *A. spectabilis* (Fig. 5.1).

Homostyly is generally regarded as derived from the breakdown of heterostyly (Baker, 1966; Bir Bahadur, 1970b; Barrett et al., 1989; Barrett and Eckert, 1990; Lloyd and Webb, 1992a). In contrast to distyly, homostyly has flowers in which anthers and stigma are positioned at almost the same level. Most homostylous plants are self-pollinating and genetically self-compatible (Ganders, 1979a; Piper et al., 1986; Boyd et al., 1990; Tremayne and Richards, 1993). From the viewpoint of floral morphometrics, it is well-known that besides the heteromorphic stigma and anther heights, many other floral traits are also more or less associated with morphs (pin, thrum and homostyle;

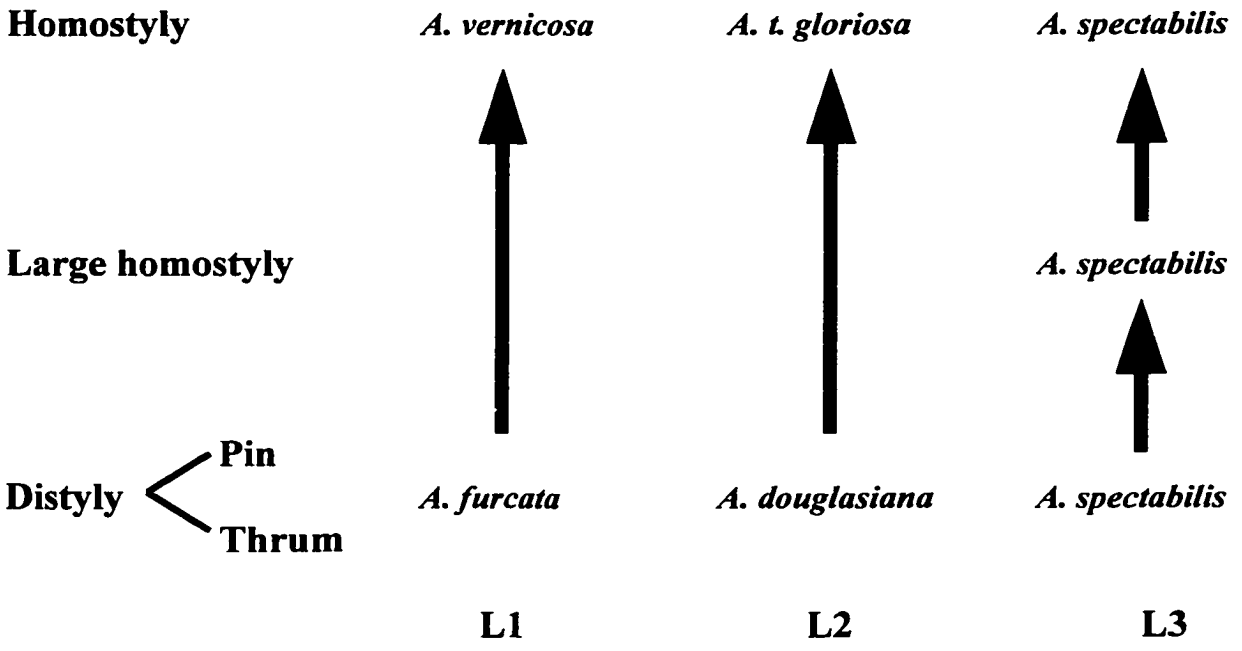

Figure 5.1. A total of 10 species-morph combinations belong to the three evolutionary lineages of *Amsinckia* studied here. L1: Lineage 1; L2: Lineage 2; L3: Lineage 3.

Ganders, 1979a; Barrett, 1992a; Barrett et al., 2000; Dulberger, 1992; Chapter 4). Furthermore, the type and degree of the relationships between floral traits and morphs often vary with different species and evolutionary lineages.

In order to find the most important traits differentiating floral morphs and lineages in *Amsinckia*, I performed Canonical Discriminant Analyses (CDA) on 19 major flower traits (Table 5.1). CDA is a multivariate statistical technique, specifically a dimension-reduction technique, related to principal component analysis and canonical correlation. It has been widely used by botanists and other researchers to determine which variables discriminate two or more naturally occurring groups by constructing a linear combination of the variables that has the highest possible multiple correlation with the groups, which thus maximize differences among groups relative to the variation within them (Astholm and Nyman, 1994; Cruz-Castillo et al., 1994; Marcoulides and Hershberger, 1997; Chandler and Crisp, 1998). The goal of this study was to find the most important floral traits that separate 1) distyly and homostyly; 2) pins, thrums and homostyles; 3) pins, thrums, large homostyles, and small homostyles within Lineage 3 (*A. spectabilis*); and 4) three evolutionary lineages of *Amsinckia*.

5.3. Materials and Methods

5.3.1. Species and floral morph types

A total of 10 species-morph combinations (groups) belonging to three evolutionary lineages of *Amsinckia* were studied (Fig. 5.1). For detailed descriptions of

Table 5.1. The 19 morphometric characters used in the CDAs. Morphometric measurements are illustrated in Figure 5.2.

Whorl	Characters	Abbreviation	Measurement scale
Corolla	Flower length	BUDL	mm
Corolla	Flower width	BUDW	mm
Corolla	Fused petal length	CFPL	mm
Corolla	Corolla lobe width	CLBW	mm
Corolla	Petal length	CPTL	mm
Corolla	Corolla tube length	CTBL	mm
Stamen	Anther length	SANL	mm
Stamen	Anther width	SANW	mm
Stamen	Free stamen filament length (i.e., portion not fused to petal)	SFIL	mm
Stamen	Stamen insertion height	SINH	mm
Stamen	Stamen height (anther height)	SSIL	mm
Pistil	Pistil length (stigma height)	PISL	mm
Pistil	Style and stigma length	PSSL	mm
Pistil	Style length	PSTYL	mm
Pistil	Stigma thickness (height)	PSTH	mm
Pistil	Stigma length	PSTL	mm
Pistil	Stigma width	PSTW	mm
Pistil	Stigma area $[PSTA = 2(PSTL \times PSTH) + 2(PSTW \times PSTH) + (PSTL \times PSTW)]$	PSTA	mm^2
(Pistil/Stamen)	Functional distance between anther and stigma	ASD	mm

species, morphs, and lineages see Chapters 3 and 4. Study samples were collected from the field in California in April and May 1995. Eight to fifteen inflorescences were studied in each species-morph group. Every inflorescence was taken from a different individual plant. Inflorescences were fixed in formalin-acetic acid-acohol (FAA), and then stored in 70% ethanol for later studies.

5.3.2. Measurements

At least three fully opened flowers on each inflorescence were dissected under an OLYMPUS SZH10 stereo microscope. Images of dissected floral parts were recorded using a video imaging system and computer that were connected to the microscope. Measurements of floral traits were performed on recorded images using the public domain NIH Image program (version 1.62, developed at the U.S. National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image/). For statistical analyses, only the flower with the largest traits on each inflorescence was used. The 19 quantitative traits used in the canonical discriminant analysis (CDA) are listed in Table 5.1. All measurements and traits are defined or illustrated in Figure 5.2.

5.3.3. Statistical analysis

I conducted a series of canonical discriminant analyses (CDA) using the two, three or four morphs (depending on comparison) or the three lineages as categories. In the CDA, individuals were used as replicates and floral styles, morphs or evolutionary lineages were used as groups. CDA determines the linear combination of input variables that maximizes the ratio of variance among groups to variance within groups. This

Figure 5.2 Dissected *Amsinckia* flowers, showing the morphometric characters and the measurement positions of various floral traits. All abbreviations in the figure are explained in Table 4.1 of Chapter 4 and Table 5.1. Magnifications vary among graphs. **a.** Longitudinal section of a live pin flower with natural shape (4x). **b.** Top view of a live thrum flower (2.5x). **c.** Stamen attached to corolla tube in a dissected thrum flower bud (20x). **d.** Anther (16x). **e.** Dissected flatted pin corolla (4x). **f.** Pistil (6x). **g.** Stigma surface [scanning electron micrograph (SEM; 120x)]. **h.** Top view of a stigma (SEM; 45x). **i.** Stigma (SEM; 60x). **j.** Longitudinal section of a portion of style [projected image from confocal laser scanning microscope (LSM; 180x)]. **k.** Cross section of a style (LSM; 200x).

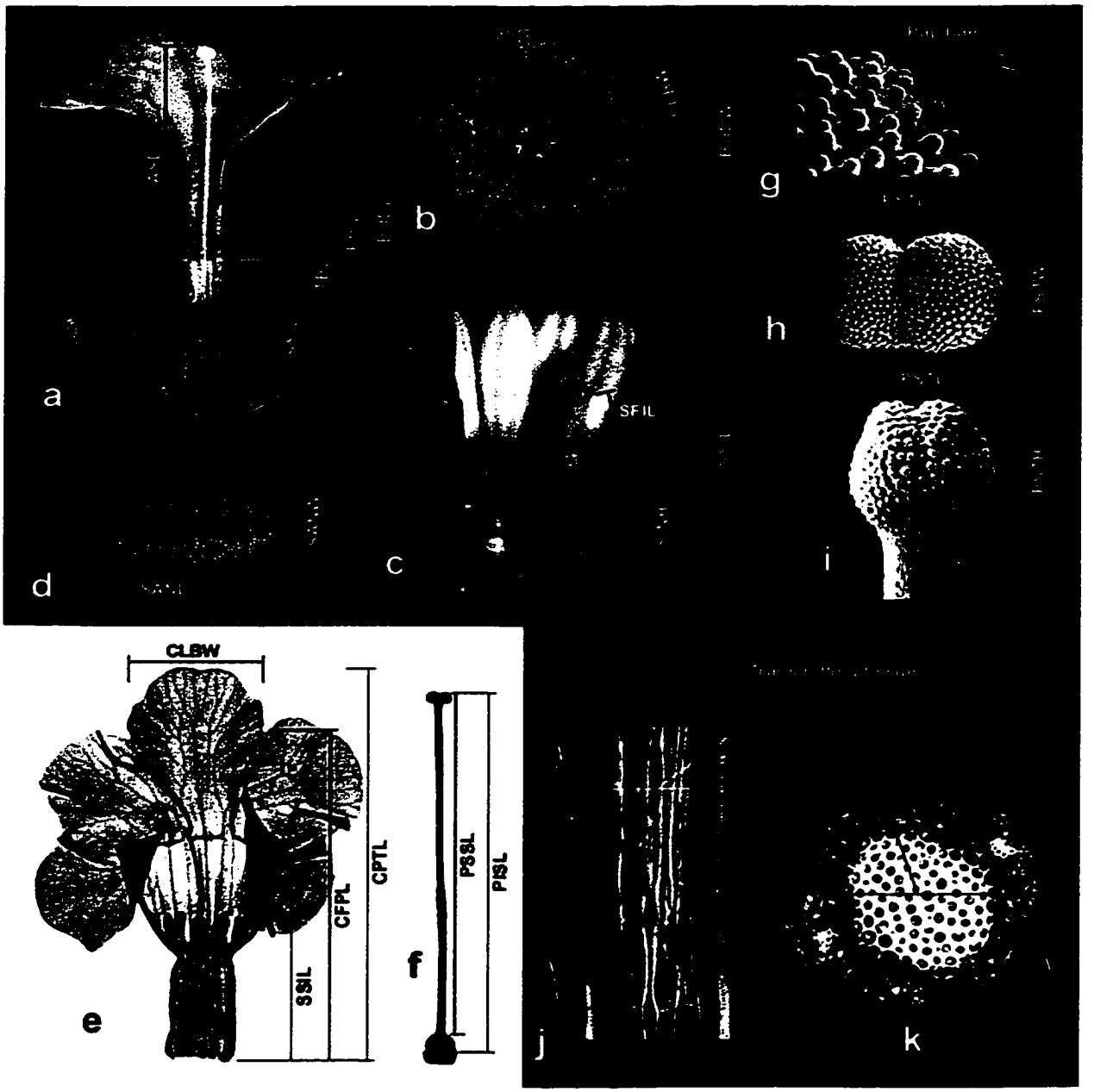

Figure 5.2

algorithm is repeated $N-1$ times, where N is the number of groups, each time with the restriction that each axis be uncorrelated with all previous axes. CDA generally requires multivariate normal distribution of variables and equality of within-group covariance matrices. However, CDA is to a large extent robust to violations of these assumptions (Sneath and Sokal, 1973; Thorpe, 1976; Klecka, 1980; Marhold, 1996). The study included 90 individuals. Sample size were often less than 90 because either the comparison did not include all morphs, or individuals had missing values for some traits, causing CDA to exclude that individual. Some of the 19 traits used for the CDA were more or less correlated. Redundancy, however, is not a problem in the statistical analysis, because CDA will automatically downweight correlated variables and thus a group of correlated variables will only contribute once to the discrimination on the canonical axes (Eriksen, 1997). Canonical discriminant functions (CDFs) are the canonical weights of the original variables. They provide information about the discriminatory power of each variable. An attempt was made in interpreting each CDF and evaluating the contribution of each original variable to that CDF, as follows: Absolute values and signs of pooled within-groups canonical structure coefficients were used to rank variables in the order of their contribution and to characterize the function (Marcoulides and Hershberger, 1997; Chapter 5). A one-way univariate analysis of variance (ANOVA) was performed in order to partition the variance among and within comparison groups for the major discriminating characters.

Both Wilks' lambda (Λ) and the canonical correlation coefficients indicate the degree of contribution of CDF to the overall separation of groups. The larger the value of Λ, the greater is the within-groups variation as a proportion of the total variation, and the

less successful is the CDF in separating the groups. The canonical correlation indicates the ratio of between-groups to total variance estimates along the CDF. As this correlation approaches one, the function is more successful in separating the groups.

CDA was performed using three different data sets: "All," "All excluding LH" and "Nondefinitional." Data "All" included all available species, populations and traits. Data "All excluding LH" was similar to the data "All" but excluded a population with large homostylous flowers in *Amsinckia spectabilis*, because it did not represent the typical homostylous condition. Ganders (1975a) termed it a "mixed population." In general, because of the very definition of pins, thrums and homostyles the differences of floral morphometrics among pins, thrums and homostyles were primary associated with pistil height, stamen height and flower size (Ornduff, 1976; Ganders, 1979a; Barrett, 1992a; Dommee et al., 1992; Dulberger, 1992; Richards and Koptur, 1993; Chapter 4). In order to investigate how other floral traits, which are here called "nondefinitional traits," differed among the groups, a third data set named "Nondefinitional" that omitted pistil height, stamen height, and flower-size-related traits based on the data "All excluding LH" was also created and analyzed. The CDAs were performed using the CANDISC procedure of SAS (version 6.12, Cary, North Carolina, 1999) on the mainframe computer of Dalhousie University. The ANOVAs were carried out with SYSTAT for the Macintosh (version 5.2, Evanston, Illinois, 1992).

5.4. RESULTS

5.4.1. The most important traits differentiating distyly and homostyly

In order to find the characteristics that best distinguished distyly from homostyly in *Amsinckia*, CDA was performed on three different data sets. The functional distance between anther and stigma heights, as well as characters related to flower size, particularly the corolla size, in both analyses on "All" and "All excluding LH" provided the best discrimination between distyly and homostyly (Table 5.2). The functional anther-stigma distance (ASD), flower length (BUDL), flower width (BUDW), petal length (CPTL), corolla lobe width (CLBW) and fused petal length (CFPL) in both all-traits analyses including or excluding LH played the largest roles in the canonical discriminant function (CDF), while CLBW had the highest value and contributed most to the CDF in all-traits analysis that excluded LH.

The discriminant analysis on nondefinitional traits, which excluded not only large-flowered homostyle morph (LH) in *A. spectabilis* but also all traits related to floral-morph-definition, found stigma length (PSTL) and anther length (SANL) to be the most important variables discriminating distyly from homostyly in *Amsinckia*. By examining the group means on the single discriminant function shown in Table 5.3, it was clear that the two groups of flowers, distylous and homostylous, were well separated, especially in all-trait analysis and in the analysis on all traits excluding LH.

In comparisons of three discriminant analyses using three different data sets on distyly and homostyly, stigma length (PSTL) and anther length (SANL) in nondefinitional-trait analysis were able to discriminate distyly from homostyly (Figs. 5.3c

and 5.4). This was supported by its high canonical correlations (0.80) and low Wilks' lambda ($\Lambda = 0.36$) which gave the proportion of the total variance in a CDA that was due to variation within groups – in this case floral morphs (Table 5.4). The null-hypothesis, that all means across floral morphs are equal, should therefore be rejected ($p < 0.0001$). This indicated that the CDFs in the nondefinitional-trait analysis were successful in separating distyly and homostyly in *Amsinckia*. However, flower size and particularly corolla size at both all-trait analyses (including or excluding LH) were obviously much more effective in differentiating the two styles (canonical correlation = 0.97, $\Lambda = 0.05$ and canonical correlation = 0.97, $\Lambda = 0.06$, respectively; Figs. 5.3a-b, 5.4; Table 5.4). Furthermore, the average size of floral traits in a homostylous flower was smaller when LH were excluded from the analyses, compared with that including LH in the analyses, and distyly was more effectively separated from homostyly (Fig. 5.4).

5.4.2. The most important traits differentiating pin, thrum and homostyle

With all groups and traits included (data "All"), the CDA on pins, thrums and homostyles first separated thrums from the other two groups, and next separated pins from homostyles (Fig. 5.5a). Stamen insertion height (SINH) was the trait most responsible for the first discriminant function (CDF_1; Table 5.5). The combined length of style and stigma (PSSL), especially style length (PSTYL), was important to CDF_1 as well (Table 5.5). On the other hand, the functional anther-stigma distance (ASD) was the most important contributing trait to the second discriminant function (CDF_2; Table 5.5). Flower-size, corolla-size, and pistil-height related variables also had higher positive structure canonical coefficients ranging from 0.22 to 0.36, which indicated that they were

Table 5.2. Structure canonical coefficients on one axis from canonical discriminant analyses based on the variables between distyly and homostyly in *Amsinckia* from three different data sets. See Figure 5.3 for the distribution of stylous types on the axis. Abbreviations of variables correspond to those in Table 5.1.

Variable		Traits included		
		All	**All excluding LH**	**Nondefinitional**
		CDF	**CDF**	**CDF**
Flower size	BUDL	**0.35**	**0.38**	–
	BUDW	**0.37**	**0.50**	–
Corolla	CTBL	0.27	0.26	–
	CPTL	**0.35**	**0.38**	–
	CLBW	**0.34**	**0.54**	–
	CFPL	**0.35**	**0.37**	–
Pistil	PISL	0.06	0.08	–
	PSSL	0.07	0.09	–
	PSTYL	0.06	0.09	–
	PSTH	0.12	0.11	0.33
	PSTL	0.25	0.27	**0.84**
	PSTW	0.12	0.11	0.34
	PSTA	0.19	0.18	0.55
Stamen	SSIL	0.06	0.07	–
	SINH	0.05	0.05	–
	SFIL	0.06	0.10	0.31
	SANL	0.24	0.22	**0.68**
	SANW	0.15	0.16	0.49
	ASD	**0.43**	**0.42**	–

Table 5.3. Group means on the single canonical discriminant function separating distyly from homostyly in *Amsinckia*.

Groups	All traits	All traits excluding LH	Nondefinitional traits
Distyly	3.14	2.68	0.86
Homostyly	-5.39	-6.14	-2.00

Table 5.4. General information from the canonical discriminant analyses of distyly and homostyly in *Amsinckia* (*df*, degrees of freedom; Num *df*, numerator degrees of freedom; Den *df*, denominator degrees of freedom; $P > F$, the probability level associated with the F statistic).

		Traits included		
		All	**All excluding LH**	**Nondefinitional**
Variables		19	19	7
Classes		2	2	2
N	Distyly	55	55	56
	Homostyly	32	24	24
	Total	87	79	80
df	Total	86	78	79
	Within classes	85	77	78
	Between classes	1	1	1
Wilks' lambda (Λ)	Value	0.054	0.056	0.363
	F	61.03	52.38	18.00
	Num *df*	19	19	7
	Den *df*	67	59	72
	$P > F$	0.0001	0.0001	0.0001
Canonical correlation	CDF_1 ($P > F$)	0.97 (0.0001)	0.97 (0.0001)	0.80 (0.0001)
Discriminatory power	CDF_1	1.0	1.0	1.0

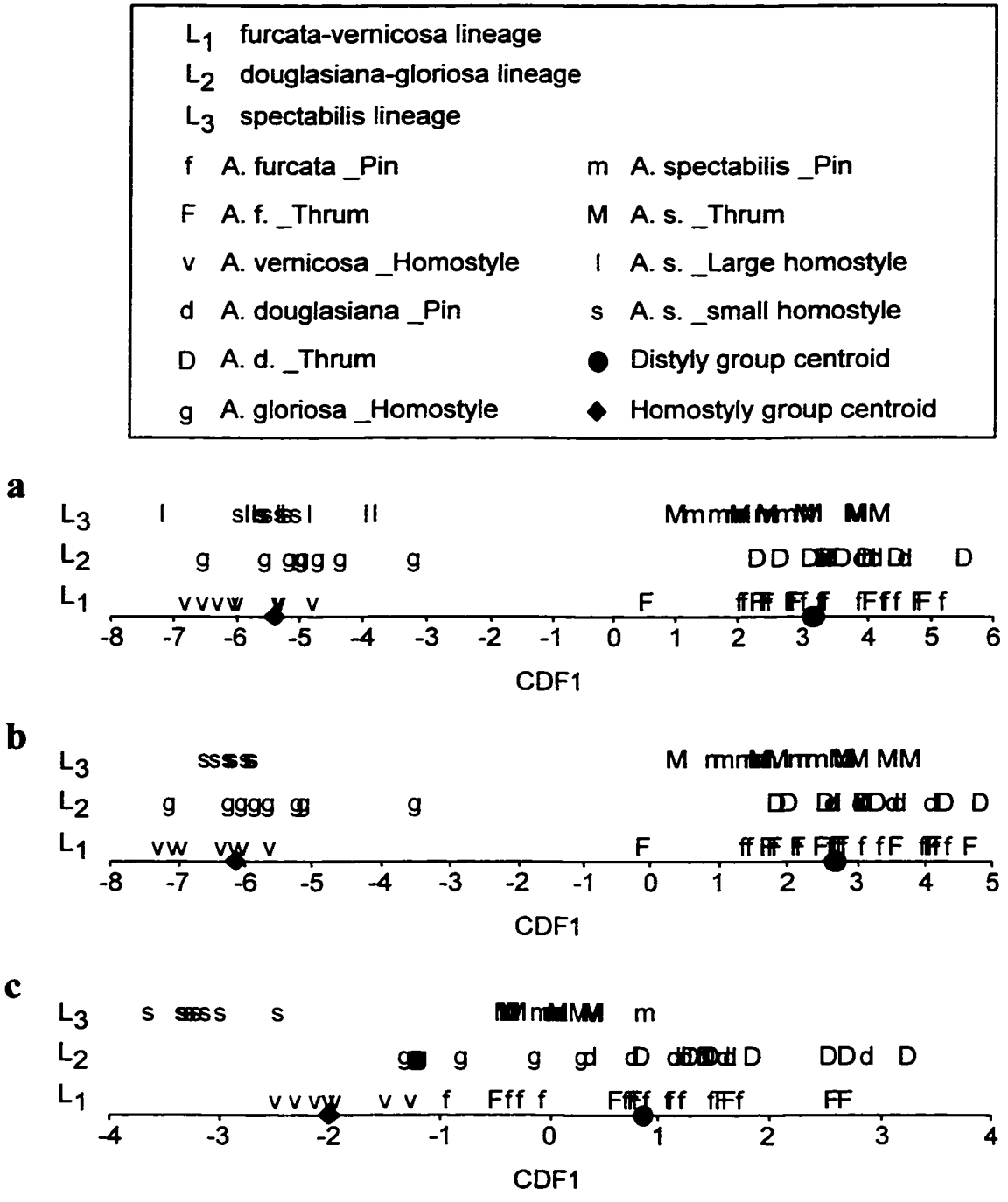

Figure 5.3. Scatter diagrams of species and floral morphs of *Amsinckia*, represented by the first canonical discriminant function (CDF_1). Each diagram shows the separation of distyly (pins and thrums) from homostyly. (a) CDA on all traits. (b) CDA on all traits excluding those from large-flowered homostylous *A. spectabilis*. (c) CDA on nondefinitional traits. See Table 5.2 for structure canonical coefficients.

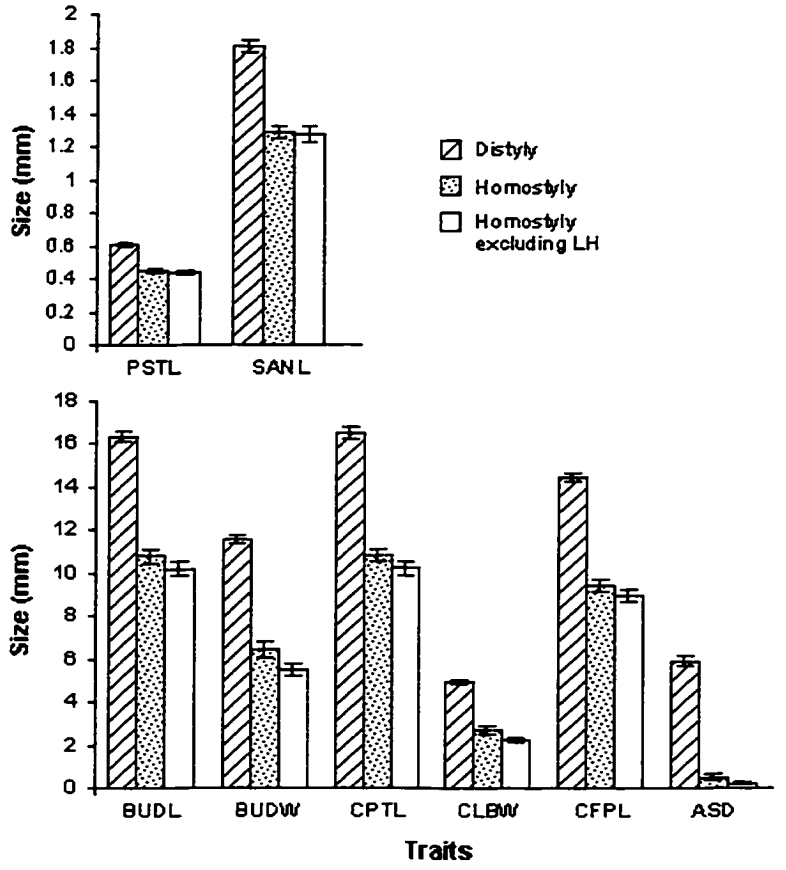

Figure 5.4. A comparison of the size (mean ± SE) of eight floral traits contributing most to the canonical discriminant function in separating distyly from homostyly in *Amsinckia*.

associated with the CDF_2 as well (Table 5.5). In the analyses with all-traits data, the CDF_1 and CDF_2 had discriminatory powers of 65.35% and 34.65%, respectively (Table 5.7). The high canonical correlation coefficients of the two CDFs (0.98 and 0.97 for CDF_1 and CDF_2, respectively; Table 5.7) indicated a high correlation between each function and the original variables.

When CDA was performed on "All excluding LH" data, two stamen-height traits (SSIL and SINH) and two pistil-height traits (PSSL and PSTYL) were the most important traits to CDF_1 (Table 5.5). Stamen height (SSIL) and especially the stamen insertion height (SINH) were actually the major variables responsible for the CDF_1. Thus, SSIL and SINH played the most important roles in separating the three floral morphs (pin, thrum, and homostyle).

The CDA on "ALL excluding LH" further showed that bud width (BUDW), corolla lobe width (CLBW), and anther-stigma distance (ASD) in a flower contributed most to CDF_2, although other flower-size, corolla-size, and pistil-height related variables were also associated with the CDF_2 (Table 5.5). The CDF_1 and CDF_2 explained 67% and 33% of the group separations, respectively (Table 5.7). The CDA had extremely low Wilks' lambda ($\Lambda = 0.002$) and high canonical correlation (0.99 and 0.97 for CDF_1 and CDF_2, respectively; Table 5.7). This indicated a great success in separating the three floral morphs by the discriminant functions, which was also supported by the group means of the morphs on two discriminant functions (Table 5.6). Furthermore, the effectiveness of discrimination was easily visualized on the scatter diagram of the distribution of three floral morphs on two discriminant functions (Fig. 5.5b). It was also supported by the enormous difference in actual size of these floral traits among the three

Table 5.5. Structure canonical coefficients on two axes from canonical discriminant analyses based on the variables among pin, thrum and homostylous flowers in *Amsinckia* from three different data sets. See Figure 5.5 for the distribution of morph types on the two axes.

Variables		Traits included					
		All		All excluding LH		Nondefinitional	
		CDF_1	CDF_2	CDF_1	CDF_2	CDF_1	CDF_2
Flower size	BUDL	-0.21	0.30	-0.15	0.38	–	–
	BUDW	-0.14	0.34	-0.09	**0.49**	–	–
Corolla	CTBL	-0.21	0.22	-0.16	0.26	–	–
	CPTL	-0.20	0.30	-0.15	0.38	–	–
	CLBW	-0.13	0.32	-0.10	**0.53**	–	–
	CFPL	-0.22	0.30	-0.17	0.37	–	–
Pistil	PISL	0.22	0.31	0.26	0.27	–	–
	PSSL	0.25	0.36	**0.34**	0.37	–	–
	PSTYL	0.26	0.36	**0.35**	0.37	–	–
	PSTH	-0.03	0.12	-0.01	0.11	0.12	0.34
	PSTL	-0.15	0.21	-0.11	0.26	**0.56**	**0.65**
	PSTW	-0.05	0.10	-0.03	0.11	0.19	0.28
	PSTA	-0.09	0.17	-0.05	0.17	0.30	**0.45**
Stamen	SSIL	**-0.38**	-0.12	**-0.38**	0.01	–	–
	SINH	**-0.38**	-0.16	**-0.39**	-0.04	–	–
	SFIL	-0.19	-0.04	-0.22	0.08	**0.77**	-0.15
	SANL	-0.12	0.21	-0.07	0.21	0.39	**0.55**
	SANW	-0.18	0.09	-0.16	0.15	**0.61**	0.19
	ASD	-0.10	**0.50**	-0.01	**0.50**	–	–

Table 5.6. Group means on the two canonical discriminant functions separating pin, thrum and homostylous flowers in *Amsinckia*.

Groups	Traits included					
	All		All excluding LH		Nondefinitional	
	CDF_1	CDF_2	CDF_1	CDF_2	CDF_1	CDF_2
Pin	3.36	4.71	4.95	3.67	-0.82	1.34
Thrum	-8.33	0.01	-8.12	1.13	2.45	-0.29
Homostyle	3.35	-4.42	2.28	-5.77	-1.50	-1.43

Table 5.7. General information of canonical discriminant analyses of pin, thrum and homostylous flowers in *Amsinckia*.

		Traits included		
		All	**All excluding LH**	**Nondefinitional**
Variables		19	19	7
Classes		3	3	3
N	Pin	30	30	31
	Thrum	25	25	25
	Homostyle	32	24	24
	Total	87	79	80
df	Total	86	78	79
	Within classes	84	76	77
	Between classes	2	2	2
Wilks' lambda (Λ)	Value	0.002	0.002	0.107
	F	73.46	70.84	20.85
	Num *df*	38	38	14
	Den *df*	132	116	142
	P > *F*	0.0001	0.0001	0.0001
Canonical correlation	CDF_1 (*P* > *F*)	0.98 (0.0001)	0.99 (0.0001)	0.86 (0.0001)
	CDF_2 (*P* > *F*)	0.97 (0.0001)	0.97 (0.0001)	0.76 (0.0001)
Discriminatory power	CDF_1	0.65	0.67	0.68
	CDF_2	0.35	0.33	0.32

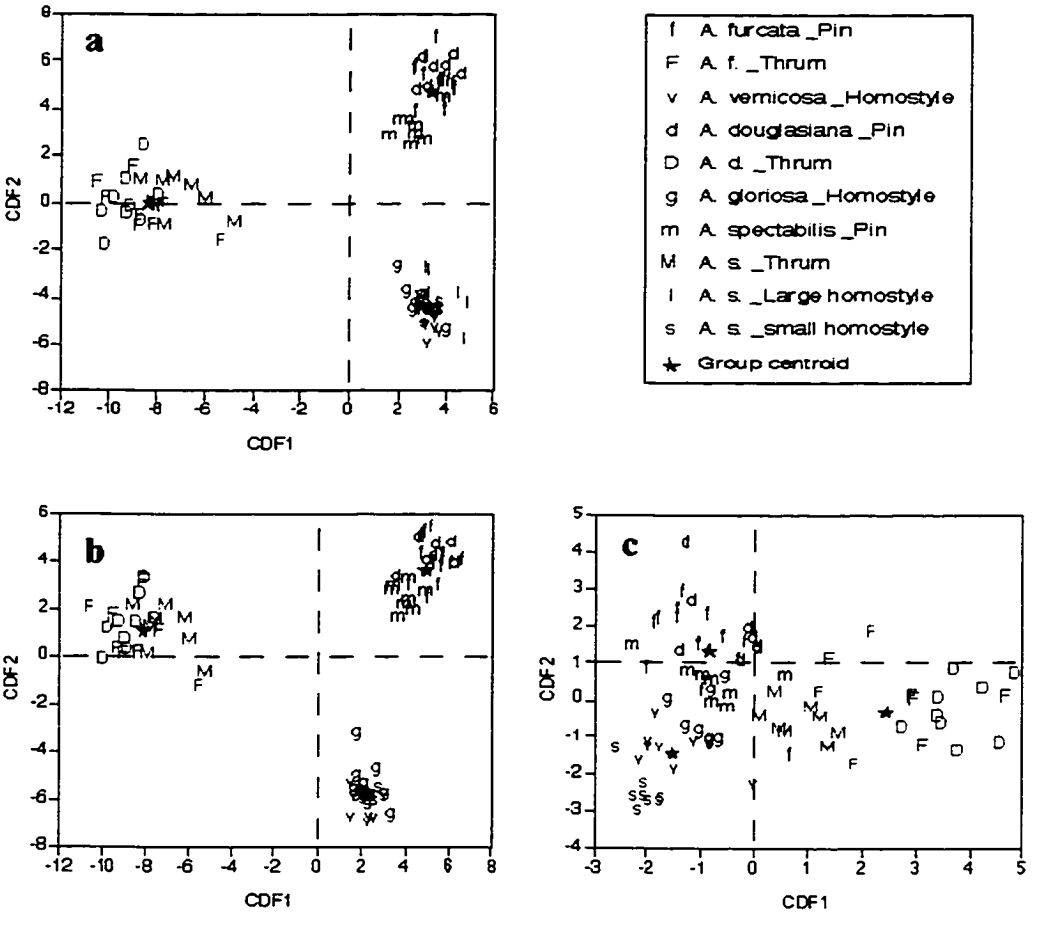

Figure 5.5. Scatter diagrams of species and floral morphs of *Amsinckia*, represented by the two canonical discriminant functions (CDF_1 and CDF_2). Each diagram shows the separation of the three floral morphs: pins, thrums and homostyles. (a) CDA on all traits. (b) CDA on all traits excluding those from large-flowered homostylous *A. spectabilis*. (c) CDA on nondefinitional traits. See Table 5.5 for structure canonical coefficients.

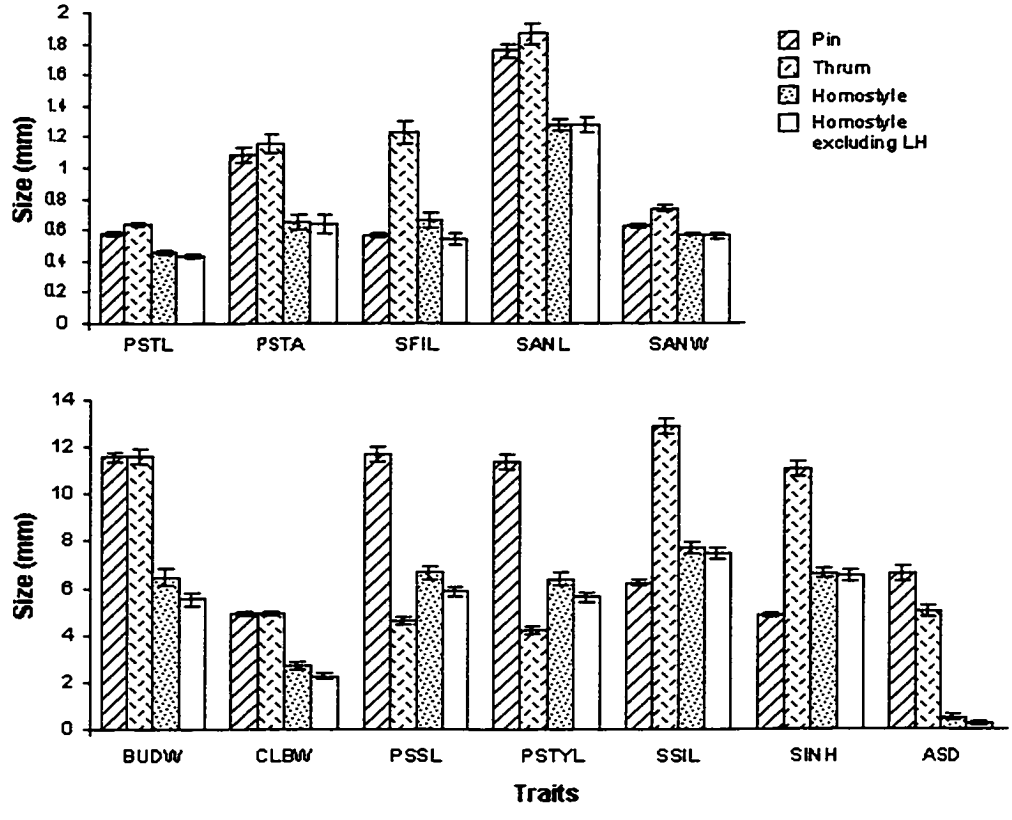

Figure 5.6. A comparison of the size (mean ± SE) of 12 floral traits contributing most to the canonical discriminant functions in separating pin, thrum, and homostylous flowers in *Amsinckia*. Note: The unit of PSTA is in mm^2.

different floral morphs (Fig. 5.6).

A similar discriminant analysis on nondefinitional traits showed that stamen filament length (SFIL), anther width (SANW) and stigma length (PSTL) in a flower were the major variables responsible for CDF_1, whereas PSTL, anther length (SANL) and stigma area (PSTA) for CDF_2 (Table 5.5). Similar to the results from CDA on "All excluding LH," CDF_1 and CDF_2 explained 68% and 32%, respectively, of the group separation among pin, thrum and homostyle in this analysis using only the nondefinitional traits (Table 5.7).

In comparison of the results from two CDAs on "All" and "All excluding LH," both CDAs were able to effectively separate pins, thrums and homostyles in *Amsinckia*. However, the Wilks' lambda, the canonical correlation of CDF, the discriminatory power (Table 5.7), the separation of group means on CDFs (Table 5.6), and the distribution of morphs on two CDF's axes (Fig. 5.5a-b), all indicated that the CDA on the data excluding LH could more effectively discriminate the three floral morphs in *Amsinckia*. This was supported by the lower means of most discriminating floral traits in the homostyle that excluded LH, compared with the means of the same traits in the homostyle that included LH (Fig. 5.6).

The CDA based on nondefinitional traits showed that its discrimination among the three groups was not as effective as the CDA using all traits either including or excluding LH, and there was some overlap on the two CDFs among three floral morphs (Fig. 5.5c). Both relatively higher Wilks' Lambda ($\Lambda = 0.13$) and lower canonical correlation (0.82 and 0.78 for CDF_1 and CDF_2, respectively) of CDA using nondefinitional traits indicated a weak separation of pins, thrums and homostyles.

5.4.3. The most important traits differentiating pin, thrum, large homostyle and small homostyle in *A. spectabilis*

There are four floral morphs within the species *A. spectabilis*. CDA on all traits showed that CDF_1 was mostly associated with traits related to pistil height (PISL), especially the style length (PSTYL) or style-stigma height (PSSL; Table 5.8). CDF_2, on the other hand, was mainly associated with the functional anther-stigma distance (ASD; Table 5.8). ASD was also one of the most important traits responsible for the third discriminant function (CDF_3), in which stamen height (SSIL), including stamen insertion height (SINH) and especially the filament length (SFIL), played the major roles (Table 5.8).

With data for four groups, the maximum number of CDFs is three. With all traits included, CDF_1 and CDF_2 together accounted for about 95% of the among-group variation (67% and 28% for CDF_1 and CDF_2, respectively) in *A. spectabilis* (Table 5.10). CDF_3 explained the remaining 5% of among-group variation. As CDF_3 accounted for negligible proportions of among-group variation, only CDF_1 and CDF_2 required further examination. The extremely low Wilks' lambda ($\Lambda = 0.000002$) and high canonical correlation (0.998, 0.995 and 0.976 for CDF_1, CDF_2 and CDF_3, respectively) indicated that the discrimination of four different floral morphs in *A. spectabilis* using CDFs was successful. This result was also supported by the means of each floral morph group on canonical discriminant functions (Table 5.9) and demonstrated in scatter diagram of floral morphs on three CDF axes (Fig. 5.7a). Since the first two discriminant functions (CDF_1 and CDF_2) accounted for 95% of the morph separations, it suggested that the height of

pistil, mostly of style, and the functional anther-stigma distance, among all traits, were the major discriminant variables in separating the four floral morphs in *A. spectabilis*. In discriminating the four floral morphs of *A. spectabilis* using nondefinitional floral traits, CDA showed that CDF_1 separated groups based on stigma-size-related traits, especially stigma width (PSTW), whereas CDF_2 separated groups mostly based on stamen filament length (SFIL; Table 5.8). CDF_3 separated morphs mainly relied on anther width (SANW) and stigma area (PSTA) in which mostly, in this case, contributed by PSTW (Table 5.8), although it accounted for only about 1% of the group separations (Table 5.10). CDF_1 alone explained more than 91% of the floral morph separation, while CDF_2 accounted for about 7% of the among-morph variation (Table 5.10). Due to their low discriminatory powers, CDF_2 and CDF_3, or at least CDF_3, are almost negligible. The CDA results further showed that in addition to PSTW, the structure canonical coefficients of three other stigma-size-related variables (PSTA, PSTL and PSTH) were also higher on CDF_1 (Table 5.8). This probably suggested that the size of the stigma was the major variable that separated the four different floral morphs in *A. spectabilis* when the traits directly related to floral-morph definition were excluded from consideration. This was supported by the evidence from Figure 5.7b, where CDF_1 separated the small homostyle from the other three larger-flowered morphs.

Nondefinitional traits could effectively discriminate four floral morphs in *A. spectabilis* (Table 5.9, Fig. 5.7b), although some overlap between pin and thrum morphs were present (Fig. 5.7b). Moreover, this analysis also had relatively higher Wilks' lambda ($\Lambda = 0.001$) and relatively lower canonical correlation (0.99, 0.91 and 0.71 for CDF_1, CDF_2 and CDF_3, respectively), compared to those from all traits analysis (see Table

Table 5.8. Structure canonical coefficients on three axes from canonical discriminant analyses based on the variables among pin, thrum, large homostylous and small homostylous flowers in *Amsinckia* from two different data sets. See Figure 5.7 for the distribution of morph types on the three axes.

Variable		Traits included					
		All			Nondefinitional		
		CDF_1	CDF_2	CDF_3	CDF_1	CDF_2	CDF_3
Flower size	BUDL	0.08	0.19	-0.02	–	–	–
	BUDW	0.14	0.18	-0.01	–	–	–
Corolla	CTBL	0.03	0.19	-0.02	–	–	–
	CPTL	0.09	0.19	-0.02	–	–	–
	CLBW	0.16	0.19	-0.01	–	–	–
	CFPL	0.08	0.19	-0.03	–	–	–
Pistil	PISL	**0.24**	-0.16	0.15	–	–	–
	PSSL	**0.29**	-0.21	0.18	–	–	–
	PSTYL	**0.29**	-0.22	0.18	–	–	–
	PSTH	0.09	0.16	0.16	0.28	-0.14	0.31
	PSTL	0.09	0.20	0.04	0.30	0.13	0.40
	PSTW	0.11	0.23	0.05	**0.36**	0.15	0.48
	PSTA	0.09	0.21	0.08	0.32	0.07	**0.52**
Stamen	SSIL	-0.02	0.22	**0.30**	–	–	–
	SINH	-0.04	0.20	**0.29**	–	–	–
	SFIL	0.04	0.10	**0.35**	0.18	**-0.60**	0.46
	SANL	0.08	0.14	-0.06	0.23	0.28	0.05
	SANW	0.04	0.13	0.12	0.19	-0.10	**0.56**
	ASD	0.11	**0.31**	**-0.31**	–	–	–

Table 5.9. Group means on the three canonical discriminant functions separating pin, thrum, large homostylous and small homostylous flowers in *A. spectabilis*.

Groups	Traits included					
	All			Nondefinitional		
	CDF_1	CDF_2	CDF_3	CDF_1	CDF_2	CDF_3
Pin	18.14	-1.66	-4.94	4.23	2.28	-1.10
Thrum	-8.24	15.36	0.01	5.08	0.75	1.43
Large Homostyle	9.14	-3.70	6.51	3.27	-3.26	-0.43
Small Homostyle	-19.04	-10.00	-1.58	-12.58	0.23	0.10

Table 5.10. General information from canonical discriminant analyses of pin, thrum, large homostylous and small homostylous flowers in *A. spectabilis*.

		Traits included	
		All	**Nondefinitional**
Variables		19	7
Classes		4	4
N	Pin	8	8
	Thrum	8	8
	Large Homostyle	8	8
	Small Homostyle	8	8
	Total	32	32
df	Total	31	31
	Within classes	28	28
	Between classes	3	3
Wilks' lambda (Λ)	Value	0.000002	0.0014
	F	50.62	26.66
	Num *df*	54	21
	Den *df*	34	64
	P > *F*	0.0001	0.0001
Canonical correlation	CDF_1 (*P* > *F*)	1.00 (0.0001)	0.99 (0.0001)
	CDF_2 (*P* > *F*)	1.00 (0.0001)	0.91 (0.0001)
	CDF_3 (*P* > *F*)	0.98 (0.0001)	0.71 (0.0037)
Discrimina-tory power	CDF_1	0.67	0.91
	CDF_2	0.28	0.07
	CDF_3	0.05	0.01

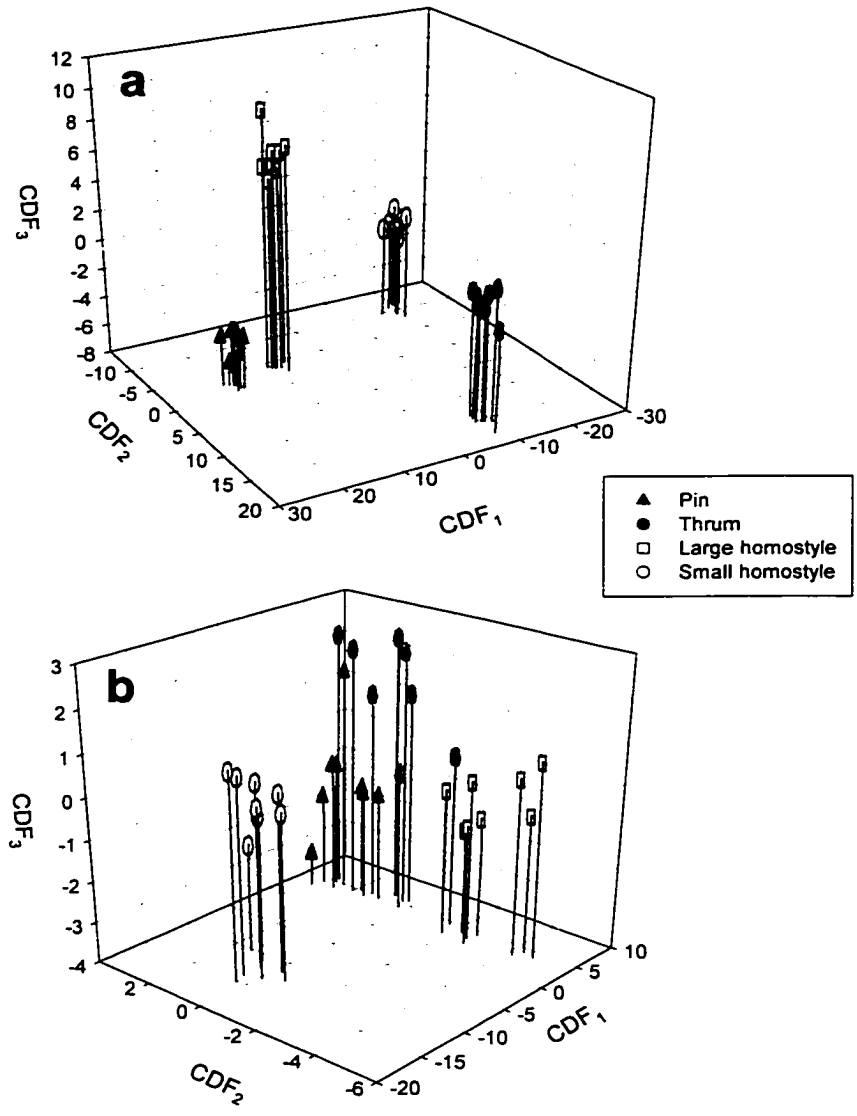

Figure 5.7. Three-dimensional scatter diagrams of the separation of four floral morphs (pin, thrum, large homostyle and small homostyle) of *A. spectabilis*, represented by the three canonical discriminant functions (CDF_1, CDF_2, and CDF_3), derived using (a) all morphometric variables, and (b) seven nondefinitional morphometric variables. See Table 5.8 for structure canonical coefficients.

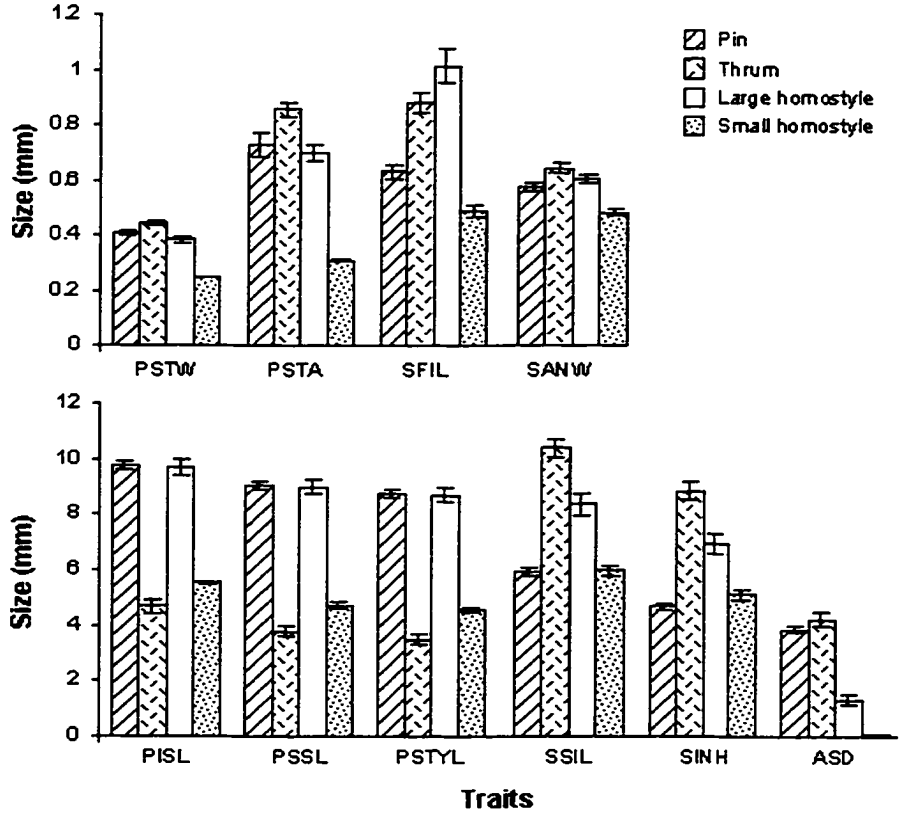

Figure 5.8. A comparison of the size (mean ± SE) of 10 floral traits contributing most to the canonical discriminant functions in separating pin, thrum, large homostylous, and small homostylous flowers in *A. spectabilis*. Note: The unit of PSTA is in mm^2.

5.10). Therefore, in this case, using all traits could more effectively discriminate four floral morphs in *A. spectabilis*, compared to use nondefinitional traits only. This was evidenced by the large size difference of all major discriminating traits among four floral morphs (Fig. 5.8).

5.4.4. The most important traits differentiating three evolutionary lineages

The CDA based on the data with all floral traits from all studied species and floral morphs showed that one of the stigma-size variables, stigma thickness (PSTH), was the single most important variable that was responsible for CDF_1. It accounted for more than 85% of the group separation among the three evolutionary lineages in *Amsinckia* (Tables 5.11, 5.13).

CDF_1 discriminated the *furcata–vernicosa* lineage from the other two lineages (Fig. 5.9a). The corolla-tube length (CTBL), anther length (SANL), and stigma length (PSTL) had highest values on CDF_2, which was responsible for almost 15% of the group variations among the three lineages (Tables 5.11, 5.13). CDF_2 separated the *A. douglasiana –A. t. gloriosa* lineage from the *A. spectabilis* lineage (Fig. 5.9a). The high canonical correlation (0.97 and 0.84 for CDF_1 and CDF_2, respectively) and low Wilks' Lambda ($\Lambda = 0.02$; Table 5.13) indicated that the two CDFs were successful in discriminating three evolutionary lineages in *Amsinckia*, in which the trait of stigma thickness played the most important role. This effective discrimination could also be seen from the mean CDF for each lineage (Table 5.12) and from the scatter plot of distribution of plants from three lineages on two CDF axes (Fig. 5.9a). The big difference in these floral traits among the three lineages supported that these traits were the major

contributing ones in separating the lineages in the view of flower morphometrics (Fig. 5.10).

When large-flowered homostylous *A. spectabilis* was excluded from CDA, the results showed that both CDFs were almost contributed by the same variables as they were in the analyses with all-traits data (Table 5.11). CDF_1 was mostly contributed by PSTH, whereas CDF_2 was by CTBL and PSTL. CDF_1 and CDF_2 explained about 87% and 13% of the group separations, respectively (Table 5.13). Comparing to the results of CDA on all traits, CDF_1 and CDF_2 from the analysis that excluded LH had a similar or even a little higher canonical correlation (0.98 and 0.86 for CDF_1 and CDF_2, respectively) and lower Wilks' Lambda ($\Lambda = 0.01$; Table 5.13). This suggested that the three lineages of *Amsinckia* might get a little better discriminations by CDA using the data that excluded LH. The suggestion was more or less supported by the group means (Table 5.12), and the distributions of plants from all three lineages (Fig. 5.9b), on two CDF's axes.

Using nondefinitional traits only, CDA showed that stigma thickness (PSTH) was also the only major variable that was responsible for the CDF_1, whereas anther size (SANL and SANW) and stigma length (PSTL) contributed most to the CDF_2 (Table 5.11). CDF_1 accounted for almost 97% of the separations among the three lineages in *Amsinckia* (Table 5.13). Whereas CDF_2 explained only about 3% of the group separations. CDF_1 had relatively high canonical correlation (0.95; Table 5.13), it separated the *furcata-vernicosa* lineage from the other two lineages pretty well (Fig. 5.9c). The CDF_2, however, had lower canonical correlation (0.4893), it failed to effectively discriminate *A. douglasiana – A. t. gloriosa* lineage from *A. spectabilis*

Table 5.11. Structure canonical coefficients on two axes from canonical discriminant analyses based on the variables among three lineages in *Amsinckia* from three different data sets. See Figure 5.9 for the distribution of lineages on the two axes.

Variable		Traits included					
		All		All excluding LH		Nondefinitional	
		CDF_1	CDF_2	CDF_1	CDF_2	CDF_1	CDF_2
Flower size	BUDL	-0.09	0.18	0.07	0.14	–	–
	BUDW	-0.07	0.06	0.06	0.05	–	–
Corolla	CTBL	-0.09	**0.30**	0.07	**0.24**	–	–
	CPTL	-0.09	0.18	0.07	0.14	–	–
	CLBW	-0.03	0.03	0.03	0.03	–	–
	CFPL	-0.08	0.22	0.07	0.17	–	–
Pistil	PISL	-0.13	0.06	0.13	0.07	–	–
	PSSL	-0.09	0.04	0.10	0.06	–	–
	PSTYL	-0.08	0.04	0.09	0.06	–	–
	PSTH	**-0.50**	0.06	**0.42**	-0.03	**0.60**	0.54
	PSTL	-0.06	**0.27**	0.06	**0.23**	0.05	**0.77**
	PSTW	-0.11	0.17	0.09	0.13	0.11	0.51
	PSTA	-0.25	0.18	0.21	0.12	0.28	0.64
Stamen	SSIL	-0.04	0.15	0.04	0.14	–	–
	SINH	-0.03	0.16	0.03	0.14	–	–
	SFIL	0.03	0.15	0.00	0.18	-0.02	0.49
	SANL	-0.19	**0.28**	0.16	0.20	0.20	**0.81**
	SANW	-0.12	0.23	0.11	0.20	0.13	**0.73**
ASD		-0.09	0.17	0.06	0.11	–	–

Table 5.12. Group means on the two canonical discriminant functions separating three lineages of *Amsinckia*.

Groups	Traits included					
	All		All excluding LH		Nondefinitional	
	CDF_1	CDF_2	CDF_1	CDF_2	CDF_1	CDF_2
A. furcata – *A. vernicosa* (L1)	-4.81	-0.49	5.08	-0.69	3.77	-0.10
A. douglasiana – *A. t. gloriorosa* (L2)	1.40	2.41	-1.43	2.45	-1.84	0.74
A. spectabilis (L3)	3.61	-1.33	-5.14	-1.56	-2.96	-0.65

Table 5.13. General information of canonical discriminant analysis of flowers in three lineages of *Amsinckia*.

		Trait size		
		All	**All excluding LH**	**Nondefinitional**
Variables		19	19	7
Classes		3	3	3
N	L1	31	31	31
	L2	24	24	25
	L3	32	24	24
	Total	87	79	80
df	Total	86	78	79
	Within classes	84	76	77
	Between classes	2	2	2
Wilks' lambda (Λ)	Value	0.019	0.013	0.072
	F	21.47	24.12	27.64
	Num *df*	38	38	14
	Den *df*	132	116	142
	P > *F*	0.0001	0.0001	0.0001
Canonical correlation	CDF_1 (*P* > *F*)	0.97 (0.0001)	0.98 (0.0001)	0.95 (0.0001)
	CDF_2 (*P* > *F*)	0.84 (0.0001)	0.86 (0.0001)	0.49 (0.0025)
Discrimina-tory power	CDF_1	0.85	0.87	0.97
	CDF_2	0.15	0.13	0.03

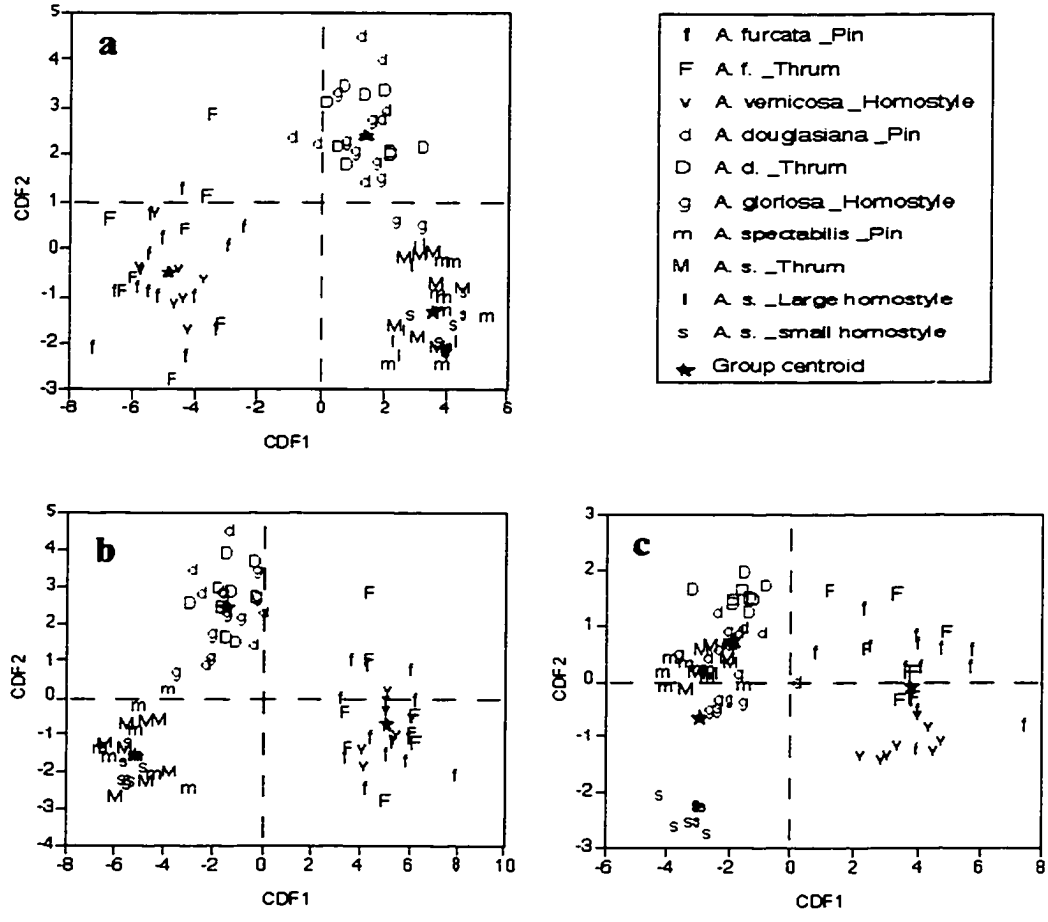

Figure 5.9. Scatter diagrams of species and floral morphs of *Amsinckia*, represented by the two canonical discriminant functions (CDF_1 and CDF_2). Each diagram shows the separation of the three evolutionary lineages: *A. furcata – A. vernicosa*, *A. douglasiana – A. t. gloriosa*, and *A. spectabilis*. (a) CDA on all traits. (b) CDA on all traits excluding those from large-flowered homostylous *A. spectabilis*. (c) CDA on nondefinitional traits. See Table 5.11 for structure canonical coefficients.

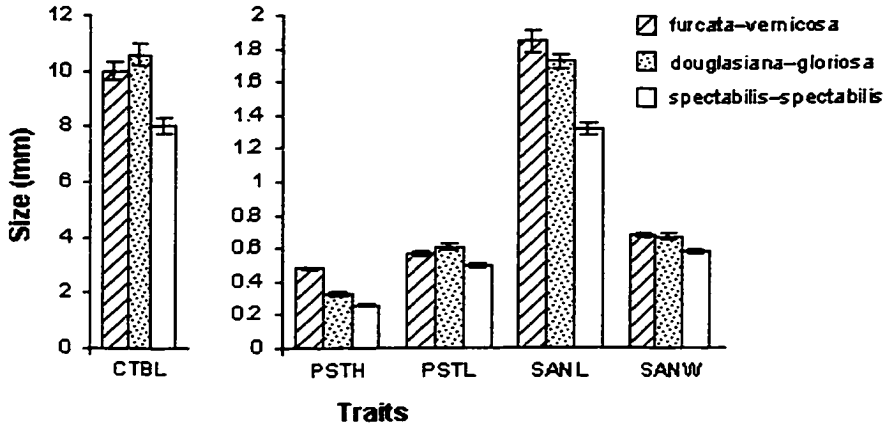

Figure 5.10. A comparison of the size (mean ± SE) of five floral traits contributing most to the canonical discriminant functions in separating flowers of three evolutionary lineages in *Amsinckia*.

lineage. Figure 5.9c showed that there were a lot of overlaps in the distribution of plants between the two lineages on two CDF's axes, especially between the homostylous *A. t. gloriosa* and the distylous *A. spectabilis*. The relative higher Wilks' Lambda ($\Lambda = 0.07$; Table 5.13) and the not far-apart group means on the two CDFs (Table 5.12) also indicated that the CDA based on nondefinitional traits did not discriminate the three lineages well.

5.5. Discussion

5.5.1. Differentiating distyly from homostyly

There is no doubt that the functional anther-stigma distance (ASD) in a flower is the key discriminating character between distyly and homostyly from both floral morphometrics and mating-system-related floral syndrome aspects. The ASD in a distylous flower is approximately 6 mm while it is close to zero in a homostylous flower of *Amsinckia* (Fig. 5.4; Li and Johnston, submitted). In addition, when all floral traits were included in a discriminant analysis, the size of some non-sexual directly related parts in a flower, particularly the flower size, was the most important trait in discriminating distyly from homostyly (Tables 5.2, 5.14). The flower length and width in a distylous flower is about 1.5 and 1.8 times larger than that in a homostylous flower, respectively (Fig. 5.4). Generally speaking, the size of a flower mostly depends on or is directly related to the size of the corolla, as this is also true in *Amsinckia*. The traits related to corolla size thus actually played the most important role in separating distylous

Table 5.14. The most important floral traits responsible for the discrimination of floral morphs and lineages in *Amsinckia*. Only the traits that contributed most to the CDF_1 are included.

Traits		All traits				All traits excluding LH			Nondefinitional traits only			
		D-H	P-T-H	P-T-LH-SH	L1-L2-L3	D-H	P-T-H	L1-L2-L3	D-H	P-T-H	P-T-LH-SH	L1-L2-L3
Flower size	BUDL	•				•						
	BUDW	•				•						
Corolla	CTBL											
	CPTL	•				•						
	CLBW	•				•						
	CFPL	•				•						
Pistil	PISL			•								
	PSSL			•			•					
	PSTYL			•			•					
	PSTH				•			•				•
	PSTL								•	•		
	PSTW										•	
	PSTA											
Stamen	SSIL		•				•					
	SINH		•				•					
	SFIL									•		
	SANL								•			
	SANW									•		
	ASD	•				•						

flowers from homostylous ones in *Amsinckia*. On the other hand, the sexual-reproduction directly related parts in a flower, i.e. the pistil and stamen, had very low values to the discriminant function (Table 5.2) because their overall sizes or heights in distyly is similar to those of homostyly (Li and Johnston, submitted). Consequently, they were not the most important traits in discriminating distyly from homostyly.

When large-flowered homostylous *A. spectabilis* was excluded from all-traits analysis, the traits that were largely responsible for the discriminant functions were not only similar to those from all-traits analysis but also often had higher values to the functions (Tables 5.2, 5.14). This suggested that the same floral traits could discriminate distyly from homostyly better when the large-flowered homostylous plants were excluded from the analyses. Furthermore, because the large-flowered homostylous plants in *A. spectabilis* are of a special type of homostyly in which some of its floral traits are very much different from those of regular homostyly, i.e., small-flowered homostyly, the study results will probably be much more meaningful and comparable to other homostylous plants only when the large-flowered homostylous plants are excluded from the analyses. Since the same reasoning and similar results also occurred in the rest of the analyses and comparisons in *Amsinckia*, the results of CDA from all-traits that excludes the large-flowered homostylous ones, therefore, will not be discussed further in this paper.

CDA using the nondefinitional floral traits, sometimes called "ancillary characters" (Richards and Barrett, 1992) or "ancillary features" (Lloyd and Webb, 1992a), unexpectedly showed that the stigma length (PSTL), along with the anther length (SANL), is most highly associated with the discriminant functions (Tables 5.2, 5.14). In other words, PSTL and SANL are the best morphometric variables in discriminating

distylous flowers from homostylous ones in *Amsinckia* besides the functional anther-stigma distance and the flower-size traits, specifically, the size of petals. Both PSTL and SANL in a distylous flower are approximately 1.4 times larger than those of a homostylous flower (Fig. 5.4).

In short, functional anther-stigma distance and flower size are the major discriminating traits separating distyly from homostyly in *Amsinckia*. The stigma length and the anther length are the best nondefinitional floral traits in discriminating the two style morphs in the genus. This is evidenced in all distylous and homostylous plants, i.e., a distylous flower has a conspicuous vertical spatial separation between its anthers and stigma while a homostylous flower lacks such vertical separation between its anthers and stigma. The fact that a distylous flower is larger than a homostylous one in *Amsinckia* is consistent with the results from other distylous and homostylous taxa (Ganders, 1979a; Dulberger, 1992). This also falls well into the generalization that the out-cross-pollinated flowers are usually larger than the self-pollinated ones (Ornduff, 1969; Wyatt, 1983). Both the large corolla size and high anther-stigma distance, especially the reciprocal positioning of anthers and stigma in pin and thrum flowers of distyly, can play important roles in insect-mediated outcross pollination between the two morphs in distylous species (Barrett, 1992a; Lloyd and Webb, 1992b; Richards, 1997). The spatial separation of anthers and stigma in a distylous flower have often been viewed as an "anti-selfing" device that can efficiently reduce self-pollination, self-fertilization, inbreeding depression, and thus increase female reproductive success (Webb and Lloyd, 1986; Barrett, 1992a). The small flower size in self-pollinated homostylous plants may indicate a reduced resource allocation to pollinator attraction in these plants, compared to

distylous plants. Furthermore, the close positioning of anthers and stigma in homostylous flowers tends to promote self-pollination or at least optimizes the precision of pollen transfer from anthers to stigma.

From the evolutionary point of view, the small self-pollinated flowers are usually believed to be derived from the large out-cross-pollinated flowers (Stebbins, 1957, 1974; Jain, 1976; Niklas, 1997), and homostyly often results from the breakdown of distyly (Ernst, 1955; Baker, 1966; Ganders, 1975a, 1979a; Ganders et al., 1985; Barrett, 1989a). From the adaptation point of view, it is generally believed that the large flower in outcross-pollinated plants can attract insect for pollinating while the small flower is a consequence of the evolution of self-fertilization (Guerrant, 1989; Delph et al., 1996). From flower development point of view, the small-sized flower may result from an evolutionary juvenilization, or paedomorphic ontogeny by progenetic process – earlier developmental offest (Guerrant, 1989). The formation of the small homostylous flowers in *Amsinckia* can either result from paedomorphic ontogeny through progenesis and neoteny (decreased developmental rate; e.g., in Lineages 1 and 2), or caused by both paedomorphic and peramorphic ontogenies through neoteny and hypermorphosis (delayed developmental offset; e.g., in Lineage 3; Li and Johnston, 2000; Chapter 6).

5.5.2. Differentiating pin, thrum and homostyle

Stamen height (SSIL) and especially its insertion height (SINH) are the key traits discriminating among all studied floral traits in separating the three floral morphs (pin, thrum and homostyle) in *Amsinckia* (Tables 5.5, 5.14). Both traits of SSIL and SINH in

thrums are approximately 1.7 times larger than those of homostyles, and about 2-2.5 times larger than those of pins (Fig. 5.6).

Similar to most distylous and homostylous plants whose flowers usually are sympetalous and often have a corolla tube (Ganders, 1979a), stamens are epipetalous in flowers of *Amsinckia*. Thus, both the stamen-insertion-height on corolla tube and the length of the corolla tube itself can contribute to the anther height, as is true in *Amsinckia*, in which the stamen-insertion-height is positively correlated with anther height and their sizes are in the order of thrum (T) > homostyle (H) > pin (P) in all three lineages. The situation that the stamen-insertion-height determines anther height was also observed in some other distylous plants, such as *Hedyotis caerulea* (Rubiaceae; Ornduff, 1980) and *Primula* spp. (Primulaceae; Richards, 1986). The corolla tube length, however, in thrums is significantly larger than that in both pins and homostyles and in the order of T > P > H in *Amsinckia* (Li and Johnston, submitted).

On the other hand, the polymorphism of anther-height among the three morphs are positively correlated with their pollen-size polymorphism in *Amsinckia* (Li and Johnston, submitted), which is in accordance with the results found in other distylous plants (Dulberger, 1992). Unlike in many other distylous species (Dulberger, 1992), however, the anther-height polymorphism in distylous *Amsinckia* did not show the negative correlation with the pollen-production dimorphism except in *A. douglasiana* (Li and Johnston, submitted). In *Amsinckia*, pins and thrums produced similar amount of pollen grains but approximately twice as much as homostyles. The pollen production in *A. furcata* and *A. spectabilis* did not show the significant difference between pin and thrum flowers found in many other distylous plants (Ganders, 1979a; Dulberger, 1992).

This may be related to the fact that pollen production varies from year to year even for the same population and the health condition of the plant (Ganders, 1979a). Repeated studies of pollen production within and among populations in different years should be able to clarify this. Nevertheless, pollen production was reported only in very limited distylous species, and it could vary among species as well.

The functional anther-stigma distance (ASD) is also important to the discrimination of the three morphs, but mostly it contributed to the separation of homostyles from pins and thrums. This is because stigma and anthers are positioned almost at the same level in a homostylous flower, but at different and reciprocal heights in pin and thrum flowers. The ASD is significantly larger in pins than in thrums in *A. furcata* and *A. douglasiana*, but the difference is not significant in *A. spectabilis* (Li and Johnston, submitted). The ASD, as seen in *A. spectabilis*, is supposed to be similar in pin and thrum flowers in distylous plants. This is because reciprocal dimorphism in stigma and anther heights is the distinctive feature of the distyly, although the reciprocity is often not perfect, as is the case in *A. furcata*, *A. douglasiana*, and many other distylous species (Ganders, 1979a; Dulberger, 1992; Li and Johnston, submitted). The ASD, therefore, does not differ significantly between pins and thrums in most distylous plants. As a result, it will not be seen as one of the major discriminating traits between the two morphs of distyly.

There is no doubt, as shown in the analyses of all floral traits, that pistil length and stamen height in a flower are the most important characters in distinguishing pin and thrum morphs in distylous plants. These two traits, however, have lower values in differentiating the three morphs: pin, thrum and homostyle. This mainly because the

stamen height is similar in pin and homostylous flowers, whereas the pistil length is similar in thrum and homostylous flowers (Li and Johnston, submitted).

When flower size, pistil length, and stamen height, which are directly related to floral morph definition, are excluded from the analysis, filament length, anther width, and stigma length are the major distinguishing floral traits in discriminating pins, thrums, and homostyles (Tables 5.5, 5.14). The size of the filament length is similar in pins and homostyles but it is significantly larger in thrums in all three lineages of *Amsinckia*, whereas both anther width and stigma length show the order of T > P > H in all three lineages (Fig. 5.6; Li and Johnston, submitted). The filament length plays an important role in positioning the anther level in a flower. Similar results were also reported in some other distylous species, such as *Oxalis suksdorfii* (Oxalidaceae; Ornduff, 1964), *Jepsonia heterandra* (Saxifragaceae; Ornduff, 1971), and *Erythroxylum coca* (Erythroxylaceae; Ganders, 1979b). In addition, stigmatic surface area and anther length are also important to the discriminations of the three morphs among nondifinitional floral traits in *Amsinckia*.

The discrimination among pins, thrums, and homostyles using all traits showed great success (Fig. 5.5). The same discrimination by using nondefinitional traits was relatively weak, and there was some overlap of the distribution of plants based on their canonical scores among the three morphs. This suggested that stamen height is the most discriminative trait among the three floral morphs, and the nondefinitional traits play supportive roles in the discrimination of the three morphs.

5.5.3. Differentiating pin, thrum, large homostyle, and small homostyle in *A. spectabilis*

A. spectabilis is a species that not only has typical distylous and homostylous populations, but also has a special type of population that Ganders (Ganders, 1975a) called "mixed population" and I call "large-flowered homostylous population." Plants in the large-flowered homostylous population have an intermediate floral form characterized by larger flowers that are similar to distylous ones, with pin type pistil length (stigma height does not differ significantly between larger-flowered homostylous and pin morphs), and mid-sized anther height (anther position in a larger homostylous flower is significantly higher than that in a pin and homostylous flower but significantly lower than that in a thrum flower; Chapter 4). Overall, the floral morph of this special population looks more like larger-sized homostylous flowers with a small protrusion of stigma above the anthers.

Among all floral traits, pistil length, particularly the length of the style or style plus stigma, is the major responsible floral trait that discriminates the four floral morphs (pin, thrum, large homostyle, and small homostyle) in *A. spectabilis* (Tables 5.8, 5.14), though the difference between pin and large homostyle was not significant (Fig. 5.8; Li and Johnston, submitted). The functional anther-stigma distance is also one of the major contributing characters in separating the four floral morphs. Specifically, it well separated large homostyle from pin, thrum and small homostyle, because it was significantly smaller in large homostyle than in pin and thrum but significantly larger than that in small homostyle (Fig. 5.8; Li and Johnston, submitted). Stamen-height-related traits play only limited roles in differentiating the four floral morphs. This is mostly because anther

height, stamen insertion height and filament length do not differ significantly between pin and small homostyle, while filament length also does not differ significantly between thrum and larger homostyle (Fig. 5.8; Li and Johnston, submitted).

Stigma width among nondefinitional traits is the trait that best discriminates the four floral morphs (Tables 5.8, 5.14). In addition, stamen filament length plays a supportive role in the discrimination. The discrimination of the four floral morphs, however, using the nondefinitional traits, was not very effective, as shown in Figure 5.7. It only separated the small homostyle from the other three floral morphs, because the major discriminating trait, stigma width, was not significantly different between pin and thrum, or between pin and large homostyle (Li and Johnston, submitted).

In short, style length is the most discriminative trait among all traits, while stigma width is the most important one among nondefinitional traits, in differentiating the four floral morphs in *A. spectabilis*. Using all traits, mainly the style length, can effectively separate the four floral morphs.

5.5.4. Differentiating three evolutionary lineages

Each of the three evolutionary lineages in *Amsinckia* consists of both distylous and homostylous species or populations, and thus has pin, thrum, and homostylous floral morphs. I was interested in finding the similarities and differences among the three lineages of *Amsinckia*, in terms of floral morphometrics, from which, furthermore, I wished to look for the clues of the evolutionary relationships among the three lineages. My study shows that one of the nondefinitional floral traits, stigma thickness (PSTH), is the single most important discriminative trait to the three evolutionary lineages, from all

three canonical discriminant analyses with three different data sets (Tables 5.11, 5.14). The overall size of PSTH among the three lineages is in the order of L1 > L2 > L3. However, the PSTH of homostylous flowers in L2 and L3 is similar. The analyses also suggest that a few other traits, including corolla tube length, stigma length, and anther length, play roles in separating the three lineages.

With regard to other floral traits, interestingly, except for a few anther- and stigma-height directly related traits which showed intermediate size in homostylous flowers compared with those in pin and thrum flowers, most studied floral traits are smaller in homostyle than in pin and thrum, in all three lineages of *Amsinckia* (see Chapter 4 for details). Moreover, most traits were in the same size order among pins, thrums and homostyles in all three lineages. In addition, the sizes of primary or definitional traits for the same type of floral morph across lineages are similar, and thus the three lineages can only be discriminated mainly by nondefinitional traits. Therefore, the distylous flowers of different species across the three evolutionary lineages of *Amsinckia* are similar, as are the homostylous flowers. This suggests that either the separate derivations of homostyly from distyly have occurred in similar ways or the different evolutionary pathways have led to similar morphologies in *Amsinckia*. Comparative floral development studies in the genus indicate that the later is probably true (see Chapter 6).

CHAPTER 6

COMPARATIVE FLORAL DEVELOPMENT AND EVOLUTION OF HOMOSTYLY FROM DISTYLY IN THREE EVOLUTIONARY LINEAGES OF *AMSINCKIA*

6.1. ABSTRACT

The developmental changes associated with the evolution of homostyly from distyly and with the differentiation of two distylous floral morphs have been studied by comparing floral ontogenies of homostylous and distylous flowers within and among three evolutionary lineages in *Amsinckia*, using floral morphometrics. Paedomorphosis through neoteny and progenesis was the major developmental mechanism responsible for the evolution of homostyly from distyly in all three lineages. Contributions from the extent of paedomorphosis, developmental dissociation, and changes of ontogenetic trajectories, in conjunction with some other developmental processes such as peramorphic ontogeny by acceleration, have resulted in the evolution of homostyly in different ways among lineages. Similar developmental mechanisms have led to the differentiation of pins from thrums in distyly independently in three evolutionary lineages of *Amsinckia*. Contrasting growth rates of stamen and pistil heights in distylous flowers have caused pin and thrum flowers to have the reciprocal arrangement of anther and stigma heights. The self-compatible distyly in *Amsinckia* is likely derived from some unidentified self-incompatible distyly by loss of self-incompatibility system. The unique ontogenetic

patterns of the large-flowered homostyly in lineage of *A. spectabilis* suggested that it may represent an intermediate morph in the evolution of homostyly from distyly. In general, multiple heterochronic processes were involved in the mosaic development and evolution of homostylous flowers. Convergence and parallelism may have also been involved in the evolution of homostyly and differentiation of two distylous flower morphs. Although comparative floral ontogenetic results support the assumption that the small self-pollinated homostyly was derived independently from the large outcross-pollinated distyly in three evolutionary lineages of *Amsinckia*, some similarities on patterns of ontogenetic differences between homostyly and distyly in lineages of *A. furcata – A. vernicosa* and *A. douglasiana – A. t. gloriosa* pose a question whether these two lineages might have been originated from a recent common ancestor.

6.2. INTRODUCTION

The evolution of selfing from outcrossing ancestors is one of the most common and important evolutionary transitions among flowering plants (Stebbins, 1974; Barrett and Eckert, 1990). It is also believed that the self-pollinated homostylous plants have evolved from the breakdown of outcross-pollinated heterostylous plants, especially in the taxa where there are mixed mating systems (Baker, 1966; Ornduff, 1972; Charlesworth, 1979; Charlesworth and Charlesworth, 1979a; Richards, 1986; Barrett, 1989a, 1995; Barrett et al., 1989; Barrett and Richards, 1990; Lloyd, 1992; Husband and Barrett, 1993; Johnston and Schoen, 1996).

A variety of evolutionary transitions have been recognized in distylous plants (Ganders, 1979a; Richards, 1986; Barrett, 1988), including the shift from outcrossing to different degrees of selfing through the evolution of homostyly [e.g., *Amsinckia* (Ganders et al., 1985); *Psychotria* (Hamilton, 1990); *Turnera ulmifolia* (Barrett and Shore, 1987)]. The frequent breakdown of floral polymorphism to monomorphism in distylous groups represents a model system for studies of the evolution of self-fertilization in plants (Barrett, 1992).

From a genetic point of view, distyly is hypothesized to be controlled by a supergene which consists of at least three loci (*gpa/gpa* for pin and *GPA/gpa* or *GPA/GPA* for thrum; Dowrick, 1956; Charlesworth and Charlesworth, 1979a; Lewis and Jones, 1992; Richards and Barrett, 1992). The shift from distyly to homostyly is often caused by crossing over within the supergene (*gPA/gpa* for most homostyle – long homostyly and *Gpa/gpa* for short homostyly) or sometimes by changes at modifer loci outside the supergene (Charlesworth and Charlesworth, 1979a; Ganders, 1979a; Lewis and Jones, 1992; Wedderburn and Richards, 1992; Tremayne and Richards, 1993; Fenster and Barrett, 1994). The genetic basis for the evolution of homostyly from distyly in *Amsinckia* is still uncertain (Ganders, 1979a; Barrett, 1992b), although it has been suggested that instead of crossing over, unlinked modifier genes may be responsible for the derivation of homostyly in the genus (Ganders, 1975a, 1979a).

The evolutionary shift from outcrossing to high levels of selfing in angiosperms is usually accompanied by major changes in floral morphology, which include reduction in flower number, flower size, floral organ size, and probably most importantly the anther-stigma distance (Wyatt, 1988; Barrett and Harder, 1992). These shifts also involve

changes in the timing of floral developmental processes (Guerrant, 1989; Diggle, 1992; Stewart, 1998 #1196). Most of this knowledge, however, is based on studies of relationships between mating system and mature floral morphology. Therefore, the mechanism of floral modifications in those plants and how it is related to the evolution of selfing from outcrossing is still not really understood. It has been suggested, however, that different developmental mechanisms are involved in the phenotypic variation or evolutionary change in floral traits both within heterostylous species and between heterostylous and homostylous plants, as well as between self-pollinated flowers and their herkogamous ancestors, although there are still few detailed structural analyses or developmental studies with which to test this prediction (Barrett and Harder, 1992; Richards and Barrett, 1992; Richards and Koptur, 1993; Stewart and Canne-Hilliker, 1998).

"Evolutionary developmental biology" (Hall, 1998) is a relatively new discipline that investigates the mechanistic relationship between "ontogeny and phylogeny" (Gould, 1977) and focuses on the influence of developmental mechanisms on morphological evolution and how ontogenetic processes are modified in phylogeny. It has been stated that, as a result of selection, "ontogenies evolve to produce phylogenies" (McKinney and Gittleman, 1995).

There are several different developmental mechanisms that can lead to phenotypic evolution, and heterochrony is perhaps the best known (Gould, 1977; McKinney and Gittleman, 1995; McNamara, 1995; Alberch and Blanco, 1996; Hall, 1998; Eble, in press). Heterochrony is a change in the relative timing of developmental processes (rate, initiation and/or termination) in a descendant relative to its ancestor. It has long been recognized as the developmental mechanism responsible for evolutionary change in

morphology, and is a major paradigm for understanding the role of developmental processes in evolution (Gould, 1977; Hall, 1983, 1998, 2001; Gould, 1992; Kluge, 1985; Fink, 1988; Swan, 1990; Diggle, 1992; McKinney and Gittleman, 1995; McNamara, 1995; Alberch and Blanco, 1996; Zelditch and Fink, 1996; Raff, 1996; Klingenberg, 1998). In other words, "rate and timing changes in ontogeny produce evolution" (McKinney and Gittleman, 1995).

Comparative floral developmental studies among related species thus can provide unique ontogenetic-phylogenetic information that is not deducible from their mature morphology. Quantitative approaches have been found to be useful sources of evidence for phylogenetic reconstruction and elucidation of mechanisms of floral evolution (Lord and Hill, 1987; Hufford, 1988a, 1997; Kampny et al., 1993). Heterochrony has been proposed as the evolutionary mechanism underlying the origin of the small, self-pollinating flowers from their large, outcrossing progenitors (Hill and Lord, 1990; Hill et al., 1992; Stewart and Canne-Hilliker, 1998). Comparative floral ontogenetic studies in a taxon with both distylous and homostylous plants, and/or with various mating systems, will have the best chance to be able to identify the kinds of ontogenetic modifications and mechanisms that underlie the evolution of flowers and mating systems. Although it is well-known that developmental studies can offer new insights for evolution, "there have been few applications to plants and what is missing, most of all, are concrete examples of the evolution of development" (Sachs, 1992).

The genus *Amsinckia* (Boraginaceae) is a particularly appropriate group for the study of mating-system evolution. Besides the diversity of distyly and homostyly, the genus exhibits a great diversity of mating systems, ranging from predominant cross-pollination, to intermediate cross-pollination to predominant self-pollination to nearly

complete self-pollination (Ray and Chisaki, 1957a, 1957b; Ganders, 1975b; Johnston and Schoen, 1996; Schoen et al., 1997). On the basis of morphology and chromosome number studies, Ray and Chisaki (1957b) proposed a phylogenetic tree in *Amsinckia* which consists of four separate evolutionary transitions from predominant outcrossing to predominant selfing. A recent phylogenetic study in *Amsinckia* using cpDNA data (restriction site variation in the chloroplast DNA) has supported this phylogenetic tree, and further suggested that the selfing taxa are recently derived from outcrossing ancestors, which occurred in each of the four lineages in *Amsinckia*, in comparison with amount of time separating the different outcrossing taxa (Schoen et al., 1997). A more-recent phylogenetic analysis using both chloroplast and nuclear DNA sequences has further supported these conclusions (M.O. Johnston and W.J. Hahn, unpublished results). These four lineages are *A. furcata* to *A. vernicosa*; *A. douglasiana* to *A. tessellata gloriosa* (and *A. t. tessellata)*; large-flowered, distylous *A. spectabilis* to large-flowered, homostylous *A. spectabilis* to small-flowered, homostylous *A. spectabilis*; and large-flowered *A. lunaris* to small-flowered *A. lunaris* (Fig. 3.7).

The purpose of this study is to understand, for each of the first three evolutionary lineages of *Amsinckia*, the developmental changes in floral form that separate (a) highly self-fertilizing taxa from their outcrossing ancestors and (b) the pin and thrum floral morphs within distylous taxa (Fig. 3.7 or Fig. 4.1). Specifically, I will employ quantitative comparisons of floral development in *Amsinckia* 1) to find out what kinds of heterochronic processes are responsible for differences in floral structure and floral morphs; and 2) to evaluate my hypothesis that all pin flowers in different lineages within same clade may have the same developmental pathway, while all thrum flowers in

different lineages within a clade may have another common developmental pathway. The study will also explore whether homostyly evolved differently in three lineages within the genus. Most importantly, for the first time this research will lead to an understanding of the developmental mechanisms involved in the evolution of homostylous selfing from distylous outcrossing based on comparative floral ontogeny, heterochrony, and floral trait shifts associated with mating patterns.

6.3. Materials and Methods

6.3.1. Study species and floral morphs

A total of 10 species-morph combinations representing three evolutionary lineages in *Amsinckia* were studied (see section 4.3.1 of Chapter 4 for details). Study samples were collected from the field in California in 1995 by Mark Johnston. Eight to fifteen inflorescences taken from different individual plants were studied for each floral morph of each species or population.

6.3.2. Measurements

In order from distal to proximal, each coiled *Amsinckia* inflorescence consists of flower primordia, flower buds, newly-opened flower, fully-opened flowers, and senescing flowers. For each inflorescence studied, at least three fully opened flowers, the newly opened flower, and all flower buds that were larger than 0.2-0.3 mm in length were dissected under an OLYMPUS SZH10 stereo microscope. This study therefore does not

include the earliest flower development, occurring in buds smaller than 0.2-0.3 mm in length. Images of all dissected floral parts from each flower and bud were recorded and saved using a video imaging system and computer, which were connected to the microscope, for later measurements. Floral traits were measured using the public domain NIH Image program (version 1.62, developed at the U.S. National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image) on images of dissected floral parts.

The 21 quantitative traits used in this study are listed in Table 6.2. For reference on the definitions and measurements of these traits please refer to Table 4.1 and Fig. 4.2 in Chapter 4. Most traits were named using a four-letter abbreviation with the first letter of K, C, S, or P indicating the whorl of calyx, corolla, stamen or pistil, respectively. For the measurements of stigma area (PSTA) and functional anther-stigma distance (ASD), please see section 4.3.2 of Chapter 4.

6.3.3. Flower developmental timing and duration

Most published floral ontogenetic studies used the length of the corolla or ontogeny of the pistil as indicators of developmental age (Stewart and Canne-Hilliker, 1998). This method cannot provide the real flower developmental time or age information. Such studies are thus actually based on allometry, not heterochrony (see Chapter 2 section 2.6 for discussion; McKinney, 1988a). "Allometric patterns cannot be used to infer the underlying heterochronic processes" (Klingenberg and Spence, 1993). Because allometry ignores developmental processes, it is not suitable for studying morphological evolution (Blackstone, 1987; Strauss, 1987). In order to use the concept of

heterochrony to distinguish the types of developmental processes and patterns in ontogenetic studies, information on real developmental age is necessary. I will describe the way I obtained the actual flower developmental age information in this study below.

In *Amsinckia*, flowers and buds mature from base to tip along the determinate inflorescence, in a chronological sequence,. The developmental time of any specific bud or flower on an inflorescence [units: days/bud position; often termed "plastochron" (Lamoreaux et al., 1978)] was determined as the inverse of the rate of flower opening (units: bud positions/day), under the assumption that the plastochron remains constant as the inflorescence grows. To obtain the rate of flower opening in the field, the newly opened flower on an inflorescence was marked with paint; five to seven days later, the inflorescence was collected and placed in FAA. The number of flowers that opened between the time of painting and the time of collection defined the flower-opening rate. Inflorescences were dissected to determine the total number of bud positions between the youngest primordium (confirmed by scanning electron microscopy using standard SEM method) and the newly opened flower. Each bud on an inflorescence was numbered starting from the youngest floral primordium (zero) and ending with the newly opened flower (Fig. 7.1).

The floral developmental duration, i.e., the days needed for a flower to develop from primordium to anthesis (opening), was obtained by multiplying the total number of buds on an inflorescence by the time interval of flower opening (days/bud) on that inflorescence. The actual age or developmental time elapsed from the primordium initiation to a particular bud or flower was calculated by multiplying the position of that bud or flower by the time interval of flower opening (days/bud) on that inflorescence.

The relative age or developmental time of a floral bud was calculated as the ratio of the position of that bud to the total number of buds on the inflorescence. The relative developmental time of a bud expresses the time elapsed from primordium to the bud as a proportion of the total time from primordium to flower opening. The relative developmental time does not depend on the actual time separating successive buds, but does assume that this does not change as the inflorescence grows. The flower developmental rate or the growth rate of a floral trait was calculated by dividing the size of the trait at flower opening by the flower developmental duration.

Meiosis timing was used as a reference point for flower development. The best and as well as an easy indication of the time when pollen mother cells (microsporocyte) "finish" meiosis is the formation of microspore tetrads in anthers. To identify which bud(s) contained microspore tetrads, and thus the timing of microspore tetrad formation, anthers of individual buds were stained with safranin-O or aceto-carmine after their images were taken for measurement purpose, then squashed and observed under a compound microscope. Microspore tetrads usually occurred in only one bud on an inflorescence in *Amsinckia*. When more than one bud on an inflorescence contained microspore tetrads, the one adjacent to the bud having microsporocyte meiosis was chosen for calculating the tetrad formation time.

6.3.4. Standardizing bud positions among inflorescences

The total number of buds from the primordium to the newly opened flower varied among inflorescences. Thus, the buds at the same position on different inflorescences were often at different absolute developmental ages. This made it difficult to compare the

relationships between trait size and developmental time among inflorescences either within a group, i.e., floral morph in this study, or among groups. I therefore standardized bud positions to a relative scale ranging from zero to one, by dividing the absolute bud position by the total number of buds. Growth trajectories must be compared at identical relative positions, which were not shared by different inflorescences because of the variable number of total buds. Therefore, the actual measurements were used to interpolate trait sizes at increments of 0.05 between the smallest bud measured (usually position 0.3) and one. The interpolation of trait size was performed using a program (Appendix 6.2) written by Mark Johnston with True BASIC (version 2.61, True BASIC, Inc., West Lebanon, NH, 1993).

6.3.5. Statistical analysis

Flower developmental duration and growth rate were analyzed using ANOVA with SYSTAT for the Macintosh (version 5.2, Evanston, Illinois, 1992). In order to compare floral ontogenetic trajectories among floral morphs, species and lineages, the developmental data were analyzed by employing Repeated Measures ANOVA (RM), a specific type of MANOVA, with SAS (version 6.12, Cary, North Carolina, 1999) on the mainframe computer of Dalhousie University. For these RM analyses, the within-subjects factor was the relative age of the flower during development, and two between-subjects (grouping) factors were floral morph type and evolutionary lineage of *Amsinckia*. A multivariate approach was adopted for within-subjects tests. Among the test output, only Wilks' Lamba and *P*-value are presented in this paper. The Wilks' Lamba is the likelihood ratio statistic for testing the hypothesis that the means of the groups on the

selected variables are equal in the population. The value of Wilks' Lamba is close to zero if any two groups are well separated. General Linear Models (GLM) procedure was used for between-subjects tests, and statistical significance was indicated with *P*-value. In order to pinpoint the relative age, if any, at which growth trajectories diverged, RM was performed on Helmert contrast variables (Carey, 1998). For a particular relative age, a Helmert contrast the difference between size at that age and the mean of the remaining ages.

6.4. RESULTS

6.4.1. Flower developmental duration, number of buds and RAFT

Flower developmental duration did not differ between pins and thrums within any lineages or among pins, thrums and homostyles in Lineages 1 and 2 (Table 6.1). In Lineage 3 (*A. spectabilis*), however, small homostyles had significantly longer developmental duration than pins, thrums and large homostyles, which did not differ. The pattern for number of buds per inflorescence was the opposite of that for developmental duration. Pins, thrums and large and small homostyles did not differ in Lineage 3, but homostyles had significantly fewer buds per inflorescence than pins and thrums in both Lineages 1 and 2.

At the time of opening, homostylous flowers were smaller than pins and thrums in all lineages (Table 6.1; see Chapter 4 for details). In Lineage 3, the large homostylous flower was smaller than pins and thrums, but larger than the small homostyle (Table 6.1).

Table 6.1. Mean ±SE of several inflorescence and flower traits in *Amsinckia*. Means with different letters were significantly different ($P < 0.05$) among floral morphs within each lineage. Except for RAFT in Lineage 1, all the differences with a $P < 0.05$ were significant (marked with *) after tablewide correction ($\alpha = 0.05$) for multiple comparisons using the sequential Bonferroni technique (Rice, 1989). Units: flower size: mm; flower developmental duration and AAFT: day. L1 = lineage of *A. furcata* – *A. vernicosa*; L2 = lineage of *A. douglasiana* – *A. t. gloriosa*; L3 = lineage of *A. spectabilis*. P = pin; T = thrum; H = homostyle; LH = large-flowered homostyle; SH = small-flowered homostyle; dev. = development; PMC = pollen-mother-cell; AAFT = actual age of flower when microspore tetrads were formed; RAFT = relative age of flower when microspore tetrads were formed.

Table 6.1.

Line -age	Species_morph & statistics	*N*	Flower dev. duration	# of buds per inflorescence	PMC meiosis termination time		Flower size at opening	
					AAFT	RAFT	Length (BUDL)	Width (BUDW)
L1	*A. furcata*_P	15	19.35 ±0.73[a]	25.07 ±0.80[a]	9.22 ±0.34[a]	0.47 ±0.01[a]	13.70 ±0.27[a]	10.12 ±0.28[a]
	*A. furcata*_T	8	19.79 ±0.73[a]	26.63 ±1.10[a]	8.85 ±0.35[a]	0.45 ±0.01[b]	16.26 ±0.49[b]	10.87 ±0.44[a]
	*A. vernicosa*_H	8	19.79 ±1.44[a]	17.00 ±1.05[b]	9.00 ±0.56[a]	0.46 ±0.01[ab]	7.48 ±0.34[c]	3.17 ±0.22[b]
	F-ratio (2, 28)		0.08	25.46	0.21	3.48	131.35	145.43
	P-value		0.92	$< 10^{-7}$*	0.81	< 0.05	$< 10^{-14}$*	$< 10^{-15}$*
L2	*A. douglasiana*_P	8	20.73 ±0.53[a]	31.63 ±0.98[a]	10.04 ±0.91[a]	0.45 ±0.01[a]	12.43 ±0.55[a]	8.50 ±0.75[a]
	*A. douglasiana*_T	11	22.97 ±1.44[a]	31.64 ±1.14[a]	10.24 ±0.61[a]	0.45 ±0.01[a]	15.75 ±0.28[b]	9.98 ±0.36[a]
	*A. t. gloriosa*_H	8	19.90 ±1.41[a]	25.00 ±0.78[b]	9.02 ±0.82[a]	0.45 ±0.01[a]	10.86 ±0.32[c]	6.54 ±0.31[b]
	F-ratio (2, 24)		1.64	12.89	0.75	0.02	44.94	12.80
	P-value		0.22	< 0.0002*	0.48	0.99	$< 10^{-8}$*	< 0.0002*
L3	*A. spectabilis*_P	8	18.09 ±1.35[a]	30.13 ±2.17[a]	8.08 ±0.57[a]	0.45 ±0.01[a]	12.21 ±0.22[ab]	9.53 ±0.29[a]
	*A. spectabilis*_T	8	17.37 ±0.93[a]	26.88 ±1.57[a]	7.71 ±0.35[a]	0.45 ±0.01[a]	13.36 ±0.50[a]	9.36 ±0.36[a]
	*A. spectabilis*_LH	8	14.69 ±0.89[a]	27.75 ±2.14[a]	6.36 ±0.35[a]	0.44 ±0.01[a]	11.47 ±0.30[b]	8.16 ±0.17[b]
	*A. spectabilis*_SH	8	23.08 ±0.94[b]	29.88 ±1.27[a]	10.47 ±0.49[b]	0.45 ±0.00[a]	8.20 ±0.22[c]	4.53 ±0.09[c]
	F-ratio (3, 28)		11.28	0.76	14.42	0.65	45.34	86.51
	P-value		< 0.00005*	0.51	< 0.00001*	0.59	$< 10^{-10}$*	$< 10^{-13}$*

Similar to the flower developmental duration, the actual age when pollen-mother-cells (PMC) finished meiosis (AAFT) was not significantly different between distylous and homostylous flowers in both Lineages 1 and 2 (Table 6.1). There was also no difference between distylous and large homostylous flowers in Lineage 3, although meiosis terminated significantly later in small homostylous flowers (Table 6.1). The relative age when pollen-mother-cells finish meiosis (RAFT), however, was almost the same (0.45) among all floral morphs, species and lineages in *Amsinckia* (Table 6.1; see Chapter 7 for details).

6.4.2. Developmental rate

6.4.2.1. *Distyly vs. homostyly*

The developmental rate of sepal length (KSL) was not significantly different between homostyly and distyly in Lineages 1 and 2 (Table 6.2). In Lineage 3, KSL developed approximately 50% slower in the small homostyles than in both distyles and large-flowered homostyles (Table 6.2).

The degree of developmental rate differences for flower-size-related traits between distyly and homostyly varied among the three lineages. All flower-size-associated traits (BUDL, BUDW, CFPL, CLBW, CPTL and CTBL) of distyly grew approximately 2-3 times faster than those of homostyly in Lineage 1, and about twice as fast as those of small homostyly but no difference to those of large homostyly in Lineage 3 (Table 6.2). In Lineage 2, however, the developmental rate of these flower-size traits of

Table 6.2. Mean ±SE of developmental rate of floral traits in three evolutionary lineages of *Amsinckia*. Means with different letters were significantly different ($P < 0.05$) among floral morphs within each lineage. Morphs: P = pin of *A. furcata*, *A. douglasiana*, or *A. spectabilis*; T = thrum of *A. furcata*, *A. douglasiana*, or *A. spectabilis*; LH = large homostyle of *A. spectabilis*; H = homostyle of *A. vernicosa*, *A. t. gloriosa*, or small homostyle of *A. spectabilis*.

Trait	Morph & statistics	Developmental rate (mm/day)		
		A. furcata* – *A. vernicosa	***A. douglasiana* – *A. t. gloriosa***	***A. spectabilis***
KSL	P	0.40 ±0.01^{a}	0.34 ±0.01^{a}	0.28 ±0.02^{a}
	T	0.40 ±0.01^{a}	0.34 ±0.02^{a}	0.27 ±0.01^{a}
	LH	–	–	0.28 ±0.01^{a}
	H	0.36 ±0.02^{a}	0.35 ±0.02^{a}	0.12 ±0.01^{b}
	F-ratio	1.70	0.10	30.47
	P-value	0.20	0.90	$< 10^{-8}$*
	R^2	0.11	0.01	0.77
BUDL	P	0.72 ±0.02^{a}	0.60 ±0.02^{ab}	0.70 ±0.05^{a}
	T	0.82 ±0.02^{b}	0.71 ±0.05^{a}	0.79 ±0.05^{a}
	LH	–	–	0.80 ±0.04^{a}
	H	0.39 ±0.02^{c}	0.56 ±0.04^{b}	0.36 ±0.02^{b}
	F-ratio	81.68	4.07	23.24
	P-value	$< 10^{-11}$*	< 0.05	$< 10^{-7}$*
	R^2	0.85	0.25	0.71
BUDW	P	0.53 ±0.01^{a}	0.41 ±0.03^{ab}	0.55 ±0.04^{a}
	T	0.55 ±0.02^{a}	0.45 ±0.03^{a}	0.55 ±0.04^{a}
	LH	–	–	0.57 ±0.03^{a}
	H	0.16 ±0.01^{b}	0.34 ±0.02^{b}	0.20 ±0.01^{b}
	F-ratio	162.74	4.21	29.24
	P-value	$< 10^{-15}$*	< 0.05	$< 10^{-8}$*
	R^2	0.92	0.26	0.76

Table 6.2. Continued.

Trait	Morph & statistics	Developmental rate (mm/day)		
		A. furcata – *A. vernicosa*	*A. douglasiana* – *A. t. gloriosa*	*A. spectabilis*
CFPL	P	0.62 ±0.02^{a}	0.53 ±0.02^{ab}	0.62 ±0.03^{a}
	T	0.71 ±0.01^{b}	0.62 ±0.03^{a}	0.69 ±0.04^{a}
	LH	–	–	0.67 ±0.03^{a}
	H	0.34 ±0.01^{c}	0.49 ±0.03^{b}	0.30 ±0.01^{b}
	F-ratio	81.21	4.10	28.51
	P-value	$< 10^{-11}$*	< 0.05	$< 10^{-7}$*
	R^2	0.85	0.26	0.75
CLBW	P	0.21 ±0.01^{a}	0.16 ±0.01^{ab}	0.25 ±0.02^{a}
	T	0.20 ±0.01^{a}	0.19 ±0.01^{a}	0.23 ±0.01^{a}
	LH	–	–	0.25 ±0.01^{a}
	H	0.06 ±0.00^{b}	0.14 ±0.01^{b}	0.08 ±0.00^{b}
	F-ratio	129.14	7.70	30.44
	P-value	$< 10^{-14}$*	< 0.005	$< 10^{-8}$*
	R^2	0.90	0.39	0.77
CPTL	P	0.72 ±0.02^{a}	0.61 ±0.03^{ab}	0.71 ±0.05^{a}
	T	0.82 ±0.02^{b}	0.70 ±0.04^{a}	0.79 ±0.05^{a}
	LH	–	–	0.80 ±0.04^{a}
	H	0.37 ±0.01^{c}	0.56 ±0.04^{b}	0.36 ±0.02^{b}
	F-ratio	92.94	3.46	24.48
	P-value	$< 10^{-12}$*	< 0.05	$< 10^{-7}$*
	R^2	0.87	0.22	0.72
CTBL	P	0.45 ±0.01^{a}	0.39 ±0.02^{a}	0.42 ±0.03^{a}
	T	0.52 ±0.02^{b}	0.46 ±0.03^{a}	0.52 ±0.03^{a}
	LH	–	–	0.49 ±0.03^{a}
	H	0.27 ±0.01^{c}	0.40 ±0.03^{a}	0.25 ±0.01^{b}
	F-ratio	53.48	1.88	21.20
	P-value	$< 10^{-9}$*	0.17	< 0.000001*
	R^2	0.79	0.14	0.69

Table 6.2. Continued.

Trait	Morph & statistics	Developmental rate (mm/day)		
		A. furcata – *A. vernicosa*	*A. douglasiana* – *A. t. gloriosa*	*A. spectabilis*
SANL	P	0.10 ±0.00[a]	0.08 ±0.00[a]	0.08 ±0.00[a]
	T	0.11 ±0.00[a]	0.09 ±0.01[a]	0.09 ±0.00[a]
	LH	–	–	0.09 ±0.00[a]
	H	0.07 ±0.00[b]	0.08 ±0.01[a]	0.05 ±0.00[b]
	F-ratio	25.17	2.50	19.92
	P-value	< 0.000001*	0.11	< 0.000001*
	R^2	0.64	0.19	0.68
SANW	P	0.04 ±0.00[a]	0.03 ±0.00[a]	0.03 ±0.00[a]
	T	0.04 ±0.00[b]	0.04 ±0.00[a]	0.04 ±0.00[ab]
	LH	–	–	0.04 ±0.00[b]
	H	0.03 ±0.00[a]	0.03 ±0.00[a]	0.02 ±0.00[c]
	F-ratio	6.53	3.36	16.53
	P-value	< 0.005*	0.05	< 0.00001*
	R^2	0.32	0.23	0.64
SFIL	P	0.03 ±0.00[a]	0.02 ±0.00[a]	0.03 ±0.00[a]
	T	0.05 ±0.00[b]	0.06 ±0.01[b]	0.05 ±0.00[b]
	LH	–	–	0.06 ±0.00[c]
	H	0.02 ±0.00[a]	0.03 ±0.00[a]	0.02 ±0.00[d]
	F-ratio	52.75	27.20	41.46
	P-value	$< 10^{-9}$*	< 0.000001*	$< 10^{-9}$*
	R^2	0.79	0.69	0.82
SINH	P	0.23 ±0.01[a]	0.16 ±0.01[a]	0.23 ±0.01[a]
	T	0.53 ±0.01[b]	0.46 ±0.03[b]	0.47 ±0.03[b]
	LH	–	–	0.44 ±0.02[b]
	H	0.27 ±0.01[a]	0.35 ±0.02[c]	0.20 ±0.01[a]
	F-ratio	187.52	36.80	42.56
	P-value	$< 10^{-15}$*	$< 10^{-7}$*	$< 10^{-9}$*
	R^2	0.93	0.75	0.82

Table 6.2. Continued.

Trait	Morph & statistics	Developmental rate (mm/day)		
		A. furcata – *A. vernicosa*	*A. douglasiana* – *A. t. gloriosa*	*A. spectabilis*
SSIL	P	0.31 ±0.01[a]	0.22 ±0.01[a]	0.30 ±0.02[a]
	T	0.63 ±0.01[b]	0.57 ±0.03[b]	0.55 ±0.03[b]
	LH	–	–	0.54 ±0.02[b]
	H	0.33 ±0.01[a]	0.40 ±0.03[c]	0.25 ±0.01[a]
	F-ratio	158.05	45.92	42.92
	P-value	$< 10^{-15}$*	$< 10^{-8}$*	$< 10^{-9}$*
	R^2	0.92	0.80	0.82
PISL	P	0.61 ±0.02[a]	0.54 ±0.02[a]	0.52 ±0.03[a]
	T	0.25 ±0.01[b]	0.20 ±0.01[b]	0.23 ±0.01[b]
	LH	–	–	0.62 ±0.03[c]
	H	0.31 ±0.01[b]	0.36 ±0.02[c]	0.21 ±0.01[b]
	F-ratio	118.76	92.80	71.51
	P-value	$< 10^{-13}$*	$< 10^{-11}$*	$< 10^{-12}$*
	R^2	0.90	0.89	0.89
POVH	P	0.04 ±0.00[ab]	0.03 ±0.00[a]	0.03 ±0.00[ab]
	T	0.03 ±0.00[a]	0.03 ±0.00[a]	0.03 ±0.00[a]
	LH	–	–	0.04 ±0.00[b]
	H	0.04 ±0.00[b]	0.04 ±0.00[b]	0.02 ±0.00[c]
	F-ratio	3.71	20.45	10.88
	P-value	< 0.05	< 0.00001*	< 0.0001*
	R^2	0.21	0.63	0.54
PSSL	P	0.58 ±0.02[a]	0.50 ±0.02[a]	0.49 ±0.03[a]
	T	0.22 ±0.01[b]	0.18 ±0.01[b]	0.21 ±0.01[b]
	LH	–	–	0.58 ±0.02[c]
	H	0.27 ±0.01[b]	0.32 ±0.02[c]	0.19 ±0.01[b]
	F-ratio	138.76	105.10	76.05
	P-value	$< 10^{-14}$*	$< 0.1 \times 10^{-11}$*	$< 10^{-12}$*
	R^2	0.91	0.90	0.89

Table 6.2. Continued.

Trait	Morph & statistics	Developmental rate (mm/day)		
		A. furcata – A. vernicosa	*A. douglasiana – A. t. gloriosa*	*A. spectabilis*
PSTYL	P	0.55 ±0.02[a]	0.49 ±0.02[a]	0.48 ±0.03[a]
	T	0.19 ±0.01[b]	0.16 ±0.01[b]	0.19 ±0.01[b]
	LH	–	–	0.56 ±0.02[c]
	H	0.25 ±0.01[b]	0.31 ±0.01[c]	0.18 ±0.01[b]
	F-ratio	146.51	112.11	80.41
	P-value	$< 10^{-14}$*	$< 0.1 \times 10^{-12}$*	$< 10^{-13}$*
	R^2	0.91	0.90	0.90
PSTH	P	0.02 ±0.00[a]	0.02 ±0.00[a]	0.02 ±0.00[a]
	T	0.02 ±0.00[a]	0.02 ±0.00[a]	0.02 ±0.00[a]
	LH	–	–	0.02 ±0.00[a]
	H	0.02 ±0.00[a]	0.01 ±0.00[b]	0.01 ±0.00[b]
	F-ratio	0.84	5.19	15.19
	P-value	0.44	< 0.02	< 0.00001*
	R^2	0.06	0.31	0.62
PSTL	P	0.03 ±0.00[ab]	0.03 ±0.00[a]	0.03 ±0.00[a]
	T	0.03 ±0.00[a]	0.03 ±0.00[a]	0.03 ±0.00[a]
	LH	–	–	0.03 ±0.00[a]
	H	0.02 ±0.00[b]	0.02 ±0.00[b]	0.02 ±0.00[b]
	F-ratio	5.35	5.43	15.23
	P-value	< 0.02	< 0.02	< 0.00001*
	R^2	0.28	0.32	0.62
PSTW	P	0.02 ±0.00[a]	0.02 ±0.00[a]	0.02 ±0.00[a]
	T	0.02 ±0.00[a]	0.02 ±0.00[a]	0.02 ±0.00[a]
	LH	–	–	0.03 ±0.00[a]
	H	0.02 ±0.00[b]	0.02 ±0.00[a]	0.01 ±0.00[b]
	F-ratio	9.79	2.42	14.60
	P-value	< 0.001*	0.11	< 0.00001*
	R^2	0.41	0.17	0.61

Table 6.2. Continued.

Trait	Morph & statistics	Developmental rate (mm/day)		
		A. furcata – A. vernicosa	*A. douglasiana – A. t. gloriosa*	*A. spectabilis*
PSTA	P	0.03 ±0.00[a]	0.04 ±0.00[a]	0.04 ±0.00[a]
	T	0.06 ±0.00[a]	0.05 ±0.00[a]	0.04 ±0.00[a]
	LH	–	–	0.05 ±0.00[a]
	H	0.04 ±0.00[b]	0.02 ±0.00[b]	0.01 ±0.00[b]
	F-ratio	6.85	11.13	16.30
	P-value	< 0.005*	< 0.0005*	< 0.00001*
	R^2	0.34	0.49	0.64
ASD	P	0.30 ±0.01[a]	0.32 ±0.01[a]	0.22 ±0.02[a]
	T	0.28 ±0.01[a]	0.27 ±0.01[a]	0.23 ±0.02[a]
	LH	–	–	0.08 ±0.01[b]
	H	0.02 ±0.00[b]	0.04 ±0.01[b]	0.04 ±0.00[c]
	F-ratio	145.56	189.35	69.81
	P-value	$< 10^{-14}$*	$< 10^{-13}$*	$< 10^{-12}$*
	R^2	0.91	0.95	0.88

* Significant after tablewide correction ($\alpha = 0.05$) for multiple comparisons using the sequential Bonferroni technique (Rice, 1989) in the analyses across species, morphs and traits within each lineage.

$N = 15$, 8, and 8 for P, T and H, respectively, in lineage of *A. furcata – A. vernicosa*.

$N = 8$, 11, and 8 for P, T and H, respectively, in lineage of *A. douglasiana – A. t. gloriosa*.

$N = 8$ for each morph (P, T, LH, and H) in lineage of *A. spectabilis*.

homostyles was similar to that of pins but significantly lower than that of thrums, excluding CTBL where there was no difference between homostyly and distyly (Table 6.2).

The growth rate of the stamen-height traits (SFIL, SINH and SSIL) in all three lineages was highly significantly lower in homostyles than in thrums, and also lower than in large homostyles of Lineage 3 (Table 6.2). Of these three traits, stamen insertion height (SINH) and stamen height (SSIL) had similar developmental rates in homostyles (small homostyles in L3) and pins in Lineages 1 and 3, but were significantly faster in homostylous flowers in Lineage 2. The filament length (SFIL) developed at a similar rate between homostyle and pin in Lineages 1 and 2, but was significantly slower in small homostyles than in pins in Lineage 3 (Table 6.2).

The developmental rate of pistil-height traits (PISL, PSSL and PSTYL excluding POVH) did not much differ between homostyles (small homostyles in L3) and thrums, but it was significantly lower in homostyles (small homostyles in L3) than in pins, in both Lineages 1 and 3 (Table 6.2). In Lineage 2, however, these three pistil-height traits in homostyles developed significantly faster than in thrums but slower than in pins (Table 6.2). The relative developmental rate of ovary height (POVH), one of the pistil-height-related traits, varied among the homostyles of the three lineages (Table 6.2). POVH in homostyly developed at a similar rate as in distyly in Lineage 1, but significantly faster than in distyly in Lineage 2, and significantly slower than in both distyly and large homostyly in Lineage 3.

In addition, anthers (SANL and SANW) grew significantly faster in distylous flowers than in homostylous flowers in both Lineages 1 and 3, but the developmental rate

of SANW was similar between homostyles and pins in Lineage 1 (Table 6.2). In Lineage 2, the developmental rate of these two traits was similar between homostyly and distyly (Table 6.2).

The developmental rate of most stigma-size-related traits was significantly lower in homostyly (small homostyly in L3) than in distyly in Lineages 1 and 3, while the difference was generally not significant between the two styles in Lineage 2 (Table 6.2).

In terms of the anther-stigma distance, its developmental rate was extremely significantly lower in homostyly than in distyly in all three lineages as one would expect.

Within Lineage 3, large homostyly was similar to distyly, because both had very similar developmental rates in traits associated with sepal length, flower size, stigma size and anther length (Table 6.2). In addition, the traits SANW, SINH and SSIL in large homostyly also had similar developmental rates as those in thrum flowers, although they were higher than those in pin flowers (Table 6.2). All pistil-height-related traits in large homostyly, however, had significant higher developmental rate compared to those of distyly (Table 6.2). On the other hand, large homostyly developed approximately 2-3 times faster than small homostyly in all studied floral traits (Table 6.2).

6.4.2.2. *Pin vs. thrum*

The rate of sepal length increase was similar between pin and thrum flowers in all three lineages of *Amsinckia* (Table 6.2).

For flower-size-related traits, the developmental rate was not different between pins and thrums in both Lineages 2 and 3 (Table 6.2). In Lineage 1, however, the traits associated with flower length (BUDL, CFPL, CPTL and CTBL) in thrums grew

significantly faster than in pins, while the rate for flower-width-related traits (BUDW and CLBW) was similar between the two morphs (Table 6.2).

Anther-height-related traits (SFIL, SINH and SSIL) of thrums grew about twice as fast as those of pins, whereas the rate for pistil-length associated traits (PISL, PSSL and PSTYL, excluding POVH) in thrums was approximately 60% slower than those of pins, in all three lineages (Table 6.2).

The growth rate of anther size (BUDL and BUDW), ovary size (POVH), stigma size (PSTH, PSTL, PSTW and PSTA), and functional anther-stigma distance (ASD) between the two distylous morphs was similar in all three lineages (Table 6.2).

6.4.3. Developmental trajectories

6.4.3.1. *Distyly vs. homostyly*

The developmental trajectory of sepal length (KSL) between homostyly and distyly was similar in Lineages 1 and 2 (Fig. 6.1). In Lineage 3, KSL in small homostyly grew relatively slower than in distyly, and the developmental trajectories of the two style morphs diverged at an early stage of flower ontogeny. On the other hand, the developmental trajectory of KSL was not only lineage dependent but also floral-morph dependent. In thrum and homostylous (small homostylous in L3) flowers, the growth curve of KSL was significantly lower in Lineage 3 than in Lineages 1 and 2, and they diverged probably before PMC meiosis (relative age of 0.45; Fig. 6.8; Tables 6.3-6.5). In thrum and homostylous flowers, the growth trajectories of KSL between Lineages 1 and

2 were similar until their late development (Fig. 6.8). A later developmental divergence of KSL's growth among three lineages was observed in pin flowers (Fig. 6.8).

The developmental trajectories of flower-size-related traits (BUDL, BUDW, CFPL, CLBW and CPTL) differed between distyly and homostyly in all three lineages (Figs. 6.1, 6.2). The trajectories in homostyly were much lower than those in distyly, especially during later development, due to a steeper increase of the relative growth rate in distylous flowers. The divergence of these traits' development between the two style morphs mostly occurred before or around the time of PMC meiosis (Figs. 6.1, 6.2; Table 6.5). The trajectories of most flower-size-related traits among the three lineages were not much different until the later developmental stage or even until flower opening in both pin and thrum flowers (Figs. 6.8, 6.9). The trajectories, however, differed among the lineages probably around relative age of 0.4-0.6 in homostyle (Figs. 6.8, 6.9; Table 6.5).

The growth trajectories of stamen-height-related traits (SFIL, SINH and SSIL) between homostylous and distylous flowers differed among lineages. The specific time when their trajectories diverged, however, varied among both traits and lineages (Figs. 6.3, 6.4; Table 6.5). For example, the divergence of filament length (SFIL) growth between homostyly and distyly occurred after PMC meiosis in Lineage 1, at meiosis time in Lineage 2, and far before meiosis in Lineage 3 (Fig. 6.3). For stamen insertion height (SINH) and stamen height (SSIL) in Lineage 1, the separation of growth curves between homostyles and pins was much later than between homostyles and thrums (Fig. 6.4). In Lineage 2, the SINH growth curve in homostyles diverged from those in pins earlier than from those in thrums; and the opposite is true for SSIL growth curve (Fig. 6.4). On the other hand, the developmental trajectories of stamen-height-related traits, especially

Table 6.3. Statistical significance levels for effects of lineage, floral morph, and interactions between lineage and floral morph on mean floral trait size in *Amsinckia* (results of Repeated Measures ANOVA). The analysis excludes the LH morph of A. spectabilis.

Trait	*P*-value		
	Lineage	**Morph**	**Lineage x morph**
Calyx			
KSL	0.0001	0.0001	0.0562
Corolla			
BUDL	0.0001	0.0001	0.0004
BUDW	0.0178	0.0001	0.0030
CFPL	0.0001	0.0001	0.0001
CLBW	0.0001	0.0001	0.0001
CPTL	0.0014	0.0001	0.0001
Stamen			
SANL	0.0001	0.0001	0.0051
SANW	0.0001	0.0001	0.0001
SFIL	0.0973	0.0001	0.0027
SINH	0.0001	0.0001	0.0001
SSIL	0.0001	0.0001	0.0001
Pistil			
PISL	0.0001	0.0001	0.0001
POVH	0.0001	0.0002	0.0015
PSSL	0.0045	0.0001	0.0001
PSTYL	0.0232	0.0001	0.0001
PSTH	0.0001	0.0002	0.0017
PSTL	0.0032	0.0001	0.0001
PSTW	0.0134	0.0001	0.2267
PSTA	0.0001	0.0001	0.0072

df for lineage, morph, and lineage x morph is 2, 2, and 4, respectively.

Table 6.4. Effects of developmental age and interactions between age and lineage, age and floral morph, age and lineage and floral morph on mean floral trait size across developmental ages in Amsinckia (MANOVA results). The analysis excludes the LH morph of A. spectabilis.

Trait	Wilks' Lambda (*P*-value)			
	Relative age	Relative age x lineage	Relative age x morph	Relative age x lineage x morph
Calyx				
KSL	0.006 (0.0001)	0.076 (0.0001)	0.377 (0.0001)	0.535 (0.1714)
Corolla				
BUDL	0.007 (0.0001)	0.151 (0.0001)	0.179 (0.0001)	0.295 (0.0001)
BUDW	0.008 (0.0001)	0.389 (0.0001)	0.239 (0.0001)	0.286 (0.0001)
CFPL	0.006 (0.0001)	0.475 (0.0002)	0.126 (0.0001)	0.381 (0.0023)
CLBW	0.011 (0.0001)	0.339 (0.0001)	0.164 (0.0001)	0.335 (0.0003)
CPTL	0.006 (0.0001)	0.595 (0.0122)	0.140 (0.0001)	0.300 (0.0001)
Stamen				
SANL	0.004 (0.0001)	0.204 (0.0001)	0.196 (0.0001)	0.232 (0.0001)
SANW	0.006 (0.0001)	0.534 (0.0092)	0.240 (0.0001)	0.343 (0.0001)
SFIL	0.005 (0.0001)	0.215 (0.2124)	0.043 (0.0010)	0.128 (0.0482)
SINH	0.005 (0.0001)	0.389 (0.0001)	0.037 (0.0001)	0.190 (0.0001)
SSIL	0.005 (0.0001)	0.365 (0.0001)	0.054 (0.0001)	0.207 (0.0001)
Pistil				
PISL	0.005 (0.0001)	0.136 (0.0001)	0.011 (0.0001)	0.041 (0.0001)
POVH	0.006 (0.0001)	0.259 (0.0001)	0.335 (0.0001)	0.379 (0.0058)
PSSL	0.006 (0.0001)	0.255 (0.0001)	0.030 (0.0001)	0.068 (0.0001)
PSTYL	0.007 (0.0001)	0.289 (0.0001)	0.035 (0.0001)	0.087 (0.0001)
PSTH	0.017 (0.0001)	0.197 (0.0001)	0.613 (0.0038)	0.466 (0.0035)
PSTL	0.018 (0.0001)	0.655 (0.0047)	0.472 (0.0001)	0.578 (0.0303)
PSTW	0.014 (0.0001)	0.627 (0.0096)	0.350 (0.0001)	0.640 (0.3028)
PSTA	0.023 (0.0001)	0.416 (0.0001)	0.317 (0.0001)	0.482 (0.0091)

Table 6.5. Effects of lineage, floral morph, and interactions between lineage and floral morph on mean developmental trajectory of floral trait in *Amsinckia* (results of Repeated Measures ANOVA). A relative age at which developmental trajectories diverged among groups was identified when the means of the trait size both at that age and at the subsequent ages differed significantly among the groups.

Trait	Developmental trajectories differ prior to relative age (*P*-value)		
	Lineage	Morph	Lineage x morph
Calyx			
KSL	0.4 (0.0034)	0.4 (0.0013)	1.0 (0.4276)
Corolla			
BUDL	0.4 (0.0008)	0.4 (0.0083)	1.0 (0.0001)
BUDW	0.5 (0.0004)	0.4 (0.0297)	0.5 (0.0309)
CFPL	0.5 (0.0050)	0.4 (0.0002)	0.6 (0.0245)
CLBW	0.8 (0.0171)	0.4 (0.0018)	0.6 (0.0075)
CPTL	0.6 (0.0312)	0.4 (0.0038)	0.6 (0.0014)
Stamen			
SANL	0.6 (0.0001)	0.4 (0.0065)	0.6 (0.0017)
SANW	0.5 (0.0137)	0.4 (0.0015)	0.6 (0.0005)
SFIL	1.0 (0.2659)	0.8 (0.0376)	1.0 (0.0777)
SINH	0.5 (0.0023)	0.4 (0.0068)	0.6 (0.0006)
SSIL	0.5 (0.0065)	0.4 (0.0068)	0.6 (0.0001)
Pistil			
PISL	0.4 (0.0001)	0.4 (0.0001)	0.4 (0.0001)
POVH	0.8 (0.0002)	0.6 (0.0011)	0.8 (0.0170)
PSSL	0.6 (0.0168)	0.7 (0.0001)	0.8 (0.0001)
PSTYL	0.6 (0.0138)	0.7 (0.0001)	0.6 (0.0116)
PSTH	0.6 (0.0001)	0.6 (0.0017)	0.6 (0.0002)
PSTL	1.0 (0.0269)	0.6 (0.0374)	0.7 (0.0201)
PSTW	0.7 (0.0002)	0.6 (0.0001)	1.0 (0.0779)
PSTA	0.7 (0.0001)	0.6 (0.0001)	0.6 (0.0004)

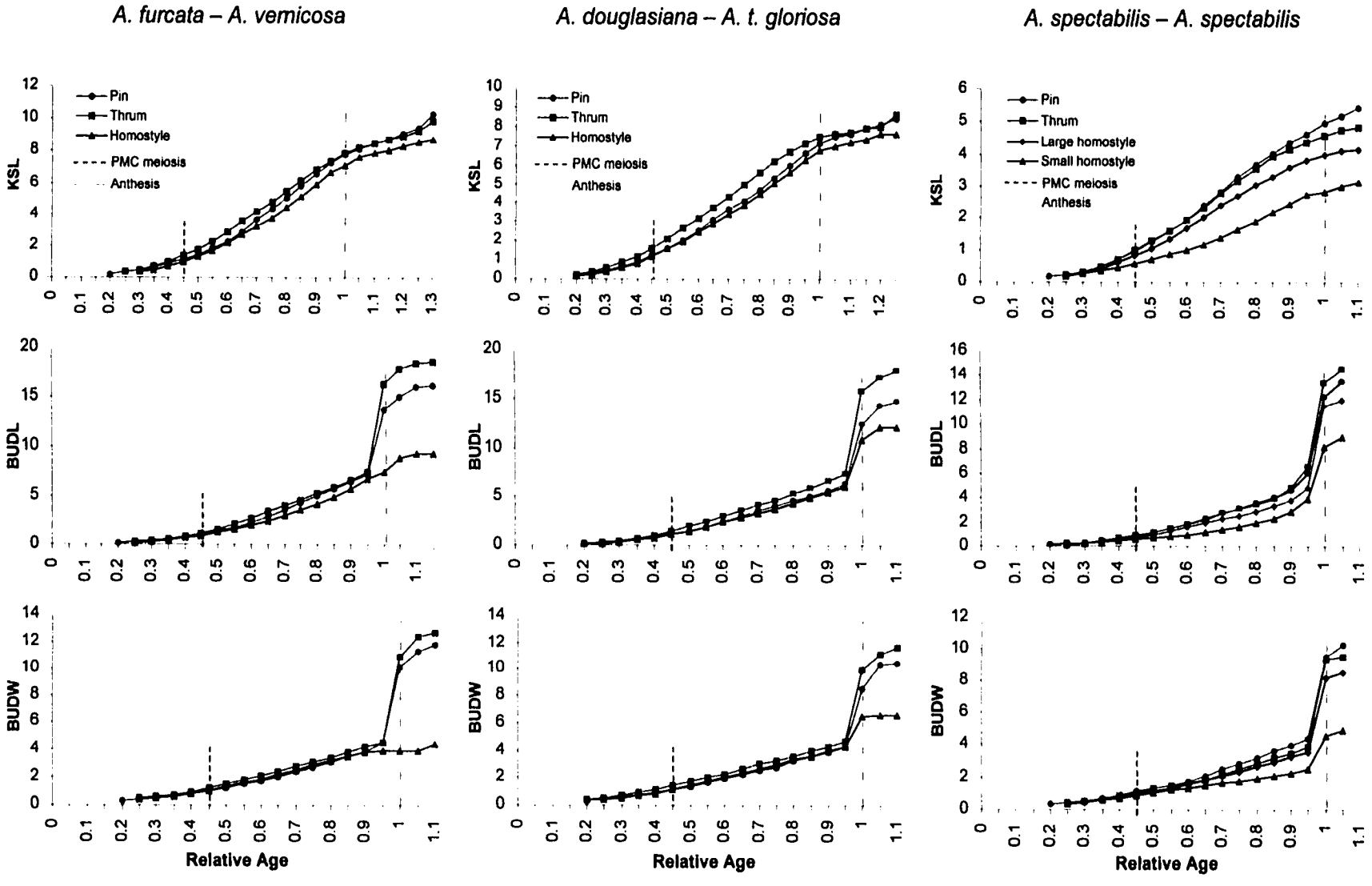

Figure 6.1. Growth of floral traits in *Amsinckia* (Part I). Unit of trait size: mm.

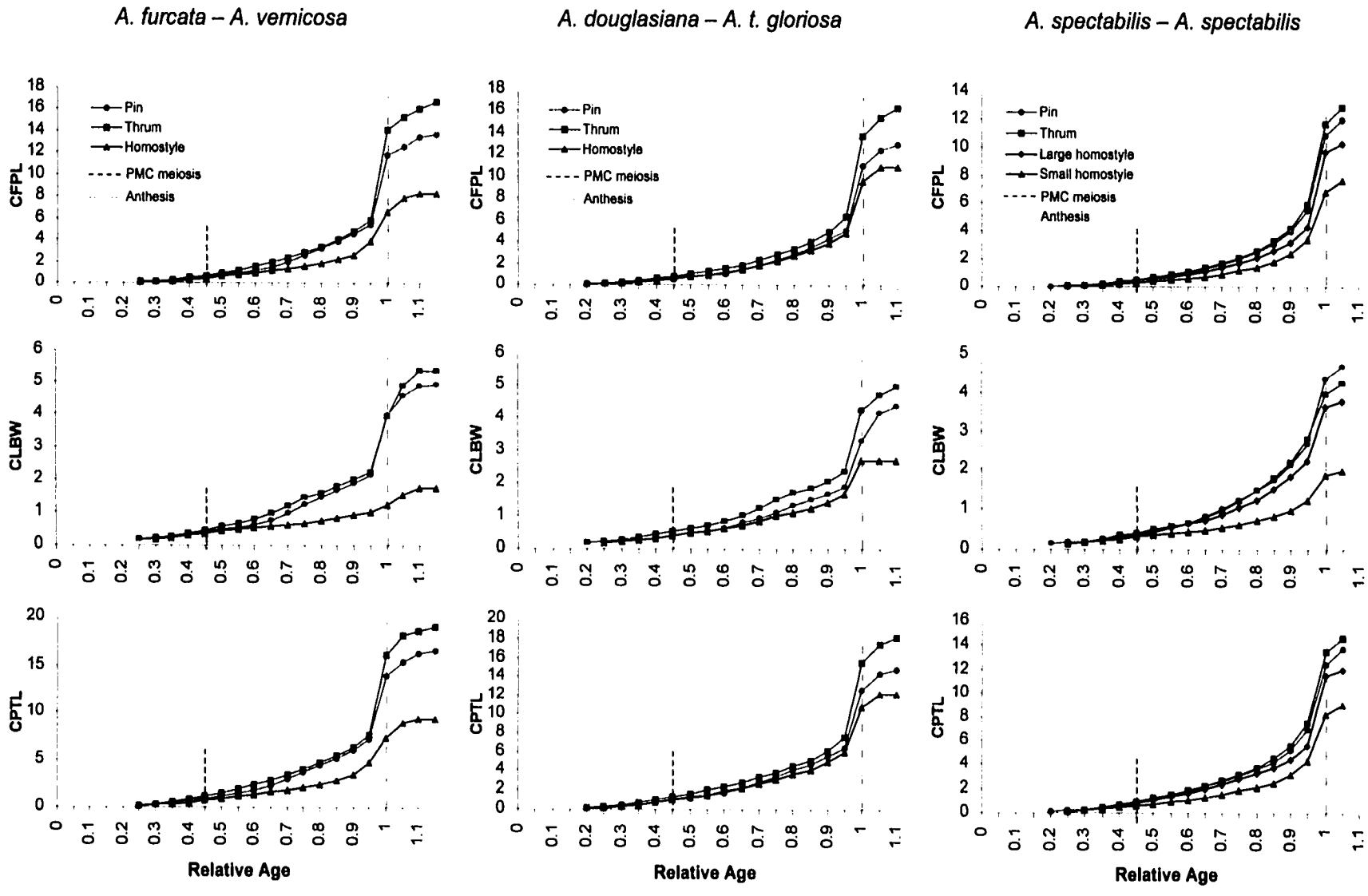

Figure 6.2. Growth of floral traits in *Amsinckia* (Part II). Unit of trait size: mm.

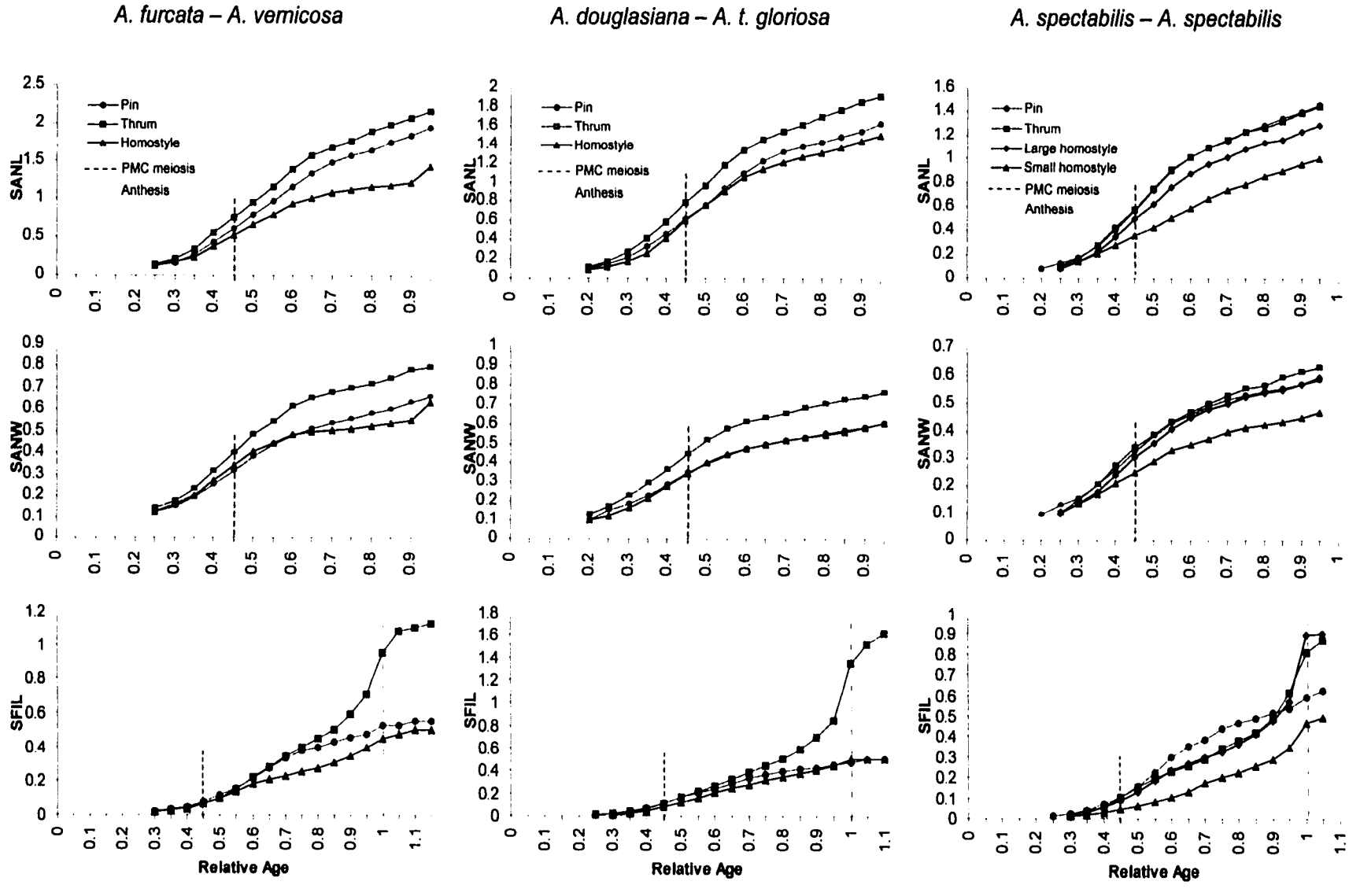

Figure 6.3. Growth of floral traits in *Amsinckia* (Part III). Unit of trait size: mm.

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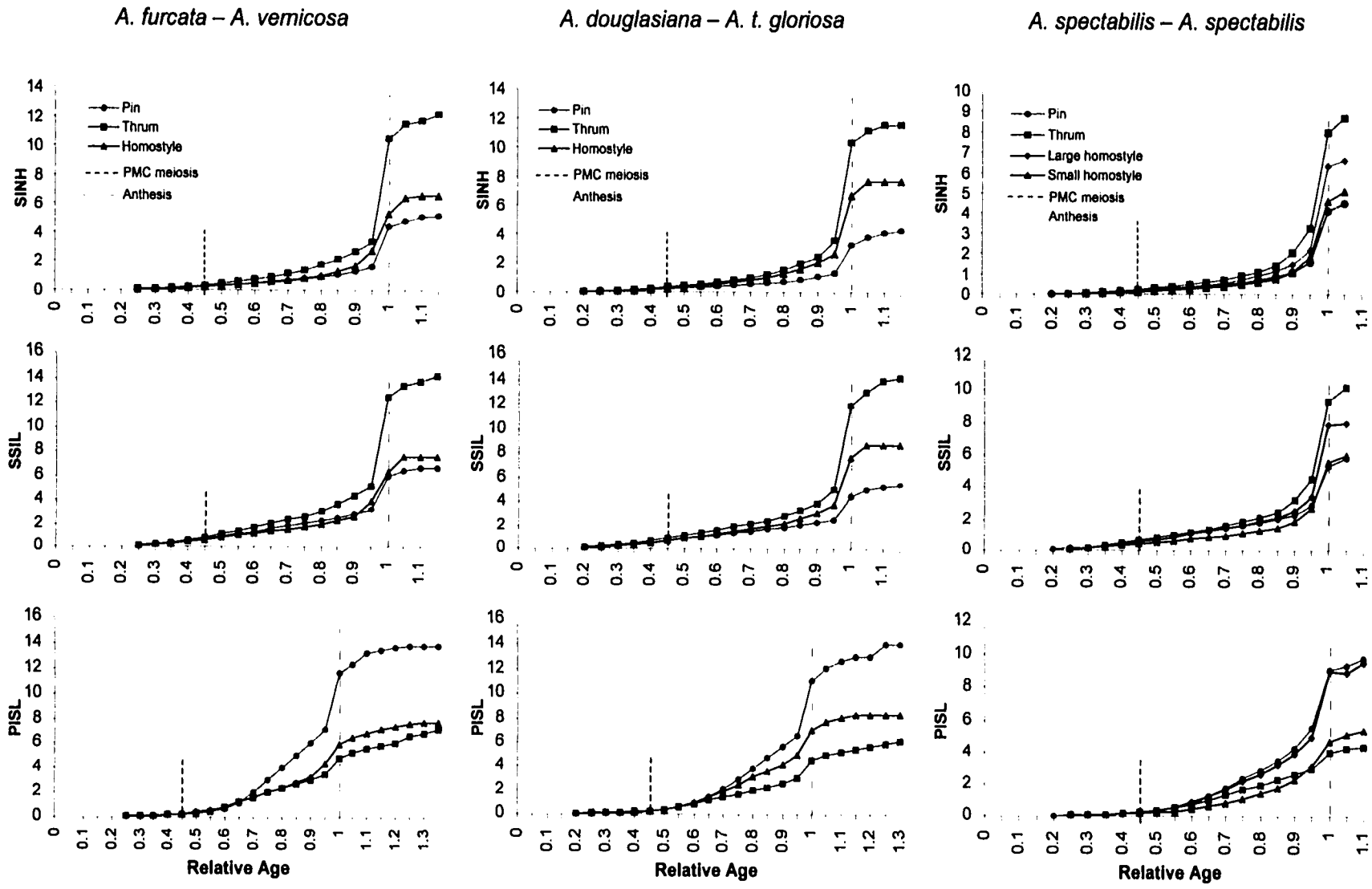

Figure 6.4. Growth of floral traits in *Amsinckia* (Part IV). Unit of trait size: mm.

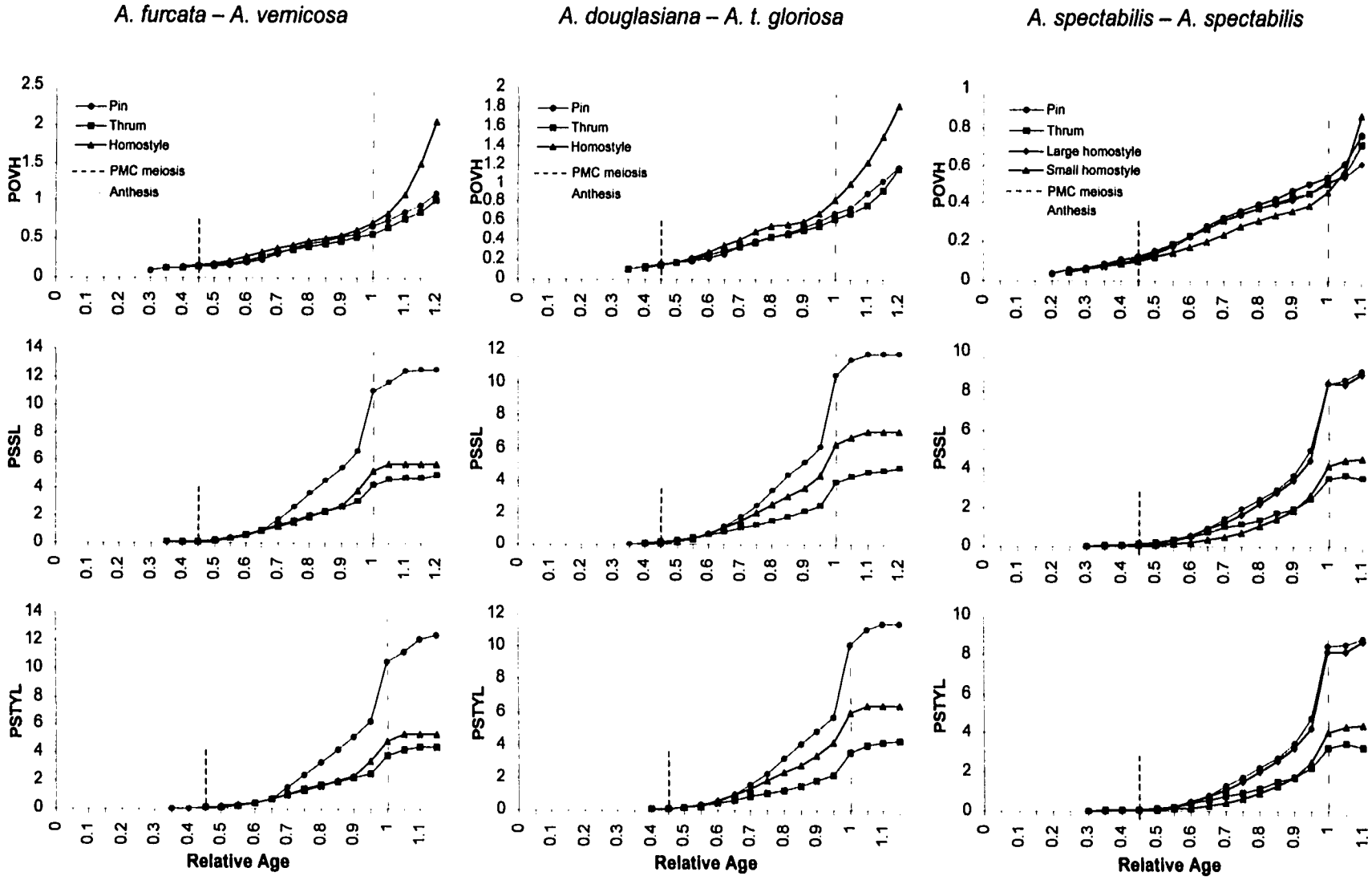

Figure 6.5. Growth of floral traits in *Amsinckia* (Part V). Unit of trait size: mm.

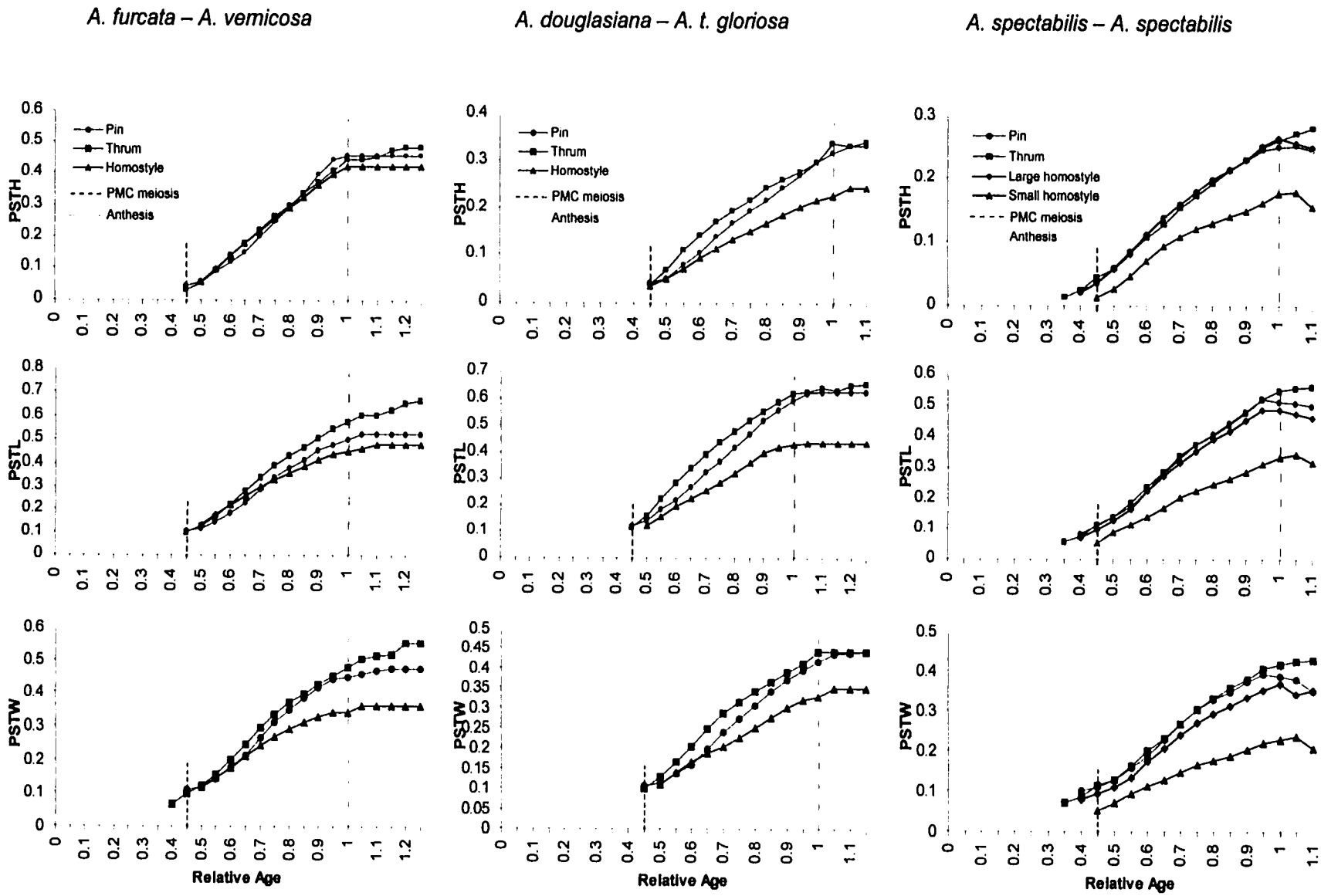

Figure 6.6. Growth of floral traits in *Amsinckia* (Part VI). Unit of trait size: mm.

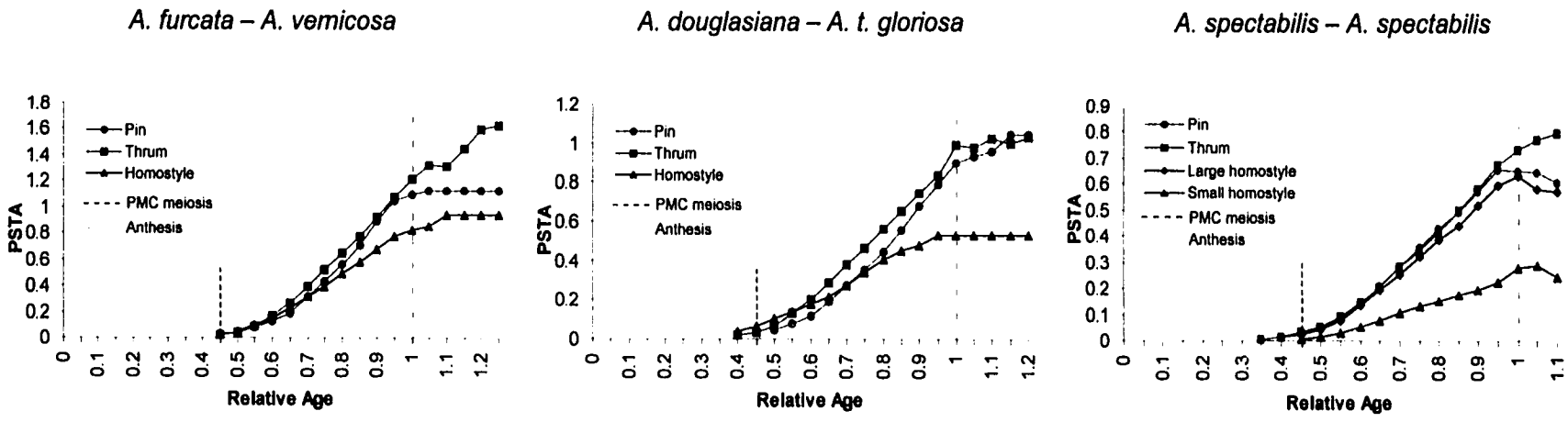

Figure 6.7. Growth of floral traits in *Amsinckia* (Part VII). Unit of trait size: mm^2.

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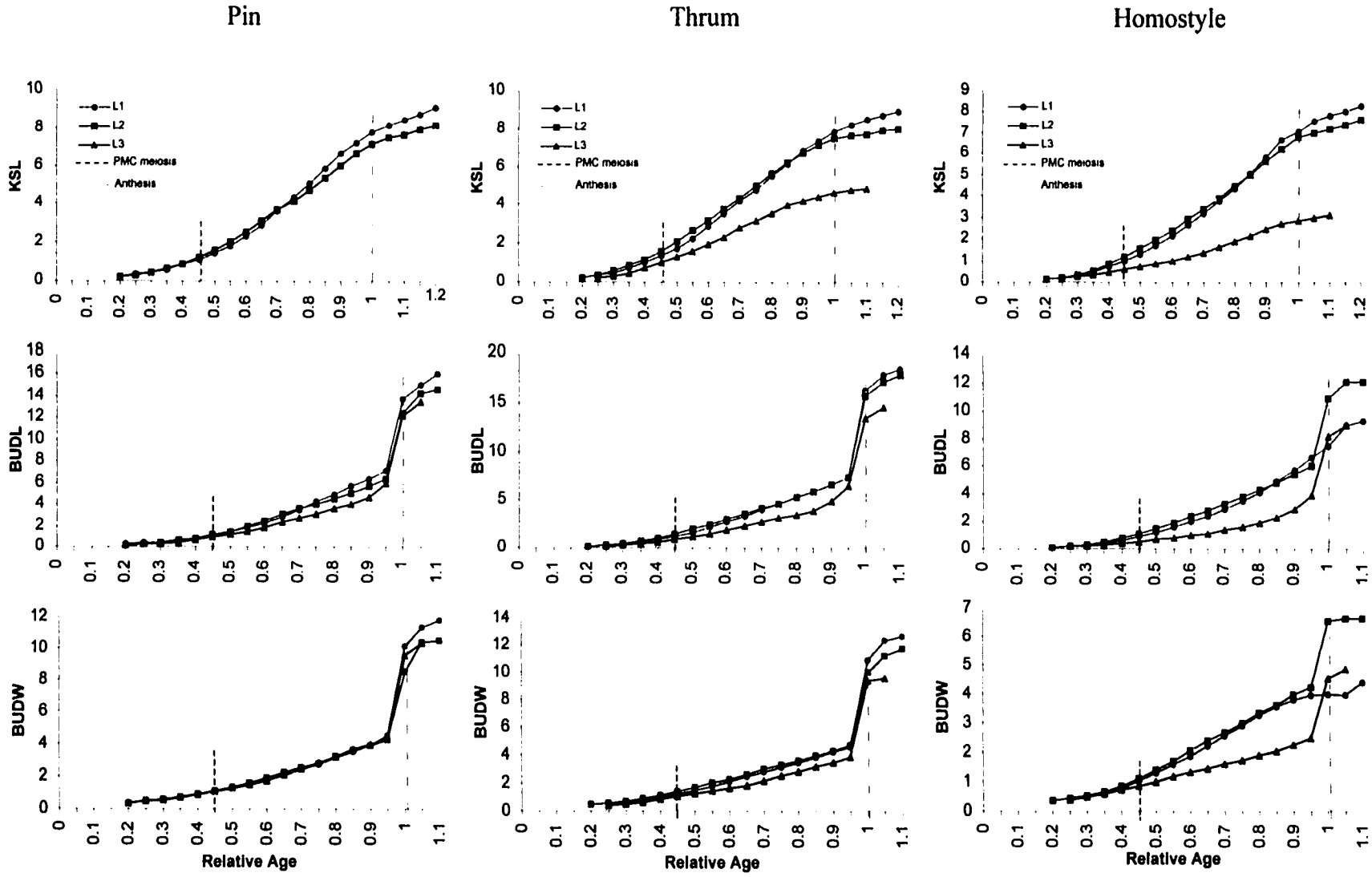


Figure 6.8. Growth of floral traits in *Amsinckia* (Part VIII). Unit of trait size: mm. L1: lineage of *A. furcata – A. vernicosa*; L2: lineage of *A. douglasiana – A. t. gloriosa*; L3: lineage of *A. spectabilis*. Homostyle in L3 is small homostyle only.

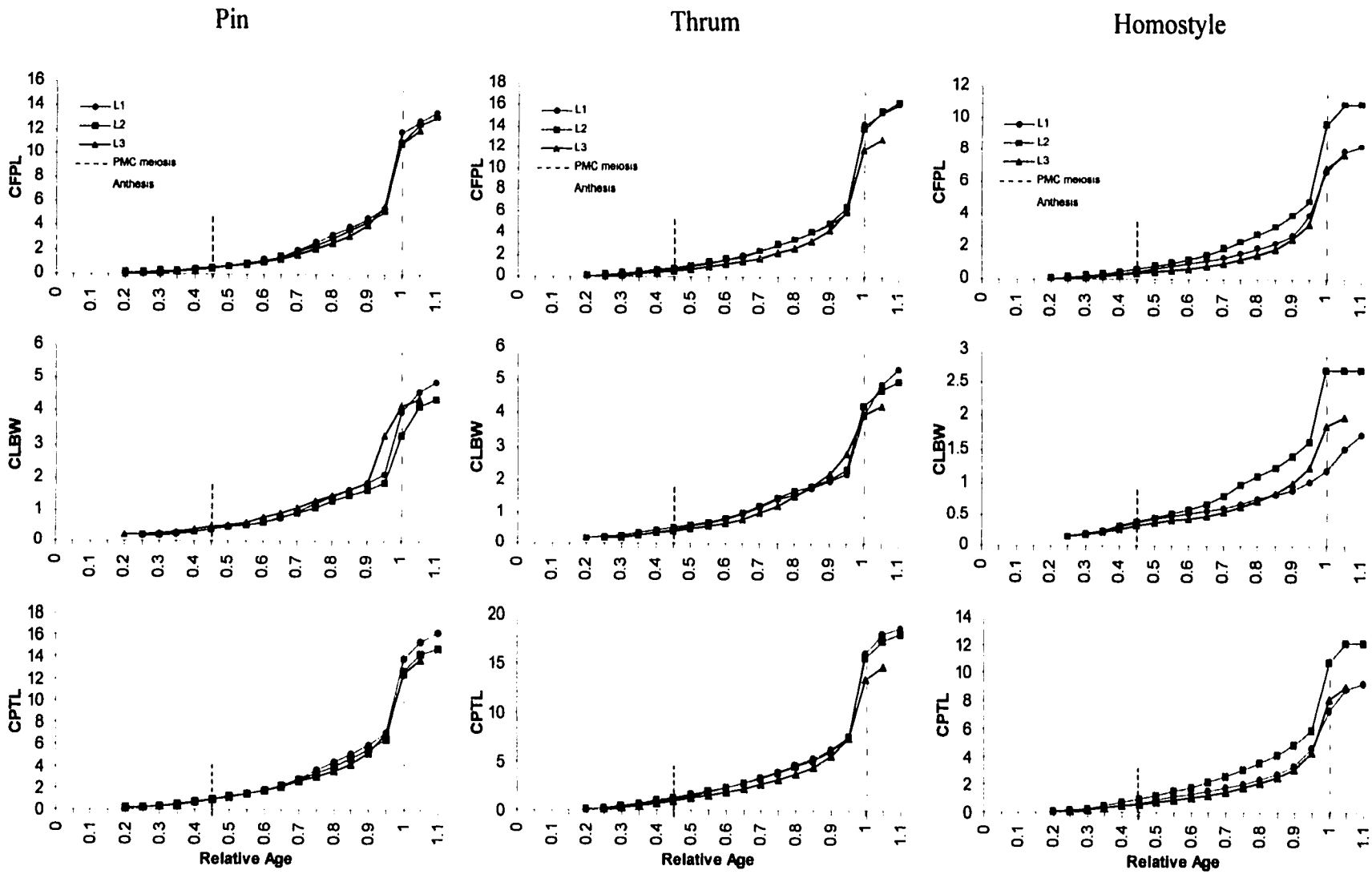


Figure 6.9. Growth of floral traits in *Amsinckia* (Part IX). Unit of trait size: mm. L1: lineage of *A. furcata – A. vernicosa*; L2: lineage of *A. douglasiana – A. t. gloriosa*; L3: lineage of *A. spectabilis*. Homostyle in L3 is small homostyle only.

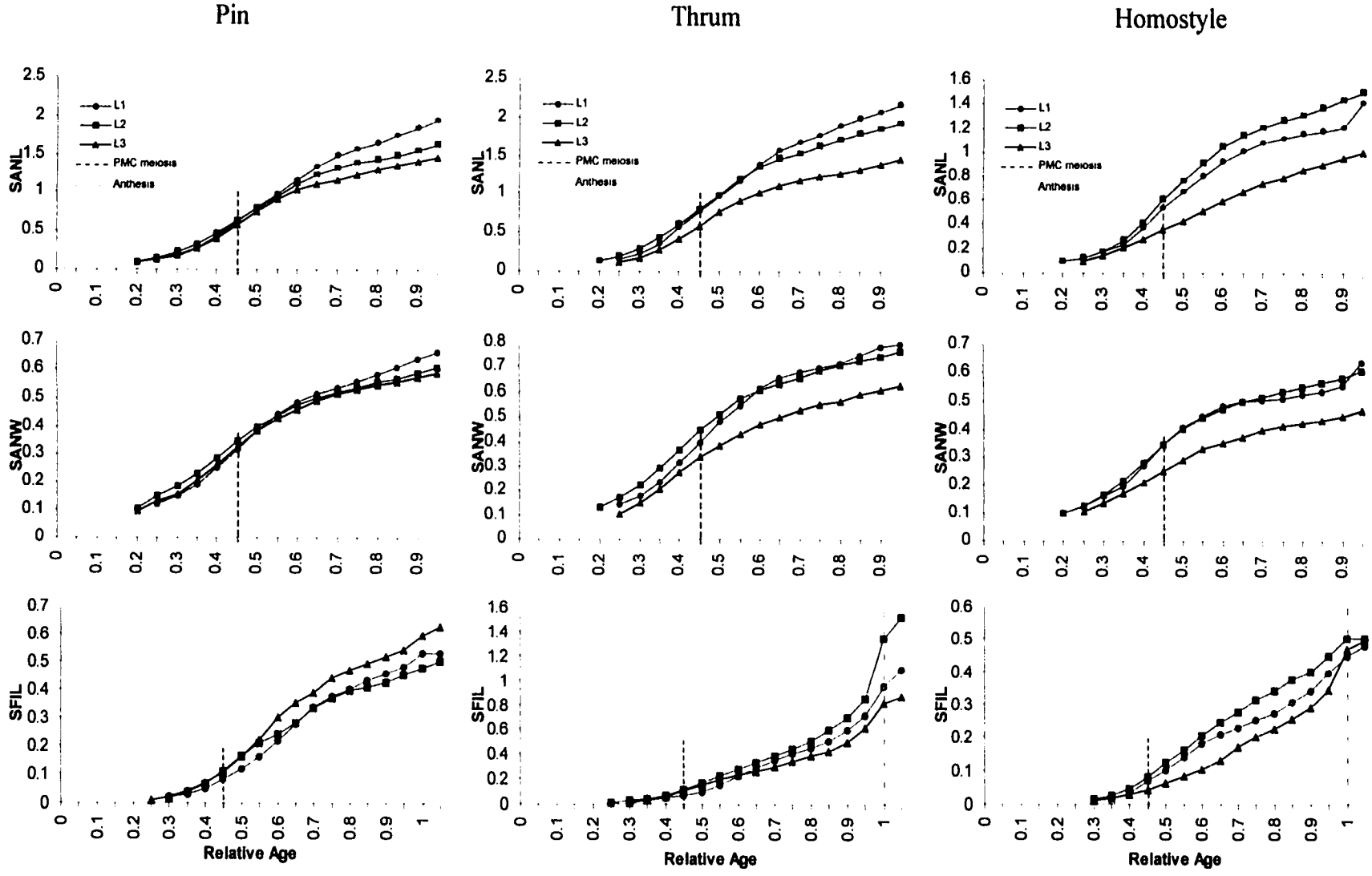


Figure 6.10. Growth of floral traits in *Amsinckia* (Part X). Unit of trait size: mm. L1: lineage of *A. furcata – A. vernicosa*; L2: lineage of *A. douglasiana – A. t. gloriosa*; L3: lineage of *A. spectabilis*. Homostyle in L3 is small homostyle only.

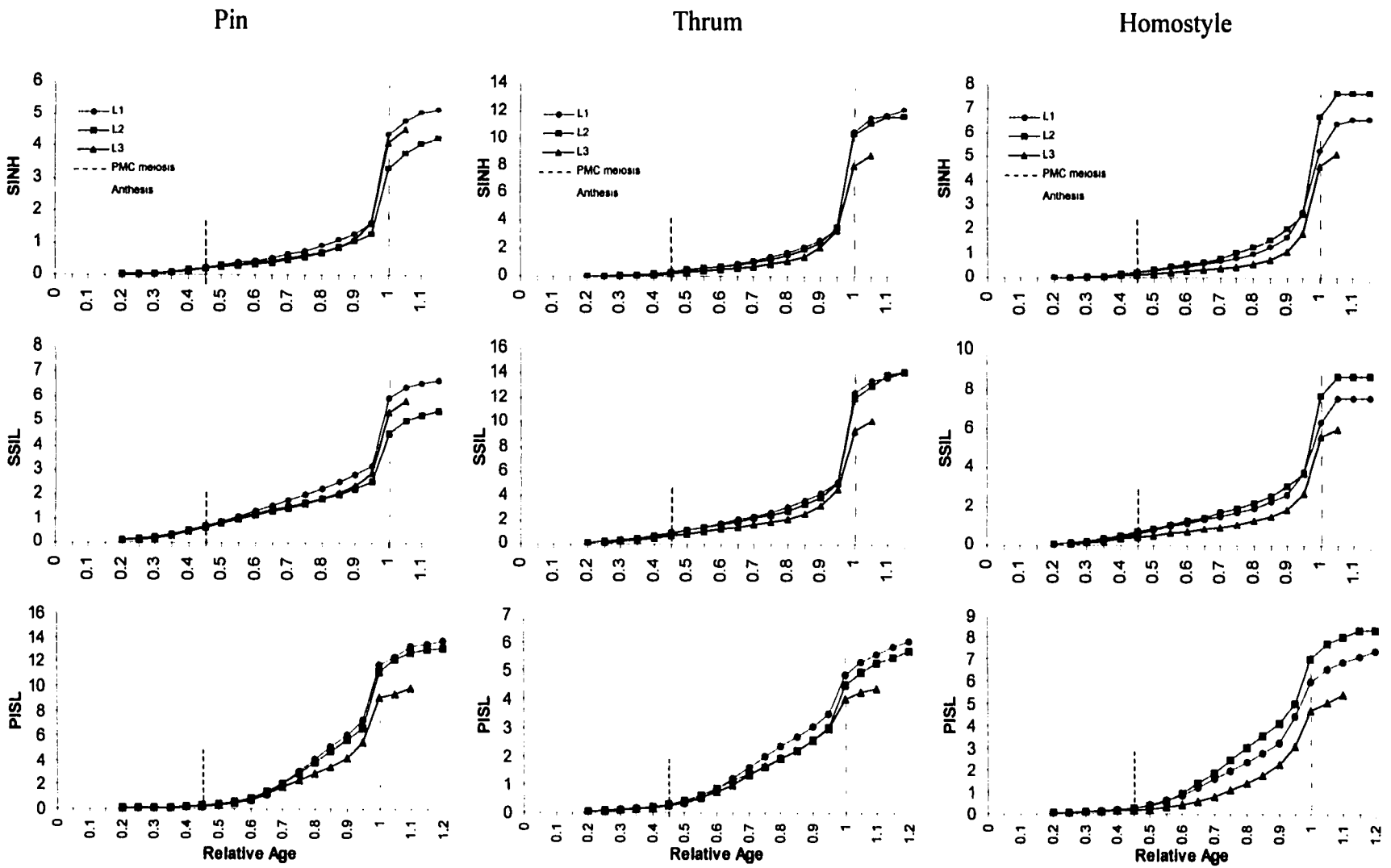


Figure 6.11. Growth of floral traits in *Amsinckia* (Part XI). Unit of trait size: mm. L1: lineage of *A. furcata – A. vernicosa*; L2: lineage of *A. douglasiana – A. t. gloriosa*; L3: lineage of *A. spectabilis*. Homostyle in L3 is small homostyle only.

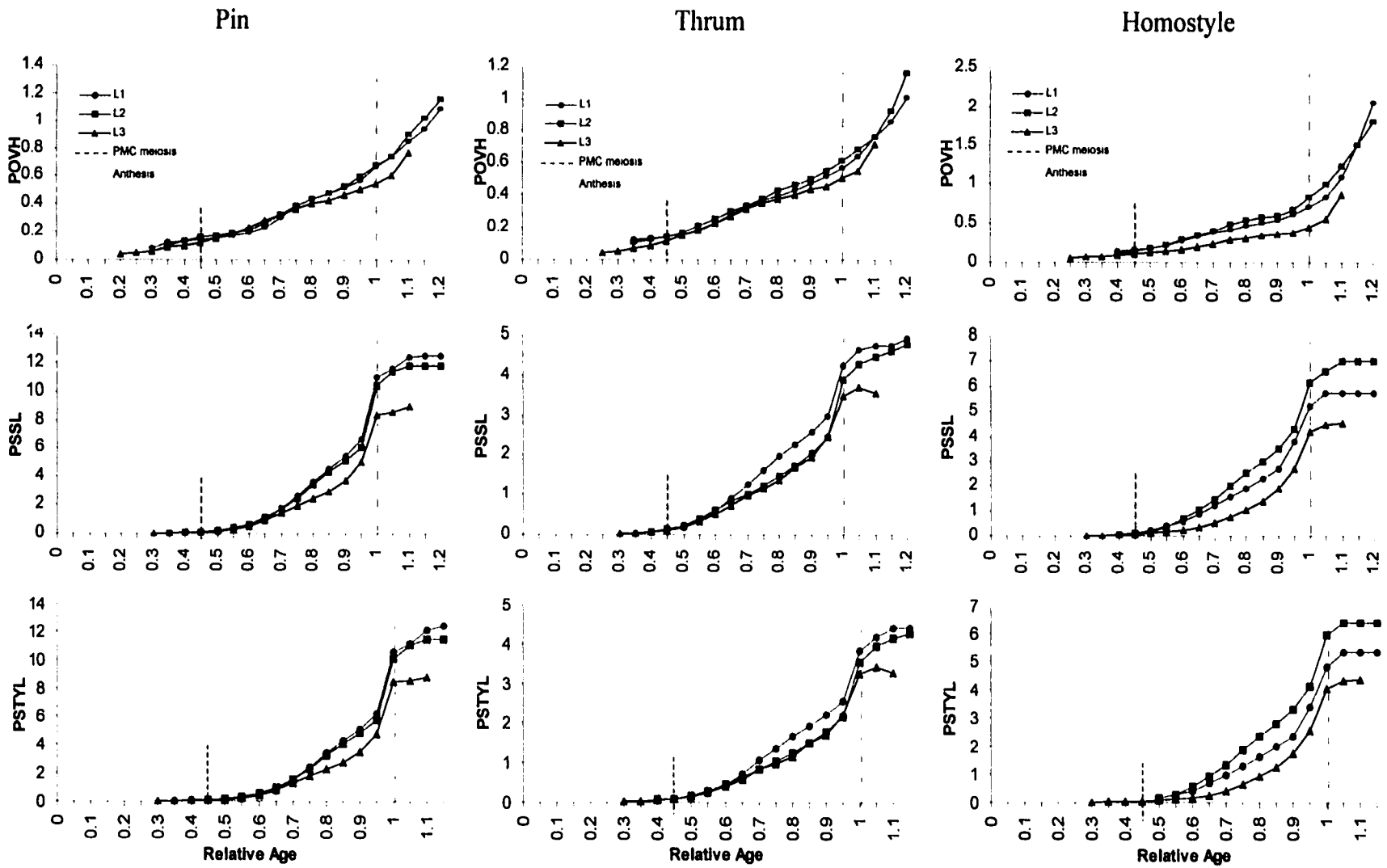


Figure 6.12. Growth of floral traits in *Amsinckia* (Part XII). Unit of trait size: mm. L1: lineage of *A. furcata – A. vernicosa*; L2: lineage of *A. douglasiana – A. t. gloriosa*; L3: lineage of *A. spectabilis*. Homostyle in L3 is small homostyle only.

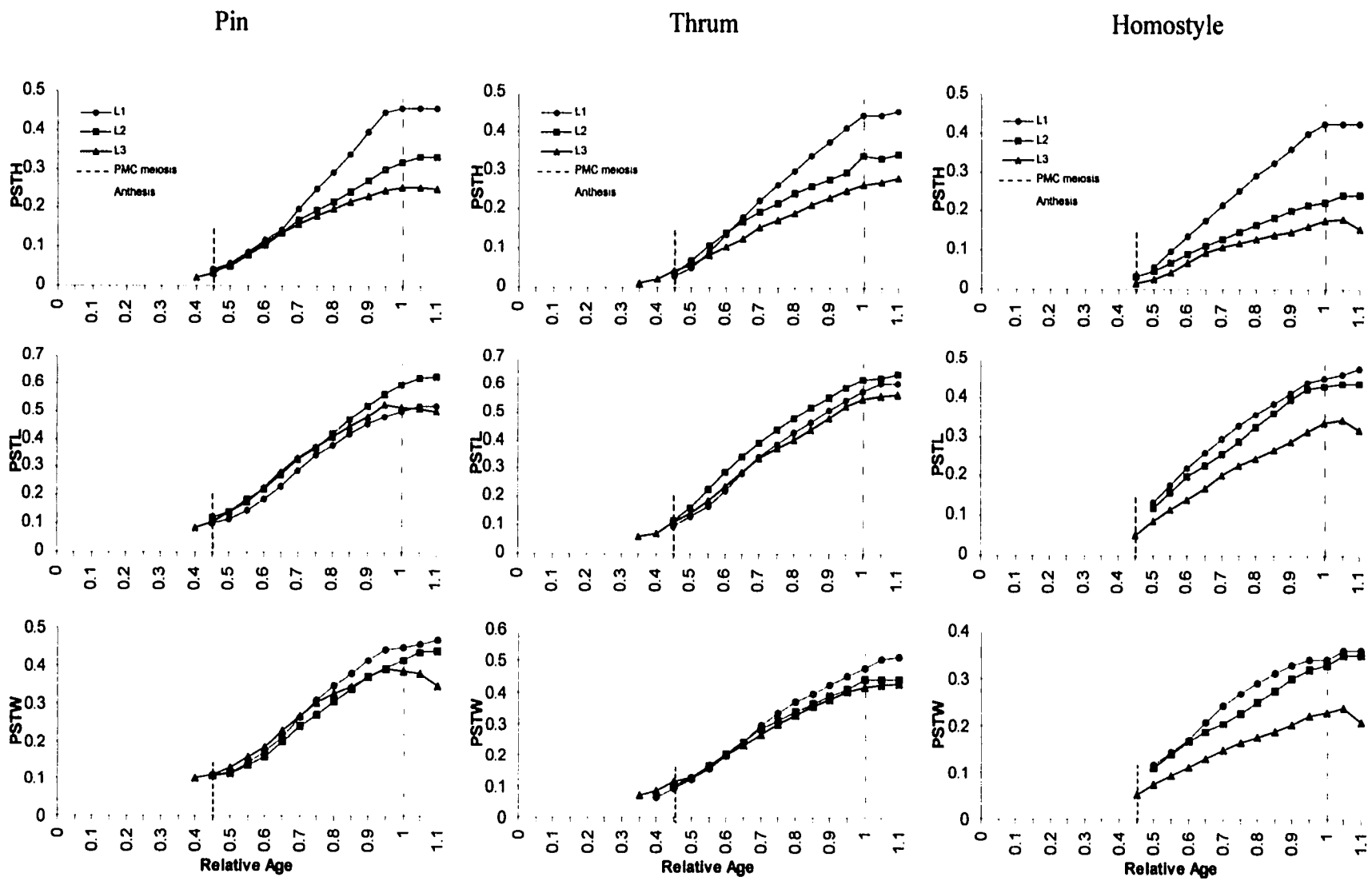


Figure 6.13. Growth of floral traits in *Amsinckia* (Part XIII). Unit of trait size: mm. L1: lineage of *A. furcata – A. vernicosa*; L2: lineage of *A. douglasiana – A. t. gloriosa*; L3: lineage of *A. spectabilis*. Homostyle in L3 is small homostyle only.

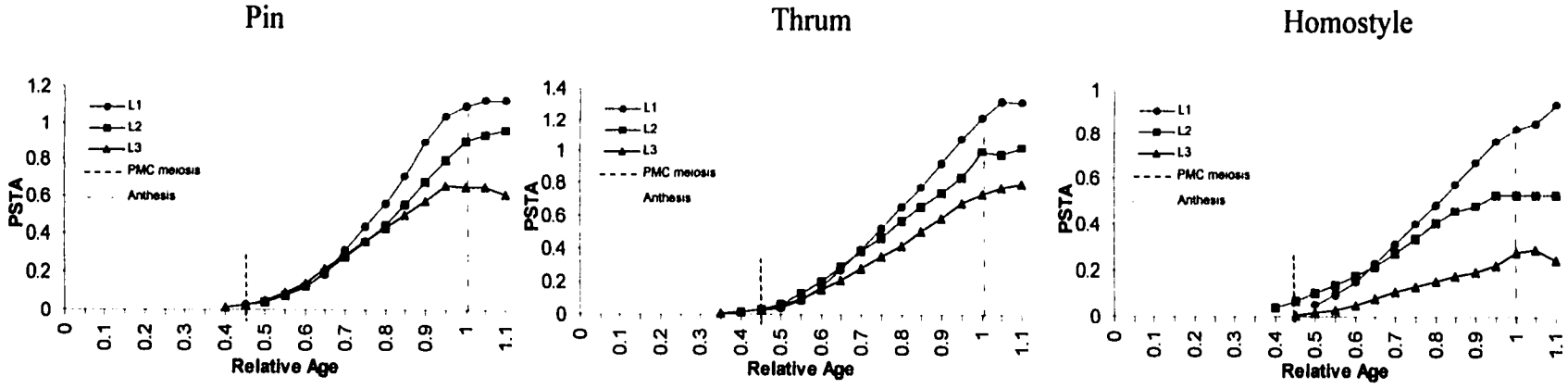


Figure 6.14. Growth of floral traits in *Amsinckia* (Part XIV). Unit of trait size: mm^2. L1: lineage of *A. furcata – A. vernicosa*; L2: lineage of *A. douglasiana – A. t. gloriosa*; L3: lineage of *A. spectabilis*. Homostyle in L3 is small homostyle only.

SFIL, also varied among lineages within each morph (Figs. 6.10, 6.11). These variations were also indicated by the repeated measures ANOVA (Tables 6.3, 6.5). It showed that the amount by which morphs differ depended on lineage, and vice versa, and this difference (interaction of morph and lineage on trait size) also depended upon the trait's developmental age (Table 6.4).

The developmental divergence for pistil-height-associated traits (PISL, POVH, PSSL and PSTYL) between homostyly (small homostyle in L3) and distyly mostly occurred some time after PMC meiosis in all three lineages (Figs. 6.4, 6.5; Table 6.5). Variations in diverging time, however, did exist among lineages. In both Lineages 1 and 3, the growth trajectories of pistil-height-associated traits (except POVH) in homostyles (small homostyle in L3) diverged from those in thrums much later than from those in pins (Figs. 6.4, 6.5). In addition, the growth trajectories of pistil height also varied among the lineages within each floral morph, particularly in homostylous flowers (Figs. 6.11-6.12; Tables 6.3-6.4). The variations among lineages were mostly caused by the difference in trait's growth rate among species or populations.

The developmental divergence of anther size (SANL and SANW) growth trajectories between homostyly and distyly occurred earlier than any other floral trait's divergence in this study. In Lineage 3, the divergence initiated far before the PMC meiosis time (Fig. 6.3; Table 6.5). Whereas in Lineages 1 and 2, the trajectory diverged between homostyles and thrums probably prior to the relative age of 0.2-0.3; and the separation between homostyles and pins occurred after the PMC meiosis (Fig. 6.3). In Lineage 2, the ontogenetic trajectories of anther width (SANW) in homostyles and pins were the same. Anthers of same type of floral morph from different lineages had very

different developmental trajectories (Fig. 6.10; Table 6.5). This was especially obvious in thrum and homostylous flowers in which the trajectories differed among lineages since the early development.

The developmental trajectories of stigma size (PSTH, PSTL, PSTW and PSTA) varied both among floral morphs and among lineages (Figs. 6.6-6.7, 6.13-6.14; Tables 6.3-6.5). In all three lineages, especially in Lineages 2 and 3, the growth curves of stigma-size-related traits were much lower in homostyly (small homostyly in L3) than in distyly (Figs. 6.6, 6.7). The separation of the curves between the two styles occurred at or after PMC meiosis time in Lineages 1 and 2, but before meiosis in Lineage 3 (Figs. 6.6, 6.7; Table 6.5). The developmental trajectories of stigma length (PSTL) and width (PSTW) in pin and thrum flowers were similar among the three lineages, but in homostylous flowers they were much lower in Lineage 3 than in the other two lineages (Fig. 6.13). The developmental trajectories of stigma thickness (PSTH) and area (PSTA) were similar among the three lineages until sometime after PMC meiosis, at least in pin and thrum flowers (Figs. 6.13, 6.14, Table 6.5).

In the *A. spectabilis* lineage, the development of large homostyly was similar to that of distyly in many traits, including those associated with sepal length, flower size, anther size, and stigma size (Figs. 6.1-6.3, 6.6-6.7). Of three major stamen-height-related traits, filament length (SFIL) and stamen height (SSIL) in large homostyle were similar to those of thrum in terms of their developmental trajectories, while the third trait, stamen insertion height (SINH), was almost same as in small homostyle (Figs. 6.3, 6.4). On the other hand, pistil-height-associated traits (PISL, PSSL and PSTYL excluding POVH) of the large homostyle developed in a similar way as those of pin (Figs. 6.4, 6.5). The large

homostyly differed from small homostyly, in terms of developmental trajectories, in all floral traits except SINH (Figs. 6.1-6.7). The divergence of developmental trajectories between large homostyly and distyly occurred mostly after the PMC meiosis or during a later development except in traits of KSL, SANL, SANW and PSTW in which the two styles separated before the meiosis time. On the other hand, the developmental divergence between the two homostyles, in almost every trait except SINH, initiated before or around the PMC meiosis time and it was much earlier than between the large homostyly and distyly (Figs. 6.1-6.7).

6.4.3.2. *Pin vs. thrum*

Flower developmental trajectories between the two distylous floral morphs, pin and thrum, varied depending on the lineages and the traits. The developmental trajectories of sepal length (KSL) between the two morphs diverged before PMC meiosis, but they converged again by the time when they reached flower opening or their mature size in Lineages 1 and 2 (Fig. 6.1). In Lineage 3, the sepal's growth curves were not divergent until later developmental stages and the divergence gap between the two morphs increased as development proceeded.

The difference in flower size between pin and thrum appears to have initiated around the time of PMC meiosis, but the major separation of growth trajectories tended to occur later, and often at the time right before flower opening (Figs. 6.1, 6.2). This was particularly notable in the development of BUDL, BUDW, CFPL and CPTL in Lineages 1 and 2.

Stamen- and pistil-height-related traits were the major traits discriminating the two distylous morphs. The developmental trajectories of these traits between pins and thrums usually diverged sometime after the PMC meiosis in all three lineages (Figs. 6.3-6.5). For stamen-height traits, the developmental divergence between the two morphs was mostly caused by the dramatic growth-rate increase in thrum flowers after the relative age of 0.4-0.7. In contrast, the separation of growth curves of pistil-height traits between the two morphs was mainly due to the steep acceleration of the trait's growth rate in pin flowers after the relative age of 0.5-0.6.

The development of anther size among the three lineages was different. The developmental trajectories of anther length (SANL) and width (SANW) were well separated between pin and thrum prior to relative age of 0.2-0.3 in both Lineages 1 and 2, and that led to the final anther size being significantly different between the two morphs. In Lineage 3, however, the growth curves of SANL were almost exactly the same between pin and thrum flowers, while growth curves of SANW diverged between the two morphs only when they reached an approximate relative age of 0.6 (Fig. 6.3).

The trajectories of stigma development varied among the three lineages. In Lineages 1 and 2, the developmental divergence of all four traits (PSTH, PSTL, PSTW and PSTA) between pin and thrum occurred around relative age of 0.4-0.5. The gaps between the two trajectories in Lineage 1, however, were very small in PSTH, PSTW and PSTA, and it led to the size being similar in pin and thrum at flower opening. The separate trajectories in Lineage 2 converged again in all four traits by the time of flower opening or during post-anthesis development. In contrast, the growth of stigma size in the two morphs in Lineage 3 shared the same ontogenetic trajectory until they reached

relative age of 0.95, right before flower opening, and then the traits in pin flower ceased growth (Figs. 6.6, 6.7). The late divergence of growth trajectories in this case, however, was not large enough to render the stigma statistically different in size between the two morphs in Lineage 3.

The results on variations of developmental trajectories among floral morphs and lineages in *Amsinckia* were also well supported by the multivariate repeated-measures analysis. These analyses indicated that almost every trait studied here differed in mean size among lineages (except SFIL) and floral morphs, and the difference among lineages also depended on floral morphs and vice versa (Table 6.3). The analyses further found that the change in mean trait size across developmental ages differed among lineages (except SFIL) and floral morphs, and the difference among floral morphs that depended upon lineage (and vice versa) also relied on the developmental age (except PSTW; Table 6.4). The analyses also showed that the overall mean developmental trajectory of each trait differed among lineages (except SFIL) and floral morphs, and the difference of the mean developmental trajectory among floral morphs also depended on lineages and vice versa (except KSL, SFIL and PSTW; Table 6.5).

6.5. Discussion

6.5.1. Development and evolution of homostyly

6.5.1.1. *Developmental time and rate effects*

It is common that self-pollinating flowers are smaller than outcross-pollinating ones. Self-pollinated homostylous flowers in *Amsinckia* are significantly smaller than their ancestral, predominately outcross-pollinated distylous flowers. Statistically, almost every studied floral trait was significantly smaller in homostyly than in distyly in all three lineages of *Amsinckia*, except sepal length (not different between homostyly and distyly), pistil-height-related traits (smaller than in pin but larger than in thrum) and stamen-height-associated traits (smaller than in thrum but larger than in pin; Table 4.2). To understand how homostyly has evolved from distyly or the way the small selfing flowers were produced compared to the large outcrossing flowers, from the viewpoint of flower development and evolution, floral ontogenies were compared between flower morphs and among evolutionary lineages in *Amsinckia*.

The flower developmental duration from the initiation of a floral primordium to anthesis, i.e., flower opening, is not different between homostylous and distylous flowers in Lineages 1 and 2 (Table 6.1). This suggests that the developmental duration prior to anthesis is not the major cause that leads to the homostylous flower being smaller in these two lineages. The small homostylous flowers in Lineage 3, however, have significantly longer developmental duration, compared to the distylous and large homostylous ones. This means that the small homostylous flowers in Lineage 3 delayed their offset time (the flower opening), because the onset time (the initiation of floral primordium) are presumably the same for all floral morphs in terms of the flower ontogeny. Based on the heterochrony concept, this is a peramorphic ontogeny caused by hypermorphosis. In general, longer developmental duration will lead to a larger flower or a larger floral organ. In *A. spectabilis*, however, the flower size and the developmental duration (up to

anthesis) are inversely related. The small homostyly with longer developmental duration does not produce flowers of larger size, because of a decreased developmental rate (approximately 50-60% slower than distyly, more details below). Thus, the increased duration (hypermorphosis) is not sufficient to counterbalance the decreased growth rate (neoteny).

The developmental duration discussed above is only up to anthesis. I use anthesis as the end of the developmental duration because it is one of the best timing reference points or marks for flower development. However, one must bear in mind that the size of the flower and some floral organs at anthesis is smaller than their final size because development continues. Furthermore, modifications of post-anthesis development are often important in species-level differentiation in terms of flower morphology (Hufford, 1988a). It is therefore important to include the post-anthesis development for the purpose of comparing the complete floral ontogeny.

Comparison of mature sizes (Table 4.2) and post-anthesis development (Figs. 6.1-6.7, some data not shown) shows that approximately 14 of 21 studied floral traits had relatively earlier developmental offset in homostylous flowers than in distylous ones, while the remaining traits had similar offset time in the two styles in Lineages 1 and 2 (Fig. 6.15). This indicates that although the developmental time before anthesis is the same for both homostylous and distylous flowers in these two lineages, the earlier cessation of most floral traits during their post-anthesis development in homostylous flowers (progenesis) may have led to an overall shorter developmental duration in homostylous flowers compared to distylous ones. A similar result has been observed in Lineage 3, in which about seven of 21 traits in small homostylous flowers cease

Figure 6.15. A summary of heterochronic changes in homostyly compared with ancestral distyly in 20 floral traits in three evolutionary lineages of *Amsinckia*. Comparisons are based on relative age, where zero is floral primordium and one is flower opening, and are made to both distylous morphs without regard to statistical significance. Because onset of growth is defined at floral primordium, delayed onset (postdisplacement) and earlier onset (predisplacement) are excluded as possibilities. Offset times are defined as the relative age at which maximum size is reached. It means no difference on growth offset time between two compared morphs if there is no result entry in both progenesis and hypermorphosis columns. The large homostyle of *A. spectabilis* is excluded. Figure Abbreviations: All abbreviations of traits are explained in Table 4.1 of Chapter 4; L1, Lineage 1; L2, Lineage 2; L3, Lineage 3.

Trait and Lineage		Change in Homostyle															
		Compared to Pin							Compared to Thrum								
			Paedomorphosis			Peramorphosis				Paedomorphosis			Peramorphosis				
		None	Neoteny	Progenesis	Postdisplacement	Acceleration	Hypermorphosis	Predisplacement	None	Neoteny	Progenesis	Postdisplacement	Acceleration	Hypermorphosis	Predisplacement		
KSL	L1		•	•						•	•						
	L2			•		•					•		•				
	L3		•							•							
BUDL	L1		•	•						•	•						
	L2		•	•						•	•						
	L3		•							•							
BUDW	L1		•	•						•	•						
	L2		•	•						•	•						
	L3		•							•							
CFPL	L1		•	•						•	•						
	L2		•	•						•	•						
	L3		•							•							
CLBW	L1		•							•							
	L2		•	•						•	•						
	L3		•	•						•	•						
CPTL	L1		•	•						•	•						
	L2		•	•						•	•						
	L3		•							•							
CTBL	L1		•							•							
	L2					•				•							
	L3		•							•							
SANL	L1		•							•							
	L2		•							•							
	L3		•							•							
SANW	L1		•							•							
	L2					•				•							
	L3		•							•							
SFIL	L1		•							•	•						
	L2					•				•	•						
	L3		•	•						•	•						
SINH	L1			•		•				•	•						
	L2			•		•				•	•						
	L3		•							•	•						
SSIL	L1			•		•				•	•						
	L2			•		•				•	•						
	L3		•							•	•						
PISL	L1		•										•				
	L2		•	•							•		•				
	L3		•							•							
POVH	L1					•							•				
	L2					•							•				
	L3		•							•							
PSSL	L1		•	•							•		•				
	L2		•								•		•				
	L3		•							•					•		
PSTYL	L1		•	•							•		•				
	L2		•	•							•		•				
	L3		•							•					•		
PSTH	L1		•							•	•						
	L2		•							•	•						
	L3		•							•	•						
PSTL	L1		•	•						•	•						
	L2		•	•						•	•						
	L3		•				•			•	•						
PSTW	L1		•	•						•	•						
	L2		•							•					•		
	L3		•				•			•	•						
PSTA	L1		•				•			•	•						
	L2		•	•						•	•						
	L3		•				•			•	•						

Fig. 6.15

development earlier than in distylous flowers in terms of their relative developmental age, and another three traits are in between the two distylous morphs (earlier than one of the distylous morph but later than the other morph) while the remaining 11 traits show no difference between the small homostyly and distyly (Fig. 6.15). The differences of ontogenetic offset times between the two styles imply that the overall relative developmental time in small homostylous flowers is probably similar to or a little shorter than that in the distylous flowers in Lineage 3, although the actual developmental time before flowering is longer in the small homostylous flowers.

Overall, the earlier offset of development in most of the floral traits in homostyly suggests that the small, homostylous flowers in *Amsinckia* at least partially result from paedomorphic ontogeny through progenesis (Figs. 6.15, 6.16). Progenesis is known to be one of the major developmental processes that lead to the modification and evolution of flowers or floral organs in angiosperms (Takhtajan, 1976, 1991; Runions and Geber, 2000).

Developmental rate and timing changes in a descendant compared to its ancestor are the core of the heterochronic concept, and are the source of evolution on development. Self-pollinated flowers often grow more slowly than outcross-pollinated flowers (Hill and Lord, 1990). My study also shows that most floral traits in homostylous flowers have a slower growth rate than distylous flowers in all three lineages of *Amsinckia*, especially in the lineage of *A. spectabilis* in which all of the floral traits in the small homostylous flowers are highly significantly slower than in distylous flowers (Table 6.2; Fig. 6.15). The results suggest that the small homostylous flowers in *Amsinckia* are produced through a reduction in the relative developmental rate of the

Figure 6.16. **a-c**. Models for the effect of heterochrony on morphological evolution of homostyly (descendant) from distyly (ancestral) in lineages *Amsinckia furcata* – *A. vernicosa* and *A. douglasiana* – *A. t. gloriosa*. **d-f**. Models for the effect of heterochrony on morphological evolution of homostyly (descendant) from distyly (ancestral) in lineage *A. spectabilis*. **a**. Homostyle diverged from distyly earlier than the divergence between two distylous floral morphs: pin (P) and thrum (T). Homostyle also had lower developmental rate (neoteny) and earlier offset (progenesis) compared with pin and thrum. This paedomorphic ontogeny results in the homostylous flower being smaller than the distylous flower. **b**. The developmental rate of pistil height in homostyle is slower than in pin (neoteny) but faster than in thrum (acceleration). The pistil-height growth trajectories diverged earlier between homostyle and pin than between homostyle and thrum. The medium developmental rate and early offset in homostyle result in its final pistil height being lower than in pin but higher than in thrum. **c**. The growth of stamen height in homostyle is faster than in pin but slower than in thrum. The developmental divergence of stamen-height growth between homostyle and thrum is earlier than between homostyle and pin. The early developmental offset and medium growth rate in homostyle results in its stamen height being higher than in pin but shorter than in thrum. **d**. A slower developmental rate in small homostyle results in the final size of a homostylous flower being smaller than that of pin, thrum and large homostyle (LH). Pin, thrum and large homostyle have a similar growth trajectory in flower size. **e**. The slow pistil-height growth in small homostyle is similar to that of thrum. It leads to the pistil-height in small homostyle being lower than that in pin and large homostyle but similar to that in thrum. To the pistil-height growth, small homostyle shares a similar growth trajectory with pin while large homostyle and thrum share another similar growth trajectory. **f**. The slow stamen-height growth in small homostyle is similar to that of pin but differs from those of thrum and large homostyle. Both small homostyle and pin share a similar growth trajectory and cease their stamen-height growth earlier while large homostyle shares another growth trajectory with thrum but has an early offset. Paedomorphosis through neoteny and progenesis in small homostyle results in stamen height in small homostylous flower being lower than that in thrum and large homostylous flowers. Mosaic development with multi-heterochronic processes (neoteny and

acceleration) has also led anther height in large homostylous flower to be shorter than in thrum but higher than in pin flowers. The above results indicate that multiple heterochronic processes are involved in the mosaic development and evolution of homostyly. Note: The development of most floral traits diverges among floral morphs around microsporocyte meiosis time (*m*), and ceases during post-anthesis period (*pa*). When different morphs in Lineage 3 share a same line in the models above it means only that their ontogenetic trajectories are similar and they are not necessary to be the same due to variations among different traits of the same floral organ. The relative time of 0 is the onset time and 1 is the flowering (anthesis) time. Lineage 1: lineage of *A. furcata* – *A. vernicosa*; Lineage 2: *A. douglasiana* – *A. t. gloriosa*; Lineage 3: *A. spectabilis*.

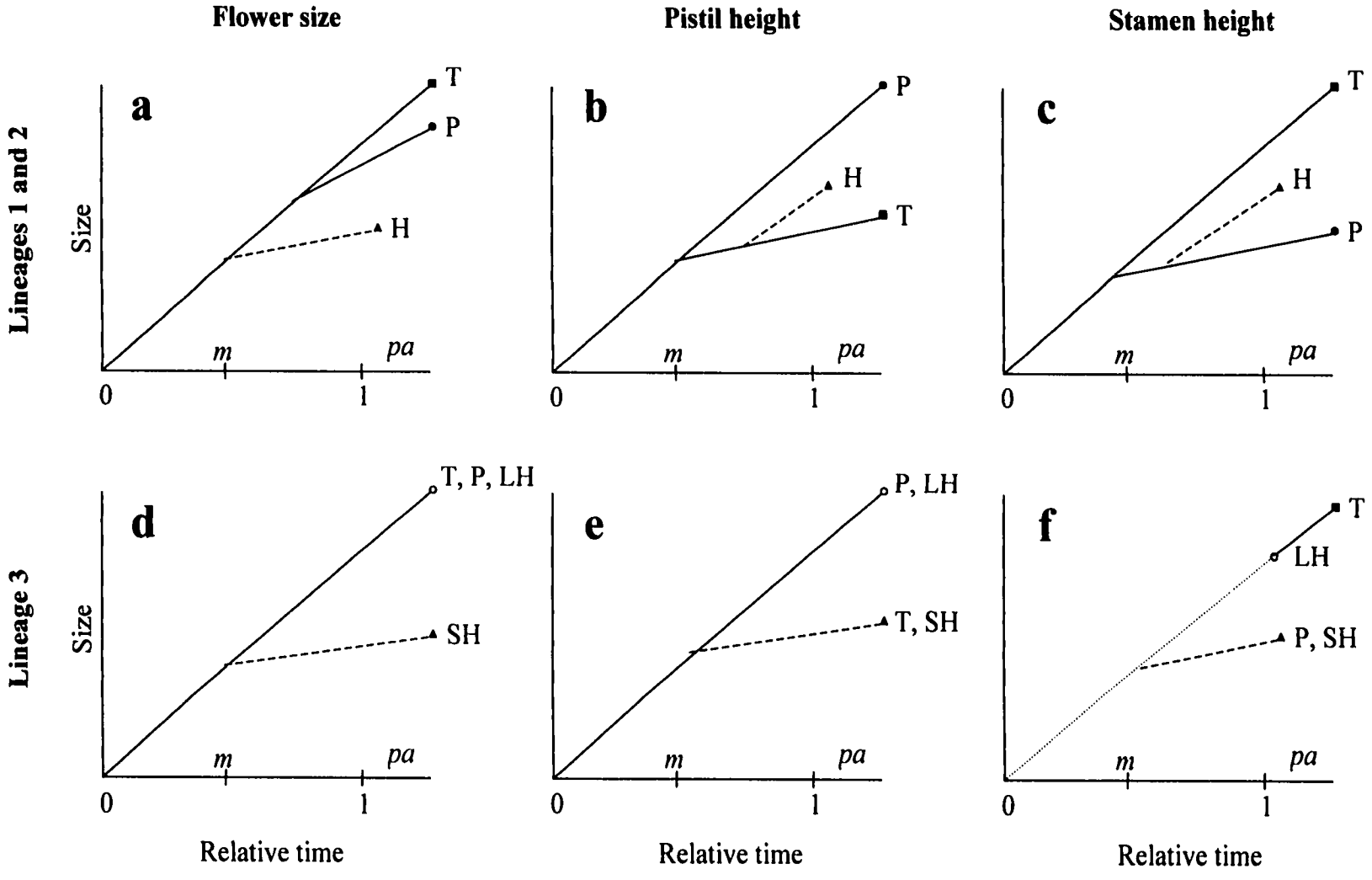

Flower size
Pistil height
Stamen height
Lineages 1 and 2
Lineage 3
a
b
c
d
e
f
Size
T
P
H
T, P, LH
P, LH
T, SH
SH
LH
P, SH
m
pa
0
1
Relative time

Fig. 6.16

majority of floral traits, with either no change (in L1 and L2) or longer (in L3) developmental duration (Note: post-anthesis development is excluded here because the calculation of developmental rate does not include the post-anthesis period). This means that the derivation of homostyly from distyly in *Amsinckia* is strongly associated with the paedomorphic ontogeny through neotony (decreased rate; Fig. 6.16).

From the viewpoint of evolution of selfing, the slow development of homostylous flowers in *Amsinckia* would not be agreeable to the hypothesis of selection for rapid maturation. It, however, does support another hypothesis that selfing flowers evolve as a consequence of direct selection for the ability to self-pollinate or the greater reproductive assurance in the geographically or ecologically marginal environments colonized by the self-pollinated homostylous plants (Barrett, 1989a). The geographical distribution of homostylous species and populations of *Amsinckia* corresponds well with the hypothesis. While the distylous *Amsinckia* species "typically occur in natural habitats such as chaparral borders, serpentine soils, and Pleistocene sand dunes, ... most homostylous species and populations of *Amsinckia* are found in ecologically marginal habitats such as roadsides and grazed fields" (Schoen et al., 1997), particularly, the homostylous *A. spectabilis* "is strictly coastal in distribution and ranges interruptedly from Guadalupe Island off Baja California to the Queen Charlotte Islands in British Columbia" (Ganders, 1975a). From the ecological developmental biology point of view, although differential gene expression results in the formation of morphology, environmental factors can produce specific morphology by changing gene expression patterns (Gilbert, 2001). Thus, changes of flower development under the environmental pressure including the marginal

habitats and limitation of pollinator availability may have played important roles in the evolution of homostylous flowers.

The role of neoteny in flower development and evolution has also been reported in other studies, such as in *Arenaria uniflora* in which the self-pollinated flowers had longer developmental duration but a decreased developmental rate compared with their putative outcross-pollinated ancestor (Wyatt, 1984a, 1984b; Hill et al., 1992). It is believed that the development and evolution of most small, self-fertilizing flowers is associated with neoteny (Hill and Lord, 1990; Diggle, 1992), but there are very few studies. On the other hand, shorter developmental duration and accelerated developmental rate were often reported in the development and evolution of selfing flowers, such as in *Agalinis neoscotica* (Stewart and Canne-Hilliker, 1998), *Clarkia xantiana* ssp. *parviflora* (Runions and Geber, 2000), and *Limnanthes floccosa* (Guerrant, 1984, 1988), including the derivation of self-pollinated cleistogamous flowers from outcross-pollinated chasmogamous flowers in *Astralagus cymbicarpos* (Gallardo et al., 1993), *Collomia grandiflora* (Minter and Lord, 1983), *Lamium amplexicaule* (Lord, 1982), and *Viola odorata* (Mayers and Lord, 1983a).

6.5.1.2. *Ontogenetic trajectory effects*

The differences in developmental rate and overall duration between distylous and homostylous flowers are significant for the majority of the floral traits in all three lineages. This implicates the creative role of heterochrony in the evolution of the homostyly. But the differences between the two styles or three morphs (pin, thrum and homostyle) are more than just in rate and time; they also differ in their ontogenetic

transformations in almost every trait. Rather than developing along the same ontogenetic trajectory at different rates and varied offset times, the two styles and three floral morphs follow different ontogenetic trajectories at different rates and offset times (Figs. 6.15, 6.16).

For most floral traits, the divergence of developmental trajectories between distylous and homostylous flowers has initiated by the time PMC meiosis finishes (at a relative age of 0.45) due to a steep increase of developmental rate in distylous flowers in most cases. Nevertheless, the major differences or separations of the developmental curves between the two styles usually occurred right before flower opening because of a much dramatic developmental rate increase in distylous flowers than in homostylous ones (Figs. 6.1-6.7). This earlier developmental divergence can also be indicated from the differences in the size of floral traits between distylous and homostylous flowers at the PMC meiosis time. Approximately 12-13 of 19 studied floral traits are significantly smaller in homostylous flowers than in distylous flowers while the other six to seven traits have no difference in size between the two styles at their relative developmental age of 0.45 in both Lineages 1 and 2 (data not shown). In Lineage 3, all 19 studied floral traits are highly significantly smaller in small homostylous flowers than in distylous ones at the relative age of 0.45. The results suggest that the difference of homostyly from distyly may have occurred during the early stages of flower development based on a developmental time scale proposed by Hufford (1988b), i.e., "early ontogeny was considered to be that phase between floral inception and microsporocyte meiosis. Late ontogeny was considered to be that phase from the end of microsporocyte meiosis until corolla-androecium abscission."

Therefore, the derivation of homostyly from distyly is the result of multiple heterochronic processes. It is not simply a result of a decrease in overall developmental duration or earlier offset (progenesis) and relative growth rate (neoteny). It is also partly the outcome of the modified ontogenetic trajectories (Figs. 6.1-6.15).

Besides flowers being smaller in homostyly, the stigma and anther heights in a flower are the most important discriminating characters for homostyly, compared with distyly. As it is common that the development of gynoecium and androecium is independent (Lloyd and Bawa, 1984; Goldman and Willson, 1986). The relative developmental rate of pistil-height-related traits in homostylous flowers is significantly lower than in pins but similar to (in L1 and L3) or even higher than (in L2) in thrums. In other words, in homostyles the pistil height has paedomorphic ontogeny by neoteny compared to those in pins while it can either have no difference (in L1 and L3) or have peramorphic ontogeny by acceleration (in L2) compared to thrums (Figs. 6.15, 6.16).

In contrast, stamen-height-associated traits in homostylous flowers develop as fast as (in L1 and L3) or faster than (in L2) in pins but significantly more slowly than in thrums. This indicates that stamen height in homostyles has paedomorphic ontogeny by neoteny compared to that in thrum while it can either be similar to (in L1 and L3) or have peramorphic ontogeny by acceleration (in L2) compared with that in pin (Figs. 6.15, 6.16). The contrary development of pistil and stamen heights in homostyly in comparison with that in distyly shows the presence of dissociated heterochrony with multi-heterochronic processes, and this is common in evolutionary development (Fink, 1982; Reilly, 1997; Zelditch et al., 2000). It has led to the homostylous flowers having stigma and anthers positioned at a similar height in contrast to the distylous flowers in which the

stigma and anther heights are reciprocally positioned in pin and thrum morphs. Hence it has created a favorable spatial condition for homostylous flowers to self-pollinate.

6.5.1.3. *Function of the large homostyly in A. spectabilis*

The large homostylous flower in *A. spectabilis* lineage has distinct characteristics and developmental patterns. All 21 studied floral traits are highly significantly larger in large homostyle than in small homostyle, the regular homostyle of Lineages 1 and 2 (Table 4.2). However, twelve traits, including all flower-size-related traits in large homostylous flowers, are significantly smaller than in distylous flowers, another eight of them have the same size as distylous ones, and only one trait, filament length, is significantly longer than in distylous flowers. These results suggest that, in terms of morph characters, the large homostylous flower is intermediate between the distylous and the regular homostylous flowers, and generally more similar to the distylous ones.

The developmental duration, from primordium to anthesis, of the large homostylous flower is a little shorter but statistically does not differ from that of distylous flower, although it is significantly shorter than in the small homostylous flower (Table 6.1). On the other hand, the relative developmental offset times are similar among the three (distyly, large homostyly and small homostyly) in about 12 of 19 studied floral traits (Figs. 6.1-6.7). The offset times of the other seven traits in large and small homostyles are much the same but are earlier than in distylous flowers or at least earlier than the pin morph. This suggests that the overall relative developmental time from primordium initiation to reaching maximum size of the flower or floral trait is similar in both homostylous flowers. However, about one third of the floral traits, including stamen

height in large homostylous flowers, may have truncated development compared to the thrum flowers (Figs. 6.15, 6.16). It thus implies that progenesis has caused paedomorphic ontogeny in at least some parts of the large homostylous flower compared to its ancestor, the distylous flower, in *A. spectabilis*. In this aspect, the large homostylous and small homostylous flowers are very much similar.

Changes of developmental rate between a descendant and its ancestor are one of the major components of the heterochrony. The developmental rate in large homostylous flowers is about 2-3 times higher than in small homostylous flowers in all 19 floral traits (Table 6.2). Comparatively about 11 traits in large homostylous flowers have no growth rate difference compared with distylous flowers. The pistil height in larger homostyles grows significantly faster than in thrums but about the same rate as or a little faster than in pins. The stamen height in larger homostyles, in contrast, develops faster than that in pin but similar to that in thrum. This scenario suggests that the large homostylous flowers are more or less similar to the distylous ones, and they differ from each other in about one half of the floral traits in terms of the developmental rate. These changed developmental rates, incorporating the changes of developmental offset time, may have produced the large homostylous flower with its unique characters, the distyly-like flower size and the homostyly-like positioning of reproductive organs. More importantly, the results indicate that the small homostylous flowers are not only paedomorphic to the distylous flowers but also paedomorphic to the large homostylous flowers by neoteny from the view of heterochronic changes in ontogeny (Fig. 6.16).

Based on the distinct floral morphology and ontogeny, it is more likely that the large homostylous flower is functioning as a transitional morph during the evolution of

the typical small homostyly from the distyly in the lineage of *A. spectabilis*. The large homostyly is the closest descendant of distyly in the lineage. This is also supported by their characterized ontogenetic trajectories that the large homostyly developmentally diverged from the small homostyly much earlier than from the distyly (Figs. 6.1-6.7). This assumption is also consistent with the selfing rate (Fig. 3.1) and the phylogenetic position of the large homostyle (Ray and Chisaki, 1957b; Johnston and Schoen, 1996; Schoen et al., 1997), which all shows that the large homostyle is in between the distyly and small homostyle, in the lineage of *A. spectabilis*.

6.5.2. Differentiation of pin and thrum in distyly

Depending on lineage, nine to 16 of the 21 studied floral traits differ significantly between pins and thrums (Table 4.2). The common differences between the two morphs in all distylous species are the anther and stigma heights in the flowers. Thus, all traits contributing to their heights are highly significantly different between the two morphs. Flower size, anther size, stigma size and some other floral traits also differ distinctively between the two morphs. From a floral development point of view, there are various developmental processes and modifications that can differentiate two distylous floral morphs. Changes in developmental rate, however, are usually the major cause that leads to the contrasted floral morphology of pin and thrum in distylous species (Stirling, 1936; Riveros et al., 1987; Richards and Koptur, 1993). This must be true whenever final size differ but developmental durations do not.

Floral development duration, from primordium to anthesis, does not differ between the two floral morphs within each distylous taxon in *Amsinckia* (Table 6.1).

Developmental offset times of the two morphs are also similar except the stigmas of *A. furcata*, which had earlier offset in pin and thus resulted in the stigma being smaller in pin than in thrum. This suggests that the mechanisms distylous flowers involved to promote out-crossing is their spatial differences in anther-stigma positioning within a flower, it does not involve any significant temporal changes either within or between the two floral morphs. In tristylous *Eichhornia paniculata* and *Pontederia cordat*, it has been reported that a change in developmental duration, in association with changes in growth rate, has resulted in the differences in anther and stigma heights among morphs (Richards and Barrett, 1984, 1987).

As has been reported in studies of some other distylous plants [e.g. *Quinchamalium chilense* (Riveros et al., 1987) and *Guettarda scabra* (Richards and Koptur, 1993)], changes in growth rate have resulted in the reciprocal positioning of anthers and stigmas in pin and thrum flowers in distylous species of *Amsinckia*. It is the same in all three studied distylous species of *Amsinckia* that the relative developmental rate for all stamen-height-associated traits are highly significantly slower in pins than in thrums (Table 6.2). In contrast, all pistil-height-related traits (except POVH, which has no difference between morphs,) develop highly significantly faster in pins than in thrums in all three distylous species. Clearly, the relative slower developmental rate of stamen height in pin flowers has caused the stamens to be positioned lower than that in thrum flowers. In contrast, the relative higher growth rate of pistil height in pin flowers has led the stigma to be positioned higher in pin flowers than in thrum flowers. It is evident that the contradictory growth rates of stamen and pistil heights in distylous flowers caused the

two morphs to have the reciprocal arrangement of anther and stigma heights in their flowers.

Because the stamens in distylous species are usually epipetalous (stamens arise from the petals), the extent of corolla tube growth will have an important effect on the height of anthers. This has been reported in several other studies such as *Cordia sebestena* (Percival, 1974), *Gaertnera vaginata* (Pailler and Thompson, 1997), *Guettarda scabra* (Richards and Koptur, 1993), and *Myosotis* (Robertson and Lloyd, 1991). Exceptions, however, do exist. Gibbs and Taroda (1983) reported that corolla tube length in distylous *Cordia alliodora* and *C. trichotoma* is not associated with differences in anther heights. Due to a lack of a reliable positional reference point on the corolla tube after flower opening, I stopped measurement of the corolla tube length after flower opening. Therefore, I do not know exactly when the corolla tube ceases its growth. However, for the purpose of detecting changes or contributions of corolla tube to the anther height, I measured the stamen insertion height (SINH, where the filament is attached to the corolla tube) during the whole floral development period. This is actually a better and more precise way for analyzing the effects of corolla tube on anther height compared with using a whole corolla tube length that is often used by some other researchers. The results in this study clearly indicate that the slower relative growth rate of both the stamen insertion height on corolla tube and the filament length in pin flowers is the major cause differentiating pin from thrum flowers in anther-height development.

Among those traits related to stigma height in a flower, style length (PSTYL) is the major or even probably the only trait that has led to the difference of stigma height between pin and thrum flowers in distylous species of *Amsinckia*. This is because two of

the three pistil-height components, ovary height (POVH) and stigma thickness (PSTH), are statistically not different in their relative growth rates and mature sizes between the two distylous floral morphs. Style length, in contrast, is highly significantly different in relative growth rate and mature size between pin and thrum flowers. Thus, it is evidenced that the slower relative developmental rate of style length in pin flower has resulted in the pin having a shorter pistil compared with thrum, in all three studied distylous species of *Amsinckia*. A study in tristylous *Pontederia cordata* (Richards and Barrett, 1987) also suggested that "morph-dependent variation in stigma height depends on differences in style length, not ovary length."

Studies of ontogenetic trajectories of floral traits show that the developmental divergence of the two distylous flowers can occur any time from a very early stage to right before anthesis, depending on the floral traits and species or lineages. The most dramatic separations between pin and thrum, however, occur when flowers are just about to open due to a trait's steep increase of its relative developmental rate in one of the morph. This is especially evident in the growth of flower length, pistil and stamen heights.

The divergences of both anther and stigma heights between pin and thrum flowers are initiated around PMC meiosis time or a little later. This is consistent with some other studies such as distylous *Guettarda scabra* (Richards and Koptur, 1993), *Primula* spp. and *Menyanthes trifoliata* (Stirling, 1936), and tristylous *Eichhornia paniculata* (Richards and Barrett, 1984).

The developmental trajectories of stigma size between pin and thrum in *A. douglasiana* diverge prior to PMC meiosis. Interestingly, the diverged growth curves of

the two morphs gradually get closer due to changes in the relative growth rate during late development and finally merge again right after anthesis. This "unusual" stigma ontogeny in *A. douglasiana* suggests that either the diverged ontogenetic trajectories may converge again as development proceeds or different developmental processes can lead to the same end.

Distylous flowers are often genetically self-incompatible (Lewis and Jones, 1992; Riveros et al., 1995). Some distylous species, including distylous species of *Amsinckia*, however, are self-compatible. The evolution of distyly has been studied for over a century, beginning with Darwin's (Darwin, 1877) early work. Unfortunately, it is still uncertain with regard to the evolutionary pathways of distyly and its adaptive significance (Barrett, 1992a; Barrett et al., 1996). Various explanations, models and arguments on the evolution of distyly have been presented in last twenty or thirty years (Charlesworth, 1979; Charlesworth and Charlesworth, 1979b; Ganders, 1979a; Richards, 1986; Barrett, 1990; Barrett et al., 1996, 2000; Lloyd and Webb, 1992a, 1992b).

The debates on the evolution of distyly mainly have focused on what is the presumed ancestral condition, a homostylous flower or an approach herkogamous flower (a flower with an exerted stigma) and which syndrome of distyly arose first, the dialletic self-incompatibility or the reciprocal herkogamy. Currently there are two major models for the evolution of distyly. One is proposed by Charlesworth and Charlesworth (1979b). The model proposes that the ancestral condition for distyly is a self-compatible homostyly-like phenotype with pistil and stamens of equal height. The establishment of diallelic self-incompatibility as an inbreeding avoidance mechanism would be the first step of evolution towards distyly, followed by the development of reciprocal herkogamy

to promote efficient pollen transfers between morphs. The other model (Lloyd and Webb, 1992a, 1992b) proposes that approach herkogamy is the ancestral condition of distyly. The initial step during the evolution of distyly is the establishment of reciprocal herkogamy, followed by the development of dialletic self-incompatibility system. Both of these models have been well recognized by evolutionary biologists. More tests in various distylous plants, however, are probably needed before we can be sure about the evolutionary pathways of distyly. It may also be possible that both models have been the true pathways in the evolution of distyly and the pathways may be different among different distylous groups. Or, even as Mather and de Winton (1941) postulated that reciprocal herkogamy and self-incompatibility may arose together in some cases.

It has been reported that in some cases distylous species may have lost their self-incompatibility while their stigmas and anthers still kept reciprocal arrangement in pin and thrum flowers, such as *Malochia pyramidata*, a self-compatible distylous species which is known to be derived from self-incompatible distylous species (Martin, 1967). A similar situation might also have occurred in the distylous *Nivenia* (Goldblatt and Bernhardt, 1990). Goldblatt and Bernhardt (1990) believe that the distylous *Nivenia* is derived from self-incompatible ancestors which are perhaps now extinct. Five of the nine extant species of *Nivenia* have dimorphic flowers with the dimorphism only on pistil and stamen heights, but their self-incompatibility response has been lost or weakened. The residual self-compatibility found in dimorphic *N. capitata* leads Goldblatt and Bernhardt (1990) to conclude that the self-compatible *Nivenia* is derived from self-incompatible ancestors.

Self-compatible distylous species of *Amsinckia* were also suggested to have evolved from self-incompatible ancestors (Ganders, 1979a) through the "relaxation and eventual loss of the incompatibility system" (Barrett and Richards, 1990). Furthermore, if Charlesworth and Charlesworth's (1979b) model applies to *Amsinckia*, the dimorphic features of self-compatible distylous flowers are unlikely to have developed without a pre-existing self-incompatibility system. On the other hand, Lloyd and Webb's (1992a, 1992b) model cannot explain the evolution of the self-compatible distyly in *Amsinckia* either. This is because the distylous taxa in *Amsinckia* are breaking down to homostylous species (Ray and Chisaki, 1957a, 1957b, 1957c; Schoen et al., 1997), and it appears unlikely that distylous taxa in *Amsinckia* will develop a self-incompatibility system. Therefore, it is more likely that the self-compatible distyly in *Amsinckia* is derived from some unidentified self-incompatible ancestors by secondary loss of their incompatibility system (Barrett, 1988). The cryptic self-incompatibility character of distylous species in *Amsinckia* (Weller and Ornduff, 1977) may also indirectly support this conclusion.

6.5.3. Differences between lineages

Distyly has been reported in at least 28 angiosperm families (Barrett et al., 2000) and the evolution of homostyly from distyly is also known to have occurred in many different taxa (Ray and Chisaki, 1957b; Ganders, 1979a; Piper et al., 1986; Kelso, 1987; Wedderburn and Richards, 1992; Tremayne and Richards, 1993). It is reasonable to believe that both the origin of distyly and the evolution of homostyly from the breakdown of distyly are polyphyletic. My studies on flower ontogenies in both distylous and

homostylous species among three evolutionary lineages of *Amsinckia* provide strong support to this hypothesis.

The floral morphology of both distyly and homostyly among three lineages of *Amsinckia* is more or less similar. The major floral developmental processes or the mechanisms that lead to the formation of distyly and homostyly are also more or less similar. Paedomorphic ontogeny through neoteny and progenesis is the major developmental cause that is responsible for the evolution of homostyly from distyly in all three lineages of *Amsinckia*. However, the extent of paedomorphosis, the degree of developmental dissociation, and changes of ontogenetic trajectories in homostyly compared to its ancestor, in conjunction with some other developmental processes or mechanisms such as peramorphic ontogeny by acceleration in some cases, have resulted in the evolution of homostyly in different ways in different lineages.

The pattern of floral development differences between homostyly and distyly varied greatly among the three evolutionary lineages of *Amsinckia*. The differences of this pattern are an indication of the differences that how homostyly has evolved independently in three lineages. This pattern in Lineages 1 and 2 is somewhat similar, but they are very different from that in Lineage 3. For example, the actual flower developmental duration of homostyly, from primordium to anthesis, is significantly longer in Lineage 3 than in Lineages 1 and 2. The duration in Lineages 1 and 2 is same. More importantly, the actual flower developmental time for distyly and homostyly is not different in Lineages 1 and 2, but it is significantly different in Lineage 3. Likewise, the actual PMC meiosis time (AAFT) between the two styles in Lineages 1 and 2 do not differ. Whereas the AAFT differ significantly between the two styles in Lineage 3, although their relative meiosis time (RAFT) is the same.

The extent of difference in relative flower developmental rate between distyly and homostyly, for most floral traits, is also lineage dependent. For example, flower length and width of both distylous flowers grow more than twice as fast as that of homostylous flowers in Lineages 1 and 2, while the same traits only in thrum grow faster than in homostyle in Lineage 3. This varied growth rate pattern between distyly and homostyly among lineages may have led to another lineage dependent pattern, i.e., the difference in relationships between distyly and homostyly in terms of the number or amount of floral primordia and buds on an inflorescence. The difference in the number of floral primordia and buds on each inflorescence between the two styles is significant in Lineages 1 and 2, but not in Lineage 3 (Table 6.1).

The pattern of changes of relative growth rate in pistil and stamen heights between homostyle and two distylous flower morphs also varies among three lineages. For instance, the developmental rate of ovary in homostyly is no different from, faster than, and slower than that in distyly in Lineage 1, 2 and 3, respectively. For almost every floral trait, the pattern of changes of relative growth rate between homostyle and distyly is lineage dependent.

It is obvious that the differences among lineages depend on not only the patterns of developmental difference between distyly and homostyly, but also on individual floral traits. Many of the individual floral traits are developmentally lineage and flower morph dependent. In other words, the difference among lineages, in large extent, depends upon floral morphs and vice versa. For example, sepal length in Lineages 1 and 2 grows more than twice as fast as that in Lineage 3 in homostylous flowers, about 60% faster than that in Lineage 3 in thrum flowers, and same as that in Lineage 3 in pin flowers (Fig. 6.8).

The same floral trait often has different ontogenetic trajectory among species and lineages. More interestingly, different ontogenetic trajectories can lead to the same end product, a trait of same size in this study. For example, the growth trajectories of flower size (length and width) of homostyly differ greatly among three lineages (Fig. 6.8). The trajectories in Lineages 1 and 2 are almost the same until right before flowering. After a relative age of 0.95 the two lineages diverge due to a dramatic increase of growth rate in Lineage 2 while the rate in Lineage 1 is almost unchanged. On the other hand, the divergence of the growth trajectories between these two lineages (L1 and L2) and Lineage 3 exist from an early developmental stage, probably prior to a relative age 0.4, due to a growth rate increase in Lineages 1 and 2. The growth in Lineage 3, however, has a steep increase after a relative age of 0.95, which leads its growth curve goes up and converges with Lineage 1's growth curve by the time of flowering. Therefore, two different growth trajectories in Lineages 1 and 3 produce flowers of the same size. This may be regarded as a result of convergence, and it appears to be common in floral development and evolution, because different developmental pathways can lead to similar mature morphologies (Tucker, 1992; Douglas and Tucker, 1996), a small, self-pollinated, homostylous flower in this case.

Considering that the ontogenetic differences between homostyly and distyly are so different among the three lineages of *Amsinckia*, it is reasonable to believe that homostyly evolved from distyly in the three lineages independently. However, there are some similarities between Lineages 1 and 2 in terms of how homostyly is different from distyly in some traits' ontogenies. These ontogenetic similarities might suggest that these two lineages are very closely related in phylogeny. This is consistent with the recently

proposed phylogeny in *Amsinckia* that Lineages 1 and 2 are from the same branch while Lineage 3 is independent, based on molecular-phylogenetic studies (Schoen et al., 1997; M.O. Johnston and W.J. Hahn, unpublished results). On the other hand, the similarities between lineages also suggest developmental parallelism.

The ontogenetic relationships between the two distylous floral morphs are also lineage dependent in *Amsinckia*. For example, the actual flower developmental time prior to anthesis between pin and thrum is almost the same within a lineage, but differs among three evolutionary lineages. Similarly, the patterns of relative developmental rate changes between the two morphs in many floral traits differ among the three lineages. The same is true for changes in ontogenetic trajectories between pins and thrums among lineages. It is interesting that not only the pattern of ontogenetic differences between pin and thrum varied among lineages, but also the differences between Lineage 3 and the other two lineages (L1 and L2) is greater than between Lineages 1 and 2. Actually, the relationships between the two distylous floral morphs in some traits' ontogenies are more or less similar between Lineages 1 and 2. For example, the pattern of differences in anther-size growth between the two morphs is similar between Lineages 1 and 2, but differs greatly from that in Lineage 3 (Fig. 6.3). The differential ontogenetic changes between pin and thrum among three lineages suggest that they are independent in evolution, especially for the distylous species in Lineage 3. The more or less similar pattern in ontogenetic differences between the two distylous morphs in Lineages 1 and 2 support the assumption that they are very closely related and probably recently diverged species or they may have originated from a common ancestor. This may also explain that the way homostyly evolved from the distyly in these two lineages are somewhat similar.

The developmental processes and pathways leading to the formation of the same floral morph (pin, thrum or homostyle) among three lineages in *Amsinckia* are similar, especially for pin and thrum flowers. The floral ontogenetic trajectories, however, varied greatly among the three lineages. This variation is mostly caused by the differences of developmental duration and more importantly the differential growth rate changes along the developmental pathways. A similar scenario that the differences of ontogenetic changes of distyly between Lineages 1 and 2 is smaller than between these two lineages and Lineage 3 is also present here (Figs. 6.8-6.14). Once again, this supports the conclusion that the evolution of distyly and homostyly are independent among the three evolutionary lineages in *Amsinckia*. Nevertheless, based on flower ontogenetic studies I cannot rule out the possibility that the distylous species in Lineages 1 and 2, *A. furcata* and *A. douglasiana*, might have a recent common ancestor, as proposed from recent molecular-phylogenetic studies in the genus (Schoen et al., 1997; M.O. Johnston and W.J. Hahn, unpublished results). Future studies on comparative early flower ontogenies in the genus can enhance our understandings on the evolution of both distyly and homostyly.

CHAPTER 7

EVOLUTION OF MEIOSIS TIMING DURING FLORAL DEVELOPMENT

7.1. ABSTRACT

Meiosis divides the haploid and diploid portions of the life cycle in all sexual organisms. In angiosperms meiosis occurs during floral development, the duration of which varies widely among species and is affected by environmental conditions within species. For 36 species representing 13 angiosperm families, I determined the time at which meiosis ceased in the anthers as a fraction of the total time from floral primordium initiation (beginning of development) to flower opening (end). It was found that this fraction, rather than being continuously distributed among species, occurred in three discrete classes despite wide variations within and among species in absolute developmental durations. Each species was characterized by a single timing class. For all species within a given timing class, therefore, the durations before and after the end of microsporocyte meiosis existed in constant ratio. Each timing class was found in phylogenetically distant species, and, conversely, a plant family often contained more than one class. Timing class was not related to ploidy level, inflorescence architecture, pollination syndrome or mating system. These findings show that either the durations before and after microsporocyte meiosis are regulated by the same exogenous process, or one duration

determines the other. They further imply that the underlying developmental processes have evolved in a limited number of ways among flowering plants.

7.2. INTRODUCTION

In angiosperms, microsporocyte (pollen-mother-cell) meiosis occurs in the anthers during floral development. The proximate causes of meiotic onset and offset are not yet fully understood for plants or any other organism (Sauter, 1971; Luomajoki, 1986; Dickinson, 1987, 1994; McLeod and Beach, 1988; John, 1990; Stern, 1990; Riggs, 1997), although several genes necessary for onset have been identified in yeast, a lily and maize (Walters, 1985; McLeod and Beach, 1988; Golubovskaya et al., 1993; Riggs, 1994, 1997; Sheridan et al., 1996; Bogdanov, 1998). While the onset of meiosis has been more intensively studied, there are two aspects in which its offset has greater significance. First, in plants, the end of meiosis defines the beginning of the gametophytic generation. Second, pollen tetrad formation corresponds to the end of cell division in the anther and corolla (Hill, 1996). Following microsporocyte meiosis, all growth, including corolla expansion (flower opening), occurs by cell enlargement.

Events in different whorls of the developing flower are often highly temporally correlated. This is particularly evident between whorls two and three, the corolla and stamens (Erickson, 1948; Minter and Lord, 1983; Kiss and Koning, 1989; Koltunow et al., 1990; Scott et al., 1991; Goldberg et al., 1993; Greyson, 1994), where the correlation can result from developmental processes at several levels. For example, some substances

produced in the anther are transferred to the corolla and other whorls, where they influence corolla expansion and various other processes (Erickson, 1948; Minter and Lord, 1983; Mohan Ram and Rao, 1984; Raab and Koning, 1988). The effects of the stamen on other whorls appear to cease at anther dehiscence (Marre, 1946; Mohan Ram and Rao, 1984). Other substances, such as homeotic gene products, are regulated transcriptionally or post-transcriptionally (Coen, 1991; Ma, 1994; Meyerowitz, 1994, 1997). For example, *AGAMOUS* (*AG*) and *FORAL BINDING PROTEIN1* (*FBP1*) are homeotic genes that code for putative transcription factors (MADS domain proteins) in *Arabidopsis* and *Petunia*, respectively. Transcription of *AG* in the anther and in most ovule-primordia cells ceases when microsporocytes differentiate (Drews et al., 1991). In contrast, *FBP1* transcription continues in the anther and corolla throughout development, but the protein becomes undetectable in most anther cells at a specific stage (Canas et al., 1994).

Consider a developmental event, for example in the anther, that occurs between initiation of the floral primordium and flower opening. The absolute durations preceding and following the event will clearly vary among species. The absolute durations will also vary among individuals within species according to environmental conditions such as temperature and light level. On the other hand, the relative timing of the event (measured as a fraction of total developmental duration) is expected to exhibit less variation within species simply because of the stereotyped and contingent nature of developmental processes. Mathematically, a constant relative timing means that the durations preceding and following the event exist in a constant ratio, independent of absolute developmental times. Developmentally, a constant relative timing means either that the durations before

and after the event are controlled by the same process or that one duration controls the other. All species with the same relative timing of an event are also expected to share the same developmental relationships between primordium initiation, the event and flower opening.

Although the absolute timing of tetrad formation in the anther varies among species (Bennett, 1977; Bennett et al., 1971; Bhandari, 1984; Luomajoki, 1986), the timing of tetrad formation relative to total floral developmental duration has not previously been studied. The goal of this study was to measure, in flowers of phylogenetically diverse species, the developmental duration preceding tetrad formation as a fraction of total developmental duration. I tested the null hypothesis that species vary continuously in this fraction. A contrary finding of a few discrete timing classes would suggest an evolutionary constraint on the developmental relation between pre- and post-tetrad durations. As defined here, floral development encompasses the period from primordium initiation to flower opening. In species where the flowers are produced sequentially from base to tip (i.e., acropetally) along an inflorescence, the flowers and buds form a chronological sequence. For any developmental event, the time elapsed since primordium initiation can be measured by the number of positions separating the primordium and the event-containing bud. Absolute times can be calculated with knowledge of the plastochron (Erickson, 1976; Lamoreaux et al., 1978), the time separating consecutive positions (Fig. 7.1). Times expressed relative to the total developmental period can be measured only when floral positions representing both beginning and end of development exist concurrently on an inflorescence. This study was

therefore restricted to species in which floral primordia continued to be initiated while flowers opened at older positions on the inflorescence.

7.3. Materials and Methods

To determine the timing of microspore tetrad formation, the position of each bud on an inflorescence was numbered starting from the newly initiated floral primordium ($P_{primordium} = 0$) and ending with the youngest open flower ($P_{opening}$, Fig. 7.1). Anthers of individual buds pre-stained with safranin-O or aceto-carmine were squashed and observed under a compound microscope to determine the position, P_{tetrad}, at which microspore tetrads formed. RAFT, the relative age of a floral bud with tetrads (no units), was calculated as the ratio of the bud position with tetrads to the total number of buds, $P_{tetrad} / P_{opening}$. RAFT expresses the time elapsed from primordium initiation to tetrad formation as a proportion of the total time from primordium initiation to flower opening. This method of calculating RAFT assumes that the plastochron remains constant as the inflorescence grows. This has been confirmed for *Amsinckia spectabilis* (M.O.J., pers. obs.)

The absolute age of a floral bud at tetrad formation, AAFT (days), was calculated when possible (Fig. 7.1). To calculate AAFT, the plastochron (days/bud position) was obtained by painting the youngest open flower on inflorescences in the field. *D* days later (typically five to seven), the inflorescences (one per plant) were collected and fixed in FAA (formalin, acetic acid, ethanol). Plastochron = *D* / (number of flowers opened

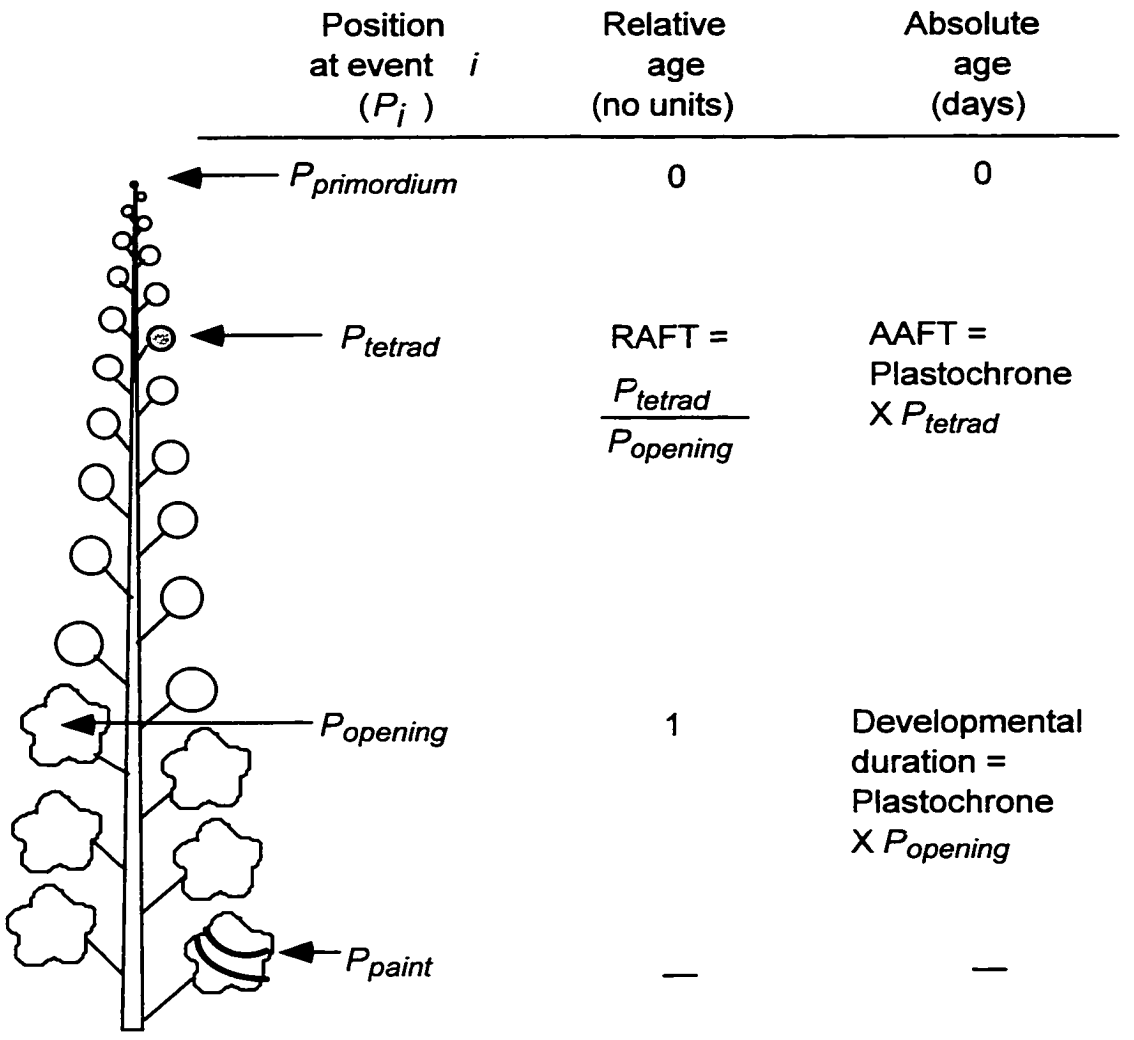


Figure 7.1. Methods for calculation of RAFT (relative age) and AAFT (absolute age) of a floral bud when its microsporocyte meiosis terminates, indicated by formation of pollen tetrads in the anther. RAFT was calculated for all species, while AAFT was calculated for a subset. Floral developmental duration measures the time required for a flower to develop from primordium initiation to corolla opening. Distances between youngest floral buds are greatly exaggerated for clarity.

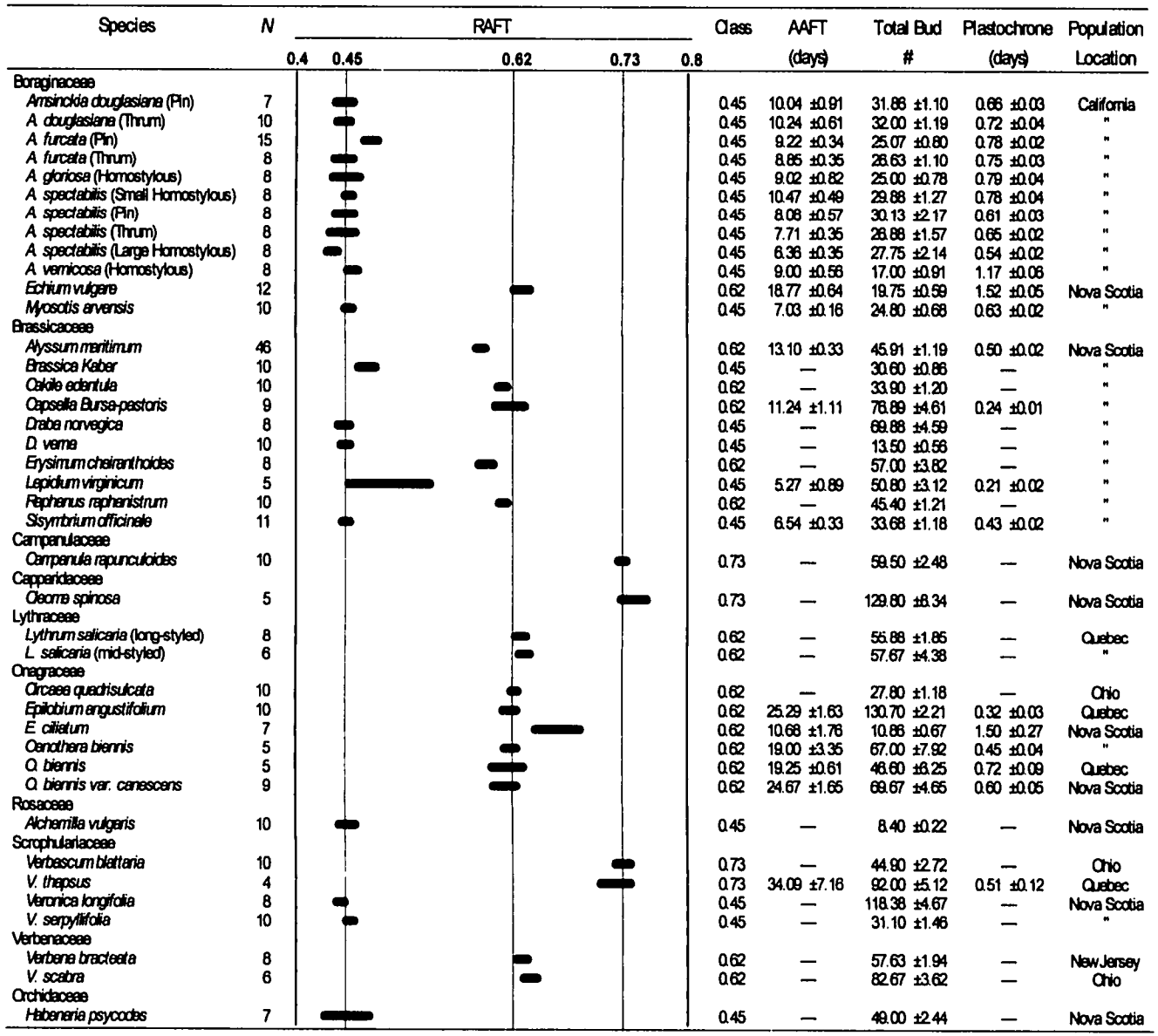
during D). Fixed inflorescences were dissected as described to determine P_{tetrad} and $P_{opening}$. In the cases where more than one bud on an inflorescence contained microspore tetrads, the one adjacent to the bud having microsporocyte meiosis was chosen for calculating the tetrad formation time.

I examined 32 species representing 23 genera and 10 families. Data were analyzed using SYSTAT (1992, Macintosh version 5.2.1). Inflorescence types included racemes (Brassicaceae, Campanulaceae, Capparidaceae, Lythraceae, Onagraceae, Rosaceae), spikes (Orchidaceae, Scrophulariaceae, Verbenaceae) and helicoid cymes (Boraginaceae). Racemes and spikes are indeterminate inflorescences; helicoid cymes are determinate. The literature provided data from which RAFT could be calculated in four additional species: *Arabidopsis thaliana* (Brassicaceae), *Lamium amplexicaule* (Lamiaceae), *Cornus officinalis* (Cornaceae) and *Viola odorata* (Violaceae).

7.4. RESULTS

RAFT varied significantly among the 32 species analyzed (Anova $P < 10^{-20}$, $N = 375$ inflorescences, $F_{31,343} = 133$, $R^2 = 0.92$). Visual inspection of data (Fig. 7.2) suggested that mean RAFT fell into three classes. This was confirmed by a three-means cluster analysis using the 32 species means as observations (Anova $P < 10^{-25}$, $N = 32$; $F_{2,29} = 659$, $R^2 = 0.98$). Mean RAFT for each of these classes (clusters) was 0.45, 0.62 and 0.73, whether determined from all individuals or from species means. A second cluster analysis using all 375 individuals without regard to species, population or style

Figure 7.2. Mean RAFT and associated developmental traits in 32 species of flowering plants. Bar width is ± 1 standard error. Separate analyses are presented for populations within species as well as floral morphs within populations. RAFT theoretically ranges from zero to one; for clarity only the region from 0.4 to 0.8 is shown.



Species	N	Class	AAFT (days)	Total Bud #	Plastochrone (days)	Population Location
Boraginaceae						
Amsinckia douglasiana (Pin)	7	0.45	10.04 ±0.91	31.86 ±1.10	0.66 ±0.03	California
A. douglasiana (Thrum)	10	0.45	10.24 ±0.61	32.00 ±1.19	0.72 ±0.04	"
A. furcata (Pin)	15	0.45	9.22 ±0.34	25.07 ±0.80	0.78 ±0.02	"
A. furcata (Thrum)	8	0.45	8.85 ±0.35	26.63 ±1.10	0.75 ±0.03	"
A. gloriosa (Homostylous)	8	0.45	9.02 ±0.82	25.00 ±0.78	0.79 ±0.04	"
A. spectabilis (Small Homostylous)	8	0.45	10.47 ±0.49	29.88 ±1.27	0.78 ±0.04	"
A. spectabilis (Pin)	8	0.45	8.08 ±0.57	30.13 ±2.17	0.61 ±0.03	"
A. spectabilis (Thrum)	8	0.45	7.71 ±0.35	26.88 ±1.57	0.65 ±0.02	"
A. spectabilis (Large Homostylous)	8	0.45	6.36 ±0.35	27.75 ±2.14	0.54 ±0.02	"
A. vernicosa (Homostylous)	8	0.45	9.00 ±0.56	17.00 ±0.91	1.17 ±0.06	"
Echium vulgare	12	0.62	18.77 ±0.64	19.75 ±0.59	1.52 ±0.05	Nova Scotia
Myosotis arvensis	10	0.45	7.03 ±0.16	24.80 ±0.68	0.63 ±0.02	"
Brassicaceae						
Alyssum maritimum	46	0.62	13.10 ±0.33	45.91 ±1.19	0.50 ±0.02	Nova Scotia
Brassica Kaber	10	0.45	—	30.60 ±0.86	—	"
Cakile edentula	10	0.62	—	33.90 ±1.20	—	"
Capsella Bursa-pastoris	9	0.62	11.24 ±1.11	76.89 ±4.61	0.24 ±0.01	"
Draba norvegica	8	0.45	—	69.88 ±4.59	—	"
D. verna	10	0.45	—	13.50 ±0.56	—	"
Erysimum cheiranthoides	8	0.62	—	57.00 ±3.82	—	"
Lepidium virginicum	5	0.45	5.27 ±0.89	50.80 ±3.12	0.21 ±0.02	"
Raphanus raphanistrum	10	0.62	—	45.40 ±1.21	—	"
Sisymbrium officinale	11	0.45	6.54 ±0.33	33.68 ±1.18	0.43 ±0.02	"
Campanulaceae						
Campanula rapunculoides	10	0.73	—	59.50 ±2.48	—	Nova Scotia
Capparidaceae						
Cleome spinosa	5	0.73	—	129.80 ±8.34	—	Nova Scotia
Lythraceae						
Lythrum salicaria (long-styled)	8	0.62	—	55.88 ±1.85	—	Quebec
L. salicaria (mid-styled)	6	0.62	—	57.67 ±4.38	—	"
Onagraceae						
Circaea quadrisulcata	10	0.62	—	27.80 ±1.18	—	Ohio
Epilobium angustifolium	10	0.62	25.29 ±1.63	130.70 ±2.21	0.32 ±0.03	Quebec
E. ciliatum	7	0.62	10.68 ±1.76	10.86 ±0.67	1.50 ±0.27	Nova Scotia
Oenothera biennis	5	0.62	19.00 ±3.35	67.00 ±7.92	0.45 ±0.04	"
O. biennis	5	0.62	19.25 ±0.61	46.60 ±6.25	0.72 ±0.09	Quebec
O. biennis var. canescens	9	0.62	24.67 ±1.65	69.67 ±4.65	0.60 ±0.05	Nova Scotia
Rosaceae						
Alchemilla vulgaris	10	0.45	—	8.40 ±0.22	—	Nova Scotia
Scrophulariaceae						
Verbascum blattaria	10	0.73	—	44.90 ±2.72	—	Ohio
V. thapsus	4	0.73	34.09 ±7.16	92.00 ±5.12	0.51 ±0.12	Quebec
Veronica longifolia	8	0.45	—	118.38 ±4.67	—	Nova Scotia
V. serpyllifolia	10	0.45	—	31.10 ±1.46	—	"
Verbenaceae						
Verbena bracteata	8	0.62	—	57.63 ±1.94	—	New Jersey
V. scabra	6	0.62	—	82.67 ±3.62	—	Ohio
Orchidaceae						
Habenaria psycodes	7	0.45	—	49.00 ±2.44	—	Nova Scotia

Figure. 7.2

morph, assigned only five (1.3%) to a class not otherwise representing their species. Only two species differed in mean RAFT from that of the nearest class by more than 0.03: *Lepidium virginicum* (0.04 units from class 0.45) and *Epilobium ciliatum* (0.05 from class 0.62).

Within species, the relative measure RAFT exhibited small standard errors (typically < 0.01) despite often great variability in the absolute measures of growth, such as total developmental duration, total bud number and plastochron (Fig. 7.2, Table 7.1). Within each class, RAFT was generally unrelated to any of these three absolute measures. The sole exception was a correlation between RAFT and plastochron in class 0.62 (Pearson correlation = 0.44, Bonferroni $P < 0.01$, $N = 103$).

In contrast to the general lack of correlations between RAFT and other variables within classes, higher RAFT classes exhibited statistically greater mean bud number (Tukey test, $P < 10^{-15}$) and floral developmental duration ($P < 10^{-18}$). A positive correlation between AAFT and RAFT therefore also occurred, arising directly as a result of this correlation between RAFT class and developmental duration. Plastochron, the time separating buds, did not differ among classes ($P > 0.6$).

Four previous studies of floral development supply data from which it is possible to calculate RAFT. All support the present results that RAFT falls into a few, narrowly defined classes. RAFT in wild-type *Arabidopsis thaliana* is 0.735 (AAFT = 161 hours, floral developmental duration = 219 hours; Crone and Lord, 1994); RAFT in chasmogamous flowers of *Lamium amplexicaule* is 0.466 (AAFT = 7 days, floral developmental duration = 15 days; Lord, 1979); RAFT in *Cornus officinalis* is approximately 0.453 (AAFT ≈ 145 days, floral developmental duration ≈ 320 days; Li et

Table 7.1. Means and coefficients of variation (# species in parentheses) of relative and absolute floral developmental traits. Values are calculated from the species means and are presented separately for the three RAFT classes. Within each RAFT class, means are presented above coefficients of variation and numbers of species.

Trait				
RAFT	**AAFT**	**Total bud number**	**Plastochron**	**Total developmental duration**
0.45	8.0	37.2	0.67	16.7
2.8%	20%	73%	42%	30%
(15)	(8)	(15)	(8)	(8)
0.62	16.7	54.3	0.78	26.9
3.2%	36%	57%	75%	37%
(13)	(6)	(13)	(6)	(6)
0.73	34.1	81.6	0.51	45.5
1.1%	–	46%	–	–
(4)	(1)	(4)	(1)	(1)

al., 1991); and RAFT in *Viola odorata* is approximately 0.718 (AAFT ≈ 43 days, floral developmental duration ≈ 59.9 days) for chasmogamous flowers (Mayers and Lord, 1983a). Inflorescence types for these species are, respectively, racemes, axillary cymes, corymbs and none (flowers solitary).

7.5. Discussion

7.5.1. Relation to phylogeny, mating system and ploidy

Among the 36 species included in this study, RAFT class was highly evolutionarily labile. A particular RAFT class was found in distantly related genera, families and orders (Fig. 7.2). Furthermore, class 0.45, common among dicots, was found in the single monocot analyzed, the orchid *Habenaria psycodes*. It thus appears that the control of meiosis offset timing relative to flower opening is similar in monocots and dicots at least those studied. Although RAFT class often differed among species within a family, there was no evidence of differences in RAFT class at lower taxonomic levels: within the seven genera for which more than one species was analyzed (*Amsinckia*, *Draba*, *Epilobium*, *Oenothera*, *Verbascum*, *Verbena*, *Veronica*); among the three analyzed populations of *Oenothera* (one population representing a varietal form); or between the two style-length morphs examined in tristylous *Lythrum salicaria*.

Seven of the populations used in this study belong to *Amsinckia*, a genus of yellow- to orange-flowered annuals possessing a variety of mating systems and associated floral traits (Ray and Chisaki, 1957a; Ganders et al., 1985; Johnston and

Schoen, 1995, 1996; Schoen et al., 1997). Distylous species/populations contain two floral morphs, pin (stigma is positioned higher than anthers in flower) and thrum (anthers are higher than stigma). The remaining species/populations were homostylous, bearing stigmas and anthers at similar heights in the flower. Compared to distylous populations, homostylous populations have higher rates of self-fertilization and in most cases smaller flowers. Molecular, morphological and karyological data suggest that *A. vernicosa* is derived from *A. furcata*, *A. gloriosa* (a tetraploid) from *A. douglasiana*, and homostylous *A. spectabilis* (both large- and small-flowered forms) from distylous *A. spectabilis*. If the duration of meiosis was shorter in *A. gloriosa* than in *A. douglasiana*, as has been reported for polyploids compared to related diploids (Bennett and Smith, 1972; Bennett, 1977; Bennett et al., 1971; John, 1990), then there was no consequent effect on RAFT. Within *Amsinckia*, therefore, RAFT class appeared to be unaffected by floral size, floral morph, rate of self-fertilization and ploidy.

7.5.2. Significance of discrete classes

The existence of narrowly defined RAFT classes indicates at least two facts concerning the control of floral development. First, within each class, the ratio of time (both absolute and relative) preceding tetrad formation to time following is constant and independent of total developmental duration. Second, the end of microsporocyte meiosis is not simply a cue that initiates or potentiates subsequent processes. Instead, one of the following must hold: either the absolute time required for pre-tetrad events determines the time required for post-tetrad events, or the two processes are regulated by an exogenous factor that maintains them in constant temporal ratio.

7.5.3. Causes of the three RAFT fractions

The two facts above follow directly from the existence of discrete RAFT classes. The reasons why the classes possess particular numerical values, however, are less certain, because the genetic, cellular and biochemical processes controlling floral development are not sufficiently well known. Furthermore, because 0.45 = 0.62 X 0.73, the number of independent RAFT classes is unknown; two developmental processes might act in combination to produce the third class. Despite current ignorance of developmental details, some simple mathematical and developmental possibilities suggest themselves. Below I present two such possibilities and provide evidence against one of them. It is hoped that this brief presentation will spur further modeling and testing of the role of microsporocyte meiosis in floral development.

One plausible scenario is that the complementary fractions indicating relative time before and after tetrad formation exist in simple exponential relationship, such that $\mathrm{RAFT} = 1 - \mathrm{RAFT}^k$, or $k = \log(1 - \mathrm{RAFT}) / \log(\mathrm{RAFT})$. Here, the logarithms, to any base, of the relative durations after versus before tetrad formation exist in constant ratio k. The values $k = 2$ and 4 correspond to RAFT ≈ 0.618 and 0.724, respectively. In this scenario, RAFT class 0.62 divides total floral development by the golden ratio, $\tau = (1+\sqrt{5})/2 = 1.618...$, and RAFT class 0.45 can be produced by $k = 3/4$ (if this class is independent of the other two classes, RAFT ≈ 0.450), or by dividing class 0.73 by the golden ratio (if this class is the product of the other two, RAFT ≈ 0.448).

The golden ratio was not explicitly included in the above model, which was based only on simple exponential relations between complementary fractions. Patterns in plant

morphology based on the golden ratio are conspicuous and have long been the subject of investigation (Jean, 1994; Guerreiro and Rothen, 1995; Douady and Couder, 1996; Green et al., 1996). When an object is divided according to the golden ratio, the ratio of the smaller to the larger part equals the ratio of the larger to the whole. The golden cut of a unit measure results in complementary proportions 0.381966... and 0.618034.... It is also the ratio, in the limit, of two successive members of the Fibonacci series (1, 1, 2, 3, 5, 8, 13, ...), the Lucas series (1, 3, 4, 7, 11, 18, ...) and indeed any series constructed by summing the two previous values to obtain the next.

The most conspicuous appearance of the golden ratio in plant morphology concerns phyllotaxis, the spiral or whorled arrangement on an axis bearing structures such as flowers, leaves, branches or scales. A number of clockwise spirals and a different number of counterclockwise spirals are especially evident on sunflower capitula, pineapple fruits, conifer cones, palm trunks, etc. The number of such spirals winding in each direction is usually a pair of consecutive members of either the Fibonacci or Lucas series (Jean, 1994). The type of phyllotaxis is determined primarily by the divergence angle, d (< 0.5 or < 180°), the angular separation of two successive primordia with respect to the apical center (Richards, 1951; Jean, 1994). Fibonacci phyllotaxis arises from divergence angles near $1-\tau^{-1}=\tau^{-2}\approx 0.382\approx 137.5^{\circ}$, and Lucas phyllotaxis arises from angles near $\left(3+\tau^{-1}\right)^{-1}=(5-\sqrt{5})/10\approx 0.276\approx 99.5^{\circ}$. On a given plant specimen, one can readily estimate the divergence angle by locating two nodes on approximately the same line parallel to the axis, determining the number of turns around the axis when proceeding through each successive node and dividing by the number of nodes. Typical

fractions in spiral phyllotaxis are 2/5, 3/8, 5/13, etc. (approximating 0.382) for Fibonacci patterns and 2/7, 3/11, 5/18, etc. (approximating 0.276) for Lucas patterns.

A second causal possibility therefore is suggested by the fact that the RAFT classes bear striking relations to the two most common divergence angles causing spiral arrangements of flowers and leaves. The RAFT classes found in this study are related to these two common divergence angles, as follows: $0.45 \approx 1 - 2d_{Lucas}$, $0.62 \approx 1 - d_{Fibonacci}$ and $0.73 \approx 1 - d_{Lucas}$. Thus, in this study it was found that the proportion of time a developing flower spends between meiosis termination and flower opening approximates common divergence angles (or double) between successive primordia. The phyllotactic divergence angle does not refer to processes within individual flowers, but instead to the disposition of separate floral primordia. Therefore, the divergence angle would be able to determine RAFT only as a result of establishing a particular lattice geometry in the inflorescence (Jean, 1994). In this scenario RAFT would be determined by the effects of lattice geometry on morphogen diffusion and transport.

At least two empirical facts argue against this hypothesized causal connection between RAFT and divergence angle. First, the explanation applies only to spiral inflorescences, and the present study included two types of nonspiral inflorescence architecture that nevertheless expressed RAFT values in the same three classes as the spiral inflorescences: Boraginaceae and single flowers. In the Boraginaceae primordia are initiated in a zig-zag fashion along one side of the inflorescence. In such cases divergence angles are unrelated to the golden ratio, but classes 0.45 and 0.62 were found in this family. In *Viola odorata* (class 0.73), flowers are borne singly. Because singly borne flowers are not part of an inflorescence lattice, the timing of meiosis termination in such

plants cannot be determined by developmental cues from other floral buds. Second, I determined the divergence angles separating floral positions in seven of the species of Figure 7.2 and found that all approximated the Fibonacci angle: *Alyssum maritimum*, *Epilobium angustifolium*, *Verbena scabra*, *Capsella bursa-pastoris*, *Brassica kaber*, *Cakile edentula* and *Campanula rapunculoides*. Because these species represented all three RAFT classes, it is clear that RAFT was often related to a floral divergence angle not used by the plant. Therefore, if there is a relationship between RAFT and the golden ratio, it is not simply a consequence of developing buds existing in a τ-based cylindrical lattice. This leaves as more probable the scenario of a constant exponential relation between RAFT and 1 - RAFT, with very simple exponents.

Other mathematical sequences that approximate the three RAFT classes of course exist, but none is as straightforward as that based on simple exponents. Distinguishing among the possibilities will in general not be achieved by measuring RAFT on a large number of species, because many of the competing mathematical sequences will differ only by a degree of precision greater than that measurable in plants. Instead, the correct mathematical relations among the three classes will be revealed by a mechanistic understanding of the genetic, cellular and biochemical processes of meiosis and floral development. The existence of a small number of discrete RAFT classes suggests that these processes have been highly conserved in angiosperm evolution.

CHAPTER 8

GENERAL DISCUSSION AND CONCLUSIONS

Development is a process that leads to the formation of various floral morphologies; thus, the evolution of floral morphology is actually the result of evolutionary changes in developmental processes. Several different developmental mechanisms can lead to evolution. Heterochrony, however, is perhaps the best-known mechanism responsible for evolutionary changes of flower morphology through its ontogeny.

Heterochrony is a change in the relative timing and/or rate of developmental processes, or alteration in sequences of developmental events in ontogeny, in a descendant relative to its ancestor. In Chapter two I reviewed the concept and application of heterochrony in plant evolutionary studies. It seems that most heterochronic changes in plant evolution involve more than one of the six classic pure heterochronic processes. Of these processes, neoteny (decreased developmental rate in descendant), progenesis (earlier offset) and acceleration (increased rate) have been more commonly reported than hypermorphosis (delayed offset) and predisplacement (earlier onset). No postdisplacement (delayed onset) was found in published studies. I noticed one of the particularly important aspects about heterochrony that has not been described in any other heterochronic models, that is the phenotypic effects of evolutionary changes in onset or offset timing can be exaggerated, suppressed or reversed by changes in rate. This is evident in my study on evolution of the small flowered homostyly from its ancestor, the

large flowered distyly, in *Amsinckia spectabilis*. Homostyly has a much longer developmental duration (delayed flowering time) than distyly. The homostylous flower, however, does not get any larger than distylous flower, as we would normally have expected according to the heterochrony concept. Instead, it is actually significantly smaller than the distylous flower. This is because the developmental rate in a small homostylous flower is less than 50% of that in a distylous flower. The extremely slower growth rate (paedomorphosis by neoteny) in the small homostylous flower totally reversed its potential effect of longer developmental duration (peramorphosis by acceleration).

In the review I also discussed the relationships between heterochrony and some other developmental mechanisms that can also lead to evolution, such as heterotopy and homeosis. Because not all-developmental changes responsible for evolution are the result of heterochrony, I propose that it is better to integrate these different developmental mechanisms in plant evolutionary studies.

The main project of this study is on comparative floral morphometrics, development, and evolution of homostyly and distyly in three lineages of *Amsinckia*. Twenty-six floral traits were studied. In two distylous flower morphs, stamen and pistil heights varied as expected from their close relationship to the definition of pins and thrums, with the stamen-height-related traits greater in thrums and the pistil-height-related traits greater in pins. Thrums make larger but fewer pollen grains in all lineages. Thrums also tend to have larger values for corolla size (six traits measured), stigma size (four traits), style cross-sectional area and style transmission tissue cross-sectional area. In two of three lineages, pins exceed thrums in functional anther-stigma distance and in

stigmatic papilla length and width. The size order of a trait in pins versus thrums is consistent in all lineages for 18 of 26 traits; in seven of the eight remaining traits *A. spectabilis* is the unusual lineage. In homostyles, traits related to anther height and pistil height are intermediate between pins and thrums in all lineages; for other traits homostyles generally have the smallest values.

Functional anther-stigma distance and flower size are the two key characters in discriminating distyly from homostyly. A distylous flower is about 1.5-1.8 times larger than a homostylous flower. The functional anther-stigma distance in a distylous flower is approximately 6 mm while it is close to zero in a homostylous flower. Stamen height (SSIL) and especially its insertion height (SINH) are the major discriminating traits in separating the three floral morphs (pin, thrum and homostyle) in *Amsinckia*. Both traits of SSIL and SINH in thrums are approximately 1.7 times larger than that of homostyles, and about 2-2.5 times larger than that of pins. Pistil length (PISL), particularly the style length (PSTYL) is the major responsible floral trait that discriminates the four floral morphs (pin, thrum, large homostyle, and small homostyle) in *A. spectabilis*. Surprisingly, the study shows that one of the non-definitional floral traits, the stigma thickness (PSTH), is the single most important discriminative trait to the three evolutionary lineages in *Amsinckia*. The overall size of PSTH among the three lineages is in the order of L1 > L2 > L3.

Comparative flower ontogenetic studies between homostyly and distyly both within and among evolutionary lineages suggest that homostyly evolved from distyly. Paedomorphosis through neoteny and progenesis is the major developmental mechanism responsible for the evolution of homostyly from distyly in all three lineages. The

evolution of homostyly is lineage dependent in *Amsinckia*. This is caused by differences in the extent of paedomorphosis, developmental dissociation, and changes of ontogenetic trajectories in homostyly compared to its ancestral distyly, in association with some other developmental processes or mechanisms such as peramorphic ontogeny by acceleration in some cases, among lineages. Similar developmental mechanisms have led to the differentiation of pins from thrums in distyly independently in three evolutionary lineages of *Amsinckia*. Contradictory growth rates of stamen and pistil heights in distylous flowers have resulted in pin and thrum flowers having reciprocal positioning of anther and stigma heights. The self-compatible distyly in *Amsinckia* is more likely derived from some unidentified self-incompatible distyly by losing their self-incompatibility system. The unique ontogenetic patterns of the large-flowered homostyly in lineage of *A. spectabilis* suggest that it may represent an intermediate morph in the evolution of homostyly from distyly. It is common that multiple heterochronic processes are involved in the mosaic development and evolution of homostylous flowers. Convergence and parallelism may have also been involved in the evolution of homostyly and differentiation of two distylous flower morphs. Although comparative floral ontogenetic results support the assumption that the small self-pollinated homostylous flower was derived independently from the large outcross-pollinated distylous flower in three evolutionary lineages of *Amsinckia*, some similarities in patterns of ontogenetic differences between homostyly and distyly in lineages of *A. furcata* – *A. vernicosa* and *A. douglasiana* – *A. t. gloriosa* suggest that these two lineages might have originated from a recent common ancestor.

Although early development is believed to be subjected to constraint and highly conserved in evolution (Raff et al., 1991), it has been reported many times that the initial

size of a floral primordium (and floral organ primordia) is an important developmental determinant of size differences seen among mature flowers and floral organs. This suggested a cause and effect relationship between the initial and final sizes (Sinnott, 1921; Houghtaling, 1935; Whaley, 1939; Guerrant, 1988). Therefore, studies on early flower ontogeny in homostylous and distylous plants will be certainly helpful to see if there is any and what kind of early ontogenetic modification during the evolution of homostyly from distyly. This is one of the research projects I would like to pursue in future.

Development is a process for the production of phenotype or morphology. On the other hand, development is subjected to the regulations of differential gene expression. Therefore, in the future it is necessary to integrate developmental (including developmental anatomy) and genetic (including molecular genetics) studies in order to fully understood the mechanisms underlying the evolution of homostyly from distyly and thus the evolution of self-fertilization.

Microsporocyte meiosis time, especially the microspore-tetrad formation time is one of the major timing reference points in flower developmental studies. It is generally believed that floral development is a continuous process, and that the timing of meiosis, which results in plants switching from diploid to haploid phase during their life cycles, varies widely among species. For 36 species representing 13 angiosperm families, it was found that microsporocyte meiosis terminated at only three discrete relative times during flower development (from primordium to anthesis) despite wide variations within and among species in absolute developmental durations. A single timing class characterized each species. Thus, for all species within a given class, the durations before and after the

end of the meiosis existed in a constant ratio. Interestingly, the three timing classes are related to fractions based on the golden ratio. Each timing class was found in phylogenetically distant species, and, conversely, a plant family often contained more than one class. Timing class is not related to ploidy level, inflorescence architecture, pollination syndrome or mating system. These findings suggest that a single exogenous process may have regulated the timing of premeiotic and postmeiotic floral development, or that one rate determines the other. They further imply that the underlying developmental processes have evolved in a limited number of ways among flowering plants. It will be my future interest to investigate meiosis timing in more species from a wider range of taxa to see if these three timing classes still hold.

APPENDIX 1

HETEROCHRONY IN PLANTS

Entries are restricted to cases of reasonably certain phylogeny plus some fossils. See Chapter 2 for further explanation.

			Paedomorphosis			Peramorphosis			
Ancestor, Descendant	**Structure or event**	**Derived morphology**	**Neoteny**	**Progenesis**	**Postdisplacement**	**Acceleration**	**Hypermorphosis**	**Predisplacement**	**References, Notes**
REPRODUCTIVE TRAITS									
Gymnosperms, Angiosperms	flower generally	flower (from ancestral reproductive shoot)		X					Takhtajan (1976, 1991)
Gymnosperms, Angiosperms	gametophyte	reduced size, reduced complexity, loss of gametangia		X		X			Takhtajan (1976, 1991), Friedman & Carmichael (1998)
Angiosperms generally	whole flower	zygomorphic (from actinomorphic)						X	Tucker (1987), Stebbins (1992)

Appendix 1. Continued.

Ancestor, Descendant	Structure or event	Derived morphology	Paedomorphosis: Neoteny	Paedomorphosis: Progenesis	Paedomorphosis: Postdisplacement	Peramorphosis: Acceleration	Peramorphosis: Hypermorphosis	Peramorphosis: Predisplacement	References, Notes
Amsinckia douglasiana (distylous), *A. t. gloriosa* (homostylous)	whole flower	reduced size	X						Li & Johnston, unpubl.
Amsinckia furcata (distylous), *A.vernicosa* (homostylous)	whole flower	reduced size	X						Li & Johnston, unpubl.
Amsinckia spectabilis (distylous), *A.s.* (homostylous)	whole flower	reduced size	X		X		X		Li & Johnston, unpubl.
Arenaria uniflora Outcrossing flower, Selfing flower	whole flower	reduced size	X				X		Hill et al. (1992)

Appendix 1. Continued.

Ancestor, Descendant	Structure or event	Derived morphology	Paedomorphosis: Neoteny	Paedomorphosis: Progenesis	Paedomorphosis: Postdisplacement	Peramorphosis: Acceleration	Peramorphosis: Hypermorphosis	Peramorphosis: Predisplacement	References, Notes
Astragalus cymbicarpos CH flowers, CL flowers	whole flower	reduced size		X		X			Lord (1979, 1982) Acceleration occurred after PMC meiosis
Bromus carinatus CH flowers, CL flowers	anther and flower maturation	earlier		X					Harlan (1945)
Bromus unioloides CH flowers, CL flowers	anther	reduced size	X	X					Langer & Wilson (1965)
Clarkia xantiana ssp. Xantiana, C. x. ssp. parviflora	plant/flowering time/pollination type	smaller/earlier/selfing		X		X			Runions & Geber (1998)
Collomia grandiflora CH flowers, CL flowers	anther	reduced size						X	Lord et al. (1989), Hill & Lord (1990), Minter & Lord (1983)

Appendix 1. Continued.

Ancestor, Descendant	Structure or event	Derived morphology	Paedomorphosis			Peramorphosis			References, Notes
			Neoteny	Progenesis	Postdisplacement	Acceleration	Hypermorphosis	Predisplacement	
Collomia grandiflora CH flowers, CL flowers	pollen	reduced number						X	Lord et al. (1989), Hill & Lord (1990), Minter & Lord (1983)
Collomia grandiflora CH flowers, CL flowers	whole flower	reduced size				X			Minter & Lord (1983) Acceleration occurred before PMC meiosis. PMC meiosis earlier onset
Cucurbita argyrosperma sororia, *C.a. argyrosperma*	timing (nodal position) of first flower	earlier (lower nodal position)		X					Jones (1992, 1993)
Delphinium decorum, *D. nudicaule*	sepals and nonnectariferous petals	resemble buds of ancestral form	X						Guerrant (1982) applies to whole flower externally viewed

Appendix 1. Continued.

Ancestor, Descendant	Structure or event	Derived morphology	Paedomorphosis			Peramorphosis			References, Notes
			Neoteny	Progenesis	Postdisplacement	Acceleration	Hypermorphosis	Predisplacement	
Delphinium decorum, *D. nudicaule*	nectariferous petal	increased size				X	X		Guerrant (1982)
Ephedra, *Gentum gnemon*	female gametophyte matures sexually (fertilized)	earlier		X		X			Friedman & Carmichael (1998)
Lamium amplexicaule CH flowers, CL flowers	whole flower	reduced size		X		X			Gallardo et al. (1993) Acceleration occurred after PMC meiosis
Limnanthes alba, *L. floccosa*	whole flower	reduced size		X					Guerrant (1984, 1988)
Limnanthes alba, *L. floccosa*	flower maturation	earlier		X		X			Guerrant (1984, 1988)

Appendix 1. Continued.

Ancestor, Descendant	Structure or event	Derived morphology	Paedomorphosis			Peramorphosis			References, Notes
			Neoteny	Progenesis	Postdisplacement	Acceleration	Hypermorphosis	Predisplacement	
Salpiglossis sinuata CH flowers, CL flowers	corolla	reduced size		X					Lee et al. (1979)
Sigillaria, *Chaloneria* (both fossils)	time of reproduction	earlier	X	X					Bateman (1994)
Veroniocastrum virginicum, *Veronica chamaedrys*	sepals	increased size	X			X	X		Kampny et al. (1993) Neoteny in early stages, acceleration later
Viola odorata CH flowers, CL flowers	maturation time	earlier				X			Mayers (1983a,b)
Viola odorata CH flowers, CL flowers	whole flower	reduced size				X			Mayers (1983a,b) CL floral primordium is smaller

Appendix 1. Continued.

Ancestor, Descendant	Structure or event	Derived morphology	Paedomorphosis: Neoteny	Paedomorphosis: Progenesis	Paedomorphosis: Postdisplacement	Peramorphosis: Acceleration	Peramorphosis: Hypermorphosis	Peramorphosis: Predisplacement	References, Notes
VEGETATIVE TRAITS									
Cucurbita argyrosperma sororia, *C.a. argyrosperma*	leaf	reduced lobing	?	?	?				Jones (1992, 1993) Paedomorphosis plus allometric growth
Lepidodendron, *Hizemodendron* (both fossils)	stem	reduced height		X					Bateman & DiMichele (1991), Bateman (1994)
Lyginopteridop-sida, Magnoliales	leaf	simple, entire		X					Takhtajan (1976, 1991)
Pseudopanax crassifolius mature leaves, juvenile leaves	leaf	increased length, decreased width				X			Clearwater & Gould (1993)

Appendix 1. Continued.

Ancestor, Descendant	Structure or event	Derived morphology	Paedomorphosis			Peramorphosis			References, Notes
			Neoteny	Progenesis	Postdisplacement	Acceleration	Hypermorphosis	Predisplacement	
Rhinanthus glacialis populations from alpine grassland, populations from subalpine hay meadows	onset of vegetative growth	earlier						X	Zopfi (1995)
R. glacialis populations from alpine grassland, populations from subalpine limestone grassland	offset of vegetative growth	later					X		Zopfi (1995)

Appendix 1. Continued.

Ancestor, Descendant	Structure or event	Derived morphology	Paedomorphosis: Neoteny	Paedomorphosis: Progenesis	Paedomorphosis: Postdisplacement	Peramorphosis: Acceleration	Peramorphosis: Hypermorphosis	Peramorphosis: Predisplacement	References, Notes
R. glacialis population from grassland on rocks, population from litter meadows	offset of vegetative growth			X					Zopfi (1995)
Sigillaria, *Chaloneria* (both fossils)	whole plant	shorter, lacking branches	X	X					Bateman (1994)
Viola odorata CH plants, CL plants	leaf, petiole	increased size				X			Mayers (1983a)

APPENDIX 2

PROGRAM FOR TRAIT SIZE INTERPOLATION

by Dr. Mark Johnston

```
! *************Infl.slope.MSE.PGM.DSCNDG.8************
! WILL calculate A NEW LINE OF AVERAGE Y'S AT APPROPR. X's (X's=AT DESIRED
!    INCREMENTS SUCH AS 0.3, 0.4, ETC) as well as THE MEAN SQUARED ERROR
!    BETWEEN THIS LINE AND THE ACTUAL Y-VALUES, PRINTED IN OUTPUT WINDOW
! THE 3 INPUT COLUMNS MUST BE AS FOLLOWS. C1: INFLORESCENCE ID (SORTED)
!    C2: SIZE OF FLOWER PART (Y).  C3: RELATIVE POSITION (X)
!    (SORTED HIGH TO LOW WITHIN EACH INFLORESCENCE)
! IT IS ASSUMED THAT FOR COL.3 OF INPUT, THE FINAL VALUE WITHIN AN
!    INFLOR. < THE 1ST VALUE OF THE SUCCEEDING INFLOR.
! IT IS NOT NECESSARY FOR EVERY INFLOR TO HAVE ITS HIGH X-VALUE >= UPPER BOUND
!   AND ITS LOWEST X-VALUE <= LOWER BOUND
! WHEN CONSECUTIVE VALUES IN INPUT COL.3 SPAN BY MORE THAN ONE INCREMENT,
!   THEN APPROP. NEW INCREMENTAL X'S AND EST'D Y'S ARE FILLED IN
!
DIM X(1,3),Y(1,1),Z(1,1),M(1,1),MM(1,2)
CLOSE #2
!LIBRARY "Macintosh HD:True BASIC:TB Library:MacTools*"     ! loads library
!LIBRARY "Macintosh HD:TrueBASIC:TB Library:MacTools*"     ! loads library
LIBRARY "7100/80AV:True BASIC:TB Library:MacTools*"     ! loads library
DECLARE DEF MacGetFile$           ! so it knows its not an array
DECLARE DEF MacPutFile$           ! so it knows its not an array
!
PRINT "WHEN PROMPTED FOR MULTIPLE INFO., USE COMMAS BETWEEN RESPONSES "
PRINT
!INPUT PROMPT "ENTER TRAIT NAME ": TRAIT$
PRINT
INPUT PROMPT "ENTER A NUMBER GREATER THAN #OBSERV. ":GT
PRINT
PRINT "ENTER LOWER,UPPER **BOUNDS** OF X-AXIS "
PRINT " TO BE USED EVERY RUN.  Note: UPP. BOUND ENTERED "
PRINT "  MUST BE INCLUDED WITHIN THE VALUES OF AT LEAST ONE"
PRINT "   INFLORESCENCE!!!!"
```

```
INPUT PROMPT "   ":LB,UB
PRINT
PRINT "ENTER LEAST,GREATEST **INCREMENT** "
INPUT PROMPT "    TO BE EXPLORED IN SEPARATE RUNS ":INCR1,INCR2
PRINT
PRINT "IN THE FOLLOWING, IF WANT TO RUN ONLY ONE INCREMENT (ONE RUN),  "
PRINT " THEN can just ENTER A STEP-VALUE GREATER THAN DIFFERENCE IN "
PRINT "  INCREMENT SIZE BETW SUCCESSIVE RUNS "
INPUT PROMPT "ENTER DIFF BETW SUCCESSIVE INCREMENTS TO BE EXPLORED ":DELTA
MAT REDIM X(GT,3),Y(GT,2)
LET INFILE$=MacGetFile$(0,0,TEXTPICT$,"Open input")    ! OPENS DIALOG BOX SO CAN OPEN ANY FILE
LET OUT1$=MacPutFile$(3,0,"Save output all inflor as","OUT1.all","Save me")   !OPENS DIALOG BOX SO CAN save to ANY FILE
LET OUT2$=MacPutFile$(3,0,"Save your means output as","OUT.means","Save me")  !OPENS DIALOG BOX SO CAN save to ANY FILE

OPEN #1: NAME INFILE$,  create old, ORG TEXT
OPEN #2: NAME OUT1$, create new
! SET #2: MARGIN 9*16+1
!PRINT #2,USING "$$$$$$$$$$ ,":TRAIT$
OPEN #3: NAME OUT2$, create new
!
! 1st WE INPUT DATA
LET R=0
DO WHILE MORE #1
   LET R = R+1
   INPUT #1:X(R,1),X(R,2),X(R,3)
LOOP
CLOSE #1
LET NINFLOR = X(R,1)
LET NOBSX = R
PRINT
PRINT "_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _"
PRINT "NOBSX= ";R
PRINT "# INFLORESCENCES = "; NINFLOR
PRINT "_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _"
PRINT
PRINT

! following probably not needed.  Probably need to round at k only (in sub make_y)
!FOR I=1 TO NOBSX
!    FOR J=1 TO 3
!        LET X(I,J)=ROUND(X(I,J),8)
!   NEXT J
```

```
!NEXT I

FOR INCR=INCR1 TO INCR2 STEP DELTA
    MAT REDIM X(NOBSX+2,3),Y(NOBSX*12,3)     ! MAKING Y-MAT OVERLY LONG FOR NOW
    FOR I=NOBSX+1 TO NOBSX + 2     ! ADDING 2 ROWS TO X-MATRIX.  REALLY NEED ONE
        FOR J=1 TO 3
            LET X(I,J)=888
        NEXT J
    NEXT I
    LET X(NOBSX+1,1)=X(NOBSX,1)
    LET X(NOBSX+2,1)=X(NOBSX,1)

    CALL make_y

    PRINT "INCREMENT= " ;INCR
    PRINT "NOBSY= ";NOBSY
    !PRINT "HERE'S Y MATRIX "
    !MAT PRINT Y

    CALL make_m

    PRINT "Maximum possible NOBSM=NOBSM = ";NOBSM
    !PRINT "HERE'S M MATRIX ALL DONE "
    !MAT PRINT M
    PRINT "Actual NOBSM=NM = ";NM

    CALL SSE_MSE


NEXT INCR
SOUND 444, .2
SOUND 600, .1
SOUND 888, .2


SUB make_y
    MAT Y=999
    LET CNT=0
    FOR xx = 1 TO NOBSX
        FOR K = UB TO LB STEP -1*INCR
            ! the above statement w/0 rounding does not grab the FINAL (=SMALLEST) value of an inflor
            !    if it exactly = the min. K--This failure occurs only for some cases of UB and LB,
```

```
            !    which remain baffling.
            ! NEED TO AT LEAST ROUND K BELOW.  THE FOLLOWING MIGHT ALSO BE TRUE:
            ! ROUNDING: NEED TO ROUND INPUT DATA TO # OF DECIMAL PLACES GREATER THAN K BELOW.  IF
            ! OUTPUT HAS TOO MANY/FEW DATA POINTS (AND CHECK OUTPUT!!!!!!!!) THEN TRY CHANGING
            ! THE ROUNDING FCTN DECIMAL PLACES, ALWAYS KEEPING THE ONE FOR K LOWER THAN THE ONE
            ! FOR X-MATRIX (I THINK, ANYWAY)

            LET k=round(k,6)
            DO
               IF X(xx,3)>=K and X(xx+1,3)<K THEN
                  LET CNT=CNT+1
                  LET Y(CNT,1)=X(xx,1)
                  LET Y(CNT,2)=X(xx,2)-(X(xx,3)-K)*(X(xx,2)-X(xx+1,2))/(X(xx,3)-X(xx+1,3))
                  LET Y(CNT,3)=K
                  PRINT #2, USING " #####.#######,":Y(CNT,1),Y(CNT,2),Y(CNT,3)
               ELSE IF X(xx,3)=K and x(xx+1,1)<>x(xx,1) THEN
                  LET CNT=CNT+1
                  LET Y(CNT,1)=X(xx,1)
                  LET Y(CNT,2)=X(xx,2)
                  LET Y(CNT,3)=K
                  PRINT #2, USING " #####.#######,":Y(CNT,1),Y(CNT,2),Y(CNT,3)
               ELSE IF X(xx,3)=K and x(xx+1,1)=x(xx,1) THEN
                  LET CNT=CNT+1
                  LET Y(CNT,1)=X(xx,1)
                  LET Y(CNT,2)=X(xx,2)
                  LET Y(CNT,3)=K
                  PRINT #2, USING " #####.#######,":Y(CNT,1),Y(CNT,2),Y(CNT,3)
               END IF
               EXIT DO
            LOOP
         NEXT K
      NEXT xx
      LET NOBSY=CNT
      MAT REDIM Y(NOBSY,3)
   END SUB


   SUB make_m
      LET NOBSM=INT(1.00000001+((UB-LB)/INCR))
      !LET NOBSM=1+((UB-LB)/INCR)
      MAT REDIM M(NOBSM,4)
      FOR MROW=1 TO NOBSM
```

```
        LET M(MROW,1)=0            ! COLUMN 1 IN M SHOULD LATER ADD UP TO NINFLOR
        LET M(MROW,2)=0            ! COLUMN 2 IN M SHOULD LATER ADD UP TO SUM Y-VALUES
        LET M(MROW,3)=UB-INCR*(MROW-1)      ! COLUMN 3 IN M IS STANDARD X-VALUES
        LET M(MROW,3)=round(M(MROW,3),6)
        LET M(MROW,4)=999          ! COLUMN 4 IN M WILL BE AVERAGE Y-VALUES
    NEXT MROW

    FOR I=1 TO NOBSY
        DO
           FOR J=1 TO NOBSM
               IF ROUND(Y(I,3),6)=ROUND(M(J,3),6) THEN
                  LET M(J,2)=M(J,2)+Y(I,2)  ! SUMMING THE Y-VALUES FOR EACH STAND. X
                  LET M(J,1)=M(J,1)+1  ! COUNTING THE NUMBER OF INFLOR'S
                  EXIT DO
               END IF
           NEXT J
        LOOP
    NEXT I

    LET nm=0
    FOR I=1 TO NOBSM
        IF M(I,1)>0 and M(i,3)<>999 THEN
           LET M(I,4)=M(I,2)/M(I,1)    ! MEAN Y-VALUES
           LET nm=nm+1
        END IF
    NEXT I
    MAT redim m(nm,4)              ! so output does not have any extra unused lines
    MAT PRINT #3, USING " #####.#######,":M
END SUB


SUB SSE_MSE
    LET SUMSQE=0
    LET A=0
    FOR I=1 TO NOBSY
        DO
           FOR J=1 TO nm           !   NOBSM
               IF ROUND(Y(I,3),6)=ROUND(M(J,3),6) THEN
                  LET SUMSQE=SUMSQE+(M(J,4)-Y(I,2))^2
                  LET A=A+1
                  EXIT DO
               END IF
```

```
            NEXT J
          LOOP
      NEXT I
      IF A<>NOBSY THEN PRINT "*PROBLEM*: COUNTS FOR MSE= ";A, "BUT NOBSY= ";NOBSY
      LET MSE=SUMSQE/NOBSY
      PRINT "INCREMENT= " ;INCR
      PRINT "SUMSQE= ";SUMSQE
      PRINT "MSE= ";MSE
      PRINT
END SUB

CLOSE #2
CLOSE #3

END
```

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