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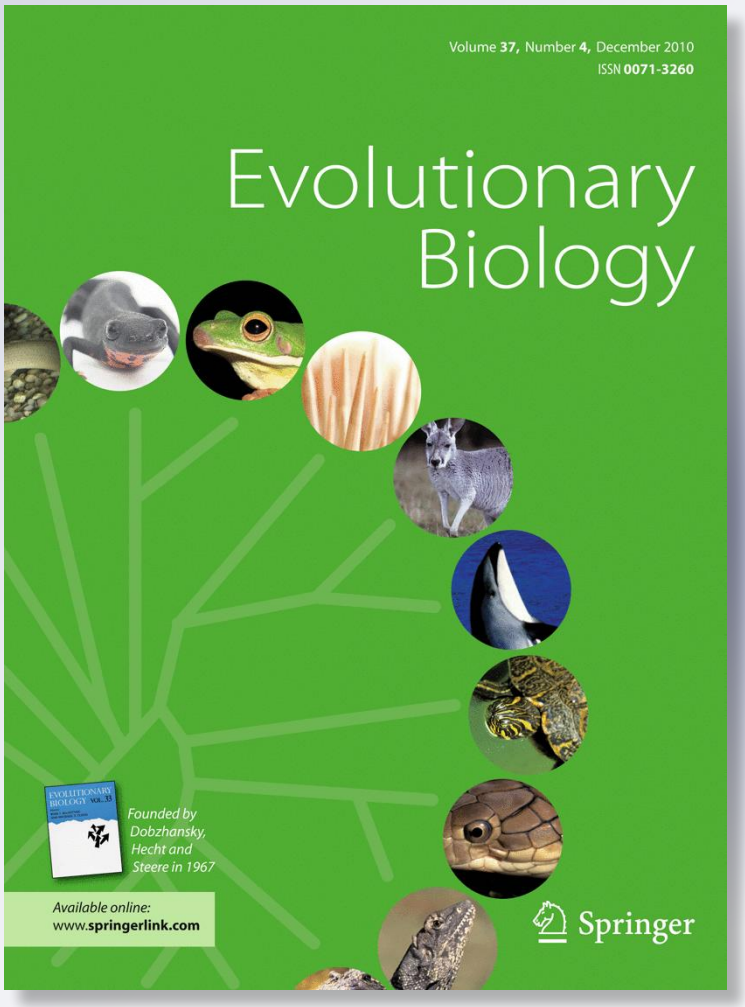

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RESEARCH ARTICLE

Flower Development and the Evolution of Self-fertilization in *Amsinckia*: The Role of Heterochrony

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Abstract We studied the development of 26 flower traits under natural conditions in three clades of the genus *Amsinckia* (Boraginaceae). Each clade contained both a derived highly self-fertilizing taxon and an ancestral more highly outcrossing taxon. The more outcrossing taxa contained two flower morphs—pins and thrums—with opposite positioning of the sex organs (heterostyly). The highly selfing taxa had smaller flowers with sex organs in close proximity (homostyly). Growth trajectories were quantified over the entire or nearly the entire period from primordium initiation to flower opening. These trajectories were compared in the heterochronic framework and, in contrast with previous studies, character size was tracked over time rather than relative to another character. We focused on three hypotheses: (1) The distinct developmental trajectories leading to pins and thrums should be similar in all clades, while the trajectories leading to homostylous flowers might differ among clades. This was supported. Specifically, contrasting growth rates of stamen and pistil heights in heterostylous flowers caused pin and thrum flowers to have the reciprocal arrangement of anther and stigma heights. From the viewpoint of heterochrony, the decreased size (paedomorphosis) of the homostylous morph, compared to pins and thrums, resulted from decreased growth rate (neoteny) and earlier offset (progenesis) in all clades. Nevertheless, multiple heterochronic processes were involved in the mosaic development and evolution of homostylous flowers. (2) We tested the hypothesis that small, self-fertilizing flowers have reduced development times, one of the proposed selective advantages of increased self-fertilization rates. We found in contrast that developmental duration of homostylous flowers was either the same (two clades) or longer (one clade) compared to duration of pins and thrums. (3) Finally, we tested von Baer's Law, which proposes that developmental differences among closely related taxa should arise later in development than differences among more distantly related taxa. Von Baer's Law was supported strongly among homostyles, moderately among thrums and weakly among pins.

Keywords Development · Distyly · Flower size · Growth curve · Heterochrony · Homostyly · Heterostyly

Introduction

The evolution of self-fertilization from outcrossing is one of the most common and important evolutionary transitions among flowering plants (Stebbins 1957; Ornduff 1969; Stebbins 1970). The evolutionary shift from outcrossing to high levels of selfing in angiosperms is usually accompanied by major changes in flower morphology, which include reductions in flower size, flower organ size, pollen production and anther-stigma distance (Ornduff 1969; Wyatt 1988; Barrett and Harder 1992; Sherry and Lord 2000). These shifts also involve changes in the timing of flower developmental processes (Guerrant 1989; Hill and Lord 1990; Diggle 1992; Hill et al. 1992; Stewart and Canne-Hilliker 1998; Runions and Geber 2000;

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Armbruster et al. 2002; Georgiady and Lord 2002). With few exceptions (e.g., Georgiady and Lord 2002), these conclusions about heterochronic changes are based on late or even mature developmental stages. In many cases, an increase or decrease in rate is found by dividing final size by total development time, ignoring all intervening development. While such an approach correctly tests for change in developmental rate, it cannot determine whether particular organs in the descendant stop development earlier or later, nor can it pinpoint the age at which the developmental trajectories first differ. For these, it is necessary to study a larger portion of the developmental process. Furthermore, most published flower ontogenetic studies used the length of the corolla or pistil as indicators of developmental age (e.g., Stewart and Canne-Hilliker 1998; Sherry and Lord 2000), a method that does not necessarily provide true developmental time or age information. Such approaches are thus actually studies of allometry rather than heterochrony (McKinney 1988a; Klingenberg and Spence 1993; Li and Johnston 2000), and the two methods can give different results whenever growth rate of the reference structure differs among taxa.

Mature phenotypes evolve because the underlying developmental processes evolve. Understanding the precise ways that the developmental process changes as the mature phenotype evolves remains a fundamental goal of evolutionary biology (Hall 1998). Heterochrony is a change in the rate or timing of development in a descendant relative to the ancestor or among closely related taxa (McKinney 1988b; McKinney and McNamara 1991; Smith 2003). Because much of developmental evolution necessarily involves changes in rates and timings of events, heterochrony is often proposed to be the most common, or even the only, mechanism of phenotypic evolution (McKinney 1988b; McKinney and McNamara 1991; Georgiady and Lord 2002). For example, a trait may be smaller in a descendant because of a lower growth rate or an earlier termination of development. Furthermore, a complex structure might change shape because its constituent traits are developmentally altered to different degrees. There are two general classes of heterochrony: paedomorphosis, in which the descendant structure is smaller than that of the ancestor, and peramorphosis, in which the descendant structure is larger (Alberch et al. 1979; Li and Johnston 2000). Paedomorphosis can result from a decrease in developmental rate (neoteny), earlier offset (progenesis) or later onset (postdisplacement). Peramorphosis on the other hand can result from an increased developmental rate (acceleration), delayed offset (hypermorphosis) or earlier onset (predisplacement). Despite its undoubted ubiquity, we still know little about the relative frequency of the different types of heterochrony within either animals (McNamara and McNamara 1997) or plants (Guerrant 1988; Li and Johnston 2000).

The genus *Amsinckia* (Boraginaceae) exhibits a diversity of mating systems, ranging from predominant outcrossing, to intermediate outcrossing to predominant self-pollination to nearly complete self-pollination (Ray and Chisaki 1957a, b; Ganders 1975; Johnston and Schoen 1996; Schoen et al. 1997). The predominantly outcrossing taxa contain two flower morphs, and are thus distylous (a type of heterostyly): in "pins" the stigma is positioned above the five anthers, at or above the flower throat, while "thrums" have the opposite arrangement. Taxa that have lost the distylous condition are termed "homostylous" and bear anthers and stigma in close proximity, resulting in high selfing rates. The repeated evolution of homostyly and increased selfing in *Amsinckia* (Fig. 1) allows us to test several hypotheses concerning the evolution of flower development. First, all evidence suggests that distyly is ancestral, while homostyly has evolved independently several times. Therefore, we hypothesize that the developmental pathways of pins and of thrums should be similar in all clades, while the pathways leading to homostyly might differ among clades. Second, one can address the hypothesis that small, self-fertilizing flowers have reduced development times, one of the proposed selective advantages favoring the evolution of increased self-fertilization rates (Stebbins 1974). Finally, it is possible to test the applicability of "von Baer's Law," the proposition that developmental differences among closely related taxa should arise later in development than differences among more distantly related taxa (von Baer 1828; see Takhtajan 1976, 1991; Raff 1996; Tucker 1997; Arthur 2004). If von Baer's Law holds for *Amsinckia*, we can make two predictions. First, developmental events causing pins and thrums to differ should arise later within species than among species. Second, developmental events differentiating homostyles from pins and thrums should arise later within each (distyle-homostyle) sister group than among these clades. That is, pins, thrums and homostyles within a clade should share developmental traits until late ontogeny, while clade differences should arise earlier in ontogeny. The goal of this study is to describe flower development from very early stages and to test the hypotheses described

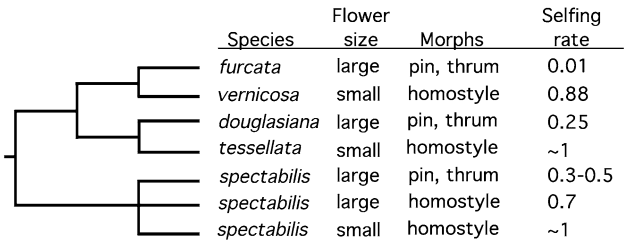

Fig. 1 Cladogram of the *Amsinckia* species used in this study (from Ray and Chisaki 1957b; Schoen et al. 1997; Johnston and Hahn, unpublished)

above. Our focus is on heterochronic changes, and we do not exclude other mechanisms. We are not aware of any other comparative studies covering nearly all of flower development in related plants differing in mating system.

Materials and Methods

Study Species and Flower Morphs

On the basis of morphology and chromosome number, Ray and Chisaki (1957b) proposed a phylogeny of *Amsinckia* consisting of four separate 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 phylogeny, and further suggested that the selfing taxa are recently derived from outcrossing ancestors, which occurred in each of the four clades (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 (Johnston and Hahn, unpublished results). These four clades are *A. furcata* and *A. vernicosa*; *A. douglasiana* and *A. tessellata gloriosa* (and *A. t. tessellata)*; *A. spectabilis* comprising large-flowered distylous, large-flowered homostylous and small-flowered, homostylous morphs; and large- and small-flowered *A. lunaris* (Fig. 1).

We studied 10 species-morph combinations representing three clades of *Amsinckia* (Fig. 1). These species-morph combinations were the pins (P), thrums (T) and homostyles (H) of the three clades (nine combinations), plus an additional large-flowered homostylous morph (LH) in *A. spectabilis*. Specifically, the *furcata-vernicosa* clade included the pins and thrums of *A. furcata* (population 91007, New Idria Road, San Benito County) and the derived homostylous *A. vernicosa* (population 91006, Catway, Santa Barbara Co.). Clade 2 included the pins and thrums of *A. douglasiana* (population 91002, Jolon Road, Monterey Co.) and the derived homostylous tetraploid *A. tessellata gloriosa* (population 95005, Jolon Road, Monterey Co.; this taxon was termed "*A. gloriosa*" in several earlier papers). Clade 3, which is wholly *A. spectabilis*, included the pins and thrums of a distylous population (91004, Nipomo, San Luis Obispo Co.); a derived large-flowered, homostylous population (88017, La Purisima, Santa Barbara Co.); and a derived homostylous population (91011, Zmudowski State Beach, Monterey Co.). (In some areas outside of the collection area, the large-flowered homostylous *A. spectabilis* population contains pinlike and thrumlike individuals, possibly actual pins and thrums; this population as a whole is thus best referred to as "mixed" rather than "homostylous." See Li and Johnston (2001) for further details on the populations studied.) All specimens were collected from the field in California in 1995. Eight to 15 inflorescences (average 9.0), each from a different individual, were studied for each species-morph combination. We analyzed 26 traits, 21 of which were morphological aspects of developing and mature flowers, on an average of 12.8 buds per inflorescence, resulting in more than 29,000 data points.

Traits Measured

In order from proximal to distal, each coiled *Amsinckia* inflorescence consists of senescing flowers, fully opened flowers, newly opened flowers, flower buds and flower primordia. 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 a stereo microscope. This study therefore included all but the earliest flower development. Images of dissected flower parts were recorded with a video camera and saved to computer for later measurement with the program NIH Image (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).

The 21 morphological traits analyzed in this study are listed in Table 1 and shown in Fig. 2. Most traits were named using a four-letter abbreviation with the first letter indicating the whorl: K, calyx; C, corolla; S, stamen; or P, pistil. In this study we used the absolute value of functional anther-stigma distance (AVASD). Functional anther-stigma distance (ASD), as originally defined in our morphometric study of mature flowers (Li and Johnston 2001), had positive values when the stigma was above the anthers, had negative values when it was below and was zero if there was any overlap. Use of the absolute value here allowed us to investigate development of anther-stigma separation, and thus autogamous self-fertilization, without regard to which organ was more distal. In addition to the 21 morphological traits, we measured or calculated the following five quantities: flower developmental duration from primordium initiation to opening (DEVDUR), number of buds per inflorescence from primordium to first open flower (TOTBUDS), time separating adjacent buds or flowers of equal development (DAYPFLR), and absolute (AAFT) and relative (RAFT) ages of a flower bud at the termination of anther meiosis (Table 1). We calculated developmental rate for all 21 morphological traits as trait size at flower opening divided by developmental duration. Measurements and calculations are described more fully below.

Table 1 The 26 traits analyzed in this study and their abbreviations

Abbreviation	Character (units)
Timing traits	
DAYPFLR	Days per flower (time separating adjacent positions [=plastochron, days])
TOTBUDS	Total number of bud positions from floral primordium to first open flower
DEVDUR	Developmental duration from floral primordium to first open flower (days)
RAFT	Relative age of a floral bud at meiosis termination in the anther (no units)
AAFT	Absolute age of a floral bud at meiosis termination in the anther (days)
Morphological traits	
Calyx whorl	
KSL	Sepal length
Corolla whorl	
BUDL	Flower or bud length in natural position
BUDW	Flower or bud width in natural position
CFPL	Fused petal length
CLBW	Corolla lobe width
CPTL	Petal length
CTBL	Corolla tube length
Stamen whorl	
SANL	Anther length
SANW	Anther width
SFIL	Free stamen filament length (i.e., portion not fused to petal)
SINH	Stamen insertion height (on corolla)
SSIL	Stamen height (to top of anther)
Pistil whorl	
PISL	Pistil length (to top of stigma)
POVH	Ovary height
PSSL	Style and stigma length
PSTYL	Style length
PSTH	Stigma thickness
PSTL	Stigma length
PSTW	Stigma width
PSTA	Stigma surface area [five-sided box = 2(PSTL × PSTH) + 2(PSTW × PSTH) + (PSTL × PSTW)]
Pistil and stamen whorls	
AVASD	Absolute value of functional distance between anther and stigma

The 21 morphological traits are illustrated in Fig. 2. All morphological measurements are in mm, except stigma area (PSTA), which is in mm^2

Determining Developmental Age

For every bud and flower on an inflorescence we calculated developmental age as follows. In *Amsinckia*, flowers and buds mature from base to tip along the determinate inflorescence, in a chronological sequence. To obtain the rate of flower opening in the field, the most newly opened flower on an inflorescence was marked with paint; 5–7 days later the inflorescence was collected and placed in formalin—acetic acid—ethanol (FAA). The number of flowers that opened between the time of painting and the time of collection defined the flower-opening rate (positions/day), and the inverse (days/position) gave the plastochron (Lamoreaux et al. 1978), which is the developmental time separating adjacent flowers, and is abbreviated here as DAYPFLR. Inflorescences were then dissected to determine the total number of bud positions between the youngest primordium (confirmed by scanning electron microscopy) and the most newly opened flower. Each bud on an inflorescence was numbered starting from the youngest flower primordium (zero) and ending with the newly opened flower (Li and Johnston 1999).

The flower 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 the inflorescence by the time separating positions (days/position) on that inflorescence. The absolute developmental age of a given bud or flower was calculated by multiplying the position of that bud or flower by the time interval of flower opening (days/position) on that inflorescence.

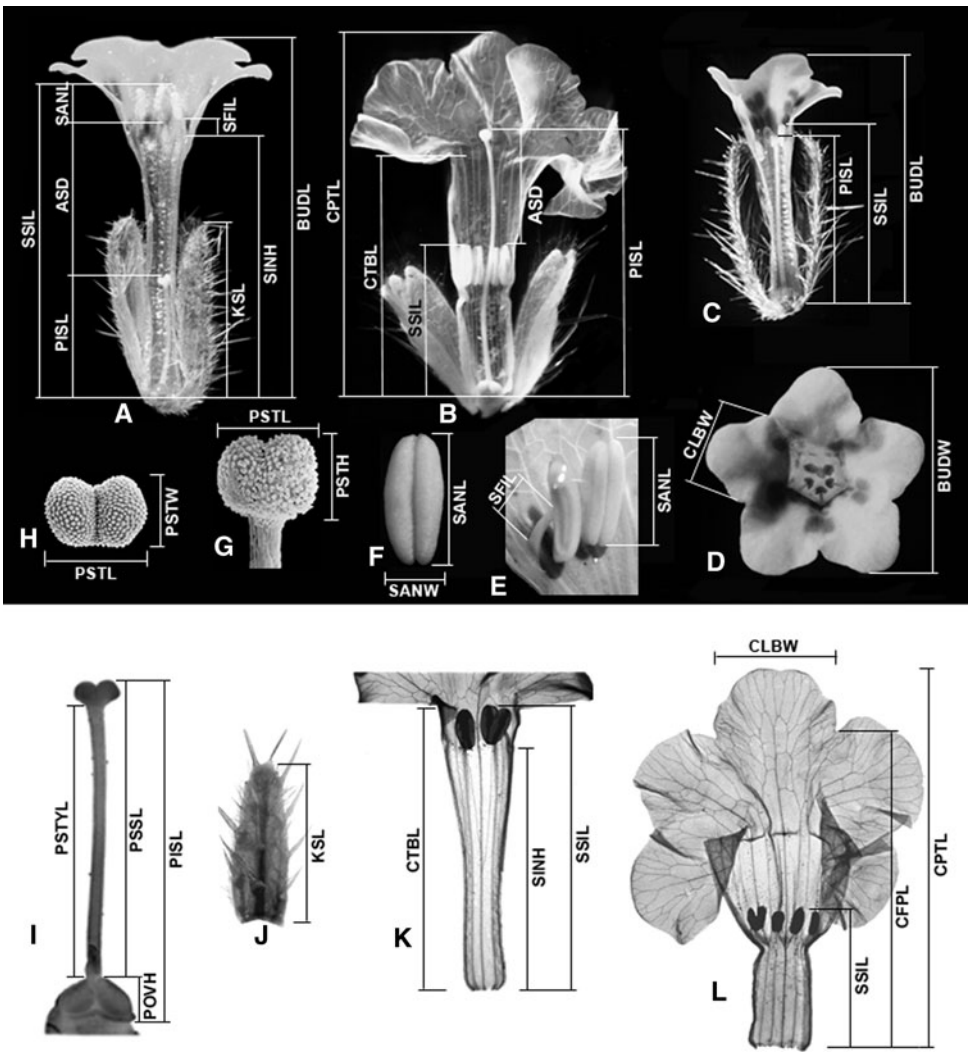

Fig. 2 Dissected *Amsinckia* flowers, showing the morphometric characters and the measurement positions of various floral traits. All abbreviations in the figure are explained in Table 1. Magnifications vary among photographs. **a** Longitudinal section of a freshly cut thrum flower with natural shape. **b** Longitudinal section of a flattened fixed pin flower. **c** Longitudinal section of a freshly cut homostylous flower with natural shape. **d** *Top view* of a freshly cut homostylous flower. **e** Stamen attached to corolla tube. **f** Anther. **g** Stigma [scanning electron micrograph (SEM)]. **h** *Top view* of a stigma (SEM). **i** Pistil. **j** Sepal. **k** Longitudinal section of a flattened fixed corolla tube. **l** Dissected flattened pin corolla. Modified from Li and Johnston (2001)

The relative developmental age of a flower bud or flower was calculated as the ratio of the position of that bud or flower to the total number of buds on the inflorescence. The relative developmental age of a bud thus expresses the time elapsed from primordium to that 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 growth rate of a flower trait was calculated by dividing the size of the trait at flower opening by the flower developmental duration.

In addition to primordium initiation and flower opening, we used meiosis termination in the anther as a developmental reference point (Li and Johnston 1999). The formation of microspore tetrads signals the end of meiosis in the pollen mother cells (microsporocytes). To identify which bud contained microspore tetrads, anthers of individual buds were stained with safranin-O or aceto-carmine after their images were recorded for measurement purposes, then squashed and viewed under a compound microscope. Microspore tetrads usually occurred in only one bud on an inflorescence. When more than one bud on an inflorescence contained tetrads, the younger one, adjacent to the bud having microsporocyte meiosis, was chosen for calculating the tetrad formation time.

Comparing Size-at-Age Among Inflorescences

The total number of buds from the primordium to the newly opened flower varied among inflorescences. Thus, the set of relative ages (relative positions) was generally not the same in different inflorescences. For example, an inflorescence with 25 positions separating primordium from first open flower would have buds at relative ages 0 (primordium), 0.04, 0.08, etc., while one with 20 positions would have relative ages 0, 0.05, 0.10, etc. Growth trajectories must be compared using size at identical relative ages. Therefore, the original measurements were used to

interpolate trait sizes at increments of 0.05 between the smallest bud measured (usually position 0.3) and at least the youngest open flower (position one), and beyond for some traits. The interpolation of trait size was performed using a computer program.

Statistical Analysis

The primary aim of this study was to determine how morphs and clades (lineages) differed, first, in mean values of the 26 developmental traits and, second, in developmental trajectories for 19 flower-morphological traits. We therefore compared trait means among morphs within clades, and among clades, using a priori contrasts (*t* tests) followed by adjustments to maintain the overall familywise type-I error rate below 0.05. This was accomplished using the BOOTSTRAP option (20,000 iterations) in the MULTTEST procedure of SAS software (version 6.12, Cary, North Carolina, 1999) on the mainframe computer of Dalhousie University. Bootstrapping incorporates correlations among variables, and is therefore more appropriate than the sequential Bonferroni correction for multiple comparisons (Westfall and Young 1993). The following families of comparisons were conducted (note that "H" indicates small homostyly and thus excludes the LH form of *A. spectabilis*):

(i) morphs over all clades: P versus T, P versus H, T versus H and distyly (P and T combined) versus H (104 tests);
(ii) morphs within clade 1 (*furcata-vernicosa*): four comparisons as above (104 tests);
(iii) morphs within clade 2 (*douglasiana-tessellata*): four comparisons as above (104 tests);
(iv) morphs within clade 3 (*spectabilis*): four comparisons as above (104 tests);
(v) morphs within *spectabilis* including the LH morph: P versus T, P versus LH, P versus H, T versus LH, T versus H, LH versus H, and distyly (P and T combined) versus H (LH and H combined, 182 tests); and
(vi) clades including all morphs: clade 1 versus 2, clade 1 versus 3, clade 2 versus 3 and clade 1 and 2 combined versus 3 (104 tests).

Developmental trajectories of 19 traits were compared among the relevant groups (morphs and/or clades) using repeated measures ANOVA (RM) implemented in the GLM procedure of SAS software. We omitted two of the 21 traits in these analyses: corolla tube length (CTBL), because there is no evident flower tube in early development, and the absolute value of functional anther-stigma distance (AVASD). The SAS model for each trait was:

$$\text{size-at-relative-age-1.0}\ \text{size-at-relative-age-0.9}\ldots \text{size-at-relative-age-0.3} = \text{morph} + \text{clade} + \text{morph} \times \text{clade},$$

which also analyzed all other interaction terms (see below). For these RM analyses, the subjects were individual inflorescences, the within-subjects factor was the relative age of the flower (bud) during development, and two between-subjects (grouping) factors were flower morph and clade. A multivariate approach was adopted for within-subjects tests, and among the test output only Wilks' Lamba and *P* value are presented here. Wilks' Lamba is the likelihood ratio statistic for testing the hypothesis of equal group means. The value of Wilks' Lamba is close to zero if any two groups are well separated. In order to pinpoint the relative age, if any, at which growth trajectories diverged, RM was performed on reverse Helmert contrast variables using the SUMMARY option in SAS software (Carey 1998). For a particular relative age, a reverse Helmert contrast variable is the difference between size at that age and the mean size at the earlier ages.

In the RM analysis the within-subjects main effect was relative age, and the between-subjects main effects were morph and clade. Statistical significance tests of these three main effects were of limited interest for the following reasons. First, relative age is necessarily significant in studies such as this that span nearly all of development. Second, the test for whether morphs differ in mean trait size takes averages over clades, and the test for whether clades differ takes averages over morphs. Similarly of limited interest was the "morph × clade" term, the between-subjects interaction effect. This term asked whether the influence of morph depended on clade. This test includes sizes at all ages, thus ignoring age information, and so would be better asked by using only the mature-trait sizes (as done in Li and Johnston 2001). The questions of greatest interest were addressed by the three within-subjects-by-between-subjects interaction effects. The term "relative age × morph" asked whether the influence of relative age on trait size depended on morph. That is, "Do morphs differ in growth trajectories when clade identity is ignored?" This term should be statistically significant for morphs that differ in final size. The term "relative age × clade" asked whether the influence of relative age on trait size depended on clade. That is, "Do clades differ in growth trajectories when morph identity is ignored?" Finally, of greatest interest, the three-way interaction term "relative age × morph × clade" asked, "Does the influence of morph on trait size depend on clade and relative age?" That is, "Do particular combinations of morph and clade differ in growth trajectory?" (ACITS 1997).

Results

The comparisons of means reported in the first two sections below were assessed following the bootstrap correction for tablewide pairwise comparisons (raw and bootstrapped *P* values provided in Appendix Tables 6, 7, 8).

Timing Traits: Developmental Duration, Bud Number, Plastochron, AAFT and RAFT

Flower developmental duration (DEVDUR) did not differ between pins and thrums within any clade (Table 2 and Appendix). Developmental duration also did not differ among pins, thrums and homostyles in the *furcata-vernicosa* and *douglasiana-tessellata* clades. In *A. spectabilis*, however, homostyles had significantly longer developmental duration than pins, thrums and large homostyles. Homostyles had significantly fewer buds (positions) per inflorescence (TOTBUDS) than pins and thrums in both the *furcata-vernicosa* and *douglasiana-tessellata* clades, but morphs did not differ in the *spectabilis* clade (Table 2). Plastochron (DAYPFLR), the time separating successive flowers of identical stage, was highest in homostyles in all three clades, but homostyles differed statistically from pins and thrums only in the *furcata-vernicosa* clade and from large homostyles in the *spectabilis* clade (Table 2).

Similar to floral developmental duration, the number of days from primordium initiation to meiosis termination (AAFT) did not differ significantly between distylous and homostylous flowers in either the *furcata-vernicosa* or *douglasiana-tessellata* clade (Table 2). In the *spectabilis* clade, meiosis terminated significantly later in homostylous flowers. RAFT, the relative age when pollen-mother-cells finish meiosis, in contrast, did not differ among morphs in any clade (Table 2). That is, the proportion of development time before (and after) pollen-mother-cell meiosis was the same in all clades and morphs.

Developmental Rate of the 21 Morphological Traits

The developmental rate of sepal length (KSL) did not differ significantly among morphs within the *furcata-vernicosa* and *douglasiana-tessellata* clades. In *spectabilis*, KSL developed approximately 50% slower in the homostyles than in pins, thrums and large-flowered homostyles (Table 2 and Appendix).

All six corolla traits (BUDL, BUDW, CFPL, CLBW, CPTL and CTBL) grew approximately two to three times faster in pins and thrums than in homostyles in the *furcata-vernicosa* clade, and about twice as fast as in homostyles in *spectabilis* (Table 2). In the *douglasiana-tessellata* clade, in contrast, the developmental rate of only CLBW differed among morphs.

The developmental rate of anther length (SANL) was significantly lower in homostyles than in the other two morphs in the *furcata-vernicosa* clade and lower in homostyles than the other three morphs in *spectabilis* (Table 2). These two rates did not differ among morphs within the *douglasiana-tessellata* clade. Developmental rate of anther width (SANW) did not differ among morphs, with the exception of a lower rate in the homostyle of *spectabilis*. The developmental rates of the three stamen-height traits—filament length (SFIL), insertion height (SINH) and anther height (SSIL)—were significantly greater in thrums than in pins or homostyles in the *furcata-vernicosa* clade. In the *douglasiana-tessellata* clade, growth rates were greater in thrums than in the other two morphs for SFIL and greater in both thrums and homostyles than in pins for SINH and SSIL (Table 2). In *spectabilis*, SINH and SSIL had similar growth rates in thrums and large homostyles, these rates being greater than in pins and homostyles. Growth rate of filament length (SFIL) in *spectabilis*, on the other hand, was lowest in homostyles, intermediate and similar in pins and thrums and highest in large homostyles.

The developmental rate of stigma height (PISL) was significantly greater in pins than in the other morphs in all three clades, and thrums had a lower rate than homostyles in the *douglasiana-tessellata* clade. Ovary height (POVH) developmental rate did not differ among morphs in the *furcata-vernicosa* clade, was greater in homostyles than in thrums in the *douglasiana-tessellata* clade and was greater in large homostyles than in homostyles in *spectabilis*. Growth rate in ovary height was similar in pins and homostyles in all clades. The growth rates of combined style and stigma length (PSSL) and of stigma length itself (PSTYL) were greater in pins than in thrums in all clades. Thrums also had a significantly lower rate than homostyles in the *douglasiana-tessellata* clade, and large homostyles had greater rates than thrums and homostyles in *spectabilis*. In *douglasiana-tessellata*, developmental rate of stigmatic area (PSTA) was approximately 44% lower in homostyles than in pins and thrums, although none of the three linear dimensions of stigmatic area—height (PSTH), length (PSTL) and width (PSTW)—differed among morphs in growth rate. In the *furcata-vernicosa* clade, developmental rate of stigmatic area was 30% lower in homostyles than in pins and thrums, although this comparison was not statistically significant despite homostyles having the lowest growth rate in stigma width. In *spectabilis*, area growth rate was 73% lower in homostyles than in the other three morphs, which did not differ among themselves, and homostyles also exhibited lower growth rates for stigma height, length and width (Table 2).

Table 2 Timing of development: the five timing traits plus developmental rate for the 21 morphological traits (means ± standard errors)

Trait	Clade 1			Clade 2			Clade 3			
	furcata		*vernicosa*	*douglasiana*		*tess. glor.*	*spectablilis*			
	Pin	Thrum	Homostyle	Pin	Thrum	Homostyle	Pin	Thrum	Large homostyle	Homostyle
DAYPFLR	0.78 ± 0.02^{a}	0.75 ± 0.03^{a}	1.17 ± 0.06^{b}	0.66 ± 0.03^{a}	0.73 ± 0.04^{a}	0.79 ± 0.04^{a}	0.61 ± 0.03^{ab}	0.65 ± 0.02^{ab}	0.54 ± 0.02^{a}	0.78 ± 0.04^{b}
TOTBUDS	25.0 ± 0.80^{a}	26.63 ± 1.10^{a}	17.00 ± 0.91^{b}	31.63 ± 0.98^{a}	31.64 ± 1.14^{a}	25.00 ± 0.78^{b}	30.13 ± 2.17^{a}	26.88 ± 1.57^{a}	27.75 ± 2.14^{a}	29.88 ± 1.27^{a}
DEVDUR	19.35 ± 0.73^{a}	19.79 ± 0.73^{a}	19.79 ± 1.44^{a}	20.73 ± 0.53^{a}	22.97 ± 1.44^{a}	19.90 ± 1.41^{a}	18.09 ± 1.35^{a}	17.37 ± 0.93^{a}	14.69 ± 0.89^{a}	23.08 ± 0.94^{b}
AAFT	9.22 ± 0.34^{a}	8.85 ± 0.35^{a}	9.00 ± 0.56^{a}	8.78 ± 1.48^{a}	9.31 ± 1.08^{a}	9.02 ± 0.82^{a}	8.08 ± 0.57^{a}	7.71 ± 0.35^{a}	6.36 ± 0.35^{a}	10.47 ± 0.49^{b}
RAFT	0.47 ± 0.01^{a}	0.45 ± 0.01^{a}	0.46 ± 0.01^{a}	0.48 ± 0.03^{a}	0.45 ± 0.01^{a}	0.45 ± 0.01^{a}	0.45 ± 0.01^{a}	0.45 ± 0.01^{a}	0.43 ± 0.01^{a}	0.45 ± 0.00^{a}
Developmental rates of floral morphological traits										
Calyx										
KSL	0.40 ± 0.01^{a}	0.40 ± 0.01^{a}	0.37 ± 0.02^{a}	0.34 ± 0.01^{a}	0.34 ± 0.02^{a}	0.35 ± 0.02^{a}	0.28 ± 0.02^{a}	0.27 ± 0.01^{a}	0.28 ± 0.01^{a}	0.12 ± 0.01^{b}
Corolla										
BUDL	0.72 ± 0.02^{a}	0.82 ± 0.02^{a}	0.34 ± 0.02^{b}	0.60 ± 0.02^{a}	0.71 ± 0.05^{a}	0.56 ± 0.04^{a}	0.70 ± 0.05^{a}	0.79 ± 0.05^{a}	0.80 ± 0.04^{a}	0.36 ± 0.02^{b}
BUDW	0.53 ± 0.01^{a}	0.55 ± 0.02^{a}	0.16 ± 0.01^{b}	0.41 ± 0.03^{a}	0.45 ± 0.03^{a}	0.34 ± 0.02^{a}	0.55 ± 0.04^{a}	0.55 ± 0.04^{a}	0.57 ± 0.03^{a}	0.20 ± 0.01^{b}
CFPL	0.62 ± 0.02^{a}	0.71 ± 0.01^{a}	0.34 ± 0.01^{b}	0.53 ± 0.02^{a}	0.62 ± 0.03^{a}	0.49 ± 0.03^{a}	0.62 ± 0.04^{a}	0.69 ± 0.04^{a}	0.67 ± 0.03^{a}	0.30 ± 0.01^{b}
CLBW	0.21 ± 0.01^{a}	0.20 ± 0.01^{a}	0.06 ± 0.00^{b}	0.16 ± 0.01^{ab}	0.19 ± 0.01^{a}	0.14 ± 0.01^{b}	0.25 ± 0.02^{a}	0.23 ± 0.02^{a}	0.25 ± 0.01^{a}	0.08 ± 0.00^{b}
CPTL	0.72 ± 0.02^{a}	0.82 ± 0.02^{a}	0.37 ± 0.01^{b}	0.61 ± 0.03^{a}	0.70 ± 0.04^{a}	0.56 ± 0.04^{a}	0.71 ± 0.05^{a}	0.79 ± 0.05^{a}	0.80 ± 0.04^{a}	0.36 ± 0.02^{b}
CTBL	0.45 ± 0.01^{a}	0.52 ± 0.02^{a}	0.27 ± 0.01^{b}	0.39 ± 0.02^{a}	0.45 ± 0.03^{a}	0.40 ± 0.03^{a}	0.42 ± 0.03^{a}	0.52 ± 0.03^{a}	0.49 ± 0.03^{a}	0.25 ± 0.01^{b}
Stamen										
SANL	0.11 ± 0.00^{a}	0.11 ± 0.00^{a}	0.07 ± 0.00^{b}	0.08 ± 0.00^{a}	0.09 ± 0.01^{a}	0.08 ± 0.01^{a}	0.09 ± 0.01^{a}	0.09 ± 0.01^{a}	0.09 ± 0.01^{a}	0.05 ± 0.00^{b}
SANW	0.04 ± 0.00^{ab}	0.04 ± 0.00^{a}	0.03 ± 0.00^{a}	0.03 ± 0.00^{a}	0.04 ± 0.00^{a}	0.03 ± 0.00^{a}	0.04 ± 0.00^{a}	0.04 ± 0.00^{a}	0.04 ± 0.00^{a}	0.02 ± 0.00^{b}
SFIL	0.03 ± 0.00^{a}	0.05 ± 0.00^{b}	0.02 ± 0.00^{a}	0.02 ± 0.00^{a}	0.06 ± 0.01^{b}	0.03 ± 0.00^{a}	0.04 ± 0.00^{a}	0.05 ± 0.00^{a}	0.06 ± 0.00^{b}	0.02 ± 0.00^{c}
SINH	0.23 ± 0.01^{a}	0.53 ± 0.01^{b}	0.27 ± 0.01^{a}	0.16 ± 0.01^{a}	0.46 ± 0.03^{b}	0.35 ± 0.03^{b}	0.23 ± 0.01^{a}	0.47 ± 0.03^{b}	0.44 ± 0.02^{b}	0.20 ± 0.01^{c}
SSIL	0.31 ± 0.01^{a}	0.63 ± 0.01^{b}	0.33 ± 0.01^{a}	0.22 ± 0.01^{a}	0.54 ± 0.04^{b}	0.40 ± 0.03^{b}	0.30 ± 0.02^{a}	0.55 ± 0.03^{b}	0.54 ± 0.02^{b}	0.25 ± 0.01^{c}
Pistil										
PISL	0.61 ± 0.02^{a}	0.25 ± 0.01^{b}	0.31 ± 0.01^{b}	0.54 ± 0.02^{a}	0.20 ± 0.01^{b}	0.36 ± 0.02^{c}	0.52 ± 0.03^{a}	0.23 ± 0.01^{b}	0.28 ± 0.01^{b}	0.21 ± 0.01^{b}
POVH	0.04 ± 0.00^{a}	0.03 ± 0.00^{a}	0.04 ± 0.00^{a}	0.03 ± 0.00^{a}	0.03 ± 0.00^{a}	0.04 ± 0.00^{b}	0.03 ± 0.00^{ab}	0.03 ± 0.00^{ab}	0.04 ± 0.00^{a}	0.02 ± 0.00^{b}
PSSL	0.58 ± 0.02^{a}	0.22 ± 0.01^{b}	0.27 ± 0.01^{b}	0.50 ± 0.02^{a}	0.18 ± 0.01^{b}	0.32 ± 0.02^{c}	0.47 ± 0.03^{ab}	0.21 ± 0.01^{c}	0.58 ± 0.02^{b}	0.19 ± 0.01^{ac}
PSTYL	0.55 ± 0.02^{a}	0.19 ± 0.01^{b}	0.25 ± 0.01^{b}	0.49 ± 0.02^{a}	0.16 ± 0.01^{b}	0.31 ± 0.02^{c}	0.48 ± 0.03^{ab}	0.19 ± 0.01^{c}	0.56 ± 0.02^{b}	0.18 ± 0.01^{ac}
PSTH	0.02 ± 0.00^{a}	0.02 ± 0.00^{a}	0.02 ± 0.00^{a}	0.02 ± 0.00^{a}	0.02 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.02 ± 0.00^{a}	0.02 ± 0.00^{a}	0.01 ± 0.00^{b}
PSTL	0.03 ± 0.00^{a}	0.03 ± 0.00^{a}	0.02 ± 0.00^{a}	0.03 ± 0.00^{a}	0.03 ± 0.00^{a}	0.02 ± 0.00^{a}	0.03 ± 0.00^{a}	0.03 ± 0.00^{a}	0.03 ± 0.00^{a}	0.01 ± 0.00^{b}
PSTW	0.02 ± 0.00^{a}	0.02 ± 0.00^{a}	0.02 ± 0.00^{b}	0.02 ± 0.00^{a}	0.02 ± 0.00^{a}	0.02 ± 0.00^{a}	0.02 ± 0.00^{a}	0.02 ± 0.00^{a}	0.03 ± 0.00^{a}	0.01 ± 0.00^{b}
PSTA	0.06 ± 0.00^{a}	0.06 ± 0.00^{a}	0.04 ± 0.00^{a}	0.04 ± 0.00^{a}	0.04 ± 0.00^{a}	0.02 ± 0.00^{b}	0.04 ± 0.00^{a}	0.04 ± 0.00^{a}	0.05 ± 0.00^{a}	0.01 ± 0.00^{b}
AVASD	0.30 ± 0.01^{a}	0.28 ± 0.01^{a}	0.01 ± 0.00^{b}	0.32 ± 0.01^{a}	0.26 ± 0.02^{a}	0.00 ± 0.00^{b}	0.22 ± 0.02^{ab}	0.23 ± 0.02^{a}	0.07 ± 0.02^{c}	0.00 ± 0.00^{bc}

Means with different superscripts within a clade differ statistically after bootstrap correction for multiple pairwise tests (original and adjusted *P* values in Appendix)

Developmental Trajectories

General Results

Growth trajectories for nine of the 19 traits are shown in Fig. 3. The complete set is provided as Electronic Supplementary Material arranged both by clade (S1) and by morph (S2) to facilitate different kinds of comparison. References to Fig. 3 in the following text should be construed as referring to the complete set of 19 trajectories.

As explained in "Materials and Methods" section, some results of the repeated measures analysis are almost necessarily true and are of limited interest here. First, mean trait size during development differed among morphs, among clades and among morph-clade combinations (Table 3). Second, traits changed size with relative age (Table 4, relative age column).

Of primary interest are the ways growth trajectories differ among morphs and clades (Fig. 3; Table 4). These questions are addressed by the MANOVA interaction terms of the repeated measures analysis (Table 4), and there are three general results. First, clades differed in growth trajectory for all traits except free stamen filament length (Table 4, SFIL, relative age × clade column; Fig. 3). Second, morphs differed in growth trajectory for all traits (Table 4, relative age × morph column; Fig. 3). Finally, the influence on trait size at a particular age depended on both clade and morph in different ways (Table 4, relative age × clade × morph column; Fig. 3). Specific trait comparisons are presented in more detail next. We first compare developmental trajectories of distyles and homostyles, and then pins and thrums.

Distyly vs. Homostyly

The developmental trajectory of sepal length (KSL) was similar in homostyly and distyly in both the *furcata-vernicosa* and *douglasiana-tessellata* clades (Fig. 3; Table 2). In *spectabilis*, sepals grew more slowly in homostyly than in distyly (Table 2), and the developmental trajectories of the two style morphs diverged at an early stage of flower ontogeny, probably before PMC meiosis at relative age 0.45 (Fig. 3; Table 5).

The developmental trajectories of flower-size traits (BUDL, BUDW, CFPL, CLBW and CPTL) differed between distyly and homostyly in all three clades (Fig. 3). 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

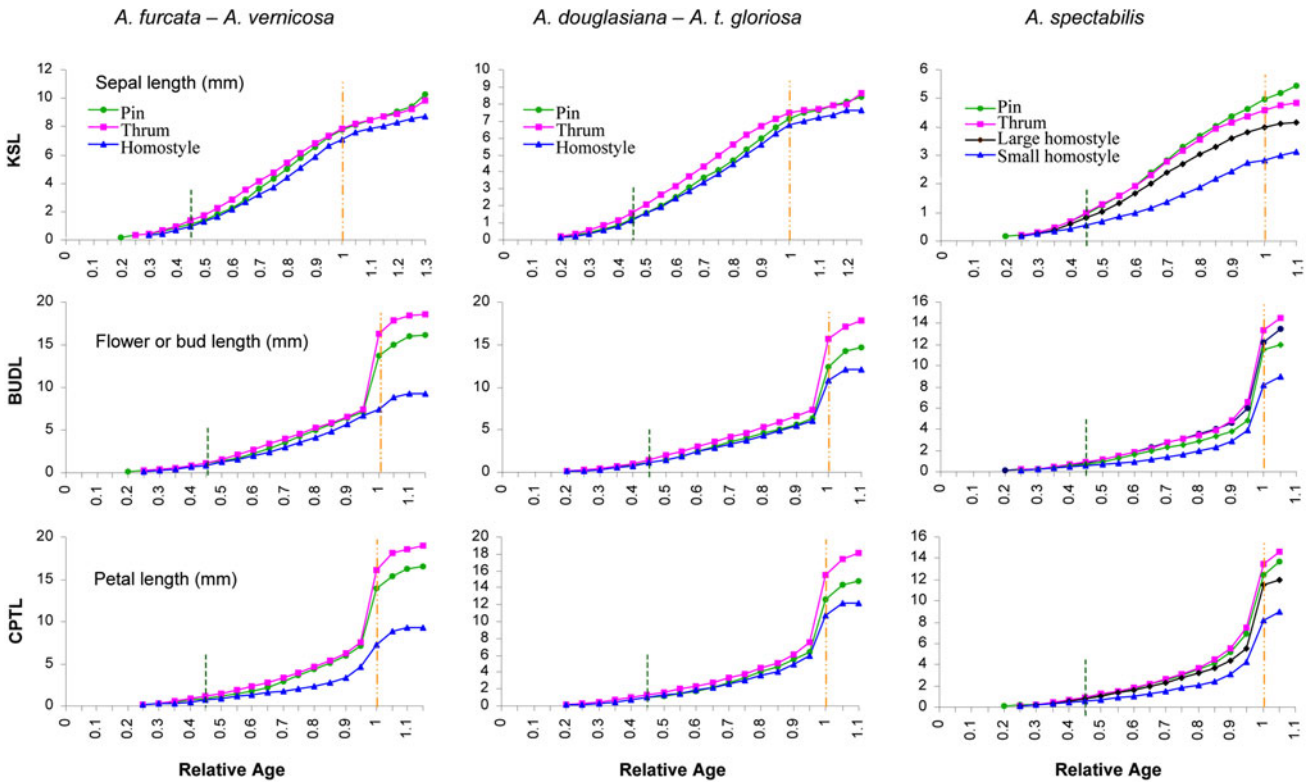

Fig. 3 Developmental trajectories for nine of the 19 morphological traits. The two vertical *dashed lines* indicate relative age of pollen mother cell meiosis (0.45) and flower opening (1.0). See S1 and S2 (Electronic Supplementary Material) for trajectories of all traits

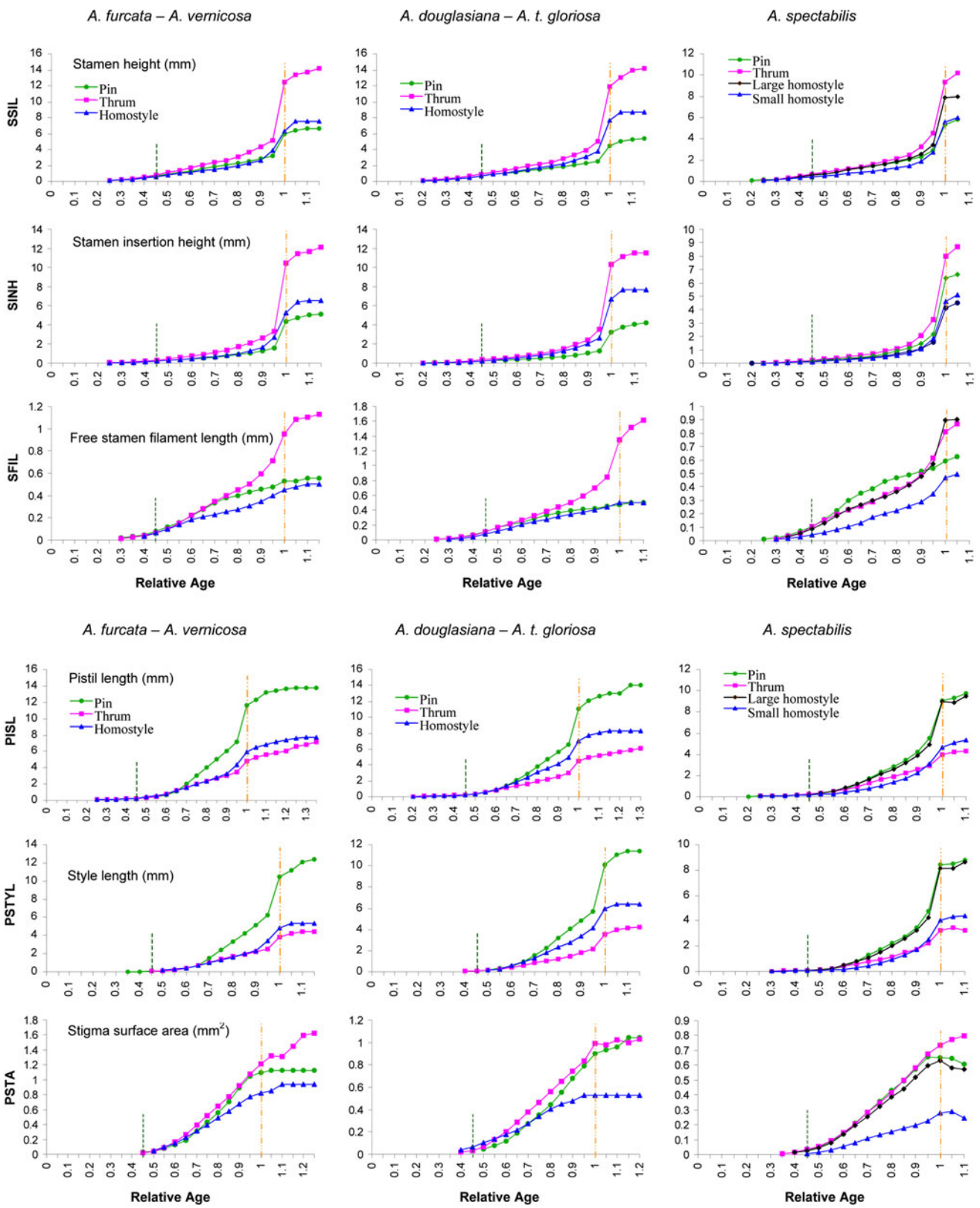

Fig. 3 continued

Table 3 Mean floral trait size during development: statistical significance levels for effects of clade, floral morph, and interaction between clade and floral morph (results of between-subjects ANOVA in the repeated measures analysis)

Trait	*P* value		
	Clade	Morph	Clade × 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

The analysis excludes the large homostylous (LH) morph of *A. spectabilis*. Degrees of freedom for clade, morph, and clade × morph were 2, 2, and 4, respectively

between the two style morphs mostly occurred before or around the time of PMC meiosis (Fig. 3; Table 5). In both pin and thrum flowers, the trajectories of most flower-size-related traits were not much different among the three clades until the later developmental stages (Fig. 3). In homostyles, in contrast, the trajectories differed among the clades probably around relative age of 0.4–0.6 (Fig. 3; Table 5).

The growth trajectories of stamen-height-related traits (SFIL, SINH and SSIL) between homostylous and distylous flowers differed among clades. The specific time when their trajectories diverged, however, varied among both traits and clades (Fig. 3; Table 5). For example, the divergence of filament length (SFIL) growth between homostyly and distyly occurred after PMC meiosis in the *furcata-vernicosa* clade, at meiosis time in the *douglasiana-tessellata* clade, and far before meiosis in *spectabilis* (Fig. 3). For stamen insertion height (SINH) and stamen height (SSIL) in the *furcata-vernicosa* clade, the separation of growth curves between homostyles and pins was much later than that between homostyles and thrums (Fig. 3). In *douglasiana-tessellata*, 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. 3). On the other hand, the developmental trajectories of stamen-height-related traits, especially SFIL, also varied among clades within each morph (Fig. 3; Tables 4, 5).

The developmental divergence for pistil-height-associated traits (PISL, POVH, PSSL and PSTYL) between homostyly and distyly mostly occurred some time after PMC meiosis in all three clades (Fig. 3; Table 5). Variations in divergence time, however, did exist among clades. In both the *furcata-vernicosa* and *douglasiana-tessellata* clades, growth trajectories of pistil-height-associated traits (except POVH) in homostyles diverged from those in thrums much later than from those in pins (Fig. 3). In addition, the growth trajectories of pistil height also varied among the clades within each flower morph, particularly in homostylous flowers (Fig. 3; Tables 3, 4). The variations among clades were mostly caused by the difference in trait 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 flower trait's divergence in this study. In *spectabilis*, the divergence initiated far before the PMC meiosis time (Fig. 3). In *furcata-vernicosa* and *douglasiana-tessellata*, the trajectory diverged between homostyles and thrums probably prior to relative age 0.2–0.3; and the separation between homostyles and pins occurred after PMC meiosis (Fig. 3). In the *douglasiana-tessellata* clade, the ontogenetic trajectories of anther width (SANW) in homostyles and pins were the same. Anthers of same type of flower morph from different clades had very different developmental trajectories (Fig. 3). This was especially obvious in thrum and homostylous flowers in which the trajectories differed among clades since the early development.

The developmental trajectories of stigma size (PSTH, PSTL, PSTW and PSTA) varied both among flower morphs and among clades (Fig. 3; Tables 3, 4, 5). In all three clades, especially in *furcata-vernicosa* and *spectabilis*, the growth curves of stigma-size-related traits were much lower in homostyly than in distyly (Fig. 3). The separation of the curves between the two styles occurred at or after PMC meiosis in *furcata-vernicosa* and *douglasiana-tessellata*, but before meiosis in *spectabilis*. The developmental trajectories of stigma length (PSTL) and width (PSTW) in pin and thrum flowers were similar among the three clades, but in homostylous flowers they were much lower in *spectabilis* than in the other two clades. The

Table 4 Developmental trajectories: MANOVA results of repeated measures analysis (Wilks' lambda and statistical significance levels)

Trait	Wilks' Lambda (*P* value)			
	Relative age	Relative age × clade	Relative age × morph	Relative age × clade × 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)

The three interaction terms test for differences in developmental trajectory among clades, morphs and clade-morph combinations. The analysis excludes the large homostylous (LH) morph of *A. spectabilis*

developmental trajectories of stigma thickness (PSTH) and area (PSTA) were similar among the three clades until sometime after PMC meiosis, at least in pin and thrum flowers (Fig. 3).

In the *A. spectabilis* clade, 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 (Fig. 3). 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 homostyles. On the other hand, pistil-height-associated traits (PISL, PSSL and PSTYL excluding POVH) of the large homostyle developed in a way similar to those of pin. Large homostyly differed from homostyly, in terms of developmental trajectories, in all flower traits except SINH (Fig. 3). The divergence of developmental trajectories between large homostyly and distyly occurred mostly after PMC meiosis except in 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 PMC meiosis and was much earlier than between large homostyly and distyly (Fig. 3).

Pin vs. Thrum

Flower developmental trajectories between the two distylous flower morphs, pin and thrum, varied depending on the clades 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 Clades 1 and 2 (Fig. 3). In *spectabilis*, 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 immediately before flower opening (Fig. 3). This was particularly evident in the development of BUDL, BUDW, CFPL and CPTL in *furcata-vernicosa* and *douglasiana-tessellata*.

Stamen- and pistil-height-related traits were the major traits discriminating the two distylous morphs. The developmental

Table 5 Relative age at which developmental trajectories diverge: effects of clade, floral morph, and interaction between clade and morph on mean developmental trajectory of floral traits (results of repeated measures ANOVA)

Trait	Developmental trajectories differ prior to relative age (P value)		
	Clade	Morph	Clade × 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)

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. Relative age 0 is primordium initiation and age 1.0 is flower opening

trajectories of these traits between pins and thrums usually diverged sometime after the PMC meiosis in all three clades (Fig. 3). 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 differed among the three clades. The developmental trajectories of anther length (SANL) and width (SANW) were well separated between pin and thrum prior to relative age 0.2–0.3 in both *furcata-vernicosa* and *douglasiana-tessellata*, leading to the final anther size differing significantly between the two morphs. In *spectabilis*, 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. 3).

The trajectories of stigma development varied among the three clades. In *furcata-vernicosa* and *douglasiana-tessellata*, the developmental divergence of all four traits (PSTH, PSTL, PSTW and PSTA) between pin and thrum occurred around relative age 0.4–0.5. The gaps between the two trajectories in the *furcata-vernicosa* clade, however, were very small in PSTH, PSTW and PSTA, leading to similar pin and thrum size at flower opening. The separate trajectories in the *douglasiana-tessellata* clade 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 *spectabilis* shared the same ontogenetic trajectory until they reached relative age of 0.95, just before flower opening, and then the traits in the pin ceased growth (Fig. 3). 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 *spectabilis*.

Finally, it should be noted that the developmental trajectories were analyzed and displayed in Fig. 3 on a relative scale, with 0 representing primoridum initiation and 1 representing flower opening. Because most developmental durations did not differ within a clade (Table 2), these developmental trajectories would appear essentially the same if expressed in absolute time.

Discussion

Von Baer's Law

Von Baer's Law proposes that closely related taxa differ by changes later in development than do more distantly related taxa. This pattern should emerge if later changes are less disruptive to proper development than earlier changes. Von Baer's "Law" has received mixed support (Poe 2006 and references therein). Furthermore, the applicability of the law to flower development may be limited because most development occurs by expansion of cells that completed replication early. Thus, early development (cell division and early replication) will probably not greatly affect later development (cell expansion), a motivating assumption of von Baer's Law. The law can be tested in *Amsinckia* by comparing differences within clades to differences among clades. The most straightforward approach is to compare developmental trajectories, separately for each of the three morphs, within the clade comprising *furcata-vernicosa* and *douglasiana-tessellata*, and between this clade and *A. spectabilis* (see Fig. 1). To compare the trajectories among clades, it is helpful to view all clades on a single graph, with a separate graph for each morph. We therefore present a modified version of Fig. 3, with trajectories grouped by clade, as Fig. 3 S2 (see electronic supplemental material).

Visual inspection of growth trajectories in Fig. 3 shows strong support for von Baer's Law in homostylous flowers, some support in thrums, and little support in pins. This finding is surprising for two reasons: first, there is strong support at least among homostyles; second, the support differs greatly among morphs. Specifically, among homostylous flowers, developmental trajectories diverge earlier between *spectabilis* and the *furcata-vernicosa/douglasiana-tessellata* clade than within the latter for all traits except the three corolla-size traits (CFPL, CLBW, CPTL) and style length (PSTYL; see Fig. 3 S2 electronic supplementary material, trajectories grouped by morph). Among thrums, the pattern occurs in all traits except stamen filament length (SFIL) and six pistil traits (PISL, PSSL, PSTYL, PSTH, PSTL, PSTW). Among pins, the pattern occurs only in CLBW, SFIL (questionably), PISL, POVH, PSSL and PSTYL. Thus, agreement with the von Baer prediction occurs for approximately 15 of 19 traits in homostyles (79%), 12 of 19 traits in thrums (63%) and 6 of 19 traits in pins (32%).

Developmental Evolution of Homostyly

Developmental Time and Rate Effects

Self-pollinated homostylous flowers in *Amsinckia* are significantly smaller than their ancestral, predominately outcross-pollinated distylous flowers (Li and Johnston 2001). Statistically, almost every studied flower trait was significantly smaller in homostyly than in distyly in all three clades 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) (Li and Johnston 2001). To understand how small and highly selfing homostylous flowers have evolved from larger distylous ones, from the viewpoint of flower development, flower ontogenies were compared between flower morphs and among evolutionary clades in *Amsinckia*.

Flower developmental duration from the initiation of a flower primordium to anthesis, i.e., flower opening, did not differ between homostylous and distylous flowers in the *furcata-vernicosa* and *douglasiana-tessellata* clades (Table 2). The developmental duration prior to anthesis is therefore not the major cause leading to the smaller size in homostyly in these two clades. Furthermore, in contrast to the expectation that small selfing flowers have shorter developmental duration than large outcrossing flowers (Stebbins 1974), as reported in *Lycopersicon pimpinellifolium* (Georgiady and Lord 2002) (see also Runions and Geber 2000), the homostylous flowers in *A. spectabilis* have significantly longer developmental duration, compared to the distylous and large homostylous ones. This means that the homostylous flowers in *spectabilis* delayed their offset time (flower opening), because the onset time (the initiation of flower primordium) is the same by definition for all flower morphs. In general, longer developmental duration will lead to a larger flower or a larger flower organ. In *A. spectabilis*, however, the flower size and the developmental duration (up to anthesis) are inversely related. Homostyly with longer developmental duration does not produce flowers of larger size, because of a decreased developmental rate (approximately 50–60% slower than distyly). Thus, the increased duration (hypermorphosis) is not sufficient to counterbalance the decreased growth rate (neoteny). Small flower size in homostyles is caused by neoteny in all three clades, and the decreased rate is most pronounced in *A. spectabilis*.

The developmental duration discussed above was only up to anthesis. We used anthesis as the end of the developmental duration because it is ecologically important and readily identifiable. However, one must bear in mind that growth of some organs, including the corolla, continues after anthesis (see Fig. 3). Furthermore, modifications of post-anthesis development are often important in species-level differentiation (Hufford 1988). If we include post-anthesis development, then approximately 14 of 21 traits in the *furcata-vernicosa* and *douglasiana-tessellata* clades and seven of 21 traits in *spectabilis* had relatively earlier

developmental offset in homostylous flowers than in distylous ones, while the remaining traits had similar offset time in the two style morphs (Figs. 3, 4).

Although the major cause of the homostylous flower morph is decreased growth rate (neoteny), particularly up to the point of anthesis, the earlier offset in homostyles for most flower traits indicates that progenesis also has a role (Figs. 4, 5). Progenesis is hypothesized to be one of the major developmental processes that lead to the modification and evolution of flowers or flower organs in angiosperms (Takhtajan 1976, 1991; Li and Johnston 2000; Runions and Geber 2000). The role of neoteny in flower development and evolution has 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

Trait and Clade		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	C1		•	•								•	•				
	C2			•		•							•		•		
	C3		•									•					
BUDL	C1		•	•								•	•				
	C2		•	•								•	•				
	C3		•									•					
BUDW	C1		•	•								•	•				
	C2		•	•								•	•				
	C3		•									•					
CFPL	C1		•	•								•	•				
	C2		•	•								•	•				
	C3		•									•					
CLBW	C1		•									•					
	C2		•	•								•	•				
	C3		•	•								•	•				
CPTL	C1		•	•								•	•				
	C2		•	•								•	•				
	C3		•									•					
CTBL	C1		•									•					
	C2					•											
	C3		•									•					
SANL	C1		•									•					
	C2		•									•					
	C3		•									•					
SANW	C1		•									•					
	C2					•											
	C3		•									•					
SFIL	C1		•									•	•				
	C2					•							•				
	C3		•	•								•	•				
SINH	C1			•		•						•	•				
	C2			•		•							•				
	C3		•									•	•				
SSIL	C1			•		•						•	•				
	C2			•		•							•				
	C3		•									•	•				
PISL	C1		•												•		
	C2		•	•									•		•		
	C3		•									•					
POVH	C1					•									•		
	C2					•									•		
	C3		•									•					
PSSL	C1		•	•									•		•		
	C2		•										•		•		
	C3		•									•				•	
PSTYL	C1		•	•									•		•		
	C2		•	•									•		•		
	C3		•									•				•	
PSTH	C1		•									•	•				
	C2		•									•	•				
	C3		•									•	•				
PSTL	C1		•	•								•	•				
	C2		•	•								•	•				
	C3		•				•					•	•				
PSTW	C1		•	•								•	•				
	C2		•									•				•	
	C3		•				•					•	•				
PSTA	C1		•				•					•	•				
	C2		•	•								•	•				
	C3		•				•					•	•				

Fig. 4 A summary of heterochronic changes in homostyly compared with ancestral distyly in 20 floral traits in three evolutionary clades 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. No entry in both the progenesis and hypermorphosis columns indicates no difference in offset time. The large homostyle of *A. spectabilis* is excluded. *Figure Abbreviations*: All abbreviations of traits are explained in Table 1. C1, Clade 1; C2, Clade 2; C3, Clade 3

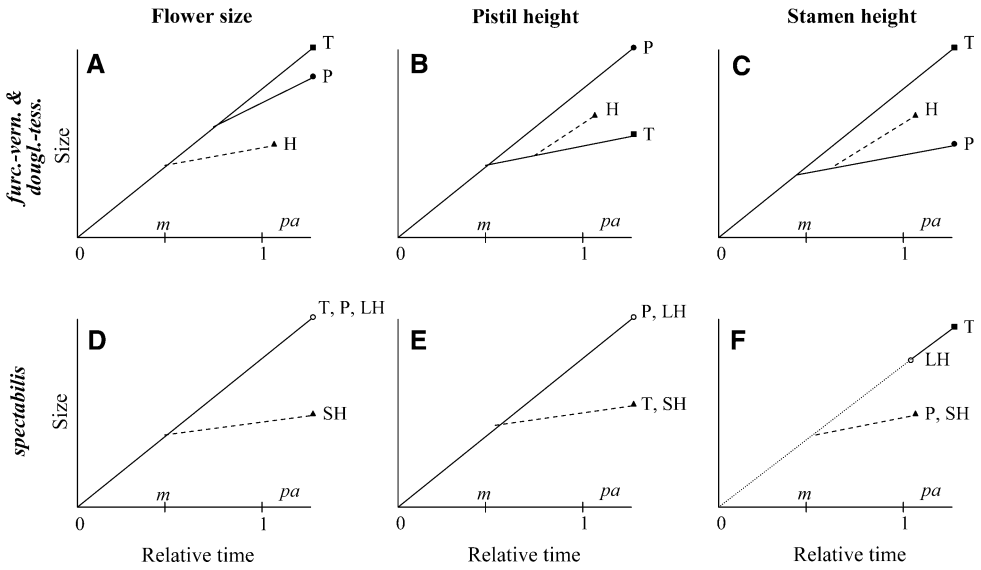

Fig. 5 Models for the effect of heterochrony on morphological evolution of homostyly (descendant) from distyly (ancestral) in clades *Amsinckia furcata—A. vernicosa* and *A. douglasiana—A. t. gloriosa* (**a**–**c**) and in the *A. spectabilis* clade (**d**–**f**). **a** Homostyle morph diverges from distyly earlier than the divergence between the two distylous floral morphs, pin (P) and thrum (T). Homostyle also has 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 diverge 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 the homostyle results in its stamen height being higher than in pin but shorter than in thrum. **d** A slower developmental rate in the 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 the homostyle is similar to that of thrum, leading to the pistil-height in homostyle being lower than that in pin and the large homostyle but similar to that in thrum. In the pistil-height growth, 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 the homostyle is similar to that of pin but differs from those of thrum and the large homostyle. Both homostyle and pin have a similar growth trajectory and cease their stamen-height growth earlier, while the large homostyle shares another growth trajectory with thrum but has an early offset. Paedomorphosis through neoteny and progenesis in the homostyle results in homostylous stamen height being lower than in thrum and large homostylous flowers. Mosaic development with multi-heterochronic processes (progenesis and acceleration) has also led anther height in the 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 the time of microsporocyte meiosis (*m*), and ceases during post-anthesis period (*pa*). The relative time of 0 is the onset time and 1 is the flowering (anthesis) time. Clade 1: *A. furcata—A. vernicosa*; Clade 2: *A. douglasiana—A. t. gloriosa*; Clade 3: *A. spectabilis*

outcross-pollinated ancestor (Wyatt 1984a, b; Hill et al. 1992). It is believed that the development and evolution of small, self-fertilizing flowers is often 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 are also 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 floccose* (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 1983).

Ontogenetic Trajectory Effects

For most flower traits, the divergence of developmental trajectories between distylous and homostylous flowers has begun by the time PMC meiosis finishes (at relative age [RAFT] 0.45) due to a steep increase of developmental rate in distylous flowers in most cases. Nevertheless, the major separation of the developmental curves usually occurred just before flower opening because of a much more dramatic developmental rate increase in distylous flowers than in homostylous ones (Fig. 3). This earlier developmental

divergence can also be seen from the differences in the size of flower traits between distylous and homostylous flowers at PMC meiosis. In both the *furcata-vernicosa* and *douglasiana-tessellata* clades, approximately 12–13 of the studied flower traits were significantly smaller in homostylous than in distylous flowers at relative age 0.45, while the remainder showed no difference (data not shown). In *spectabilis*, all 19 studied flower traits were highly significantly smaller in homostylous flowers than in distylous ones at the relative age of 0.45. The results suggest that the divergence of homostyly from distyly may have occurred by the time of microsporocyte meiosis.

Stigma and anther heights are the characters distinguishing homostyly from distyly, and it is common for gynoecium and androecium development to be relatively independent (Lloyd and Bawa 1984; Goldman and Willson 1986). In homostyles, the relative developmental rate of pistil-height-related traits was significantly lower than in pins but similar to (in *furcata-vernicosa* and *spectabilis*) or even higher than (in *douglasiana-tessellata*) in thrums. In other words, in homostyles the pistil height displayed paedomorphic development by neoteny compared to those in pins, while it showed either no difference (two clades) or peramorphic development by acceleration (in *douglasiana-tessellata*) compared to thrums (Figs. 4, 5).

In contrast, stamen-height-associated traits in homostylous flowers developed as fast as (in *furcata-vernicosa* and *spectabilis*) or faster than (in *douglasiana-tessellata*) in pins but significantly more slowly than in thrums. Thus stamen height in homostyles showed paedomorphic ontogeny by neoteny compared to thrums, and either no difference (two clades) or peramorphic ontogeny by acceleration (in *douglasiana-tessellata*) compared with pins (Figs. 4, 5). The contrasting development of pistil and stamen heights in homostyly in comparison with that in distyly shows the presence of dissociated heterochrony with multiple heterochronic processes, as may be common (Fink 1982; Reilly 1997; Zelditch et al. 2000). These developmental changes have led to 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.

Large Homostyly in A. spectabilis

The large homostylous flower in the *spectabilis* clade had distinct characteristics and developmental patterns. All 21 studied flower traits developed more quickly in the large than the regular homostylous *A. spectabilis* flowers (Table 2). However, 12 traits in large homostylous flowers, including all flower-size-related traits, were significantly smaller than in distylous flowers, another eight were the same size, and only one trait, filament length, was 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 was a little shorter but did not differ statistically from that of distylous flowers, although it was significantly shorter than in the regular homostylous flower (Table 2). On the other hand, the relative developmental offset times were similar among the three (distyly, large homostyly and homostyly) in about 12 of 19 studied flower traits (Fig. 3). The offset times of the other seven traits in large and regular homostyles were much the same but were 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 flower trait is similar in both homostylous flowers. However, about one-third of the flower traits, including stamen height in large homostylous flowers, may have truncated development compared to the thrum flowers (Figs. 4, 5). It thus implies that progenesis has caused paedomorphic development 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 regular small homostylous flowers are 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 two to three times higher than in regular small homostylous flowers in all 19 flower traits (Table 2). Additionally, about 11 traits in large homostylous flowers showed no growth rate difference compared with distylous flowers. Pistil height in larger homostyles grew significantly faster than in thrums but at about the same rate as or a little faster than in pins. Stamen height in larger homostyles, in contrast, developed faster than in pins but similarly in thrums. 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 flower traits in terms of 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 regular 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. 5).

Based on the distinct flower morphology and ontogeny, it is likely that the large homostylous flower of *A. spectabilis* is a transitional morph during the evolution of typical small homostyly from the distyly. This is also supported by ontogenetic trajectories showing that large homostyly diverged from small homostyly much earlier than from distyly (Fig. 3). This conclusion is further consistent with a selfing rate intermediate between distylous and (small) homostylous populations (Johnston and Schoen 1996).

Differentiation of Pin and Thrum in Distyly

Depending on clade, nine to 16 of the 21 flower traits differed significantly between pins and thrums (Table 2; see Li and Johnston 2001 Appendix A for comparisons of mature trait sizes). 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 flower traits also differ distinctively between the two morphs. From a flower developmental point of view, there are various developmental processes and modifications that can differentiate two distylous flower morphs. Changes in developmental rate, however, are usually the major cause that leads to the contrasted flower 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.

Flower developmental duration, from primordium to anthesis, did not differ between the two flower morphs within each distylous taxon in *Amsinckia* (Table 2). Developmental offset times of the two morphs were also similar except for the stigmas of *A. furcata*, which had earlier offset in pin, resulting in smaller final size. 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). An earlier termination and slower growth rate of stylar growth in thrum than in pin were also observed in distylous *Palicourea padifolia* (Hernández and Ornelas 2007).

Changes in growth rate caused the reciprocal positioning of anthers and stigmas in pins and thrums in distylous species of *Amsinckia*, as has been reported in some other distylous plants (e.g. *Quinchamalium chilense* Riveros et al. 1987 and *Guettarda scabra* Richards and Koptur 1993). In all three distylous species of *Amsinckia*, the developmental rate for all stamen-height-associated traits was highly significantly slower in pins than in thrums (Table 2). In contrast, all pistil-height-related traits (except POVH, which did not differ between morphs), developed highly significantly faster in pins than in thrums in all three distylous species. Clearly, the slower developmental rate of stamen height and faster rate of pistil height in pin flowers has caused the reciprocal positioning of sex organs.

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 species, 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, we stopped measurement of the corolla tube length after flower opening. Therefore, we 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, we measured the stamen insertion height (SINH, where the filament is attached to the corolla tube) during the whole flower developmental period. This is actually a more precise way to analyze the effects of corolla tube on anther height compared with using the entire corolla tube length. 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 in stigma height between pin and thrum flowers of *Amsinckia*. This is because two of the three pistil-height components, ovary height (POVH) and stigma thickness (PSTH), are not statistically different in their relative growth rates or mature sizes between the two distylous flower morphs. 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." Furthermore, longer style cells in pin than in thrum may have contributed to the differentiation of the style length as seen in distylous *Primula vulgaris* (Heslop-Harrison et al. 1981; Webster and Gilmartin 2006).

The developmental trajectories of stigma size between pin and thrum in *A. douglasiana* diverge prior to PMC meiosis. Interestingly, the divergent growth curves of the two morphs gradually get closer due to changes in the

relative growth rate during late development and finally converge again right after anthesis.

Studies of ontogenetic trajectories of flower traits showed that the developmental divergence of the two distylous flowers can occur at any time from a very early stage to right before anthesis, depending on the flower traits and species or clades. The most dramatic separations between pin and thrum, however, occured when flowers were just about to open due to a trait's steep increase of its relative developmental rate in one of the morph. This was especially evident in the growth of corolla length, pistil height and stamen height. The divergences of both anther and stigma heights between pin and thrum flowers were 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 trifoliate* (Stirling 1936), and tristylous *Eichhornia paniculata* (Richards and Barrett 1984).

Differences Among Clades

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 1979; Piper et al. 1986; Kelso 1987; Wedderburn and Richards 1992; Tremayne and Richards 1993). It is clear that both the origin of distyly and the evolution of homostyly through the breakdown of distyly are polyphyletic. The mature flower morphology of both distyly and homostyly is similar among three clades of *Amsinckia*. Furthermore, the major flower developmental processes—neoteny and progenesis—that lead to the formation of distyly and homostyly are also broadly similar. Nevertheless, the extent of paedomorphosis, the degree of developmental dissociation, and changes in ontogenetic trajectories has resulted in the evolution of homostyly in different ways in the different clades.

It was often found that the developmental trajectories in the *furcata-vernicosa* and *douglasiana-tessellata* clades were somewhat similar, but different from those in *spectabilis*. For example, homostylous flowers required a longer development time in *A. spectabilis*, but morphs did not differ in the other two clades. Similarly, the actual time of PMC meiosis (AAFT) was later for homostyles of *A. spectabilis*, but did not differ among morphs of other clades. Relative meiosis time (RAFT) did not differ among morphs or clades (see also Li and Johnston 1999). These developmental differences mirror the differences in final form: most traits in the mature flower do not differ between *furcata-vernicosa* and *douglasiana-tessellata*, but do differ between these two clades and *spectabilis* (see Appendix C of Li and Johnston 2001). The difference in the number of flower primordia and buds on each inflorescence between the two styles is significant in *furcata-vernicosa* and *douglasiana-tessellata*, but not in *spectabilis* (Table 2). Differences among clades in the time when trajectories diverge are discussed above with regard to von Baer's Law.

The same flower trait often had a different ontogenetic trajectory among species and clades. More interestingly, different trajectories can lead to the same end. For example, the growth trajectories of flower size (length and width) of homostyles differed greatly among the three clades. The trajectories in *furcata-vernicosa* and *douglasiana-tessellata* were very similar until just prior to flower opening. After relative age 0.95 the two clades diverged due to a dramatic increase in growth rate in the *douglasiana-tessellata* clade, while the rate in the *furcata-vernicosa* clade was almost unchanged. On the other hand, the divergence of the growth trajectories between these two clades (*furcata-vernicosa* and *douglasiana-tessellata*) and *spectabilis* existed from an early developmental stage, probably prior to a relative age 0.4, due to a growth rate increase in *furcata-vernicosa* and *douglasiana-tessellata*. The growth in *spectabilis*, however, had a steep increase after relative age 0.95, so that its growth curve converged with the *furcata-vernicosa* growth curve by the time of flowering.

The ontogenetic relationships between the two distylous flower morphs were also clade dependent in *Amsinckia*. For example, the actual flower developmental time prior to anthesis between pin and thrum was almost the same within a clade, but differed among clades. Similarly, the patterns of relative developmental rate changes between the two morphs in many flower traits differed among the clades. The same was true for changes in ontogenetic trajectories between pins and thrums among clades.

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Appendix

See Tables 6, 7, and 8.

Table 6 Comparisons of morphs: significance levels for pairwise comparisons of timing traits and developmental rates of pins, thrums and homostyles

Trait	Di vs. H		P vs. T		T vs. H		P vs. H	
	Raw *P*	Boot *P*	Raw *P*	Boot *P*	Raw *P*	Boot *P*	Raw *P*	Boot *P*
All clades								
DAYPFLR	0.0001	0.0001	0.8192	1.0000	0.0001	0.0004	0.0001	0.0001
TOTBUDS	0.0006	0.0245	0.6177	1.0000	0.0013	0.0507	0.0042	0.1344
DEVDUR	0.2393	0.9969	0.3069	0.9994	0.5880	1.0000	0.1235	0.9433
RAFT	0.5398	1.0000	0.0198	0.4197	0.5535	1.0000	0.0907	0.8810
AAFT	0.1851	0.9869	0.8527	1.0000	0.2169	0.9939	0.2729	0.9983
KSL	0.0033	0.1096	0.3329	0.9999	0.0331	0.5756	0.0021	0.0745
BUDL	0.0001	0.0001	0.0097	0.2576	0.0001	0.0001	0.0001	0.0001
BUDW	0.0001	0.0001	0.7963	1.0000	0.0001	0.0001	0.0001	0.0001
CFPL	0.0001	0.0001	0.0080	0.2211	0.0001	0.0001	0.0001	0.0001
CLBW	0.0001	0.0001	0.9606	1.0000	0.0001	0.0001	0.0001	0.0001
CPTL	0.0001	0.0001	0.0299	0.5448	0.0001	0.0001	0.0001	0.0001
CTBL	0.0001	0.0001	0.0031	0.1060	0.0001	0.0001	0.0001	0.0001
SANL	0.0001	0.0001	0.6188	1.0000	0.0001	0.0001	0.0001	0.0001
SANW	0.0001	0.0051	0.0129	0.3162	0.0001	0.0006	0.0193	0.4124
SFIL	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0601	0.7652
SINH	0.0001	0.0013	0.0001	0.0001	0.0001	0.0001	0.0020	0.0698
SSIL	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.1016	0.9067
PISL	0.0001	0.0001	0.0001	0.0001	0.0015	0.0546	0.0001	0.0001
POVH	0.2809	0.9985	0.0150	0.3504	0.0383	0.6221	0.8156	1.0000
PSSL	0.0001	0.0001	0.0001	0.0001	0.0014	0.0516	0.0001	0.0001
PSTYL	0.0001	0.0001	0.0001	0.0001	0.0005	0.0191	0.0001	0.0001
PSTH	0.0017	0.0603	0.1762	0.9840	0.0324	0.5694	0.0006	0.0245
PSTL	0.0001	0.0001	0.2460	0.9973	0.0001	0.0001	0.0001	0.0001
PSTW	0.0001	0.0001	0.7241	1.0000	0.0001	0.0001	0.0001	0.0001
PSTA	0.0001	0.0001	0.8931	1.0000	0.0001	0.0001	0.0001	0.0001
AVASD	0.0001	0.0001	0.0602	0.7656	0.0001	0.0001	0.0001	0.0001
Clade 1: *furcata—vernicosa*								
DAYPFLR	0.0001	0.0001	0.5991	1.0000	0.0001	0.0001	0.0001	0.0001
TOTBUDS	0.0001	0.0001	0.2425	0.9945	0.0001	0.0001	0.0001	0.0001
DEVDUR	0.8624	1.0000	0.7421	1.0000	1.0000	1.0000	0.7421	1.0000
RAFT	0.6325	1.0000	0.0186	0.4008	0.4947	1.0000	0.0985	0.8886
AAFT	0.9472	1.0000	0.5336	1.0000	0.8272	1.0000	0.7078	1.0000
KSL	0.0819	0.8448	0.7208	1.0000	0.1902	0.9832	0.0685	0.7922
BUDL	0.0001	0.0001	0.0023	0.0829	0.0001	0.0001	0.0001	0.0001
BUDW	0.0001	0.0001	0.3344	0.9996	0.0001	0.0001	0.0001	0.0001
CFPL	0.0001	0.0001	0.0014	0.0546	0.0001	0.0001	0.0001	0.0001
CLBW	0.0001	0.0001	0.4083	1.0000	0.0001	0.0001	0.0001	0.0001
CPTL	0.0001	0.0001	0.0060	0.1783	0.0001	0.0001	0.0001	0.0001
CTBL	0.0001	0.0001	0.0029	0.1004	0.0001	0.0001	0.0001	0.0001
SANL	0.0001	0.0001	0.1316	0.9424	0.0001	0.0001	0.0001	0.0001
SANW	0.0859	0.8561	0.0082	0.2255	0.0109	0.2770	0.7912	1.0000
SFIL	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0694	0.7954
SINH	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0171	0.3785
SSIL	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.4688	1.0000
PISL	0.0001	0.0036	0.0001	0.0001	0.0456	0.6711	0.0001	0.0001

Table 6 continued

Trait	Di vs. H		P vs. T		T vs. H		P vs. H	
	Raw *P*	Boot *P*	Raw *P*	Boot *P*	Raw *P*	Boot *P*	Raw *P*	Boot *P*
POVH	0.0783	0.8310	0.0299	0.5415	0.0177	0.3879	0.5587	1.0000
PSSL	0.0001	0.0010	0.0001	0.0001	0.0571	0.7374	0.0001	0.0001
PSTYL	0.0001	0.0008	0.0001	0.0001	0.0480	0.6859	0.0001	0.0001
PSTH	0.6313	1.0000	0.3565	0.9997	0.9945	1.0000	0.3605	0.9997
PSTL	0.0079	0.2206	0.0874	0.8605	0.0038	0.1273	0.0778	0.8293
PSTW	0.0001	0.0021	0.9310	1.0000	0.0003	0.0121	0.0001	0.0037
PSTA	0.0008	0.0357	0.3856	0.9999	0.0016	0.0622	0.0042	0.1372
AVASD	0.0001	0.0001	0.3279	0.9994	0.0001	0.0001	0.0001	0.0001
Clade 2: *douglasiana—tessellata*								
DAYPFLR	0.0458	0.5961	0.1931	0.9730	0.2239	0.9856	0.0243	0.4013
TOTBUDS	0.0001	0.0016	0.9938	1.0000	0.0001	0.0043	0.0003	0.0098
DEVDUR	0.2428	0.9900	0.2236	0.9856	0.0993	0.8419	0.6691	1.0000
RAFT	0.5648	1.0000	0.2378	0.9895	0.9218	1.0000	0.2986	0.9969
AAFT	0.9846	1.0000	0.7460	1.0000	0.8574	1.0000	0.8932	1.0000
KSL	0.7216	1.0000	0.8064	1.0000	0.6552	1.0000	0.8510	1.0000
BUDL	0.0787	0.7740	0.0553	0.6623	0.0131	0.2551	0.5425	1.0000
BUDW	0.0150	0.2818	0.3228	0.9981	0.0080	0.1738	0.0931	0.8230
CFPL	0.0646	0.7129	0.0647	0.7138	0.0116	0.2323	0.4668	1.0000
CLBW	0.0070	0.1546	0.0292	0.4564	0.0008	0.0240	0.1685	0.9554
CPTL	0.0657	0.7181	0.1195	0.8915	0.0171	0.3128	0.3866	0.9997
CTBL	0.5253	1.0000	0.1581	0.9455	0.2008	0.9771	0.8964	1.0000
SANL	0.3738	0.9995	0.1970	0.9755	0.1496	0.9366	0.8818	1.0000
SANW	0.7630	1.0000	0.0489	0.6190	0.2009	0.9772	0.4869	1.0000
SFIL	0.0060	0.1367	0.0001	0.0002	0.0001	0.0004	0.6640	1.0000
SINH	0.2846	0.9960	0.0001	0.0001	0.0031	0.0793	0.0001	0.0024
SSIL	0.6317	1.0000	0.0001	0.0001	0.0042	0.1024	0.0010	0.0292
PISL	0.7709	1.0000	0.0001	0.0001	0.0001	0.0002	0.0001	0.0002
POVH	0.0001	0.0017	0.0643	0.7116	0.0001	0.0006	0.0027	0.0708
PSSL	0.3774	0.9996	0.0001	0.0001	0.0001	0.0002	0.0001	0.0001
PSTYL	0.4665	1.0000	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
PSTH	0.0028	0.0724	0.8282	1.0000	0.0075	0.1638	0.0075	0.1645
PSTL	0.0030	0.0777	0.6906	1.0000	0.0099	0.2054	0.0065	0.1466
PSTW	0.0229	0.3867	0.8897	1.0000	0.0425	0.5723	0.0444	0.5861
PSTA	0.0001	0.0038	0.8156	1.0000	0.0002	0.0075	0.0008	0.0241
AVASD	0.0001	0.0001	0.0035	0.0867	0.0001	0.0001	0.0001	0.0001
Clade 3: *spectabilis*								
DAYPFLR	0.0005	0.0131	0.3199	0.9936	0.0065	0.1202	0.0006	0.0141
TOTBUDS	0.5200	1.0000	0.1948	0.9401	0.2300	0.9658	0.9189	1.0000
DEVDUR	0.0006	0.0148	0.6427	1.0000	0.0013	0.0289	0.0040	0.0774
RAFT	0.6638	1.0000	0.8751	1.0000	0.6493	1.0000	0.7654	1.0000
AAFT	0.0003	0.0064	0.5902	1.0000	0.0005	0.0130	0.0020	0.0419
KSL	0.0001	0.0001	0.4619	0.9998	0.0001	0.0001	0.0001	0.0001
BUDL	0.0001	0.0001	0.1595	0.9020	0.0001	0.0001	0.0001	0.0003
BUDW	0.0001	0.0001	0.9272	1.0000	0.0001	0.0001	0.0001	0.0001
CFPL	0.0001	0.0001	0.1668	0.9115	0.0001	0.0001	0.0001	0.0001
CLBW	0.0001	0.0001	0.4298	0.9996	0.0001	0.0001	0.0001	0.0001
CPTL	0.0001	0.0001	0.2105	0.9530	0.0001	0.0001	0.0001	0.0001

Table 6 continued

Trait	Di vs. H		P vs. T		T vs. H		P vs. H	
	Raw *P*	Boot *P*	Raw *P*	Boot *P*	Raw *P*	Boot *P*	Raw *P*	Boot *P*
CTBL	0.0001	0.0001	0.0148	0.2338	0.0001	0.0001	0.0001	0.0030
SANL	0.0001	0.0001	0.7789	1.0000	0.0001	0.0001	0.0001	0.0001
SANW	0.0001	0.0002	0.2429	0.9729	0.0001	0.0003	0.0002	0.0062
SFIL	0.0001	0.0001	0.0026	0.0534	0.0001	0.0001	0.0009	0.0203
SINH	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.2694	0.9829
SSIL	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0996	0.7736
PISL	0.0001	0.0001	0.0001	0.0001	0.4063	0.9991	0.0001	0.0001
POVH	0.0001	0.0018	0.5039	1.0000	0.0009	0.0203	0.0002	0.0046
PSSL	0.0001	0.0001	0.0001	0.0001	0.4950	1.0000	0.0001	0.0001
PSTYL	0.0001	0.0003	0.0001	0.0001	0.6987	1.0000	0.0001	0.0001
PSTH	0.0001	0.0001	0.8166	1.0000	0.0001	0.0007	0.0001	0.0014
PSTL	0.0001	0.0001	0.5339	1.0000	0.0001	0.0003	0.0001	0.0013
PSTW	0.0001	0.0001	0.6748	1.0000	0.0001	0.0001	0.0001	0.0001
PSTA	0.0001	0.0001	0.7141	1.0000	0.0001	0.0001	0.0001	0.0001
AVASD	0.0001	0.0001	0.5400	1.0000	0.0001	0.0001	0.0001	0.0001

The LH form of *A. spectabilis* is not included

Di distyly (pins and thrums), *H* homostyly, *P* pin, *T* thrum

Table 7 Comparisons of clades: significance levels for pairwise comparisons of timing traits and developmental rates

Trait	Clades 1 and 2 vs. 3		Clade 1 vs. 2		Clade 1 vs. 3		Clade 2 vs. 3	
	Raw *P*	Boot *P*	Raw *P*	Boot *P*	Raw *P*	Boot *P*	Raw *P*	Boot *P*
DAYPFLR	0.0028	0.0902	0.0010	0.0375	0.0001	0.0015	0.2859	0.9981
TOTBUDS	0.0353	0.5704	0.0001	0.0001	0.0001	0.0016	0.5906	1.0000
DEVDUR	0.2666	0.9965	0.0579	0.7233	0.9461	1.0000	0.0650	0.7596
RAFT	0.2056	0.9858	0.4434	1.0000	0.1306	0.9306	0.4680	1.0000
AAFT	0.5746	1.0000	0.9969	1.0000	0.6147	1.0000	0.6282	1.0000
KSL	0.0001	0.0001	0.0055	0.1581	0.0001	0.0001	0.0001	0.0001
BUDL	0.5605	1.0000	0.7709	1.0000	0.5089	1.0000	0.7131	1.0000
BUDW	0.7776	1.0000	0.3786	0.9997	0.8612	1.0000	0.5107	1.0000
CFPL	0.4948	1.0000	0.7347	1.0000	0.4393	1.0000	0.6653	1.0000
CLBW	0.1743	0.9713	0.8341	1.0000	0.2649	0.9962	0.2024	0.9844
CPTL	0.5805	1.0000	0.6128	1.0000	0.4604	1.0000	0.8093	1.0000
CTBL	0.3207	0.9990	0.9595	1.0000	0.3617	0.9996	0.4027	0.9998
SANL	0.0003	0.0114	0.0088	0.2270	0.0001	0.0005	0.0444	0.6430
SANW	0.0301	0.5225	0.0275	0.4964	0.0030	0.0977	0.3934	0.9998
SFIL	0.7334	1.0000	0.0826	0.8296	0.5867	1.0000	0.2657	0.9963
SINH	0.4378	1.0000	0.5582	1.0000	0.6814	1.0000	0.3444	0.9992
SSIL	0.3523	0.9994	0.8824	1.0000	0.4471	1.0000	0.3817	0.9997
PISL	0.0705	0.7857	0.0406	0.6151	0.0098	0.2481	0.5431	1.0000
POVH	0.0002	0.0102	0.7417	1.0000	0.0005	0.0208	0.0021	0.0711
PSSL	0.0686	0.7774	0.0352	0.5689	0.0088	0.2275	0.5551	1.0000
PSTYL	0.1439	0.9476	0.0607	0.7377	0.0279	0.5001	0.7012	1.0000
PSTH	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.1515	0.9538
PSTL	0.7364	1.0000	0.9088	1.0000	0.8062	1.0000	0.7299	1.0000
PSTW	0.2811	0.9975	0.0172	0.3696	0.0348	0.5662	0.8404	1.0000
PSTA	0.0001	0.0048	0.0001	0.0019	0.0001	0.0001	0.1372	0.9400
AVASD	0.0604	0.7362	0.6718	1.0000	0.0600	0.7348	0.1510	0.9535

The LH form of *A. spectabilis* is not included

Clade 1: *furcata—vernicosa*, Clade 2: *douglasiana—tessellata*, Clade 3: *spectabilis*

Table 8 Comparisons within *A. spectabilis* of the four morphs P, T, LH and H: significance levels for pairwise comparisons of timing traits and developmental rates

Variable	P & T vs. H & LH		P vs. T		T vs. H		P vs. H	
	Raw *P*	Boot *P*	Raw *P*	Boot *P*	Raw *P*	Boot *P*	Raw *P*	Boot *P*
DAYPFLR	0.2759	0.9988	0.2695	0.9986	0.0024	0.0963	0.0012	0.0531
TOTBUDS	0.8656	1.0000	0.2196	0.9954	0.2562	0.9979	0.1531	0.9761
DEVDUR	0.2775	0.9988	0.6266	1.0000	0.0006	0.0291	0.0002	0.0092
RAFT	0.6338	1.0000	0.8629	1.0000	0.6172	1.0000	0.2415	0.9971
AAFT	0.2611	0.9983	0.5660	1.0000	0.0002	0.0101	0.0001	0.0021
KSL	0.0001	0.0003	0.4644	1.0000	0.0001	0.0001	0.0001	0.0017
BUDL	0.0006	0.0313	0.1605	0.9798	0.0001	0.0001	0.0001	0.0001
BUDW	0.0001	0.0018	0.9265	1.0000	0.0001	0.0001	0.0001	0.0003
CFPL	0.0001	0.0025	0.1601	0.9796	0.0001	0.0001	0.0001	0.0001
CLBW	0.0001	0.0027	0.4381	1.0000	0.0001	0.0001	0.0001	0.0009
CPTL	0.0003	0.0161	0.2050	0.9937	0.0001	0.0001	0.0001	0.0001
CTBL	0.0007	0.0330	0.0136	0.3622	0.0001	0.0001	0.0001	0.0001
SANL	0.0010	0.0450	0.7883	1.0000	0.0001	0.0001	0.0001	0.0018
SANW	0.0738	0.8489	0.2761	0.9988	0.0001	0.0011	0.0001	0.0003
SFIL	0.9010	1.0000	0.0021	0.0858	0.0001	0.0001	0.0001	0.0001
SINH	0.1631	0.9810	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
SSIL	0.2004	0.9927	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
PISL	0.0001	0.0001	0.0001	0.0001	0.3640	0.9999	0.0001	0.0018
POVH	0.3343	0.9997	0.5730	1.0000	0.0031	0.1183	0.0012	0.0547
PSSL	0.0515	0.7490	0.0001	0.0001	0.5175	1.0000	0.0062	0.2040
PSTYL	0.1401	0.9671	0.0001	0.0001	0.7021	1.0000	0.0483	0.7290
PSTH	0.0526	0.7557	0.8392	1.0000	0.0001	0.0065	0.0002	0.0092
PSTL	0.0060	0.1977	0.5487	1.0000	0.0001	0.0004	0.0001	0.0047
PSTW	0.0049	0.1718	0.7354	1.0000	0.0001	0.0009	0.0002	0.0098
PSTA	0.0007	0.0356	0.7292	1.0000	0.0001	0.0001	0.0001	0.0017
AVASD	0.0001	0.0001	0.5459	1.0000	0.0001	0.0001	0.0087	0.2595

Variable	T vs. LH		P vs. LH		H vs. LH	
	Raw *P*	Boot *P*	Raw *P*	Boot *P*	Raw *P*	Boot *P*
DAYPFLR	0.0073	0.2295	0.0883	0.8917	0.0001	0.0001
TOTBUDS	0.7378	1.0000	0.3666	0.9999	0.4185	1.0000
DEVDUR	0.0801	0.8693	0.0286	0.5658	0.0001	0.0002
RAFT	0.3200	0.9996	0.2453	0.9973	0.1403	0.9672
AAFT	0.0427	0.6936	0.0115	0.3206	0.0001	0.0001
KSL	0.6597	1.0000	0.7689	1.0000	0.0001	0.0001
BUDL	0.8469	1.0000	0.1130	0.9366	0.0001	0.0001
BUDW	0.7076	1.0000	0.6406	1.0000	0.0001	0.0001
CFPL	0.7306	1.0000	0.2827	0.9990	0.0001	0.0001
CLBW	0.3047	0.9994	0.7975	1.0000	0.0001	0.0001
CPTL	0.9002	1.0000	0.1654	0.9818	0.0001	0.0001
CTBL	0.4439	1.0000	0.0739	0.8495	0.0001	0.0001
SANL	0.5151	1.0000	0.3601	0.9999	0.0001	0.0001
SANW	0.1587	0.9788	0.0162	0.4035	0.0001	0.0001
SFIL	0.0011	0.0490	0.0001	0.0001	0.0001	0.0001
SINH	0.3541	0.9999	0.0001	0.0001	0.0001	0.0001
SSIL	0.8958	1.0000	0.0001	0.0001	0.0001	0.0001

Table 8 continued

Variable	T vs. LH		P vs. LH		H vs. LH	
	Raw *P*	Boot *P*	Raw *P*	Boot *P*	Raw *P*	Boot *P*
PISL	0.1589	0.9789	0.0001	0.0001	0.0249	0.5224
POVH	0.0223	0.4911	0.0751	0.8529	0.0001	0.0003
PSSL	0.0001	0.0001	0.0015	0.0636	0.0001	0.0001
PSTYL	0.0001	0.0001	0.0171	0.4185	0.0001	0.0001
PSTH	0.1565	0.9778	0.1079	0.9292	0.0001	0.0001
PSTL	0.5705	1.0000	0.2475	0.9974	0.0001	0.0001
PSTW	0.5693	1.0000	0.3669	0.9999	0.0001	0.0001
PSTA	0.5860	1.0000	0.3754	0.9999	0.0001	0.0001
AVASD	0.0001	0.0001	0.0001	0.0001	0.0021	0.0871

There are seven kinds of pairwise comparison

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