

# Comparative floral morphometrics of distyly and homostyly in three evolutionary lineages of *Amsinckia* (Boraginaceae)

Ping Li and Mark O. Johnston

**Abstract:** Using three ancestor-descendant lineages of *Amsinckia* (Boraginaceae), we studied changes in floral morphology associated with evolution of the breeding system. Each lineage comprised a distylous ancestor and a homostylous descendant: (i) *Amsinckia furcata* Suksd. – *Amsinckia vernicosa* Hook. & Arn.; (ii) *Amsinckia douglasiana* A. DC. – *Amsinckia tessellata* var. *gloriosa* (Suksd.) Hoover; and (iii) *Amsinckia spectabilis* Fischer & C. Meyer. Comparisons of 26 floral traits were made between pins and thrums within the distylous groups, between distyly (pins and thrums combined) and homostyly as well as among pins, thrums, and homostyles. Differences among the morphs were also compared across the three lineages. In distylous flowers, the six traits directly related to stamen height or pistil height varied as expected from their close relationship to the definition of pins and thrums, with the stamen-height-related characters greater in thrums and the pistil-height-related characters greater in pins. Thrums had larger pollen grains in all lineages. Pollen production was either similar in the two distylous morphs or lower in thrums than in pins, depending on species. Thrums also tended to have larger style cross-sectional area and style transmission tissue cross-sectional area. In two of three lineages, thrums had longer corollas, whereas pins exceeded thrums in functional anther–stigma separation and in stigmatic papilla size. The size order of a trait in pins versus thrums was consistent in all lineages for 18 of 26 traits; in seven of the eight remaining traits, *A. spectabilis* was the unusual lineage. Sepal length, corolla width, and stigma size did not differ significantly between the two distylous morphs. In homostyles, traits related to anther height and pistil height were intermediate between pins and thrums in all lineages; for other traits, including anther–stigma separation and overall size, homostyles generally had the smallest values. For most traits, lineages differed in the degree of differentiation among the three morphs as well as between distyly and homostyly. Thus, in *Amsinckia*, the evolution of homostyly involves a general reduction in flower size, but by an amount that varies both among traits and among lineages.

*Key words:* *Amsinckia*, dimorphism, distyly, floral evolution, homostyly, mating system.

**Résumé :** En utilisant trois lignées ancêtres-descendants du genre *Amsinckia* (Boraginaceae), les auteurs ont étudié les changements de la morphologie florale associés avec l'évolution du système de croisement. Chaque lignée comporte un ancêtre distyle et un descendant homostyle : (i) *Amsinckia furcata* Suksd. – *Amsinckia vernicosa* Hook & Arn.; (ii) *Amsinckia douglasiana* A. DC. – *Amsinckia tessellata* var. *gloriosa* (Suksd.) Hoover; et (iii) *Amsinckia spectabilis* Fischer & C. Meyer. Ils ont comparé 26 caractères chez les fleurs longistyles (pins) et les fleurs brévistyles (thrums) dans les groupes distyles, entre distyles (longistyles et brévistyles combinées) et homostyles, ainsi qu'entre longistyles, brévistyles et homostyles. Ils ont également comparé les différences entre les morphes pour l'ensemble des lignées. Chez les fleurs distyles, les six caractères directement liés à la hauteur des étamines ou à la hauteur des pistils varient comme prévu à partir de leur étroite relation avec la définition de longistylie et de brévistylie, les caractères reliés à la hauteur des étamines étant plus grands chez les brévistyles et les caractères liés à la hauteur du pistil étant plus élevés chez les longistyles. Les fleurs brévistyles ont des pollens plus gros dans toutes les lignées. La production de pollen est soit similaire dans les deux morphes distyles ou encore plus faible chez les brévistyles que chez les longistyles, selon l'espèce. En section transverse, chez les fleurs brévistyles, les superficies du style et celle du tissu de transmission ont tendance à être plus grandes. Chez deux des trois lignées, les fleurs brévistyles ont des corolles plus longues, alors que les fleurs longistyles dépassent les fleurs brévistyles en termes d'espace fonctionnel anthères–stigmates, ainsi que dans la dimension des papilles des stigmates. L'ordre de grandeur d'un caractère chez les fleurs longistyles versus les fleurs brévistyles est congru dans toutes les lignées pour 18 des 26 caractères; pour sept des 8 caractères résiduels, l'*A. spectabilis* constitue le cas particulier. La longueur des sépales, la largeur de la corolle et la dimension de stigmates ne diffèrent pas significativement entre les deux morphes distyles. Chez les homostyles, les caractères liés à la hauteur des anthères et la hauteur des pistils sont intermédiaires entre ceux des fleurs longistyles et brévistyles, dans toutes les lignées; pour les autres caractères, incluant l'espace anthères–stigmates et la dimension d'ensemble, on retrouve généralement les valeurs les plus

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petites chez les homostyles. Pour la plupart des caractères, les lignées diffèrent quant au degré de différenciation parmi les trois morphes ainsi qu'entre les formes distyles et homostyles. Ainsi, chez les *Amsinckia*, l'évolution de l'homostylie comporte une réduction générale de la dimension des fleurs, mais selon une importance qui varie d'après les caractères et au sein des lignées.

*Mots clés* : *Amsinckia*, dimorphisme, distylie, évolution florale, homostylie, système de croisement.

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## Introduction

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

Many genera or species with distylous members also contain other species or populations lacking distyly (Dowrick 1956; Ganders 1975a, 1979a; Barrett 1989). In most instances, such homostylous species or populations probably evolved from distylous ancestors (Ganders et al. 1985; Barrett 1988 1992; Schoen et al. 1996, 1997), a process initiated by a crossover or mutation in the distyly supergene (Lewis and Jones 1992; Richards and Barrett 1992). Homostylous populations are usually highly self-fertilizing (Shore and Barrett 1985; Piper et al. 1986; Boyd et al. 1990; Johnston and Schoen 1996) because of the reduced or non-existent anther–stigma separation and the loss of self-sterility caused by disruption of the supergene.

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

to pins and thrums within distylous taxa. An understanding of changes in relationships among traits during evolution is best achieved by measuring traits in pins, thrums, and homostyles within an evolutionary lineage.

Here we report measurements of approximately 26 floral traits in pins, thrums, and related homostyles in three evolutionary lineages of *Amsinckia* (Boraginaceae). Within each lineage, homostyly is thought to have evolved from distyly (Ray and Chisaki 1957b; Schoen et al. 1997). The objectives of the study were, firstly, to identify differences in floral traits among the three morphs (pin, thrum, homostyle) and, secondly, to determine whether these differences are consistent among the three lineages. This study therefore examines differences, both within and among lineages, in a large number of single traits of mature flowers.

## Materials and methods

### Species and floral morphs

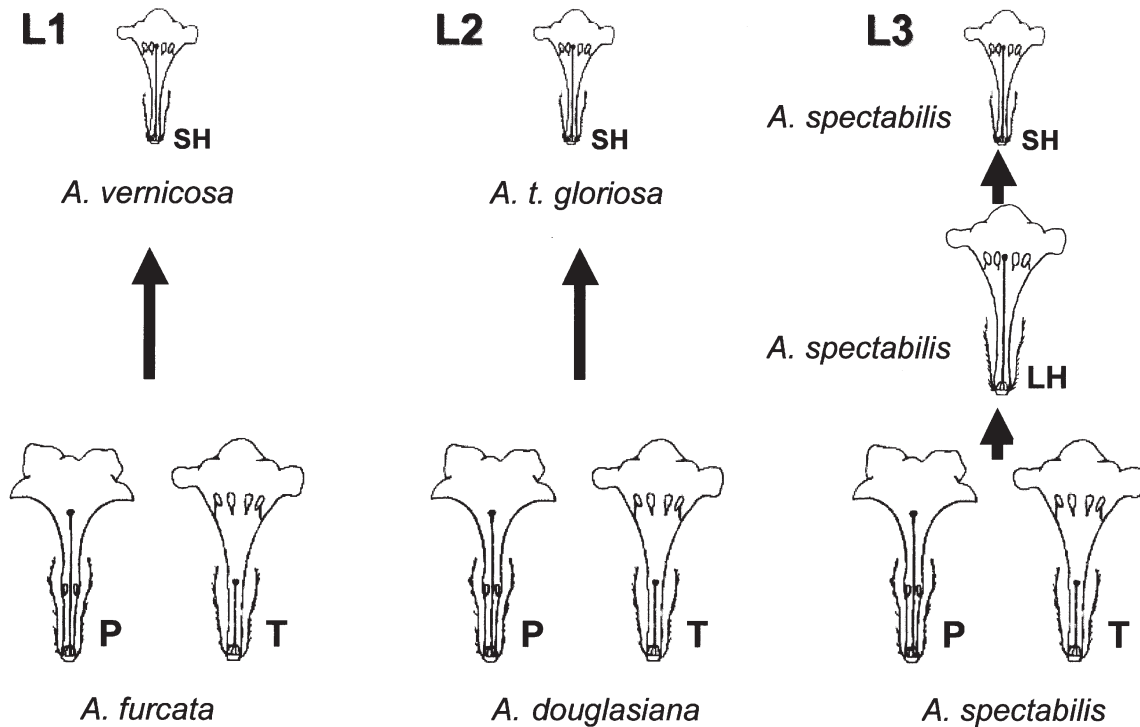
The species and populations studied are classified into three evolutionary lineages (Ray and Chisaki 1957a, 1957b; Ganders et al. 1985; Schoen et al. 1997). These lineages are *Amsinckia furcata* Suksd. – *Amsinckia vernicosa* Hook. & Arn. (lineage 1 or L1), *Amsinckia douglasiana* A. DC. – *Amsinckia tessellata* var. *gloriosa* (Suksd.) Hoover (lineage 2 or L2), and *Amsinckia spectabilis* Fischer & C. Meyer (lineage 3 or L3; see Fig. 1).<sup>2</sup> Each lineage consists of a distylous taxon, the presumed ancestor, and a homostylous taxon, the presumed descendant (Ray and Chisaki 1957a, 1957b; Schoen et al. 1997). Lineages 1 and 2 are in section *Tessellatae*, while lineage 3 is in section *Microcarpae* (Ray and Chisaki 1957b). *Amsinckia tessellata*, the homostylous species of lineage 2 is tetraploid (Ray and Chisaki 1957b). The *A. spectabilis* lineage (L3) additionally contains populations with an intermediate floral form characterized by large flowers that are not distinctly distylous. These populations are termed “mixed” (Ganders 1975a) or “large-flowered homostylous” (see Fig. 1). Therefore, this study consisted of pins (P), thrums (T), and homostyles (H) in each of these lineages, plus the large-flowered homostyle in *A. spectabilis*, giving a total of 10 lineage–morph combinations. In this paper, homostyly of lineage 3 (*A. spectabilis*) includes both large-flowered homostyly (LH) and small-flowered homostyly (SH). All study samples were collected from the field in California between 28 April and 6 May 1995. Eight to 15 inflorescences were used for each floral morph of each species or population in the study. Each inflorescence was taken from a different individual plant. All inflorescences were fixed in formalin – acetic acid – ethanol (FAA) for about 1 week and then stored in 70% ethanol for later studies.

### Measurements

In order from distal to proximal, the coiled *Amsinckia* inflorescence consists of unopened flowers (buds), fully opened flowers available for pollination, and senescing flowers. A typical inflores-

<sup>2</sup>*A. tessellata gloriosa* (Ganders 1993) is equivalent to *A. gloriosa* (Ray and Chisaki 1957a, 1957b, 1957c).

**Fig. 1.** Three evolutionary lineages of *Amsinckia*. Modified from Ray and Chisaki (1957b). The diagrams of flowers were modified from Ganders (1975b). L1, lineage 1; L2, lineage 2; L3, lineage 3; P, pin; T, thrum; LH, large homostylous flower; SH, small homostylous flower.



cence has two to eight fully opened flowers. For each inflorescence studied, at least three fully opened flowers were dissected and measured under an Olympus SZH10 stereo microscope, which was connected to a video imaging system and computer. Measurements of floral traits were performed using the public domain NIH Image program (version 1.62, developed at the U.S. National Institutes of Health and available on the Internet at <http://rsb.info.nih.gov/nih-image>) on images of dissected floral parts. To pinpoint precisely the parts or segments of each floral organ that differ among morphs and lineages and provide basic information for floral developmental studies (a separate study), 26 quantitative traits were used in the statistical analyses (Table 1). All measurements or traits are defined or illustrated 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; and P, pistil). From among the flowers dissected and measured, the largest (named "final-sized" in the rest of the text) was used in the statistical analyses. Stigma papilla size (papilla length, PAPIL, and width, PAPIW), style cross-sectional area (STYLECA), and style transmission tissue cross-sectional area (TRANSCA) were measured on sections made from paraffin-embedded stigmas and styles. STYLECA and TRANSCA were measured on cross sections cut from the midportion of the styles. Stigma area (PSTA) was estimated as a five-sided box and was calculated as

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

where PSTL is stigma length, PSTH is stigma thickness, and PSTW is stigma width. Functional anther–stigma distance (ASD) measured the minimum distance separating the top of the stigma from the anthers. If the stigma top was within the anthers, that is, above the anther bottom and below the anther top (pistil length (PISL) > stamen height (SSIL) – anther length (SANL) and PISL < SSIL), then ASD = 0. Otherwise, ASD was positive and was calculated as follows for the two possible situations. If the stigma was

below the anther bottom (PISL < SSIL – SANL), then ASD = SSIL – SANL – PISL. If the stigma was above the anther top (PISL > SSIL), then ASD = PISL – SSIL (see Fig. 2).

### Statistical analysis

The primary aim of this study was to determine how individual traits differed among morphs and clades. In addition to all pairwise comparisons among morphs and lineages for each trait, we were also interested in comparing distyly (pins and thrums combined) to homostyly, as well as section *Tessellatae* (*furcata–vernica* and *douglasiana–tessellata*) to section *Microcarpae* (*spectabilis*). Therefore, data were analyzed using a priori contrasts (*t* tests) followed by adjustments to maintain the maximum familywise error rate below 0.05. For all pairwise comparisons, we used the bootstrapping option in the MULTTEST procedure of SAS (20 000 iterations; Westfall and Young 1993), which incorporates correlations between variables and, therefore, can be more powerful than the sequential Bonferroni technique. The following families of pairwise comparisons were carried out (H includes LH of *A. spectabilis*): (i) morphs over all lineages: P versus T, P versus H, T versus H, and distyly (P and T combined) versus H (104 tests); (ii) morphs within L1: four comparisons as above (104 tests); (iii) morphs within L2: four comparisons as above (104 tests); (iv) morphs within L3: four comparisons as above (104 tests); (v) morphs within L3: P versus T, P versus SH, P versus LH, T versus SH, T versus LH, LH versus SH, and distyly (P and T combined) versus H (LH and SH combined, 182 tests); (vi) lineages over all morphs: L1 versus L2, L1 versus L3, L2 versus L3, and L1 and L2 combined versus L3 (104 tests). In addition to the above contrasts, factorial ANOVAs were used to test for effects of morph (levels P, T, H) and lineage (levels L1, L2, L3), as well as their interaction, on the 26 traits. Both lineage and morph were considered fixed factors. ANOVA *p* values were adjusted for multiple comparisons using the sequential Bonferroni technique in the MULTTEST procedure of SAS.

**Table 1.** List of the 26 studied floral morphometric characters in three lineages of *Amsinckia*.

Abbreviation	Measurement scale	Whorl	Character
KSL	mm	Calyx	Sepal length
BUDL	mm	Corolla	Flower length in natural position
BUDW	mm	Corolla	Flower width in natural position
CFPL	mm	Corolla	Fused petal length
CLBW	mm	Corolla	Corolla lobe width
CPTL	mm	Corolla	Petal length
CTBL	mm	Corolla	Corolla tube length
POLN	No./flower	Stamen	Pollen number per flower
POLS	$\mu\text{m}$	Stamen	Pollen size (diameter on long axis)
SANL	mm	Stamen	Anther length
SANW	mm	Stamen	Anther width
SFIL	mm	Stamen	Free stamen filament length (i.e., portion not fused to petal)
SINH	mm	Stamen	Stamen insertion height
SSIL	mm	Stamen	Stamen height (anther height)
PAPIL	mm	Pistil	Stigma papilla length
PAPIW	mm	Pistil	Stigma papilla width
PISL	mm	Pistil	Pistil length (stigma height)
PSSL	mm	Pistil	Style and stigma length
PSTYL	mm	Pistil	Style length
PSTH	mm	Pistil	Stigma thickness
PSTL	mm	Pistil	Stigma length
PSTW	mm	Pistil	Stigma width
PSTA	$\text{mm}^2$	Pistil	Stigma area
STYLECA	$\mu\text{m}^2$	Pistil	Style cross-sectional area
TRANSCA	$\mu\text{m}^2$	Pistil	Style transmission tissue cross-sectional area
ASD	mm	Pistil or stamen	Functional distance between anther and stigma

Note: Morphometric measurements are illustrated in Fig. 2.

## Results

### Combined lineages: overall comparison of morphs

We first compare morphs without regard to lineage, that is, with lineages combined. Homostylous and distylous (i.e., P and T combined) flowers differed in 25 of the 26 traits (Appendix A, all lineages). The single exception was pollen size (POLS). In all cases, the trait was larger in the distyles (Fig. 3).

The three pairwise comparisons between pins, thrums and homostyles indicated that all 26 traits differed between at least two of the three morphs (Appendix A, all lineages). This result was corroborated by ANOVA (Appendix B, morph). The differences between morphs were lineage dependent in 20 of 26 traits (Appendix B, morph  $\times$  lineage; Fig. 3). The exceptions to this interaction were sepal length (KSL), anther width (SANW) and all four traits measuring stigma size (PSTH, PSTL, PSTW, and PSTA; see also Fig. 3).

Each morph differed from each other morph for 15 of the traits, when lineages were combined (Appendix A, all lineages). These included flower length (bud length (BUDL), fused petal length (CFPL), petal length (CPTL), and corolla tube length (CTBL)), pollen number (POLN), POLS, SANL, stamen height (SSIL and stamen insertion height (SINH)), PAPIL, pistil length (PISL, style and stigma length (PSSL), and style length (PSTYL)), STYLECA, and ASD. The remainder of the traits, however, differed significantly between only two of the three morphs. For example, pins and thrums did not differ in KSL, flower width (bud width (BUDW) and corolla lobe width (CLBW)), SANL, PSTH, PSTL, PSTW, or PSTA. Pins did not differ from homostyles in

SANW or stamen filament length (SFIL). Finally, thrums did not differ from homostyles in PAPIW.

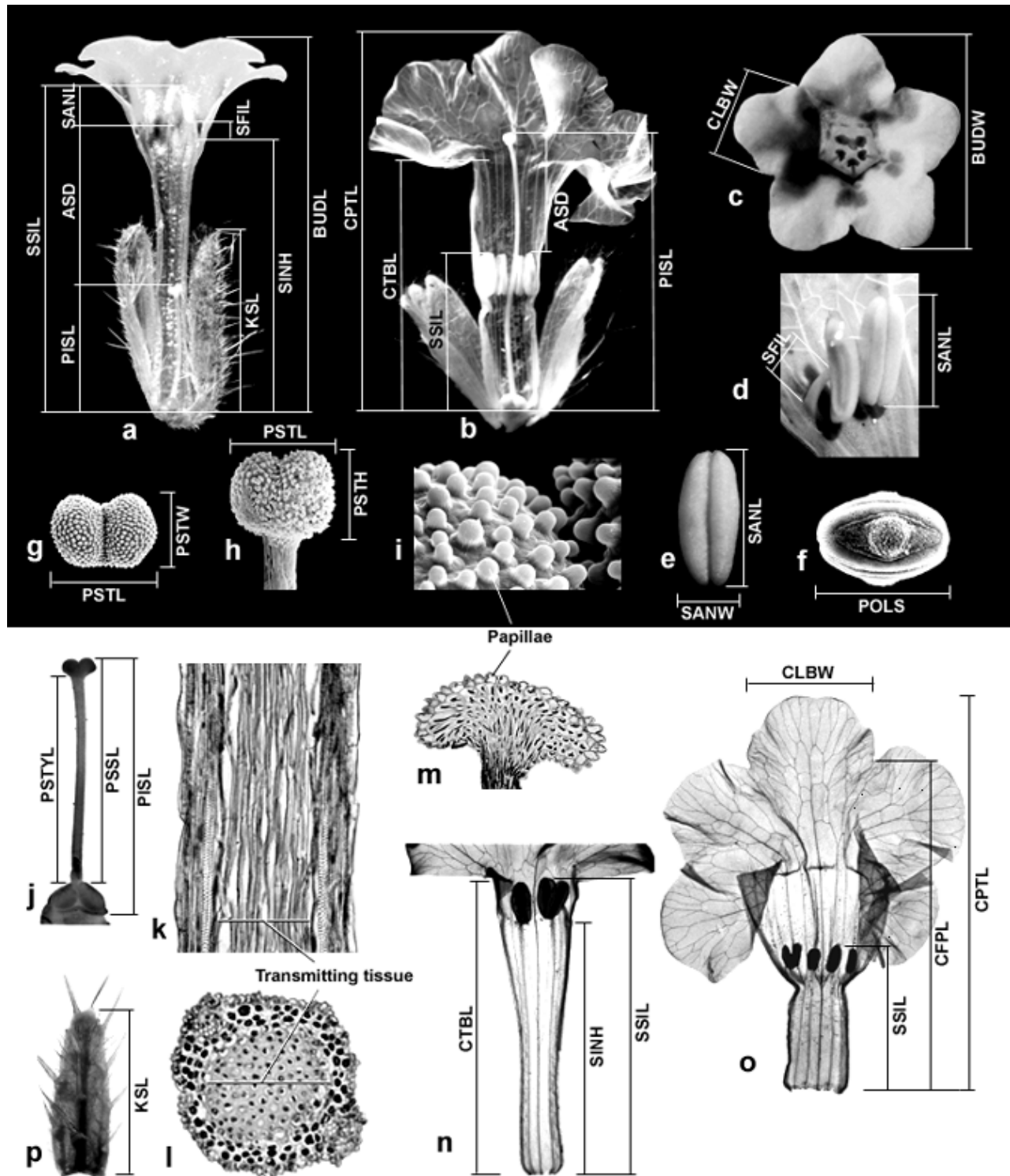
### Differences among lineages

When all morphs were combined, 13 traits differed significantly between at least two of the three lineages. Eight traits showed no difference between any lineage: BUDW, CLBW, SFIL, SINH, SSIL, PAPIL, PSSL, and PSTYL (Appendix C). Five traits (PAPIW, PISL, PSTL, PSTW, and ASD) differed between only one pair of lineages, and this pair always included *A. spectabilis* (L3). Only two traits, PSTH and PSTA, differed between all three pairs of lineages. The remaining 11 traits differed between two of the three pairs of lineages. In 9 of these 11 traits, members of the Tessellatae (L1 and L2) were the similar pair. No trait differed between only L1 and L2. Overall, most (17) floral traits differed significantly between sections Tessellatae (L1 and L2) and Microcarpae (L3). These traits were KSL, flower-length-related traits (BUDL, CFPL, CPTL, CTBL), POLN, anther size (SANL and SANW), PISL, stigma size (PSTH, PSTL, PSTW, PSTA), PAPIW, style cross-sectional size (STYLECA and TRANSCA), and ASD. In contrast, only four traits (POLS, PSTH, PSTA, TRANSCA) differed significantly between L1 and L2 within the Tessellatae (Appendix C).

### Differences among the three morphs within each lineage

The mean size of a trait in a final-sized flower for each of the 10 lineage-morph combinations in five species of *Amsinckia* is presented in Fig. 3. The results of the three

**Fig. 2.** Dissected *Amsinckia* flowers, showing the morphometric characters and the measurement positions of various floral traits. Abbreviations of the traits are given in Table 1. Magnifications vary among photomicrographs. (a) Longitudinal section of a freshly cut thrum flower with natural shape. (b) Longitudinal section of a flattened fixed pin flower. (c) Top view of a freshly cut homostylous flower. (d) Stamen attached to corolla tube. (e) Anther. (f) Scanning electron micrograph (SEM) of pollen grain. (g) Top view of a stigma (SEM). (h) Stigma (SEM). (i) Stigma surface (SEM). (j) Pistil. (k) Longitudinal section of a style (micrograph). (l) Cross section of a style (micrograph). (m) Longitudinal section of a stigma (micrograph). (n) Longitudinal section of a flattened fixed corolla tube. (o) Dissected flattened pin corolla. (p) Sepal.



pairwise comparisons for each trait among the three floral morphs within a lineage are presented in Appendix A.

### Pin versus thrum

Some traits showed the same kinds of difference between pins and thrums in all three lineages. For example, thrums were always larger than pins in the mean value of CTBL, SSIL, SINH, SFIL, TRANSCA, and POLS (Fig. 3; Appendix A). On the other hand, PISL, PSSL, and PSTYL were significantly longer in pins than in thrums. Some traits, such as PAPIL and PAPIW, reversed their size order between pins and thrums depending on lineage. PAPIL and PAPIW in pin flowers were significantly larger than those in thrum flowers in both the lineages *A. furcata* – *A. vernicosa* (L1) and *A. douglasiana* – *A. t. gloriosa* (L2). The size order was reversed in *A. spectabilis* (L3). Some traits were highly significantly different between pins and thrums in one or two lineages but not in another. For instance, BUDL, CPTL, and CFPL in thrums were significantly larger than those in pins in the first two lineages. The ASD in pins was highly significantly larger than that in thrums in the first two lineages. These same traits, however, were not significantly different between pin and thrum morphs in L3 (Appendix A). In contrast, STYLECA was larger in thrums than in pins only in L2 and L3, while a larger SANL was seen only in thrums of L2 (Fig. 3; Appendix A). In addition, POLN produced in pin flowers was significantly greater than that in thrum flowers in L2 but not in the other two lineages (Fig. 3; Appendix A).

Some of the traits did not differ between pins and thrums in any lineage, including KSL, BUDW, CLBW, and PSTH (Fig. 3; Appendix A).

### Distyly versus homostyly

Many of the traits in homostylous flowers were significantly smaller than those in both pin and thrum flowers in all three lineages. They were BUDL, BUDW, CFPL, CLBW, CPTL, CTBL, POLN, and ASD (Fig. 3; Appendix A). These traits, except POLN and ASD, reflect overall flower size, which is smaller in homostyles.

Other traits of homostylous flowers were intermediate in size compared with the same traits of pin and thrum. For example, PISL of homostyles was smaller than that of pins but was larger than that of thrums in all lineages (Fig. 3). Conversely, POLS of homostyles was larger than that of pins but was smaller than that of thrums (Fig. 3).

In all lineages, SFIL of the homostylous flowers was similar to that in pin flowers (Fig. 3). The relative size of some homostylous floral traits, however, varied in different lineages in relation to the size of the same traits of pin and thrum. The size of a trait in a homostylous flower could be similar to the same trait in either a pin or a thrum (e.g., KSL of homostyle was similar to that of both pin and thrum in L1 but similar to only that of pin in L2; in L2, KSL, SANW and STYLECA of the homostyle were similar to those of pin, whereas TRANSCA was similar to that of thrum). Furthermore, homostylous trait size might be similar to the same trait of a pin flower in one lineage but to the same trait of a thrum flower in another lineage (e.g., TRANSCA of homostyle was similar to that of pin in L1 and to that of thrum in L2; Fig. 3).

### The four morphs of *A. spectabilis*

Within *A. spectabilis* 23 of 26 traits were significantly larger in the large homostylous flower than in the small homostylous flower; the exceptions were POLN, PAPIW, and TRANSCA (Fig. 3; Appendix D). In addition, 20 of 26 traits were significantly larger in pins and thrums than in homostyles; the exceptions were SFIL, SINH, SSIL, PISL, PSSL, and PSTYL. Comparison of the 26 traits in large homostylous flowers to those in pins and thrums showed that only 6 (BUDW, SANL, SANW, PSTH, PSTL, and PSTA) were statistically indistinguishable from traits in both pins and thrums, 2 (CLBW and SFIL) were different from pins but not from thrums, 11 (BUDL, CFPL, CPTL, CTBL, PAPIL, PAPIW, PISL, PSSL, PSTYL, PSTW, and STYLECA) were different from thrums but not from pins, 6 (KSL, POLN, SINH, SSIL, TRANSCA, and ASD) were different (all smaller) from both pins and thrums, and 1 (POLS) was larger than in pins but smaller than in thrums.

## Discussion

### Distyly versus homostyly

In the limited number of taxa studied, the distylous flower was larger than the descendant homostylous flower (Ganders 1979a), and this was found in all three lineages of *Amsinckia*. This significance included those traits related to corolla size, anther length, stigma length and surface area, stigma papilla length, pollen size and number, as well as functional anther–stigma distance (Appendix A; Figs. 3 and 4). A few traits, however, including some traits related to pistil length and stamen height, were similar between distyly and homostyly in one or two lineages. The lack of significant difference for these traits between distyly and homostyly resulted from the fact that the size for distyly was averaged from pin and thrum and because the trait size in homostylous flowers was often smaller than that in pins but larger than that in thrums, or vice versa. This averaging diminished or cancelled the actual differences between homostyly and the two floral morphs of distyly in some lineages.

Among those traits that were significantly larger in distyly than in homostyly, many differed in degree among the three lineages of *Amsinckia*. These traits were mostly those associated with flower size, anther length, functional anther–stigma distance, and pollen size and production. Pollen size is particularly interesting, because the pollen grain comprises only a few cells, and polyploidy is often associated with increased cell size. In all three lineages, pollen size was largest in thrums and was smallest in either the pin (L1 and L2) or the homostyle (L3, SH). Compared with distyly, that is, the mean of pins and thrums, pollen size of (small) homostyles was 10.3% smaller in L3, 4.0% smaller in L1, but 12.8% larger in L2, where homostyles are tetraploid.

The literature contains few detailed floral comparisons between distyly and homostyly within the same evolutionary lineage, so it is difficult to compare the evolution of homostyly in *Amsinckia* to that in other species. Nevertheless, within *Amsinckia*, the existence of lineage-specific differences between homostyles and distyles, especially the variation of stigma- and anther-height-related traits between the two styles among lineages, suggests that each of these

**Fig. 3.** Means of mature flower trait sizes among morphs and lineages in *Amsinckia*. See appendices for statistical comparisons. P, pin; T, thrum; LH, large-flowered homostyle; H, homostyle (small-flowered homostyle for L3); L1, lineage of *A. furcata* – *A. vernicosa*; L2, lineage of *A. douglasiana* – *A. t. gloriosa*; L3, lineage of *A. spectabilis*.

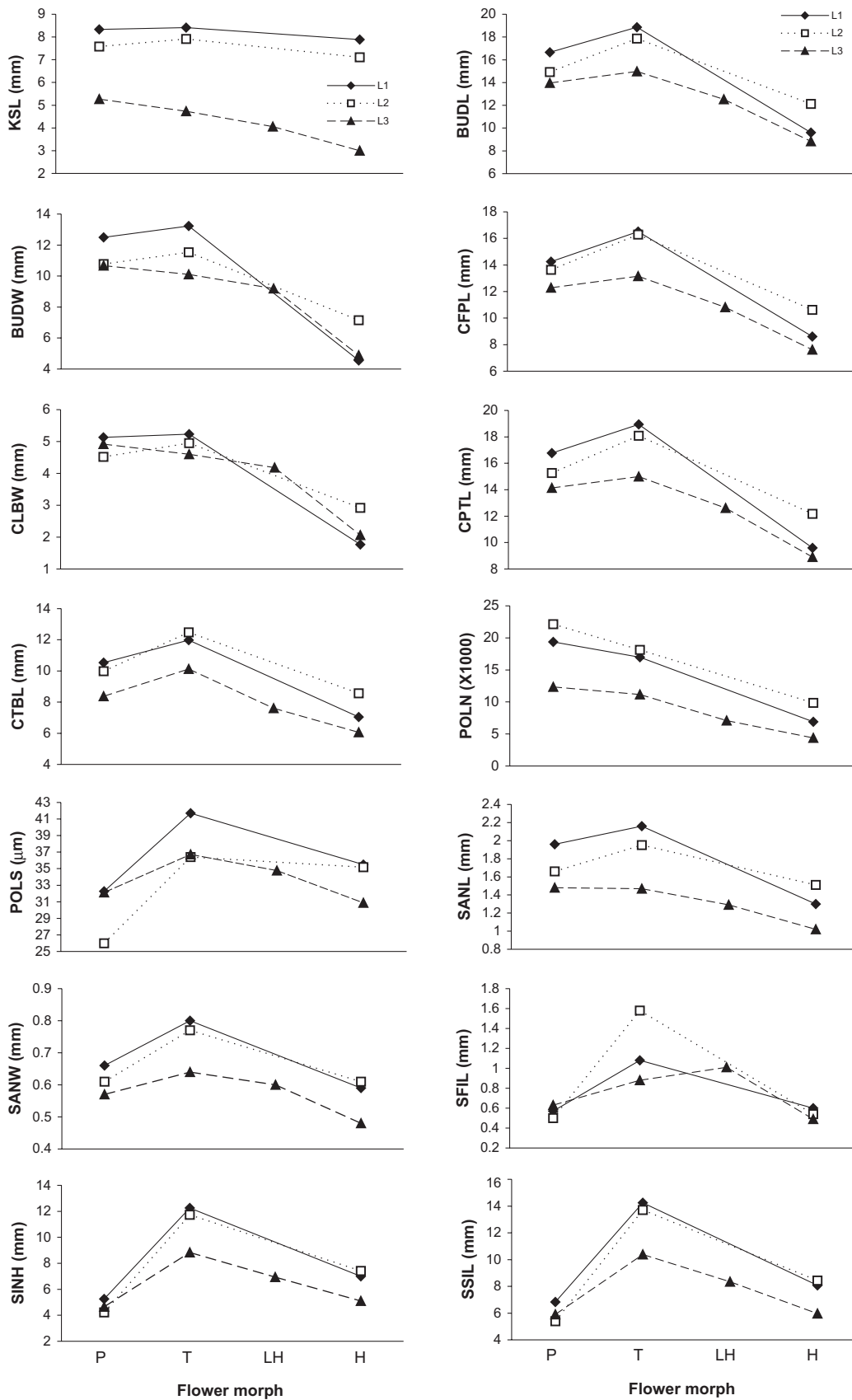
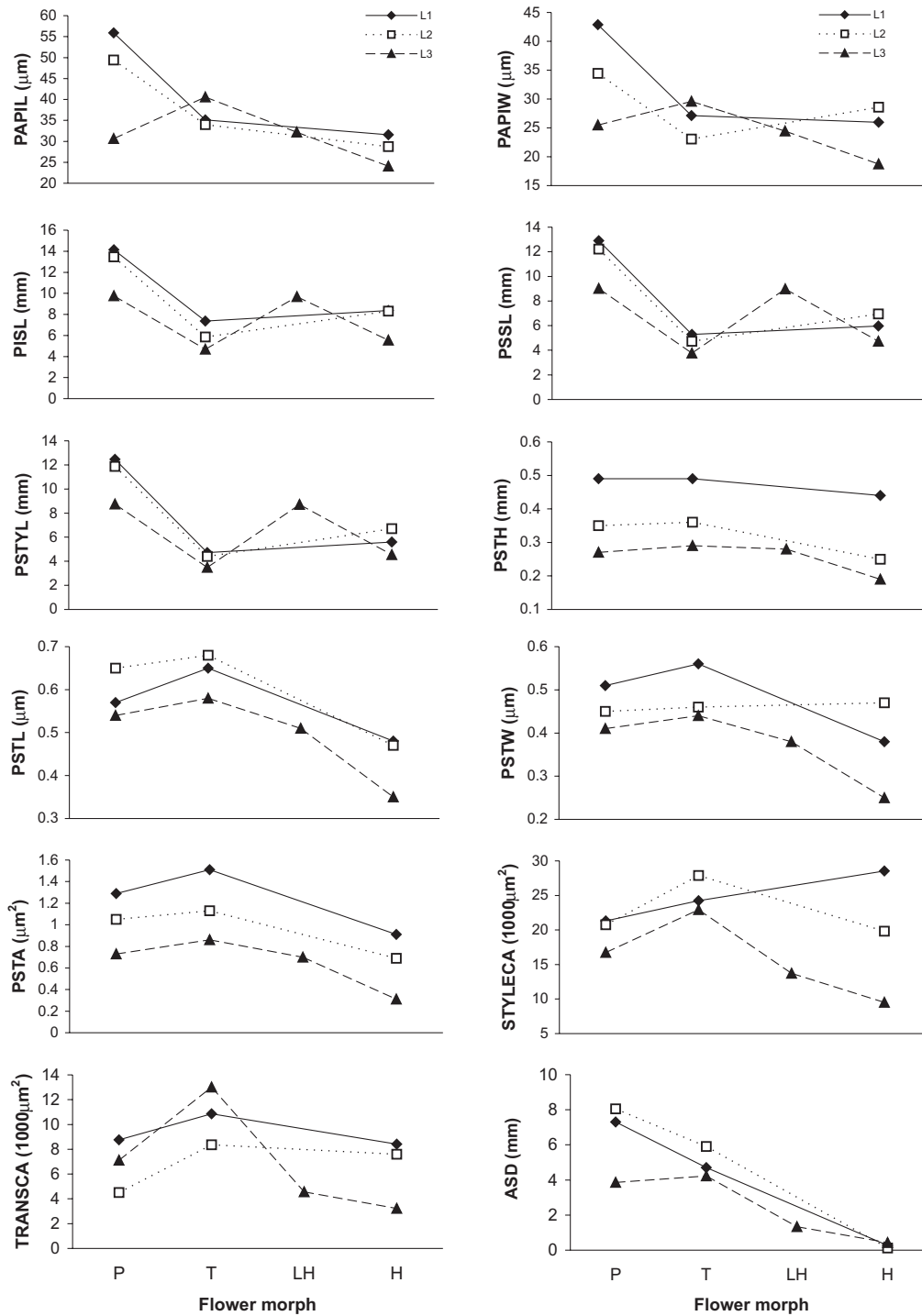


Fig. 3 (concluded).



three lineages is at a different evolutionary stage in the evolution of homostyly, or that the genetic basis differs.

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

**Evolution of homostyly**

Phylogenetic analyses of *Amsinckia* to date suggest that homostyly is derived from distyly (Ray and Chisaki 1957b; Schoen et al. 1997; M.O. Johnston and W.J. Hahn, unpublished results). This is similar to the situation in the genera *Armeria* and *Limonium* (Plumbaginaceae), where monomorphic self-compatible species have derived repeatedly from dimorphic self-incompatible ancestors (Baker 1966). The evolutionary breakdown of distyly to homostyly has been modeled by several authors, including Charlesworth and Charlesworth (1979), who considered in-



**Fig. 4.** A summary of relative size of 26 floral traits among floral morphs and three evolutionary lineages in *Amsinckia*. Note that the trait sizes are based on numerical order without regard to statistical significance. The large homostyle of *A. spectabilis* is omitted. Abbreviations of traits are given in Table 1. L1, lineage 1; L2, lineage 2; L3, lineage 3; P, pin; T: thrum; H, homostyle.

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

breeding depression as well as dominance relations of alleles at three loci that determine stigma height, anther height, and incompatibility reactions. These models probably apply to *Amsinckia*, with the exception that distylous populations are self-compatible and intramorph compatible. When it occurs, self-incompatibility and intramorph incompatibility in *Amsinckia* is limited to cryptic self-incompatibility (Weller and Ornduff 1977; Casper et al. 1988).

The similar anther and stigma heights within the small homostyles are not achieved by combining the ancestral thrum stigma position with the ancestral pin anther position. Instead, the small homostyles possessed values intermediate between pins and thrums for those traits determining anther and stigma height. Specifically, this study included six such traits, three directly related to anther height (SFIL, SINH, SSIL) and three to stigma height (PISL, PSSL, PSTYL). Among these six traits, homostyles were intermediate between pins and thrums within a lineage in 17 of the 18 comparisons (Fig. 4). The single exception was free filament length (SFIL) in *A. spectabilis*. For the remaining 20 traits not directly related to anther or stigma height, small homostyles possessed smaller values than both pins and thrums in 54 of the 60 comparisons (Fig. 4).

The evolution of the homostylous flower can be compared among lineages both in kind and in degree. As described, homostylous *Amsinckia* flowers were intermediate between pins and thrums in absolute anther and stigma height but smaller in almost all other traits. In fact, the size order of traits in pins, thrums, and homostyles of all three lineages was identical in 13 of the 26 studied traits (Fig. 4). Among the remaining 13 traits, eight had the same size order among three morphs between only L1 and L2; two traits showed the same size order between only L1 and L3; two traits showed the same size order only between L2 and L3; and one trait had different size order among three lineages. Despite the similarities in kind among the three lineages, the differences in degree are statistically significant in the majority of traits (interaction term in Appendix B). These results indicate that homostylous flowers differ from distylous flowers in the same direction but to different degrees. Furthermore, the similarities of most floral traits between L1 and L2 may suggest that the evolution of homostyly from distyly in L1 and L2 is similar but differs from that in L3. This conclusion is in accord with the sectional delimitations of Ray and Chisaki (1957b), as well as with a molecular phylogeny of *Amsinckia*, indicating that L1 and L2 are in the same clade, while L3 is in a separate clade (Schoen et al. 1997; M.O. Johnston and W.J. Hahn, unpublished results). Quantitative comparisons of flower development between homostyly and distyly among the three evolutionary lineages also strongly support this (P. Li and M.O. Johnston, unpublished results).

**Pin versus thrum**

There are many more published comparisons between pins and thrums than between pins and thrums on one hand and homostyles on the other. *Amsinckia furcata*, *A. douglasiana*, and distylous *A. spectabilis* exhibit the typical floral morphological syndrome of distylous species. Each of these species consists of two forms of individuals that reciprocally differ in both stigma and anther heights in the flowers. The pin flower has a relatively high stigma and low

**Table 2.** Floral trait size dimorphisms between pin (P) and thrum (T) flowers in three distylous species of *Amsinckia*.

Trait	General	<i>A. furcata</i>	<i>A. douglasiana</i>	<i>A. spectabilis</i>
KSL	— <sup>a</sup>	P ≈ T <sup>b</sup>	P ≈ T	P ≈ T
BUDL	—	T > P <sup>c</sup>	T > P	P ≈ T
BUDW	—	P ≈ T	P ≈ T	P ≈ T
CFPL	—	T > P	T > P	P ≈ T
CLBW	—	P ≈ T	P ≈ T	P ≈ T
CPTL	—	T > P	T > P	P ≈ T
CTBL	T > P	T > P	T > P	T > P
POLN	P > T	P ≈ T	P > T	P ≈ T
POLS	T > P	T > P	T > P	T > P
SANL	T > P <sup>d</sup>	P ≈ T	T > P	P ≈ T
SANW	—	T > P	T > P	P ≈ T
SFIL	T > P	T > P	T > P	P ≈ T
SINH	—	T > P	T > P	T > P
SSIL	T > P	T > P	T > P	T > P
PAPIL	P > T	P > T	P > T	T > P
PAPIW	P > T	P > T	P > T	T > P
PISL	P > T	P > T	P > T	P > T
PSSL	—	P > T	P > T	P > T
PSTYL	—	P > T	P > T	P > T
PSTH	—	P ≈ T	P ≈ T	P ≈ T
PSTL	—	T > P	P ≈ T	P ≈ T
PSTW	—	T ≈ P	P ≈ T	P ≈ T
PSTA	—	T ≈ P	P ≈ T	P ≈ T
STYLECA	T > P	T ≈ P	T > P	T > P
TRANSCA	T > P	T > P	T > P	T > P
ASD	P ≈ T <sup>d</sup>	P > T	P > T	P ≈ T

**Note:** Character traits are defined in Table 1. A general dimorphic status of floral traits in most other studied distylous species (listed as "General") found in the literature is included for comparison. For comparisons without regard to statistical significance, see Fig. 3.

<sup>a</sup>—, either no common results or no information was found.

<sup>b</sup>≈, there was no statistically significant difference between the flower morphs.

<sup>c</sup>>, there was a statistically significant difference between the flower morphs.

<sup>d</sup>Exceptions or opposite results exist in some cases.

anthers, while the thrum flower has high anthers and a low stigma. The difference in pistil length or the height of the stigma between the two floral morphs is caused almost wholly by a difference in style length. The other two components of pistil length, stigma thickness and ovary height (the latter is not included in this paper), were not significantly different between the two morphs in this study, similar to results in other distylous species (Richards and Barrett 1992). The differences of the two floral morphs in the three distylous species also include most of other traits (Appendix A; Fig. 4). The dimorphism of many traits was in agreement with what has been observed in other distylous species (Table 2; Bir Bahadur 1968; Ornduff 1971, 1976; Dulberger 1973, 1974, 1992; Ganders 1979a, 1979c; Philipp and Schou 1981; Murray 1990; Riveros et al. 1995; Thompson and Dommée 2000). In particular, stigma papilla size tended to be larger in pins than in thrums, whereas style cross-sectional area, style transmitting-tissue area, stamen filament length, and pollen were usually larger in thrums.

### Papillae, style, and stigma

The dimorphism of stigma papilla size ( $P > T$  in both papilla length and width) in *Amsinckia furcata* and *A. douglasiana* is similar to that in most other distylous spe-

cies (Table 2; Appendix A), such as *Anchusa hybrida* (Boraginaceae; Dulberger 1970), *Linum pubescens*, *Linum mucronatum* (Linaceae; Dulberger 1967, 1973, 1974), *Linum grandiflorum* (Dulberger 1992), *Lythrum curtisii* (Lythraceae; Ornduff 1978), *Menyanthes trifoliata* L. (Menyanthaceae; Nic Lughadha and Parnell 1989), *Pentstemon lanceolata* (Bir Bahadur 1970a), *Primula malacoides* (Pandey and Troughton 1974), *Primula obconica* (Dowrick 1956), and *Pulmonaria obscura* (Boraginaceae; Olesen 1979). In contrast, the stigma papilla size in *Amsinckia spectabilis* has an opposite dimorphism ( $T > P$  in both papilla length and width). Larger thrum papillae have also been reported in the distylous species *Amsinckia grandiflora* (Ornduff 1976), *Luculia gratissima* (Rubiaceae; Murray 1990), *Reinwardtia indica* (Linaceae; Bir Bahadur et al. 1984), and the stigma-height dimorphic species *Anchusa officinalis* L. (Schou and Philipp 1984). The contrasting results found in *Amsinckia douglasiana* and *A. grandiflora* are interesting because of their very close phylogenetic relationship (Ray and Chisaki 1957b; Schoen et al. 1997).

Because pin flowers in most studied distylous species have longer papillae, it has been suggested that the length of stigma papillae is associated with the degree of style elongation and that the elongation of both stigma papillae and style

may have the same physiological basis (Dulberger 1992). The existence, however, of a negative correlation between stigma papilla length and style length found in *A. spectabilis* and the four other species mentioned above shows that the relationship between papilla and style differs among species. It thus appears doubtful that a common physiological basis underlies the elongation of papilla and style.

Similar to the relationship between stigma papilla size and style length, the relationship between stigma size (surface area) and style length also varies among distylous species. In the three distylous *Amsinckia* species studied here, stigma size did not differ significantly between the two distylous morphs (Table 2; Appendix A), although thrums possessed larger stigmas than pins (Fig. 4). The thrum stigma is larger in several other distylous species, including *A. grandiflora* (Ornduff 1976), *Gelsemium sempervirens* (Loganiaceae; Bir Bahadur et al. 1984), *Hedyotis caerulea* (Ornduff 1980), *M. trifoliata* (Nic Lughadha and Parnell 1989), *Neanotis montholoni* (Rubiaceae; Bir Bahadur et al. 1984), *Primula malacoides* (Primulaceae; Pandey and Troughton 1974), and *Palicourea lasiorrachis* (Rubiaceae; Feinsinger and Busby 1987). In contrast, the pin stigma is larger in *Jepsonia heterandra* (Saxifragaceae; Ornduff 1971), *Linum grandiflorum*, *Linum mucronatum*, *Linum pubescens*, *Plumbago capensis* (Plumbaginaceae; Dulberger 1992), and many other distylous species (Dulberger 1992; Richards and Barrett 1992). The statement that "morph-specific differences in stigma size are closely linked to the size of the stigmatic papillae" (Hermann et al. 1999) is, therefore, not true of all species. The evidence from a variety of distylous species thus suggests that both papilla size and stigma surface area can be modified independently of style length. The degree to which natural selection shapes the relationships among these three characters is unknown.

In contrast to style length, STYLECA and TRANSCA are significantly larger in thrums than in pins in almost all three distylous species of *Amsinckia* except in L3 in which STYLECA does not differ significantly between the two distylous morphs (Table 2). Although information regarding the size of style structures in other distylous species is very limited, the results found in *Amsinckia* are almost the same as what has been observed in *Primula obconica* (Dowrick 1956) and *Linum pubescens* (Dulberger 1992). This could indicate the existence of opposite dimorphisms between the style length and diameter. The inverse correlation between the length and the cross-sectional size (area) of the style, especially the transmitting-tissue size, may have a physical effect on pollen tube growth, perhaps promoting or allowing more pollen-tube growth in thrums. Thus, it could be a factor associated with a lower seed set in pins of some distylous species, such as *Primula obconica* (Dowrick 1956).

### Anther and filament

As in most other heterostylous species (Ganders 1979a), *Amsinckia* flowers are sympetalous. The filaments of stamens are inserted on the corolla tube (Fig. 2d). Thus, filament length, corolla tube length, and stamen insertion height can contribute to anther height in a flower. Filaments are significantly longer in thrum than in pin within L1 and L2, although the difference is not significant in L3. The other two anther-height-related traits are highly significantly larger in

thrum than pin flowers in all three distylous species of *Amsinckia* (Table 2). This indicates that all three traits play important roles in the dimorphism of anther height in the three distylous species. The situation is similar to that in many other heterostylous species (Richards and Barrett 1992) but is different from that in *Jepsonia heterandra* (Ornduff 1971), *Erythroxylum coca* (Ganders 1979b), *Cordia alliodora*, and *Cordia trichotoma* (Gibbs and Taroda 1983) in which the filament is the trait primarily responsible for anther height. It also differs from that in *Hedyotis caerulea* (Ornduff 1980), where stamen insertion height determines the anther height, and in *Cordia sebestena* (Percival 1974), *Gaertnera vaginata* (Rubiaceae; Pailler and Thompson 1997), *Bouvardia ternifolia*, and *Psychotria chiapensis* (Rubiaceae; Faivre 2000), where anther height mainly depends on corolla tube length. Collectively, these studies suggest that the major contributing traits to anther height in a flower differ among heterostylous species.

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

### Pollen

Pollen is larger in thrums than in pins in all three distylous species of *Amsinckia*. Pollen size dimorphism occurs in most distylous plants and thrum pollen is usually larger than pin pollen (Ganders 1979a; Dulberger 1992; McKenna 1992; Richards and Barrett 1992). The ratio of thrum to pin pollen size in most distylous species varies from 1.06 to 1.80 (Dulberger 1992). The ratios in *A. furcata*, *A. douglasiana*, and *A. spectabilis* are about 1.29, 1.40 and 1.14, respectively. The results in *A. furcata* and *A. douglasiana* are similar to what Ray and Chisaki (1957a) and Ganders (1976) found in the same species. Ornduff (1976) also observed similar pollen-size dimorphism in *A. grandiflora*. Our result in *A. spectabilis*, however, differs from Ray and Chisaki's (1957a) observation, which showed no pollen-size dimorphism. It is known that exceptions to pollen-size dimorphism do exist. The absence of pollen-size dimorphism was reported in some distylous plants, such as in *Goniolimon tataricum* (Plumbaginaceae), *Limonium vulgare* (Plumbaginaceae; Weber 1981), and *Linum pubescens* (Dulberger 1973). It was also reported that thrum pollen was larger than pin pollen in one of the two studied populations of *Fauria crista-galli* (Menyanthaceae), but pin pollen was larger than thrum pollen in another nearby population of the same species (Ganders 1979a). A significant difference in pollen size between populations of the same species was also observed in *A. furcata* (Ganders 1976). Recent studies in tristylous species of the Lythraceae (*Decodon verticillatus* (Eckert and Barrett 1994)

and *Lythrum salicaria* (Mal and Hermann 2000)) showed significant effects of populations on pollen size as well. Thus, the difference of the observations within *A. spectabilis* between this study and Ray and Chisaki (1957a) could be an indication of pollen-size variation among populations.

### Differences among lineages

As discussed above, most studied traits had similar dimorphisms between pins and thrums in all three lineages of *Amsinckia* (Table 2, Fig. 4; Appendix A). There were, however, exceptions. Flower length (including petal length and fused petal length) and functional anther–stigma distance were larger in thrum than pin flowers in *A. furcata* and *A. douglasiana* but were not dimorphic in *A. spectabilis*. Differences in flower length were previously reported in *A. furcata*, *A. douglasiana* (Ganders 1976), and *A. grandiflora* (Ornduff 1976). Stigma length, width and area were dimorphic ( $T > P$ ) only in *A. furcata*. The difference in pollen production between pin and thrum occurred only in *A. douglasiana*, in which pins produced more pollen than thrums. Pollen production in *A. furcata* and *A. spectabilis* was not significantly different between pin and thrum morphs in this study, although pins produced slightly more pollen than thrums. Ganders (1975b), however, reported that thrum flowers produced more pollen in these two species. The reason for the contradictory results is not known. In most distylous species, pins produce more pollen than thrums (Ganders 1979a; Dulberger 1992). Sepal length is monomorphic in all three studied distylous species of *Amsinckia*, which is different from what Ganders (1979c) found in another member of the Boraginaceae, *Lithospermum cobrense*, whose sepals were longer in thrum than in pin flowers. Degree of dimorphism between pins and thrums differed among lineages for 13 of 26 traits (13 traits that differed only in one or two of three lineages; Appendix A).

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

Overall, the size order of a trait in pins versus thrums was consistent in all lineages for 18 of 26 traits without regard to statistical significance (Fig. 4). In seven of the eight remaining traits, *A. spectabilis* was the unusual lineage. This pattern is consistent with phylogenies based on chloroplast DNA restriction-site divergence, in which the *A. furcata* and *A. douglasiana* lineages cluster in one clade, and the *A. spectabilis* lineage, in another (Schoen et al. 1997).

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## Appendix A

**Table A1.** Comparisons of pins (P), thrums (T), and homostyles (H) (including the LH form of *A. spectabilis*).

Trait	P and T versus H		P versus T		T versus H		P versus H	
	Raw $p^a$	Boot $p^b$	Raw $p$	Boot $p$	Raw $p$	Boot $p$	Raw $p$	Boot $p$
<b>All lineages</b>								
KSL	0.0001	0.0014	0.8312	1.0000	0.0004	0.0193	0.0001	0.0069
BUDL	0.0001	0.0001	0.0002	0.0104	0.0001	0.0001	0.0001	0.0001
BUDW	0.0001	0.0001	0.9478	1.0000	0.0001	0.0001	0.0001	0.0001
CFPL	0.0001	0.0001	0.0001	0.0010	0.0001	0.0001	0.0001	0.0001
CLBW	0.0001	0.0001	0.9656	1.0000	0.0001	0.0001	0.0001	0.0001
CPTL	0.0001	0.0001	0.0004	0.0211	0.0001	0.0001	0.0001	0.0001
CTBL	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
POLN	0.0001	0.0001	0.0001	0.0066	0.0001	0.0001	0.0001	0.0001
POLS	0.6537	1.0000	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
SANL	0.0001	0.0001	0.1253	0.9761	0.0001	0.0001	0.0001	0.0001
SANW	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0022	0.0972
SFIL	0.0001	0.0047	0.0001	0.0001	0.0001	0.0001	0.1531	0.9902
SINH	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
SSIL	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0015
PAPIL	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
PAPIW	0.0001	0.0013	0.0001	0.0001	0.4259	1.0000	0.0001	0.0001
PISL	0.0002	0.0122	0.0001	0.0001	0.0001	0.0007	0.0001	0.0001
PSSL	0.0001	0.0010	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
PSTYL	0.0001	0.0031	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
PSTH	0.0001	0.0011	0.4627	1.0000	0.0008	0.0396	0.0001	0.0024
PSTL	0.0001	0.0001	0.0015	0.0674	0.0001	0.0001	0.0001	0.0001
PSTW	0.0001	0.0016	0.5484	1.0000	0.0001	0.0062	0.0005	0.0278
PSTA	0.0001	0.0001	0.3065	1.0000	0.0001	0.0001	0.0001	0.0001
STYLECA	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
TRANSCA	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
ASD	0.0001	0.0001	0.0001	0.0012	0.0001	0.0001	0.0001	0.0001
<b>Lineage 1: <i>furcata-vernica</i></b>								
KSL	0.0460	0.7534	0.7661	1.0000	0.0711	0.8741	0.0800	0.9008
BUDL	0.0001	0.0001	0.0001	0.0009	0.0001	0.0001	0.0001	0.0001
BUDW	0.0001	0.0001	0.0745	0.8856	0.0001	0.0001	0.0001	0.0001
CFPL	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
CLBW	0.0001	0.0001	0.6745	1.0000	0.0001	0.0001	0.0001	0.0001
CPTL	0.0001	0.0001	0.0001	0.0015	0.0001	0.0001	0.0001	0.0001
CTBL	0.0001	0.0001	0.0001	0.0003	0.0001	0.0001	0.0001	0.0001
POLN	0.0001	0.0001	0.0291	0.6102	0.0001	0.0001	0.0001	0.0001
POLS	0.0001	0.0078	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
SANL	0.0001	0.0001	0.0063	0.2201	0.0001	0.0001	0.0001	0.0001
SANW	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0057	0.2050
SFIL	0.0131	0.3719	0.0001	0.0003	0.0001	0.0043	0.6982	1.0000
SINH	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
SSIL	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
PAPIL	0.0001	0.0001	0.0001	0.0001	0.0132	0.3750	0.0001	0.0001
PAPIW	0.0001	0.0001	0.0001	0.0001	0.4349	1.0000	0.0001	0.0001
PISL	0.0001	0.0001	0.0001	0.0001	0.0193	0.4812	0.0001	0.0001
PSSL	0.0001	0.0001	0.0001	0.0001	0.0335	0.6566	0.0001	0.0001
PSTYL	0.0001	0.0001	0.0001	0.0001	0.0325	0.6478	0.0001	0.0001
PSTH	0.0177	0.4579	0.7960	1.0000	0.0575	0.8201	0.0176	0.4549
PSTL	0.0001	0.0001	0.0004	0.0220	0.0001	0.0001	0.0001	0.0094
PSTW	0.0001	0.0001	0.0014	0.0638	0.0001	0.0001	0.0001	0.0001
PSTA	0.0001	0.0001	0.0075	0.2514	0.0001	0.0001	0.0001	0.0033
STYLECA	0.0002	0.0125	0.0215	0.5141	0.0113	0.3375	0.0001	0.0012
TRANSCA	0.0431	0.7333	0.0005	0.0260	0.0020	0.0862	0.6286	1.0000
ASD	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

Table A1 (concluded).

Trait	P and T versus H		P versus T		T versus H		P versus H	
	Raw $p^a$	Boot $p^b$	Raw $p$	Boot $p$	Raw $p$	Boot $p$	Raw $p$	Boot $p$
<b>Lineage 2: douglasiana-tessellata</b>								
KSL	0.0527	0.8114	0.1611	0.9919	0.0165	0.4498	0.3030	1.0000
BUDL	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0003
BUDW	0.0001	0.0001	0.0809	0.9166	0.0001	0.0001	0.0001	0.0001
CFPL	0.0001	0.0001	0.0001	0.0005	0.0001	0.0001	0.0001	0.0003
CLBW	0.0001	0.0001	0.0343	0.6777	0.0001	0.0001	0.0001	0.0001
CPTL	0.0001	0.0001	0.0001	0.0006	0.0001	0.0001	0.0001	0.0005
CTBL	0.0001	0.0006	0.0001	0.0032	0.0001	0.0001	0.0127	0.3823
POLN	0.0001	0.0001	0.0004	0.0249	0.0001	0.0001	0.0001	0.0001
POLS	0.0001	0.0001	0.0001	0.0001	0.0020	0.0936	0.0001	0.0001
SANL	0.0001	0.0013	0.0001	0.0056	0.0001	0.0001	0.0220	0.5373
SANW	0.0001	0.0004	0.0001	0.0001	0.0001	0.0001	0.9766	1.0000
SFIL	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.5411	1.0000
SINH	0.0579	0.8399	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
SSIL	0.0012	0.0622	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
PAPIL	0.0001	0.0001	0.0001	0.0001	0.0016	0.0805	0.0001	0.0001
PAPIW	0.9264	1.0000	0.0001	0.0003	0.0093	0.3077	0.0020	0.0940
PISL	0.0001	0.0053	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
PSSL	0.0001	0.0011	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
PSTYL	0.0001	0.0024	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
PSTH	0.0001	0.0001	0.4331	1.0000	0.0001	0.0001	0.0001	0.0007
PSTL	0.0001	0.0001	0.3245	1.0000	0.0001	0.0001	0.0001	0.0008
PSTW	0.8614	1.0000	0.9097	1.0000	0.9185	1.0000	0.8412	1.0000
PSTA	0.0004	0.0228	0.4782	1.0000	0.0004	0.0233	0.0043	0.1755
STYLECA	0.0809	0.9166	0.0001	0.0007	0.0034	0.1440	0.7248	1.0000
TRANSCA	0.0476	0.7802	0.0001	0.0001	0.2213	0.9993	0.0001	0.0004
ASD	0.0001	0.0001	0.0001	0.0013	0.0001	0.0001	0.0001	0.0001
<b>Lineage 3: spectabilis</b>								
KSL	0.0001	0.0001	0.1497	0.9763	0.0001	0.0007	0.0001	0.0001
BUDL	0.0001	0.0001	0.2496	0.9977	0.0001	0.0005	0.0001	0.0074
BUDW	0.0001	0.0012	0.5334	1.0000	0.0005	0.0225	0.0001	0.0045
CFPL	0.0001	0.0001	0.2692	0.9987	0.0001	0.0004	0.0001	0.0045
CLBW	0.0001	0.0011	0.4636	1.0000	0.0005	0.0226	0.0001	0.0034
CPTL	0.0001	0.0001	0.3320	0.9998	0.0001	0.0006	0.0001	0.0063
CTBL	0.0001	0.0001	0.0011	0.0426	0.0001	0.0001	0.0010	0.0389
POLN	0.0001	0.0001	0.1044	0.9298	0.0001	0.0001	0.0001	0.0001
POLS	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0803	0.8766
SANL	0.0001	0.0001	0.8682	1.0000	0.0001	0.0025	0.0001	0.0017
SANW	0.0042	0.1410	0.0279	0.5555	0.0006	0.0249	0.2393	0.9972
SFIL	0.9572	1.0000	0.0350	0.6294	0.1967	0.9929	0.2275	0.9967
SINH	0.0432	0.6969	0.0001	0.0001	0.0001	0.0001	0.0033	0.1153
SSIL	0.0237	0.5032	0.0001	0.0001	0.0001	0.0001	0.0181	0.4261
PAPIL	0.0001	0.0007	0.0001	0.0001	0.0001	0.0001	0.3635	1.0000
PAPIW	0.0001	0.0011	0.0006	0.0266	0.0001	0.0001	0.0153	0.3814
PISL	0.5118	1.0000	0.0001	0.0001	0.0003	0.0144	0.0053	0.1687
PSSL	0.4380	1.0000	0.0001	0.0001	0.0002	0.0087	0.0053	0.1696
PSTYL	0.3851	1.0000	0.0001	0.0001	0.0001	0.0067	0.0051	0.1632
PSTH	0.0033	0.1153	0.2308	0.9968	0.0025	0.0901	0.0669	0.8301
PSTL	0.0001	0.0015	0.2387	0.9972	0.0001	0.0023	0.0013	0.0529
PSTW	0.0001	0.0007	0.2287	0.9967	0.0001	0.0017	0.0008	0.0349
PSTA	0.0001	0.0027	0.1377	0.9669	0.0001	0.0028	0.0043	0.1429
STYLECA	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
TRANSCA	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
ASD	0.0001	0.0001	0.2822	0.9991	0.0001	0.0001	0.0001	0.0001

**Note:** Character traits are defined in Table 1.

<sup>a</sup>Raw  $p$  values are the original value from each comparison of means.

<sup>b</sup>Boot  $p$  values are adjusted for a maximum familywise error rate of  $\alpha = 0.05$  using the bootstrapping procedure of Westfall and Young (1993), where the family consists of 104  $p$  values (26 traits  $\times$  4 pairwise contrasts/trait).

**Appendix B**

**Table B1.** Probability values from ANOVA for effect of morph (P, T, H), lineage (L1, L2, L3) and interaction on each of 26 traits.

Trait	Morph		Lineage		Morph × lineage	
	Raw <i>p</i>	Adjusted <i>p</i>	Raw <i>p</i>	Adjusted <i>p</i>	Raw <i>p</i>	Adjusted <i>p</i>
KSL	<<0.000 01	<<0.000 01	<0.000 01	<0.000 01	0.071 98	0.282 40
BUDL	<<0.000 01	<<0.000 01	<0.000 01	<0.000 01	<0.000 01	<0.000 01
BUDW	<<0.000 01	<<0.000 01	0.057 01	0.114 02	<0.000 01	<0.000 01
CFPL	<<0.000 01	<<0.000 01	<0.000 01	<0.000 01	0.000 01	0.000 11
CLBW	<<0.000 01	<<0.000 01	0.618 08	0.618 08	0.000 03	0.000 23
CPTL	<<0.000 01	<<0.000 01	<0.000 01	<0.000 01	<0.000 01	<0.000 01
CTBL	<<0.000 01	<<0.000 01	<0.000 01	<0.000 01	0.001 16	0.008 10
POLN	<<0.000 01	<<0.000 01	<0.000 01	<0.000 01	0.000 06	0.000 49
POLS	<<0.000 01	<<0.000 01	<0.000 01	<0.000 01	<0.000 01	<0.000 01
SANL	<<0.000 01	<<0.000 01	<0.000 01	<0.000 01	<0.000 01	<0.000 01
SANW	<<0.000 01	<<0.000 01	<0.000 01	<0.000 01	0.010 29	0.061 74
SFIL	<<0.000 01	<<0.000 01	0.031 29	0.093 86	<0.000 01	<0.000 01
SINH	<<0.000 01	<<0.000 01	<0.000 01	<0.000 01	<0.000 01	<0.000 01
SSIL	<<0.000 01	<<0.000 01	<0.000 01	<0.000 01	<0.000 01	<0.000 01
PAPIL	<<0.000 01	<<0.000 01	<0.000 01	<0.000 01	<0.000 01	<0.000 01
PAPIW	<<0.000 01	<<0.000 01	<0.000 01	<0.000 01	<0.000 01	<0.000 01
PISL	<<0.000 01	<<0.000 01	<0.000 01	<0.000 01	0.000 02	0.000 18
PSSL	<<0.000 01	<<0.000 01	<0.000 01	0.000 01	<0.000 01	<0.000 01
PSTYL	<<0.000 01	<<0.000 01	0.000 01	0.000 06	<0.000 01	<0.000 01
PSTH	<<0.000 01	<<0.000 01	<0.000 01	<0.000 01	0.056 48	0.282 40
PSTL	<<0.000 01	<<0.000 01	<0.000 01	0.000 06	0.213 46	0.426 93
PSTW	0.000 96	0.000 96	0.001 83	0.007 34	0.060 74	0.282 40
PST	<<0.000 01	<<0.000 01	<0.000 01	<0.000 01	0.315 29	0.426 93
STYLECA	<<0.000 01	<<0.000 01	<0.000 01	<0.000 01	<0.000 01	<0.000 01
TRANSCA	<<0.000 01	<<0.000 01	<0.000 01	<0.000 01	<0.000 01	<0.000 01
ASD	<<0.000 01	<<0.000 01	<0.000 01	<0.000 01	<0.000 01	<0.000 01

**Note:** Character traits are defined in Table 1.

<sup>a</sup>Raw *p* values are the original value from each ANOVA.

<sup>b</sup>Adjusted *p* values are adjusted for a maximum familywise error rate of  $\alpha = 0.05$  for each effect using the Bonferroni procedure.

**Appendix C**

**Table C1.** Comparisons of lineages (clades), including all morphs.

Trait	L1 and L2 versus L3		L1 versus L2		L1 versus L3		L2 versus L3	
	Raw <i>p</i>	Boot <i>p</i>	Raw <i>p</i>	Boot <i>p</i>	Raw <i>p</i>	Boot <i>p</i>	Raw <i>p</i>	Boot <i>p</i>
KSL	0.0001	0.0001	0.0584	0.8348	0.0001	0.0001	0.0001	0.0001
BUDL	0.0001	0.0048	0.8897	1.0000	0.0004	0.0192	0.0009	0.0444
BUDW	0.0133	0.3816	0.4130	1.0000	0.0098	0.3077	0.0895	0.9300
CFPL	0.0001	0.0021	0.5299	1.0000	0.0006	0.0331	0.0001	0.0080
CLBW	0.2703	0.9996	0.8388	1.0000	0.2846	0.9999	0.4072	1.0000
CPTL	0.0001	0.0046	0.9856	1.0000	0.0004	0.0245	0.0007	0.0336
CTBL	0.0001	0.0001	0.2470	0.9992	0.0001	0.0045	0.0001	0.0002
POLN	0.0001	0.0001	0.0030	0.1231	0.0001	0.0001	0.0001	0.0001
POLS	0.0047	0.1752	0.0001	0.0001	0.0001	0.0001	0.0013	0.0631
SANL	0.0001	0.0001	0.1099	0.9599	0.0001	0.0001	0.0001	0.0001
SANW	0.0001	0.0002	0.6648	1.0000	0.0001	0.0006	0.0001	0.0065
SFIL	0.3451	1.0000	0.0154	0.4210	0.6459	1.0000	0.0438	0.7485
SINH	0.0155	0.4229	0.3209	1.0000	0.1048	0.9544	0.0114	0.3441
SSIL	0.0106	0.3250	0.4300	1.0000	0.0647	0.8617	0.0109	0.3324
PAPIL	0.0058	0.2068	0.1824	0.9950	0.0026	0.1109	0.0781	0.9039
PAPIW	0.0004	0.0194	0.2777	0.9997	0.0003	0.0183	0.0090	0.2877
PISL	0.0004	0.0215	0.0108	0.3309	0.0001	0.0012	0.0757	0.8980
PSSL	0.0151	0.4172	0.0700	0.8808	0.0024	0.1030	0.2468	0.9992



**Table C1** (concluded).

Trait	L1 and L2 versus L3		L1 versus L2		L1 versus L3		L2 versus L3	
	Raw <i>p</i>	Boot <i>p</i>	Raw <i>p</i>	Boot <i>p</i>	Raw <i>p</i>	Boot <i>p</i>	Raw <i>p</i>	Boot <i>p</i>
PSTYL	0.0241	0.5571	0.1026	0.9508	0.0052	0.1899	0.2720	0.9996
PSTH	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0002
PSTL	0.0001	0.0020	0.0861	0.9231	0.0039	0.1526	0.0001	0.0011
PSTW	0.0001	0.0027	0.3835	1.0000	0.0001	0.0041	0.0020	0.0886
PSTA	0.0001	0.0001	0.0002	0.0120	0.0001	0.0001	0.0001	0.0006
STYLECA	0.0001	0.0001	0.8343	1.0000	0.0001	0.0001	0.0001	0.0001
TRANSCA	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.2547	0.9993
ASD	0.0002	0.0098	0.9549	1.0000	0.0007	0.0352	0.0015	0.0731

**Note:** Character traits are defined in Table 1. L1, lineage 1; L2, lineage 2; L3, lineage 3.

<sup>a</sup>Raw *p* values are the original value from each comparison of means.

<sup>b</sup>Boot *p* values are adjusted for a maximum familywise error rate of  $\alpha = 0.05$  using the bootstrapping procedure of Westfall and Young (1993), where the family consists of 104 *p* values (26 traits  $\times$  4 pairwise contrasts/trait).

## Appendix D

**Table D1.** Comparisons within *A. spectabilis* of the four morphs P, T, LH, and SH.

Variable	P and T versus SH and LH													
	SH and LH		P versus T		T versus SH		P versus SH		T versus LH		P versus LH		SH versus LH	
	Raw <i>p</i>	Boot <i>p</i>	Raw <i>p</i>	Boot <i>p</i>	Raw <i>p</i>	Boot <i>p</i>	Raw <i>p</i>	Boot <i>p</i>	Raw <i>p</i>	Boot <i>p</i>	Raw <i>p</i>	Boot <i>p</i>	Raw <i>p</i>	Boot <i>p</i>
KSL	0.0001	0.0001	0.0054	0.2700	0.0001	0.0001	0.0001	0.0067	0.0001	0.0055	0.0001	0.0001	0.0001	0.0001
BUDL	0.0001	0.0001	0.0699	0.9455	0.0001	0.0001	0.0001	0.0003	0.0001	0.0089	0.0121	0.4696	0.0001	0.0002
BUDW	0.0001	0.0001	0.1957	0.9996	0.0001	0.0001	0.0001	0.0003	0.0424	0.8476	0.0018	0.1141	0.0001	0.0001
CFPL	0.0001	0.0001	0.0904	0.9744	0.0001	0.0001	0.0001	0.0005	0.0001	0.0055	0.0055	0.2741	0.0001	0.0002
CLBW	0.0001	0.0001	0.1033	0.9840	0.0001	0.0001	0.0001	0.0002	0.0378	0.8193	0.0006	0.0456	0.0001	0.0001
CPTL	0.0001	0.0001	0.1252	0.9926	0.0001	0.0001	0.0001	0.0004	0.0001	0.0127	0.0088	0.3800	0.0001	0.0002
CTBL	0.0001	0.0001	0.0001	0.0122	0.0001	0.0001	0.0001	0.0005	0.0001	0.0002	0.0635	0.9313	0.0006	0.0459
POLN	0.0001	0.0001	0.1000	0.9820	0.0001	0.0001	0.4443	1.0000	0.0001	0.0107	0.0001	0.0002	0.0108	0.4344
POLS	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0002	0.0001	0.0001	0.0001	0.0001
SANL	0.0001	0.0001	0.8177	1.0000	0.0001	0.0001	0.0013	0.0858	0.0028	0.1636	0.0015	0.1000	0.0001	0.0018
SANW	0.0001	0.0077	0.0018	0.1150	0.0001	0.0001	0.0001	0.0002	0.0572	0.9127	0.1546	0.9976	0.0001	0.0004
SFIL	0.9138	1.0000	0.0001	0.0111	0.0001	0.0002	0.0001	0.0001	0.0217	0.6496	0.0001	0.0002	0.0001	0.0001
SINH	0.0071	0.3276	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0017	0.0001	0.0002	0.0001	0.0020
SSIL	0.0012	0.0798	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0018	0.0001	0.0002	0.0001	0.0003
PAPIL	0.0001	0.0002	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0002	0.3419	1.0000	0.0004	0.0311
PAPIW	0.0001	0.0002	0.0004	0.0285	0.0001	0.0001	0.0001	0.0026	0.0003	0.0225	0.4455	1.0000	0.0032	0.1798
PISL	0.0785	0.9616	0.0001	0.0001	0.0081	0.3605	0.0360	0.8053	0.0001	0.0001	0.7921	1.0000	0.0001	0.0001
PSSL	0.0165	0.5609	0.0001	0.0001	0.0008	0.0574	0.0102	0.4206	0.0001	0.0001	0.8874	1.0000	0.0001	0.0001
PSTYL	0.0086	0.3748	0.0001	0.0001	0.0003	0.0200	0.0043	0.2308	0.0001	0.0001	0.8438	1.0000	0.0001	0.0001
PSTH	0.0001	0.0002	0.0211	0.6419	0.0001	0.0001	0.0001	0.0001	0.3000	1.0000	0.1765	0.9988	0.0001	0.0001
PSTL	0.0001	0.0001	0.0404	0.8357	0.0001	0.0001	0.0001	0.0002	0.0012	0.0783	0.1523	0.9973	0.0001	0.0001
PSTW	0.0001	0.0001	0.0162	0.5556	0.0001	0.0001	0.0001	0.0001	0.0002	0.0146	0.0874	0.9712	0.0001	0.0001
PSTA	0.0001	0.0001	0.0061	0.2970	0.0001	0.0001	0.0001	0.0001	0.0010	0.0703	0.4870	1.0000	0.0001	0.0001
STYLECA	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0011	0.0753	0.0001	0.0013
TRANSCA	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0002	0.0068	0.3185
ASD	0.0001	0.0001	0.1378	0.9953	0.0001	0.0001	0.0001	0.0047	0.0001	0.0001	0.0001	0.0001	0.0001	0.0017

**Note:** Character traits are defined in Table 1. P, pin; T, thrum; LH, large homostylous flower; SH, small homostylous flower.

<sup>a</sup>Raw *p* values are the original value from each comparison of means.

<sup>b</sup>Boot *p* values are adjusted for a maximum familywise error rate of  $\alpha = 0.05$  using the bootstrapping procedure of Westfall and Young (1993), where the family consists of 182 *p* values (26 traits  $\times$  7 pairwise contrasts/trait).