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EVOLUTION OF THE SELF-POLLINATING FLOWER IN *CLARKIA XANTIANA* (ONAGRACEAE). I. SIZE AND DEVELOPMENT OF FLORAL ORGANS[1]

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Clarkia xantiana has two subspecies that differ in breeding system: ssp. *xantiana*, which is outcrossing, and ssp. *parviflora*, which is self-fertilizing. Outcrossing is the ancestral breeding system for the genus *Clarkia*. Flowers of ssp. *parviflora* have characteristics commonly associated with selfing taxa: they are smaller and have little temporal and spatial separation between mature anthers and stigma (dichogamy and herkogamy, respectively). Flower morphology and development were studied in four populations of each subspecies to establish the developmental changes that occurred in the evolution of selfing. In particular, we sought to evaluate the hypothesis that the selfing flower may have arisen as a byproduct of selection for rapid maturation in the arid environment occupied by ssp. *parviflora*. This hypothesis predicts that development time should be reduced in spp. *parviflora* relative to ssp. *xantiana*. We also sought to compare the pattern of covariation of flower morphology and development between subspecies to that within subspecies. Similar within vs. between patterns of covariation could be indicative of developmental or functional constraints on the independent evolution of floral parts. In spite of significant variation among populations within subspecies, the subspecies clearly differ in flower morphology and development. All floral organs, except ovaries, are smaller in ssp. *parviflora* than in ssp. *xantiana*. The flower plastochron, the duration of flower development from bud initiation to anthesis, and the duration of protandry are all shorter in ssp. *parviflora* than in ssp. *xantiana*. Maximum relative growth rates are higher for all organs in ssp. *parviflora* than in ssp. *xantiana*. Thus, progenesis (i.e., via a reduction in development time) is combined with growth acceleration in the evolution of the selfing flower. Since reduced development time and growth acceleration both allow selfing flowers to mature earlier than outcrossing ones, selection for early maturation may have contributed to the evolution of the selfing flower form. The pattern of trait covariation differs within spp. *parviflora* relative to the patterns within spp. *xantiana* and between the two subspecies, suggesting that floral parts can and have evolved independently of one another.

Key words: *Clarkia*; development; growth rate; heterchrony; outcrossing; progenesis; selfing; trait covariation.

The evolution of self-pollinating taxa from outcrossing progenitors is well documented in angiosperms (Stebbins, 1970; Jain, 1976; Lloyd, 1979; Wyatt, 1983; Lande and Schemske, 1985; Wyatt, 1988; Diggle, 1992). Flowers of selfing taxa typically have smaller corollas, reduced herkogamy (spatial separation between mature anthers and stigma), and reduced dichogamy (temporal separation between anther dehiscence and stigma receptivity) compared to flowers of related outcrossing taxa (Ornduff, 1969; Wyatt, 1983). Reduced herkogamy and dichogamy facilitate self-pollination because mature pollen is brought into close contact with the receptive stigma (Schoen, 1982; Gottlieb, 1984; Holtsford and Ellstrand, 1989). Small corolla size is thought to evolve secondarily in selfing taxa as a means of limiting costly expenditures on pollinator attraction.

Related outcrossing and self-pollinating taxa also often differ in life history and ecology (Ornduff, 1969; Hill, Lord, and Shaw, 1992). For example, selfing taxa commonly reach reproductive maturity at an earlier age and at a smaller vegetative size compared to related outcrossers (Stebbins, 1974; Schoen, 1982; Wyatt, 1983; Holtsford and Ellstrand, 1992), and they occur in habitats at the geographic or ecological limit of the outcrosser's range (Stebbins, 1950; Baker, 1955; Lewis and Lewis, 1955; Vasek, 1964, 1968; Lloyd, 1965; Solbrig and Rollins, 1977; Schoen, 1982; Wyatt, 1988). One hypothesis for the evolution of self-pollination in geographically or ecologically marginal environments is the greater reproductive assurance afforded self-pollinating plants in habitats where pollinators may be scarce (Stebbins, 1950; Baker, 1955). An alternative hypothesis is that small flower size and self-fertilization arise as by-products of selection for rapid maturation in marginal environments (Arroyo, 1973; Guerrant, 1989). In marginal environments, selection may favor individuals that can complete reproduction early, in advance of impending stress (Arroyo, 1973; Gould, 1977; Guerrant, 1989; Eckhart, Geber, and Jonas, 1996). One way of achieving early reproduction is through a decrease in developmental time, both of individual organs (e.g., flower organs) and of whole organisms (Gould, 1977). In flowers, shorter organ development time is likely to reduce herkogamy. In addition, in the absence of other developmental changes, a decrease in development time results in smaller floral organs and hence smaller flowers. Under this second hypothesis, the characteristic features of the self-pollinating flower evolve as a by-product of selection for early maturation, and the evolution of self-pollination follows from the changes in floral form (Arroyo, 1973; Guerrant, 1989). The rapid maturation hypothesis can also explain the differences in flowering time and whole-plant size that often distinguish related self-pollinating and outcrossing taxa.

Ancestor-descendant changes in mature size and form, such as those that distinguish the flowers of self-pollinating and outcrossing taxa, often arise through changes in the timing or

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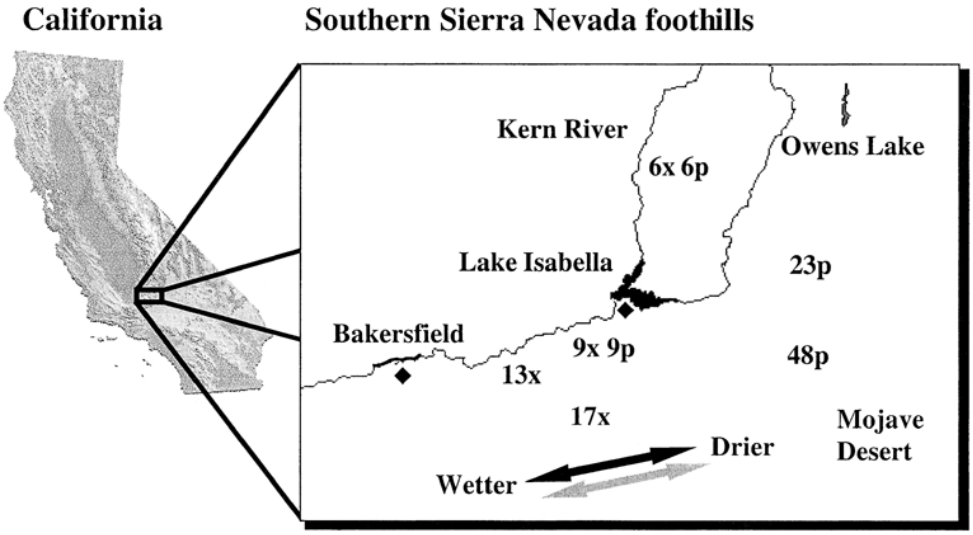


Fig. 1. *Clarkia xantiana* is distributed on a southwest-to-northeast transect in Kern and Tulare counties in the Southern Sierra Nevada Mountains of California. Outcrossing populations are from the wetter, western zone of allopatry (13x and 17x) and the central sympatric region (6x and 9x). Selfing populations are from the central zone of sympatry (6p and 9p) and the drier, eastern allopatric zone (23p and 48p).

rate of development, i.e., through heterochrony (deBeer, 1958; Gould, 1977; Alberch et al., 1979; Lord and Hill, 1987; Guerrant, 1988; Poethig, 1988; Lord, Eckard, and Crone, 1989; Kellogg, 1990; Hill, Lord, and Shaw, 1992; Gallardo, Dominguez, and Muñoz, 1993; Sherry, 1994; Itoh et al., 1998; Klingenberg, 1998). A reduction in the duration of organ development is a form of heterochrony that results in progenesis, i.e., in the early maturation of organs at a smaller size and juvenilized form. If self-pollination evolves from outcrossing as a result of selection for rapid maturation, then the selfing flower should be a progenetic form of the outcrossing flower. On the other hand, the reproductive assurance hypothesis for the evolution of self-pollination does not make any specific prediction about the nature of the developmental differences between flowers of related outcrossing and self-pollinating taxa.

In this study, we compare floral organ size and development between the self-pollinating subspecies *Clarkia xantiana* ssp. *parviflora* and its outcrossing progenitor *C. x.* ssp. *xantiana* to address whether progenesis accounts for subspecific differences in flower size and form. *Clarkia xantiana* ssp. *parviflora* inhabits marginal (arid) environments and flowers earlier and at a smaller plant size than its outcrossing progenitor (Eckhart and Geber, 2000). It also displays floral characters that are typical of the selfing habit, namely small petals and reduced herkogamy and protandry. Lastly, its mature flowers resemble juvenile stages of the flower buds of *C. x.* ssp. *xantiana* in several respects. For example, close proximity of anthers and stigma is a characteristic of selfing flowers at anthesis and of outcrossing flower buds pre-anthesis. The smaller petals of selfing flowers also resemble developing petals of outcrossing buds.

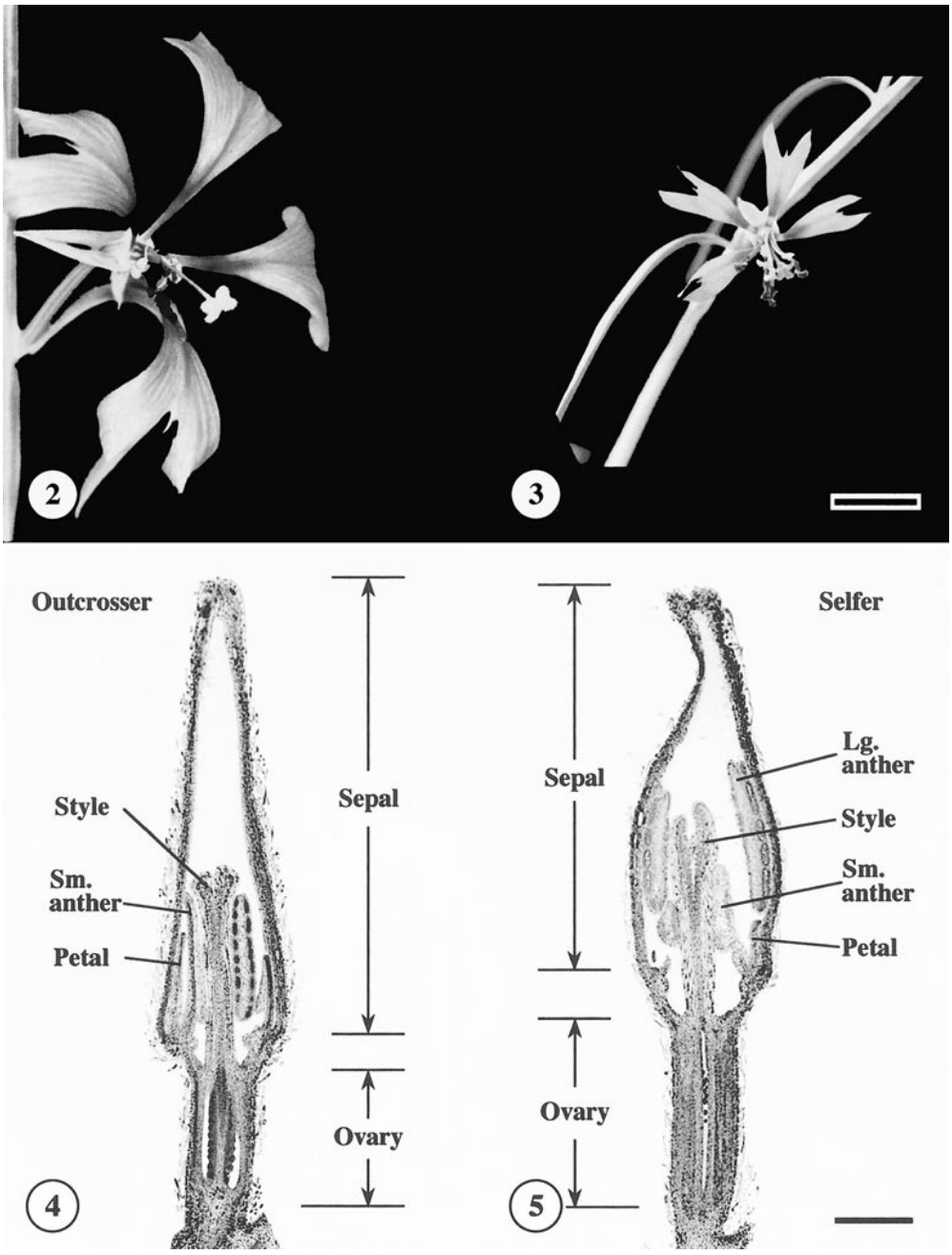
We chose to examine flower size and development in multiple populations of the two subspecies because both subspecies exhibit variation in floral characters, and it is not known which outcrossing lineage might have given rise to self-pollinating forms (Gottlieb, 1984), or even whether selfing has evolved more than once in *C. xantiana.*

Another reason for quantifying variation among populations was to compare the within-subspecies pattern of covariation in morphological and developmental traits to the pattern of covariation between subspecies. Such comparisons can give a sense of the degree of developmental or functional integration among flower parts (Armbruster and Schwaegerle, 1996). For example, similar patterns of covariation within and between subspecies would be indicative either of developmental constraints on the independent evolution of floral parts, or of strong functional integration between floral parts, while dissimilar patterns of covariation would indicate that floral organs can and have changed independently of one another.

MATERIALS AND METHODS

Clarkia is a genus of winter annuals native to the west coast of North America (Lewis and Lewis, 1955). Systematic studies of the genus indicate that self-pollinating taxa are evolutionarily derived from outcrossing ones (Lewis and Lewis, 1955; Vasek, 1964; Moore and Lewis, 1965; Vasek and Harding, 1976; Vasek and Weng, 1988) and that self-pollinating species, subspecies, or populations have arisen independently from outcrossing taxa a minimum of ten times (Lewis and Lewis, 1955; Lewis and Raven, 1958; Moore and Lewis, 1965; Vasek and Harding, 1976; Allen, Ford, and Gottlieb, 1991).

The species—*Clarkia xantiana* Gray is native to the southern Sierra Nevada Mountains in Kern and Tulare Counties of California and occurs at a limited number of sites in the Transverse Ranges (Liebre and San Gabriel Mountains) east of Los Angeles (Lewis and Lewis, 1955; Eckhart and Geber, 2000) (Fig. 1). Flowers of *C. xantiana* have an inferior ovary, a small hypanthium, a whorl of four sepals that enclose a whorl of four petals, a whorl of four large stamens, a whorl of four smaller stamens, and a style. *Clarkia xantiana* ssp. *xantiana* (hereafter referred to as ''outcrosser'') has flowers with larger sepals, petals, anthers and styles (Figs. 2–3), and greater herkogamy and protandry than those of *C. x.* ssp. *parviflora* (hereafter referred to as ''selfer'') (Moore and Lewis, 1965; Gottlieb, 1984; Sytsma, Smith, and Gottlieb, 1990; Lewis and Raven, 1992). Although outcrossing rates have not been measured in the two subspecies, the selfer readily sets seed whereas the outcrosser has much lower fruit set in the absence of pollinators. Electrophoretic studies also reveal that populations of the selfer are either monomorphic or have very low levels of polymorphism at enzyme loci, whereas populations of the outcrosser have



Figs. 2–5. Outcrossing and selfing flowers of *Clarkia xantiana*. At stigma maturation, the outcrossing subspecies (ssp. *xantiana*) has flowers with longer petals and styles (Fig. 2) than those of the selfing subspecies (ssp. *parviflora*) (Fig. 3). Outcrossers have several days of protandry so anthers have dehisced and shriveled several days prior to the stage shown in Fig. 2. The selfer stigma matures amid the dehiscent anthers thus ensuring self-pollination (Fig. 3). Bar = 1.0 cm. 4–5. Equivalent-sized flower buds of outcrossing and selfing subspecies of *Clarkia xantiana*. At equivalent sizes, flowers of the outcrossing subspecies are older (plastochron 10, age = 11.2 d; Fig. 4) than those of the selfing subspecies (plastochron 8, age = 7.4 d; Fig. 5). Growth of all floral organs is accelerated in the selfer. In particular, ovaries of the selfers develop very quickly relative to those of the outcrosser so that the ovary:sepal ratio is greater in the selfer. Large anthers are out of the plane of section in Fig. 4 but overtop the style by approximately the same margin as seen in Fig. 5. Bar = 0.5 mm.

much higher levels of polymorphism (Gottlieb, 1984; Travers and Geber unpublished data). These results are consistent with the notion that *C. x.* ssp. *parviflora* is highly selfing.

In the Sierra Nevada, *C. xantiana* is distributed along a south–west to north–east transect from the western foothills to the Pacific Crest Divide on the eastern end of the Kern River Basin (Eckhart and Geber, 2000). This transect corresponds to a west-to-east moisture gradient. Outcrossers occur alone (allopatrically) in the wetter, western end of the species range; selfers are allopatric in the more arid eastern region. A zone of sympatry is found along the western shore of Isabella Lake and the North Fork of the Kern River where the two subspecies frequently co-occur in mixed populations. At these sites, selfers always flower earlier than outcrossers, with little overlap in flowering time (Eckhart and Geber, 2000).

Populations—We selected four populations of each subspecies that spanned the range of sepal and ovary lengths measured during an earlier greenhouse

study of 45 populations. Each population is referred to by a site number (e.g., 6). Outcrossing populations of subspecies *xantiana* are designated by an ''x'' (e.g., 6x), while selfing populations of subspecies *parviflora* are designated by a ''p'' (e.g., 6p). Within each subspecies, two out of four populations were selected from the zone of sympatry and two from allopatric regions (Fig. 1). Sympatric populations were chosen from sites where the two subspecies naturally grow side by side or in close proximity (sites 6 and 9). The two allopatric outcrossing populations (13x and 17x) come from the western, wetter end of the species' distribution in the Sierra Nevada, while the two allopatric selfing populations come from the eastern, drier end of the distribution. Subspecies *parviflora* occurs in two flower colors, pink (as in the outcrosser) and white. This study was limited to an examination of pink-flowered individuals, although white-flowered plants occurred at three of the four *parviflora* sites (6p, 9p, 23p).

Plant culture—Seeds were collected in the wild in 1996 and 1997 and stored dry at 5°C. Prior to sowing in December 1997, we bulked 2–3 seeds per maternal parent for a total of 216 seeds for each population. The seeds from each population were then sown onto the surface of moist Metro Mix 360 (Scotts-Sierra Horticultural Products Co., Maryville, Ohio, USA) in flats, and placed covered in a refrigerator at 5°C for 7 d. Flats were then transferred to growth chambers where germination took place under short daylength (11 h), low temperature (22°C day/15°C night), and high (90%) relative humidity. After 1 wk, 49 plants were selected at random from each population and were transplanted into a planting mixture (1 Metro Mix: 1 fritted clay, Oil Dri, Softco Mead, Elmira, New York, USA) in 156-mL plastic tubes, and growth chamber conditions were changed to longer daylength (15 h), higher temperature (26°C day/20°C night), and lower relative humidity (60%). Transplants were watered from below for the first few weeks. Later on, plants were watered from above 1–2 times per day and were fed a dilute solution of Excel 15–5–15 (Peters) once every 2–3 d.

Experimental design—Transplanted seedlings were split at random into two cohorts: six plants per population were assigned to a macroscopic study of flower development and the rest were reserved for a microscopic study of floral development. Plants for both studies were subdivided between two growth chambers with identical settings. Growth chamber groups were treated as blocks in statistical analyses.

Macroscopic study of flower development—The purpose of this study was to provide estimates of the duration of flower development and of the sizes of mature flower organs. In order to calculate estimates of development time, floral organs were measured daily on the six plants per population. Prior to flower opening, only sepals and ovaries were measured. Measurements began when sepals were first visible, usually at 1–2 mm length, and continued through stigma receptivity, and were made on the first, third, fifth, seventh, and ninth flower of each plant.

Three measures of development time were obtained: (1) the flower plastochron (i.e., the time interval between the initiation of successive flowers on the main stem of each plant), (2) the duration of flower bud development from a size of ~1–2 mm until flower opening, and (3) the duration of protandry (i.e., the length of time between large anther dehiscence and stigma receptivity). The flower plastochron is a measure of development time that pertains to inflorescence ontogeny, while flower bud development time and protandry pertain more directly to the flower itself.

For floral organ size we used (1) the lengths of the ovary, sepal, petal, large anther, large filament, and style at flower opening, and (2) the maximum lengths of these organs. For ovary, petal, and style, maximum lengths were obtained at stigma maturation. Sepals attain their maximum length at flower opening and so we only use this one measurement of size. Large anthers and filaments in outcrossing populations reach maximum length just prior to anther dehiscence, a few days before stigma receptivity. In selfing populations, we were usually unable to measure anther lengths, because they had already begun to dehisce at flower opening.

Microscopic study of flower development—The purpose of this study was to obtain measures of floral organ length from bud initiation to flower opening in order to construct organ growth curves and estimate the relative growth rates of organs. In order to harvest buds of known age from each population, it was necessary that plants from a given population be at approximately equivalent stages of development at the outset of the study. Toward this end, we selected from each population a subset of 12 individuals on a single day when the sepal length of their third flower was between 2.5 and 3.5 mm. This day was designated as day 1 for that population. Day 1 was not the same for all populations because of population differences in the time of initiation of the first flower bud and in the rate and duration of flower bud development. The selection of 12 individuals of similar developmental stage undoubtedly eliminated some of the variation present in a population, but this could not be avoided. Developmental phenology is in fact very uniform among plants within populations, especially of the selfer: under greenhouse or growth chamber conditions, plants from a given population begin flowering within a few days of each other (Geber, unpublished data).

The 12 plants from each population were randomly assigned to one of six harvests, with two plants harvested on day 1, and two plants harvested at successive 5-d intervals until the last harvest on day 26. The 26-d duration was selected so that the oldest (most basal) flowers would have opened on plants assigned to the last harvest. For the early harvests, all flower buds and the apical region of the main inflorescence of each plant were collected. At later harvests, on rare occasions, the youngest buds and apical region were discarded if the inflorescence showed signs of slowed development and only the oldest buds were collected. The mean nodal positions of buds ($\pm$ SE) at each of the six harvests were: 14.6 $\pm$ 0.8 at harvest 1; 19.5 $\pm$ 0.7 etc. at harvest 2; 23.4 $\pm$ 1.2 at harvest 3; 27.9 $\pm$ 1.3 at harvest 4; 31.2 $\pm$ 0.9 at harvest 5; and 29.0 $\pm$ 1.9 at harvest 6. The range of nodal positions from which buds were collected did not differ between populations.

All buds up to 15 mm in length and all apices were fixed by vacuum infiltration of 4% glutaraldehyde in 0.1 mol/L cacodylate buffer in 0.15 mol/L sucrose. For all but the smallest buds, one sepal was removed to allow better penetration of the fixative to internal organs. Fixed buds were rinsed, dehydrated through an ethanol series, and embedded in JB-4 resin (Electron Microscopy Sciences, Ft. Washington, Pennsylvania, USA). Flower buds were sectioned at 2–3 μm using an Olympus CUT 4060 rotary microtome equipped with a glass knife holder. Sections were stained with 0.1% toluidine blue in 0.005% sodium carbonate (pH 5.8). We measured the lengths of the sepal, ovary, petal, style, and the combined length of the large anther and filament (anther and filament are indistinguishable during their earliest developmental stages) to the nearest 0.05 mm using a Zeiss Stemi 2000C dissecting microscope equipped with an eyepiece micrometer (Figs. 4 and 5). Flower buds >15 mm were too large to embed and were fixed in formalin–acetic acid–ethanol, and floral organs were measured after dissection, as described above.

For each organ measured, length data from successive flower buds within plants and from different plants from the six harvests were pooled in order to construct a plot of organ length vs. time throughout the period of bud development. In pooling data within and between plants, we made the assumption that flower buds at successive nodes and from different plants were representative of different ages of a flower at the same node position. This assumption is justified only if floral bud development is the same for all buds, independent of node position (see Analysis section).

Analysis—All statistical analyses were performed with SAS statistical software (JMP Statistics and Graphics Guide, 1996; or SAS Statistical Software, 1996).

Development time and floral organ sizes—Flower plastochron was calculated after the method of Hill and Lord (1990), using data from the macroscopic study of flower development. Plots of $\log_e$(sepal length + 1) vs. time were linear during the period that included sepals of 3 mm length, indicating that growth was exponential during this phase. The 3 mm length was chosen as the reference length. The time interval (t_{ref}) from bud initiation to attainment of the reference length was calculated as follows:

$$t_{ref} = t_2 - (t_2 - t_1)\left(\frac{\log_e[L(n_i, t_2)] - \log_e(R)}{\log_e[L(n_i, t_2)] - \log_e[L(n_i, t_1)]}\right)$$

where n_i = node of the youngest organ (sepal) with length exceeding the reference length, t_1 = time when organ at n_i was just short of reference length, t_2 = time when organ at n_i first equaled or exceeded the reference length, $L(n_i, t_1)$ = length of organ at n_i at time when it was just short of the reference length, $L(n_i, t_2)$ = length of organ at n_i at time when it first equaled or exceeded the reference length, and R = reference length.

The plastochron is estimated as the difference in t_{ref} between flowers at successive nodes. In this case, where flowers were measured at every other node, the plastochron was calculated as the difference in t_{ref} divided by 2. For each population, an estimate of the plastochron was obtained based on the intervals between the first and third, third and fifth, fifth and seventh, and seventh and ninth flowering nodes.

Each of the three developmental time parameters, and the size of each organ, was analyzed by a repeated-measures analysis of variance, where the repetition consisted of measurements at successive flower nodes. The statistical model included the following effects: block, subspecies, population nested within subspecies, node position of the flower, and interactions between node position and subspecies, and between position and population. Block was treated as a random effect, and the other factors were treated as fixed effects. Population was treated as fixed because populations were selected explicitly to span the known range of floral variation rather than at random. The covariance structure of the data was assumed to be compound symmetric, because no improvement in fit was obtained with an autoregressive structure (Crowder and Hand, 1993). For anther length, we only analyzed data from outcrossing populations, and the subspecies and subspecies by position interaction were therefore dropped from the statistical model.

In assessing the statistical significance of a particular effect on a trait, we accounted for the fact that we were performing multiple analyses on a set of nonindependent traits by adjusting the significance levels of our tests by the sequential Bonferonni procedure (Rice, 1989).

Test of flower bud equivalency—To test the assumption of equivalency of floral organ development at successive node positions, we compared the allometry of sepal to ovary length of buds from the different harvests of the microscopic study of flower development. We reasoned that if the allometry of sepal to ovary did not differ between harvests, then flower development was likely to be similar across node positions, because, for a given bud size, buds from earlier harvests originated from more basal node positions compared to buds from later harvests. Allometric relationships were determined by least-squares linear regression of $\log_e$(sepal length) vs. $\log_e$(ovary length).

Because the relationship between log-transformed sepal and ovary lengths was curvilinear when flower buds in all size classes were included in a single regression, we divided the data into three size classes of flower buds: sepal $\leq$ 6 mm, 6 mm $<$ sepal $\leq$ 12 mm, sepal $>$ 12 mm. Only outcrosser buds were included in the largest sepal size class.

To compare organ allometry among harvests in each of the three bud size classes, we compared the intercepts and slopes of the sepal-to-ovary allometric relationship using multivariate analysis of covariance, with block, subspecies, and population nested within subspecies as main effects, and the mean flower node position of buds from each harvest as the covariate, since mean node position increases with later harvests. A statistically significant effect of the covariate would indicate that floral allometry, and by inference, flower development, varies across node positions.

In pooling data from several buds within plants in the regression analyses, the assumption of statistical independence of observations was violated since flowers within plants are likely to be correlated. We do not consider this to be a serious problem because of the strong intrinsic relationship between sepal and ovary sizes (Niklas, 1994).

Analysis of organ relative growth rate—To construct curves of organ growth it was necessary to establish the age of each fixed flower bud. The age of a bud was determined from our estimates of flower plastochron obtained in the macroscopic study of flower development and the bud's nodal position relative to the youngest bud primordium on the inflorescence axis from which the bud was collected. The youngest bud was arbitrarily assigned an age of 1. The bud and its floral organs at j node positions basal to the youngest bud was assigned a plastochron age equal to the product of the plastochron and j ($1 \leq j \leq 36$). We used the population mean value of the plastochron to determine the plastochron ages of the buds collected from plants from that population.

Data from all flowers of each population and block were pooled, and for each organ, organ size was plotted against plastochron age. To reduce the systematic increase in variance associated with larger organs, organ size was log-transformed [$\log_e$(organ size + 1)]. Gompertz curves were fit to the data for each population and block, as follows:

$$\log_e(\text{organ size} + 1) = ae^{-be^{-rt}}$$

where a, b, and r are nonlinear curve parameters and t is the plastochron age of the organ. Estimates of the parameters a, b, and r were obtained by nonlinear curve fitting with JMP statistical software (SAS, 1996). The maximum relative growth rate (natural log transformed of data taken in millimetres per day) of an organ occurs at the inflection point of the Gompertz curve and is estimated as (ra/e) (Tipton, 1984). For each organ (ovary, sepal, petal, combined long anther and filament, and style), two estimates of ra/e were obtained per population, with one estimate from each block.

To compare maximum relative growth rates of all organs among populations and subspecies, we performed a multivariate analysis of variance of relative growth rate estimates for the five organs. The statistical model included effects of block (random) and of subspecies and population nested within subspecies (fixed). We also performed univariate analyses of variance on each organ because the multivariate analysis indicated significant effects of subspecies and population on organ growth rates.

Morphological and developmental covariation—We used principal components analysis to ask whether subspecific differences in developmental time and organ growth rate paralleled differences in flower size. For a set of p characters (e.g., organ sizes), principal component analysis defines a new set of p axes (principal component axes or PCA), which are linear combinations of the original traits, i.e., $\text{PCA}_i = \Sigma a_{ij}\text{X}_j$, where X_j is the jth original trait (i, $j = 1$ to p). The absolute value of the coefficient (or loading), a_{ij}, measures the importance of the jth character in defining the direction of the ith axis. The first PCA axis defines the direction of greatest trait variation among individuals in the data set; the second PCA axis is orthogonal to the first in the direction of the second highest level of variation, and so on, up to the pth PCA (Harris, 1985). Principal components analysis is especially useful if the first few axes account for a major portion of variation in traits, since this variation can then be summarized with respect to a smaller set of composite variables defined by the axes. Plotting of individuals (in our case, populations) with respect to these first few axes allows one to visualize their location in the space defined by the axes (e.g., ''morphological'' or ''developmental space''). In analyses of organ size or growth rate variation, the first PCA often has positive loadings for all traits and can therefore be thought of as a composite measure of overall size or magnitude. Our purpose in doing principal components analysis was (1) to determine how well subspecies were differentiated in ''morphological'' and ''developmental'' space and (2) to ask whether the differentiation with respect to flower development (growth rate and duration) paralleled the differentiation in flower size.

We conducted separate principal components analyses on organ size traits (PCA_{SZ}), on organ growth rates (PCA_{GR}), and on development time (duration of bud development and protandry, PCA_{DT}). For analyses of size and growth rates we only included data on the lengths of ovaries, sepals, petals, and styles, since these were the organs for which we had both final size and growth rate data in both subspecies. In the analysis of development time, we excluded the plastochron, since this is a developmental character of the whole plant, rather than of individual flower buds. The principal component axes were derived from the covariances of mean trait values for each population and block. We report results on the first one or two axes from each analysis, because, in every case, these accounted for $>$ 90% of the trait variation.

TABLE 1. Tests of fixed effects in repeated-measures analysis of variance of (a) the three traits of floral development time, (b) floral organ sizes at flower opening, (c) and maximum floral organ sizes. The denominator degrees of freedom for *F* tests were as follows: for plastochron, df = 135; for bud development time and sepal length, df = 198; for protandry and ovary length, petal length, petal width, filament length and style length, df = 181; for anther length, df = 85. Numerator degrees of freedom are indicated as Num df for each effect. The statistical significance (*) of *F* tests has been adjusted by the sequential Bonferroni procedure at tablewide $\alpha = 0.05$).

A) Developmental time

Source	Plastochron until opening		Bud development		Protandry	
	Num df	*F*	Num df	*F*	Num df	*F*
Subspecies	1	61.71*	1	441.13*	1	374.57*
Population (subspecies)	6	6.74*	6	4.67*	6	2.13
Node	3	6.63*	4	4.11*	4	1.44
Node × Subspecies	3	3.84*	4	2.46	4	0.31
Node × Population (subspecies)	18	2.33*	24	1.46	24	1.07

B) Organ sizes at flower opening

Source	Num df	Ovary length *F*	Sepal length *F*	Petal length *F*	Petal width *F*	Filament length *F*	Anther length *F*	Style length *F*
Subspecies	1	8.02	206.42*	248.02*	441.14*	34.85*	—	93.30*
Population (Ssp.)	6	16.91*	618.73*	18.00*	31.94*	10.13*	11.36*	14.34*
Node	4	5.09*	1.80	14.74*	5.59*	0.85	5.86*	2.64
Node × Subspecies	4	5.16*	0.95	0.70	0.85	1.05	—	0.28
Node × Pop. (Ssp.)	24	1.54	1.78	1.33	1.04	1.11	2.39*	1.26

C) Maximum floral organ sizes up to the time of stigma maturation

Source	Num df	Ovary length *F*	Petal length *F*	Petal width *F*	Filament length *F*	Anther length *F*	Style length *F*
Subspecies	1	5.29	413.59*	596.09*	30.06*	—	411.88*
Population (Ssp.)	6	17.02*	16.28*	38.82*	16.88*	4.85	15.73*
Node	4	4.72*	15.71*	11.32*	3.87*	1.01	2.27
Node × Subspecies	4	4.41*	2.10	0.88	2.41	—	1.57
Node × Pop. (Ssp.)	24	1.24	0.97	1.27	2.10*	2.04	0.54

RESULTS

Development time and flower size—Subspecies differed in virtually all traits related to development time and organ size (Tables 1 and 2). Compared to outcrossers, selfers had shorter plastochron, bud development time, and protandry (Tables 1A, 2A) and smaller sepals, petals, anthers, and styles (Figs. 4, 5, Tables 1B, 2B). Stamen filaments were longer in the selfer than in the outcrosser at flower opening (Table 2B) but this relationship was reversed by the time of stigma maturation (Table 2C). Ovaries also tended to be longer in the selfer at flower opening, but smaller at stigma maturation than outcrossers (Table 2B, C), but the differences were not statistically significant (Table 1B, C). Floral organs grew less between flower opening and stigma maturation in selfers than outcrossers (Table 2B vs. C). Fourteen out of the 16 traits also differed significantly among populations within subspecies (Tables 1 and 2).

Ten traits differed among flowers at different node positions. The only exceptions were protandry, filament and anther lengths (at flower opening and at stigma maturation, respectively), and style length (Tables 1 and 2). In spite of the position effect and the occasional position by subspecies or position by population interaction effects on development time and organ size, there did not appear to be a consistent pattern of effect across node positions. In other words, development time and organ sizes did not increase or decrease consistently with node position. In particular, plastochron interval did not differ consistently across nodes (Fig. 6). Block effects were never significant in the analyses (results not shown).

Test of flower bud equivalency—Within each size class of buds, plots of log-transformed sepal vs. ovary lengths were linear, and the coefficients of determination from least-squares linear regressions were almost always > 0.90. Univariate analyses of slopes and intercepts were performed for each flower bud size class because multivariate analyses revealed significant subspecies and population effects on the sepal-to-ovary allometric parameters. There was no effect of mean flower node position on allometric parameters in any of the three size classes of buds (Table 3).

Organ relative growth rate—Gompertz curves provided good fits to the data on log-transformed organ size vs. time (see Fig. 7 for illustration of curve fit to sepal growth). Univariate (and multivariate) analyses revealed strong subspecies differences in maximum relative growth rates of organs (Table 4). Relative growth rates of all organs were higher in selfers than in outcrossers (Table 5). In addition, there was significant variation in relative growth rates among populations within subspecies for all organs but petals (Table 4).

Morphological and developmental differences between subspecies—In the three sets of principal component axes for organ size, relative growth rates, and development time, the loadings of traits on the first axis were positive, indicating that these axes reflect variation in overall flower size, relative growth rate, and development time (Table 6). In all three cases the first axis accounted for nearly 90% or more of the variation in traits, and it is along this first axis that much of the differentiation between subspecies occurs. For organ size (Fig. 8a) all outcrossing populations have higher scores on the first axis ($PCA1_{SZ}$), except for population 6x, which is very similar in overall size to 48p. These two populations are separated along $PCA2_{SZ}$ which is principally a measure of ovary size (see high loading for ovary, Table 6). Population 48p has relatively large ovaries, while population 6x has relatively small ovaries. For organ relative growth rates, the second axis reflects the dif-

TABLE 2. Means (± 1 SE) of (a) developmental time parameters and floral organ sizes at (b) flower opening and at (c) stigma maturation.

A) Developmental time parameters (days)

Pop./Subspecies	Flower plastochron	Bud development until opening	Protandry
6p	0.93 ± 0.06	22.69 ± 0.27	1.17 ± 0.08
9p	0.57 ± 0.07	24.83 ± 0.40	1.77 ± 0.10
23p	0.73 ± 0.03	22.13 ± 0.17	1.97 ± 0.09
48p	0.85 ± 0.04	24.07 ± 0.26	2.77 ± 0.12
Selfer mean	0.76 ± 0.03	23.44 ± 0.17	1.97 ± 0.07
6x	0.89 ± 0.04	30.83 ± 0.38	6.92 ± 0.29
9x	1.14 ± 0.06	33.47 ± 0.30	7.17 ± 0.23
13x	1.06 ± 0.04	31.40 ± 0.24	6.60 ± 0.14
17x	1.09 ± 0.07	31.23 ± 0.61	6.89 ± 0.25
Outcrosser mean	1.05 ± 0.03	31.73 ± 0.22	6.89 ± 0.12

B) Floral organ measurements (mm) at flower opening

Pop./Subspecies	Ovary length	Sepal length	Petal length	Petal width	Lg. filament length	Lg. anther length	Style length
6p	15.37 ± 0.31	12.45 ± 0.10	10.22 ± 0.21	5.74 ± 0.18	6.71 ± 0.12	n/a	5.72 ± 0.13
9p	13.82 ± 0.35	11.25 ± 0.10	10.37 ± 0.15	6.23 ± 0.13	6.63 ± 0.13	n/a	6.12 ± 0.14
23p	15.97 ± 0.19	10.43 ± 0.10	10.97 ± 0.12	6.93 ± 0.10	5.63 ± 0.09	n/a	4.43 ± 0.14
48p	17.35 ± 0.23	14.65 ± 0.14	12.75 ± 0.18	8.48 ± 0.14	7.47 ± 0.15	5.50 ± 0.50	6.48 ± 0.17
Selfer mean	15.71 ± 0.18	12.19 ± 0.16	11.16 ± 0.13	6.94 ± 0.12	6.60 ± 0.09	n/a	5.67 ± 0.11
6x	9.44 ± 0.18	14.00 ± 0.23	12.68 ± 0.26	9.40 ± 0.21	4.78 ± 0.12	6.79 ± 0.12	5.92 ± 0.21
9x	16.58 ± 0.48	17.27 ± 0.21	18.47 ± 0.23	18.78 ± 0.54	5.80 ± 0.13	7.90 ± 0.12	7.98 ± 0.24
13x	16.23 ± 0.31	18.07 ± 0.28	17.20 ± 0.31	16.20 ± 0.32	6.10 ± 0.19	8.00 ± 0.10	9.35 ± 0.20
17x	14.88 ± 0.52	16.35 ± 0.35	16.84 ± 0.41	13.80 ± 0.24	6.13 ± 0.17	7.77 ± 0.09	10.25 ± 0.36
Outcrosser mean	14.49 ± 0.34	16.42 ± 0.19	16.45 ± 0.25	14.79 ± 0.37	5.73 ± 0.09	7.64 ± 0.07	8.45 ± 0.20

C) Maximum floral organ measurements (mm) up to the time of stigma maturation

Pop./Subspecies	Ovary length	Petal length	Petal width	Lg. filament length	Lg. anther length	Style length
					n/a	
6p	15.59 ± 0.30	10.46 ± 0.19	5.83 ± 0.18	6.83 ± 0.14	n/a	5.96 ± 0.15
9p	14.63 ± 0.39	11.54 ± 0.19	6.52 ± 0.16	7.42 ± 0.16	n/a	6.77 ± 0.14
23p	16.58 ± 0.20	12.05 ± 0.13	7.20 ± 0.10	5.85 ± 0.12	5.50	5.68 ± 0.09
48p	18.75 ± 0.29	15.18 ± 0.16	8.97 ± 0.14	8.93 ± 0.14	± 0.50	8.57 ± 0.14
Selfer mean	16.50 ± 0.21	12.45 ± 0.19	7.23 ± 0.13	7.28 ± 0.13	n/a	6.79 ± 0.13
6x	11.80 ± 0.32	18.82 ± 0.47	11.16 ± 0.23	7.04 ± 0.16	6.92 ± 0.14	10.86 ± 0.40
9x	21.05 ± 0.54	27.72 ± 0.28	22.65 ± 0.50	8.28 ± 0.11	8.05 ± 0.20	14.12 ± 0.21
13x	19.48 ± 0.34	25.05 ± 0.44	18.90 ± 0.36	8.92 ± 0.16	8.54 ± 0.37	15.18 ± 0.21
17x	18.39 ± 0.68	23.86 ± 0.70	15.86 ± 0.31	9.77 ± 0.22	8.46 ± 0.38	16.68 ± 0.54
Outcrosser mean	17.92 ± 0.40	24.08 ± 0.38	17.43 ± 0.43	8.54 ± 0.12	8.02 ± 0.16	14.31 ± 0.26

ference between ovary and petal relative growth rates compared to sepal and style relative growth rates (Table 6). For development time, the second axis reflects the difference between the lengths of bud development time vs. protandry, but only accounts for 1.3% of the variation (Table 6).

The first principal component axes of flower development time ($PCA1_{DT}$) and organ relative growth rate ($PCA1_{GR}$) separate populations of the two subspecies into nonoverlapping clusters and correlate well with the subspecific differences in final flower size. For example, variation in overall flower size between subspecies ($PCA1_{SZ}$) is positively correlated with variation in the duration of flower development ($PCA1_{DT}$) (Fig. 8b), with longer durations leading to larger flowers. Subspecies differences in overall flower size are also related to variation in the magnitude of organ growth rates ($PCA1_{GR}$), but contrary to intuition, overall flower size and organ relative growth rate are negatively correlated (Fig. 8c). Populations with faster organ relative growth rates do not produce flowers of larger size because it is these same populations that have the shortest flower development (Fig. 8d). Populations 48p and 6x achieve a similar overall size by different means: 48p has a higher overall rate of organ growth (Fig. 8c) but matures sooner than 6x (Fig. 8b).

Trait covariation within vs. between subspecies—Patterns of covariation in floral morphology and development across the two subspecies are not always matched by within-subspecies patterns. For example, while the correlations between overall flower size ($PCA1_{SZ}$) and organ growth rates ($PCA1_{GR}$) and between organ growth rates ($PCA1_{GR}$) and development time ($PCA1_{DT}$) are strongly negative (Fig. 8c, $r_{SZ,GR} = -0.83$. $P < 0.0001$; Fig. 8d, $r_{GR,DT} = -0.85$, $P < 0.0001$), these same correlations are more weakly negative in ssp. *xantiana* ($r_{SZ,GR} = -0.47$, $P = 0.24$; $r_{GR,DT} = -0.38$, $P = 0.36$) and are weakly positive in ssp. *parviflora* ($r_{SZ,GR} = 0.43$, $P = 0.29$; $r_{GR,DT} = 0.49$, $P = 0.22$). Differences in the sign of these correlations between the two subspecies are not statistically significant, perhaps due to the small sample size of populations.

DISCUSSION

Flower morphology and phenology—Flowers of the self-pollinating *Clarkia xantiana* ssp. *parviflora* are smaller in all

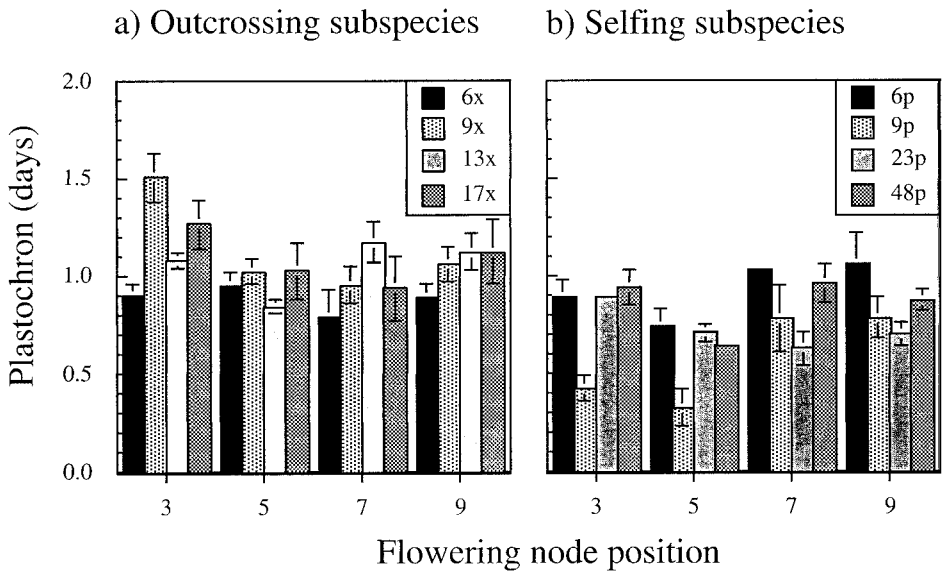


Fig. 6a,b. The relationship between flower plastochron and flower node position in the (a) outcrossing subspecies and (b) selfing subspecies of *Clarkia xantiana.*

respects, except ovary length, than those of its outcrossing progenitor, *C. x.* ssp. *xantiana* (Tables 1, 2; Fig. 8a). The mature style of selfing flowers is both shorter in absolute length and shorter relative to stamen filament length compared to the style of the outcrosser (Table 2C). Stamens of the selfing flower also tend to remain erect at flower opening, while those of the outcrosser tend to reflex away from the style. As a result, herkogamy, i.e., the spatial separation between anther and receptive stigma, is greatly reduced and dehiscing anthers are brought into closer contact with the receptive stigma in selfing compared to outcrossing flowers. Protandry is either absent or much reduced in selfing compared to outcrossing flowers (Table 2A). Indeed, in a 1998 survey of flower morphology and phenology in 125 natural populations (75 selfing, 53 outcrossing), reduced herkogamy and protandry were the two most diagnostic characters of subspecific differences, more so even than petal size (Eckhart and Geber, 2000).

Reduced flower size, herkogamy, and duration of protandry are the norm in selfing *Clarkia* taxa compared to their outcrossing relatives (Lewis and Lewis, 1955; Vasek, 1958, 1964; Moore and Lewis, 1965; Holtsford and Ellstrand, 1992; Sherry, 1994; Sherry and Lord, 1996). Moore and Lewis (1965) studied two of the *C. xantiana* populations included in this study (6x and 6p) and reported that the selfers had smaller organs (they did not measure ovary length) and reduced protandry and herkogamy compared to outcrossers. Sherry (1994) studied the development of flowers from selfing and outcrossing populations of *Clarkia tembloriensis* and reported that, except for ovaries, flower organs were smaller in selfing flowers. The morphology and phenology of selfing flowers appear to be effective in promoting selfing. Outcrossing rates are lower in populations of *C. tembloriensis* with the self-pollinating flower form than in populations with larger flowers (Holtsford and Ellstrand, 1989) and are strongly negatively correlated with the degree of protandry and herkogamy (Holtsford and Ellstrand, 1992). In *C. xantiana,* populations of the white- and pink-flowered ssp. *parviflora* are much less genetically variable at allozyme loci than populations of spp. *xantiana* at the same and other locations, as would be expected if selfing rates are higher in spp. *parviflora* (Gottlieb, 1984; Travers and Geber, unpublished data).

Flower development—In order to determine the developmental basis of differences in mature flower form between the two subspecies of *C. xantiana,* we compared flower development time and organ relative growth rates between selfing and outcrossing flowers from four populations of each subspecies. Measures of developmental time were determined from daily

TABLE 3. Univariate analyses of the slope and intercept from the regression of $\log_e$(sepal) $\times$ $\log_e$ (ovary) for the three flower bud size classes. Mean flower position was included as a covariate in the analyses to test the hypothesis of flower bud equivalency at different nodes. * indicates a statistically significant effect at $\alpha = 0.05$.

Sepal size class	Effect	Num df/Den df	Slope *F*	Intercept *F*
Sepal $\leq$ 6 mm	Subspecies	1/77	14.11*	509.53*
	Population (Ssp.)	6/77	1.24	10.72*
	Mean flower position	1/77	0.51	0.34
6 mm $<$ sepal $\leq$ 12 mm	Subspecies	1/45	38.33*	12.13*
	Population (Ssp.)	6/45	2.17	2.43*
	Mean flower position	1/45	0.51	0.52
Sepal $>$ 12 mm	Population	1/16	1.27	0.00
	Mean flower position	1/16	0.45	0.00

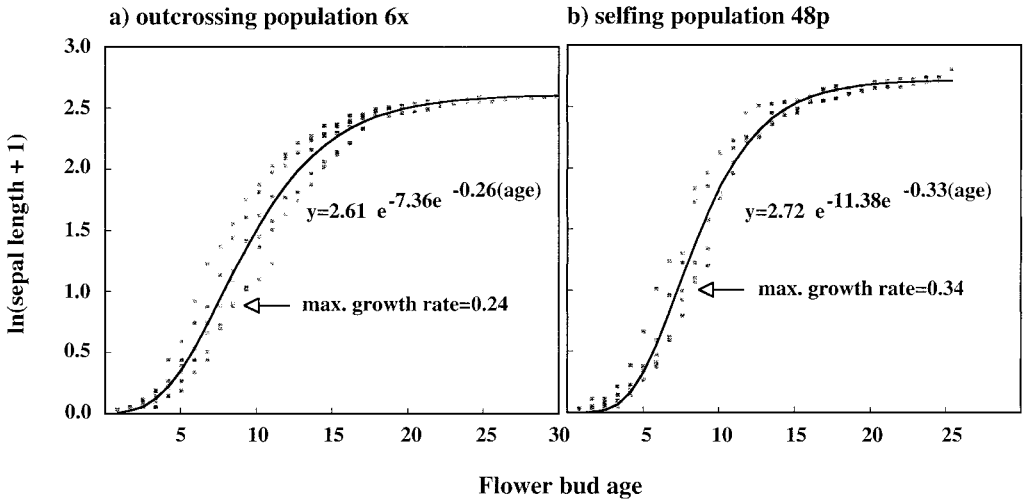


Fig. 7. Representative plots of log-tranformed sepal length (in mm) vs. flower bud age (in days) and the estimated Gompertz growth curves for outcrossing (6x) and selfing (48p) populations of *Clarkia xantiana.* Maximum growth rates are calculated from parameters *a* and *r* of the Gompertz equation.

measurements of the same flower buds, from bud sizes of 1–2 mm to stigma presentation in open flowers. Organ relative growth rates, on the other hand, were derived from measurements of sectioned flower buds at different stages of development and collected from successive node positions on inflorescences. The accuracy of the relative growth rate estimates depends on the validity of several assumptions. First, flower buds at successive nodes and from different plants must be representative of different ages of a flower at the same node position. In other words, flower bud development must not differ with node position. We tested this assumption by comparing the allometry of sepal to ovaries in buds from six successive harvests that included buds drawn from progressively later nodes. Because there was no evidence of an effect of node position on allometry (Table 4), we concluded that bud development was independent of node position, at least within the range of nodes included in this study. The second assumption underlying the estimates of organ relative growth rate is that flower bud age for a population of plants can be accurately estimated from the population's mean flower plastochron. Plastochron interval was determined for flowers from node positions 1–9, while the buds used in the estimation of organ relative growth rates came from node positions 1–36. We cannot be certain therefore that plastochron values (and hence flower age estimates) from lower nodes are accurate for buds at higher nodes. On the other hand, in our analysis of variation in flower plastochron, we found no consistent effect of node position on plastochron interval (Table 1, Fig. 6). We therefore felt that the mean flower plastochron over nodes 1–9 was our best estimate of a population's plastochron for all nodes.

TABLE 4. Univariate analyses of floral organ growth rates (natural log transformation of data taken in mm per day). * indicates a statistically significant effect at $\alpha = 0.05$.

Source	df	Ovary length *F*	Sepal length *F*	Petal length *F*	Lg. anther/ filament length *F*	Style length *F*
Subspecies	1/7	316.12*	169.89*	56.72*	25.97*	16.62*
Population (ssp.)	6/7	4.67*	13.96*	1.21	4.50*	5.70*
Block	1/7	0.01	0.49	0.20	1.70	1.33

Developmental basis of subspecific differences in flower form—The developmental comparisons between selfing and outcrossing flowers of *C. xantiana* were motivated by contrasting hypotheses about the evolution of self-pollination. The first hypothesis states that the selfing flower form evolved as a consequence of direct selection for the ability to self-pollinate in the arid environments occupied by ssp. *parviflora.* According to the second hypothesis, selfing arose indirectly as a by-product of selection for rapid maturation. The first hypothesis does not make any specific predictions concerning the developmental basis of subspecific differences in flower form, whereas the second hypothesis predicts that a reduction in flower development time (i.e., truncation and progenesis) should account, at least in part, for the smaller size and altered phenology of *parviflora* flowers. An alternative heterochronic mechanism for producing a smaller flower is through a reduction in the rate of organ development (e.g., slower relative growth rate), with no change in development time. This latter mechanism, which is termed deceleration and which results in neotony, would not be consistent with selection for rapid maturation.

There is clear evidence of progenesis in ssp. *parviflora* relative to ssp. *xantiana.* Selfing flowers required 26% less time to develop from a bud size of ~1–2 mm to flower opening and the period of protandry was 71% shorter (Table 2A). The flower plastochron interval is also shorter in the selfers compared to outcrossers (Table 2A). These findings are consistent therefore with the hypothesis that selection favored rapid development in ssp. *parviflora.*

There is no evidence of a deceleration in development rate and of neotony in ssp. *parviflora* relative to ssp. *xantiana.* To the contrary, the relative growth rates of all flower organs are accelerated in the selfer compared to the outcrosser (Table 5). Acceleration ranged from a 23% increase in anther/filament relative growth rate to a 57% increase in ovary relative growth rate.. Acceleration of development rate is consistent with selection for rapid maturation to the extent that it allows flower

TABLE 5. Population mean maximum floral organ growth rates (*ra*/*e*) (natural log transformed of data taken in mm per day). Parameters *a* and *r* were generated by fitting the Gompertz equation to $\log_e$(organ size + 1) by plastochron age. Mean ± 1 SE.

Pop./Subspecies	Maximum organ growth rate				
	Ovary length	Sepal length	Petal length	Lg. anther + filament length	Style length
6p	0.21 ± 0.01	0.31 ± 0.01	0.26 ± 0.03	0.27 ± 0.00	0.24 ± 0.00
9p	0.22 ± 0.00	0.30 ± 0.01	0.27 ± 0.00	0.30 ± 0.00	0.26 ± 0.01
23p	0.22 ± 0.01	0.26 ± 0.00	0.26 ± 0.01	0.23 ± 0.01	0.19 ± 0.01
48p	0.22 ± 0.01	0.34 ± 0.00	0.26 ± 0.03	0.30 ± 0.01	0.26 ± 0.00
Selfer mean	0.22 ± 0.00	0.30 ± 0.01	0.26 ± 0.01	0.27 ± 0.01	0.24 ± 0.01
6x	0.15 ± 0.00	0.24 ± 0.00	0.19 ± 0.01	0.26 ± 0.01	0.24 ± 0.02
9x	0.12 ± 0.00	0.17 ± 0.00	0.15 ± 0.00	0.18 ± 0.01	0.16 ± 0.01
13x	0.16 ± 0.00	0.25 ± 0.01	0.20 ± 0.01	0.23 ± 0.01	0.22 ± 0.01
17x	0.13 ± 0.01	0.20 ± 0.02	0.15 ± 0.02	0.19 ± 0.04	0.17 ± 0.03
Outcrosser mean	0.14 ± 0.01	0.22 ± 0.01	0.17 ± 0.01	0.22 ± 0.01	0.19 ± 0.01

to achieve a mature size in a minimum amount of time. Selection for small flower size in selfing flowers would not necessarily be expected to generate selection for accelerated organ growth rates. Indeed, accelerated organ growth rates mean that flowers of ssp. *parviflora* are larger than they would otherwise have been had growth rates not differed between the subspecies. Final organ sizes are smaller in ssp. *parviflora* than in ssp. *xantiana*, because of shorter development time (Fig. 8b) and in spite of higher organ relative growth rates (Fig. 8d).

In sum, selection for rapid maturation may have caused or contributed to the evolution of selfing in *C. xantiana*. However, because direct selection for self-pollination in ssp. *parviflora* could also have caused floral development to change by the same heterochronic means, definitive answers about the selective basis of the selfing flower form await measurement of natural selection in the wild on development time vs. on the ability to self-pollinate. There are reasons to think that the ability to self-fertilize may be directly advantageous, and hence directly selected, in arid regions of *C. xantiana*'s geographic distribution. Members of the genus *Clarkia* are pollinated by an array of specialist bees that use *Clarkia* pollen to provision their young (McSwain, Raven, and Thorpe, 1973). The specialist pollinators are absent from arid areas where ssp. *parviflora* occurs alone (Fausto, Eckhart, and Geber, 1999). Field studies of natural selection on development time vs. the ability to self-pollinate are now ongoing (Geber and Eckhart, unpublished data).

A review of the literature on comparisons of outcrossing and selfing flower forms indicates that a variety of heterochronic mechanisms have been involved in the evolution of self-pollination. Shorter development time is found in buds of selfing *Mimulus guttatus* (Fenster et al., 1995) and in selfing *Limnanthes alba* flowers (Guerrant, 1988). By contrast, flowers from selfing populations of *Arenaria uniflora* develop over a longer period of time than their outcrossing counterparts (Wyatt, 1984a, b; Hill, Lord, and Shaw, 1992). In comparisons of cleistogamous and chasmogamous flowers, developmental differences appear to be more consistent, at least with respect to the flower bud. Cleistogamous flower buds are reported to mature in less time than chasmogamous buds in *Viola odorata* (Mayers and Lord, 1983), *Lamium amplexicaule* (Lord, 1982), *Collomia grandiflora* (Minter and Lord, 1983), and *Astralagus cymbicarpos* (Gallardo, Dominguez, and Muñoz, 1993).

There is also no universal pattern with respect to differences in flower plastochron between selfing and outcrossing taxa. Cleistogamous flowers of *V. odorata* have a shorter plastochron than chasmogamous flowers (Mayers and Lord, 1983), but flower plastochron is longer in selfing populations of *Eichhornia paniculata* (Morgan and Barrett, 1989) and *A. uniflora* (Hill, Lord, and Shaw, 1992). Lastly, both acceleration and deceleration of development rate are observed in selfing flowers compared to outcrossing ones. As in *C. xantiana*, faster growth rates are reported in buds of selfing *M. guttatus* (Fenster et al., 1995) and in selfing *L. alba* flowers (Guerrant, 1988). Faster growth rates also characterize cleistogamous relative to chasmogamous flower buds (Lord, 1982; Mayers and Lord, 1983; Minter and Lord, 1983; Gallardo, Dominguez, and Muñoz, 1993). However, selfing flowers of *A. uniflora* develop at a slower rate than outcrossing flowers (Wyatt, 1984a, b; Hill, Lord, and Shaw, 1992).

Taxonomic variation in the developmental mechanisms underlying the transition from outcrossing to selfing may reflect in part the diversity of ecological causes that have favored this transition (Diggle, 1992). For example, selfing flowers of *Ar-*

TABLE 6. Character loadings on the first two principal components axes (PC1 and PC2) from analyses of (1) maximum floral organ sizes, (2) floral organ growth rates, and (3) duration of developmental stages (bud development time to opening and protandry).

Character loadings	(1) Maximum organ size		(2) Floral organ growth rate		(3) Duration of floral developmental stages	
	$PC1_{SZ}$	$PC2_{SZ}$	$PC1_{GR}$	$PC2_{GR}$	$PC1_{DT}$	$PC2_{DT}$
Ovary length						
Sepal length	0.233	0.954	0.470	−0.439	—	—
Petal length	0.308	0.028	0.568	0.269	—	—
Style length	0.781	−0.124	0.554	−0.423	—	—
Bud dev. to opening	0.492	−0.272	0.386	0.746	—	—
Protandry	—	—	—	—	0.870	−0.493
Variation accounted by PCA	—	—	—	—	0.493	0.870
	89.3	7.9	88.7	8.1	98.7	1.3

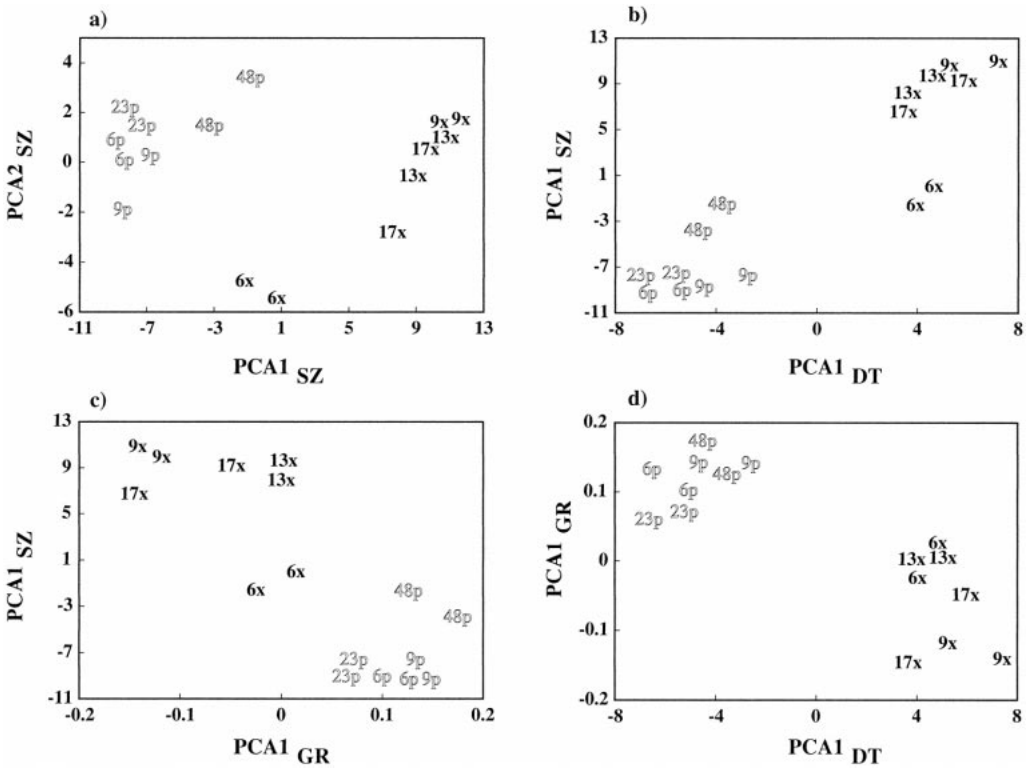


Fig. 8. Locations of populations in the coordinate space defined by principal component s axes of organ size, organ growth rates, and development time of the flowers of *Clarkia xantiana.* (a) Populations are plotted with respect to the first two principal components axes of organ size ($PCA2_{SZ}$ vs. $PCA1_{SZ}$). (b) The relationship between overall flower size and flower development time is shown by plotting populations with respect to the first principal components axes for size ($PCA1_{SZ}$) and for development time ($PCA1_{DT}$). (c) The relationship between overall flower size and flower development time is shown by plotting populations with respect to the first principal components axes for size ($PCA1_{SZ}$) and for organ growth rate ($PCA1_{GR}$). (d) The relationship between overall growth rate and development time is shown by plotting populations with respect to the first principal components axes for growth rate ($PCA1_{GR}$) and for development time ($PCA1_{DT}$).

enaria uniflora are not progenetic forms of outcrossing ones since the duration of development is longer and the rate of development is slower in selfing compared to outcrossing flowers. Selfing is thought to have evolved in marginal populations of *A. uniflora,* not as a by-product of selection for rapid maturation, but because of pollinator competition from *A. glabra,* or as a means of avoiding hybridization with *A. glabra,* a congener that is found at the margins of *A. uniflora*'s range (Wyatt, 1986; Fishman, 1996).

Developmental constraint and selection in the evolution of the selfing flower—In spite of taxonomic variation in the nature of outcrosser-selfer differences in flower development, one pattern does emerge from this collection of studies. Outcrosser-selfer shifts in flower bud development time, development rate, and plastochron are correlated among taxa. In other words, where the shift to a selfing flower form has involved a reduction in flower bud development time, it is more likely than not to have also involved an increase in flower bud development rate and a decrease in flower plastochron.

Developmental correlations among flower parts and across taxa can be a sign either of development and genetic constraints or of past selection (Berg, 1960; Armbruster, 1991; Armbruster and Schwaegerle, 1996; Hufford, 1997; Cresswell, 1998). Developmental correlations can be caused by pleiotropic effects of genes such that change in one aspect of development (e.g., developmental duration) forces change in other aspects of development (e.g., developmental rate). Alternatively selection to improve the functional integration of flower parts can also lead to the evolution of strong genetic and developmental correlations.

In order to address the cause of correlated shifts in development processes (or size of flower parts) between ssp. *xantiana* and *parviflora,* we examined trait covariation among populations within each subspecies. Similar patterns of trait covariation within and between subspecies would bolster an argument of developmental constraint. Dissimilar patterns of trait covariation, on the other hand, would suggest that correlated changes in development processes (or size of parts) are the result of selection for a flower that is developmentally and/or functionally integrated.

In *Clarkia xantiana,* there is a suggestion that the pattern of trait covariation differs within subspecies as compared to between subspecies. Thus, the correlations between organ size and relative growth rate and between developmental duration and rate are negative between subspecies and within ssp. *xantiana,* but these correlation are positive within ssp. *parviflora* (Fig. 8d). The shift in the sign of these correlations between ssp. *xantiana* and ssp. *parviflora* suggests that developmental duration can be uncoupled from developmental rate, and these two aspects of development are not inextricably linked by pleiotropic effects of genes. The uncoupling of developmental timing and organ growth is also supported by findings of character covariation among populations of *C. unguiculata* (Jonas

and Geber, 1999), a species that is in the sister clade to *C. xantiana*. In *C. unguiculata*, the positive correlation between herkogamy and protandry that is characteristic of interspecific differences in the genus (Lewis and Lewis, 1955; Vasek, 1964, 1968; Moore and Lewis, 1965; Holtsford and Ellstrand, 1992) appears to have been disrupted in some populations; in these populations, plants have long-duration protandry but virtually no herkogamy. Since herkogamy in *Clarkia* is primarily the result of continued growth of the style after flower opening (Table 1), it appears that the association between organ growth and developmental duration can also be disrupted. Fenster et al. (1995) in studies of outcrossing and selfing species of *Mimulus* have also found evidence that the duration of floral development is genetically independent of the rate of development.

There are now quite a few studies of genetic variation and covariation in floral form (Shore and Barrett, 1990; Meagher, 1992; Conner and Via, 1993; Mitchell and Shaw, 1993; O'Neil and Schmitt, 1993; Carr and Fenster, 1994; Robertson, Diaz, and MacNair, 1994; Stanton and Young, 1994; Young et al., 1994; Bradshaw et al., 1995; Andersson, 1996, 1997; Campbell, 1996; Galen, 1996; Mazer and Delesalle, 1996). While the verdict is still out on the relative strengths of constraint vs. selection in shaping the genetic and developmental architecture of floral form, it is clear that developmental constraints are not so large as to prevent the independent evolution of floral characters under the appropriate selective regime (Stanton and Young, 1994; Delph, Galloway, and Stanton, 1996; Eckhart, 1999).

LITERATURE CITED

ALBERCH, P., S. J. GOULD, G. F. OSTER, AND D. B. WAKE. 1979. Size and shape in ontogeny and phylogeny. *Paleobiology* 5: 296–317.

ALLEN, G. A., V. S. FORD, AND L. D. GOTTLIEB. 1990. A new subspecies of *Clarkia concinna* (Onagraceae) from Marin County, California. *Madroño* 37: 305–310.

ANDERSSON, S. 1996. Floral variation in *Saxifraga granulata*: phenotypic selection, quantitative genetics and predicted response to selection. *Heredity* 77:2 17–223.

———. 1997. Genetic constraint on phenotypic evolution in *Nigella* (Ranunculaceae). *Biological Journal of the Linnean Society* 62: 519–532.

ARMBRUSTER, W. S. 1991. Multilevel analysis of morphometric data from natural populations: insights into ontogenetic, genetic, and selective correlations in *Dalechampia scandens*. *Evolution* 45: 1229–1244.

———, AND K. E. SCHWAEGERLE. 1996. Causes of covariation of phenotypic traits among populations. *Journal of Evolutionary Biology* 9: 261–276.

ARROYO, M. T. K. 1973. Chiasma frequency evidence on the evolution of autogamy in *Limnanthes floccosa*. *Evolution* 27: 679–688.

BAKER, H. G. 1955. Self-compatibility and establishment after 'long-distance' dispersal. *Evolution* 9: 347–348.

BERG, R. L. 1960. The ecological significance of correlation pleiades. *Evolution* 14: 171–180.

BRADSHAW, H. D. JR, S. M. WILBERT, K. G. OTTO, AND D. W. SCHEMSKE. 1995. Genetic mapping of floral traits associated with reproductive isolation in monkeyflowers (*Mimulus*). *Nature* 376: 762–765.

CAMPBELL, D. R. 1996. Evolution of floral traits in a hermaphroditic plant: field measurements of heritabilities and genetic correlations. *Evolution* 50: 1442–1453.

CARR, D. E., AND C. B. FENSTER. 1993. Levels of genetic variation and covariation for *Mimulus* (Scrophulariaceae) floral traits. *Heredity* 72: 606–618.

CONNER, J. K., AND S. VIA. 1993. Patterns of phenotypic and genetic correlations among morphological and life history traits in wild radish, *Raphanus raphanistrum*. *Evolution* 47: 704–711.

CROWDER, M. J., AND D. J. HAND. 1993. Analysis of repeated measures. Chapman and Hall, New York, New York, USA.

CRESSWELL, J. E. 1998. Stabilizing selection and the structural variability of flowers within species. *Annals of Botany* 81: 463–473.

DEBEER, G. R. 1958. Embryos and ancestors. Clarendon Press, Oxford, UK.

DIGGLE, P. K. 1992. Development and evolution of plant reproductive characters. *In* R. Wyatt [ed.], Ecology and evolution of plant reproduction: new approaches, 326–355. Chapman and Hall, New York, New York, USA.

DELPH, L. F., L. F. GALLOWAY, AND M. L. STANTON. 1996. Sexual dimorphism in flower size. American Naturalist 148: 299–320.

ECKHART, V. M. 1999. Sexual dimorphism in flowers and inflorescences. *In* M. A. Geber, L. F. Delph, and T. E. Dawson [eds.], Gender and sexual dimorphism in flowering plants, 123–148. Springer-Verlag, Berlin, Germany.

———, AND M. A. GEBER. 2000. Character variation and geographic range in *Clarkia xantiana* (Onagraceae): breeding system and phenology distinguish two common subspecies. *Madroño* 46: 117–125.

———,———, AND C. S. JONAS. 1996. Developmental controls of flowering-time evolution in *Clarkia* (Onagraceae). *American Journal of Botany* 83 (supplement): 81 (Abstract).

FAUSTO, J. A., V. M. ECKHART, AND M. A. GEBER. 1999. Reproductive assurance and the evolution of autogamy in *Clarkia xantiana* (Onagraceae). *Ecology* (Supplement) 80: 249.

FENSTER, C. B., P. K. DIGGLE, S. C. H. BARRETT, AND K. RITLAND. 1995. The genetics of floral development differentiating two species of *Mimulus* (Scrophulariaceae). *Heredity* 74: 258–266.

FISHMAN, L. 1996. The evolution of autogamy in *Arenaria uniflora*: Reproductive insurance against interspecific pollination? *American Journal of Botany* 83: (Supplement) 65 (Abstract).

GALEN, C. 1996. Rates of evolution: adaptation to bumblebee pollination in an alpine wildflower, *Polemonium viscosum*. *Evolution* 50:120–125.

GALLARDO, R., E. DOMINGUEZ, AND J. M. MUÑOZ. 1993. The heterochronic origin of the cleistogamous flower in *Astragalus cymbicarpos* (Fabaceae). *American Journal of Botany* 80: 814–823.

GOTTLIEB, L. D. 1984. Electrophoretic analysis of the phylogeny of the self-pollinating populations of *Clarkia xantiana*. *Plant Systematics and Evolution* 147: 91–102.

GOULD, S. J. 1977. Ontogeny and phylogeny. Belknap Press of Harvard University Press, Cambridge, Massachusetts, USA.

GUERRANT, E. O. 1988. Heterochrony in plants: the intersection of evolution, ecology, and ontogeny. *In* M. L. McKinney [ed.], Heterochrony in evolution, 111–133. Plenum Press, New York, New York, USA.

———. 1989. Early maturity, small flowers and autogamy: a developmental connection? *In* J. H. Bock and Y. B. Linhart [eds.], The evolutionary biology of plants, 61–84. Westview Press, Boulder, Colorado, USA.

HARRIS, R. J. 1985. A primer of multivariate statistics. Academic Press, Orlando, Florida, USA.

HILL, J. P., AND E. M. LORD. 1990. A method for determining plastochron indices during heteroblastic shoot growth. *American Journal of Botany* 77: 1491–1497.

———, AND R. G. SHAW. 1992. Morphological and growth rate differences among outcrossing and self-pollinating races of *Arenaria uniflora* (Caryophyllaceae). *Journal of Evolutionary Biology* 5: 559–573.

HOLTSFORD, T. P., AND N. C. ELLSTRAND. 1989. Variation in outcrossing rate and population genetic structure of *Clarkia tembloriensis* (Onagraceae). *Theoretical and Applied Genetics* 78: 480–488.

———, AND ———. 1992. Genetic and environmental variation in floral traits affecting outcrossing rate in *Clarkia tembloriensis* (Onagraceae). *Evolution* 46: 216–225.

HUFFORD, L. 1997. The roles of ontogenetic evolution in the origins of floral homoplasies. *International Journal of Plant Sciences* 158: S65-S80.

ITOH, J.-I., A. HASEGAWA, H. KITANO, AND Y. NAGATO. 1998. A recessive heterochronic mutation, *plastochron1*, shortens the plastochron and elongates the vegetative phase in rice. *Plant Cell* 10: 1511–1521.

JAIN, S. K. 1976. The evolution of inbreeding in plants. *Annual Review of Ecology and Systematics* 7: 469–495.

JMP STATISTICS AND GRAPHICS GUIDE. 1996. Statistical software for the Macintosh from SAS Institute Inc., Cary, North Carolina, USA.

JONAS, C. S., AND M. A. GEBER. 1999. Variation among populations of *Clarkia unguiculata* (Onagraceae) along altitudinal and latitudinal gradients. *American Journal of Botany* 86: 333–343.

KELLOGG, E. A. 1990. Ontogenetic studies of florets in *Poa* (Gramineae): allometry and heterochrony. *Evolution* 44: 1978–1989.

KLINGENBERG, C. P. 1998. Heterochrony and allometry: the analysis of evolutionary change in ontogeny. *Biological Review* 73: 79–123.

LANDE, R., AND D. W. SCHEMSKE. 1985. The evolution of self-fertilization and inbreeding depression in plants. I. Genetic models. *Evolution* 39: 24–40.

LEWIS, H., AND M. E. LEWIS. 1955. The genus *Clarkia*. *University of California Publications in Botany* 20: 241–392.

———, AND P. H. RAVEN. 1958. Rapid evolution in *Clarkia*. *Evolution* 12: 319–336.

———, AND ———. 1992. New combinations in the genus *Clarkia* (Onagraceae). *Madroño* 39: 163–169.

LLOYD, D. G. 1965. Evolution of self-compatibility and racial differentiation in *Leavenworthia* (Cruciferae). *Contribution to Gray Herbarium* 195: 3–134.

———. 1979. Some reproductive factors affecting the selection of self-fertilization in plants. *American Naturalist* 113: 67–79.

LORD, E. M. 1982. Floral morphogenesis in *Lamium amplexicaule* L. (Labiatae) with a model for the evolution of the cleistogamous flower. *Botanical Gazette* 143: 63–72.

———, K. J. ECKARD, AND W. CRONE. 1989. Development of the dimorphic anthers in *Collomia grandiflora*; evidence for heterochrony in the evolution of the cleistogamous anther. *Journal of Evolutionary Biology* 2: 81–93.

———, AND J. P. HILL. 1987. Evidence for heterochrony in the evolution of plant form. *In* R. A. Raff and E. C. Raff [eds.], Development as an evolutionary process, 47–70. Alan R. Liss, New York, New York, USA.

MAYERS, A. M., AND E. M. LORD. 1983. Comparative flower development in the cleistogamous species *Viola odorata*. I. A growth rate study. *American Journal of Botany* 70: 1548–1555.

MAZER, S. J., AND V. A. DELESALLE. 1996. Floral trait variation in *Spergularia marina* (Caryophyllaceae): ontogenetic, maternal family, and population effects. *Heredity* 77: 269–281.

MCSWAIN, J. W., P. H. RAVEN, AND R. W. THORP. 1973. Comparative behavior of bees and Onagraceae. IV. *Clarkia* bees of the western United States. *University of California Publication in Entomology* 70: 1–87.

MEAGHER, T. R. 1992. The quantitative genetics of sexual dimorphism in *Silene latifolia* (Caryophyllaceae). I. Genetic variation. *Evolution* 46: 445–457.

MINTER, T. C., AND E. M. LORD. 1983. A comparison of cleistogamous and chasmogamous floral development in *Collomia grandiflora* Dougl. ex lindl. (Polemoniaceae). *American Journal of Botany* 70: 1499–1508.

MITCHELL, R. J., AND R. SHAW. 1993. Heritability of floral traits for the perennial wild flower *Penstemon centranthifolius* (Scrophulariaceae): clones and crosses. *Heredity* 71:185–192.

MOORE, D. M., AND H. LEWIS. 1965. The evolution of self-pollination in *Clarkia xantiana*. *Evolution* 19: 104–114.

MORGAN, M. T., AND S. C. H. BARRETT. 1989. Reproductive correlates of mating system variation in *Eichhornia paniculata* (Spreng.) Solms (Ponteridaceae). *Journal of Evolutionary Biology* 2: 183–203.

NIKLAS, K. J. 1994. Plant allometry: the scaling of form and process. University of Chicago Press, Chicago, Illinois, USA.

O'NEILL, P., AND J. SCHMITT. 1993. Genetic constraints on the independent evolution of male and female reproductive characters in the tristylous plant *Lythrum salicaria*. *Evolution* 47: 1457–1471.

ORNDUFF, R. 1969. Reproductive biology in relation to systematics. *Taxon* 18: 121–133.

POETHIG, R. S. 1988. Heterochronic mutations affecting shoot development in maize. *Genetics* 119: 959–973.

RAVEN, P. H. 1964. Catastrophic selection and edaphic endemism. *Evolution* 18: 336–338.

RICE, W. R. 1989. Analyzing tables of statistical tests. *Evolution* 43: 223–225.

ROBERTSON, A. W., A. DIAZ, AND M. R. MACNAIR. 1994. The quantitative genetics of floral characters in *Mimulus guttatus*. *Heredity* 72: 300–311.

SAS. 1996. Statistical software, SAS Institute Inc. Cary, North Carolina, USA.

SCHOEN, D. J. 1982. The breeding system of *Gilia achilleifolia*: variation in floral characteristics and outcrossing rate. *Evolution* 36: 352–360.

SHERRY, R. A. 1994. Developmental stability and floral ontogeny in self- and cross-pollinating populations of *Clarkia tembloriensis* (Onagraceae). Ph.D. dissertation, University of California, Riverside, California, USA.

———, AND E. M. LORD. 1996. Developmental stability in flowers of *Clarkia tembloriensis* (Onagraceae). *Journal of Evolutionary Biology* 9: 911–930.

SHORE, J. S., AND S. C. H. BARRETT. 1990. Quantitative genetics of floral characters of homostylous *Turnera ulmifolia* var. *angustifolia* Willd. (Turneraceae). *Heredity* 64: 1 05–112.

SOLBRIG, O. T., AND R. C. ROLLINS. 1977. The evolution of autogamy in species of the mustard genus *Leavenworthia*. *Evolution* 31: 265–281.

STANTON, M. L., AND H. J. YOUNG. 1994. Selecting for floral character associations in wild radish, *Raphanus sativus* L. *Journal of Evolutionary Biology* 7: 271–285.

STEBBINS, G. L. 1950. Variation and evolution in plants. Columbia University Press, New York, New York, USA.

———. 1970. Adaptive radiation in angiosperms. I. Pollination mechanisms. *Annual Review of Ecology and Systematics* 1: 307–326.

———. 1974. Flowering plants: evolution above the species level. Belknap Press, Cambridge Massachusetts, USA.

SYTSMA, K. J., J. F. SMITH, AND L. D. GOTTLIEB. 1990. Phylogenetics in *Clarkia* (Onagraceae): restriction site mapping of chloroplast DNA. *Systematic Botany* 15: 280–295.

TIPTON, J. L. 1984. Evaluation of three growth curve models for germination data analysis. *Journal of the American Society of Horticultural Science* 109: 451–454.

VASEK, F. C. 1958. The relationships of *Clarkia exilis* to *Clarkia unguiculata*. *American Journal of Botany* 45: 150–162.

———. 1964. The evolution of *Clarkia unguiculata* derivatives adapted to relatively xeric environments. *Evolution* 18: 26–42.

———. 1968. The relationships of two ecologically marginal, sympatric *Clarkia* populations. *American Naturalist* 102: 25–40.

———, AND J. HARDING. 1976. Outcrossing in natural populations. V. Analysis of outcrossing, inbreeding, and selection in *Clarkia exilis* and *Clarkia tembloriensis*. *Evolution* 30: 403–411.

———, AND V. WENG. 1988. Breeding systems of Clarkia Sect. Phaeostoma (Onagraceae). I. Pollen-ovule ratios. *Systematic Botany* 13: 336–350.

WYATT, R. 1983. Pollinator-plant interactions and the evolution of breeding systems. *In* L. Real [ed.], Pollination biology, 51–95. Academic Press, Orlando, Forida, USA.

———. 1984a. The evolution of self-pollination in granite outcrop species of *Arenaria* (Caryophyllaceae). I. Morphological correlates. *Evolution* 38: 804–816.

———. 1984b. The evolution of self-pollination in granite outcrop species of *Arenaria* (Caryophyllaceae). IV. Correlated changes in the gynoecium. *American Journal of Botany* 71: 1006–1014.

———. 1986. The ecology and evolution of self-pollination in *Arenaria uniflora* (Caryophyllaceae). *Journal of Ecology* 74: 403–418

———. 1988. Phylogenetic aspects of the evolution of self-pollination. *In* L. D. Gottlieb and S. K. Jain [eds.], Plant evolutionary biology, 109–131. Chapman and Hall, New York, New York, USA.

YOUNG, H. J., M. L. STANTON, N. C. ELLSTRAND, AND J. M. CLEGG. 1994. Temporal and spatial variation in heritability and genetic correlations among floral traits in *Raphanus sativus*, wild radish. *Heredity* 73: 298–308.