DISSERTATION

PATTERNS AND CONSEQUENCES OF FLORAL FORMULA VARIATION IN *PHLOX* (POLEMONIACEAE)

Submitted by

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY MELANIE BROOKE BYERLEY ENTITLED "PATTERNS AND CONSEQUENCES OF FLORAL FORMULA VARIATION IN *PHLOX* (POLEMONIACEAE)" BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

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ABSTRACT OF DISSERTATION

PATTERNS AND CONSEQUENCES OF FLORAL FORMULA VARIATION IN *PHLOX* (POLEMONIACEAE)

The numbers of organs produced in each of the floral whorls often follow highly consistent and predictable patterns within species, genera, and often higher taxonomic groups. The floral formula is assumed to exhibit low variability due to historically strong selection pressures on suites of integrated reproductive traits. This dissertation examines natural levels and patterns of floral formula variation, the reproductive consequences of formula variation, and the selection potential for a new floral formula. When analyzed for floral organ number variation, most wild *Phlox longifolia* and greenhouse-grown *Phlox drummondii* plants were abnormal for at least one flower, with abnormal organ numbers occurring least often in *P. longifolia* gynoecia and most often in *P. drummondii* corollas. The gynoecium was the most independent whorl, whereas organ numbers in the other whorls were correlated. Direction of abnormality was largely subnumerary in all whorls of *P. longifolia* and supernumerary in *P. drummondii*. Abnormal whorls were often coordinated for direction of variation and for equal organ numbers. Abnormalities increased in frequency over time in *P. longifolia* and decreased in *P. drummondii*. *Phlox drummondii* abnormalities were more frequent among low order branch positions than higher orders. Early floral development in *Phlox* may be unstable, becoming more stable

over time in the absence of environmental stress. Organ number variation within the reproductive whorls significantly affected reproductive output. Stamen and carpel number were positively correlated with pollen and seed number, respectively. Gynoecia with more carpels yielded more seeds per fruit and greater total seed mass per fruit than gynoecia with fewer carpels, suggesting plants producing more carpels per flower would yield greater lifetime seed volume. One round of upward and stabilizing selection on mean carpel number in *Phlox drummondii* did not result in carpel number divergence but did indirectly affect the frequency of abnormality in the corolla and the whole floral formula, suggesting that selection on organ number within a specific floral whorl may act to alter the sensitivity of stable organ number expression, thus increasing overall instability. Overall, this dissertation represents (1) the first formal documentation of floral formula variation in *Phlox longifolia,* (2) the first extensive analysis of patterns of variation with respect to degree and direction in all four whorls simultaneously, (3) the first evidence of effect of organ number variation on reproductive output in *Phlox*, and (4) the first attempt at carpel number selection in *Phlox*.

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CHAPTER 1

DISSERTATION OVERVIEW

In introductory botany courses, it is often taught that the parts of a monocot flower come in multiples of three while dicot flowers have organs in multiples of four or five. An eager instructor might further expound that within Class Dicotyledoneae, flowers of the Onagraceae family are tetramerous while those of Geraniaceae are pentamerous. Sweeping generalizations such as these are universally applied time and again because they are largely accurate. But why? Why do the organs of a flower follow any sort of coordinated pattern? Why not make as many organs as possible without sacrificing functionality and fitness? And why do groups of related plants often share the same patterns? Is it because we classify them based on those patterns or are the pathways of floral development fundamentally constrained? What does it take to evolve away from multiples of five and toward multiples of four or some other pattern? These questions have motivated the investigations within this dissertation.

On a smaller, intra-species or intra-plant level, variation for the expected pattern of floral organ numbers – the "floral formula" – is readily observable, whether among wild plants lining a forest trail or horticultural species in a manicured garden. The presence of such variation suggests there may be room for new floral formulae to evolve

given the right circumstances: selection pressures of adequate intensity, a genetically heritable component, and surmountable trade-offs among any integrated traits. The studies described in this dissertation were intended to quantify levels and patterns of existing floral formula variation and to investigate the potential for formula changes by way of organ number variation within the reproductive whorls. Plants within the *Phlox* genus were chosen for study due to both the purported stability of floral formula among members of the Polemoniaceae and the amount of comparative data already available regarding organ number variation among family members.

The first study included here (**Chapter 2**) describes the results from a survey of natural populations of *Phlox longifolia*. Particular attention was paid to simultaneous patterns of abnormality among all floral whorls and to the direction of organ number variation. Regarding the latter, I use the terms **supernumerary** and **subnumerary** to describe whorls with greater than or fewer than the normal or expected number of organs, respectively. The second study (**Chapter 3**) is similar to the first but additionally investigates the patterns of floral formula variation with respect to both flowering time and flowering position within plants. This analysis was conducted on both wild *Phlox longifolia* and greenhouse-grown *Phlox drummondii*.

The second half of the dissertation addresses the elements needed for a floral formula to evolve to a new state. **Chapter 4** attempts to quantify the effects that organ number variation in the reproductive whorls has on reproductive output. Specifically, the relationships between stamen number and total pollen production and between carpel number and various seed traits were examined for wild *P. longifolia* (male output) and greenhouse *P. drummondii* (female output). It was hypothesized that at least female

output would positively correspond to female organ number, which prompted the following questions: If having more carpels is advantageous through higher seed yield per fruit, then why are supernumerary gynoecia infrequent? Is heritability low? Do pleiotropic effects or correlated responses among other floral traits limit the capacity for carpel number increase? These questions led to the study described in **Chapter 5**, which attempts to select both for greater carpel number and for less variable (stabilized) carpel number among flowers of *Phlox drummondii* while noting any indirect selection responses among other floral whorls.

Given that different floral formulae within taxonomic groups presumably diverged via selection on variation of ancestral formulae, the formula variation still in existence could provide substrate for future selection events. The examination of when, where, and to what extent floral formula variation occurs will hopefully shed light on both the constancy of floral morphology and the potential evolutionary trajectories of flowers within *Phlox* and other polemoniaceous species.

CHAPTER 2

NATURAL VARIATION FOR FLORAL FORMULA IN *PHLOX LONGIFOLIA* NUTT.

ABSTRACT

Organ numbers in each of the four floral whorls were quantified for five wild populations of *Phlox longifolia* Nutt. (Polemoniaceae) to determine the natural distribution of floral formula variation and potential patterns therein. Eighty-seven percent of plants sampled had at least one abnormal flower, and 24% of flowers scored had at least one abnormal whorl. Across all populations, there was a mean of 14% abnormal calyces per plant, 16% abnormal corollas, 15% abnormal androecia, and 11% abnormal gynoecia. The gynoecium was the most independent whorl, whereas the calyx, corolla, and androecium were all highly correlated. Abnormal whorls within the same flower almost always varied in the same direction, simultaneously having either more than or less than the normal number of organs. The gynoecium tended to have more organs when abnormal while the other whorls had fewer organs when abnormal. Few plants exhibited either complimentary abnormalities, with total organ production across all its flowers remaining constant, or high formula polymorphism, producing four or more unique abnormal formulae. In addition, some types of abnormal formulae were

much more common than others. This study represents the first formal documentation of floral formula variation in *Phlox longifolia* and the first extensive analysis of patterns of variation with respect to degree and direction in all four whorls simultaneously.

INTRODUCTION

Phenotypic characters that are uniformly expressed within and among populations and are predominantly present in a single state may be referred to as canalized (Waddington, 1942; Dobzhansky, 1970). Variation can still exist for such a trait, but it may be masked by genetic or developmental processes. When previously hidden phenotypic variation for a normally invariant character is exposed, the process is called decanalization. Decanalization of phenotypic plant characters can result from artificial selection (Huether, 1968; Wilson and Stapp, 1979), allopolyploidy (Grant, 1956), inbreeding (Lehmann, 1987), hybridization (Levin, 1970; Bachmann and Chambers, 1978), and environmental stress (Heslop-Harrison, 1959; Huether, 1969; Waddington, 1975; Wilson and Stapp, 1979).

The numbers of different floral organs that comprise a flower – collectively referred to as the "floral formula" – are thought to be among the most constant of all canalized characters in angiosperms (Stebbins, 1951; Berg, 1959; Bradshaw, 1965; Cronquist, 1968), yet inconstancy of floral formula does exist. Intra-specific inconstancy of floral formula has evolutionary potential in that if a particular genetically-based deviation exhibits adaptive value, it can become favored by selection (Levin, 2000). For example, variation within the perianth whorls – fewer or greater than normal numbers of sepals and petals – might affect overall flower structure and thereby pollination. And

abnormalities in the reproductive whorls – the androecium (stamens) and the gynoecium (carpels) – might affect total reproductive output and thereby male and female fitness. Thus, it is highly probable that due to its involvement in reproductive function, the floral formula is subject to various selective pressures, most likely ones that act to optimize and ensure constancy of floral form (Huether, 1968; Waddington, 1975; Fenster and Galloway, 1997; Cresswell, 1998). However, selection could also lead to a stable new floral formula through an increase in formula variation, exposure of novel adaptive variants, and recanalization at the new formula state.

The objective of this study was to quantify the amount of floral formula variation in natural populations of *Phlox longifolia* and to identify potential patterns within the abnormal formulae. By looking at patterns of floral formula variation, we can gain insight into how genetic variation for a canalized character is simultaneously suppressed and preserved. More specifically, in an attempt at complete deconstruction of the floral formula, this study looked at: (1) incidence and degree of abnormality, (2) tendencies toward greater than normal (supernumerary) and fewer than normal (subnumerary) numbers of organs, (3) correlation among whorls, (4) uniqueness (rarity) of specific formulae, and (5) abnormal formula polymorphism.

MATERIALS AND METHODS

Study organism – *Phlox longifolia* Nutt. is a perennial native plant of the western United States. The modal floral formula (5 sepals, 5 petals, 5 stamens, 3 carpels) is shared by most of the genera in the Polemoniaceae family, and with the exception of carpel number, the same formula is also highly prevalent among the order Polemoniales

(Dawson, 1936; Grant, 1959, 1998; Grant and Grant, 1965; Cronquist, 1968; Stebbins, 1974). *Phlox longifolia* blooms from April at lower elevation to July at higher elevation, each individual producing from several tens to several hundred flowers. Flowers range in color from lavender to pink-purple and are pollinated by various lepidopterans. The spreading horizontal stems from which the upright flowering stems arise are commonly buried beneath pine needles or other ground litter, and neighboring plant individuals can be distinguished by consistent differences in corolla color, size, and shape. As with many other *Phlox* species, *P. longifolia* is commonly found in disturbed sites next to roads, cattle and horse trails, hiking trails, and bike paths. The populations observed in this study were all roadside and/or trailside on north to northwest facing slopes among stands of predominantly Ponderosa pine (*Pinus ponderosa* Laws.) and Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) (Weber, 1976).

The five populations used in this study were located in Roosevelt National Forest in the Hewlitt Gulch area (HGA and HGB; approx. 40° 42.85' N, 105° 18.91' W) and Cherokee Park area (CPA, CPB, CPD; approx. 40° 53.46' N, 105° 27.46' W), approximately 30 miles northwest and north-by-northwest of Fort Collins, Colorado, USA. A sixth population (CPC) was destroyed when a private landowner mowed the roadside right-of-way. These populations are at the eastern edge of the species range and, prior to this study, had not been mapped to any national or local databases. Voucher specimens were deposited at the Colorado State University Herbarium.

Survey methods – During peak flowering in the spring of 2003, five natural populations of *Phlox longifolia* Nutt. were surveyed for floral formula inconstancy. At each site, plants were sampled at one meter intervals along three 10m transects for a total

of 30 plants per population (N = 150 plants). Depending on the spatial arrangement of the plants, transects were either longitudinally aligned or staggered in parallel rows, whichever best sampled the entire breadth of the population. Ten flowers were scored for each plant (N = 1500 flowers). If more than ten flowers were present, flowers were sampled haphazardly within the plant relative to branching and inflorescence position to minimize temporal or developmental bias. Scoring consisted of counting the numbers of sepals, petals, stamens, and stigmatic lobes for each flower relative to the modal organ values for the *Phlox* genus: five sepals, five petals, five stamens, and three carpels. Stigmatic lobe number was considered a direct estimate of carpel number. This was verified by collecting and dissecting flowers with various lobe numbers (see Appendix I). Reduced or deformed organs, when encountered, were counted as full parts.

Data management – Raw count data were treated in three main ways. To investigate the frequency of abnormal organ production, counts were converted to binomial data where whorls with abnormal numbers of organs were scored as 1 and normal whorls were scored as 0. Similarly, whole flowers and whole plants were scored as either normal or abnormal. These binomial data will hereafter be referred to as "incidence" of abnormality. In addition, the total proportion of abnormal calyces, corollas, androecia, and gynoecia for each plant was calculated (i.e., the proportion of 1's per total flowers scored). These data were then transformed by taking the arcsine square root to satisfy the assumption of homogeneity of variance. These proportional data will hereafter be referred to as "degree" of abnormality.

To investigate the tendencies of plants, when abnormal, to produce fewer versus greater numbers of floral organs, abnormalities at the whorl, flower, and plant levels were

partitioned into two categories: "supernumerary" (having more than the normal number of organs) and "subnumerary" (having fewer than the normal number of organs). Specifically, the normal organ values at each level were as follows: 5 sepals, 5 petals, 5 stamens, 3 carpels, 18 total organs per flower, and 180 total floral organs per plant (assuming 10 flowers scored). The two higher level analyses (flower and plant) were included to determine whether abnormal organ number in whorls and/or flowers is potentially balanced out to some degree over the greater floral development of the entire flower and/or plant.

To investigate the incidence of both supernumerary/subnumerary whorls and simple abnormal/normal whorls in relation with the other floral whorls simultaneously, the raw organ counts for the four whorls of each flower were converted into a four-digit number that will hereafter be referred to as the "count-formula". This number served as a potentially unique identifier for the entire floral formula as a singular trait and was used in analyses regarding patterns of concurrent variation among the four whorls and floral formula polymorphism. The latter can be indicative of the type of developmental aberration behind deviant floral organ numbers. For example, plants may either produce many abnormal flowers all of the same formula type (e.g., 4-4-4-3) or of several different formula types (e.g., 4-4-4-3, 4-4-5-3, 5-6-5-3, etc.). To investigate the involvement of different types of developmental instability in floral formula deviation, plants were sorted into one of two classes of abnormal formula polymorphism: high polymorphism (four or more unique abnormal formulae) and low polymorphism (three or less unique abnormal formulae). An abnormal floral formula was anything other than 5-5-5-3.

Statistical analyses – The FREQUENCY and GLIMMIX procedures in SAS software (Version 9.1, 2002-2003 SAS Institute Inc.) were used to analyze binomial data at the plant and flower levels both by population and by whorl. Specifically, the GLIMMIX procedure was used for the by-whorl analyses because the units of comparison represent measures from the same plant individuals and are therefore not independent. This procedure allows for a generalized linear mixed model while specifying a particular distribution and link function (here, binomial and logit, respectively). The GLIMMIX procedure was used to determine if ratios of abnormal to normal flowers and plants for each population differed among whorls (i.e., within-population differences among whorls), and the FREQUENCY procedure was used to determine if ratios of abnormal to normal flowers and plants for each whorl differed among populations (i.e., within-whorl differences among populations).

Analyses of variance were used where appropriate to determine if any of the populations or whorls differed for degree of abnormality. If F-tests were significant, pairwise Tukey's studentized range tests (HSD) were performed to determine which of the populations or whorls differed. During early analyses, there appeared to be two population groupings for several of the traits, with all members of each group being different from all members of the opposing group but similar to members of the same group. These two groups corresponded to geographical locations, with the Cherokee Park plants comprising one group and the Hewlitt Gulch plants the other. Therefore, *a posteriori* contrast statements were designed to test the following specific hypotheses: (1) do plants of the three Cherokee Park populations differ for the trait?, (2) do plants of

the two Hewlitt Gulch populations differ for the trait?, and (3) do the Cherokee Park plants differ from the Hewlitt Gulch plants?

RESULTS

Eighty-seven percent of the 150 plants sampled were abnormal for at least one flower (Table 2.1). Incidence of abnormal plants did not significantly differ either among the whorls within each population or among the populations within each whorl. However, when all the populations were pooled, the whorls significantly differed ($F = 3.10$, $P = 0.0295$), owing mostly to low incidence of plants with abnormal gynoecia relative to the other three whorls (Fig. 2.1).

There was no significant population effect on degree of abnormality within any of the four whorls. Across all populations, the mean degree of abnormal flowers (abnormal for at least one whorl) was 24%, with 74% of plants producing between one and four abnormal flowers out of ten. Only 13% of plants had zero abnormal flowers, and no plants had more than 8 abnormal flowers (Table 2.1). The degree of abnormality differed among the four whorls ($F = 3.79$, $P = 0.0104$), with a mean of 14% abnormal calyces per plant, 16% abnormal corollas, 15% abnormal androecia, and 11% abnormal gynoecia (Fig. 2.2). A similar trend among the whorls was seen in the individual populations, though none proved statistically significant. Furthermore, there was no correlation between the level of within-population abnormality and within-plant abnormality, i.e., the populations with the highest and lowest proportions of abnormal plants did not necessarily contain plants with the highest and lowest degrees of abnormality, respectively.

There was, however, a significant population effect, whorl effect, and population*whorl effect on the tendency to produce either subnumerary or supernumerary floral organs (Table 2.2). In three of the five populations, abnormal calyces, corollas, and androecia were significantly more subnumerary than supernumerary (Fig. 2.3). By contrast, the gynoecium tended toward supernumerary abnormalities, although this was only significant in two of the five populations. Only one population, HGB, showed no significant tendencies toward either sub- or supernumerary abnormalities in any of the whorls.

The patterns of sub- and supernumerary organ production at the flower level and the whole plant level were similar. Differences among populations at both levels were strongly influenced by geographical location. Cherokee Park populations produced more subnumerary flowers and plants, while the Hewlitt Gulch populations produced more normal flowers and plants, with the degree of deviation from normality being less extreme than in the Cherokee Park populations (Fig. 2.4). However, significant within-population variation was present at the plant level; this fact, coupled with the geographical differences, could suggest both a genetic (among-plant) and an environmental (among-population) component to total organ production among all the flowers within an individual plant.

Several distinct trends emerged when analyzing the floral formula as a singular trait. Across all populations and the 362 total flowers that had an abnormal floral formula, 36 different types of count-formulae were represented. Just six of these comprised 70% of all abnormal flowers (Fig. 2.5), and these six were all formulae in which the outer three whorls were all identically abnormal, the gynoecium was abnormal, or both. More

specifically, abnormal flowers which were simultaneously deviant for at least the calyx, corolla, and androecium were much more common (51% of all abnormal flowers) than flowers that were deviant for either two of the outer three whorls (10%) or one of the outer three whorls (16%). The floral formulae 4-4-4-3 and 5-5-5-4 were the two most common abnormal formulae in four of the five populations. Abnormal flowers in the fifth population, HGB, were mostly either 6-6-6-3 or 5-5-5-2.

As the outer three whorls varied together, they also tended to vary in the same direction and to the same degree. Out of the 186 flowers in which at least the outer three whorls were abnormal, 184 had identical organ counts for those three whorls – either all identically supernumerary or identically subnumerary – and the remaining two flowers had whorls that were deviant in the same direction, though not with equal organ counts. Similarly, out of the 37 flowers in which just two of the outer three whorls were abnormal, 35 of these had equal organ counts in the deviant whorls, one had whorls that were deviant in the same direction, and only one flower had whorls that were deviant in opposite directions (both a subnumerary whorl and a supernumerary whorl).

Although organ number and variation in the gynoecium were less correlated with the outer three whorls than the outer three whorls were with each other (Table 2.3), abnormal gynoecia still varied, more often than not, in the same direction as other abnormal whorls. Out of 88 flowers in which the gynoecium and at least one other whorl were abnormal, 68 flowers had a gynoecium that varied in the same direction as the other abnormal whorls (Fig. 2.6). So although incidence of abnormality in the gynoecium appears at least partially independent, the over-/under-production of organs is probably linked among all four whorls. In addition, on the limited occasions when abnormalities in

the outer whorls did not match the direction of abnormality in the gynoecium, it was overwhelmingly when the gynoecium was supernumerary. This might be related to the fact that in these instances the supernumerary gynoecium and the subnumerary outer whorl(s) both produced four organs. Thus, secondary to correlation of direction of abnormalities, there may also be an intrafloral developmental tendency to produce the same number of organs, even within the gynoecium.

Lastly, the level of abnormal formula polymorphism did not differ among populations. Eighty-eight percent of all abnormal plants exhibited low polymorphism with three or fewer different abnormal formulae. Only a small number of plants showed high polymorphism with four or more unique abnormal formulae, and these were distributed randomly among the populations.

DISCUSSION

Overall variation and comparative conclusions – This study represents the first formal documentation of floral formula variation in *Phlox longifolia* and the first extensive analysis of patterns of variation with respect to degree and direction in all four whorls simultaneously. Overall, wild *Phlox longifolia* was highly variable for floral formula with roughly 90% of all plants producing at least one abnormal flower. Given that the current study represents a snapshot of the total flowering period and thus only a portion of total developmental time, *P. longifolia* plants with 100% perfect floral development are likely rare in nature. Ellstrand (1983) found that wild populations of the polemoniaceous *Ipomopsis aggregata* sampled during peak flowering produced only 33% abnormal plants. That number jumped to 93% in a later study where the same plants

were scored over the entire flowering season (Ellstrand and Mitchell, 1988). Furthermore, it appears that at least for the plants for which sufficient organ number data exists, degree of within-plant abnormality varies with plant duration. Only 7% of flowers in the annual *Phlox drummondii* (Lehmann, 1987) and 2% of flowers among various annual species of *Linanthus* (Huether, 1969) were abnormal for organ number, while floral abnormality in the biennial *Ipomopsis aggregata* was slightly greater at 10% (Ellstrand, 1983). By comparison, the perennial shrubs *Nyctanthes arbor-tristis* and *Jasminum multiflorum* were reported to have 31% and 50% abnormal flowers, respectively (Roy, 1963), and two populations of perennial *Drosophyllum lusitanicum* had 59% and 87% abnormal flowers (Olivencia et al., 1995). In addition, Wilson and Stapp (1979) observed over 50% of flowers within a primitive race stock of cotton to have abnormal numbers of involucral bracts. *Phlox longifolia*, an herbaceous perennial, fits neatly into this apparent continuum of longevity with an intermediate 24% abnormal flowers.

Where comparative values for all four floral whorls are available, the extent of abnormality within each of the whorls of polemoniaceous species is inconsistent. The current study found that roughly one in ten *P. longifolia* flowers is abnormal, with a greater likelihood that the aberration is in one (or all) of the outer three whorls. Alternatively, Lehmann (1987) found that the most abnormal whorl in *Phlox drummondii* was most often the gynoecium. Data for *Ipomopsis aggregata* are contradictory, with the gynoecium most abnormal in one study (Ellstrand, 1983) and least abnormal in another (Ellstrand and Mitchell, 1988).

Genetics versus environment – The five populations of *Phlox longifolia* analyzed here were not significantly different for either the production of abnormal plants, the

degree to which those plants were abnormal, or the relative extent to which each whorl was abnormal and integrated with the other whorls. The only significant population-level difference involved direction of whorl abnormality. The Cherokee Park populations all produced large proportions of subnumerary calyces, corollas, and androecia but either supernumerary or balanced gynoecia, resulting in plants that were subnumerary overall for total organ production. The plants from Hewlitt Gulch, by contrast, produced abnormal whorls that were mostly random with respect to directionality, with mean total organ production indistinguishable from fully normal plants. Plants from both areas experience fairly similar general environmental conditions such as exposure, elevation, co-occurring species, and weather. The biggest distinguishing feature between the two geographic regions is level of disturbance. The Cherokee Park populations are exposed to constant road dust and intermittent trampling from livestock herding, trail riding, and hiking, while the Hewlitt Gulch populations are considerably more sheltered and likely suffer infrequent and minor disturbance, if any. In addition, given that coincident supernumerary and subnumerary whorls within the same abnormal flower were extremely rare in all populations, we can assume that total floral organ number is not constrained at the level of individual flowers. Yet abnormal organ production in the Hewlitt Gulch populations was random enough such that total organ number at the plant level was indistinguishable from that of normal plants. Perhaps then, variation among these plants represents some base level of developmental "noise" while directional variation among Cherokee Park plants is environment-dependent. This contradicts evidence claiming petal number variation in *Phlox drummondii* is unaffected by

experimental stresses (Schlichting and Pigliucci, 1998), suggesting a need for further experimentation in this area.

The reproductive whorls – Special consideration should be given to directional deviations within the reproductive whorls. If stamen and carpel number are directly proportional to gamete number, then super/subnumerary organs at the plant level could affect total reproductive output. Few other studies report both meristic variation in a reproductive whorl as well as the direction of abnormality. Abnormal androecia in *Collomia grandiflora* were mostly subnumerary (Ellstrand et al., 1984). *Ipomopsis aggregata* produced more supernumerary gynoecia over a season while the direction of deviant androecia was population-dependent (Ellstrand and Mitchell, 1988). Results in *Phlox longifolia* were similar, with general trends toward supernumerary gynoecia and subnumerary androecia in all but one population. It is unclear what might cause simultaneous overproduction in one whorl and underproduction in another. It is possible that subnumerary variation in the androecium is more "acceptable" in natural populations given the large number of pollen grains produced by even a single stamen, and thus reduced stamen number is not heavily acted upon by selection. Supernumerary variation in gynoecia, on the other hand, may be allowed to persist given the more independent nature of its development and the assumption that more ovules, whenever possible, are advantageous. This hypothesis is in agreement with results from Lehmann's survey of greenhouse-grown lines of *Phlox drummondii* (1987) where all four whorls were overwhelmingly supernumerary. Developmental variation may have been influenced by presumably plentiful resources, and instability was therefore expressed predominantly as overproduction of floral organs rather than underproduction.

Evolution and the floral formula – The evolutionary implications of floral formula variation are complex. The few interpopulational differences among wild-growing *P. longifolia* might suggest that the mere capacity for meristic variation – the capacity to exhibit some level of plasticity for a meristic phenotype – may be inherent to the complex developmental pathways occurring within a floral meristem and thus may ultimately be driven by genetics. Slight population leanings, on the other hand, suggest that perhaps the degree and direction to which that plasticity for meristic phenotype is manifest is driven by environment. If such were true, highly variable environments might favor more plastic genotypes. Through expression of a range of formulae, certain plants may be more likely to contribute to subsequent generations under unpredictable circumstances. Thus, preservation of formula plasticity would be favored over stabilization of a single beneficial formula. For example, it at first seems reasonable that having more androecia to produce pollen, more gynoecia to produce ovules, and more perianth parts to attract pollinators would be advantageous. However, an individual having more of these organs might also be likely to require more resources to maintain the extra parts or suffer maladaptive changes to floral architecture.

In his review of floral structure variability versus stabilizing selection, Cresswell (1998) found that characters related to floral architecture and pollinator-flower mechanics exhibit the least variation. Alternatively, the characters most likely to be affected by environment and age, such as measures of biomass and nectar production, are often the most variable. Meristic traits like organ number were reported to be intermediate in variability. However, this does not account for the direct effects of variable organ number on either of the other two categories. For example, the number of petals in a flower might

easily affect measures of corolla size, which was reported as an "advertising" trait with low variability, while the number of carpels is likely related to number of ovules per flower, a purported "gender" trait with high variability. Thus under Cresswell's schematic, meristic traits, themselves of collective intermediate variation, could be partitioned differentially among the low-variable and high-variable trait categories, with the low-variable traits constrained by pollinator-mediated selection and the high-variable traits selected for plasticity of expression. In *Phlox longifolia*, this translates to maintenance of variation among the reproductive whorls and dueling forces within a perianth shaped by both pollination-related preferences for high structural constancy and unpredictable environmental conditions.

Further confounding the evolution of floral formula is the fact that although *Phlox longifolia* exhibited plasticity for formula phenotype, that range of phenotypes was constrained by significant correlation among the whorls (some more so than others). According to Scharloo (1989), such correlation is usually caused by shared genetic control, shared resource base, shared functional role, and/or correlated response to within-environment variability. For the number of organs within at least the outer three whorls of *P. longifolia*, all could be true. In addition, the inter-whorl correlation observed here for organ number is of a different type and follows a different pattern than inter-whorl developmental correlation relative to organ identity. Specifically, patterns of organ identity as defined by the ABC model of gene action (Coen and Meyerowitz, 1991) show pair-wise homeotic gene associations between the perianth whorls (A class), between the reproductive whorls (C class), and between the corolla and androecium (B class). By

contrast, the organ number patterns observed here show close associations among the outer three whorls only, with a more independently acting gynoecium.

Simple organ number correlation relates to a greater developmental bias toward full floral merosity. Similar overriding tendencies to maintain merosity have been documented in species of the legume *Gleditsia* where flowers with different merosity (trimerous, tetramerous, and pentamerous) were often found within the same inflorescence of an individual, yet variable organ number among the whorls within each meristic morphotype was rare (Tucker, 1991). This suggests strong developmental links between the whorls during early organogenesis. Indeed, a number of studies have shown that the patterns of organ initiation in the first-formed whorl set the pattern for organ initiation in later whorls (reviewed in Greyson, 1994). In *Phlox drummondii* (and presumably for all species within the genus), the developmental sequence of organogenesis begins with sepal primordia, followed by initiation of the androecium, gynoecium, and corolla in that order (Miller and Wetmore, 1946). In the current study, number of sepals was an accurate predictor of the number of petals and stamens 84% of the time. The independent nature of the gynoecium, on the other hand, possibly speaks to the importance of having its developmental regulation separate from the regulation of perianth and androecium development. Ovules are often a better reproductive investment than pollen (Willson, 1983), and by partitioning reproductive development in two separate modules (outer three whorls versus gynoecium), a plant gets two independent chances at reproduction for every flower made, reducing the effects of ephemeral developmental instability on total reproductive output and fitness.

The usefulness of a floral formula as a taxonomic diagnostic tool is predicated upon the assumption that developmental and/or selective constraints maintain its constancy. Inconstancy of floral formulae, however, can serve as potential fodder for diversification among lineages, especially given its probable association with reproductive fitness. Using descriptive studies such as this as a starting point, further analyses that focus on fitness consequences and heritability should advance our understanding of developmental constraints and unravel the adaptive nature of floral merism.

Table 2.1. Frequency distributions and percentages of *Phlox longifolia* flowers and plants with abnormal numbers of organs.

		No. abnormal flowers (out of 10 per plant)											% abnormal	% abnormal
	Population	0	1	2	3	4	5	6	7	8	9	10	flowers	plants
		No. plants												
Any whorl	CPA	4	4	6	4	3	4	2	2	1	.	.	31.3	86.7
	CPB	4	8	6	2	8	1	.	1	.	.	.	23.3	86.7
	CPD	4	6	10	3	3	3	1	.	.	.	.	22.7	86.7
	HGA	2	8	7	5	5	1	.	2	.	.	.	25.3	93.3
	HGB	6	9	6	7	.	.	.	1	1	.	.	19.0	80.0
	All pops	20	35	35	21	19	9	3	6	2	.	.	24.3	86.7
Calyx	CPA	11	5	3	3	4	2	1	1	.	.	.	19.7	63.3
	CPB	8	9	6	5	2	.	.	.	.	.	.	14.7	73.3
	CPD	11	9	6	1	2	1	.	.	.	.	.	12.3	63.3
	HGA	7	11	9	1	1	1	.	.	.	.	.	13.7	76.7
	HGB	12	6	7	5	.	.	.	.	.	.	.	11.7	60.0
	All pops	49	40	31	15	9	4	1	1	.	.	.	14.4	67.3
Corolla	CPA	6	6	6	3	4	4	1	.	.	.	.	23.0	80.0
	CPB	5	12	7	2	4	.	.	.	.	.	.	16.0	83.3
	CPD	10	10	5	4	1	.	.	.	.	.	.	12.0	66.7
	HGA	7	10	7	3	1	1	1	.	.	.	.	16.0	76.7
	HGB	12	5	7	5	1	.	.	.	.	.	.	12.7	60.0
	All pops	40	43	32	17	11	5	2	.	.	.	.	15.9	73.3
Androecium	CPA	7	7	6	2	4	3	.	1	.	.	.	21.0	76.7
	CPB	4	16	4	3	3	.	.	.	.	.	.	15.0	86.7
	CPD	7	9	9	2	2	1	.	.	.	.	.	15.3	76.7
	HGA	6	12	8	2	1	1	.	.	.	.	.	14.3	80.0
	HGB	13	5	8	3	1	.	.	.	.	.	.	11.3	56.7
	All pops	37	49	35	12	11	5	.	1	.	.	.	15.4	75.3
Gynoecium	CPA	12	9	5	.	3	.	1	.	.	.	.	12.3	60.0
	CPB	12	7	7	.	3	.	1	.	.	.	.	13.0	60.0
	CPD	12	12	4	1	.	1	.	.	.	.	.	9.3	60.0
	HGA	9	12	4	4	1	.	.	.	.	.	.	12.0	70.0
	HGB	14	11	3	.	.	1	.	.	1	.	.	10.0	53.3
	All pops	59	51	23	5	7	2	2	0	1	.	.	11.3	60.7

Table 2.2. ANOVA for ratio of subnumerary to supernumerary abnormalities at the whorl level for five populations of *Phlox longifolia*.

Effect	Num DF	Den DF	F value	Pr > F
Population	4	145	3.08	0.0182
Whorl	3	5835	29.94	<0.0001
Pop* Whorl	12	5835	2.49	0.0030

Table 2.3. Pearson correlation coefficients for (A) degree of abnormality per plant, (B) number of organs per whorl within all flowers scored, and (C) number of organs per whorl within abnormal flowers only. All coefficients are significantly greater than zero ($P < 0.05$).

A) Degree of abnormality per plant (N = 150)

	Calyx	Corolla	Androecium	Gynoecium
Calyx	---	0.90	0.88	0.30
Corolla		---	0.90	0.32
Androecium			---	0.32
Gynoecium				---

B) Organ number per whorl (all flowers; N = 1500)

	Calyx	Corolla	Androecium	Gynoecium
Calyx	---	0.84	0.84	0.25
Corolla		---	0.87	0.25
Androecium			---	0.27
Gynoecium				---

C) Organ number per whorl (abnormal flowers only; N = 362)

	Calyx	Corolla	Androecium	Gynoecium
Calyx	---	0.83	0.84	0.32
Corolla		---	0.86	0.34
Androecium			---	0.35
Gynoecium				---

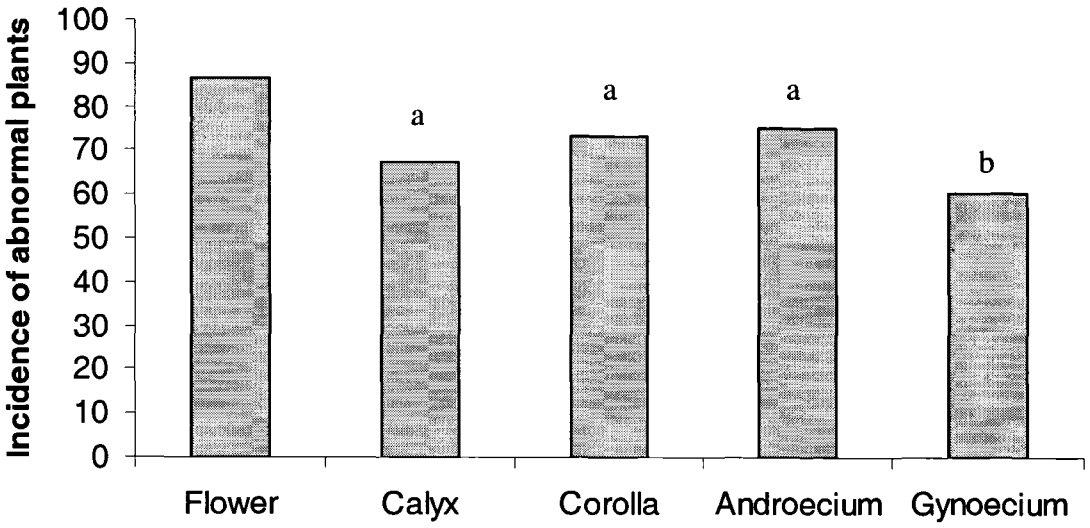

Figure 2.1. Incidence of abnormal plants for each trait. Populations did not significantly differ for any of the whorls so plants from all populations were pooled. The y-axis represents the percentage of total plants that have at least one flower abnormal for that trait. The "flower" trait denotes plants with at least one flower abnormal for at least one whorl. Whorls with different letters are significantly different ($P < 0.05$).

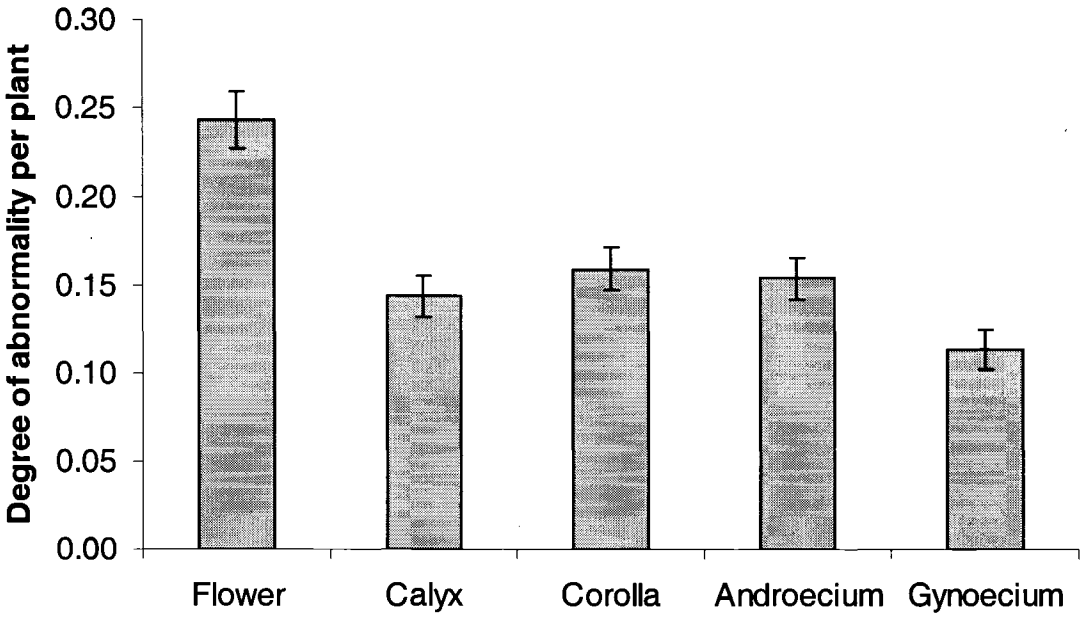

Figure 2.2. Mean degree of abnormality per plant for each trait. Populations did not significantly differ for any of the whorls, so plants from all populations were pooled. The "flower" trait represents the proportion of flowers out of total flowers scored that were abnormal for at least one whorl. Y-error bars = standard error of the mean.

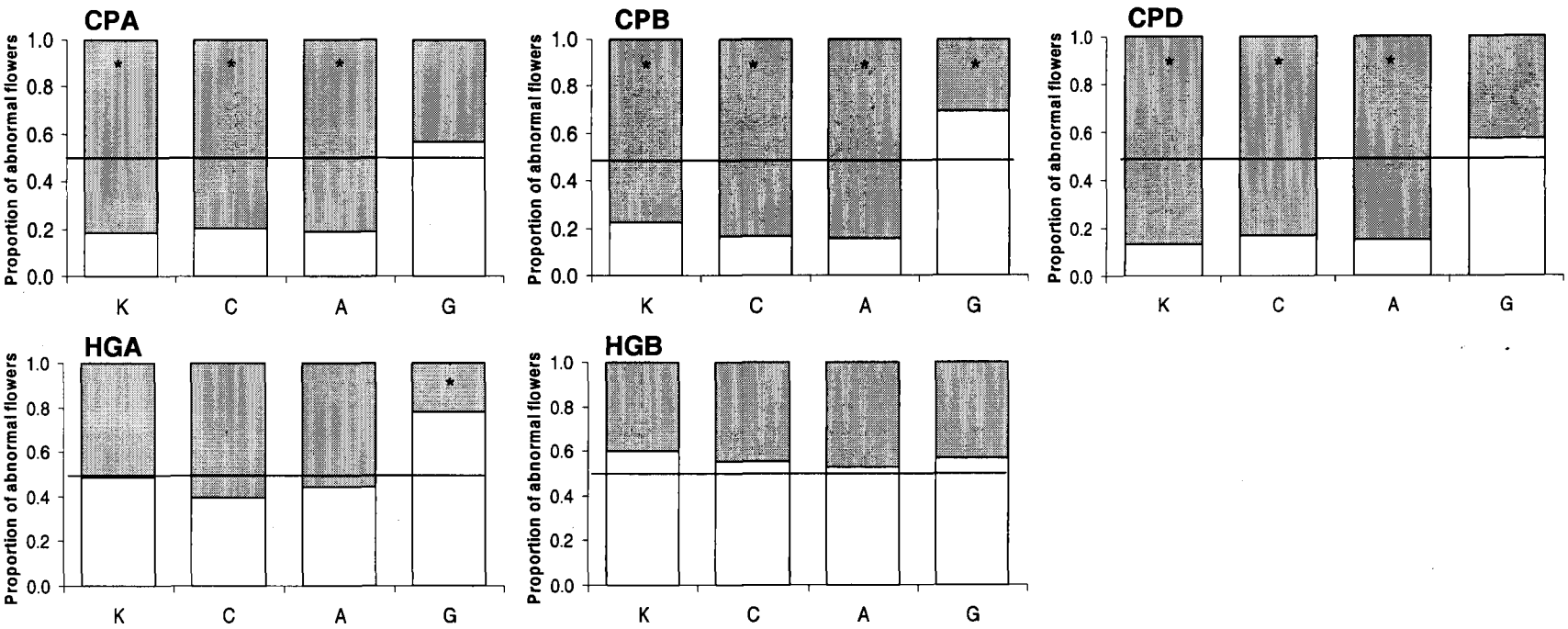

Figure 2.3. Relative proportions of subnumerary (grey) versus supernumerary (white) whorls in five populations of *Phlox longifolia*. Subnumerary whorls have fewer than the normal number of organs. Supernumerary whorls have greater than the normal number of organs. K = calyx; C = corolla; A = androecium; G = gynoecium. Bars with asterisks have proportions significantly different than 0.5 as denoted by the horizontal lines.

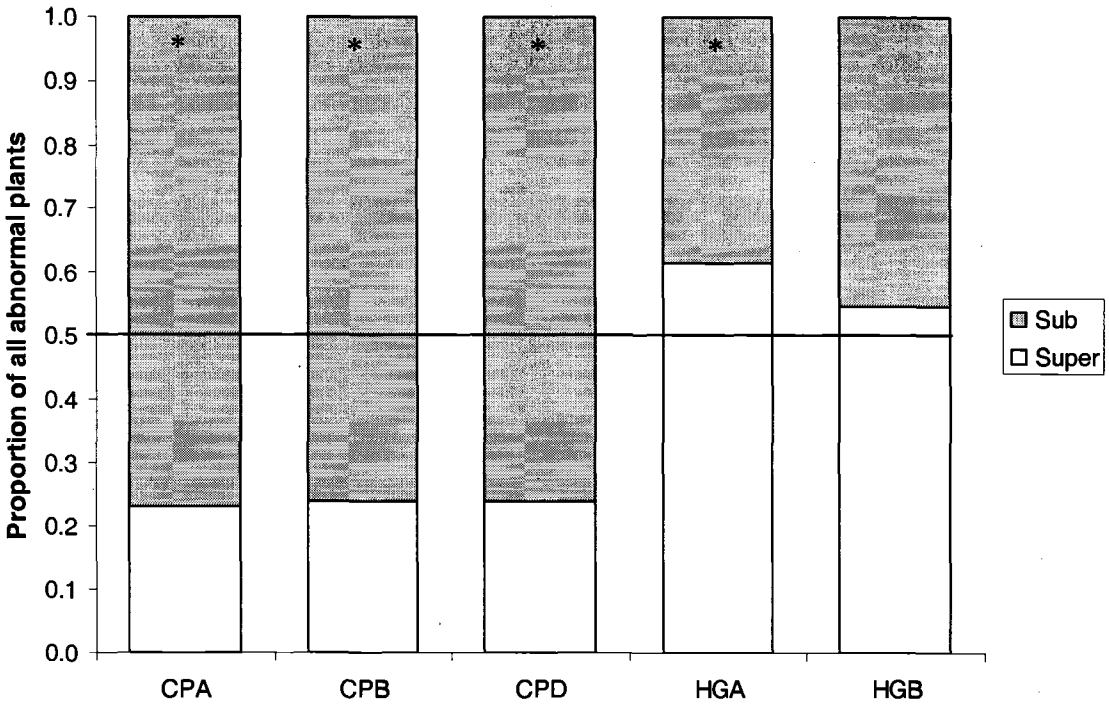

Figure 2.4. Relative proportions of subnumerary versus supernumerary plants for five populations of *Phlox longifolia*. Subnumerary plants have fewer than 180 total floral organs. Supernumerary plants have greater than 180 total floral organs. Bars with asterisks have proportional ratios that are significantly different than 0.5 as denoted by the horizontal line.

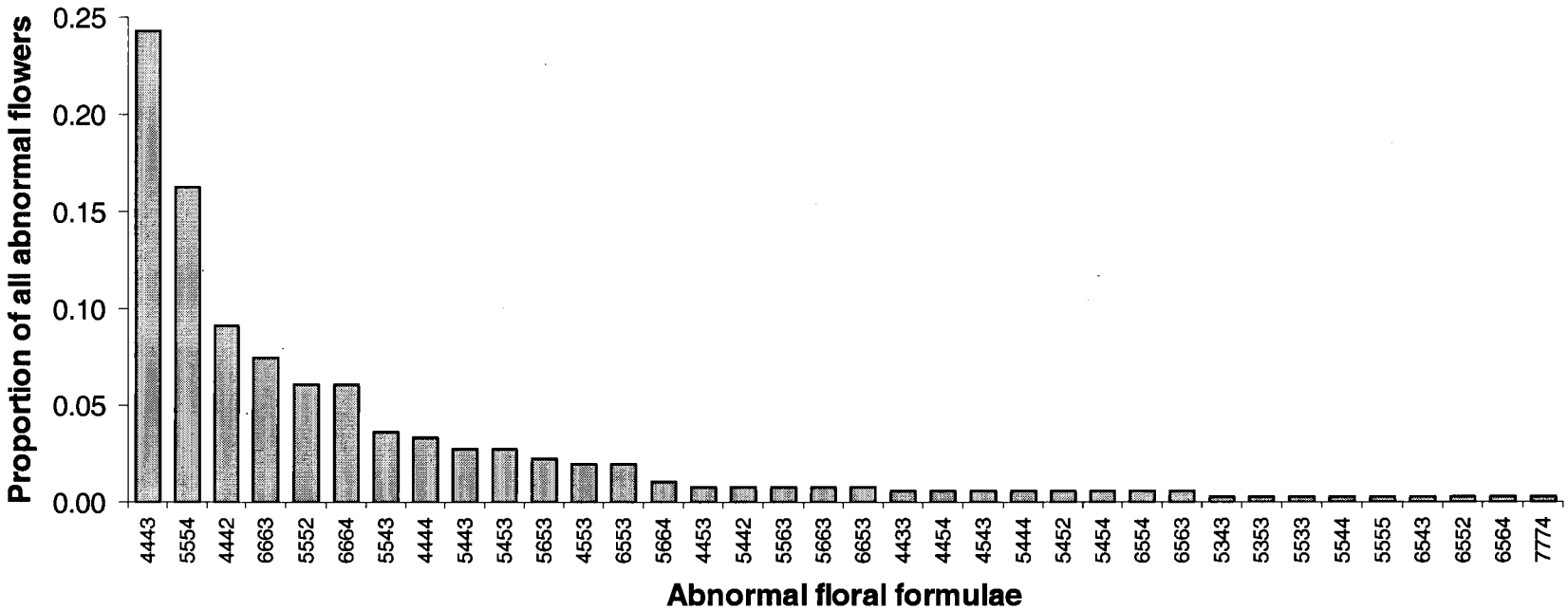

Figure 2.5. Frequency of different abnormal floral formulae across all populations of *Phlox longifolia*. Within each formula, the order of the whorls, left-to-right, is calyx, corolla, androecium, gynoecium. Each digit within a formula is an organ count for that whorl. The first six formulae together represent 70% of all of abnormal flowers ($N = 362$). The normal floral formula is 5553.

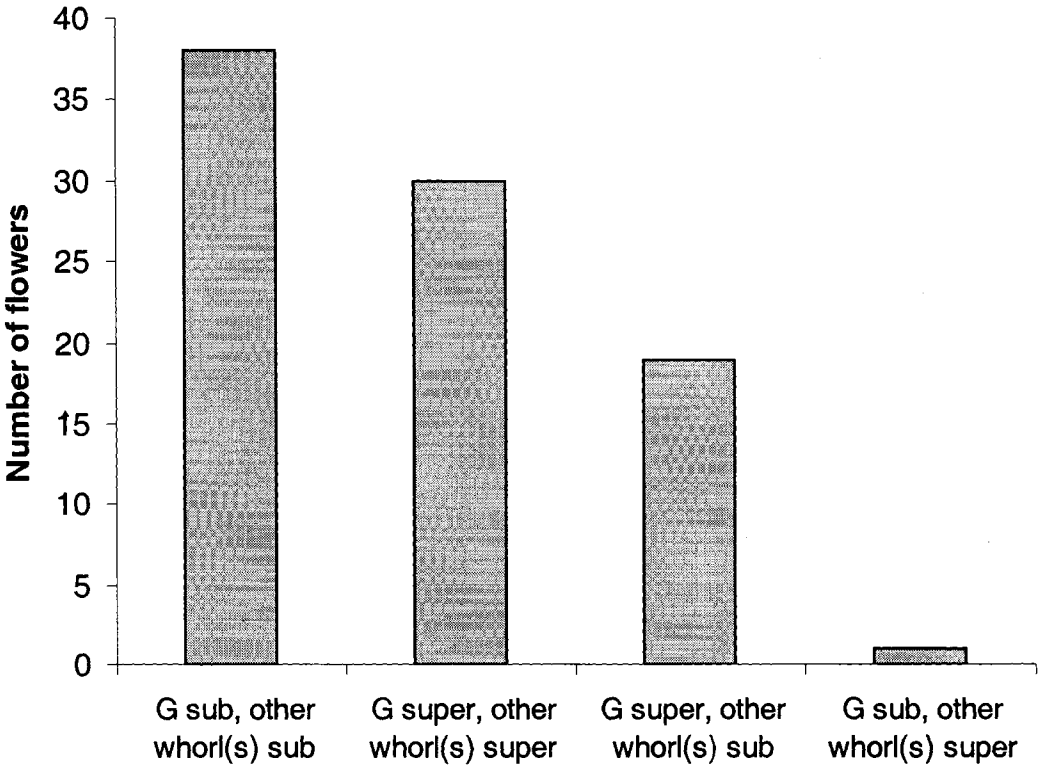

Figure 2.6. Frequency and directionality of abnormalities in *Phlox longifolia* gynoecia (G) relative to directional abnormalities in the other floral whorls. Sub = subnumerary or possessing fewer than normal number of organs. Super = supernumerary or possessing greater than normal number of organs.

CHAPTER 3

TEMPORAL AND SPATIAL PATTERNS OF FLORAL FORMULA VARIATION IN *PHLOX* (POLEMONIACEAE)

ABSTRACT

Two species of *Phlox* were quantified for temporal and spatial patterns of floral formula variation. Natural populations of *Phlox longifolia* exhibited relatively high proportions of flowers with abnormal formulae, with abnormality increasing over the course of the flowering season. A greenhouse population of *Phlox drummondii* exhibited only moderate levels of abnormality among early flowers, with abnormality decreasing over time. In addition, *Phlox drummondii* flowers within branches initiated early in development were more abnormal than those of later developed branches. These results suggest that early floral development is unstable but becomes more stable over time in the absence of environmental stress. Patterns of directional abnormalities in wild *Phlox longifolia* also suggest some plants may be predisposed to either greater or fewer organs when abnormal, which could potentially affect reproductive output and thereby fitness.

INTRODUCTION

Several features of the angiosperm flower are considered highly conserved within taxonomic groups. Such features include floral organ fusion (adnation/connation), organ

insertion (hypogyny/epigyny/perigyny), inter-whorl organ arrangement (opposite/alternate), and organ number per whorl (i.e., the floral formula) (Smyth, 2005). Little is currently known about how the pathways of floral development specify organ patterning both with regularity within taxonomic groups but differentially among taxonomic groups (Zik and Irish, 2003; Smyth, 2005; Aida and Tasaka, 2006). Only one gene to date, *PERIANTHIA*, is known to directly affect floral merosity without a concomitant increase in meristem size or other pleiotropic effects (Running, 1996; Chuang et al., 1999). Mutations of this gene cause *Arabidopsis* flowers to exhibit an ancestral pentamerous floral formula (5 sepals, 5 petals, 5 stamens, and 2 carpels) rather than the normal formula of 4-4-6-2.

Similar variation for coordinated floral merosity has been observed for a number of other plants (Ellstrand, 1983; Lehmann, 1987; Tucker, 1991; Donnison and Francis, 2002; Byerley, Chapter 2). Existing variation is evolutionarily informative given that the patterns now so highly conserved among taxonomic groups may have originally evolved by selective forces acting upon similar variation. Unfortunately, there is a large gap of knowledge concerning the potential effects of time and whole-plant developmental progression on floral formula constancy. Very few studies to date have documented levels of organ number variation within the same plants over time (but see Ellstrand and Mitchell, 1988).

The objective of this study was to observe patterns of intra-plant floral formula variation over an entire flowering period. This was achieved through a longitudinal organ number census for both wild and greenhouse-grown plants. It was hypothesized that if more abnormal flowers are produced during early flowering, then perhaps initial

activation of the floral meristem pathway is developmentally imprecise, but stability increases over time. Conversely, if later flowers are more abnormal, it may suggest that components of the system are breaking down over time, perhaps as a result of resource limitations. In addition to the temporal aspect, it was also of interest to determine whether abnormalities occur as independent events within the plant architecture or whether they can be correlated to flowering position and thus developmental timing. Because effects of development and environment are not mutually exclusive in natural settings, spatial effects of floral formula variation were only observed for greenhouse-grown plants.

MATERIALS AND METHODS

Study organisms – *Phlox longifolia* Nutt. is a perennial native plant of the western United States. The *P. longifolia* populations used in this study are located in the Cherokee Park area of Roosevelt National Forest (40° 53.46' N, 105° 27.46' W), approximately 30 miles north-by-northwest of Fort Collins, Colorado, USA. Due to low germination of naturally collected *P. longifolia* seeds, the greenhouse part of the study was conducted on *Phlox drummondii* Hook., a winter annual found primarily in the southeastern United States. A greenhouse common garden of *P. drummondii* was started from seeds acquired from Native American Seed Company (Junction, Texas, USA) where natural populations are maintained on private land. The modal floral formula of both *Phlox* species (five sepals, five petals, five stamens, and a tricarpellate gynoecium) is shared by most of the genera in the Polemoniaceae family (Grant and Grant, 1965; Cronquist, 1968; Stebbins, 1974; Grant, 1998). Both species are predominantly outcrossing (Appendix II; Byerley, unpublished data), producing flowers from early

spring to early summer. Lepidopteran activity is responsible for the bulk of pollination, after which flowers produce spherical capsules that explosively dehisce when mature (Grant and Grant, 1965; personal obs.).

Temporal variation in natural populations – Five randomly marked *Phlox longifolia* plants each at six different study sites (CPA1, CPA2, CPB, CPD1, CPD2, CPD3) were scored weekly for all open flowers from first bloom (19 May 2004) through the end of the natural flowering period (17 June 2004). Scoring consisted of counting the number of sepals, petals, stamens, and carpels for each flower. Carpel number was estimated using the number of stigmatic lobes (see Appendix I), and reduced or deformed organs were counted as whole. Organ number variation at the whorl and flower levels was analyzed for effects of population, date, and a population-by-date interaction using the GLIMMIX procedure in SAS software (Version 9.1, SAS Institute Inc. 2002-2003). These methods were used to investigate patterns of both the incidence of abnormality (normal versus abnormal) and the direction of organ number variation, where whorls with greater than the modal organ number were classified as "supernumerary", and whorls with fewer, "subnumerary".

Temporal and spatial variation in greenhouse plants – Thirty greenhouse-grown *Phlox drummondii* plants were scored for floral formula in every flower produced over a period of roughly 70 days. This length of time approximates 2.5 times the natural flowering duration of these plants. In addition to being scored for organ numbers in the manner described above, flowers were also scored for spatial position within each plant.

The hierarchical ordination methods used here to define spatial floral position were adapted from those originally used to define geomorphological systems and later by

botanists to describe plant architecture (reviewed in Steingraeber, 1980). Under this method, flowers and cymose inflorescences were nested within branching units which were each categorized as first, second, third, and higher orders within the entire plant body (Fig. 3.1). For example, all branches arising from the main first order stem were classified as second order and were ordered alphabetically from the base of the main stem upwards (2a, 2b, 2c, etc.). Similarly, any branches arising from a second order branch were classified as third order and acropetally alphabetized (3a, 3b, etc.). Groups of flowers were thus mapped within an entire plant according to the full name of the branch at whose terminus they arose (e.g. 1a:2b:3c:4a). This method allowed for flowers within the same inflorescence to serve as repeated measures for a specific position, and for all inflorescences to be collapsed into larger inflorescences as defined by their supported branching network.

These data were tested for effects of date and position on floral formula variation using the GLIMMIX procedure in SAS software (Version 9.1, SAS Software Inc. 2002-2003). Constant greenhouse conditions allowed for a significant date effect to be interpreted as an effect of developmental age or progression. Such an effect could then be further described within the plant architecture by examining effects of floral position. Specifically, position effects were analyzed in two ways: by order and by branching system. A significant order effect would indicate that developmental timing, distance from the main stem, and/or meristem age was involved in the stability of floral organ patterning. A significant branching system effect, by contrast, would indicate that abnormal flowers occurred more often within some branch systems than in others. This might be expected if organ number abnormalities are influenced by a somatic

chromosomal defect that spontaneously arises and then is passed along to all subsequent mitotic products, as is seen with cases of leaf and petal chimeras (Tilney-Bassett, 1986; Klekowski, 1988).

RESULTS

Temporal variation in natural populations – Floral formula variation was readily observed in natural populations of *Phlox longifolia*. All plants were abnormal for at least one flower over five weeks of observation (Table 3.1). Of 1800 total flowers, 333 (19%) were abnormal for at least one whorl. The degree of abnormal flowers per plant over the entire flowering season ranged from 2% to 48%. The most variable whorl was the corolla, and the least variable whorl was the gynoecium; these were abnormal in 55% and 16% of all abnormal flowers, respectively. Over 40% of abnormal flowers were simultaneously deviant for the calyx, corolla, and androecium, and three-quarters of these had the specific formula 4-4-4-3. An additional 25% of abnormal flowers were deviant for either the gynoecium-only or for all four whorls simultaneously.

Both population and date had a significant effect on the proportion of abnormal flowers per plant ($F_{pop} = 9.51$, $P < 0.0001$; $F_{date} = 6.27$, $P = 0.0002$). Although earlier flowers were typically more normal than later flowers (Fig. 3.2), a significant interaction term indicates that the extent to which calyces, corollas, and androecia varied over time was not identical in all populations. One population, CPA2, was most abnormal during early flowering, with abnormalities becoming less frequent with time.

With respect to the direction of abnormalities, the outer three whorls were generally much more subnumerary than supernumerary (Fig. 3.3). The only exceptions

were early season flowers from CPA1 and CPA2 which were more supernumerary or had equal proportions. The gynoecium, by contrast, was more variable over both time and population, with some populations in some weeks having only supernumerary gynoecia while others had only subnumerary gynoecia (Fig. 3.4). Across all plants, populations, and whorls, supernumerary abnormalities were less frequent during early and late flowering than during peak flowering. In addition, there were more unique types of abnormal floral formulae produced during early flowering than late flowering.

Temporal and spatial variation in greenhouse plants – Of 21608 total *Phlox drummondii* flowers, 793 (4%) were abnormal for at least one whorl (Table 3.2). Total flower production per plant ranged from 99 to 1139 flowers. Abnormal flowers per plant ranged from 0.5% to 21%; half of all plants had less than 2% abnormal flowers, while only two plants had greater than 8% (18% and 21%). Sixty percent of all abnormal flowers were deviant for either the corolla or the gynoecium. Although most plants consistently produced abnormal whorls that were supernumerary, two plants more heavily favored subnumerary deviations. Over all plants, the androecium was the least variant whorl but had the highest frequency of subnumerary abnormalities. The most common abnormal formulae were 5-5-5-4 and 5-6-5-3. Of the 141 abnormal flowers that were simultaneously deviant for at least the calyx, corolla, and androecium, 137 were deviant in the same direction (all supernumerary or all subnumerary), and 128 had matching organ numbers in all deviant whorls.

In general, all 30 plants followed a similar pattern of flower production over time (Fig. 3.5-A). Daily flower emergence – as measured by the number of new flowers emerging on each flowering day – steadily increased up to Day 25 and then decreased

over the following week as new vegetative shoots and floral buds developed. As this next batch of flowers sequentially emerged, total flower number peaked for a second time around Day 45, and was approaching a third peak when data collection ended around Day 65. Only the first peak and trough (Days 1-32) were assumed to approximate the natural flowering cycle. The subsequent 1.5 cycles were assumed to represent a range of unnatural, extended flowering and longevity.

As total flower numbers increased over time, the proportion of abnormal flowers decreased (Fig. 3.5-B). Total flowers and proportion of abnormal flowers by date were negatively correlated (r = -0.36, P = 0.0042). Despite the significance of all GLIMMIX model effects (a consequence of the enormous sample size), much more of the variability for floral organ production can be explained by among-plant differences, probably genotypic in nature, than by date or plant-by-date interaction (F_{plant} = 24.6, F_{date} = 3.1, $F_{plant*date}$ = 1.4).

Relative to the spatial positioning of flowers, order level had a significant effect on the frequency of abnormal flowers (F = 11.84, $P < 0.0001$). First and second orders had the highest proportions of abnormal flowers, regardless of the branching network in which they were embedded, and proportions decreased as order levels increased (Fig. 3.6). There was no evidence that frequency of abnormal flowers varied among specific branching systems.

DISCUSSION

The overall floral formula variation seen here in *Phlox longifolia* is similar to variation observed in a previous study where plants were sampled during peak flowering

only (Byerley, Chapter 1). One notable difference, however, involves the direction of gynoecium abnormalities. When observed only during peak flowering in the previous study, many plants produced more supernumerary than subnumerary abnormal gynoecia. Plants in the present study showed a similar trend during peak flowering, but by the end of the entire season, subnumerary gynoecia comprised most of the total abnormal gynoecia for more than half of the plants. Such results contrast with earlier theories that used the incongruent directionality of the gynoecium relative to the other whorls as evidence supporting its independent development. Because these two studies took place during different years and involved different plants, further interpretation of the disparity is limited.

Significant temporal trends for floral formula variation were apparent among both *Phlox* species. Early-season flowers were more normal in wild *P. longifolia* and more abnormal in greenhouse-grown *P. drummondii*. Upward temporal trends in abnormal flower production in the wild might be attributed to environmental stress. As temperature, light intensity, and competition all presumably increase with time, the stability of organ development may become compromised. A similar explanation was given for the temporal increase in petal number variation observed in natural populations of *Linanthus androsaceus* (Huether, 1969); late germination, delayed floral development, and an increase in temperature and day-length were listed as the specific primary causes, although among-plant differences in susceptibility were also offered as evidence of a genetic component to variation.

Alternatively, a seasonal increase in floral formula variation could be a consequence of a seasonal increase of within-plant competition. Although both the ovary

tissue and its persistent calyx tube are photosynthetic, maturing fruits and ovules from early flowers almost certainly act as resource sinks and may as a consequence divert resources away from later flowers still in development (Waller and Steingraeber, 1995). Limited resources might affect localized transcription of various inhibitory or promoting factors involved in delineating positions of primary organ initiation within a floral meristem (Running and Hake, 2001; Aida and Tasaka, 2006). Fewer or greater regions of such factors could result in fewer or greater areas of cell division and thus a supernumerary or subnumerary organ pattern. This hypothesis is highly speculative, though, given that the suite of biochemical cues and transcription pathways associated with organ patterning are still largely unknown (Zik and Irish, 2003; Smyth, 2005; Aida and Tasaka, 2006).

Downward temporal trends in organ number variation, on the other hand, might reflect a level of background instability inherent to early flower development, causing variation to be greatest among the first-made flowers. When early-season flowers of wild *Ipomopsis aggregata* were found to have more variable formulae than later flowers (Ellstrand and Mitchell, 1988), the authors proposed that the early flowering environment is stressful, allowing for unstable floral production. Development then stabilizes as the season progresses, via either relief from environmental pressures or an increase in genetically-based ecological tolerance (i.e., better developmental "buffering"). A similar developmental pattern was observed in jellyfish populations where medusa symmetry was more variable during early clonal production but later stabilized (Gershwin, 1999). Environmental treatments had no effect on medusa variation, so temporal stabilization was explained by genetically-based self-correction mechanisms.

The data from this study suggest a similar situation may be true for *Phlox* where early flowering is unstable, and developmental precision increases with time given no environmental stress. Decreasing abnormality among greenhouse plants and a large initial degree of abnormality among wild plants both support this explanation. Specifically for *P. longifolia*, the added stress of its perennial duration and new floral growth from old tissue may explain why the starting values are so high in these plants, with further increase in floral abnormality being attributed to external environmental factors. In addition, an observed greater frequency of formula polymorphism in early *P. longifolia* flowers than in later flowers also supports an early-season instability hypothesis. The production of many different types of abnormal formulae (e.g., 5-6-5-3, 4-5-5-2, 6-6-5-3) may indicate a less precise, less integrated program of development than consistent production of just a few coordinated formula types (e.g., 4-4-4-3, 6-6-6-4).

For *P. drummondii*, greater floral abnormality among lower order branches is in line with the early-season hypothesis. Lower order flowers almost always developed before higher order flowers, although this sequential correspondence tended to break down over time. Huether (1968) reported similar results for *Linanthus androsaceus* where more petal number abnormalities were found among earlier terminal flowers than among flowers in lateral positions. Furthermore, although the greenhouse-grown *P. drummondii* plants experienced multiple "waves" of flowering, only the first of which can be expected to occur in wild plants, there was no evidence that extended longevity or an extended flowering period affected stability of floral development. Proportions of abnormal flowers in the later flowering cycles were still lower than in the first flowering cycle and much lower than in the first few days of flowering. Again, this pattern supports

the idea that in the absence of environmental stress or resource limitations, stability of floral organ development increases over time rather than breaking down.

Regarding temporal effects on direction of abnormality, the results here are inconclusive. The fact that abnormalities among the greenhouse-grown plants were mostly supernumerary while wild plants were only marginally supernumerary, and this only during peak flowering, may lead one to at first suggest a possible role of resource availability in the over- or under-production of floral organs. Results from previous studies, however, contradict this notion; deviant androecia from greenhouse-grown *Collomia grandiflora* (Ellstrand et al., 1984) and deviant corollas from greenhouse-grown *Linanthus androsaceus* (Huether, 1968) were largely subnumerary. Conversely, deviant involucral bracts of wild cotton (Wilson and Stapp, 1979) and deviant corollas and androecia in wild *Thryptomene calycina* plants (Beardsell et al., 1993) were mostly supernumerary.

Rather than a consequence of resource partitioning, directional variation may be largely driven by genetic factors. Pappus (calyx) parts in flowers of *Microseris* species proved mostly supernumerary when abnormal, with abnormal variation around the mode drastically increasing among later inflorescences (Vlot et al., 1992). Based on patterns of segregation from extensive breeding experiments, the authors speculated that differential tendencies among genetic lines for direction of pappus deviation arise from separate genetic events and that numerical canalization of pappus parts may be largely under the control of a single major gene. Whether a similar system operates within *Phlox* is unknown, but the fact that some individuals of both species produced abnormalities in

only one direction while others varied in both directions suggests a genetic component to directionality is not an unreasonable assumption.

Perhaps more notable, however, is the presence of individuals of both species that produced abnormalities in only one direction within the reproductive whorls. If organ number variation in these whorls carries with it any reproductive consequences, then negative fitness impacts may follow in plants with uneven variation. For example, if plants producing only subnumerary abnormal androecia experience decreased pollen output from those flowers, then a lack of any compensatory pollen increases from supernumerary androecia elsewhere within the plant might result in lower lifetime pollen production than a neighboring plant that produces abnormal androecia in both directions. Thus, production of equal proportions of directional abnormalities, at least for the reproductive whorls, should be advantageous given a possible buffering effect of balanced organ numbers on total gametic output.

Current research in the area of floral formulae and floral organ numbers is limited. More studies are needed that examine both natural variation and its influencing genetic and environmental components. By examining how, when, and where current floral organ number abnormalities occur, we can more fully understand the nature of floral evolution and the constancy of floral morphology.

Table 3.1. Summary of longitudinal flower census of 30 wild *Phlox longifolia* plants.

Population	Plant	No. total flowers	No. abn flowers	% abn flowers	No. flowers with abnormal organ number (supernumerary : subnumerary)			
					Calyx	Corolla	Androecium	Gynoecium
CPA1	1	70	19	27.1	14 (9:5)	16 (11:5)	17 (11:6)	2 (0:2)
	2	40	1	2.5	0 -----	0 -----	0 -----	1 (0:1)
	3	86	14	16.3	5 (2:3)	6 (3:3)	6 (2:4)	11 (11:0)
	4	51	3	5.9	1 (0:1)	1 (0:1)	2 (0:2)	2 (1:1)
	5	9	1	11.1	1 (0:1)	1 (0:1)	1 (0:1)	0 -----
CPA2	6	96	15	15.6	13 (11:2)	10 (9:1)	11 (9:2)	4 (4:0)
	7	57	9	15.8	7 (1:6)	9 (1:8)	7 (1:6)	3 (1:2)
	8	41	1	2.4	0 -----	0 -----	0 -----	1 (0:1)
	9	73	10	13.7	9 (5:4)	8 (5:3)	10 (5:5)	5 (3:2)
	10	63	6	9.5	0 -----	4 (0:4)	0 -----	2 (2:0)
CPB	11	98	18	18.4	7 (1:6)	11 (1:10)	8 (1:7)	12 (3:9)
	12	25	2	8.0	2 (0:2)	2 (0:2)	2 (0:2)	1 (1:0)
	13	23	4	17.4	1 (1:0)	2 (2:0)	1 (1:0)	2 (2:0)
	14	23	4	17.4	3 (0:3)	4 (0:4)	3 (0:3)	2 (1:1)
	15	107	6	5.6	4 (1:3)	4 (1:3)	2 (0:2)	2 (1:1)
CPD1	16	62	13	21.0	7 (1:6)	8 (1:7)	8 (0:8)	7 (3:4)
	17	50	15	30.0	6 (6:0)	7 (2:5)	2 (1:1)	4 (4:0)
	18	27	7	25.9	4 (4:0)	4 (3:1)	5 (3:2)	2 (1:1)
	19	69	23	33.3	17 (0:17)	21 (0:21)	17 (0:17)	8 (7:1)
	20	35	11	31.4	9 (6:3)	8 (5:3)	7 (3:4)	6 (5:1)
CPD2	21	24	8	33.3	2 (0:2)	5 (0:5)	3 (0:3)	4 (0:4)
	22	38	3	7.9	2 (2:0)	3 (2:1)	2 (1:1)	0 -----
	23	58	6	10.3	4 (4:0)	3 (3:0)	3 (2:1)	3 (3:0)
	24	77	18	23.4	14 (1:13)	15 (2:13)	13 (1:12)	7 (4:3)
	25	59	4	6.8	0 -----	0 -----	1 (0:1)	3 (1:2)
CPD3	26	15	4	26.7	3 (0:3)	4 (0:4)	4 (0:4)	1 (0:1)
	27	196	43	21.9	21 (8:13)	29 (5:24)	20 (7:13)	10 (7:3)
	28	100	15	15.0	11 (1:10)	11 (1:10)	12 (1:11)	5 (4:1)
	29	96	46	47.9	36 (0:36)	44 (0:44)	40 (0:40)	14 (1:13)
	30	32	4	12.5	1 (1:0)	2 (2:0)	3 (1:2)	1 (1:0)
Total		1800	333	mean 17.8%	204 (65:139)	242 (59:183)	210 (50:160)	125 (71:54)

Table 3.2. Summary of longitudinal flower census of 30 greenhouse-grown *Phlox drummondii* plants.

Plant	No. total flowers	No. abn flowers	% abn flowers	No. flowers with abnormal organ number (supernumerary : subnumerary)			
				Calyx	Corolla	Androecium	Gynoecium
1	1048	33	3.1	5 (3:2)	7 (6:1)	3 (2:1)	26 (26:0)
2	190	40	21.1	32 (32:0)	28 (27:1)	20 (19:1)	17 (17:0)
3	500	16	3.2	13 (11:2)	8 (6:2)	7 (4:3)	3 (2:1)
4	209	1	0.5	0 -----	0 -----	0 -----	1 (1:0)
5	776	40	5.2	20 (20:0)	19 (19:0)	11 (11:0)	21 (21:0)
6	936	50	5.3	22 (21:1)	22 (22:0)	14 (12:2)	30 (30:0)
7	248	15	6.0	0 -----	6 (6:0)	1 (0:1)	10 (10:0)
8	99	1	1.0	1 (1:0)	0 -----	0 -----	0 -----
9	733	8	1.1	4 (2:2)	3 (2:1)	1 (0:1)	4 (4:0)
10	605	7	1.2	4 (0:4)	6 (1:5)	5 (0:5)	3 (2:1)
11	1139	22	1.9	7 (5:2)	7 (5:2)	6 (4:2)	16 (16:0)
12	356	2	0.6	0 -----	0 -----	0 -----	2 (2:0)
13	234	2	0.9	1 (1:0)	2 (1:1)	1 (0:1)	0 -----
14	634	14	2.2	9 (8:1)	8 (7:1)	5 (4:1)	3 (2:1)
15	726	48	6.6	0 -----	46 (44:2)	3 (0:3)	2 (2:0)
16	704	54	7.7	45 (45:0)	22 (22:0)	21 (20:1)	14 (14:0)
17	710	4	0.6	3 (1:2)	2 (0:2)	3 (0:3)	1 (1:0)
18	718	15	2.1	12 (12:0)	9 (9:0)	3 (3:0)	1 (1:0)
19	1012	6	0.6	4 (2:2)	5 (3:2)	6 (2:4)	1 (0:1)
20	880	6	0.7	5 (3:2)	4 (3:1)	4 (0:4)	0 -----
21	920	9	1.0	3 (0:3)	0 -----	1 (0:1)	5 (5:0)
22	818	41	5.0	10 (7:3)	11 (9:2)	10 (8:2)	34 (34:0)
23	705	14	2.0	3 (3:0)	9 (9:0)	2 (2:0)	5 (5:0)
24	1006	18	1.8	12 (6:6)	8 (7:1)	7 (4:3)	8 (8:0)
25	878	15	1.7	3 (2:1)	9 (4:5)	3 (2:1)	7 (6:1)
26	831	15	1.8	7 (2:5)	9 (5:4)	8 (5:3)	8 (8:0)
27	1053	191	18.1	11 (9:2)	169 (167:2)	3 (1:2)	24 (24:0)
28	1064	23	2.2	6 (4:2)	11 (10:1)	2 (0:2)	7 (6:1)
29	868	29	3.3	5 (5:0)	3 (3:0)	1 (1:0)	25 (25:0)
30	1008	54	5.4	39 (12:27)	35 (18:17)	30 (6:24)	17 (13:4)
Total	21608	793	mean 3.8%	286 (217:69)	468 (415:53)	181 (110:71)	295 (285:10)

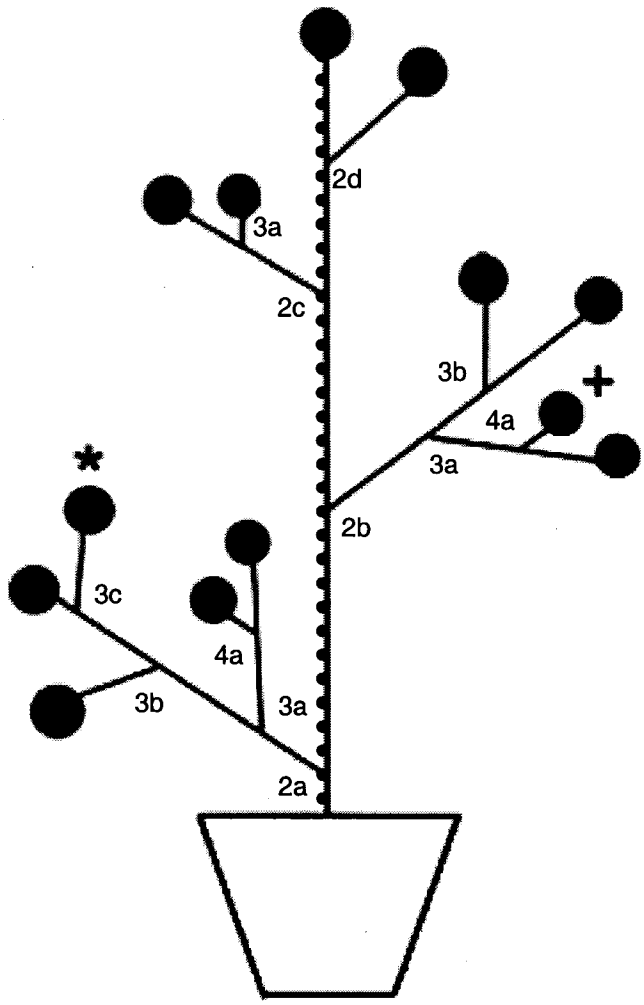

Figure 3.1. Branching ordination schematic for *Phlox drummondii*. Sequential numbering denotes relationships among insertions of branch axes. Letters are assigned acropetally to denote position along a parent axis. The main parent axis is represented by a dotted line and is assigned the label of "1a". Each darkened circle represents a cymose cluster of approximately four flowers. Flowers at the asterisk (*) position would be labeled as "1a:2a:3c", while flowers at the plus (+) would be labeled as "1a:2b:3a:4a".

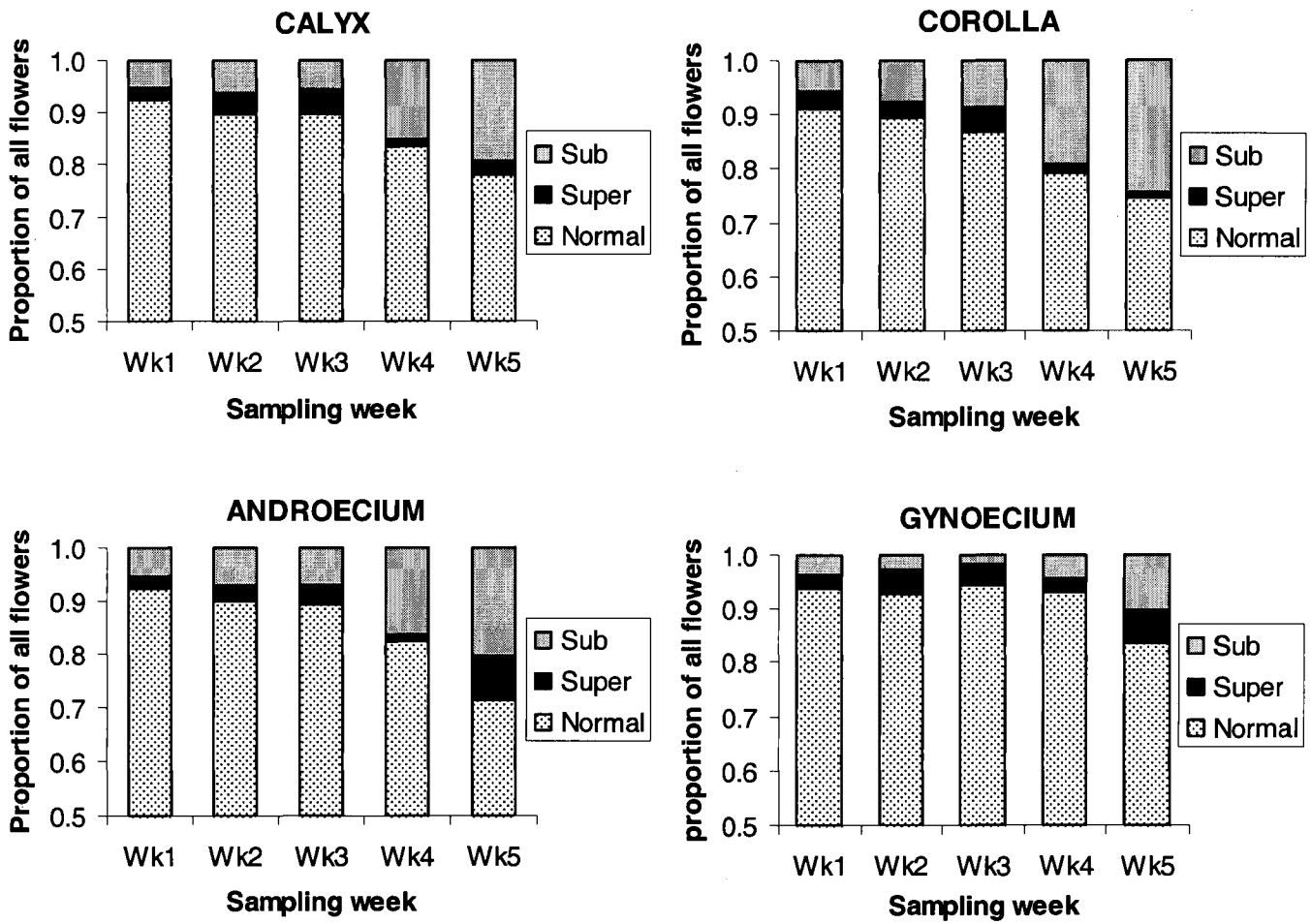

Figure 3.2. Seasonal trends in whorl abnormalities summed over six populations of *Phlox longifolia.* Each bar shows the relative proportions of normal, subnumerary, and supernumerary whorls out of all whorls counted for a specific week.

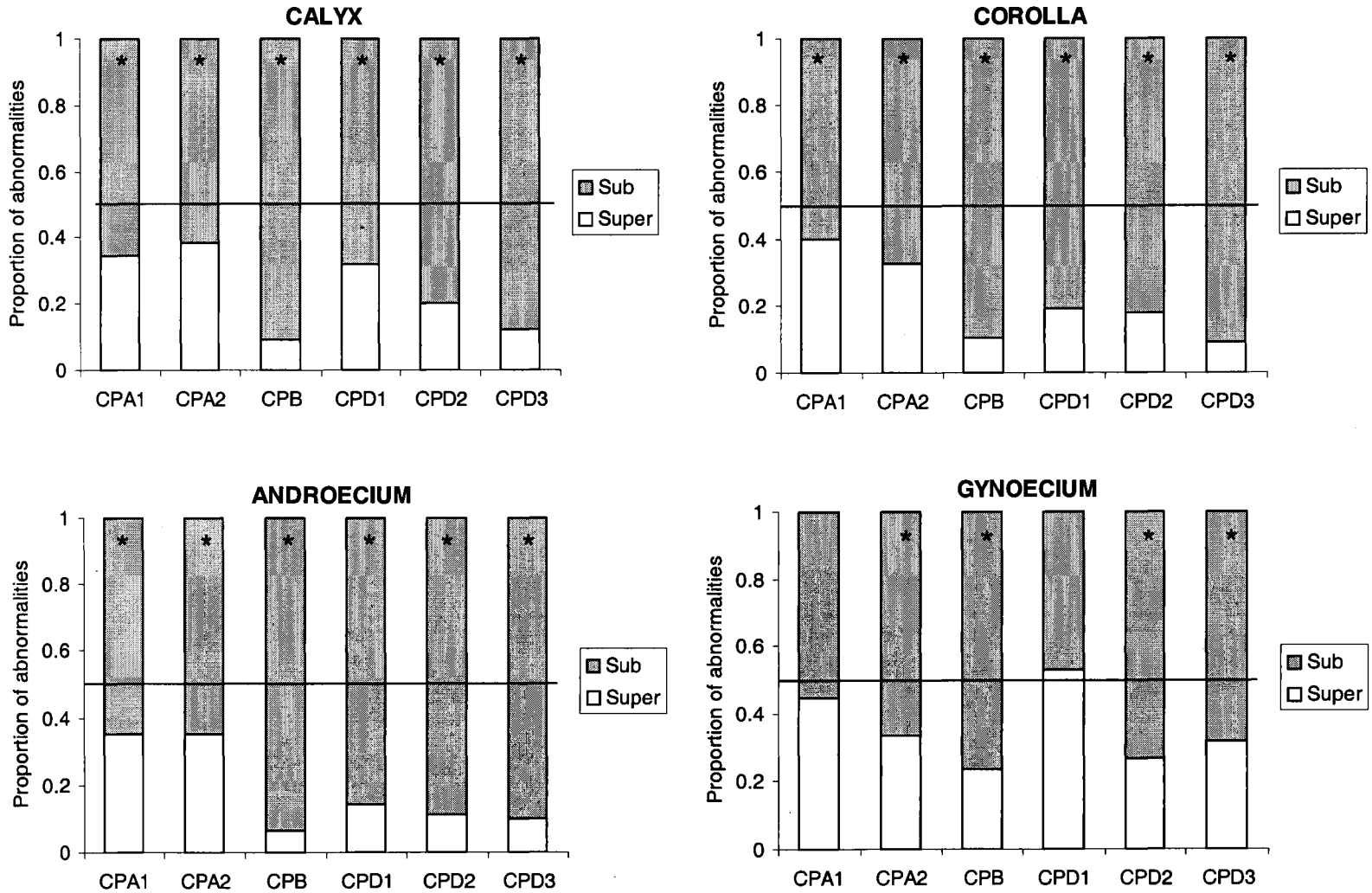

Figure 3.3. Proportions of supernumerary versus subnumerary abnormalities in each whorl for six populations of *Phlox longifolia.* Data were summed over all sampling weeks. Bars with asterisks have proportions significantly different than 0.5 as denoted by the horizontal line.

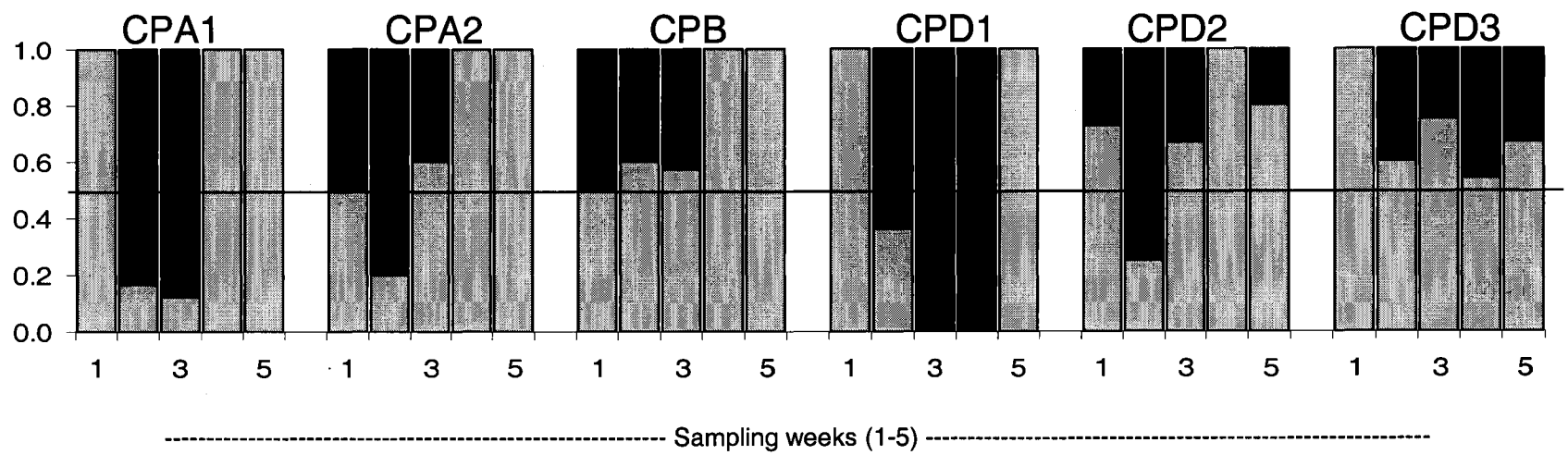

Figure 3.4. Relative weekly proportions of subnumerary (grey) and supernumerary (black) gynoecia for six populations of *Phlox longifolia.*

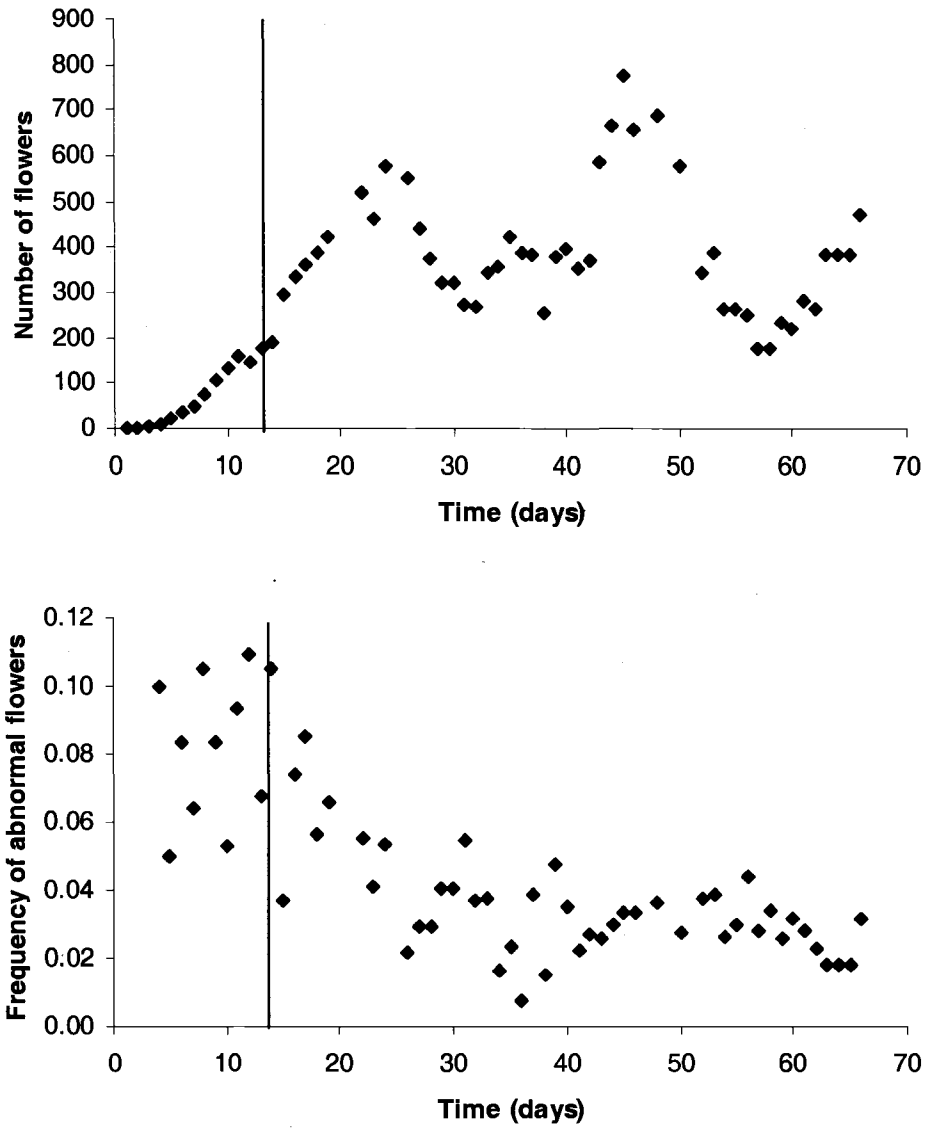

Figure 3.5. Trends in daily flower emergence for 30 greenhouse-grown *Phlox drummondii* plants. (A) Total daily flower counts over time with each day representing only newly emerged flowers. (B) Frequency of abnormal flowers over time. The vertical bar at Day 13 signifies the point at which all plants had begun flowering.

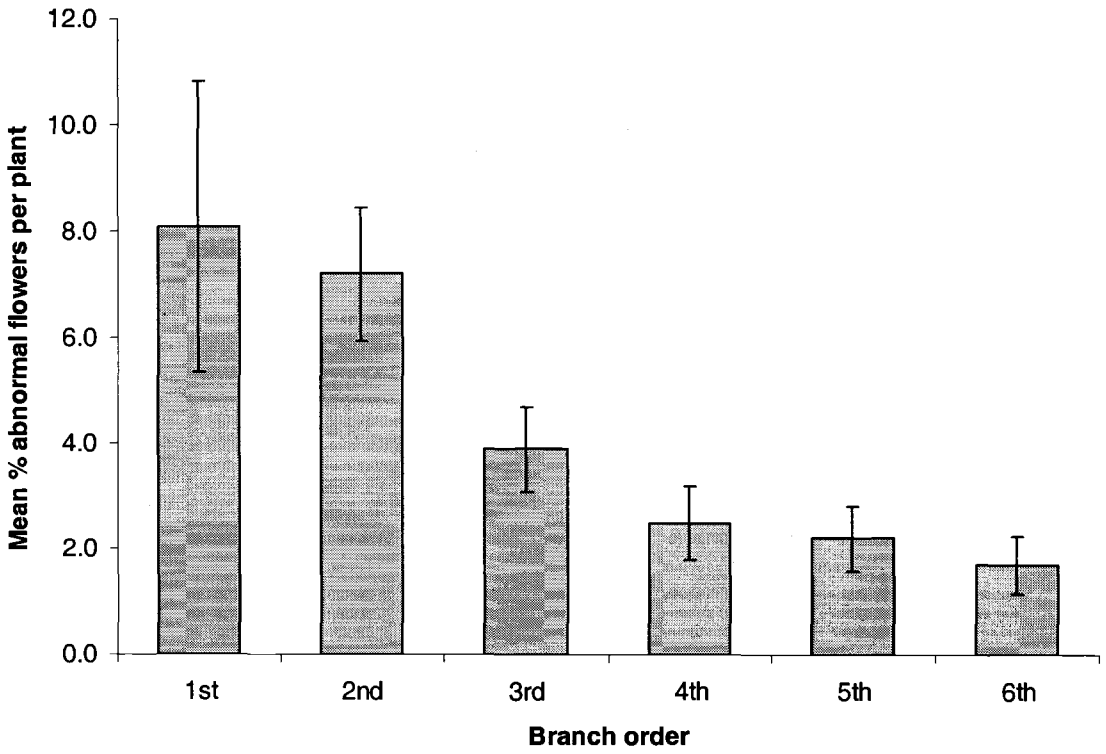

Figure 3.6. Mean percent abnormal flowers per *Phlox drummondii* plant relative to branching position. Second order branches develop from first order branches; third order branches develop from second order branches, etc. Y-error bars = standard error of the mean.

CHAPTER 4

FLORAL FORMULA VARIATION AND REPRODUCTIVE OUTPUT IN *PHLOX* (POLEMONIACEAE)

ABSTRACT

The number of organs comprising the four whorls of a flower is thought to be invariant in most plants. Natural variation for these numbers does exist, and when that variation occurs in the androecium or gynoecium, reproduction may be affected. To test this possibility, reproductive output was quantified using two *Phlox* species with variable numbers of stamens and carpels. Male reproductive output was estimated for wild *Phlox longifolia* using pollen counts, while female reproductive output was estimated for greenhouse-grown *Phlox drummondii* using seed counts and seed mass. Total pollen production was found to increase proportionally with the number of stamens per flower although the range within androecial classes was so large as to suggest a large effect of unknown origin. Tetracarpellate gynoecia yielded greater mean seed counts and total seed mass per fruit than tricarpellate gynoecia, suggesting plants producing more tetracarpellate flowers would yield greater lifetime seed volume. Average single seed mass decreased inversely with seeds per fruit over both gynoecia types. This study

provides evidence that a consequence of meristic variation in the androecium and gynoecium of *Phlox* flowers is variation in total gametic output and therefore fecundity.

INTRODUCTION

The number of organs in each of the four whorls of a flower (calyx, corolla, androecium, gynoecium) can be collectively referred to as the floral formula. The specific floral formula of a plant species is often conserved among genera and even higher taxonomic levels (Stebbins, 1951; Berg, 1959; Bradshaw, 1965; Cronquist, 1968). This is especially true for the Polemoniaceae family where the floral formula rarely deviates from the basic plan of five sepals, five petals, five stamens, and three carpels (Grant and Grant, 1965). However, natural variation for this floral formula does exist, often appearing in coordinated fashion across the different whorls (Ellstrand, 1983; Lehmann, 1987; Ellstrand and Mitchell, 1988; Byerley, Chapter 2). Assuming that organ number variation in the reproductive whorls significantly affects reproductive output through variable gamete production, evolution of a new stable floral formula might be possible through organ number selection in either the androecium or the gynoecium.

The objective of this study was to investigate the capacity for differential male and female reproductive output of polemoniaceous flowers with supernumerary, subnumerary, and normal androecia and gynoecia. More specifically, this study attempts to determine whether there are gamete-related consequences of meristic variability in the androecium of *Phlox longifolia* and the gynoecium of *Phlox drummondii*. Assuming that some aspect of floral organ number determination is heritable, then variation in total

gametic output due to variation in stamen and/or carpel number may be a factor on which selective forces can act.

MATERIALS AND METHODS

Study organisms – *Phlox longifolia* Nutt. is a perennial native plant of the western United States. *Phlox drummondii* Hook. is a winter annual found primarily in the southeastern United States. Both species are predominantly outcrossing (Appendix II; Byerley, unpublished data), producing flowers from early spring to early summer. Lepidopterans are responsible for the bulk of pollination, after which plants bear fruit in the form of spherical capsules that explosively dehisce when mature (Grant and Grant, 1965; personal obs.). Wild populations are commonly found in disturbed sites next to roads, cattle and horse trails, hiking trails, and bike paths (personal obs.). The *P. longifolia* populations from which samples were collected are located in the Cherokee Park area of Roosevelt National Forest (40° 53.46' N, 105° 27.46' W), approximately 30 miles north-by-northwest of Fort Collins, Colorado, USA. A greenhouse common garden of *P. drummondii* was started from seeds acquired from Native American Seed Company (Junction, Texas, USA) where natural populations are maintained on private land. The modal floral formula of both *Phlox* species (five sepals, five petals, five stamens, and a tricarpellate gynoecium) is shared by most of the genera in the Polemoniaceae family, and with the exception of carpel number, the same formula is highly prevalent among the entire order Polemoniales (Dawson, 1936; Grant, 1959, 1998; Grant and Grant, 1965; Cronquist, 1968; Stebbins, 1974).

Male reproductive output – Anthers were collected from flowers of wild *Phlox longifolia* to calculate the relative pollen production of normal androecia (5 stamens), subnumerary androecia (< 5 stamens), and supernumerary androecia (> 5 stamens). Since flowers with either four or six stamens were far more common than other abnormalities, only these androecium types were measured along with a normal androecium control. For each type, a minimum of 66 flowers from different plants were sampled across four sites and pooled to account for common environmental variance. Anthers were excised from buds just prior to anthesis and allowed to shed pollen in small centrifuge tubes. Pollen samples were suspended in a 0.5 ml fixed volume of 40:1 ethanol-lactophenol aniline blue according to the methods described in Kearns and Inouye (1993) and sonicated to ensure the release of all grains from anther locules. Only a very small proportion of grains remained within locules using this method. Three to five 15 μl subsamples per 0.5 ml pollen suspension (i.e. per flower) were aliquoted onto separate gridded microscope slides. With the aid of a tally counter, subsamples were microscopically observed at 40X magnification, and pollen grains were counted and averaged within flowers. Variation between subsamples was minimal and homogenous across flowers. The mean pollen count per 15 μl sample was then scaled up to match the initial starting volume, yielding an estimate of total pollen production per flower. Analysis of variance methods were used to compare pollen counts among the three androecium types using SAS software (Version 9.1, SAS Institute Inc. 2002-2003).

Female reproductive output – Expected seed number per fruit in *Phlox* plants is relatively low with an average of three seeds per fruit or fewer in most species (Grant and Grant, 1965). To control for resource limitations and other environmental factors that

could affect fruit and seed production in the wild, measures of female reproductive output were performed on greenhouse-grown plants. Unfortunately, the perennial duration of *Phlox longifolia* and extremely low germinability of naturally collected seeds precluded successful establishment of a greenhouse common garden, and thus *Phlox drummondii* plants were used instead.

Manual pollinations were performed on flowers of plants with both normal tricarpellate gynoecia and abnormal tetracarpellate gynoecia. Bicarpellate or other gynoecium types were rare in the greenhouse population and thus not included in the experiment. Approximately fifty pollinations on each gynoecium type were performed such that each abnormal gynoecium was paired with a normal gynoecium within the same plant to account for maternal effects on seed set and mass. Pollinations were achieved by first scoring mature unopened buds for carpel number each day, and performing planned pollination pairs only once tetracarpellate flowers were found. Stigma lobe number was used as a 1:1 predictor of both carpel and ovule number (see Appendix I). Pollen from at least five different conspecifics was used per pollination, ensuring a full pollen load and controlling for pollen limitation and genetic incompatibilities. Seed number and seed mass were measured per fruit and analyzed for the following effects: carpel number, plant, and carpel number*plant interaction. This was done using the MIXED procedure in SAS software (Version 9.1, SAS Institute Inc. 2002-2003) where carpel number was a fixed effect. Plant and carpel number*plant were random effects.

In addition, to test for within-plant resource limitations caused by simultaneous production of flowers and fruit, plants were randomly assigned to one of four treatment

classes: (1) pollinations within the same inflorescence with removal of superfluous flowers and fruits; (2) pollinations in spatially separated inflorescences with removal of superfluous flowers and fruits; (3) pollinations within the same inflorescence with natural maturation of other flowers and fruits; and (4) pollinations in spatially separated inflorescences and natural maturation of other flowers and fruits. A significant treatment effect would indicate that fruits compete as resource sinks, while pair-wise comparisons of the four treatment types would determine whether the strength of competition is influenced by spatial proximity of developing fruits.

During collection of seed data, an apparent trend emerged regarding carpel number and placental features, whereby the number of placental grooves and septa within harvested fruits appeared to directly correspond to carpel number as determined by stigmatic lobe number. In order to verify use of such features for determining carpel number post-fertilization (when styles and stigmatic lobes have abscised), the number of placental grooves and septa were recorded for all harvested fruits.

RESULTS

Meristic variation in both reproductive whorls affected flower-level reproductive output. In *Phlox longifolia*, stamen number significantly affected total pollen production per flower ($F = 30.1$, $P < 0.0001$) (Fig. 4.1). Flowers with more stamens produced more total pollen grains than flowers with fewer stamens. Although standard errors were small, the range of total pollen production within each androecium type was quite large, with differences in stamen number only accounting for 15% of the variation, indicating strong influence of other variables (e.g. plant or environment) on overall pollen production.

It must be noted that the methods used here for analysis of variance for pollen count assume the data are normally distributed, which these data were not. A slight left skew or heavy left tail within the 5-stamen class is largely to blame for this non-normality. That is, there were more 5-stamen flowers with low pollen counts than expected under a normal distribution. However, when these observations were removed and the analysis repeated, effect of stamen number on pollen count was still highly significant.

For female reproductive output in *Phlox drummondii*, the effect of carpel number on seed number was highly significant ($F = 8.33$, $P = 0.009$), suggesting that among flowers of the same plant, tetracarpellate gynoecia yield more seeds than tricarpellate gynoecia (Fig. 4.2). Effect of carpel number on total seed mass per fruit was only marginally significant ($F = 3.23$, $P = 0.088$). Greater sample size and statistical power would better resolve the nature of such a comparison. Carpel number did not significantly affect mean seed mass, and local competition from developing fruits and flowers had no significant effect on seed number, mean seed mass, or total seed mass per fruit.

Regardless of carpel number, as seed number per fruit increased, individual seed masses decreased, and the inverse correlation of the two was significant ($r = -0.29$, $P = 0.0211$) (Fig. 4.3-A). Mean individual seed masses within each seed number class were significantly different ($F = 6.86$, $P = 0.0006$), with only 2-seeded fruits and 3-seeded fruits having similar mean single seed masses. Conversely, total seed mass per fruit was positively correlated with seed number per fruit ($r = 0.70$, $P < 0.0001$) (Fig. 4.3-B), with all pair-wise comparisons of seed number classes being significantly different for total seed mass except 1-seeded versus 2-seeded fruits.

Concerning the correlation of carpel number and placental features among *Phlox drummondii* fruit, the number of septa and placental grooves equaled expected carpel number in all bicarpellate, tricarpellate, and tetracarpellate fruits examined (Fig. 4.4). Ovule number also equaled expected carpel number in all fruits. Where seed set was not 100%, aborted ovules/seeds were readily observable.

DISCUSSION

Implications of variable androecia – These results suggest that stamen number in *Phlox longifolia* is directly associated with male fecundity. More experiments are needed to determine the success of those pollen grains produced by *Phlox* flowers with abnormal organ numbers, i.e., whether trends in reproductive success match the trends seen here in reproductive output. For example, pollen size and performance might vary with pollen quantity, thereby offsetting stamen number effects among the different androecium types.

If no such tradeoff exists, then more pollen grains represent more chances to distribute the genotype into successive generations. However, the fact that the range of total pollen production within each androecium type was quite large could indicate a potential plant or environment effect on overall pollen production. More specifically, the large amount of variation might suggest that (1) certain genotypes are capable of producing more total pollen per flower than other genotypes regardless of stamen number and/or (2) micro-environmental conditions experienced by some plants contribute to greater pollen production than other conditions. As long as pollen count is still positively correlated with stamen number within individuals, the interpretation remains that supernumerary stamens are potentially advantageous, perhaps even more so for plants

with lower overall pollen production. Supernumerary stamens in such plants could be compensatory, allowing for competitive pollen numbers when compared to robust neighboring plants that may be subnumerary but still highly productive.

The implications of variable stamen number go beyond simple measures of fecundity. When pollen produced by a plant serves not only for fertilization purposes but also as a reward to pollinators, an increase in pollen output could be adaptive relative to pollinator constancy and/or efficiency. In general, a pollen output increase can be obtained via either production of additional stamens, as seen here in *P. longifolia*, or production of more grains per anther. Although the latter strategy may be less constrained by developmental processes (Willson, 1983), the former might result in a more dramatic effect from a pollinator's perspective and perhaps elicit a responsive behavioral preference for extra stamens. More stamens could increase the frequency of beneficial outcrossing events, especially if the new stamens are positionally unique or current pollination mechanisms are inefficient. For example, Grant and Grant (1965) make the distinction between positions of anther insertion in the different sections within the *Phlox* genus where anthers are either clustered near the corolla tube opening or are scattered at different depths within the corolla tube. The former arrangement facilitates face and tongue-base pollen transfer to lepidopteran pollinators while the latter facilitates tongue-tip pollination. The authors note that tongue-tip pollination is a more efficient strategy as it better serves a suite of pollinators with different tongue lengths. Supernumerary stamens, then, have a potential two-fold effect on male reproductive fitness via both an increase in total pollen output and a possible increase in effective pollinator quantity and service.

Implications of variable gynoecia – *Phlox drummondii* plants producing more tetracarpellate than tricarpellate flowers are potentially more fecund via greater seed production. And although single seed mass decreased with the number of seeds per fruit, the greater total seed mass per fruit among tetracarpellate flowers suggests a greater total offspring investment. This could hypothetically offset any possible seed quantity/quality tradeoffs, the most common of which is the highly documented – but not absolute – relationship between seed mass and seedling success/vigor (Westoby and Rice, 1982; Willson, 1983; Willson and Burley, 1983; Lovett Doust and Lovett Doust, 1988).

In *Phlox drummondii*, both tricarpellate and tetracarpellate gynoecia produced single seeds of similar mass whether fruits were 1-seeded, 2-seeded, or 3-seeded as most of the variation within seed classes was attributed to maternal identity. Thus, if seed mass is in fact a prevailing selective force in nature, then the effects of seed set on seed mass would not necessarily negatively impact gynoecia with more carpels, but rather all gynoecia, no matter the carpel number, might be constrained to an optimal number of seeds of a certain size. Alternatively, if seed number is the greater selective force, then one would expect carpel number to play a positive role in gynoecium evolution as gynoecia with more carpels yield more seeds.

Another notable conclusion from these results is that previous reports of seed set or any measure related to expected seed number in *Phlox* species should be interpreted with caution. Most *Phlox* researchers use a maximum of three ovules per fruit when performing seed set calculations, likely presuming that other possible ovule counts are rare or negligible, though seldom is this last caveat ever stated in the text. The present study, however, shows that if tetracarpellate flowers are indeed present to any moderate

extent within either a plant or population, then figures based on that assumption will overestimate the true value. For instance, if all gynoecia in the present study are assumed to be tricarpellate, overall seed set is inflated from 82% to 95%. This dramatic increase is partially due to a high 1:1 ratio of tri-/tetracarpellate fruits and may be considered an extreme scenario. Further, Byerley (Chapter 2; Chapter 3) found that in addition to normal tricarpellate gynoecia, several *Phlox longifolia* populations produced both tetracarpellate and bicarpellate gynoecia, and these were not evenly distributed within individuals or populations. Therefore, if measures of seed set are to be accurately assessed, then the possibility of differential production of non-tricarpellate flowers within separate individuals should be considered. If carpel number cannot be determined prior to fertilization using stigmatic lobe number, it can be determined retroactively by examining placental remnants from mature harvested fruits as demonstrated here.

Implications for evolution of floral formulae – For stamen and carpel number variation to drive evolution of the entire floral formula, three main obstacles must be overcome: sex allocation tradeoffs, low heritability, and developmental integration among the whorls. Conflicts in resource partitioning between male and female traits may limit the extent to which flowers with supernumerary organs functionally impact an entire population of plants. A recent review of genetic constraints of floral evolution (Ashman and Majetic, 2006) found that while male-female traits are commonly positively correlated within flowers, inflorescence- or plant-level correlation can be negative, possibly due to negative pleiotropy across flowering modules. Evidence of significant plant-level genetic covariance of pollen output and seed number within *Phlox* individuals would suggest that the genes affecting male output also affect female output. Absence of

plant-level covariance would suggest that both stamen number and carpel number are free to evolve independently to separate optima.

In order to effect evolutionary change in the floral formula, fecundity selection (or pollinator-mediated selection for stamen number) must be coupled with some factor of organ number development which is heritable. Although petal number in the polemoniaceous *Linanthus androsaceus* was found to have a readily selectable genetic component (Huether, 1968), life history traits such as fecundity are largely assumed to have low heritability (Falconer and Mackay, 1996). Ashman and Majetic (2006) found heritability estimates for male allocation traits (e.g. pollen number and size) to be centered around 0.4 while heritabilities of female allocation traits (e.g. seed number and mass) were closer to 0.3. Price and Schluter (1991), though, point out that variation in life-history traits are often a consequence of variation in underlying metric traits, and that the same genetic and environmental components of the latter will affect the former. Thus, because fecundity seen here in *Phlox* is a direct consequence of variation of another trait with its own, perhaps greater, level of heritability, the assumed low heritability of the life-history trait could be circumvented.

Even if the capacity to produce extra organs is not passed on to successive progeny, a fecundity increase experienced by parents elevates the inclusive fitness of all progeny and their descendants. This is true provided there is no severe reduction in offspring viability corresponding to measures related to an increase in reproductive output (e.g., seedlings from lighter seeds from tetracarpellate gynoecia fare poorly).

A survey of several natural populations of *P. longifolia* reported high positive within-flower correlation for organ number and within-plant correlation for organ

variation (Byerley, Chapter 2). That is, in addition to normal flowers expressing the modal 5 sepals-5 petals-5 stamens-3 carpels formula, flowers with a formula of 6-6-6-3 or 4-4-4-3 were frequent among deviant flowers. Similar patterns involving equal or matching organ numbers in at least the outer three whorls were reported for *Phlox drummondii* (Lehmann, 1987) and the polemoniaceous *Ipomopsis aggregata* (Ellstrand, 1983; Ellstrand and Mitchell, 1988). Such developmental integration among the androecium and perianth whorls may limit formula evolution either through conflicting selective pressures brought about by strict pollinator discrimination or through greater resource demands for more organ tissue in several whorls at once. Cresswell (1998) claims that although it is unlikely to sufficiently restrict minor alterations to continuous floral traits, developmental integration might sufficiently constrain "major" changes acting on, for example, meristic traits.

The converse, however, could also be true. Intrafloral organ number integration could facilitate floral formula changes. Just as fecundity-based selection could exploit the greater heritability of related reproductive traits, selection for perianth changes could "unintentionally" boost fecundity via corresponding changes in stamen number. Whether the pollinator fauna can or would respond to organ number variability among the floral display of an entire population is unknown, but with both a genetic component for organ number in a perianth whorl and strong covariance among the perianth and androecium, upward evolution of the floral formula could occur via dual impacts of both pollinator preference for perianth traits and fecundity benefits from supernumerary stamens.

Selection experiments that focus separately on organ number in the four floral whorls (giving heritability estimates) coupled with selection for specific floral formulae

(e.g. 6-6-6-4 and 4-4-4-2) would shed light on the constraints of floral integration and the possible evolutionary trajectories of *Phlox* and other polemoniaceous floral formulae. It is currently unclear whether such experiments would represent selection for instability of trait expression (e.g. variable petal number) or for stable development of an alternative phenotype (e.g. six-petalled flowers). It could be that any latter-type change must first progress through a stage involving the former. The following chapter (Chapter 5) presents the results of such a selection experiment on carpel number in *Phlox drummondii*.

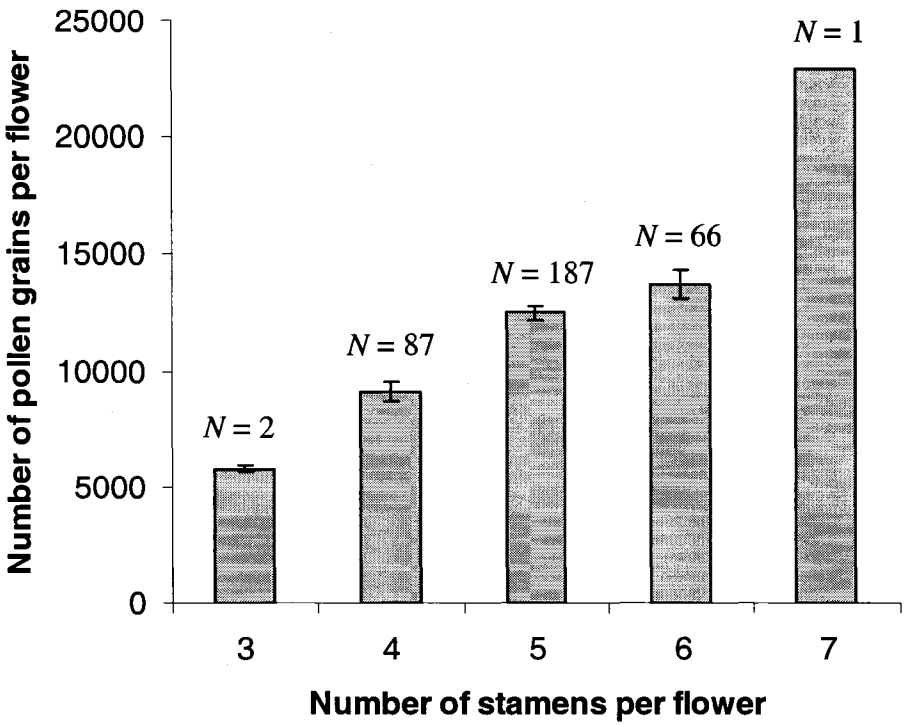

Figure 4.1. Mean pollen grains per flower versus stamen number per flower for *Phlox longifolia.* The three total flowers comprising stamen classes 3 and 7 were not included in the statistical analyses but are represented here as supporting evidence of the same trend extending across these two rare androecium types. Y-error bars = standard error of the mean.

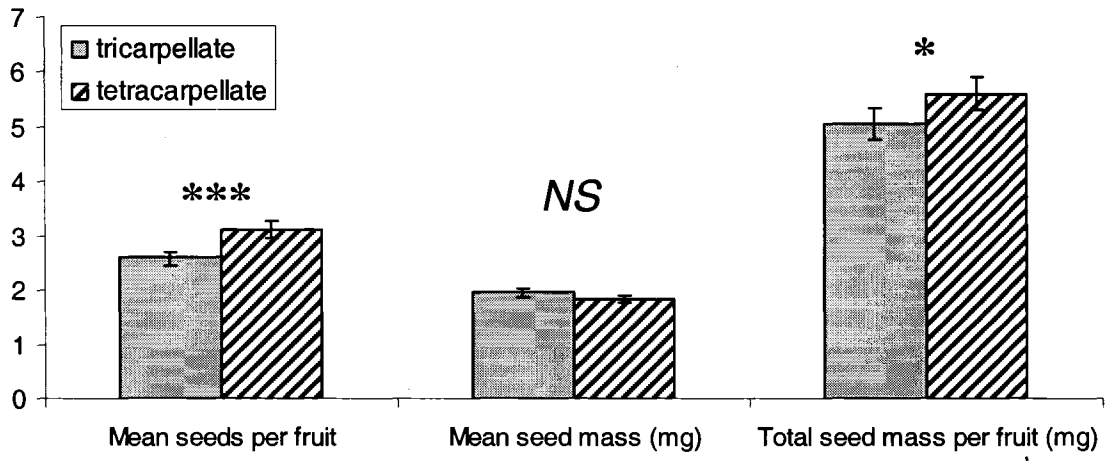

Figure 4.2. Comparison of female reproductive traits for tricarpellate and tetracarpellate flowers of *Phlox drummondii*. * denotes $P < 0.10$; *** denotes $P < 0.01$; *NS* denotes nonsignificance.

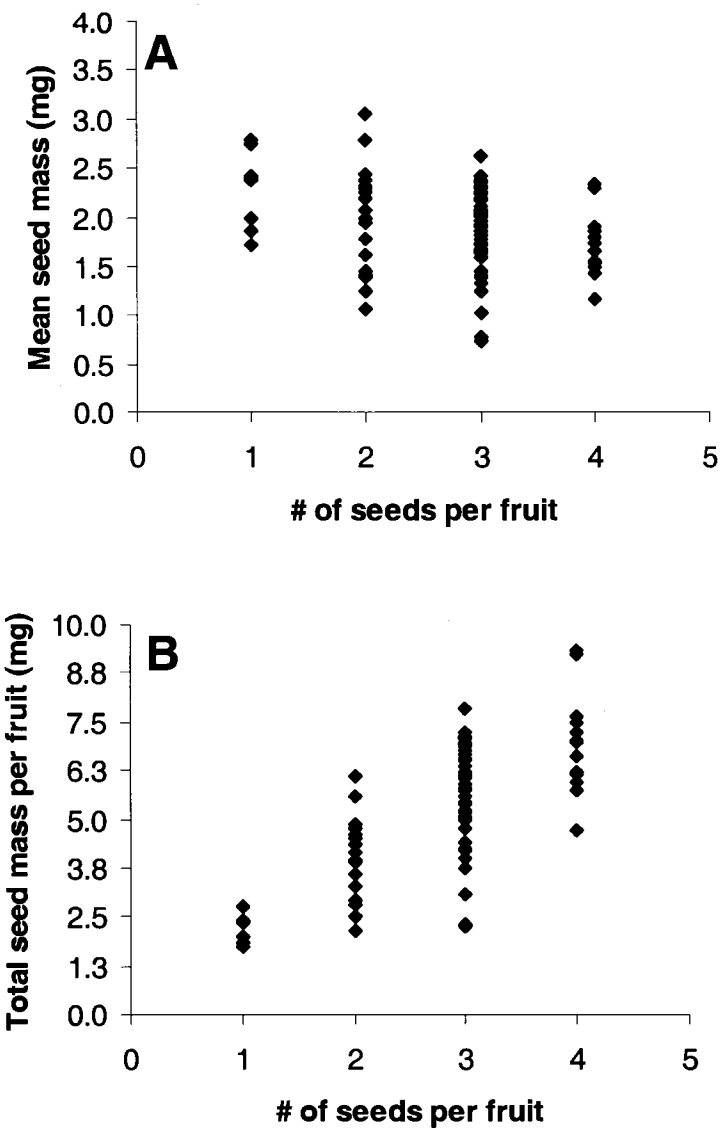

Figure 4.3. Distribution of mean individual seed mass (A) and total seed mass per fruit (B) relative to seed number per fruit for both tricarpellate and tetracarpellate flowers of *Phlox drummondii*.

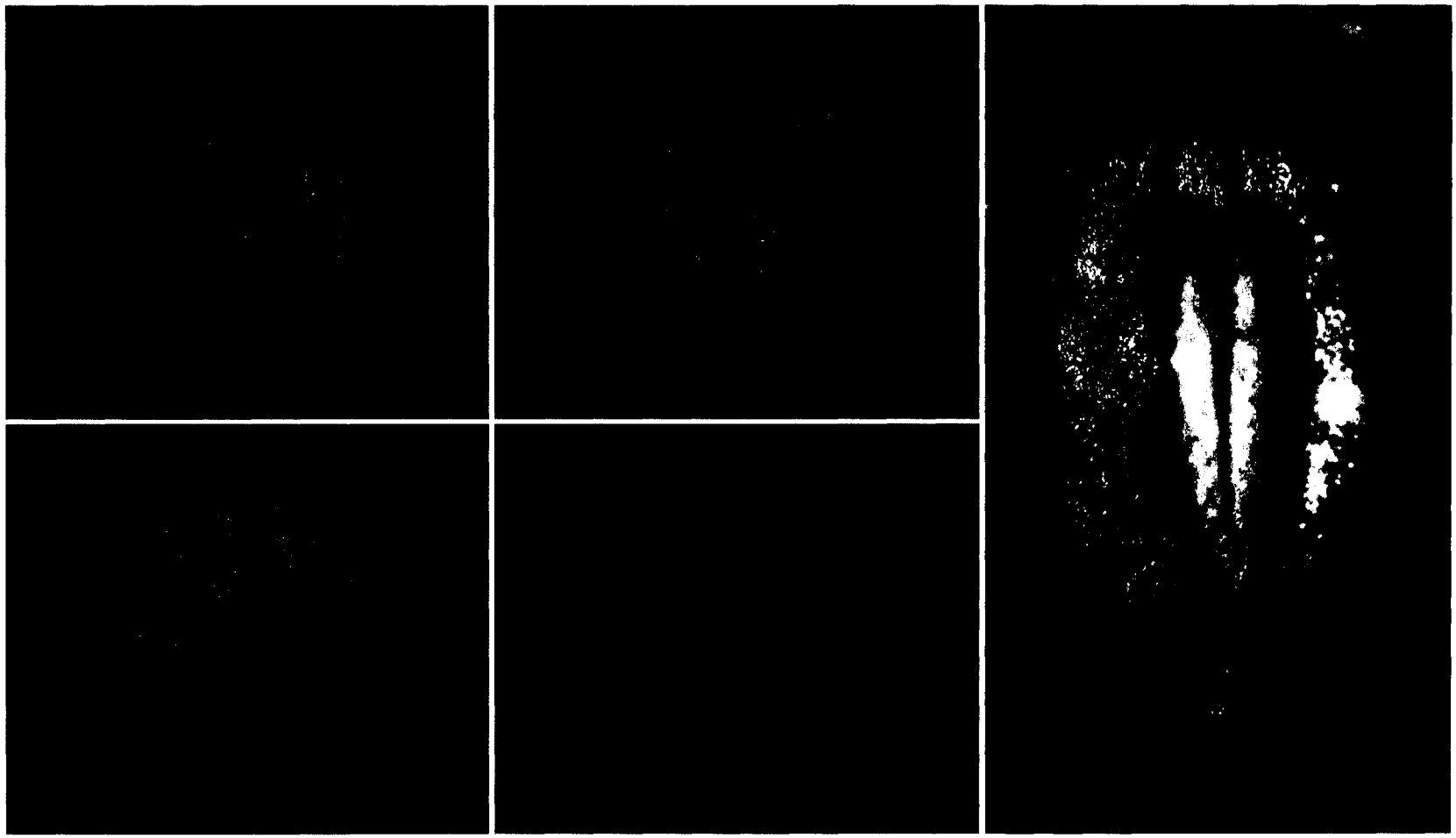

Figure 4.4. Comparison of placental features from tricarpellate and tetracarpellate gynoecia. A-B, placental and septal remnants from a tricarpellate gynoecium as seen from (A) the stylar end and (B) the receptacle end. C-D, placental and septal remnants from a tetracarpellate gynoecium as seen from (C) the stylar end and (D) the receptacle end. E, aborted ovule/seed retained within the placental apparatus of a tricarpellate gynoecium. Scale bars = 2mm.

CHAPTER 5

SELECTION FOR CARPEL NUMBER IN *PHLOX DRUMMONDII* HOOK.

ABSTRACT

One round of selection to alternatively increase and stabilize carpel number per gynoecium was performed on *Phlox drummondii*. Mean carpel number per plant did not diverge after a single generation but rather increased among both selection lines. Other floral whorls exhibited an indirect selection response as frequency of abnormality in the corolla and the floral formula as a whole increased for the upward selection line and decreased or remained constant for the stabilizing selection line. These initial results suggest that selection on organ number within a specific floral whorl may act to alter the sensitivity of stable organ number expression and decouple the floral integration among all the whorls, thus increasing overall instability.

INTRODUCTION

Floral traits have long been the subject of plant experimenters as floral modifications in nature can easily lead to reproductive isolation and species formation (Levin, 2000). Despite this fact, or perhaps as a consequence, several floral characters are considered largely invariant among taxonomic groups. Included in such a group of

characters are floral organ fusion, organ insertion, inter-whorl organ arrangement, and organ number per whorl (Smyth, 2005). In particular, organ number per whorl, often collectively referred to as the floral formula, is invariant to the extent that it is commonly used to identify plants at the family level.

Divergence of floral traits is presumably one of the major forces that historically drove the rapid adaptive radiation of angiosperms (Levin, 2000), and subsequent stabilizing selection is often assumed to have reduced any remaining genetic variation to minimal levels (Cresswell, 1998). Multiple studies have documented floral organ number variation in both natural and controlled populations (Roy, 1958, 1963; Huether, 1968, 1969; Wilson and Stapp, 1979; Ellstrand, 1983; Ellstrand et al., 1984; Lehmann, 1987; Ellstrand and Mitchell, 1988; Tucker, 1991; Vlot et al., 1992; Beardsell et al., 1993; Olivencia et al., 1995; Donnison and Francis, 2002; Byerley, Chapter 1, Chapter 2), suggesting that perhaps there still remains an adequate source of variation upon which natural selection may act. Given this notion that variation does in fact exist, it is of interest to determine what factors may be involved in further evolution of an entire floral formula for a particular species.

Members of the Polemoniaceae family are highly consistent for floral formula (Grant and Grant, 1965), but within-species and within-plant organ number variation has been reported on several occasions (Huether, 1968, 1969; Ellstrand, 1983; Ellstrand et al., 1984; Lehmann, 1987; Ellstrand and Mitchell, 1988). More recently it was determined that (1) organ number variation among the reproductive whorls directly affects total gametic output in the polemoniaceous *Phlox drummondii* and *Phlox longifolia* (Byerley, Chapter 4) and (2) variation of organ numbers in the same species is often exhibited in a

coordinated fashion across multiple floral whorls (Byerley, Chapter 2, Chapter 3). If variable floral organ numbers are correlated among the whorls in *Phlox* species and have the potential to influence reproductive fitness at the plant level, then selection on organ number variation could alter the entire floral formula. Directional selection for petal number in *Linanthus androsaceus* (Huether, 1968) and for floral bract number in cotton (Wilson and Stapp, 1979) were both successful, suggesting that organ number in *Phlox* species may also be heritable. This study was designed to determine (1) whether *Phlox drummondii* would respond to selection on carpel number and (2) how the other floral whorls and the floral formula as a whole would indirectly respond to such selection.

MATERIALS AND METHODS

Study organism – *Phlox drummondii* Hook. is a winter annual found primarily in the southeastern United States. The modal floral formula consists of five sepals, five petals, five stamens, and a tricarpellate gynoecium and is shared by most other genera in the Polemoniaceae family (Grant and Grant 1965; Cronquist 1968; Stebbins 1974; Grant 1998). *Phlox drummondii* is predominantly outcrossing and produces flowers from early spring to early summer. Lepidopteran activity is responsible for the bulk of pollination, after which flowers produce spherical capsules that explosively dehisce when mature (personal obs.).

Experimental design – An experimental greenhouse population of *P. drummondii* was established using seeds from Native American Seed Company (Junction, Texas, USA) where natural populations are maintained on private land. Separate seed lots are purportedly harvested from thousands of maternal plants (Jay Kane, personal comm.),

and thus are assumed to contribute adequate genetic diversity to the base population ($Generation_0$).

To create a base population, seeds were germinated in flats of fine vermiculite in the lab under grow lights with 14 hour days. Two to three weeks after germination, seedlings were transferred to 6-inch pots of potting soil and were randomized with respect to position on the greenhouse bench. As plants flowered, they were scored for floral formula up to the first 50 flowers produced. Formula scoring consisted of counting the number of sepals, petals, stamens, and carpels. Stigmatic lobe number was used to directly estimate carpel number (see Appendix I). Of the 121 plants that survived beyond production of 10 flowers, the 30 plants with the highest mean carpel number were chosen for an upward selection line and 30 plants with a mean carpel number of exactly three were chosen for a stabilizing selection line. Subsequent mortality reduced both selection lines to 23 individuals, the upward selection line having an average mean carpel number of 3.06 across all flowers. A downward selection line was not possible given the extremely low frequency of bicarpellate flowers.

Approximately 130 manual pollinations were performed within each of the two selection lines. This process involved gathering and mixing pollen from a minimum of five plants and distributing it to the stigmas of within-group flowers emasculated three days prior. Although *Phlox drummondii* has been thoroughly documented as predominantly self-incompatible (gametophytic), emasculation was performed to eliminate all possibility of self-pollination.

Prior to capsule dehiscence, seeds were collected according to maternal parent and stored in coin envelopes at 4°C to break winter dormancy. After approximately 18

months, the 377 seeds were randomized within flats of fine vermiculite and germinated under grow lights with 14 hour days. Germination was uniformly high; less than 12% of seeds failed to germinate after 1+ years of cold storage. After germinating, seedlings were transferred to 6-inch pots of potting soil and moved to the greenhouse. Plants were randomized with respect to position and assigned arbitrary numbers to mask selection group identities and minimize observational bias. Pre-flowering mortality was minimal (3%), with 310 plants surviving to comprise Generation$_1$. The first 20 flowers on these plants were scored for floral organ number in the same manner described above. This number is smaller than the number of flowers scored in Generation$_0$ plants as it better served to synchronize the timing of progeny scoring and selection of new parent lines in Generation$_1$.

Statistical analyses – The realized heritability (h_R^2) of carpel number was calculated using the equation $h_R^2 = R / S$, where the selection differential (S) is the difference between mean carpel number of the base population and mean carpel number of a selected line, and the selection response (R) is the difference between mean carpel number of the base population and mean carpel number of the offspring from a selected line. The narrow-sense heritability of carpel number (h_N^2) was estimated using mother-mid-offspring regression, where heritability equals two times the regression coefficient (β) (Falconer and Mackay, 1996) and mid-offspring values are the mean of all offspring values for a particular maternal parent. Although maternal parents contributed unequal numbers of offspring (range: 1-63), possible bias from within-family correlation was dismissed by a lack of significant association between maternal mean carpel number and number of offspring ($r = 0.05$) (Lendvai and Levin, 2003).

To determine the extent to which organ numbers in the other floral whorls (calyx, corolla, and androecium) were indirectly affected by selection on carpel number, one-way ANOVAs from the GLM procedure in SAS software (Version 9.1, SAS Institute Inc. 2002-2003) were used to look for differences between trait means of the selection lines after one generation. Because these data were proportional (no. flowers abnormal for organ number / total flowers), they were arcsine-square-root transformed prior to analysis to satisfy assumptions of normality and homogeneity of variance.

RESULTS

Direct selection for carpel number – After one round of selection, mean carpel number per flower increased for both the upward and stabilizing selection lines (Fig. 5.1). Mean carpel number did not significantly differ between selection lines in $Generation_1$ ($F = 0.59$, $P = 0.4433$). Variation among maternal parents was significant ($F = 1.71$, $P = 0.0376$), and maternal identity explained 23% of the variation among maternal families for mean offspring carpel number. Figure 5.2 depicts the distribution of mean carpel numbers among $Generation_1$ plants. Although the highest individual mean carpel numbers were among plants in the stabilizing line, the upward line contained a greater total proportion of plants with at least one tetracarpellate gynoecium among the first 20 flowers. The realized heritabilities of carpel number within the upward and stabilizing lines were 1.44 and -0.39, respectively (Table 5.1-A).

Regression analysis of mid-offspring carpel number on maternal carpel number was significant ($\beta = 0.50$, $P = 0.035$) and yielded a narrow-sense heritability of 1.00 (Fig. 5.3), suggesting that maternal plants with more carpels per flower produced offspring that

on average also had more carpels per flower. Although the heritability estimate was significantly different than zero (95% CI = 0.08 – 1.93), it at best represents an upper limit to carpel number heritability. The positive y-intercept of the linear regression function ($y = \beta x + 1.5$) and the difference between correlation and regression coefficients both indicate that supernumerary carpels were overall more frequent in $Generation_1$ than in $Generation_0$.

Indirect responses to selection – Although carpel number did not diverge after one round of selection, other whorls exhibited an indirect selection response with values increasing within the upward line and either decreasing or remaining constant in the stabilizing line (Fig. 5.4). The proportion of abnormal corollas per plant statistically differed between selection lines in $Generation_1$ ($F = 7.48$, $P = 0.0066$), with proportions of abnormal calyces and androecia showing similar but insignificant trends. Overall variability of floral formula also diverged as indicated by the proportion of flowers with at least one abnormal whorl ($F = 9.51$, $P = 0.0022$). Realized heritabilities for organ number variation were calculated for each whorl from indirect selection responses (Table 5.1-B). Where heritability was positive for both selection lines, trait means significantly differed in $Generation_1$.

DISCUSSION

After only a single round of selection, all conclusions regarding carpel number selection and floral formula evolution are tentative. Potentially, these results suggest that while variability of the floral formula likely has a heritable component, an evolutionary shift to a new stable number of carpels per gynoecium in *Phlox drummondii* may or may

not be constrained by correlated responses among the other floral whorls. There was some evidence that plants with more variable floral formulae produced offspring with more variable floral formulae, but an initial increase in carpel number among both selection lines suggests a greater inherent lability in the trait than originally expected. Ultimately, what was intended as selection for an alternative trait phenotype (tetracarpellate vs. tricarpellate) is not mutually exclusive of selection for instability of trait expression (variable carpel number) and expression of correlated characters. The situation seen here in *Phlox drummondii* may be similar to that observed for floral bract number in cotton where selection for abnormal numbers was manifest as selection for a decrease in the threshold for abnormal expression (Wilson and Stapp, 1979).

One possible explanation for the carpel number increase among both selection lines could involve the different sampling methods used among generations. A recent study of temporal variation in floral organ number among greenhouse-grown *Phlox drummondii* found variation to be highest at initial flowering and to decrease over time (Byerley, Chapter 3). In the current study, Generation_0 plants were scored for up to the first 50 flowers while Generation_1 plants were only scored for the first 20 flowers. If later flowers are inherently less variable than early flowers, then mean carpel number among Generation_0 plants would be diluted by the greater sample size while Generation_1 values would be inflated relative to parent values. This could result in a general increase in offspring values regardless of selection effects. Further rounds of selection must be careful to account for possible declines in abnormal flowering over time.

Other selection studies on *Phlox drummondii* indicate that reproductively related characters can be readily altered in this species. Self-incompatibilty levels responded to

both upward and downward selection after two generations (Bixby and Levin, 1996), interspecific-compatibility levels with a closely related species were artificially increased after two generations (Fritz, 1997), and corolla diameter was both increased and decreased after three generations (Lendvai and Levin, 2003). In addition, *Phlox roemeriana*, a species presumed to have recently diverged from a *P. drummondii*-like ancestor, is one of the few species in the genus that has 3-5 ovules per locule rather than 1 ovule per locule, indicating the capacity for major meristic changes in the reproductive structures of *Phlox* (Lendvai and Levin, 2003).

Effects of sample size on selection results cannot be dismissed. The relatively small number of plants within each selection line not only reduces statistical power but also introduces the possibility of random effects due to genetic drift. Indeed, the simultaneous increase in carpel number among both selection lines could represent random phenotypic variability exacerbated by a small number of initial genotypes. If the effect of carpel number selection on evolution toward a stable new floral formula is to be determined, then future rounds of selection must be of a greater sample size. Further, once data from additional rounds of selection are obtained, the heritability estimates over all generations can be averaged to give a more reliable estimate that is less influenced by random generational fluctuations of unknown origin (Falconer and Mackay, 1996). Regardless of the methods, continued investigation of this subject is valuable in that it sheds light on the constraints of floral integration and the possible evolutionary trajectories of *Phlox* and other polemoniaceous floral formulae.

Table 5.1. Selection responses and estimated realized heritabilities (h^2) for two lines of *Phlox drummondii* over one generation of selection. Proportional data were calculated out of 20 flowers per plant. "Abnormal" = greater/fewer organs than the modal value.

(A) Direct selection for carpel number

	Mean carpel number per flower			
Selection line	$Generation_0$	Selected Parents	$Generation_1$	h^2
Upward	3.04	3.06	3.06	1.44
Stabilizing	3.04	3.00	3.06	-0.39

(B) Indirect selection for abnormal organ number among the other floral whorls

	Mean proportion of abnormal calyces per plant			
Selection line	$Generation_0$	Selected Parents	$Generation_1$	h^2
Upward	0.03	0.03	0.04	5.00
Stabilizing	0.03	0.00	0.03	-0.14

	Mean proportion of abnormal corollas per plant			
Selection line	$Generation_0$	Selected Parents	$Generation_1$	h^2
Upward	0.06	0.08	0.09	2.00
Stabilizing	0.06	0.00	0.05	0.30

	Mean proportion of abnormal androecia per plant			
Selection line	$Generation_0$	Selected Parents	$Generation_1$	h^2
Upward	0.03	0.02	0.03	-1.50
Stabilizing	0.03	0.01	0.02	0.54

	Mean proportion of abnormal flowers per plant (any whorl abnormal)			
Selection line	$Generation_0$	Selected Parents	$Generation_1$	h^2
Upward	0.09	0.13	0.14	1.28
Stabilizing	0.09	0.05	0.09	0.02

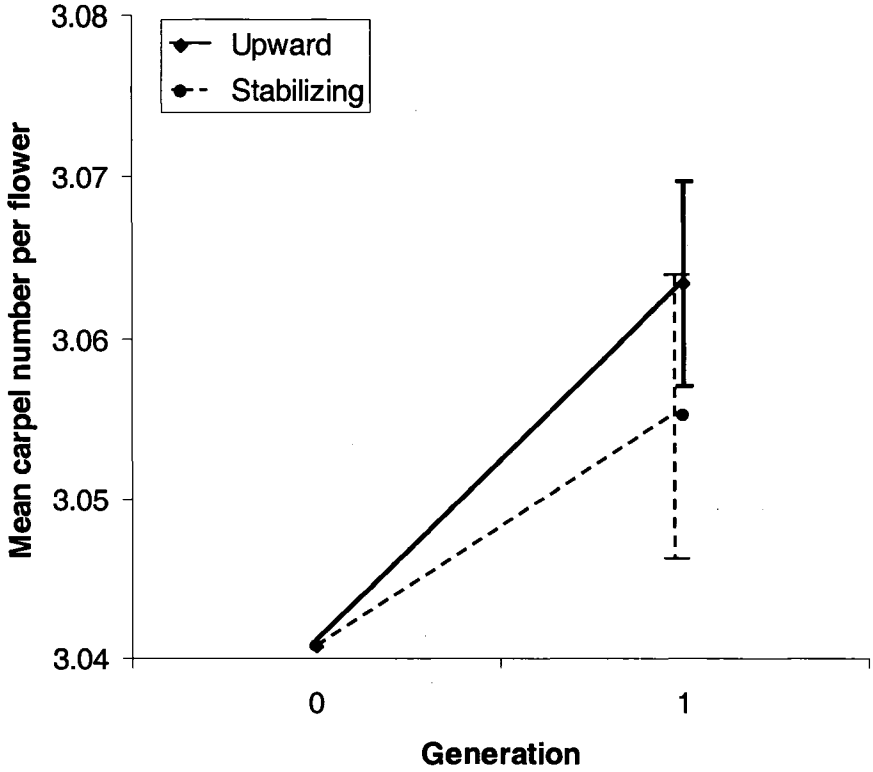

Figure 5.1. Response to one round of upward and stabilizing selection for carpel number in *Phlox drummondii*. Y-error bars = standard error of the mean.

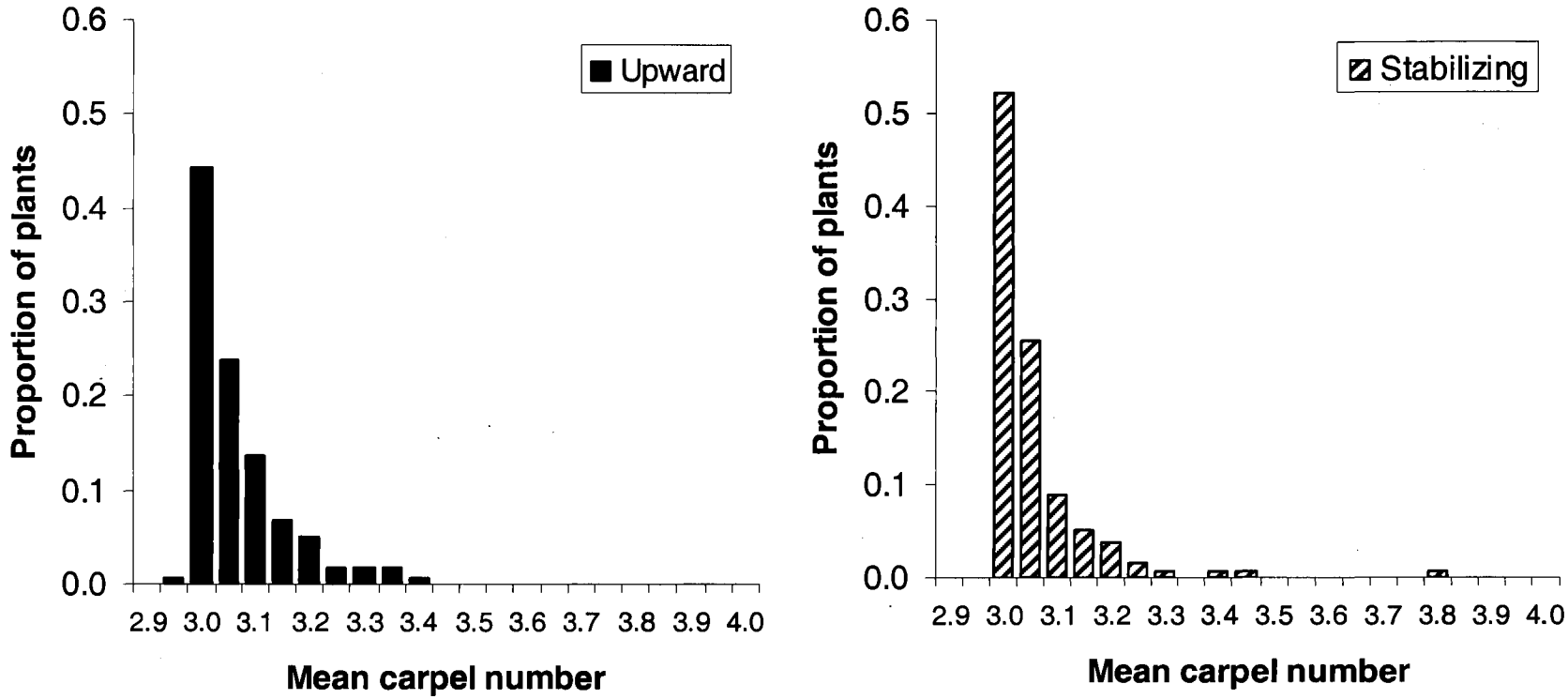

Figure 5.2. Distribution of mean carpel number per flower for Generation$_1$ plants in two selection lines of *Phlox drummondii*.

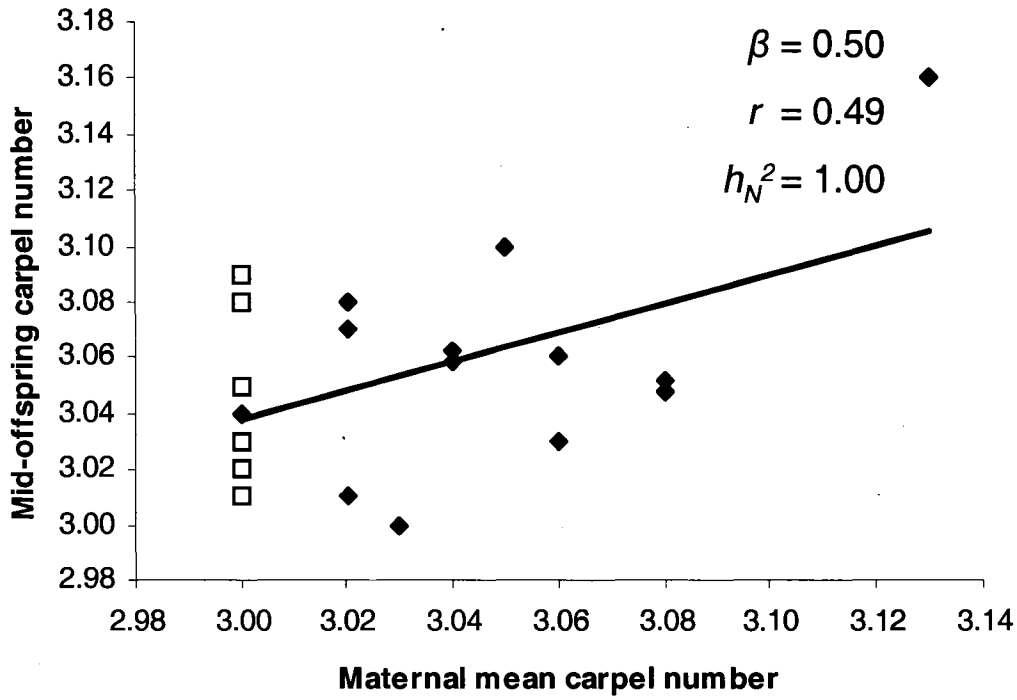

Figure 5.3. Regression of mid-offspring carpel number on maternal mean carpel number in *Phlox drummondii*. Open squares (□) denote plants in the stabilizing selection line; closed diamonds (♦) denote plants in the upward selection line. Regression coefficient (β); correlation coefficient (r); narrow-sense heritability (h_N^2).

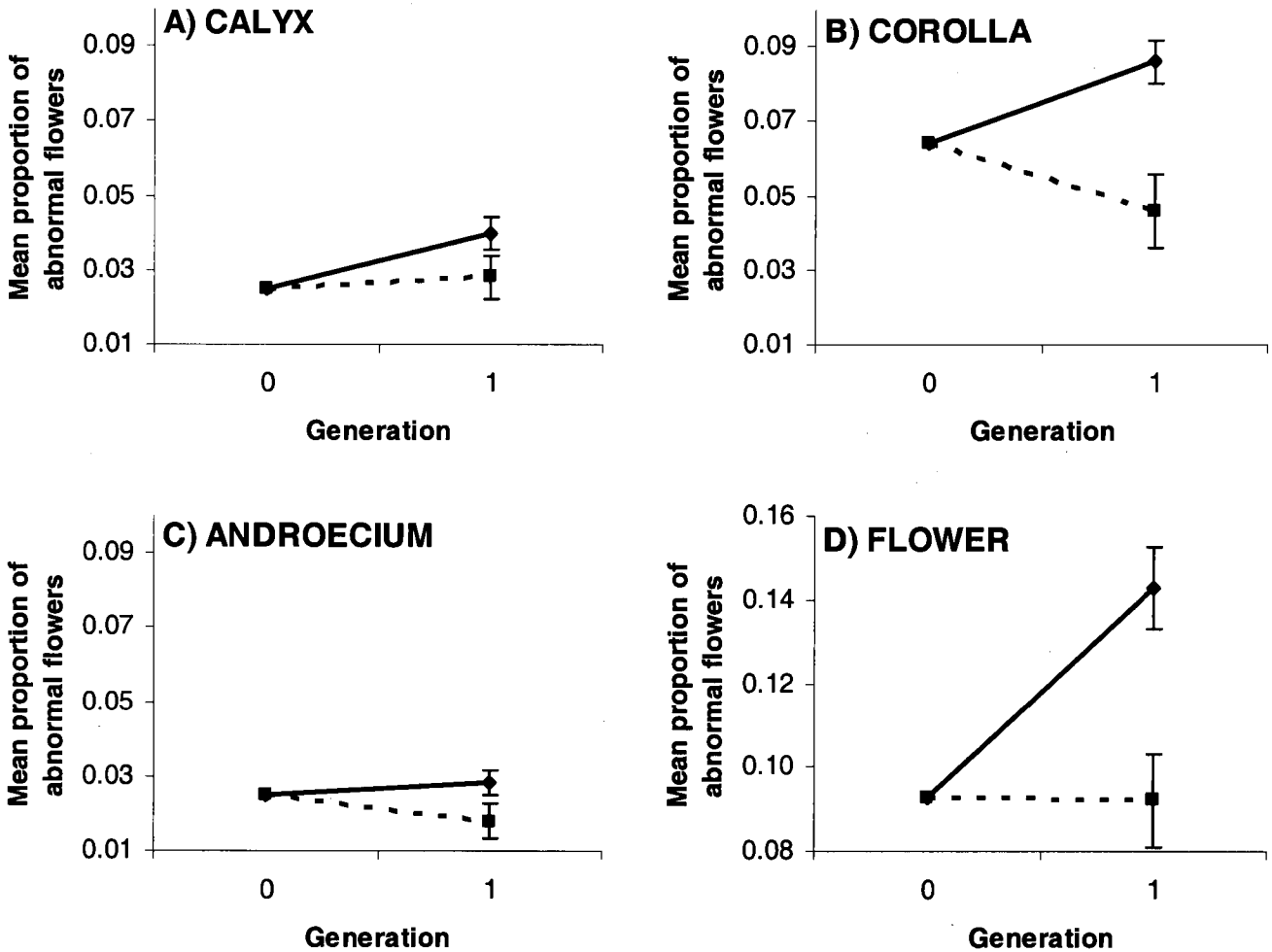

Figure 5.4. Indirect responses to selection on carpel number as observed in the calyx (A), corolla (B), and androecium (C), and at the whole flower level (D). Solid lines represent upward selection. Broken lines represent stabilizing selection. Abnormal flowers (D) are flowers that have deviant organ numbers in at least one of the four whorls. Y-error bars = standard error of the mean.

CHAPTER 6

DISSERTATION CONCLUSIONS

The results of this dissertation indicate that there is potential for a presumably stable character such as the floral formula to evolve through selection pressures: (1) variation for floral formula is present in both natural and greenhouse populations, (2) this variation can lead to differential reproductive output at the flower level, and (3) there may be at least some heritable component to organ number determination. Additionally, the temporal trends in abnormal formula frequency suggest that formula evolution may likely operate by decreasing the threshold for unstable expression, thereby increasing the total variation upon which selection may act.

It is of interest to note that the intra-plant floral formula variation observed here in *Phlox* can be viewed as a product of both developmental instability and phenotypic plasticity. Fenster and Galloway (1997) and Schlichting and Pigliucci (1998) both make a distinction between the two as separate phenomena. Specifically, developmental instability refers to a lack of precision or repeatability of a specific phenotype, while plasticity refers to inconsistency of a phenotype by a given genotype across different environments. Further, plasticity and canalization represent opposite ends of the same spectrum. In *Phlox*, because flowers produced by a single plant are subjected to

potentially different internal and external environments during floral development, meristic variation of floral organs can be considered a symptom of both instability and plasticity. This is highlighted by wild *Phlox longifolia* plants that exhibited moderate abnormality early in the flowering season (instability) and even higher abnormality late in the season (plasticity), presumably as a result of environmental stress. If a decrease in frequency of abnormality were observed in the same plants under controlled conditions, the above notion could be verified.

Evidence that certain plants are predisposed to produce abnormalities largely in a single direction while other plants exhibit abnormalities equally in both directions indicates that floral formula variation likely stems from multiple sources, each of which could be acted upon by selection to different degrees. For example, plants which are randomly unstable such that formula variation appears to merely be developmental noise, may be subject to selection for increased homeostasis and canalization of development, but only if that variability carries with it negative fitness impacts (Schlichting and Pigliucci, 1996). Conversely, plants which are non-randomly unstable such that formula variation is unidirectional may experience selection for decreased homeostasis and greater plasticity of development if the direction of abnormality is reproductively beneficial.

Lastly, a statement that floral formula variation simply exists contributes little to the collective knowledge base. The work included in this dissertation provides empirical evidence and a level of detail regarding floral formulae that will hopefully add to our current understanding of patterns of floral morphology as a whole and the evolution of floral form.

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APPENDIX I

CORRELATION OF STIGMATIC LOBES AND CARPELS IN *PHLOX* GYNOECIA

Among researchers who study plants of the Polemoniaceae family, the number of stigmatic lobes within a single flower is commonly used as a noninvasive estimate of the number of carpels comprising the gynoecium. However, this assumption is only weakly validated in the current literature, usually through reference to an unknown or small number of preliminary dissections. This appendix attempts to serve as documented empirical evidence supporting this widely held assumption, eliminating the need for future researchers to rely on anecdotal evidence or to cite "unpublished data".

Methods – Gynoecia with 2-lobed, 3-lobed, and 4-lobed stigmas (Fig. AI.1, A-C) were collected from wild populations of *Phlox longifolia* Nutt. (Larimer County, Colorado, USA). In addition, four rare gynoecia with 5-lobed stigmas (Fig. AI.1, D) were collected from greenhouse-grown *Phlox drummondii* Hook. Gynoecia were dissected and scored for carpel number and ovule number.

Results and Discussion – Out of a total of 171 total gynoecia, only one did not match for numbers of stigmatic lobes and carpels (Table AI.1). This single *P. drummondii* gynoecium had five stigmatic lobes but only three carpels. Despite the single

deviation, the probability that lobe number will accurately predict carpel number within *Phlox longifolia* was equal to one. All gynoecia examined had one ovule per carpel, thus stigmatic lobes should also predict total ovule number with the same accuracy in this species.

Table AI.1. Number of stigmatic lobes and carpels for 171 different *Phlox* gynoecia. The gynoecia with five stigmatic lobes are from *Phlox drummondii*. All others are from *Phlox longifolia*.

No. of lobes per gynoecium	No. of carpels per gynoecium				
	Two	Three	Four	Five	Total
	No. of gynoecia				
Two	17	--	--	--	17
Three	--	100	--	--	100
Four	--	--	50	--	50
Five	--	1	--	3	4

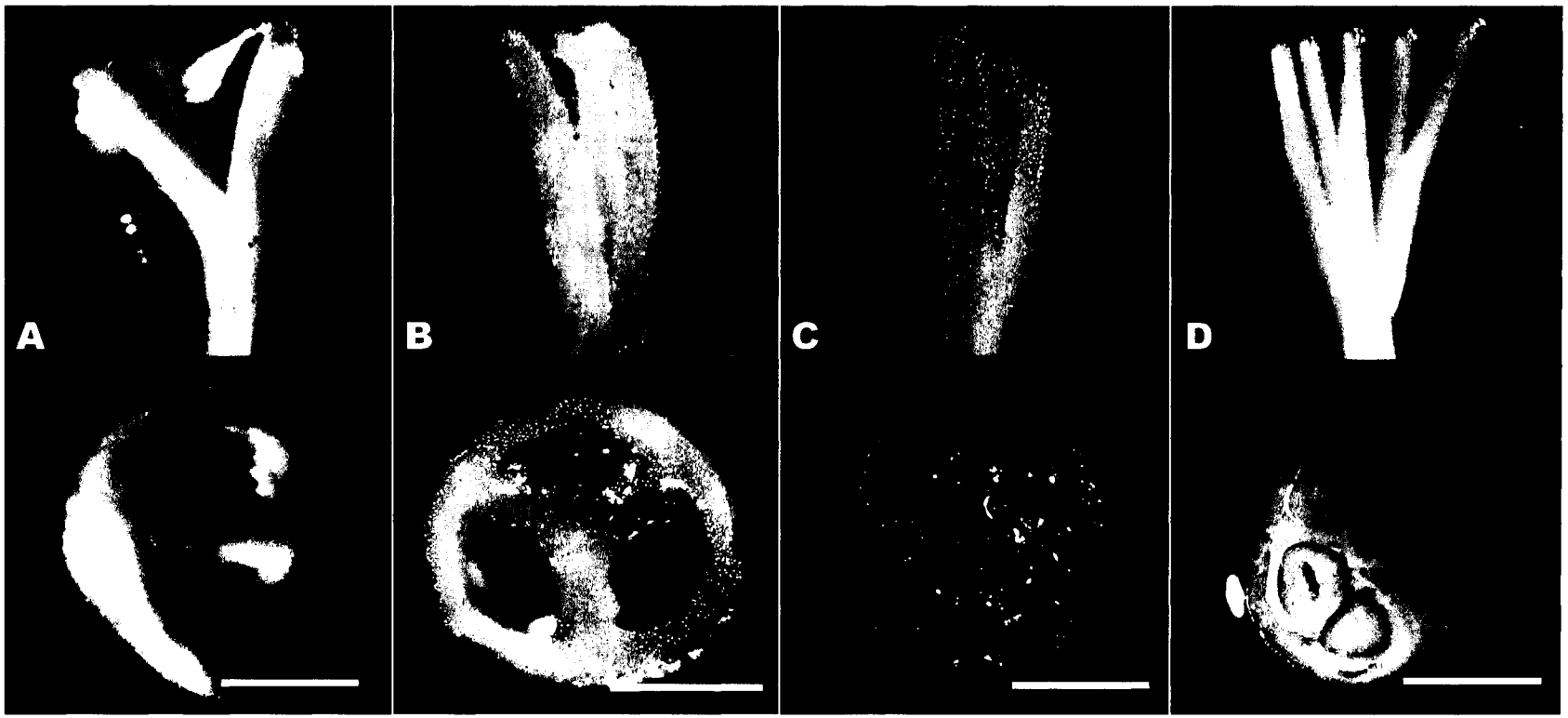

Figure AI.1. Correlation among numbers of stigmatic lobes and carpels within *Phlox* gynoecia. The top row depicts stigmatic lobes, the bottom row, ovary cross sections. Ovules have been removed from the ovary sections of A-C, leaving empty locules. (A) 2-lobed stigma and bicarpellate gynoecium. (B) 3-lobed stigma and tricarpellate gynoecium. (C) 4-lobed stigma and tetracarpellate gynoecium. (D) 5-lobed stigma and pentacarpellate gynoecium with one ovule per locule. Bars = 0.5mm.

APPENDIX II

THE BREEDING SYSTEM OF *PHLOX LONGIFOLIA* NUTT.

Although gametophytic self-incompatibility has been documented for multiple species within the *Phlox* genus, both pseudo-incompatible and fully autogamous species do exist. Because evolutionary interpretations for selfing species are likely to be different from those of obligate outcrossers, it was essential to this dissertation to determine the breeding system of wild *Phlox longifolia* Nutt., which had yet to be reported elsewhere in the literature.

Methods – Twenty-five plants were chosen at each of three natural populations located in the Cherokee Park area of Roosevelt National Forest (Larimer County, Colorado, USA). Each plant received the following four pollination treatments: (1) within-flower selfing, (2) within-plant (geitonogamous) selfing, (3) manual outcrossing, and (4) open pollination (unmanipulated). Where possible, each treatment was repeated three times within each plant for a maximum of 12 pollinated flowers per plant. The treatments were staggered slightly over time and the order randomized to limit the effect of within-plant competition for resources. All pollinations were performed on normal flowers with tricarpellate gynoecia.

These methods yielded a total of ~175 pollinations for each of the two autogamous treatments and ~215 pollinations each for the outcrossed and open treatments. Successful pollinations were measured by the production of a fruit, and total fruit set and seed set for each treatment were used to approximate the relative breeding strategy success.

Results and Discussion – Although one population had a higher overall fruit set (One-way ANOVA, $F = 5.42$, $P = 0.0065$), all three populations showed similar trends in fruit set over the four pollination treatments. Specifically, outcross and open pollinations yielded fruit sets that were roughly five times the fruit set of the autogamous treatments (Fig. AII.1). Seed set was similar in all treatments; 85% of all matured fruits had 1-2 seeds. These results suggest that given the similarly high success rates of conspecific and open pollination treatments, the breeding system of wild *Phlox longifolia* is likely to be predominately outcrossing.

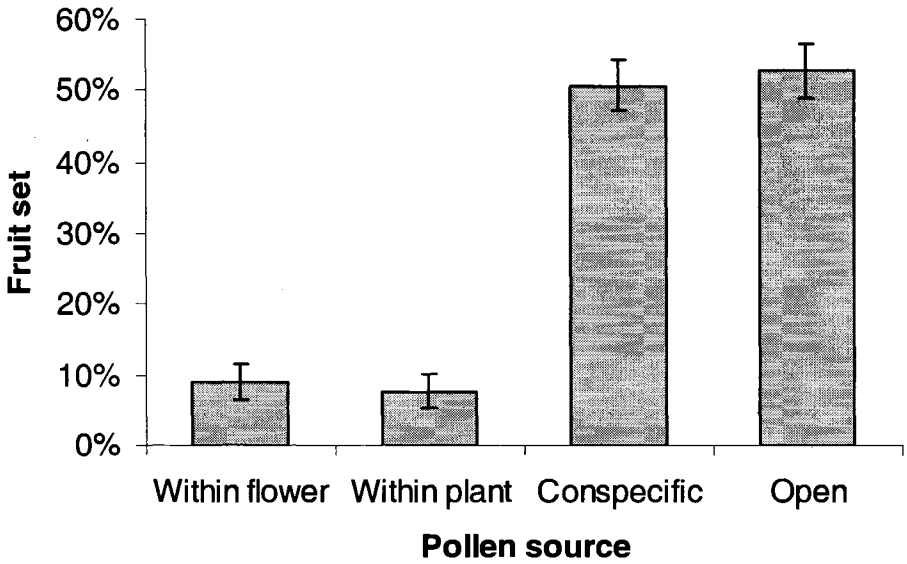

Figure AII.1. Fruit set results from four pollination treatments on wild *Phlox longifolia* plants. Fruit set is the percentage of successful pollinations (as measured by production of a fruit) relative to the number of attempted pollinations. Y-error bars = standard error of the mean.