CLARKIA: *GENETIC BASIS OF SISTER SPECIES DIVERGENCE*

CLARKIA CONCINNA X CLARKIA BREWERI

by

Amanda Ann (Shaffer) Henry

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APPROVAL

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Amanda Ann (Shaffer) Henry

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the Division of Graduate Education.

Dr. Norman F. Weeden

Approved for the Department of Plant Sciences & Plant Pathology.

Dr. John Sherwood

Approved for the Division of Graduate Education.

Dr. Joseph J. Fedock

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ABSTRACT

The genetic changes accompanying speciation provide insight into the mode and tempo of phenotypic divergence. A genetic linkage map of *Clarkia* was used to investigate the genetic basis of several morphological differences between two closely related plant taxa with divergent pollination syndromes, *C. concinna* and *C. breweri.* The genetic linkage map of the hybrid genome contained 40 markers on 11 linkage groups. In this study, the genetics of eight floral and vegetative traits were analyzed in a segregating *C. concinna* x *C. breweri* F_2 population (*N*=95) and in replicates of the parentals, F_1 hybrids, and backcrosses. The genetic linkage map was constructed as the framework for locating loci underlying the divergence in the eight morphological traits. The results suggest that genes of large effect contribute to species divergence in *Clarkia.*

THESIS FOCUS

The genetic change accompanying speciation was the main focus of this mapping study on *Clarkia*. The objectives of this project were two-fold; create a set of primers that could be utilized across *Clarkia* species and explore the genetic relationship between the two species of the mapping population, *Clarkia concinna* and *C. breweri*. The results of previous studies have indicated a need for tools to bridge the gaps between *Clarkia* species for meaningful comparative/syntenic studies (Gottlieb, 2003; Levin et al., 2003).

To study the differing floral characteristics of the cross *C. concinna* x *C. breweri,* five segregating phenotypic traits were recorded in the F_2 generation: flower aroma, petal shape, hypanthium length, flower hue and days to flower. There were also three vegetative characteristics that were recorded leaf shape, stem anthocyanin, and apical dominance. The divergent morphological traits between the parent species, *C. concinna* and *C. breweri* were related to the genetic map by gene tagging and quantitative trait loci (QTL) mapping analyses. These results were compared to similar analyses in several other genera, including *Mimulus, Petunia,* and *Aquilegia.*

There is little known about the nature of the genes controlling adaptive evolution and speciation in natural systems (Fishman et al., 2001). Using the genetic map produced for the *Clarkia* crosses, I explored the hypothesis that the morphological and physiological changes diverging between the species *C. concinna* and *C. breweri* are each the result of simple genetic changes involving one or two genes. The genetic map constructed contains 20 sequenced tagged sites (STS) further specified as cleaved amplified polymorphic sequences (CAPS) in several cases, and 16 random amplified

polymorphic DNA (RAPD) markers with the addition of the eight morphological markers. The genetic linkage map is necessary for the identification of QTL involved in phenotype differences and can also provide insight into patterns of genomic divergence (Rieseberg et al., 1995, 2000; Whitkus, 1998).

LITERATURE REVIEW

Introduction

The availability of linkage maps and sequence data now allow studies to be conducted in many genera to help identify genetic mechanisms for controlling evolutionary divergence (Turelli et al., 2001). Those genera in which divergence is found between floral characteristic are of special interest. This interest is due to one of the major tenets of pollination biology; floral characteristics attract different pollinators and therefore differentially affect pollen carryover from visitations (Stuurman et al., 2004). Speciation studies conducted on highly speciose plant taxa have found that many genera show divergence of these floral characteristics, as compared to vegetative characters, which suggests that adaptation to pollinators has played a major role in some species formation (Johnson, 1996). The genetic control of floral characters affecting pollinators have been studied in *Mimulus* (hummingbird and bee pollinators), *Petunia* (bee and moth), and *Aquilegia* (hummingbird and moth) (Bradshaw Jr. et al., 1998; Stuurman et al., 2004; Hodges et al., 2002). The genus *Clarkia* (Onagraceae) is ideal for the study of diverging floral characteristic because it is very species rich and affords several differing relationships and breeding structure study opportunities (Lewis, 1953; Lewis and Lewis, 1955; Systma et al., 1990; Levin, 2002).

Clarkia Evolutionary Studies

Taxonomy

Onagraceae is composed of about 650 species in 20-24 genera with worldwide distribution, although the family is most species-rich in the New World (Levin, 2003, Table 1.; Raven, 1988; Mabberley, 1997). The family belongs to the division Magnoliophyta in the class Magnoliopsida. Onagraceae is in the subclass Rosidae. The Fabaceae (legumes) and the Brassicaceae (mustards) are also among the Rosidae. Some families, not included in this subclass, that were related close enough to the Rosidae for use in primer sequence design are the Vitaceae (grapes) and the Solanaceae (nightshade). The closest relatives of the Onagraceae are all in the order Myrtales (Figure 1).

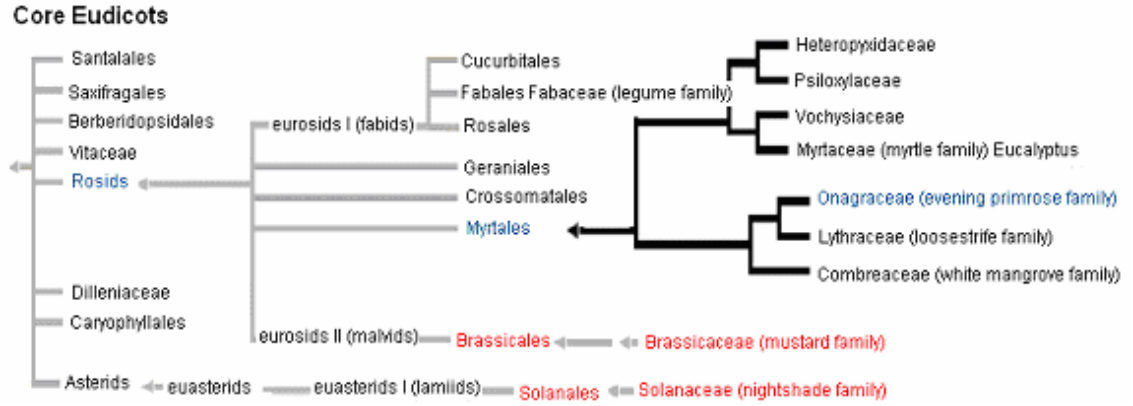
Table 1.[1] Tribes and genera of Onagraceae with number of section, species, and geographical distribution

Taxon	No. sections/ spp.	Distribution
Megacorax Gonzalez & W.L. Wagner (incertae sedis)	–/1	Central Durango, Mexico
Tribe Jussiaeeae		
Ludwigia L.	23/81	Pantropical, extending to temperate North America and Asia
Tribe Hauyeae		
Hauya D.C.	–/2	Southern Mexico to Costa Rica
Tribe Fuchsieae		
Fuchsia L.	10/105	Andean South America, extending to Mexico and Hispaniola; New Zealand and Tahiti
Tribe Ciraeeae		
Circaea L.	–/7	North temperate, especially Asia
Tribe Lopezieae		
Lopezia Cav.	6/22	Mexico, extending to Panama
Tribe Epilobieae		
Chamerion (Raf.) Raf.	2/8	North temperate, especially Asia
Epilobium L.	7/164	Cosmopolitan at high altitudes and latitudes; all seven

Table 1. continued		
		sections occur in western North America
Tribe Onagreae		
Calylophys Spach	2/6	Rocky Mountains to central U.S. and central Mexico
Camissonia Link	9/62	Western North America, mostly California; 1 sp. In temperate South America
Clarkia Pursh	11/42	Western North America, mostly California; 1 sp. In temperate South America, and another common to both Continents
Gaura L.	8/21	Southwestern to central U.S. with center Texas, extending to Atlantic coast and south to Guatemala
Gayophytum A. Juss.	−/9	Mainly western North America; 1 sp. In temperate South America, and another common to both continents
Gongylocarpus Schlecht. & Cham	−/2	One endemic on islands off coast of western Baja California, Mexico and the other in central Mexico to Guatemala
Oenothera L.	15/121	All sections in North America, esp. western U.S., with center of diversity in Arizona and Texas to northern Mexico; four sections and >50 spp. In Central to South America; 2 spp. Of European hybrid origin from North American introduced taxa
Stenosiphon Spach	−/1	Great Plains of central U.S.
Xylonagra Donn. Smith & Rose	−/1	Central Baja California, Mexico
January 2003	108	AMERICAN JOURNAL OF BOTANY

[1] Levin et al., 2003; reproduced with permission from the American Journal of Botany

Figure 1. Taxonomic relationships of the family Onagraceae (Tree of life web project, 2002; Conti et al., 1993)



The family Onagraceae contains both woody and herbaceous species, including a number of popular garden plants, such as evening primroses (*Oenothera*) and fuchsias

(*Fuchsia*). A few of the species within Onagraceae are considered wildflowers, but may be found in gardens particularly the willowherbs (*Epilobium*), e.g. fireweed. The family is typically characterized by flowers with four sepals and four petals; in some genera (e.g. *Fuchsia and Clarkia*), the sepals are brightly coloured resembling a second whorl of petals. The seeds are very small, and in some genera (e.g. *Epilobium*) they possess a tuft of hair, to aid wind-dispersal. In others (e.g. *Fuchsia*) a juicy berry, dispersed by birds, is produced (Salisbury, 1976). Anotherfeature of the family Onagraceae is the general shape and arrangement of the leaves. The leaves are usually opposite or whorled but are spirally arranged in some species. Most leaves are simple and lanceolate in shape (Watson and Dallwitz, 2005).

The main tribe within the family Onagraceae is the Onagreae, which consists of nine genera according to Raven and Mabberly (from Levin et al., 2003, Table 1.). The phylogenetic relationships within and among these genera and within the family Onagraceae are not fully understood or described. One of the drawbacks of previous analyses is the limited sampling within the large tribe Onagreae that was noted by Levin et al. (2003).

Previous efforts to describe the evolutionary relations within the family Onagraceae and *Clarkia* have been conducted using morphological features (Hoch et al., 1993) (*Clarkia* specifically: Lewis, 1955) and molecular analyses of chloroplast DNA (Systma et al., 1990) and nuclear restriction site data (Crisci et al., 1990) (*Clarkia* specifically: Gottlieb et al., 1996), 18S and 26S nuclear ribosomal RNA sequence data (Bult and Zimmer, 1993) and isozymes in *Clarkia* (Gottlieb and Weeden, 1979). These

studies were conducted to elucidate the species relationships within the family Onagraceae and the genus *Clarkia* specifically.

Clarkia is a genus consisting of annual wildflowers found primarily in California. These plants grow to between 1 dm to 20 dm in height. Upon blooming most species have slender, usually unlobed, petals of a pink to purple color. Most species of *Clarkia* are located within oak woodland, although some extend into adjacent communities and some are characteristically associated with the lower margins of the montane forest, the grassland and the chaparral (Lewis, 1953). There are two species that are found along sea bluffs, *Clarkia concinna ssp. raichei* and *C. franciscana* (Walker, 1992; Gottlieb and Edwards, 1992). Although some species share the same type of habitat, no two species of *Clarkia* have an identical range of distribution. There are approximately 44 species of *Clarkia,* and all but one, are endemic to western temperate North America (Moore and Lewis, 1965). The *Clarkia tenella* polyploid complex is found in South America where it spread to from North America by long distance dispersal (Systma et al., 1990).

In general *Clarkia* is colonial and therefore the populations are spread out in patches, each of which is called a colony, like most annuals found in similar environments. Colonies generally reoccur each year at the same site. These sites do not, as a rule, change appreciably in extent from year to year. Some colonies have been observed in the same location for at least 70 years (Gottlieb, personal communication). The flowers of *Clarkia* are bisexual and usually favor outcrossing. Outcrossing is ensured by the shorter length of the stamens, and pollen is shed before the stigma opens fully. Fertilization is followed, within a day, by a withering of the petals and abscission of the floral structures above the apex of the ovary. Despite the promotion of outcrossing

by floral anatomy, all *Clarkia* species are self-compatible. Some species of *Clarkia* including the *C. concinna ssp. concinna, C. xantiana parviflora*, and *C. exilis* have become selfing (Groom et al., 2000; Knive, 2004). This trait has developed independently at least 12 times in *Clarkia* (Lewis and Raven, 1958; Runions and Geber, 2000). Selfing taxa (whether species or subspecies) are typically distributed parapatrically (contiguous populations) to and occupying more marginal (typically drier) habitats than those of their sister outcrossing taxa (Lewis and Lewis, 1955). Understanding the genetics behind selfing subspecies may shed light on the evolution of *Clarkia*'s breeding systems and whether variation of floral features plays a role in species divergence. Selfing subspecies are under study in several other plant species besides *Clarkia,* such as *Mimulus, Petunia, Gilia,* and others (Schoen, 1982).

Clarkia ovaries give rise to a number of seeds, approximately a millimeter in diameter produced within one capsule. The number of seeds varies with the species and with the vigor of the plant. In hybrids within *Clarkia,* a lack of seed set is noted as these hybrids are mainly sterile. Capsules with an average of about 50 seeds would be representative of fertile species seed set (Lewis, 1953). However, *C. heteranthera* (previously *Heterogaura heteranthera*) has much smaller capsules containing few seeds (Lewis and Raven, 1992). Seed pods dry and dehisce during the summer months. Dissemination is not aided, so the seeds are deposited in the soil beneath the mother plant, therefore ensuring the seeds remain in the same favorable habitats as the parents. These structures and conditions seem to promote the rapid speciation seen in the *Clarkia* genus (Lewis, 1973).

The genus *Clarkia* has been reconstructed and amended several times but was originally assembled from several morphologically diverse groups originally recognized as genera (Genera: *Clarkia* plus *Eucharidium* Fisher and Meyer, *Godetia* Spach, *Heterogaura* Rothrock and *Phaeostoma* Spach), as well as species removed from *Oenothera* L. and a number of newly discovered species (Lewis and Lewis, 1955; Lewis, 1993) and a few others since that time (Gottlieb and Ford, 1996). The changes to proposed evolutionary relationships have been in response to new data or techniques that were made available in the genus *Clarkia*. With the addition of a mapping tool technique new rearrangements and inferences into species development in this group may be revealed.

As currently defined, the genus *Clarkia* has only one known autopomorphy, derived characteristic possessed by a single group and that is having unicellular papillae on their stigmas (Heslop-Harrison, 1990; Hoch et al., 1993). In addition, all *Clarkia* have basifixed anthers and the sporogenous tissue in each anther sac is divided into small packets separated by sterile tissue (Gottlieb and Ford, 1996). All three characteristics were used to delimit the genus and to separate it from *Oenothera*; however, the last two characters have now been demonstrated outside the genus.

The 44 species of *Clarkia* are divided among seven sections (Systma, et al. 1990). Lewis and Lewis initially created the seven sections to accommodate similar diploid species and related polyploids (1955). Each section is defined by shared morphological and cytological traits. Three monotypic sections for allopolyploid species considered derived by intersectional hybridization. Of these seven sections Eucharidium contains only the two species *Clarkia concinna* and *C. breweri*, which share the basal, or most

ancient, chromosome number *n*=7. Eucharidium is basally located in the family as described by research on the *PgiC* sequence and has many unique characteristics that separate this section from all others (Gottlieb, 1997). Section Eucharidium is described by Lewis and Lewis as

> "an erect herb with stems that usually branch, puberulent, or glabrate but not glaucous; leaves are entire or essentially so; rachis of the inflorescence is erect or bent to one side in bud; the buds are deflexed, becoming erect as the flowers open; the hypanthium is slender, 13-35mm. long, the ring of hairs seen here in other sections is absent; sepals remain united and deflexed to one side at anthesis; petals are conspicuously 3-lobed, the middle lobe usually exceeding the length of the lateral lobes; the stamens are reduced to 4, 8 are seen in all the other sections of *Clarkia*; the anthers are ciliate and obtuse; the ovary is subterete and sessile" (1955 p. 359)

These two species also share some novel characteristics among themselves that are not included in the other sections of *Clarkia* (Onagraceae) such as unique pollen features, anther shape, three-lobed petals, floral tubes, stamen reduction to four, and petaloid sepals. These characters represent the greatest morphological divergence from other clarkias (Lewis, 1980).

Using chloroplast and single gene sequence analysis, Gottlieb (1996) demonstrated that section Eucharidium is not closely related to any other section of *Clarkia* and represents, either alone or perhaps in conjunction with section Rhodanthos, one of the basal groups for the entire genus. Data from *PgiC1* sequence alone provides 92.5% bootstrap support for the basal placement of section Eucharidium (Gottlieb, 1996).

Lewis (1955) hypothesized and supported several cases of ancestor-derived species relationships within the genus *Clarkia*, including *C. concinna* and *C. breweri*. Chromosomal, cytogenetic, morphological, and distributional data, all suggest that *C. breweri* evolved from *C. concinna* and belongs in the same section (Lewis, 1955). Several existing progenitor-derived species pairs in *Clarkia* probably separated recently

(Systma et al., 1990). Derived species have areas of distribution adjacent to and usually much smaller than those of their respective parents (Lewis and Lewis, 1955; Futuyma and Mayer, 1980). *C. breweri* has a much smaller area of distribution in a drier location to the south of the *C. concinna* populations (Figure 2).

Clarkia concinna was first described as a species within the old genus Eucharidium by Fischer & Meyer (1835).

Though the type specimen of *C. concinna*, presumably at Leningrad, was not examined by Lewis and Lewis they felt the original description left no doubt as to the species intended (1955).

Figure 2. Distribution of *Clarkia concinna* and *C. breweri* (Lewis and Lewis, 1955).

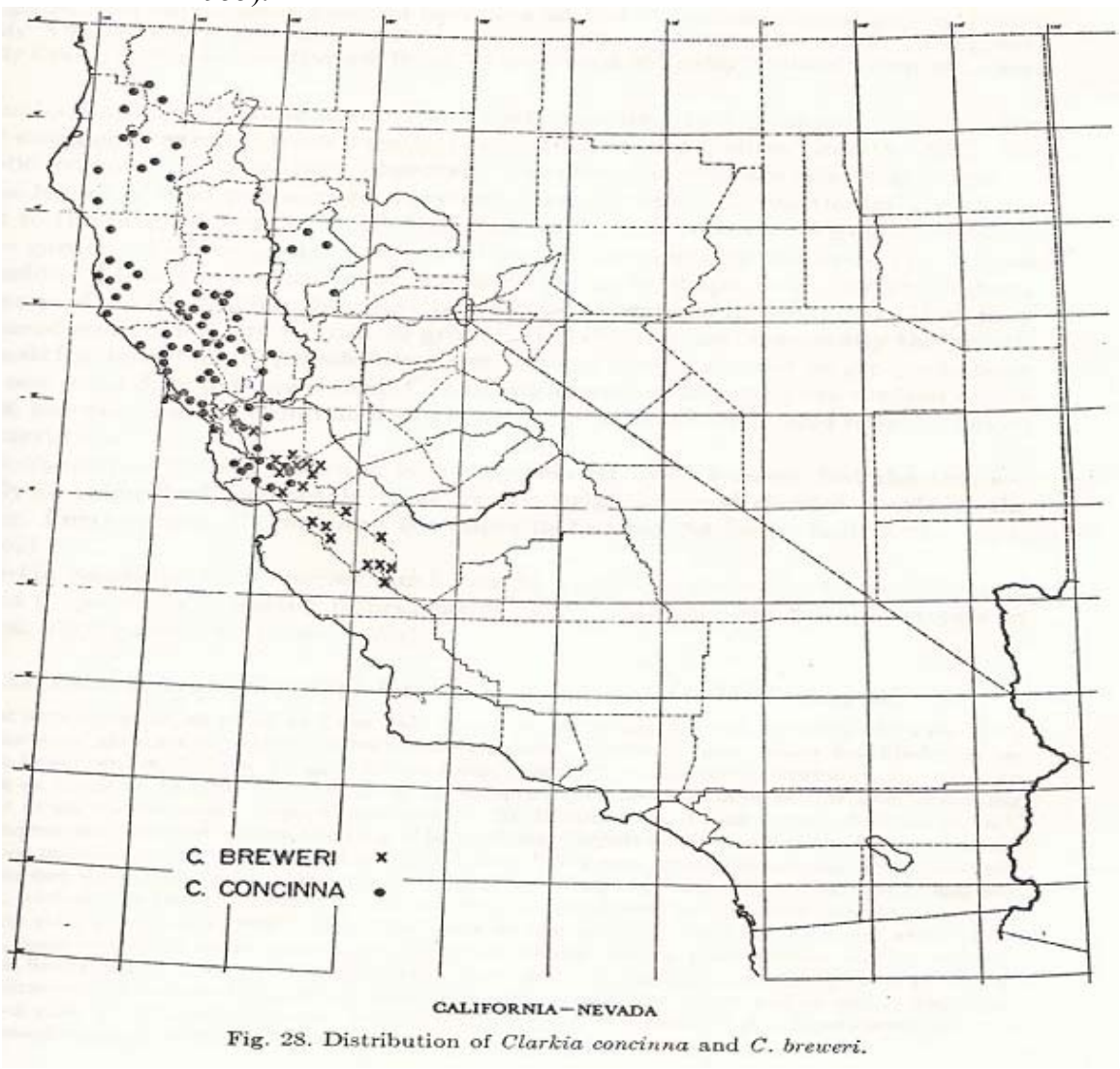


Fig. 28. Distribution of *Clarkia concinna* and *C. breweri*.

The typical habitat for *C. concinna* is along the openings in the woodland and forest of the Coast Range and occasionally in the northern Sierra Nevada below 1000m. A representative specimen and the major floral characteristics as described by Lewis and Lewis are presented in Figure 3. The characteristic petal shape, leaf width, growth habit, scent production, and hypanthium length differ between the two species.

Figure 3.

Figure 3. Floral features of *Clarkia concinna* (left) and *C. breweri* (right) taken M.S.U. greenhouse (AAH, 2003).

Clarkia concinna	*Clarkia breweri*
	
Petals Deep Bright Pink*	Petals pink to paler or white at the base*
Slender Petal Lobes*	Lateral lobes of petals are broad, overlapping*
Lower half of sepal petaloid	Sepals non-petaloid
Filaments not clavate	Filaments clavate
Nonscented*	Scented*
Broad leaves	Long thin leaves
Shorter hypanthium than *C. breweri* *	Long hypanthium*

* An asterisks indicates that the trait is affecting the pollinator directly

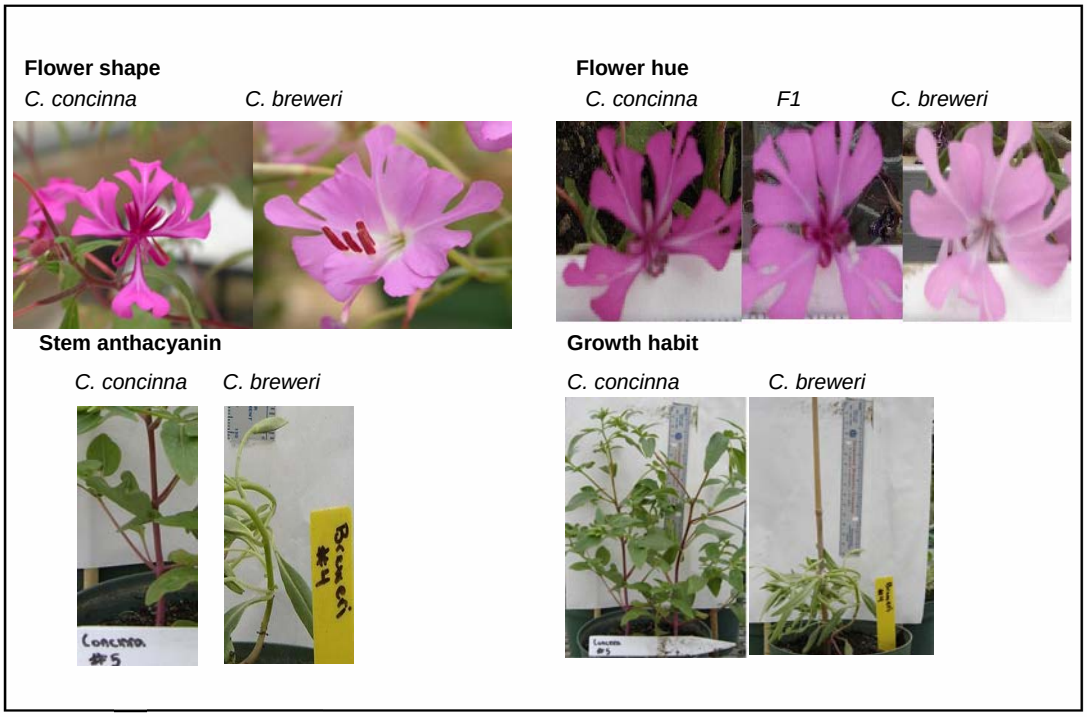
Previously, genetic material has been described for the species *C. concinna*. No meiotic irregularities were observed, and the chromosomes were described as relatively large (Lewis and Lewis, 1955). The genome size is described for *Clarkia concinna* as approximately 2900 Mbp by Bennett (1982). *C. breweri* is readily distinguished from *Clarkia concinna* by the brightly colored slender petals and petaloid sepal of the latter.

The species *C. breweri* was described by Gray (1865, 1887).

Table 2. Morphological characters differing between *C. breweri* and *C. concinna*

Character	Breweri phenotype	Concinna phenotype
Plant height (greenhouse)	10-20 cm	20-40 cm
Stem anthocyanin	Absent or weak	Strongly expressed
Leaf shape	Linear to lanceolate	Elliptic to oval
Sepal color	Green to yellowish	Pink to red
Petal hue	Pink	Red and white
Length of petal	21 mm	26 mm
Width of lateral lobe (base)	9 mm	5 mm
Length of hypanthium	20-35 mm	13-25 mm
Length of filaments at anthesis	37 $\pm$ 4.9 mm	26 $\pm$ 1.5 mm
Length of style at anthesis	48 $\pm$ 5.5 mm	31 $\pm$ 0.6 mm
Anthers	3-4 mm L, straight	2-3 mm L, scorpioid
Hairs on anthers	0.5-1 mm L	0.2-0.3 mm L
Length of capsule	1.5-4 cm	1.5-2 cm
Filament shape below anther	Clavate	Thread-like
Time of flower opening	Evening	Morning
Scent	Conspicuous	Not detectable
Primary pollinator	Hawkmoth, hummingbird	Honey bee

Figure 4. Morphological features of *Clarkia concinna* (left) and *C. breweri* (right) taken from M.S.U. greenhouse (AAH, 2004).



The chromosomes of *C. breweri* are comparable in size to those of *Clarkia concinna* (Lewis, 1955). *C. breweri* is morphologically very distinct and relatively uniform throughout its restricted area of distribution. It displays a higher degree of inbreeding depression than *Clarkia concinna* (Lewis and Lewis, 1955).

Clarkia as a Model Genus

The use of *Clarkia* for evolutionary studies revolves around the taxonomic richness of the genus including progenitor-derived species differing in: chromosome number, breeding habits, floral and vegetative morphology, and previous use as a model genus of evolutionary mechanisms (Lewis, 1953; Lewis and Lewis, 1955; Lewis, 1973; Gottlieb and Weeden, 1979; Bowman, 1984; Pichersky and Gottlieb, 1983; Odrzykoski and Gottlieb, 1984; Systma et al., 1990; Gottlieb et al., 1993; Gottlieb and Ford, 1996, 1997; Groom and Preuninger, 2000; Leong et al., 2001; Ford and Gottlieb, 2002, 2003; Levin, 2003). This large accumulation of data provides a platform from which questions about genetic change accompanying evolution may be addressed. Questions concerning allopolyploid formation from hybridization and Lewis's hypothesis on the function of chromosomal rearrangements in speciation events are just a few examples.

The relationships of *Clarkia* to other genera within family Onagraceae are not well understood. The study performed by Levin et al. in 2003 compared chloroplast *RBCL* (large subunit of ribulose bisphosphate carboxylase) and *NDHF* (NADH dehydrogenase) sequence but found that most of the evolutionary relationships within Onagreae were weakly resolved. These data suggested a rapid diversification of the

group in western North America. The results of the chloroplast DNA study also suggested that some changes in generic and tribal delimitation within Onagreae are warranted, but such revisions require results from analysis with increased taxon sampling.

L. D. Gottlieb and V. S. Ford (1996), studied the phylogenetic relations among the sections of *Clarkia* (Onagraceae) using nucleotide sequences of the cytosolic isozyme of phosophoglucose isomerase (*Pgi*c). They found that the results were in perfect accord with established relationships among the *Clarkia* sections. "From the base of the genus, the sections branch in the following order: sect. *Eucharidium*, sect. *Rhodanthos*, sect. *Myxocarpa*, sect. *Phaeostoma*, sect. *Godetia*, and sect. *Sympherica*" (Gottlieb and Ford, 1996).

Speciation in *Clarkia*

The genus *Clarkia* is an important evolutionary model for rapid speciation and has been since the pioneering work of Lewis and Lewis (1955). Cytologic evidence of polyploidy as well as varying degrees of translocation heterozygosity has been detected within the genus *Clarkia* (Lewis and Lewis, 1955; Raven, 1979). One result of Lewis's analysis is that differential accumulation of chromosomal rearrangements is the highest contributing factor to speciation within the genus *Clarkia* (1953). Chromosomal rearrangements consist of a structural change in a chromosome and may consist of a deletion, translocation, inversion, or gene amplification.

Speciation can result from chromosomal rearrangements (Levin, 2002). Differential accumulation of these chromosomal rearrangements indicates certain regions

of the chromosome will be more likely to contain rearrangements instead of random distribution of chromosomal changes. Those areas prone to rearrangement would contain a region no longer syntenic, sharing a similar gene order due to shared ancestry, in sequence to the parent species without the translocation. Individuals differing in a translocation will usually produce hybrids with reduced fertility resulting from quadrivalent or multivalent (more than two chromosomes involved in a reciprocal translocation) formation, possibly representing an initial step in the speciation process (Levin, 2002).

One main tenet of Lewis's work with *Clarkia* is that, "structural rearrangement of chromosomes is of prime importance as a mechanism which permits adapted gene combinations to persist immune from recombinations and for this reason is probably the most important single factor in the evolution of *Clarkia*, particularly with respect to species formation" (Lewis, 1953, p. 15). Lewis made note of the breeding structure in *Clarkia* as one which facilitates the accumulation of these structural rearrangements because of the prevalence of at least some degree of self-pollination in vigorous individuals of all species. Self-pollination allows the expression of homozygosity of a genetic combination, which may not be particularly well adapted as a heterozygous individual. Self-pollination and structural rearrangements may contribute to the persistence of divergent floral adapted gene combinations and possibly to rapid speciation in *Clarkia*.

Figure 2 displays the overlapping ranges of *Clarkia concinna* and *C. breweri* (Lewis and Lewis, 1953). The possible sympatry of the *Clarkia concinna* and *C. breweri* may have encouraged the two to develop prezygotic reproductive isolation barriers in

order to avoid cross-pollination and the resulting semi-sterility. However, Gottlieb indicated that the southern *C. concinna* consist of the selfing subspecies *C. concinna ssp. concinna* (personal communication). It is not known if the subspecies prevents all cross pollination between wild populations of *C. concinna* and *C. breweri*. The difference in pollinator attraction between these two species appears to allow *C. concinna* and *C. breweri* to remain separate sister species; however, it may be that the presences of the selfing subspecies sufficiently prevents wild hybrid formation. In the past, following the translocation event that arose between *C. breweri* and *C. concinna,* the partial loss of hybrid fertility likely favored the development of prezygotic isolation mechanisms in the form of differential pollinator attraction.

The difference in pollinator preference is influenced by the floral traits of the two species, one of those being scent of the flower. The difference between *C. concinna* and *C. breweri* flower scent is the production of aromatic compounds by *C. breweri.* Only one of the 44 species of *Clarkia*, hawkmoth-pollinated *C. breweri* has a scented flower while its likely progenitor of *C. concinna* is essentially non-scented and is pollinated mostly by bees (MacSwain et al., 1973).

The development of scent in *Clarkia* and other species has been studied by Raguso and Pichersky (1999). Raguso and Pichersky specifically studied the chemical volatiles present in *C. breweri.* Scent has been shown to be a strong contributing factor to moth attraction in flowers (Raguso and Willis, 2002; Raguso and Pichersky, 1995). Scent in *C. breweri* is believed to be the result of 8 to 12 different volatiles one major component making up scent is the S-linalool produced from geranyl pyrophosphate by the enzyme linalool synthase (LIS) (Table 3) (Kaiser, 1993; Knudsen et al., 1993;

Pichersky et al., 1994, 1995). S-linalool is an acyclic monoterpene alcohol and is a product of the one-step reaction. Correlation between linalool synthase levels and scent lead Raguso to investigate the activity level of LIS in flowers of the two species. Both *C. breweri* and *C. concinna* produce S-linalool, but in *C. concinna* S-linalool is present only in the stamens at very low levels, undetectable to the human nose. *C. breweri* produces high levels of S-linalool in all floral tissues excluding the ovaries and sepals (Pichersky et al., 1994). *C. breweri* production of S-linalool is upregulated and increased in the complexity of scents (Table 3, Raguso, 1995).

Table 3. [1] Scent complexity in *Clarkia concinna* X *C. breweri*

Scent compound	C. breweri	C. concinna	F1	F2
Terpenoids				
Linalool	10/10	0/6	37/37	13/20
Pyranoid Lin. Oxide	10/10	0/6	37/37	20/20
Furanoid Lin. Oxide	10/10	0/6	37/37	13/20
Trans-β-ocimene	0/10	6/6	35/37	14/20
Nerolidol	10/10	0/6	0/37	ND
Aromatics				
Benzyl acetate	10/10	0/6	37/37	8/20
Benzyl benzoate	10/10	0/6	0/37	15/20
Eugenol	10/10	0/6	2/37	3/20
Isoeugenol	10/10	0/6	0/37	ND
Methyl eugenol	10/10	0/6	27/37	ND
Methyl isoeugenol	10/10	0/6	0/37	ND
Methyl salicylate	10/10	0/6	37/37	17/20

[1] Produced with permission from R.A. Raguso, 1995

Raguso has shown that LIS activity is correlated with (and probably responsible for) production of scent. The structural gene *Lis* has been isolated and sequenced from both *C. concinna* and *C. breweri* and possibly has a large effect influencing pollinator preference between *C. breweri* and *C. concinna* (Cseke et al., 1998). The presence of genes with large phenotypic effects argues for the possibility of rapid divergence of characters between species (Stuurman et al., 2004). However, Raguso was not able to

find a difference in the coding sequence of *Lis* leaving open the possibility that the genetic defect causing a loss of scent is in another gene that affects the activity of LIS. Genetic studies are necessary to further explore this possibility.

Three major factors govern speciation in *Clarkia*: high amounts of chromosomal rearrangement (Lewis and Lewis, 1955), decreased hybrid fertility (Lewis and Lewis, 1955), and the presence of genes of large phenotypic effect (Gottlieb, 1984). The first two factors are know to occur between the cross *C. concinna* x *C. breweri* (Lewis and Lewis, 1955). The last factor, genes of large effect, may be tested by QTL analysis of floral traits diverging between two species (Bradshaw et al., 1998; Fishman et al, 2002; Hodges et al., 2002; Stuurman et al., 2004).

Genetic Studies on Plant Speciation

Speciation Mechanism

The study of speciation is becoming a very active area of evolutionary biology and substantial progress has been made (Turelli et al., 2001). The generally accepted mechanism of speciation is allopatric in which the evolution of reproductive isolating mechanisms occurs during physical separation of the populations. Sympatric speciation, in which populations diverge to form different species while occupying the same territory, is less widely accepted. However, the common presence of two or more translocations within populations of *Clarkia* species provides a favorable environment for sympatric speciation. Though many factors may affect the pattern of speciation of an organism, it seems likely that similar types of organisms; annuals with restricted ranges

of seed deposition, comparable breeding habit, and ecological niches would have a similar pattern of speciation as *Clarkia*.

Formation of pre/postzygotic isolation mechanisms occur during both allopatric and sympatric speciation. Prezygotic barriers seem to largely explain the lack of wild hybrids found between isolated species. The importance of prezygotic barriers is demonstrated in the study within *Chamerion angustifolium* (fireweed) where 97.6% of the overall isolation was caused by prezygotic genetic barriers (Husband and Sabara, 2003). However, post-zygotic isolation due to deleterious interactions between heterospecific genes has been described in *Eucalyptus* (Myburg, 2004). It has been suggested that chromosomal changes increase hybrid incompatibilities resulting from low levels of migration among small populations (Lewis and Lewis, 1955; Rieseberg et al., 1995, 2001, 2003; Quillet et al., 1995; Fishman and Willis, 2001). The incompatibilities result in speciation through lack of gene flow.

Chromosomal rearrangements can lead to coadapted complexes (Grant, 1971). Coadapted complexes are a group of genetic traits that have high fitness when they occur together, but which without each other have low fitness. The coupling of a gene complex with a translocation would create a protected group of genes within a low recombinant environment and is the type of scenario Lewis envisioned would account for rapid speciation in the *Clarkia* genus (Lewis, 1953). This hypothesis is in contrast to Futuyma and Mayer (1980) who felt chromosomal rearrangements were unlikely to facilitate speciation. Lynch and Force (2000) found that incompatibilities of species were often unassociated with chromosomal rearrangements. There exists divergent floral characters between the species *C. concinna* and *C. breweri*, and their pollinators, bees or

hawkmoths, prefer to pollinate only one or the other of the parental species aiding current isolation and speciation. Further genetic studies will reveal whether floral traits are clustered together forming coadapted complexes and if these complexes are located within a translocation.

A "pollination syndrome" is a group of co-adapted characters that influence pollinator behavior. In 1949 Grant noted that the evolution of floral characters had long been considered a major avenue for achieving reproductive isolation and generating species and floral traits may effect reproductive isolation either through differential pollination due to pollinator behavior or through differential pollen transfer. The effects of floral traits influencing pollinator visitation were found to occur in both temperate (Galen and Kevan, 1983; Galen, 1985; Pellmyr, 1986) and tropical plant species (Dodson et al., 1969). The favorable visitation of an efficient pollinator carrying pollen from plants of the same species result in viable seed set. According to Pichersky et al. (1994), scent-based pollinator discrimination affects population structure and the reproductive isolation of species. Pollinator syndromes in other species such as *Petunia* or *Mimulus* (monkey flower) and *Aquilegia* (columbine) occur between the hawkmoth and the bee or the bee and the hummingbird, respectively. The species chosen for this study represent a probable progenitor/derivative relationship, which exhibit striking differences in their pollination syndrome, *C. concinna* (bee) and *C. breweri* (hawkmoth), which may have influenced the divergence of these species.

The importance of floral evolution in speciation may be widespread as indicated by the temperate and tropical studies as reported by Dodd et al. (1999) in a recent phylogenetic analysis of the flowering plants that found a significant correlation between

pollination mode and diversification. *Petunia axillaris* is pollinated by the hawkmoths and *P. integrifolia* is pollinated mainly by diurnal bees (Stuurman et al., 2004). Some of the characteristic differences in petal morphology between these two species of *Petunia* are in the "petal color, corolla shape, reproductive organ morphology, nectar quantity, nectar quality and fragrance" (Stuurman et al., 2004). These types of floral characteristics may be used to determine if the pollination syndrome of *Clarkia* is also a coadapted complex of genes which may have lead to reproductive isolation through prezygotic means.

Genetic Studies of Floral Traits in Species Crosses

According to Gottlieb's 1984 review, divergence may often be the result of changes in only one or two genes. Stebbins had reported earlier that mutations leading to striking phenotypic divergence were more likely to occur in plants than was previously thought (1950, p.102; 1974, p. 7). This statement was supported by studies in *Nicotiana, Antirrhinum, Aquilegia* and other species in which one or two gene loci affect flower phenotypes, which may influence the pollinator behavior (Gottlieb, 1984). An example is demonstrated in differences between the bee pollinated petunia to that pollinated by the hawkmoth. The changes between these two species were shown to not be as complex as the morphology that resulted in striking floral morphology. The entire genetic control of two floral syndrome traits scent and color were described as "not very extensive and may involve a limited number of enzymatic activities" (Stuurman et al., 2004, pg. 1597).

Studies have been conducted in *Mimulus, Petunia,* and *Aquilegia* using techniques of mapping and QTL analysis to evaluate the genetic basis of floral characteristics (Stuurman et al., 2004; Bradshaw et al. 1995, 1998; Fishman et al., 2002; Hodges et al., 2002). "Interspecific crosses of bird- and bee-pollinated species (Bradshaw et al., 1995; Bradshaw et al., 1998) have suggested a relatively simple genetic basis of major QTL that can influence pollinator foraging" (Schemske and Bradshaw, 1999; Bradshaw and Schemske, 2003) (Stuurman et al., 2004, p.1585).

Floral QTL studies in species such as *Mimulus* have revealed several QTL affecting single traits, such as the lateral petal width, anthocyanin of petal, corolla width, and stamen length, indicating a striking polygenic basis for floral divergence in the *Mimulus* system (Fishman et al., 2002). The polygenic nature of floral differences between *Mimulus* species would indicate that these species may take longer to diverge than species where floral characters are controlled by single or fewer genes. Accumulated changes are needed in several genes to change a floral character instead of a change to a single gene causing large phenotypic changes. The amount and magnitude of quantitative trait loci effects may reflect variation in the genetic control of diverging phenotypes. Work by Orr (1998a, b) has generated predictions about the distribution and directionality of QTL effects underlying adaptation, but there is still little information on "magnitude, pleiotropic effects, and interactions of genetic factors recruited by natural selection" (Fishman et al., 2002).

Wild systems studies have supported a large number of minor QTL effecting important divergent morphological traits between sister taxa (True et al., 1997; MacDonald and Goldstein, 1999; Zeng et al. 2000; Fishman et al., 2002; Westerbergh

and Doebley, 2002; for review see Orr, 2001). The study conducted between the outcrossing *Mimulus guttatus* and the selfing *M. nasutus* indicated that the QTL were of minor effect (Fishman et al., 2001, 2002).

However, several other studies of wild systems identified QTL of large additive effect for diverging traits (Bradshaw et al., 1995, 1998 (*Mimulus*); Schemske and Bradshaw, 1999; Sucena and Stern, 2000; Voss and Schafer, 1997; support for floral character effects Lexer et al., 2005). The disparity found here between major and minor QTL of traits within the same genus (*Mimulus*) may be real or the result of the different species under study or as suggested by Lexer et al. (2005) the difference may be a result of the different ways in which QTL are reported.

In *Mimulus,* suites of characters are seen between the floral genes of the flowers pollinated by birds and those pollinated by bees. Some of the floral characters studied were anthocyanin concentration in the petals, lateral petal width, and corolla height. Between 1 and 6 major QTL affect each of the 12 floral traits studied in *Mimulus* (Bradshaw et al., 1998). Bradshaw et al. (1998) found that most (9/12) traits appear to be controlled in part by at least one major QTL explaining at least 25% of the total phenotypic variance. Some of the floral characters studied in *Petunia were* petal color, corolla tube length, and fragrance. Data gathered by Stuurman et al. (2004) has shown that QTL studies of the major floral characteristics of *Petunia* concerning flower color, fragrance, nectar volume and morphology all were loci of large additive effect.

Anthocyanin

Anthocyanin production in pea (*Pisum sativum* L.) can be influenced by the genes controlling chalcone synthase (CHS), flavanone 3-hydroxylase, and dihydroflavonol 4-reductase, or any of the other 15-20 loci controlling anthocyanin production in pea (Uimari, 1998). Similarly, in *Antirrhinum* (snapdragon) intensity and color pattern of the flower are controlled by several genes regulated by two genes *Delili* (Del) and *Eluta* (El) (Martin et al., 1991). Differential expression of CHS is determined by stress (Kreuzaler et al., 1983), light (Koes et al., 1989), phytopathogens (Ryder et al., 1984), or wounding (Bell et al., 1986; Ryder et al., 1987) in petunia and beans. In contrast to the findings by Ryder et al. (1984) *Petunia* flower hue was governed by a single gene AN2 which causes a color shift from violet to white flowers (Quattrocchio et al., 1999). Literature on anthocyanin is too extensive to describe here, but note that in the given examples, anthocyanin phenotypes are influenced by multiple copies of genes, environmental factors and regions of chromosomes. However, in each example cases of single genes of large effect have been demonstrated (Uimari, 1998; Martin et al., 1991; Stuurman, 2004). These examples seem to agree with the results from *Mimulus* that anthocyanin may be controlled by several genes, but single genes of large effect play an important role in morphological divergence in most species (Bradshaw et al., 1998).

Hypanthium Length

Hypanthium length has been discussed as a cause of reproductive isolation due to hybrid floral divergence (Levin, 1975). Hypanthium and corolla length have been studied in several species (Breedlove, 1969; Levin, 1971; Stuurman, 2004; Raguso, 2005,

unpublished data). Hypanthium length differs in two species of *Fuchsia* found in sympatry. The species *F. parviflora* has a shorter, broader hypanthium and *F. encliandra* is pollinated mainly by hummingbirds in the sympatric zone due to the longer narrower hypanthium which precludes bees from feeding effectively (Breedlove, 1969). Data reported by Raguso (unpublished) found that the hypanthium length appeared to be of intermediate length in the F_1 and gave a normal distribution in the F_2 cross of *Clarkia concinna x C. breweri.* This study found that the hypanthium trait was not a complete barrier to pollination by either animal. The longer hypanthium seen in *C. breweri* favors the long proboscis of the hawkmoth over the shorter mouthpart of a bee. The hypanthium length was measured in another study on *Clarkia breweri x C. concinna* population, and was shown to display polygenic behavior in an F_2 and backcross populations (Raguso, unpublished data). The corolla height in the *Mimulus* study conducted by Bradshaw et al. (1998) indicated that the trait was likely controlled by several genes, but only one of large additive effect. The study conducted by Fishman et al., indicates that corolla tube length in *Mimulus* is a highly polygenic trait with no indication of a major QTL. These studies indicate that the hypanthium length may be influenced by several genes in *Clarkia.*

Petal Shape

The genetic analysis for flower shape polymorphism has also been investigated in several wild species. The size of the petal in carnation is controlled by the gene invertase (beta-fructosidase). In carnation (*Dianthus caryophyllus*) the action of invertase affects sugar uptake of the cell, influencing cell size and ultimately petal size. Petal width was investigated in *Mimulus,* and it was found that the amount the petal was reflexed may

have affected the petal width and influenced the pollinator. A QTL explaining greater than 25% of the phenotypic variance was found for this floral trait between, *Mimulus lewisii* and *M. cardinalis* (Bradshaw et al., 1998). The number of cells making up the corolla tube in *Petunia* was governed by three major QTL of 52%, 21%, and 18% of the total difference between the parental species (Stuurman et al., 2004).

Leaf Shape

In the study conducted by Fishman et al. (2002) characters such as leaf shape, days to flower, and tube length were recorded. It was found that the floral characteristics recorded in the F_1 hybrids and F_2 hybrids had trait means greater than the midparent values suggesting partial dominance of the one parent (*M. guttatus*) (Fishman et al., 2002). Fishman et al. report that neither parental extreme was reconstituted possibly due to the segregation of many genes of small effect.

Byrne et al.'s (2001) review indicated that leaf shape was controlled by several genes such as *ROTUNDIFOLIA and ANGUSTIFOLIA,* which cause extreme changes in the width and formation of the leaf. The leaf shape was measured by several ways but blade width may be affected by those genes of large effect mentioned above. The study on *Mimulus* indicated that leaf length was controlled by several genes (Fishman et al., 2002), and it may be found in *Clarkia* that several genes also control the leaf width.

Scent

In *Petunia*, fragrance analysis has shown an increased qualitative complexity and intensity in *P. axillaris parodii* (hawkmoth-pollinated) as compared to *P. integrifolia*

inflata (bee-pollinated). These scent compounds are produced in the bee pollinated *Petunia* (normally lacking in certain scent compounds) when two of the bee pollinated species chromosomes were replaced by an intermediate species copy of the same chromosomes. The low number of chromosomes involved suggests that, as with S-linalool in *Clarkia*, the differences between the species are not as complex as was believed (Stuurman et al. 2004).

The scent in *C. breweri* appears to be correlated with the presence of S-linalool in the flower structure (Raguso, 1995, Table 3; Pichersky et al., 1994). Work done to elucidate the *Lis* (linalool synthase) gene has been done, as several groups have created *Petunia* and carnation (*Dianthus caryophyllus*) plants that express the *Lis* gene from *C. breweri* (Lavy et al., 2002; Lucker et al., 2001). Although the transgenic plants did create small amounts of linalool (not normally made by carnations and petunias), the level of expression was below the threshold of detection for the human nose and thus these plants as with the *C. concinna*, still seem scentless (Pichersky, 2004). The transgenomic studies did show that S-linalool is easily produced even in plants which do not normally produce the compound.

Scent appears to be a relatively easily acquired trait. No specialized scent glands or similar structures have been found in *C. breweri* (Raguso and Pichersky, 1995). The changes seen in *Petunia* and indicated by the simple difference in scent compounds separating *C. concinna* from *C. breweri* indicate that divergence of scent may have occurred very swiftly through simple genetic changes (Stuurman et al. 2004).

Stuurman et al. (2004) found scent, in *Petunia* to map to a similar location in two independent data sets, which provided confidence that fragrance mapping is relatively

resistant to subjective odor perception. The data revealed a single QTL allele, which functioned to enhance fragrance in both species, *Petunia i. inflate* and *P. a. parodii* (Stuurman et al., 2004). These data lead to the conclusion that scent is controlled by one major gene of large effect in *Petunia*. These results were found in moth pollinated *Petunia* and are similar to the enhancing effect on scent that occurs in *Clarkia breweri* (Table 3).

Flower Orientation

In *Aquilegia* (columbines) the orientation and length of the nectar spurs were found to influence visitations by nocturnal hawkmoths (Hodges et al., 2002). Strong genetic control was found for each of the nectar spur traits analyzed (Hodges et al., 2002). Color and odor of the flowers also influenced visitations, but QTL of these traits were not analyzed (Hodges et al., 2002). Data that was found in relation to floral traits specifically the nectar spurs of *Aquilegia* indicate that changes in floral traits promote floral isolation between species (Fulton and Hodges, 1999). The angle of flower orientation in *Aquilegia* was found to diverge between the species *A. formosa* and *A. pubescens.* It was discovered in *Aquilegia* that the QTL for different floral traits were often closely associated, indicating that linkage or pleiotropy cause at least some of the integration seen in the species investigated (Hodges et al., 2002). The angle of flower orientation may be similar to the effect of apical dominance in *Clarkia.*

In summary the results of the previous studies in floral trait analysis were mixed. The floral features under scrutiny in the studies of other genera outside *Clarkia* appeared to be controlled by many genes (True et al., 1997; MacDonald and Goldstein, 1999; Zeng

et al. 2000; Fishman et al., 2002; Westerbergh and Doebley, 2002; for review see Orr, 2001) and by single genes in other studies (Bradshaw et al., 1995, 1998; Schemske and Bradshaw, 1999; Sucena and Stern, 2000; Voss and Schafer, 1997; support for floral character effects Lexer et al., 2005). The fact that these floral traits influence pollinator behavior in these species remains. Thus, the floral traits influence the passage of genetic material leading to some cases of isolation (Grant, 1948; Johnson, 1996; Bradshaw and Schemske, 2003).

The possible result of monogenic control of traits is a swift change in pollinator preference through mutations of large effect as evinced in the *Petunia* and one of the *Mimulus* studies (Bradshaw et al., 1998; Stuurman et al., 2004). This change may have occurred long ago giving the lack of gene flow between the two species time to accumulate morphological differences in other traits other than those that affect pollinator preference. Or, as Stuurman et al. (2004) suggest, these traits may have occurred in a swift separation of species and accumulation of drift over a much smaller time frame could have caused the divergent morphology that is apparent.

Clarkia Genetic Markers/Maps

Genetic analyses in *Clarkia* have demonstrated independent assortment of specific genes (Gottlieb, 1977; Weeden and Gottlieb, 1979; Systma et al., 1990; Gottlieb and Ford, 1996) or have identified very short linkage groups. Only one previous study attempted to develop a linkage map in *Clarkia.* Leong et al. (2001) used random amplified polymorphic DNA (RAPD) markers to examine the genetic variation between

the wild-type petal and the *crinkled petal* populations. Two very short linkage groups were identified one of which contained a RAPD which cosegregated with the crinkled petal phenotype (Leong et al., 2001). In Leong et al.'s (2001) map the lack of genetic sequence data prevented the construction of a genetic map with significantly large linkage groups. By utilizing the few available sequence data provided in *Clarkia* and designing primers from consensus sequence a much more complete genetic linkage map may be assembled.

Gottlieb and Ford (1996) reported the sequence of *Pgi*C1 and *Pgi*C2, which encode the cytosolic isozymes of phosphoglucose isomerase (PGIC; EC 5.3.1.9). The aim of this study was to elucidate evolutionary relationships within the genus *Clarkia* by comparing genetic sequence between several *Clarkia* species including *C. concinna.* It was found that the genes encoding PGIC1 and 2 assort independently (Gottlieb, 1977; Gottlieb and Weeden, 1979). *PgiC1* and *PgiC2* should be located on separate linkage groups within the *Clarkia concinna* x *C. breweri* map.

The gene for Isopentenyl pyrophosphate isomerase (*Ipi*) is another gene on which sequence was available for *Clarkia breweri* (Blanc and Pichersky, 1995). This gene is responsible for the production of the volatile compound isopentenyl pyrophosphate isomerase which is emitted from *Clarkia breweri* flowers. This compound may be associated with the aroma of the flowers and result in a second QTL for scent if the product of *Ipi* affects scent detected by the human nose.

Triose phosphate isomerase (TPI) was first studied with the use of isozymes (TPI, EC 5.3.1.1.) (Gottlieb and Pichersky, 1983). There are several duplications of *Tpi* within the genus *Clarkia.* The species *C. breweri* contains several more duplications to yield

five plastid TPIs that are not seen in any of the other section of the genus *Clarkia*. *Tpi* primers may amplify several bands of duplicated *Tpi* sequences.

Linalool synthase was first isolated and sequences by (Cseke, et al. 1998). The sequences for both *Clarkia concinna* (AF067602) and *C. breweri* (AF067601) are known.

Translocations

Translocations may have similar effects as inversions, varying degrees of sterility associated with suppression of recombination in affected chromosomal regions. However, translocations have more complex effects on fertility and linkage relationships over parts of their component chromosomes (Livingstone et al., 2000). These complications lead to skewing and repression of recombination. The genomes of *C. concinna* and *C. breweri* are known to differ by a reciprocal translocation (Lewis and Lewis, 1955). The translocation between the *C. concinna* and *C. breweri* does not cause a major loss of fertility in the hybrid, but it will affect the segregation of at least some chromosomes and hence modify the linkage map generated for the population. A reciprocal translocation would cause pseudolinkage between markers near the interchange breakpoints on the chromosomes involved (Burnham 1991; Livingstone et al., 2000). Ambiguous marker order indicates the location of quadrivalents that are formed and cause pseudolinkage.

The translocation may be mapped by assessing the correlation of markers to quadrivalent formation (Rieseberg and Carney, 1998). All plants with reduced pollen viability would score as heterozygous for the translocation and all those with restored

pollen viability would be homozygous for the translocation. To test this hypothesis, Tadmore et al. (1987) used a segregating F_2 population to generate a map and four isozyme markers were found to correlate with the translocation.

Review of Genetic Marker Techniques

There are two types of marker used in this study, sequenced tagged site (STSs) (Olsen et al., 1989) and RAPDs (Williams et al., 1990). The first type of marker uses two sequence specific primers. The RAPDs do not require gene-specific primers designed from sequence and may be used to acquire markers across linkage groups.

Polymorphism may occur between STS marker amplified DNA due to differences in the sizes of the amplified regions between the two priming sequences. If no size polymorphism is observed from the initial polymerase chain reaction (PCR) amplification, restriction enzymes may be used to cleave the amplified fragments of STS markers. Providing that DNA sequence differences between the two alleles modify the restriction sites available, different restriction patterns known as cleaved amplified polymorphic sequences (CAPS) will be generated and can be used as segregating markers (Koniecyzn and Ausubel, 1993; Jarvis et al., 1994). Generation of the primers used in the PCR of STS markers commonly require cloning and/or sequencing for the construction of the specific primer sequence (Staub et al., 1996). The STS and CAPS markers are usually co-dominant and therefore able to distinguish heterozygotes from both homozygous genotypes.

The RAPD primers unlike the previous type are arbitrary sequences of nucleotides. RAPD analysis usually results in several amplified fragments after being separated by electrophoresis on agarose and visualized by staining with ethidium bromide. Due to the random nature of the bands, the sequence relation to each band is not known. RAPDs are usually a dominant type of marker where polymorphisms are determined by the presence or absence of a particular RAPD band within the agarose gel. The RAPD markers have been used to increase map coverage to verify linkage groups, but these markers are not particularly useful for comparative mapping projects. The occurrence of artificial bands and the lack of reproducibility create difficulties in using the RAPDs as comparisons between genetic maps.

QTL Mapping Techniques

Tanksley (1993) described QTL as a powerful and increasingly accessible tool for describing the genetic control of adaptive radiation of species. QTL analysis provides a rough estimate of the genetic basis of a polygenic trait and aids in the identification of candidate genes for the morphological characters (Paterson et al., 1991; Mackay, 2001). When running a QTL test the likelihood ration (LR) test statistic for each interval is -2 ln (L_0/L_1), where L_0/L_1 is the ratio of the likelihood under the null hypothesis of no QTL to the likelihood under the hypothesis that there is a QTL in the interval (Fishman et al., 2002). The LR statistic at a genomic position is distributed as χ^2 with 3 df under the null hypothesis (Jiang and Zeng, 1995). The lower the LR statistic the more likely a QTL is present at a given locus.

The F_2 generation of 95 individuals was a single population and was chosen to conduct the first set of QTL studies. Traits controlled by only one or two genes should be easily resolved in an F_2 population with 95 individuals; however, if major genes are not responsible for most of the observed variation in phenotype, the F_2 may not be sufficiently large to resolve the genetic basis (Fishman et al., 2002). In addition QTL effects in small populations, like the *Clarkia* mapping population, can be subject to overestimation by selection bias (Beavis, 1994; Broman, 2001). The best solution to this dilemma is to use inbred populations to provide a permanent mapping source. The mapping population of *C. concinna* x *C. breweri* is being inbred further into the F_5 generation as of 2006. The QTL data collected in this study represents a preliminary evaluation of the genetic basis of the floral characters investigated. The further inbred lines resulting from selfing the F_4 generation may be used to maintain this map for further studies.

Statistical Tools for Mapping

The tools employed for mapping the population were the Quikmap EXCEL macro, Joinmap and QTL Cartographer programs (Vaillancourt, Weeden, and Barnard**,** 1993; Stam, 1993; Basten et al. 1998; Wang, Basten, and Zeng, 2001-2005).

STS markers are often used as 'anchor markers' because they amplify orthologous sequences in different populations or taxa. One of the most powerful aspects of genetic mapping with DNA markers, particularly STS markers, is the fact that markers mapped in one genus or species can often be used to construct parallel maps in related but genetically incompatible taxa (Young, 2000). Due to this fact STS markers have been

used in comparative mapping by Bradshaw Jr. et al. (1994), Ganal (1998), Cervera (2001), and others. The extended use of STS markers in comparative mapping studies displays there usefulness in this capacity. The STS marker may be used across several species as in the studies by Ganal et al. (1998) and Cervera et al. (2001) or to elucidate differences between closely related species (Bradshaw et al., 1994).

Distorted Segregation

Segregation distortion may result from several sources within a genome. Distortion in segregation ratios may be caused by chromosomal rearrangements (Bonhomme et al., 1998), directional chromosome loss, segregation of a pollen lethal allele, conflicts between genetic factors that isolate the parental species, inbreeding depression as a result of genetic load (Bradshaw Jr. and Stettler, 1994), and genetic conversion events (Ky et al., 2000; for review see, Rieseberg and Carney, 1998). Those distortions due to chromosomal rearrangements are seen in several hybrids of *Clarkia* (Lewis, 1953; Lewis and Lewis, 1955). The distortions discussed here involve translocations and other pre and post-zygotic segregation distortions, though pre-zygotic barriers to gene flow are likely to be found in *Clarkia concinna* and *C. breweri* species, there is a known postzygotic barrier, a translocation.

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MAPPING THE GENES

Abstract

Chapter 3 is focused on the research conducted to explore the heritability of traits and the possibility of complexes of genes within the progeny of the cross *Clarkia concinna* x *C. breweri*. Standard segregation, backcross and QTL analyses were performed to elucidate the genetic control of morphological and physiological traits in *Clarkia.* In addition, the type of marker developed and used in this study will be used to amplify product in other *Clarkia* species permitting further testing of genetic and evolutionary hypothesis in the genus. The map presented represents initial results from the use of these markers on the F_2 population. Several of the floral traits measured appeared to be primarily controlled by single loci. The genetic basis for other characteristics, such as leaf width, were found to show weak linkages, polygenic pattern of inheritance and the single trait analysis provided weak QTL peaks. These results indicate that the saltational speciation event believed to have given rise to *C. breweri* proceeded at least partly via simple genetic changes with major phenotypic effects. A number of these major genes have remained or become clustered, forming two co-adapted complexes within *C. breweri*.

Introduction

The divergence of *C. breweri* from *C. concinna* is interesting due to a difference in pollinators and their ability to produce fertile hybrids. These two species have been described as having a progenitor/derivative relationship with *C. breweri* having arisen from its progenitor since the last Ice Age and perhaps much more recently (Lewis and Lewis, 1955). These parents were also chosen due to the fact that these parents produce viable hybrid seed set to provide a large number of plants in the mapping population. My research focused on the examination of the genetic control of phenotypic change accompanying speciation between *C. concinna and C. breweri*. Two important characters modified during divergence of the two species are the hypanthium length,

flower shape and aroma of the flowers because these features affect pollination. By focusing on some of the pollinator-influenced traits some understanding of how these traits interact may become apparent. Similar work has been done in *Mimulus, Petunia,* and *Aquilegia* (Schemske and Bradshaw, 1999; Stuurman et al., 2004; Fulton and Hodges, 1999). These studies have produced mixed results with reference to the number of genes controlling the floral characteristics.

The goal of the mapping project is to provide a set of mapped loci, to provide data on the translocation location and to identify the location and markers tightly linked to genes influencing important phenotypic traits.

Mapping the genes began with the construction of primers. Few genes have been sequenced in either *C. concinna* or *C. breweri*; however, I was able to find sequences for seven genes (X89391, Gottlieb and Ford, 1996; AF121851 AF121852 AF121853 AF121854, Nam et al., 1999; AF067601, AF067602, Cseke et al., 1998). Sequence data is also available in the form of expressed sequence tags (ESTs) compiled from cDNA from a study on *C. breweri* scent production (Dudareva and Pichersky, 2000). Primers for other genes were needed to construct the genetic map. To develop these, primers were designed from sequence alignments from different available species databases, and six of these primers pairs included ambiguous (variable nucleotide sites) and are referred to as degenerate primers. Other primers were previously available or designed from the available *Clarkia* DNA sequences and ESTs.

Materials and Methods

Plant Materials

The original *C. concinna* ssp. *concinna* parent was collected near Conn Dam, Napa Co, CA. by L.D. Gottlieb (pop. 8740). The *C. breweri* parent was collected on Rd. J17, west of I-5, Stanislaus Co, CA. (pop. 8802). The cross *C. concinna x C. breweri* was made at the University of California, Davis by L.D. Gottlieb and the F_1 plants were grown in spring of 1998 ten more have been grown since that time for DNA and morphological analysis. Approximately 200 seeds were obtained from the hybrid plants. The F_2 population (95 plants) was grown in the summer and fall of 2003 in the greenhouse at Montana State University under natural light, supplemented with artificial lights to extend day length as necessary. Each of the F_2 plants was self-pollinated and seed collected. The F_3 generation (several seeds from each F_2 plant) was germinated in spring 2004 and scored again for the segregating traits. The F_3 were intercrossed within families in order to avoid inbreeding depression in later generations. At least two plants from each F_4 family were grown in spring of 2005, examined for uniformity of phenotype and intercrossed within each family.

Backcross populations were also grown and used to verify genetic analysis. New parental crosses were made and F_1 plants were grown in the spring of 2005. These F_1 individuals were then backcrossed to *C. concinna* or *C. breweri.* The seed from these backcrosses were grown in fall 2005. There were two backcross populations the first consisted of and F_1 individual backcrossed to the *C. concinna* parent and the second population consisted of the F_1 individual backcrossed to the *C. breweri* parent. There

was a set of backcross individuals in which the pollen parent was the F_1, a set in which the pollen parent was *C. concinna,* and a set in which the pollen parent was *C. breweri.* However, those backcrosses in which the F_1 was the pollen parent and *C. breweri* the pollen acceptor failed to germinate. At least five plants for each type of F_1 backcross were scored for the segregating traits. The different populations of backcrosses consisted of lines. The line contains all the seed germinated as the result of a single cross. The data reported from the STS markers for the backcross populations are the result of fragment size polymorphisms scored on agarose gels, and the traits were scored from the germinated backcross plant individuals.

Seeds were germinated in a 50:50 by volume mix of perlite to vermiculite at a temperature between 10 to 15^{o}C in paper cups. Four seedlings resulting from seeds of the previous generation were then transferred to 13 cm pots filled with pasteurized soil and grown in the greenhouse. The soil mix was M.S.U. Mix (one third each peat, soil, and sand). Though some seeds did not germinate within the 19 days, no explicit measures of germination rates or subsequent mortality were taken. The F_2 plants were grown in the Plant Growth Center of Montana State University on a 16 hour light, 8 hour dark cycle maintained with supplemental lighting. Greenhouse conditions were similar to those used during F_1 formation and in previous experiments with similar populations (Raguso and Pichersky, 1995).

Molecular Biology Tools

There were only three previously tested DNA markers available for this project: *PgiC1* and *C2, Ipi* and *Tpi*. New "consensus" primers were designed first by L. D.

Gottlieb and V. D. Ford and later by N.F. Weeden and A.A. Henry. These "consensus" primers were designed using sequences from *Arabidopsis, Medicago, Prunus, Lycopersicon, Pisum, Solanum* or other species for which cDNA or EST sequence was available. The list of all primer pairs tested is located in Appendix D. Additional primer pairs used in previous projects on *Pisum sativum* were designed by N.F. Weeden and M. Moffet and aided in the constructing the *Clarkia* genetic map. Primers were from 20-27 base pairs in length and each contained no more than 4 degenerate sites.

Due to the availability of *Arabidopsis thaliana* DNA sequence the primers were often designed using the intron locations of *Arabidopsis thaliana* and a consensus of the EST and mRNA sequence available from other species. In areas of the primers where variation occurred between nucleotides, those nucleotides which occurred in the sequence most taxonomically related to *Clarkia* were chosen. Those areas with a high amount of variability in the nucleotide among all the sequences were the sites of degenerate nucleotides.

DNA was isolated from young leaves using the protocol from Torres et al. (1992). The optimization of RAPD reactions run on the extracted DNA included the use of a touchdown program from 56^{o}C down to 46^{o}C to ensure little low temperature binding and minimized the formation of irreproducible bands. For these reasons only the most prominent and dependable bands in a RAPD reactions were used for mapping.

The CAPS and STS markers form the foundation and framework around which the map is based but these types of markers are more difficult to generate than the RAPD markers. The RAPD markers were then used to fill in the map and verify areas of the map of low resolution.

The Invitrogen TOPO TA cloning kit with Top 10 chemically competent cells was used to clone and amplify the fragments. Sequencing was performed courtesy of D. Pouchnik at the Department of Molecular Bioscience sequencing lab, Washington State University. The Genebank, NCBI site program BLAST was then used to confirm identity with *Arabidopsis* and some other listed gene sequences (Altschul et al., 1990, 2005 updated). The other amplified fragments were those paired in fragment size to *Pisum sativum* to make a rough determination if the fragments were the genes of interest.

Scoring of Phenotypic Characters

Gathering qualitative data was done by AAH and NFW at different times on each plant. The scent was scored base on the individuals ability to differentiate non-scented from scented. This scoring for presence or absence of scent was not difficult; however there were plants that had a faint odor or a slightly different odor and determining which, if any of these scent traits, was heterozygous was not possible. The data taken from several flower on the same plant in different stages of flowering were averaged to attempt to record the presence of scent accurately. Classifying petal shape was initially done by visual observation, but was then quantified by measuring the petal lobes. Petal lobe lengths were measured from the base of the petal to the highest point on the lateral lobe. Petal lobe widths were measured from the middle vein of the petal to the outer most part of the lateral lobe where the lateral and middle lobes join together. A test of the cell size hypothesis was obtained by counting cells across the microscope field of view at 40x magnification.

Hypanthium length was measured from the base of the petal to the apex of the ovary. These measurements were conducted the same for the F_2, F_1, and the two parents. The leaf shape was determined to be correlated most with the width of the leaf. All leaf widths were measured across the lateral edges at the midpoint of the length of the leaf. The midpoint was determined by measuring the total length of the leaf and then measuring across the width from the midpoint obtained from measuring the length. Stem anthocyanin was scored on the basis of the length of stem that was red in hue. Days to flower was the number of days from germination until the first opened flowers appeared on the individual. The flower was considered open once the four petals were separated totally, to the apex of the ovary. The color or hue of the flowers was scored in comparison to the shade of the parent plants and F_1 grown at the same time. Pictures were taken of all individuals so a record of flower hue is available for comparison. The apical dominance was strikingly divergent between the two parents and was recorded as the height of the main stem.

The pollen fertility test was done with Melzer's KI (potassium iodide) dye (Lancashire, 1980; Pedersen et al., 2004). The freshly collected pollen grains were placed on slides and stained with KI for one minute. The slides were then put under a light microscope and the dark staining (fertile) numbers of pollen were counted and compared with those of the light staining (infertile) pollen grains.

The Linkage Map

The linkage map was constructed by performing joint segregation analysis on the genotypes of the F_2 population at 40 STS and RAPD markers. The statistical thresholds

for the construction of the linkage map were an LOD $\geq$ 4.0 for linkage analysis and the logarithmic odds (LOD) $\geq$ 2.0 for order, as calculated using the Joinmap (version 3.0) program (Stam, 1993). Mapping was completed by using the Quikmap and QTL Cartographer programs already available in the lab (Vaillancourt, et al., 1993; Wang, et al., 2001-2005). Quikmap calculations of recombination frequencies were used to identify possible linkages where skewing was too large for the Joinmap program to identify cosegregation of markers. These markers were denoted by a gray color instead of the red and black used to designate significant linkages supported by Joinmap. Those morphological markers that did not posses any QTL above the threshold of 2.5 LOD were compared using Quikmap to analyze segregation ratios. The scoring of the heterozygous individuals for the phenotypic traits was ambiguous in several cases. In those cases the χ^2 value was calculated for the segregation data with heterozygous individuals scored among the parents and also separately. Traits found to have significant matches among segregation data were noted on the map in a gray color instead of the black used to designate the significant QTL found.

Segregation Analysis

The segregation of all traits and markers was investigated by calculating a χ^2 analysis and determining if the segregation of the character or marker differed from 1:2:1 or 3:1 ratio. Morphological traits which were scored at first, by eye to determine a rough estimate of segregation within the F_2 population were then analyzed using Student's t-test to determine which measurement was most correlated with the scored characteristic. This was done to determine the most useful measurement for divergence of the petal

shape between *C. concinna* and *C. breweri.* For instance, petal shape score was found to be most correlated with the measurement of the petals lateral lobe width. These measurements of the lateral lobe of the petals were then used for the QTL analysis of the petal shape trait. For some characteristics, such as the aroma of the flower, direct measurement could not be taken at that time and so the qualitative score was used to estimate the QTL. The single marker QTL analysis in combination with the analysis of the segregation data using Quikmap were used to determine the most likely location of major QTL for each of the traits scored.

Quikmap was used in this study to compare segregating marker data to previously scored segregating markers. The information from the comparison was used to determine those markers that may share linkage groups, to determine which marker segregation patterns correlated with the segregation of morphological traits, and to estimate distances between the markers. The Quikmap program is used to compare the number of matching individuals to those that represent crossing over between the two markers. The lower the number of crossover and the higher the number of matches the more closely the two markers are linked. From these data the □2 and a rough linkage distance may be calculated. The Joinmap program was then used to calculate actual distances between markers within the linkage groups. Quikmap was again used to add on those markers to linkage groups were skewing appeared to limit Joinmap's ability to detect cosegregation of markers. Finally, the QTL Cartographer program was used to identify possible QTL and to calculate the LOD scores of the resultant QTL.

QTL Analysis

QTL were mapped for five floral traits and three vegetative traits with interval mapping using QTL Cartographer software (version 2.5) (Wang et al., 2001-2005). For each trait, the interval mapping procedure tests the hypothesis that an interval between adjacent markers contains a QTL affecting the trait. Tests were performed at 2-cM intervals with a flanking window size of 10 cM used to exclude potential cofactors tightly linked to the test interval. The likelihood ratio (LR) test statistic for each interval is -2 in (L_0/L_1). A rough LR threshold corresponding to a Type I error rate of □= 0.05 was used (□$^2_{0.05/M}$, where *M* is the number of mapped intervals; Zeng, 1994). Those interval mapping results for traits which did not have a normal distribution could not be considered significant. These traits possible loci were located using the single marker analysis function of QTL Cartographer.

Results

Development of STS Primers

The PCR data show that primers designed as a consensus of *Arabidopsis thaliana, Pisum sativum, Medicago truncatula, Lycopersicon esculentum*, and other sequences often amplify *Clarkia* DNA (Appendix D). Table 4 lists the primers that have been designed during the course of the mapping project.

Out of 74 primer pairs tested on *Clarkia,* 36 gave an amplified product that was in the expected size range (Appendix D). Of the primer pairs tested, 42 were designed from consensus sequence and 26 of these successfully amplified a product in *Clarkia;* 10 were

designed to *Clarkia* sequences and 9 of these gave amplification products, and 21 were designed to legume (usually *Medicago truncatula*) sequences and 10 of these amplified products (Appendix4). In total, portions of 38 different genes (Table 4) were successfully amplified using this approach.

Table 4. Genes for which STS markers have been developed in *Clarkia*

Product	**LG**	**Product**	**LG**
ADP/ATP translocator		Linalool synthase	II
ADP glucose pyrophosphorylase (small)		Malate dehydrogenase	VII
ADP glucose pyrophosphorylase (large)	XI	Mitochondrial elongation factor	
Aldo-keto redcuctase (c subunit)	IV	Nucleoside diphosphate kinase	IV
Ascorbate peroxidase		Pentose-5-phosphate 3-epimerase	
Atpase (delta prime subunit)		Phenylalanine ammonia-lyase	
Benzoyl CoA benzoyl transferase	I	Pyrol	V
Benzoyl alcohol acetyl transferase	X	Phosphoenolpyruvate carboxylase	
Cinnamoyl CoA reductase	III	Phosphofructokinase (beta subunit)	
1-D-deoxyxylulose 5-phophate synthase	V	Phosphoglucose isomerase cytosolic	1 IV
Enolase		Phosphoglucose isomerase cytosolic	2 I
Epoxide hydrolase	VIII	Phosphoglycerate kinase	
Fructokinase		Phosphoglyceromutase	
Fructose 1,6 bisphosphatase		Protochlorophyllide reductase	
□-Glucosidase		RBC activase	
Glutamine synthetase (cytosolic form)	IX	Salicilic acid methyltransferase	III
Glutamine synthetase (plastid form)		Shikimate-3-phosphate synthase	
Glyceraldehyde 3-phosphate dehydrogenase	II	Sodium citrate transporter	I
Glycine decarboxylase complex (T protein)		Starch branching enzyme	
Granule-bound starch synthase		Sucrose 6-phosphate synthetase	
Invertase	I	Thioredoxin reductase	
Isopentenyl pyrophosphate isomerase	V	Transketolase (plastid specific)	
		Triose phosphate isomerase	I

In most cases only a single fragment with the expected size (usually 1000-2000 bp) was amplified in *Clarkia.* Five displayed size polymorphisms between *C. concinna* and *C. breweri* alleles and could be directly mapped using the F_2 DNA. However, most

of the amplified fragments were of the same size in both parents and a set of restriction enzymes had to be screened in order to reveal polymorphism (Table 5). For a minority of the STS markers, CAPS analysis failed to reveal a polymorphism and the gene could not be amplified (Appendix D).

The fragments produced by several primer pairs were cloned using the TOPO TA cloning kit and sequenced. The *Gapc* sequence of Arabidopsis has a low expected value (1e-29) when compared to the cloned *Gapc* sequence from *C. concinna* (Altschul et al., 1990, 2005 updated). The e-value (expected value) describes the likelihood that a sequence with a similar score will occur in the database by chance. The smaller the e-value, the more significant the sequence alignment is because they are less likely to match by chance. The level of similarity (93%) gave confidence in the effectiveness of consensus primer design. Twenty STS markers were used in the *C. concinna x C. breweri* mapping project to provide 'anchor marker' locations and to allow future studies of comparative mapping with the list of STS markers provided in this study.

Map Construction

A total of 40 markers were used to construct the genetic map for the *Clarkia concinna* x *C. breweri* population. Markers consisted of 22 STS/CAPS markers with the other 18 markers consisting of RAPDs. The 22 STS markers gave single fragments in each parent, with no evidence of heterozygosity in the initial fragment or in the STS analysis.

Table 5. Sequences of primers developed for *Clarkia*

Gene		Primer sequence																	
Invertase	*Invr*	F	A	T	C	A	C	T	T	T	C	A	A	C	C	T	C	T	C	A
		R	T	T	C	C	C	C	A	T	A	C	A	g	C	A	C	C	T	T
Malate dehydrogenase	*c_MDH*	F4	G	A	T	G	T	C	A	M	C	C	A	T	g	C	A	A	C	T
	MDH	R3	C	T	C	T	T	C	g	G	C	T	g	T	C	A	A	g	T	C
glyceraldehyde 3-phosphate dehydrogenase	*GAPC*	F3	G	K	T	C	A	A	g	g	A	C	T	C	g	A	A	g	A	C
		R3	C	A	A	C	A	T	C	R	T	C	g	T	T	C	W	g	A	G
phosphglucose isomerase 1	*PGIC1*	F	G	C	A	G	T	T	A	g	T	A	C	A	A	A	T	C	T	T
		R	C	C	T	G	C	T	C	T	C	T	A	T	C	A	T	g	C	A
phosphglucose isomerase 2	*PGIC2*	F	G	T	T	A	T	g	T	g	g	A	C	T	A	T	C	A	g	G
		R	G	A	T	A	C	A	T	T	C	C	A	T	A	C	A	C	T	T
benzoyl-CoA: benzyl benzoyl transferase	*BEBT*	F3	G	C	T	T	C	g	T	T	T	C	C	A	g	A	T	A	C	C
		R3	G	A	A	C	C	C	A	g	C	T	C	g	T	g	T	C	A	C
salicilic acid methyl-transferase	*SAMT*	F1	C	g	g	C	A	A	g	T	T	C	T	T	C	A	C	A	T	g
		R1	G	A	A	C	C	T	g	A	g	A	T	A	g	C	C	A	C	A
isopentenyl pyrophosphate isomerase	*IPI2*	NF	C	A	A	C	C	A	A	C	C	T	g	A	A	C	C	A	A	g
		NR	G	C	T	G	g	g	A	T	g	g	A	C	g	C	T	g	T	C
linalool synthase	*LIS*	F2	G	T	C	T	T	C	T	A	T	C	A	A	C	C	T	T	G	G
		R1	C	t	g	C	t	t	c	a	t	t	a	c	a	a	g	t	c	t
sodium citrate transporter	*NaCT*	F1	G	T	T	G	G	A	A	T	G	G	G	A	C	A	A	A	A	A
		R1	C	G	A	T	G	G	C	T	G	G	A	A	C	A	G	A	A	C
Aldo-keto reductase C	*AldoC*	F3	A	T	T	C	T	T	G	C	T	G	C	T	G	A	T	G	A	G
		R3	T	G	T	C	A	A	C	C	T	T	G	A	T	A	C	C	A	G
ADP glucose pyrophosphorylase (large subunit only)	*AGPL*	F	T	g	g	T	T	Y	C	A	R	g	g	I	A	A	I	g	C	I
		R	G	T	I	C	C	I	A	T	R	T	C	Y	T	C	C	C	A	R
triose phosphate isomerase	*TPI*	F2	T	T	C	G	T	C	g	g	Y	g	g	Y	A	A	C	T	g	g
		R2	C	C	W	G	T	C	C	W	C	C	A	T	R	g	C	C	C	A

Table 5 continued

Cinnamoyl CoA reductase	*CRS*	F	G	g	C	R	T	C	T	g	g	g	A	T	g	T	g	T	g	C
		R	T	A	C	A	C	g	g	A	g	A	T	g	C	A	g	C	G	T
ADP-glucose pyrphosphorylase	*Pyrol*	F1	G	T	A	T	T	g	A	T	C	T	T	g	g	C	g	g	g	A
		R1	C	T	T	T	T	C	C	C	A	g	T	A	g	T	C	T	C	C
benzoyl alcohol acetyl transferase	*BEAT*	F2c	C	T	g	A	C	C	C	T	g	g	T	g	C	g	A	g		
		R2c	C	C	T	T	A	C	A	A	A	C	A	A	g	C	T	T	A	T
epoxide hydrolase	*ExH*	F1	G	T	T	T	C	g	g	A	T	C	A	C	A	T	T	C	T	T
		R1	C	g	T	G	C	A	A	T	A	T	C	C	A	T	C	g	A	C
Nucleoside diphosphate Kinase	*NDK*	F1	C	C	C	A	T	A	g	A	T	C	C	A	C	T	T	C	T	C
		R1	G	C	A	G	C	A	g	T	T	T	A	C	A	T	g	C	T	C
Glutamine synthase	*GS*	F2	G	A	T	G	C	T	g	g	C	C	T	C	C	T	A	T	C	T
		R2	C	C	A	A	g	T	A	g	g	T	C	C	A	T	C	C	g	T
1-D-deoxyxylulose 5- phosphate synthase	*DxPS*	F2	C	g	A	G	T	C	A	T	C	A	C	C	g	A	g	A	A	g
		R2	G	T	A	T	C	C	C	C	T	T	C	C	A	C	C	A	A	T

Table 6. Annealing temperatures, fragment size, polymorphic enzyme, and linkage map position of STS primers in *Clarkia*[1]

Symbol	**Anealing Temp oC**	**Expected Size**	**Actual Size**	**Enzyme**	**Linkage Group**
Invr	59	500	500	*Alu I*	1
c_Mdh Mdh	61	1300	1000	*Alu I*	7
Gapc	59	2000+	1600	*Msp I*	2
Pgic1	58	1350	1350	*RSA*	4
Pgic2	59	1200	1200	*Hae III*	1
Bebt	53	1000	1300	*Cfo I*	1
Samt	62	700	700	*Alu I*	3
Ipi2	67	2200	2000	*Cfo I*	5

Table 6. continued

Lis	62	1400	1000	*Hinf*	2
NaCT	54.5	850	800	-	1
Aldoc	63	600	500	-	4
Agpl	63	1000	1000	*Mbo1*	11
Tpi	60	2000	1800	*Hae III*	1
Crs	66.5	700	1000	-	3
Pyrol	59.5	1200	1000		5
Beat	53.5	800	900	*Hae III*	10
ExH	60	1200	2000	-	8
Gs	60	1200	1000	*HypC4H IV*	9
DxPS	60	1200	1400	-	5
Ndk	60	1200	750	*Dde1*	4

[1] Note that enzymes were not always needed to obtain a size polymorphism between the parent amplified fragments.

The morphological markers values for the parent species and F_1 as well as the ranges for the F_2, F_4, and backcross populations are recorded in Table 7. Traits were scored as measurements, plus/minus or as values. In four of the traits the score of the phenotypic trait is recorded as a value, such as flower color for which flowers of a hue resembling *C. concinna* were scored as a 2.

Table 7. Value ranges for morphological markers in parents, F_1 and segregation progenies

Marker	Aroma[1]	Petal	Hypm[3]	Flower hue	Days to flower	Leaf	Stem[4]	Apical[5]
Scoring[2]	+ / -	ratio	cm	1 / 1.5 / 2	1.5/ 2.0/3.0	cm	0 / 1 / 2	0 / 1 / 2
Individual								
C. breweri	+	> .50	> 2.2	1-1.5	1.5	< .9	0	0
C. concinna	-	< .30	< 2.0	2	3	> 1.1	2	2
F1	Faint scent (+)	.30-.50	1.6-2.5	1.0-2.0	1.5	.7-1.5	1	1
F2	22 / 56	.19-.86	1.4-3.2	32/28 /34	38 / 44 / 11	0.3-1.2	25 /20 / 43	44/ 40 / 9
F4	19 / 48	.21-.75	1.4-3.0	28/27 /23	24 / 24 / 10	0.4-1.2	19 / 11 / 49	38/22 /15
Backcross								
F1x *C. breweri*	5 / 0	.43-.59	2.5-3.1	0 / 5 / 0	3 /2 / 0	.6-.9	0 / 5 / 0	3 / 0 / 2
F1x *C. concinna*	4 / 18	.26-.43	1.5-2.5	3 / 6 / 13	4 / 9 / 8	.9-1.7	0 / 8 / 13	2 / 3 / 17

[1] + = aroma present - = aroma lacking
[2] These divisions are based on visual observations and comparisons to parental types.
[3] The abbreviations used in the table headings are Hypm (Hypanthium length), [4] Stem (Stem anthocyanin), and [5] Apical (Apical dominance).

Segregation of DNA markers is shown in Appendix I. The data are represented as c = *C. concinna* like amplified band fragment sizes b = *C. breweri* like amplified band fragment sizes and h = heterozygote was represented by the presence of both parental band sizes. The data at the bottom of the columns reports the band sizes for each of the parents. Table 8 presents the actual segregation ratios for each marker. The segregation ratios expected were 1:2:1 or 3:1 depending on whether the marker was an STS or RAPD being scored. Table 8 also reports the segregation χ^2 value of each marker or trait mapped.

Table 8.

Segregation analysis of individual markers or traits

Marker	flsp	flsp No hets[1]	arom	arom	dtf	dtf	Fhue	Fhue
Pairwise	---------	---------	---------	no hets	----------	no hets	----------	no hets
b-b	29	29	22	22	44	82	32	32
h-h	22	0	12	0	38	0	28	0
c-c	40	62	56	68	12	12	34	62
2	27	2	74	0	25	8	15	4

	stat	stat	Apdm	apdm	CMDh	Invr	LiS	Pgi1	Pgi2
Pairwise	---------	no hets	---------	no hets	----------	----------	----------	----------	----------
b-b	25	25	9	49	19	28	25	21	20
h-h	20	0	40	0	30	42	30	49	13
c-c	42	62	44	44	42	25	24	15	37
2	32	1	28	25	22	2	5	3	36

	NaCT	GAPc	AldC	AGPl	TPI	CRS	Pyrol	25r1	25r2
Pairwise	---------	---------	---------	---------	----------	----------	----------	----------	----------
b-b	47	7	11	12	9	17	23	35	24
h-h	22	15	11	0	4	4	3	0	0
c-c	19	22	9	22	38	3	2	52*[2]	62*
2	40	15	3	40	69	27	49	11	0

	ApyF1	ApyF2	ApyF3	ApyR1	ApyR2	BEAT	hypm	hypm	leaf
Pairwise	---------	---------	---------	---------	----------	----------	----------	no hets	----------
b-b	23	9	62*	28	79*	25	17	17	20
h-h	0	0	0	0	0	23	7	0	12
c-c	65*	81*	20	61*	11	29	29	36	21
2	0	11	0	2	8	13	34	17	16

	leaf	BEBT	IPI2	SAMT	13A1	13A2	P4F1	P4F2	P4F3
Pairwise	no hets	---------	--------	---------	----------	----------	----------	----------	----------
b-b	20	56	62	50	37*	29	64*	46*	40*
h-h	0	14	4	22	0	0	0	0	0
c-c	33	9	7	22	55	61*	26	41	51
2	19	89	141	43	59	2	1	23	17

	PER1	PER2	PER3	1OB1	1OB2	1OB3	19OB1	19OB2	15OB1
Pairwise	---------	---------	---------	---------	----------	----------	----------	----------	----------
b-b	23	44*	72*	70*	42	44*	53*	29	61*
h-h	0	0	0	0	0	0	0	0	0
c-c	67*	46	18	11	39*	37	34	57*	30
2	0	27	2	6	23	19	9	4	3

Table 8. continued

	15OB2	OD4	OD19	ExH	DxPS	GS	NDK		
Pairwise	---------	---------	---------	---------	----------	----------	----------	----------	----------
b-b	38	42	32*	43	58	74	15		
h-h	0	0	0	28	11	9	14		
c-c	50*	38*	40	11	6	2	45		
2	16	22	36	33	110	175	53		

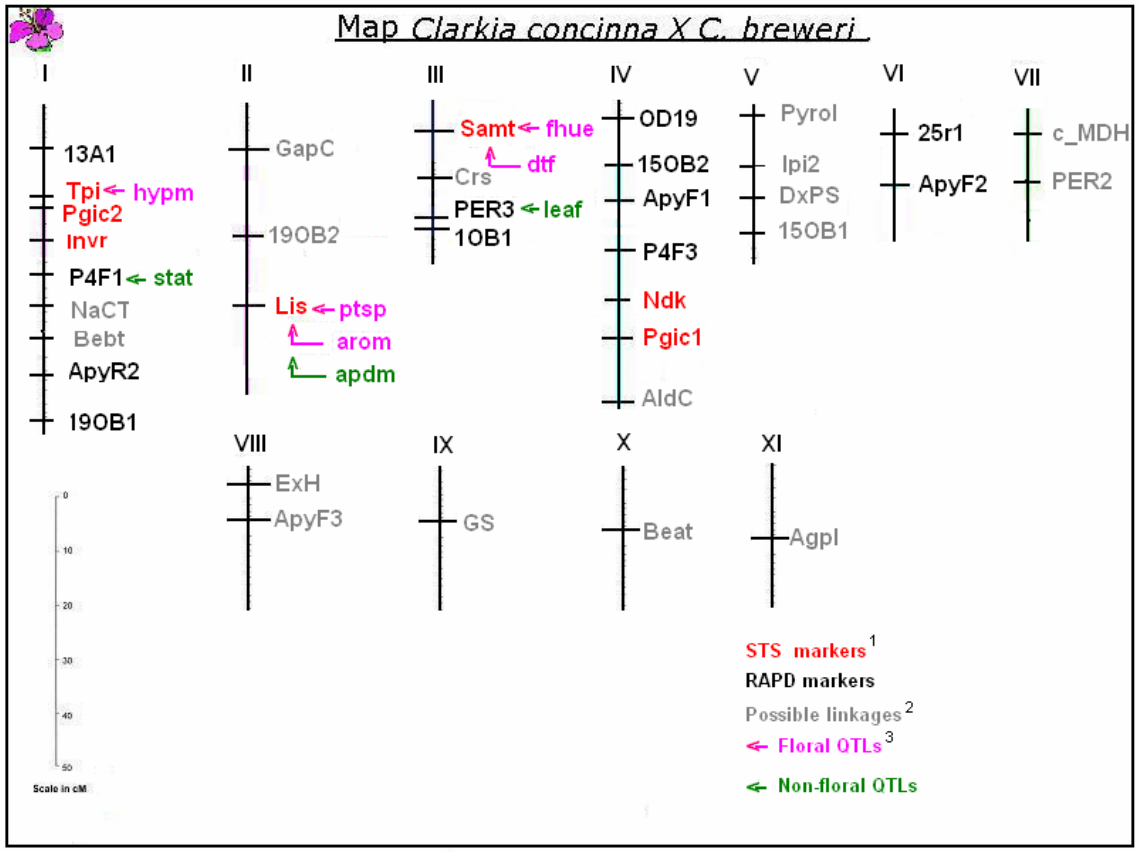
[1] The heterozygotes for the traits were difficult to identify and so the □[2] and segregation data are reported with and then without the heterozygote class. The traits scored include the aroma of the flowers (arom), petal shape (ptsp), hypanthium length (hypm), the flower hue (fhue), and days to flower (dtf), leaf shape (leaf), stem anthocyanin (stat), and apical dominance (apdm).

[2] An asterisk represents the fragment present category in the RAPD polymorphisms (i.e. dominant phenotype).

From Table 8 it can be seen that some of the markers are highly skewed to one or the other of the parental types and therefore standard joint segregation analysis is of little use in determining linkage with other markers. The linkage of those markers which displayed skewing was then confirmed by aligning the segregation data in Quikmap to determine if the parent that was skewed toward a deficit aligned to rare occurrences in the other skewed marker. If both of these conditions were met the linkage was considered possible.

Associations between markers are made using the mapping programs and linkage groups are assigned to create the genetic map (Figure 5). The depiction of the linkage map displays 11 linkage groups total (Figure 5). The total length of the map is approximately 465 cM.

Figure 5. Linkage map of *Clarkia concinna* x *C. breweri*



[1] For the STS marker abbreviations refer to Table 5.
[2] The linkages marked in grey are those that were identified using Quikmap and were found to be less than significant matches using the Joinmap program.
[3] For the abbreviations of the morphological traits refer to Table 8.

Tpi and *Pgic2* were the most closely linked markers at 2.3 cM. *Tpi*, *Invr* and the RAPDs P4F1, 19OB1, 13A1, and ApyR2 also belong in the *PGIc2* linkage group. Tentatively, *Bebt* and *NaCT* are included on this linkage group due to ambiguous marker order found around the *Tpi* marker. These ambiguous marker orders do not seem to be explained by poor data and may reflect the formation of a quadrivalent at this location. This linkage group is designated linkage group I in Figure 5. Linkage group II contains *Lis* and with the use of Quikmap the marker *Gapc*, and the RAPD 19OB2. The third linkage group contains the marker *Samt* and *Crs* along with two RAPDs: 1OB1, Per3.

The fourth linkage group contains the markers *Pgic1* and *Ndk* and with the use of Quikmap data the *AldC* is added. The RAPDs ApyF1, 15OB2, and P4F3 are also located on linkage group IV. There exist a high number of heterozygotes in the segregation data around the *Pgic1* marker.

Linkage group V consists of potential linkages revealed using Quikmap to compare the segregation of markers. The loci on this linkage group displayed distorted segregation favoring the *C. breweri* genotype (or phenotype in the case of dominant markers) and could not be adequately analyzed with Joinmap. The markers found on linkage group V are *Ipi, Dxps* and the RAPD 15OB1.

The arrangement of markers is the best match of markers among one another and do not show linkage to any markers outside this chromosome arm. The only exception is that *Pyrol* appears to show some linkage to group IV. The ambiguity in map position for *Pyrol* is probably due to the relatively few data available for this marker and the distorted segregation displayed by this data favoring the *C. breweri* allele. There is also high amount of skewing toward the *C. breweri* parent associated with *Ipi* on this linkage group.

Within linkage group 6 are located the two RAPDs 25r1 and ApyF2. The linkage group, 7 is made up of the possible linkage between *c_MDH* and the RAPD Per2. The last linkage group to have markers linked together was linkage group 8 where *Exh* and the RAPD ApyF3 were located.

Three STS markers: *Gs, Beat* and *Agpl,* segregated independently of the designated linkage groups. There may not be adequate marker coverage to link these to the other linkage groups. The primers *Beat* and *Gs* may not be specific to one gene.

Although the segregation data were scored from what appeared to be a single amplified band, both genes belong to a small family of sequences. If more than one of this family of genes was being amplified these data may not be a fair measure of linkage for *Beat* and *Gs*. There are 13 RAPD markers included in Appendix A that were scored but did not display linkage to any of the linkage groups identified above. These results may be due to improperly scored RAPD bands or there may not be adequate map coverage to identify linkage.

Plants obtained from backcrossing were confirmed to be the results of backcrosses by examination of marker phenotypes (Table 8). The segregation of the STS/CAPS markers revealed that the plants from the various backcross populations all possessed expected genotypes and segregated as expected (Table 9). For instance there were no backcrosses of the F_1 x C. *breweri* which displayed STS/CAPS PCR amplification bands identical the *C. concinna* parent and vice versa.

Table 9. Confirmation of predicted genotypes at four marker loci in each backcross and phenotypic segregation in the populations

STS markers		**Backcrosses**	***Invr***	***Bebt***	***Beat***	***c_Mdh***
Backcross Line	**# Plants**	**Cross**				
1	2	C. con x F1	H	C	H	H
2	1	C. con x F1	.	H	.	H
3	2	C. con x F1	H/C	C	.	C
4	5	F1 x C. con	H	C	C	C
5	5	F1 x C. con	H/C	H/C	H	H
6	3	F1 x C. con	H	C	C	H/C
7	5	F1 x C. brew	B	B	H	B
Morphological Traits			**Ptsp**	**arom**	**fhue**	**Hypm**
Backcross Line	**# Plants**	**Cross**				
1	3	C. con x F1	H	H	C/B	C/H
2	1	C. con x F1	H	C	C	B
3	3	C. con x F1	C	C	C/B	C/H
4	6	F1 x C. con	C	C	B	C/B
5	5	F1 x C. con	H	H	C	C
6	4	F1 x C. con	C	C	C/B	C
7	5	F1 x C. brew	B	B	C/B	B

1 The type of cross is identified as: an F_1 backcrossed with *C. concinna* pollen (F_1 x C. con), a *C. concinna* individual backcrossed with F_1 pollen (C. con x F_1), or an F_1 backcrossed with *C. breweri* pollen (F_1 x C. brew).

2 The DNA fragments for the STS markers are represented by the size fragment seen in each parent *C. concinna* fragment is represented by C, *C. breweri,* B. The H represents those individuals were both C and B fragments were observed. For the morphological traits the C and B represent the condition observed in the parent plants while a heterozygous individual was one that has some intermediate between the two parent's conditions and was labels H.

If the plant is not a backcross then homozygotes of the opposite parent are seen and it is known that the plant did not result from the desired backcross pollination event. None of the plants believed to be derived from backcrosses display such incongruities. All the backcrosses that involved *C. breweri* had genetic markers which did not amplify the *C. concinna* fragment. The heterozygous condition can occur in any of the backcross populations and it is seen in Table 9 that 'H' fragments (one of each parental type) do occur in all the populations. Morphological traits scored on a backcross population should reveal only one type of parental trait scores and the other trait scores should be the

heterozygous condition. The traits of petal shape and aroma reveal heredity patterns resembling the genetic markers. The other traits flower hue and hypanthium length occur as a range across all the populations. It appeared that the gene was not being controlled in the same way as the genetic markers (Table 9). Those traits that display a wide variety of scores in the backcross population, such as flower hue and hypanthium, may be controlled by several genes and not subject to Mendalian inheritance. The backcross data are seen in Table 8.

Another feature of the map that was investigated was the position of the translocation between *C. concinna* and *C. breweri.* Theoretically, F_2 plants heterozygous for the translocation should display about 50 sterile pollen. However, the KI stain of pollen revealed that the *C. breweri* parent had a high percentage of inviable pollen (Appendix C), a characteristic that could interfere with the analysis. The *C. breweri* parent displayed only 42% pollen fertility, whereas the value observed in the F_1 was slightly higher than 50% fertility, and the *C. concinna* parent displayed approximately 99% pollen fertility. Nearly all the F_2 and F_4 individuals examined gave 40-60% viable pollen, and none gave the high level of pollen viability observed in the *C. concinna* parent. The mechanism that is maintaining the range of pollen fertility seen in the F_2 to F_4 generations may in some part be due to the translocation. However, the presence of low pollen viability in the *C. breweri* precludes finding the position of the translocation.

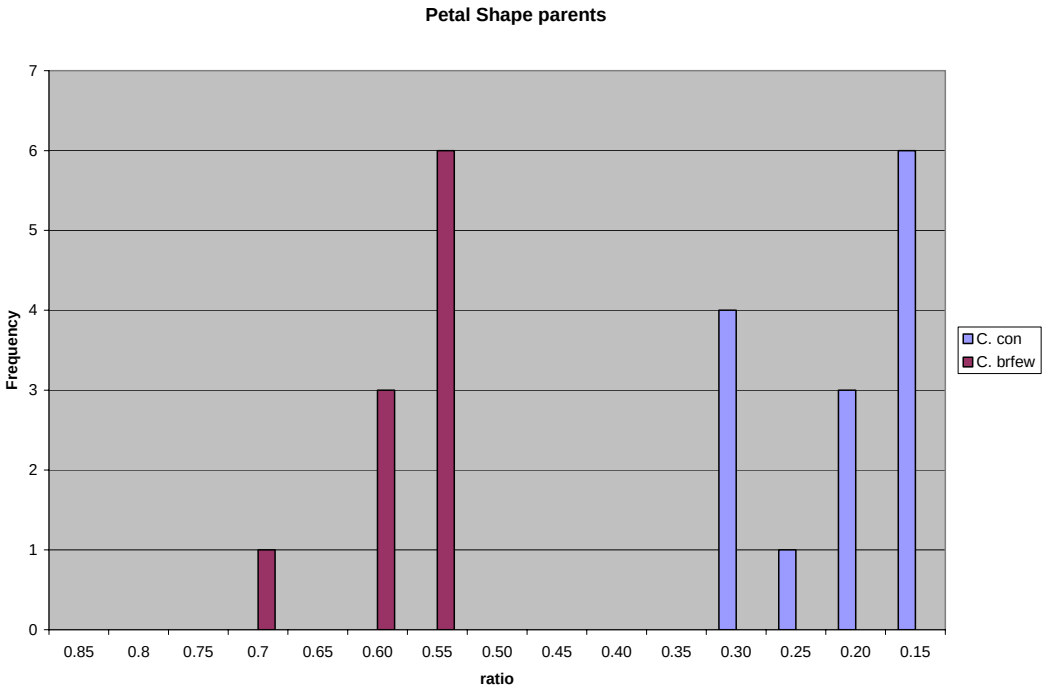
Morphological Segregation

Five floral traits were scored in this *Clarkia* study. The traits scored include the aroma of the flowers, petal shape, hypanthium length, the flower hue, and days to flower.

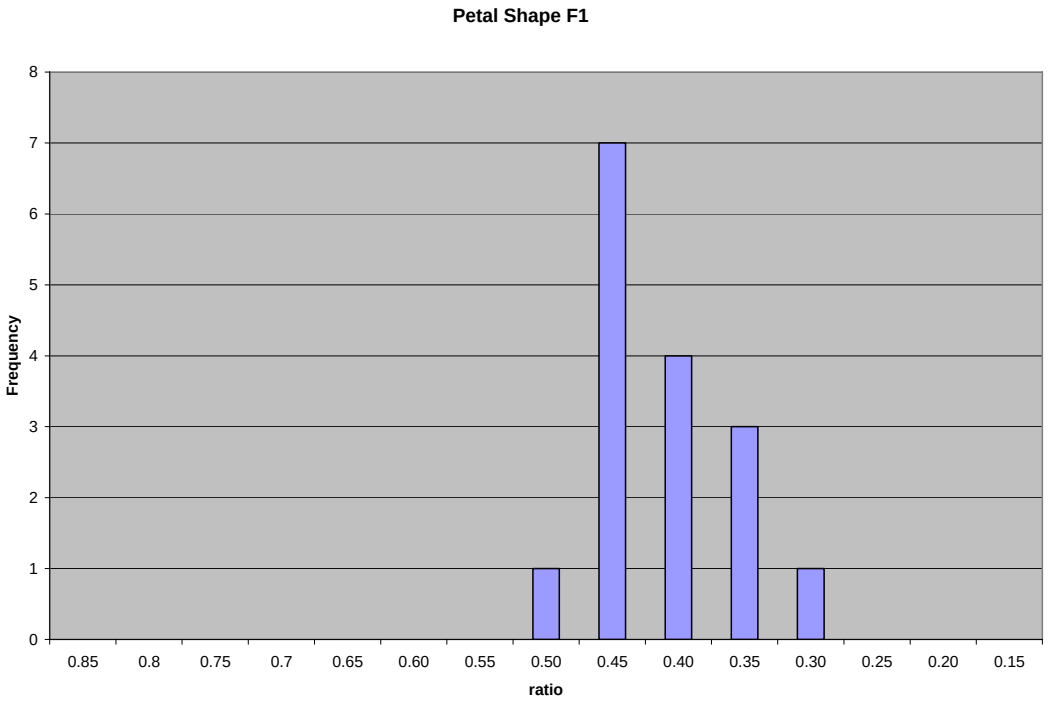
Three other vegetative traits were also scored in the F_2 individuals including leaf shape, stem anthocyanin, and apical dominance. Segregation of morphological/physiological markers is shown in Figure 6 and Appendix A. The parental types for all but days to flower and apical dominance were in the majority of individuals when compared with the number of intermediate individuals, tentatively scored as heterozygotes. In Appendix A the morphological data are represented by types (c, b and h for *C. concinna*, *C. breweri* and heterozygous or intermediate types) to allow Quikmap to analyze the data. The numerical values of the morphological traits are presented in Appendix B. These numerical values were used in the analysis done with QTL Cartographer.

Figure 6. Population trait distributions

Clarkia breweri *C. concinna*

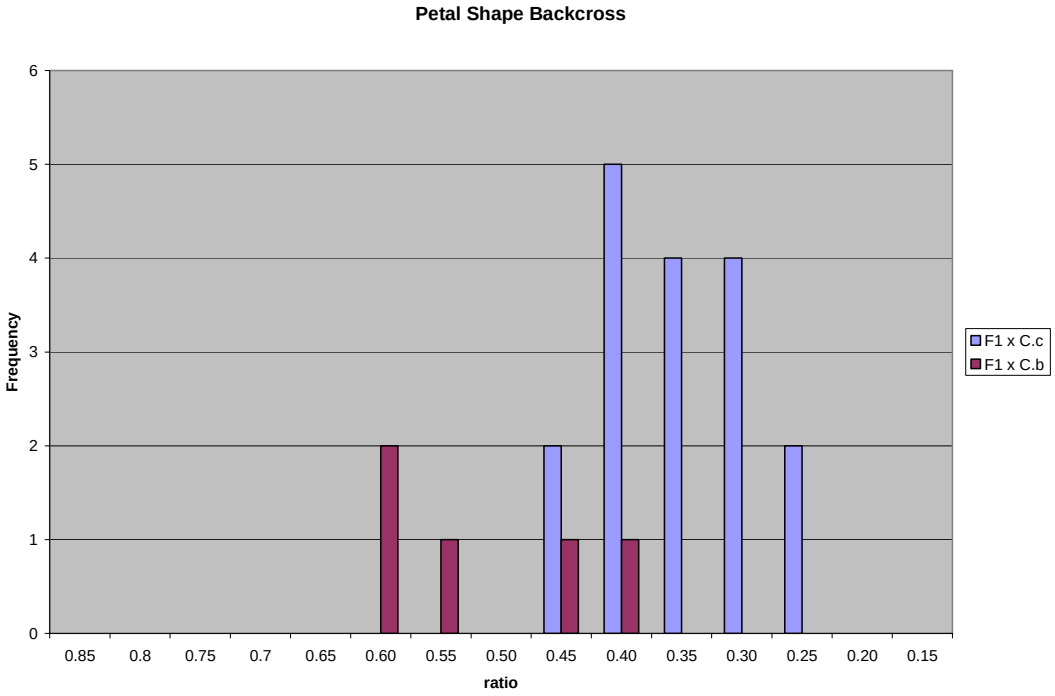


A

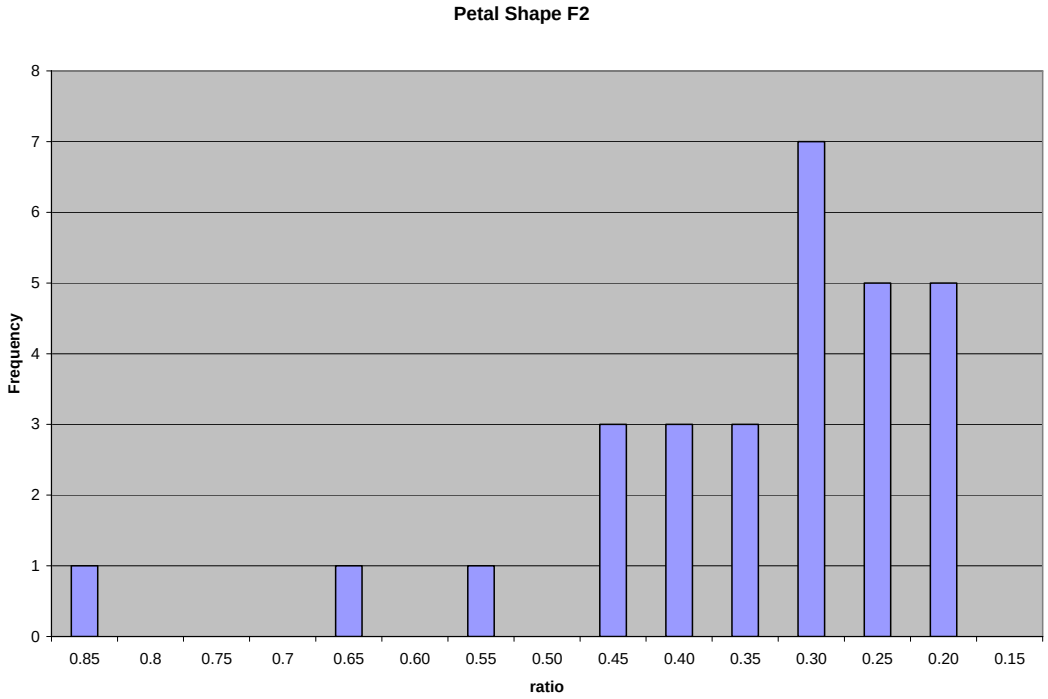


B

Clarkia breweri *C. concinna*

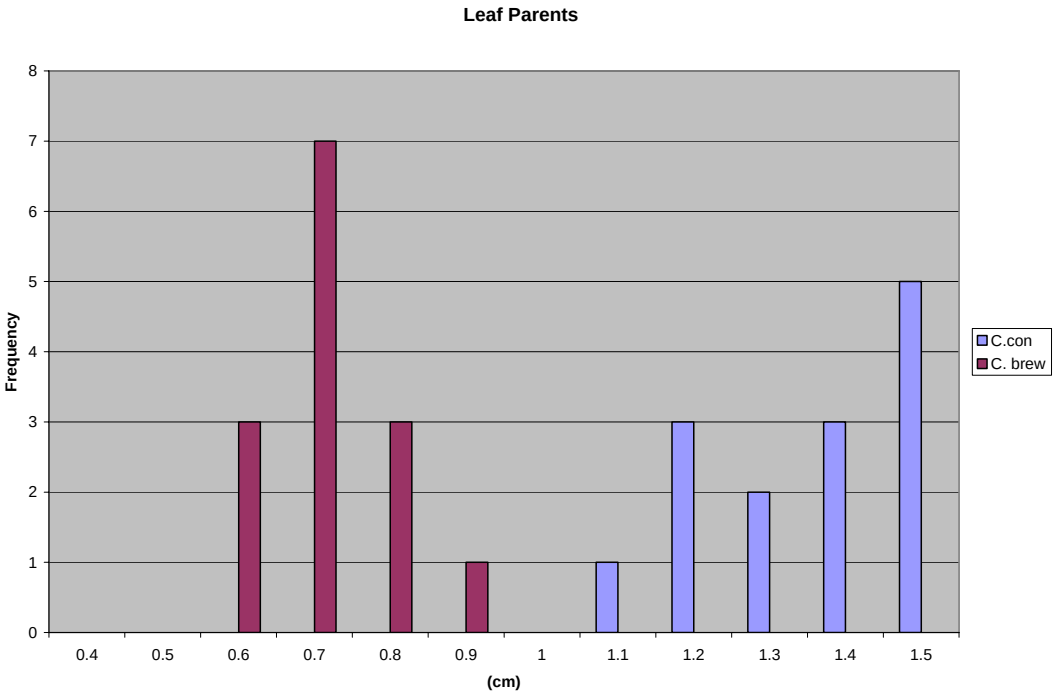


C



D

Clarkia breweri *C. concinna*



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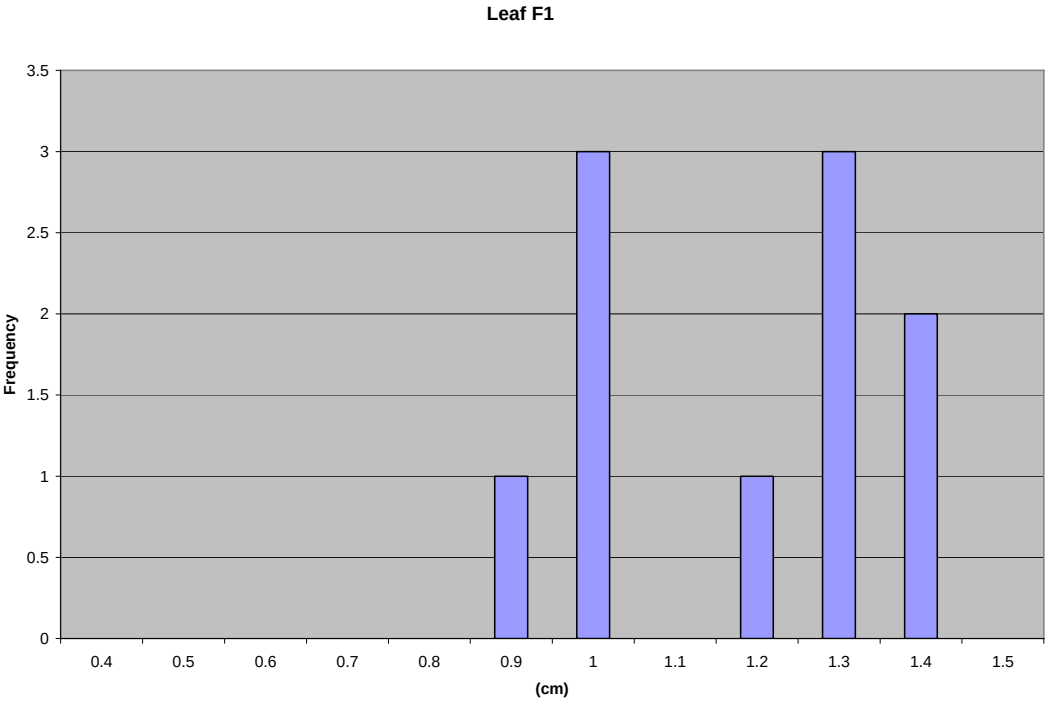
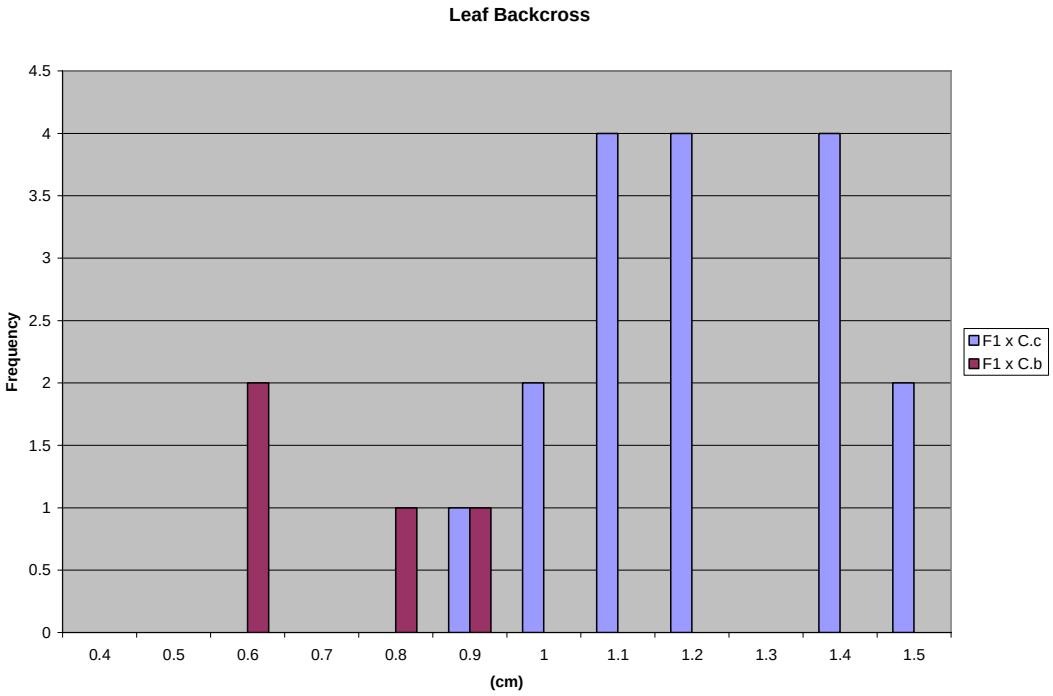


F

Clarkia breweri *C. concinna*



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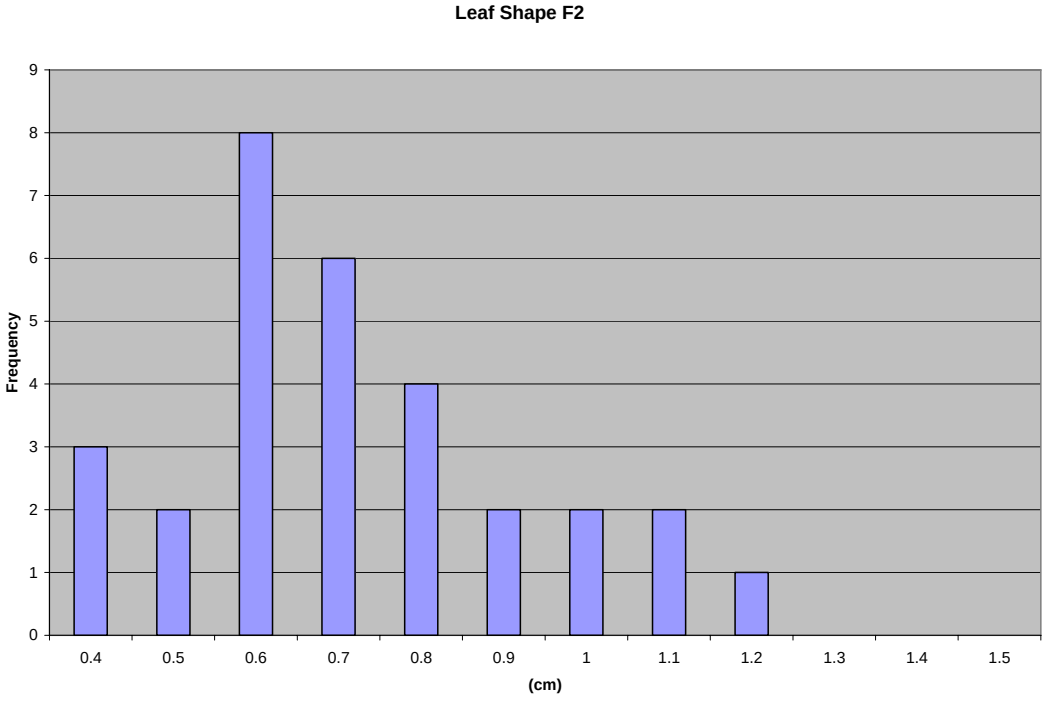
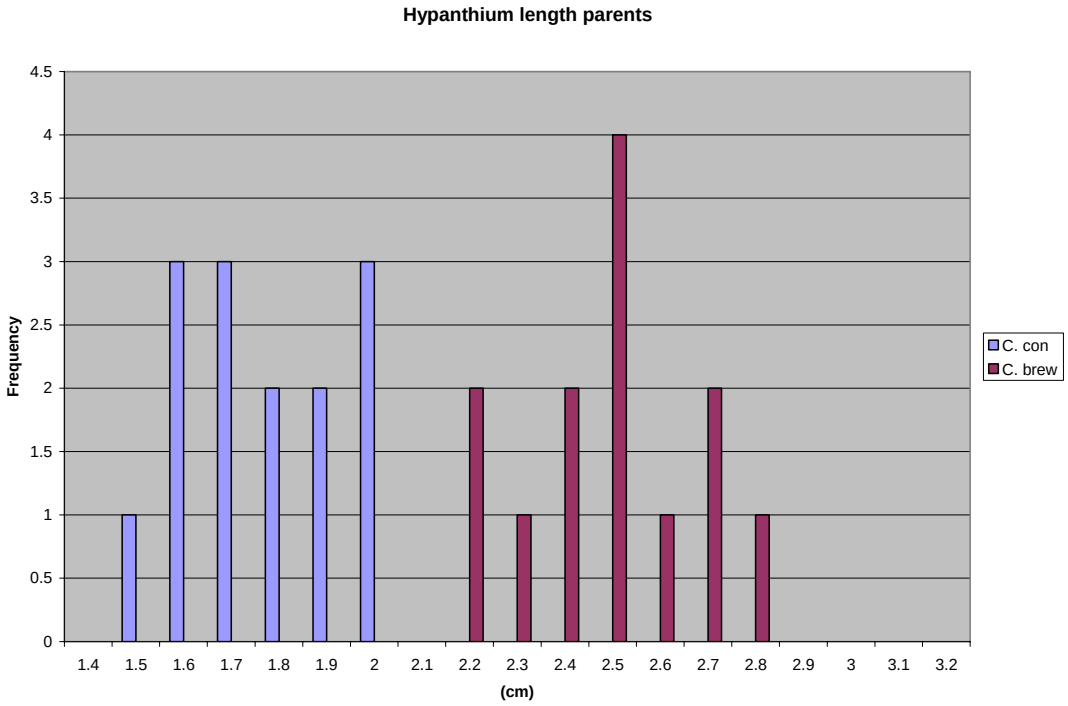


H

Clarkia concinna *C. breweri*



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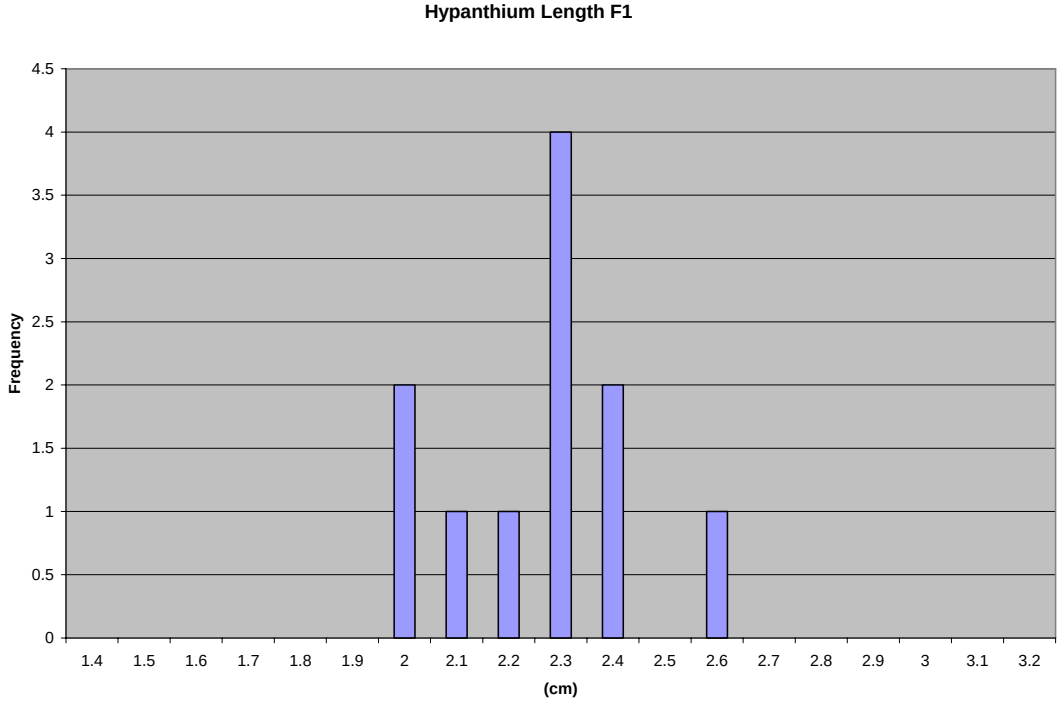
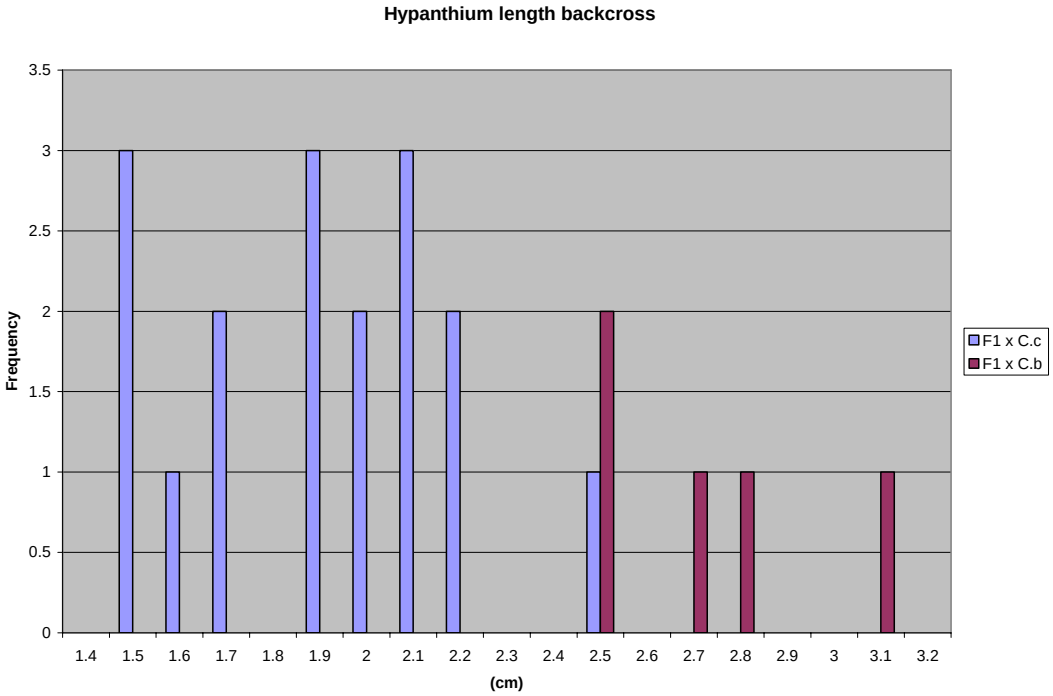


J

Clarkia concinna *C. breweri*



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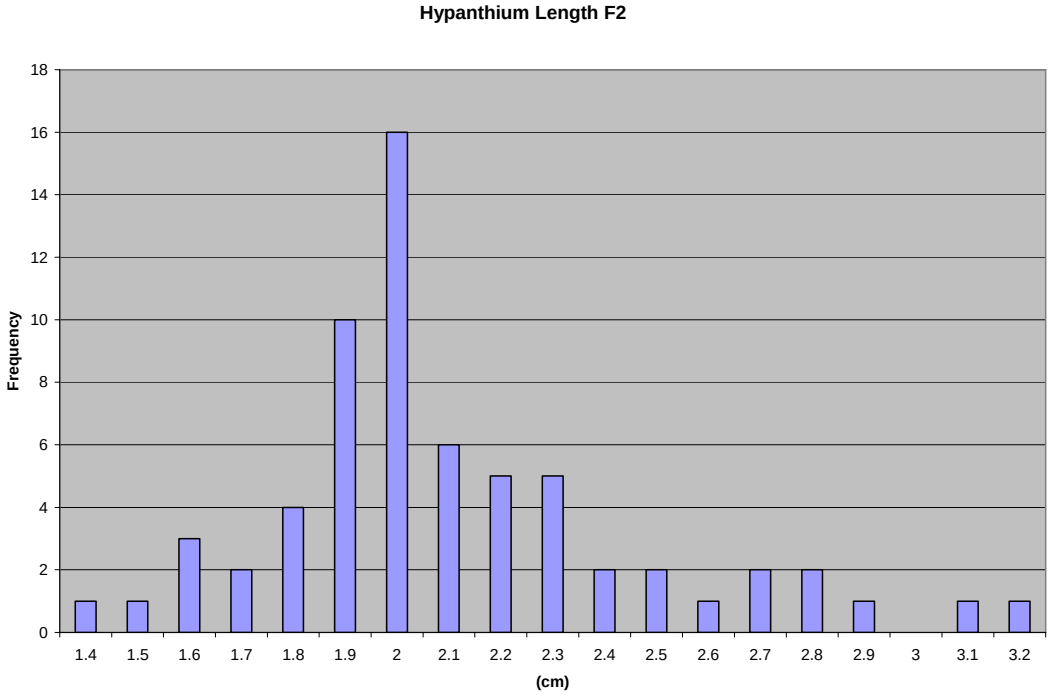
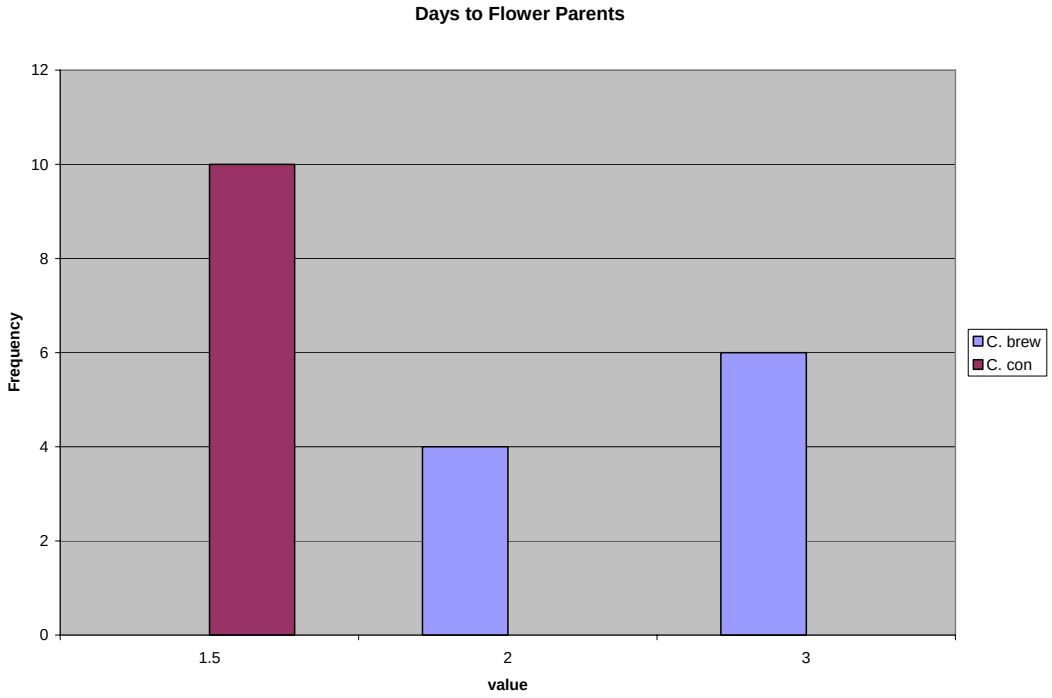


L

Clarkia concinna *C. breweri*



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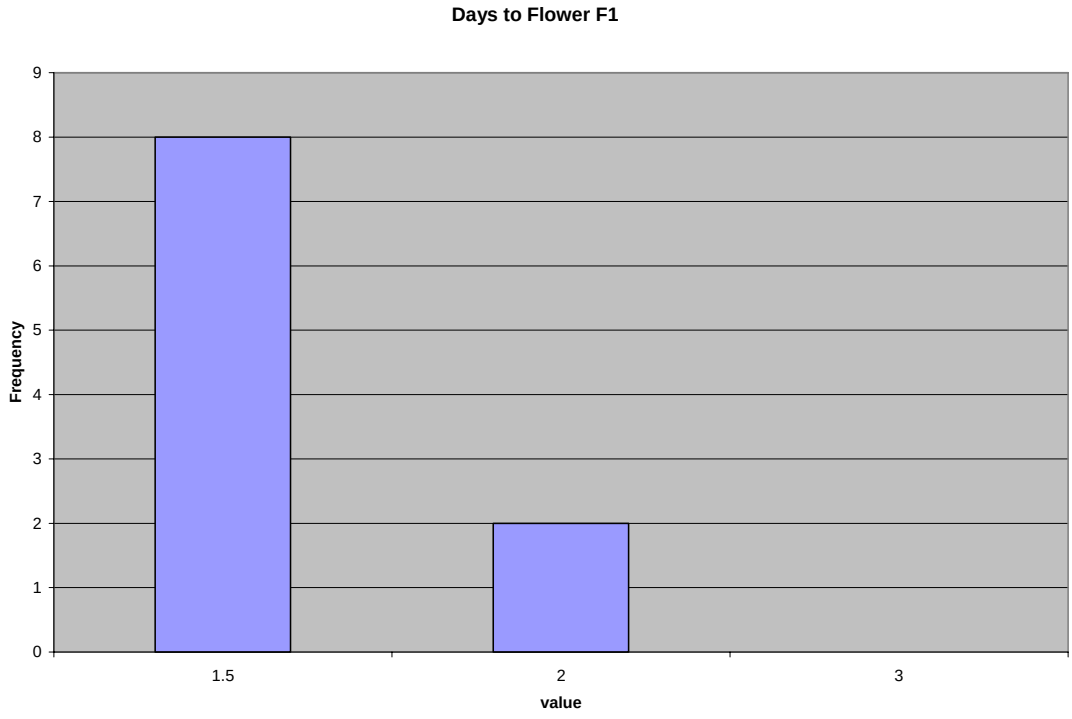
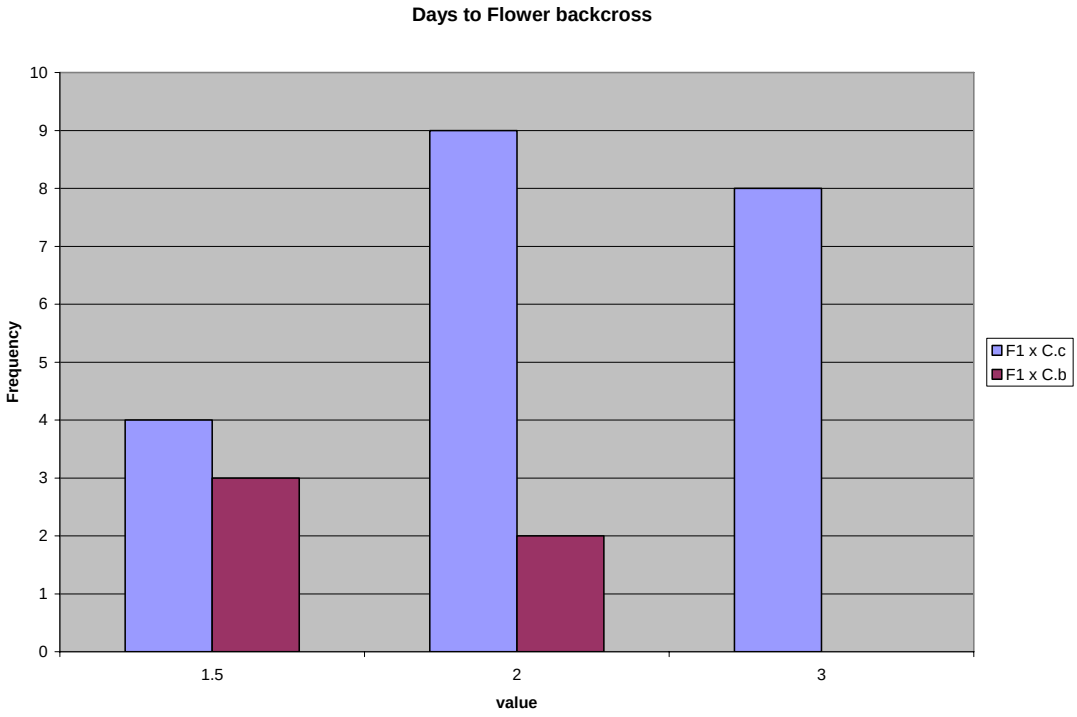
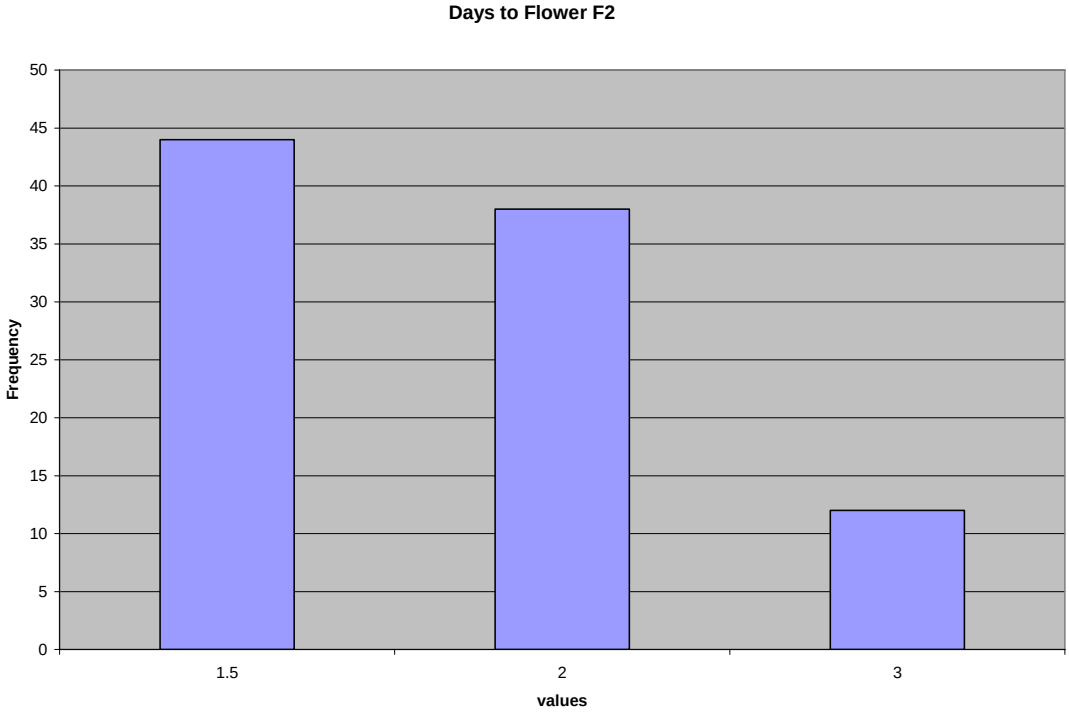


N

Clarkia concinna *C. breweri*

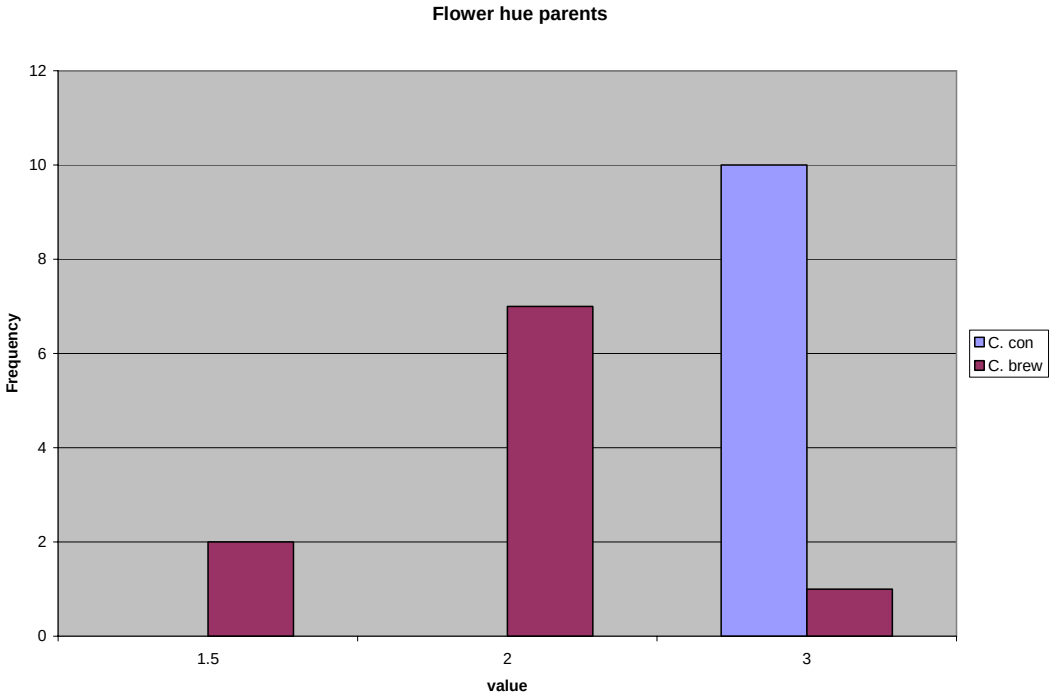


O



P

Clarkia breweri *C. concinna*



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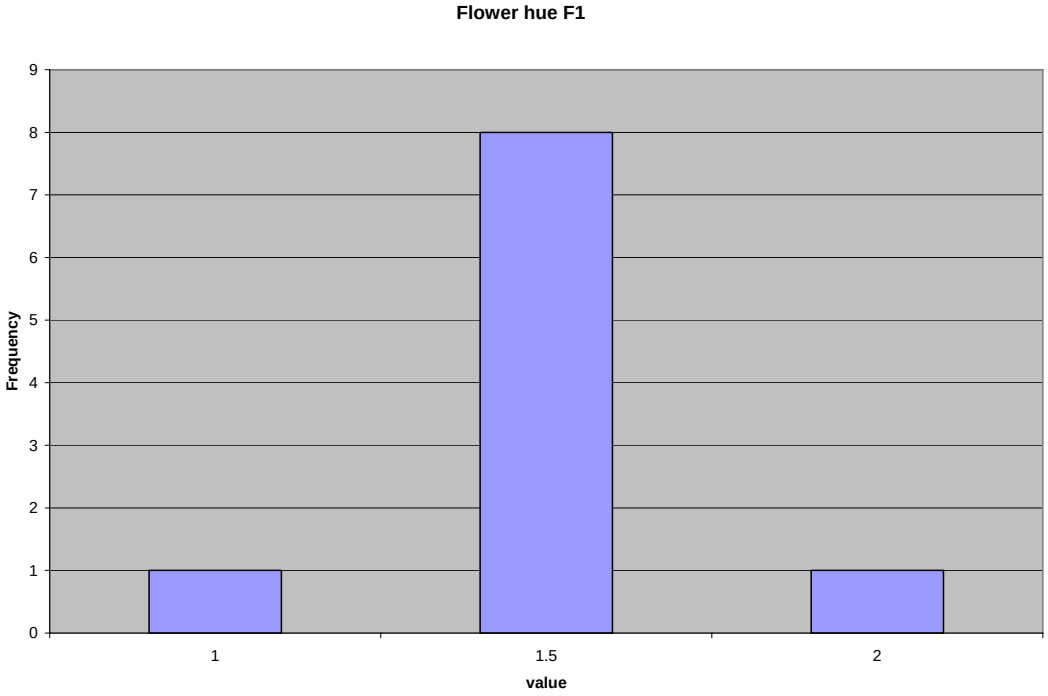
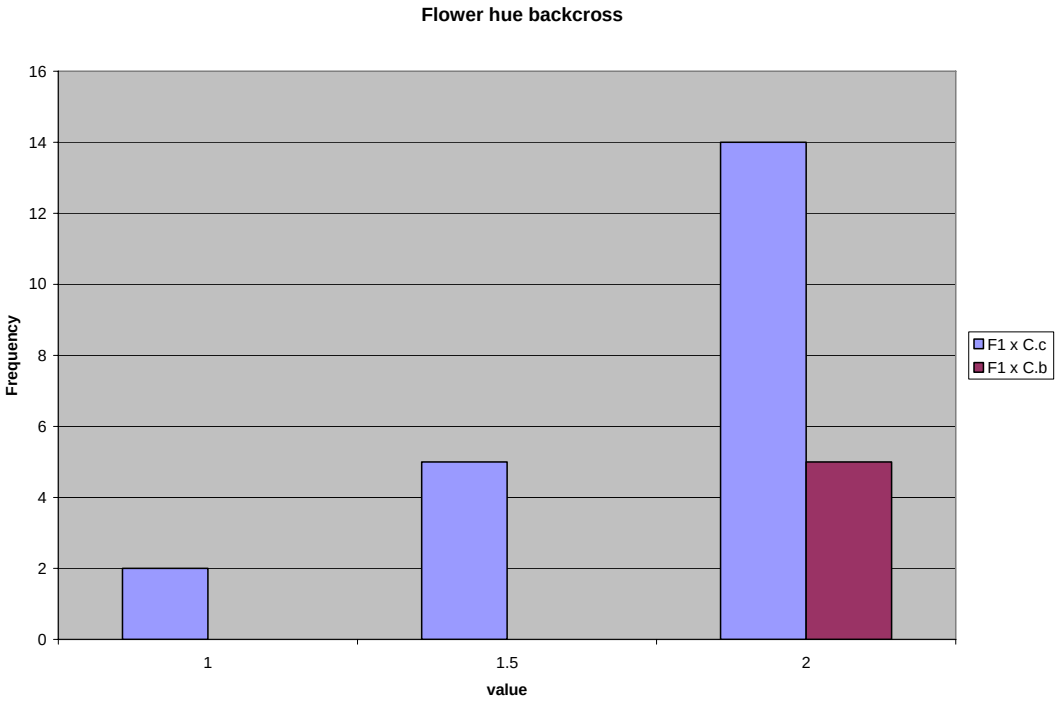


R

Clarkia concinna *C. breweri*



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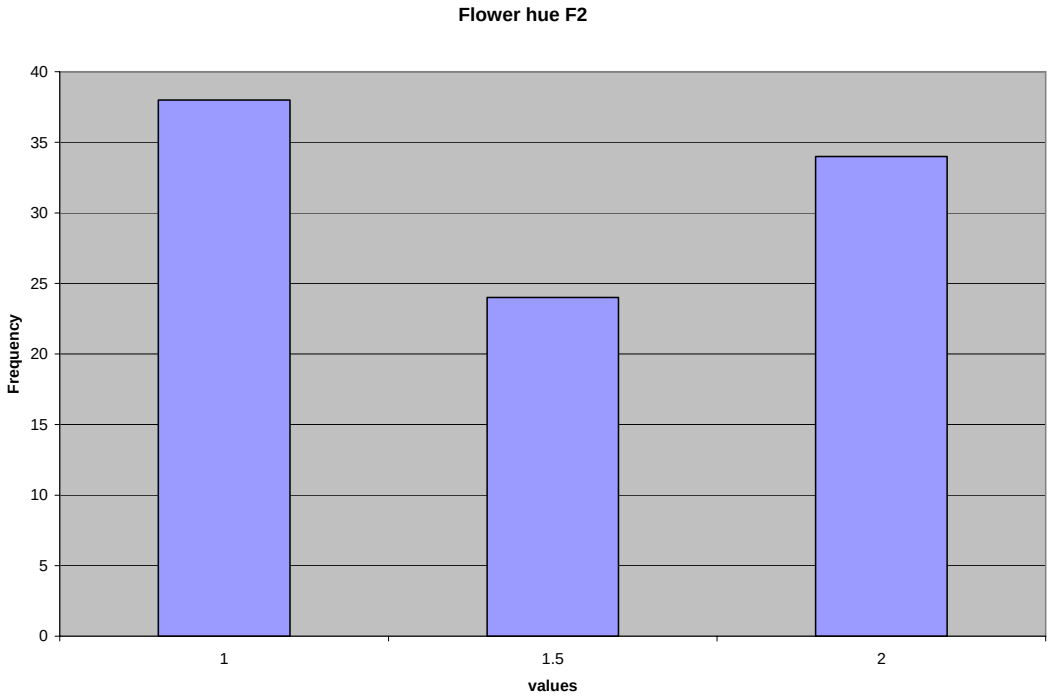
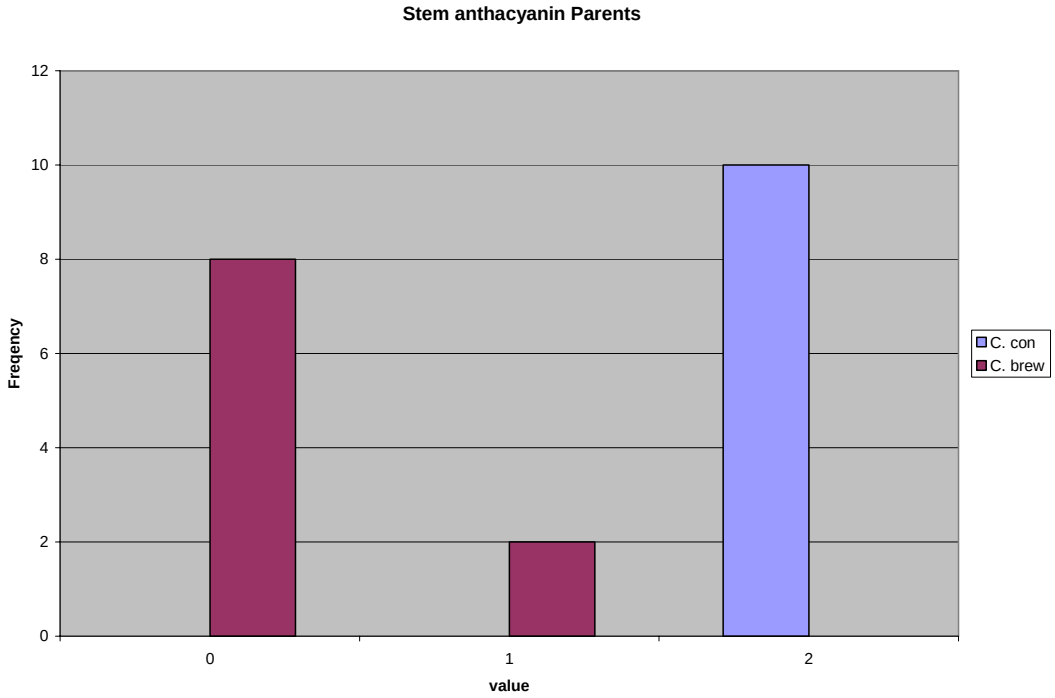


T

Clarkia breweri *C. concinna*



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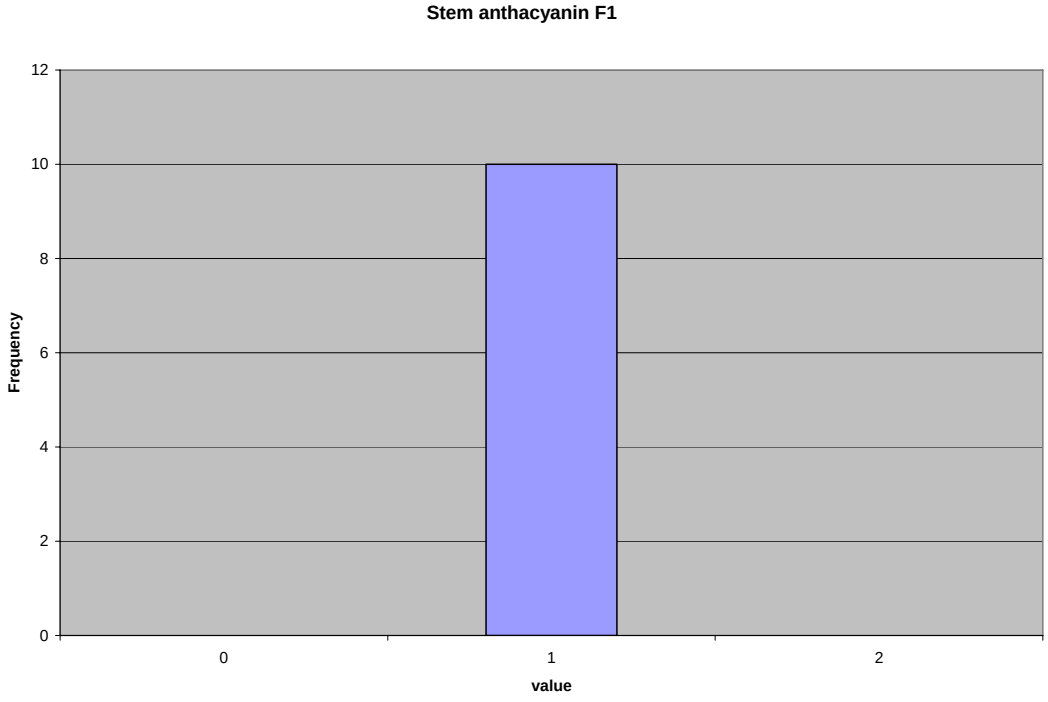
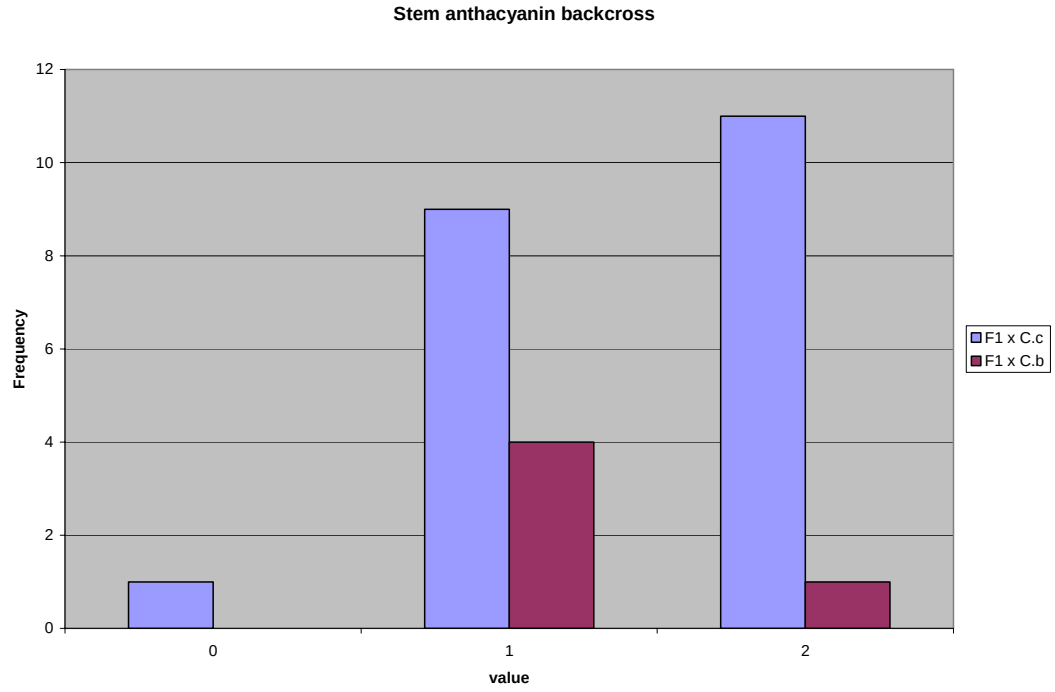


V

Clarkia concinna *C. breweri*



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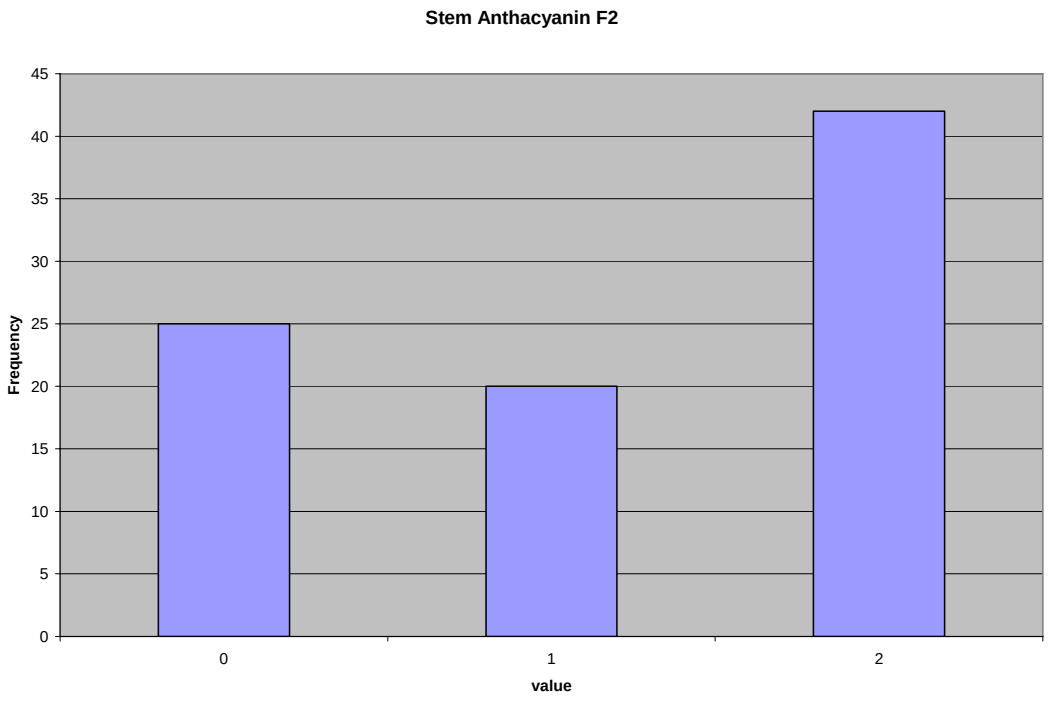
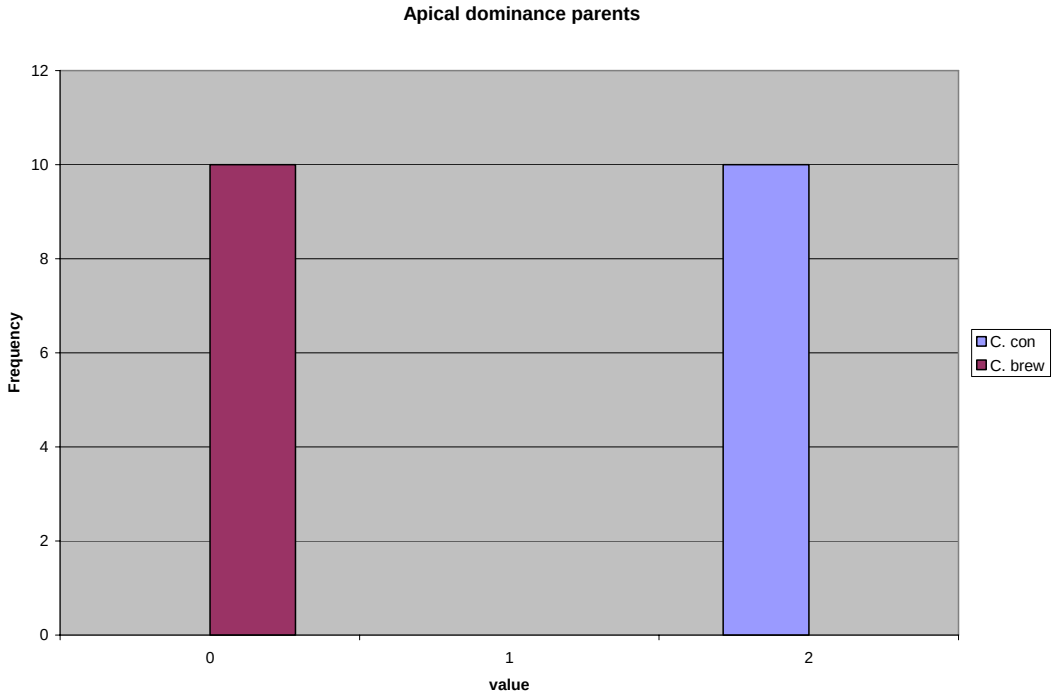


X

Clarkia breweri *C. concinna*



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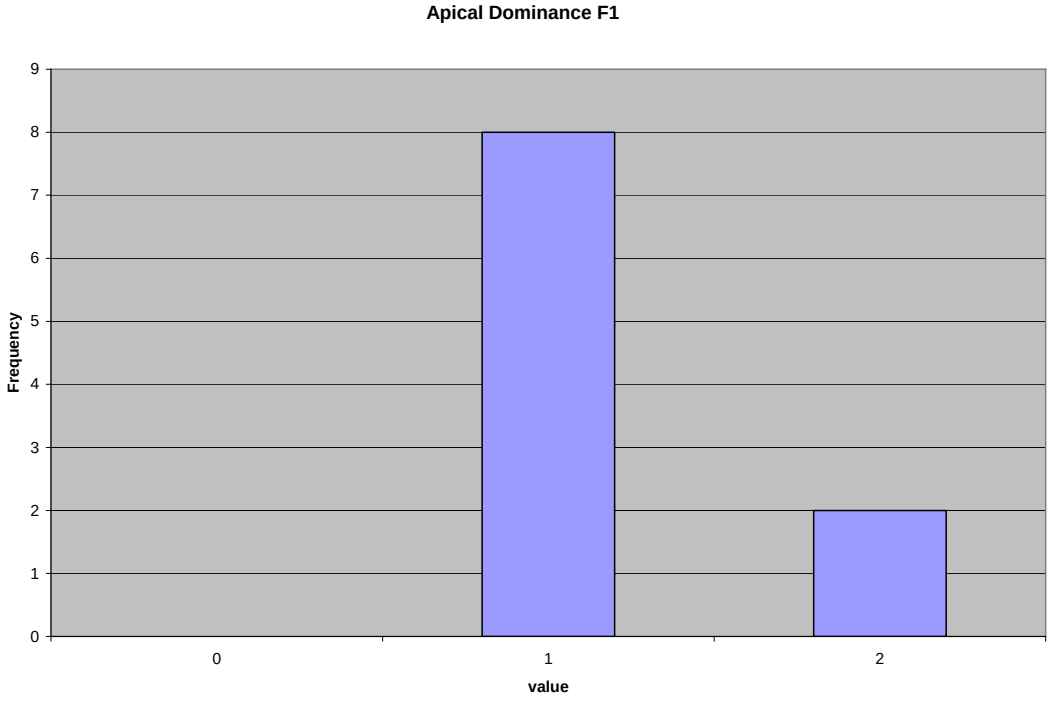

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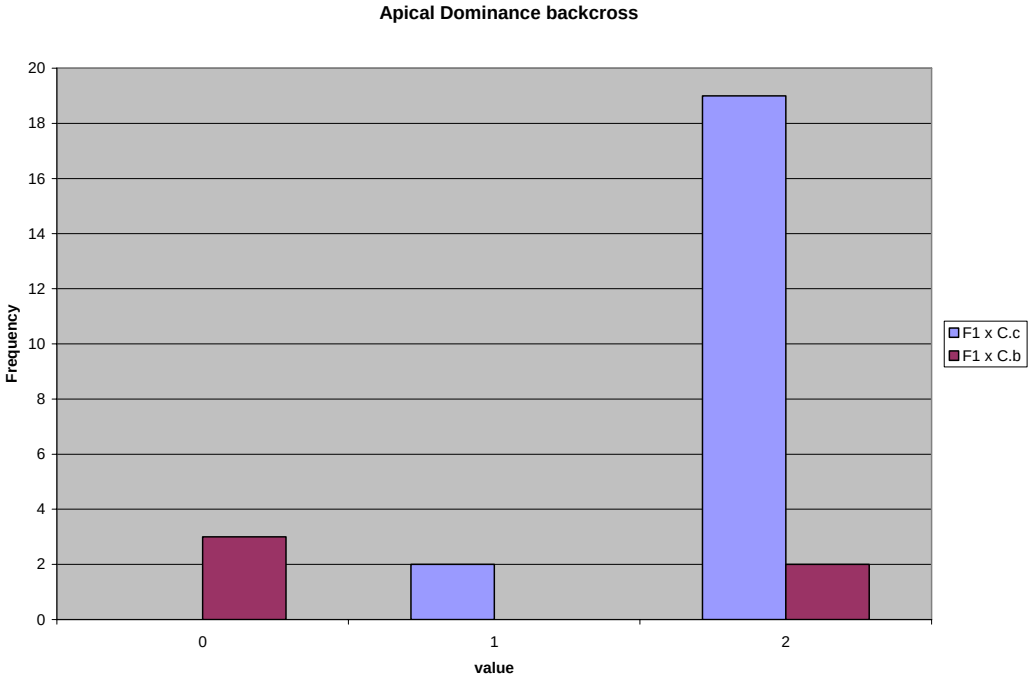
Clarkia breweri *C. concinna*



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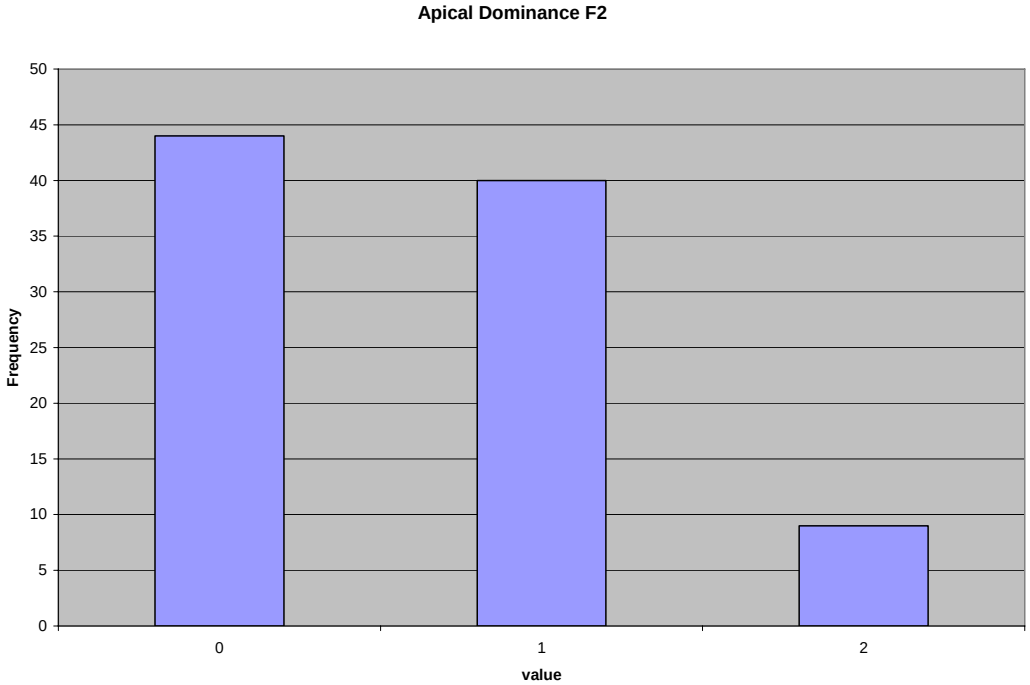
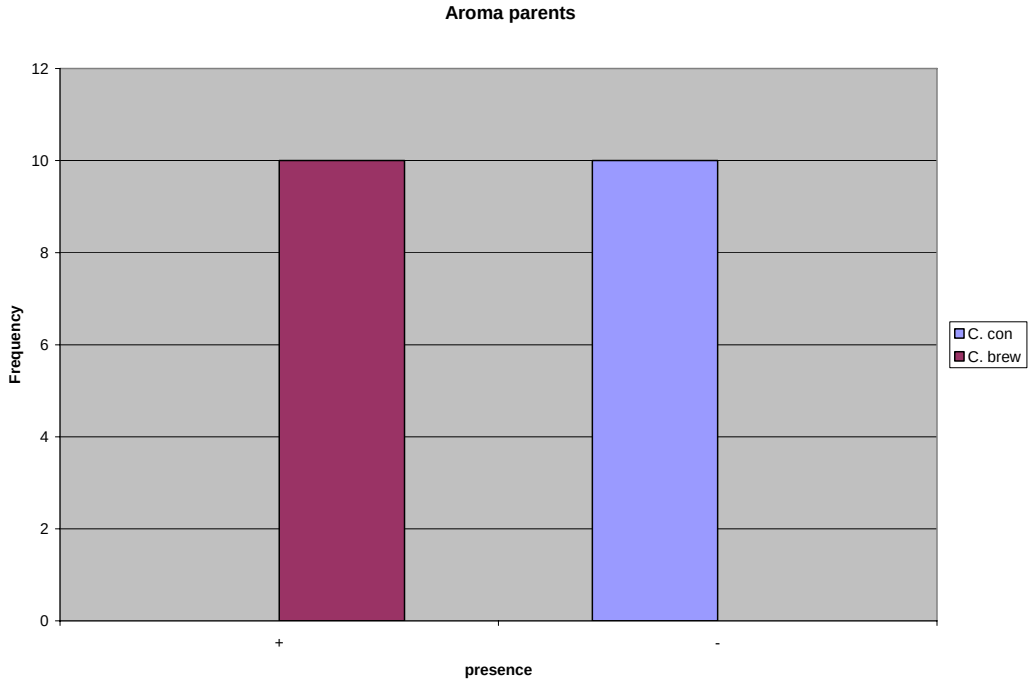
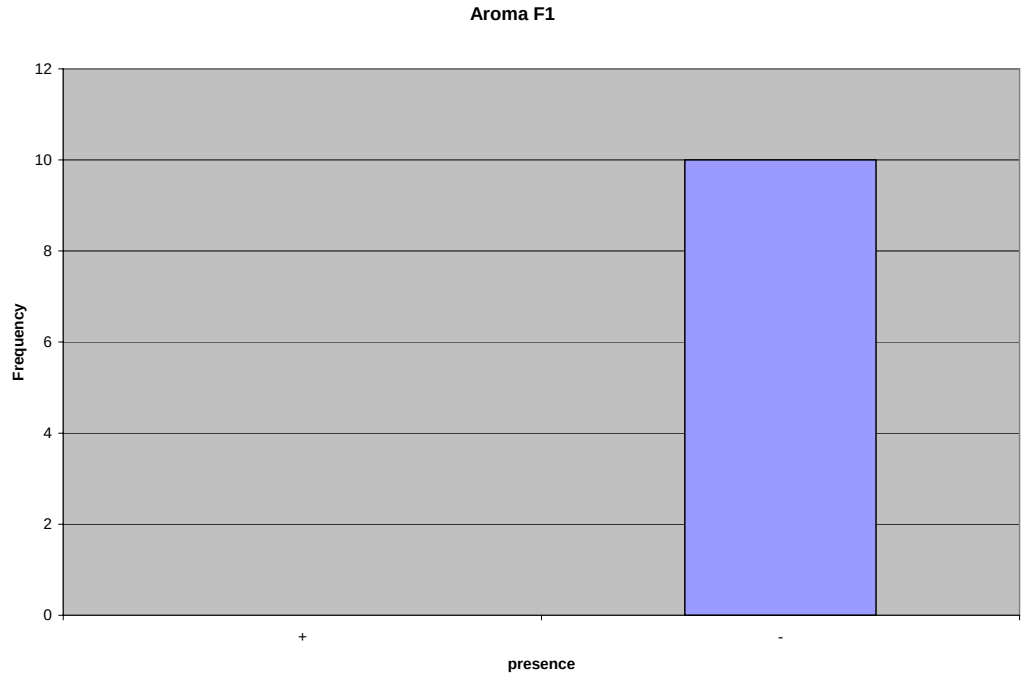


BB

Clarkia breweri *C. concinna*

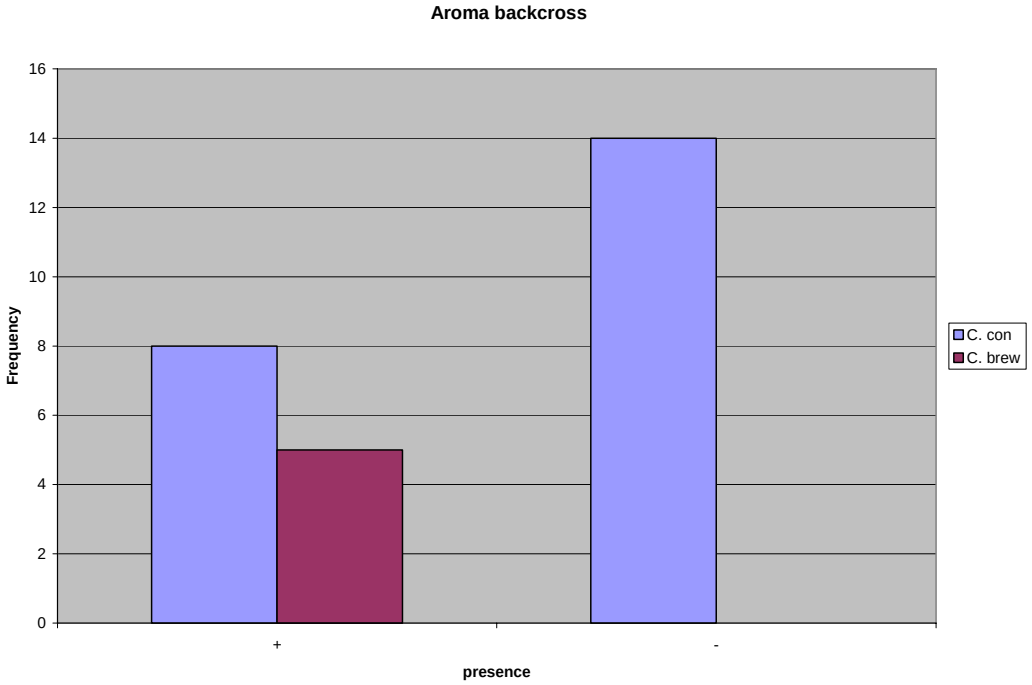


CC

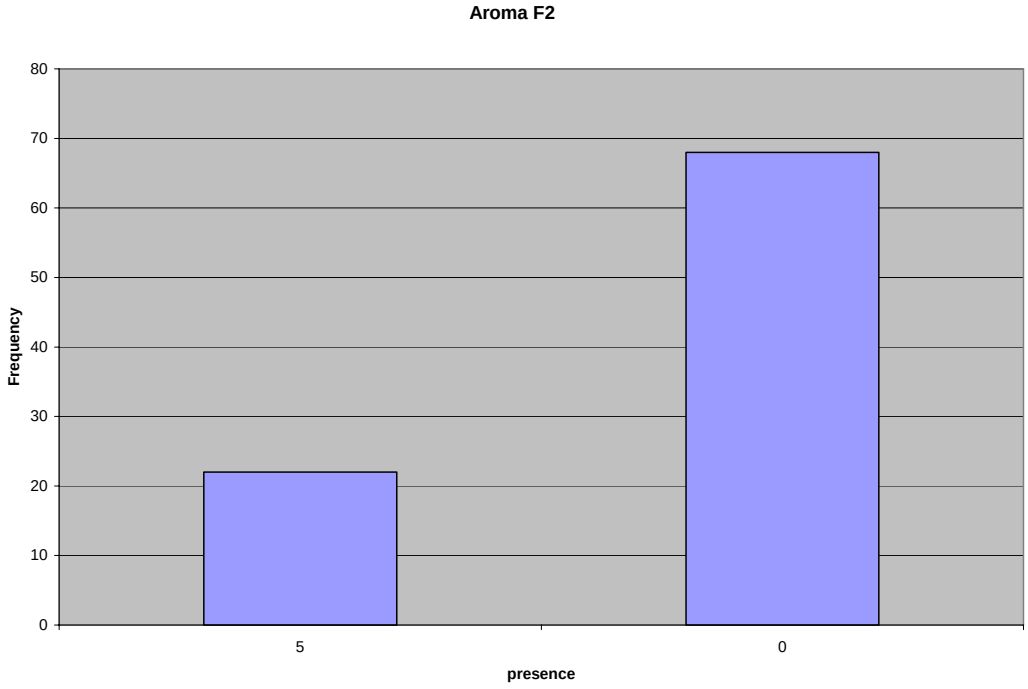


DD

Clarkia breweri *C. concinna*



EE



FF

Flower scent detectable to the human nose was found to be recessive. The F_1 is only faintly scented unlike the *C. breweri* parent and that the unscented individuals are far more numerous in the F_1 and F_4 hybrids (Table 6). The backcross data (Table 6) also supported the fact that scent is a recessive trait due to the lack of heterozygotes found in the *C. breweri* backcross population and the presence of the scented individuals in the *C. concinna* backcross population.

The second gene explored was invertase and its relationship to flower size in *Clarkia*. The gene controlling invertase in carnations is directly related to flower petal cell size (Woodson and Wang, 1987). The test of the cell size in the petals was done to determine if the same type of mechanism might be operating in *Clarkia*. From several photos of different plant petals from each parent type it was determined that the number of cells across the petals was not significantly different. A similar number of cells, 72-77 were counted across identical magnified areas of lateral petal lobes for both *Clarkia concinna* and *C. breweri* petals. The petal shape contains an intermediate range of individuals in the F_1 population (Table 9). In the backcross populations the ranges of the *C. breweri* backcross individuals cover the range of *C. breweri* and the F_1 individuals, the range of values in the *C. concinna* backcross did cross over into the more intermediate petal shapes also, and these data indicate incomplete dominance as the type of inheritance for the petal shape trait (Figure 6).

The hypanthium length was found to have both parental types in the backcross individuals. Hypanthium length was also scored in the parents and F_1 and found to have ranges in the F_2 generation (Table 9, Figure 6i-l). The ranges of the parents do not overlap. The F_1 individuals are intermediate. The hypanthium length was found to have

a range of values in both backcross populations which were similar to the parental ranges of hypanthium length (Table 8), however it was noted that one individual in the *C. concinna* backcross populations displayed *C. breweri* type hypanthium lengths. These data indicate that hypanthium length is not inherited in the same monogenic way as the previous two traits.

Flower hue was determined by eye with the use of the color designations shown in Figure 5. The flower hue ranges observed appeared independent of the other floral traits which seemed to segregate together as a complex of traits (Table 8). The F_1 hybrids and *C. breweri* parents spread across the entire color range while the *C. concinna* parent displayed only the darkest pink color of petals. The segregation data did not clarify the inheritance of flower hue, the *C. breweri* backcross was dark in color and the *C. concinna* backcross colors cover the entire range of petal colors (Tables 7 and 8 and Figure 6q-t).

Days to flower ranges were recorded as short, middle or long days to flowers. The days to flower of the parental plants was complicated due to the apparent hybrid vigor and early germination of some F_1 plants (Figure 6m and n).

The broad leaf shape is seen to be the more prominent parental type (*C. concinna*). The leaf shape ranges of the F_1 are intermediate and like the *C. concinna* parent (Table 7, Figure 6e and f). Those plants resulting from the backcross to the *C. breweri parent* were similar to the *C. breweri* leaf type and the intermediate F_1 and the *C. concinna* backcross also ranged over the parental and F_1 value (Figure 6e-h).

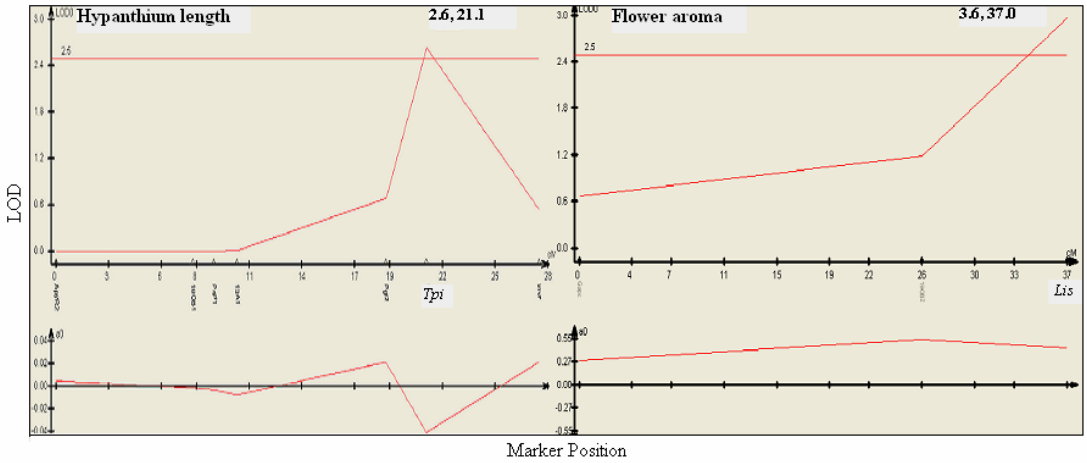
The *C. breweri* parent has almost no anthocyanin in the stem and as a result is green in color along the whole length of the stem. The *C. concinna* parent was found to have a red colored stem along the entire stem length often out to the base of the leaves

along the branches save in seedlings. In the F_1 individuals the stem anthocyanin was present only up to the midpoint of the stems length. Stem anthocyanin was considered *C. concinna* type if the complete stem up to the base of the flower was red in color, *C. breweri* in type if green in color (Figure 4). The F_1 hybrid type that displayed a red color only up half the stem represented heterozygotes or intermediates (Figure 4). The *C. concinna* backcross individuals ranged over all three values of stem anthocyanin. The *C. breweri* backcross individuals ranged over the present value (*C. concinna*) and intermediate value (F_1) (Table 7, Figure 6u-x).

Apical dominance difference can be seen in Figure 4 on the individual plants and the distribution over different populations can be seen in Figure 6y-bb. The F_1 individuals were of intermediate value and 2 were like the *C. concinna* parent type (Table 7). The *C. concinna* backcross individuals ranged over intermediate and *C. concinna* parent like values. The *C. breweri* backcrosses consisted of those individuals that appeared like the *C. breweri* parent and those that appeared to have similar apical dominance to the *C. concinna* parent.

The difference between the parental species for all the divergent traits discussed above can be seen in Figure 6 and Table 7.

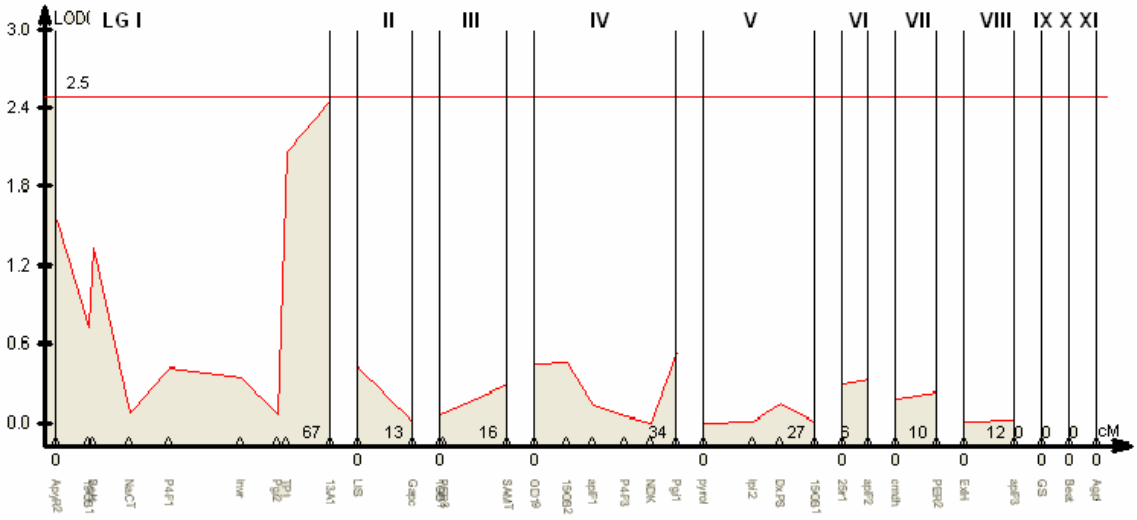
Figure 7. Quantitative trait analysis from QTL Cartographer on the traits hypanthium length and flower aroma



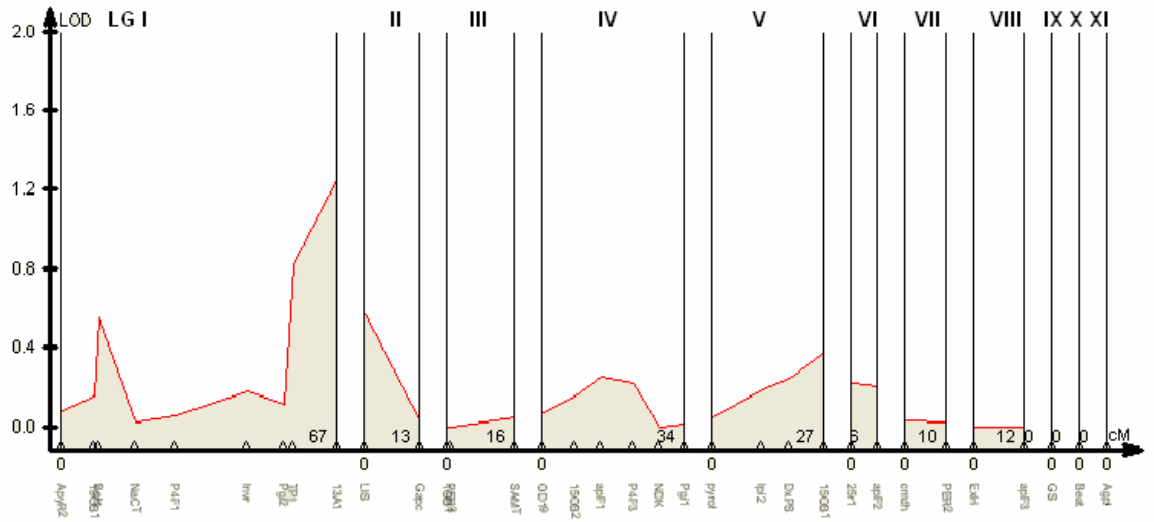
[1] The LOD scores are reported here for those traits that had significant QTL with an LOD >2.5. The trait name and the LOD, marker position are reported at the top of the figure. The peak LOD values are labeled at the top of the figures for hypanthium length and flower aroma.
[2] The linkage groups not identified by Joinmap could not be included in the QTL analysis, except as single genes. No linkage was found to these unincluded linkage groups and the segregation data did not suggest that either of these traits were linked to markers found on linkage groups VII to XI.

Figure 8. Quantitative trait analysis from QTL Cartographer over all linkage groups for hypanthium length, petal shape, and leaf shape

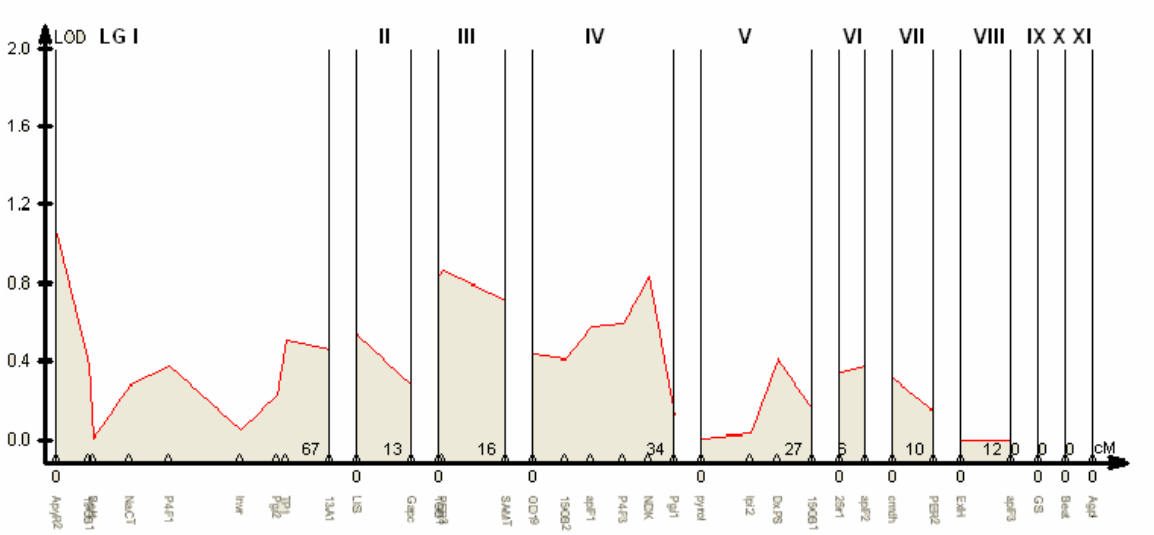
Hypanthium Length



Petal Shape



Leaf Shape



DISCUSSION

Summary

The mapping of the F_2 population *Clarkia concinna* x *C. breweri* has given rise to the first map within the *Clarkia* genus that contains STS/CAPS markers that will be useful for other *Clarkia* species. The list of primers in Appendix D reports all those primers which have been shown to amplify fragments using *Clarkia* DNA. These primers will amplify orthologous fragments in other species of *Clarkia.* The formation of gene complexes (groups of tightly linked genes controlling related aspects of a syndrome) is supported by the single marker analysis of the floral traits. Excepting the hypanthium length all other floral traits were at least partially controlled by genes associated with only two loci, *Samt* and *Lis*. At least two traits (aroma and flower shape) appear to be controlled by one major gene. The two clusters of genes controlling critical floral traits provide some supporting evidence for the formation of gene complexes to preserve the floral syndromes that attract specific pollinators to the flower.

Evaluation of Population and Linkage Map

The drawback of this F_2 population used as a mapping population is that the F_2 generation is ephemeral, the seed derived from selfing these individuals will not breed true. However, this is to be a preliminary map to provide a general outline of the

relationship between these two species and to explore how useful the CAPS markers and consensus sequence markers created are in a mapping project. In an F_2 population it is difficult to measure QTL mapping in several locations or over several years of F_2s (Young, 2000). The QTL mapping population in this project were all grown in the same location in the same year.

The mapping project presented here builds on previous available sequence data that was employed to explore relatedness between *Clarkia* species. In the future the STS markers placed on the *C. concinna* x *C. breweri* map may be used to compare taxa related in Onagraceae.

One main tenet of Lewis's work with *Clarkia* has been that, "structural rearrangement of chromosomes is of prime importance as a mechanism which permits adapted gene combinations to persist immune from recombinations and for this reason is probably the most important single factor in the evolution of *Clarkia*, particularly with respect to species formation" (Lewis, 1953, p. 15). Lewis made note of the breeding structure in *Clarkia* as one which facilitates the accumulation of structural rearrangements because of the prevalence of at least some degree of self-pollination in vigorous individuals of all species.

The high levels of chromosomal rearrangement observed in the genus *Clarkia* may be the result of spontaneous chromosomal breakage and heterozygote advantage (Levin, 2002). Translocation effects could explain how a group of genes such as a group of floral characteristics remain associated with low recombination frequency within the translocation. The gene group accumulations seen in other species do not seem to be the result of translocations (Bradshaw Jr. et al., 1998; Fishman et al., 2001, 2002; Hodges et

al., 2002; Stuurman et al., 2004). In *C. concinna* and *C. breweri* the floral traits are very conspicuous, but I was unable to determine if the traits were associated with a translocation that kept the gene combinations intact. *Samt* and *Lis* are on different linkage groups and they are presumably not both associated with the translocation. We expected to observe from the translocation a high number of heterozygous segregation out in a certain location, but this was not observed.

In the absence of the complicated pollen fertility affects from the *C. breweri* parent the fertility of the F_1 is expected to be 50% and the F_2 population would be expected to segregate out into those plants with high pollen grain viability (~100%) and those with low pollen grain viability (~50%). In this instance the translocation would have been mapped by analyzing the segregation of markers and those linked to the translocation that would display an increase in the amount of heterozygote individuals. The complications caused by the *C. breweri* parent prevent identifying this region or the ambiguous region around *Tpi* in linkage group I positively as the site of the translocation.

Several areas of this map display skewing toward one parent or the other (*C. concinna Tpi* and *GapC, C. breweri Samt, Ipi2, DxPS,* and *Bebt*) or to the heterozygous condition (*Pgic1*). The linkage distortion from the effect of the *C. breweri* parent may be the cause of the high amount of skewing seen in Appendix A and Table 7. These areas of skewing provide several places where the gene affecting *C. breweri* pollen fertility might be located.

Due to the skewing of these areas it may not be determined at this time where the translocation between *Clarkia concinna* and *C. breweri* is located. The most probable site is around the marker *Tpi* due to the ambiguous marker distances which are found

between the surrounding markers and *Tpi,* but the high amount of *C. breweri* types located around the *Ipi* marker is another possible location although there is little ambiguity of marker placement noted in close associating with *Ipi.* The areas where a high amount of skewing is present are not predicted by theory but are evident in previous studies, as stated by Burr and Burr (1991). The areas of skewing observed in this linkage map were not predicted originally, but are believed to be partially due to the lack of fertility in the *C. breweri* parent.

The pollen fertility test revealed that the translocation may be maintained in the F_2 through F_4 population, but there are complicating effects present in the parent *C. breweri* which prevent mapping of the translocation using pollen fertility studies. The range of fertility observed in the F_2 hybrid represents an interaction between the mechanism of pollen viability loss in *C. breweri* and the loss of fertility that would be expected in the presence of a single translocation (Appendix C). The amount of heterozygosity in the fourth filial generation appears to have been maintained at the same level (slightly higher than 50%) as that of the F_1 generation. However, there are more parental types recovered in the F_4 generation (Table 7). An increase in the number of parental types could indicate some genetic control of the skewing of the *C. breweri* parent. There was one center of high heterozygosity that the translocation should cause was seen in the fourth linkage group, containing the markers *Pgi, Ndk,* and *Aldc*; however, there was another area near *Tpi* where the markers had ambiguous distances from one another suggesting the arms of a quadrivalent either of these areas may be the site of the translocation.

The QTL analysis of traits revealed that the floral traits were not all controlled by single genes. With the use of further inbred lines in the future eventually greater power

of statistical analysis should elucidate those genes controlling these traits to a greater degree. Using the map in *Clarkia* the distances between many more loci may be elucidated once larger portions of the genome are cloned and aligned with the aid of the mapping markers.

Incorporation/Evaluation of Previous Findings

Pgic1 and *Pgic2* were previously found to assort independently of one another and also segregated as unlinked loci in the *C. concinna* x *C. breweri* F_2 population. The map (Figure 5) places *Pgic1* on linkage group (LG) IV and *Pgic2* on LG I. Evidence for multiple *Tpi* duplications was not found with the use of the *Tpi* primer pairs that were utilized in this study. The primer pairs appear to amplify a single gene of the *Tpi* duplication set.

Floral morphology traits showing tight linkage to markers are the scent cosegregating with the STS marker *Lis* (Linalool synthase), petal shape also segregating with *Lis* and hypanthium length which has a major QTL at the marker *Tpi*. Some QTL included in the large linkage group I are hypanthium length and stem anthocyanin. Raguso demonstrated that *Lis* appears to be highly upregulated in *C. breweri* and that control of the scent of the *C. breweri* flowers may be monogenic in nature. I have found that scent cosegregates with the *Lis* marker while the other markers and traits (flower shape) are farther in distance from the aroma loci and *Lis*. Cosegregation between scent and *Lis* confirms the hypothesis that the change in linalool synthase expression is primarily responsible for the difference in scent between *C. concinna* and *C. breweri*.

The link between LIS and scent is also supported by data from previous work with *C. breweri*'s scent (Pichersky et al., 1994; Dudareva et al., 1996). These data support those found earlier by Raguso and are consistent with the hypothesis that the *Lis* or a closely linked enhancer sequence is responsible for the difference in scent between *C. breweri* and *C. concinna*. Flower shape is also tightly linked to *Lis*, but *Lis* expression would not be expected to affect flower shape, so a different gene located close to the *Lis* loci may be responsible.

The third linkage group has three possible trait loci. Leaf shape as determined by single marker segregation analysis is associated with the RAPD *Per3* while days to flower and flower hue associate with the marker *Samt;* these are indicated on the map (Figure 5). There were many QTL peaks for leaf shape and none of them were significant (above an LOD of 2.5) and only one came close to approaching an LOD of 1.0 and that is the RAPD *Per3*. The other peaks under LOD of 1.0 are not appreciably smaller than the tallest peak these other peaks may be real QTL affecting the leaf width; however, without a larger data set this cannot be determined.

Though only two of the QTL present have an LOD above 2.5 and the other trait's QTL were not significant, this deficiency of significant QTL can be attributed to the small number of available markers (40) and relatively low number of individuals in the population (95). For these reasons, the highest non-significant QTL were noted for each trait and marked on the map if the peaks corresponded to peaks under single marker analysis (Figure 5). The QTL with significant LOD, above 2.5, were those scoring scent and hypanthium length (Figure 7). The hypanthium length scored a significant QTL that was located on linkage group 1 near *Tpi* and the QTL explains 14% of the variation in

hypanthium length. Though this is a large percentage for this study it is expected that other loci also control the hypanthium length and have not yet been located. Though scent segregated normally without the heterozygous class included, a 3:1 ratio (Table 8), QTL interval mapping can not be considered significant due to the present/absent scoring method that was utilized. However, it is interesting that both the segregation data and single marker analysis of the scent place this trait with the marker *Lis* and the QTL analysis also recognized the QTL near *Lis.* Table 8 describes flower scent and shape as single genes reflecting simple Mendelian heredity. The F_1 and backcross data demonstrate that the aroma trait appears to be dominant in the *C. breweri* parent due to the presence of scent in some *C. concinna* backcrossed individuals (Table 7 and 9). However, the flowers of the F_1 did not appear to be scented (as would be expected if scent were dominant) or were so faintly scented that it was not detectable reliably (Figure 6dd). The QTL data agreed with the single marker analysis data for scent that demonstrated that *Lis* explains a large percent of the scent trait detected by the human nose. There were two other QTL peaks found by QTL Cartographer, but these peaks were not significant (Figure 7).

The petal shape appears to be an incomplete dominant trait because the intermediate petal shape is observed in the F_1 and in both backcrossed populations, *C. breweri* and *C. concinna* backcrosses (Figure 6a-d, Table 9). The petal shape QTL analysis did not resolve any QTL of large effect, but three possible QTL loci were detected using the single marker analysis, one peak belonged to *Lis* as was expected, but the highest was linked to LG I. The QTL analysis did not resolve the relationship that the single marker analysis elucidated between *Lis* and the petal shape trait because none

of the QTL peaks were significant. This may be due to the miss-scoring of heterozygous petal shape, which is noted in Table. However, despite these problems a peak was found at the proposed location associating with *Lis*.

The hypanthium length segregation from Figure 6i-l indicate that the long *C. breweri* like hypanthium is more prominent but, from the backcross data and the F_2 population segregate toward the shorter hypanthium length suggesting that this gene is under polygenic and/or environmental control. The fact that the F_1 is of intermediate length also supports this conclusion of environmental control (Figure 6j, Table 9, and 7).

The flower hue, as indicated in Table 9 appears to be environmental in control because the segregation of the flower color does not follow a pattern. In Figure 6e it can be seen that in the backcross population the flower hue that was typical of the *C. breweri* parent now has a large amount of *C. concinna* backcross individuals also of that color and not of the intermediate color as would be expected. Also to the *C. breweri* backcross individuals share none of the colors of the *C. breweri* parent in the flower hue parent population distribution. All of these factors lead to the conclusion that flower hue is under the control of environmental factors and possible several genes. Despite this I have marked in Figure 5 the location of the largest peak obtained under single marker analysis located on LG III.

The days to flower trait also did not follow a pattern of inheritance, but appears to be explained by the control of several genes. In Table 7 it was seen that the F_1 and the *C. breweri* parent both flower earlier than the *C. concinna* parent and most of the *C. concinna* backcross individuals as well. The F_2 and F_4 populations were biased more toward the shorter number of days to flower as was the *C. breweri* backcross population.

These data indicate that the shorter days to flower is more prominent than the longer days to flower which appears to be more common in the *C. concinna* parental types (Figure 6m-p). Polygenic control of flower hue and days to flower explain the ratios seen in Table 7 where the F_1 heterozygote often has scores above both of the parents, having lighter and darker colors, and blooming much earlier than either parent.

The vegetative traits that were scored were compared with the floral trait behavior. These traits were not expected to be clustered together on the linkage groups as was expected and observed in the floral traits. The segregation data and single marker QTL analysis support polygenic control of the leaf width due to the presence of several small possible QTL and the observation that F_2 individuals range over a number of leaf widths and do not appear to be contained in discreet groups (Figure 6h). The backcross data do not show a mix of parental types in their populations. However, only three individuals clearly displayed parental phenotypes. The others were all of intermediate width comparable to the F_1 individual's leaf width (Table 7). Data on further inbred lines may elucidate the leaf width QTL.

Another vegetative trait is stem anthocyanin content. Due to a lack of any other single marker QTL analysis peaks the two very small signals found may represent actual minor QTL for stem anthocyanin the largest of which was located on LG 1 in Figure 5. Thus, the trait may be scored wrong or the map did not cover the region containing the gene responsible for stem anthocyanin. Scoring of the F_5 generation using a plus minus method may shed light on this trait.

The last trait analyzed was the apical dominance of the F_2 plants. Apical dominance is associated with the marker *Lis*. The presence of a single peak which is not

above an LOD of 2.5 was found using single marker QTL analysis and located in Figure 5 on LG II. The apical dominance in Figure 6y-bb, indicates that the gene is under polygenic control due to the distribution of individuals in the backcross population being contrary to that of the parental individuals.

Those traits that appear to be under control of a single gene were scent, and petal shape. The hypanthium length appears to be under polygenic control, but a major QTL for hypanthium length was located using QTL analysis near *Tpi* on LG I (Figure 7a). The other traits were not found to have significant QTL above and LOD of 2.5. Single marker analysis was used to identify those loci that affected each trait the most and these loci were identified with the trait in Figure 5. Figure 6 displayed the distribution of each trait in the several populations and determined that the other traits: flower hue, days to flower, leaf shape, stem anthocyanin and apical dominance appeared to be under environmental control and/or the control of several genes. Of those traits under polygenic control such as flower hue, days to flower, and apical dominance had mapped loci that explained some part of their segregation pattern. The other traits, leaf width and stem anthocyanin did not display significant correlations with other loci mapped and may be explained better by genes that have not yet been mapped.

Comparison Among the Floral QTL from Other Species

The hypothesis was, as Gottlieb (1984) stated, that divergence of species may be explained by changes in one or two genes, and this was tested for eight traits that diverged within the genus *Clarkia*. It appears that in, several cases, the traits are

governed by only one major gene (aroma, petal shape) or several genes (hypanthium, flower hue, days to flower, and apical dominance). The other traits, leaf shape and stem anthocyanin did not resolve QTL peaks, but they did display distributions indicating environmental effects in the F_2 and backcross populations.

Due to the pollinator syndromes evinced by *C. concinna* and *C. breweri,* several genes may be affecting the preference of the pollinators. The QTL analysis, single locus analysis, and backcross data of the morphological traits provides insight into how these traits are controlled genetically. The morphological trait inheritance may be explained by single genes (major QTL, tight linkage) or they may be polygenic traits controlled by many factors (many minor QTL). Understanding how a floral trait is controlled will provide information on how the trait may have changed over time and how swiftly that change may have occurred. The single marker analysis data may suggest if the divergence of the traits occurred before the speciation event or after. Those characteristics that are controlled simply have an increased likelihood of diverging at a faster pace than those characters that are controlled by several genes. Those traits under simple genetic control and that may have changed swiftly are more likely to affect traits that have diverged more quickly than traits under polygenic control that may have diverged after the species were separated at a slower pace (Gottlieb, 1984). The hypothesis was proposed earlier that the floral traits might be expected to be under monogenic control while those traits which did not affect the pollinators would be under polygenic control. The results reflect this partially. Not all of the floral characters appear to be controlled by single genes; however, none of the traits that did not affect the pollinators were monogenic. The results appear to support the hypothesis that those traits

affecting the floral divergence of a species are more likely to be under the control of only one or two genes.

Complexes, groupings of the floral traits, were also expected. The traits that affected the pollinators may have become linked by translocations. It was hypothesized by Lewis that these complexes of traits would remain together due to the more favorable arrangement of certain combinations of alleles in these locations, such as a suite of characters making up a pollinator syndrome. For example, it was discovered in *Aquilegia* that the QTL for different floral traits were often closely associated, indicating that linkage or pleiotropy cause at least some of the integration seen in the species investigated (Hodges et al., 2002). The QTL effect described in *Aquilegia* may also occur in the linked *Clarkia* traits flower shape, scent and flower hue. These complexes of traits could be indicators of swift speciation which provided stongly associated floral traits to allow the divergence of the species pollinator syndrome.

The scent of the *Clarkia* flowers segregated in an unexpected manner. The backcross populations with all *C. breweri* types being scented and most of the *C. concinna* backcrossed individuals having no or faint scent. Scent was thought to be a dominant trait; however, it has proven difficult to detect scent of the F_1 individuals, so some individuals that are scented may be scored as lacking scent (Table 3). The *Petunia* species scent QTL studies indicated that though several genes in the pathway for scent were known, the QTL for the scented petunia appeared to be explained by an enhancer of scent. The QTL seen in Figure 7 for flower aroma has a peak at 3.6 associating with the marker *Lis.* The data strongly indicate that *Lis* is the gene responsible for scent in *Clarkia breweri*.

The change required to produce linalool synthase within *C. breweri* from unscented *C. concinna* appears to be uncomplicated as discussed by Raguso, Pichersky, and others. From studies on other species such as *Petunia* it is apparent that the scent produced by *C. breweri* could have appeared very swiftly. The primary difference in scent that was scored (e.g. ability of the human nose to detect the scent of the flower) also appears to have a monogenic basis. Data collected by Raguso (1995), the QTL analysis, and the backcross data all indicated that scent is inherited monogenically even when using unsophisticated qualitative techniques of measuring the morphological trait such as scent detection as a plus or minus score.

The single gene analysis and the backcross data indicate that the shape of the lateral lobes of the flower may be controlled by a single gene of large affect. Counts to measure the petal cell size revealed that, unlike carnation, it is the number of cells, not the size of the cells which differs between the *C. concinna* small petal and the larger *C. breweri* petal lobes. These data were also supported later when the *Invr* marker responsible for petal cell size in other species did not show a QTL peak (Figure 8). These data are not in agreement with the several QTL that were found to control petal shape in *Mimulus* (Fishman et al., 2002). However, it was expected that for petal shape a significant QTL on LG II would be found and was not. This may be due to the small population number affecting the ability of QTL Cartographer to recognize significant linkage. The data in Appendix A show that the petal shape for a *C. breweri* type flower was always present when there was scent and the petal shape for *C. concinna* was always present when there was no scent. The heterozygotes were ambiguous as stated earlier, but this finding of no recombination between the *C. breweri* and *C. concinna* scores

indicates very tight linkage between flower scent and petal shape, which QTL Cartographer was unable to recognize.

The hypanthium length gave mixed results between the QTL analysis and the backcross data. Data indicated by the segregation ratio of the hypanthium in the F_2 and F_4 and backcrosses revealed that the hypanthium trait is controlled by more than one gene which segregates over all of these populations. The gene, though having a possible impact on the pollinator syndrome, is controlled by a several genes and/or the environment as was postulated from the results in studies on corolla length in *Petunia* (Stuurman et al., 2004) and *Mimulus* (Fishman et al., 2002) and the data from Raguso (1995). However, the QTL data revealed only one significant QTL present near *Tpi* (Figure 7 and 8). All the other QTL found are under an LOD 1.0. These results suggest that if the hypanthium length is controlled by more than one gene, as the backcross data indicated, the other genes responsible have yet to be identified and mapped on the present linkage groups.

Days to flower and hue appeared to be controlled by environmental factors, though the flower hue may not have been expressed consistently. Flower hue and days to flower were the last two floral traits which QTL analysis were conducted and it was found that the only QTL above an LOD of 1.0 were associated with the marker *Samt* for both traits. In *Petunia* the flower color was controlled by a single gene, while in *Mimulus* it appeared that it was controlled by several minor QTL. The single marker analysis of this trait indicates that these two traits are controlled by several genes in *Clarkia* and do not disagree with the *Mimulus* results. If only a single gene were responsible, one should be able to observe the segregation pattern. Because a clear pattern was not observed it is

suspected that two or more gene and perhaps primarily environmental factors are responsible for the phenotype observed. The closely linked traits could represent a complex and control of these characters may be maintained together through their association with *Samt* or another gene close by that enables the syndrome to be inherited more completely than if the major QTL of the floral traits were spread over several linkage groups. More data is needed to expose trait complexes in *Clarkia.*

Leaf width varied greatly over the F_2 population indicating polygenic control and /or environmental effects are operating on the trait. The distribution of the F_2 appeared normal and the QTL analysis of the leaf width trait revealed many insignificant peaks the largest of which is located on LG III. The number of genes possibly affecting leaf shape in other species makes it difficult to nominate a candidate gene which, if any may be affecting the phenotypic difference seen between *C. concinna* and *C. breweri.* The data from the study on *Mimulus* do not disagree with the data indicating that leaf shape was controlled by several genes and/or environmental factors (Fishman et al., 2002).

Stem anthocyanin gave mixed results between the single marker analysis and the backcross data. The control of the stem anthocyanin trait appeared to be monogenic in the F_2 population with clearly segregating parental morphologies. The backcross did not dispute a monogenic inheritance. The QTL analysis of stem anthocyanin revealed that there were two peaks not above a significance level of an LOD of 2.5, but the only peaks seen through any of the linkage groups. Again, this gene may be controlled largely by a gene which is not yet mapped in *Clarkia*. However, due to the break in the pattern of inheritance between the parental and backcross distributions polygenic control of this trait is likely. The data gathered so far indicate a trait which is controlled by several

genes. Single gene control by an unmapped loci of major effect does not seem likely due to the distribution data. These results agree with the results from *Mimulus* that anthocyanin may be controlled by several genes.

The last trait measured was the divergence seen between *C. concinna* and *C. breweri* for apical dominance. This trait appeared highly variable in the F_2 population, reflecting polygenic control of the growth habit of the plant. Floral position has been implicated in the preference of moths to certain other flowering species and may be a trait affecting pollinator preference, but these studies were in reference to a deflexed flower and not differing heights of the flowers compared with the ground. It is more probable that the lack of a large data set of markers and a highly inbred population are affecting the statistical power of the QTL analysis to pinpoint major genes controlling these floral traits.

The single marker QTL analysis revealed that depending on the gene single major QTL (scent and petal shape) were found, along with several traits explained by minor or insignificant QTL only (hypanthium, flower hue, time, stem anthocyanin and leaf width). Those genes affected by several QTL were in the majority. Whether a trait was floral or not did not seem to determine the number of insignificant QTL found, but this may be due to low resolution power causing artificially high LOD scores. These results indicate that whether a trait affected pollinators or not, its expression may still be controlled by several genes. Swift speciation due to smaller number of affecting loci does not seem to be a mechanism that is operating in all the floral traits of *Clarkia*. However, the scent and petal shape, which are highly significant factors in attracting moth pollinators in

other species, may have differentiated very swiftly due to their association on the same chromosome and the single gene control proposed for both traits.

The clustering of floral genes into a complex of traits was observed in *Petunia* and *Aquilegia* (Stuurman et al., 2004; Hodges et al., 2002). The floral genes do appear to cluster around certain areas of the genome in *Clarkia*. The clusters of floral morphology traits in other species are not associated with known translocations. Those clusters of traits found in *Aquilegia* are held together by some other mechanism, which may simply involve selection and drifting to fixation. The past results seem to indicate that a translocation may not be necessary for the preservation of gene complexes. The floral trait QTL were found associated with only three linkage groups and only one marker per linkage group was found to have a cluster of floral traits, LG I contained *Tpi* and hypanthium length, LG II contained *Lis* linked to scent and petal shape, and possibly LG III has a linkage containing *Samt* with flower hue and days to flower (Figure 5). These findings suggest that these traits are sufficiently linked to maintain the floral syndromes needed for pollination to occur. Hypanthium length (the only floral trait not found associating with other floral traits) may not affect the bees' ability to retrieve nectar from the flower. The hypanthium length though it is a part of floral morphology appears to be controlled by more than one gene, unlike scent and petal shape. If this is true, then the only QTL shown to affect pollinators in other species, flower hue, flower position (apical dominance), days to flower and flower aroma are clustered around only two markers, *Samt* and *Lis*. These findings support the formation of tightly linked groups of floral characteristics, but it is not known if they occur as Lewis (1953) suggested within a translocation. The gene complexes may be linked tightly enough that floral syndromes

remain coherent to the pollinator. Or it may be these traits are explained by a single gene in these regions and do not form a complex with other genes, but at this time the density of the *Clarkia* map does not allow testing of this hypothesis. What can be concluded is that the traits affecting pollinator visitation in *Clarkia* do appear in complexes of related floral traits. These findings indicate that the speciation of *C. concinna* and *C. breweri* may have occurred quickly with significant pressure to require syndrome formation for species isolation, or as Lewis (1953) suggested they may be congregating in regions of low recombination created by chromosomal rearrangements.

Future Prospects

One drawback of the previous phylogenetic studies in *Clarkia* is that not all recognized genera were included in the studies. Specifically sampling within the complex tribe Onagreae was limited to 1-3 genera out of nine. The sampling of these genera must be broadened to clarify intergeneric relationships (Levin, 2003). Molecular analysis has been conducted in several genera of the Onagreae tribe including *Fuchsia* (fuchsia), *Lopezia*, *Clarkia*, *Epilobium* (willowherb), Oenothera (evening primrose), *Chamerion* and *Gaura* (Levin et al., 2003). None of these studies looked beyond the tools needed to examine a single species and/or were not specific enough to identify linkages among the section of species. The map of *Clarkia* will provide linkage data for synteny comparison to many genera using degenerate/consensus primer design. Our ability to develop gene-specific STS primers in *Clarkia* without the availability of a closely related model plant demonstrates that STS/CAPS markers can be used to

construct a map even when sequence for a species and, in this study, an entire genera is not available. The use of conserved intron-targeted markers within single copy genes possibly expands the use of the primers used in the study to other *Clarkia* species. The primers created will allow for many other species relationships to be studied within the genus *Clarkia* and possibly other genera within the family Onagraceae.

Broader sampling of genera must be obtained to clarify intergeneric relationships within the family Onagraceae (Levin et al., 2003). Use of the STS markers designed for this experiment possibly provides several genera within Onagraceae with genetic markers. Comparisons made between all nine genera using such materials and methods as are outlined here will aid in clarifying evolutionary relationship within the family Onagraceae. Molecular analysis has been conducted in several genera of the Onagreae tribe including *Fuchsia* (fuchsia), *Lopezia*, *Clarkia* (red ribbons), *Epilobium* (willow herb), *Oenothera* (evening primrose), *Chamerion* and *Gaura* (Levin, 2003). None of these studies looked beyond the tools needed to examine a single species and/or were not specific enough to identify linkages among the section of species. The map of *Clarkia* using STS/CAPS markers will provide linkage data for synteny comparison to many species using degenerate primer design.

Lewis and Raven (1958) suggested that there may be a heritable trait underlying chromosome breakage, but there have been no direct measures of spontaneous breakage rates, the fragments indicating breakage seem to be rare. The amount of phenotypic evolution is not a good indicator of the amount of karyotypic evolution that has taken place between two species and, in general, annual species seem to possess more chromosomal differences (Grant, 1981). Mapping different populations with known

breakages and strong genetic isolation from other varieties would allow a glimpse at the areas of breakage along the chromosomes to ascertain what type of rearrangements are separating these species and what type of genetic control may be responsible for the breakages. These chromosomal rearrangements still lack a generally accepted mechanism of explanation. How they become established and whether they contribute to speciation is not known. The tools for comparative genetic mapping, including the STS primer bank begun for *Clarkia* will provide understanding by overcoming some of the shortcomings of cytological studies, such as gene disruption of meiotic pairing or indistinguishable chromosomes (Livingstone and Rieseberg, 2003). Comparative mapping is of use not only in comparing species orthologs, but genera and even family orthologous sequence to some extent, depending on the marker types available. Finding a gene or mechanism underlying genetic control leading to chromosomal breakage in *Clarkia* would explain how these rearrangements come about and mapping these areas with available markers would provide a detailed view of the breakage areas of interest.

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APPENDICES

APPENDIX A:

SEGREGATION DATA

Appendix A. Linkage group segregation data

L1	F2 gen	19OB1	ApyR2	BEBT	NaCT	P4F1	stat
	c-1	c	b	b	h	b	b
	c-2	c	b	.	h	b	c
	c-3	b	b	b	b	b	c
	c-4	.	c	.	h	.	b
	c-5	b	b	b	b	b	h
	c-6	b	b	b	h	b	h
	c-7	b	b	b	h	b	c
	c-8	b	b	h	b	c	c
	c-9	c	b	b	b	c	b
	c-10	c	b	c	b	b	c
	c-11	c	b	b	h	c	.
	c-12	c	c	c	h	b	b
	c-13	b	b	b	b	b	b
	c-14	c	b	h	b	b	b
	c-15	b	.	b	b	.	c
	c-16	c	b	b	b	b	b
	c-17	c	b	h	b	c	b
	c-18	b	b	b	b	c	c
	c-19	b	b	b	b	b	b
	c-20	c	b	.	b	c	c
	c-21	.	b	.	b	c	c
	c-22	c	b	b	h	c	c
	c-23	b	b	b	b	b	c
	c-24	b	b	b	b	b	b
	c-25	c	b	b	b	c	c
	c-26	c	b	b	b	c	c
	c-27	b	b	b	b	b	c
	c-28	c	b	h	b	c	c
	c-29	c	b	b	b	c	c
	c-30	c	b	b	b	.	c
	c-31	.	b	.	.	b	b
	c-32	b	b	b	.	b	c
	c-33	b	b	b	.	c	b
	c-34	b	b	b	h	b	b
	c-35	c	b	b	b	c	c
	c-36	b	b	c	h	b	b
	c-37	b	b	b	.	b	b
	c-38	c	b	b	.	b	b
	c-39	c	b	b	h	.	b
	c-40	c	b	b	.	c	c
	c-41	b	b	b	b	b	b
	c-42	.	b	.	b	b	h
	c-43	b	b	h	c	b	c
	c-44	c	b	h	b	b	h
	c-45	b	b	b	b	b	c
	c-46	c	b	b	c	b	.

c-47	b	b	b	h	b	c
c-48	b	b	b	h	b	h
c-49	b	b	b	c	b	h
c-50	.	b	b	b	b	c
c-51	c	c	c	b	c	b
c-52	c	b	c	c	c	c
c-53	c	b	.	c	b	c
c-54	c	b	c	c	c	h
c-55	c	b	h	c	b	b
c-56	b	b	b	b	b	b
c-57	b	b	b	h	b	b
c-58	b	b	b	h	b	h
c-59	b	b	b	h	b	c
c-60	b	c	.	h	b	h
c-61	.	c	.	h	.	h
c-62	b	b	b	h	c	b
c-63	b	b	b	b	b	b
c-64	c	c	b	c	b	c
c-65	b	b	b	b	b	c
c-66	b	b	b	b	b	.
c-67	b	b	b	h	b	h
c-68	b	b	.	h	b	h
c-69	b	b	b	b	b	b
c-70	c	b	b	c	c	c
c-71	b	b	h	b	b	c
c-72	b	b	b	b	b	c
c-73	c	c	h	c	c	h
c-74	c	c	.	c	c	h
c-75	b	b	h	b	b	h
c-76	b	b	b	b	b	c
c-77	b	b	b	b	b	h
c-78	b	b	.	b	b	h
c-79	b	b	b	b	b	c
c-80	.	c	b	c	c	h
c-81	c	b	c	c	b	h
c-82	b	.	h	c	b	c
c-83	c	b	b	c	b	c
c-84	b	.	h	b	b	.
c-85	b	b	b	c	b	c
c-86	b	c	b	c	c	c
c-87	b	b	h	b	c	c
c-88	b	b	.	b	b	.
c-89	.	b	.	b	b	c
c-90	b	b	h	c	b	.
c-91	c	c	c	b	b	.
c-92	b	b	c	c	b	h
c-93	b	b	.	h	c	.
c-94	b	.	.	b	b	c
c-95	b	.	b	.	b	c

	19OB1	**ApyR2**	**BEBT**	**NaCT**	**P4F1**	**stat**
C. concinna	abs	abs	750+450	700	abs	anthocy
C. breweri	2200	650	1300	650	700	absent
			1300	~700		

F2 gen	Invr	Pgi2	TPI	Hyp.	13A1
c-1	h	c	c	b	b
c-2	c	c	c	.	c
c-3	b	b	b	b	b
c-4	c	c	c	c	c
c-5	b	b	b	.	b
c-6	h	h	c	.	b
c-7	h	c	c	.	b
c-8	h	h	c	c	c
c-9	h	h	c	c	c
c-10	c	c	c	b	c
c-11	h	c	c	c	b
c-12	c	c	c	.	c
c-13	b	b	b	b	b
c-14	h	c	c	h	c
c-15	b	b	b	c	c
c-16	b	b	b	b	b
c-17	h	c	c	c	c
c-18	b	h	c	.	b
c-19	b	b	b	.	b
c-20	c	c	.	c	c
c-21	c	c	c	c	c
c-22	h	h	c	c	c
c-23	b	b	h	c	b
c-24	b	b	b	b	b
c-25	h	.	c	c	c
c-26	h	b	c	c	c
c-27	b	b	b	.	b
c-28	c	b	c	.	c
c-29	h	h	c	.	c
c-30	h	.	c	.	b
c-31	b	.	c	.	b
c-32	b	.	h	.	b
c-33	h	.	c	.	c
c-34	h	h	.	h	c
c-35	b	.	h	.	c
c-36	h	.	c	c	c
c-37	b	b	b	c	c
c-38	h	c	c	b	c
c-39	h	.	c	.	c
c-40	h	.	c	b	c
c-41	h	b	c	.	c
c-42	c	c	c	b	c
c-43	h	b	.	c	b
c-44	c	c	c	.	c
c-45	b	b	c	c	b
c-46	h	h	c	.	c

c-47	**h**	c	c	.	b
c-48	h	c	c	c	b
c-49	h	h	h	c	b
c-50	h	c	c	.	b
c-51	c	c	c	h	c
c-52	c	c	c	b	c
c-53	c	h	c	.	c
c-54	c	c	c	.	c
c-55	c	c	.	.	b
c-56	b	b	.	h	b
c-57	h	b	.	b	c
c-58	b	c	.	c	c
c-59	h	c	.	h	c
c-60	c	c	.	.	c
c-61	c	.	.	c	.
c-62	h	c	.	c	c
c-63	h	c	.	b	c
c-64	c	c	.	.	c
c-65	b	c	.	.	c
c-66	c	.	.	.	c
c-67	b	.	.	.	b
c-68	h	.	.	.	b
c-69	b	.	.	c	.
c-70	c	c	.	h	b
c-71	b	b	.	.	b
c-72	h	.	.	b	c
c-73	c	c	.	.	c
c-74	c	c	.	h	c
c-75	b	.	.	c	b
c-76	b	.	.	.	b
c-77	h	.	.	.	c
c-78	b	c	.	b	c
c-79	b	c	.	.	c
c-80	h	.	.	c	b
c-81	b	.	.	b	b
c-82	h	h	.	c	b
c-83	h	h	.	.	b
c-84	h	b	.	c	c
c-85	c	b	.	.	b
c-86	c	c	.	c	c
c-87	b	h	.	.	c
c-88	b	c	.	b	b
c-89	c	.	.	c	c
c-90	c	ch	.	.	.
c-91	h	c	.	.	c
c-92	h	c	.	.	b
c-93	h	.	.	c	c
c-94	h	.	.	.	c
c-95	h	.	.	b	c

Invr	Pgi2	TPI	Hyp.	13A1
275+200	850	1800	short	abs
450	1200	900+850	long	1200
500	1200	1800		

L2	GAPc	19OB2	ptsp	LiS	arom	apdm
c-1	c	c	b	b	b	b
c-2	c	c	c	c	c	c
c-3	c	c	h	c	b	b
c-4	c	.	h	h	c	b
c-5	h	c	h	c	c	b
c-6	c	c	h	h	h	b
c-7	c	c	h	c	h	b
c-8	c	c	b	h	b	c
c-9	h	c	h	h	c	b
c-10	h	c	c	c	c	c
c-11	h	c	b	h	b	.
c-12	h	c	c	c	c	b
c-13	b	b	b	b	h	b
c-14	c	c	h	c	c	h
c-15	h	c	b	b	h	b
c-16	h	c	h	b	b	h
c-17	c	c	h	b	c	h
c-18	c	c	h	c	b	h
c-19	c	b	h	b	b	b
c-20	b	c	c	h	h	h
c-21	b	c	c	c	c	b
c-22	c	c	c	h	h	c
c-23	c	c	b	h	h	h
c-24	.	c	h	b	b	h
c-25	.	c	c	c	c	c
c-26	.	c	h	h	h	h
c-27	.	c	b	h	c	h
c-28	c	c	.	h	.	h
c-29	.	c	c	h	c	h
c-30	.	c	c	h	c	h
c-31	.	.	.	.	c	b
c-32	.	c	h	.	h	b
c-33	.	c	.	.	c	h
c-34	.	c	h	.	h	h
c-35	.	c	h	c	c	c
c-36	.	c	h	.	c	b
c-37	.	c	b	.	h	b
c-38	.	c	h	.	h	b
c-39	.	c	h	c	c	h
c-40	.	c	c	c	c	b
c-41	.	b	b	b	c	h
c-42	b	.	c	b	c	h
c-43	b	b	b	b	b	h
c-44	.	c	b	b	c	h
c-45	.	.	b	b	b	b
c-46	.	.	b	h	c	h

c-47	b	b	c	b	c	h
c-48	c	c	b	b	b	b
c-49	.	c	c	c	c	h
c-50	h	b	c	h	c	h
c-51	b	b	b	.	c	b
c-52	c	c	c	c	c	h
c-53	h	c	c	h	b	b
c-54	c	.	b	b	c	b
c-55	h	c	c	h	c	h
c-56	h	b	c	h	b	h
c-57	c	c	c	h	c	h
c-58	h	b	c	h	b	b
c-59	h	b	b	c	c	h
c-60	h	c	c	h	c	h
c-61	h	.	c	c	c	h
c-62	c	c	c	h	c	h
c-63	c	b	c	h	.	h
c-64	.	c	c	.	c	b
c-65	c	c	b	h	b	h
c-66	c	c	c	c	c	b
c-67	.	b	c	h	c	b
c-68	.	b	b	b	b	b
c-69	.	b	c	h	c	h
c-70	.	c	c	c	c	b
c-71	.	b	b	b	b	b
c-72	.	c	c	h	c	b
c-73	.	c	c	.	c	c
c-74	.	c	c	c	c	h
c-75	.	b	b	b	b	b
c-76	.	b	c	.	c	h
c-77	.	b	c	c	c	b
c-78	.	b	b	b	b	h
c-79	.	b	b	c	c	b
c-80	.	.	h	c	c	c
c-81	.	b	b	b	c	b
c-82	.	c	h	.	b	b
c-83	.	b	c	c	c	h
c-84	.	c	b	.	c	h
c-85	.	b	b	h	b	b
c-86	.	c	c	.	c	b
c-87	.	c	c	h	c	b
c-88	.	c	h	h	b	c
c-89	.	.	b	b	c	b
c-90	.	b	b	b	.	b
c-91	.	b	.	b	.	.
c-92	.	b	c	b	c	b
c-93	.	b	b	b	.	h
c-94	.	b	c	.	c	b
c-95	.	b	c	.	c	b

GAPc	19OB2	flsp	LiS	arom	apdm
800+625+175	650	skinny	600	no scent	tall
1600	abs	broad	400+200	scent	bushy
1600			1000		

L3	fltm	fhue	SAMT	CRS	leaf	PER3	1OB1
c-1	h	b	b	b	b	b	.
c-2	h	b	b	b	.	b	.
c-3	h	c	b	b	b	b	.
c-4	c	h	c	.	b	.	.
c-5	b	h	b	b	.	c	.
c-6	h	h	b	b	.	b	.
c-7	h	b	b	b	.	b	.
c-8	b	b	c	c	c	c	.
c-9	c	h	c	h	c	.	.
c-10	b	b	b	h	c	b	.
c-11	.	c	c	.	c	c	.
c-12	b	b	b	.	.	b	.
c-13	h	c	h		b	b	b
c-14	b	c	h	.	c	b	b
c-15	h	b	b	.	c	b	b
c-16	c	b	b		b	c	c
c-17	b	b	h	.	h	b	b
c-18	h	b	b	.	.	b	b
c-19	b	b	b	.	.	b	b
c-20	b	b	b	.	c	b	b
c-21	h	h	h	.	b	b	b
c-22	b	h	b	.	h	b	b
c-23	b	h	c	.	b	c	c
c-24	b	b	b	.	b	b	b
c-25	h	h	b	.	c	c	c
c-26	h	b	c	.	c	c	c
c-27	h	c	b	.	.	b	b
c-28	c	c	c	.	.	b	b
c-29	b	h	b		.	b	b
c-30	b	c	b	.	.	b	b
c-31	c	c	c	.	.	.	.
c-32	c	b	b	.	.	b	b
c-33	c	c	b	.	.	b	b
c-34	h	h	b	.	b	b	b
c-35	b	b	b	.	.	b	b
c-36	b	c	b	.	h	b	b
c-37	b	b	b	.	b	c	b
c-38	h	b	b	.	b	b	b
c-39	h	h	b	.	.	b	b
c-40	b	h	b	.	c	b	b
c-41	h	c	b	b	.	b	b
c-42	c	h	b	b	c	b	b
c-43	b	c	c	b	c	b	c
c-44	b	h	h	b	.	b	b
c-45	h	b	b	b	b	b	b

c-46	h	c	b	.	.	b	b
c-47	h	b	b	b	.	b	b
c-48	h	c	c	b	b	b	b
c-49	h	b	h	.	c	b	b
c-50	b	c	c	c	.	c	c
c-51	h	h	b	b	h	b	b
c-52	h	b	b	b	b	b	b
c-53	b	c	b	b	.	b	b
c-54	b	h	c	b	.	c	c
c-55	b	b	h	h	.	b	b
c-56	b	c	b	h	c	b	b
c-57	b	h	c	c	h	c	c
c-58	b	c	c	.	h	b	c
c-59	h	c	b	.	c	b	b
c-60	b	h	h	.	.	b	b
c-61	b	c	.	.	h	.	.
c-62	b	h	c	.	h	c	b
c-63	b	c	h	.	c	b	b
c-64	b	h	h	.	.	b	b
c-65	b	b	h	.	.	b	b
c-66	b	h	h	.	.	b	b
c-67	b	c	b	.	.	b	b
c-68	b	b	b	.	.	b	b
c-69	c	b	b	.	c	b	b
c-70	b	c	c	.	c	c	c
c-71	h	c	b	.	.	b	b
c-72	b	c	h	.	b	b	b
c-73	h	b	h	.	.	b	b
c-74	h	b	c	.	b	c	c
c-75	h	h	b	.	b	b	b
c-76	b	b	b	.	.	b	b
c-77	b	h	b	.	.	b	b
c-78	h	c	h	.	h	b	b
c-79	h	b	c	.	.	b	b
c-80	c	c	c	.	h	.	b
c-81	h	h	c	.	c	c	b
c-82	h	h	b	.	c	b	b
c-83	h	b	h	.	.	b	b
c-84	c	c	c	.	c	c	b
c-85	h	c	b	.	.	b	b
c-86	b	h	h	.	h	b	b
c-87	b	c	h	.	.	c	b
c-88	h	h	b	.	b	b	b
c-89	b	h	h	.	b	b	b
c-90	h	c	h	.	.	b	b
c-91	b	.	h	.	.	b	b
c-92	h	c	h	.	.	b	b
c-93	c	c	c	.	h	c	b
c-94	h	c	b	.	.	b	b

c-95	b	b	b	.	b	b	b
	fltm	**fhue**	**SAMT**	**CRS**	**leaf**	**PER3**	**1OB1**
	mid	dk pink	350+475	1300	lanceolate	abs	abs
	late	pink	800	1000	lobate	325	700
			800	~1200			

L4	OD19	15OB2	ApyF1	P4F3	NDK	Pgi1	AldC
c-1	.	b	c	b	.	h	.
c-2	b	c	c	c	b	h	.
c-3	b	.	c	c	h	h	
c-4	.	.	c	.	.	h	.
c-5	b	c	c	b	c	h	.
c-6	b	c	c	c	.	h	.
c-7	c	b	b	c	.	b	.
c-8	c	c	c	c	.	h	.
c-9	c	c	c	c	.	h	.
c-10	c	c	c	c	.	h	.
c-11	b	b	c	c	c	h	.
c-12	b	c	c	c	c	h	.
c-13	c	b	c	c	c	h	.
c-14	c	c	c	c	.	h	.
c-15	b	b	b	.	b	b	.
c-16	c	.	.	b	c	h	
c-17	c	c	c	c	c	h	.
c-18	.	c	c	c	h	h	.
c-19	c	c	c	c	h	h	.
c-20	b	b	c	c	c	h	.
c-21	b	b	c	c	c	h	.
c-22	b	b	c	c	c	h	.
c-23	c	b	c	b	.	h	.
c-24	.	b	c	b	c	h	.
c-25	c	b	c	c	c	h	.
c-26	c	c	c	b	c	h	.
c-27	c	c	c	c	c	h	.
c-28	b	c	c	b	c	h	.
c-29	c	c	c	c	h	h	.
c-30	c	c	c	c	c	c	.
c-31	.	.	c	c	.	h	.
c-32	c	c	b	b	b	b	.
c-33	b	b	c	c	.	b	.
c-34	.	b	b	b	b	.	.
c-35	.	c	c	c	c	b	.
c-36	c	c	c	c	c	b	.
c-37	c	.	b	c	c	b	.
c-38	c	c	c	c	c	b	.
c-39	c	c	c	.	c	.	.
c-40	.	c	c	c	.	.	.
c-41	c	c	c	b	h	h	b
c-42	c	b	b	b	b	b	b
c-43	.	b	c	b	c	c	c
c-44	.	b	b	b	b	b	h
c-45	.	c	c	c	c	c	c

c-46	.	b	b	b	c	h	h
c-47	.	c	b	b	h	h	b
c-48	.	c	c	c	c	c	b
c-49	.	c	c	c	c	c	h
c-50	.	c	c	b	h	h	h
c-51	.	c	c	b	h	h	h
c-52	.	b	c	b	.	h	h
c-53	.	c	c	c	c	c	c
c-54	.	c	c	c	h	b	b
c-55	c	c	c	b	c	c	c
c-56	b	c	c	c	c	c	c
c-57	c	b	b	b	b	b	b
c-58	b	c	c	b	c	c	c
c-59	b	b	c	b	.	h	c
c-60	b	b	b	b	b	b	b
c-61	b	.	c	.	c	c	c
c-62	c	b	b	b	c	b	h
c-63	b	c	c	b	.	h	b
c-64	c	c	.	b	c	.	.
c-65	b	b	.	c	b	.	h
c-66	b	b	.	b	c	b	h
c-67	b	c	c	b	.	h	h
c-68	c	b	b	b	.	b	b
c-69	b	c	b	c	c	c	h
c-70	c	b	c	c	c	b	b
c-71	c	c	c	c	h	h	c
c-72	c	c	c	b	c	h	b
c-73	c	c	c	c	c	.	.
c-74	c	c	c	c	c	h	.
c-75	c	c	c	c	c	h	.
c-76	c	c	c	c	c	.	.
c-77	c	b	b	b	.	.	.
c-78	c	c	c	b	.	h	.
c-79	c	c	c	c	c	c	.
c-80	.	.	b	b	b	b	.
c-81	b	b	b	b	b	b	.
c-82	c	b	.	b	b	h	.
c-83	c	b	b	c	h	h	.
c-84	.	c	.	b	b	h	.
c-85	b	b	b	b	b	.	.
c-86	c	b	b	b	h	h	.
c-87	b	b	c	c	c	h	.
c-88	b	b	c	c	h	h	.
c-89	b	c	b	c	.	b	.
c-90	b	c	b	b	b	b	.
c-91	b	b	b	b	.	c	.
c-92	b	c	c	c	c	h	.
c-93	b	b	.	c	h	c	.
c-94	.	b	c	c	c	c	.

c-95	b	b	c	c	c	.	.
	OD19	**15OB2**	**ApyF1**	**P4F3**	**NDK**	**Pgi1**	**AldC**
	abs	700	1450	abs	800+775	.	500
	600	abs	abs	1300	600+350+300		425
					~750	1350	~500

L5	Pyrol	IPI2	DxPS	15OB1	L6	25r1	ApyF2
c-1	h	.	.	b	**c-1**	c	c
c-2	.	b	b	b	**c-2**	c	c
c-3	h	h	b	c	**c-3**	c	c
c-4	b	.	.	.	**c-4**	b	c
c-5	b	.	b	b	**c-5**	b	c
c-6	b	b	b	b	**c-6**	c	c
c-7	b	b	.	b	**c-7**	c	c
c-8	b	.	b	b	**c-8**	b	.
c-9	h	.	b	b	**c-9**	b	c
c-10	b	.	b	b	**c-10**	c	c
c-11	.	b	b	b	**c-11**	b	c
c-12	.	b	b	b	**c-12**	b	c
c-13		b	b	b	**c-13**	b	.
c-14	.	b	b	b	**c-14**	c	c
c-15	.	b	h	b	**c-15**	c	c
c-16	.	b	b	b	**c-16**	c	.
c-17		b	b	c	**c-17**	c	c
c-18	.	b	b	b	**c-18**	c	c
c-19	.	b	b	b	**c-19**	b	c
c-20	.	b	b	b	**c-20**	.	c
c-21	.	b	b	b	**c-21**	.	c
c-22	.	b	b	b	**c-22**	c	c
c-23	.	b	.	b	**c-23**	c	c
c-24	.	b	h	b	**c-24**	b	b
c-25	.	b	b	c	**c-25**	b	b
c-26	.	b	.	b	**c-26**	c	c
c-27	.	b	h	b	**c-27**	b	c
c-28	.	c	h	c	**c-28**	c	c
c-29	.	b	.	b	**c-29**	b	c
c-30		b	b	b	**c-30**	c	c
c-31	.	b	.	.	**c-31**	c	c
c-32	.	b	b	b	**c-32**	b	c
c-33	.	.	h	c	**c-33**	b	c
c-34	.	.	.	b	**c-34**	c	c
c-35	.	b	c	b	**c-35**	b	c
c-36	.	.	b	c	**c-36**	c	c
c-37	.	.	b	b	**c-37**	b	c
c-38	.	.	b	b	**c-38**	b	c
c-39	.	.	.	b	**c-39**	c	c
c-40	.	.	b	b	**c-40**	b	c
c-41	.	b	b	c	**c-41**	b	c
c-42	.	b	b	b	**c-42**	c	c
c-43	.	b	b	c	**c-43**	c	c
c-44	.	b	b	b	**c-44**	c	c
c-45	.	c	h	b	**c-45**	b	c

c-46	.	b	.	c	**c-46**	c	c
c-47	.	b	h	c	**c-47**	c	c
c-48	.	h	b	b	**c-48**	c	c
c-49	.	h	.	c	**c-49**	b	c
c-50	.	b	.	c	**c-50**	c	c
c-51	.	b	b	b	**c-51**	b	b
c-52	.	b	b	c	**c-52**	c	c
c-53	.	c	b	c	**c-53**	c	c
c-54	.	c	c	c	**c-54**	b	c
c-55	c	.	h	c	**c-55**	c	c
c-56	c	c	c	c	**c-56**	c	c
c-57	b	b	c	b	**c-57**	c	c
c-58	.	c	b	c	**c-58**	c	c
c-59	b	b	.	b	**c-59**	c	c
c-60	b	b	b	b	**c-60**	.	c
c-61	.	.	b	.	**c-61**	b	c
c-62	b	b	.	b	**c-62**	c	c
c-63	b	b	b	b	**c-63**	.	c
c-64	.	b	b	b	**c-64**	b	b
c-65	.	b	.	b	**c-65**	b	c
c-66	b	b	b	b	**c-66**	c	c
c-67	.	c	b	c	**c-67**	c	c
c-68	.	b	b	b	**c-68**	c	c
c-69	b	.	c	c	**c-69**	c	c
c-70	b	b	b	b	**c-70**	c	c
c-71	b	.	b	b	**c-71**	c	c
c-72	b	b	b	b	**c-72**	c	c
c-73	.	b	b	b	**c-73**	c	c
c-74	.	b	b	c	**c-74**	b	c
c-75	.	b	b	b	**c-75**	c	c
c-76	b	b	.	b	**c-76**	c	c
c-77	b	b	b	b	**c-77**	c	c
c-78	.	b	b	b	**c-78**	c	c
c-79	.	.	c	c	**c-79**	.	b
c-80	.	b	b	.	**c-80**	.	c
c-81	b	b	b	b	**c-81**	.	c
c-82	.	b	h	b	**c-82**	b	.
c-83	b	b	b	c	**c-83**	c	c
c-84	.	h	b	c	**c-84**	.	.
c-85	.	b	b	b	**c-85**	c	c
c-86	.	b	h	c	**c-86**	b	b
c-87	.	b	b	c	**c-87**	b	b
c-88	b	b	b	b	**c-88**	b	b
c-89	b	b	b	c	**c-89**	b	c
c-90	b	b	h	b	**c-90**	c	c
c-91	.	b	b	b	**c-91**	c	c
c-92	.	.	.	b	**c-92**	b	b
c-93	.	.	.	c	**c-93**	b	c
c-94	.	.	.	c	**c-94**	b	c

c-95	.	.	.	c	**c-95**	c	c
	Pyrol	**IPI2**	**DxPS**	**15OB1**		**25r1**	**ApyF2**
	1300	1100+~700	1500	abs		325	1000
	900	1900	1300	900		abs	abs
	~1000	~2000	~1400				

L7	CMDh	PER2	L8	ExH	ApyF3
c-1	b	b	**c-1**	.	b
c-2	b	b	**c-2**	h	b
c-3	b	b	**c-3**	b	c
c-4	h	.	**c-4**	.	b
c-5	b	b	**c-5**	.	c
c-6	b	b	**c-6**	b	c
c-7	b	b	**c-7**	.	c
c-8	b	b	**c-8**	c	.
c-9	h	.	**c-9**	h	b
c-10	h	b	**c-10**	h	.
c-11	b	b	**c-11**	b	b
c-12	h	b	**c-12**	c	b
c-13	b	b	**c-13**	b	.
c-14	.	c	**c-14**	b	b
c-15	c	c	**c-15**	h	b
c-16	c	c	**c-16**	h	.
c-17	h	b	**c-17**	c	b
c-18	b	b	**c-18**	b	b
c-19	b	b	**c-19**	b	.
c-20	h	b	**c-20**	h	b
c-21	h	b	**c-21**	h	b
c-22	c	c	**c-22**	h	b
c-23	h	b	**c-23**	c	b
c-24	h	b	**c-24**	h	c
c-25	h	b	**c-25**	h	b
c-26	h	b	**c-26**	c	c
c-27	h	b	**c-27**	h	.
c-28	b	b	**c-28**	h	b
c-29	h	b	**c-29**	h	c
c-30	h	b	**c-30**	h	b
c-31	h	.	**c-31**	.	b
c-32	h	b	**c-32**	c	b
c-33	c	c	**c-33**	.	c
c-34	c	c	**c-34**	h	b
c-35	h	b	**c-35**	h	b
c-36	c	c	**c-36**	h	b
c-37	h	b	**c-37**	h	b
c-38	c	c	**c-38**	b	b
c-39	.	b	**c-39**	.	b
c-40	h	b	**c-40**	b	b
c-41	b	c	**c-41**	h	b
c-42	c	c	**c-42**	h	b
c-43	h	c	**c-43**	b	b
c-44	c	c	**c-44**	h	b
c-45	b	c	**c-45**	b	c

c-46	c	c	**c-46**	b	b
c-47	h	c	**c-47**	.	c
c-48	b	c	**c-48**	b	b
c-49	h	c	**c-49**	b	b
c-50	c	c	**c-50**	b	b
c-51	h	b	**c-51**	b	b
c-52	c	c	**c-52**	b	b
c-53	c	c	**c-53**	b	b
c-54	c	c	**c-54**	b	b
c-55	c	c	**c-55**	h	b
c-56	c	c	**c-56**	b	c
c-57	c	c	**c-57**	b	b
c-58	c	c	**c-58**	b	b
c-59	c	c	**c-59**	h	b
c-60	c	c	**c-60**	c	b
c-61	c	.	**c-61**	c	b
c-62	c	c	**c-62**	b	b
c-63	c	c	**c-63**	b	c
c-64	c	c	**c-64**	b	b
c-65	h	b	**c-65**	b	.
c-66	c	c	**c-66**	h	.
c-67	c	c	**c-67**	h	b
c-68	c	c	**c-68**	b	b
c-69	c	c	**c-69**	b	b
c-70	c	c	**c-70**	b	b
c-71	h	b	**c-71**	b	b
c-72	c	c	**c-72**	b	b
c-73	c	b	**c-73**	b	b
c-74	c	c	**c-74**	b	b
c-75	c	c	**c-75**	b	.
c-76	c	c	**c-76**	b	b
c-77	c	c	**c-77**	c	c
c-78	h	b	**c-78**	b	c
c-79	c	c	**c-79**	c	c
c-80	.	.	**c-80**	.	b
c-81	h	b	**c-81**	.	.
c-82	c	c	**c-82**	.	.
c-83	b	b	**c-83**	b	c
c-84	c	c	**c-84**	b	.
c-85	.	b	**c-85**	h	c
c-86	c	c	**c-86**	b	b
c-87	b	b	**c-87**	b	b
c-88	c	c	**c-88**	b	b
c-89	h	b	**c-89**	h	b
c-90	h	b	**c-90**	b	c
c-91	c	c	**c-91**	c	b
c-92	h	b	**c-92**	b	b
c-93	c	b	**c-93**	h	.
c-94	b	b	**c-94**	.	c

c-95	b	b	**c-95**	.	c
	CMDh	**PER2**		**ExH**	**ApyF3**
	300+200	abs		2200	abs
	500	600		1800	800
	1000			~2000	

L9	GS	L10	BEAT	L11	AGPl
c-1	.		.		c
c-2	b		c		b
c-3	h		h		c
c-4	.		.		.
c-5	b		.		c
c-6	b		.		c
c-7	b		c		b
c-8	b		h		.
c-9	b		.		c
c-10	b		b		c
c-11	h		c		c
c-12	b		c		b
c-13	b		h		b
c-14	b		h		c
c-15	b		c		c
c-16	h		h		b
c-17	b		.		c
c-18	b		h		b
c-19	b		h		b
c-20	b		c		b
c-21	b		c		c
c-22	b		c		c
c-23	b		h		c
c-24	c		h		b
c-25	b		c		b
c-26	b		b		c
c-27	b		b		c
c-28	b		c		c
c-29	b		.		c
c-30	b		.		b
c-31	.		.		.
c-32	b		c		.
c-33	.		.		c
c-34	b		c		.
c-35	b		.		b
c-36	b		.		c
c-37	b		c		c
c-38	b		.		c
c-39	b		.		c
c-40	b		c		.
c-41	h		h		.
c-42	b		h		.
c-43	b		c		.
c-44	h		c		.
c-45	b		c		.

c-46	h	b	.
c-47	b	c	.
c-48	.	c	.
c-49	b	c	.
c-50	b	b	.
c-51	b	c	.
c-52	b	c	.
c-53	b	b	.
c-54	b	b	.
c-55	b	.	.
c-56	b	b	.
c-57	b	b	.
c-58	h	b	.
c-59	b	b	.
c-60	b	h	.
c-61	.	.	.
c-62	.	b	.
c-63	c	b	.
c-64	h	c	.
c-65	b	c	.
c-66	b	b	.
c-67	b	b	.
c-68	b	b	.
c-69	b	h	.
c-70	b	b	.
c-71	b	b	.
c-72	b	b	.
c-73	b	h	.
c-74	.	h	.
c-75	b	.	.
c-76	b	c	.
c-77	b	b	.
c-78	b	h	.
c-79	b	h	.
c-80	b	.	.
c-81	.	c	.
c-82	b	h	.
c-83	b	h	.
c-84	b	b	.
c-85	b	b	.
c-86	b	h	.
c-87	h	b	.
c-88	b	b	.
c-89	.	h	.
c-90	b	h	.
c-91	b	c	.
c-92	b	c	.
c-93	b	c	.
c-94	b	b	.

c-95	b	h	.
	GS	**BEAT**	**AGPl**
	275+300+400	600+300	.
	225	900	
	~1000	900	1000

singles	25r2	25r3	25r4	13A2	P4F2	PER1	1OB2	1OB3
c-1	c	c	b	c	c	c	.	.
c-2	c	c	c	b	c	c	.	.
c-3	b	b	c	c	c	b	.	.
c-4	c	c	b	c	.	.	.	.
c-5	c	b	c	b	b	c	.	.
c-6	c	c	b	c	b	c	.	.
c-7	c	c	c	c	b	c	.	.
c-8	c	b	c	c	c	c	.	.
c-9	c	c	c	c	c	.	.	.
c-10	b	c	c	c	c	c	.	.
c-11	c	c	c	c	c	c	.	.
c-12	c	c	c	c	b	c	.	.
c-13	c	b	b	c	b	c	b	c
c-14	c	c	b	c	b	b	c	b
c-15	c	c	c	c	.	b	b	b
c-16	c	c	b	b	.	b	c	b
c-17	c	c	b	c	c	c	b	c
c-18	b	c	c	c	c	c	b	c
c-19	b	b	b	b	c	c	b	c
c-20	.	.	.	b	c	c	c	b
c-21	.	.	.	c	c	c	c	b
c-22	c	c	b	c	b	c	c	b
c-23	c	c	b	b	b	b	c	c
c-24	b	c	b	b	c	c	c	b
c-25	c	c	b	c	b	c	c	b
c-26	c	c	b	c	b	c	c	c
c-27	c	b	c	c	.	b	c	b
c-28	b	c	c	c	c	c	b	c
c-29	c	c	c	c	.	c	b	c
c-30	c	c	b	c	.	c	c	c
c-31	c	b	b	b	c	.	.	.
c-32	c	c	b	b	b	c	b	b
c-33	c	c	c	b	c	c	b	c
c-34	c	c	b	c	c	c	b	b
c-35	b	c	b	c	c	b	c	c
c-36	c	b	b	c	c	c	b	b
c-37	b	c	b	c	b	b	c	b
c-38	c	c	c	c	b	c	c	b
c-39	.	.	.	c	.	b	b	c
c-40	.	.	.	c	c	c	b	b
c-41	c	c	c	c	b	b	b	c
c-42	c	c	c	c	b	b	b	c
c-43	c	c	b	b	b	c	c	b
c-44	c	c	c	b	b	c	c	c
c-45	b	b	c	c	c	c	c	c

c-46	c	c	c	b	b	c	b	c
c-47	.	c	b	b	b	c	b	c
c-48	b	b	b	.	c	c	c	c
c-49	c	c	c	c	c	c	c	b
c-50	c	c	b	b	b	c	c	c
c-51	c	c	c	c	b	b	c	c
c-52	c	c	c	b	b	c	b	b
c-53	b	c	c	b	c	c	c	c
c-54	c	c	c	b	c	c	c	b
c-55	b	c	c	c	b	c	b	c
c-56	c	c	c	b	c	b	c	c
c-57	c	c	b	c	b	c	c	b
c-58	c	c	c	c	b	c	c	c
c-59	b	c	b	c	b	c	c	c
c-60	c	c	c	c	b	c	c	c
c-61	b	c	c	.	.	.	.	.
c-62	b	c	b	c	b	b	b	b
c-63	.	b	c	c	b	c	b	b
c-64	.	c	c	c	b	c	b	b
c-65	c	b	b	c	c	c	b	b
c-66	c	b	c	c	b	c	b	b
c-67	c	c	c	c	b	c	b	b
c-68	c	c	b	.	b	c	c	c
c-69	c	c	c	.	c	b	c	b
c-70	c	c	b	b	b	c	c	b
c-71	b	b	b	b	b	c	c	c
c-72	b	b	c	c	b	c	b	c
c-73	c	b	c	b	b	c	b	c
c-74	b	b	c	b	b	b	c	c
c-75	c	c	b	c	b	c	b	c
c-76	c	b	c	c	b	b	b	c
c-77	c	b	c	c	b	c	b	c
c-78	b	b	c	c	c	b	b	c
c-79	c	b	b	c	b	b	c	b
c-80	c	c	b	b	b	.	b	c
c-81	c	c	b	c	c	c	c	b
c-82	.	.	c	c	b	c	b	b
c-83	c	.	b	c	c	c	b	b
c-84	.	.	.	b	c	b	c	b
c-85	b	.	c	c	c	c	b	b
c-86	b	b	c	b	c	c	c	b
c-87	c	b	c	c	c	b	b	b
c-88	c	c	c	c	b	c	b	b
c-89	c	b	c	b	c	c	c	b
c-90	b	b	c	.	c	b	b	b
c-91	c	b	c	c	c	c	b	b
c-92	c	b	c	c	c	c	c	b
c-93	b	b	c	c	b	b	b	b
c-94	b	c	c	b	c	c	b	b

c-95	c	b	c	b	c	c	b	b
	25r2	**25r3**	**25r4**	**13A2**	**P4F2**	**PER1**	**1OB2**	**1OB3**
	550	700	900	abs	abs	1300	650	abs
	abs	abs	abs	900	400	abs	abs	575

singles	1OB4	OD4	ADh	ApyR1	PPE	OD11	15OB3
c-1	.	.	h	b	.	.	b
c-2	.	c	h	c	.	b	b
c-3	.	c	b	b		b	b
c-4	.	.	c	c	.	.	.
c-5	.	c	b	b	.	b	b
c-6	.	b	h	b	.	c	c
c-7	.	c	h	c	.	c	c
c-8	.	c	h	c	.	b	b
c-9	.	c	h	b		b	c
c-10	.	b	h	c	.	b	b
c-11	.	b	h	b	.	b	b
c-12	.	b	h	c	.	b	b
c-13	b	c	c	b	.	b	b
c-14	b	c	h	b	.	b	b
c-15	b	c	h	.	.	b	b
c-16	b	c	b	b		b	b
c-17	b	b	b	c	.	b	b
c-18	b	b	h	c	.	b	b
c-19	b	b	h	b	.	b	b
c-20	b	b	c	c	.	b	c
c-21	b	b	h	c	.	b	b
c-22	b	c	b	b	.	b	b
c-23	b	c	h	b	.	b	b
c-24	b	b	c	b	.	b	b
c-25	b	c	h	c	.	b	c
c-26	b	b	h	b	.	b	b
c-27	b	c	c	b	.	b	b
c-28	c	b	h	c	.	b	b
c-29	b	c	c	b		c	c
c-30	b	c	c	b	.	c	c
c-31	.	.	c	b	.	.	.
c-32	b	b	c	b	.	b	b
c-33	b	b	h	c	.	c	c
c-34	b	c	h	c	.	c	b
c-35	b	b	c	b	.	b	b
c-36	b	c	.	c	.	.	b
c-37	b	c	.	c	.	.	b
c-38	b	c	.	c	.	.	b
c-39	b	b	.	c	.	c	c
c-40	b	b	.	c	.	c	c
c-41	b	.	.	c	b	.	c
c-42	b	.	.	c	b	b	c
c-43	b	b	.	b	b	b	c
c-44	b	c	.	c	b	b	b
c-45	b	c	.	b	b	b	b

c-46	b	c	.	c	b	.	c
c-47	b	b	.	c	c	.	c
c-48	b	c	.	c	c	b	b
c-49	b	b	.	c	b	b	c
c-50	b	b	.	c	c	b	c
c-51	b	b	.	c	c	c	b
c-52	b	c	.	b	b	c	c
c-53	c	b	.	c	c	b	b
c-54	b	c	.	.	c	b	c
c-55	b	c	.	c	b	b	b
c-56	b	b	.	b	b	b	b
c-57	c	b	.	c	b	b	b
c-58	b	b	.	c	.	b	b
c-59	b	b	.	c	.	b	c
c-60	b	b	.	c	.	c	c
c-61	b	.	.	c	.	.	.
c-62	b	c	.	c	.	c	c
c-63	b	b	.	b	.	b	b
c-64	c	c	.	c	.	c	b
c-65	b	.	.	c	.	c	c
c-66	b	c	.	c	.	c	c
c-67	b	b	.	c	.	b	c
c-68	b	b	.	b	.	b	b
c-69	b	b	.	c	.	b	b
c-70	c	b	.	b	.	b	b
c-71	b	c	.	c	.	b	b
c-72	b	b	.	c	.	b	c
c-73	b	b	.	c	.	c	c
c-74	b	c	.	c	.	b	c
c-75	b	c	.	c	.	b	b
c-76	b	c	.	c	.	b	c
c-77	b	c	.	c	.	b	c
c-78	b	c	.	c	.	b	b
c-79	b	b	.	c	.	b	c
c-80	b	.	.	c	.	.	.
c-81	b	.	.	c	.	c	.
c-82	b	b	.	.	.	b	b
c-83	b	c	.	c	.	b	b
c-84	b	.	.	.	.	b	c
c-85	b	.	.	c	.	b	b
c-86	b	.	.	c	.	b	b
c-87	b	b	.	b	.	b	b
c-88	b	c	.	c	.	b	.
c-89	b	b	.	c	.	b	b
c-90	b	b	.	c	.	b	c
c-91	b	.	.	c	.	c	.
c-92	b	b	.	c	.	c	.
c-93	b	.	.	c	.	b	c
c-94	b	.	.	.	.	b	b

c-95	b	b	.	.	.	c	c
	1OB4	**OD4**	**ADh**	**ApyR1**	**PPE**	**OD11**	**15OB3**
	475	abs	800	abs	475	abs	530
	abs	650	750	1800	500	1500	abs
			~800		~500		

APPENDIX B:

MORPHOLOGICAL MEASUREMENT DATA AND VALUES FOR SINGLE MARKER ANALYSIS

Appendix B. Morphological measurement data and values chosen for single marker analysis

QTL traits	Leaf	Stat	Apdm	Arom	Fhue	Fltm	Ptsp	Hypm
c-1	0.5	0	0	2	1	1.5	0.86	2.7
c-2	.	2	2	0	1	1.5	0.19	1.8
c-3	0.6	2	0	2	2	1.5	0.38	2.3
c-4	0.7	0	0	0	1.5	3	0.33	1.8
c-5	.	1	0	0	1.5	2	0.63	2.2
c-6	.	1	0	1	1.5	1.5	0.25	2
c-7	.	2	0	1	1	1.5	0.29	1.9
c-8	1	2	2	2	1	2	0.25	2
c-9	1.1	0	0	0	1.5	3	0.29	1.7
c-10	0.7	2	2	0	1	2	0.25	2.1
c-11	0.9	.	.	2	2	.	0.33	1.9
c-12	.	0	0	0	1	2	0.25	1.8
c-13	0.4	0	0	1	2	1.5	.	3.2
c-14	1.2	0	1	0	2	2	0.19	2
c-15	0.8	2	0	1	1	1.5	0.39	1.7
c-16	0.7	0	1	2	1	3	0.24	2.4
c-17	0.6	0	1	0	1	2	0.33	1.9
c-18	.	2	1	2	1	1.5	0.31	1.9
c-19	.	0	0	2	1	2	0.45	2.7
c-20	0.6	2	1	1	1	2	0.22	1.9
c-21	0.4	2	0	0	1.5	1.5	0.21	1.9
c-22	0.7	2	2	1	1.5	2	.	2
c-23	0.6	2	1	1	1.5	2	.	1.9
c-24	0.5	0	1	2	1	2	0.45	2.8
c-25	1.1	2	2	0	1.5	1.5	.	2
c-26	0.8	2	1	1	1	1.5	0.53	2
c-27	.	2	1	0	2	1.5	0.44	2.1
c-28	.	2	1	.	2	3	.	.
c-29	.	2	1	0	1.5	2	.	.
c-30	.	2	1	0	2	2	0.21	2
c-31	.	0	0	0	2	3	0.29	2
c-32	.	2	0	1	1	3	0.32	2.8
c-33	.	0	1	0	2	3	.	2.5
c-34	0.6	0	1	1	1.5	1.5	0.38	2.3
c-35	.	2	2	0	1	2	0.31	2.1
c-36	0.7	0	0	0	2	2	0.28	1.6
c-37	.	0	0	1	1	2	0.44	2
c-38	0.6	0	0	1	1	1.5	0.40	2.1
c-39	.	0	1	0	1.5	1.5	.	.
c-40	0.9	2	0	0	1.5	2	0.38	2.3
c-41	.	0	1	0	2	1.5	.	.
c-42	0.8	1	1	0	1.5	3	0.50	2.3
c-43	1	2	1	2	2	2	0.38	1.6
c-44	.	1	1	0	1.5	2	.	.

c-45	**0.6**	**2**	**0**	**2**	**1**	**1.5**	**0.33**	**2.1**
c-46	**.**	**.**	**1**	**0**	**2**	**1.5**	**.**	**.**
c-47	**.**	**2**	**1**	**0**	**1**	**1.5**	**.**	**.**
c-48	**0.6**	**1**	**0**	**2**	**2**	**1.5**	**0.33**	**2**
c-49	**0.7**	**1**	**1**	**0**	**1**	**1.5**	**0.30**	**1.9**
c-50	**.**	**2**	**1**	**0**	**2**	**2**	**.**	**.**
c-51	**0.8**	**0**	**0**	**0**	**1.5**	**1.5**	**0.43**	**1.5**
c-52	**0.4**	**2**	**1**	**0**	**1**	**1.5**	**0.33**	**2.4**
c-53	**.**	**2**	**0**	**2**	**2**	**2**	**.**	**.**
c-54	**.**	**1**	**0**	**0**	**1.5**	**2**	**.**	**.**
c-55	**.**	**0**	**1**	**0**	**1**	**2**	**.**	**.**
c-56	**1**	**0**	**1**	**2**	**2**	**2**	**0.44**	**2**
c-57	**0.8**	**0**	**1**	**0**	**1.5**	**2**	**0.31**	**2.3**
c-58	**0.6**	**1**	**0**	**2**	**2**	**2**	**0.36**	**1.8**
c-59	**.**	**2**	**1**	**0**	**2**	**1.5**	**0.44**	**2.2**
c-60	**.**	**1**	**1**	**0**	**1.5**	**2**	**.**	**.**
c-61	**0.9**	**1**	**1**	**0**	**2**	**2**	**0.50**	**2**
c-62	**0.6**	**0**	**1**	**0**	**1.5**	**2**	**0.33**	**1.9**
c-63	**0.8**	**0**	**1**	**.**	**2**	**2**	**0.35**	**.**
c-64	**.**	**2**	**0**	**0**	**1.5**	**2**	**.**	**.**
c-65	**.**	**2**	**1**	**2**	**1**	**2**	**.**	**.**
c-66	**.**	**.**	**0**	**0**	**1.5**	**2**	**.**	**.**
c-67	**.**	**1**	**0**	**0**	**2**	**2**	**.**	**.**
c-68	**.**	**1**	**0**	**2**	**1**	**2**	**.**	**.**
c-69	**1**	**0**	**1**	**0**	**1**	**3**	**0.47**	**1.9**
c-70	**1.1**	**2**	**0**	**0**	**2**	**2**	**0.47**	**3.1**
c-71	**.**	**2**	**0**	**2**	**2**	**1.5**	**.**	**.**
c-72	**0.6**	**2**	**0**	**0**	**2**	**2**	**0.39**	**.**
c-73	**.**	**1**	**2**	**0**	**1**	**1.5**	**.**	**.**
c-74	**0.5**	**1**	**1**	**0**	**1**	**1.5**	**0.41**	**2.2**
c-75	**0.3**	**1**	**0**	**2**	**1.5**	**1.5**	**0.37**	**1.4**
c-76	**.**	**2**	**1**	**0**	**1**	**2**	**.**	**.**
c-77	**.**	**1**	**0**	**0**	**1.5**	**2**	**.**	**.**
c-78	**0.6**	**1**	**1**	**2**	**2**	**1.5**	**0.32**	**2.6**
c-79	**.**	**2**	**0**	**0**	**1**	**1.5**	**.**	**.**
c-80	**0.8**	**1**	**2**	**0**	**2**	**3**	**0.38**	**1.6**
c-81	**0.9**	**1**	**0**	**0**	**1.5**	**1.5**	**0.43**	**2.2**
c-82	**1**	**2**	**0**	**2**	**1.5**	**1.5**	**.**	**2**
c-83	**.**	**2**	**1**	**0**	**1**	**1.5**	**.**	**.**
c-84	**1.1**	**.**	**1**	**0**	**2**	**3**	**0.38**	**2**
c-85	**.**	**2**	**0**	**2**	**2**	**1.5**	**.**	**.**
c-86	**1.1**	**2**	**0**	**0**	**1.5**	**2**	**0.40**	**2**
c-87	**.**	**2**	**0**	**0**	**2**	**2**	**.**	**.**
c-88	**0.6**	**.**	**2**	**2**	**1.5**	**1.5**	**0.40**	**2.5**
c-89	**0.3**	**2**	**0**	**0**	**1.5**	**2**	**0.50**	**2.2**
c-90	**.**	**.**	**0**	**.**	**2**	**1.5**	**.**	**.**
c-91	**.**	**.**	**.**	**.**	**.**	**2**	**.**	**.**
c-92	**.**	**1**	**0**	**0**	**2**	**1.5**	**.**	**.**
c-93	**1.1**	**.**	**1**	**.**	**2**	**3**	**0.32**	**2.1**

c-94	**.**	**2**	**0**	**0**	**2**	**1.5**	**.**	**.**
c-95	**0.6**	**2**	**0**	**0**	**1**	**2**	**0.30**	**2.9**
con	**>1.2**	**2**	**2**	**0**	**2**	**3**	**<0.32**	**<2.1**
brew	**<0.9**	**0**	**0**	**2**	**1**	**2**	**>0.52**	**>2.5**
F1	**.7-1.5**	**1**	**1**	**1**	**1.0-2.0**	**1.5**	**0.26-.43**	**1.5-2.5**

APPENDIX C:

POLLEN STAINING DATA

Appendix C.	Pollen counts		
line	**good**	**bad**	**% fertility**
C. concinna	144	18	0.89
	32	1	0.97
C. breweri	45	49	0.48
	33	62	0.35
	44	55	0.44
F1	49	33	0.60
	43	15	0.74
	74	31	0.70
	40	36	0.53
	42	14	0.75
F4	58	12	0.83
	48	54	0.47
	33	20	0.62
	53	11	0.83
	37	12	0.76
Bk3: F1 x C. con	89	11	0.89
Bk7: F1 x C. con	37	30	0.55
Bk8: F1 x C. brew	25	39	0.39
	45	53	0.46

APPENDIX D:

PRIMER SEQUENCES

Appendix D. Primers constructed and used to amplify *Clarkia* DNA

	PRIMER		Sequence																											
			5'	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	>	3'								
1	AAIR-F1		C	A	C	A	C	A	T	C	C	C	G	T	T	T	C	G	C	T	C									
	AAIR-R1		G	A	A	T	G	C	C	C	C	T	T	C	T	T	C	C	T	C	A	A	G							
	ACC-F3		T	C	C	C	A	T	T	A	C	T	G	T	A	G	C	A	C	C	T	C	C	A						
2	ACC-F1	*	T	A	C	T	A	C	T	T	T	T	T	A	G	A	G	T	T	G	A	A	C	C	C					
	ACC-R1	*	G	C	C	C	A	C	A	C	A	T	T	T	G	G	C	T	T	G	C	T								
	ACC-F2		G	T	G	G	A	A	G	T	A	C	C	T	G	G	T	G	G	G	A	C	C							
	ACC-R2		G	C	W	G	C	C	A	T	A	G	A	T	A	T	A	G	C	A	G	G	A	G	G					
	ACC-F3		T	C	C	C	A	T	T	A	C	T	G	T	A	G	C	A	C	C	T	C	C	A						
	ACC-R3		G	G	A	G	G	T	G	C	T	A	C	A	G	T	A	A	T	G	G	G	A							
3	ACL-F1		A	A	G	G	T	T	A	A	G	A	C	C	G	T	A	T	T	T	A	T	T	C	C	A	A	C	A	
	ACL-R1		A	G	T	C	C	A	A	A	T	T	C	G	T	C	C	C	C	A	C	T	G							
	ACL-F2		G	C	A	A	A	G	G	A	C	C	C	A	T	A	A	C	A	A	C									
	ACL-R2		T	C	T	A	A	T	C	C	A	T	G	A	A	T	G	A	A	A	G	A								
	ACL-F3		C	A	T	C	C	C	A	C	A	C	A	A	T	G	A	A	G	A	G	T								
4	ADH-F		M	A	C	T	G	C	T	G	G	T	C	A	G	G	T	C	A	T										
	ADH-F1	*	A	T	G	G	G	A	G	G	C	T	G	G	G	A	A	G												
	ADH-R1	*	G	G	A	C	T	C	C	C	C	T	T	T	C	A	A	C	A	T	G									
	ADH-F2	*	C	A	G	A	C	T	C	C	A	T	T	G	T	T	T	C	C	T	C	G								
	ADH-R2	*	A	A	C	A	G	C	A	A	C	A	C	C	C	C	A	A	C	C										
	ADH-F3		T	C	A	A	G	A	T	A	C	T	C	T	T	C	A	C	C	T	C	C								
	ADH-R3		C	T	T	G	C	C	C	C	T	T	C	A	G	C	A	G	C	C										
	ADH-F4		C	A	A	G	A	T	M	C	T	C	T	W	C	A	C	C	T	C										
5	AGPL-F	**	G	G	T	T	Y	C	A	R	G	G	I	A	C	I	G	C	I	G	A	Y	G	C						
	AGPL-R	**	T	I	C	C	I	A	T	R	T	C	Y	T	C	C	C	A	R	T	A	R	T	C						
6	AGPS-F	*	G	G	T	T	Y	C	A	R	G	G	I	A	C	I	G	C	I	G	A									
	AGPS-R	*	C	T	C	R	T	A	R	T	A	R	T	C	I	G	C	I	C	C	C	A	T							

7	ALDOC-F	*	T	G	A	G	A	A	T	G	T	T	G	A	A	T	C	C	A	A	C	A	G	A	C						
	ALDOC-R	*	C	A	A	G	A	C	C	C	T	G	A	G	T	G	G	T	G	G											
	ALDOC F3	**	T	T	C	T	T	G	C	T	G	C	T	G	A	T	G	A	G	T	C	A	A	C							
	ALDOC R3	**	G	T	C	A	A	C	C	T	T	G	A	T	A	C	C	A	G	G	A	A	G								
8	ALAT-F2		C	T	G	A	C	C	T	T	C	C	C	T	C	G	C	C	A	G											
	ALAT-R2		C	C	T	T	G	C	T	C	G	C	A	C	C	A	T	C	A	G	T										
9	ANT-F	*	C	T	I	A	T	G	G	G	W	G	G	I	G	T	K	T	C	I	G	C									
	ANT-R	*	G	R	A	T	G	T	T	I	G	C	W	C	C	I	G	C	I	C	C	Y	T	T							
10	APX1-F1		A	T	C	G	C	T	G	A	G	A	A	G	A	A	A	T	G	C											
	APX1-F2		C	C	G	A	T	T	A	C	C	A	G	A	A	G	G	C	C	A	T	T	G								
	APX1-R1		C	A	A	G	A	G	G	G	C	G	G	A	A	T	A	C	A	G											
11	APYRASE-F1	*	G	C	A	A	T	C	A	C	T	T	C	C	T	C	C	C	A	A	T	A									
	APYRASE-F2		G	C	C	T	G	C	W	A	T	C	W	C	T	T	C	C	T	C	C	C									
	APYRASE-R1	*	C	A	A	A	A	T	A	C	A	T	C	A	A	T	C	G	C	T	C	A									
	APYRASE-R2		G	C	C	T	A	R	A	G	G	C	C	A	T	G	C	R	G	C	T	T	C	C							
12	BEAT F2c	**	T	g	A	C	C	C	T	g	g	T	g	C	g	A	g														
	BEAT R2c	**	C	T	T	A	C	A	A	A	C	A	A	g	C	T	T	A	T	T	T	A	T	C	C	C					
13	BEBT F3	**	g	C	T	T	C	g	T	T	T	C	C	A	g	A	T	A	C	C	A	g									
	BEBT R3	**	g	A	A	C	C	C	A	g	C	T	C	g	T	g	T	C	A	C	g										
14	BFRU-F2	*	A	C	T	T	T	C	A	A	C	C	T	C	T	C	A	A	R	A	A	T	T	G	G						
	BFRU-R2	*	C	C	C	A	C	A	C	A	A	T	R	T	T	Y	C	C	C	C	A	T	T	Y	C	C	C	C	A		
15	BFRUCT1-F1	*	A	T	C	A	C	T	T	T	C	A	A	C	C	T	C	T	C	A	A	G	A	A							
	BFRUCT1-R1	*	T	T	C	C	C	C	A	T	A	C	A	G	C	A	C	C	T	T	T	A	G								
16	BPMF	*	A	A	Y	A	G	Y	G	A	A	G	T	I	G	G	I	C	A	Y	A	A	Y	G	C						
	BPMR	*	C	C	I	G	A	I	C	G	R	T	T	I	C	C	R	T	T	C	C	A	R	A							
17	CCR-F1	*	G	C	T	C	T	T	G	A	C	C	G	T	G	T	C	G	T	A	C										
	CCR-F2	*	G	C	T	G	T	C	A	A	T	A	C	G	A	A	G	G	G	C											
	CCR-R1	*	C	A	A	T	G	C	T	T	G	T	A	C	G	A	C	A	C	G	G										
18	c_MDH F4	**	g	A	T	g	T	C	A	M	C	C	A	T	g	C	A	A	C	T	g	T									
	MDH R3	**	C	T	C	T	T	C	g	g	C	T	g	T	C	A	A	g	T	C											
	c_MDH-R4		C	A	T	C	A	C	T	C	C	C	C	T	A	G	C	A	A	T	C										

19	CFBF-F		G	A	Y	T	G	Y	G	G	T	G	T	I	T	C	I	A	T	I	G	G	M	A	C				
	CFBF-R		G	I	C	C	I	C	C	I	G	C	Y	T	G	Y	T	C	C										
20	CIPOR-F1		A	C	T	G	C	T	A	A	G	G	C	T	T	T	G	G	C	T	G	A								
	CIPOR-F2		G	C	T	A	C	T	G	C	T	A	A	G	G	C	W	T	T	G	G	C	K	G						
	CIPOR-R1		A	G	A	T	T	T	T	G	T	T	A	G	G	C	T	T	G	G	A	T	C	A	C	T				
	CIPOR-R2		C	C	W	G	A	T	T	T	T	G	T	T	A	G	G	C	T	T	G	G	A	T	C	A	C			
21	COMT-F1	*	G	T	T	C	G	C	C	A	T	G	C	A	G	C	T	G	G	C	C	A	G							
	COMT-R1	*	C	C	T	C	C	A	A	C	A	T	G	C	T	C	C	A	C	A	C	A	C	C	A	G				
	COMT-F2		C	A	A	T	C	T	G	T	T	G	G	C	C	A	T	G	C	A	G	C								
	COMT-R2		G	T	A	C	A	G	C	C	T	C	T	C	C	A	C	C	T	T	C	C	C	G						
22	CRS-F	**	G	G	C	R	T	C	T	G	G	G	A	T	G	T	G	T	G	C	T	A								
	CRS-R	**	T	A	C	A	C	G	G	A	G	A	T	G	C	A	G	C	G	T	T	T	T	C						
23	DBE-F		T	I	T	C	I	G	C	T	C	A	Y	G	A	Y	A	A	Y	G	A	R	A	C	I	C				
	DBE-R		Y	A	A	A	C	G	R	A	A	I	A	G	I	G	G	I	G	A	R	T	A							
24	DCOP-F		G	A	R	A	A	Y	G	T	I	C	G	I	T	A	T	G	T	T	T	A	Y	C	A	I	C	C		
	DCOP-F2	*	C	C	T	C	C	C	G	C	A	G	C	A	A	A	T	C	C	T										
	DCOP-R		C	A	T	C	Y	T	C	Y	T	G	G	T	T	I	A	G	R	A	T	Y	T	G	I	A	G			
	DCOP-R2	*	A	G	G	C	T	G	A	A	G	A	A	C	C	A	C	G	T	C	A	G								
25	DPEP-F		A	G	Y	G	A	R	A	C	I	G	G	I	C	A	R	C	T	I	T	G	G							
	DPEP-R		A	G	I	A	C	I	G	C	C	A	T	Y	C	C	A	G	G	I	G									
26	DxPS F2	**	G	A	G	T	C	A	T	C	A	C	C	G	A	G	A	A	G	G										
	DxPS R2	**	T	A	T	C	C	C	C	T	T	C	C	A	C	C	A	A	T	A	T	C	C							
27	ENOL-F2		T	T	C	C	A	T	C	A	A	G	G	C	C	C	G	T	C	A	G	A								
	ENOL-R2		T	T	G	C	A	C	C	A	A	C	C	C	C	A	T	T	C	A	T	T								
28	ExH F1	**	t	t	t	c	g	g	a	t	c	a	c	a	t	t	c	t	t	g	a	c								
	ExH R1	**	g	t	g	c	a	a	t	a	t	c	c	a	t	c	g	a	c											
29	FKF	*	A	A	R	G	C	I	C	C	I	G	G	I	G	G	Y	G	C											
	FKF2		G	T	S	T	C	C	C	T	Y	G	C	A	G	A	A	G	C											
	FK-F3		A	G	A	T	C	A	G	C	A	C	A	C	T	T	G	A	A	G	G	C								
	FKR	*	T	R	G	T	I	G	T	R	A	T	I	G	C	T	C	C	R	C	A	I	G	C						
30	GAPA-F	*	C	Y	G	G	I	G	G	Y	G	T	I	A	A	R	C	A	R	G										

	GAPA-R	*	R	T	C	I	A	C	I	A	C	Y	C	T	Y	C	T	Y	T	G	I	G	A	R	T	A	I	C	C
31	GAPB-F	*	A	A	R	C	T	I	C	C	I	T	G	G	G	C	I	G	A	R	C	T								
	GAPB-R	*	C	A	Y	T	C	R	T	T	R	T	C	R	T	A	C	C	A	I	G	C	I	A	C					
32	GAPC-F	*	G	T	Y	G	C	I	G	T	I	A	A	Y	G	A	Y	C	C	I	T	T	Y	A	T					
	GAPC-R	*	G	C	Y	T	T	I	G	C	R	T	C	R	A	A	I	A	T	R	C	T	I	G	A					
	GAPC-F2	*	C	A	C	Y	A	C	C	A	C	C	G	A	K	T	A	C	A	T	G									
	GAPC-F3	**	G	K	T	C	A	A	G	G	A	C	T	C	G	A	A	G	A	C	T	C	Y							
	GAPC-R2	*	C	T	G	A	G	T	A	T	C	C	S	A	R	A	A	T	T	C	C	C	T							
	GAPC-R3	**	C	A	A	C	A	T	C	R	T	C	G	T	T	C	W	G	A	G	T	A	T	C	C					
33	GBSS-F	*	C	G	I	G	T	Y	T	T	Y	G	T	I	G	A	Y	C	A	Y	C	C								
	GBSS-R	*	R	T	C	I	G	A	I	C	C	Y	T	T	Y	T	G	C	T	C	Y	T	C							
34	GCPE-F		G	C	I	G	A	R	T	G	C	T	T	Y	G	A	Y	A	A	R	A	T	I	C	G					
	GCPE-R		A	T	I	A	G	A	A	C	R	G	A	I	C	C	R	T	C	I	C	G	R	T	G					
35	GPT F		G	G	I	G	A	AG	C	C	T	G	C	I	T	T	CT	A	G	CT	TC									
	GPT R		C	AG	A	T	I	G	C	I	G	C	AT	C	C	AG	A	G	I	G	C									
36	GLOW F		A	G	Y	T	G	K	C	G	I	G	C	I	C	C	R	T	G											
	GLOW R		G	C	R	T	C	I	G	C	W	G	C	I	G	G	I	A	C											
37	GMDH-F		C	T	I	C	A	R	C	T	I	T	A	Y	G	A	T	G	T	I	G	T	Y	A	A					
	GMDH-R		G	T	T	G	C	I	G	A	I	C	C	I	G	C	Y	C	C											
38	GS F2	**	A	T	G	C	T	G	G	C	C	T	C	C	T	A	T	C	T	T	C									
	GS R2	**	C	A	A	G	T	A	G	G	T	C	C	A	T	C	C	G	T	T	G									
39	GSP-F	*	C	C	I	A	A	R	T	G	G	A	A	Y	T	A	Y	G	A	Y	G	G	I	T	C					
	GSP-R	*	G	C	Y	T	C	I	A	G	I	G	T	I	G	G	Y	T	C	C	C	A								
40	HSP70-F1	*	C	G	G	N	A	T	Y	G	A	T	C	T	C	G	G	N	A	C	S	A	C							
	HSP70-R1	*	C	A	T	C	T	T	N	G	C	R	A	T	G	A	T	N	G	G	R	T	T	G	C					
41	INVR F	**	A	T	C	A	C	T	T	T	C	A	A	C	C	T	C	T	C	A	A									
	INVR R	**	T	T	C	C	C	C	A	T	A	C	A	g	C	A	C	C	T	T	T									
42	IPI2-F	**	G	C	T	G	G	G	A	T	G	G	A	C	G	C	T	G	T	C										
	IPI2-R	**	C	A	A	C	C	A	A	C	C	T	G	A	A	C	C	A	A	G	G	C								
43	KTC-F		C	Y	T	G	G	G	A	W	G	G	I	T	C	I	G	T	I	A	A	Y	C	C						
	KTC-R		C	W	G	G	R	T	C	I	A	C	W	C	C	I	A	C	I	G	C									

44	LIS F1	*	C	C	T	C	A	T	C	A	T	C	A	C	C	A	T	C	A	C	C										
	LIS F2	**	T	C	T	T	C	T	A	T	C	A	A	C	C	T	T	G	G	C	T	T	G								
	LIS R1	**	C	T	G	C	T	T	C	A	T	T	A	C	A	A	G	T	C	T	C	T	C								
45	MMDH-F	*	G	G	I	G	T	I	G	C	Y	G	C	Y	G	A	Y	G													
	MMDH-R	*	C	Y	G	T	I	C	C	W	C	C	R	T	C	Y	T	G	I	G	T										
46	NACP-F	*	T	C	A	A	A	G	C	A	A	A	G	C	A	G	A	A	G	Y	G	A	G								
	NACP-R	*	G	A	A	S	A	C	A	T	C	A	G	G	T	T	T	T	G	A	G	A	T	G							
	NACP-F2	*	T	G	G	C	T	C	C	A	G	G	T	C	C	A	G	T	T	R	T	T	G	A							
47	NACT-F		G	T	T	G	G	A	A	T	G	G	G	A	C	A	A	A	A	A	G	A									
	NACT-R		C	G	A	T	G	G	C	T	G	G	A	A	C	A	G	A	A	C											
	NaCT F1	**	T	T	G	G	A	A	T	G	G	G	A	C	A	A	A	A	A	G	A										
	NaCT R1	**	G	A	T	G	G	C	T	G	G	A	A	C	A	G	A	A	C												
48	NDK F1	**	C	C	A	T	A	G	A	T	C	C	A	C	T	T	C	T	C	T	G	C									
	NDK R1	**	C	A	G	C	A	G	T	T	T	A	C	A	T	G	C	T	C	C											
49	P450-F1		G	T	C	A	C	G	C	C	C	T	A	A	A	C	C	A	G	T	C										
	P450-R1		C	A	A	A	A	C	C	T	C	A	A	G	T	G	G	A	G	T	A	G									
	P450-R2		C	A	G	C	A	C	A	C	A	T	T	C	T	T	C	T	C	C	C	A									
50	PECTIN ME-F1		G	T	T	C	A	A	A	A	C	T	G	T	G	G	C	T	G	A	G	G	C	T							
	PECTIN ME-R1		G	T	G	T	T	C	T	G	G	T	T	T	G	G	G	T	C	T	T	C	T	C							
51	PEPCN F		G	C	A	A	A	A	G	T	G	A	G	T	G	A	A	G	A	T	G										
	PEPCN R		G	G	T	A	C	G	G	A	A	T	G	C	A	G	C	T	T	G											
52	PFK-F-1		G	A	A	G	A	T	T	G	G	T	G	T	T	G	T	T	T	T	G	T	C	Y	G						
	PFK-F-2		G	G	T	T	T	G	T	G	G	A	A	G	A	A	G	A	A	R	C	T	C	A	C						
	PFK-F3		G	A	T	G	C	C	C	I	A	A	R	A	C	C	A	T	I	G	A	T	G	G	W	G	A				
	PFK-R-1		C	A	G	T	T	T	C	T	T	C	A	G	C	T	T	G	T	T	T	A	A	A	C	A	G				
	PFK-R-2		C	A	G	G	C	T	T	G	A	A	Y	T	T	A	C	C	R	T	G	T	C	T	C						
	PFK-R3		C	A	T	G	Y	C	T	C	C	T	C	T	C	I	A	C	R	T	C	C	A	T							
53	PGIC1 F	**	G	C	A	G	T	T	A	G	T	A	C	A	A	A	T	C	T	T	C	C	G								
	PGIC1 R	**	C	C	T	G	C	T	C	T	C	T	A	T	C	A	T	G	C	A	A										
54	PGIC2-F	**	G	G	T	T	A	C	A	G	C	G	C	T	T	C	T	G	C	T											
	PGIC2-R	**	G	A	T	A	C	A	T	T	C	C	A	T	A	C	A	C	T	T	A	A	C	A	A	A	C	C			

55	PGIP-F	*	G	G	I	A	T	Y	G	A	T	C	A	T	C	A	R	A	A	T	G	C	W	C	A	R	C	T
	PGIP-F2	*	G	G	I	A	T	Y	G	A	Y	C	A	T	C	A	R	A	T	I	G	C	I	C	A	R	C	
	PGIP-R	*	G	T	G	C	T	T	C	C	Y	T	T	R	T	T	T	C	C	A	T	A	S	A	C			
56	PGKCF		G	T	I	A	C	I	A	A	R	T	A	C	A	G	C	T	T	R	A	A	R	C				
	PGKCR		C	C	I	G	T	I	G	A	I	A	T	G	T	G	G	C	T	C	A	T	C	T	T			
	PGKPF		G	G	C	I	A	A	G	A	A	G	A	G	Y	G	T	I	G	G	I	G	A					
	PGKPR		C	C	A	C	I	A	T	I	G	C	I	G	C	A	A	A	T	G	G	Y	C					
57	PGLM-F	*	T	T	T	G	G	A	T	G	G	K	T	G	G	G	G	Y	G	A								
	PGLM-F2	*	C	R	T	T	A	C	C	T	T	G	T	T	T	C	T	C	C	M	C	C						
	PGLM-R	*	C	T	C	C	A	A	T	N	G	C	A	A	T	T	G	G	C	A	C							
	PGLM-R2	*	T	C	T	C	Y	G	G	K	G	G	A	G	A	A	A	C	A	A	G	G						
58	PI4P-F	*	C	T	G	G	A	G	A	G	C	A	G	A	C	C	C	A	T	T	C	A	A	T				
	PI4P-R	*	G	C	G	A	A	A	G	C	C	T	C	C	A	A	T	C	C	A	C							
59	PPE-F3	**	G	G	C	T	G	G	T	T	G	T	G	A	T	T	G	G	A	T	T	C	A					
	PPE-R3	**	G	G	R	G	T	A	C	C	A	G	G	G	T	T	T	A	G	G	A							
60	PYROLS-F	*	G	T	A	T	T	G	A	T	C	T	T	G	G	C	G	G	G	A	G							
	PYROLS-R	*	C	T	T	T	T	C	C	C	A	G	T	A	G	T	C	T	C	C	G	A						
	Pyrol F1	**	T	A	T	T	G	A	T	C	T	T	G	G	C	G	G	G	A	G								
	Pyrol R1	**	T	T	T	T	C	C	C	A	G	T	A	G	T	C	T	C	C	G	A							
	PYROL-F2		C	T	C	T	G	G	T	T	G	T	G	T	G	A	G	C	T	T	G							
	PYROL-R2		T	T	C	A	A	G	G	G	A	G	A	G	G	A	T	A	T	A	G							
61	RPL22 F		C	T	C	T	C	T	C	T	T	T	A	G	C	C	A	T	T	A	A	C						
	RPL22 R		C	T	T	C	C	T	T	G	T	C	A	G	A	C	T	C	A	T	C							
62	RNAH-F	*	G	C	T	T	C	C	A	C	C	A	G	C	T	G	A	T	A	C	A	C	G					
	RNAHR	*	A	G	C	C	C	T	A	G	C	A	A	G	A	A	T	G	T	C	A	C	T	G				
63	SAHH-F1	*	G	A	T	T	G	G	G	G	T	C	C	T	G	G	T	G	G	T	G							
	SAHH-R1	*	A	A	G	A	G	C	A	G	C	A	A	C	C	T	T	C	T	C	A	T	C	A	A			
64	SAMT F1	**	C	G	G	C	A	A	G	T	T	C	T	T	C	A	C	A	T	G	A	A	G	G	G			
	SAMT R1	**	G	A	A	C	C	T	G	A	G	A	T	A	G	C	C	A	C	A	T	G	A	G	G			
65	SBE1-F	*	G	C	Y	C	C	I	C	G	I	A	T	M	T	A	Y	G	A	R	G	C						
	SBE1-R	*	R	T	T	G	C	C	Y	T	C	Y	C	T	I	G	G	R	A	A	R	T	C					

66	SBE2-F		C	G	I	A	T	I	A	A	A	A	A	I	C	T	W	G	G	I	T	A	Y	A	A	Y	G	C
	SBE2-R		G	C	C	A	T	R	T	G	Y	A	G	I	C	G	R	T	A	R	T	C							
67	SHMT-F	*	A	C	C	A	C	A	A	C	T	C	A	C	A	A	G	T	C	A	C	T	T	C					
	SHMT-R	*	C	T	T	C	C	A	A	C	A	C	C	T	T	C	T	C	A	A	C	T	C						
68	SucS F		G	G	G	A	A	A	G	G	G	A	T	T	T	T	G	C	A	A	C								
	SucS R		A	G	G	C	A	G	G	C	T	G	M	A	C	G	A	A	A	G	C								
69	THIOP-F		A	G	A	T	G	G	A	G	A	A	G	T	C	A	C	C	A	C	A	C							
	THIOP-R		C	C	A	T	G	A	G	T	T	C	T	T	C	A	Y	T	A	T	C	C							
70	TKPF	*	G	G	I	G	A	Y	G	G	I	T	G	Y	C	A	R	A	T	G	G	A							
	TKPR	*	G	A	I	G	C	R	A	G	R	T	C	I	G	C	R	C	T	I	C								
71	TPI-F	*	T	T	C	G	T	Y	G	G	I	G	G	I	A	A	Y	T	G	G	A	A							
	TPI-R	*	C	C	I	G	T	I	C	C	A	A	T	R	G	C	C	C	A	M	A	C							
	TPI F2	**	T	C	G	T	C	G	G	Y	G	G	Y	A	A	C	T	G	G	A	A								
	TPI R2	**	C	W	G	T	C	W	C	C	A	T	R	G	C	C	C	A	R	A	C								
72	TKPF		G	G	I	G	A	Y	G	G	I	T	G	Y	C	A	R	A	T	G	G	A							
	TKPR		G	A	I	G	C	R	A	G	R	T	C	I	G	C	R	C	T	I	C								
73	TUFMF	*	G	A	Y	G	G	I	C	C	I	A	T	G	C	C	I	C	A	R	A	C							
	TUFMF2		G	A	T	G	G	A	C	C	W	A	T	G	C	C	T	C	A	A	A	C							
	TUFMF3		G	A	A	C	T	A	T	T	G	G	A	C	A	C	G	T	G	G	A	T	C						
	TUFMR	*	C	C	A	C	C	Y	T	C	A	T	C	Y	T	T	I	G	T	I	A	G	I	A	C				
	TUFMR2		A	C	C	C	T	C	A	T	C	C	T	T	T	G	T	G	A	G	T	A	C						
74	UNIV-PAAL-F	*	G	A	G	G	T	G	A	A	R	C	G	Y	A	T	G	G	T	G	G								
	UNIV-PAAL-R	*	G	G	T	T	T	Y	C	C	T	T	G	C	A	T	A	A	C	T	T	C	A	G	C				

* = amplyfied *Clarkia* DNA but was not polymorphic

** = polymorphic bands bands mapped