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The Evolution of Sexual Reproduction in *Cuscuta* (Convolvulaceae)

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The Evolution of Sexual Reproduction in *Cuscuta* (Convolvulaceae)

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Abstract

Cuscuta (Convolvulaceae), the dodders, is a genus of ca. 200 species of obligate stem parasites distributed across a great diversity of habitats worldwide. The existence of a handful of species that are dangerous crop weeds has led researchers to historically focus on their growth and control. Consequently, there is a dearth of information about their biodiversity, ecology, and in particular their reproductive biology. This thesis surveys aspects of sex allocation, floral evolution, floral rewards for pollinators, and mechanisms of reproductive assurance across the genus. I demonstrate that *Cuscuta* has evolved a broad spectrum of breeding systems, from obligate selfing to obligate outcrossing. Predictions made by sex allocation theory of negative correlations between pollen number and pollen grain size, and between male investment and female investment are shown to be false in *Cuscuta.* Histological examination of the floral nectary demonstrates that it is typical in structure, and I predict that it is functional in most facultatively and obligately xenogamous species. *Cuscuta* pollen is variable in the proportions of lipid and starch reserves, and has a sticky external pollenkitt. The role of the infrastaminal scales is narrowed to 1) defense against seed predators, and 2) a shield against early self-pollination in some strongly protandrous species. Lastlly, I demonstrate that the evolution of two styles, followed by unequal styles in *Cuscuta*, were critical for the radiation of the genus. The more flexible floral design enabled *Cuscuta* to evolve different mechanisms of reproductive assurance in coordination with their exploitation of novel host species and new pollination environments.

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1. Introduction

1.1 Problem setting

Although the vast majority of plants on Earth are autotrophic, approximately 4200 species or ~1% of all angiosperms are parasitic, meaning they are partly or fully dependent on their host plant(s) for water, nutrient, and organic carbon acquisition (Kuijt, 1969; Nickrent, 2002). Parasitic plants invade root or shoot tissues of the host using specialized organs called haustoria, which form a physiological bridge for the transfer of nutrients (Kuijt, 1969). Remarkably, this form of life has arisen independently in at least 11 lineages during the evolution of the flowering plants (Nickrent, 2002; Nickrent et al., 1998). As a result, parasitic plants exhibit a number of lifeforms from woody trees and shrubs (e.g. *Nuytsia*, Loranthaceae), to vines (e.g. *Cassytha*, Lauraceae), herbs (e.g. *Pedicularis*, Orobanchaceae), and more specialized forms with endoparasitic tissue inside the host and emerging flowering stems (e.g. *Arceuthobium*, Viscaceae).

Cuscuta (Convolvulaceae), dodder, is a genus of highly modified stem parasites, with twining filiform stems, leaves reduced to tiny scales, and a complete lack of functional root tissue (Kuijt, 1969; Figure 1.1). Not only is *Cuscuta* the only parasitic lineage among the Convolvulaceae, it has also been very successful in its diversification. With ca. 200 species described in the literature, *Cuscuta* is one of the most diverse lineages in the family overshadowed only by the morning glories of tribe *Ipomoeeae* and similar in numbers to the bindweeds of the *Convolvulus*-

Calystegia alliance. *Cuscuta* are distributed worldwide ranging from 60°N to 47°S and occupy myriad habitats (Yuncker, 1932). Like other parasitic plants, *Cuscuta* can act as keystone species and ecosystem engineers that increase community diversity through the selective suppression of their host species (Pennings and Callaway, 1996). Due to the intimacy of their haustorial connection to the host, *Cuscuta* can act as vectors for plant-to-plant spread of pathogens (Bennett, 1944; Chiykowski, 1988; Heintz, 1989), and have been implicated in the horizontal transfer of mitochondrial genes between host species (Mower et al., 2004).

Despite these fascinating attributes, *Cuscuta* suffer from a negative perception due to their parasitic nature and the fact that a minority of *Cuscuta* species are significant agricultural pests (Dawson et al., 1994; Sandler et al., 1997; Costea and Tardif, 2006). The genus as a whole is listed on many national and regional noxious weed lists that require immediate destruction upon discovery (Costea and Stefanovic, 2009). Further, the introduction of generalist and possibly invasive *Cuscuta* species outside their native range is a looming issue, as *Cuscuta* seeds can easily be passed along with seeds of a desirable crop plant (Costea and Tardif, 2006) and the seeds of certain Asian *Cuscuta* are used in traditional medicines that are now becoming popular in the West both among the immigrant communities and more broadly. *Cuscuta japonica,* for example, has been discovered in Southern California and New Jersey, USA in recent years. As a consequence of all this, most research on these plants has focused on topics related to their growth and control (Dawson

et al., 1994, Costea and Tardif, 2006).

What is less often addressed is that many *Cuscuta* species have significant conservation problems. Although a number of *Cuscuta* species are currently endangered and face the threat of extinction (e.g. Weeda et al., 1988; Cheffings and Farrel, 2005; Costea et al., 2006a, b, c, d; Van Landuyt et al., 2006; Costea et al., 2008; Costea and Stefanovic, 2009), this has not had an impact on the legal status of these species, or the genus more broadly. Although there has been much recent interest in modernizing the taxonomy of the genus (e.g. Costea et al., 2005, 2006a, b, c, d; García and Martín, 2007; Stefanovic et al., 2007; Costea et al., 2008, 2009a, b), we still lack much fundamental knowledge of *Cuscuta* biodiversity, ecology, and in particular reproduction. Not only does this hinder efforts to build adequate conservation strategies, but the study of reproduction would give a strong complement to recent taxonomic studies by exposing possible mechanisms of speciation that have led to the diversity of *Cuscuta* species and floral forms we see today. There is also very little work in the literature examining how the parasitic lifestyle impacts reproduction and sexual systems in parasitic plants, providing further rationale for study.

1.2 Outline of the thesis

My thesis examines several facets of reproduction in *Cuscuta*. In the next chapter I will discuss current theoretical background on angiosperm reproduction, and bring that theory into the context of what is already known about reproduction in *Cuscuta.* I will then outline the objectives of my studies, and present the hypotheses I developed. Following that, I will present the materials and methods I have used to perform this survey. The fifth chapter examines the results of my investigation of sex allocation and the coevolution of floral form with reproductive strategy. The sixth chapter presents pollinator reward in *Cuscuta* with an examination of the floral nectary, and a brief look at the histochemical constitution of the pollen. My final results chapter will discuss field and herbarium studies conducted to examine the timing of male and female sexual function. I will then discuss the implications of my data and give a brief description of the limitations of my research and avenues for future work. A brief summary of my main conclusions is provided at the end, followed by a glossary of terms to aid readers unfamiliar with this branch of botany, a list of the references I have cited, and two appendices containing a list of the specimens used in my studies and the data matrix of pollen counts, pollen volumes, and all other floral characters that I measured. Throughout the thesis I will refer to the major lineages of *Cuscuta* subg. *Grammica* using the informal clade names of Stefanovic and colleagues (2007).

Figure 1.1 - The orange-yellow mass of twining stems growing on this roadside vegetation shows the typical habit of a *Cuscuta* species. It possesses no attachment to the ground and extracts all its metabolic needs from the host plants it is growing on. Since this *Cuscuta* is currently in the vegetative phase (i.e. there are no flowers), it is impossible to identify to species due to the lack of useful vegetative characteristics for the identification of *Cuscuta*. Photo by Dr. M. Costea.

2. Background

2.1 Reproduction in the flowering plants

Mating in flowering plants is as much an ecological process as it is a reproductive process due to its dependence on both pollination and non-pollination factors (Harder and Barrett, 2006). This dependence allows environmental variation to alter the mode and magnitude of selection on reproductive traits and promotes reproductive diversification through adaptation to local pollination environments and resource regimes (Harder and Routley, 2006). It also means that mating in plants is very context dependent and stochastic (Herrera, 2002, 2004; Johnson et al., 2005).

The diversification of floral form and function among angiosperms is connected to an extensive range of mating strategies and sexual systems, from various modes of self-pollination, to heterostyly and the evolution of dioecy (Lloyd and Barrett, 1996). Plants influence their mating by adapting their floral traits to affect the movement of pollen within and among their own flowers and those of other plants as well as through physiological mechanisms that determine the fate of pollen after it reaches a stigma (Harder and Barrett, 2006). Furthermore, floral form can be adapted to both a generalized set of pollinators, or can limit pollination to a few species of most-effective pollinators (Stebbins, 1974; Gómez and Zamora, 2006). Mating systems are also greatly affected by differential investment in male and female sexual function, with large fitness consequences (Charnov, 1982). Since re-

productive mode can have a huge impact on population genetic processes and the path of life-history evolution (Lloyd, 1980a; Takebayashi and Morrell, 2001), understanding the variety and distribution of mating systems in *Cuscuta* may give us insight into the feedbacks between parasite and host life histories, and how *Cuscuta* has evolved to overcome reproductive limitations. In particular, I am interested in exploring strategies that allow *Cuscuta* to deal with mating uncertainty and the disturbances that are common in the habitats they frequent, and in particular the maintenance of alternative tactics.

2.2 Mating strategies and reproductive assurance

Flowering plants facilitate and promote outcrossing through morphological, developmental and physiological adaptations that coevolve with pollinators and work to ensure both pollinator fidelity (Kiester et al., 1984) and the accuracy of pollen transfer (Cresswell, 1998; Fenster et al., 2004). Flower visitation is solicited through the release of floral scents, the elaboration of floral displays to make a specific and recognizable search images, and with rewards such nectar, resins, or extra pollen for consumption or provisioning by the pollinator (Dafni et al., 2005). Functional interference in the form of self-pollination is reduced or prevented through modifications to floral form and development that isolate male and female function within the flower (Darwin, 1876). Dichogamy and herkogamy are strategies that use temporal and spatial separation, respectively, of male and female sex function to promote outcrossing (Lloyd and Webb, 1986; Webb and Lloyd, 1986). The many compo-

nents of these individual strategies have long been recognized as integrated into specialized 'pollination syndromes' that favour specific groups of pollinators to increase pollination efficiency and, consequently, overall fitness (Fenster et al., 2004).

While most angiosperms reproduce predominantly through outcrossing (Goodwillie et al., 2005), self-fertilization has arisen from outcrossing ancestors in myriad lineages (Stebbins, 1974). Despite Darwin's (1876) assertion that 'nature abhors perpetual self-fertilization', there is a dynamic balance between the costs and advantages of selfing. The disadvantages of selfing comprise inbreeding depression (Charlesworth and Charlesworth, 1987; Uyenoyama et al., 1993; Carr and Dudash, 2003), pollen discounting (e.g. Holsinger et al., 1984; Harder and Wilson, 1998; Barrett, 2003); seed discounting (Herlihy and Eckert, 2002); loss of genetic diversity (Charlesworth and Charlesworth, 1995), and the potential evolutionary dead end of purely selfing lineages (Takabayashi and Morrell, 2001).

However, increased homozygosity caused by selfing may purge the deleterious mutations from a selfing population after a few generations (Lande and Schemske, 1985; Charlesworth and Charlesworth, 1987). Further, in terms of individual fitness, uniparental reproduction can be highly profitable. Lloyd (1980b) demonstrated that an allele for selfing can have an advantage over an allele for outcrossing in populations of hermaphrodites because it doubles the probability of transmission of an allele from parent to offspring, thereby avoiding Lloyd's (1988) 'cost of meiosis'. Even more critical, however, is that selfing can assure reproductive success

even if there is a lack of mating partners or adequate pollen vectors (Darwin, 1876). Insufficient pollination is a frequent limiter of seed production in outcrossing populations (Burd, 1994; Ashman et al., 2004), but self-fertilization can reduce these fitness losses (Larson and Barrett, 2000). Furthermore, it has been argued that self-compatible plants have an advantage in dispersal and colonization, with self-fertilization facilitating the establishment and persistence of new populations until densities increase (Baker, 1955; Cox, 1989; Rambuda and Johnson, 2004). Strong support for this hypothesis is shown by the predominance of selfing found in populations in ecologically and geographically marginal habitats (Jain, 1976; Lloyd, 1980a; Elle, 2004; Geber and Moeller, 2006).

In early theoretical models of plant reproduction (e.g. Nagylaki, 1976), the presence of strong positive feedbacks led to only two stable reproductive strategies: predominant selfing and predominant outcrossing, while intermediate states exist only transiently. Instead of this bimodal distribution, however, empirical work has demonstrated the widespread occurrence of mixed-mating systems (Vogler and Kalisz, 2001; Eckert et al. 2006). New models that incorporate ecological factors, population dynamics and life history characteristics (e.g. Holsinger, 1991; Cheptou and Dieckmann, 2002; Tsitrone et al., 2003) indicate that mixed-mating systems can be evolutionarily stable, particularly when reproductive assurance is important (Goodwillie et al., 2005).

An important factor to consider is that the timing and method of pollen

deposition in selfing has an impact on how much pollen is available for export and consequently on individual male fitness (Lloyd, 1979, 1992). Selfing can occur either autonomously or with facilitation by a pollen vector, and may occur before, during or after the period when outcross pollination is possible (Lloyd, 1979). Further, self-pollination may happen within a single flower (autogamy) or involve transfer between flowers on the same plant (geitonogamy) (Lloyd, 1979). Lloyd (1992) found that delayed autogamy provides the most selective benefits because it does not reduce the amount of pollen that could have been exported for outcross siring nor does it cause seed discounting by reducing the number of ovules available for outcross fertilization. Delayed autogamy as part of a robust mixed-mating system has obvious fitness benefits in habitats where there is much spatial and/or temporal variation in the availability of pollination vectors (Schoen and Brown, 1991; Morgan and Wilson, 2005; Morgan, 2006). This means that when the pollinator availability is constant and adequate, plants can maximize their outcrossed seed set and then use delayed autogamy to fertilize any remaining ovules, while if pollinators are absent the ovules may still be fertilized by autogamy and still retain a high relative fitness if inbreeding depression is low. The effects of reproductive assurance on population and metapopulation dynamics, and the feedbacks, equilibria and multiple levels of selection that this introduces are only beginning to be explored from a theoretical perspective (Pannell and Barrett, 2001; Cheptou, 2004; Morgan et al., 2005, Morgan, 2006; Pannell, 2006).

2.3 Floral gender, resource allocation, and breeding system

Shifts to selfing are usually accompanied by shifts in plant gender expression, the relative 'maleness' or 'femaleness' of their reproductive effort (Lloyd, 1979). Since the vast majority of pollen never reaches conspecific stigmas, even with very efficient pollination, outcrossing requires significantly more investment in pollen production than autogamous selfing (e.g. Harder, 2000). Pollen-ovule ratios (P/Os) can therefore be used to contrast the level of male and female investment in reproduction, and are known to provide a conservative indirect estimate of the breeding system (Cruden, 1977).

In species that reproduce via autogamous selfing, pollinator visitation becomes less of a necessity for reproductive success as the rate of selfing increases. Therefore costly traits related to pollinator attraction and fidelity, such as high nectar volume and quality, large floral display, and scent production should be under negative selection when autogamous selfing rate increases (Galen, 1999, 2000; Ashman and Schoen, 1997; Andersson, 2005). This is borne out in studies of the relationship between floral morphology, pollinator attraction and outcrossing rate, which show that typically species with larger flowers are highly outcrossing, while those with smaller flowers tend to rely more on selfing (Holtsford and Ellstrand, 1992; Fausto et al., 2001; Elle and Hare, 2002; Elle, 2004). For this reason, overall floral size and floral traits used to attract pollinators can also be used as an indirect estimate of mating system.

It would be remiss to consider changes in gametophyte number without also considering changes in per-unit investment. An assumption of resource allocation theory stipulates that, given a static resource base, changes in the number of any module should have a genetically-based negative correlation with resource investment per module (Burd, 1999). When applied to plant reproduction, this would suggest that an increase in pollen number should have a concomitant decrease in pollen size (Charnov, 1982). A number of studies have found both intra- and interspecific pollen size-number trade-offs (e.g. intraspecific: Stanton and Young, 1994; Vonhof and Harder, 1995; Worley and Barrett, 2000; Sarkissian and Harder, 2001; interspecific: Mione and Anderson, 1992; Vonhof and Harder, 1995; Yang and Guo, 2004).

Pollen grain size should also be examined as a component of integrated floral design that coevolves with floral morphological traits, and in particular those of the gynoecium (e.g. Plitmann and Levin, 1983; Harder, 1998; Roulston et al., 2000; Torres, 2000; Sarkissian and Harder, 2001; Aguilar et al., 2002). It was predicted more than thirty years ago that pollen grain size should have a correlation to pistil length because of the greater resources needed for pollen tube growth in species with longer pistils (Baker and Baker, 1979; Plitmann and Levin, 1983). A number of studies have found a positive correlation between pollen size and pistil length in various plant groups (e.g. Baker and Baker, 1982; Plitmann and Levin, 1983; Torres, 2000; Roulston et al., 2000; Aguilar et al., 2002), but not all (e.g. Cruden and

Miller-Ward, 1981; Cruden and Lyon, 1985). Welsh (2009) did examine the relationship between pollen length and style length in *Cuscuta*, and although he did not find a significant correlation, he did not examine the relationship between pollen volume and style length. As pollen volume is a better estimate of pollen energy reserves than the equatorial axis (Cruden and Lyon, 1985), a reexamination of this may provide different insight.

2.4 Pollinator fidelity and floral rewards

While the various features of floral and inflorescence architecture, colour and scent serve to attract pollinators, floral rewards must fulfill some essential need of the pollinator to ensure the repeated visitation and fidelity that are necessary for adequate pollination (Dafni, 1992). Both nectar and pollen are common nutritive rewards, but stigmatic fluid, fatty oils, general floral tissues or more specialized structures (e.g. food scales, non-fertile pollen, pseudo-pollen) can also be used for pollinator nutrition or larval provisioning (Dafni, 2005). Flowers also can provide non-nutritive rewards such as sexual attractants and mating sites, shelter and warm resting places, and nest materials in the form of trichomes, resins, waxes and parts of the corolla (Dafni, 1992).

Nectar is often considered to be the most important reward offered by angiosperms to their animal visitors (Galetto and Bernardello, 2005). A specialized tissue or organ called the nectary converts pre-nectar, a sucrose dominated phloem fluid, into a mixture of glucose, fructose, and sucrose in different proportions that is

offered as a major energy source to floral visitors (Baker and Baker, 1983b). Other substances can be found in nectar, such as phenols, antioxidants, amino acids and lipids, but these are usually only in trace quantities (Baker and Baker, 1975, 1983b). The chemical composition of nectar varies among species (Davis et al., 1998), and specific components can give nectar tastes or odours that are thought to be important for attracting floral visitors (Southwick, 1990). Nectars may also include toxic or repellent secondary compounds, such as alkaloids, saponins, or non-protein amino acids that narrow the number of floral visitors (Adler, 2000).

Histologically, nectaries usually consist of an epidermis that covers a specialized nectariferous parenchyma, and, moving deeper towards the floral axis, a more loosely packed, large-celled subnectary parenchyma (Nepi, 2007). Vascular bundles may directly supply the nectary, or may be found only in the subnectary parenchyma (Nepi, 2007). While nectaries can be morphologically obvious or hidden, in some species structurally distinct nectaries can be vestigial and non-secretory as in *Catalpa* and some other Bignoniaceae (Rivera, 2000). Secretion can occur through modified stomata, or the epidermis can be more directly involved through specialized flat secretory cells or trichomes; these modalities can occur simultaneously (Nepi et al., 2001). When secretion occurs through modified stomata, there is evidence that the guard cells are unable to regulate nectar flow by opening and closing, and do not react to turgor- and ion- stimuli the way leaf stomata do (Davis and Gunning, 1993; Razem and Davis, 1999).

The nectary parenchyma is formed of several layers of small cells with generally thin walls, relatively large nuclei, and dense cytoplasm (Nepi, 2007). Vacuoles are small in the pre-secretory phase but quickly grow in size after secretion (Nepi, 2007). Nectar carbohydrates may be photosynthesized in the nectary itself or any other part of the plant, and may require temporary storage in the nectariferous parenchyma (Pacini et al., 2003). After secretion, the fate of the nectary parenchyma varies. It may be involved in nectar reabsorption (Nepi et al., 1996), differentiate into another (parenchymatous) tissue (Cecchi Fiordi and Palandri, 1982), or degenerate (Nepi, 2007).

Pollen is also consumed as a floral reward by many different kinds of animals, including bees, wasps, flies, butterflies, mites, thrips, springtails, and even vertebrates such as bats, birds, rodents and marsupials (Roulston, 2005). Chemical analyses and nutritional bioassays have revealed a range of nutrient concentrations in pollen but little is known on the influence of pollen chemistry on pollinator preferences or the relative nutritional value of different pollen to consumers (Roulston, 2005). Protein, starch, lipids, free amino acids and total caloric content are thought to influence pollen consumer performance, but only protein concentration has been studied in any detail (Roulston, 2005). For instance, protein content is associated with larval survival (Levin and Haydak, 1957), adult body size (Levin and Haydak, 1957; Greenberg, 1982; Regali and Rasmont, 1995; Roulston and Cane, 2002), and longevity (Schmidt et al., 1987) in a number of bee species.

The presence of external pollen lipids has been quantified for many taxa (Roulston and Cane 2000), but internal lipids remain relatively unexplored (Roulston, 2005). External lipids can also be involved in the adherence of pollen grains to one another, to pollinators, and to the target stigma (Hesse 1979). Further, volatile species-specific chemical signals may act as a pollinator attractant (Dobson, 1988). Both the concentration range of internal and external lipids across species and the composition of lipids stored in pollen are also unknown (Roulston, 2005).

Baker and Baker (1979) suggested that all angiosperm pollen be divided into two classes; 'starchy and 'starchless'. Although starch is present in all immature pollen grains, some or all of it is transformed into other mono-, di- and/or polysaccharides before anther dehiscence (Pacini, 2000). Starch concentrations in mature pollen range from 0-23% (Roulston and Buchmann, 2000). Grayum (1985) recognized a tendency for whole families to be characterized by either starchy or starchless pollen, and therefore considered this character to be potentially useful for phylogenetics. It is known that some pollen collecting bees are specialized for plants with starchy pollen (e.g. Linsley et al., 1973). It has also been suggested that pollinating beetles may be another consumer, since they are known to eat other starchy floral tissues (Roulston, 2005).

Interestingly, Baker and Baker (1979) consider starchy pollen to be characteristic of wind-pollinated (anemophilous) species, whereas pollen with lipids or sugars replacing part or all of the starch is characteristic of insect pollination (ento-

mophily). An important hypothesis is that selection favours starchy pollen when pollen is not used in insect nutrition, whereas bee and fly pollination favours starchless, oil-rich pollen (Baker and Baker, 1983a), which emphasizes how pollinator preferences and requirements influence changes in resource allocation. When costly lipid provisions are not necessary to satisfy a pollen vector, they are eliminated. However, it has also been argued that the selection of starchy pollen can occur in response to a need to deter non-pollinating invertebrates from pollen feeding (Baker and Baker 1979, 1983a). It is plausible that species that are capable of autogamous selfing may also evolve starchy pollen. Unfortunately, no preference tests have been made among pollen consumers, and there has not been any effort to quantify whether there is a trade-off between pollen starch and lipid concentrations among plant species (Roulston, 2005).

2.5 The study system: sexual reproduction in *Cuscuta*

Cuscuta flowers are hermaphroditic and actinomorphic with a typical Convolvulaceae ground plan: *(K5)[(C5)-A5](G2) (Yuncker, 1932; Kuijt, 1969; see Figure 2.1). The perianth ranges in colour from white and cream tones to faint pinks and deeper purplish-reds (Yuncker, 1932). While the ovary can also take on these colours, it is usually green and perhaps photosynthetic (McNeal, 2005), which some authors relate to heavy metabolic needs for lipid synthesis during ovule and seed production (McNeal et al. 2007). Pink and red colours seem to be limited to subgenera *Monogynella, Cuscuta*, and members of the more basal clades of subgenus

Grammica (Yuncker, 1932). Floral scents have been noted, particularly for the relatively large and showy members of subg. *Grammica* Clade O (e.g. *C. odorata* and *C. foetida*; Stefanovic et al., 2007).

There is a floral nectary on the basal portion of the ovary wall, where a band of modified stomata has been observed in many species (Welsh, 2009). This region is pigmented red or orange in some species, such as *C. reflexa* (Heide-Jorgensen, 2008). While Heide-Jorgensen (2008) indicates that there is no known nectar production, Prenner and colleagues (2002) claim that the nectary of *C. reflexa* is secretory, and McNeal (2005) reports copious nectar in the flowers of *C. chilensis*. Cronquist (1981) believed the nectary to be functional in 'at least some' *Cuscuta* species. Histological examination of different developmental stages of the nectary and nectar quantification will elucidate whether or not functional nectaries do exist in other *Cuscuta* species.

Cuscuta also possess a set of fimbriate appendages, called infrastaminal scales, fused to the corolla-stamen tube adaxial to the stamens (Yuncker, 1932). These appendages are unique to *Cuscuta* and serve an undetermined function (Stefanovic et al., 2007). The scales are not a whorl of staminodia; rather, they are thought to be basal outgrowths of the staminal tissue in the corolla-stamen tube (Heide-Jorgensen, 2008). The scales may be solitary or united by tissue bridges that form a pocket between the bridge tissue and the corolla tube, and between the bridge tissue and the base of the ovary (Yuncker, 1932). Hollow structures similar to

the laticifers present in other parts of the plant have been observed at the tips of the fimbriae in most scales, and have been identified as laticifers (Yuncker, 1932; Beliz, 1986; Costea, unpublished observations). Authors have suggested the scales play a protective role and/or that they are somehow involved in pollination or pollinator reward (Heide-Jorgensen, 2008). Mechanisms of pollinator reward suggested include secondary nectar presentation in species where the scales are tightly appressed to the surface of the gynoecium, and secretions from the fimbriae (Costea, personal communication). An assessment of the chemical nature of the secretory product is necessary to weigh the likelihood of these scenarios.

Aspects of stylar and stigmatic morphology are synapomorphic to the subgeneric level in *Cuscuta* (Yuncker 1932; Welsh 2009; see Figure 2.2) but it is unclear how these alterations affect the breeding system. The inflorescences of *Cuscuta* are cymose in structure, but presentation ranges from open cymes to dense, globular clusters, and "ropes" of flowers twining around the host stems. Although not all flowers in a single inflorescence open at once, *Cuscuta* often present hundreds of open flowers at a time in many inflorescences (e.g. Gómez, 1994).

With some exceptions, the floral morphology of the genus is 'generalist,' not targeted towards specific pollinators. Exceptional species (e.g. *C. polyanthemos, C. prismatica, C. chapalana*) have long corolla tubes that point at specialization for pollination by insects with long mouthparts (e.g. Lepidopterans). There are few reports detailing the insect visitors and pollinators of *Cuscuta* species, and most are un-

published anecdotes. Field observations taken by Costea and Welsh in southwestern Mexico during February and December of 2007 demonstrate that *Cuscuta* species have a large and varied cohort of insect visitors, including flies, moths, beetles and predators such as spiders and larger insects (Costea and Welsh, unpublished data). Not all of these insects are mere visitors. Meulebrouck (2009), during her survey of the metapopulation biology and management of the endangered *C. epithymum*, observed insects from eight families of Hymenoptera and Diptera that were clearly feeding at the flowers and could be considered pollinators because of significant contact with the anthers and stigmas. Similarly, McNeal (2005) reported frequent pollinator visitation and nectar feeding at natural populations of *C. chilensis*, including species of Lepidoptera, Hymenoptera and Diptera.

McNeal also reported self-incompatibility in *C. chilensis* and *C. rostrata*. Natural seed set for *C.* chilensis is low, with less than 5% of flowers maturing seed capsules. Beliz (1986) observed similar low seed set in the large-flowered members of subsection Subulatae (i.e. Clade G; Stefanovic et al. 2007) in Central America. Like *C. chilensis*, these species can survive on perennial and arborescent hosts year round and this may alleviate the cost of low seed set, and suggests they are similarly self-incompatible (McNeal, 2005). The ability to perennate does not seem to be a requirement for self-incompatibility, however. Strong fragrance and large flowers are also found in *C. rostrata* (Clade D, Stefanovic et al. 2007), an annual but self-incompatible species that shows ample seed set in the wild (McNeal, 2005).

Sporophytic self-incompatibility systems are present in a number of Convolvulaceae (e.g. species of *Ipomoea*, *Jaquemontia*, and *Merremia*, Martin, 1970; Koyama et al., 2008), and it is likely that this is the mechanism used in *Cuscuta* as well.

By contrast, self-compatibility is known to occur in most genera of the Convolvulaceae, particularly in small annual species (Martin, 1970), and *Cuscuta* is not an exception to this rule. Many *Cuscuta* are suspected or known to be self-fertile (e.g. *C. pacifica,* Beliz 1986; *C. attenuata*, Prather and Tyrl, 1993). This is unsurprising given the typically small and disjunct populations of *Cuscuta* species across their distribution. The evolution of selfing should give a measure of reproductive assurance to both isolated individuals and small populations. In *C. obtusiflora*, it has been shown that the anthers begin dehiscing while the flower is still in bud stage, both self-pollinating and fertilizing the ovules before there is even a chance at outcrossing (Rodriguez-Pontes, 2009). This phenomenon of early anther dehiscence has been noted during gross dissection of rehydrated floral buttons from herbarium specimens of other *Cuscuta* species (Costea et. al., 2006a; Wright, unpublished observations). However, the drying and rehydration process puts anther walls under significant water stresses that could provoke artifactual dehiscence in herbarium specimens. The prevalence of pre-anthesis anther dehiscence and fertilization therefore requires verification using fresh or fixed specimens and through further anatomical study.

It has also been noted that some *Cuscuta* have significant growth of their

styles/stigmas after anthesis (Costea, unpublished data). This was postulated to be a mechanism for 'second chance' outcross pollination after early selfing in bud or during anthesis (Costea, personal communication). These patterns of growth deserve further attention in terms of their timing and spatial orientation to confirm whether there truly is a reproductive role or if the growth is a secondary feature of the post-fertilization growth of the developing fruit.

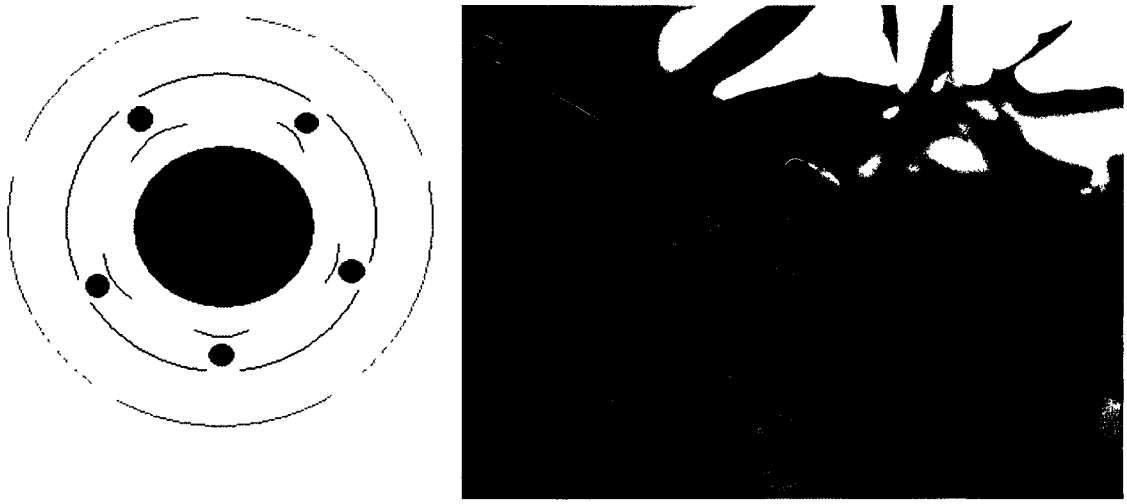

Figure 2.1 – a) General floral plan of *Cuscuta*. Green = calyx, blue = corolla, orange = stamen, red = infrastaminal scale, purple = carpel. b) *Cuscuta 'volcanica'*, a typical member of the genus, showing its twining habit and different floral stages.

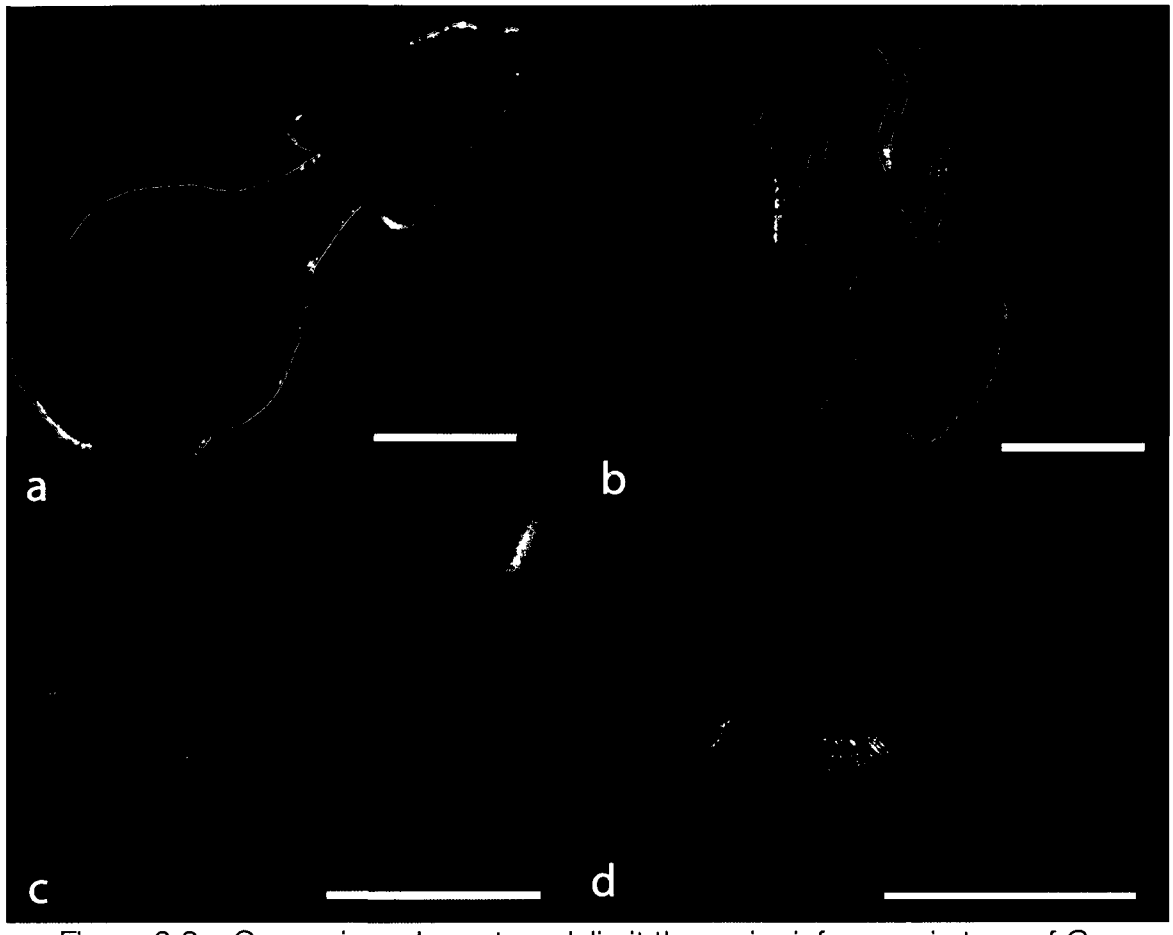

Figure 2.2 – Gynoecium characters delimit the major infrageneric taxa of *Cuscuta:* a) subg. *Monogynella* (*C. santapaui*), showing the fused styles; b) subg. *Cuscuta* (*C. triumvirati)*, with free styles and elongate stigmas; c) subg. *Cuscuta* subsect. Pachystigma (*C. nitida*), with elongate stigmas thicker than the styles; and d) subg. *Grammica* (*C. indecora*), showing free styles and capitate stigmas. All scale bars = 1 mm.

3. Objectives

a. Determine major trends in the evolution of reproductive strategies in *Cuscuta*

b. Determine whether the theoretically predicted tradeoffs between male and female investment in reproduction, and between pollen grain size and number are present in *Cuscuta*

c. Determine the tightness of correlation between floral morphology and male investment in *Cuscuta*, and in particular the strength of the relationship between overall floral size, floral tube length, herkogamy and self-pollination

d. Investigate the structure and morphology of the floral nectary in *Cuscuta*

e. Examine the nutrient constituents of pollen in *Cuscuta* and consider suitability its as a pollinator reward

f. Examine the plausibility of the hypothesis that the infrastaminal scales have a role in pollinator reward

g. Determine the timing of stigma receptivity in relation to anther dehiscence and anthesis in species representing a range of male investment strategies

4. Materials & Methods

4.1 Sex allocation & floral morphology

Male investment was assessed in 142 taxa through total pollen counts. Unopened flower buds were sampled from herbarium specimens and rehydrated in 50% ethanol on a hotplate until just at the boiling point. After cooling, the anthers were dissected from the unopened flower buds and placed on a grid slide and sliced into several pieces in a drop of 50% ethanol. Anther slices were manipulated to remove all pollen grains from the anther thecas. Remains of the anther were removed, and the pollen was spread over the grid surface. The slide was then imaged on a Nikon SMZ1500 stereomicroscope using a Pax-Cam arc digital camera and Pax-it! 7.0 imaging software (MIS Incorporated, 2009). Pollen grains were then enumerated on the images using the counter feature in Pax-it! or ImageJ (Rasband, 2010). Species assessed are marked 'p' in Appendix A. Multiple counts were done whenever the herbarium material available allowed. Counts for each species were averaged together and standard error calculated. To assess whether a size/number tradeoff exists in *Cuscuta* pollen production, pollen grain polar and equatorial diameters from Welsh and colleagues (2009) were mined to calculate species' average pollen grain volumes. Finally, average pollen counts were multiplied by the average pollen grain volume for the corresponding species to give an average total volume of pollen per flower, which should represent a more accurate assessment of male in-

vestment than pollen count numbers alone.

Female investment was approximated as the total volume of the ovary, as mature seed set data were unable to be collected for too high a proportion of the herbarium specimens. Average ovary lengths and widths were mined from the data in Mark Welsh's MSc thesis (2009), and subjected to the spheroidal volume equation:

$$(4\pi/3)a*b^2$$

where *a* is the average ovary length and *b* is the average ovary width. Seed lengths were also mined from Yuncker's (1932) monograph when available to provide a further measure of comparison. After ln-transformation, the female investment data were subjected to the same analyses described above in combination with the three male investment variables.

A morphometric approach was taken to examine floral morphology. Measurements of 7 characters (Figure 4.1) were taken from digital images of whole flowers found in the Digital Atlas of *Cuscuta* using Pax-it! and/or ImageJ (Rasband, 2010). When possible, images of type specimen material were used. Measurements of five style and stigma characters from the thesis of Mark Welsh (2009) were also appended to this data.

Perianth data were then subjected to an unrotated principal components analysis to generate a composite variable from the first principal component scores

representative of overall perianth size (Runions and Geber, 2000). The first principal component generated accounted for 54.7% of the total variation in the perianth data, with contributions from each of the perianth variables. The second and third components only accounted for 13.46 and 11.64 percent of the variation, and had contributions from three and one variables, respectively, and were dismissed from further consideration. The rationale for creating this variable was to build a way to assess the degree of correlation between overall floral magnitude and sex allocation via regression analysis. The correlation of individual traits with floral magnitude was also examined, but these results should be taken with a grain of salt, as they are biased due to autocorrelation: each perianth variable made a contribution to the forming of the first principal component scores.

One further composite variable, herkogamy, was estimated by the following formula:

$$\text{herkogamy} = \left(|S-A|^2 + (M/2)^2\right)^{1/2}$$

where *S* and *A* are the heights of the tips of the stigma and anthers, respectively, measured from the base of the calyx, and *M* is the diameter of the mouth of the corolla tube. *A* is calculated by adding the two measured variables floral tube length and anther height, while *S* was calculated by adding together ovary length, style length, and stigma length. Corolla shapes were categorized following Yuncker (1932) into an eight character states ranging from rotate to campanulate to cylindri-

cal to urceolate (Figure 4.2).

All continuous data were normalized using ln-transformation, as this reduces the statistical problems associated with typical log-normal distributions associated with biological data (Niklas, 1994). Linear regression, Pearson's correlation, and Spearman's rank correlation analyses were conducted in NCSS 2007 (Hintze, 2007) to examine the relationship between each of the sex allocation variables and the floral morphological characters. Corolla shape was compared with the continuous characters using the one-way ANOVA procedure in NCSS 2007.

4.2 Floral Rewards

Investment in pollinator reward was explored through two routes: 1) a structural study of the floral nectary and infrastaminal scales, and 2) histochemical staining of pollen grains and infrastaminal scales. Although attempts were made to quantify nectar secretion in a local population of *Cuscuta gronovii*, the volume of nectar secreted at the time of collection was too small for isolation using microcapillary tubes or a centrifugation method.

To determine the number and arrangement of nectary stomata at the base of the ovary, gynoecia were dissected out of rehydrated flowers and stained in Lugol's Iodine or Neutral Red, or a combination of the two stains. Lugol's Iodine rapidly stains starches blue-black and can be used to differentiate guard cells from the surrounding tissue (Galetto and Bernardello, 2004). Neutral Red is traditionally used to transiently stain secretory structures in plants, and is usually quickly taken up by nectary tissue (Nepi, 2007). Stomata were then enumerated, the pattern of distribution was noted, and older gynoecia/capsules were inspected for signs of tissue degeneration or collapse. The nectaries were further dissected from some gynoecia, placed onto glass slides and mounted in water for inspection on a Nikon Eclipse 50i brightfield microscope. The arrangement of subsidiary cells surrounding the modified stomata was noted.

For anatomical study of the nectary and infrastaminal scales, field collections of *C. costaricensis*, *C. cotijana*, *C. gracillima, C. gronovii*, and *C. strobilacea* were

fixed in FAA, dehydrated through an ethanol series, moved to xylene, and then infiltrated and embedded in paraffin. Sections of whole flowers were cut at 5μm on an American Optical Co. microtome, and divided sequentially among four groups of slides for staining with safranin-fast green FCF, alcian blue-PAS, Coomassie brilliant blue, or decolourized aniline blue respectively (Ruzin, 1999). Observation and imaging of all slides was conducted on Nikon Eclipse 50i brightfield and Nikon Eclipse E600 epifluorescence microscopes using a PaxCam digital arc camera and Pax-It 7.0 software. The anatomy of the nectary vasculature was also examined in cleared and stained whole mounts of 50 species (marked 'V' in Appendix 1). The wholemount specimens were cleared in 8N NaOH overnight at 60°C. Once translucent, they were rinsed in deionized water, and then stained in 1% basic fuchsin for 8-12h. Specimens were rinsed in water overnight or until the staining was stable. Specimens were then stained for 2-5min in decolourized 0.1% aniline blue in phosphate buffer (pH 8.5), rinsed in buffer, and then dissected. Gynoecia were mounted on glass slides in 50% glycerol and observed under a Nikon SMZ1500 stereomicroscope and a Nikon Eclipse E600 epifluorescence microscope. Xylem was visualized as red under brightfield, while the callose of the phloem sieve plates fluoresced green under epifluorescence UV illumination.

Histochemical staining was used to give a qualitative assessment of energy and material reserves in the pollen of 20 species, marked 'H' in Appendix 1. Specimens were collected fresh and fixed in FAA or Farmer's fluid. Anthers were

dissected off of 3-4 flowers and separated into four groups. Each group of anthers was dissected in a small drop of stain on a microscope slide. The stains used were Lugol's Iodine to visualize starch inclusions, Sudan IV for external lipids, and Nile Blue Sulfate for internal lipids (Passarelli 1999), while the fourth group of anthers was used as a control. Slides were mounted with a cover slip, and then excess stain was drained and replaced with 50% ethanol, or 7% acetic acid in the case of Nile Blue Sulfate. Slides were examined under a Nikon Eclipse E600 epifluorescence microscope using brightfield for the slides stained with Lugol's Iodine and Sudan IV, while the remainder were examined under brightfield and epifluorescence using both UV- and green- excitation settings.

Lastly, Neutral Red, Lugol's Iodine, and Nile Blue Sulfate stains were used to assess whether or not the glands found on the infrastaminal scales produce some lipid product, a common component of latex, or some other class of compound. The same FAA-fixed and Farmer's-fixed collections as in the pollen histochemistry experiment were used for this experiment. For each stain, five whole flowers of each available species were rinsed briefly in 50% ethanol and then water. The corolla tube, with the infrastaminal scales still attached, was dissected from each flower and stained for two to five minutes. Corolla tubes were flattened and mounted on glass slides adaxial side up in water or, in the case of Nile Blue Sulfate stainings, in 7% acetic acid. Observations were made on an SMZ1500 stereomicroscope and Nikon Eclipse 50i brightfield microscope.

4.3 Timing of sexual function & patterns of stylar growth

Field studies were conducted in Ontario, Canada and Jalisco, Michoacan, and Nayarit, Mexico. Observations of the timing of anther dehiscence, length of the styles, and position of the stigma were taken for flowers at different developmental stages in five species from subg. *Grammica*. Peroxidase activity of the stigma, an indicator of receptivity, was tested using a colorimetric method modified from Dafni and Motte-Maués (1998). Peroxtesmo KO test papers (Macherey-Nagel, Germany) were cut into 0.5 mm lengths, inserted into bullet tubes with 0.5 mL deionized water, macerated and left to sit for 5 minutes. The stigmas and styles were dissected from fresh flowers representing eight developmental stages of *C. campestris* var. *glandulosa* (subgenus *Grammica* Clade B), *C. gronovii* (Clade D), *C. cotijana* (Clade G), *C. costaricensis* and *C. strobilacea* (Clade K). Each stigma/style was placed individually into a prepared bullet tube. After 3 minutes, the intensity of the blue colour reaction was recorded. Voucher secimens for all the populations studied were deposited in the CIMI and/or WLU herbaria. Herbarium materials and digital images of flowering *Cuscuta* were studied for other representative species of each subgenus, using floral position in the cyme as a proxy for the age of each flower in an inflorescence.

4.4 Phylogenetic and evolutionary contextualizations

To bring data into a phylogenetic context, I utilized the Ancestral State Reconstruction Package in the program Mesquite v. 2.73 (Maddison and Maddison,

2010). The parsimony and likelihood functions of the Trace Character History module was used to map character states onto a phylogeny for the genus (Stefanovic et al., unpublished data), with a maximum of 10 visualized character states. The parsimony function reconstructs a character history using a maximum parsimony algorithm, with a linear cost assumption for state transition (i.e. to change from state x to state y, the cost is |x-y|). Analyses were considered in light of the relationships between species and important infrageneric lineages: the subgenera of Yuncker (1932), with the unique section *Pachystigma* of subg. *Cuscuta* and the 15 major clades of subg. *Grammica* identified by Stefanovic and colleagues (2007).

In order to examine the phylogeny explicity for changes in the rate of diversification, the Lineages over Time module in Mesquite (Midford and Maddison 2010) was utilized to generate a chart of the change in number of lineages over time, using branch lengths as a proxy for time. This methodology therefore assumes that the rate of mutation was fixed between the root and tips of the phylogeny. This chart was then examined for major changes to the rate of diversification to determine if these changes could be matched to the origins of novel floral characters.

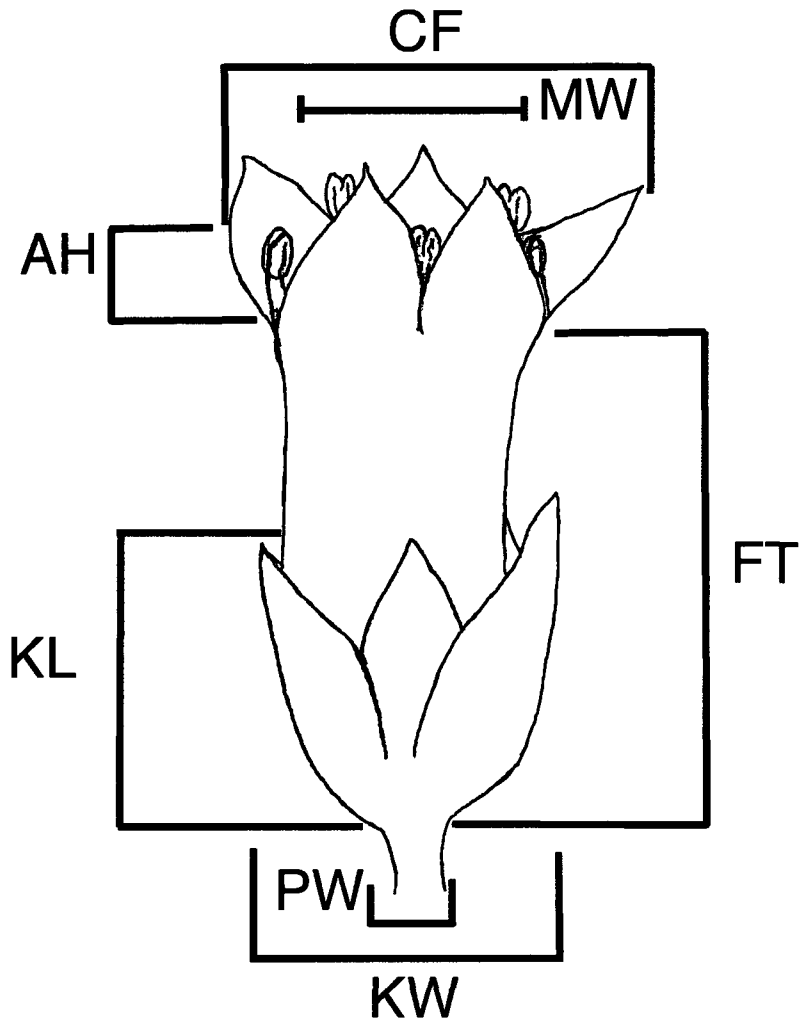

Figure 4.1 - Floral character measurements. FT = floral tube length, MW = tube mouth width, CF = corolla flare, AH = anther height, KL = calyx length, KW = calyx width, PW = pedicel width. *C. subinclusa*. Drawing after Yuncker (1932).

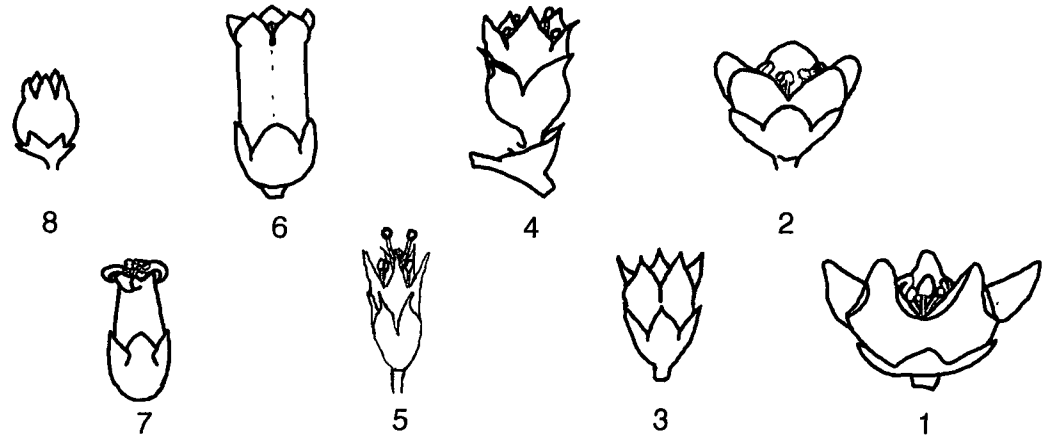

Figure 4.2 - Corolla Shape Categories. 1 – Cupulate (*C. argentinana*); 2 – broadly campanulate (*C. grandiflora*); 3 – campanulate (*C. pacifica*); 4 – campanulate-cylindric (*C. ortegana*); 5 – cylindric-campanulate (*C. choisiana*); 6 – cylindrical (*C. chilensis*); 7 – cylindrical-saccate or cylindrical-globular (*C. parodiana*); 8 - globular/urceolate (*C. jepsonii*). Drawings after Yuncker (1932).

5. Results I: Sex Allocation & Floral Morphology

5.1 Male & Female Reproductive Investment

Pollen production in *Cuscuta* varies over three orders of magnitude, with numbers extending from the low hundreds to over 20,000 grains in some individuals of *C. 'volcanica'*. The distribution is log-normal, typical of biological data (Figure 5.1). Ovule production is constant at four ovules/flower, but seed set is much more variable. Both high and low pollen/ovule ratios are distributed across the genus; some clades have both higher variances and higher average P/Os (e.g. Clade G, Clade I), while others are much more restricted (e.g. Clade H) (Figure 5.2).

No pollen size-number tradeoff could be found. Pollen count and pollen volume were not correlated positively *or* negatively (r^2= 0.0024, Pearson's r= 0.0486; p=0.5598) (Table 5.1, Figure 5.3). It is worth noting that, with the exception of *C. cassytoides*, the species of subg. *Monogynella* form a cluster somewhat separate from the rest of the data on the volume axis (Figure 5.4). Pollen count and pollen grain size were each regressed against total pollen volume to examine whether these characters had similar levels of deviation from perfect linear correlation in their contribution to total pollen volume (Figures 5.5 & 5.6). It is clear that total pollen volume is more tightly correlated with changes in pollen count (r^2= 0.6700, Pearson's r=0.8186, p<0.0001) than with pollen grain size (r^2= 0.3767, Pearson's r= 0.6135, p<0.0001), meaning that pollen count contributes more of the variation found in to-

tal male reproductive output than pollen volume does.

Ovary volume similarly has a log-normal distribution, and size variation is fairly uniform across the genus, save in subg. *Grammica* clades G and O where the ovaries tend to be larger. Pollen count is only weakly positively correlated with ovary volume, while pollen grain volume and total pollen volume per flower show moderately correlated positive relationships; total pollen volume per flower shows the strongest relationship (r^2= 0.1600, Pearson's r= 0.4000, p<0.0001; Figure 5.7). Seed length is most strongly correlated with pollen volume (r^2= 0.3017, Pearson's r= 0.5493, p<0.0001), has moderate correlation with ovary volume and total pollen volume, but is not correlated with pollen count.

5.2 Floral morphology

Each category of corolla shape was fairly dispersed throughout the phylogeny (Figure 5.8). Exceptionally homogeneous clades include subg. *Monogynella*, and subg. *Grammica* Clades H and J, which all have cylindrical to cylindrical-globular corollas, and subg. *Grammica* Clade N, which has campanulate corollas. Using one-way ANOVA, comparisons between the mean floral tube length of each corolla shape group were significantly different (F=7.31, p<0.0001; Figure 5.9). However, mean perianth sizes for each corolla shape were not significantly different (F=0.42; p=0.8880), nor were the means of any other floral character. Floral size, generally speaking, is largest in subg. *Grammica* clades G and O, with a few out-of-the ordinary large species in other clades, for example *C. macrocephala* in Clade I. The

composite variable, perianth size, has very significant ($p<0.0001$), moderate to strong correlations with all of the floral characters measured save style length, which was weakly correlated and only marginally significant (Table 5.2).

5.3 Correlations between morphology and sex allocation

Pollen count was most strongly correlated with stigma surface area (r^2= 0.2540, Pearson's r= 0.5040, p<0.0001), while all other correlations were weak to moderate-weak or not significant (Table 5.3). Interestingly, when subg. *Monogynella* is taken on its own, the correlation between pollen count and herkogamy is both strong and significant (r^2= 0.8925; r= 0.9447; p= 0.0013; Figure 5.10). Pollen volume, by contrast, was most strongly correlated to stigma length (r^2= 0.1732, Pearson's r= 0.4162, $p<0.0001$; Figure 5.11) and floral tube length (r^2= 0.1560, Pearson's r= 0.4126, $p<0.0001$), and showed moderate-weak correlations to perianth size, stigma surface area, and floral tube mouth width (Table 5.4). Pollen volume did not have a significant relationship with style length (Figure 5.12). Total pollen volume was, unsurprisingly, most strongly correlated to stigma surface area (r^2= 0.3897, Pearson's r= 0.6243, $p<0.0001$), floral tube length (r^2= 0.3035, Pearson's r= 0.5509, $p<0.0001$) and stigma length (r^2= 0.2544, Pearson's r= 0.5044, $p<0.0001$) (Table 5.5). Other significant moderate to moderate-weak correlations include perianth size and floral tube mouth width. Both pollen count and total pollen count have a significant correlation with herkogamy, but pollen volume does not.

Ovary volume showed a pattern of moderate correlations to stigma surface

area, perianth size, calyx flare, floral tube length, floral tube mouth width, and number of nectary stomata (Table 5.6). Stigma surface area is the strongest of these (r^2= 0.3290, Pearson's r= 0.5737, p<0.0001). Like pollen volume, ovary volume is not correlated with herkogamy.

Seed length shows a different pattern of correlations compared to the other sex allocation variables (Table 5.7). In general, correlations are weaker overall, while the number of marginally significant and insignificant relationships is much increased. The variables with clearly significant correlations to seed length include floral tube length, stigma surface area, number of nectary stomata, perianth size, and calyx length.

One-way ANOVA comparisons of corolla shape and sex allocation variable means were significant for pollen volume (F=3.26, p=0.0032) and total pollen volume (F=4.58, p=0.0002; Figure 5.13), but only marginally significant for pollen count (F=2.51, p=0.0189). Both seed length (F=1.14, p=0.3454) and ovary volume (F=1.03, p=0.4137) means were not significantly different between corolla shape groups.

Table 5.1 – Regression and correlation coefficients for sex allocation variables in *Cuscuta*. Values marked * = marginally significant, ** = not significant.

Sex allocation variables		*Regression & Correlation*				*Spearman's rank correlation*	
		slope	r^2	r	p	r_s	p
Total pollen volume	Pollen count	1.0353	0.6700	0.8186	<0.0001	0.8170	<0.0001
	Pollen volume	1.0668	0.3764	0.6135	<0.0001	0.5978	<0.0001
	Ovary volume	0.3437	0.1600	0.4000	<0.0001	0.3841	<0.0001
	Seed length	1.1731	0.2064	0.4543	0.0001	0.3650	0.0015
Pollen count	Pollen volume	0.0668	0.0024	0.0486	0.5998**	0.0820	0.3753**
	Ovary volume	0.1320	0.0369	0.1921	0.0388*	0.1876	0.0438*
	Seed length	0.3430	0.0271	0.1646	0.1583**	0.1381	0.2373**
Pollen volume	Ovary volume	0.1941	0.1565	0.3956	<0.0001	0.3731	<0.0001
	Seed length	0.8662	0.3017	0.5493	<0.0001	0.3835	0.0003
Ovary volume	Seed length	0.1721	0.2674	0.5171	<0.0001	0.5118	<0.0001

Table 5.2 – Perianth size vs. floral features regression and correlation coefficients. Values marked * = marginally significant, ** = not significant.

Floral variable	*Regression & Correlation*				*Spearman's rank correlation*	
	slope	r^2	r	p	r_s	p
Floral tube length	0.3162	0.5593	0.7479	<0.0001	0.7336	<0.0001
Tube mouth width	0.3173	0.7802	0.8833	<0.0001	0.8626	<0.0001
Herkogamy	0.3691	0.5822	0.7631	<0.0001	0.7507	<0.0001
Stigma surface area	0.4040	0.3132	0.5596	<0.0001	0.5166	<0.0001
Stigma length	0.1929	0.1684	0.4104	<0.0001	0.4587	<0.0001
Style length	0.1170	0.0350	0.1870	0.0426*	0.2143	0.0198*
Nectary stomata	0.2632	0.1907	0.4367	<0.0001	0.4292	<0.0001
Corolla flare	0.2781	0.4724	0.6873	<0.0001	0.6488	<0.0001
Calyx flare	0.3303	0.7875	0.8874	<0.0001	0.8591	<0.0001
Calyx length	0.3272	0.5038	0.7098	<0.0001	0.7225	<0.0001
Anther projection	0.1383	0.1382	0.3717	<0.0001	0.3970	<0.0001

Table 5.3 – Pollen count vs. floral features regression and correlation coefficients. Values marked * = marginally significant, ** = not significant.

Floral variable	*Regression & Correlation*				*Spearman's rank correlation*	
	slope	r^2	r	p	r_s	p
Perianth size	0.2178	0.0801	0.2830	0.0024	0.2803	0.0026
Floral tube length	0.5801	0.1132	0.3364	0.0002	0.3256	0.0004
Tube mouth width	0.3458	0.0308	0.1754	0.0597**	0.2007	0.0307*
Herkogamy	0.5066	0.1064	0.3262	0.0007	0.3119	0.0012
Stigma surface area	0.5045	0.2540	0.5040	<0.0001	0.5093	<0.0001
Stigma length	0.5177	0.1120	0.3347	0.0003	0.4108	<0.0001
Style length	0.2586	0.0514	0.2268	0.0148*	0.2608	0.0049
Nectary stomata	0.3549	0.0706	0.2657	0.0041	0.2611	0.0048
Corolla flare	0.4933	0.0730	0.2701	0.0035	0.2918	0.0016
Calyx flare	0.2223	0.0124	0.1115	0.2336**	0.1322	0.1571**
Calyx length	0.2199	0.0199	0.1412	0.1305**	0.1853	0.0464*
Anther projection	0.3826	0.0395	0.1987	0.0341*	0.2410	0.0098

Table 5.4 – Pollen volume vs. floral characters regression & correlation coefficients. Values marked * = marginally significant, ** = not significant.

Floral variable	*Regression & Correlation*				*Spearman's rank correlation*	
	slope	r^2	r	p	r_s	p
Perianth size	0.2175	0.1560	0.3949	<0.0001	0.3878	<0.0001
Floral tube length	0.5006	0.1702	0.4126	<0.0001	0.4203	<0.0001
Tube mouth width	0.5379	0.1212	0.3481	<0.0001	0.3349	0.0001
Herkogamy	0.1925	0.0301	0.1734	0.0582**	0.2027	0.0264*
Stigma surface area	0.2682	0.1541	0.3925	<0.0001	0.3885	<0.0001
Stigma length	0.4499	0.1732	0.4162	<0.0001	0.4541	<0.0001
Style length	0.1104	0.0199	0.1411	0.1080**	0.1878	0.0317*
Nectary stomata	0.2548	0.0882	0.2970	0.0009	0.3019	0.0007
Corolla flare	0.1548	0.0140	0.1182	0.1788**	0.1180	0.1796
Calyx flare	0.4299	0.0853	0.2921	0.0007	0.3332	0.0001
Calyx length	0.2043	0.0609	0.2468	0.0043	0.3352	0.0001
Anther projection	0.0130	0.0001	0.0096	0.9146**	0.0805	0.3667**

Table 5.5 – Total pollen volume vs. floral characters regression & correlation coefficients. Values marked * = marginally significant, ** = not significant.

Floral variable	*Regression & Correlation*				*Spearman's Rank Correlation*	
	slope	r^2	r	p	r_s	p
Perianth size	0.4189	0.1810	0.4254	<0.0001	0.4082	<0.0001
Floral tube length	1.2040	0.3035	0.5509	<0.0001	0.5235	<0.0001
Tube mouth width	1.0040	0.1407	0.3751	0.0001	0.3378	0.0003
Herkogamy	0.6432	0.1079	0.3284	0.0007	0.3212	0.0009
Stigma surface area	0.7719	0.3897	0.6243	<0.0001	0.6233	<0.0001
Stigma length	0.9662	0.2544	0.5044	<0.0001	0.5890	<0.0001
Style length	0.4120	0.0838	0.2894	0.0021	0.3748	0.0001
Nectary stomata	0.5896	0.1402	0.1402	0.0001	0.3666	0.0001
Corolla flare	0.7182	0.0933	0.3055	0.0014	0.3087	0.0012
Calyx flare	0.7800	0.0833	0.2885	0.0025	0.2904	0.0023
Calyx length	0.6257	0.1002	0.3165	0.0008	0.3639	0.0001
Anther projection	0.4105	0.0271	0.1647	0.0915**	0.2444	0.0116*

Table 5.6 – Ovary volume vs. floral features regression and correlation coefficients. Valu marked * = marginally significant, ** = not significant.

Floral variable	*Regression & Correlation*				*Spearman's rank correlation*	
	slope	r^2	r	p	r_s	p
Perianth size	0.5691	0.2840	0.5329	<0.0001	0.5215	<0.0001
Floral tube length	1.0807	0.1997	0.4466	<0.0001	0.4050	<0.0001
Tube mouth width	1.3057	0.1904	0.4363	<0.0001	0.4163	<0.0001
Herkogamy	0.2446	0.0125	0.1118	0.2203**	0.0988	0.2790**
Stigma surface area	0.8020	0.3290	0.5737	<0.0001	0.5577	<0.0001
Stigma length	0.6973	0.0993	0.3152	0.0002	0.4306	<0.0001
Style length	0.3996	0.0626	0.2503	0.0033	0.2896	0.0006
Nectary stomata	0.7934	0.1823	0.4270	<0.0001	0.4222	<0.0001
Corolla flare	0.6937	0.0715	0.2675	0.0029	0.2154	0.0172*
Calyx flare	1.3927	0.2430	0.4929	<0.0001	0.5112	<0.0001
Calyx length	0.8797	0.1548	0.3935	<0.0001	0.3725	<0.0001
Anther projection	-0.0833	0.0009	-0.0295	0.7498**	-0.0075	0.9358**

Table 5.7 – Seed length vs. floral features regression and correlation coefficients. Values marked * = marginally significant, ** = not significant.

Floral variable	*Regression & Correlation*				*Spearman's rank correlation*	
	slope	r^2	r	p	r_s	p
Perianth size	0.1226	0.1036	0.3219	0.0043	0.3354	0.0029
Floral tube length	0.3754	0.1985	0.4456	<0.0001	0.4327	0.0001
Tube mouth width	0.2732	0.0681	0.2610	0.0202*	0.2913	0.0092
Herkogamy	0.1929	0.0649	0.2548	0.0285*	0.2929	0.0113*
Stigma surface area	0.1757	0.1537	0.3920	0.0003	0.3888	0.0004
Stigma length	0.1953	0.0739	0.2719	0.0141*	0.2914	0.0083
Style length	0.0289	0.0030	0.0548	0.6272**	0.1833	0.1014**
Nectary stomata	0.2079	0.1397	0.3737	0.0008	0.3856	0.0005
Corolla flare	0.1396	0.0269	0.1642	0.1509**	0.2264	0.0462*
Calyx flare	0.2581	0.0574	0.2396	0.0334*	0.2503	0.0261*
Calyx length	0.2397	0.0916	0.3026	0.0067	0.3028	0.0067
Anther projection	0.0332	0.0013	0.0364	0.7550**	0.0223	0.8487**

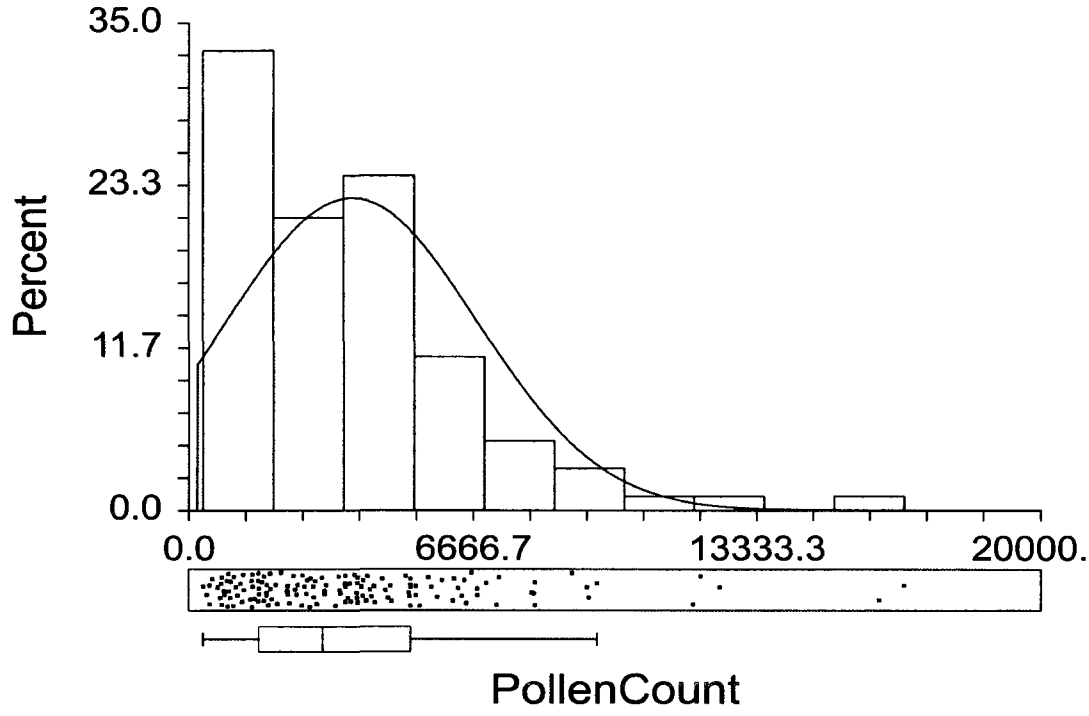

Figure 5.1 – Histogram, dot and box plots of the pollen counts. Pollen counts show a log-normal distribution with a long right tail. The dots of the dot plot each represent a species average. The vertical lines of the box plot represent the quartiles while the whiskers show the outer bounds for outliers.

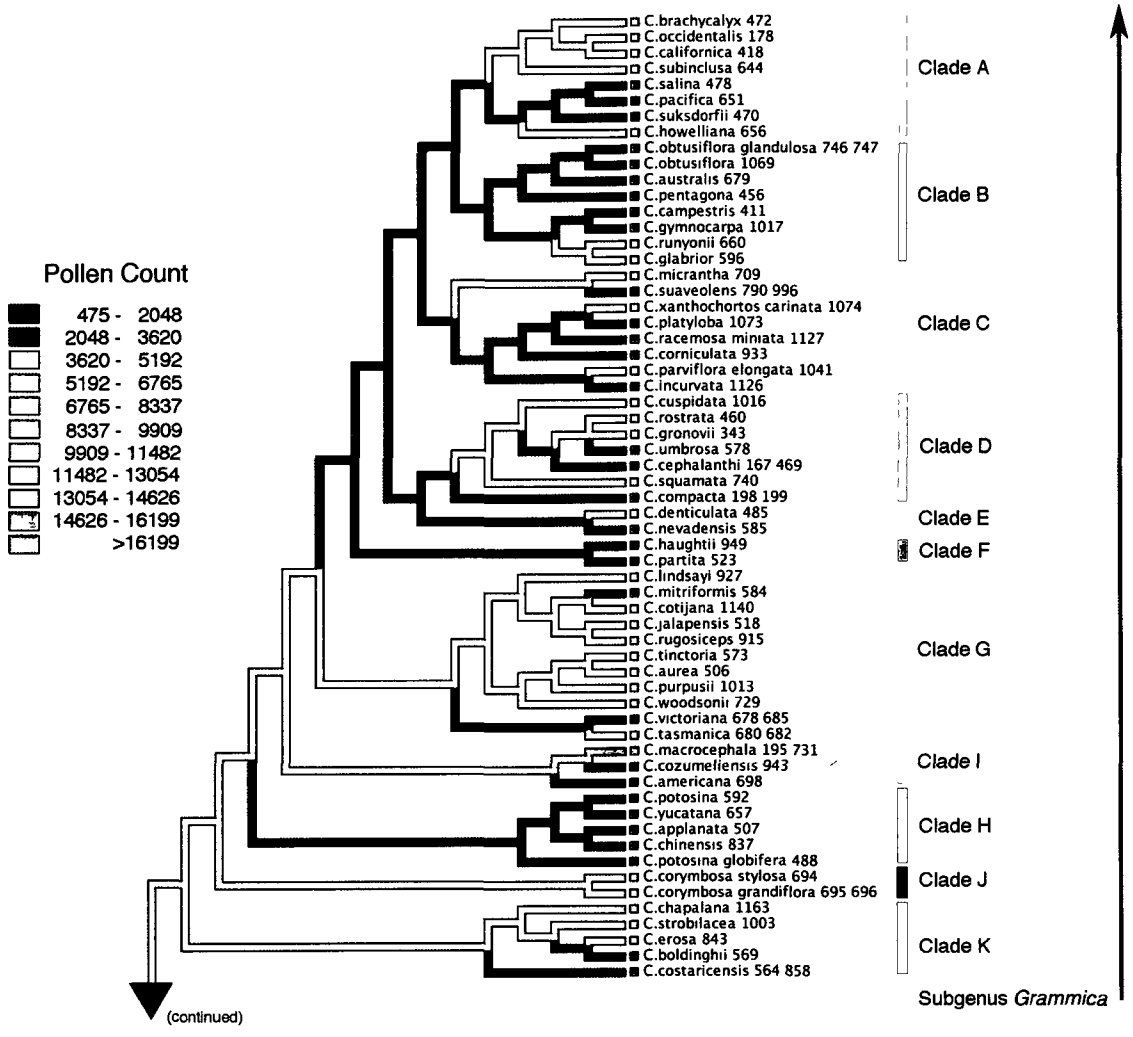

Figure 5.2 – Parsimony reconstruction of the evolution of pollen production across the genus. Mesquite identified eleven ranges of pollen production. Clades G and O tend to have higher pollen production than other clades. No other consistent or obvious patterns in the evolution of pollen production in *Cuscuta* can be gleaned. Numbers beside species names refer to the published DNA accessions used by Stefanovic and colleagues (2007) to generate this phylogeny. The figure continues on next page with the basal branches of the genus.

Figure 5.2 (cont'd) – Parsimony reconstruction of the evolution of pollen production across the genus. Clades G and O tend to have higher pollen production than other clades. No other consistent or obvious patterns in the evolution of pollen production in *Cuscuta.* Mesquite identified eleven ranges of pollen production.

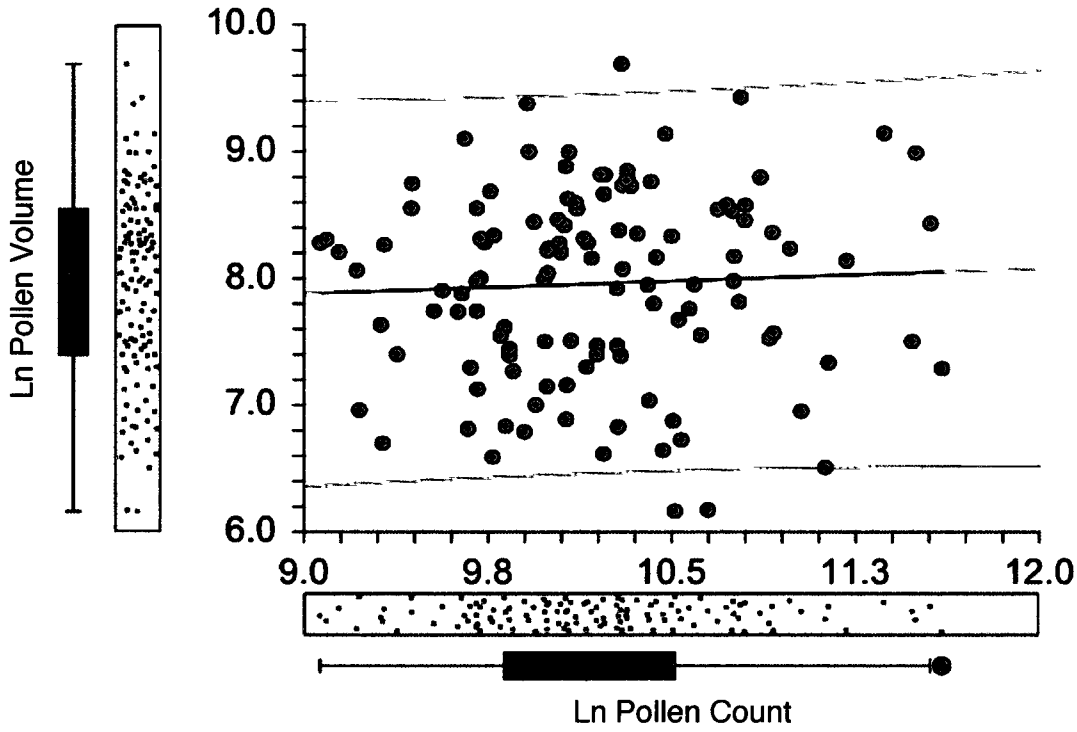

Figure 5.3 – Regression of pollen volume against pollen count, showing the regression line and 95% confidence interval. The variables are found to be uncorrelated at the level of the whole genus (r^2=0.0024, r=0.0486, p=0.5598). Box and dot plots are included to help illustrate the distribution of each individual variable. Circles outside of the whiskers of the box plots are outliers.

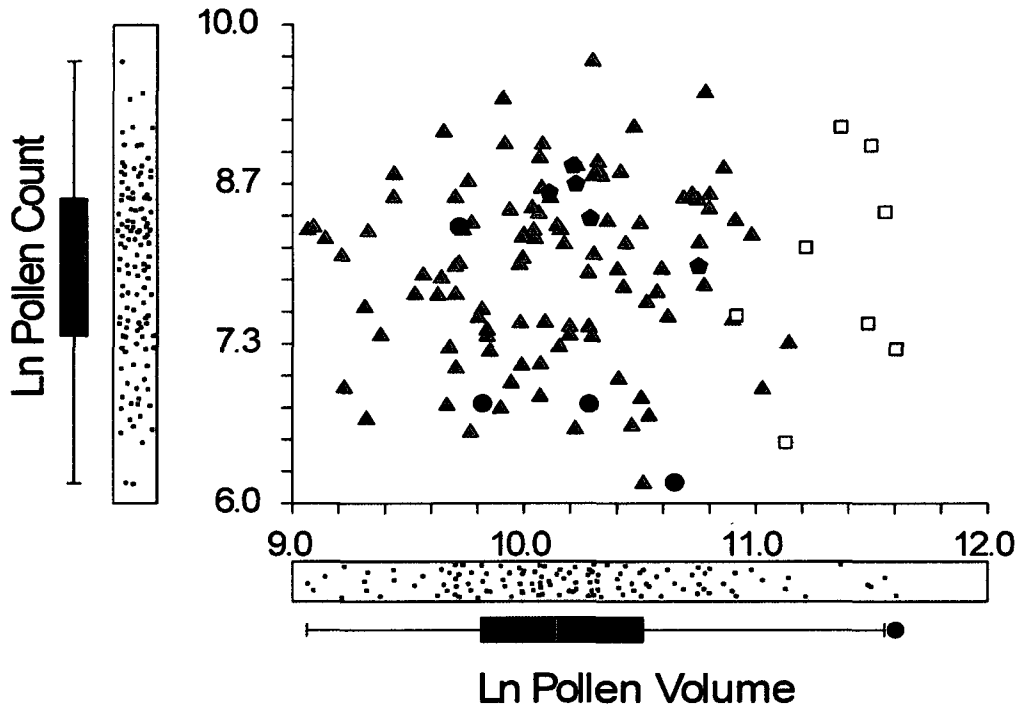

Figure 5.4 - Scattergram of Ln Pollen Count vs Ln Pollen Volume. Species of subg. *Monogynella* segregate out at the high end for pollen volume in this comparison. Both subg. *Cuscuta* and subg. '*Pachystigma*' could, within group, form a negative relationship between pollen count and pollen volume but there are too few points to achieve significance. Grey squares - subg. *Monogynella*, black circles - subg. *Cuscuta*, black pentagons - subg. '*Pachystigma*', grey triangles - subg. *Grammica*.

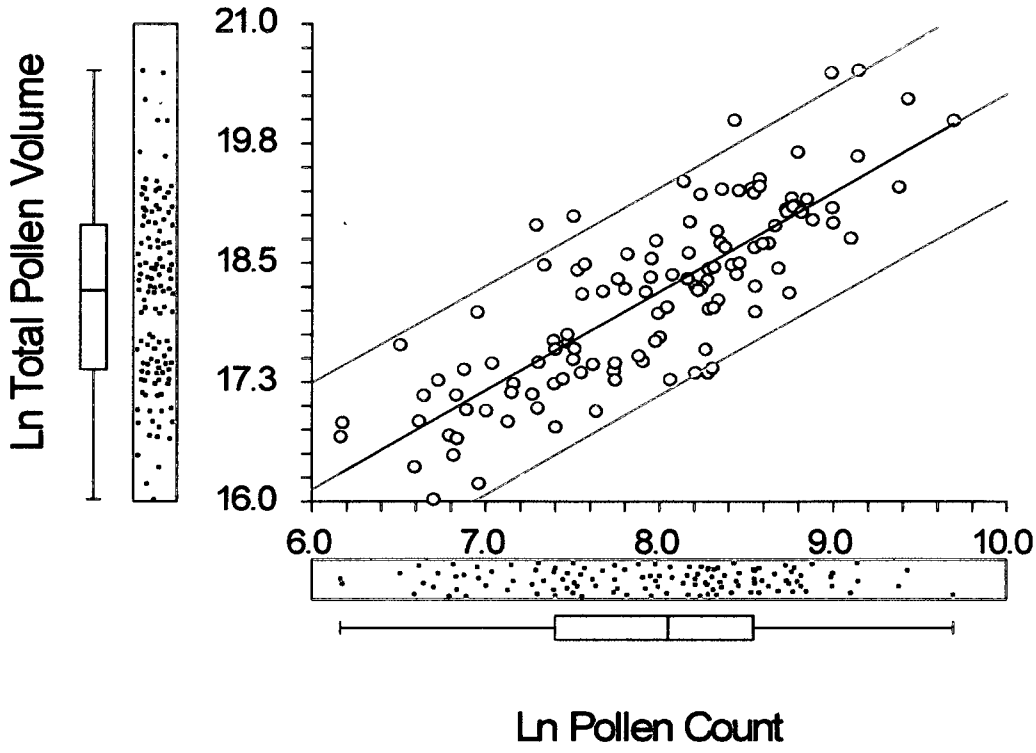

Figure 5.5 - Pollen count vs. total pollen volume (r^2= 0.6700, Pearson's r=0.8186, p<0.0001). Pollen count contributes the bulk of the variation in total pollen volume, compared to pollen grain volume.

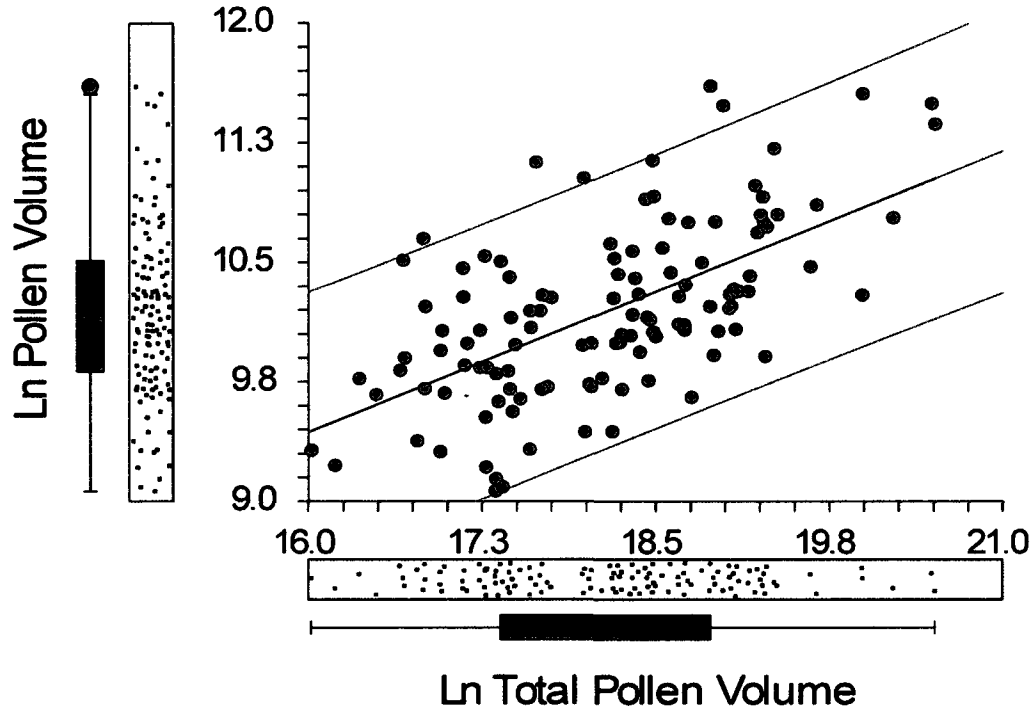

Figure 5.6 - Pollen volume vs. total pollen volume size (r^2= 0.3767, Pearson's r= 0.6135, p<0.0001). Pollen volume contributes less to the variation in male reproductive output than pollen count does.

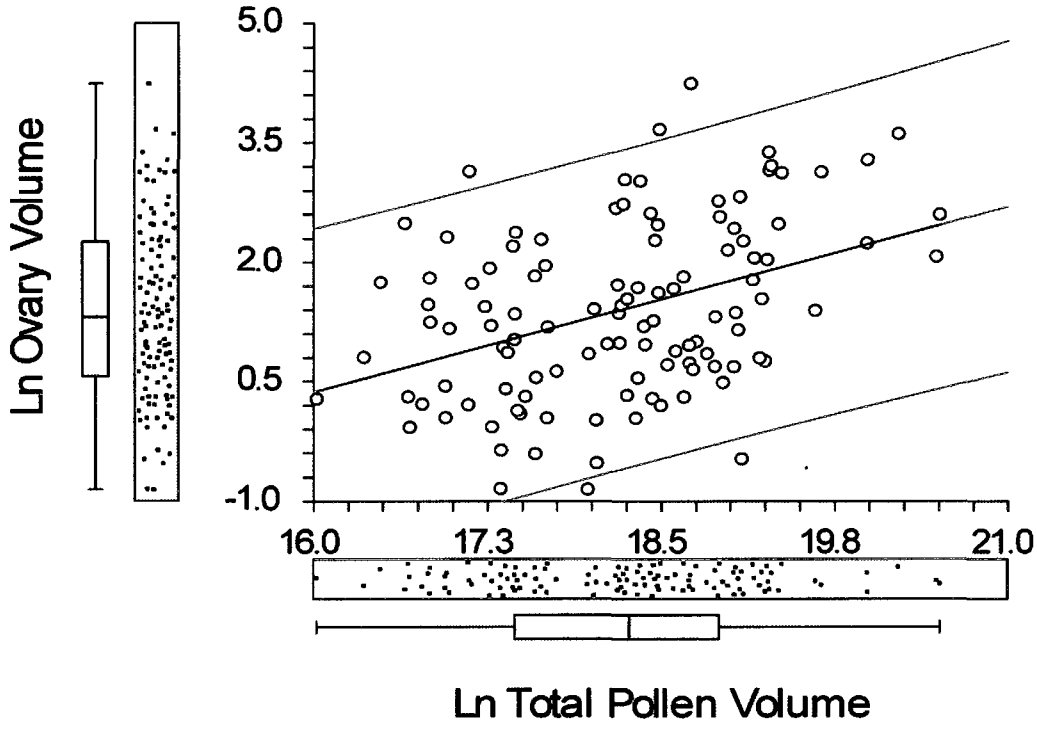

Figure 5.7 - Total pollen volume vs. ovary volume (r^2= 0.1600, Pearson's r= 0.4000, p<0.000). Although the relationship isn't particularly strong, it goes against hypotheses of tradeoffs between male and female sexual function.

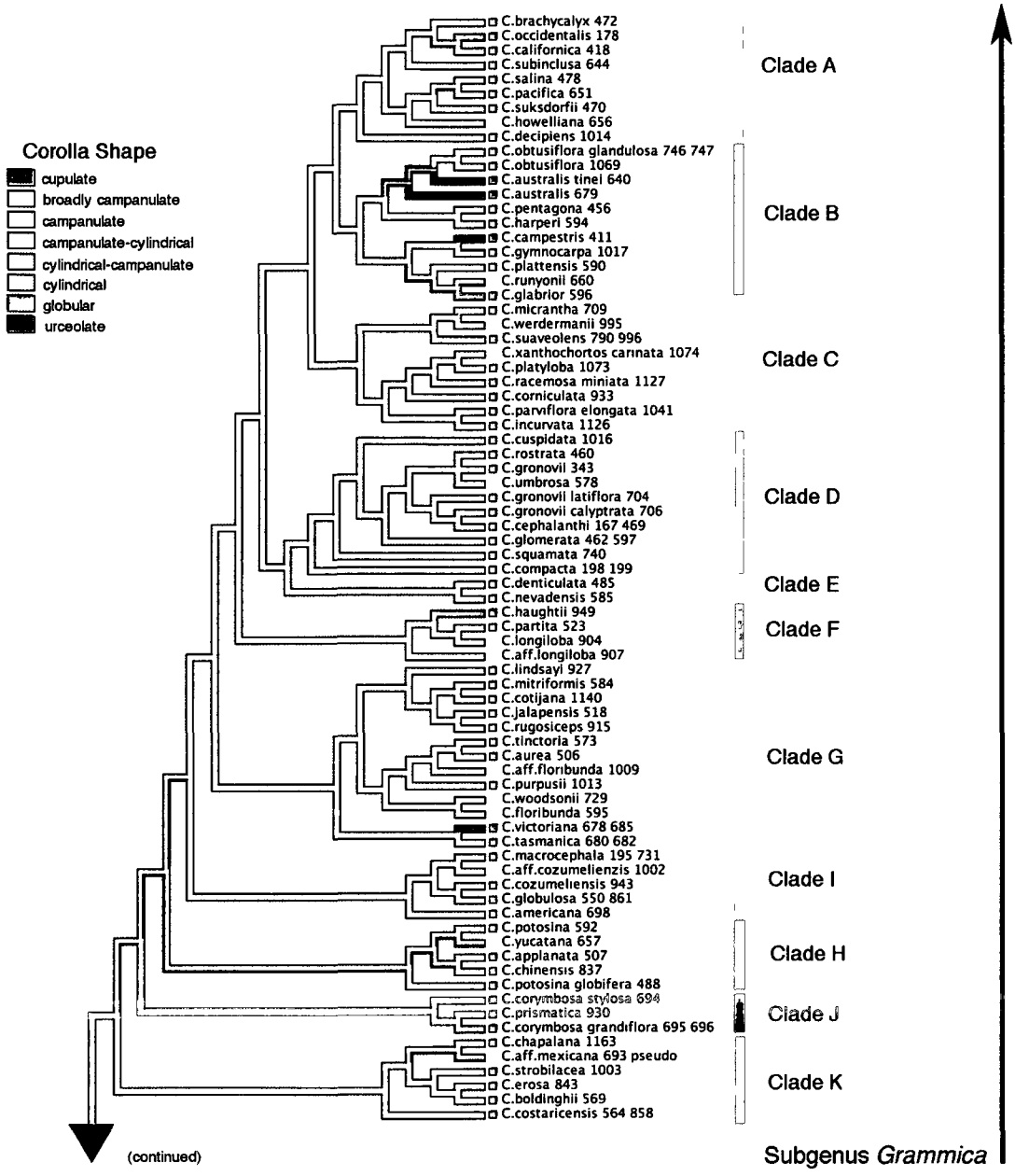

Figure 5.8 – Parsimony reconstruction of the evolution of corolla shape traced onto the phylogeny of genus *Cuscuta*. Cylindric to tubular corollas have been lost and regained numerous times over the evolution of *Cuscuta*. Figure continues on the next page.

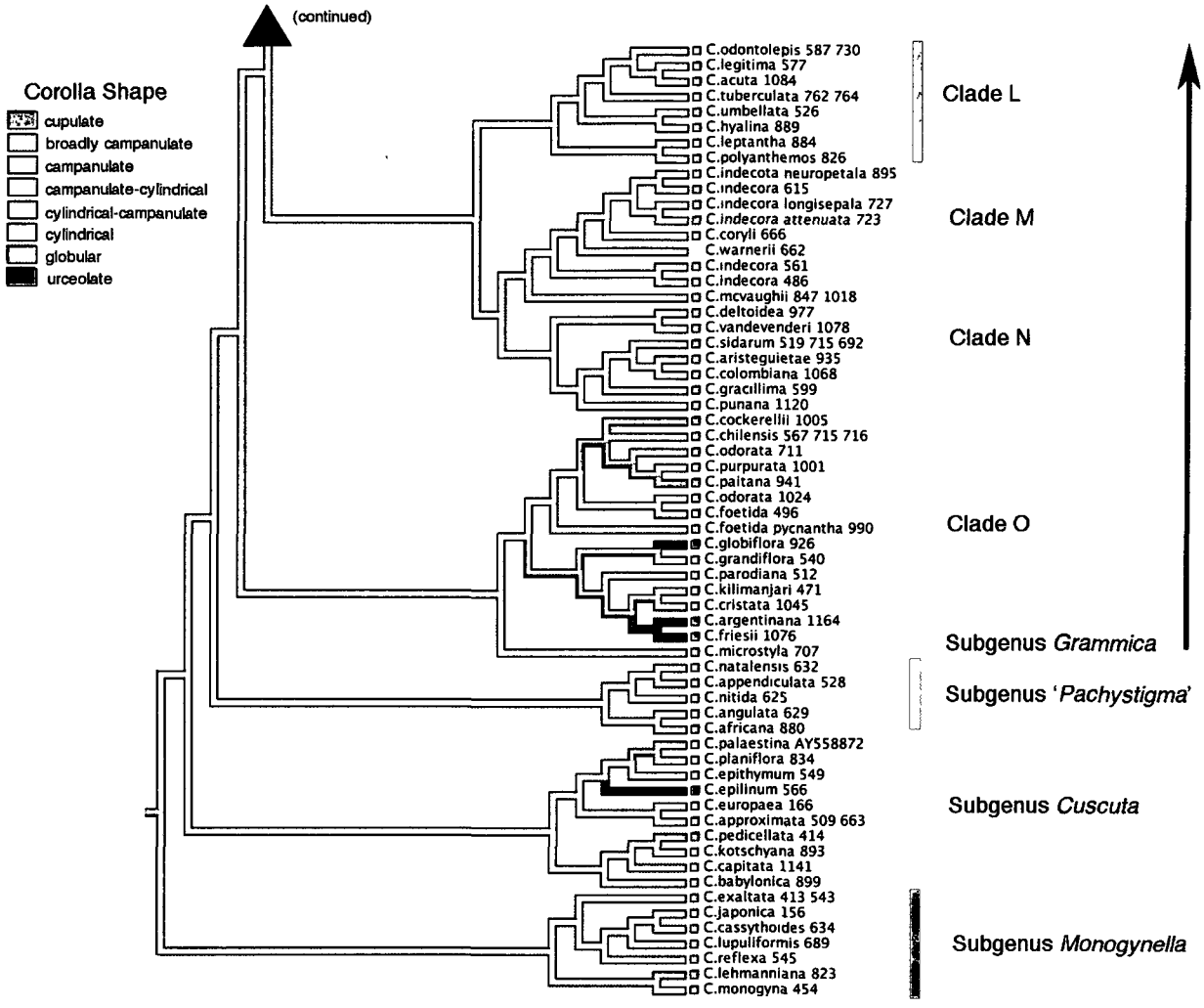

Figure 5.8 (cont'd) – Parsimony reconstruction of the evolution of corolla shape traced onto the phylogeny of genus *Cuscuta*. Cylindric to tubular corollas have been lost and regained numerous times over the evolution of *Cuscuta*. A more cylindrical to tubular shape is found in most of subg. *Monogynella*, and the presence of long corolla tubes in most Convolvulaceae suggest that a cylindric-tubular corolla is ancestral for *Cuscuta*.

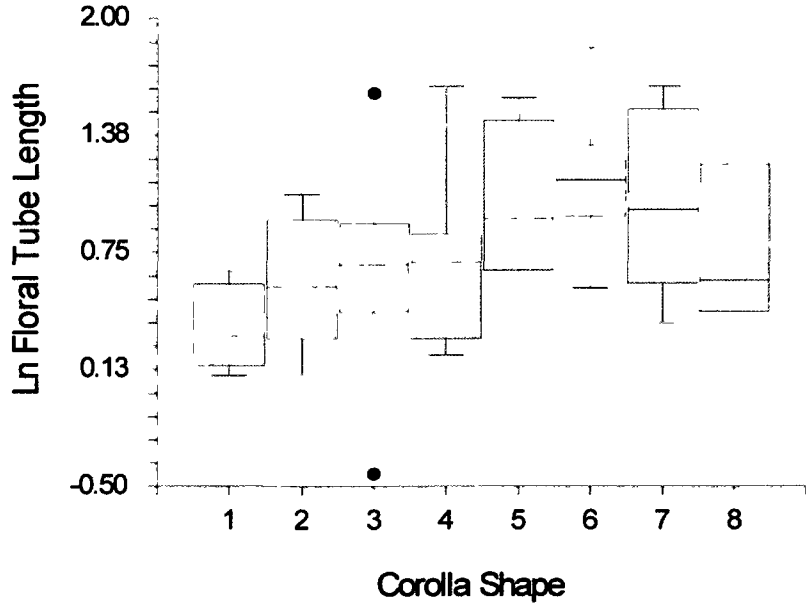

Figure 5.9 – Box plot from ANOVA comparisons of corolla tube lengths for each corolla shape group, as classified by Yuncker (1932). Means were found to be significantly different (F=7.31, p<0.0001), falling into two visually identifiable groups. Box plots show the mean, and quartiles while the whiskers represent range of variation. Green dots represent outliers.

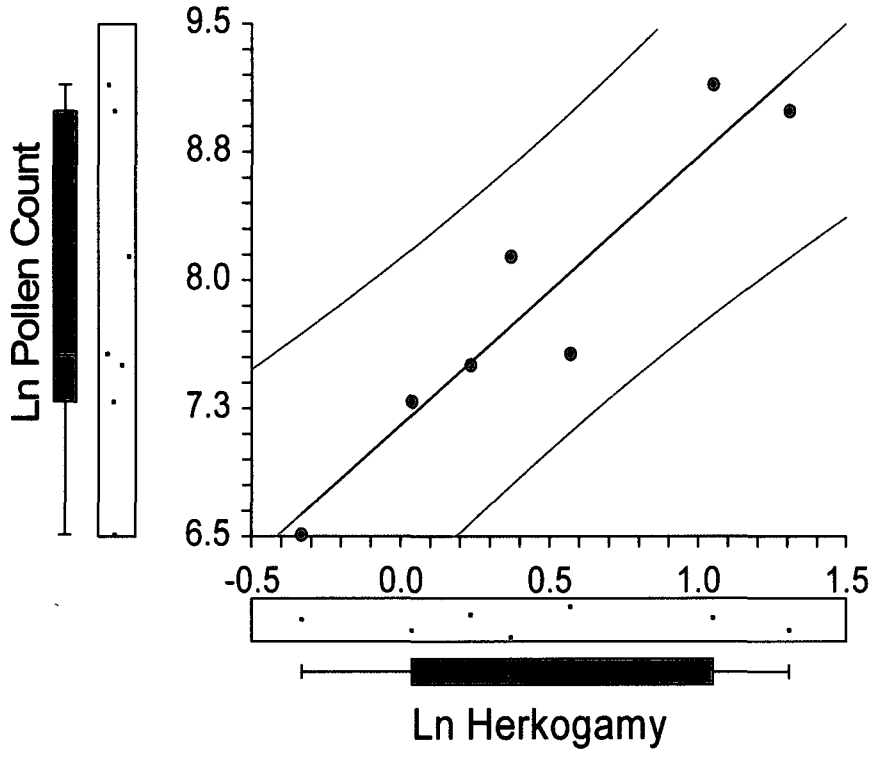

Figure 5.10 - Significant positive relationship between pollen count and herkogamy in subg. *Monogynella* (r^2= 0.8925; r= 0.9447; p= 0.0013). Their single style and tubular corollas likely limit the kinds of reproductive strategies that other *Cuscuta* have developed.

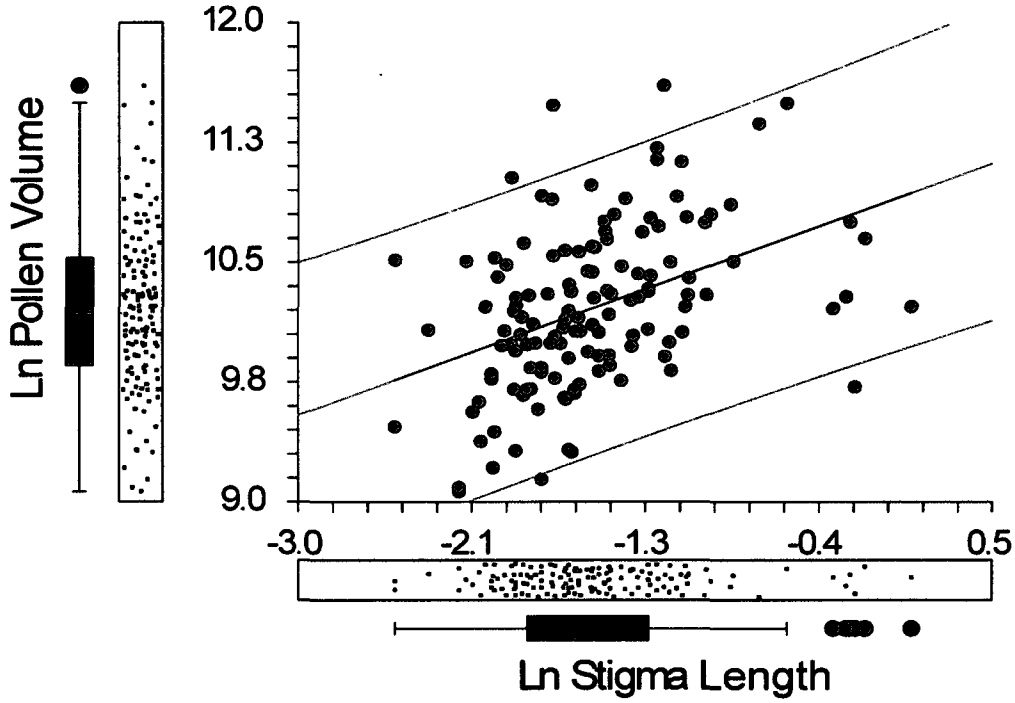

Figure 5.11 - Regression of pollen volume vs. stigma length (r^2= 0.1732, Pearson's r= 0.4162, p<0.0001). All the points that are considered 'outliers' on the stigma length variable are members of subg. *Cuscuta* (including '*Pachystigma*').

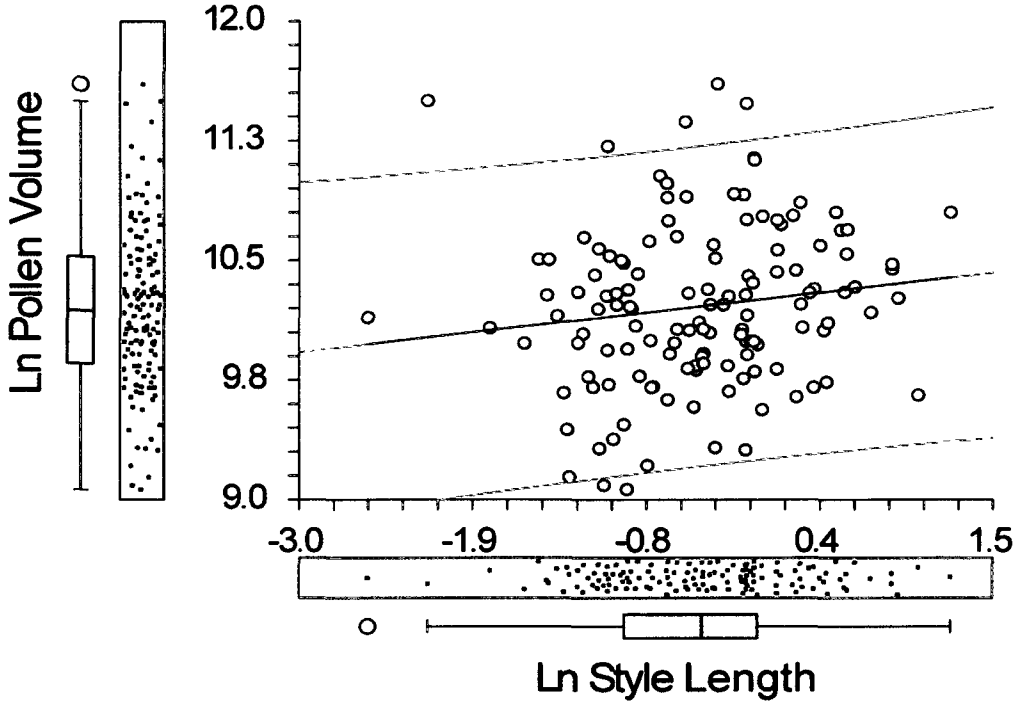

Figure 5.12 – Despite the hypothesis that pollen volume is correlated to style length, no significant relationship exists between the two characters across the genus (r^2= 0.0199, Pearson's r= 0.1411, p=0.1080).

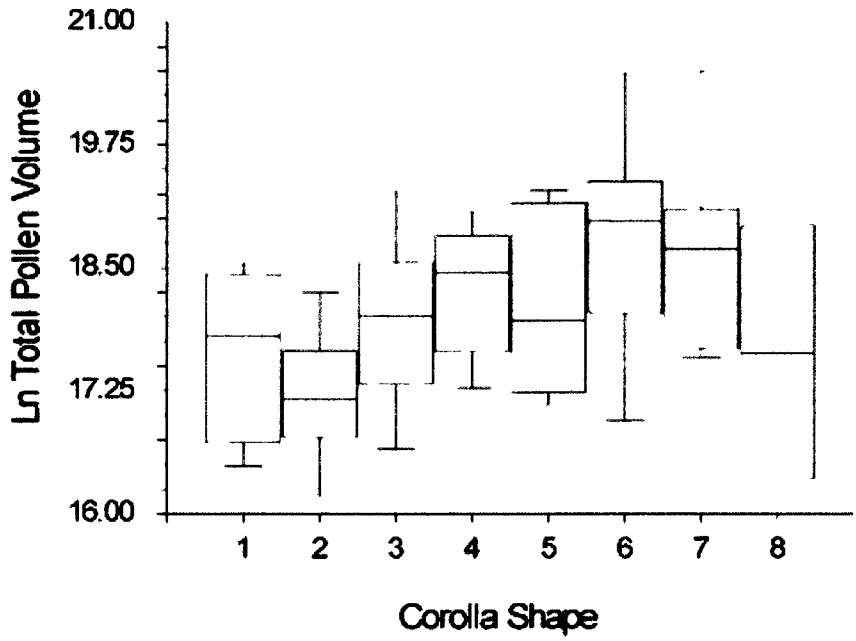

Figure 5.13 - Box plot from ANOVA comparisons of mean total pollen volume for the different corolla shape groups returned significant differences (F= 4.58, p=0.0002). Box plots show the mean, and quartiles while the whiskers represent range of variation.

6. Results II: Floral Rewards

6.1 Morphology and anatomy of the floral nectary

The nectary in *Cuscuta* consists of a nectariferous parenchyma overlain by a cuticularized epidermis with modified stomata in a distinct band (Figures 6.1 & 6.2). Stomata were anomocytic, with the subsidiary cells indistinguishable from the cells of rest of the epidermis. The number and surface arrangement of the stomata varies greatly between lineages and species, with stomata numbers ranging from 7 to over 140 (Figure 6.3). Stomata may occur singly or clustered together in groups of two to four, but the amount and closeness of clustering varies from flower to flower and species to species. Guard cells could be rounded to angular, and have significant starch reserves that were easily stained using Lugol's iodine to distinguish the guard cells from the rest of the epidermis. Neutral red staining was inconsistent. Abnormal stomata were witnessed with extra guard cells (Figure 6.2b), or where two stomata abutted against one another along their longitudinal axes and appeared to share a common, larger opening.

The nectary is vascularized by radial branches from the central vascular cylinder of the ovary that end at the juncture with the ovary wall apical to the nectary. These branches are composed entirely of phloem, except in subgenus *Monogynella* and a couple exceptional species in subgenus *Grammica*, where xylem is present in the bundles representing the carpelar dorsals. Since the vasculature is not inti-

mately associated with the nectariferous parenchyma, I hypothesize that in *Cuscuta* phloem sap is taken up and modified by the nectariferous parenchyma prior to the release of nectar during anthesis, as opposed to a system where the phloem sap is released directly. Histological analysis of the nectary at different developmental stages supports this hypothesis. In *C. gracillima*, *C. gronovii*, and *C. costaricensis* the nectariferous parenchyma becomes loaded with starch granules before anthesis, but these deplete from this tissue after the flower opens (Figures 6.4, 6.5 and 6.6). Furthermore, in some species, the nectariferous tissue collapses as development proceeds, leading to the formation of pits (e.g. *Cuscuta epithymum*, *Cuscuta ortegana*) or depressions with isolated circular or continuous linear shapes (e.g. *C. tinctoria*), although this character is not always consistent within species (Figure 6.7).

6.2 Pollen histochemistry

Staining of the pollen reveals that *Cuscuta* possess both the starchy and non-starchy pollen types, and a substantial pollenkitt (Table 6.1; Figures 6.8 and 6.9). In fixed specimens of species from Mexico and southern Ontario, the pollenkitt was golden yellow to deep orange in colour indicating the presence of carotenoid pigments. In none of the species with starchy pollen did internal starch granules dominate the interior. Lipid deposits were similarly diffuse. Species with high pollen counts tend to have substantial external lipids and strong internal staining with Nile blue sulfate, but the presence of starch does not seem to have a pattern.

6.3 Infrastaminal scales

Staining of the infrastaminal scales revealed a similar staining pattern to that of the corolla more broadly. In each species the fimbrial laticifers of the infrastaminal scales along with the laticifers of the corolla tube were deeply stained blue to purple-pink with Nile blue sulfate, while Lugol's iodine produced a golden to orangish colour in these structures (Figure 6.10). Lugol's iodine also demonstrated the presence of starch grains in non-laticiferous cells of the corolla tube and infrastaminal scales. Neutral red staining was inconsistent and will not be reported on here. This staining pattern indicates that the latex of the fimbrial glands has a large neutral lipid component. The secretory cell appears to gradually protrude from between the epidermal cells at the end of the fimbria.

In cross section, the fimbriae consisted of a set of five to six epidermal cells surrounding a central cell in the species examined (Figure 6.11). Very little cytoplasm was noted in any of the cells, though starch grains were infrequently seen in the periphery of the epidermal cells. The epidermal cells have a ridged and cutinized external surface. Unfortunately, the infiltration and embedding method chosen, while suitable for general histological study, is not suitable for examining structures that are sites of lipid synthesis and storage, as the xylene and paraffin media will solubilize and remove lipids from the specimens. Therefore, no detail was recovered in the secretory cells of the fimbriae.

Table 6.1 – Pollen & Pollenkitt Histochemistry. Staining intensity of pollen grains in Lugol's Iodine (starch), Sudan IV (external lipids), and Nile Blue Sulfate (lipids), and amount of pollenkitt lipids present are indicated by number of +'s. Species marked with an asterisk were sampled from herbarium materials. Pollen counts and pollen grain volume are supplied for context

Species	Average Pollen Count	Pollen Grain Volume (μm^3)	Pollenkitt Lipids	Pollen Grain Staining Intensity		
				Lugol's Iodine	Sudan IV	Nile Blue Sulfate
C. americana	1100.20	20846	+	++		
C. chapalana	9325.80	35358	+++		+	+
C. corymbosa var. *grandiflora*	6497.75	30333	+++	+++		
C. corymbosa var. *stylosa*	6180.67	30714	+++	++	+	+
C. costaricensis	3524.33	34090	+++	+++		
C. cotijana	11830.00	20117	++++		+	+++
*C. epithymum**	4077.00	16639	+++		+	++
C. gracillima	8972.00	15566	++		+	
C. gronovii	5162.00	24645	++		+	+
*C. indecora**	3772.00	58739	+		+	+++
*C. howelliana**	3942.00	8641	++		+	++
C. jalapensis	5072.00	46492	+++		+	+
*C. japonica**	3425.60	74113	+++		+	+
C. lindsayi	12457.00	48048	++++		+	++++
*C. natalensis**	6759.00	27177	+		+	++
C. obtusiflora var. *glandulosa*	3212.00	29739	+++			++
C. pacifica	1626.00	18743	+++	+	++	++
C. rugosiceps	3796.00	21989	+++	+	+	
*C. stenolepis**	2294.00	15156	+		+	+
C. strobilacea	6966.00	30264	+++			+
*C. suksdorfii**	748.25	27464	++		+	++
C. tinctoria	6392.00	33346	+	+++		
*C. xanthochortos**	2846.17	39786	++		+	
C. 'volcanica'	16788.00	n/a	++++		++	++++

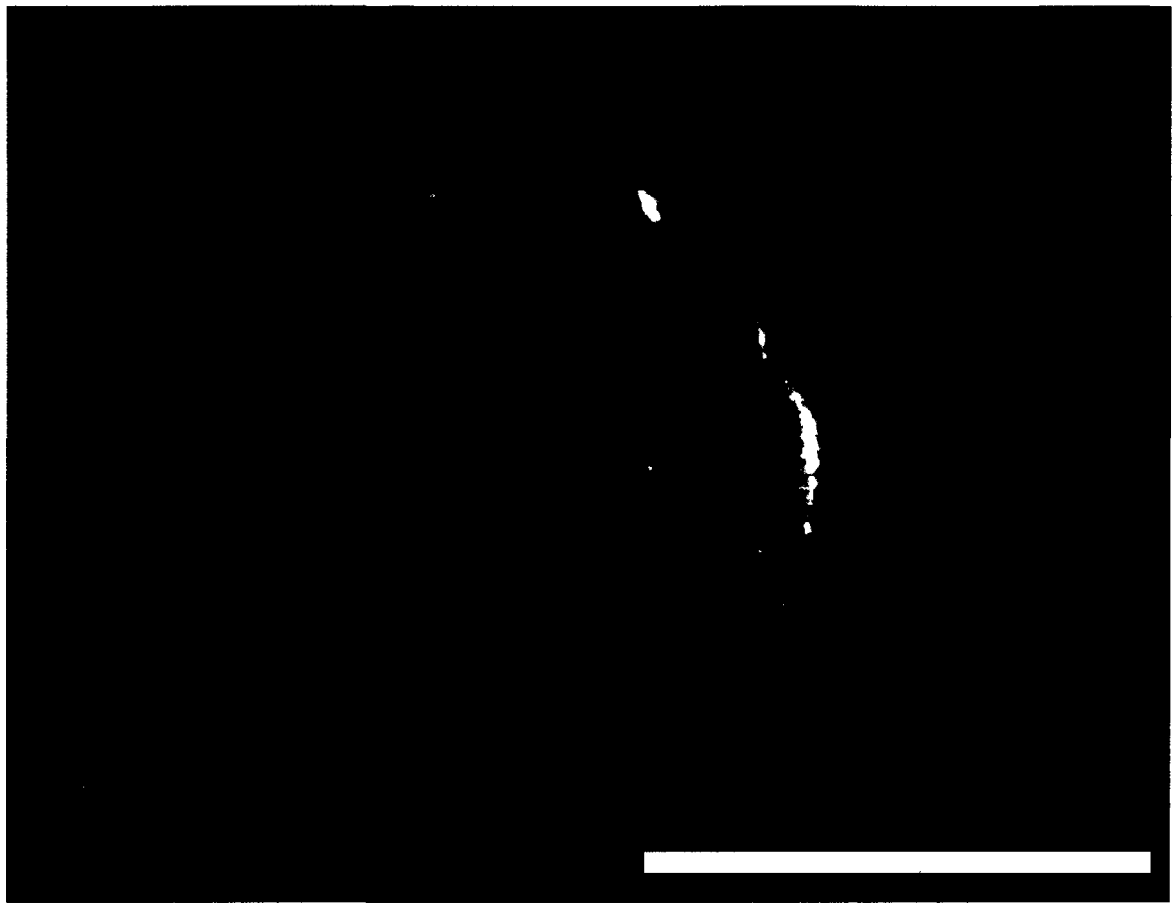

Figure 6.1 - *C. macrocephala*. Brief staining with Lugol's iodine reveals the band of modified stomata near the base of the ovary (black dots). Note the orange staining of the laticifers near the future line of dehiscence above the nectary. Scale = 1 mm.

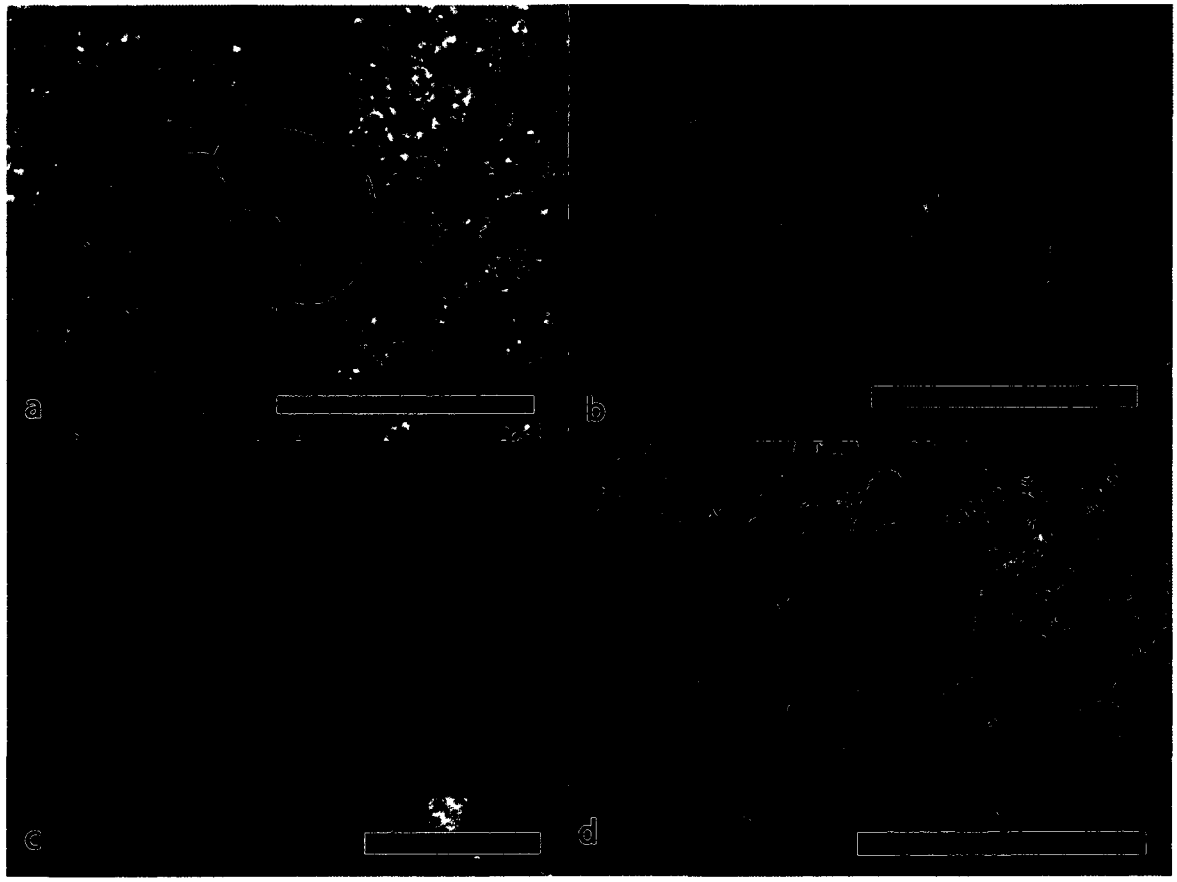

Figure 6.2 – Form of the nectary stomata. a) *C. japonica* - The modified stomata of the nectary are anomocytic in development and are always open once mature. They are often rich in starch, stained black with Lugol's iodine in this micrograph. Scale bar = 100 µm. b) *C. foetida* reveals one of the many forms of abnormal stomata that can be found on the nectary bands. Scale bar = 100 µm. c) *C. suaveolens* displays both clustered stomata and guard cells with more angular borders. Scale bar = 100 µm. d) *C. platyloba* has extremely C-shaped, starch-rich guard cells and a very wide stomatal aperture. Scale bar = 250 µm.

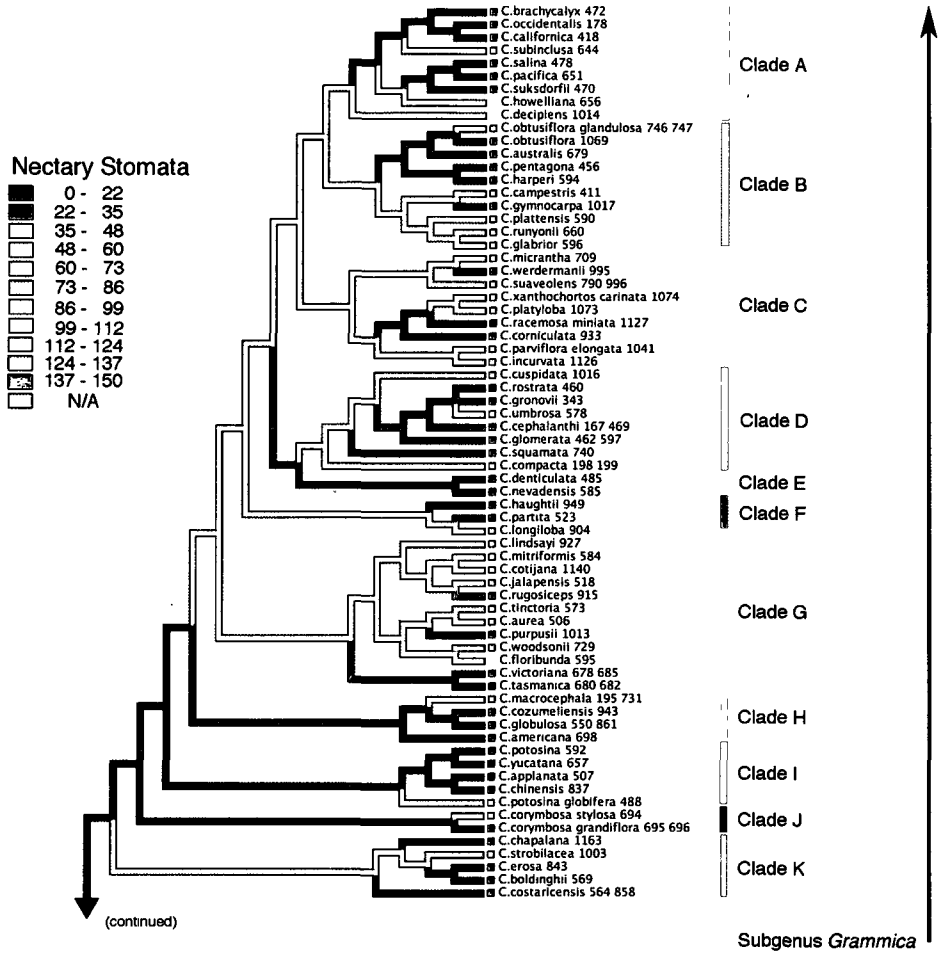

Figure 6.3 - Parsimony reconstruction of the evolution of nectary stomata number per ovary mapped onto the phylogeny of the genus. Clades G and O of subg. *Grammica* have higher relative numbers. Counts unavailable for grey branches. Figure continued on next page.

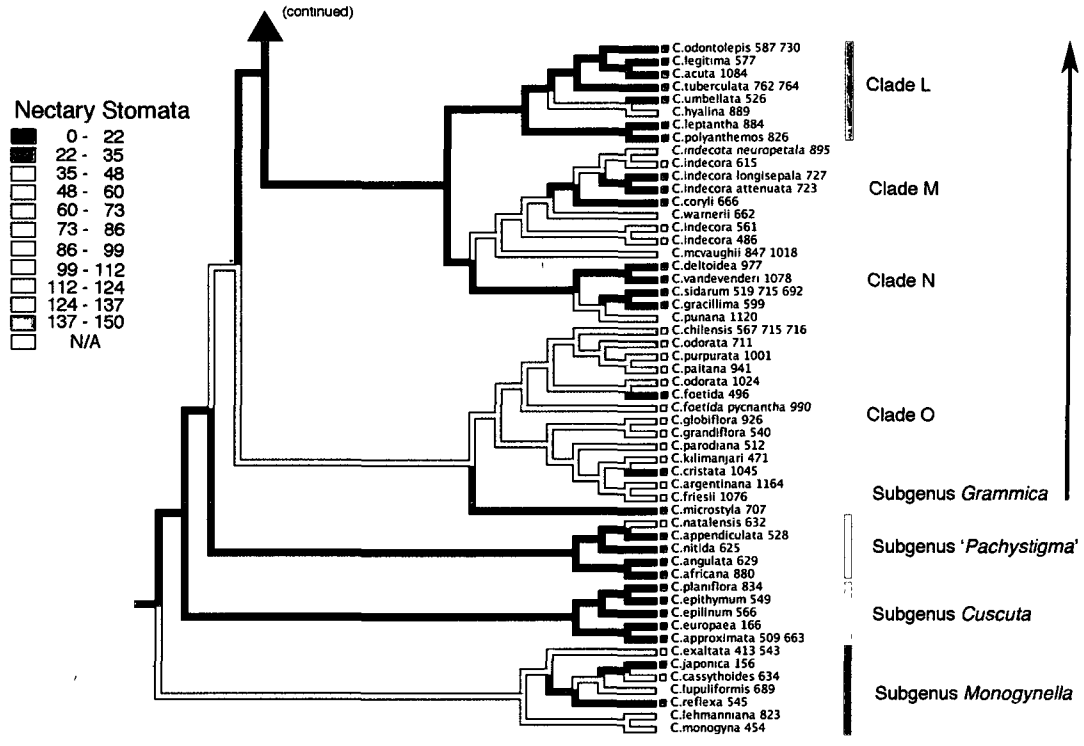

Figure 6.3 (cont'd) – Parsimony reconstruction of the evolution of nectary stomata number per ovary mapped onto the phylogeny of the genus. Clades G and O of subg. *Grammica* have higher relative numbers. Counts unavailable for grey branches.

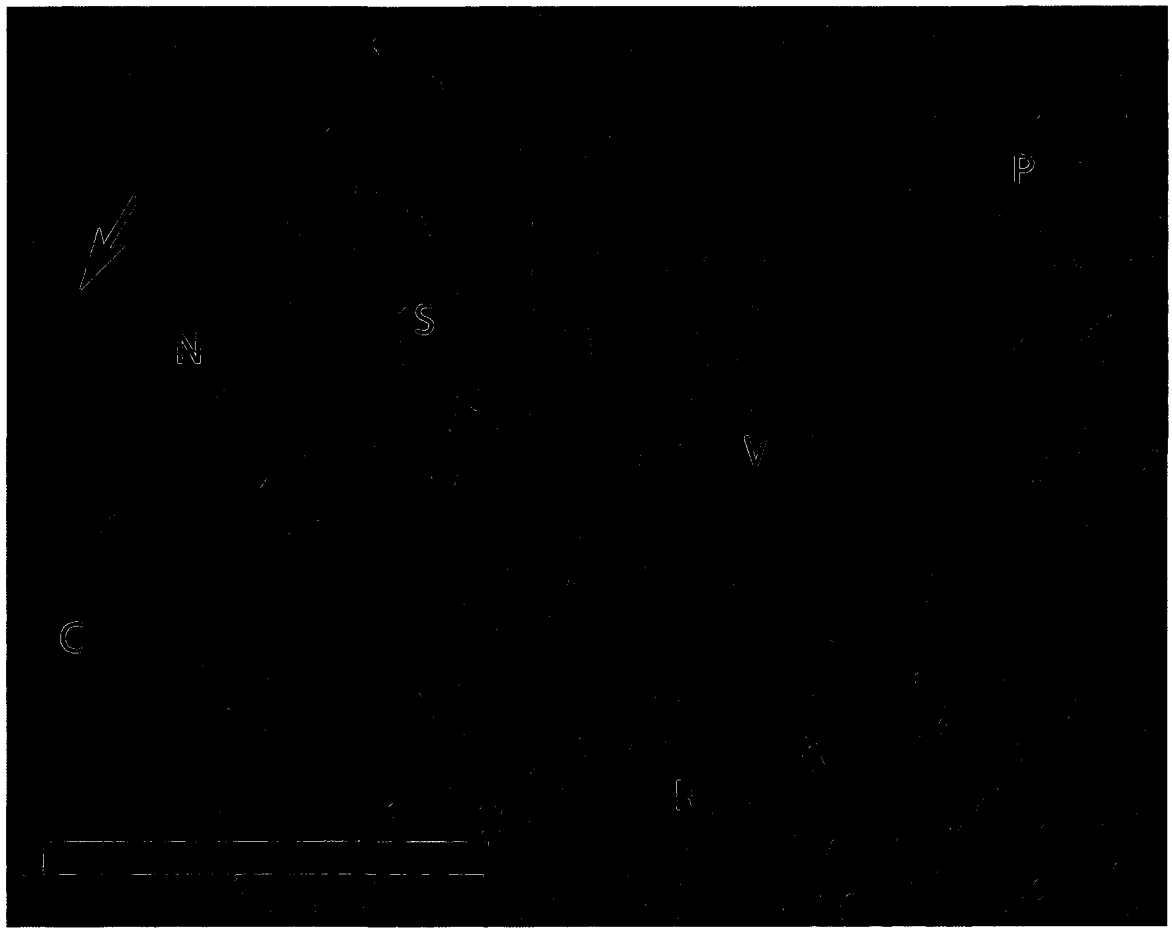

Figure 6.4 - Longitudinal section of the nectary from a mature *C. gracillima* bud of just prior to anthesis. Note the abundant starch granules (magenta) in the nectariferous (N) and subnectary (S) parenchyma. C - corolla tube, R - receptacle, V – central vascular cylindar of the ovary, P - placenta, large arrow - modified stomata, small arrows - starch granules. PAS-Alcian blue stain. Scale bar = 250 µm.

Figure 6.5 - Longitudinal section of the nectary from an open *C. gracillima* flower. Note the reduction in the size and number of starch granules in the nectariferous (N) and, to a lesser extent, the subnectary (S) parenchyma. (R) - receptacle, (V) – radial vasculature of the nectary and ovary wall, (P) - placenta, (O) - obturator, W, ovary wall, large arrow - tangential section of a modified stomata. PAS-Alcian blue stain. Scale bar = 250 μm.

Figure 6.6 - Longitudinal section of the nectary from an old *C. gracillima* flower where the corolla has begun to wither. Note the absence of starch granules in the nectariferous (N) and subnectary (S) parenchyma. (C) - corolla, (L) - laticifer, (P) - placenta, (X) - xylem, large arrow - modified stomata. PAS-Alcian blue stain.

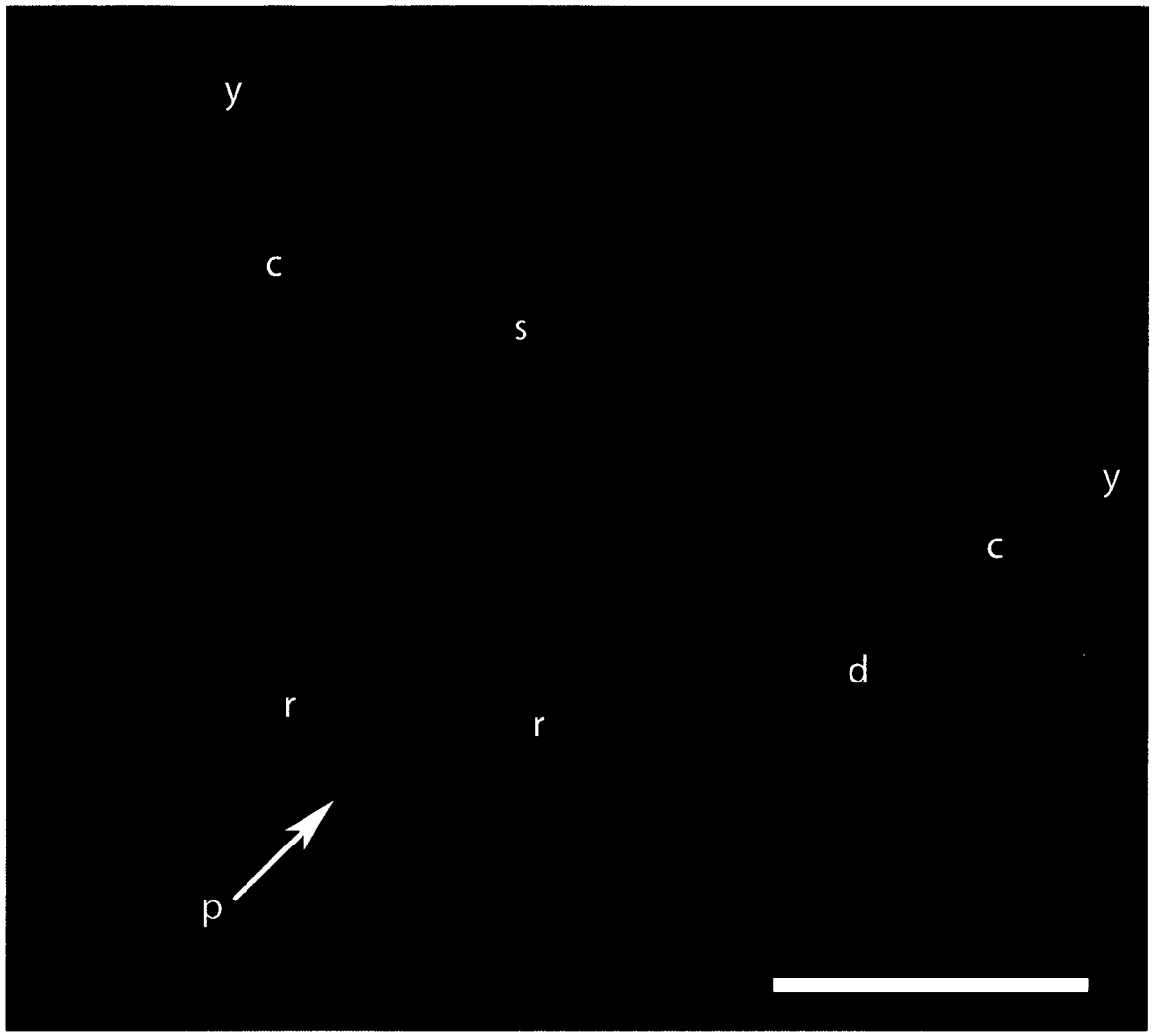

Figure 6.7 - This developing capsule of *C. chapalana* was stained with decolourized aniline blue, UV excitation. Callose binds aniline blue and fluoresces green. Cyan and pink are autofluorescence of lignins and lipids, respectively, in the sclerified caps of each locule (c) and seeds (s). The nectary stomata show much callose deposition and have collapsed to form deep pits (p). The radial phloem (r) that supply the nectary and meristematic zone of the ovary wall, and one of the carpelar dorsals (d) extends into the style (y). Scale bar = 1 mm.

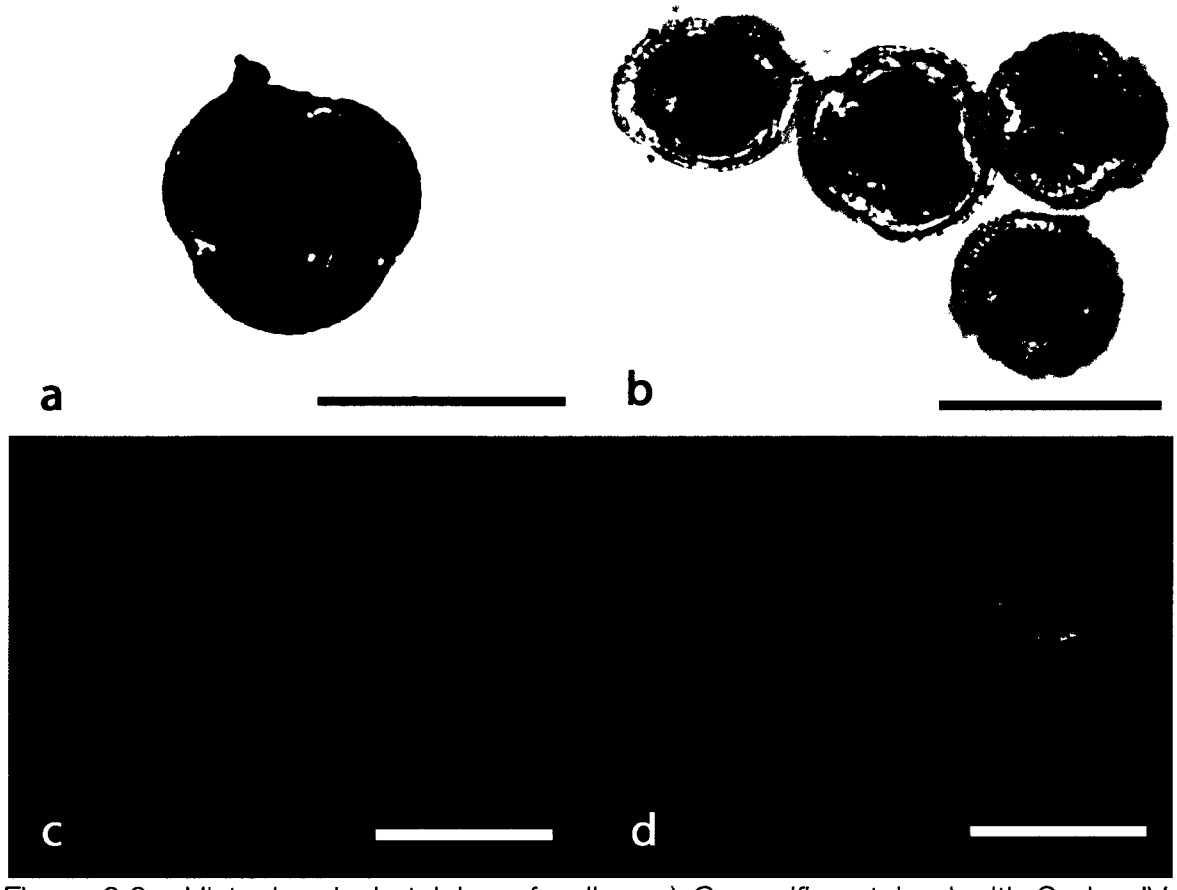

Figure 6.6 - Histochemical staining of pollen. a) *C. pacifica* stained with Sudan IV demonstrating the presence of lipid deposits (red globules). b) *C. tinctoria* stained with Lugol's Iodine demonstrates starchy pollen (black stained material). c, d) *C. chapalana* stained with Nile Blue sulphate to demonstrate lipids. c, UV excitation (acidic lipids pink; neutral lipids/sporopollenin blue-white); d, green excitation (acidic lipids yellow-orange). Scale bar = 35μm.

Figure 6.7 - Copious pollenkitt lipid oozes from the edge of a crushed *Cuscuta 'volcanica'* anther after staining with Nile blue sulfate. UV excitation. Scale bar = 150 µm.

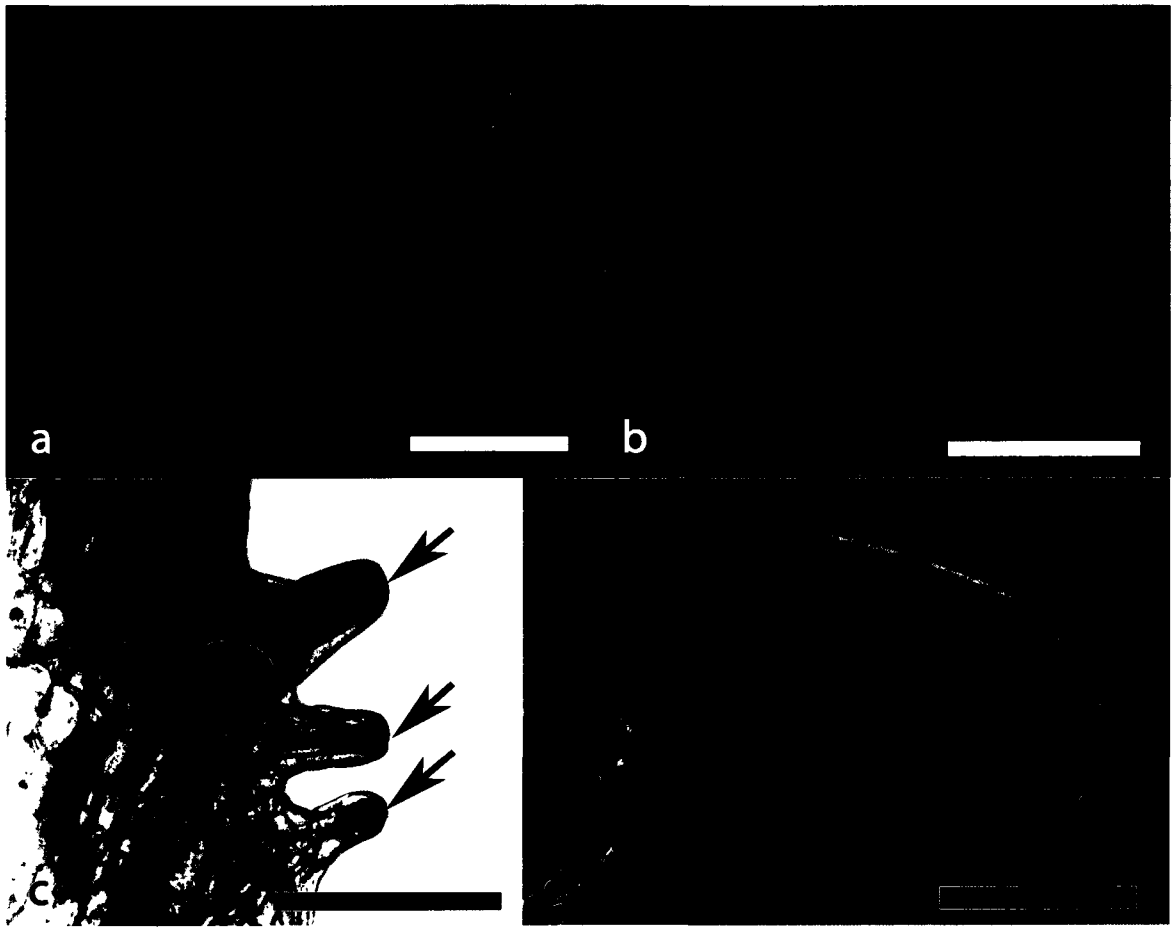

Figure 6.8 - Infrastaminal scales stained with Lugol's iodine or Nile Blue Sulfate to reveal the presence of inclusions. a) dissected corolla tube of *C. macrocephala* stained briefly with Lugol's iodine. Laticifers in the petals, the anthers, and the fimbrial glands of the scales are stained a deep amber. Scale bar = 2 mm. b) Closer view of a Lugol's iodine-stained scale from *C. ortegana*. Scale bar = 250 µm. c) Nile Blue sulfate localized to the glands of several short scale fimbriae in *C. mitriformis*. Sacle bar = 500 µm. d) detail of a stained fimbrial gland from *C. cotijana.* Scale bar = 50 µm.

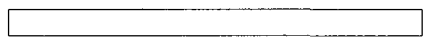

Figure 6.9 - Cross section through the fimbriae of an infrastaminal scale in *C. gracillima*. Tissue in the left portion of the field is part of the corolla tube. PAS-alcian blue stain, scale bar = 100 µm.

7. Results III: Stigma Receptivity & Spatial-Temporal Orientation

Both field and herbarium observations indicate that the orientation and position of the stigma change over the course of flower development, but each of the subgenera show a different general pattern of development. In all species, anther dehiscence begins either in bud or at the beginning of anthesis. The anthers are bent inwards towards the floral axis in bud and early in anthesis, and the dehiscent surface is oriented towards or even in direct contact with the stigmas. This means that in species whose anthers dehisce in bud or early in anthesis, pollen is easily and autonomously transferred to the stigmas (Figure 7.1).

In subgenus *Monogynella*, the styles may briefly grow until stigmas have reached their final position, but there is little change in the size or orientation of the stigma. By contrast, after anthesis the stigmas of subgenus *Cuscuta* may continue to elongate (e.g. in *C. natalensis, C. epithymum*; Figure 7.2). In subg. *Grammica*, the unequal styles can elongate considerably after anthesis, and in many species they continue to elongate after the anthers have dehisced and withered (e.g. *C. costaricensis*, *C. 'volcanica'*; Figure 7.3). At anthesis the stigmas may initially be fully or partly hidden beneath the infrastaminal scales (e.g. *C. tasmanica, C. strobilacea*; Figure 7.4), or may be exposed (e.g. *C. gronovii*, *C. obtusiflora* var. *glandulosa;* Figure 7.5). In a few species (e.g. *C. cephalanthi*, Clade D; *C. gracillima*, Clade N), the

stigmas may even advance past the anthers very quickly as anthesis begins and before anther dehiscence, but this condition is much more rare (Figure 7.6). It is not known whether this condition represents protogyny in either of these species, or if stigma receptivity is delayed until full anthesis.

In my tests of stigma receptivity, the onset and peak of receptivity varied with respect to both anthesis and anther dehiscence between the species tested (Table 7.1). The smaller-flowered *C. campestris* var. *glandulosa*, for example, becomes receptive in bud and peaks at the beginning of anthesis while larger-flowered *C. cotijana* and *C. strobilacea* become receptive at the beginning of anthesis and do not peak until after the anthers have begun to wither. Importantly, the timing of peak reactivity was delayed in the lower stigma for all species tested.

I will now highlight three examples of strategies evolved in *Cuscuta* that use combinations of differential timing of stigma receptivity, differences in stigma height, and/or stigma or style elongation to provide reproductive assurance. Further, each of these examples represents a different level of pollen production and lineage. The first two example strategies highlight reproductive assurance through prior selfing, while the latter example illustrates a strategy that facilitates and priviledges outcrossing.

In *C. epithymum* (subg. *Cuscuta*), the anthers dehisce right at the time of anthesis and are bent inwards against the stigma, autonomously transferring pollen to the distal part of the stigma (Figure 7.1). The anthers later become erect while the

stigmas elongate a little and change orientation from vertical to much more horizontal (Figure 7.2). A similar but more extreme pattern can be seen in *C. obtusiflora* (subg. *Grammica* Clade B), a weedy species where anther dehiscence and stigma receptivity both occur in bud, allowing fertilization to take place before the flower opens (Rodrigues-Pontes 2009). My observations of the Mexican *C. obtusiflora* var. *glandulosa* confirm a similar phenology of anther dehiscence and stigma receptivity. However, I observed that the stigmas in this variety remain receptive after anthesis. While the upper stigma is in contact with the dehiscing anthers in bud, the lower stigma has delayed receptivity and is partly shielded from self-pollen by the upper stigma, allowing a later 'second chance' for competitive outcross pollination to occur after the flower opens. Interestingly, *C. obtusiflora* var. *obtusiflora* was found to have a much lower average pollen count than var. *glandulosa* (912 vs. 3212).

C. strobilacea (subg. *Grammica* Clade K) contrasts with the previous examples. In this species I have observed a strong separation of male and female sex function that facilitates outcrossing. Both stigmas remain hidden beneath the infrastaminal scales until well after anther dehiscence occurs at the initiation of anthesis (Figure 7.3). The anthers begin to wither when the stigmas grow beyond the mouth of the corolla tube (Figure 7.7). The styles further lengthen and diverge until they come into contact with the collapsing anthers where they may pick up remaining self-pollen just before withering (Figure 7.7).

Table 7.1 – Peroxidase test of stigma receptivity across floral stages. Stage 1 – immature bud; Stage 2 – later bud; Stage 3 – mature bud; Stage 4 – anthesis begins; Stage 5 – flower fully open; Stage 6 – anthers begin to wither; Stage 7 – anthers fully withered; Stage 8 – stigmas wither. Number of +'s = intensity of color reaction; U = upper stigma, L = lower stigma; * = timing of anther dehiscence

Species	Stigma	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Stage 6	Stage 7	Stage 8
C. obtusiflora var. *glandulosa*	U	+	+++	+++++	++++	+++	+	+	
	L	+	++	+++*	+++++	++++	++	+	+
C. gronovii	U	+	+	++	+++++	+++++	+++	++	+
	L		+	+	+++*	+++++	++++	+++	+
C. cotijana	U			+	++	+++*	+++++	+++++	++
	L				+	++	++++	+++++	+
C. 'volcanica'	U				++*	+++	++++	+++++	++
	L					++	+++	+++++	++
C. costaricensis	U		+	++	++++	+++++	++++	+++	+
	L		+	+	++*	++++	+++++	+++++	+
C. strobilacea	U			+	++*	+++	+++++	++++	+
	L				+	++	+++++	+++++	++

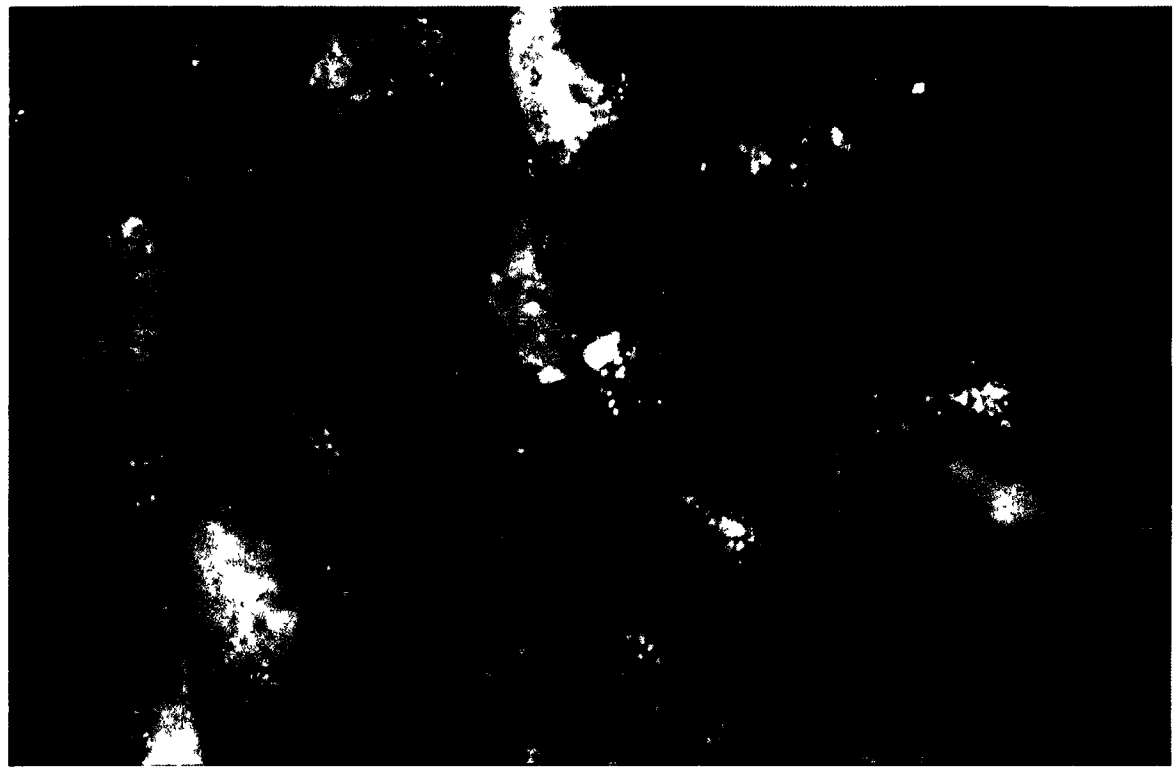

Figure 7.1 - *C. epithymum* flowers early in anthesis. Note that the dehisced anthers are clustered together around the emerging tip of the two free stigmas. Photo by Miguel A. García, used with permission.

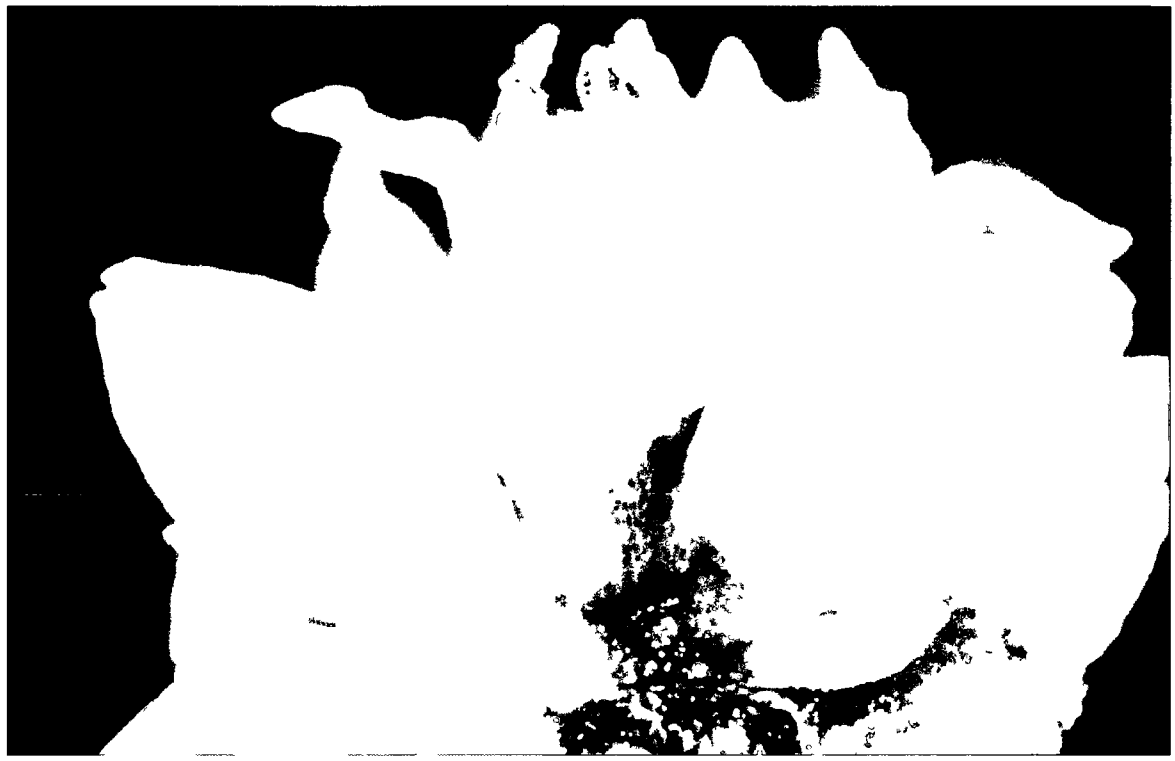

Figure 7.2 - *C. epithymum* flowers later in anthesis. Note that the dehisced anthers are pulling away from the tip of the two free stigmas in the centre flower, while in the older flower on the right the anthers are now fully erect and the stigmas and styles are growing longer while the stigma begins to bend laterally. Photo by Miguel A. García, used with permission.

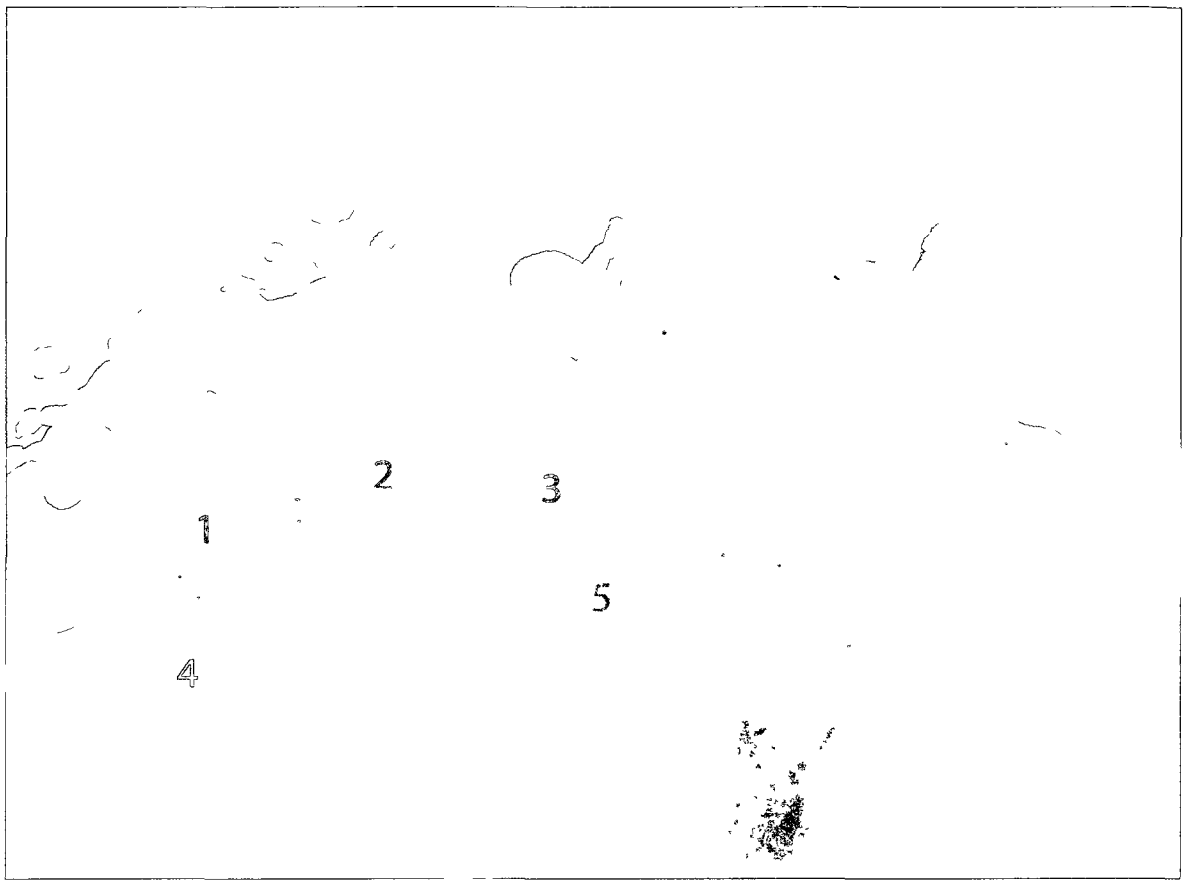

Figure 7.3 - *C. 'volcanica'* inflorescence, with bloom order marked for the cluster on the right. The stigmas can be seen growing out of the floral tube (3, 2, 1). Stigma exposure does not occur until the anthers have begun to wither. Photo by Dr. Mihai Costea.

Figure 7.4 - *C. strobilacea* flower at mid-anthesis. Note that the dehisced anthers are fully erect but the stigmas remain hidden beneath the infrastaminal scales. Photo by Dr. Mihai Costea.

Figure 7.5 - *C. obtusiflora* inflorescence with bloom order marked. The stigmas are well exposed at anthesis (4). Note how there is very little change to the position and length of the styles from early anthesis until they collapse (4, 3, 2, 1).

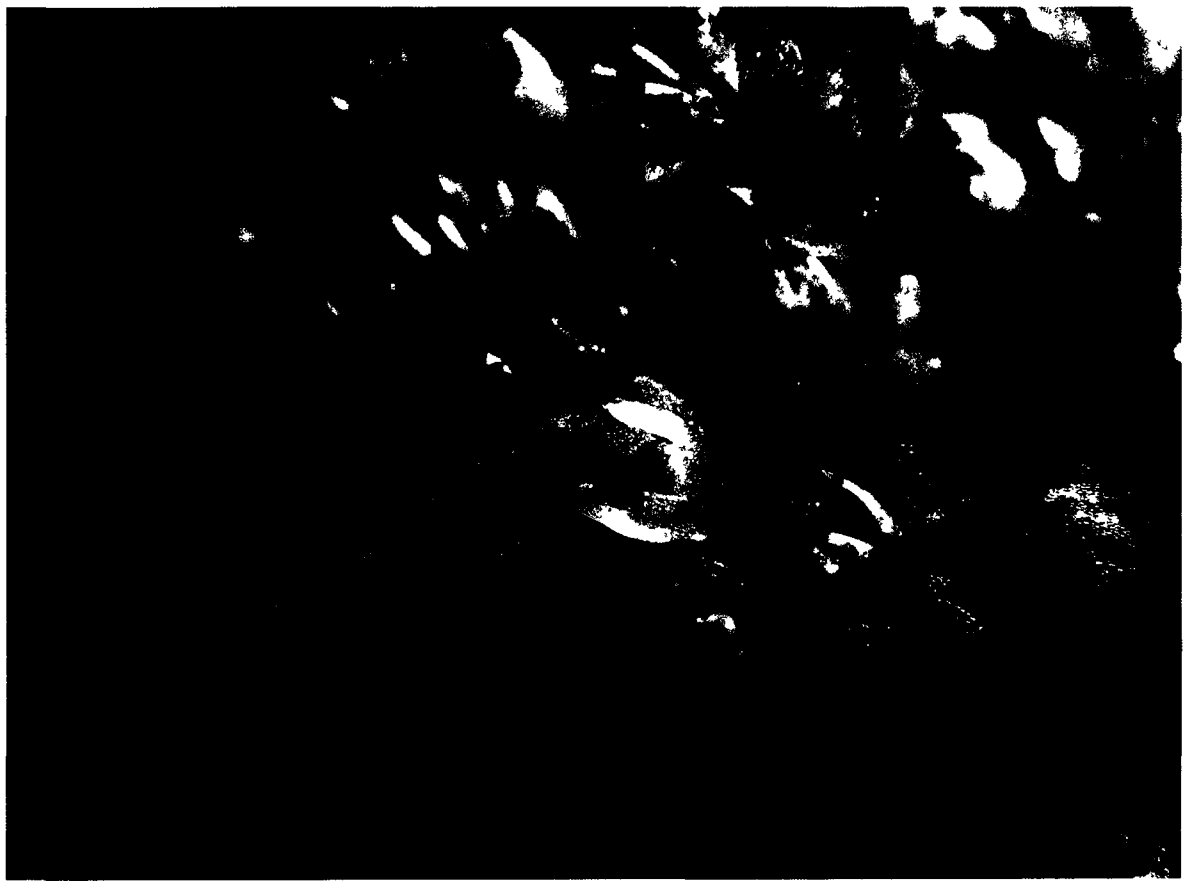

Figure 7.6 - *C. gracillima* inflorescence showing early growth of the styles exposes the stigma before the anthers dehisce. Arrows - opening floral buds with stigmas protruding. Photo by Dr. Mihai Costea.

Figure 7.7 - *C. strobilacea* inflorescence. Note how the stigmas have grown out between the withered anthers on the left side. Late in flower development, the anthers collapse and come into contact with the diverging styles, giving one final chance for self pollination. Photo by Dr. Mihai Costea.

8. Discussion

8.1 Breeding systems in *Cuscuta* – within expectations?

Cruden (1977) asserted that P/O ratios range from 2.7-6.7 for cleistogamous flowers, from 18.1 to 39.0 for obligate autogamous flowers, from 31.9-396.9 in facultatively autogamous flowers, from 244.7-2558.6 in facultatively xenogamous flowers, and from 2108 and up for fully xenogamous flowers. None of the *Cuscuta* species I have sampled have P/O ratios that fit into Cruden's cleistogamous or obligate autogamous range. At the other end of the spectrum, a total of nine species fall into the fully xenogamous range (*C. gracillima*, *C. chapalana*, *C. lehmanniana*, *C. gigantea*, *C. cotijana*, *C. rotundiflora*, *C. lindsayi*, *C. macrocephala*, and *C. 'volcanica'*). Of the remainder, only thirty-two species fit into the facultatively autogamous category, while the last 103 are considered facultatively xenogamous. It is clear that mixed mating systems are the norm in *Cuscuta*, but the degree to which outcrossing vs. sefling is used shows much variation.

In accordance with expectations, many of the species that are widely distributed and/or are considered weedy have low P/O ratios that fall into Cruden's facultatively autogamous range (e.g. *C. approximata*, *C. obtusiflora* var. *obtusiflora*, *C. planiflora*, *C. europea* var. *europaea* and var. *halophyta*, *C. americana*, *C. campestris*, *C. australis*, *C. umbrosa*). Low P/O ratios are characteristic of plants occupying marginal habitats, and disturbed habitats at early successional stages

(Cruden, 1977), and this fits with the picture of these weedy *Cuscuta* that grow on roadsides, in agricultural fields, and near water. However, I suspect that Cruden's ranges don't quite fit the realities of mating and gene flow in *Cuscuta*, particularly for those species where xenogamy is more important. *C. chilensis* (P/O=1654.44) and *C. rostrata* (P/O=1132.5) are known to be self-incompatible, at least in some populations (McNeal, 2005), and therefore are not able to self-fertilize. Despite that, their P/O ratios fall in Cruden's 'facultative xenogamy' range. Extending the limit for obligate outcrossing down to *C. rostrata* results in a larger group of 43 species. It is known that specialized pollen transport mechanisms such as pollinia, polyads and viscin threads result in lower P/O ratios because of their increased pollination efficiency (Cruden, 1977; Cruden and Jensen, 1979; Vasek and Weng, 1988; Wyatt et al. 2000). The presence of the substantial sticky lipid pollenkitt that I observed in the pollen histochemistry studies is likely the factor increasing pollination efficiency in *Cuscuta*. This sticky substance should enhance the transfer and retention of pollen grains on pollinators, and more importantly allows clumps of multiple pollen grains to be transferred to and from pollinators as single cohesive units (Pacini & Hesse, 2005). This could explain why xenogamy seems to be taking place at a lower P/O than suggested by Cruden.

Comparison of *Cuscuta* P/Os to those of other members of the Convolvulaceae demonstrate that, despite their less elaborate flowers, *Cuscuta* falls well within the norms of the family (Table 8.1). Interestingly, *Ipomoea* has a large propor-

tion of species that are facultatively autogamous, despite the presence of colourful and showy flowers that are, at minimum, ten times larger than average *Cuscuta* flowers. Though the sampling here is quite biased towards weedy (and some ornamental) species, one interesting point can be extrapolated. The numbers indicate that the self-incompatible *Ipomoea* spp., similar to *Cuscuta*, seem to have more efficient pollination than Cruden's category ranges allow for obligately xenogamous species.

8.2 Breeding system evolution and floral morphology

My phylogenetic tracing of floral shape confirms Stefanovic and colleagues' (2007) conclusion that the genus displays much morphological homoplasy. The many independent transitions between species with short- and long- tubed corollas emphasize the importance of pollinators and pollinator transitions as agents of selection. Although most of the obligately xenogamous species have large flowers, there are some smaller-flowered species present (e.g. *C. gracillima* and *C. micrantha*). In the case of *C. gracillima*, while the flowers are only ca. 2mm long, the stamens and styles are, relatively to floral size, very long for a *Cuscuta*. These lengths perhaps proxy for the larger, wider floral tube found in most outcrossers and perhaps represent adaptation to the morphology of a class of pollinators different from those that are generally the effective pollinators of large-flowered xenogamous *Cuscuta*. At the other end of the spectrum, there are also exceptional species that have both moderate to large flowers and have a low P/O. *Cuscuta grandiflora* (4-6 mm; P/O=

358.69) and *C. corymbosa* var. *corymbosa* (4 mm; P/O = 208.75), are two good examples with very different morphologies. *C. grandiflora* has broad, shallowly campanulate corolla with a distinct red-purple zone around the exposed ovary. *C. corymbosa* var. *corymbosa* possesses a cylindrical corolla tube, and its sister varieties are both xenogamous (P/O= 1545.17, 1624.44). For both of these species, the low P/O is unexpected. It is possible that my P/O estimates for these species are incorrect; if the specimens sampled for generating the P/O came from marginal habitats, those individuals should be more strongly selfing than the species average.

In terms of features with the most evolutionary lability, both style length and pollen count seem to be the least constrained. This is unsurprising for pollen count, which is known to vary in a significant way between populations due to differences in habitat marginality and successional stage (Cruden, 1977). Changes in pollen/ovule ratio and self-compatibility often precede more substantial evolutionary changes (Lloyd 1965). Style length is the only floral character measured that had a marginally significant correlation to perianth size; all other correlations were strongly significant. This makes intuitive sense. Regardless of the form or size of the corolla, the style is responsible for stigma placement, and is under selection to meet several different, often conflicting needs: to be optimally placed for pollen receipt from the most efficient pollinator, to be positioned to enable (autogamous) selfing for reproductive assurance, and to maximize pollen competition. The lack of correlation

could also be explained, however, by the continued growth of the styles after anthesis begins. These temporal changes made it difficult to select an equivalent stage of floral development between species.

8.3 Do the predictions of sex allocation theory hold up?

In this study, neither the predicted negative correlation between pollen size and number, nor a negative correlation between male and female investment was found to be present in *Cuscuta*. Pollen number and pollen volume showed no correlation, suggesting that they are shaped by different selective pressures and constraints. Other studies with broad interfamilial sampling (e.g. Cruden and Miller-Ward, 1981), sampling of a single family (e.g. Polemoniaceae; Plitmann and Levin, 1983) and within a single tribe (e.g. Solanaceae: Lycieae; Aguilar et al., 2002) or genus (e.g. *Tarasa,* Malvaceae; Tate and Simpson 2004) have each failed to find this expected negative correlation. However, Vonhof and Harder (1995) were able to recover a clear inverse relationship between pollen size and number from Cruden and Miller Ward's data when phylogenetic relatedness (family) was taken into account. They also suggested that if pollinator diversity had been taken into account in Plitmann and Levin's survey of Polemoniaceae, the size-number tradeoff would have been recovered.

Unfortunately, this does not explain why the correlation has been recovered for *Pedicularis* (Yang and Guo, 2004) but not for Lycieae (Aguilar et al. 2002), as both of these constitute small monophyletic groups with the same or similar pollina-

tors (Yang and Guo, 2004). Götzenberger and colleagues (2007) suggest that when pollen is used as a floral reward for pollen-feeders, as in the Fabeae, selection can act to increase both pollen grain size and number. Regardless, accounting for pollinator guilds may succeed in recovering the relationship from *Cuscuta*, since it is clear that a variety of pollinator guilds visit *Cuscuta* (McNeal, 2005; Meulebrouck, 2009). As mentioned above, however, these data are unavailable for most *Cuscuta* species at present.

Another, perhaps more significant, factor that should be accounted for is nuclear genome size. A number of studies have suggested that there is a positive relationship between genome size and pollen size in angiosperms (e.g. Gould, 1957; Orjeda et al., 1990; Altmann et al., 1994; Dewitte et al., 2009). McNeal (2005) found that nuclear genome size in *Cuscuta* shows large variation (e.g. *C. exaltata* =41.86 pg/2C, *C. europaea* =2.15 pg/2C, *C. gronovii* =4.37 pg/2C to 13.81 pg/2C). Importantly, this variation is considerable even between closely related species, such as in Clade B (Stefanovic et al., 2007) where measured genome sizes vary by a factor of 10 between the smallest and largest (McNeal, 2005). If genome size and pollen size are correlated in *Cuscuta*, the variation in genome size is likely sufficient to obscure correlation of pollen grain size to any trait that is not itself scaled by genome size. This may explain why no correlation was found between pollen volume and style length.

Male and female sex allocation in *Cuscuta* were found to be correlated, but

this relationship is positive and not negative. This correlation was found between the male variables total pollen volume and pollen grain volume and the female variables ovary volume and seed length. Pollen count, however, was only marginally correlated to ovary volume and did not have a significant correlation to seed length. This is perhaps indicative of shared genetic size constraints for gametes (and cells in general) in contrast to the more labile pollen count.

8.4 Nectary structure & pollen composition

The nectary in *Cuscuta* is much reduced in size compared to most Convolvulaceae, where most species have a distinct disk with its own vascular supply (Govil 1972). *Humbertia* and *Ericybe* also have a nectary restricted to the ovary wall (Deroin 1992, 2002), and a reduction or even absence of the nectary has evolved independently within a number of other lineages in Convolvulaceae, including species of *Evolvulus*, *Cressa*, and *Porana* (Govil 1972). It seems that, despite the reductive trends affecting most aspects of *Cuscuta* form, the nectary still has an important enough role in pollinator reward to remain functional in at least some species of *Cuscuta*.

I did not expect the range of pollen reserve strategies, from starchy to lipidic, that we see in the data. In particular, I find the starchiness of *C. tinctoria* and the two varieties of *C. corymbosa* to be confusing, since their pollen counts would suggest that these species are facultatively xenogamous. Perhaps the external pollenkitt lipids are more important than internal reserves for meeting pollinator's nutri-

tional needs. The (putative) presence of carotenoids in the pollenkitt is significant, as carotenoids can shield the pollen grains from harmful UV radiation and act as a pollinator attractant (Stanley and Linskens 1974). The weaker reaction of the pollen grains with Sudan IV compared to Nile blue staining is similar to that reported by Passarelli (1999) for *Solanum* sect. *Basarthrum*. Although Grayum (1985) and Zona (2001) have confirmed Baker and Baker's (1979) prediction that starchy pollen should tend to have larger diameters than non-starchy pollen in the Araceae and the commelinoid monocots, Wang and colleagues (2004) did not find a similar trend when looking at the Zingiberaceae alone. I note that my results seem to agree with Wang and colleagues (2004), but I consider my sample size to be too limited for formal statistical testing. The diversity I encountered in my small sampling of pollen reserves deserves further exploration.

8.5 Infrastaminal Scales

The lipid staining pattern of the fimbriae is similar to that of the corolla laticifers and confirms that the secretory cells of the fimbriae are laticifers. The evolutionary origins of the scales and their laticifers can be traced to the existence of glandular hairs on the staminal bases in most lineages of Convolvulaceae (Wilkin, 1999). In *Lepistemon*, the anther bases are also modified to possess scale-like appendages (Wilkin, 1999). The glandular hairs in *Convolvulus* and *Calystegia* are morphologically similar to the fimbriae of *Cuscuta* infrastaminal scales (Figure 8.1, compare with Figure 6.10). Furthermore, the glands on these hairs contain a secretion that is

visually similar to the latex in the laticifers of *Convolvulus* and *Calystegia*, and stainable with Nile blue sulfate and Lugol's iodine (Figure 8.2). This indicates that the infrastaminal scales are part of the suite of plant defenses, and their position is particularly suited defense against seed predators such as the *Smicronyx* weevils.

What is not clear is whether or not the laticifers actively secrete their product onto the surface of the ovary, or if the scales just serve as an added layer of covering until the laticifers of the ovary wall are more developed. The contents of laticifers are usually under pressure (Pickard, 2008); the thin walls of the laticifer cells perhaps break upon disturbance by a floral visitor, releasing the latex. Secretion onto the surface of the ovary could contaminate the nectar, making it either unpalatable or toxic, and thereby narrow the range of floral visitors that could make use of the nectar (Adler, 2000; Galetto and Bernardello, 2005).

While protection of seeds is important, the infrastaminal scales do also play a role in pollination, at least in those protandrous species that have delayed stylar growth (e.g. *C. strobilacea*, *C. tasmanica*). In these cases, the infrastaminal scales shield both stigmas from self-pollination until the female phase begins. Species with this phenology are also good candidates to examine for self-incompatibility and the factors that maintain it in *Cuscuta*. This is a topic of significant consequence because of the increased survival risks self-incompatibility places on a parasitic plant.

8.6 Fitting of stigma receptivity and growth patterns to host life-cycle and community structure

Cuscuta have adapted the timing of sexual function and organ positioning to a wide range of pollination conditions. Welsh (2009) noted that in other angiosperm genera with linear or filiform stigmas, growth of the stigma continues until pollination occurs and posited that this mechanism likely occurs in subg. *Cuscuta*. Based on my observations of *C. epithymum,* this seems to be true. However, I hypothesize that it is the combination of stigma growth *and* vertical to horizontal orientation change in subg. *Cuscuta* that provides the fullest fitness advantage. This dual-purpose mechanism gives reproductive assurance through guaranteed autogamous self-pollination, but also awards late-deposited (outcross) pollen on the lateral stigma surfaces a competitive advantage over early-deposited self-pollen found closer to the tips of the stigmas. There is therefore greater probability that those self-pollen tubes reaching the ovule have a fit enough genotype to be worth the effort of maturing to seed. In particular, this minimizes the fitness losses accrued by early selfing's reduction of pollen available for export and the dangers of inbreeding depression. A laboratory study of the location of the zone of growth, the pattern of onset of receptivity along the length of the stigma, and pollen tube growth rates from controlled self and cross pollinations are needed to verify this prediction.

This mechanism described for *C. epithymum* fits with what is known about its population dynamics in the Belgian heathlands. This species has a very patchy

distribution in its habitats, because it is very sensitive to successional stage (Meulebrouck, 2009). *C. epithymum* is reliant on disturbances to 'reset' the successional stage to one of low vegetation cover, a stage where it can successfully parasitize its preferred host, *Calluna vulgaris*. Although it can overwinter on *Calluna*, eventually the host becomes too woody for the parasite to successfully use it (Meulebrouck et al. 2009). It must make contributions to the persistent seed bank each growing season in order to survive between-disturbance periods where the heath succession has moved to too late a stage (Meulebrouck et al. 2009). This mechanism ensures that seed is produced and further serves to allow *C. epithymum* to keep inbreeding depression at bay by competitively disadvantaging poor self-pollen genotypes.

In subg. *Grammica*, the delayed receptivity and semi-shielded position of the lower stigma provide a similar mechanism of reproductive assurance for species like *C. obtusiflora*, the anthers of which dehisce in bud or early in anthesis. The shorter distance that pollen tubes have to travel through the lower stigma and style could give a limited equalization of fertilization opportunities between early-selfed pollen on the upper stigma and later outcross pollen deposited on the lower stigma during anthesis. This phenology is particularly well suited for species like *C. obtusiflora* that parasitize annual hosts and must set seed every growing season in order to persist.

The strong protandry and stigmatic shielding seen in *C. strobilacea*, by con-

trast, strongly favours outcrossing while still providing an opportunity for late self-pollination when the styles begin to diverge horizontally late in floral development. It would be interesting to know if *C. strobilacea* has a system of waning self-incompatibility that prevents self-pollination until the end of floral development, or if it is constitutively self compatible once the stigma becomes receptive. The mechanism of reproductive assurance in *C. strobilacea* is more suited to habitats where the parasites are able to take advantage of a good host for several growing seasons, or where *Cuscuta* population density and pollination efficiency are high enough to guarantee successful outcrossing. The ability to perenniate, hidden in the host during adverse seasons, is definitely essential for the persistence of these xenogamous species but the observation that populations of these *Cuscuta* seem to go through multi-year boom and bust cycles (Costea, pers. obs) indicates that the situation is perhaps more precarious than it looks at first glance. Unfortunately, it is these xenogamous, tree-dwelling, perennial *Cuscuta* that we have the weakest understanding of from an ecological perspective. Elucidating the life history and population dynamic characteristics of these species should be one of the top priorities for future research in the genus.

8.7 The evolution of separate and unequal styles

It is clear that the evolution of separate and unequal styles was profoundly freeing for *Cuscuta.* These two transitions are the key innovations that spurred the development of the increasing diversity in subg. *Cuscuta,* and later, subg. *Grammica*.

The diversity of habitats and hosts seen in populations of *Cuscuta* in subg. *Grammica* in particular has challenged these plants to adapt to generalist or specialist pollination, to different host life-cycles and community parameters. The *Cuscuta* of subg. *Grammica* have evolved multiple modes of reproductive assurance as a result. Two unequal styles offer a more flexible base-plan for the evolution of reproductive phenology in response to host- and pollinator-driven selection, and consequently allow subg. *Grammica* to take advantage of novel hosts and habitat niches more easily than the other subgenera. I tested this hypothesis by examining the change in number of lineages over time (Figure 8.3). The two rapid periods of radiation seen near the beginning of the curve match well with the split between the ancestors of subg. *Monogynella* and the two-styled species, and the split between the ancestors of subgenera *Cuscuta* and *Grammica*.

8.8 Limitations of this study

While the broad sampling of the genus that I pursued brings the evolutionary context of the data to the forefront, I was not able to achieve this broad sampling in each category of data I gathered. Sampling within species was very limited. My sampling could easily have been biased by human error, the limitations of specimen availability. In many ways, the broad scope of this study has produced many hinderances in developing a greater understanding of morphological evolution of the flowers in *Cuscuta*. If smaller clades are studied with greater sampling within spe-

cies, allometric data could be produced that give a fuller picture of what parts of the flower are under selection in a particular group and provide a basis for studying coevolution of floral morphology with habitat-specific pollination conditions in *Cuscuta*.

It is unfortunate that I was not able to put together ovule mass data. There are weaknesses inherent to the female sex-allocation characters I have used in this study. The seed length data I mined from Yuncker is fairly incomplete and length is a problematic measure to use because it does not accurately reflect the amount invested maternally because *Cuscuta* species vary in the number of seeds they typically mature. Ovary volume is also problematic because it is affected not only by ovule/seed size, but also other factors like genome size and the thickness of the wall needed to adequately protect the ovules.

While general linear model-based statistical analyses are commonly used for this kind of data, most authors recognize that biological data such as the characteristics examined in this thesis inherently fail the criterion of independence due to the differing degrees of relatedness between species. They therefore use different methods of statistical analysis to account for the relatedness of species when analyzing data. Although I attempted to use Felsenstein's (1985) phylogenetically independent contrasts, a commonly used technique for analyzing continuous comparative biological data, I was unable to use the data produced by the analysis. This technique assumes a Brownian-motion type model of evolution, and this assump-

tion was shown to be violated when model-testing the phylogenetic trees available for *Cuscuta*.

8.8 Future Directions

Several short-, medium-, and long-term goals can be extrapolated from my work here. First, mature seed/ovule weight data are required for more thorough assessment of female investment in reproduction in *Cuscuta*. At that point, interesting questions about how sex allocation varies with plant age, population size, and host life history could begin to be considered. Second, the morphometric data should be expanded to include many samples per species within selected clades where a large proportion of specimens are available. The data may then be assessed using allometry methodologies to give further insight into what floral structures are under the most intense selection at the species level, at the clade level, and in the genus more broadly.

In the medium term, further characterization of the ultrastructure and chemistry of the infrastaminal scales' laticifers will give us a better understanding of their role in *Cuscuta* reproduction. For example, are they passive defense structures, or do they react to floral damage by increasing secretion? What are the constituents of the latex secretion? How toxic are these chemicals? Do these secretions narrow the number of potential pollinators?

A direct assessment of gene flow and breeding system using molecular population genetic tools such as microsatellite markers would be useful in develop-

ing our ability to create long-term management plans for threatened species and narrow endemics. The work I have done in this thesis is largely inferential; validating my results for a number of *Cuscuta* representing different host life-histories and diverse habitats and evaluating levels of gene flow, homozygocity and minimum effective population sizes are a crucial next step in this process.

Table 8.1 – P/Os and self-compatability in other Convolvulaceae.

Species	P/O	Self Compatible	Source
Calystegia soldanella	3758.1	Yes	Ushimaru and Kikuzawa 1999
Calystegia hederacea	2703.9	Yes	
Calystegia japonica	3759.4	Yes	
Calystegia sepium	2263.8	Yes	
Evolvulus nummularium	242	Yes	Sarma et al. 2007
Evolvulus alsinoides	312.5	Yes	Sing et al. 2010
Ipomoea ampullacea	3016.6	No	Jaramillo and Bullock 2002
Ipomoea bracteata	507.1	No	
Ipomoea chamelana	135.0	Yes	
Ipomoea clavata	1465.9	Yes	
Ipomoea hederifolia	145.4	Yes	
Ipomoea meyeri	122.1	Yes	
Ipomoea muricata	169.1	Yes	
Ipomoea nil	155.9	Yes	
Ipomoea pedicellaris	1613.8	Yes	
Ipomoea quamoclit	87.9	Yes	
Ipomoea trifida	795.0	Yes	
Ipomoea triloba	177.1	Yes	
Ipomoea pes-caprae	1648.5	No	Devall and Thien 1992
Ipomoea cairica	1227.1	No	Maimoni-Rodella and Yanagizawa 2007
Ipomoea grandifolia	194.9	Yes	
Ipomoea nil	199.9	Yes	
Ipomoea hederacea	206.1	Yes	Stucky 1985
Ipomoea purpurea	165.5	Yes	
Ipomoea habeliana	1407.0	Yes	McMullen 2009

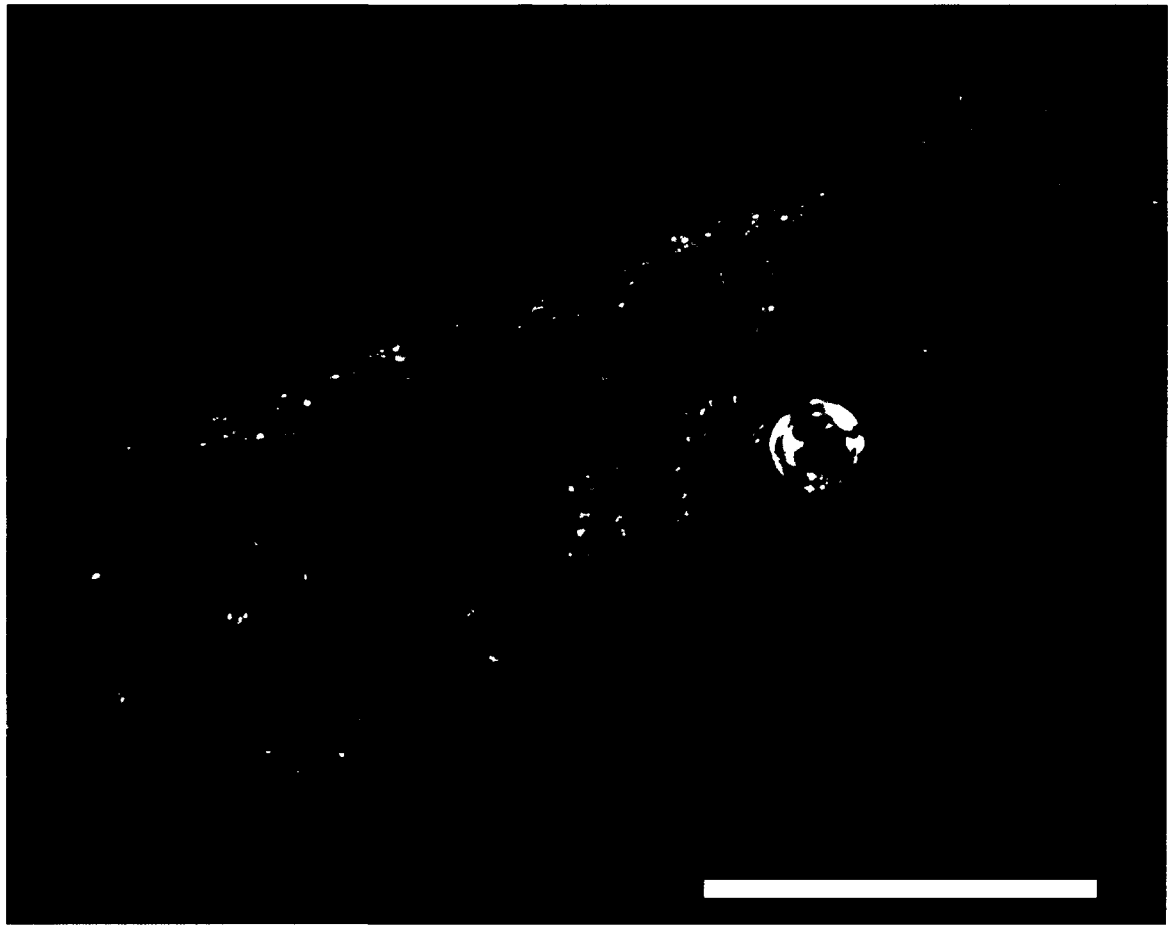

Figure 8.1 - Anther base from *Convolvulus arvensis,* moving basal to apical from left to right. Note the similarity of the epidermal surface architecture and, critically, the glandular hairs to that of the infrastaminal scale in *Cuscuta*.

Figure 8.2 - Glandular hairs of a) *Convolvulus arvensis*, unstained, and b) *Calystegia sepium*, stained briefly with Nile Blue sulfate. The secretion has two distinct phases, the hydrophobic phase took up a mild amount of Nile Blue sulfate in *C. sepium*. The subsidiary cells below the gland cell have an outer wall that is visually different from that of the gland cell. These cells also stain pink, indicating that they are likely involved in the synthesis of the secretory product, or at the very least form a reserve of precursor lipid. Scale bars a) 50 µm, b) 100 µm.

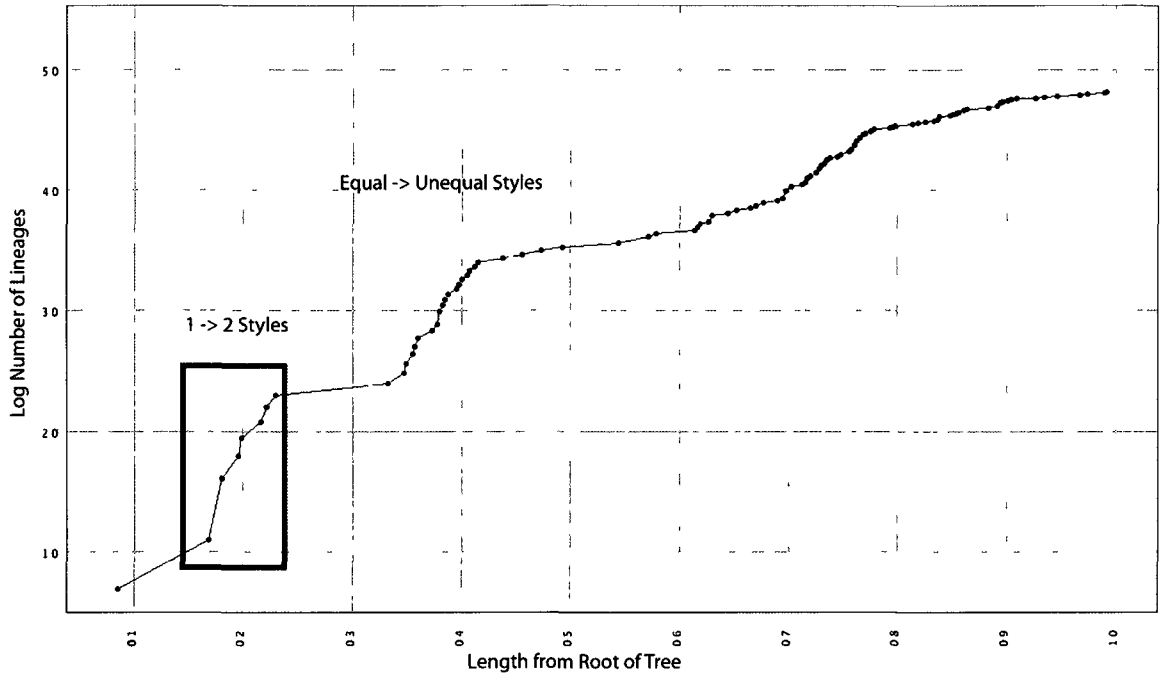

Figure 8.3 - Graph of log-lineages over time. The red and yellow boxes represent two periods of intensive diversification which I hypothesize correspond to the advent of free styles in the common ancestor of subg. *Cuscuta* and *Grammica*, and the evolution of unequal styles in the group ancestral to subg. *Grammica*, respectively.

9. Summary

This broad survey of reproduction in *Cuscuta* has revealed a striking diversity of breeding systems and adaptive strategies. Pollen production varies over three orders of magnitude in response to the unique habitat and host-driven pressures that shape each species. No negative tradeoffs between male and female sex allocation nor between pollen grain size and pollen volume were found. Although genome size can be raised as a significant confounding factor, these results cast further doubt on the universality of the assumptions made in sex allocation theory. Overall, floral morphology in *Cuscuta* forms a well-integrated unit, with style length and pollen grain number being the most flexible components under selection.

Cuscuta reward their pollinators with both nectar and pollen. Although the internal provisions of the pollen grain species examined showed a wide range of variation in stored energetic compounds, the presence of a sticky lipid pollenkitt was universal. This pollenkitt may be increasing *Cuscuta*'s pollination efficiency beyond the outcrossing thresholds identified by Cruden (1977). The role of the infrastaminal scales can be narrowed with some confidence to defense against seed predators and participation in maintaining the physical separation of male and female reproductive function in a number of outcrossing *Cuscuta* species.

Cuscuta alter the timing and spatial positioning of male and female functions to ensure outcross success, to provide reproductive assurance, or in some cases both simultaneously. Although protandry is common, there may be some protogy-

nous species present in *Cuscuta* as well. The evolution of two distinct styles followed by unequal style lengths represent two major evolutionary transitions that resulted in a burst of adaptive radiation, particularly in subg. *Grammica* due to the flexibility and precision with which this gynoecial morphology can be adapted to different pollination environments and host life histories. There are still many unaddressed questions about reproductive biology in *Cuscuta* that need to be addressed, in particular regarding the life histories and population dynamics of perennial, outcrossing species in the tropics. Future work that integrates spatial ecology and molecular genetic techniques will arm us with a better understanding of these fascinating plants, and what needs to be done to help them persist in our world.

10. Glossary

Anomocytic – 'irregular-celled' or 'ranunculaceous' type of stoma where the guard cells are surrounded by a limited number of subsidiary cells that are indistinguishable in form or size from the surrounding epidermis [1]

Autogamy – self-fertilization following intra-floral transfer of viable pollen from the anthers to the receptive stigmas.[2]

Autonomous autogamy – self-fertilization following self-pollination within a single flower without the activity of a pollen vector. Can often arise from contact between dehisced anthers and receptive stigmas at some point during floral development. May occur before, during or after the period during which outcross pollen is deposite don the stigmas. All timings provide a degree of reproductive assurance. [2]

Callose – a polysaccharide of glucose linked through ß-1,3 linkages, produced in plants as a response to wounding, during pollen tube development and often laid down at sieve plates [3]

Cleistogamous – a flower that self-pollinates autonomously and never opens. Usually contrasted with *chasmogamous* (open) flowers accessible to pollien carried by a pollination vector [2]

Cost of meiosis – the reduction of the genetic contribution of the female to offspring because of 'gene sharing' with an unrelated mate during ssexual reproduction. [2]

Dichogamy – differences in the timing of stigma receptivity and anther dehiscence within a flower; can also occur at the inflorescence or plant level. Dichogamy is common in the angiosperms. Generally reduces intra-floral self-pollination and can reduce geitonogamous selfing if the floewrs are arranged so that pollinators tend to visit female-phase flowers before male-phase flowers. [2]

Geitonogamy – self-pollination resultant from the transfer of pollen between flowers on the same individual. Common in species with mass-flowering and genetically equivalent to autogamous selfing. Thought to be a common cause of complete pollen and seed discounting. [2]

Herkogamy – the spatial separation of receptive stigmas and dehiscing anthers within flowers. Common in angiosperms and, like dichogamy, generally reduces

intra-floral self-pollination. [2]

Inbreeding depression – reduction of viability and/or fertility of inbred offspring when compared with those from outcross matings due to the expression of deleterious recessive alleles in homozygous genotypes. Inbreeding depression is most strongly expressed in predominantly outcrossing species and can occur t-hrough out the life cycle [2]

Latex – a suspension or emulsion of many small particles in a liquid with a different refractive index, secreted in the cells of *laticifers*. The dispersed phase of the latex may consist of polyisoprene hydrocarbons, triterpenols and sterols, fatty acids, aromatic acids, carotenes, phospholipids, proteins and inorganic constituents, or various combinations of the above. Latex may also contain sugars, starch grains, tannins, and alkaloids. [4]

Laticifer – A specialized cell or row of such cells containing *latex*. They may be branched or unbranched, and may be derived from embryonic cells ('unarticulated') or from cells in the apical meristem ('articulated') [4]

Mating system – in sexual reproduction, the method of transmission of genes from one generation to the next. Determinants of plant mating systems are the maternal (ovule) selfing rate and male (pollen) siring success or fertility. [2]

Metapopulation – a population of populations characterized by colonization-extinction dynamics and more or less connected by gene flow. [2]

Nectary – a specialized tissue that secretes a sugary solution involved in plant-animal interactions. Nectaries have diverse constructions across the angiosperm families, and may be found both in flowers and on extrafloral tissues [5]

Outcrossing rate – proportion of seeds produced by an individual or population that are cross-fertilized (contrast with *selfing rate*). Usually refers to the female outcrossing rate, the proportion of cross-fertilized seeds produced. An individual's male and female outcrossing rates may differ when the number of outcrossed seeds an individual sires on other plants is not equal to the number of outcrossed seeds it produces. [2]

Ovule discounting – female fertility is reduced by the disabiling of some ovules by self-pollen tubes. Reported from species with self-incompatibility systems that act at the level of the ovary. [2]

Ovule limitation – a constraint on seed production occuring when all ovules are

fertilized but too few embryos survive predation and genetic death to compete for maternal resources. Ovule limitation may be alleviated by increasing the number of ovules produced. [2]

Pollen discounting – the loss of outcross siring success due to self-pollination. This reduces the transmission advantage of selfing and represents a major cost of selfing, along with *inbreeding depression* [2]

Pollen limitation – reduction of potential seed production when some ovules remain unfertilized, causing too few embryos to survive predation and genetic death to compete for maternal resources [2]

Pollenkitt – viscous, sticky substance containing lipids, carotenoids and other compounds that acts as an adherent. Often absent from pollen of wind-polliinated plants, but ubiquitous among angiosperm families. [6]

Pollination syndrome – a correlated suíte of floral traits that are adapted to the behaviour and morphology of a specific class of pollen vector (e.g. butterflies, wind, bees, hummingbirds, bats, hawk moths, flies). [2]

Protandrous – a form of *dichogamy* expressing male gender before female. In the context of hermaphroditic plant species, protandry indicates that a flower or whole plant functions as a male (anther dehiscence and pollen export) before functioning as a female (stigma receptivity and pollen receipt) [2]

Protogynous – a form of *dichogamy* expressing female gender before male. In the context of hermaphroditic plant species, protogyny indicates that a flower or whole plant functions as a female (stigma receptivity and pollen receipt) before functioning as a male (anther dehiscence and pollen export) [2]

Pseudopollen – a yellow-white white farinaceous powder on the labella of certain orchids formed by the fragmentation of multicellular trichomes with cells rich in food reserves [7]

Reproductive assurance – an increase in seed production caused by self-fertilization when an absence of mates or pollinators causes conditions unfavourable for outcrossing. Requires self-compatibility and usually the capability for *autonomous autogamy*. [2]

Resource limitation – a constraint on seed production when an ovary contains more embryos than can be matured to seed with the available maternal resources. [2]

Seed discounting – reduced production of outcrossed seeds due to self-fertilization when self-fertilization pre-empts ovule or because selfed seeds consume maternal resources that would have been used to produce outcrossed seeds. It can occur within flowers or between flowers produced at different times on the same plant. [2]

Self-incompatibility – inability of a fertile hermaphroditic individual to set abundant seed following self-pollination. Self-incompatibility is the most common anti-selfing mechanism in angiosperms, and involves diverse physiological mechanisms that typically operate pre-zygotically to prevent self-pollen germination, self-pollen tube growth, or self-pollen fertilizing ovules. [2]

Selfing rate – the proportion of an individual's offspring produced by self-fertilization (contrast *outcrossing rate*). Often used to refer to the proportion of self-fertilized seeds. [2]

Sex allocation – differential investment of limited reproductive resources in male versus female function in hermaphroditic individuals [2]

Viscin threads – thin, sporopollenin-containing threads that bind pollen grains together and assist in adherence of groups of pollen to pollinators through friction alone. Have arose independently in a handful of unrelated lineages (Onagraceae, Fabaceae: Caesalpinioideae,), and can be considered analogous to the pollenkitt. Some plants with viscin threads also have pollenkitt. [6]

Xenogamy – outcross-fertilization following the transfer of viable pollen of one individual to the receptive stigma of another individual. Contrast with *autogamy* and *geitonogamy*. [2]

Definitions adapted from the following sources:

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Appendix A – List of Specimens

Specimens are sorted by subgenus and clade. Specimens are marked with the procedures they were used for: [f] – voucher of fresh/fixed material [h] – pollen histochemistry. [n] – nectary stomata counts, [p] – pollen counts, [s] – stigma receptivity. Specimen data for those images used in morphometric analysis is available on the Digital Atlas of *Cuscuta* (Costea, 2007-onwards).

Subgenus *Grammica*:

Clade A

C. brachycalyx – Howell 38877 (NY) [n, p]; *C. californica* var. *californica* – W. Martin s.n. (RSA) [n, p]; *C. californica* var. *papillosa* – Gander 7838 (RSA) [n]; *C. draconella* – Spellenberg & Mahrt 10497 (NMC) [n]; *C. howelliana* – True 6716 (CAS) [h, p], True 7407 (DS) [n, p]; *C. jepsonii* – A.A. Heller 5981 (WTU) [p], Munz 16560 (RSA) [n]; *C. occidentalis* – O. Arnow 4550 (RSA) [p], Rowen 3720 (RSA) [n]; *C. pacifica* – Lawrence 4641 (OSC) [n, p], Wright & Welsh 2008-34 (WLU) [f, h, n, p]; *C. salina* – D. Galway sn (UC) [n, p]; *C. subinclusa* – Sanders 17902 (RSA) [n], Twisselman 2394 (RSA) [p]; *C. suksdorfii* – A. C. Colwell 05-213 (UC) [h, p], Oswald & Ahart 5874 (CHICO) [n]

Clade B

C. australis – W.R. Sykes CH99 (CHR) [p], Town Clerk s.n. (NSW) [n]; *C. campestris* – Oldham et al. 8621 (DAO) [n]; *C. gymnocarpa* – Fagerlind & Wibom 3641 (S) [n, p]; *C. harperi* – Demaree 46295 (NY) [n]; *C. pentagona* – Demaree 50393 (SMU) [p], Parks s.n. (RSA) [n]; *C. polygonorum* – Demaree 22402 (NY) [n]; *C. obtusiflora* var. *glandulosa* – I. Garcia-Ruiz 8054 (CIMI) [f, m, n, p, s]; *C. obtusiflora* var. *obtusiflora* – Guaglianone 138 (SI) [n]; *C. plattensis* – Nelson 2741 (MO) [n]; *C. runyonii* – Flyr 368 (SMU) [n, p]; *C. stenolepis* – Jaramillo & Caravajal 2307 (AAU) [p], Holm-

Nielsen et al. 29089 (AAU) [h, n]

Clade C

C. corniculata – Stannard et al. 51861 (F, G) [n, p]; *C. incurvata* – Anisitis 2395 (S) [n, p]; *C. micrantha* var. *latiflora* – Worth & Morrison 16235 (K) [n, p]; *C. parviflora* – Oliveira, Filgueiras, Fonseca & Santos 745 (IBGE) [n]; *C. platyloba* – Ibarrola s.n. (SP) [n], Krapovickas 2911 (K) [p]; *C. racemosa* var. *calycina* – 4559 (MO) [p]; *C. racemosa* var. *miniata* – Cordeiro et al. 8211 (K) [n], Richon 7835 (S) [p]; *C. racemosa* var. *racemosa* – Smith et al. 14478 (F) [n]; *C. suaveolens* – Abrams s.n. (RSA) [n]; *C. xanthochortos* var. *carinata* – Bernardi 18758 (MO) [n, p]; *C. xanthochortos* var. *lanceolata* – P.R. Reitz 4524 (NY) [p] *C. xanthochortos* var. *xanthochortos* – Burkart 14287 (SI) [n], Arbo et al. 6953 (MO) [h, p]; *C. werdermannii* – Werdermann 880 (G) [n]

Clade D

C. cephalanthi – Farwell 5124 (NY) [n], Hill 34308 (BRIT) [p]; *C. compacta* – Lundell 11862 (SMU) [n, p]; *C. cuspidata* – Deam 333011 (IND) [n], Runyon 2828 (SMU) [p]; *C. glabrior* – Hendrickson 13676c (RSA) [n]; *C. glomerata* – Deam 49868 (NY) [n]; *C. gronovii* – Masson 1101 (QUE) [n], Mellinger s.n. (SMU) [p], Wright & Bols 2009-01 (WLU) [f, h, n, s]; *C. rostrata* – S.W. Leonard 2053 (ARIZ) [n, p]; *C. squamata* – Wooton s.n. (NMC) [n]; *C. umbrosa* – Fields s.n. (DAO) [n], Lundell 128939 (CAS) [p]

Clade E

C. denticulata – B. Schwenn sn (UCR) [n, p]; *C. nevadensis* – Haynie 3601 (RSA) [n] Sanders 2094 (UCR) [p]; *C. veatchii* – Johnston 3430 (GH) [n, p];

Clade F

C. haughtii – Asplund 5618 (K) [n], Asplund 15974 (NY) [p]; *C. longiloba* - A.

Krapovickas & A. Schinini 31255 (F) [n]; *C. partita* – Lindman 3481 (S) [p], Marg Ma-Baros 120 (RB) [n];

Clade G

C. aurea – Palmer 87 (S) [n, p]; *C. cotijana* – I. Garcia-Ruiz & M. Costea 7557 (CIMI, WLU) [f, h, n, p]; *C. jalapensis* – Waterfall & Wallis 14213 (SMU) [n, p]; *C. lindsayi* – I. Garcia-Ruiz & M. Costea 7571 (CIMI, WLU) [f, h, n, p]; *C. mitriformis* – E. Carranza 5658 (IEB) [n, p]; *C. purpusii* – Meyer & Rogers 2878 (GH) [n, p]; *C. rugosiceps* – I. Garcia-Ruiz & M. Costea 7567 (CIMI, WLU) [f, h, n, p], McVaugh 13419 (MICH) [n]; *C. tasmanica* – B.J. Lepschi 908 (CANB) [n], N.G. Walsh 3045 (MEL) [p]; C. *tinctoria* – I. Garcia-Ruiz & M. Costea 7575 (CIMI, WLU) [f, h, p], McVaugh 592 (MICH) [n]; *C. victoriana* – Newbey 10073 (WLU) [p], H.T. Smyth 261 (CANB) [n]; *C. 'volcanica'* – I. Garcia-Ruiz & M. Costea 8029 (CIMI, WLU) [f, h, n, p, s]; *C. woodsonii* – Hammel 1567 (MO) [n], Shary 44637 (NY) [p]

Clade H

C. applanata – Wright 1623 (NY) [m, n]; *C. chinensis* – B.J. Carter 628 (CANB) [n, p]; *C. potosina* var. *globifera* – Pringle 6575 (GH) [n]; *C. potosina* var. *potosina* – M. Medina 2493 (F) [p], Pringle 6575 (S) [n]; *C. sandwichiana* var. *kailuona* – Degener & Wiebke 3261 (MO) [n]; *C. sandwichiana* var. *sandwichiana* – Fosberg 44019 (RSA) [n], Legener 24212 (RSA) [p]; *C. yucatana* – Nee & Taylor 29575 (MO) [n], Steere 1695 (MICH) [p]

Clade I

C. americana – I. Garcia-Ruiz & M. Costea 7582 (CIMI, WLU) [f, h, n, p]; *C. cozumeliensis* – E. Cabrera 15612 (MEXU) [n], Standley 62142 (F) [p]; *C. globulosa* Broadway s.n. (K) [n]; *C. macrocephala* – Alava & Cook 1694 (MICH) [p], Van Devender 2006-872 (WLU) [n];

Clade J

C. corymbosa var. *corymbosa* – Standley 81967 (F) [n, p]; *C. corymbosa* var. *grandiflora* – I. Garcia-Ruiz & M. Costea 8053 (CIMI, WLU) [f, h, p], Johnston 3222 (GH) [n]; *C. corymbosa* var. *stylosa* – I. Garcia-Ruiz & M. Costea 8027 (CIMI, WLU) [f, h, n, p], Moore 2157 (GH) [n]; *C. prismatica* Mille 112 (F) [n]

Clade K

C. boldinghii – Ekman 10455 (S) [n, p]; *C. 'bonafortuna'* – I. Garcia-Ruiz (CIMI) [n]; *C. chapalana* – I. Garcia-Ruiz 7942 (CIMI) [f, h, n, p], Pringle 5349 (GH) [m]; *C. costaricensis* – I. Garcia-Ruiz & M. Costea (CIMI, WLU) [f, h, p], Van Devender 98-1789 (ARIZ) [n]; *C. erosa* – Shreve 6632 (RSA) [p], Van Devender 2004-1199 (WLU) [n]; *C. ortegana* – Hinton 2604 (K) [n], Van Devender 2006-74 (WLU) [n, p]; *C. strobilacea* – I. Garcia-Ruiz & M. Costea 8274 (CIMI, WLU) [f, h, n, p, s]

Clade L

C. acuta – Anderson s.n (GH) [m], Howell 10140 (G) [n], Sparre 19700 (S) [p]; *C. desmouliniana* – C.G. Pringle s.n. (NY) [m], Van Devender 98-1120 (WLU) [n, p]; *C. legitima* –Van Devender 2005-1661 (WLU) [n, p]; *C. leptantha* – Johnston 3494 (GH) [p], Van Devender 2000-993 (WLU) [n]; *C. membranacea* – Nee 46609 (NY) [n, p]; *C. odontolepis* – Hartman 52 (K) [n], Van Devender 2006-467 (WLU) [p]; *C. polyanthemos* – Van Devender 2006-809 (WLU) [m, n, p]; *C. tuberculata* – Reina 2001-623 (ARIZ) [m], Van Devender 92-1386 (WLU) [n, p]; *C. umbellata* var. *umbellata* – Juan M. Escobedo 760 (IEB) [p], Moran 24758 (SD) [n], Nabhan & Rea 167 (ARIZ) [m]

Clade M

C. coryli – Boivin & Champagne 13869 (QFA) [n]; *C. indecora attenuata* – Palmer

333 (F) [n]; *C. indecora* var. *indecora* – Austin 7599 (RSA) [h, n], Bestley s.n. (RSA) [p]; *C. indecora* var. *longisepala* – Burkart s.n. (K) [n, p]

Clade N

C. deltoidea – Palmer 948 (NY) [n]; *C. gracillima* – I. Garcia-Ruiz 8033 (CIMI) [f, h, n, p]; *C. mcvaughii* – Hinton et al. 12098 (G) [p]; *C. punana* – Madsen 83850 (AAU) [p]; *C. saccharata* – Standley 24370 (F) [n]; *C. serruloba* – Orcutt 4338 (K) [p]; *C. sidarum* – Hammel 18763 (MO) [n], Haught 4535 (K) [p], Standley 12359 (S) [p]; *C. vandevenderi* – Van Devender 98-1434 (UCR) [n, p]

Clade O

C. argentinana – Brücher s.n. (S) [n, p]; *C. bella* – MacBride 3993 (S) [p]; *C. boliviana* – K. Fiebrig 2880 (NY) [n, p]; *C. chilensis* – Buchtien 446 (CHSC) [p], Savatier 1750 (K) [n]; *C. cristata* – Krapovickas et al. 26901 (G) [n], Rotvon & Betla s.n. (SI) [p]; *C. flossdorfii* var. *flossdorfii*– Teodoro Mayer s.n. (NY) [p]; *C. flossdorfii* var. *pampagrandensis* - Mendoza & Acebo 919 (MO) [n]; *C. foetida* var. *foetida* – Asplund 6447 (NY) [n, p]; *C. foetida* var. *pycnantha* – Balsley 1907 (NY) [p], T. Plowmar, et al. 14291 (F) [n]; *C. friesii* – Toledo et al. 12993 (CTES) [n, p]; *C. grandiflora* – Aquilor s.n. (MO) [n], Miguel Bang 115 (NY) [p]; *C. globiflora* – Lewis 35298 (MO) [n], Mulgura et al. 1199 (MD) [p]; *C. kilimanjari* – Lacroix 4559 (MO) [n, p]; *C. lucidicarpa* – Killip & Smith 21909 (NY) [p]; *C. microstyla* var. *bicolor*– Boelcke 10243 (CTES) [p], Burkart et al. 6968 (SI) [n]; *C. odorata* var. *botryoides* – Hatschboch 22109 (F) [p]; *C. odorata* var. *odorata* – Klitgaaard et al. 185 (AAU) [n, p]; *C. odorata* var. *squarrulosa* – Killip & Smith 21684 (NY) [p]; *C. orbiculata* – Harley et al. 21452 (AAU) [n]; *C. paitana* – Madsen 62940 (AAU) [p], Madsen 63940 (AAU) [n]; *C. parodiana* – Krapovickas 35879 (G) [n, p]; *C. purpurata* – Taylor et al. 10697 (MO) [n]

Subgenus '*Pachystigma*'

C. africana – J. Muir 156 (GRA) [n] Till Wurb 472 (NBE) [p]; *C. angulata* – E.R. Orchard 469 (NBG) [p], I. Williams 2690 (NBG) [n, p]; *C. appendiculata* – P. Bohnen 7827 (NBG) [n], G.E. Hofmeyr s.n. (GRA) [p]; *C. natalensis* – O.M. Hilliard & B.L. Burt 14528 (NU) [h, p] H. Rudatis 2412 (NBG) [n]; *C. nitida* – H.G. Taylor s.n. (NBG) [m, n, p]

Subgenus *Cuscuta*

C. abyssinica var. *ghindensis*– Fred Eyles 327 (BOLM) [p]; *C. approximata* –, Kennedy 16422 (CAS) [p]; *C. epilinum* – Cartier s.n. (QFA) [n], *C. epithymum* – Baki Karapligil 2609 (ORE) [p], K. Meulebrouck s.n. (WLU) [m], Spregazzini s.n. (BAR) [h, n, p]; *C. epithymum* ssp. *kotschyi* – Duvigneanol & Lumbinon 74E687 (QUE) [n]; *C. europaea* var. *europaea* – Asplund 1314 (RSA) [n], T. Lempiainen & O. Ravanko s.n. (ORE) [p]; *C. europaea* var. *halophyta* – Kalevi Alho et al. s.n. (ORE) [n, p]; *C. palaestina* – M.A. Garcia 981 (WLU) [p]; *C. planiflora* – Easkins s.n. (WLU) [n, p]; *C. triumvirati* – M.A. Garcia 1077 (WLU) [p]

Subgenus *Monogynella*

C. cassytoides – I. Garland s.n. (NU) [n, p]; *C. exaltata* – Can 12341 (BRIT) [p], Reverdion 663 (MO) [n]; *C. gigantea* – Koie 2597 (NY) [m]; *C. japonica* var. *formosana* – Zhang 220 (MO) [m], *C. japonica* var. *japonica* – Hill 22616 (MO) [m, n, p]; *C. lehmanniana* – Vvedansky s.n. (MEL) [m]; *C. lupuliformis* – Barta 2004-302 (NY) [m]; *C. monogyna* – Greuter 11495 (CANB) [m], Sintenis [p]; *C. reflexa* – Koelz 21955 (NY) [m], J.D.H. s.n. (MO) [n]; *C. santapaui* – Nicolson 2796 (CANB, MO) [m, n, p]

Appendix B - Data Matrix

Taxon	Subgenus	Clade	Corolla Shape	Average Pollen Count	Pollen Volume	Total Pollen Volume	Ovary Volume	Floral Tube Length (mm)	Calyx Length
C. acutiloba	g	?	3	1286	23669.92	30439521.63			
C. alatoloba	g	?		2538					
C. andina	g	?	6						
C. dentatasquamata*	g	?	3	1273	21824.1	27782084.12	5.66	2.00	2.65
C. goyaziana	g	?	5		24003.66			4.28	3.93
C. insquamata	g	?	1	335					
C. orbiculata	g	?			31214.56			4.18	3.80
C. pauciflora	g	?			25040.69				
C. peruviana	g	?	6					2.42	2.32
C. polygonoides	g	?			22780.13				
C. rotundiflora aff.	g	?		12000					
C. serrata	g	?	6		23408.43		2.03	2.66	2.23
C. brachycalyx	g	a	3	3950	25735.93	101656911.60	1.33	2.06	0.42
C. californica	g	a	4	6756	27580.57	186334300.30	3.91	2.24	1.59

C. californica var. papillosa	g	a	4	3956					
C. decipiens	g	a	3		18244.85		5.40	2.50	2.23
C. draconella	g	a			14464.7		2.32	1.69	1.45
C. howelliana	g	a		3942	8641.893	34066342.45	0.43	1.43	1.43
C. jepsonii	g	a	8	729	17487.36	12748286.41	2.23	1.84	0.81
C. occidentalis	g	a	7	5601	23745.01	132995810.90	2.08	1.67	1.12
C. pacifica	g	a	2	1626	18743.73	30477312.29	4.21	2.19	2.19
C. pacifica papillata	g	a	2	1055.5	10145.58	10708664.93			
C. salina	g	a	4	3171.67	10031.85	31817720.66	3.33	2.14	2.14
C. subinclusa	g	a	6	3959.17	16899.26	66907024.14	4.08	3.72	2.79
C. suksdorfii	g	a	3	748.25	27464.36	20550210.55	3.47	1.11	1.92
C. australis	g	b	1	1246	16431.46	20473593.28	6.06	1.35	1.85
C. campestris	g	b	1	1138.33	33105.7	37685212.87	2.79	1.18	1.18
C. glabrior	g	b	7	3888.5	11222.27	43637803.12	6.20	1.94	1.54
C. gymnocarpa	g	b	3	1758.5	26758.98	47055666.95	7.05	1.00	1.00
C. harperi	g	b	3		12966.71		3.29	0.65	0.48
C. obtusiflora var. glandulosa	g	b	1	3212	29739.02	95521731.41	3.29	3.06	2.38
C. obtusiflora var. obtusiflora	g	b	1	912	15802.5	14411880.05	5.74	1.92	1.56

C. pentagona	g	b	3	1638	11836.32	19387890.39	1.24	1.07	1.06
C. plattensis	g	b	2		21434.77		17.79	2.89	1.94
C. polygonorum	g	b	1		23669.92			1.38	1.32
C. runyonii	g	b		4186	17551.28	73469667.56	2.63	2.27	0.86
C. stenolepis	g	b	5	2294	15156.48	34768954.82	2.52	1.95	1.33
C. corniculata	g	c	3	1754.5	29065.16	50994824.42	1.87	1.83	1.56
C. incurvata	g	c	2	1478.33	15965.57	23602386.79	3.20	1.78	1.41
C. micrantha	g	c	3	8101.33	20268.51	164201876.60	15.89	1.42	1.13
C. micrantha latiflora	g	c	3	8101.33				1.73	1.70
C. parviflora	g	c	2	3666.67					
C. parviflora var. elongata	g	c	2	3666.67	23081.21	84631164.88	1.38	1.11	0.89
C. platyloba	g	c	3	2069	11061.02	22885258.91	1.56	2.63	2.34
C. racemosa var. calycina	g	c	3	2767.75				1.50	1.50
C. racemosa var. miniata	g	c	3	890	19910.71	17720532.34	0.93	1.47	1.06
C. racemosa var. racemosa	g	c	3	3651.33			1.44	2.44	2.00
C. suaveolens	g	c	4	2032	18349.62	37286427.03	9.04	1.98	1.39
C. werdermanii	g	c			21669.03		1.22	2.06	1.55
C. xanthochortos var. carinata	g	c		3930.5	22936.14	90150496.77	1.04	2.69	2.44

C. xanthochortos var. lanceolata	g	c	4	2986	16621.39	49631463.36		2.11	1.44
C. xanthochortos var. xanthochor-tos*	g	c	1	2846.17	39786.13	113238090.90	2.03	1.10	1.03
C. cephalanthi	g	d	5	1046	61581.78	64414546.03	2.33	1.75	0.91
C. compacta	g	d	6	2753	28997.55	79830265.09	3.88	3.35	1.76
C. cuspidata	g	d	6	4657	20689.27	96349942.29	2.62	1.75	1.86
C. glomerata	g	d	6		34325.88		2.41		
C. gronovii var. gronovii	g	d	3	5162	24645.02	127217611.80	6.15	2.67	1.96
C. gronovii var. latifolia	g	d	3				3.53		
C. rostrata	g	d	3	4530	23435.24	106161628.90	5.03	4.14	2.19
C. squamata	g	d	6	7206.5	23547.77	169697004.60	1.63	3.40	2.99
C. umbrosa	g	d		1485	25628.69	38058610.53	10.74	2.55	1.73
C. denticulata	g	e	3	4047	8865.357	35878098.33	2.37	1.48	1.31
C. nevadensis	g	e	3	2909.67	16352.84	47581355.97	1.04	1.80	1.80
C. veatchii	g	e	3	1898.5	18041.09	34251017.45		1.69	1.56
C. burellii	g	f			20199.6		1.34	1.46	2.45
C. haughtii	g	f	7	1622	29497.66	47845207.20	3.26	1.46	1.30
C. longiloba	g	f			40008.02		1.76	3.50	2.87

C. partita	g	f	3	1815.33	21636.67	39277700.11	1.10	2.46	2.31
C. aurea	g	g	3	5924	17297.13	102468207.70	3.54	1.38	1.38
C. cotijana	g	g	5	11830	20117.3	237987678.30	23.40	4.84	2.85
C. floribunda	g	g	6		27479.72		5.69	4.95	3.75
C. jalapensis	g	g	3	5072	46492.95	235812237.50	29.34	2.39	1.37
C. lindsayi	g	g	6	12457.67	48048.77	598575778.20	36.92	4.21	2.00
C. mitriformis	g	g	3	1534.33	68869.19	105668064.00	11.84	2.98	2.61
C. purpusii	g	g	6	5143	43767.76	225097610.40	4.65	2.68	2.47
C. rugosiceps	g	g	3	3796	21989.74	83473057.28	20.77	2.36	1.77
C. tasmanica	g	g	3	3720.33	21865.48	81346819.23	4.29	2.47	1.45
C. tinctoria	g	g	3	6392	33346.64	213151698.90	7.80	2.77	2.20
C. victoriana	g	g	1	475.67	36808.51	17508702.99	1.36		
C. volcanica	g	g	5	16788					
C. woodsonii	g	g	6	4278	54716.07	234075356.00	7.64	3.69	3.00
C. applanata	g	h	3	2959	21574.92	63840178.95	0.43	1.68	1.68
C. chinensis	g	h	7	2345	39017.34	91495664.25	1.72	2.63	1.92
C. potosina var. globifera	g	h	4	2153	37297.52	80301569.40	2.68	1.23	1.23
C. potosina var. potosina	g	h	4	969	36502.9	35371312.26	1.50	1.27	1.27

C. sandwichiana*	g	h	3	1909.67	40890.12	78086626.71	14.50	2.55	1.65
C. yucatana	g	h		813.2	11159.93	9075251.54	1.33	1.12	1.12
C. americana	g	I	6	1100.2	20846.91	22935765.00	1.05	2.60	2.24
C. cozumeliensis	g	I	6	3502.5	26150.67	91592712.86	5.36	3.29	2.93
C. globulosa	g	I	6		43928.81		9.13	3.98	3.20
C. macrocephala	g	I	6	16199	29556.1	478779323.00	9.39	4.90	4.00
C. corymbosa var. corymbosa	g	j	6	835	37757.23	31527288.11	6.85	2.83	2.01
C. corymbosa var. grandiflora	g	j	7	6497.75	30333.04	197096540.90	9.66	5.14	3.69
C. corymbosa var. stylosa	g	j	6	6180.67	30714.74	189837696.60	3.15	3.14	2.46
C. prismatica	g	j	6		28606.82		7.93		
C. boldinghii	g	k	3	1636.5	26738.55	43757632.87	0.66	1.89	1.89
C. bonafortuna	g	k						1.88	1.88
C. chapalana	g	k	6	9325.8	35358.15	329743014.30	4.02	3.25	2.33
C. costaricensis	g	k	3	3524.33	34090.11	120144793.20	2.41	3.49	3.19
C. erosa	g	k	3	4738.5	22800.42	108039803.50	1.22	2.01	2.01
C. ortegana	g	k	6	2309	13732.01	31707221.64		2.92	2.92
C. strobilacea	g	k	4	6966	30264.38	210821655.80	5.91	1.31	1.31
C. desmouliniana	g	I	3	2708	14232.17	38540712.80	1.14	1.56	0.84

C. fasciculata	g	l	3	5676					
C. hyalina	g	l	3		35696.97		1.26		
C. legitima	g	l	3	5184.67	16360.41	84823301.11	4.65	2.01	2.01
C. leptantha	g	l	6	3114.17	21871.47	68111477.68	0.59	2.21	1.14
C. membranacea	g	l		419					
C. odontolepis	g	l	5	6210	29714.96	184529871.40	11.31	3.07	2.57
C. polyanthemos	g	l	6	4715	48882.76	230482226.40	2.13	3.65	1.86
C. tuberculata	g	l	6	3664.5	9331.954	34196946.75	0.69	2.24	1.30
C. umbellata var. umbellata	g	l	3	1710	18743.73	32051785.98	0.93	1.07	1.07
C. corylii	g	m	5		53793.34		6.10	1.93	1.36
C. indecora var. attenuata	g	m	3		38715.68		5.23	2.10	2.04
C. indecora var. indecora	g	m	3	3772	58739.93	221567010.60	2.22	1.71	1.62
C. indecora var. longisepala	g	m	3	2445.33	33735.51	82494457.02	15.24		
C. indecora var. neuropetala	g	m	2		41886.65		2.72	1.63	1.36
C. warneri	g	m			23590.63		5.95	2.53	1.73
C. acuta	g	l	3	982.4	23614.99	23199363.74	10.13	1.67	2.10
C. aristeguietae	g	n			20726.8				
C. choisiana	g	n	5		23597.88			2.55	2.55

C. columbiana	g	n			15057.93			1.44	1.32
C. deltoidea	g	n	3		18068.66		1.78	1.06	0.92
C. gracillima	g	n	4	8972	15566.64	139663910.60	2.71	1.25	1.40
C. mcvaughii	g	n	3	6447	30174.43	194534535.60	0.62	2.46	0.35
C. punana	g	n	3	1828	24074.8	44008740.97	1.73	1.64	0.78
C. serruloba	g	n	3	2516					
C. sidarum	g	n	3	2645.5	15406.31	40757386.55	1.36	3.61	2.36
C. vandevenderi	g	n	3	1490			3.19	1.45	1.45
C. argentiniana	g	o	1	6303	12559.33	79161478.62	5.55	1.74	0.76
C. bella	g	o	3	1858.5	54045.45	100443462.60	13.63	3.93	3.13
C. boliviana*	g	o	4	2479	47796.98	118488708.60	5.30	1.59	1.19
C. chilensis	g	o	6	6617.75	52075.8	344624611.80	22.85	4.79	3.31
C. cockerellii	g	o	7		32399.66			4.53	250.00
C. cristata	g	o	2	2309	16375.25	37810452.47	3.85		
C. flossdorfii	g	o	3	769				4.96	4.96
C. flossdorfii pampagrandensis	g	o	3	769	35030.75	26938645.00			
C. foetida var. foetida	g	o	4	2832.33	32901.24	93187163.41	20.39	5.14	4.34
C. foetida var. pycnantha	g	o	6	5310	48985.15	260111143.10	22.62	4.49	2.69

C. friesii	g	o	1	4085.5	25366.67	103635523.70	9.73	1.76	1.54
C. globiflora	g	o	8	3546	46832.35	166067512.90	13.05	3.39	2.95
C. grandiflora	g	o	2	1434.75	19018	27286069.09	23.07		
C. killimanjari	g	o	4	4242	31517.25	133696188.60	69.39	5.14	4.34
C. lucidicarpa	g.	o	4	1619			5.44		
C. microstyla	g	o	4	5183	12529.93	64942617.47		1.34	1.00
C. microstyla var. bicolor	g	o	4	5183			2.67		
C. odorata var. botryoides	g	o	4	4336.5					
C. odorata var. odorata	g	o	4	8071	23841.72	192426542.70	16.74	2.03	1.59
C. odorata var. squarrulosa	g	o	4	7264					
C. paitana	g	o	7	4159	36261.82	150812911.90	2.34	4.56	6.02
C. parodiana	g	o	6	5310.5	45425.34	241231243.20	24.67	3.75	2.30
C. parodiana tucumana	g	o	6	5190					
C. purpurata	g	o	3		29598.74		27.19	2.38	2.01
C. rubella	g	o	7		28184.74			2.68	1.95
C. abyssinica ghindensis	p	p	4	2659				1.69	1.00
C. africana	p	p	4	2921.5	46664.2	136329446.20	1.92	2.33	1.30
C. angulata	p	p	3	4357	29279.31	127569946.70	1.35	2.61	2.61

C. appendiculata	p	p	3	5404.67	24523.12	132539378.70	2.60	2.16	1.62
C. arabica	p	p	3					1.56	1.17
C. natalensis	p	p	4	6759	27177.14	183690281.80	1.98	2.91	2.04
C. nitida	p	p	3	5808.5	27521.19	159856828.50	1.99	2.21	1.50
C. approximata	c	q	3	480.67	42128.05	20249691.58	4.34	2.17	1.19
C. epilinum	c	q	8		36472.38		4.25	1.55	2.81
C. epithymum	c	q	3	4077	16639.16	67837874.98	1.01	1.83	1.83
C. europea	c	q	3	930.4	18427.18	17144643.67	12.06	2.00	1.75
C. europea var. halophyta	c	q	3	1203.75					
C. palaestina	c	q	6	547					
C. planiflora	c	q	5	926	29174.5	27015591.50	1.23	2.00	1.38
C. triumvirati	c	q	3	3173				1.92	1.53
C. cassytoides	m	r	6	1938.67	54992.35	106612015.00	39.02	2.95	2.55
C. exaltata	m	r	6	1816	96940.94	176044749.20	8.60	2.43	2.67
C. gigantea	m	r	6	9561.67				2.71	2.24
C. japonica	m	r	6	3425.6	74113.25	253882347.10	11.95	2.05	1.26
C. lehmanniana	m	r	7	9366.25	86414.81	809382703.40	13.43	4.11	2.85
C. lupuliformis	m	r	6	671.67	68009.83	45680159.83	9.83	2.58	1.98

C. monogyna	m	r	6	1465.33	109593	160589876.60	3.72	2.46	3.46
C. reflexa	m	r	6	8025.5	98348.92	789299217.90	7.95	4.16	2.05
C. santapaui	m	r	6	4600	104507.1	480732653.10	26.64	6.34	2.39

Taxon	Floral Tube Mouth Width	Floral Tube Width at Calyx Tips	Max Width of Calyx	Corolla Flare	Width of Pedicel at Calyx Base	Anther Projection	StigmaHeight	AntherHeight	Herkogamy	Perianth Size
C. acutiloba										
C. alatoloba										
C. andina										
C. dentatasquamata*	2.21	2.21	2.90	2.22	0.78	0.78	1.84	2.78	1.45	0.02
C. goyaziana	1.89	1.97	2.86	4.55	1.11	1.02		5.30		
C. insquamata										
C. orbiculata	2.91	2.92	4.20	1.09	1.25	1.01		5.19		0.82
C. pauciflora										
C. peruviana	2.12	2.13	2.74	2.23	1.24	1.18		3.60		0.23
C. polygonoides										
C. rotundiflora aff.										

C. serrata	1.65	1.62	2.29	2.06	0.52	0.67	2.34	3.33	1.29	-0.50
C. brachycalyx	1.18	1.35	1.35	2.95	0.71	0.87	1.58	2.92	1.47	-1.35
C. californica	1.72	1.59	1.95	4.79	0.94	1.38	2.22	3.62	1.64	-0.24
C. californica var. papillosa										
C. decipiens	2.52	2.39	2.51	2.93	0.71	0.83	1.79	3.33	1.99	0.22
C. draconella	2.10	1.97	2.31	3.71	0.41	0.91	1.43	2.60	1.57	-0.57
C. howelliana	0.86	0.86	1.41	1.55	0.43	0.68	0.98	2.11	1.21	-2.09
C. jepsonii	1.96	2.01	1.75	2.36	0.45	0.63	1.13	2.47	1.66	-0.84
C. occidentalis	1.59	1.57	1.40	1.71	0.64	0.60	1.04	2.27	1.47	-1.21
C. pacifica	2.42	2.42	2.32	4.01	0.97	0.88	1.90	3.07	1.68	0.39
C. pacifica papillata										
C. salina	1.36	1.36	1.82	3.36	0.48	0.60	1.65	2.74	1.29	-0.86
C. subinclusa	1.86	1.98	3.28	4.17	1.14	0.85	2.51	4.57	2.26	0.50
C. suksdorfii	1.96	2.14	3.07	2.41	0.71	1.15	1.44	2.26	1.28	-0.29
C. australis	2.41	2.41	2.41	2.43	0.84	0.91	1.47	2.26	1.44	-0.16
C. campestris	2.59	2.59	2.61	3.23	1.44	1.28	1.14	2.46	1.85	0.11
C. glabrior	1.63	1.88	2.03	2.74	0.44	0.84	1.68	2.78	1.37	-0.80
C. gymnocarpa	1.78	1.78	1.81	2.06	0.92	0.82	1.65	1.82	0.91	-0.94

C. harperi	1.11	1.07	1.10	1.41	0.57	0.50	1.29	1.15	0.57	-2.55
C. obtusiflora var. glandulosa	3.03	2.86	3.10	3.99	1.02	1.47	1.12			
C. obtusiflora var. obtusiflora	2.47	2.40	2.67	4.08	1.07	1.05	1.40	2.97	2.00	0.26
C. pentagona	1.44	1.44	1.86		0.92	0.65	1.06	1.73	0.98	
C. plattensis	2.83	2.76	3.43	3.75	1.15	0.92	2.56	3.81	1.89	0.93
C. polygonorum	2.30	2.12	2.38	2.68	1.01	0.90		2.28		-0.22
C. runyonii	2.87	1.93	2.35	3.67	0.56	1.06	1.11	3.34	2.65	-0.18
C. stenolepis	0.96	1.15	1.78	2.05	0.69	0.66	1.48	2.60	1.22	-1.27
C. corniculata	2.44	2.24	2.66	2.85	0.98	1.11	1.12	2.95	2.20	0.10
C. incurvata	2.60	2.47	2.58	4.03	1.21	1.22	1.75	3.00	1.80	0.30
C. micrantha	1.63	1.61	1.82	1.78	0.39	0.44	2.03	1.86	0.83	-1.30
C. micrantha latiflora	2.94	2.75	3.22	2.98	1.17	1.36		3.09		0.51
C. parviflora										
C. parviflora var. elongata	1.14	1.13	1.27	1.09	0.43	0.96	1.49	2.07	0.81	-2.25
C. platyloba	2.16	2.07	2.45	3.44	0.89	1.94	1.59	4.57	3.17	0.23
C. racemosa var. calycina	1.26	1.26	1.87	2.34	0.38	0.80		2.30		-1.38
C. racemosa var. miniata	1.25	1.29	1.47	1.71	0.59	0.79	1.26	2.26	1.17	-1.55
C. racemosa var. racemosa	1.79	1.70	1.70	3.11	0.70	0.90	1.58	3.34	1.97	-0.46

C. suaveolens	2.34	2.36	2.60	2.76	0.57	1.46	2.53	3.44	1.49	-0.12
C. werdermanii	2.43	2.47	2.72	3.35	1.20	-1.27	1.22	0.79	1.29	
C. xanthochortos var. carinata	1.90	1.68	2.41	4.00	1.17	0.85	0.90	3.53	2.80	0.17
C. xanthochortos var. lanceolata	1.78	1.48	3.20	4.53	0.50	1.28		3.39		-0.31
C. xanthochortos var. xanthochor-tos*	1.95	1.92	2.40	2.53	1.28	0.45	2.35	1.55	1.26	-0.46
C. cephalanthi	1.19	1.33	1.55	1.47	0.89	0.73	1.39	2.49	1.24	-1.43
C. compacta	2.00	2.09	2.54	2.00	0.83	0.81	1.91	4.16	2.47	-0.10
C. cuspidata	1.54	1.54	1.74	1.60	0.73	0.79	1.29	2.54	1.47	-0.94
C. glomerata							3.77			
C. gronovii var. gronovii	2.84	2.48	2.30	4.14	1.04	1.53	2.06	4.20	2.57	0.47
C. gronovii var. latifolia							1.51			
C. rostrata	3.79	2.83	3.23	4.61	1.32	1.80	2.10	5.94	4.28	1.35
C. squamata	1.99	1.85	2.26	3.44	0.71	1.21	1.87	4.60	2.91	0.18
C. umbrosa	2.57	2.67	2.60	3.17	1.20	0.94	1.91	3.49	2.04	0.47
C. denticulata	1.07	1.07	1.50	1.62	0.48	0.37	1.32	1.85	0.75	-1.79
C. nevadensis	1.31	1.31	1.63	4.25	0.35	0.68	1.25	2.48	1.39	-0.93
C. veatchii	1.69	1.52	1.96	2.36	0.53	0.53		2.22		-0.89

C. burellii	1.57	1.57	2.02	2.10	0.48	0.36	1.23	1.82	0.98	-0.88
C. haughtii	2.31	2.30	2.32	3.31	0.59	0.67	1.36	2.13	1.39	-0.37
C. longiloba	3.78	3.67	5.73	6.80	1.28	1.61	1.56	5.11	4.02	1.87
C. partita	2.10	2.09	3.45	3.86	0.61	1.31	0.98	3.77	2.98	0.19
C. aurea	1.77	2.04	2.04	2.56	1.15	1.09	1.82	2.48	1.10	-0.43
C. cotijana	3.69	3.54	5.04	5.57	1.84	1.32	2.62	6.16	3.99	1.90
C. floribunda	2.81	3.10	4.68	6.06	1.55	1.16	1.89	6.11	4.45	1.73
C. jalapensis	2.43	2.42	2.62	3.28	1.10	0.87	2.32	3.26	1.54	0.32
C. lindsayi	2.85	4.26	4.35	3.89	1.59	0.98	3.29			
C. mitriformis	3.52	3.61	3.65	4.24	0.78	1.11	2.21	4.09	2.57	1.19
C. purpusii	1.54	1.52	2.11	4.21	0.93	1.15	2.66	3.83	1.40	-0.11
C. rugosiceps	2.57	2.42	2.61	3.64	0.89	1.25	2.24	3.60	1.87	0.35
C. tasmanica	2.74	2.51	2.53	4.73	0.80	1.51	1.91	3.98	2.48	0.38
C. tinctoria	3.42	3.54	3.99	6.72	0.70	1.78	1.61	4.55	3.40	1.14
C. victoriana							1.42			
C. volcanica										
C. woodsonii	4.20	4.54	5.51	4.23	2.72	1.32	2.06	5.01	3.62	2.30
C. applanata	2.32	2.32	2.24	2.71	0.75	0.99	0.64	2.67	2.34	-0.16

C. chinensis	2.47	2.62	3.50	3.91	0.64	1.15	1.08	3.78	2.97	0.50
C. potosina var. globifera	2.03	2.03	2.20	3.00	1.15	0.93	1.18	2.15	1.41	-0.34
C. potosina var. potosina	2.28	2.28	2.23	2.77	1.01	0.90	0.89	2.17	1.71	-0.27
C. sandwichiana*	3.12	2.87	3.21	3.41	1.41	1.24	1.88	3.79	2.47	0.87
C. yucatana	1.34	1.34	1.78	3.24	0.71	0.66	0.99	1.79	1.04	-1.00
C. americana	1.30	1.29	1.96	1.57	1.27	0.69	1.03	3.29	2.35	-0.68
C. cozumeliensis	2.24	2.32	3.09	2.43	1.37	0.63	3.33	3.91	1.26	0.58
C. globulosa	3.00	3.00	4.03	3.02	1.12	0.69	3.19	4.67	2.11	1.11
C. macrocephala	3.09	3.20	4.03	3.21	1.63	1.10	1.97	6.00	4.31	1.55
C. corymbosa var. corymbosa	1.62	1.51	2.50	1.62	1.10	0.41	3.10	3.24	0.82	-0.39
C. corymbosa var. grandiflora	1.67	2.74	3.16	1.80	0.99	0.56	2.88	5.70	2.94	0.54
C. corymbosa var. stylosa	1.82	2.44	2.33	2.20	1.13	1.01	2.86	4.15	1.58	0.09
C. prismatica							3.69			
C. boldinghii	1.67	1.67	1.52	3.88	0.77	1.17	0.80	3.05	2.40	-0.56
C. bonafortuna	1.66	2.07	2.07	3.26	0.59	0.76		2.64		-0.38
C. chapalana	1.53	1.48	3.61	3.13	0.93	0.66	3.41	3.91	0.91	0.09
C. costaricensis	2.89	2.76	3.18	3.73	1.15	1.23	2.06	4.72	3.02	1.02
C. erosa	1.96	1.96	2.24	4.55	0.76	1.43	1.49	3.44	2.18	-0.14

C. ortegana	1.47	1.47	3.69	3.81	1.37	0.89		3.81		0.30
C. strobilacea	1.34	1.54	1.54	2.72	0.62	0.98	1.94	2.29	0.75	-1.17
C. desmouliniana	0.81	1.04	1.53	1.27	0.77	0.83	1.62	2.40	0.88	-1.90
C. fasciculata										
C. hyalina							1.26			
C. legitima	2.37	2.37	3.93	5.65	0.93	1.41	2.43	3.41	1.54	0.58
C. leptantha	2.02	1.46	1.44	2.81	0.41	1.03	1.58	3.25	1.95	-0.91
C. membranacea										
C. odontolepis	2.78	2.47	3.28	3.75	1.17	1.50	3.02	4.57	2.08	0.77
C. polyanthemos	1.44	1.30	1.34	3.15	0.41	1.24	4.45	4.90	0.85	-0.96
C. tuberculata	1.23	1.00	1.86	1.67	0.43	0.81	0.76	3.05	2.37	-1.46
C. umbellata var. umbellata	1.67	1.67	1.66	2.99	0.54	1.29	1.45	2.35	1.23	-1.00
C. corylii	2.14	2.28	2.75	1.99	1.43	1.25	1.76	3.18	1.78	0.01
C. indecora var. attenuata	3.70	3.62	5.49	5.26	0.58	1.40	2.33	3.50	2.19	1.17
C. indecora var. indecora	2.87	2.71	2.44	3.28	0.73	1.44	1.35	3.16	2.31	0.19
C. indecora var. longisepala							2.66			
C. indecora var. neuropetala	3.10	2.88	2.84	3.53	0.95	1.05	1.19	2.68	2.15	0.41
C. warneri	1.85	3.00	4.61	2.07	0.73	0.91	1.66	3.44	2.00	0.32

C. acuta	1.99	2.26	2.82	2.26	0.81	0.94	1.68	2.61	1.37	-0.11
C. aristeguietae										
C. choisiana	1.78	1.78	2.84	3.78	1.12	1.03		3.58		0.18
C. columbiana	1.46	1.50	2.95	1.91	0.48	0.98		2.42		-0.95
C. deltoidea	1.60	1.45	1.39	2.78	0.31	1.10	1.61	2.16	0.97	
C. gracillima	1.88	1.63	2.55	2.07	0.40	1.52	3.55	2.77	1.22	-0.94
C. mcvaughii	1.94	1.87	1.92	3.30	0.52	1.78	0.98	4.24	3.40	-0.68
C. punana	1.13	1.60	3.02	3.10	0.27	0.76	1.18	2.40	1.35	-1.25
C. serruloba										
C. sidarum	2.96	3.08	5.71	4.62	0.90	1.80	1.93	5.41	3.79	1.38
C. vandevenderi	1.19	1.19	1.39	1.40	0.44	1.33	2.12	2.78	0.89	-1.81
C. argentiniana	3.54	2.65	2.15	5.50	1.04	0.98	1.22	2.72	2.33	0.42
C. bella	2.73	2.76	3.53	3.86	0.82	0.77	1.97	4.70	3.05	0.91
C. boliviana*	1.48	2.04	2.56	2.48	0.74	0.51	2.07	2.10	0.74	-0.63
C. chilensis	2.83	2.96	3.91	5.21	1.88	0.47	3.04	5.26	2.63	1.64
C. cockerellii	1.69	2.12	2.67	3.01	1.30	0.34		4.87		1.50
C. cristata							1.25			
C. flossdorfii	8.33	8.33	9.60	8.33	2.12	2.13		7.09		3.77

C. flossdorfii pampagrandensis										
C. foetida var. foetida	3.27	3.41	4.75	5.47	1.35	0.88	2.64	6.02	3.75	1.84
C. foetida var. pycnantha	2.15	2.21	4.01	4.46	1.05	0.94	3.58	5.43	2.14	0.85
C. friesii	3.09	3.09	3.23	4.22	1.31	0.86	1.31			
C. globiflora	2.89	3.64	4.53	2.75	0.94	0.87	2.05	4.26	2.64	1.15
C. grandiflora							2.17			
C. killimanjari	3.27	3.41	4.75	5.47	1.63	1.18	3.31	6.32	3.42	1.81
C. lucidicarpa							1.55			
C. microstyla	1.42	1.49	1.87	2.61	0.66	0.71		2.05		-1.12
C. microstyla var. bicolor							0.82			
C. odorata var. botryoides										
C. odorata var. odorata	2.02	1.91	2.65	5.32	0.66	1.68	2.79	3.71	1.37	-0.04
C. odorata var. squarrulosa										
C. paitana	2.33	2.33	5.42	1.95	1.42	1.30	1.28	5.85	4.72	1.24
C. parodiana	2.29	2.54	3.04	2.77	1.22	1.51	3.01	5.26	2.52	0.55
C. parodiana tucumana										
C. purpurata	3.33	3.44	4.92	5.49	2.00	1.41	2.95	3.79	1.87	1.48
C. rubella	1.55	2.00	2.47	2.46	0.73	0.73		3.41		-0.32

C. abyssinica ghindensis	1.92	2.11	2.80	3.51	0.67	0.91		2.60		-0.24
C. africana	2.95	2.36	2.31	4.38	0.49	1.18	1.70	3.51	2.34	
C. angulata	2.09	2.09	2.81	2.55	0.46	1.15	1.48	3.76	2.51	-0.08
C. appendiculata	2.20	2.24	2.55	3.40	0.97	1.08	2.31	3.24	1.44	0.09
C. arabica	2.45	2.53	2.55	2.72	0.70	0.83		2.39		-0.16
C. natalensis	3.40	2.90	3.31	5.41	0.64	1.23	1.17	4.14	3.42	0.81
C. nitida	2.03	2.10	1.92	3.74	0.64	1.38	1.42	3.59	2.39	-0.21
C. approximata	1.19	1.38	1.93	2.88	0.93	0.59	1.63	2.75	1.27	-0.85
C. epilinum	2.11	1.74	3.57	2.11	1.25	0.00	1.51	1.55	1.06	0.05
C. epithymum	2.17	2.17	2.37	3.93	0.93	1.09	1.02	2.92	2.19	0.05
C. europea	1.57	1.86	2.95	1.84	1.35	0.57	1.89	2.57	1.04	-0.19
C. europea var. halophyta										
C. palaestina										
C. planiflora	1.51	2.01	2.53	1.44	0.46	0.62	0.82			
C. triumvirati	2.05	1.86	1.63	3.09	0.80	1.01		2.93		-0.42
C. cassytoides	2.34	2.42	3.44	4.76	1.72	0.98	2.60	3.93	1.77	1.00
C. exaltata	2.00	2.13	2.98	2.14	1.11	0.48	2.12	2.90	1.27	0.17
C. gigantea	2.83	2.71	3.00	4.48	1.41	1.10		3.81		0.95

C. japonica	1.61	1.37	1.72	2.59	0.62	0.94	1.79	2.99	1.45	-1.01
C. lehmanniana	2.58	2.34	2.78	3.53	0.94	0.97	2.53	5.08	2.86	0.77
C. lupuliformis	1.26	1.22	1.68	1.40	0.73	0.00	2.24	2.58	0.72	-1.12
C. monogyna	1.66	1.93	2.42	1.67	1.06	0.00	1.84	2.46	1.04	-0.18
C. reflexa	2.79	2.14	2.30	4.28	1.36	0.73	1.47	4.89	3.70	0.73
C. santapaui	3.30	2.19	3.02	5.35	1.54	0.39		6.73		1.26