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Coexistence of sympatric taxa of *Chamaecrista* section *Xerocalyx*: addressing the interplay between morphology and biotic interactions

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University of Missouri Saint Louis

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COEXISTENCE OF SYMPATRIC TAXA OF *CHAMAECRISTA* SECTION *XEROCALYX*: ADDRESSING THE INTERPLAY BETWEEN MORPHOLOGY AND BIOTIC INTERACTIONS

by

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A dissertation submitted to the Graduate School at the University of Missouri – St. Louis in partial fulfillment of the requirements for the degree
Doctor of Philosophy in Biology, with an emphasis in Environmental Studies

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Dissertation Abstract

Animal-plant interactions may prevent gene flow and promote divergent selection among closely related plants, ultimately leading to formation of new species. This may be the case for *Chamaecrista* sect. *Xerocalyx*, in which two or more of the 24 varieties often are encountered in the same area, with marked morphological and phenological differences among them. Over a broad geographical range, however, the morphological gaps among varieties disappear, and they cannot be distinguished clearly.

Several biotic interactions contribute to the fitness of *Chamaecrista* species. The flowers are pollinated by bees that remove pollen as a reward. Leaves, flowers, fruits, and seeds are attacked by herbivores that can directly and indirectly reduce reproductive success. In addition, all *Xerocalyx* species have extrafloral nectaries on the leaves. The nectar produced by these structures attracts ants that may attack herbivores and improve plant fitness. Thus, dissimilarities in the morphology of co-occurring *Chamaecrista* can potentially reduce plant-plant competition for pollinators and mutualistic ants, reduce the number of shared herbivores, and/or reflect diverging strategies for resource acquisition and defense against herbivory.

Hand pollination experiments demonstrated that production of hybrid seeds among syntopic varieties of *C. desvauxii*, a species within section *Xerocalyx*, was severely limited by slower pollen tube growth in foreign styles, differences in style length between pollen donor and pollen receiver, and abortion of developing fruits and seeds by the mother plant. In addition to the existence of reproductive isolation mechanisms, co-occurring varieties were also clearly distinguishable based on morphological traits, including the sizes of flowers, leaves, and extrafloral nectaries. Variation in nectary size modifies the role of ants as a defensive mechanism. In the field, the variety bearing the largest nectary also had the higher amount of nectar production and the highest levels of visitation by ants. Removal of the extrafloral nectaries resulted in decreased fruit and seed set, but only for that variety. However, the benefit of attracting ants was greatly reduced when the ecological context was modified by the exclusion of seed predators. Overall, these results provide support for separating the varieties into different species, and suggest a role for interactions with mutualists and herbivores in shaping morphological traits of sympatric taxa in this group.

To understand the patterns of taxa co-occurrence in this group, a novel approach was used to assess local morphological dissimilarity across a wide geographic scale using collection data. Interactions between co-occurring varieties may result in a pattern of morphological divergence within sympatric communities. Both vegetative and reproductive traits were more dissimilar between pairs of sympatric individuals of different varieties than between pairs of allopatric individuals. Based on permutation tests, this pattern is more likely to stem from competitive exclusion and ecological sorting than from character divergence following competitive interactions.

This work provides new insight into the patterns and processes of coexistence in phenotypically continuous taxa, from local to broad geographic scales. In particular, it shows how widespread patterns of locally differentiated assemblages of taxa can be formed and maintained in groups that are phenotypically continuous over their full range. *Chamaecrista* section *Xerocalyx* is now well positioned as a valuable system to further investigate the evolution of morphological diversification, and how trait divergence influences coexistence.

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Chapter 1. Reproductive isolation among sympatric and allopatric taxa of *Chamaecrista* sect. *Xerocalyx* (Leguminosae)

Abstract

Several varieties of *Chamaecrista desvauxii* occur syntopically in some areas but are clearly distinguished based on vegetative morphology. These varieties occupy similar habitats and attract generalist bees searching for pollen, raising the possibility that postmating mechanisms, rather than premating ones, are responsible for reproductive isolation among them. We used hand pollination between four varieties of *C. desvauxii* and one variety of a closely related species in two field sites in the Brazilian cerrado to quantify the relative role of several postmating mechanisms of reproductive isolation. We found that, although some crosses between varieties produced viable hybrids, several mechanisms acted in sequence to reduce the likelihood of hybridization, including differences in pollen tube growth and style length, and postzygotic abortion of ovaries and ovules.

Introduction

Several mechanisms may lead to the formation of completely or partially isolated populations, such as geographic barriers, adaptation to different habitats, chromosomal rearrangements, hybridization, and polyploid formation (Baker, 1959; Grant, 1981; Levin, 1993; Coyne and Orr, 2004; Rieseberg and Wendel, 2004). These lineages can only persist separately in sympatry if there is some barrier to gene flow (Coyne and Orr, 2004), and species are often defined based on reproductive isolation (Coyne and Orr, 2004; Grundt et al., 2006; Rieseberg et al., 2006; Rieseberg and Willis, 2007; Okuyama and Kato, 2009). Isolating barriers can also be a side-effect of the accumulation of genetic differences among geographically isolated populations (Barton, 2001; Rieseberg et al., 2003; Coyne and Orr, 2004), resulting in divergence among genotypes (incongruence) (Hogenboom, 1984), and reinforcement may increase reproductive isolation among sympatric species (Van der Niet et al., 2006; Kay and Schemske, 2008). However, despite their importance for the study of speciation, relatively few studies have examined the separate contribution of different isolating barriers for reproductive isolation in plants (Widmer et al., 2009). In the present paper, we quantify the relative importance of different pre- and postzygotic mechanisms, and their degree of asymmetry, among several co-occurring legume varieties that largely overlap in habitat and phenology.

Typically, several pre- and postzygotic isolating barriers work together to prevent gene flow between species (Coyne and Orr, 2004). Although the relative contribution of prezygotic mechanisms for reproductive isolation is often stronger, because they happen earlier in reproductive events (Ramsey et al., 2003; Coyne and Orr, 2004), postzygotic isolating mechanisms may be essential to fully prevent hybridization (Widmer et al., 2009). One prezygotic mechanism in plants is variation in floral morphology, color and odor that lead to attraction of different pollinators (ethological isolation) or differential pollen placement on the visitor (mechanical isolation) (Bradshaw et al., 1995; Fulton and Hodges, 1999; Wolf et al., 2001). These mechanisms are usually insufficient to prevent pollen flow between taxa (Armbruster and Muchhala, 2009), and several additional factors may limit the opportunity for heterospecific pollen deposition. For instance, divergence in flowering phenology can reduce the potential for pollen transfer among species (Gottsberger and Silberbauer-Gottsberger, 1988; Madeira and Fernandes, 1999). Post-mating, prezygotic barriers occur after pollen deposition on the stigma, but before the formation of the zygote, and include lower pollen germination rates, slower pollen tube growth rates, and/ or reduced ability to fertilize ovules when compared to conspecific pollen (Hauser et al., 1997; Van Creij et al., 1997). After ovule fertilization, postzygotic barriers may be expressed through higher abortion rates of hybrid seeds within fruits and of fruits containing hybrid seeds within plants (Hauser et al., 1997). In addition, viable hybrid individuals can have reduced fitness (Fishman and Willis, 2001; Campbell and Waser, 2007; Tierney and Wardle, 2008).

Reproductive isolation between plants is often asymmetric, i.e., rates of hybrid formation and hybrid sterility depend on which species is the pollen donor, resulting in unilateral incompatibility (Tiffin et al., 2001). In crosses among species with differences in flower morphology, pollen from short-styled flowers may either be unable to reach ovules of long-styled flowers or be outcompeted by faster-growing conspecific pollen (Buchholz et al., 1935; Kiang and Hamrick, 1978; Wolf et al., 2001). Unilateral incompatibility can also result from the “SI x SC rule” (Lewis and Crowe, 1958), a common pattern in which heterospecific pollen tube growth is arrested in pistils of self-incompatible (SI) species, but no inhibition occurs when the pistil belongs to a self-compatible (SC) species (Harder et al., 1993; Onus and Pickersgill, 2004, but see Sorensson and Brewbaker, 1994 for an exception). After fertilization, nuclear-cytoplasmic interactions may result in hybrid inviability or sterility (Tiffin et al., 2001). Even when a hybrid is formed, its fitness may be lower in one direction of the cross (Tiffin et al., 2001; Campbell and Waser, 2007).

The genus *Chamaecrista* includes more than 240 species in the Americas (Irwin and Barneby, 1982), and 188 of those species are found in the cerrado biome of Brazil (Mendonça et al., 2008). In the last thorough taxonomic review of the American species of this genus, Irwin and Barneby (1982) noted that section *Xerocalyx* is characterized by a high degree of intergradation among taxa and proposed that this section actually constitutes "one enormous macrospecies in which complex evolutionary processes are currently active but not yet so advanced as to give rise to truly discrete units". Currently, this section includes 25 varieties separated into three species (*C. desvauxii* (Collad.) Killip, *C. ramosa* (Vogel) H.S. Irwin & Barneby and *C. diphylla* (L.) Greene) (Irwin and Barneby, 1982). Despite the amount of morphological variation within *Xerocalyx*, this section is well circumscribed within *Chamaecrista* through sepal, leaf, and seed morphological traits and a chromosome count of $2n = 14$, while the rest of the genus has $2n = 16$ or multiples of 16. In addition, a molecular phylogeny of *Chamaecrista* that includes *C. diphylla* and four varieties of *C. desvauxii* supports the monophyly of this group (Conceição et al., 2009). Nevertheless, it is common to find two or more varieties coexisting in one location, often occurring side by side, and distinguished by locally uniform morphological traits (Irwin and Barneby, 1982; Gottsberger and Silberbauer-Gottsberger, 1988; Madeira and Fernandes, 1999). In the only study examining reproductive isolation between sympatric varieties of *C. desvauxii*, Costa et al. (2007) found considerable overlap in phenology and the identity of flower visitors, but inter-taxa crossings yielded no seeds, and genetic distance between taxa was high. This work demonstrated that reproductive isolation between sympatric taxa can occur, but the study was limited to two taxa in one location. Given the morphological diversity and extensive geographic distribution of section *Xerocalyx*, the varieties of this section have the potential to represent different steps in the process of speciation. To begin to address this question, however, it is necessary to expand the number of taxa examined and include areas with a higher diversity of co-occurring taxa.

Here, we quantify mechanisms of reproductive isolation among several sympatric varieties of *C. desvauxii* and one variety of *C. ramosa.* These varieties occupy similarly open habitats and overlap in flower phenology. We used artificial crosses among flowers bagged in the field to measure the degree of postmating reproductive isolation among sympatric and allopatric varieties in two cerrado areas. Given that sympatric varieties largely overlap in habitat, flower morphology, and phenology, levels of premating reproductive isolation are likely low, and we predicted that postmating mechanisms should be in place to prevent hybridization between these taxa. Viable seeds were formed with relatively high frequency in only one combination of pollen donor and seed plant, between varieties from sites 20 km apart, suggesting that these two varieties could be in the early stages of divergence. However, for sympatric varieties we found that postmating barriers reduced the formation of hybrid seeds in all of the crosses, resulting in high levels of reproductive isolation. Thus, the occurrence of intrinsic postmating barriers is likely essential to maintain the integrity of those co-occurring varieties.

Materials and Methods

Study System— The present study focused on three varieties of one species of the Neotropical *Chamaecrista* section *Xerocalyx* (Leguminosae). *Chamaecrista desvauxii* is distributed from Argentina to Mexico. Considerable morphological variation is observed throughout the range of this species, justifying the recognition of 17 varieties (Irwin and Barneby, 1982). In addition, we included one undescribed variety of *C. desvauxii* and one individual of *C. ramosa* var. *parvifoliola* in the hand pollination treatment as pollen donors. Although *C. desvauxii* has relatively larger leaflets and longer petioles, in all other aspects it is morphologically similar to *C. ramosa*. Leaves of both species have four leaflets and, as a rule, one extrafloral nectary on the petiole (Irwin and Barneby, 1982). The flowers have no nectar but produce large amounts of pollen, which is harvested by bees through buzz-pollination (Gottsberger and Silberbauer-Gottsberger, 1988; Costa et al., 2007). The pods open explosively to disperse the seeds, and there are no specialized structures for secondary dispersal.

The work was conducted in two field sites 21 km apart: the cerrado reserve of Clube Caça & Pesca Itororó (hereafter "CCPIU", 18º55' S, 48º17' W) and the Estação Ecológica do Panga ("Panga", 19º11' S, 48º24' W), both in the outskirts of Uberlândia, Brazil. Both areas have open savanna areas with scattered trees (campo cerrado) and areas dominated by trees and shrubs (cerrado *sensu stricto*; see (Oliveira-Filho and Ratter, 2002). Using the Köppen classification system, the climate of the region is Aw (tropical savanna climate); average temperatures range from 18ºC to 23ºC throughout the year, but rainfall is concentrated in the wet season, between the months of November and April. Annual precipitation fluctuates around 1550 mm (Silva et al., 2008). In August 2004, an accidental fire burned a large portion of CCPIU, and ca. 40% of the area of Panga burned in September 2006. In both cases, the largest populations of all varieties included in this study were observed in the rainy season following each fire.

Five of the seven taxa in section *Xerocalyx* recorded from Uberlândia were included in this study (Table 1-1, Fig. 1-1). All are predominantly annual, germinating at the start of the rainy season and ripening fruits over several months until the beginning of the dry season. A few individuals were observed to persist during the dry season and flower again in the following rainy season, but no individuals lived for more than two years. *Chamaecrista desvauxii* var. *brevipes* (Benth.) Irwin & Barneby and *C. desvauxii* var. *mollissima* (Benth.) Irwin & Barneby are subshrubs distributed from Central to most of South America, and are distinguished primarily by the length of the pedicel ((1-)2-8(-12) mm and (12-)15-35(-42) mm, respectively) (Irwin and Barneby, 1982). The distribution of var. *brevipes* is scattered within var. *mollissima*'s range, and these varieties are occasionally sympatric. In fact, var. *brevipes* was considered by Irwin and Barneby (1982) as "an assembly of minor variants that independently have acquired abbreviated pedicels". In Uberlândia, a population of *C. desvauxii* with average pedicel lengths intermediate between vars. *brevipes* and *mollissima* is commonly observed in CCPIU but not found in Panga. In contrast, a population of typical var. *mollissima* is abundant in Panga. The two morphotypes are easily distinguished by differences in pedicel length, flower size, foliage and ovary pubescence, and leaf shape. In addition, the leaves of var. *mollissima* in Panga often have an additional extrafloral nectary between the two pairs of leaflets, which was never observed in CCPIU, and seedlings grown from seed in the greenhouse retained the vegetative characters of each variety (Baker, pers. obs). Thus, for the purposes of this study, the population in CCPIU is referred to as var. *brevipes*, while the population in Panga is identified as var. *mollissima.* Voucher specimens for both populations are deposited in HUFU.

The third variety included in the study, *C. desvauxii* var. *modesta* Irwin & Barneby, is restricted to central Brazil, and is characterized by a more erect habit, linear-elliptic leaflets, and flowers of reduced size (Irwin and Barneby, 1982). In contrast to the more uniform distribution of var. *brevipes* and var. *mollissima,* this variety occurs in clear patches at both field sites. *C. desvauxii* var. 1 (undescribed), the fourth variety, has a prostrate habit and glabrous leaflets that in shape resemble those of var. *brevipes*. This variety is characterized by extrafloral nectaries that are clearly stipitate and reduced in size, long pedicels and small flowers, usually with only six stamens, although some dissected flower buds had 10, the norm for section *Xerocalyx*. Unlike the other varieties, scattered individuals flower throughout the year, although reproduction is concentrated in the wet months. No collections resembling this variety were observed in herbarium collections at GH, MO, NY, TEX, UB, and US (Baker, pers. obs.). In 2005 and 2006, this variety was extremely abundant in CCPIU, with an estimated population in the thousands of individuals, and records from the local herbarium (HUFU) include numerous collections of this variety over the past 15 years. In the region of Uberlândia, this variety was also located in Fazenda Água Limpa (19.09º S, 48.35ºW), a cerrado area between CCPIU and Panga; voucher specimens are deposited in HUFU (record numbers 48823-5). The fifth taxon to be included in the study is *C. ramosa* var. *parvifoliola* (Irwin) Irwin & Barneby, a subshrub distributed in cerrado areas of central-eastern Brazil. Its flowers are intermediate in size and are supported by long pedicels, and the leaves are glabrous and small. Over the period of three years of field work, only three individuals of this variety were found in CCPIU, and none in Panga. The inclusion of this variety in the study was opportunistic and, due to its local rarity, no voucher specimens were collected.

In CCPIU, we used var. *brevipes* and var. *modesta* as seed plants, but included var.1 and *C. ramosa* as pollen donors for var. *brevipes*, and var.1 as pollen donor for var. *modesta*. In Panga, we used var. *mollissima* and var. *modesta* both as pollen donors and as seed plants. Due to differences in phenology, it was not possible to perform crosses between individuals of var. *modesta* in CCPIU and those in Panga.

Varieties traits—One flower and one leaf were collected from five individuals of each variety for measurement of morphological traits. Because only one individual of *C. ramosa* was found during the course of this study, all flowers and leaves of this variety were collected from the same individual. Differences in morphological traits among varieties were tested using ANOVAs followed by Tukey's HSD (Honest Significant Differences) tests. Due to limited population sizes, we surveyed a subset of the individuals used as seed plants in each variety to estimate the number of individuals that had at least one flower in each month during the period of this study; observations on the phenology of the remaining taxa are based on informal field observations.

Chromosome counts—Root tips from germinated seeds of all varieties included in the study were pretreated in paradichlorobenzene (PDB) and fixed in Carnoy's fluid (3 96% ethanol : 1 glacial acetic acid). Root meristems were squashed in 45% acetic acid solution, frozen in liquid nitrogen, and stained with 2% Giemsa stain. Chromosome numbers were determined from cells at mitotic metaphase using a light microscope. In addition, small flower buds from all *C. desvauxii* varieties in CCPIU were collected in January 2007 and fixed in Carnoy's fluid. Pollen grains from each variety were smeared in carmine solution to observe chromosomes in meiotic cells.

Hand-pollination treatments—Branches with flower buds were bagged one to five days before a flower opened. Bagged branches were checked every morning for open flowers. A tuning fork was used to remove pollen from flowers by vibrating the stamens to spread the pollen grains onto a glass slide. Pollen used in cross-pollination treatments came from at least three individuals, with the exception of pollen from *C. ramosa*, in which case only one flowering individual was available. To enable crosses among individuals from CCPIU and Panga, flower buds were cut from several individuals one day prior to anthesis and kept in humid chambers. Pollen was removed from open flowers the following day. In all crosses, pollen was mixed on the slide using a toothpick, and applied by tapping the style and pushing the stigma onto the slide several times. Several slides were used each day to prevent contamination among pollen sources, and all slides were cleaned with ethanol between trials.

Treatments were distributed among available individuals and throughout the flowering season (from January to May 2007). A total of 479, 339, 248 and 135 flowers from 40, 72, 46 and 24 individuals were treated for vars. *brevipes*, *mollissima*, *modesta* (CCPIU) and *modesta* (Panga), respectively. Each open flower was individually marked and received one of the following treatments: a) automatic selfing: flower bagged but otherwise untreated; b) self-pollination: pollen was removed and applied to the same flower; c) cross-pollination: pollen from other individuals of the same variety was applied onto the stigma; and d) inter-taxa: pollen from one of the other taxa was applied (see Fig. 1-1 for an overview of hand-pollination treatments). After artificial crosses, flowers were rebagged. In addition, open flowers (no treatment) were marked for monitoring.

Pollen tube growth—Between 2–3 flowers were collected after 6, 12 and 24 h for each artificial cross treatment in 70% ethyl alcohol. Pistils were soaked in 5% NaOCl for 20 to 120 minutes and washed with distilled water. Pistils were opened along the sutures, mounted on slides, stained with 0.25% aniline blue, and placed under an epifluorescence microscope to observe pollen tube growth and ovule penetration (Martin, 1959). We expected that pollen grains from inter-taxa crosses would take longer to reach the ovary and would fertilize fewer or no ovules when compared to grains from intra-taxa crosses.

Flower fate—Treated flowers were censused at least once a week throughout the reproductive season. Bagged, untreated flowers (automatic selfing treatment) aborted after two days. In treated and open flowers most reproductive organs fell off within this period, leaving the ovary behind. The fate of each treated flower was classified as follows: “aborted flower”, “aborted ovary” (ovary retained after abscission of corolla and stamens, indicating successful ovule fertilization, but aborted before any fruit development began), “aborted fruit” (enlarged ovary, often with hardened valves, either aborted or containing no filled seeds) or “viable fruit” (mature fruit containing loose, filled seeds). Developing fruits were bagged individually to prevent pre-dispersal seed predation. Fruit and seed maturation required about one month to complete. The number of aborted flowers (indicating failure to fertilize ovules) and the number of aborted ovaries and fruits (indicating selective fruit abortion) were compared among treatments using Fisher’s exact test. Mature fruits were collected to count the number of viable (filled) and non-viable seeds and ovules per pod. Ovules that showed no increase in size were considered non-fertilized; fertilized, unfilled ovules were considered aborted. The number of fertilized ovules, aborted ovules, and viable seeds per fruit were compared among treatments using negative binomial GLMs, with the cross-pollinated treatment (treatment ‘c’ above) as the baseline for comparison. We predicted that more flowers and ovaries/ developing fruits would be aborted following inter-taxa crosses than from intra-taxa crosses, and that fruits of inter-taxa crosses would have fewer fertilized ovules, more aborted ovules and fewer viable seeds.

The relative position of the most basal fertilized ovule was noted for each fruit; ovules were numbered sequentially from the distal to the proximal end of the fruit, and position was calculated by dividing the number of the most basal fertilized ovule (i.e., the fertilized ovule with the highest number) by the total number of ovules. Thus, a relative position of 1 indicates an ovule close to the pedicel, while a relative position near zero indicates an ovule near the style. In addition, we calculated a style length ratio as the mean style length of the pollen donor taxon divided by the mean style length of the maternal taxon. We used linear regression to determine if style length ratio affected mean relative ovule position. Data were pooled for large- and small-flowered varieties to increase sample sizes. We predicted that style length ratio would correlate with the position of the most basal fertilized ovules in seed plants with large flowers (var. *mollissima* and var. *brevipes*), since pollen tubes from small flowers are less likely to reach the proximal end of the fruit. For the seed plants with small flowers (var. *modesta*), we expected that pollen tubes from all varieties would be able to reach the base of the fruit, yielding a non-significant correlation between ovule position and style length ratio.

Germination trials—Up to 30 seeds from each treatment were weighed, scarified, and placed in Petri dishes with filter paper and distilled water under a 12:12 light cycle for 30 days. Seeds were observed daily for radicle emergence, and germinated seeds were removed from the dishes and planted on soil from either Panga or CCPIU. Both the percent of germinated seeds and the Germination Speed Index (Maguire, 1962), based on the cumulative number of germinated seeds per day, were calculated for each treatment. We predicted that seeds from inter-taxa crosses would show reduced fitness through little or no germination and increased time to germinate. Cotyledon length was measured after the first true leaf had matured. Seedling development was followed for 50 days after germination, at which point up to 14 seedlings per treatment were harvested. We counted the total number of leaves and measured maximum length of roots and stem, and length and width of both leaflets in the largest leaf. Differences among treatments were assessed using ANOVAs followed by Tukey HSD tests, if appropriate.

Crossability Index—We calculated a Crossability Index (McDade and Lundberg,

1982) using a composite estimate of seedling number per flower for each treatment, and dividing the value by the value obtained for the cross-pollination treatment for each taxon. We estimated the number of seedlings per flower by multiplying the probability of fruit formation (number of viable fruits divided by total number of treated flowers) by the mean proportion of filled seeds per fruit and mean percent germination.

Results

Variety traits—The five varieties were significantly different in leaflet size and in the size of their floral parts, supporting the idea that they represent separate lineages (Table 1-1). In addition, no individuals of intermediate form, evidence for hybridization, were encountered over the course of four years in the field (BB, pers. obs.).

The leaves of *C. desvauxii* var. *brevipes* are pubescent, and relatively large with large extrafloral nectaries (Table 1-1). The flowers of this variety have relatively large petals, medium to short pedicels, and long styles (Table 1-1). Individuals flowered between January and June, with a peak on March (Fig. 1-2). The flowers open after sunrise, between the hours of 0600 and 0700, and close around 1300 hours on sunny days and 1400-1500 hours on cloudy days. As in all other taxa included here, flowers open sequentially every two to three days within a branch, but anthesis lasts only half a day.

The leaves of var. *mollissima* are similar to those of var. *brevipes* in size and in the obovate shape of the leaflets (Table 1-1), but are entirely glabrous and may occasionally contain a second extrafloral nectary. The flowers are slightly larger, with a longer pedicel, but the style length and number of ovules are similar (Table 1-1). Flowering of this variety also peaked in March during the study period (Fig. 1-2). Unlike var. *brevipes*, flowers of this variety opened about one hour before sunrise, around 0500 hours, and closed relatively early, by 1100 hours.

The leaves of var. *modesta* are glabrous with linear-elliptic leaflets, similar in size at both study sites. At both sites the pedicels are short and the flowers are relatively small (Table 1-1). However, there was little overlap in the flowering phenology of this variety between CCPIU and Panga; in CCPIU, the peak flowering period for this variety occurred in February, but in Panga most individuals flowered in March and April (Fig. 1-2), thus precluding crosses between individuals from the two locations during the study year. At both sites flowers opened about half an hour after sunrise, from 0600 to 0700 hours, and closed around 1100-1200 hours.

The other two varieties used as pollen donors for this study were relatively rare during the course of this study. Var. 1, however, was extremely common in CCPIU in 2005 and 2006. This variety is characterized by relatively small, glabrous leaves, obovate leaflets, long pedicels, and very small flowers (Table 1-1). Scattered flowering individuals were found throughout the study season. Flowers of var.1 open at sunrise, around 0600 hours, and by 1100 hours all are closed. In 2005, eight of nine bagged flowers set seeds spontaneously (without treatment). Only one individual of *C. ramosa* was found during the period of the study, and it had small, glabrous leaves, medium to long pedicels, and medium-sized flowers with a short style (Table 1-1). This individual flowered from the beginning of March to early April.

Chromosome counts—All taxa included in this study were diploid, with $2n = 14$. No abnormalities were observed in the meiotic slides.

Pollen tube growth—Pollen germination occurred in all crosses, thus no mechanisms are in place to prevent self or inter-taxon pollen germination or arrest pollen tube growth in the style. Generally, however, pollen tubes took longer to reach ovaries in inter-taxa crosses compared to intra-taxa pollen. In CCPIU, pollen tubes reached the ovary and fertilized the first ovules of var. *brevipes* pistils within 6 h for most crosses, and by 24 h tubes reached the base of the ovary. However, a minimum of 12 h was necessary for pollen tubes to reach the base of the style in pistils treated with pollen from small-flowered varieties (var. 1, varieties *modesta* and *ramosa*). When var. *modesta* was used as seed plant, most ovules were fertilized within 6 h after intra-taxon crosses, and only a few or no ovules were fertilized after 24 h after inter-taxa crosses. In Panga, pollen tubes reached the first ovules of var. *mollissima* within 6 h for flowers treated with intra-taxon cross-pollen, but not for flowers treated with self- and inter-taxon pollen. With the exception of var. *modesta* pollen, however, pollen tubes reached the bottom of the *mollissima* ovaries within 24 h. Similarly to what was observed for var. *modesta* in CCPIU, most ovules from intra-taxon pollen for var. *modesta* in Panga were fertilized within 6 h. Although pollen tube growth was slower for inter-taxa crosses, pollen tubes reached the bottom of the ovary within 12 h.

Flower fates—With the exception of bagged, untreated flowers (automatic selfing treatment), fruits initiated development in all hand-pollination treatments (Fig. 1-3), again pointing to a potential for hybridization. No difference in fruit set between self- and cross- pollinated flowers was observed for any of the varieties ($P > 0.1$), suggesting that all are self-compatible (but see results for var. *mollissima* below) and discarding the SC X SI rule as a mechanism of incompatibility between varieties.

Contrary to expectation, the proportion of flowers treated with inter-taxa pollen that initially retained the ovary was comparable to those of open flowers for all varieties, suggesting similar levels of fertilization. However, subsequent abortion of both unexpanded ovaries and developing fruits led to significant reductions in fruit set, conforming to our prediction, with the exception of var. *mollissima* flowers treated with pollen from var. *brevipes* ($P < 0.05$, black bars in Fig. 1-3).

Fertilized ovules and seed set—The number of fertilized ovules in self- and cross-pollinated flowers was similar for varieties *brevipes* and *modesta*, but self-pollinated flowers from var. *mollissima* tended to have fewer fertilized ovules, and had significantly more aborted ovules and fewer filled seeds than cross-pollinated flowers, indicating some level of self-incompatibility in this variety (Table 1-2).

When varieties with long styles (*brevipes* and *mollissima*) were used as seed plants (pollen receptors), the position of the farthest ovule fertilized was correlated with the length of the style of the pollen donor ($R^2 = 0.799$, $P < 0.002$, $N = 8$; Fig. 1-4), suggesting that pollen tube growth was limited by the style length of the paternal plant, as predicted. For flowers with short styles, on the other hand, the correlation was not significant ($R^2 = 0.063$, $P = 0.312$, $N = 6$; Fig. 1-4), and pollen from both long- and short-styled flowers reached the most basal ovules.

Consistent with our original hypothesis, in almost all cases flowers treated with inter-taxa pollen had fewer fertilized ovules and more aborted ovules compared to cross-treated flowers, producing few or no viable seeds (Table 1-2). The clear exception was var. *mollissima*, which when treated with var. *brevipes* pollen had a similar number of fertilized ovules and produced only slightly fewer filled seeds than cross-pollinated flowers (Table 1-2). Flowers of var. *modesta* in Panga treated with pollen from var. *mollissima* also had a similar proportion of fertilized ovules as cross-pollinated flowers, but most of those were subsequently aborted and few hybrid seeds were produced.

Germination trials and seedling growth—In general, if seeds were formed, their viability did not differ between intra- and inter-taxon crosses, contrary to expectation. Seed weight was significantly lower than cross-treated seeds in only one combination, var. *brevipes* ♀ x var. *mollissima* ♂. All seeds had relatively high rates of germination (above 80%; Table 1-3). Exceptions were seeds from var. *brevipes* ♀ x var. *mollissima* ♂, which are also significantly smaller than cross-treated seeds (Table 1-3), and from cross-pollinated var. *modesta* in Panga (Table 1-3). However, the only seed obtained from the crossing of var. *modesta* from Panga and var. *mollissima* did not germinate after 30 days. Overall, seeds from var. *brevipes* germinated faster than those of other varieties, especially those from Panga, and seeds from self-pollination tended to germinate slower than those from cross-pollination or open flowers in all cases (Table 1-3).

After 50 days of seedling growth, there were few differences in morphology among different treatments within each variety. Seedlings from open, self- and cross-pollinated flowers generally attained the same size for all varieties (Table 1-4), but self-pollinated *mollissima* seedlings had shorter stems and roots and smaller leaflets than cross-pollinated ones (Table 1-4), suggesting some degree of inbreeding depression. Seedlings from crosses of var. *brevipes* ♀ x var. *mollissima* ♂ had shorter cotyledons relative to cross-pollinated flowers (Table 1-4), but there were no significant differences in this trait among treatments for any of the other varieties. In addition, seedlings of var. *brevipes* treated with pollen from *C. ramosa* were significantly taller, and had more leaves and shorter leaflets than seedlings from cross-pollinated flowers (Table 1-4), while seedlings from crosses with var. *mollissima* had significantly fewer leaves (Table 1-4).

Seedlings of var. *mollissima* ♀ x var. *brevipes* ♂ had significantly more leaves than cross-pollinated *mollissima* seedlings (Table 1-4).

Crossability Index—The only inter-taxa cross that had a high Crossability Index, indicating relatively high formation of seeds when compared to intra-variety crosses, was var. *mollissima* ♀ x var. *brevipes* ♂ (Table 1-5).

Discussion

The goal of this study was to quantify levels of reproductive isolation among sympatric and allopatric taxa of *Chamaecrista* section *Xerocalyx* through hand-pollination treatments. Our original hypothesis was that postmating mechanisms would prevent hybridization between the so-named varieties in this study. We found such post-mating mechanisms in the form of reduced pollen tube growth and differential fruit and seed abortion in inter-taxon compared to intra-taxon crosses (Fig. 1-1). Although most inter-taxa crosses produced no viable seeds, those between *C. desvauxii* var. *mollissima* ♀ x var. *brevipes* ♂ produced a relatively high number of seedlings, demonstrating the potential for hybridization *sensu* Arnold (1997) between those two varieties (Fig. 1-1). However, the long-term viability and fertility of those hybrids were not determined. Along with the lack of putative hybrids in the field, these observations support our hypothesis that sympatric taxa represent populations with significantly reduced gene flow, which may eventually lead to complete genetic isolation (Rieseberg and Willis, 2007).

Significant differences in flower size between sympatric varieties (Table 1-1), with likely effects on pollinator visitation, suggest that some degree of premating reproductive isolation does occur. Flowering phenology does not appear to be a premating isolating mechanism, given the general overlap in flowering phenology between the different varieties (Figure 1-2; but see below). The low number of flowering individuals during the period of the study prevented systematic observations of flower visitation, but opportunistic observations allowed us to conclude that the varieties included in this study are visited by generalist bees in search of pollen. Although larger-bodied bees in the families Andrenidae (subfamily Oxaeinae) and Apidae (tribes Meliponini, Bombini and Xylocopini) were only observed visiting flowers of vars. *brevipes* and *mollissima*, smaller bees in the families Halictidae and Apidae (tribe Euglossini) were seen visiting flowers from all varieties. It is unlikely that larger bees would be able to extract pollen from the flowers of var. *modesta* and var. 1, because both the stamens and the opening of these flowers are extremely small (maximum flower diameter is less than 15 mm). The long stamens and large flowers of vars. *mollissima* and *brevipes* (up to 39 mm in diameter), on the other hand, are adequate for buzz pollination by larger-bodied bees, while smaller bees may subsequently remove pollen deposited on the petals. Those smaller bees, however, are unlikely to touch the stigmatic surface, located at the tip of the style, while removing pollen. The flowers of *C. ramosa* are intermediate in size (ca. 25 mm in diameter), and are likely to be visited by at least some of the larger bees that visit var. *brevipes*.

Var. *modesta* has a patchy distribution, and phenology was synchronized within patches but not always among them (BB, pers. obs.). Although during the period of this study there was little overlap in flower production among the two field sites for this variety, in previous years patches of flowering individuals were observed in CCPIU as early as December and as late as April, so that some overlap in phenology between sites is expected to occur in some years. Flight distance in bees increases with body size (Gathmann and Tscharntke, 2002; Greenleaf et al., 2007). Although solitary bees on average fly less than 600 m (Gathmann and Tscharntke, 2002), Janzen (1971) reported that largebodied euglossine bees *Eufriesea surinamensis* were able to fly up to 23 km back to their nests, and *Xylocopa* bees were observed to fly up to 12.5 km (Rau, 1929). The distance of pollen dispersal, however, depends not only on the maximum distance bees are able to fly, but also on their behavior and foraging pattern, the density and composition on the local flora, and characteristics of the landscape connecting populations (Ghazoul, 2005; Sork and Smouse, 2006; Pasquet et al., 2008). In addition, plants in dense populations like those of section *Xerocalyx* tend to receive pollen over shorter distances (Ward et al., 2005). Thus, pollen transfer between the two study areas is possible, but unlikely, especially for those varieties visited only by small-bodied bees, which have a shorter flight range.

In general, pollen tubes grew more slowly in inter-taxa styles than intra-taxon cross-pollen, but in all crosses the pollen tubes eventually reached the ovary. Even if the inter-taxa pollen tubes are able to reach all ovules, however, natural pollen loads usually contain pollen of mixed origin. Simultaneous deposition of both intra- and interspecific pollen grains on the stigma may increase pod abortion, and lead to formation of fewer hybrid seeds than expected based on the pollen ratio (Carney et al., 1994; Hauser et al., 1997; Klips, 1999). In section *Xerocalyx*, it is likely that both conspecific and heterospecifc pollen regularly reach the stigma because the varieties attract generalist bees. In this case, competition among pollen tubes within the style and among developing seeds within the fruit may select against heterospecifc pollen or hybrid zygote, further reducing hybrid formation rates. When the pollen donor is rare, as was the case with var. 1 and *C. ramosa* during the period of this study, it is unlikely that its pollen grains would be the only ones to reach the stigma of flowers of a different taxon. When this relationship is reversed, however, it is possible that no flowers from the same variety are visited before pollen deposition, increasing the odds of inter-taxa fertilization (but see Zhou *et al.*, 2008).

In addition to conspecific pollen precedence, differences in style length contribute to prezygotic reproductive isolation. Varieties with short styles (*modesta*, var. 1, and *C. ramosa*) fertilized few or no ovules from the varieties with long styles (*brevipes* and *mollissima*), while pollen from the latter group fertilized most or all of the ovules from the short-styled flowers. This pattern has been observed in interspecific crosses involving flowers of different sizes in several genera (e.g. among ten species of *Datura* _ Buchholz et al., 1935, *Ipomopsis aggregata* x *I. arizonica* _ Wolf et al., 2001, and *Mimulus lewisii* x *M. cardinalis*_ Ramsey et al., 2003). Levin (1958; 1978) proposed that pollen grains accumulate just enough metabolites for tube growth from the stigma to the embryo sac, so that pollen from short-styled species does not have sufficient nutrients to sustain pollen tube growth in longer styles. In these cases, heterospecific crosses will always produce a very limited number of seeds.

Even when heterospecific pollen tube growth is not arrested in the style, fertilization rates and selective abortion can limit the number of viable hybrid seeds that are formed (Carney et al., 1994). The frequent occurrence of fruits from inter-taxa pollen crossings with fertilized ovules but no filled seeds (contrast dark gray and black bars in Fig. 1-3) and the significant increases in ovule abortion in inter-taxa crosses (Table 1-2) suggest that in section *Xerocalyx* abortion of developing seeds is an important post-zygotic mechanism preventing hybrid formation. A similar pattern was observed in crosses between sympatric *Chamaecrista desvauxii* var. *graminea* H.S. Irwin & Barneby and *C. desvauxii* var. *latistipula* (Benth.) G.P.Lewis, in which inter-taxa pollen took longer to penetrate ovules and yielded high levels of fruit development but no seeds

(Costa et al., 2007).

All varieties in section *Xerocalyx* examined to date have 2*n* = 14 chromosomes (Irwin and Turner, 1960; Ormond et al., 1976; Ormond et al., 1977; Biondo et al., 2005; Biondo et al., 2006), and the same was true for the taxa included in this study. None of the varieties used as seed plants set seeds spontaneously, and all of them can be considered self-compatible, although in the case of var. *mollissima* some level of self incompatibility was indicated by the lower number of filled seeds per fruit and smaller seedling size in selfed plants when compared to cross-pollinated ones. Under these circumstances, differences in ploidy and self-incompatibility mechanisms cannot explain reproductive isolation in this group. However, it is common for several flowers to open on the same individual on any given day, thus geitonogamous pollen deposition may further reduce the likelihood of ovule fertilization by foreign pollen.

In contrast to the other taxa, var. 1, which was only used as pollen donor, was found previously to form fruits without pollinator visitation (eight out of nine bagged, untreated flowers yielded fruits). In addition to the small flower size, the number of stamens is reduced, anthers are located immediately above pistils, and visits to flowers are rarely observed. Those observations suggest that this variety may have a predominantly selfing reproductive system (Coyne and Orr, 2004), and could potentially be isolated by its mating system (Gottlieb, 1973; Levin, 1978). More details on the reproductive biology of this variety are necessary to address this question.

Asymmetries in seed siring success are common and may be caused by differences in style length, mechanisms involved in self-incompatibility, pollen competition, differential fruit abortion, nuclear-cytoplasmic and triploid endoplasm interactions (Tiffin et al., 2001; Turelli and Moyle, 2007). In the present study, differences in style length partially explain the low fruit and seed set observed when short-style taxa were used as pollen donors for long-styled ones, but incompatibility in the reciprocal crosses and in crosses between similar-sized flowers are also caused by post-zygotic processes that result in differential fruit and seed abortion. Nuclear-cytoplasmic interactions or dysfunctional interactions between the male and female genetic components within the triploid endosperm (Tiffin et al., 2001; Turelli and Moyle, 2007) are the most likely explanations for the observed asymmetries in the success of reciprocal reproductive crosses between vars. *mollissima* and *brevipes*, both long-styled taxa, and may be responsible for the reduced seed set in the remaining crosses.

This study demonstrated the occurrence of strong postmating reproductive isolation among sympatric and allopatric varieties of *Chamaecrista* section *Xerocalyx*. The results from the present work and from Costa et al. (2007) suggest that section *Xerocalyx* includes more species than recognized by Irwin and Barneby in their 1982 revision, and that various isolating mechanisms may act in concert to maintain morphological diversity in this group. Population sizes of all varieties were highly variable over the course of three years of field work, ranging from a few individuals several meters apart to densely populated patches including 3 or 4 individuals per square meter. Both in CCPIU and in Panga, the densest populations of all varieties over the period of four years were observed in the rainy season immediately following a fire. Because these plants have no underground regeneration structures, these population explosions are best explained by regeneration from the seed bank, with dormancy broken by fire-related cues; heat-shock frequently triggers germination in legumes, and extended periods of warm temperatures explain the higher frequency of *C. desvauxii* individuals on the edge of dirt roads and in similarly open areas in years with low population density (Keeley, 2000). While the postmating mechanisms detected in the present study are likely to maintain reproductive isolation between syntopic varieties, site-to-site differences in the timing of fire events could act as a diversification mechanism by decoupling local increases in flowering intensity from the regional climate. This would reduce pollen flow between sites when population densities peak in different years, allowing these populations to accumulate differences that can result in incongruence and reproductive isolation. This could be the case of var. *brevipes* in CCPIU and var. *mollissima* in Panga, which are similar in morphology but are only partially compatible. This scenario is also in line with Irwin and Barneby's suggestion (1982) that var. *brevipes* comprises several populations of var. *mollissima* that independently acquired shorter pedicels. Future studies should further expand the number of varieties examined and incorporate genetic data to quantify gene flow and reconstruct phylogenetic relationships among the currently recognized taxa. Broadly, this study represents a vital first step in understanding the evolutionary mechanisms that shape section *Xerocalyx*, a group with considerable potential to become a model system to address the role of different isolating barriers on plant speciation.

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Table 1-1. Flower and leaf measurements for all varieties. Mean ± standard deviation (n = 5 for all varieties except for number of ovules in *ramosa*, where n = 1). All measurements in mm. Same letters indicate values that are not significantly different after a Tukey HSD test ($\alpha = 0.05$).

Variety	Geographic range	Distal leaflet length	EFN length	Pedicel length	Largest petal length	Largest petal width	Longest stamen length	Style length	Number of ovules
C. desvauxii var. *brevipes* (CCPIU)	Widespread	20.9 ± 3.2^{a}	1.32 ± 0.25^{a}	8.9 ± 2.5^{a}	14.9 ± 1.8^{a}	20.9 ± 2.6^{a}	11.1 ± 0.7^{a}	7.3 ± 1.2^{a}	18.0 ± 1.7^{a}
C. desvauxii var. *mollissima* (Panga)	Widespread	$18.5 \pm 4.8^{a,b}$	1.30 ± 0.23^{a}	15.1 ± 3.8^{b}	16.3 ± 1.5^{a}	26.8 ± 3.0^{b}	10.6 ± 1.0^{a}	7.3 ± 0.8^{a}	17.6 ± 1.1^{a}
C. desvauxii var. *modesta* (CCPIU)	Narrow	$18.9 \pm 3.3^{a,b}$	0.59 ± 0.17^{b}	3.3 ± 1.0^{c}	6.9 ± 0.4^{b}	$9.2 \pm 0.7^{c,d}$	$5.0 \pm 0.4^{b,c}$	3.1 ± 0.4^{b}	10.4 ± 0.5^{b}
C. desvauxii var. *modesta* (Panga)	Narrow	22.2 ± 1.4^{a}	0.68 ± 0.24^{b}	2.9 ± 1.4^{c}	8.2 ± 1.5^{b}	$12.2 \pm 2.2^{c,e}$	5.4 ± 0.8^{b}	$3.2 \pm 0.4^{b,c}$	11.0 ± 0.7^{b}
C. desvauxii var.1 (CCPIU)	Local	15.0 ± 0.8^{b}	0.41 ± 0.08^{b}	24.9 ± 4.2^{d}	7.1 ± 0.7^{b}	6.0 ± 2.1^{d}	3.6 ± 0.8^{c}	$3.3 \pm 0.5^{b,c}$	9.0 ± 1.6^{b}
C. ramosa var. *parvifoliola* (CCPIU)	Regional	7.1 ± 0.9^{c}	0.41 ± 0.09^{b}	15.8 ± 2.7^{b}	8.7 ± 0.7^{b}	15.3 ± 1.8^{e}	7.5 ± 0.6^{d}	4.4 ± 0.2^{c}	10
		$F_{5,24}=18.73$, P<0.001*	$F_{5,24}=24.24$, P<0.001*	$F_{5,24}=43.96$, P<0.001*	$F_{5,24}=56.75$, P<0.001*	$F_{5,24}=63.02$, P<0.001*	$F_{5,24}=84.18$, P<0.001*	$F_{5,24}=46.53$, P<0.001*	$F_{5,20}=49.14$, P<0.001*

Notes: see text for details on geographic range; EFN = extrafloral nectary

Table 1-2. Number of ovules, fertilized ovules, aborted ovules and filled seeds per fruit [mean ± standard deviation (sample size)]; asterisks indicate treatments in which numbers of fertilized or aborted ovules or number of filled seeds were significantly different when contrasted against numbers for cross-pollinated flowers of each variety, as indicated by negative binomial GLMs with number of ovules, number of fertilized ovules, and number of ovules , respectively. *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; • $P < 0.10$.

Variety	Treatment	Number of ovules	Number of fertilized ovules	Number of aborted ovules	Number of filled seeds (viable fruits only)
C. desvauxii var. *brevipes*	**Cross**	**18.1 ± 1.7 (19)**	**15.3 ± 3.0 (19)**	**6.2 ± 4.2 (19)**	**9.1 ± 5.5 (19)**
	Open	17.9 ± 1.6 (46)	13.1 ± 4.4 (46) *	6.4 ± 4.2 (46)	7.0 ± 4.1 (44) •
	Self	18.1 ± 1.2 (24)	14.1 ± 3.1 (24)	6.9 ± 2.9 (24)	7.9 ± 2.7 (22)
	Var. *mollissima*	17.8 ± 1.2 (8)	9.5 ± 3.0 (8) ***	6.6 ± 3.5 (8) *	4.6 ± 3.4 (5) *
	Var. *modesta*	18.3 ± 0.6 (3)	3.3 ± 1.5 (3) ***	3.3 ± 1.5 (3) *	(0)
	Var. 1	19.0 ± 0.0 (2)	3.5 ± 2.1 (2) ***	1.5 ± 2.1 (2)	2.0 ± 0.0 (2) **
	C. ramosa	16.2 ± 1.8 (6)	5.2 ± 2.9 (6) ***	3.0 ± 1.4 (6)	3.2 ± 2.6 (4) **
C. desvauxii var. *mollissima*	**Cross**	**18.4 ± 1.7 (23)**	**14.5 ± 3.9 (23)**	**5.7 ± 4.8 (23)**	**10.6 ± 4.4 (19)**
	Open	19.0 ± 2.9 (22)	12.8 ± 5.5 (22) •	7.5 ± 5.8 (22) *	5.8 ± 4.9 (19) **
	Self	18.7 ± 3.4 (26)	12.7 ± 5.5 (26) •	10.7 ± 5.8 (26) ***	3.5 ± 2.8 (15) ***
	Var. *brevipes*	18.2 ± 2.6 (24)	12.8 ± 4.3 (24)	6.9 ± 4.6 (24) •	7.5 ± 4.5 (19) •
	Var. *modesta*	17.9 ± 2.4 (10)	3.8 ± 1.5 (10) ***	3.8 ± 1.5 (10) ***	(0)
C. desvauxii var. *modesta* (CCPIU)	**Cross**	**8.9 ± 1.0 (8)**	**7.6 ± 1.8 (8)**	**2.9 ± 3.1 (8)**	**5.4 ± 1.7 (7)**
	Open	9.4 ± 0.9 (23)	8.1 ± 1.6 (23)	4.7 ± 3.3 (23)	3.9 ± 3.0 (18)
	Self	9.4 ± 1.2 (16)	8.4 ± 1.3 (16)	3.2 ± 2.5 (16)	5.2 ± 2.8 (16)
	Var. *brevipes*	9 (1)	3 (1)	3 (1)	(0)
	Var. 1	9.4 ± 1.3 (5)	4.2 ± 2.6 (5) **	4.2 ± 2.6 (5) *	(0)
C. desvauxii var. *modesta* (Panga)	**Cross**	**10.9 ± 0.8 (15)**	**9.7 ± 1.2 (15)**	**6.3 ± 3.3 (15)**	**4.4 ± 3.1 (11)**
	Open	11.3 ± 0.9 (11)	7.7 ± 3.2 (11) *	5.3 ± 2.5 (11)	2.5 ± 1.3 (11)
	Self	11.5 ± 0.9 (15)	9.1 ± 2.7 (15)	5.3 ± 4.2 (15)	4.7 ± 2.9 (12)
	Var. *brevipes*	12.0 ± 1.4 (4)	4.5 ± 3.3 (4) ***	4.2 ± 2.9 (4)	1 (1)
	Var. *mollissima*	11.2 ± 0.6 (12)	8.4 ± 2.4 (12)	7.8 ± 2.4 (12) *	1.5 ± 0.7 (2) •

Table 1-3. Average seed weight ± standard deviation, percent germination rates and germination speed index (GSI) for each treatment. Sample sizes in parentheses. Asterisks indicate significant differences in seed weight or number of germinated seeds compared to cross-pollen plants, using linear contrasts or pair-wise Fisher's exact tests, respectively.

Variety	Treatment	Seed weight (mg)	% germination	GSI[a]
C. desvauxii var. *brevipes*	**Cross**	**7.4 ± 2.2 (30)**	**80**	**13.4**
	Open	8.6 ± 1.5 (30)*	100*	14.0
	Self	8.0 ± 1.6 (30)	87	10.7
	Var. *mollissima*	5.8 ± 1.5 (22)*	59	3.0‡
	Var. *modesta*	no seeds	-	-
	Var. 1	7.8 ± 2.2 (4)	100	1.6‡
	C. ramosa	7.8 ± 1.6 (12)	92	6.5‡
		$F_{5,124}=7.5$, P<0.001*		
C. desvauxii var. *mollissima*	**Cross**	**5.1 ± 1.8 (30)**	**80**	**6.6**
	Open	5.2 ± 1.8 (28)	93	6.0‡
	Self	5.1 ± 1.6 (30)	93	4.1
	Var. *brevipes*	4.9 ± 1.5 (30)	90	5.4
	Var. *modesta*	no seeds	-	-
		$F_{3,115}=0.20$, P=0.894		
C.desvauxii var. *modesta* (CCPIU)	**Cross**	**8.5 ± 1.5 (30)**	**100**	**9.2**
	Open	8.0 ± 2.0 (30)	87	9.2
	Self	7.9 ± 2.3 (30)	90	8.8
	Var. *brevipes*	no seeds	-	-
	Var. 1	no seeds	-	-
		$F_{2,87}=0.76$, P=0.470		
C. desvauxii var. *modesta* (Panga)	**Cross**	**9.2 ± 3.0 (29)**	**62**	**2.1‡**
	Open	9.3 ± 2.9 (21)	100*	4.8‡
	Self	9.6 ± 1.8 (30)	87*	2.6
	Var. *brevipes*	? (1)	not tested[b]	-
	Var. *mollissima*	2.8 (1)	0	-
		$F_{2,81}=0.20$, P=0.818		

Notes: [a] GSI is proportional to the sample size; sample sizes lower than 30 are indicated by ‡; larger numbers indicate faster germination.

[b] the only filled seed obtained from this crossing was produced late in the season and was not included in the germination trial

Table 1-4. Cotyledon length and number of leaves, leaf and seedling size after 50 days for all treatments. Sample sizes in parentheses. Asterisks indicate significant differences ($P < 0.05$) in comparison to cross-pollinated plants using linear contrasts.

Variety	Treatment	Cotyledon length (mm)	Number of leaves	Proximal leaflet length (mm)	Distal leaflet length (mm)	Root length (mm)	Stem length (mm)
C. desvauxii var. *brevipes*	**Cross**	**13.8 ± 1.2 (14)**	**4.4 ± 0.5 (14)**	**13.6 ± 1.7 (14)**	**16.4 ± 2.4 (14)**	**104 ± 19 (14)**	**85 ± 16 (14)**
	Open	12.9 ± 1.4 (14)	4.9 ± 0.6 (14)	13.6 ± 0.9 (14)	16.3 ± 1.3 (14)	113 ± 19 (14)	93 ± 18 (14)
	Self	13.0 ± 1.4 (14)	4.7 ± 0.9 (14)	12.1 ± 2.4 (14)	15.1 ± 2.4 (14)	91 ± 29 (14)	82 ± 20 (14)
	Var. *mollissima*	11.0 ± 2.1 (5)*	3.3 ± 0.7 (9)*	12.3 ± 2.7 (9)	14.9 ± 3.4 (9)	102 ± 48 (9)	59 ± 22 (9)*
	Var. 1	13.0 ± 1.0 (3)	4.3 ± 1.0 (4)	11.8 ± 3.3 (4)	13.8 ± 3.4 (4)	102 ± 36 (4)	73 ± 38 (4)
	C. ramosa	12.7 ± 1.7 (11)	6.8 ± 1.1 (11)*	10.2 ± 1.0 (11)*	12.2 ± 2.3 (11)*	123 ± 35 (11)	117 ± 27 (11)*
		$F_{5,55}=2.70$, $P<0.030$*	$F_{5,60}=22.0$, $P<0.001$*	$F_{5,60}=5.10$, $P<0.001$*	$F_{5,60}=4.81$, $P<0.001$*	$F_{5,60}=1.72$, $P=0.14$	$F_{5,60}=7.35$, $P<0.001$*
C. desvauxii var. *mollissima*	**Cross**	**10.8 ± 1.3 (14)**	**3.9 ± 0.7 (14)**	**9.6 ± 1.2 (14)**	**12.4 ± 1.5 (14)**	**104 ± 27 (14)**	**55 ± 13 (14)**
	Open	11.3 ± 1.8 (14)	4.0 ± 0.8 (14)	9.1 ± 2.6 (14)	12.2 ± 1.2 (14)	102 ± 30 (14)	54 ± 14 (14)
	Self	10.1 ± 1.2 (8)	4.0 ± 0.7 (14)	7.4 ± 1.8 (14)*	9.5 ± 2.1 (14)*	73 ± 38 (14)*	43 ± 12 (14)*
	Var. *brevipes*	11.3 ± 1.3 (14)	5.3 ± 1.9 (13)*	9.8 ± 3.5 (13)	11.9 ± 3.4 (13)	114 ± 37 (14)	65 ± 13 (14)*
		$F_{3,46}=1.46$, $P=0.239$	$F_{3,51}=3.79$, $P=0.016$*	$F_{3,51}=2.76$, $P=0.051$	$F_{3,51}=5.52$, $P=0.002$*	$F_{3,51}=3.80$, $P=0.016$*	$F_{3,51}=7.43$, $P<0.001$*
C. desvauxii var. *modesta* (CCPIU)	**Cross**	**13.3 ± 0.9 (14)**	**5.9 ± 1.1 (14)**	**11.7 ± 1.1 (14)**	**15.4 ± 1.7 (14)**	**157 ± 45 (14)**	**85 ± 15 (14)**
	Open	13.5 ± 0.9 (14)	5.7 ± 0.7 (14)	11.9 ± 0.8 (14)	15.9 ± 0.9 (14)	142 ± 38 (14)	84 ± 15 (14)
	Self	13.8 ± 0.9 (14)	6.4 ± 0.7 (13)	12.0 ± 1.0 (13)	15.9 ± 1.1 (13)	175 ± 35 (13)	101 ± 31 (13)
		$F_{2,39}=0.94$, $P=0.398$	$F_{2,38}=2.29$, $P=0.115$	$F_{2,38}=0.50$, $P=0.610$	$F_{2,38}=0.55$, $P=0.580$	$F_{2,38}=2.30$, $P=0.110$	$F_{2,38}=2.67$, $P=0.082$
C. desvauxii var. *modesta* (CCPIU)	**Cross**	**14.3 ± 0.8 (11)**	**6.7 ± 0.5 (14)**	**12.2 ± 0.7 (14)**	**15.4 ± 1.1 (14)**	**134 ± 30 (14)**	**95 ± 10 (14)**
	Open	14.2 ± 1.2 (13)	6.3 ± 1.4 (14)	11.1 ± 1.9 (14)	14.5 ± 2.6 (14)	130 ± 24 (14)	85 ± 21 (14)
	Self	13.7 ± 1.8 (14)	6.5 ± 0.5 (14)	12.0 ± 0.9 (14)	15.5 ± 1.2 (14)	132 ± 30 (14)	94 ± 11 (14)
		$F_{2,39}=0.67$, $P=0.519$	$F_{2,39}=0.76$, $P=0.477$	$F_{2,39}=2.82$, $P=0.072$	$F_{2,39}=1.50$, $P=0.240$	$F_{2,39}=0.06$, $P=0.940$	$F_{2,39}=1.91$, $P=0.160$

Table 1-5. Index of Crossability (McDade and Lundberg, 1982) for all treatments

	Pollen donor				
Pollen receiver	Var. *brevipes*	Var. *mollissima*	Var. *modesta*	Var. 1	*C. ramosa*
C. desvauxii var. *brevipes* (CCPIU)	-	0.089	0.000	0.030	0.136
C. desvauxii var. *mollissima* (Panga)	0.700	-	0.000	-	-
C. desvauxii var. *modesta* (CCPIU)	0.000	-	-	0.000	-
C. desvauxii var. *modesta* (Panga)	0.062[a]	0.000	-	-	-

Notes: [a] value calculated assuming a germination rate of 100%

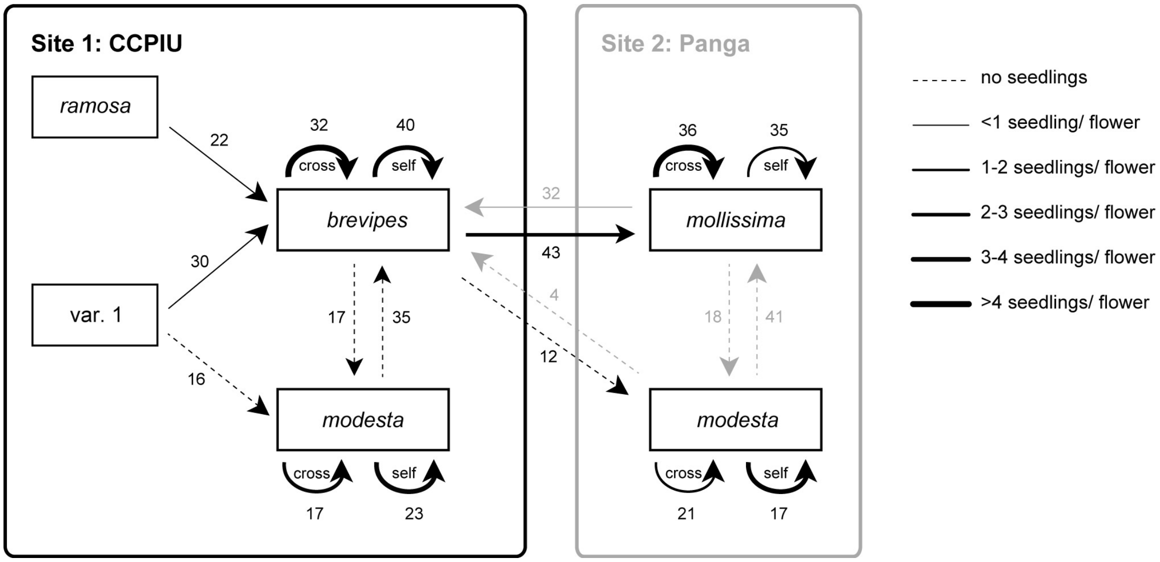

Figure 1-1. Schematic representation of the crosses carried out in this study. Non-represented treatments were automatic selfing (flowers bagged but not treated), and open flowers (no treatment). “CCPIU” and “Panga” refer to two field sites 21 km apart. Arrows point from pollen donors to pollen receivers (seed plants); arched arrows refer to self- and cross-pollination within varieties. Numbers refer to the number of hand-pollinated flowers. The thickness of the arrow is proportional to the number of seedlings obtained per treated flower; dashed arrows indicate crossings that produced no seedlings.

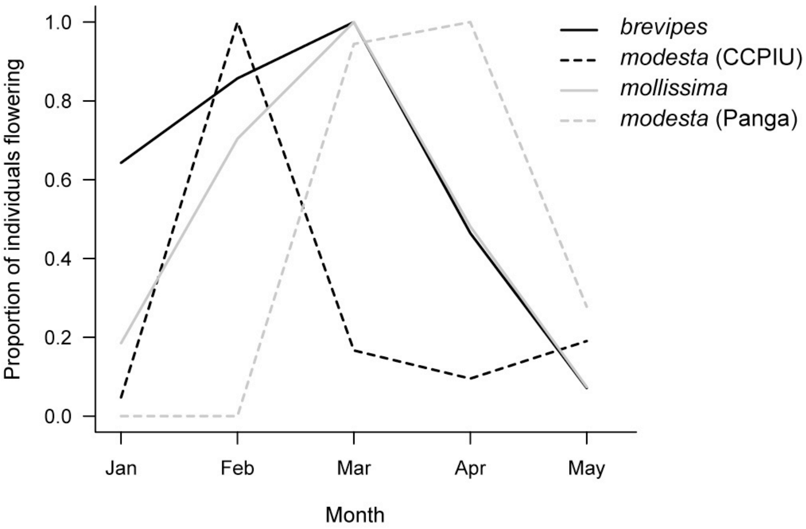

Figure 1-2. Flowering phenology of the varieties used as seed plants during the period of study (January to May 2007), calculated as number of individuals with flowers divided by total number of marked individuals.

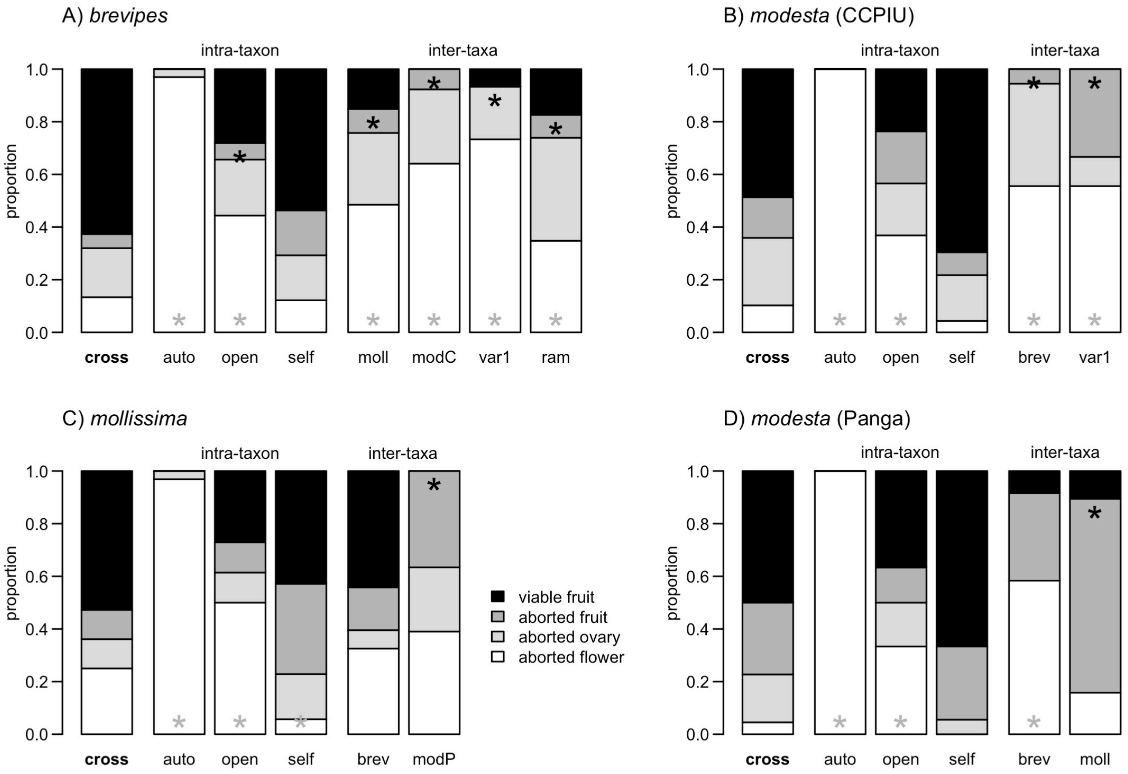

Figure 1-3. Flower fates of hand-pollinated and marked flowers of each variety. (A) *Chamaecrista desvauxii* var. *brevipes*; (B) *C. desvauxii* var. *modesta* in Caça & Pesca (CCPIU); (C) *C. desvauxii* var. *mollissima;* (D) *C. desvauxii* var. *modesta* in Panga. Flower fates were classified as follows: "aborted flowers" were aborted within 48 h since anthesis; "aborted ovaries" were retained by the plant past this period, but eventually aborted before any visible changes in size occurred; "aborted fruits" include both enlarged ovaries and fruits that reached maturity and dehisced, but had not viable seeds; "viable fruits" are fruits containing at least one viable (filled) seed. Treatments were as follows: "cross": pollen from different individuals of the same variety; "auto": bagged, untreated flowers; "open": non-bagged, marked flowers; "self": pollen removed from and applied to the same flower; "moll": pollen from *C. desvauxii* var. *mollissima*; "modC": pollen from var. *modesta* individuals from CCPIU; "var. 1": pollen from var. 1; "ram": pollen from *C. ramosa* var. *parvifoliola*; "brev": pollen from *C. desvauxii* var. *brevipes*; "modP": pollen from var. *modesta* individuals from Panga. Number of marked/treated flowers as in Fig. 1-1. Significant pairwise differences ($P < 0.05$) in abortion between the intra-taxon cross treatment and each of the other treatments are indicated by gray asterisks (flower abortion, white bars) and black asterisks (ovary and fruit abortion, gray bars), both according to Fisher's exact tests.

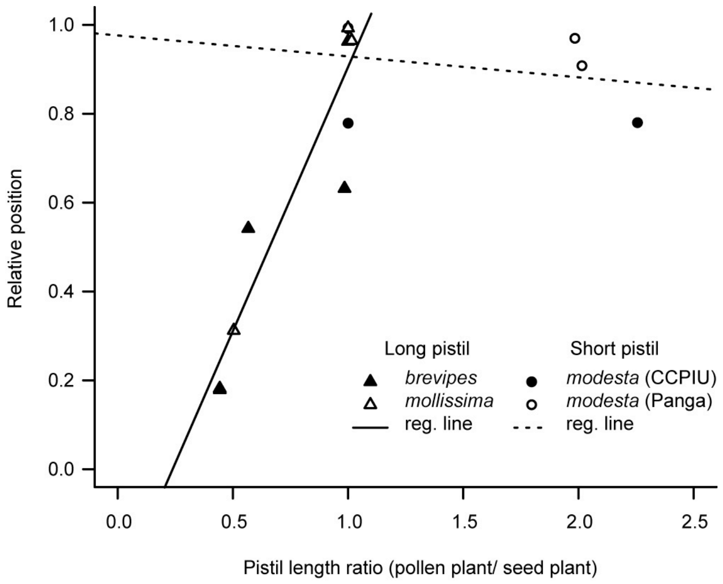

Figure 1-4. Correlation between the relative position of the most basal ovule reached by the pollen tube and the style length ratio.

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Chapter 2. Context-dependent benefits from ant-plant mutualism in three sympatric varieties of *Chamaecrista desvauxii* (Leguminosae)

Abstract

Mutualistic interactions are characterised by outcomes that depend on both the biotic and the abiotic context. However, limited information is available on the factors that affect the strength of ant-plant mutualisms among sympatric congeneric species. We compared the benefits gained from attracting ants via extrafloral nectaries in lowering herbivory and increasing seed set of three co-occurring varieties in the of *Chamaecrista desvauxii* complex (Leguminosae) in a cerrado area in Uberlândia, Brazil. Using whole-individual exclusion experiments, we tested the hypotheses (1) that the relative strength of those benefits is higher in the variety with the largest extrafloral nectaries, and (2) that those benefits are conditional on the presence of pre-dispersal seed predators. Extrafloral nectaries are larger, produce more nectar and attract more ants in var. *brevipes* than in the other two varieties included in the study. Var. *modesta* has intermediate-sized nectaries, while var. 1 has small nectaries, and both attract relatively few ants. For var. *brevipes*, ant exclusion significantly increased levels of folivory and attack to fruits by sucking insects, decreasing the relative number of flowers, fruits and seeds produced per individual. For the other two varieties, in contrast, ant effects were reduced, and ants did not significantly improve reproductive success. In addition, effects of ant exclusion were less pronounced or absent when seed predators were excluded from fruits of var. *brevipes*. We showed experimentally that benefits from interactions of three co-occurring varieties of *Chamaecrista desvauxii* with ants are context-dependent both within and among taxa. Variation in the strength of mutualism among sympatric taxa may potentially reinforce ecological reproductive isolation and contribute to diversification in this group.

Introduction

Numerous plant traits such as tissue toughness, trichomes and secondary compounds directly reduce the impact of herbivores (Coley, 1983; Harborne, 1993; Fernandes, 1994; Diniz et al., 1999; Peeters, 2002). Indirect defences, on the other hand, are based on the attraction, nourishment or housing of herbivore enemies (Heil, 2008). Examples include protection provided by ants attracted to plants by extrafloral nectar, domatia and/or food bodies (Bentley, 1977). Ants can increase plant fitness by preying on herbivores, chasing them away or simply by disrupting their behaviour (Schemske, 1980; Costa et al., 1992; Del-Claro et al., 1996; Freitas and Oliveira, 1996; de la Fuente and Marquis, 1999), ultimately increasing the number of flowers, fruits and seeds produced by an individual (Horvitz and Schemske, 1984; Del-Claro and Oliveira, 1996; Rudgers, 2004).

Benefits from mutualistic interactions with ants within plant species often depend on biotic and abiotic context: they have been shown to depend on the abundance of ants and herbivores (Rudgers and Strauss, 2004), the identity of the ants attracted to the plant (Miller, 2007; Palmer and Brody, 2007) and resource levels (de la Fuente and Marquis, 1999; Kersch and Fonseca, 2005). This is especially true for facultative mutualisms, that is, when ants are attracted by extrafloral nectar or food bodies but do not nest on the plant (Heil, 2008; Bronstein, 2009); these associations are looser and absent mutualists can be easily replaced by alternative species (Bronstein, 1994). In addition, benefits from ant-plant mutualisms are likely to be a function of the abundance of the partners (Barton, 1986), in contrast to other mutualistic interactions where one individual can fulfil the reward or service required (Bronstein, 1994). Nevertheless, a recent meta-analysis by Chamberlain and Holland (2009) found that ant effects are consistently positive, or at most neutral, regardless of context.

When studied, congeners have been shown to vary in benefits received from ant protectors. *Tococa guianensis*, for example, has a much higher increase in herbivory after ant exclusion than *T. coronata* or *T. macrosperma* (Michelangeli, 2003). In *Macaranga*, ant-protection is more intense in obligately ant-associated species than in species that are facultatively associated with ants (Fiala et al., 1994; Heil et al., 2001). For *Macaranga* myrmecophytic species, ant exclusion led not only to increased leaf removal, but also to increased mortality (Heil et al., 2001). In this and other systems, differences in benefits from ant association may be related to interspecific differences in production of domatia, food bodies and extrafloral nectar. For instance, the relative investment into growth and production of rewards (pearl bodies and Müllerian bodies) is variable across myrmecophytic *Cecropia* species (Folgarait and Davidson, 1994, 1995).

To our knowledge, no studies have addressed how the strength of ant-plant interactions varies among taxa that have facultative interactions with ants. We might expect such variation because co-occurring, closely related species usually vary in the level of extrafloral nectar expression (Koptur, 1984; Blüthgen et al., 2004). For instance, there is great variation among species in the cotton tribe (Gossypieae) in the size and number of extrafloral nectaries (Rudgers et al., 2004). Given that extrafloral nectar production can be a heritable trait (Rutter and Rausher, 2004), loss or decrease in nectar production may be related to selective pressures stemming from the costs of producing nectar, while relatively high levels of nectar activity suggest that associations with ants are beneficial for the fitness of those plants. In this study, we address the question of context-dependency in the outcome of ant-plant mutualisms by quantifying the relative strength of ants as a defensive mechanism in three co-occurring varieties of *Chamaecrista desvauxii* (Leguminosae). Although all varieties have extrafloral nectaries, their sizes are consistently different, and different levels of ant activity on these species are evident in the field. We examined both inter- and intra-taxon context dependency. First, we predicted that benefits from ant attraction would depend on the variety (inter-taxon context), being highest in the variety with the largest nectaries, and lower or negligible in the other two varieties. Second, we predicted that the outcome of the ant-plant mutualism within each variety would be more substantial in the presence of predispersal seed predators (intra-taxon context), since seed predators have more direct impacts on plant reproduction than do leaf herbivores.

Materials and Methods

Study site–The work was conducted at the 130-ha cerrado reserve of Clube Caça & Pesca Itororó in Uberlândia, Brazil (18º55’ S, 48º17’ W). This reserve includes areas of seasonally flooded grasslands (vereda), open savanna areas with scattered trees (campo cerrado) and areas dominated by trees and shrubs (cerrado *sensu stricto*; see Oliveira-

Filho and Ratter, 2002). Temperatures are constant throughout the year, but rainfall is concentrated in the wet season, between the months of October and April.

Plant species and varieties– Section *Xerocalyx* of genus *Chamaecrista* is a Neotropical taxon that includes three species and several varieties distributed from Argentina to Mexico, except for the West Indies. Although the section is monophyletic, phylogenetic relationships among species and varieties within this section are not resolved (Conceição et al., 2009), and the number of recognised species has fluctuated from 16 to three over the years (Irwin, 1964; Irwin and Barneby, 1982). The focus of this paper, *C. desvauxii* (Coll.) Killip, is currently subdivided into 17 varieties. All varieties are short-lived perennials with relatively macrophyllous leaves, each with four leaflets and one extrafloral nectary on the petiole (Irwin and Barneby, 1982). In some varieties, an additional, smaller extrafloral nectary may occur on the rachis, between the two pairs of leaflets (BB, pers. obs.). The flowers have no nectar but produce large amounts of pollen, which is harvested by bees through vibrational movements (Gottsberger and Silberbauer-Gottsberger, 1988; Costa et al., 2007). The pods open explosively to disperse the seeds. More than one variety may be found in the same area, often side by side; co-occurring varieties may have reproductive isolating mechanisms that prevent hybridisation (Costa et al., 2007; Baker and Marquis in prep)

Three sympatric varieties of the *C. desvauxii* complex were included in the present study. Although a given variety may be more common in some portion of the study site, there were no clear differences in their distribution, and they often occurred syntopically. *C. desvauxii* var. *brevipes* (Benth.) Irwin & Barneby is a subshrub found in savanna areas from Honduras to Central Brazil and Paraguay. Its flowers are large and supported by relatively short pedicels. Although pilosity is variable throughout the range of this variety, in CCPIU the relatively broad leaflets are covered with short, soft trichomes. *C. desvauxii* var. *modesta* Irwin & Barneby is restricted to Central Brazil, and is characterised by a more erect habit, the narrow shape of its leaflets and the reduced size of its flowers. In CCPIU, the flowers have extremely short pedicels. The third variety, *C. desvauxii* var. 1, has not been described, and has only been found in one additional cerrado reserve 10 km from CCPIU (Fazenda Água Limpa, 19º5' S, 48º21' W), despite the fact that it is locally abundant. In addition, no specimens resembling this variety were found after inspection of relevant herbarium material at GH, MO, NY, UB and US. Therefore, this variety appears to be an extremely localised taxon; it has a prostrate habit, glabrous leaflets that resemble *C. desvauxii* var. *brevipes*, and small flowers supported by a long pedicel. The three varieties are primarily annuals that germinate at the beginning of the wet season (November-December) and finish fruiting early in the dry season (May), but some individuals are able survive the dry season and flower during the following wet season, and scattered individuals of var. 1 can be found flowering throughout the year.

Extrafloral nectar production–In March of 2007, five leaves were collected from each of six individuals per variety. Length and width of the extrafloral nectary on each leaf were measured using a dissecting scope. Because the shape of the top of the extrafloral nectary in all varieties is either elliptical or round, the formula [area = (length • width • π)/4] was used to estimated its area. Measurements were averaged per individual and differences among varieties were tested using an ANOVA.

Extrafloral nectar production was measured in February 2005 on seven individuals of *C. desvauxii* var. *brevipes*, seven of var. *modesta* and five of var. 1. Three hours prior to the measurement, one branch of each individual was bagged to prevent visitation to the EFNs and evaporation of the nectar. After this period the bag was removed and the number of leaves with visible nectar secretion was recorded, along with the total number of bagged leaves. The amount of nectar secreted was measured by touching a piece of chromatography paper to each nectary. The area covered by the nectar is proportional to the volume produced, which can be obtained by using the formula in Baker (1979). This method adequately measures low volumes of nectar. Measurements were carried out simultaneously for the three varieties in early afternoon (from 12 to 16 hrs) and at the beginning of the night (from 19 to 23 hrs).

EFN removal and seed predator exclusion–A factorial design was used to test the role of EFNs as one factor and the impact of pre-dispersal seed predators as the other on the reproductive success of each variety. Each treatment was randomly assigned in January 2006 to half of the individuals in each variety; more individuals were added to the experiment in March 2006, totalling 24 and 4 individuals of *C. desvauxii* var. *brevipes* marked in January and March, respectively, 24 and 12 of var. *modesta*, and 20 and 12 of var. 1. The disparity in the number of treated individuals was a consequence of the differences in size and abundance of each taxon. Marked individuals were scattered throughout the field site. Each variety had a different distribution within the field site, which prevented us from marking individuals of all varieties in the same area. Individuals that were syntopic were included whenever possible. Due to differences in distribution, relative size, phenology and sample size, responses of each variety were analysed separately, except for the ant attendance data.

EFN removal was achieved by cutting extrafloral nectaries from all leaves of the treated individuals using a hypodermic needle. This method allowed the exclusion of all arthropods attracted by the extrafloral nectaries, while allowing crawling herbivores to reach the plant (Heil, 2008). After the initial treatment was applied (between Jan 31 and Feb 5), plants were visited weekly to remove EFNs from newly produced leaves, while nectaries on leaves in the control plants were pricked briefly with the needle. Seed predators were excluded by bagging each developing fruit from treated individuals with mesh bags. Bags were added as fruits matured, and plants were visited weekly to ensure that no developing fruits were unprotected. Each fruit of control individuals was bagged briefly at the beginning of fruit development; in addition, as the fruits approached ripeness they were bagged to allow seed collection. By applying exclusion treatments at the whole-plant level, we avoided inflating our estimates of ant and seed predator effects, which tend to be higher when paired branches on the same plant are compared (Chamberlain and Holland, 2009).

At the end of February individuals of var. *brevipes* began to show an abnormal flower bud development. Initially the ovary of these buds elongated, resulting in the protrusion of the style through the top of the corolla. After a couple of weeks, the corolla and stamens of those buds fell, revealing an elongated, non-fertilised ovary narrower than developing fruits. These flower buds never opened and did not produce pollen. In some cases, after several weeks the ovary split open longitudinally and generated a new vegetative branch. In these cases, the first leaf would often have six or more leaflets (as opposed to the four leaflets that are the norm for the species), while subsequent leaves were indistinguishable from the regular leaves of var. *brevipes*. After the onset of this abnormal development, all subsequent flower buds produced by a given branch would present the same condition, which eventually spread to the whole individual and effectively terminated reproduction until the individual died. This abnormal floral bud development was also observed for var. 1 in Fazenda Água Limpa (19º5' S, 48º21' W) in 2008 and for *C. desvauxii* var. *mollissima* in the Panga Ecological Reserve (19º11' S, 48º24' W) in 2007, both in Uberlândia, Brazil. A similar pattern on was observed in the following herbarium collections, all collected in Brazil: in *C. desvauxii* var. *mollissima*: Santos 1636 (MO), 10-III-1969 and Irwin 16236 (MO, UB, GH), 29-V-1966, both collected in Rio Turvo, c. 200 km N of Xavantina, Mato Grosso; Irwin 16668 (UB), 6-VI-1966, 60 km N of Xavantina, Mato Grosso; in *Chamaecrista diphylla*: Rizzo 7 (UB), 5-III-1966, 4 km from Aparecida de Goiás, Goiás; in *Chamaecrista ramosa* var. *parvifoliola*: Eiten 10906 (UB), 10-III-1969, Serra do Cipó, Minas Gerais. The cause of this abnormal development was not determined, but its occurrence was not affected by the exclusion treatments (Fisher's Exact Test, P = 0.551). In total, 13 out of 28 marked individuals were affected, but only in four of them were more than ten percent of the flowers buds abnormal. For the statistical analyses, we excluded reproductive structures that had this abnormal development, and excluded the four individuals with more than 10 % of damaged buds (two controls, one ant-excluded, one ant- and seed-predator-excluded).

Ant attendance–Ant attendance to EFNs was measured by counting the number of ants on all marked individuals, both during the day and at night. Ant attendance was measured in March and April 2006 at four different periods of the day: early morning, between 6 and 7 hrs; midday, between 12 and 13 hrs; afternoon, between 18 and 19 hrs; and night, between 0 and 1 hr. All varieties were sampled during the same time period. In addition, ants were collected on each variety at various times of day throughout the field season for identification. Because of differences in the identity and size of ants among individuals, only presence or absence data were used to establish the success of the ant exclusion treatment and differences in ant attendance among varieties. Data from the four time periods were pooled and, due to the presence-absence nature of these data, differences among varieties and between ant treatments were tested using a generalised linear model with a binomial family and logit link, followed by contrasts among varieties.

Vegetative growth and damage to leaves–We counted the initial number of leaves and leaf scars of each individual as a measure of size. Ten weeks later, the number of leaves and leaf scars was recounted, and vegetative growth was calculated as the ratio between the size after ten weeks and the initial size. A Welch t-test was used to test for differences in growth between ant-excluded and control plants, to account for differences in variance between groups.

In the second week of February 2006, five new leaves were marked on five or six individuals per treatment per variety. Thirty days later, damage to each of the four leaflets per leaf was ranked separately for missing leaf area and area attacked by pathogen. Rank values were 0 (no damage), 1 (up to 25% damage), 2 (between 25% and 50% damage), 3 (between 50% and 75% damage) and 4 (more than 75% damage). The values for each leaflet were summed to obtain a rank value for the whole leaf, and leaf ranks were averaged per individual for statistical analyses. Leaves on fallen or dead branches and dead individuals were excluded from the analyses. Differences in rank between ant-excluded and control individuals were tested using a non-parametric Wilcoxon rank sum test.

Reproductive success–The fate of each flower bud produced by all individuals was followed by mapping those buds through their positions on marked branches. Although more than one flower bud might be found at the same position, the buds almost never developed simultaneously. Approximately 10 days were necessary for the buds to develop into flowers, which opened early morning and wilted by midday. Unpollinated flowers were aborted after two days, but fruits took between a few days and several weeks to begin development. Fruit and seed maturation took one month to complete. Individuals were censused at least once a week throughout the reproductive season, and reproductive structures were classified as bud, flower, ovary (the period between anthesis and the beginning of fruit development), new fruit (enlarged ovary with soft valves) or developing fruit (after valves harden and seeds are filled). In addition, mature fruits were collected to count the number of filled, consumed and aborted seeds per pod; the presence of small punctures on the fruit valves was used as an indicator of attack by sucking insects, while external damage to a fruit valve, presence of a large exit hole on the valve or presence of frass or chewed seeds indicated attack by chewers. The effect of each treatment in the number of flowers and fruits was estimated using Poisson or negative binomial regressions with either number of flower buds or number of developing fruits as offsets, followed by likelihood ratio tests. These models were chosen due to the count nature of data and heteroscedasticity. A negative binomial was used when there was an indication of overdispersion in the Poisson regression. In cases where many individuals had no flowers, flower buds and/or fruits attacked by herbivores, hurdle regression models were used; the zero counts were modelled with a binomial logit and the positive counts with a negative binomial distribution, and the number of flower buds or developing fruits was used as an offset (Zuur et al., 2009). The number of fruits attacked by sucking and chewing insects were modelled separately. Differences in the total number of filled seeds per individual were tested using the maximum potential number of seeds (i.e., the total number of ovules in developing fruits, directly counted or estimated) as an offset in a negative binomial regression. Two individuals of var. 1 in the ant-exclusion treatment produced no developing fruits and were excluded from the analyses involving fruits.

Filled seeds were assumed to be viable, but a seed germination experiment with up to 30 seeds per individual, depending on availability, was also carried out. Seeds were weighed and scarified and placed in Petri dishes with filter paper under a 12:12 light cycle for 30 days. Seeds were observed daily for radicle emergence, and germinated seeds were removed from the dishes. Differences in seed weight among treatments were tested using ANOVAs, while differences in the number of germinated seeds were tested using negative binomial regressions with the total number of tested seeds as offset.

The effect sizes of ants on plant performance (number of flowers, fruits and seeds) in the presence or absence of seed predators were calculated for each variety using a ln-transformed response ratio calculated as $L = \ln(E(C)/E(E))$, where E(C) are expected values for control (ant present) plants, and E(E) are expected values for ant-excluded plants. This calculation follows Chamberlain and Holland (2009), so that positive values indicate positive ant effects. All statistical analyses were conducted in R 2.10.1 (R Development Core Team, 2009), using packages stats, MASS, multcomp and pscl.

Results

Extrafloral nectar production and ant attendance–*C. desvauxii* var. *brevipes* had the largest extrafloral nectaries (3.57 ± 0.38 mm^2, $F_{2,15} = 75.45$, $P < 0.001$), while var.

modesta has nectaries of intermediate size (1.27 ± 0.18 mm^2) and var.1 had the smallest nectaries (0.54 ± 0.04 mm^2). Accordingly, nectar production was higher for *C. desvauxii* var. *brevipes* (0.073 ± 0.077 $\mu l \bullet h^{-1}$) than for the other varieties (var. *modesta*: 0.013 ± 0.017 $\mu l \bullet h^{-1}$, var. 1: 0.005 ± 0.010 $\mu l \bullet h^{-1}$; $F_{33,2} = 8.34$, $P = 0.001$). Average nectar secretion was also 23% higher during the day than at night ($F_{33,1} = 4.69$, $P = 0.038$), but there was no interaction between time period and variety ($F_{33,2} = 0.17$, $P = 0.841$).

During the course of this study, four morphospecies of the genera *Camponotus*, *Pheidole*, *Pseudomyrmex* and *Linepithema* were found on individuals of var. 1, although no ants were observed feeding at extrafloral nectaries of this variety. Var. *modesta* was visited by a total of eight morphospecies, of the genera *Camponotus* (two morphospecies), *Pheidole* (two morphospecies, one of which was also found on var. 1), *Crematogaster* (two morphospecies), *Cephalotes* and *Brachymyrmex*. Var. *brevipes* was visited by all of the morphospecies found in the other two varieties, but also by larger-bodied ants, adding up to 28 morphospecies. These belonged to the genera *Camponotus* (10 morphospecies), *Pheidole* (three morphospecies), *Pseudomyrmex*, *Crematogaster* (four morphospecies), *Ectatomma* (two morphospecies), *Gnamptogenys*, *Odontomachus*, *Solenopsis*, *Linepithema*, *Brachymyrmex* (two morphospecies), *Cephalotes* and *Paratrechina*. Overall ant attendance was lower on individuals with cut EFNs (pooled data for all time periods, LRT [likelihood ratio test] = 18.27, $P < 0.001$; Fig. 2-1), and significantly higher on var. *brevipes* than on individuals of the other varieties (LRT = 23.03, $P < 0.001$).

Herbivory levels–In general, damage to leaves was relatively low, and 71% of the individuals had summed damage ranks equal or less than 4, corresponding to damage rates below 25%. Folivory levels were higher in ant-excluded individuals of var. *brevipes* (W = 14, P = 0.008). Mean herbivory rank to leaves of EFN removal plants was 1.65, while median rank for controls was 0.47. For the other varieties, the exclusion treatment had no effects on the folivory scores (var. *modesta*: W = 46, P = 0.335, ant excluded mean = 0.39, control = 0.40; var. 1: W = 43.5, P = 0.625, ant excluded mean = 2.18, control = 1.53). Levels of pathogen attack were not affected by ant exclusion in any variety (var. *brevipes*: W = 55.5, P = 0.915, ant excluded mean = 1.61, control = 2.25; var. *modesta*: W = 51.5, P = 0.560, ant excluded = 0.9, control = 1.0; var. 1: W = 43, P = 0.605, ant excluded = 1.8, control = 2.3). Despite the differences in folivory levels between ant-present and ant-excluded plants of var. *brevipes*, vegetative growth over two months was not different among treatments for var. *brevipes* (Welch's $t_{1,15}$ = 0.00, P = 0.501, ant excluded mean = 27.1 ± 2.0 %, control = 28.6 ± 1.8 %) nor var. 1 (Welch's $t_{1,13.3}$ = -0.15, P = 0.558, ant excluded = 31.1 ± 2.5 %, control = 29.8 ± 1.3 %), but ant-excluded individuals of var. *modesta* tended to grow less than ant-attended ones (3.9 ± 0.1 % vs. 9.7 ± 1.2 %, respectively; Welch's $t_{1,13}$ = 1.74, P = 0.053).

Reproductive success–There were no differences among treatments in rates of herbivore damage to flower and flower buds for any of the varieties (all P > 0.14; Fig. 2-2a). Fruits of ant-excluded individuals of var. *brevipes* suffered more damage from sucking insects (LRT [likelihood ratio test] = 4.90, P = 0.027; Fig. 2-2b), but ants did not reduce damage from chewers (LRT = 0.10, P = 0.752; Fig. 2-2c). Seed predator exclusion significantly reduced attacks by sucking insects to fruits of var. 1 (LRT = 9.62, P = 0.002; Fig. 2-2b) and by chewers to fruits var. *modesta* (LRT = 5.59, P = 0.018) and var.1 (LRT = 7.07, P = 0.008), but the effect was only marginal on var. *brevipes* (LRT = 3.16, P =

0.076; Fig. 2-2c). Ant exclusion and the interaction between ant and seed predator treatments also had marginal effects on chewer damage on var. 1 (ant LRT = 3.07, P = 0.080; interaction LRT = 3.30, P = 0.069; Fig. 2-2c).

The number of open flowers relative to the number of initiated flower buds was significantly affected by the ant exclusion treatment in *brevipes* and *modesta*. While ant-excluded *brevipes* individuals had a smaller percentage of flower buds that opened than controls (LRT 7.82, P = 0.005, 60.8 % ± 3.3 % (largest standard deviation) for ant-excluded vs. 75.5 % ± 3.9 % for controls), more flower buds opened on ant-excluded *modesta* individuals (LRT = 4.47, P = 0.034, 49.4 % ± 4.0 % for ant-excluded vs. 38.9 % ± 3.3 % for controls). Ant exclusion did not affect flower opening in var. 1 (LRT = 0.21, P = 0.647, 62.6 % ± 4.4 % for ant-excluded vs. 59.3 % ± 4.6 % for controls), nor did other treatment combinations affect flower production in any of the varieties (P values > 0.12), although seed predator exclusion did have marginal effects on flower production for var. 1 (LRT = 2.81, P = 0.094, 69.7 % ± 4.9 % for seed-predator-excluded).

Ant exclusion significantly reduced fruit set in *brevipes*, but only in the presence of seed predators (ant LRT = 9.45, P = 0.002; interaction LRT = 7.19, P = 0.007, Fig. 2-3a, Table 2-2). For *modesta*, only seed predator exclusion had a significant effect on fruit set (LRT = 19.7, P < 0.001), while for var. 1 none of the treatments affected fruit set (P > 0.21). Similarly, ant exclusion reduced the total number of filled seeds in var. *brevipes* (LRT = 11.18, P < 0.001), but the effect was diminished when seed predators were excluded (interaction LRT = 6.10, P = 0.014). Seed predator exclusion also increased the total number of seeds in var. *modesta* (LRT = 6.03, P = 0.014), but had no significant effect on seed set of var. 1 (LRT = 1.42, P = 0.230).

There were no differences in seed weight among treatments for any of the varieties (Table 2-1; $F_{3,17} = 2.23$, P = 0.122; $F_{3,23} = 0.76$, P = 0.528; $F_{3,23} = 1.47$, P = 0.249). Fewer filled seeds of var. *brevipes* from ant-excluded, unbagged individuals germinated when compared to seeds from other treatments (Table 2-1; ant excl. LRT = 6.16, P = 0.013, interaction LRT = 4.27, P = 0.039), but for the other varieties there were no significant differences among treatments (all probabilities > 0.224).

Discussion

Our results show that the effectiveness of ants as a defence against herbivores in the *Chamaecrista desvauxii* complex depends both on the variety considered and on the presence of seed predators, as determined by the experimental exclusion. The three varieties included in the present study are sympatric and are frequently found in the same areas within the study site. Generally speaking, they are exposed to the same abiotic and biotic conditions. Differences among varieties in the size of their extrafloral nectaries and amount of nectar produced, however, resulted in a gradient of ant attractiveness. Although nectar quality was not determined in the present study, populations of the North American congeneric *Chamaecrista fasciculata* that produce the highest volume of extrafloral nectar also had nectar with the highest amount of sugar (Rios et al., 2003). In addition, differences observed in the field in ant attendance among the three *C. desvauxii* varieties were consistent with differences in nectar quantity. Only the variety with the highest level of nectar production, var. *brevipes*, showed higher reproductive success in the presence of ants. However, this benefit was dependent on the presence of predispersal seed predators: when levels of seed predation were reduced through bagging, ants did not significantly increase seed set.

Our data indicate that the relationship between the varieties of *C. desvauxii* and ants is not specific, a result typical of facultative mutualisms (Bronstein et al., 2006; Chamberlain and Holland, 2009). The most common visitors belonged to ten morphospecies of ground-nesting ants in the genus *Camponotus* and six morphospecies of *Crematogaster*. Plants were visited opportunistically by ants in the vicinity, and the identity of the visiting ants varied over time for the same individual. This pattern is common for other cerrado plants that produce extrafloral nectar, and likely stems from differences in humidity and temperature preferences among ant species (Oliveira and Pie, 1998; Oliveira and Freitas, 2004). Despite considerable overlap in the identities of ants found on the three varieties of *C. desvauxii*, the large nectaries of var. *brevipes* attracted a more diverse set of ants, including larger-bodied predatory ants in the genera *Ectatomma*, *Gnamptogenys* and *Odontomachus* that may be more aggressive towards large insect herbivores (Davidson and McKey, 1993). In addition, different ant species are effective against different types of herbivores (Miller, 2007). Thus, attracting more than one ant species may further reduce overall herbivory rates, although that is not always the case, especially for obligatory ant-plant mutualisms (Chamberlain and Holland, 2009; Rosumek et al., 2009).

Excluding ants by cutting the extrafloral nectary was generally effective. Although ant presence was detected on some individuals of var. *brevipes* in the ant exclusion treatment, this was largely due to the occurrence of several small-bodied *Crematogaster* individuals tending the nectaries of newly expanded leaves. Censuses were conducted before these nectaries were removed. In those cases, however, ant activity was restricted to the tips of the branches, and all activity ceased after the new nectaries were removed.

In addition, no larger-bodied ants were observed in any ant-excluded individuals. The success of bagging fruits to prevent seed predation was lower for two reasons. First, the timing of bagging was constrained because young fruits are difficult to manipulate and are typically supported by a thin peduncle that can easily snap with the weight of a wet bag, and some fruits were attacked before the ovary was expanded enough to allow bagging. Second, sucking insects were often able to attack fruits through the holes in the material of the bag, thus seed predator exclusion was mostly restricted to chewing insects.

As predicted based on extrafloral nectary size, extrafloral nectar secretion and ant attendance, the effect of ant exclusion was most pronounced in var. *brevipes*, for which the removal of nectaries resulted in higher folivory rates, higher damage to fruits by sucking insects, fewer flowers, fruits and filled seeds and lower germination rates per individual. In contrast, ant exclusion led to slightly lower growth and reproductive output of var. *modesta* and chewer damage on var. 1. The percentage of flower buds that opened was affected by ant exclusion in both var. *brevipes* (decrease) and var. *modesta* (increase), despite no significant difference in florivory rates. While a decrease in vegetative growth with ant exclusion is expected if ants attack folivores, an increase in the number of flower buds that reached anthesis is unexpected, although the same (but non-significant) trend was found in a meta-analysis of ant-exclusion experiments (Rosumek et al., 2009). For var. *modesta*, it is possible that, as a consequence of eliminating nectar secretion through the removal of extrafloral nectaries, resources that would be used in nectar production were reallocated to flower production, and flower buds that would be aborted were retained until anthesis instead. If this scenario is correct, this variety may be more resource-limited than var. *brevipes*. Moreover, florivory in var. *brevipes* is likely to have been underestimated because oviposition by moths in small flower buds of this variety may cause early abortion, before any damage is detected. In any case, the difference in flower production between treatments did not translate into higher fruit or seed production in control plants of var. *modesta*.

Ants had a stronger effect on post-pollination components of reproductive success of var. *brevipes* (fruit and seed set) than on flower production (Table 2-2). However, the effect of ants on fruit and seed set was mitigated by the exclusion of seed predators: when fruits were bagged to reduce seed predation, the benefits from attracting ants largely disappeared (contrast values for var. *brevipes* between the two columns on Table 2-2). The fruits of *C. desvauxii* can be attacked by sucking insects, which insert their probosces into developing seeds through the fruit valve, or by chewers, in this case either caterpillars that feed externally on the young, soft-valved fruits or caterpillars and weevil larvae that feed internally on the developing seeds. In the latter case, the developing larvae are sheltered from ants by the fruit valves, and attracting ants does little to reduce seed loss. Ants seem to be generally ineffective in reducing attack rates by internally feeding chewers (O'Dowd and Catchpole, 1983; Devall and Thien, 1989; Ruhren, 2003; Lach, 2007; Palmer and Brody, 2007), and may actually benefit seed predators by reducing parasitoid attack (Koptur and Lawton, 1988), although there are exceptions (e.g. Schemske, 1980). The protection provided by ants against herbivores thus depends on the type of attack: in var. *brevipes*, only fruit damage by sucking insects was effectively reduced by ant presence (Fig. 2-3a).

In obligate ant-plant mutualisms, in which ants nest in the associated plant, food rewards are not necessarily associated with structures that are more valuable for plant fitness, and ant distribution within the plant is often independent of those rewards (Heil, 2008). In these cases, ant-plant symbioses are re-assembled each generation, and ants may benefit more from protecting vegetative parts of the plants, which contribute to colony performance by increasing the amount of shelter and food, than by protecting reproductive parts, which have no direct impact on colony fitness unless there are extrafloral nectaries associated (Palmer and Brody, 2007). In facultative associations, in contrast, ants are usually more abundant where the food resources are more abundant (O'Dowd, 1979), and investment in extrafloral nectar secretion has shown to be adjusted according to the reproductive state of the individual (Koptur and Lawton, 1988; Miller, 2007), increasing ant activity around more valuable plant parts (i.e., fruits, Holland et al., 2009). Indeed, floral nectaries may stay active after the corolla falls, attracting ants that reduce seed predation (Keeler, 1981). Accordingly, reproductive individuals of var. *brevipes* have higher nectar production than non-reproductive individuals of similar sizes (0.090 μl h^{-1} ± 0.065 vs. 0.024 μl h^{-1} ± 0.051, respectively; unpublished data). As an extension, it is possible that extrafloral nectar production is variable among other ontogenetic stages in all varieties, and that ant defence increases survivorship and/or early growth in seedlings of vars. *modesta* and var. 1. This possibility should be addressed in future studies.

While ants may provide an effective defence against herbivores for some varieties of *Chamaecrista desvauxii*, for others the benefit of attracting these animals is likely negligible. All varieties of *C. desvauxii* have one or two extrafloral nectaries on the leaf petiole, but the size of this structure is extremely variable among varieties and throughout this species' geographic range (pers. obs.). Extrafloral nectar production is likely to be costly (O'Dowd, 1979; Rutter and Rausher, 2004), thus the reduction or loss of extrafloral nectaries are likely to occur in areas where ant populations are reduced (Bentley, 1977; Rios et al., 2003). Although the varieties included in the present study are sympatric, populations of var. 1 are often more dense in open, grassy vereda areas prone to seasonal flooding, where the other varieties are rarely encountered. Given that ants attracted by the extrafloral nectaries are mostly ground nesters, mutualistic ant populations in those sites are likely to be small, providing a possible scenario for nectary loss in those individuals. The increased relevance of ant protection for reproductive success in the presence of seed predators in var. *brevipes* illustrates an alternative mechanism for nectary loss: in areas or years with low herbivory rates, decrease in nectary size or loss altogether would have a positive effect on fitness if nectar production is costly for the individual (Bronstein et al., 2006). In the congeneric *Chamaecrista fasciculata*, benefits from ant-plant associations are likewise absent in sites in Florida where either ants or herbivores are scarce (Barton, 1986). In Missouri populations of this species, both the total volume and the sugar content of extrafloral nectar are higher in sites where herbivore damage is higher, while density of hairs on the rachis is lower, suggesting a trade-off between those kinds of defences (Rios et al., 2003).

Our results support the hypothesis that the outcomes of ant-plant mutualisms in *C. desvauxii* are variable among taxa of the *C. desvauxii* complex and depends on the biotic context. If extrafloral nectar production is costly, locally isolated populations of *C. desvauxii* that receive low benefits from ant attraction may be selected for reduced extrafloral nectar production and increased investment in alternative defences. Hybrids between closely related taxa with differing defence strategies can have intermediate defence levels that lead to higher herbivory rates and lower reproductive success than any of the parents (Léotard et al., 2008), reinforcing reproductive isolation. Extrafloral nectaries have a unique origin within the genus *Chamaecrista* but were secondarily lost in two clades (Conceição et al., 2009), in one case being replaced by glandular setae, suggesting that this scenario for nectary loss may already have occurred within this genus.

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Table 2-1. Seed weight and % germination of each variety and treatment combination; mean for % germination were calculated using arcsine-transformed values and back-transformed, range is given in parentheses.

Variety	Treatment	Seed weight (mg)	% germination
Var. *brevipes*	Control	7.29 ± 0.85	81 (43 - 94)
	Ant excluded	5.45 ± 1.65	70 (28 - 100)
	Seed predator excluded	7.12 ± 1.53	84 (42 - 97)
	Ant and seed predator excluded	7.59 ± 0.53	92 (70 - 100)
Var. *modesta*	Control	6.12 ± 1.22	79 (40 - 100)
	Ant excluded	6.24 ± 1.85	64 (0 - 100)
	Seed predator excluded	6.94 ± 0.42	95 (74 - 100)
	Ant and seed predator excluded	6.62 ± 0.76	92 (52 - 100)
Var. 1	Control	4.30 ± 0.62	84 (14 - 100)
	Ant excluded	4.28 ± 1.39	66 (0 - 100)
	Seed predator excluded	4.95 ± 0.61	95 (57 - 100)
	Ant and seed predator excluded	4.98 ± 0.30	98 (81 - 100)

Table 2-2. Mean effect sizes of ants in the presence or absence of seed predators. Effect sizes were calculated as ln(E(C)/E(E)), where E(C) and E(E) are expected values of control (ant access) and experimental (ant exclusion) groups, following Chamberlain and Holland (2009). Positive values reflect positive effects of ants on plants. Asterisks indicate significant effects of ant exclusion (predator present column) or significant interactions (predator excluded column). *** P < 0.001; ** P < 0.01; * P < 0.05.

	Variety	Ant effect (pred. present)	Ant effect (pred. excluded)
Number of flowers	Var. *brevipes*	0.201 **	0.071
	Var. *modesta*	-0.180 *	0.012
	Var. 1	-0.044	0.160
Fruit set	Var. *brevipes*	1.119 ***	-0.161 ***
	Var. *modesta*	-0.440	0.143
	Var. 1	0.124	0.002
Seed set	Var. *brevipes*	1.542 *	0.030 *
	Var. *modesta*	0.262	0.352
	Var. 1	0.241	0.026

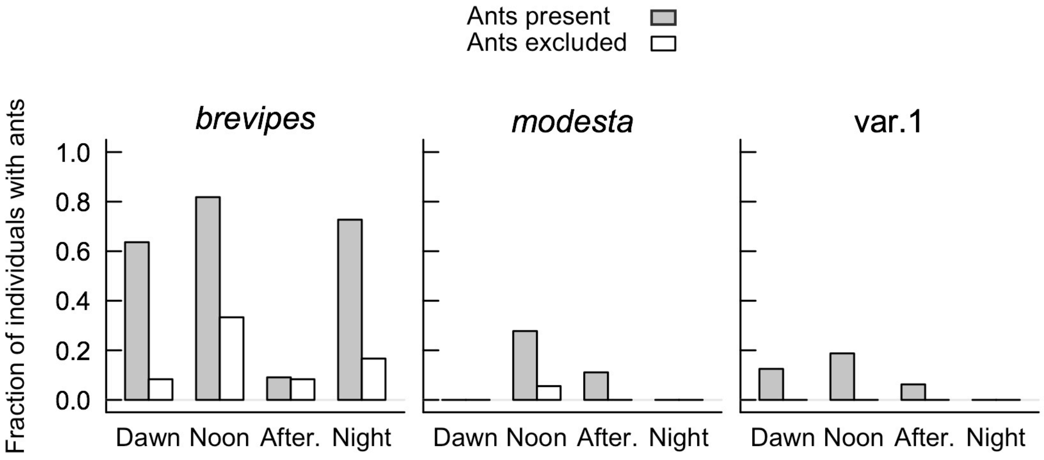

Figure 2-1. Proportion of individuals visited by ants in ant-excluded and control individuals of each variety, at four periods of the day (dawn, noon, afternoon and night).

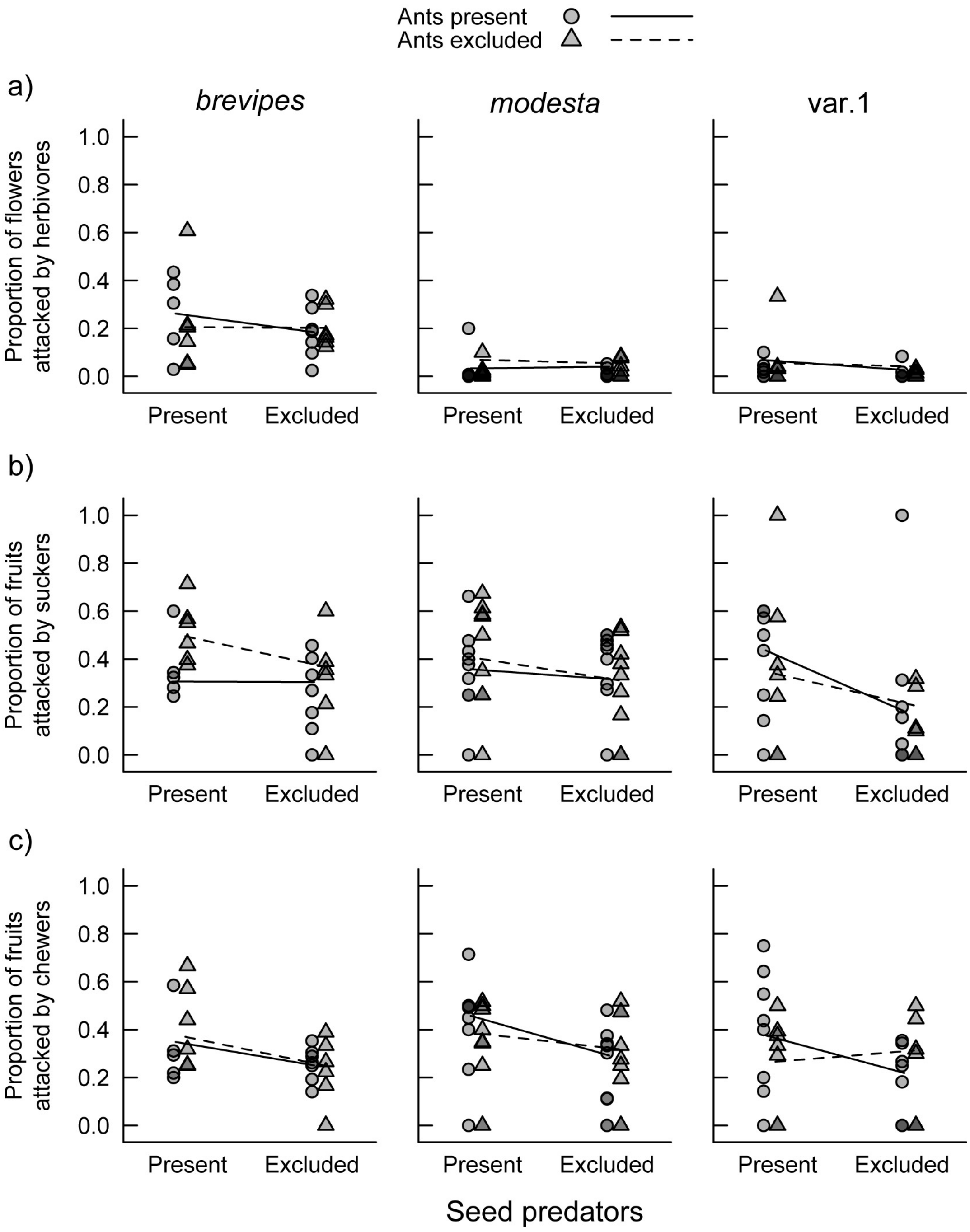

Figure 2-2. Number of flower buds, flowers and fruits attacked by herbivores relative to the total number of each, for each variety; each point represents one individual. a) flowers and flower buds attacked by chewing insects; b) fruits attacked by sucking insects; c) fruits attacked by chewing insects. Lines connect predicted values for each treatment combination, based on negative binomial models.

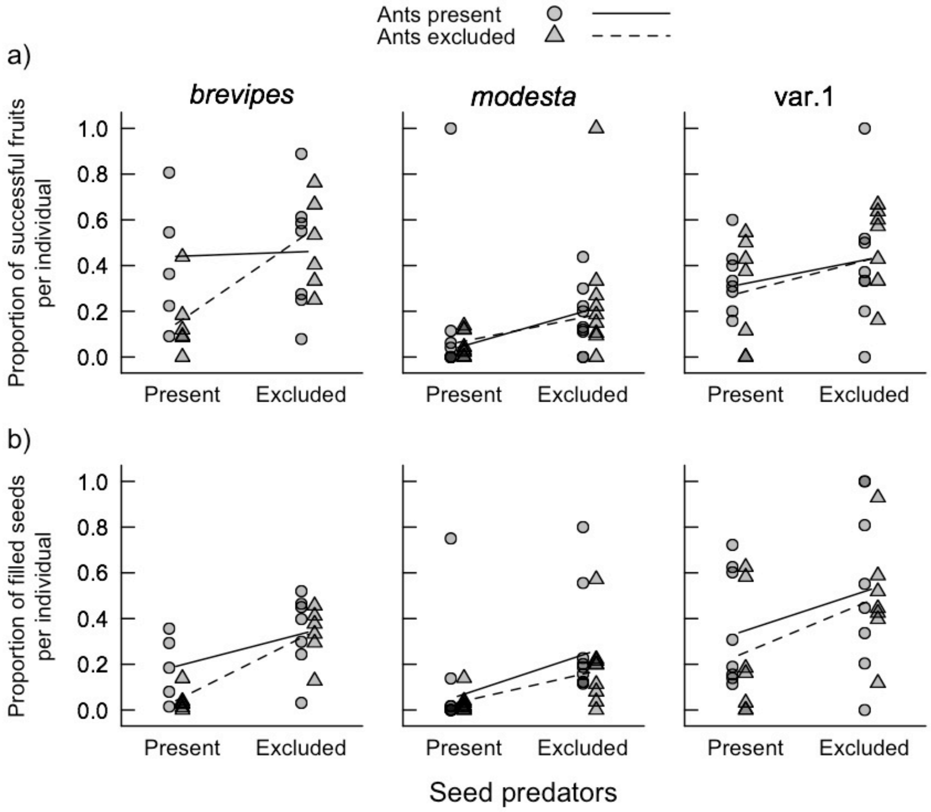

Figure 2-3. Number of successful fruits and filled seeds for each variety; each point represents one individual. a) total number of fruits containing at least one filled seed relative to the number of successfully pollinated flowers; b) total number of filled seeds relative to the potential number of seeds (i.e., total number of ovules in initiated fruits) per individual. Lines connect predicted values for each treatment combination, based on negative binomial models.

Chapter 3. Detecting local trait divergence and its causes at a broad geographic scale

Abstract

Several studies address character displacement by demonstrating that pairs of related species are more dissimilar when found in sympatry than in allopatry. Nevertheless, these studies encompass a limited geographic scale and do not take into account interactions among more than two species. We used herbarium data to detect trait divergence across the entire geographic range of several varieties of *Chamaecrista* (Leguminosae), including both vegetative and reproductive characters. We used permutations to test for greater morphological dissimilarity in sympatric pairs of different taxa, and if divergence results from ecological sorting or character displacement. Subdividing the dataset into 6º x 6º subsamples, sympatric pairs of individuals of different taxa had greater morphological dissimilarity than other pairs for vegetative and/or reproductive traits in several grids, the same pattern found using the entire dataset. Ecological sorting, rather than character displacement, is a better tentative explanation for the patterns observed.

Introduction

Sympatric species often display some degree of morphological, ecological, behavioural, or physiological divergence in relation to each other and to allopatric populations, a process termed character displacement by Brown and Wilson (1956). They proposed that intense competition over shared resources is expected when closely related species co-occur, resulting in an evolutionary divergence in character values. The presence of a second species may also lead to convergence or parallel changes in the traits of both species (Abrams, 1996). Accordingly, the concept of character displacement has been expanded to encompass all three processes (Grant, 1972; Taper and Case, 1992). 'Ecological character displacement' is often the result of competition for resources (Schluter, 2000), while 'reproductive character displacement' is recognised as a distinct phenomenon that arises from assortative mating and reproductive isolation (Slatkin, 1980; Goldberg and Lande, 2006).

An alternative explanation for morphological divergence of local communities is ecological sorting through competitive exclusion (Armbruster, 1995; Hansen et al., 2000; Dayan and Simberloff, 2005). In this scenario, only species that are sufficiently different from the established population can successfully invade a given area. On the other hand, if there is habitat filtering (phenotypic sorting), tolerance to the local environment determines which species of the regional species pool are found in a given location, and communities will tend to have phenotypically similar species (Perret et al., 2007; Sargent and Ackerly, 2008). At the community level, community composition will be a balance between habitat filtering and competition with other, similar species, but morphological divergences will be primarily determined by the species composition of a given community (Webb et al., 2002). For plants, Armbruster (1995) suggests that vegetative interactions are more likely to lead to ecological sorting, while reproductive interactions lead to character displacement.

Two mechanisms to reduce the detrimental effects of sharing pollinators have been frequently investigated within the context of character displacement: reduced overlap in flowering phenology (Armbruster, 1995) and differences in flower morphology (Whalen, 1978; Armbruster et al., 1994; Perret et al., 2007). On the one hand, competition for pollinators may cause pollen limitation and decrease plant fitness, and pollen limitation generally increases with species richness (Vamosi et al., 2006). Several studies have shown that flowering phenologies of co-occurring plants are more evenly spaced in time than expected by a null model of random distribution (e.g. Stone et al., 1998). This pattern of overdispersion is considered evidence for character displacement because it reduces the number of species with open flowers at any given time, but the specific null model used and strong seasonality in the environment both affect the outcome of this test (Aizen and Vázquez, 2006). On the other hand, plants that share pollinators are likely to suffer more losses during pollen export (Mitchell et al., 2009) and to receive more interspecific pollen, resulting in reduced seed set and loss of ovules to hybridisation (Morales and Traveset, 2008). Morphological differences among flowers of sympatric species can lead to differences in pollen placement on pollinators (as in *Asclepias*; Kephart and Theiss, 2004), reducing interspecific pollen transfer, or to specialisation on different subsets of pollinators, reducing the overlap in flower visitors (Armbruster and Muchhala, 2009).

Numerous studies address ecological character displacement in animals (reviewed in Dayan and Simberloff, 2005), but work on plant taxa has focused on reproductive character displacement. Because all terrestrial plants essentially compete for the same set of nutrients, several authors (e.g. Armbruster, 1995) believe that competition for resources is unlikely to be a driving force in plant community organisation (except when pollinators are treated as a shared resource, see above). Nevertheless, below- and above-ground competition can lead to partitioning among closely related species (Silvertown, 2004), and closely related species may respond differently to environmental variation (Schulman et al., 2004). The idea that pairwise ecological character displacement can occur in plants is also suggested by the work of Martin (1981), who showed that when two *Erodium* species are grown together, individuals from sympatric areas have higher seed set than individuals from allopatric areas. In addition, several studies demonstrated the existence of limiting similarity among co-occurring species, a pattern often attributed to community-wide character displacement (*sensu* Strong et al., 1979) that could also be caused by sorting (Dayan and Simberloff, 2005). For instance, Veech et al. (2000) showed that seed masses in pine communities, a character that probably influences seedling competition, are more evenly dispersed than expected by chance, and co-occurring plant species in a sand dune community in New Zealand are more dissimilar in traits related to rooting patterns and leaf water control than random combinations (Stubbs and Wilson, 2004).

Several criteria must be fulfilled before a robust case for character displacement can be made (Schluter and McPhail, 1992), but demonstrating a pattern of divergence in sympatry is often a starting point. Trait divergence is usually identified by testing for greater morphological distance when two species are sympatric than when one or both are found allopatrically, but the importance of taking into account the influence of geographical variation on phenotypic change was highlighted early in the literature (Grant, 1972). Indeed, recent models demonstrated that if the trait being analysed varies monotonically with the environment, sympatric populations may show *less* morphological divergence than allopatric ones. According to these models, traits directly involved in reproductive isolation are more likely to exhibit the classic pattern of higher divergence in sympatry (Goldberg and Lande, 2006).

Two approaches are normally used to characterise divergence in sympatry: comparison of the pattern of distribution of phenotypic values within local communities against a null model of random distribution (e.g. McEwen and Vamosi, 2010), or differences in phenotype between well-defined allopatric and sympatric populations of two species (e.g. Johanet et al., 2009). In the present study, we take a novel approach by using herbarium data to search for trait divergence in vegetative and reproductive traits among several co-occurring taxa of closely related plants. We define trait divergence as a pattern of increased morphological distance between sympatric pairs relative to allopatric pairs of individuals belonging to different taxa, regardless of the causal mechanism. We use three different permutation schemes to test for the presence of trait divergence and to test if significant divergence results from the identity of local pairs (ecological sorting) or from local divergence between co-occurring individuals (character displacement). Compared to methods commonly used to study character displacement, our approach is more suitable to detect divergence over a broad geographic scale and to take into account several taxa at once.

Materials and methods

Morphology measurements–We focused our study on section *Xerocalyx* of genus *Chamaecrista* (Leguminosae - Caesalpinioideae), distributed from Argentina to south of Mexico (Fig. 3-1). In the last detailed revision of this section, Irwin and Barneby (1982) based the recognition of two species (*C. desvauxii* and *C. ramosa*) on the observation of "several narrowly sympatric pairs of microphyll (*C. ramosa*) and macrophyll (*C. desvauxii*) populations which either in a given locality or over a more extensive common range maintain sharply distinct individual facies without signs of intergradation". This pattern suggests that competitive exclusion in sympatry may have constrained the morphology of co-occurring species (ecological sorting), or that competition could have caused character displacement between *C. desvauxii* and *C. ramosa*. The third species in the section (*C. diphylla*) is differentiated by having two leaflets instead of four, but has been found to be nested within *C. desvauxii* in a recent phylogenetic reconstruction based on molecular data (Conceição et al., 2009). Several varieties are recognised based on relative sizes and shapes of leaves, stipules, and flowers, making a total of 24 taxa, but there are no clear morphological discontinuities between them (Irwin and Barneby, 1982). Despite that, co-occurring varieties are easily distinguished from each other (Madeira and Fernandes, 1999; Costa et al., 2007), and in two locations investigated so far they have reproductive isolation mechanisms that prevent or reduce hybridisation (Costa et al., 2007, Baker & Marquis unpubl. data). As a result, each variety is treated here as a different entity.

We examined specimens deposited in GH, HUFU, MICH, MO, NY, TEX, UB and US (herbaria acronyms as in Thiers, continuously updated). Each specimen was identified as one of the currently recognised varieties of *Chamaecrista desvauxii* and *C. ramosa* or as *C. diphylla* using the keys in Irwin and Barneby (1982). Collections from nearby localities were compared to each other to aid in identification. Collection data was georeferenced manually using maps, online gazetteers and Google Earth. An estimate of maximum uncertainty was added to each location based on the guidelines in Chapman and Wieczorek (2006). Average uncertainty was 6.9 km ± 4.2.

We used the length and width of the largest leaflet and the length and width of the largest stipule as proxies for vegetative traits. One extrafloral nectary (EFN) was measured by taking a digital picture of the largest visible EFN next to a ruler divided into millimetres. We used ImageJ 1.42q (Rasband, 1997-2009) to estimate the length and height of the EFN using the ruler as calibration. As a proxy for reproductive traits, we measured the length of the pedicel, longest sepal and shortest sepal in the largest available flower. We avoided measuring more than one collection of the same variety from a given area, and ascensions that had no visible EFNs or that were sterile. Preference was given to collections that included latitude and longitude or UTM locations, and ascensions from localities with estimated geographical uncertainty larger than 25 km were excluded from the analyses.

Morphological distances–Vegetative (leaflet and stipule), EFN and reproductive data were analysed separately. We carried out a Principal Component Analysis for each group of traits, using both the original measurements and ratios between them. Leaf size and shape are correlated with climate and soil (Cornelissen et al., 2003; Schulman et al., 2004), thus observed morphological patterns are likely to be affected by those factors. We used six temperature and four precipitation bioclimatic variables in the 5 arc-minutes resolution from the WorldClim database (Hijmans et al., 2005) to account for the effect of climate on morphology. We used GRASS 6.4 (GRASS Development Team, 2010) to extract values of each climatic variable for each collection location. A principal component analysis was used to reduce the number of variables, and the principal component scores from each group of morphological traits containing more than 1.5% of the variance were regressed one at a time against the principal components of climatic variables using linear regressions (LM). To account for spatial autocorrelation, two additional regression models including both the climatic variables and latitude and longitude data were used: a generalised additive model (GAM) with Gaussian family and identity link and a conditional autoregressive (CAR) model with a neighbourhood of 50 km and a binary indicator of neighbourhood. To verify the presence of autocorrelation, we examined both multivariate Mantel correlograms and maps of the principal component scores and regression residuals (Legendre and Legendre, 1998). Residuals from each model were used to construct Morphological Distance Matrices calculated using Euclidean distances between each pair of points. These matrices were used in permutation tests as explained below.

Permutation tests–All analyses were carried out in two geographic scales. In the broadest scale, all measured collections were included. For the finest scale, we subdivided the sampling region into 6º x 6º grids, and carried out permutation tests within each grid with 30 or more collection points.

A Geographical Distance Matrix was constructed based on great circle distances among collection points. Uncertainty was taken into account by treating each collection point as a circle with the radius equal to the uncertainty and the centre on the location coordinates. Locations that either had central coordinates 10 km or less apart or that overlapped after the addition of uncertainty were considered sympatric.

Mantel tests of the correlation between sympatry and identity combined and morphology were carried out using two Hypothesis Matrices based on sympatry and taxon (Fig. 3-2 a-b). Hypothesis 1 was that morphological distances increase from pairs of the same variety to pairs of different varieties, that sympatric pairs of the same variety are more similar than allopatric pairs (due to similar environmental requirements among sympatric pairs), and that sympatric pairs of different varieties are more distant than allopatric pairs (due to competition). The matrix was coded 1 to 4, with increasing numbers corresponding to the expectation of increasing morphological distances: 1 for sympatric pairs of the same variety, 2 for allopatric pairs of same variety, 3 for allopatric pairs of different varieties, and 4 for sympatric pairs of different varieties (Fig. 3-3a). Hypothesis 2 was that sympatric pairs of different varieties have the largest morphological distance of all groups. In this second hypothesis matrix, all sympatric pairs of different taxa were coded as 1, while allopatric pairs or pairs of the same taxon were coded as 0 (Fig. 3-3a). We did 9,999 permutation for the complete dataset and 49,999 permutations for the subsamples.

We carried out two additional permutation tests to test for the significance of the differences in mean morphological distance between sympatric and allopatric pairs of either different varieties or the same variety (Fig. 3-2c-d). A Sympatry Matrix was constructed by coding sympatric pairs as 0 and allopatric pairs as 1. Similarly, an Identity Matrix was constructed by coding pairs of the same species and variety as 0 and pairs of different varieties as 1. The first test (P_{sort}) was designed to address the effect of sorting. Each permutation of the Morphological Distance Matrix was carried out by shuffling columns and rows without replacement, as in the Mantel test. The columns and rows of the Identity Matrix were permutated in the same order, while the Sympatry Matrix was left intact (Fig. 3-2c). This permutation scheme is equivalent to assigning individuals randomly to each location, while maintaining the geographic structure of the data and the morphological distances between each pair of individuals. With sorting, sympatric pairs of individuals are expected to be more dissimilar than pairs created by randomly distributing individuals. Differences in means between allopatric and sympatric pairs for each identity group were recalculated after each permutation and used to calculate significance. The second test (P_{chd}) was designed to address the effect of character displacement. Before constructing the Morphological Distance Matrix, individuals of each taxon were randomly assigned to locations occupied by that taxon, maintaining the taxonomic structure of the dataset (Fig. 3-2d). Permutations of the Morphological Distance Matrix were carried out as in the Mantel test, and differences between means were calculated after each permutation. Character displacement should result in greater morphological distance between sympatric pairs than between random pairs of the same varieties. All statistical analyses were conducted in R 2.10.1 (R Development Core Team, 2009), using packages maps, mgcv, spdep and vegan.

Results

We examined 2349 collections encompassing all varieties and the whole geographic extent of section *Xerocalyx* (Fig. 3-1). From those, we measured vegetative traits (leaflet and stipules) in 1198 ascensions, EFN in 712 ascensions, and reproductive traits in 1087 ascensions. The principal components of morphological traits used in the calculation of morphological distances contained 99.7 %, 99.8 % and 98.4 % of the variance for vegetative, EFN and reproductive traits, respectively. The five first principal components of the bioclimatic variables contained 97.3 % of the variance, and were used in the regressions.

Using the complete dataset, morphological distances increased from sympatric pairs of the same variety to sympatric pairs of different varieties for all morphological groups, consistently with the prediction of Hypothesis 1 (Table 3-1, Fig. 3-3a). In addition, sympatric pairs of different varieties had the largest vegetative morphological distances, consistent with a pattern of character displacement (Hypothesis 2, Table 3-1), and the largest reproductive distances using GAM and CAR residuals (Table 3-2). EFN distance, however, was significantly higher in those pairs only when using GAM residuals (Table 3-2). Although no indication of sorting or character displacement was found for morphological traits before removing the effect of spatial autocorrelation, additional permutation tests using GAM or CAR residuals showed that morphological traits of co-occurring varieties are significantly more distant than expected by chance (P_{sort}, Table 3-2), consistent with the hypothesis of ecological sorting, while sympatric pairs of varieties are more different than allopatric pairs for all three morphological groups (P_{chd}, Table 3-2), consistent with a pattern of character displacement. In addition, co-occurring individuals of the same variety were found to be more similar than allopatric individuals in all cases (Table 3-2), stemming from some level of spatial autocorrelation in the data.

Thirteen 6º x 6º grids contained 30 collection points or more (Fig. 3-1), and were selected as subsamples to be analysed separately. Within each subsample, morphological distances generally increased from sympatric pairs of the same variety to sympatric pairs of different varieties for all groups of morphological variables (Hypothesis 1, Appendix - Tables S-1–S-3). In addition, using CAR residuals the sympatric pairs of different varieties had the largest morphological distance (Hypothesis 2) in eight out of 13 grids (vegetative) and seven out of 12 grids (reproductive; Appendix - Table S-3, Fig. 3-3b-d). Among those, six and five subsamples, respectively, had significantly higher morphological distance than expected by random combinations (P_{sort}, Appendix - Table S-6). After correcting for false discovery rate, no sympatric pairs were more distant than expected by chance in any of the grids, although vegetative distance was marginally higher in grid 11 (P_{chd}, Appendix S1 - Table S-6). Despite lower spatial autocorrelation when using residuals from the CAR model, sympatric pairs of the same variety were still found to be more similar than allopatric pairs in several grids for each morphological group (Appendix - Table S-9), although this result was less frequent than using principal components or GAM residuals (Appendix - Tables S-7 and S-8).

Discussion

Our goal was to assess trait divergence between co-occurring taxa of section *Xerocalyx* throughout the range of this group using herbarium specimens and permutation tests. At a broad geographic scale, we found that co-occurring individuals of different taxa are generally more distant from each other morphologically than individuals that do not co-occur. In addition, after subdividing the dataset into 6° x 6° subsamples we detected significant morphological divergence in vegetative and/or reproductive traits in several grids. In almost all subsamples, ecological sorting, rather than character displacement, was a better explanation for the patterns observed.

Few grids showed significant trait divergence when morphological distances were calculated based on linear model (LM) residuals (Table 3-4). However, adaptation to local environmental conditions is likely to increase similarity among co-occurring individuals, obscuring the signal for trait divergence. We removed the effect of spatial autocorrelation by applying a generalised additive model (GAM) and a conditional autoregressive model (CAR) to the dataset. Inspection of maps showed that these regression models did remove some of the east-west trends in the magnitude of morphological measurements, but Mantel correlograms showed that multivariate spatial autocorrelation was still present in the data. Indeed, we found that in several subsamples sympatric pairs of the same variety were more similar than allopatric pairs, both when individuals were assigned randomly to each location and when permutations were carried out only within varieties, maintaining local community composition (Appendix - Tables S7-S9). Given the high amount of noise inherent to our method (see below), it is in fact remarkable that almost half of the grids showed evidence of trait divergence when using residuals from the CAR model (Appendix S1 - Table S-6).

Almost no plant studies have addressed reproductive character displacement over a large geographic scale (but see Hansen et al., 2000; Perret et al., 2007). The most similar approach to the one presented here is Perret *et al.*'s study of floral diversification in Gesneriaceae (2007). Although they found no correlation between floral divergence and degree of sympatry, their method ignored geographic variation, and flower measurements were carried out on a few (1 to 16) cultivated and collected specimens of unspecified geographic origin (Perret et al., 2007), thus may not reflect the range of phenotypic values found in the field. We included three flower characters that could be measured non-destructively from herbarium collections. Although we used few characters, we chose traits that are related to flower size and that are used to distinguish varieties. Flowers of taxa in section *Xerocalyx* do not differ significantly in shape (Irwin and Barneby, 1982), and are pollinated by generalist bees searching for pollen that often visit individuals of more than one taxon in a given area (Gottsberger and Silberbauer-Gottsberger, 1988; Costa et al., 2007). Post-zygotic incompatibility mechanisms reduce or prevent the formation of hybrids among varieties (Costa et al., 2007). Differences in overall flower size may reduce this overlap in flower visitors by excluding large bees from small flowers and preventing pollen deposition by small bees in large flowers, reducing pollen wastage (Gottsberger and Silberbauer-Gottsberger, 1988). In a couple of grids, we found that sympatric pairs of flowers of different varieties are more different than parapatric pairs, lending support to the possibility of character displacement among those varieties. More commonly, we found evidence that co-occurring taxa have more disparate flower morphologies than expected from random combinations. Part of these results are likely to be the result of local diversification, given that several varieties of section *Xerocalyx* have a somewhat restricted geographic distribution, such as *Chamaecrista desvauxii* var. *linearis*, *C. desvauxii* var. *chapadicola*, and *C. ramosa* var. *ventuarensis*, and may have suffered strong selection for divergent flower morphology in relation to nearby varieties.

We did not include differences in flowering phenology in our analyses because it is typically impossible to determine the flowering season based on herbarium collections, and geographic variation in climatic conditions would make direct comparisons unfeasible. As a result, we did not address a mechanism that is commonly implied in reproductive character displacement in plants (Morales and Traveset, 2008; Devaux and Lande, 2009). However, varieties in section *Xerocalyx* frequently overlap in phenology (Madeira and Fernandes, 1999; Costa et al., 2007), thus additional mechanisms are necessary to reduce competition for pollinators.

We found trait divergence of both vegetative and reproductive traits with comparable frequency. This result was surprising, given that ecological character displacement is generally believed not to occur in plants (Armbruster, 1995). However, this notion has not been tested because few studies have explicitly addressed ecological character displacement in plants, although niche differentiation has been demonstrated to occur in several systems (Silvertown, 2004), and leaf and stipule morphology reflect different resource acquisition strategies and trade-offs between photosynthetic benefits and biotic costs from herbivory (Givnish, 1987). Our results suggest that differences in vegetative traits may be important for the coexistence of closely related plants, although the specific mechanism is not clear.

Besides direct competition for resources, another mechanism driving trait divergence could be selection for enemy-free space through reduced similarity (Jeffries and Lawton, 1984; Brown and Lawton, 1991), akin to predator avoidance in animals (Taper and Case, 1992). Ricklefs and O'Rourke (1975) proposed this mechanism to explain their observation that moths in tropical communities had increased aspect diversity (a measure of variation in appearance), but subsequent analyses including more sites revealed that aspect volume is not related to species richness (Ricklefs, 2009). To our knowledge this idea has not been tested in plants, although both leaf colour and shape influence where moths and butterflies land for oviposition (Renwick and Chew, 1994). In fact, Brown and Lawton (1991) proposed that leaf shape and size of co-occurring plants, including divergence in morphology between species within the same habitat, could be a result of selective pressure from herbivores.

Vegetative and reproductive traits may not evolve independently. For instance, vegetative traits could indirectly affect reproductive success by changing the relative height and exposure of reproductive structures (Peakall and Handel, 1993; O'Connell and Johnston, 1998). In addition, reproductive and vegetative traits are pleiotropically related in some systems (reviewed in Armbruster, 2002). Thus, selective pressure towards character displacement in vegetative traits may be slowed or reversed by selection on reproductive traits, or vice versa. In *Chamaecrista fasciculata*, selection on rate of phenological development, leaf number and leaf thickness in response to a warmer climate is weaker or reversed when the prediction of evolutionary response takes into account correlations among traits as opposed to treating each trait separately (Etterson and Shaw, 2001).

Despite the large number of studies on ant-plant interactions, few of those address differentiation among closely related plant species, and none takes into consideration the possible effects of competition among plants for protective ants. If ants are viewed as a resource, competition for this biotic defence could represent an unrecognized mechanism for character displacement in plants. We found little evidence of trait divergence in EFN traits among co-occurring varieties. It must be noted, however, that we only included two EFN measurements (length and height) and their ratio, which may be insufficient to describe the variability of this structure among varieties. Differences in shape and nectar production and composition may be key to attract specific ant groups to the nectary; in addition, since EFNs are part of the plant's defensive strategies, their role in reducing inter-variety competition may be more appropriately addressed by including measurements of alternative defences, both morphological and chemical.

To maximise the scope of our study we measured herbarium collections encompassing the whole geographic range of section *Xerocalyx*, sampling only a few individuals per location. Nevertheless, it is important to acknowledge the limitations of using morphological data from herbarium specimens. As a rule, herbarium collections are aggregated in space (e.g. Schulman et al., 2007), and several areas within the extent of occurrence of *Xerocalyx* were not included in our study (Fig. 3-1). However, variation in morphological distance within subsamples showed no obvious geographical trends, thus it is unlikely that including specimens from poorly collected areas would change our conclusions significantly.

The absence of a variety from a given location in our dataset does not mean that variety does not occur there, and we used an arbitrary distance to define pairs as sympatric or allopatric. Using the distance we chose (10 km), it is likely that some varieties that occur in different microsites within an area were considered sympatric, despite occupying different ecological niches and not competing directly. However, *Xerocalyx* varieties are often encountered side by side or in close proximity (BBM, pers. obs.). In addition, using zero km or a very low distance would separate populations that do co-occur but were collected in different points of the same area as allopatric.

It is possible that our results are affected by the phylogenetic structure of *Xerocalyx*. If morphology is constrained by phylogeny, pairs of closely related taxa are likely to be more similar. However, much of the morphological variation within this section stems from variation in the relative sizes of leaves, stipules, pedicels, and flowers (Irwin and Barneby, 1982), which may be more labile than shape. In that case, trait divergence can occur despite phylogenetic clustering, as recently shown for flower colour in alpine meadows (McEwen and Vamosi, 2010). In addition, phylogenetic information can be used to distinguish between single and multiple origins of trait divergence (Rice et al., 2009); the latter scenario lends support to the role of competition and character displacement in trait evolution.

Our study provides a new approach to detect trait divergence at a large scale and including more than two species. Although detailed ecological studies are necessary to determine the cause of the patterns observed, our results suggest tentative mechanisms and provide a method based on collection data to select promising sites for further studies.

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Table 3-1. Results of Mantel correlations between morphological distance and sympatry and identity using residuals from LM, GAM and CAR models. Hypothesis 1 tests if morphological distances increase from sympatric pairs of the same variety to sympatric pairs of different varieties (Fig. 3-1). Hypothesis 2 tests if sympatric pairs of different varieties show the largest morphological distances of all groups. Significance codes: *** P < 0.001; ** P < 0.01; * P < 0.05; ˙ P < 0.10; n.s. P ≥ 0.10.

Test	Vegetative		EFN		Reproductive	
	Hyp. 1	Hyp. 2	Hyp. 1	Hyp. 2	Hyp. 1	Hyp. 2
LM residuals	0.286, P < 0.001***	0.005, P < 0.001***	0.124, P < 0.001***	-0.005, P = 0.929 n.s.	0.245, P < 0.001***	$5•10^{-5}$, P = 0.491 n.s.
GAM residuals	0.184, P < 0.001***	0.019, P < 0.001***	0.073, P < 0.001***	0.007, P = 0.030*	0.153, P < 0.001***	0.010, P < 0.001***
CAR residuals	0.236, P < 0.001***	0.016, P < 0.001 ***	0.102, P < 0.001***	0.004, P = 0.112 n.s.	0.188, P < 0.001***	0.010, P < 0.001***

Table 3-2. Number of pairs of individuals and difference D in mean morphological distance between sympatric and allopatric pairs, using residuals from LM, GAM and CAR models. Significance values derived from permutations to test for sorting (P_{sort}) or for character displacement (P_{chd}). Significance codes: *** P < 0.001; ** P < 0.01; * P < 0.05; • P < 0.10; n.s. P ≥ 0.10.

Pairs	Test	Vegetative			EFN			Reproductive		
		D	P_{sort}	P_{chd}	D	P_{sort}	P_{chd}	D	P_{sort}	P_{chd}
		(634,791 pairs)			(224,529 pairs)			(522,811 pairs)		
Different varieties	LM residuals	0.034	0.231 n.s.	0.719 n.s.	-0.159	0.997 n.s.	0.368 n.s.	-0.082	0.963 n.s.	0.591 n.s.
	GAM residuals	0.459	<0.001***	<0.001***	0.133	0.017*	0.002**	0.224	<0.001***	0.005**
	CAR residuals	0.356	<0.001***	<0.001***	0.061	0.154 n.s.	0.002**	0.191	<0.001***	0.006**
		(82,212 pairs)			(28,587 pairs)			(67,430 pairs)		
Same variety	LM residuals	-0.548	<0.001***	<0.001***	-0.391	<0.001***	<0.001***	-0.438	<0.001***	<0.001***
	GAM residuals	-0.605	<0.001***	<0.001***	-0.365	<0.001***	<0.001***	-0.528	<0.001***	<0.001***
	CAR residuals	-0.523	<0.001***	<0.001***	-0.333	<0.001***	<0.001***	-0.445	<0.001***	<0.001***

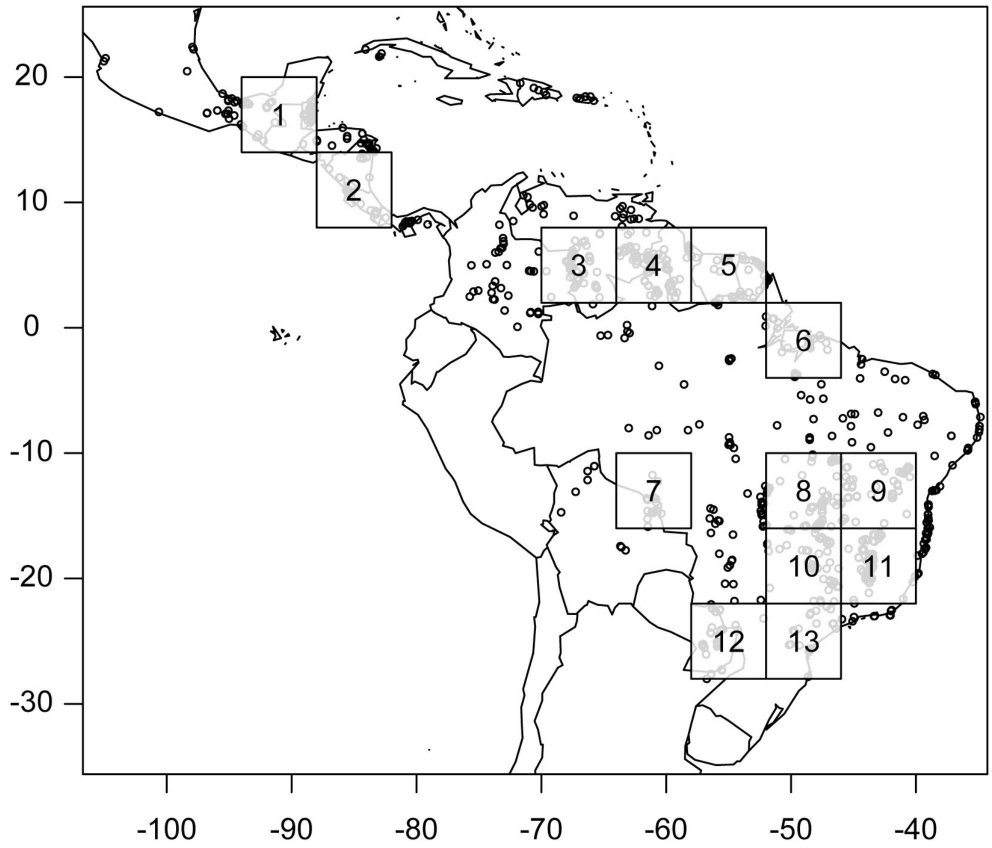
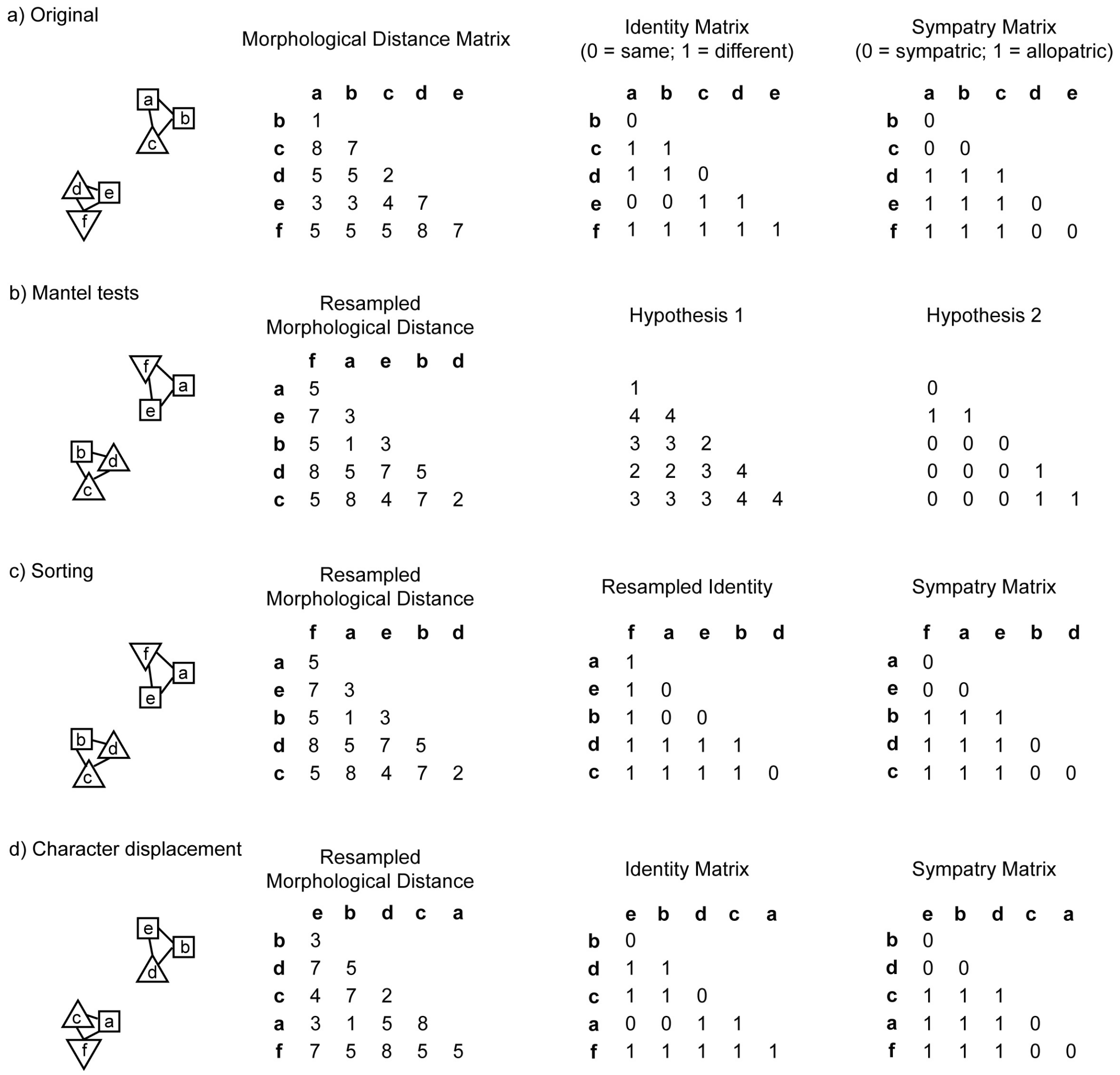


Figure 3-1. Map depicting collection sites and 6º x 6º subsample grids.



a) Morphological Distance Matrix

	a	b	c	d	e
b	1				
c	8	7			
d	5	5	2		
e	3	3	4	7	
f	5	5	5	8	7

Identity Matrix (0 = same; 1 = different)

	a	b	c	d	e
b	0				
c	1	1			
d	1	1	0		
e	0	0	1	1	
f	1	1	1	1	1

Sympatry Matrix (0 = sympatric; 1 = allopatric)

	a	b	c	d	e
b	0				
c	0	0			
d	1	1	1		
e	1	1	1	0	
f	1	1	1	0	0

b) Resampled Morphological Distance

	f	a	e	b	d
a	5				
e	7	3			
b	5	1	3		
d	8	5	7	5	
c	5	8	4	7	2

Hypothesis 1

1				
4	4			
3	3	2		
2	2	3	4	
3	3	3	4	4

Hypothesis 2

0				
1	1			
0	0	0		
0	0	0	1	
0	0	0	1	1

c) Resampled Morphological Distance

	f	a	e	b	d
a	5				
e	7	3			
b	5	1	3		
d	8	5	7	5	
c	5	8	4	7	2

Resampled Identity

	f	a	e	b	d
a	1				
e	1	0			
b	1	0	0		
d	1	1	1	1	
c	1	1	1	1	0

Sympatry Matrix

	f	a	e	b	d
a	0				
e	0	0			
b	1	1	1		
d	1	1	1	0	
c	1	1	1	0	0

d) Resampled Morphological Distance

	e	b	d	c	a
b	3				
d	7	5			
c	4	7	2		
a	3	1	5	8	
f	7	5	8	5	5

Identity Matrix

	e	b	d	c	a
b	0				
d	1	1			
c	1	1	0		
a	0	0	1	1	
f	1	1	1	1	1

Sympatry Matrix

	e	b	d	c	a
b	0				
d	0	0			
c	1	1	1		
a	1	1	1	0	
f	1	1	1	0	0

Figure 3-2. Schematic maps and example matrices to illustrate permutation schemes. a) hypothetical values for six individuals (each indicated by a different letter) belonging to three taxa (each indicated by a different geometric shape), distributed in space as shown in the figure. Lines connect pairs that were considered sympatric. Morphological Distance (MD), Identity and Sympatry matrices were constructed based on values for each pair of individuals; b) permutation scheme for Mantel tests; for each permutation, the rows and columns of the MD matrix are rearranged, and a correlation is calculated between the new MD matrix and the matrices corresponding to Hypothesis 1 (increasing morphological distance from sympatric individuals of the same taxon to sympatric individuals of different taxa) and Hypothesis 2 (sympatric individuals of different taxa have the largest morphological distance); c) permutation scheme for sorting (P_{sort}); MD matrix is randomized as previously, and Identity matrix is rearranged in the same order; this scheme corresponds to assigning individuals randomly to each position in the map; d) permutation scheme for character displacement (P_{chd}); individuals of each taxon are assigned randomly to positions where that taxon is observed; note that Identity matrix does not change.

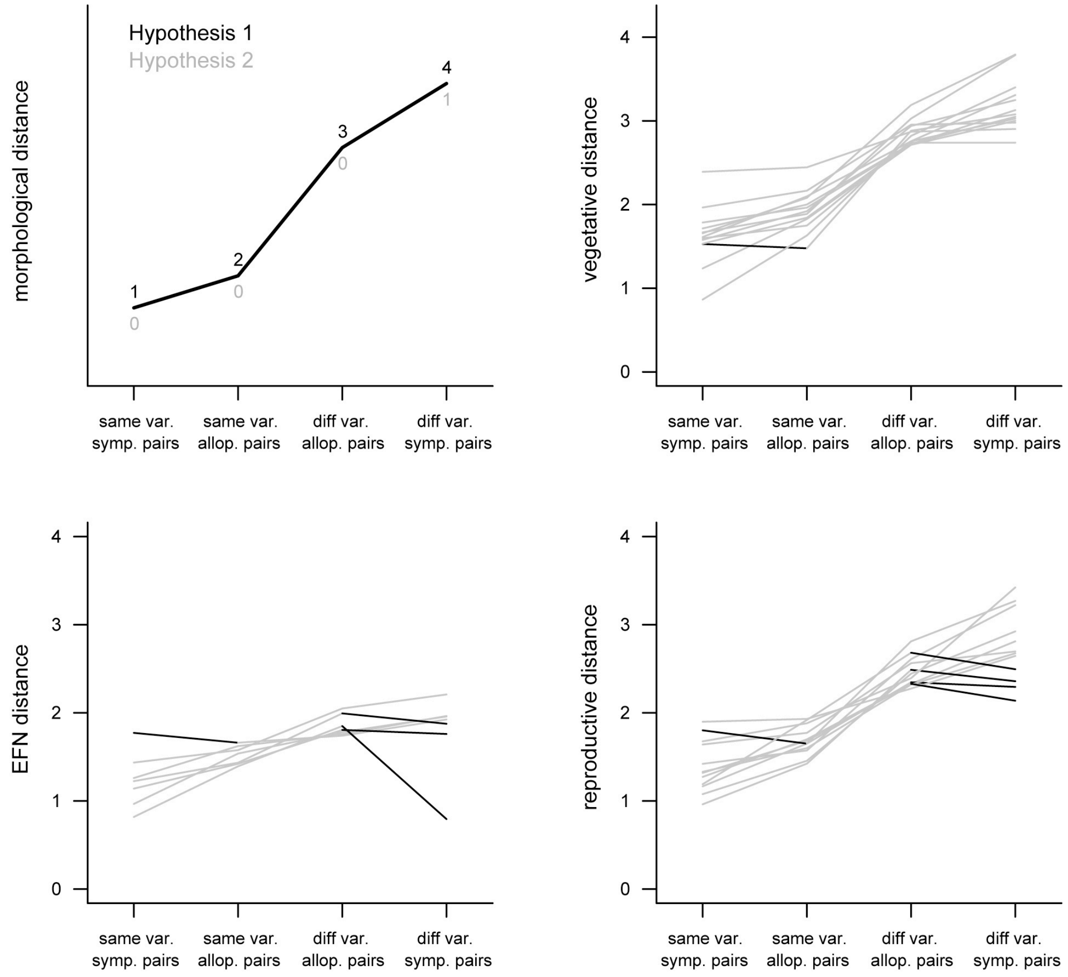


Figure 3-3. a) Coding used to construct hypotheses matrices for Mantel correlations. Hypothesis 1 codes the combination of sympatry and identity with numbers 1 to 4, representing the expectation of increased morphological distances. Hypothesis 2 codes sympatric pairs of different varieties, expected to have the largest morphological distances, as 1, and all other pairs as 0. b-d) average morphological distances between sympatric and parapatric pairs of the same variety or different varieties within 6º x 6º grids, using residuals from CAR model. Lines connect means within the same grid; black lines identify sequences within grids where distances did not follow the expectation from Hypothesis 1.

Table S-1. Location and number of measured collections per 6º x 6º subsample, and results of Mantel correlations between morphological distance and sympatry and identity using residuals of linear regressions (LM). Hypothesis 1 tests if morphological distances increase from sympatric pairs of the same variety to allopatric pairs of different varieties (Fig. 3-1). Hypothesis 2 tests if sympatric pairs of different varieties show the largest morphological distances of all groups. Significance codes for each region correspond to adjusted p-values after using the Benjamini-Hochberg correction to control for false discovery rate (Benjamini and Hochberg, 1995): *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; • $P < 0.10$; n.s. $P \geq 0.10$.

Region	Coords	# collections			Vegetative		EFN		Reproductive	
		Veg	EFN	Rep	Hyp. 1	Hyp. 2	Hyp. 1	Hyp. 2	Hyp. 1	Hyp. 2
1	91ºW, 17ºN	53	10	48	0.300, $P < 0.001$***	0.024, $P = 0.205$ n.s.			0.359, $P < 0.001$***	0.031, $P = 0.150$ n.s.
2	85ºW, 11ºN	34	1	31	0.643, $P < 0.001$***	0.125, $P = 0.001$**			0.611, $P < 0.001$***	0.098, $P = 0.018$*
3	67ºW, 5ºN	57	38	50	0.304, $P < 0.001$***	0.027, $P = 0.189$ n.s.	0.213, $P < 0.001$***	-0.010, $P = 0.554$ n.s.	0.512, $P < 0.001$***	0.044, $P = 0.069$ n.s.
4	61ºW, 5ºN	124	87	112	0.519, $P < 0.001$***	0.090, $P < 0.001$***	0.254, $P < 0.001$***	0.006, $P = 0.359$ n.s.	0.608, $P < 0.001$***	0.075, $P < 0.001$***
5	55ºW, 5ºN	46	28	43	0.485, $P < 0.001$***	0.030, $P = 0.195$ n.s.			0.307, $P < 0.001$***	0.092, $P = 0.005$*
6	49ºW, 1ºS	36	27	36	0.484, $P < 0.001$***	0.154, $P = 0.001$**			0.409, $P < 0.001$***	-0.007, $P = 0.562$ n.s.
7	61ºW, 13ºS	33	18	27	0.492, $P < 0.001$***	0.087, $P = 0.047$•				
8	49ºW, 13ºS	119	102	102	0.268, $P < 0.001$***	-0.001, $P = 0.521$ n.s.	0.088, $P < 0.001$***	0.008, $P = 0.354$ n.s.	0.257, $P < 0.001$***	0.057, $P = 0.006$*
9	43ºW, 13ºS	72	31	69	0.243, $P < 0.001$***	0.052, $P = 0.023$•	0.175, $P < 0.001$***	-0.088, $P = 0.977$ n.s.	0.269, $P < 0.001$***	-0.043, $P = 0.976$ n.s.
10	49ºW, 19ºS	98	75	90	0.326, $P < 0.001$***	0.039, $P = 0.098$ n.s.	0.153, $P < 0.001$***	0.010, $P = 0.394$ n.s.	0.266, $P < 0.001$***	-0.007, $P = 0.575$ n.s.
11	43ºW, 19ºS	120	74	100	0.298, $P < 0.001$***	0.047, $P = 0.024$•	0.060, $P < 0.001$***	0.011, $P = 0.326$ n.s.	0.164, $P < 0.001$***	0.073, $P = 0.010$*
12	55ºW, 25ºS	43	33	39	0.411, $P < 0.001$***	0.036, $P = 0.203$ n.s.	0.343, $P < 0.001$***	0.017, $P = 0.334$ n.s.	0.485, $P < 0.001$***	0.031, $P = 0.247$ n.s.
13	49ºW, 25ºS	31	25	30	0.365, $P < 0.001$***	0.131, $P = 0.013$*			0.291, $P < 0.001$***	0.094, $P = 0.069$ n.s.

Table S-2. Mantel correlations between morphological distance and sympatry and identity using residuals from generalized additive models (GAM). See Table S1 for locations of regions, number of measured collections, and explanation of hypotheses and significance symbols.

Region	**Vegetative**		**EFN**		**Reproductive**	
	Hyp. 1	**Hyp. 2**	**Hyp. 1**	**Hyp. 2**	**Hyp. 1**	**Hyp. 2**
1	0.207, P < 0.001***	0.034, P = 0.120 n.s.			0.303, P < 0.001***	0.026, P = 0.195 n.s.
2	0.554, P < 0.001***	0.132, P = 0.001**			0.584, P < 0.001***	0.112, P = 0.009*
3	0.295, P < 0.001***	0.036, P = 0.125 n.s.	0.186, P < 0.001***	-0.005, P = 0.507 n.s.	0.461, P < 0.001***	0.057, P = 0.031•
4	0.484, P < 0.001***	0.090, P < 0.001***	0.276, P < 0.001***	0.004, P = 0.405 n.s.	0.484, P < 0.001***	0.092, P < 0.001***
5	0.445, P < 0.001***	0.047, P = 0.092 n.s.			0.243, P < 0.001***	0.107, P = 0.003*
6	0.457, P < 0.001***	0.149, P = 0.001**			0.395, P < 0.001***	-0.010, P = 0.592 n.s.
7	0.490, P < 0.001***	0.089, P = 0.044•				
8	0.261, P < 0.001***	0.017, P = 0.197 n.s.	0.088, P = 0.001**	0.031, P = 0.077 n.s.	0.260, P < 0.001***	0.071, P = 0.001**
9	0.242, P < 0.001***	0.044, P = 0.047•	0.176, P < 0.001***	-0.090, P = 0.976 n.s.	0.225, P < 0.001***	-0.024, P = 0.856 n.s.
10	0.313, P < 0.001***	0.053, P = 0.044•	0.150, P < 0.001***	0.021, P = 0.299 n.s.	0.273, P < 0.001***	-0.008, P = 0.599 n.s.
11	0.292, P < 0.001***	0.064, P = 0.004*	0.053, P = 0.072•	0.025, P = 0.161 n.s.	0.167, P < 0.001***	0.077, P = 0.007*
12	0.407, P < 0.001***	0.038, P = 0.198 n.s.	0.322, P = 0.001**	0.022, P = 0.31 n.s.	0.461, P < 0.001***	0.048, P = 0.156 n.s.
13	0.368, P < 0.001***	0.141, P = 0.011*			0.304, P < 0.001***	0.108, P = 0.049•

Table S-3. Mantel correlations between morphological distance and sympatry and identity using residuals from conditional autoregressive (CAR) models. See Table S1 for locations of regions, number of measured collections, and explanation of hypotheses and significance symbols.

Region	**Vegetative**		**EFN**		**Reproductive**	
	Hyp. 1	**Hyp. 2**	**Hyp. 1**	**Hyp. 2**	**Hyp. 1**	**Hyp. 2**
1	0.210, P < 0.001***	0.031, P = 0.146 n.s.			0.423, P < 0.001***	0.026, P = 0.194 n.s.
2	0.567, P < 0.001***	0.119, P = 0.002**			0.579, P < 0.001***	0.111, P = 0.009*
3	0.269, P < 0.001***	0.048, P = 0.062•	0.163, P = 0.001**	0.006, P = 0.419 n.s.	0.427, P < 0.001***	0.077, P = 0.006*
4	0.497, P < 0.001***	0.102, P < 0.001***	0.269, P < 0.001***	0.008, P = 0.344 n.s.	0.495, P < 0.001***	0.097, P < 0.001***
5	0.456, P < 0.001***	0.053, P = 0.074•			0.261, P < 0.001***	0.106, P = 0.002**
6	0.503, P < 0.001***	0.191, P < 0.001***			0.410, P < 0.001***	0.021, P = 0.337 n.s.
7	0.490, P < 0.001***	0.109, P = 0.022*				
8	0.265, P < 0.001***	0.021, P = 0.143 n.s.	0.077, P = 0.001**	0.043, P = 0.026 n.s.	0.255, P < 0.001***	0.078, P < 0.001**
9	0.242, P < 0.001***	0.060, P = 0.011*	0.170, P = 0.001**	-0.090, P = 0.978 n.s.	0.205, P < 0.001***	-0.013, P = 0.708 n.s.
10	0.304, P < 0.001***	0.069, P = 0.015*	0.142, P < 0.001***	0.046, P = 0.135 n.s.	0.256, P < 0.001***	0.009, P = 0.381 n.s.
11	0.268, P < 0.001***	0.085, P < 0.001***	0.028, P = 0.215 n.s.	0.029, P = 0.126 n.s.	0.153, P < 0.001***	0.091, P = 0.002**
12	0.356, P < 0.001***	0.044, P = 0.163 n.s.	0.265, P = 0.002**	0.029, P = 0.258 n.s.	0.397, P < 0.001***	0.064, P = 0.085 n.s.
13	0.368, P < 0.001***	0.146, P = 0.009*			0.300, P < 0.001***	0.133, P = 0.024*

Table S-4. Number of varieties, number of pairs of different varieties, and difference D in mean morphological distance between sympatric and allopatric pairs of different varieties, using residuals of linear regressions (LM). Significance values derived from permutations to test for sorting (P_{sort}) or for character displacement (P_{chd}). See Table S1 for significance codes.

Region	Vegetative					EFN					Reproductive				
	# Vars	# Pairs	D	P_{sort}	P_{chd}	# Vars	# Pairs	D	P_{sort}	P_{chd}	# Vars	# Pairs	D	P_{sort}	P_{chd}
1	2	672	-0.140	0.693 n.s.	0.687 n.s.						2	540	-0.070	0.636 n.s.	0.629 n.s.
2	2	288	0.609	0.003*	0.023 n.s.						2	240	0.310	0.100 n.s.	0.195 n.s.
3	8	1320	0.077	0.330 n.s.	0.464 n.s.	7	560	-0.192	0.762 n.s.	0.802 n.s.	8	1016	0.154	0.181 n.s.	0.290 n.s.
4	7	4710	0.591	<0.001**	0.471 n.s.	6	1859	-0.139	0.666 n.s.	0.682 n.s.	7	3927	0.289	0.009•	0.723 n.s.
5	7	730	0.019	0.453 n.s.	0.961 n.s.						6	631	0.813	<0.001**	0.181 n.s.
6	9	521	0.420	0.024•	0.397 n.s.						9	521	-0.243	0.920 n.s.	0.453 n.s.
7	8	442	0.099	0.285 n.s.	0.784 n.s.										
8	14	6070	-0.138	0.880 n.s.	0.962 n.s.	14	4455	0.008	0.471 n.s.	0.682 n.s.	14	4476	0.228	0.036 n.s.	0.067 n.s.
9	12	2278	0.345	0.025•	0.226 n.s.	12	423	-1.040	1.000 n.s.	0.837 n.s.	12	2092	-0.508	0.999 n.s.	0.965 n.s.
10	12	4198	0.090	0.265 n.s.	0.792 n.s.	11	2421	-0.014	0.519 n.s.	0.141 n.s.	11	3503	-0.148	0.851 n.s.	0.794 n.s.
11	11	5953	0.090	0.204 n.s.	0.090 n.s.	9	2205	0.041	0.386 n.s.	0.224 n.s.	11	4127	0.281	0.022•	0.557 n.s.
12	5	527	-0.113	0.668 n.s.	0.502 n.s.	5	244	-0.106	0.645 n.s.	0.118 n.s.	5	463	-0.186	0.801 n.s.	0.464 n.s.
13	6	385	0.485	0.038•	0.193 n.s.						6	362	0.295	0.114 n.s.	0.580 n.s.

Table S-5. Difference D in mean morphological distance between sympatric and allopatric pairs of different varieties, using GAM residuals. See Table S4 for number of varieties and number of pairs in each subsample, meaning of P_{sort} and P_{chd}, and significance codes.

Region	Vegetative			EFN			Reproductive		
	D	**P_{sort}**	**P_{chd}**	**D**	**P_{sort}**	**P_{chd}**	**D**	**P_{sort}**	**P_{chd}**
1	0.065	0.395 n.s.	0.400 n.s.				-0.060	0.615 n.s.	0.610 n.s.
2	0.764	0.001**	0.015 n.s.				0.455	0.032•	0.097 n.s.
3	0.155	0.185 n.s.	0.330 n.s.	-0.135	0.688 n.s.	0.745 n.s.	0.286	0.047•	0.201 n.s.
4	0.634	<0.001**	0.401 n.s.	-0.215	0.774 n.s.	0.763 n.s.	0.585	<0.001***	0.331 n.s.
5	0.251	0.133 n.s.	0.660 n.s.				1.023	<0.001***	0.103 n.s.
6	0.427	0.030•	0.490 n.s.				-0.248	0.925 n.s.	0.563 n.s.
7	0.111	0.268 n.s.	0.760 n.s.						
8	-0.022	0.570 n.s.	0.783 n.s.	0.134	0.117 n.s.	0.316 n.s.	0.310	0.007*	0.026 n.s.
9	0.281	0.059 n.s.	0.275 n.s.	-1.054	1.000 n.s.	0.893 n.s.	-0.297	0.961 n.s.	0.809 n.s.
10	0.178	0.116 n.s.	0.617 n.s.	0.043	0.403 n.s.	0.113 n.s.	-0.156	0.876 n.s.	0.864 n.s.
11	0.189	0.044 n.s.	0.031 n.s.	0.140	0.162 n.s.	0.181 n.s.	0.292	0.015*	0.626 n.s.
12	-0.089	0.634 n.s.	0.521 n.s.	-0.016	0.527 n.s.	0.075 n.s.	-0.036	0.574 n.s.	0.288 n.s.
13	0.528	0.026•	0.153 n.s.				0.354	0.079 n.s.	0.348 n.s.

Table S-6. Difference D in mean morphological distance between sympatric and allopatric pairs of different varieties, using residuals from CAR models. See Table S4 for number of varieties and number of pairs in each subsample, meaning of P_{sort} and P_{chd}, and significance codes.

Region	**Vegetative**			**EFN**			**Reproductive**		
	D	**P_{sort}**	**P_{chd}**	**D**	**P_{sort}**	**P_{chd}**	**D**	**P_{sort}**	**P_{chd}**
1	0.032	0.440 n.s.	0.441 n.s.				-0.187	0.843 n.s.	0.839 n.s.
2	0.603	0.003*	0.022 n.s.				0.459	0.034•	0.100 n.s.
3	0.271	0.061•	0.212 n.s.	-0.046	0.545 n.s.	0.652 n.s.	0.480	0.003**	0.058 n.s.
4	0.758	<0.001***	0.119 n.s.	-0.118	0.672 n.s.	0.585 n.s.	0.613	<0.001***	0.200 n.s.
5	0.310	0.088 n.s.	0.654 n.s.				1.030	<0.001**	0.042 n.s.
6	0.580	0.003*	0.245 n.s.				-0.129	0.782 n.s.	0.362 n.s.
7	0.196	0.135 n.s.	0.752 n.s.						
8	0.001	0.490 n.s.	0.797 n.s.	0.205	0.038 n.s.	0.153 n.s.	0.354	0.002**	0.010 n.s.
9	0.406	0.009*	0.091 n.s.	-1.055	1.000 n.s.	0.944 n.s.	-0.191	0.864 n.s.	0.674 n.s.
10	0.288	0.032•	0.307 n.s.	0.177	0.168 n.s.	0.033 n.s.	-0.053	0.647 n.s.	0.612 n.s.
11	0.321	0.004*	0.006•	0.184	0.099 n.s.	0.151 n.s.	0.371	0.003**	0.420 n.s.
12	0.024	0.465 n.s.	0.503 n.s.	0.159	0.278 n.s.	0.076 n.s.	0.133	0.277 n.s.	0.304 n.s.
13	0.552	0.021*	0.115 n.s.				0.482	0.033•	0.233 n.s.

Table S-7. Number of varieties, number of pairs of the same varieties, and difference D in mean morphological distance between sympatric and allopatric pairs of same varieties, using residuals of linear regressions (LM). P values derived from permutations to test for sorting (P_{sort}) or for character displacement (P_{chd}). See Table S1 for significance codes.

Region	Vegetative					EFN					Reproductive				
	# Vars	# Pairs	D	P_{sort}	P_{chd}	# Vars	# Pairs	D	P_{sort}	P_{chd}	# Vars	# Pairs	D	P_{sort}	P_{chd}
1	2	706	0.050	0.599 n.s.	0.666 n.s.						2	588	-0.795	0.001*	0.001**
2	2	273	-0.202	0.214 n.s.	0.181 n.s.						2	225	0.207	0.806 n.s.	0.863 n.s.
3	8	276	-0.484	0.073 n.s.	0.027•	7	143	-0.282	0.255 n.s.	0.465 n.s.	8	209	-0.187	0.256 n.s.	0.326 n.s.
4	7	2916	-0.285	0.004*	0.010*	6	1882	-0.173	0.148 n.s.	0.172 n.s.	7	2289	-0.100	0.157 n.s.	0.155 n.s.
5	7	305	-0.395	0.114 n.s.	0.185 n.s.						6	272	-0.240	0.267 n.s.	0.483 n.s.
6	9	109	-0.806	0.002*	0.001**						9	109	-0.428	0.040 n.s.	0.135 n.s.
7	8	86	0.058	0.600 n.s.	0.358 n.s.										
8	14	951	-0.377	0.007*	<0.001***	14	696	-0.404	0.009*	0.001**	14	675	-0.285	0.039 n.s.	<0.001**
9	12	278	-0.184	0.295 n.s.	0.189 n.s.	12	42	-0.600	0.173 n.s.	0.072 n.s.	12	254	-0.280	0.174 n.s.	0.091 n.s.
10	12	555	-0.572	<0.001**	0.685 n.s.	11	354	-0.578	0.007*	0.428 n.s.	11	502	-0.482	0.002*	0.150 n.s.
11	11	1187	-0.279	0.010*	0.005*	9	496	0.076	0.634 n.s.	0.848 n.s.	11	823	-0.017	0.455 n.s.	0.239 n.s.
12	5	376	-0.144	0.219 n.s.	0.141 n.s.	5	284	-0.137	0.268 n.s.	0.265 n.s.	5	278	-0.127	0.223 n.s.	0.234 n.s.
13	6	80	-0.268	0.246 n.s.	0.125 n.s.						6	73	-0.570	0.085 n.s.	0.015•

Table S-8. Difference D in mean morphological distance between sympatric and allopatric pairs of same varieties, using GAM residuals. See Table S7 for number of varieties and number of pairs in each subsample, meaning of P_{sort} and P_{chd}, and significance codes.

Region	Vegetative			EFN			Reproductive		
	D	P_{sort}	P_{chd}	D	P_{sort}	P_{chd}	D	P_{sort}	P_{chd}
1	-0.043	0.429 n.s.	0.500 n.s.				-0.838	0.001**	0.001*
2	-0.350	0.088 n.s.	0.068 n.s.				0.098	0.668 n.s.	0.692 n.s.
3	-0.460	0.090 n.s.	0.041 n.s.	-0.258	0.277 n.s.	0.594 n.s.	-0.361	0.111 n.s.	0.141 n.s.
4	-0.304	0.003*	0.006*	-0.223	0.077 n.s.	0.073 n.s.	-0.205	0.024•	0.018•
5	-0.440	0.086 n.s.	0.105 n.s.				-0.227	0.284 n.s.	0.553 n.s.
6	-0.877	0.003*	0.002*				-0.491	0.022•	0.083 n.s.
7	0.058	0.599 n.s.	0.356 n.s.						
8	-0.328	0.015*	0.001**	-0.357	0.022•	0.005*	-0.255	0.061 n.s.	0.003*
9	-0.207	0.270 n.s.	0.160 n.s.	-0.626	0.175 n.s.	0.061 n.s.	-0.393	0.134 n.s.	0.041•
10	-0.580	<0.001**	0.727 n.s.	-0.563	0.009•	0.471 n.s.	-0.483	0.002*	0.172 n.s.
11	-0.251	0.019*	0.010*	0.145	0.739 n.s.	0.910 n.s.	-0.028	0.425 n.s.	0.243 n.s.
12	-0.155	0.209 n.s.	0.128 n.s.	-0.116	0.296 n.s.	0.289 n.s.	-0.152	0.205 n.s.	0.193 n.s.
13	-0.174	0.311 n.s.	0.178 n.s.				-0.497	0.087 n.s.	0.023•

Table S-9. Difference D in mean morphological distance between sympatric and allopatric pairs of same varieties, using residuals from CAR models. See Table S7 for number of varieties and number of pairs in each subsample, meaning of P_{sort} and P_{chd}, and significance codes.

Region	**Vegetative**			**EFN**			**Reproductive**		
	D	**P_{sort}**	**P_{chd}**	**D**	**P_{sort}**	**P_{chd}**	**D**	**P_{sort}**	**P_{chd}**
1	-0.054	0.412 n.s.	0.529 n.s.				-0.729	0.002*	0.003*
2	-0.423	0.059 n.s.	0.042 n.s.				0.151	0.737 n.s.	0.781 n.s.
3	-0.486	0.083 n.s.	0.039 n.s.	-0.281	0.256 n.s.	0.478 n.s.	-0.379	0.110 n.s.	0.087 n.s.
4	-0.220	0.024•	0.060 n.s.	-0.209	0.104 n.s.	0.117 n.s.	-0.152	0.080 n.s.	0.067 n.s.
5	-0.342	0.148 n.s.	0.295 n.s.				-0.205	0.307 n.s.	0.564 n.s.
6	-0.766	0.002*	0.002*				-0.462	0.030 n.s.	0.084 n.s.
7	0.051	0.591 n.s.	0.344 n.s.						
8	-0.311	0.018•	<0.001**	-0.363	0.019•	0.004*	-0.268	0.050 n.s.	0.001**
9	-0.177	0.299 n.s.	0.177 n.s.	-0.575	0.183 n.s.	0.102 n.s.	-0.363	0.150 n.s.	0.054 n.s.
10	-0.590	<0.001**	0.683 n.s.	-0.572	0.009•	0.456 n.s.	-0.485	0.002*	0.183 n.s.
11	-0.286	0.012•	0.007*	0.112	0.682 n.s.	0.880 n.s.	-0.032	0.418 n.s.	0.219 n.s.
12	-0.201	0.139 n.s.	0.066 n.s.	-0.141	0.254 n.s.	0.247 n.s.	-0.132	0.228 n.s.	0.246 n.s.
13	-0.152	0.336 n.s.	0.223 n.s.				-0.426	0.113 n.s.	0.028 n.s.