

TRIPTEROCALYX CARNEUS (NYCTAGINACEAE) IS SELF-COMPATIBLE

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ABSTRACT—Recent phylogenetic work has demonstrated that the ability of species of the angiosperm family Nyctaginaceae to self-fertilize is evolutionarily labile. However, the potential for further investigation of the evolution of mating systems in the family is limited, because there is no information on reproductive biology for several genera. I performed an experiment on a natural population of *Tripterocalyx carneus* to determine whether this species is self-compatible. Individual flowers were emasculated, bagged to exclude pollinators, or both. Flowers that were bagged but not emasculated set viable fruit in the majority of cases, which demonstrates that this species is self-compatible.

RESUMEN—Un estudio filogenético reciente ha demostrado que la capacidad de autofecundación de las especies de la familia Nyctaginaceae de las angiospermas es evolutivamente cambiante. Sin embargo, el potencial de investigaciones extensas sobre la evolución de sistemas de reproducción en la familia es limitado, porque no hay información sobre la biología reproductiva de varios géneros. Realicé un experimento sobre una población natural de *Tripterocalyx carneus* para determinarse si esta especie es autocompatible. A flores individuales se les removieron los estambres o fueron embolsadas para excluir a los polinizadores, o ambos. El grupo de flores que fue embolsado, pero al que no se le removieron los estambres, produjo frutos viables en la mayoría de los casos, lo cual demuestra que esta especie es autocompatible.

The four o'clock family (Nyctaginaceae) contains both self-compatible and self-incompatible species. As part of a recent phylogenetic study of the family (Douglas and Manos, 2007), we compiled and reviewed all known reports of mating systems of Nyctaginaceae. We found that based on current knowledge, at least three evolutionary gains, or alternatively, six losses of self-incompatibility are required to explain the current distribution of known mating systems in Nyctaginaceae. This is a minimum estimate of the number of transitions in mating systems in the family: we based our inferences on experimental reports for some species, anecdotal reports for others, and certain assumptions (i.e., that species with stylar movements that ensure self-pollination, or that possess cleistogamous flowers, are in fact self-compatible; and that dioecious species are self-incompatible). Data were not available for all genera, nor do all genera have morphological or "behavioral" traits (e.g., Spellenberg, 2000) that allow inferences about reproductive systems to be made. Some genera contain both self-compatible and self-incompatible species. For instance, *Mirabilis*

is largely self-compatible, but one section, *Quamoclidion*, is comprised of self-incompatible species (Cruden, 1973; Pilz, 1978; Hernández, 1990; Hodges, 1995; Leal et al., 2001)

Of particular interest with regard to evolution of mating system is the tribe Abronieae. Although some authors have considered Abronieae to be monogeneric (Bittrich and Kühn, 1993), most authors have maintained two genera, *Tripterocalyx* and *Abronia* (Galloway, 1975; Spellenberg, 2003). As in *Mirabilis*, both self-incompatible and self-compatible species are known in *Abronia*. Experimental evidence has shown that *Abronia macrocarpa* most likely has a sporophytic self-incompatibility system (Williamson et al., 1994; Williamson, and Bazeer, 1997). The three maritime species, *Abronia latifolia*, *A. maritima*, and *A. umbellata* failed to set seed when experimentally self-pollinated (Tillett, 1967). In a different study, *Abronia umbellata breviflora*, however, was observed to self-pollinate and set viable seed in a greenhouse (McLaughlin et al., 2002). Finally, Saunders and Sipes (2006) reported a mixed mating system for the narrowly endemic *Abronia ammophila*. Although the mat-

ing system has been experimentally examined in only six species or subspecies of *Abronia*, it is worthwhile to have information on mating systems from the probable sister genus, *Tripterocalyx*, to aid in understanding the evolution of mating systems in *Abronia* specifically, and more generally, to increase confidence and resolution in phylogenetic reconstructions of the evolution of mating systems in Nyctaginaceae.

I examined the ability of *Tripterocalyx carneus* to self-fertilize. Like *Abronia*, *Tripterocalyx* produces umbellate inflorescences of salverform flowers with a showy limb and a narrow tube, which indicates that lepidopterans are likely pollinators. Anthesis occurs in the early evening. The highly fragrant flowers are open through the night and wilt the following morning. Like nearly all other Nyctaginaceae, the fruit is an achene, surrounded by an "anthocarp," a persistent accessory fruit that develops from the lower part of the uniseriate perianth. In *Tripterocalyx*, the anthocarp enlarges greatly, hardens, and develops three or four large, membranaceous wings.

The study was conducted at a site on the U-Bar Ranch, Grant Co., New Mexico. A large population of >40 individual plants was located in sandy loam along the Gila River. The plants were arranged around a circular clearing (result of activity by harvester ants). All plants in the population were well established and each had several umbels producing flowers. I chose 12 plants adjacent to the clearing, and on a single unopened flower in each of four separate inflorescences on each plant, I performed one of the following manipulations: Treatment 1) The flower was slit down the side of the perianth with a razor blade, and with fine forceps, the filaments and anthers were removed. Afterwards, a fine-mesh drawstring bag was placed over the emasculated flower to exclude pollinators. I anticipated that no seed would mature with this treatment. Treatment 2) The flower was emasculated as above, but not bagged, so that seeds that matured would be the result of either geitonogamy (in which an ovule is fertilized by pollen from a different flower on the same plant) or xenogamy (in which the pollen is transferred from a different individual), but not intrafloral self-pollination. Treatment 3) Bags were placed over non-emasculated flowers. Any seeds that resulted from this treatment would be the result of intrafloral self-pollination and would confirm self-compatibility. Treatment 4)

Control; flowers were neither emasculated nor bagged, to provide an estimate of the proportion of flowers that would form a viable seed under field conditions.

While hand-pollinations would have been the most straightforward way of testing self-compatibility, it proved to be too difficult to make these manipulations accurately under field conditions. Subsequent to each manipulation, I tied a thread (colored according to treatment) loosely around the lower part of the perianth, which would eventually develop into the accessory fruit. This thread served to identify the fruit resulting from the manipulated flower while allowing me to avoid modifying the surrounding flowers in the umbel. All manipulations took place on the morning of 11 July 2001. I visited the population that evening to verify that the manipulated flowers opened normally, and to observe activity of pollinator in the population. The population was revisited during the morning and evening on 12 and 13 July, at which point the flowers had completely senesced. I then removed the bags. On 5 August, I returned to the population, at which point fruits were fully mature on the manipulated umbels, but had not dispersed. I relocated and collected fruits using the colored threads. In those cases where a fruit failed to develop, the small remnant of the perianth was still surrounded by the thread and all fruits or undeveloped remnants of flowers were recovered while still attached to the maternal plant. Thus, I was able to know the outcome of each manipulation of a flower. I opened all fruits in the lab to ascertain presence or absence of a mature achene. The proportion of anthocarps developing a mature achene were determined for each treatment and compared using a generalized linear model with logit link function (McCulloch and Searle, 2001) to determine whether there was a significant difference in proportion of mature achenes developed under each treatment.

The first evening after flowers were treated, all flowers opened normally. Because, in altered flowers, the slits were longitudinal along the tube of the flower, leaving the limb intact, emasculated flowers were not greatly distorted from the perspective of floral visitors. Presence of bags did not interfere with normal opening of the flower. In *Tripterocalyx*, the stamens and style are included in the floral tube; thus, the bag never contacted either organ. Numerous hawkmoths

TABLE 1—Seeds matured according to pollination treatment, and additional empty fruits produced. Proportions of matured seeds followed by same letter do not differ significantly ($P > 0.05$).

Pollination treatment	<i>n</i>	Mature seeds	Additional empty fruits	Proportion of mature seeds
Emasculated, bagged	12	1	2	0.083 a
Emasculated, not bagged	12	10	0	0.833 b
Intact, bagged	12	8	0	0.667 b
Control	12	11	1	0.917 b

(*Hyles lineata*, Sphingidae) visited flowers in the population. Moths visited both emasculated and unmodified flowers (and in several instances, probed the mesh bags). The following morning, all flowers that had opened the previous night were wilted and drooping, but the color had not faded. The second evening, many weakly reopened, including some that had been emasculated. The following evening, no treated flower opened, and the petaloid distal portion of the perianth had fallen from some flowers and was faded in the rest.

When fruits were collected, it was apparent that many had failed to mature. Whereas matured anthocarps were large (20–30 mm in length, with wings ≤ 12 mm broad), and usually filled with a large (7–10 mm) achene, the undeveloped anthocarps were small and shriveled (7 mm long, with small wings < 5 mm broad). These anthocarps were not filled with an achene, and essentially represented remnants of the lower perianth that ceased development after the flowering stage due to the ovule not being fertilized. Tillett (1967) and Galloway (1975) reported that some *Abronia* would mature anthocarps for all flowers on an umbel if even one flower on an umbel were fertilized, although most anthocarps would be empty. In this experiment, a similar phenomenon might have been at work; I found three empty, but mature, anthocarps, although in most cases where an achene failed to mature, the anthocarp was aborted also.

Table 1 shows number of mature achenes produced (and additional empty anthocarps, if any) with respect to treatment applied. The procedure for emasculating and bagging flowers was not 100% effective. In Treatment 1, one flower was still able to produce a normal seed, although this represented a significantly smaller proportion ($P < 0.05$) than in other experimental treatments. Barring apomixis, the only expla-

nation is that I accidentally disrupted an anther sac in the process of removing a stamen from the flower. Nyctaginaceae produce single-seeded fruits, and a single stray pollen grain could effectively pollinate the flower.

Despite limitations of technique, Treatment 2 showed that *T. carneus* is an effective outcrosser, because most emasculated flowers that were open-pollinated set normal fruits. This is not surprising, considering the vigorous attention paid to plants by hawkmoths during the experiment.

Treatment 3 demonstrated convincingly that the species is self-compatible. Intact bagged flowers set fruits in 8 of 12 cases. Mesh on bags I employed was much too fine to be penetrated by the proboscis of *Hyles lineata*. Drawstrings on the mesh bags enabled me to exclude crawling insects, and no insect was found inside bags when they were removed. Therefore, self-pollination is the only explanation for the high number of seeds set. It also is consistent with the unexpected results from Treatment 1; if this species was self-incompatible, I would have had to transfer outcrossed pollen to a stigma enclosed in an unopened bud, rather than simply leaving a grain of self pollen behind.

The proportion of matured achenes in Treatments 2 and 3 did not differ significantly ($P > 0.05$) from each other or from the proportion matured in Treatment 4, the open-pollinated control group. All but one of the unmodified flowers in the control group produced a mature anthocarp filled with a normal achene, indicating that with a combination of self-pollination and outcrossing, *T. carneus* is able to achieve high rates of successful pollination.

These results confirm the ability of *T. carneus* to self-fertilize and suggest that the species possesses a mixed mating system. It is not yet possible to infer the ancestral state for the tribe Abronieae based on current knowledge phylog-

eny and reproductive compatibility. However, as future studies address the phylogeny and reproductive biology of Abronieae, it will be of interest to see whether the apparent lability in mating system is evidenced at fine taxonomic levels, and to investigate correlations with life history, habitat, and pollination ecology.

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