Revisiting an old question in California botany: Why do many plant species have five-petaled flowers?

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If one takes the time to observe many different species of flowering plants, one may notice that a large proportion of them have five-petaled flowers. Not only are five-petaled flowers widespread, but petal number is remarkably constant within plant families and is often used to differentiate among families (*1, 2*). Consistently five-petaled flowers, with few shifts to other petal numbers, is one of the defining characteristics of Pentapetalae: a massive clade of ~175 plant families comprising much of flowering plant diversity (*3, 4*).

These patterns in Pentapetalae raise some questions: why is petal number so invariant in Pentapetalae, and why is five petals the predominant number? Early botanists noticed these patterns in petal number, and the popular explanation was that pollinators preferred a certain number of petals, thereby selecting for that number as well as a reduction in variation (e.g., *5-7*). In particular, during the 1950s, Elmar Leppik demonstrated that various pollinators were able to "count" by manipulating the number of petals on flowers to force pollinators to differentiate among them to gain rewards. He found that bees and butterflies could differentiate among petal numbers, but that flies, beetles and weevils could not (*6, 8*). From his observations, he noted anecdotally that bees preferred five-petaled flowers to other petal numbers (*6*).

Leppik's work was well known at the time, even making the New York Times (*9*) and it attracted the attention of an eminent California botanist and evolutionary biologist, George Ledyard Stebbins. Stebbins was in the process of writing his second treatise on plant evolution (*10*), and his graduate student, Carl Huether, had been studying petal number variation in normally five-petaled *Leptosiphon androsaceous* (Polemoniaceae) in the mountains around San Francisco. Huether found that 1-4% of *L. androsaceous* flowers deviated from five petals in natural populations, with an increasingly higher proportion of four-petaled flowers, relative to six-petaled, as environmental conditions became drier (*11*). However, field observations suggested that the pollinators (bombyliid and syrphid flies) did not discriminate against four- or six-petaled flowers (*10*). Despite this apparent lack of pollinator preference, Stebbins concluded that a fixed petal number was adaptive and maintained by natural selection (*10*). He also called for more research on pollinator preferences for petal number (*12*), since this had not been thoroughly explored. Alas, in the ensuing 45 years, the field turned elsewhere.

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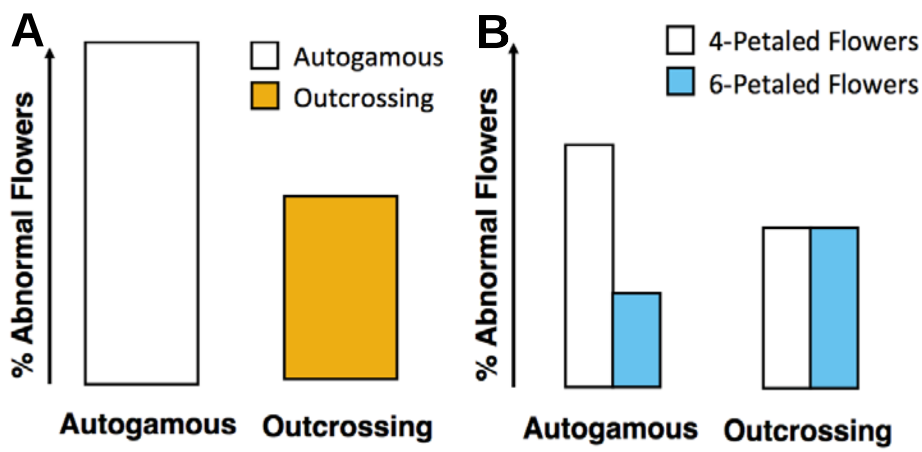

Figure 1. Hypothetical differences in the percentages of abnormally-petaled flowers between self-pollinated (autogamous) and outcrossing species under our hypotheses. In A), if pollinators select for invariant petal number, autogamous species would be expected to have a higher percentage of abnormal flowers because pollinator selection would be relaxed. In B), if higher petal numbers are costly, autogamous species would be expected to have more four-petaled flowers and perhaps also fewer six-petaled flowers than outcrossers.

We decided to revisit this old conundrum and assess the evidence for pollinator selection on petal number, or traits correlated with it, (*13*) using a different method: by comparing the amount of petal number variation between species that were pollinated by insects (outcrossing), and those that could self-pollinate without any insect assistance (autogamous). If selection by pollinators were responsible for the predominance of five-petaled flowers, then species freed from such selection, such as autogamous species, might be expected to exhibit more variation in petal number (Figure 1A). In addition, if making petals is costly to the plant – they can lose a lot of water (*14*) and can also attract herbivores (*15*) – we might expect that autogamous plants would produce flowers with fewer than five petals (Figure 1B).

We have focused on species in the Polemoniaceae because they have natural variation for petal number, and there is evidence for a genetic basis for this variation (up to 16% petal number variants: *11*, *16-18*; Figure 2). In addition, the pollination biology of the Polemoniaceae is unusually well characterized, thanks to the work of Verne and Karen Grant, who were also California botanists and contemporaries of Stebbins. In a monumental effort, even by current standards, the Grants collected detailed data on 122 species in the Polemoniaceae, identifying the primary pollinators, and testing whether species could self-pollinate (*19*). This combination of data on petal number variation, primary pollinators, and mating systems provides a useful system to test our two main hypotheses: 1) Autogamous species should have higher levels of petal number variation, compared to that of outcrossers, if pollinators are directly or indirectly selecting for five petals; and 2) Autogamous species should show a shift toward more four-petaled flowers, if having more petals is costly.

We selected two genera from the Polemoniaceae to work with: *Gilia* and *Saltugilia*. Both genera are desert annuals occurring in similar geographic regions and dry habitats, and each contains closely-related species that are autogamous or outcrossing. *Gilia cana* and *S. splendens* ssp. *splendens* are pollinated by bombyliid flies, and *G. sinuata*, *S. latimeri*, and *S. australis* are autogamous self-pollinated species (*19, 20*). For each species, petal number for a minimum of 500 flowers (# plants: 140-435) was recorded for three separate populations in April 2015 (Table 1). These populations were located across Southern California within Mojave National Preserve, Sweeney Granite Mountains Desert Research Center, Joshua Tree National Park, San Bernardino National Forest, the Pioneertown Mountains Preserve (Wildlands Conservancy), and natural areas managed by the Bureau of Land Management. Voucher specimens were collected for all the species sampled at each site and deposited in the CONN herbarium at the University of Connecticut (CONN00200743–CONN00200833). Data were summarized as per-individual proportions of each petal number.

All species we studied exhibited natural variation in petal number, ranging from < 1% of flowers that were not five-petaled in *S. splendens*, *S. australis*, and *G. sinuata*, to 4% in *S. latimeri*. In most cases, variants were four- or six-petaled, though more extreme variation was occasionally found (0.1% of flowers), particularly in *S. latimeri* (e.g., Figure 2). Since this extreme variation was rare, it was grouped with four- and six-petaled flowers for analysis. Species with more petal number variation mostly produced more six-petaled flowers; proportions of four-petaled flowers were similar across species. Despite these differences among species, autogamous species did not have greater variation in petal number compared to outcrossing species (Figure 3).

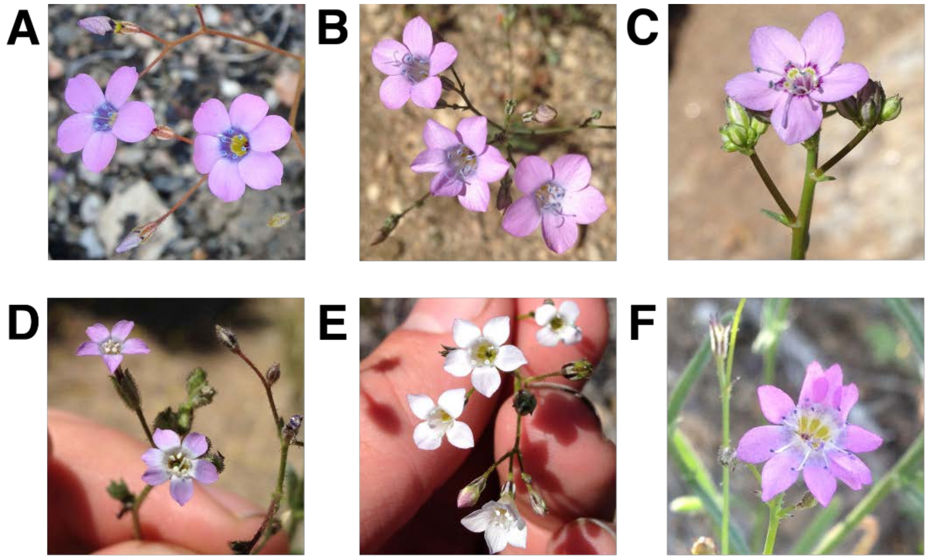

Figure 2. An assortment of variation in natural populations of petal number across species in *Gilia* and *Saltugilia* in the Polemoniaceae, which normally have five-petaled flowers. A) *Gilia cana* ssp. *speciformis*; B) *Saltugilia splendens* ssp. *splendens*; C) *S. caruifolia*; D) *G. sinuata*; E) *S. australis*; F) *S. latimeri*. Most abnormal flowers are either four-petaled or six-petaled, though more extreme variations exist (e.g., nine petals in F).

Table 1. A list of the field site locations where species were sampled across Southern California. Note that multiple species were sampled at several sites.

Site	Species	Latitude	Longitude
Granite Cove	*G. sinuata*	34.78238	-115.65548
Black Rock	*G. sinuata*, *S. latimeri*, *S. splendens* ssp. *splendens*	34.06982	-116.39351
Burns Crossroad	*G. sinuata*	34.22112	-116.62119
Kelbaker[1]	*G. cana* ssp. *speciformis*	35.20488	-115.87035
Aiken Mine	*G. cana* ssp. *speciformis*	35.18528	-115.76691
Rattlesnake Canyon	*G. cana* ssp. *bernardina*	34.23017	-116.65197
Smarts Quarry	*G. cana* ssp. *bernardina*	34.30404	-116.79989
Elata Ave.	*S. latimeri*, *S. splendens* ssp. *splendens*	34.07416	-116.41512
Elk Trail	*S. latimeri*	34.07486	-116.43531
Burns Spring	*S. splendens* ssp. *splendens*	34.20462	-116.57495
HWY 243[2]	*S. australis*	33.89241	-116.85896
S22 PCT 0.4S	*S. australis*	33.21182	-116.58227
S22 PCT 1.5S	*S. australis*	33.20837	-116.57798
S22 PCT 2.4S	*S. australis*	33.20389	-116.56817

[1] Small population with 12 plants and 53 flowers

[2] Small population with 75 plants and 124 flowers

Within genera, autogamous species exhibited more petal number variation in *Saltugilia*, but less in *Gilia* (Figure 3). Similarly, there was no overall pattern of autogamous species having more four-petaled or fewer six-petaled flowers, though autogamous species also showed divergent patterns for six-petaled flowers (Figure 3). In each case, the trends in *Saltugilia* were driven by *S. latimeri* – it had an unusually large proportion of six-petaled flowers.

Based on these results, there is no evidence that the absence of pollinators results in differences in selection on petal number in autogamous species, or that there are selective advantages to reducing petal number. Though these species were not pollinated by bees, as in Leppik's work (*6*), this calls into question the old assertion that pollinator preferences drove trait fixation on five petals. We do not find this result surprising, as it is difficult to conceive of a reason why a particular pollinator would prefer flowers with five petals over those with some other number, if other advertisement traits remained unchanged.

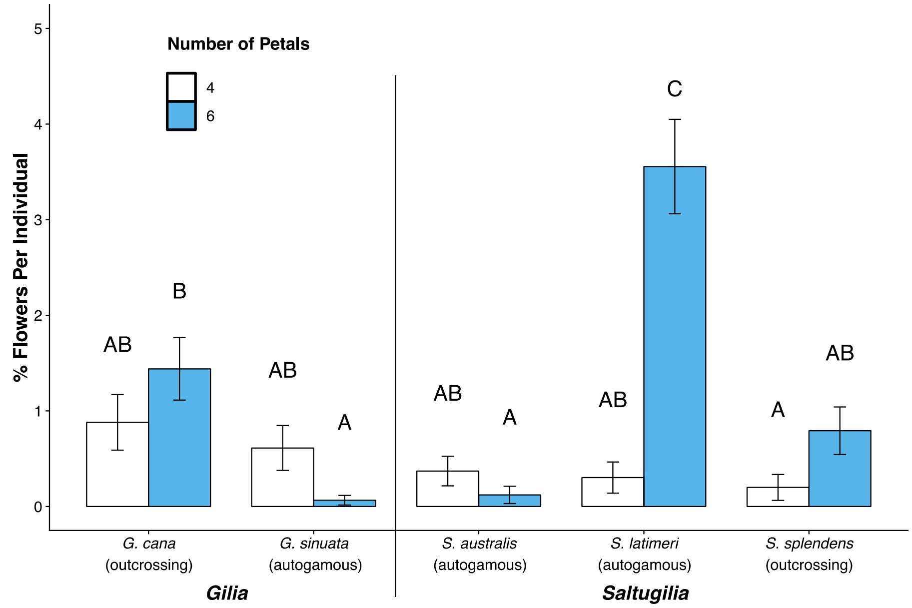

Figure 3. The difference in petal number variation for autogamous species relative to that of outcrossers within each genus. Error bars represent standard errors of the mean per-individual percentages. Letters represent significant groupings from Tukey post-hoc tests with bonferroni corrections. In *Gilia*, there is less overall petal number variation and significantly fewer six-petaled flowers in the autogamous species (*G. sinuata*). In *Saltugilia*, the trends are reversed in *S. latimeri* (more overall variation, significantly more six-petaled flowers), while *S. australis* has less overall variation and fewer six-petaled flowers than the outcrosser. No genera show differences in the percentage of four-petaled flowers between autogamous and outcrossing species.

Differences among species are present in the proportions of four- and six-petaled flowers, both among those species we sampled, and among species in other studies (*11, 17, 18*). As noted above, *S. latimeri* showed substantially more petal number variation than other species we studied, particularly for flowers with six or more petals. *Saltugilia latimeri* is a recently described and poorly-studied species with small populations and a restricted geographic range, and perhaps random genetic drift has played a role in influencing the control of petal number. More species need to be assessed to determine what drives these differences in petal number among species and whether species such as *S. latimeri* are unusual outliers, or fit within the range of normal variation among species. We were unable to include two additional *Saltugilia* species (*S. caruifolia* and *S. splendens* ssp. *grantii*) because they bloom later in the season. These two species are pollinated by different pollinators: bees and hummingbirds, respectively.

Although most research on natural variation in petal number has occurred with species in the Polemoniaceae, it is less clear what patterns of natural petal number variation (if any) are present in other plant families. While pollinators may not be the primary factor constraining plants to five-petaled flowers, having a certain petal number may still be adaptive, as Stebbins theorized (*10*), but for other reasons. One possibility is that the genetic framework that produces a certain petal number may also control other traits. In this case, changes in petal number could result in changes to other floral or vegetative traits that might be maladaptive. Petal number is correlated with the numbers of sepals, stamens, and carpels (*17, 18, 21*), and changing petal number may adversely affect traits such as pollen production or seed set (*18*). Unfortunately, little is known about specific genetic and developmental processes that predictably lead to specific petal numbers. In our future work, we hope to explore aspects of these processes to ask further questions about whether having consistently five-petaled flowers is adaptive and why this is the predominant pattern in Pentapetalae.

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In Memoriam – Robert Fulton

With great sadness we report that Robert "Rob" Fulton, outstanding manager of the California State University Desert Studies Center for 32 years, passed away at the age of 63. Rob was a true naturalist and an exceptional desert ecologist. Over the years, Rob touched the lives of thousands of students and researchers by enthusiastically sharing his keen knowledge and passion for the desert. Rob served as mentor, friend, and colleague to many in the science and land management community of the Mojave Desert, including the editors of this newsletter. He will be dearly missed.

Cards and messages to Fulton's family may be sent in care of the CSU Desert Studies Consortium, College of Natural Sciences and Mathematics, Cal State Fullerton, 800 N. State College Blvd., Fullerton, CA 92831.

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