An increase in atypical petal numbers during a shift to autogamy in a coastal sand verbena and potential evolutionary mechanisms

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Abstract

Premise:

The evolution of variation in reproductive traits is of longstanding interest in biology. In plants, meristic traits, such as petal and sepal numbers, are usually considered invariant within taxa. However, certain species consistently exhibit great variability in these traits, though the factors contributing to "atypical" counts are not well-known. The sand verbenas, *Abronia* (Nyctaginaceae), usually have five perianth lobes ('petals') in their fused corollas and are self-incompatible, thus departures from either of these norms in populations, varieties, or species are of evolutionary interest.

Methods:

To characterize and understand an increase in atypical petal numbers during a transition from xenogamy (outcrossing) to autogamy (selfing) in the coastal sand verbena *Abronia umbellata*, we integrated common garden studies with analysis of over 11,000 photographed flowers from iNaturalist, a citizen science project. Here we evaluate several adaptive and nonadaptive explanations for the production of these 'atypical' flowers.

Key results

Our photo analysis and common garden show that the nominate xenogamous variety has 5 petals with very little variation, however, an autogamous, geographically separated variety, *A. u.* var. *breviflora* has a high preponderance of four-petalled morphs. Flower morph did not affect successful autogamy, and petal numbers were not related to environmental factors, hybridization, or flower size in the ways hypothesized.

Conclusions

We conclude that this loss of petals is consistent with relaxation of selection on petal number in selfers, inbreeding leading to a loss of developmental stability, or correlated selection on another trait. This study strongly demonstrates the power of data available from public citizen databases for easily scored traits, such as petal number.

Keywords: Abronia; autogamy; citizen science; floral traits, hybridization, iNaturalist, meristic variation, Nyctaginaceae, petal number,

INTRODUCTION

Understanding variation in reproductive traits has long been a focus in evolutionary biology. Taxonomic and geographic variation in reproductive phenotypes can provide key insights into fundamental evolutionary processes such as selection, speciation, and adaptation. In plants, petal number and other meristic characters are easily quantified reproductive traits. On one hand, these characters are often considered fixed within species, genera, or families and, as such, are commonly used in species descriptions, keys, and other taxonomic and identification resources. Any departure from a 'normal' meristic character within a taxon, whether species, genus, or even family, is thus of evolutionary, developmental, morphological, and taxonomic interest (Ellstrand, 1983). On the other hand, interspecific transitions in petal number or intraspecific variants in petal number are quite common in wild plants of many families, and may take the form of a change in mean or variance within or among species (e.g. Stark, 1918; Lowndes, 1931; Saunders, 1934; Roy, 1962; Huether, 1968; Schemske, 1978; Ellstrand, 1983; Lehmann, 1987; Shepard et al., 2005). Despite interest in the development, breeding, genetics, and evolution of of meristic variation, critical examination of evolutionary drivers of patters of variation, whether adaptive or non-adaptive, are scarce. Here, we integrate lab, field, and citizen-science data to examine support for several *a priori* hypotheses (Table 1) about patterns of petal number variation using a coastal sand verbena (*Abronia umbellata*, Nyctaginaceae) where allopatric varieties show remarkably different patterns of variation in perianth lobe (hereafter petal, as functionally equivalent) number.

Several hypotheses concern petal number evolution in plants, broadly falling into adaptive and non-adaptive categories. The invariance of petal number, notably across many taxa in the ancestrally five-petalled Pentapetalae (a group which includes much of Eudicot diversity:

Cantino et al., 2007; Soltis and Soltis, 2013), suggests a highly conserved developmental program (Endress 2001), or selection maintaining constancy (Stebbins, 1974; Herrera, 2009). If the mechanism is selective, pollinator choice or innate preference would be obvious candidates for the maintenance of petal number. While pollinator selection has been long-hypothesized (e.g. Leppik, 1953), positive evidence for this hypothesis remains elusive. Certain pollinators may have the capability to 'count' petals, though many others were not found to differentiate petal numbers (e.g., Leppik, 1953; Golding et al., 1999; Mickley, 2017). In this vein, Mickley and Schlichting (2018) devised an alternate way of examining pollinator-mediated stabilizing selection on petal number. They reasoned that if pollinators were maintaining a fixed petal number, autogamous species would lack constancy due to relaxed selection on petal number. Using several closely-related species of Polemoniaceae, they found no evidence that xenogamous species had greater constancy in petal number than autogamous congeners. However, using many species in a comparative phylogenetic analysis would be necessary to robustly test this hypothesis.

An alternative, but related, hypothesis is that loss of petals in autogamous taxa may be adaptive, but unrelated to pollinator preferences, if petals are costly (e.g., Galen, 1999; Strauss & Whittall, 2007; Lambrecht 2013) or if loss of petals increases autogamous efficiency. The autogamous efficiency hypothesis was formulated by Monnaiux et al. (2016) as a potential mechanism by which *Cardame hirsuta*, an autogamous mustard, has reduced petals from the four-petals typical of Brassicaceae. Specifically, they hypothesize that petal loss slows bud opening and promotes more effective selfing, though they do not test the proposed hypothesis (Table 1: H1). However, a later paper found that a genotype with reduced petal numbers had a lower outcrossing rate in the field, but did not differ in seed set in the lab (Monnaiux et al., 2018). Therefore, it is unlikely

that low petal number increased autogamous success in *C. hirsuta*, as hypothesized, but this intriguing hypothesis certainly deserves testing in other plant species.

Non-adaptive evolutionary or phenomenological hypotheses have also been proposed to drive petal number variation. For example, inbreeding itself, independent of pollinator-mediated selection could reduce meristic stability (Table 1: H2). Another set of non-adaptive hypotheses focus on hybridization. Hybridization, especially between distantly-related taxa, may cause breakdown in the canalization of normal development due to genetic incompatibilities, e.g. outbreeding depression, Dobzhansky-Muller incompatibilities (Bachman et al., 1981; Pelabon et al., 2003). Hybridization led to an increased number of atypical petals in intrageneric hybrids of both *Gilia* and *Rubus* (Grant, 1956; Bammi and Olmo, 1966). Hybridization between *Microseris* species with 5 and 10 pappus parts respectively led to complete breakdown of canalization, with all combinations between the parents represented (Bachmann et al., 1981). However, Choi et al. (2001) found that, in Pseudostellaria, crosses between a species with variable petal number and one with invariable petal number produced hybrids without petal number variation, a similar result to certain *Solanum* hybrids (Bletsos et al., 1998). In systems where hybridization occurs, it is possible that incompatibilities due to hybridization could drive atypical petal counts (Table 1: H3); alternately, different developmental processes might cause hybridization to stabilize petal number.

Regardless of the specific hypothesized mechanism, if evolution is to change petal number in any way, it must have a heritable component. A preponderance of published evidence bears this assumption out. Petal number was found to be heritable or genetic control was demonstrated in *Begonia semiovata*, *Cardamine hirsuta*, *Dianthus cariophyllus*, *Gilia* spp., *Impatiens balsamina*, *Linanthus androsaecus*, *Portulaca grandiflora*, *Rosa wichurana*, *Spergularia maritima*, *P.*

drummondii (Grant, 1956; Alpi et al., 1968; Katsuyoshi and Harding, 1969; Stevens et al., 1972; Agren and Schemske, 1995; Delesalle and Mazer, 1995; Mazer et al., 1999; Tooke and Battey, 2000; Byerley, 2006, Pieper et al., 2015; Roman et al., 2015; Monnaiux et al., 2016, 2018; Mickley 2017). Together with the invariance of meristic traits within most taxa, these studies show that some level of heritability in petal number and other meristic traits is expected across plants (and indeed, our ability to describe and identify plants based on these characters depends on it!). If heritable, these same genes could be responsible for other traits involved in meristic development and therefore, selection on other traits could correlate with shifts in petal number (e.g. Table 1:H4).

Finally, while petal number is demonstrably heritable in most systems, several studies have additionally found evidence of plastic variation in petal number (Table 1: H5). While the genetic control of atypical petal numbers in *Cardamine hirsuta* is well-known, there is a seasonal component to the expression, with atypical petal numbers being more common in summer than spring (McKim et al., 2017). Huether (1968, 1969) found that herbivory, day length, and temperature increased petal number variation, though only in some plants, suggesting GxE interactions similar to that of *C. hirsuta*. Hoffman et al. (2009) found that an elevational gradient in atypical floral number in *Stylidium armeria* disappeared in a greenhouse common garden, suggesting an environmentally plastic component. In populations of *Spergularia maritima*, greenhouse grown plants consistently had fewer petals than plants in the populations from which seeds were collected, however, petal number had the highest broad-sense heritability of all reproductive traits they measured (Delesalle and Mazer, 1995). Roy (1962), with the help of a dedicated field assistant, one JBS Haldane (!), found that while mean petal number of individual *Nyctanthes arbor-tristis* plants did not change markedly through the season (< 5%), the variance

increased substantially (20-40%) from beginning to end of season. Developmental changes could also explain variation in certain cases. Older clones of a buttercup (*Ranunculus repens*) had higher proportions of atypical petal numbers, a result which Warren (2009) ascribes to the accumulation of somatic mutations over time, but this result could also be driven by other age-correlated factors, such as hormonal changes. The production of repeated units in an organism, i.e. many flowers on a plant, inevitably leads to some variation in these parts (Herrera, 2009). A model of developmental stochasticity over repeated floral development led to realistic variation in petal numbers (Kitazawa and Fujimoto, 2014). This suggests that no matter the characteristics of the plant; one with many flowers will have a higher likelihood of a greater range of atypical petal numbers than without (sampling effects of this sort also discussed in Roy 1962).

Despite the various hypotheses proposed for petal number variation, no investigations have addressed multiple hypotheses in a single system or characterized variation across species' entire range in the field. Petal number can be scored on live plants, herbarium specimens, or photographs. Rapidly growing georeferenced photographic databases from citizen science initiatives (e.g. iNaturalist, CalFlora, etc.), hold massive amounts of easily quantified data, extraordinarily well-suited for studies of petal number variation. Photographic citizen science data have been used recently in other studies for easily scored traits including plant pathogen infection rates (Kiddo and Hood, 2020) and lizard ectoparasite presence (Putnam et al., 2020). Therefore, these databases represent an exciting complement to herbarium and other natural history collections for scoring of characterization of traits.

However, scoring phenotypes from field photos alone cannot disentangle evolutionary and plastic drivers of trait variation. As such, integrating large, range-wide field photography datasets with carefully controlled common garden experiments is a powerful approach for evaluating

wide-scale patterns of natural field variation in light of genetic variation in the lab. Here, we employ this integrative approach to address several proposed hypotheses for atypical petal numbers (Table 1) using the naturally variable species *Abronia umbellata*. In particular, we capitalize on natural variation across two a natural transition, a southern, outcrossing variety (=subspecies in Nyctaginaceae taxonomic conventions) with typical 5-lobed flowers, and a northern selfing variety with 4-lobe flowers (any deviation from five is considered 'atypical' for our purposes). We integrated a greenhouse common garden of *A. umbellata* subspecies and hybrids, and photographic analysis of over 11,000 citizen science (iNaturalist) photos spanning the entire *A. umbellata* range to characterize the petal number variation and evaluate several adaptive and non-adaptive hypotheses (Table 1). Together, this study represents a comprehensive integrative analysis of petal number variation in a single system, and characterizes an evolutionarily compelling transition in reproductive morphology in association with the evolution of selfing.

MATERIALS AND METHODS

The geographic range of* Abronia umbellata *and its varieties — *A. umbellata* grows on beaches and coastal dunes – never more than a few hundred meters inland - from British Columbia, Canada, south to Baja California, Mexico. It has two geographically separated, genetically-distinct varieties (=subspecies) across its range on the Pacific Coast of North America (Baja California, Mexico – British Columbia, Canada) (Greer, 2016; Van Natto, 2020). The southern variety (*A. u. umbellata*) is self-incompatible, and therefore xenogamous, whereas the northern variety (*A. u. breviflora*) is self-compatible and probably nearly completely autogamous in nature (Doubleday et al., 2013; Doubleday and Eckert, 2018; LoPresti, pers. obs.). Initial observations (E.F.L.) suggested that the southern xenogamous (outcrossing) *A. u.*

umbellata has very little variation in perianth lobe (hereafter, petal) number, almost all flowers having the typical five petals. In contrast, the northern autogamous *A. u. breviflora* has strikingly more variable petal number (Fig. 1: A-D). The autogamy, and petal number variation, of this variety is likely derived. Almost all *Abronia* species are self-incompatible (Tillett, 1967; Galloway, 1975; Williamson et al., 1994; Darling et al., 2008; LoPresti, unpublished data), and thus, the shift in production of atypical flowers in *A. u. breviflora* occured with a transition to autogamy (Doubleday et al., 2012; Greer, 2016; Van Natto, 2020).

The opening of San Francisco Bay (~37.8° N) separates the northern *A. u. breviflora* from *A. u. umbellata* (Doubleday et al., 2013; Greer 2016; Van Natto 2020). Various authors have posited that specimens, especially from southern Sonoma county (~38.3° N), do not fit this break point (e.g. Tillett 1967). However, all plants from north of the bay, even if not fitting the Galloway (2003) or Murdock (2012) measurements for *A. u. breviflora*, appear to be selfing, both from common garden, and field collections (a high proportion of filled fruit is indicative of autogamy in coastal *Abronia*), a result also found by Darling et al. (2008) and Van Natto (2020). Population genetic data also shows a cluster from the north and a cluster from the south (Greer, 2016), though the break was not entirely possible to ascertain completely as it did not include any samples close to the boundary.

Darling et al. (2008), in an examination of range limits and dispersal of the species, treated the northern range limit as southern Oregon, USA (~43° N), as populations north of that point are small, rare, and protected from collecting. They considered the southern limit in Baja California, Mexico as ~30° N (these range limits also used in Samis, 2007; Greer, 2016; Van Natto 2020). We consider the range limit differently in this study, using 49° N to 32.5° S, with the caveat that our southern limit is conservative. Our northern range limit is accurate, as both historical

herbarium collections and a few extant populations range northward to British Columbia, Canada (49° N); sometimes these northernmost populations are treated as a separate variety, *A. u*. var. *aculata*, though we do not treat this as a separate variety from *A. u.* var. *breviflora* as they are morphologically indistinguishable in our experience (also supported by the analyses of Van Natto, 2020; though taxonomic priority belongs to *A. u.* var. *aculata*).

The southern range limit is truly unknown because of a gradation in morphology into *A. gracilis* and, probably, *A. villosa*, almost certainly indicative of an extensive hybrid zone (see Ann Johnson's extensive collections and determinations of hybrids in Baja California at the UC-Davis herbarium). This accords well with previous knowledge of the genus; when growing in sympatry, many sand verbenas hybridize naturally, and hybrids are easy to make and usually successful in artificial crosses (Tillett, 1967; Galloway, 1974; Johnson, 1978; Van Natto, 2020; Edwards & LoPresti, unpublished data). While we have seen herbarium specimens from as far south as 24° N have been annotated as *A. umbellata*, these are unlikely to be accurate or of non-hybrid origin. Tillett (1967), in the most comprehensive examination of coastal *Abronia* species, found *A. gracilis* at San Simon (30.5° N) and apparent hybrid *A. gracilis* x *A. umbellata* at San Quintin, Baja, MX (30.6° N; both records north of the farthest south populations in Darling et al 2008). However, from herbarium specimen examination, he notes that *A. gracilis* sometimes occurs as far north as San Diego county (i.e. north of 32.5° N), a result which we doubt. The farthest north record we find convincing is a record from iNaturalist at 31° north (record #16085273) which is morphologically indistinguishable from *A. gracilis*, though could certainly be of hybrid origin. In the only population genetics examination, Greer (2016) found that Baja populations "clustered apart from [*A. u. umbellata*] in adjacent Southern California, with very high levels of polymorphism" and Van Natto (2020) found much genetic variation in these

populations not shared by farther north populations. Neither of these works mentioned *A. gracilis* or the possibility of hybridization but is seems possible introgression or a hybrid zone a likely source of the unique SNPs found in these populations. Further complicating identifications, the only noted character differentiating *A. umbellata* and *A. gracilis* is leaf shape (Tillett 1967, Galloway 2004) which is immensely variable within and among individuals, even within the core range of *A. umbellata* (and leads some individuals of *A. umbellata* and *A. villosa* to key to *A. gracilis* in various keys, pers. obs.). For these reasons, we conservatively used the US-MX border, 32.5° N, as the southern edge of this study. Further study is necessary to determine the actual southern range limit of *A. umbellata* (Fig. 2) and any hybrid zone in that area.

***Broad survey of petal number across the range of* Abronia umbellata (~*H2)* —**To quantify patterns of petal number variation in the field, we manually extracted data from iNaturalist photographic records across the entire range of the species. All records identified as "research grade" *Abronia umbellata* as of 13-Jan-2020 were assessed (n=1096). Of these, 865 records were able to be scored, comprising 1,808 inflorescences, and 11,428 individual flowers. For each inflorescence in the photograph able to be scored, we recorded petal number of each flower, as well as record number, latitude, and the date of the record. For those records where the location was obscured by iNaturalist, the latitude listed was taken, as the obscuring occurs within a 10 km radius, a relatively small distance compared to the range-wide scale of this study and therefore, this uncertainty should not affect our results. Because interdigitation between prostrate stems of closely-packed plants is common, we were unable to conclusively assign inflorescences to individual plants therefore, multiple inflorescences in the same photograph were scored separately, but with the iNaturalist record number the same; the record number was used as a random effect in analyses when necessary (as a rough proxy for individual).

Two-hundred and two records were not used for the following reasons: the plants were not in flower, the flowers were out of focus, flowers were turned away from the camera, the photograph was too distant to count petals on individual flowers, the inflorescence had been consumed by herbivores, only one flower was visible, the photograph was duplicated across records, or the record occurred south of this study's border. Twenty-nine 'research-grade' records were also discarded because of misidentifications: one *Abronia maritima* was misidentified as *A. umbellata* and 28 records were obvious hybrids between *A. umbellata* and co-occurring congeners. EFL added the correct identifications as part of the review process and these records will likely eventually get a correct determination in the iNaturalist database (pending other users' 'votes').

To determine whether variety predicted atypical petal counts (i.e. not 5-lobed), we used a binomial mixed model (R 3.6.2: package *lme4*) of with typical, atypical petal number as a response variable and variety as a fixed effect and record number as a random factor. This model was compared to a null model without variety using a likelihood ratio test.

Common garden petal counts (~H2)— To quantify variation in petal counts and minimize the effect of plasticity, we scored plants in the shared environment of an *Abronia* common garden at Michigan State University. As part of a genus-wide growout, we grew ten var. *breviflora* individuals and six var. *umbellata* individuals from seed collected by EFL and one USDA accession of var. *breviflora* (Supplementary Table 1). These plants were maintained in the greenhouse and laboratory under artificial lights with a 12-hour light:dark cycle (though greenhouse plants got additional natural light in summer). Plants were maintained in 8-18" pots, grown in a mixture of sand and potting soil (~1:1), and kept ~70° F with 12-hour artificial lighting, though individuals in the greenhouse experience longer days naturally during spring and summer seasons. Plants were watered every 3-5 days and fertilized (Miracle-Gro, 10:10:10) once

a month. Plants were rotated between the greenhouse and laboratory often, as we took all measurements in the lab (for this and other studies). Petal number of flowers in all flowers in mature inflorescences were counted between July 2019 and May 2020 when each plant was flowering as part of the broader grow-out. We compared summed counts of petal numbers of each variety in the common garden, however statistics were not performed because of low number of individuals involved (in contrast to the iNaturalist data set).

Hybridization effects (H3) — Hybridization may alter meristic characters (e.g. Vlot et al., 1992) and therefore, may result in the production of atypical petal morphs in regions where hybridization and introgression occurs (H3). Hybridization occurs commonly in *Abronia*, especially in the xenogamous *A. u.* var. *umbellata*, which hybridizes naturally with *A. latifolia*, *A. maritima*, *A. villosa*, *A. gracilis*, and probably *A. pogonantha* (Tillett, 1967; Johnson, 1977; Van Natto, 2020; LoPresti, pers. obs.). While Tillett (1967) hypothesized that *A. u.* var. *breviflora* may be the result of a hybridization event between *A. umbellata* var. *umbellata* and *A. latifolia*, using genetic methods Van Natto (2020) found this hypothesis extremely unlikely. We experimentally crossed *Abronia* species across the phylogeny in genus-wide hybridization study; for this study we scored petal numbers of F1 hybrids, grown under the same conditions as parents, which had a parent either of *A. u. umbellata* or *A. u. breviflora* (10 combinations, n = 44 total hybrid individuals; parental collection locations in Supplementary Table 1). These plants did not necessarily reflect naturally-occurring hybrids, instead these hybrid combinations were designed to score traits across as broad a range of relatedness as we could in the genus. However, we see no reason to assume that these particular combinations would have meristic incompatibilities not representative of other combinations within the genus, and therefore we do not expect the exact combinations to affect the overall results in a particular direction. We asked

whether each hybrid combinations had a consistent pattern of production of atypical flowers compared to its' parental *umbellata* variety using a chi-squared test.

Laboratory test of autogamy success with different petal numbers (H1) — In order to the adaptive hypothesis that decreased petal number increases autogamous success, we compared the success of autogamy in flowers differing in petal number. Between 7-November-2019 and 09-March-2020 we marked all individual flowers in 58 inflorescences of the autogamous variety *A. u.* var. *breviflora* in common garden study and scored the success of autogamy when fruit had developed (Fig. 1: E, F). In total, these 58 inflorescences comprised 597 flowers (417 5-petalled, 169 4-petalled, 6 3-petalled). As all *Abronia* develop one seed per fruit/flower, the response variable was binomial (seed/no seed). We used a binomial mixed model with petal number as a factor and plant ID and inflorescence ID as random variables.

Environmental effects (H5)— To determine whether hypothesized seasonal and latitudinal factors affected production of atypical petal number morphs (H5), we tested for correlation between these environmental variables and each of atypical petal production and inflorescence mean petal number using the iNaturalist data set. Latitidue and season have been suggested to affect petal number in other plants (Mathiason, 1982; McKim et al., 2017). As the varieties showed such different patterns of petal number and do not overlap in latitude, variation is confounded with latitude (and flowering time correlates with latitude), we analyzed the varieties separately for each analysis. For seasonal effects, we used mixed models with Julian date (from Jan 1 of each year) as a continuous predictor and iNaturalist record as a random variable to account for non-independence of inflorescences in the same photo being likely from the same plant. We tested linear and second-order polynomial (in case of changes at start and end of flowering period; i.e. as found for Cardamine in McKim et al., 2017) for each variety.

Correlation of floral size and petal numbers (H4) — To test whether flower petal number correlates with flower size, we combined our iNaturalist data with previously published data. As measuring floral size is not possible from photos, and we had only one population of var. *umbellata*, and three of var. *breviflora* in the common garden, data on corolla diameter and number flowers in the inflorescence from 12 populations was extracted from Doubleday et al. (2013) using the data extraction program: (https://apps.automeris.io/wpd/). The petal number data for these populations was taken from the iNaturalist photo analysis, using +/- 0.0025 degrees from their coordinates as the range for each population, since the nearest populations in their analysis were 0.0050 apart. This minimum grain ensures that no records are individually counted in multiple populations. By this population grain, we had petal number records for 7 of Doubleday et al.'s populations, all of var. *umbellata*, with a range of 2 to 56 iNaturalist records falling within each population. We analyzed data using mixed models with petal number as a response, either floral face diameter or flowers per umbel fit as fixed effects and record as a random effect. There was not sufficient overlap between the populations in our sample and Doubleday et al.'s to perform a similar analysis for variety *breviflora*.

RESULTS

Because our hypotheses incorporated evidence from common garden and the iNaturalist data set, we have organized the results by hypothesis and were specific about from where the data came.

Characterization of petal numbers of autogamous and xenogamous varieties — Petal number counts from both common garden and field iNaturalist plants showed that the autogamous *A. u. breviflora* had far fewer 5-petalled (and more 4-petalled) flowers than the xenogamous *A. u. umbellata*. The varieties differed significantly in their production of atypical petals in the range-wide iNaturalist data set; the random-effects model including variety as a fixed effect fit

significantly better than a null model without variety (X^2=93.6, df=1, p<0.0001). A mean percentage per record of 80.2% of the autogamous *A. u. breviflora* flowers were five-petalled and 93.1% of the xenogamous *A. u. umbellata* were five-petalled (Fig. 2,3,4). In the common garden, *A. u. breviflora* had a mean percentage per individual of 74% 5 petalled, and 25% four-petalled flowers (Table 2), whereas *A. u. umbellata* had 93% 5-petalled and 7% four-petalled flowers. While lab-grown plants scored for petal number were of varying age, the fact that field results showed the same qualitative pattern as that in lab-grown plants suggest that plant age is not a large factor in petal number variation between the two varieties.

Hypothesis 1: autogamous success of differing petal numbers — Petal number had no effect on autogamous success (4: 76.3% & 5: 78.4% set a seed; n=58 inflorescences, 586 flowers: six flowers with three petals and five which did not open were excluded from analyses). A binomial mixed model with petal number as a predictor fit no better than a null with just random effects - inflorescence ID nested in plant ID (Likelihood ratio test, df=1, X^2=0.41).

Hypothesis 3: hybridization effects on petal numbers — Hybrids did not consistently produce more atypical flowers compared to parents in fact, almost all hybrids that differed significantly from their parental variety had lower than expected atypical morphs (Table 2). Contrary to the prediction of hybridization reducing meristic stability (i.e. increasing atypical morphs), it is noteworthy that most crosses involving variety *umbellata* with already low levels of atypical petal morphs, had even lower rates of atypical petals than expected (significantly different in 4 of the 8 crosses: Table 2). However, these results are not drawn from large numbers of samples (n=44 plants). Crosses of the two umbellata varieties showed atypical petal numbers which did not differ from variety *breviflora*, with 27% of flowers being four-petalled, but differed significantly from variety *umbellata*.

Hypothesis 4: correlation of flower size and petal number — Among the populations (n=11) of *A. u.* var. *umbellata* with flowers measured by Doubleday et al. (2013) for which we also had petal number data in the iNaturalist data set (n = 7), there was no correlation between corolla width and production of atypical petals. Models with either floral face diameter or flowers per umbel fit no better than a null with only a random effect of record (X^2=1.48, df=1, p=.22; X^2=1.07, df=1, p=0.30).

Hypothesis 5: environmental drivers of petal number — We found no significant effect of phenology on atypical petal number of either variety from the iNaturalist data set. Date (days after 1-Jan each year) had no correlation with atypical petal production either linearly or with a second-order polynomial (in case of changes at start and end of flowering period; i.e. as found for *Cardamine* in McKim et al., 2017) for either variety. Mixed models including only the random effect of the record ID fit better than those containing the day for both varieties (var. *breviflora*: X2=1.46, df=1, p=0.23; var. *umbellata*: X2=0.07, df=1, p=0.79).

Latitude predicted atypical petal number better than a null model in each variety (var. *breviflora*: X^2=23.2, df=1, $p < 0.001$; var. *umbellata*: X^2, df=1, $p < 0.001$); however, the amount of variance explained by latitude in these best-fitting models was small (marginal pseudo-R^2, package *sjstats*: *breviflora* = 0.055; *umbellata* = 0.016). In both models, the random effects of record (a proxy for individual) explained far more variance (conditional pseudo-R^2, *breviflora* = 0.195, *umbellata* = 0.260). Using mean petal number instead to characterize the trend, we found mean petal number decreased with increasing latitude. In *A. u.* var. *breviflora*, the relationship between mean petal number and latitude was strong; for every increase of 1° latitude, mean petal number decreased by 0.042 ($\pm$ 0.008); from the southernmost populations (~38 N) to the northernmost (~49 N), this equates to a drop in mean petal number of almost 0.5! In contrast, while the

correlation between latitude and mean petal number was significant in *A. u.* var. *umbellata* as well and this model fit better than a null; the effect was extremely small – a predicted drop of 0.01 petals per every increase of 1° latitude (i.e. 0.05 drop across the entire range) and explained only ~1% of the variance observed (r^2=0.0111). Regardless, this result demonstrates that the direction, but not magnitude, of correlation is consistent between the varieties.

DISCUSSION

Using a combination of a large citizen-science dataset, common garden grow-outs, and laboratory studies of autogamous success and of hybridization, we characterized petal number variation across the entire range of a widespread species and critically examined several published hypotheses about the evolutionary drivers of petal number variation in plants. Broadly, we found that the autogamous *Abronia umbellata* var. *breviflora* had far higher proportions of atypical flowers than the xenogamous *A. u. umbellata*. Overall, we documented a pattern consisten with relaxation of selection in petal numbers in selfers, correlated selection on another trait (but probably not flower size), or inbreeding leading to a loss of developmental stability (H2). We did not find support for the hypotheses of increase in autogamous efficiency of reduced petal numbers (H1), hybridization reducing meristic stability and increasing atypical morphs (H3), correlated selection on flower size (H4), and evidence for environmental drivers of plasticity was mixed, and the effect was too small to account for the overall pattern (H5). While we were unable to conclusively identify any mechanism as contributing to the pattern; our integration of citizen science and common garden studies was able to eliminate several hypotheses and we believe the approach we used can be applied to other systems to better understand this interesting, yet understudied, phenomenon.

Interpretation of the pattern — We found a robust pattern of increased atypical petal production in the self-compatible *Abronia umbellata* var. *breviflora* compared to the outcrossing *A. u.* var. *umbellata* in both field and lab. This pattern is consistent with several, but not all, of the hypotheses proposed in the literature to explain variation in petal number (Table 1). In particular, the increase in atypical flowers in the selfing var. *breviflora* is at least partially consistent with at least three, non-exclusive, mechanistic hypotheses: relaxation of selection on petal number in selfers (implying some adaptive function of five-petals in outcrossers, for example via pollinator-mediated selection), inbreeding leading to a loss of meristic stability (H2), or correlated selection on an unmeasured trait (H4). We were, however, able to provisionally eliminate several proposed mechanisms (autogamous efficiency: H1, hybridization: H3, seasonality/phenology: H5) in this system. Below we discuss the rationale for each of the three remaining hypotheses, including what additional evidence might be garnered to support each of them.

It is possible that inbreeding may be responsible for the observed high rates of atypical petal production in selfers. In contrast to the self-incompatible *A. u. umbellata*, *A. u.* var. *breviflora* reproduces almost solely autogamously (Doubleday et al., 2013; Van Natto, 2020), and therefore, almost all individuals in our study – in nature and the lab – were probably inbred for several generations. Greer (2016) and Van Natto (2020) found that there was little genetic variation in *A. u.* var. *breviflora*. Inbreeding may increase aberrant morphs in meristic traits (Sherry and Lord, 1996), and potentially carry little cost in this autogamous population. Therefore, it seems possible that inbreeding may be responsible for the observed high rates of atypical petal production. While this is a viable hypothesis in our system, the only other evaluation of this hypothesis that we know of, which used data from Schlichting and Levin (1986) on xenogamous *Phlox drummondii*, found that inbreeding decreased the proportion of

atypically petalled flowers (Mickley and Schlichting, unpublished data). Allowing lines of var. *breviflora* to self for several generations as well as manually outcrossing those lines could be used to rigorously evaluate this hypothesis in future studies.

A relaxation of pollinator-mediated selection maintaining five-petalled flower (Leppik, 1953, 1956), or petal number constancy (Herrera, 2009) during the evolution of autogamy in var. *breviflora* also remains a possibility as an explanatory mechanism of the high rates of atypical petal numbers in var. *breviflora*. Correlated selection on numbers of other meristic traits by pollinators also is possible, though the lack of a reduction in stamen number at the same time makes this less likely (virtually all four-petalled flowers in *Abronia* have five stamens: LoPresti, unpublished data) Any pollinator-mediated selection on petal number would require pollinators to be able to count petals, which bees have been found to be capable of (Leppik, 1953, 1956; Lehrer et al., 1995) but Lepidoptera have not (Leppik, 1954, 1955). *A. umbellata* is likely primarily moth pollinated; Doubleday and Eckert (2018) found that *A. umbellata* had higher floral visitation during the day, but all but a very small amount of pollination occurred at night. Given these lines of evidence, it seems unlikely that the lower production of atypical morphs in var. *umbellata* is due to pollinator-mediated selection, though it is consistent with our results and we cannot rule it out. Further investigations into pollination success of atypical flowers in the field would be necessary to rigorously examine this hypothesis.

The two varieties of *A. umbellata* differ greatly in floral size, and, given that close relatives are also outcrossing and large flowered, the small flowers and autogamy in var. *breviflora* are derived states (Doubleday et al., 2013). Large-flowered, close relatives, including *Abronia villosa* var. *villosa*, *Abronia villosa* var. *aurita*, and *Abronia gracilis*, have extremely low rates of atypical petal morphs (LoPresti, unpublished data), therefore, the evolution of atypical petals

occurred alongside the evolution of autogamy and small flowers in this lineage. We did not find evidence that within var. *umbellata*, petal morphs correlated with corolla width; however, the macro-evolutionary and population-level processes may be decoupled, and rigorous interpretation of those data, from seven populations in a narrow part of the range of this variety (34.22 –36.95 ° N) is limited. Because the atypical petal number is confounded with floral size differences between the morphs, analyzing across the range is inappropriate. Therefore, whether the lack of correlation within the narrow range of flower sizes within var. *umbellata* is informative in explaining the marked transition to smaller flowers and fewer petals during the evolution of var. *breviflora* remains an open question and cannot be tested with a single transition. Instead, either correlations across multiple transitions to selfing across more species or an artificial selection experiment could be used more rigorously examine this hypothesis.

No effect of petal number on autogamy — Petal expansion assists in opening of the floral bud, leading Monnaiux et al. (2016) to hypothesize that a reduction in petal number might delay bud opening and increase successful autogamous fertilization. In our laboratory study, we found no support for increased autogamous success of four-petalled morphs compared to five-petalled morphs in the autogamous *A. u.* var. *breviflora*. There is a possibility that in the field, four-petalled morphs may get fewer, shorter, or less effective pollinator visits and thus, may have lower pollen removal and therefore higher autogamous success; our laboratory study could not examine this hypothesis. Additionally, we attempted and failed to find any distinguishing characteristics in fruit resulting from flowers of differing petal numbers, meaning a *post hoc* analysis could not be done from field seed collections, as we had initially hoped. Any test for autogamy in a field setting would therefore require marking individual flowers, as we did in the laboratory. Nevertheless, this study was the first to test this broad hypothesis and we found no

support. Our study design is simple and could be easily replicated in other taxa with variable petal number (species in the Polemoniaceae, Ranunculaceae, etc.) to help draw larger conclusions than from this test alone.

Environmental or phenological effects — In other systems, changes in petal numbers occur plastically in response to environmental variables (e.g. Huether, 1968; McKim et al., 2017), the two which we could test with the large iNaturalist data set were latitude and phenology (both as proxies for climatic variables or day length). In *A. u.* var. *breviflora*, latitude was correlated with atypical petal number; as latitude increased mean petal number decreased. Whether this effect was due to environmental factors associated with latitude or some genetic variation, due to any number of factors, including drift or founder effects of those small, very isolated northern populations, is impossible to tell with certainty. Our common garden data is consistent with the observed gradient in the field, though it is extremely limited, suggesting that this gradient may not be entirely due to plasticity. Individuals from a population from Oregon (n=3) had a higher proportion of 4- (36.5%) and 3-petalled (5.3%) flowers compared to a population from Sonoma County, CA (n=6) with 23.5% and 0.8%, respectively. Further common gardens drawn from populations across the entire range could shed light on to what extent this latitudinal gradient is heritable and/or environmental.

Latitude was also a ‘significant’ negative predictor of atypical and mean petal number in *A. u.* var. *umbellata*. However, any biological meaning is difficult to interpret, given that it explained ~1% of the variation with minimal effect on petal number across the much larger range of this variety; this was a pattern that would likely not have been picked up in any smaller data set than the >11,000 flowers we were able to score from iNaturalist. We therefore hesitate to ascribe any

biologically relevancy to this small an effect, though the consistent direction between the varieties does suggest a common mechanism.

In contrast to McKim et al. (2017)'s characterization of phenology causing changes in petal number in *Cardamine hirsuta*, we did not find any effect of phenology on either variety. We analyzed this with linear and polynomial models; none fit better than a null model. Coupled with the consistent pattern between field and common garden plants, we believe that this lack of a seasonal correlation is further evidence that the observed production of petal morphs is largely heritable and the latitudinal effect should be investigated to parse out population differentiation across latitudes or a plastic response to environmental differences across latitudes.

No consistent effect of hybridization on petal number variation — Hybrids of *A. umbellata* did not consistently exhibit increases in atypical petal number (only one of 9 crosses did). While we cannot completely exclude hybridization as a mechanism behind the variable petal numbers in *A. u.* var. *breviflora*, especially given the lack of crosses involving other coastal species, it seems extremely unlikely that hybridization leads to consistent meristic instability. Most tellingly in our system, Van Natto (2020) found no measureable levels of introgression in var. *breviflora*, which they deduced was likely due to its autogamous reproduction system. Therefore, since the non-introgressed variety has the higher atypical petal morphs and hybridization does not increase atypical petal numbers in most crosses, hybridization is an unlikely mechanism behind the increased atypical petal numbers in *A. u.* var. *breviflora*.

Conversely, could hybridization reduce petal number variation in *A. u.* var. *umbellata*? Consistent with this hypothesis, most of the hybrids created in this study had reduced atypical petal numbers; somewhat contrary to this broader hypothesis is the stability of meristic traits across all *Abronia* species, a result very unlikely to be driven by hybridization (LoPresti, pers.

obs./unpublished data). Demonstrating that the low levels of introgression into this variety (Tillett, 1967; Van Natto, 2020) stabilizes petal number would require far more investigation than this survey study could provide, and would need to be investigated in far more detail. Whether the identity of the hybrid species pairs also may be informative in predicting stability or lack thereof, Pelabon et al., (2003) found that in crosses of intermediate distance (within a species) the resulting hybrids had reduced variation in floral traits. We did not find that result; our crosses of var. *umbellata* did not show a pattern with relatedness (unpublished data), however, we caution interpretation of this result both as Pelabon's comparisons were between populations of the same species, and the small sample size of our crossing data. The ongoing larger hybridization study from which these data were extracted holds the potential to test the shape of this relationship across a greater range of relatedness of many species pairs across the *Abronia* phylogeny.

More broadly, hybridization deserves increased study in *Abronia umbellata* var. *umbellata*; Van Natto's (2020) findings that "early-generation hybridization was occurring anywhere [var. *umbellata*] was sympatric with another species" suggests the importance of considering hybridization in evolution and ecology of this species. While Van Natto examined *A. umbellata* hybrids with only *A. maritima* and *A. latifolia*, Tillett (1967) also suggested hybridization with *A. villosa*. We consider this additional hybrid extremely likely in several locations where *A. villosa* var. *aurita* occurs near the coast (i.e. Camp Pendleton); material from these sites is not easily ascribable morphologically to one or the other species. We also add another intriguing possibility: specimens collected by William Palmer in 1876 from an inland site in eastern San Luis Obispo county (the 'McGinnis Ranch') were described as *Abronia minor* by Standley (1909). Tillett considered *A. minor* a hybrid of *A. umbellata* x *A. latifolia*, though neither species

occurs inland and morphologically, these specimens have no characteristics indicative of *latifolia* parentage. Most tellingly, the McGinnis ranch falls into the current and historic range of *A. pogonantha*. Most species of *Abronia* are pollinated commonly by large, migratory hawkmoths, (Keeler, 1978; Douglas, 2008; Doubleday and Eckert, 2018; LoPresti, pers. obs) and pollen movement from over this <50km distance from *A. umbellata* to *A. pogonantha*, but not fruit movement, seems most likely. For these reasons, from our examination of Standley's illustrations and scans of the series collected, we find it far more likely that this material represents backcrosses of *A. umbellata* (♂) x *A. pogonantha* (♀) with *A. pogonantha* (F1 hybrids are unlikely since Palmer collected several individual plants).

The invaluable contribution of iNaturalist citizen science data — Initial observations of atypical petal production were confined to the few plants of each variety we had in the Michigan State University greenhouse during a common garden grow-out of many *Abronia* species. Herbarium sheets often have very few easily scoreable flowers on them, and therefore, the scale of this study and conclusions reached would have been greatly reduced without the immense iNaturalist repository of identified photographs. Field sampling across the geographic range during all twelve months of the year, would have been logistically and financially impossible; yet, the analysis of these flowers from iNaturalist data took EFL less than six weeks! This study adds to a burgeoning body of literature using iNaturalist data to extract scoreable traits on varied organisms (e.g. Kido and Hood, 2020; Putman et al., 2020). Petal number could easily be scored across various clades with transitions to selfing and would be informative for more evolutionary correlations than the one transition studied here.

Conclusions — Integrating a common garden study, crossing experiment, and large-scale field citizen science data, we characterized an intriguing shift in atypical petal number during a

transition from outcrossing to autogamy in a coastal sand verbena. The increase of atypical petal number of the selfing variety was not consistent with directional selection caused by increased autogamous success, nor was it driven by seasonal environmental changes, as in other similar systems. While we did not find total support for any hypothesis, we believe the totality of the data presented suggests that some combination of inbreeding leading to loss of meristic stability, correlated selection on another trait, or relaxation of stabilizing selection on petal number in this selfing variety are potential mechanisms. We also demonstrate the power and ease of using high-quality photographs from citizen-science efforts to score easily quantifiable traits across range and seasons to generate a data set which would have been logistically difficult to get with fieldwork or herbarium specimens.

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DATA AVAILABILITY

Data sheets will be archived as supplements, see online supporting information section. Hybridization data is in Table 2; more data (including more crosses of *A. umbellata*) will be available when the whole-genus study from which those data were taken is completed.

ONLINE SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information section at the end of the article, Four supplementary data sheets and one file of code are included: (1) the iNaturalist data, (2) autogamy data, and (3) parsed iNaturalist data to the populations in Doubleday et al (2013) for the flower size comparison, (4) collection locations and herbarium specimen numbers for plants used in the common garden studies, and (5) an R script uses the attached data sheets for the analyses in the paper.

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Table 1: Evolutionary and ecological hypotheses for atypical petal counts, with predictions for *Abronia umbellata*, and references.

Table 2: Summed counts of floral morphs in common garden *Abronia umbellata* (both varieties) and hybrids of this species. Statistics not performed because of low sample sizes.

Figure 1. A: *Abronia umbellata* var. *breviflora* with many 4-petalled and a 3-petalled (arrow) flower. Doran Beach, Sonoma County, CA. B. *A. u.* var. *umbellata* with a 6-petalled flower (arrow). Morro Beach, San Luis Obispo County, CA. (C) *A. u.* var. *breviflora* with an atypically low number of 4-petalled flowers (none visible). Doran Beach, Sonoma County, CA. D. A. u. var. *umbellata* with a six-petalled flower (arrow). Laboratory plant. E. Individually marked flowers in autogamy experiment on *A. u.* var. *breviflora*. F. Developing fruit with tape denoting petal counts. All photos by EFL.

Figure 2: The range of iNaturalist records used in this study, with proportions of floral morphs as depicted for visualization as pie charts for ease of visualization of each variety.

Figure 3: Summation of iNaturalist data. Left: histograms of proportion of flowers 5-petalled for each variety, by infloresence (var. *breviflora*, n=238 inflorescences, var. *umbellata* n=1570); Right: histogram of petal numbers for all flowers of each variety comprising (var. breviflora, n=1355 flowers; var. umbellata n=10,070 flowers).

Figure 4: Box plot of mean petal numbers, per inflorescence, of each variety, by inflorescence, from the iNaturalist data set (derived from the same data as in Figure 3, left).

Figure 1:

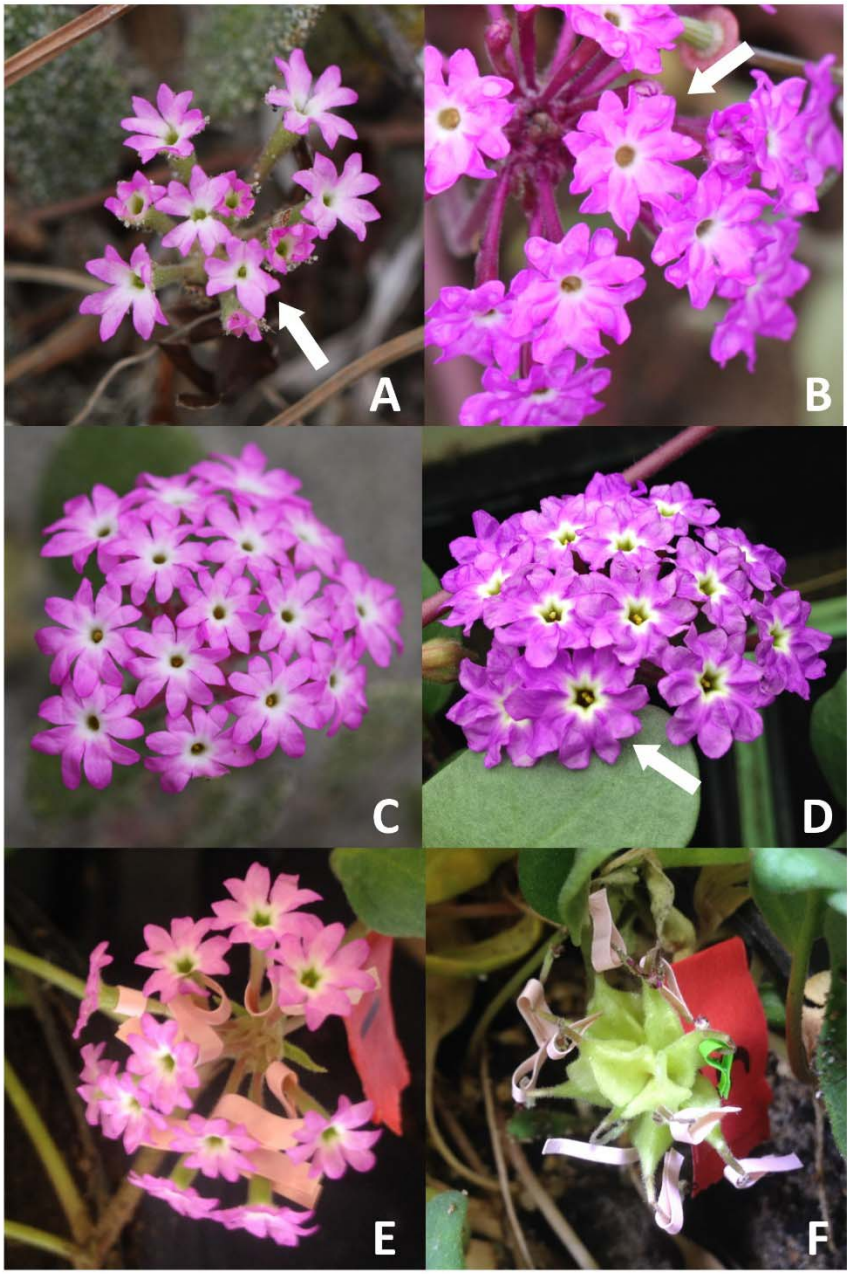


Figure 2:

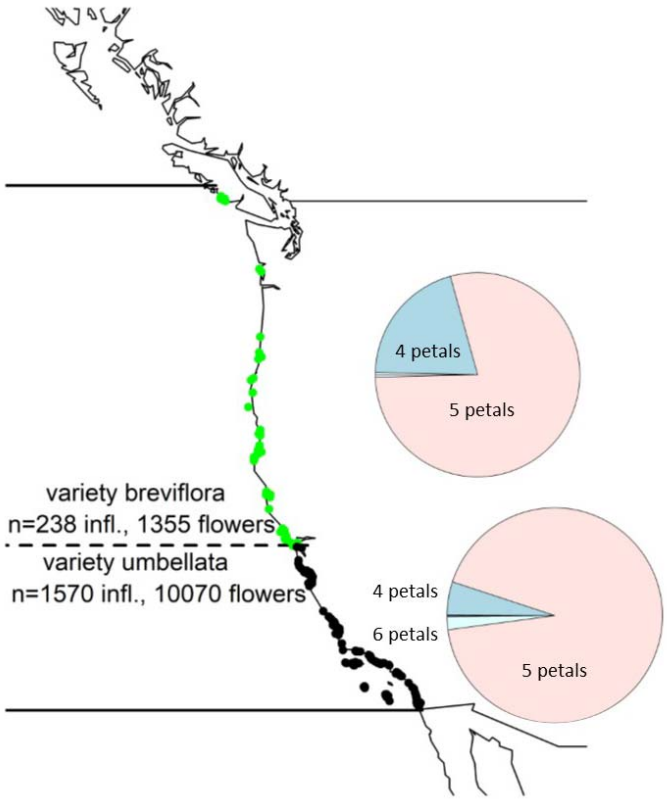
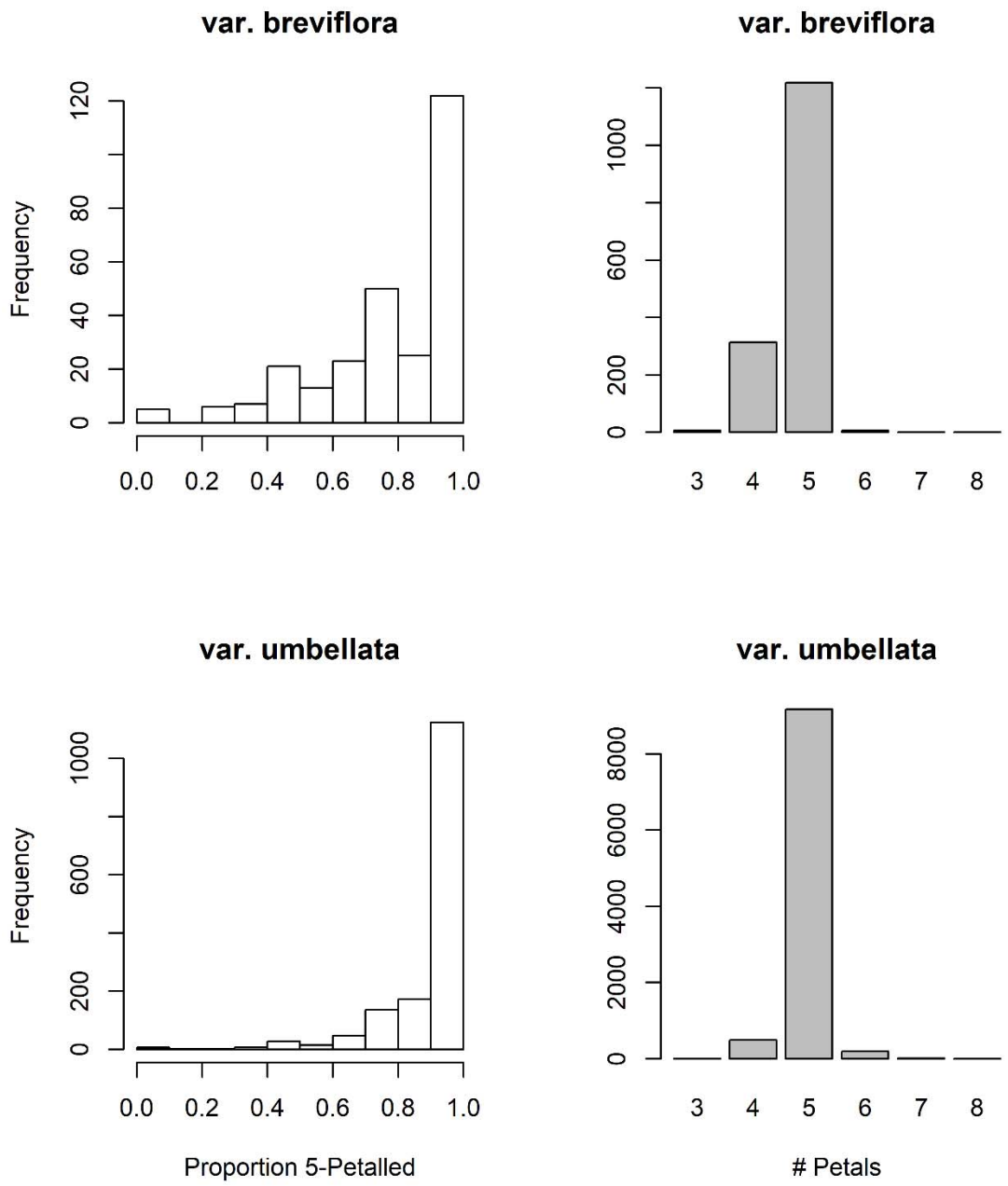

4 petals
5 petals
variety breviflora
n=238 infl., 1355 flowers
variety umbellata
n=1570 infl., 10070 flowers
4 petals
6 petals
5 petals

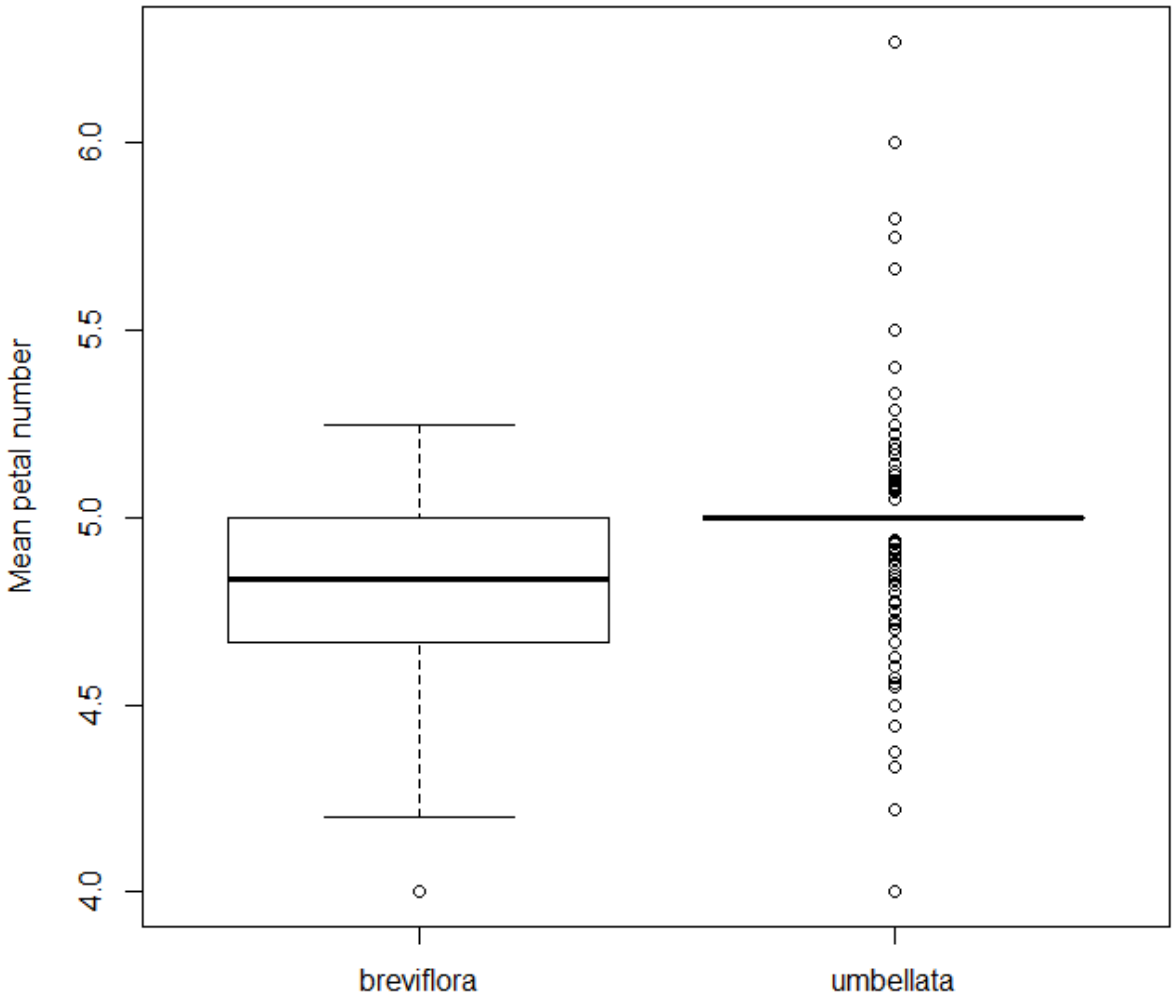
Figure 3:


var. breviflora
Frequency
0
20
40
60
80
120
0.0
0.2
0.4
0.6
0.8
1.0
var. breviflora
0
200
600
1000
3
4
5
6
7
8
var. umbellata
Frequency
0
200
600
1000
0.0
0.2
0.4
0.6
0.8
1.0
Proportion 5-Petalled
var. umbellata
0
2000
4000
6000
8000
3
4
5
6
7
8
Petals

767

768

Figure 4



Hypotheses from literature tested here:

(H1) Loss of petals increases autogamous success

(H2) Inbreeding increases developmental instability

(H3) Incompatibilities due to hybridization increase atypical petal morphs

(H4) Correlated selection on other traits (here: flower size)

(H5) Environmentally-driven

Predictions for *Abronia umbellata*

In *A. u* . var. *breviflora* , fewer petalled-flowers will have higher reproductive success in greenhouse

A. u. var. *breviflora* (autogamous) will have increased atypical petal morphs

Artificially-created hybrids will have increased atypical petal morphs

A. u. var. *umbellata* (xenogamous), which hybridizes with congeners commonly in the field, will have an increased atypical petal morphs in field compared to autogamous variety

A. u. var. *breviflora* (smaller flowered) will have fewer mean petals

Within each variety, populations with smaller flowers will have fewer mean petals

A correlation between phenology and atypical petal morphs for each variety

A correlation between latitude and atypical petal morphs for each variety

	# individuals, inflorescences	8 Petals	7 Petals	6 Petals	5 Petals
umbellata var. *breviflora*	10, 63	0	0	0	493
umbellata var. *umbellata*	6,16	0	0	0	259
hybrids					
umbellata x *breviflora*	3,3	0	0	0	33
hybrids of umbellata					
bigelovii x *umbellata*	2,3	0	0	0	56
pogonantha x *umbellata* [2,3]	12,51	1	0	3	674
umbellata x *pogonantha* [3]	1,4	0	0	0	56
umbellata x *ameliae*	3,10	0	0	1	231
umbellata x *turbinata*	9,42	0	2	11	890
umbellata x *villosa* var. *villosa* [3]	2,5	0	0	0	56
villosa var. *villosa* x *umbellata* [3]	7,19	2	0	8	241
villosa var. *aurita* x *umbellata* [3]	1,1	0	0	2	5
hybrids of breviflora					
pogonantha x *breviflora*	4,9	0	0	0	106

1: cumulative of all scored flowers, expected from the parental variety, 2: see text for information on this hyl
1967, 4: expected calculated from var. *breviflora* , 5: expected calculated from var. *umbellata* .

4 Petals	3 Petals	Proportion 5-petalled	Proportion 4-petalled	Chi-squared value[1]	p =	Greater or fewer atypical petals than expected?
163	9	0.74	0.25			
20	1	0.93	0.07			
12	0	0.73	0.27	0.70[4]	0.353	no difference
				25.96[5]	**<0.001**	greater
10	0	0.85	0.15	6.58	**0.019**	greater
2	0	0.99	0.00	50.37	**<0.001**	fewer
0	0	1.00	0.00	4.54	0.052	no difference
1	0	0.99	0.00	16.65	**<0.001**	fewer
2	0	0.98	0.00	67.37	**<0.001**	fewer
2	0	0.97	0.03	1.42	0.246	no difference
3	0	0.95	0.01	13.67	**<0.001**	fewer
0	0	0.71	0.00	0.86	0.325	no difference
2	0	0.98	0.02	32.49	**<0.001**	fewer

brid, specifically 3: hybrid occurs or is very likely to occur in nature; see above note and Tillett