Evolution of Amphibious Vernal Pool Specialist Annuals: Putative Vernal Pool Adaptive Traits in *Navarretia* (Polemoniaceae)

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ABSTRACT. The amphibious plants endemic to vernal pools of the California Floristic Province provide an excellent opportunity to study adaptive evolution for two reasons. First, most of these plants exhibit a major shift in habitat preference from that of their terrestrial ancestors. Second, in most cases, this shift apparently has occurred recently. Because this adaptive evolution has been dramatic and recent, obligate vernal pool species may be compared with relatives of other habitats to determine what developmental, morphological, and reproductive traits may have been important for vernal pool adaptation. A number of traits have been proposed by various authors as adaptive in vernal pool specialists. We test whether these traits are strictly correlated with the vernal pool habitat in *Navarretia*, a genus with closely related vernal pool specialists and terrestrial species. All vernal pool specialist taxa and their closest terrestrial relatives of the California Floristic Province were observed in their native habitats and under uniform cultivation. Adaptive hypotheses are supported for non-shattering inflorescences, outcrossing, and isoetid juvenile growth form. Contrary to prediction, spininess is reduced in the vernal pool taxa compared with their terrestrial relatives. Indehiscent fruits, small seeds, and absence of glandular hairs are perhaps advantageous traits for the vernal pool navarretias, but are not likely evolutionary adaptations in the strict sense.

INTRODUCTION

Vernal pools, ephemeral pools fed more or less exclusively by winter rains, occur in Mediterranean-like climates in many parts of the world (Keeley and Zedler, 1998). The vernal pools of the California Floristic Province (CFP) (Raven and Axelrod, 1978), however, are unusual for the species richness and high degree of endemism of their flora (Keeley and Zedler, 1998; Holland and Jain, 1981; Thorne, 1984). The relative resistance of CFP vernal pools to invasion by exotic species is additional evidence for the unique nature of this habitat. The vernal pool flora contains only 5-10% introduced species, mostly of South American and African origin, while adjacent grasslands (38% introduced) and marshes have been transformed by introduced Eurasian species (Holland and Jain, 1977). Climate (cool wet winters, hot dry summers), topography, and substrate (impervious subsurface layer due to clay or hardpan) are important environmental factors in vernal pool formation (Zedler, 1987).

The interaction of these factors influences other variables in the plant's environment, such as season and cues for germination, growth, and flowering; pH and salinity; pollinator availability; year to year moisture predictability along steep inundation gradients; exposure to herbivores and seed predators; light and gas availability; and habitat area (Zedler, 1987; Zedler, 1990; Holland and Dains, 1990).

The majority of the plants endemic to CFP vernal pools (e.g. species of *Lasthenia, Downingia, Pogogyne, Limnanthes, Navarretia, Mimulus, Eryngium, Orcuttia*) are annuals adapted to limited growth under water during the winter months, followed by maturation and flowering as the pools dry (Stone, 1990; Zedler, 1990). These amphibious plants are generally derived from terrestrial elements in the local flora rather than from aquatic species or from species of vernal pool like habitats in other parts of the world (Thorne, 1984). Many are considered to be threatened with extinction due to habitat loss. Even

though most of these species are believed to have evolved quite recently (Stebbins, 1976; Stone, 1990; Raven and Axelrod, 1978; Thorne, 1984), they frequently differ strikingly from their terrestrial relatives in morphology (Stone, 1990) and life history (Zedler, 1990), in addition to physiological differences (Keeley, 1990) that would be expected with adaptation to an amphibious environment. Adaptive hypotheses have been proposed for many of these differences (Table 1). A knowledge of the adaptive role of these differences is a key to understanding the environmental and historical factors that differentiate CFP vernal pools from other habitats and that have prompted the evolution of their rich and unique flora. Such an understanding is not only important to the appreciation of the vernal pool flora, but also to its preservation, since it is not only the obvious aspects of vernal pools, such as hydrology, topography, and substrate, that must be preserved as part of the habitat, but other environmental aspects that may have been important in the evolution of the flora, and which likely remain important in giving the vernal pool specialists a competitive advantage over alien invaders.

Adaptive hypotheses (Table 1) are a necessary beginning in this endeavor. Many of these hypotheses, however, are based on overly broad or ambiguous definitions of vernal pool habitat, and common garden studies have generally not been performed to determine whether the hypothesized adaptive traits reflect genetic or environmental differences between vernal pool and non vernal pool taxa. These putative adaptive traits should now be investigated in individual plant groups that have both vernal pool and non vernal pool species. Traits that are found to be strictly associated with the vernal pool habitat in such groups, and which are found to have a genetic basis, may then be subject to phylogenetic, genetic, and selection studies to investigate their evolution, and to fitness studies to investigate their current adaptive value. To our knowledge such studies have not been previously performed in reference to most of these adaptive hypotheses (but see Linhart, 1976; Jain, 1984).

We have selected the genus *Navarretia* as a model group to investigate vernal pool adaptive evolution. *Navarretia* is particularly well suited for this study. Its vernal pool endemics are frequently dominants in the deeper parts of vernal pools, yet they have close relatives that are exclusively terrestrial. The navarretias that frequent vernal pools are all members of *Navarretia* sect. *Navarretia*, which contains taxa of varying degrees of adaptation to vernal pools. Molecular phylogenetic

TABLE 1. Putative adaptive traits and selective forces in CFP vernal pool specialists. This list was compiled from presented papers of previous vernal pool symposia and from various treatments of vernal pool species.

Putative Adaptive Trait [Measured in This Study]	Selective Force
Mating System	
outcrossing* [P-O ratio, pollen number, corolla size]	environmental heterogeneity[g]
Seed Dispersal and Size	
mechanisms restricting dispersal* [shattering, fruit dehiscence]	localized habitat, predation, heat stress[g]
small seeds* [seed weight]	localized habitat[e]
Herbivory / Water Loss	
plant viscid, spiny*, etc. [spininess]	herbivory, transpirational water loss[b,d,f]
vegetative growth without glandular hairs* [glandular hair presence]	abundant soil moisture[a]
Juvenile Growth Form	
'isoetid' growth form* [leaf shape and orientation]	carbon limitations of pool environment[c]
Not Addressed in This Study	
elongation of inundated stems and petioles	frequent or prolonged inundation[f]
inundated plants with adventitious roots	changing water levels or anaerobic soil[f]
long seed dormancy, special germination cues	unpredictable year to year environment[b,f]
phenotypic plasticity	localized, unpredictable habitat[d,f]
altered anatomy/morphology of submerged leaves	gas exchange in pool environment[c,d]
altered carbon assimilation in submerged leaves	gas exchange in pool environment[c,d]
low nuclear DNA content	short generation time, specialization[d]

[a] Crampton 1954; [b] Crampton 1959; [c] Keeley 1990; [d] Stone 1990; [e] Zedler 1984; [f] Zedler 1987; [g] Zedler 1990.
* Traits considered in this study of *Navarretia*.

evidence indicates that *Navarretia* sect. *Navarretia* represents a monophyletic lineage (Spencer, unpubl. data) and thus must contain the closest relatives of the vernal pool specialists. Moreover, the vernal pool specialists within sect. *Navarretia* form a monophyletic lineage that is clearly derived from non vernal pool ancestors (Figure 1), a pattern that is consistent with a hypothesis of recent evolution of vernal pool adaptation in this group (Stebbins, 1976; Crampton, 1954). Recent evolution of the vernal pool specialists would increase the likelihood that major traits which differentiate them from their closest relatives originated in conjunction with, or in response to, the shift in habitat. In addition, the small size and rapid growth of these plants make them suitable for fitness and genetic studies of putative adaptations.

We first made quantitative estimates of the relative degrees of vernal pool specialization of all CFP members of *Navarretia* sect. *Navarretia* based on a narrow definition of vernal pool habitat. We then tested whether several of the hypothesized adaptive traits previously referred to (Table 1) are strictly correlated with the vernal pool habitat in *Navarretia*. Where possible we used data from plants raised in a uniform garden environment to insure that the traits of interest are based on genetic rather than environmental differences among taxa. Ongoing phylogenetic studies will investigate the evolution of those putative adaptive traits which were found to be associated with the vernal pool habitat in *Navarretia*.

METHODS

The Study Group

The genus *Navarretia* consists of about 40 species and subspecies (Day, 1993). All but one (*N. involucrata*, of Chile and Argentina) are restricted to western North America. Of the North American taxa, all but five (*N. breweri* of sect. *Masonia*, *N. leucocephala* ssp. *minima* and *N. intertexta* ssp. *propinqua* of sect. *Navarretia*, and two undescribed species of sect. *Navarretia*) occur predominantly within the CFP. All navarretias are small annuals, and most inhabit clayey, rocky, or shallow soils. Vernal pool specialists are all members of sect. *Navarretia*, which also contains non vernal pool species. Some members of this section outside the CFP in North America frequent ephemeral pools, though climatic differences affecting type and seasonality of precipitation differentiate these ephemeral pools from the CFP vernal pools. The single species of *Navarretia* from South America is a member of sect. *Navarretia*, and also frequents ephemeral pools.

In order to facilitate the comparison of habitat among taxa, we did not include the members of sect. *Navarretia* with distributions predominantly outside the Mediterranean-type climatic region defining the CFP. This left eleven members of sect. *Navarretia* occurring within the same climatic region, with ver-
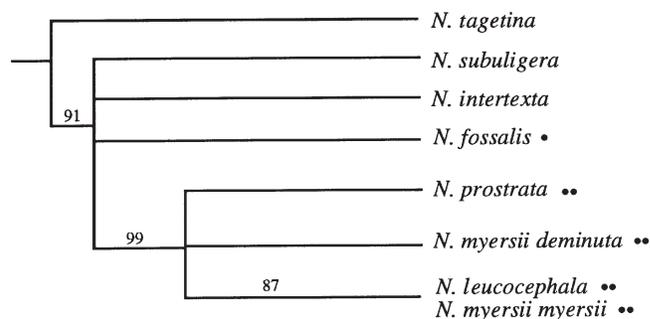

FIGURE 1. Phylogenetic relationships among the CFP members of *Navarretia* sect. *Navarretia* as inferred from sequence variation in the internal transcribed spacer of the nuclear ribosomal DNA (Spencer, unpublished). Shown is a portion of a strict consensus tree, with bootstrap percentages indicating the robustness of internal groups (see also Table 3). •• = vernal pool specialists (Day 1993; 1995), • = species of intermediate occurrence in vernal pools (Day 1993, Moran, 1977).

nal pool and non vernal pool species frequently sympatric, separated only by the steep inundation gradients of the vernal pool edges.

All members of the genus *Navarretia* are included in phylogenetic studies, to be reported separately, which investigate the evolution of the putative adaptive traits.

Estimation of Degree of Vernal Pool Specialization

In order to include information concerning historical and inaccessible sites, and in an attempt at meaningful sample sizes, we estimated the relative degree of restriction to vernal pools for each taxon by using habitat descriptions from herbarium specimens. We calculated the habitat estimate for each taxon as the number of collection sites described as vernal pools (by one or more collectors) divided by the total number of collection sites with habitat information. Collection sites within a mile of each other were considered a single site. Only collections from within the CFP, and only collections with physical descriptions of habitat (rather than mention of "associations" or "communities", or no habitat information at all) were considered in estimating degree of restriction to vernal pools.

A potential weakness of using herbarium data to estimate taxon habitat preference is the assumption that independent collectors will have equivalent concepts of vernal pools. The potential effect of collector bias in our study did not appear to be serious. Excluding the newly described taxa (both subspecies of *N. myersii*), there were three taxa in section *Navarretia* for which an unusually large proportion of habitat information came from a single collector (32% for *N. fossalis*, 26% for *N. leucocephala* ssp. *plieantha*, and 33% for *N. leucocephala* ssp.

pauciflora). In each case, however, the collector described about half of the sites as vernal pools and half as other habitats, affecting the total estimates little for these taxa.

All collections of *Navarretia* in the Rancho Santa Ana Botanic Garden (RSA) and Pomona College (POM) herbaria were surveyed for this purpose after identifications were verified. Loans of the genus from the United States National Herbarium (US) and herbaria housed at Oregon State University (OSC, ORE, WILLU) were likewise surveyed. In addition, specimens of rare taxa were borrowed from the University of California at Riverside (UCR), the California Academy of Sciences (CAS), and herbaria at the University of California, Berkeley (UC, JEPS).

We attempted to interpret herbarium label data in light of a somewhat narrow definition of vernal pools, as small natural ephemeral pools fed more or less exclusively by winter rains. We did not consider very large bodies (e.g. seasonal lakes, vernal marshes) or artificial or artificially filled depressions (e.g. cow ponds) or bodies of potentially flowing water (e.g. ditches, ephemeral streams) or margins of perennial water bodies (e.g. lake shores, stream banks) to be vernal pools. Terms (on herbarium labels) considered descriptive of vernal pool habitat include "vernal pool", "vernal pool edge", "vernal pool basin", "winter pool", "ephemeral pool", "rain pool", "dried pool", "receding pool", etc. "Swale" and "depression" were also considered as equivalent to "vernal pool" since these terms seem to be used somewhat interchangeably in describing localized basins within a grassland matrix. We realize that by restricting our definition of vernal pools to small natural depressions, we are eliminating habitats which may in other aspects be quite similar to vernal pools. However, subsurface features of vernal pools likely play a significant role in defining the habitat, and are not easily replicated artificially (Holland and Dains, 1990), and the timing, depth, and duration of standing water, all of which are more predictable in smaller than in larger depressions, are among the most important environmental factors for vernal pool organisms (Keeley and Zedler, 1998; Zedler 1987).

We also collected habitat data for two species in sect. *Mitracarpium* (*N. heterandra* and *N. nigelliformis*) that were indicated by Day (1993) and Munz (1959) as frequenting vernal pools, but that in our own experience occur predominantly in other habitats. Nor are they mentioned as occurring in vernal pools in Crampton's (1954) ecological treatment of the genus. Herbarium data failed to support a vernal pool habitat for these species, and they were not investigated further.

Putative Adaptive Trait Data Collection

Many of the putative adaptive traits (Table 1) are of a nature to make comparison between vernal pool and terrestrial species impractical, if not impossible, since their expression would presumably require levels of inundation not tolerated by terrestrial

taxa. Many of these traits may simply be a consequence of vegetative growth under water (Ridge, 1987). Differences in juvenile growth form, however, were evident in the common garden study, and will be discussed. Other traits, such as plasticity and seed viability, could be compared neither in the single common garden study performed, nor with herbarium data, nor obtained from information in published literature. The traits considered in this study are marked with an asterisk in Table 1.

One population was selected to represent each of the eleven CFP taxa of sect. *Navarretia* for the collection of putative adaptive trait data. Fruiting and voucher (except for two taxa listed by California as threatened or endangered) collections were made for each population (Table 2).

Inflorescence shattering and fruit dehiscence were observed, and seed weight was measured, in ten plants of each taxon from the fruiting collection. Mean seed weight was estimated as the combined weight of the seeds of a fruit (one per plant) divided by their number.

All other traits were measured in five cultivated plants per taxon (four in *N. subuligera*). Seeds were pretreated in distilled water at 2-4° C in low light for two weeks and then transferred to an automated growth chamber programmed for 16 hr light at 21° C and 8 hr dark at 15° C. Relative humidity was at least 45% and increased with ambient conditions. Seeds from all taxa germinated within three days, and were potted two days later into a mix of equal parts fine silica sand, peat, and vermiculite, in 3 cm diameter pots. Plants were bottom watered every 1-3 days. Nutrients (20-20-20 N-P-K) were applied with watering at recommended levels for continuous indoor feeding. Plants were harvested while in full flower.

Juvenile growth form (leaf orientation and shape) was observed in rosette plants at about ten days after germination. Leaf orientation was compared in plants never having been submerged and plants submerged in water for two days and then removed for two hours.

Spininess was measured subjectively as the stiffness and sharpness of the tips of leaves and bracts of harvested and dried plants.

Glandular hairs were recorded as either present or absent on plant vegetative surfaces.

Mating system was estimated with number of pollen grains per flower, pollen-ovule (P-O) ratio, and corolla size. Pollen-ovule ratios have been found to be useful, though lagging, predictors of mating systems (Cruden, 1977; Cruden and Lyon, 1989). Pollen grains per flower was suggested by Wyatt (1984) as being superior to P-O ratios in predicting mating system, since ovule numbers may fluctuate frequently and may be influenced more strongly by selective factors unrelated to mating system.

TABLE 2. Voucher information for sampled taxa. Taxonomy follows Day (1993) and Day (1995). The second voucher number for each taxon is a plant harvested from the common garden study. All localities are in California. All vouchers ar deposited at RSA.

Species/Subspecies	Voucher	Locality
N. fossalis Moran	Spencer 4416-21, 61c-6	Riverside Co.: Winchester E 2 mi
N. intertexta (Benth.)Hook.		
intertexta	Spencer 4514-n1, 61c-3	Lake Co.: Bogg's Lake
N. leucocephala Benth.		
bakeri (H.Mason)A.G.Day	Spencer 4514-a1, 61c-c	Lake Co.: Lower Lake S 1 mi
leucocephala	Spencer 55i-04, 61c-f	Tehama Co.: Orland N 3 mi
pauciflora (H.Mason)A.G.Day	Crampton 1403; Spencer 61c-e	Lake Co.: Manning Flat
plieantha (H.Mason)A.G.Day	Crampton 3695; Spencer 61c-d	Lake Co.: Bogg's Lake
N. myersii P.S.Allen&A.G.Day		
deminuta A.G.Day	Spencer 4514-h1, 61c-a	Lake Co.: Middletown E 2 mi
myersii	Spencer 569-01, 61c-g	Sacramento Co.: Orangeville
N. prostrata (A.Gray)Greene	Spencer 4601-12, 61c-8	Riverside Co.: Santa Rosa Plateau
N. subuligera Greene	Spencer 568-82, 61c-2	Lake Co.: Bogg's Lake
N. tagetina Greene	Spencer 4514-d1, 61c-1	Lake Co.: Manning Flat

Corolla size tends to be greater in outcrossing species (Wyatt, 1988) and has been shown to be correlated with pollinator visit rate and pollen transfer in experimental studies (e.g. *Begonia involucrata*, Schemske and Ågren, 1995; *Ipomopsis aggregata*, Campbell et al., 1991; *Polemonium viscosum*, Galen and Stanton, 1989; *Raphanus sativus*, Stanton and Preston, 1988). However, floral traits cannot be assumed to be developmentally independent, and pollen production has been shown to be correlated with traits affecting pollinator attraction, including corolla size, in *Polemonium viscosum* (Galen and Stanton, 1989) and *Raphanus sativus* (Stanton and Preston, 1988). In *Navarretia*, corolla limb diameter accounts for most of the flower surface visible to pollinators. Pollen was counted in one anther per plant without staining and multiplied by the number of stamens (five) to estimate pollen per flower; ovules were counted in one ovary per plant; and corolla limb diameter was measured in one fresh flower per plant. Despite the low sampling of these three traits (five measurements for all populations except *N. subuligera*), the three estimates of breeding system (pollen grains per flower, P-O ratio, corolla limb diameter) gave similar results (Figure 2) and prompted the same conclusion concerning the proposed hypotheses. The P-O ratio for each population was calculated as the ratio of median values for number of pollen grains and number of ovules per flower.

Statistical Analyses

Correlation of putative adaptive traits (median values) and degree of taxon restriction to vernal pools was estimated with Spearman rank correlation coefficients because some of the traits were scored with categorical values and most data did not meet the normal distribution assumption of parametric tests.

Spearman rank correlation is an appropriate nonparametric test of association when there is uncertainty about the reliability of close ranks (Sokal and Rohlf, 1981). Correlation coefficients corrected for ties are reported.

In order for probability values to be meaningful in correlation tests, the data must be statistically independent. Without sufficiently detailed phylogenetic analyses, the statistical independence of character data from related taxa cannot be assumed (Felsenstein, 1985), and in fact our preliminary phylogenetic data (Figure 1) suggest that vernal pool specialists may have a single origin in the section. We therefore report only the correlation coefficients, which indicate the degree, though not the statistical significance, of the association of the putative adaptive traits with vernal pool habitat.

In evolutionary biology, the term "adaptation" has been used in two different senses in describing fitness-related traits of organisms (Gould and Vrba, 1982): 1) as a trait that enhances fitness in a particular environment, regardless of the selective forces originally responsible for the evolution of the trait, or, 2) in a narrower sense, as a trait having evolved in consequence of the same selective forces that are the basis for its present utility. Many of the traits listed in Table 1 were proposed as adaptive only in the broader sense of being of present utility in vernal pools. The mere presence of these traits in vernal pool navarretias, regardless of their presence or absence in non vernal pool relatives, would be consistent with a broad sense adaptive hypothesis. We are primarily interested, however, in those traits that may be adaptations in the narrower sense of having originated in response to natural selection in vernal pools, and it is in this sense that we will use the term in the remainder of
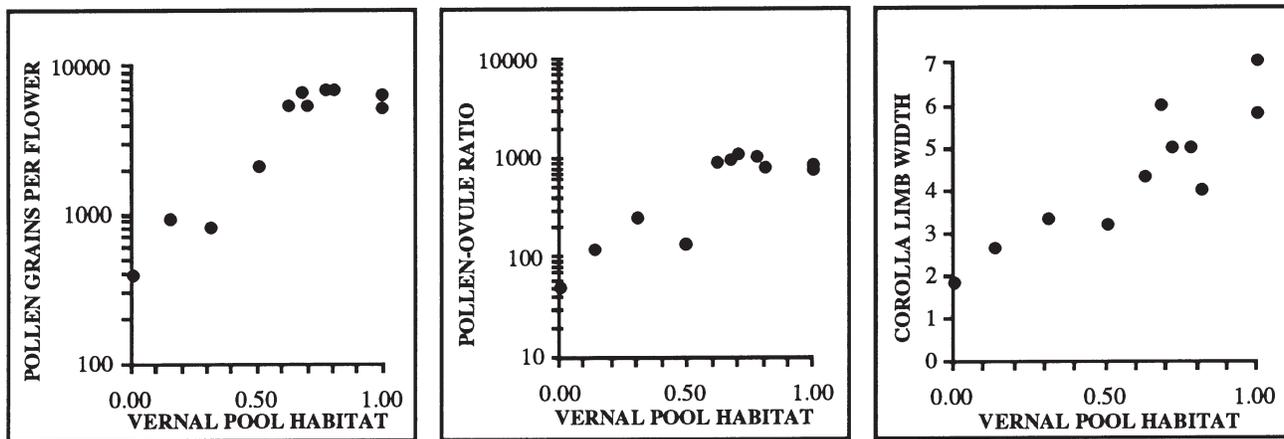
FIGURE 2. Traits estimating mating system from Table 4. The x-axis represents taxon degree of restriction to vernal pools from Table 3.

the paper. Those traits that are strongly correlated with the vernal pool habitat are considered to be the best candidates for adaptations in this narrower sense. Since the vernal pool species appear to be relatively derived in sect. *Navarretia* (Figure 1), vernal pool adaptive traits would not be expected in the non vernal pool taxa. The lack of a positive correlation would constitute evidence against a narrow sense adaptive hypothesis.

RESULTS AND DISCUSSION

Relative Degrees of Vernal Pool Specialization

Estimates of degree of restriction to vernal pools for members of sect. *Navarretia* are reported in Table 3, with taxa ordered in the table according to these estimates. Very few sites are known to exist for the rarest taxa (see footnote on Table 3), but habitat information was acquired for most existing sites. The first three species listed are generally not considered to be vernal pool species. *Navarretia fossalis* frequently occurs in habitats other than vernal pools (Moran, 1977; Spencer, pers. obs.; Bramlet, pers. comm.), but is generally considered a vernal pool species. The remaining species are usually found in vernal pools.

We found that the two species of sect. *Mitracarpium* that have been considered vernal pool species by some authors, infrequently inhabit vernal pools: at only one of six sites for *N. heterandra* (six collections, six collectors), and at only one of twelve sites for *N. nigelliformis* (seventeen collections, ten collectors; six sites for each subspecies). These species were not investigated further in this study.

Mating system

Zedler (1990) noted that, while many, or perhaps most, species that frequently inhabit vernal pools are self-pollinating, those groups apparently most specialized to vernal pools are predominantly outcrossing. He suggested that the ability of the vernal pool specialists to take advantage of both aquatic and terrestrial phases of vernal pools permits them to develop a deeper root system, which extends the growing season and increases plant size, providing better access to pollinators. Dependable pollinator access increases reproductive assurance without self-pollination, and outcrossing permits the specialists to better adapt to local conditions (presumably through the recombination of advantageous genes).

All three estimates of mating system were strongly correlated with the vernal pool habitat (Table 4, Figure 2) in a manner consistent with Zedler's observation and hypothesis. Pollen-ovule ratios of the four taxa least restricted to vernal pools (*N. subuligera*, *N. tagetina*, *N. intertexta*, and *N. fossalis*) fall within the range of predominantly to facultatively (for *N. tagetina*) selfing species (Plitmann and Levin, 1990) in the Polemoniaceae. Pollen-ovule ratios of the remaining species (those most restricted to vernal pools) are in the range of predominant to obligate outcrossing. The correlation of P-O ratio and vernal pool habitat (0.569) would be higher but for a doubling of ovule number (as discussed by Wyatt [1984]) in *N. fossalis* relative to the remaining species (see Table 4). Accordingly, pollen per flower shows a greater correlation (0.740) with vernal pool habitat than does P-O ratio. Corolla limb width shows an even greater correlation with habitat (0.862). These results are consistent with the hypothesis that outcrossing is advantageous for vernal pool specialists. This does not necessarily mean, however, that outcrossing vernal pool specialists evolved from selfing ancestors. Such a derivation of outcrossing from selfing is contrary to the trend in angiosperms (Stebbins, 1957; Jain, 1976; Wyatt, 1988) and beset with theoretical problems (Lande and Schemske, 1985; Stebbins, 1957; Bull and Charnov, 1985). Detailed phylogenetic analyses may help to clarify mating system evolution in this group.

Table 3. Degree of restriction to vernal pools in CFP taxa of *Navarretia* sect. *Navarretia*, as estimated from habitat descriptions on herbarium collection labels. Taxa are ordered by increasing degree of restriction to vernal pools.

Species/Subspecies	Collections with habitat description [Principal collectors]	Number of sites represented	Proportion of sites described as vernal pools
N. subuligera	8 [6]	7[a]	0.00
N. intertexta	74 [37]	59	0.14
N. tagetina	34 [24]	29	0.31
N. fossalis	40 [15]	22[a]	0.50
N. leucocephala			
plieantha	23 [12]	8[a]	0.63
leucocephala	40 [24]	31	0.68
pauciflora	12 [8]	7[a]	0.71
bakeri	12 [12]	9[a]	0.78
N. prostrata	31 [22]	16[a]	0.81
N. myersii			
deminuta	3 [2]	1[a]	1.00
myersii	4 [3]	4[a]	1.00

[a] Rare taxa. Only one population of *N. myersii* ssp. *deminuta* is known to exist, and only 5 sites are known for *N. myersii* ssp. *myersii*. *N. leucocephala* ssp. *plieantha* is listed as "Endangered" by the state of California, and *N. leucocephala* ssp. *pauciflora* is listed as "Threatened".

Seed Dispersal and Size

Zedler (1990) suggested that the island-like nature of the habitat would favor a reduction in seed dispersal in vernal pool specialists, and that seed retention by the parent would help protect the seeds from predation and heat stress at ground level during the desert-like summer conditions. All members of sect. *Navarretia* have indehiscent fruits (Table 5). The seeds are typically released after heavy rains as fibers on the seed surface uptake water and expand to break open the fruit (Day, 1993; Spencer, pers. obs.). Because the vernal pool specialists as well as all other species in sect. *Navarretia* have indehiscent fruits, this trait is not likely an adaptation to vernal pools, but nevertheless may be advantageous in that habitat. Lack of inflorescence shattering (Table 5) is more likely to be an adaptation to vernal pools, because it is correlated with vernal pool habitat.

Zedler (1984) postulated that small seed size in vernal pool species allows for a large number of seeds per unit biomass, which serves to increase the effective population size in small basins, making chance extinction less likely. Local extirpation would be particularly detrimental to species with limited means of dispersal. Zedler did not hypothesize that small seed size evolved in response to vernal pool environmental conditions (which would have invoked the controversial idea of group selection in this case [Goodnight et al., 1992]) but only that small seeds are presently advantageous for vernal pool inhabitants.

We did not find a correlation of seed size with vernal pool habitat in sect. *Navarretia*. Seed weights for these species are, however, lower than average for native California herbaceous plants and grassland plants (Baker, 1972).

Herbivory Defense or Water Retentive Morphology

While most species of *Navarretia* have glandular hairs on stems, leaves, and bracts (Spencer, pers. obs.), glandulat hairs are completely lacking on these organs in most members of sect. *Navarretia*. Crampton (1954) suggested that reduced glandularity in *Navarretia* may have evolved in response to the abundant moisture available in vernal pools. It is more likely, however, that glandular hairs serve to defend against herbivory rather than to reduce transpirational water loss (Duke, 1994). Regardless, two of the three non vernal pool species in sect. *Navarretia* also lack glandular hairs (Table 5), suggesting that the condition may have already existed in the ancestors of the vernal pool species.

Zedler (1987) suggested that late-blooming vernal pool species may be subject to heavy grazing if not for various deterrents such as spininess, viscid secretions, and odor. Even though many navarretias are ill-smelling and most are spiny, the vernal pool species lack obvious grazing deterrents. Spininess in particular shows a strong negative correlation with the vernal pool habitat (Table 5), in direct contradiction to Zedler's hypothesis,

TABLE 4. Reproductive traits. Values are means ± SE [medians] in seed size, and means ± SD [medians] in the remainder. Pollen-ovule ratio is the ratio of median values for pollen grains per flower and ovules per flower. Spearman (r_S) coefficients show rank correlation of putative adaptive traits with degree of restriction to vernal pools (Table 3). Taxa are arranged as in Table 3.

Species/Subspecies	Pollen grains per flower	Corolla limb width (mm)	Ovules per flower	Seed weight (mg)	P-O ratio
N. subuligera	390±43 [390]	1.8±0.2 [1.8]	7.5±1.0 [8]	0.36±0.04 [0.34]	49
N. intertexta	940±199 [920]	2.8±0.3 [2.6]	7.8±1.5 [8]	0.30±0.09 [0.32]	115
N. tagetina	731±133 [780]	3.4±0.3 [3.3]	3.6±0.9 [3]	1.15±0.26 [1.10]	260
N. fossalis	2243±284 [2110]	3.2±0.3 [3.2]	15.8±1.8 [16]	0.13±0.05 [0.13]	132
N. leucocephala					
plieantha	5271±765 [5235]	4.2±0.3 [4.2]	5.6±1.1 [6]	0.19±0.07 [0.20]	873
leucocephala	6132±814 [6490]	6.0±0.6 [6.0]	7.0±1.0 [7]	0.34±0.07 [0.34]	927
pauciflora	5772±909 [5355]	4.9±0.7 [5.0]	5.4±1.7 [5]	0.32±0.18 [0.28]	1071
bakeri	6457±735 [6790]	4.8±0.8 [5.0]	6.8±1.1 [7]	0.15±0.05 [0.15]	970
N. prostrata	6936±614 [6800]	4.1±0.9 [4.0]	8.4±0.6 [8]	0.17±0.08 [0.14]	850
N. myersii					
deminuta	6316±809 [6350]	5.7±0.4 [5.8]	7.8±1.1 [8]	0.40±0.15 [0.34]	794
myersii	5210±724 [5030]	7.2±0.5 [7.0]	7.2±1.8 [6]	0.64±0.29 [0.53]	838
Correlation (r_S):	0.743	0.826	-	-0.046	0.569

suggesting that grazing may be less important in vernal pools than in habitats occupied by most navarretias.

Juvenile Growth Form

In a study of sixteen dominant macrophytes from selected southern California vernal pools, Keeley (1990) found that a majority of these species produced an isoetid (*Isoetes*-like rosette of stiff, ascending, more or less cylindrical leaves) growth form (Den Hartog and Segal, 1964) as submerged juveniles. Keeley (1990) hypothesized that carbon limitations in the vernal pool environment may have indirectly selected for this growth form, because the cylindrical shape of the leaves would provide structural support while allowing for internal air spaces and a thinner boundary layer to facilitate gas exchange. We found that in the common garden experiment, only *N. myersii* had an unambiguous isoetid juvenile growth form, while other species had rosette forms with more decumbent to prostrate leaves (unlike the more or less erect leaf orientation in species mentioned by Keeley [1990] and Den Hartog and Segal [1964] as isoetid). In all but two of the species without initially erect leaves, leaves became more erect soon after submersion in water, and retained this orientation for several hours after removal from the water. Those taxa whose leaf orientation changed in response to submersion are reported as having an "inducible" isoetid juvenile form in Table 5. If "inducible" is considered an intermediate step, an isoetid juvenile form is strongly correlated with the vernal pool habitat, indicating that it may have evolved in response to natural selection in that habitat. It may also be noted that the three taxa estimated here to be most restricted to vernal

pools are acaulescent, remaining in a rosette form as adults, although the leaves spread before flowering. In separate aquatic garden studies (Spencer, unpubl. data), vernal pool navarretias grew slowly while the leaves were submerged, but growth rate greatly increased once the tips of the long leaves reached the water surface, suggesting that the ascending leaf orientation in vernal pool plants having an isoetid growth form may benefit the submerged plant by bringing the leaves more quickly into the aerial environment.

SUMMARY AND CONCLUSIONS

Degree of restriction to vernal pools was estimated using herbarium data, and putative vernal pool adaptive traits were measured, for all vernal pool navarretias and their nearest relatives of the CFP. These traits were tested for correlation with vernal pool habitat. Vernal pool adaptive hypotheses are contradicted in *Navarretia* for spininess. Small seeds, indehiscent fruits, and lack of glandular hairs are traits that may be advantageous in vernal pools, but are not tightly associated with that habitat. Outcrossing, non-shattering inflorescences, reduced spininess, and an isoetid growth form are better candidates for evolutionary adaptations to vernal pools, although the evolution of outcrossers from selfing ancestors is theoretically unlikely.

This correlation study included only CFP taxa because degree of restriction to vernal pools is not definable for taxa occurring outside Mediterranean-type climatic regions. All close relatives of the vernal pool navarretias, however, will be included in phylogenetic studies investigating the evolution of these traits. Be-

TABLE 5. Traits potentially influencing dispersal, herbivory, water loss, and carbon assimilation. Spearman (r_S) coefficients show rank correlation of these traits with degree of restriction to vernal pools (Table 3). Taxa are arranged as in Table 3.

Species/Subspecies	Dehiscent Fruit	Shattering	Spiny Leaves/Bracts	Glandular Trichomes	Isoetid Juvenile
N. subuligera	no	yes	yes/yes	no	no
N. intertexta	no	yes	yes/yes	no	inducible
N. tagetina	no	yes	yes/yes	yes	no
N. fossalis	no	no	no/yes	no	inducible
N. leucocephala					
plieantha	no	yes	no/no	no	inducible
leucocephala	no	no	no/no	no	inducible
pauciflora	no	yes	no/no	no	inducible
bakeri	no	no	no/no	no	inducible
N. prostrata	no	no	no/no	no	inducible
N. myersii					
deminuta	no	no	no/no	no	yes
myersii	no	no	no/no	no	yes
Correlation (r_S):	-	0.694	-0.855	0.301	0.812

cause the vernal pool navarretias are all closely related and likely have a single origin, the results of these studies will be most useful in combination with those of similar studies in other plant groups.

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