

# Population Genetics of Vernal Pool Plants: Theory, Data and Conservation Implications

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**ABSTRACT.** One goal of population genetics is to quantify and explain genetic structure within and among populations. Factors such as genetic drift, inbreeding, gene flow and selection are expected to influence levels and distribution of genetic variation. I review available data on genetic structure of vernal pool plant species with respect to these factors. Where relevant data are lacking, as is often the case for vernal pool plants, I examine how these factors are expected to influence vernal pool population genetic structure. I also consider whether the available population genetic data and theory can help provide approximate predictions of the genetic structure of unstudied vernal pool plant species and suggest reasonable approaches to conservation and management.

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## INTRODUCTION

In the early 1980's, genetic approaches were identified as potentially useful tools in conservation biology for answering questions about population viability, long-term persistence of populations and species, and maintenance of evolutionary potential (e.g. Soulé, 1980; Frankel and Soulé, 1981). Despite this awareness and despite the recognition that vernal pools are important centers of endemism and valuable laboratories for the study of population biology, evolution and conservation biology (Holland and Jain, 1981; Jain, 1994), research on the population genetics of vernal pool plants has been limited. Understanding levels and distribution of genetic variation within and among populations is necessary if effective conservation and management practices are to be implemented to preserve genetic diversity and evolutionary potential (Beardmore, 1983) in vernal pool plants.

My review addresses three issues: 1) what do available data and theory tell us about the genetic structure of vernal pool plant populations, 2) how might information on levels and distribution of genetic variation help in the design of effective conservation and management strategies for vernal pool plant taxa, and 3) what factors ought to be considered in making predictions about the probable genetic structure of unstudied species. Because theory suggests that evolutionary factors such as genetic drift, inbreeding, gene flow and selection are important in structuring genetic variation (Futuyma, 1986; Hartl and Clark,

1989), I examine the expectations of theory and available data for vernal pool plant taxa for each of these factors.

## FACTORS AFFECTING POPULATION GENETIC STRUCTURE

### *Genetic Drift*

*Expectations of theory.* Genetic drift is random change in allele frequencies that occurs because only a subset of alleles present in the parental generation is transmitted to the next generation. Genetic drift is expected to substantially influence the genetic structure of populations that are continually small in size (e.g.  $< 100$  individuals) or that undergo occasional fluctuations to small size, as in population bottlenecks or founder/colonization events (Frankel and Soulé, 1981; Futuyma, 1986). Population genetic structure is expected to be substantially altered by drift when  $1/4N_e$  is much greater than the mutation rate ( $\mu$ ) and the selection coefficient ( $s$ ) where  $N_e$  is the effective population size. Effective population size is the number of individuals in an ideal population that would have the same genetic response to random processes as a real population of size  $N$  (Wright, 1931; Crawford, 1984).  $N_e$  is difficult to measure accurately in nature but is generally expected to be less than the census size,  $N$  (e.g. Nunney and Elam, 1994).

Small populations may undergo large and unpredictable fluctuations in allele frequencies due to drift. As a result, one can expect 1) a decrease of genetic variation within populations

(exhibited by loss of heterozygosity and eventually fixation of alleles) and 2) an increase in differentiation among populations (i.e. populations become genetically more different from each other) (Futuyma, 1986; Falconer, 1989). In populations undergoing substantial fluctuations in population size, allelic variation is likely to decrease (i.e. alleles are likely to be lost) while expected heterozygosity often remains relatively unchanged as long as population size rebounds rapidly (Futuyma, 1986; Barrett and Kohn, 1991). The specific effects of drift on genetic diversity in small plant populations (Ellstrand and Elam, 1993) and the population genetic consequences of bottlenecks and founder events (Barrett and Kohn, 1991) have been reviewed elsewhere.

*Genetic drift and vernal pool plants.* For genetic drift to be an important factor influencing the amount and/or distribution of genetic variation in a species, the species must contain some small populations or populations that undergo fluctuations to small size. I asked whether this was the case for sensitive, threatened or endangered vernal pool plants by examining all occurrences of vernal pool plant taxa in the California Department of Fish and Game's RAREFIND (CDFG, 1996) computer database. For the purposes of my survey, I assumed that each occurrence constitutes a single population. There is no straightforward way to conduct such a survey for both common and rare taxa. However, RAREFIND contains substantial data for sensitive species, those species most often the focus of conservation efforts. Each taxon in RAREFIND is associated with one or more "habitat codes" which designate the habitat(s) in which the taxon typically occurs. I defined "vernal pool plant taxa" as those taxa which had "vernal pool" designated as one of their habitat codes. Population size data (i.e. number of individuals) were available for 59 vernal pool plant taxa and almost 1000 occurrences.

Several vernal pool taxa, including *Castilleja campestris* ssp. *succulenta*, *Downingia pusilla*, *Eryngium aristulatum* ssp. *parishii*, *Gratiola heterosepala* and *Neostapfia colusana*, had occurrences of 100 or fewer individuals in the year they were observed. Because they represent only one year, the data are insufficient to address whether these occurrences are continually small in size. Small population sizes have been reported in the literature for populations of *Tuctoria* and endangered taxa of *Limnanthes* (Kesseli, 1992). The RAREFIND data also provided evidence for substantial fluctuations in population size in some species (e.g. *Blennosperma bakeri*, *Gratiola heterosepala*, *Orcuttia tenuis*, *Tuctoria mucronata*). Population size fluctuations have been noted in other taxa including *Downingia cuspidata*, *D. concolor* ssp. *brevior* (Bauder, 1987, 1992 cited in Bauder, 1993), *Limnanthes* (Kesseli, 1992; J. Dole and R. Kesseli, pers. obs. cited in Dole and Sun, 1992), *Orcuttia pilosa*, *O. viscida* and *O. (=Tuctoria) greenei* (Griggs and Jain, 1983) and *Veronica peregrina* (Y. Linhart, pers. comm.). In *V. peregrina*, population size was routinely 1000 to 10,000 in years of high rainfall and less than 100 when rainfall was low

(Y. Linhart, pers. comm.). Because they provide evidence of small and/or fluctuating population sizes, these data suggest that genetic drift may influence the level and distribution of genetic variation in some populations of some vernal pool plant taxa. Populations influenced by genetic drift are expected to have reduced levels of genetic variation and high levels of among-population differentiation.

Few data are available to determine whether observed patterns of genetic variation in vernal pool plant taxa are consistent with the predictions of genetic drift (Tables 1 and 2). Four of the eight taxa for which we have data have lower frequencies of polymorphic loci and lower numbers of alleles per locus (i.e. lower levels of genetic variation) than the average plant species, dicot and annual (Table 1). A recent study of *Limnanthes floccosa* ssp. *californica* suggests that it is especially genetically depauperate. Dole and Sun (1992) found six of nine populations to lack polymorphism at all 28 loci examined; the remaining three populations were only polymorphic for a single locus. Interestingly, the four taxa with reduced levels of genetic variation are all relatively rare compared to those taxa exhibiting higher levels of genetic variation. This pattern is consistent with the observation of Karron (1987; 1991) that restricted species generally, but not always, contain less genetic variation than their widespread congeners, as measured by percentage of polymorphic loci and number of alleles per locus.

Population differentiation, the second pattern associated with drift, is often measured by  $G_{ST}$ , the proportion of total genetic variation residing among populations (Hamrick and Godt, 1990). In vernal pool taxa for which we have data,  $G_{ST}$  tends to be about 0.1 or higher.  $G_{ST}$  values of this magnitude represent some population differentiation but are lower than the average levels of genetic differentiation reported for plant taxa, dicots and annuals as a whole (Table 2). However, considering the small scale at which vernal pools are often studied versus the larger scale for many other plant species, the  $G_{ST}$  values for the two groups may be comparable.

The above studies measured levels of electrophoretically detectable variation. Quantitative variation may exhibit different patterns (Lande and Barrowclough, 1987; Hamrick, 1989). One study examined this question in a vernal pool taxon. Ritland and Jain (1984) found that there was significant among-population genetic variation for certain floral and life history characters of *Limnanthes alba* although allozyme variation among populations was low.

The available data suggest that patterns of genetic variation in some vernal pool taxa are consistent with the predictions of genetic drift. This does not mean that the observed patterns are necessarily due to genetic drift. Other factors, such as breeding system and gene flow, are also likely to influence patterns of genetic variation in these taxa (discussed below). In addition,

TABLE 1. Level of genetic variation (frequency of polymorphic loci and number of alleles per locus) for vernal pool plant taxa.

Taxon	Status <sup>a</sup>	Breeding system <sup>b</sup>	PLT <sup>c</sup>	PLP	ALT	ALP	Reference
<i>Limnanthes alba</i> ssp. <i>alba</i>	W	HO	0.75	0.63	2.56	2.04	McNeill and Jain, 1983
<i>Limnanthes alba</i> ssp. <i>versicolor</i>	W	HO	0.81	0.58	2.78	2.06	McNeill and Jain, 1983
<i>Limnanthes bakeri</i>	R	SS	0.37	0.22	1.47	1.20	Kesseli and Jain, 1984b
<i>Limnanthes douglasii</i> ssp. <i>rosea</i>	W	HO	0.84	0.58	3.05	1.90	Kesseli and Jain, 1984b
<i>Limnanthes floccosa</i> ssp. <i>californica</i>	R	PS	0.23	0.23	1.43	1.43	McNeill and Jain, 1983
<i>Limnanthes floccosa</i> ssp. <i>floccosa</i>	W	PS	0.29	0.04	1.44	1.05	McNeill and Jain, 1983
<i>Limnanthes gracilis</i> ssp. <i>parishii</i>	R	SS	0.69	0.40	2.11	1.82	McNeill and Jain, 1983
<i>Limnanthes vinculans</i>	R	SS	0.41	0.33	1.71	1.50	Kesseli and Jain, 1984b
Plant taxa (473 taxa, 468 populations)			0.51	0.34	1.96	1.53	Hamrick and Godt, 1990
Dicots (329 taxa, 338 populations)			0.45	0.29	1.79	1.44	Hamrick and Godt, 1990
Annuals (190 taxa, 187 populations)			0.51	0.30	2.07	1.48	Hamrick and Godt, 1990
Selfing (123 taxa, 113 populations)			0.42	0.20	1.69	1.31	Hamrick and Godt, 1990

<sup>a</sup> W = relatively widespread taxon, R = relatively rare taxon (Federally and/or California state listed)

<sup>b</sup> HO = highly outcrossing (>50%), SS = somewhat selfing (outcrossing = 10-50%), PS = predominantly selfing; From Brown and Jain, 1979 (*Limnanthes alba* ssp. *alba*, *Limnanthes alba* ssp. *versicolor*); Jain, 1981 (all other taxa).

<sup>c</sup> PLT = frequency of polymorphic loci in the taxon, PLP = frequency of polymorphic loci in the average population of the taxon, ALT = mean number of alleles per locus in the taxon, ALP = mean number of alleles per locus in the average population of the taxon

seed banks may maintain genetic variation in populations and buffer them against large changes in genetic composition (Baker, 1989). I am unaware of published studies addressing the maintenance of genetic variation by seed banks in vernal pool taxa (or rare taxa in general, Ellstrand and Elam, 1993). However, a number of vernal pool taxa are thought to have persistent and extensive seed banks (e.g. *Orcuttia*, Griggs and Jain, 1983; *Downingia cuspidata* and *D. concolor* ssp. *brevior*; Bauder 1987, 1992 cited in Bauder, 1993).

### ***Inbreeding / Breeding System***

*Expectations of theory.* Inbreeding is the mating of related individuals (Falconer, 1989). In plants, the level of inbreeding is strongly influenced by breeding (mating) system. Some plants are obligately or habitually selfing. Selfing, the most extreme form of inbreeding, may be reduced by patterns of flower development such as protandry (where anther dehiscence precedes stigma receptivity) or prevented by breeding systems which ensure outcrossing (e.g. self-incompatibility). Plants which both self and outcross have 'mixed mating' systems. Inbreeding in plants can also occur through biparental inbreeding when populations are small (Barrett and Kohn, 1991 and references therein) or exhibit spatial genetic structure (e.g. Turner et al., 1982).

Inbreeding increases levels of homozygosity and among-population differentiation. Because heterozygosity is lost at a rate of

$1/2N_e$  per generation, smaller populations are expected to lose heterozygosity faster than larger ones. Frequencies of heterozygotes are expected to approach zero in populations with continuous inbreeding (Wright, 1931; Futuyma, 1986). Because level of inbreeding is closely related to breeding system, the particular genetic structure of a plant population is highly influenced by the plant's breeding system (Hamrick, 1989). It is generally accepted that obligate or habitually selfing species tend to have low within-population genetic variation and high among-population differentiation (e.g. Hamrick and Godt, 1990).

Inbreeding might also be important due to its potential effect on fitness. Inbreeding depression is expressed as a loss of fitness (decreased viability and/or fecundity) with increasing homozygosity. The precise mechanism of inbreeding depression is controversial. Increased homozygosity may result in a loss of fitness because 1) heterozygotes are superior at loci affecting fitness (overdominance) or 2) increased homozygosity leads to expression of deleterious recessive alleles. In the latter case, typically inbreeding (e.g. selfing) populations are expected to exhibit little inbreeding depression if selection has purged these populations of deleterious recessives responsible for fitness losses (Charlesworth and Charlesworth, 1987). Nevertheless, some selfing species suffer from strong inbreeding depression, suggesting that the relationship between selfing rate (mating

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TABLE 2: Distribution of genetic variation (Nei's genetic diversity statistics) and gene flow for vernal pool plant taxa.

Taxon	Breeding			$H_T^c$	$H_s$	$D_{ST}$	$G_{ST}$	$Nm$	Reference
	Status <sup>a</sup>	system <sup>b</sup>	# poplns						
<i>Lasthenia burkei</i>	R	OO	4	0.209	0.177	0.032	0.157	0.76	Crawford and Ornduff, 1989
<i>Lasthenia conjugens</i>	R	OO	7	0.195	0.161	0.034	0.176	0.86	Crawford and Ornduff, 1989
<i>Lasthenia fremontii</i>	W	OO	5	0.168	0.152	0.016	0.097	1.49	Crawford and Ornduff, 1989
<i>Limnanthes bakeri</i>	R	SS	8	0.180	0.114	0.065	0.39	0.39	Dole, unpublished
<i>L. douglasii</i> ssp. <i>rosea</i>	W	HO	13	0.18	0.14	0.03	0.167	1.06	Kesseli, 1992
<i>L. floccosa</i> ssp. <i>californica</i>	R	PS	9	0.082	0.003	0.079	0.963	0.01	Dole and Sun, 1992
<i>Limnanthes vinculans</i>	R	PS	4	0.12	0.11	0.01	0.083	1.55	Kesseli, 1992
Plant taxa (406 taxa)			12.7	0.310	0.230		0.224		Hamrick and Godt, 1990
Dicots (246 taxa)			11.9	0.311	0.214		0.273		Hamrick and Godt, 1990
Annuals (146 taxa)			18.5	0.330	0.200		0.357		Hamrick and Godt, 1990
Selfing taxa (78 taxa)			20.3	0.334	0.149		0.510		Hamrick and Godt, 1990

<sup>a</sup> W = relatively widespread taxon, R = relatively rare taxon (Federally and/or California state listed)

<sup>b</sup> OO = obligately outcrossing, HO = highly outcrossing (>50%), SS = somewhat selfing (outcrossing = 10-50%), PS = predominantly selfing; From Crawford and Ornduff, 1989 (*Lasthenia burkei*, *Lasthenia conjugens*, *Lasthenia fremontii*); Jain, 1981 (all *Limnanthes* taxa)

<sup>c</sup>  $H_T$  = total genetic diversity,  $H_s$  = within population diversity,  $D_{ST}$  = between population diversity,  $G_{ST} = D_{ST}/H_T$  = proportion of total genetic variation among populations,  $Nm = ((1/G_{ST})-1)(4(n/n-1)^2)^{-1}$  = gene flow where n = number of populations

system) and inbreeding depression is not precise (Barrett and Kohn, 1991).

*Inbreeding / Breeding system and vernal pool plants.* Because breeding systems play a crucial role in shaping population genetic structure (Hamrick, 1989), knowledge of breeding systems of vernal pool taxa may provide clues to the genetic structure of vernal pool plant populations. Zedler (1990) summarized the breeding systems of plant taxa in the vernal pools of Kearny and Del Mar mesas of San Diego County and found that 60% of the 20 native taxa were self-pollinating. If this trend toward high levels of selfing is representative of vernal pool taxa in general, then we might expect vernal pool populations to often be genetically depauperate and highly differentiated. Such generalizations must be made with caution. In fact, vernal pool plant taxa exhibit the entire range of breeding systems, including cleistogamous (fully selfing) species (e.g. *Limnanthes floccosa* ssp. *floccosa*, Brown and Jain, 1979), predominantly selfing species (e.g. *Veronica peregrina*, Keeler, 1978), predominantly outcrossing but self-compatible species (e.g. species of *Orcuttia*, Griggs and Jain, 1983; *Limnanthes douglasii*, Kesseli and Jain, 1985), and obligate outcrossers (e.g. *Lasthenia burkei*, Crawford and Ornduff, 1989). Predictions of population genetic structure from breeding system are best made with reference to specific taxa and with specific knowledge of the breeding system biology of the taxon of concern.

Will specific knowledge of breeding systems lead to accurate predictions of population genetic structure for specific vernal pool plant taxa? For electrophoretically detectable variation, the available data suggest that the selfing taxa generally have lower frequencies of polymorphic loci and numbers of alleles per locus (both within species and within populations) than the outcrossing taxa (Table 1). However, the selfing taxa are also the rare taxa so status/geographic range confounds the result. In most cases, frequencies of polymorphic loci and number of alleles per locus are comparable to those observed for selfing taxa as a whole (Table 1). Moreover, two selfing taxa have relatively high  $G_{ST}$  values (discussed below) (Table 2).

Brown and Jain (1979) studied patterns of morphological variation, along with allozyme variation, in selfing *Limnanthes floccosa* and outbreeding *Limnanthes alba*. They examined 15 quantitative characters and found that *L. floccosa* had less variability overall than *L. alba*. However, several traits were exceptions including number of days to anthesis and number of basal branches. Interpopulation differentiation was not more pronounced in *L. floccosa* than in *L. alba*. Patterns of morphological variation observed were similar to allozyme variation and generally consistent with expectations based on breeding system.

The above data suggest that breeding system may sometimes be a useful predictor of population genetic structure for vernal

pool plant taxa. However, the data are few, and there can be exceptions. For example, although *Veronica peregrina* is almost completely selfing, individuals appear to be highly variable. The variation is probably explained by its polyploid (hexasomic) inheritance which may allow maintenance of relatively high variability despite selfing. Increases in homozygosity due to inbreeding are expected to occur more slowly in polyploids than in disomics (Keeler, 1978). In addition, outcrossing rates (hence levels of inbreeding) can vary substantially within and among populations (e.g. in *Limnanthes alba*, McNeill and Jain, unpublished data as cited in Jain, 1994).

Inbreeding depression has rarely been studied in vernal pool plant taxa. It was detected at several stages of the life cycle of *Limnanthes douglasii* ssp. *rosea* (Kesseli and Jain, 1984a). In addition, McNeill, Kesseli and Jain (unpublished data as cited in Jain, 1994) found greater inbreeding depression in *L. douglasii* ssp. *rosea* than in *Limnanthes alba*. This was not unexpected because *L. douglasii* ssp. *rosea* is apparently more highly outcrossing than *L. alba*. In contrast, moderate to high levels of inbreeding depression were detected in *Limnanthes bakeri* although it is a moderately selfing species (Dole, unpublished data).

### Gene Flow

*Expectations of theory.* Although many plant populations are geographically discrete, they may not be reproductively isolated. Gene flow between populations of plants occurs by interpopulation mating or by migration of seeds or vegetative propagules (Ellstrand and Elam, 1993). It can be substantial but is highly variable among species, populations, individuals and years (Ellstrand, 1992). It may occur at significant rates and considerable distances even in predominantly selfing species (Wagner and Allard, 1991). In general, we expect species with restricted gene movement (i.e. low levels of gene flow) to exhibit greater levels of among-population genetic differentiation than those with widely dispersed pollen and seeds (Hamrick, 1989). Techniques and difficulties associated with measurement of gene flow are discussed by Slatkin (1987) and Ellstrand (1992).

Interpopulation gene flow is often considered beneficial in conservation (e.g. Huenneke, 1991) because it can increase the effective size of populations, reducing the threat of depletion of genetic variation and inbreeding depression. Theory suggests that one immigrant every second generation or one interpopulation mating per generation ( $Nm = 0.5$ ) will be enough to prevent substantial differentiation among populations (Slatkin, 1987).

However, gene flow is not necessarily beneficial and may, in fact, be detrimental for at least three reasons (Ellstrand, 1992).

1) Substantial gene flow from a genetically depauperate source

may reduce the amount of local variation in a target population. 2) Gene flow of alleles from populations in different locations may prevent local adaptation if selection favors different alleles in the source populations than in the target population. Moderate gene flow rates, on the order of 1-5% per generation, are sufficient to counter selection of the same magnitude (Ellstrand and Elam, 1993). 3) Gene flow among populations may lead to fitness reductions in target populations through "outbreeding depression" (sensu Templeton, 1986). Such reductions have been attributed to disruption of co-adaptation to local conditions (Templeton, 1986) and may be common in plants. They have been documented in a number of plant species, sometimes at distances as small as 10-15 meters (e.g. Waser, 1993).

Because gene flow can be beneficial or detrimental, how do we know in any particular situation what its role will be? We expect the role of gene flow to depend largely upon its role in the recent evolutionary history of the species. Where levels of gene flow have been historically high, recent increases in gene flow are unlikely to be detrimental, but reductions may be of concern. Alternatively, where levels of gene flow have been historically low, decreased gene flow (or increased isolation) is likely to have little effect on genetic structure, but increased gene flow may be deleterious (e.g. it may cause outbreeding depression in target populations) (Ellstrand and Elam, 1993). Details of how changes in gene flow might be identified, possible management alternatives, and problems associated with interspecific gene flow are discussed elsewhere (Ellstrand, 1992; Ellstrand and Elam, 1993).

*Gene flow and vernal pool plants.* Because vernal pools have a patchy, island-like distribution (Linhart, 1976; Holland and Jain, 1981), populations of vernal pool plants, especially those highly restricted to pools, tend to be relatively isolated geographically. However, as noted above, geographic isolation does not necessarily imply reproductive isolation because of the potential for gene flow among populations. Therefore, it is useful to ask what patterns of gene flow typify vernal pool plant populations. The question can be approached directly by measuring pollen and seed dispersal or indirectly by inferring gene flow from population genetic structure (Ellstrand, 1992).

Both approaches have been used in research relevant to gene flow in vernal pool plant taxa. Observations by Leong et al. (1995) showed that the foraging movements of *Andrena blennospermatis*, a native solitary bee, on *Blennosperma nanum* var. *nanum* were relatively limited. Individuals of *A. blennospermatis* occasionally foraged between *B. nanum* var. *nanum* patches 25 meters apart but rarely foraged between patches 80-100 meters apart, suggesting that pollen transfer has the potential to be quite restricted. Specialist bees on *Limnanthes* also potentially restrict pollen-mediated gene flow within plant populations by foraging primarily within limited areas of their

host flowers (Thorp, 1990). However, Thorp (unpublished data cited in Kesseli, 1992) also documented long distance dispersal (between neighboring vernal pools) using pollen marked with fluorescent dye. Although 97% of the dye was distributed within 5 meters, occasionally dispersal up to 13 meters occurred. Similarly, in *Limnanthes douglasii* ssp. *rosea*, 85% of the plants received pollen from donors within 10 centimeters, but some (less than 5%) received pollen from parents up to 80 meters away (Kesseli, 1992).

Seed dispersal may also be restricted. Jain (1978) observed highly localized dispersal of *Limnanthes* seed within and between artificial pools. Using water flow patterns as a measure of dispersability also suggested limited seed dispersal in *Pogogyne abramsii* (Schleidlinger, 1981). Seed dispersal may be especially limited in taxa whose seed capsules remain closed until maturity (e.g. *Downingia concolor* and *Boisduvalia glabella* (= *Epilobium pygmaeum*)). In some cases, the seeds may actually germinate within the capsules. Such capsule indehiscence might be a mechanism to keep seeds from moving away from vernal pools. It would be of interest to determine how many vernal pool plant taxa have such closed, indehiscent capsules and how the frequency compares to related but non-vernal pool relatives (Y. Linhart, pers. comm.). Thus, measures and observations of pollen or seed movement in vernal pool plants imply relatively restricted gene flow (but even low rates of long distance dispersal over many years may be significant). The results are typical of studies using direct measures to quantify gene flow (Ellstrand, 1992).

Do we find a similar pattern using population genetic structure to infer levels of gene flow among populations? Average numbers of successful migrants per generation ( $Nm$ ) for vernal pool plant taxa are given in Table 2. When  $Nm$  is calculated from  $G_{ST}$ , as it is here, it estimates recent, order of magnitude gene flow (as opposed to current gene flow) (Slatkin and Barton, 1989). Only the values for *Limnanthes bakeri* and *Limnanthes floccosa* ssp. *californica* are low enough ( $Nm < 0.5$ ) to suggest extreme isolation among populations. However, the differentiation exhibited by *L. floccosa* ssp. *californica* is among the highest recorded for local, conspecific populations of plants (Dole and Sun, 1992). All other gene flow estimates for vernal pool taxa are large enough to suggest allele frequencies are homogenized among populations. It is not trivial that the two taxa with the lowest gene flow estimates have the highest selfing rates of the taxa for which we have data (Brown and Jain, 1979; Kesseli, 1992).

### Selection

*Expectations of theory.* Natural selection is differential survival and reproduction of genotypes in a specific environment. As a result of selection, alleles that promote higher fitness are over-represented in succeeding generations (i.e. they increase in fre-

quency). Because of its potential to alter allele frequencies, selection can influence population genetic structure (Futuyma, 1986; Hartl and Clark, 1989).

In general, selection is expected to be important in structuring genetic variation when the effects of gene flow and genetic drift are small (Futuyma, 1986; Hartl and Clark, 1989). Theory predicts that gene flow will prevent local adaptation when  $m > s$  where  $m$  is the fraction of immigrants per generation and  $s$  is the local selective coefficient. However, this relationship also implies that selection may be important even in the presence of significant gene flow if selection is strong (Slatkin, 1987). In small populations or those fluctuating to small size, advantageous alleles may be lost or deleterious alleles fixed by genetic drift, in spite of natural selection. When selection is important in structuring genetic variation, the precise outcome will depend upon the mode of selection involved. Various modes of selection and their consequences are discussed by Futuyma (1986) and Hartl and Clark (1989).

*Selection and vernal pool plants.* Vernal pool plants grow in patchy environments at the geographic (among-population) scale and the local (within-population) scale (Linhart, 1974; 1976; Holland and Jain, 1981). When patchiness reflects variation in selection (e.g. due to differences in soils), plant populations often form "races" or biotypes at the geographic scale and exhibit intrapopulation differentiation at the local scale (Linhart and Grant, 1996). There is evidence of both patterns in vernal pool plant taxa. Weiler (1962 according to Bauder, 1993) suggested that *Downingia* may exist as local races or varieties. Bauder's (1993) germination studies of coastal and foothill populations of *Downingia cuspidata*, a widely distributed vernal pool endemic, support Weiler's hypothesis. In addition, data indicate that soils are especially strong agents of selection in short lived plants. This means that vernal pools located in soils with different characteristics (e.g. of pH, mineral contents) are very likely to contain genetically differentiated populations of various plant species (Linhart and Grant, 1996). Research quantifying among-population differentiation in vernal pool plant taxa occupying different soil types would be of great interest.

Strong evidence also suggests that several vernal pool plant taxa exhibit intrapopulation differentiation over distances of 2 to 5 meters, from pool center to pool periphery (Linhart, 1974; 1976; 1988; Linhart and Baker, 1973). *Veronica peregrina* is the most extensively studied example. The conditions experienced by *V. peregrina* individuals in the pools studied by Linhart varied from pool center to periphery with respect to competitive regime, moisture availability and environmental predictability. Individuals in the center of pools grew with high densities of conspecifics and in a relatively predictable, high moisture environment. In contrast, individuals growing on the periphery were subject to interspecific competition with grassland species (*Agrostis alba*, *Avena barbata* and *Lolium temulentum*) as well as to a

more unpredictable, low moisture regime. Linhart and Baker (1973) identified significant differences in malate accumulation (a physiological response to saturated soil conditions) between greenhouse grown families from the center and the periphery of the population. Linhart (1974) also documented differences in a number of characters between progeny of center and periphery plants raised in non-competitive, greenhouse conditions. The observed differences could be interpreted in adaptive terms. For example, individuals from the center of the pool produced fewer, heavier, more rapidly germinating seeds, all features expected to be advantageous under intraspecific competition. Significant differences between center and periphery plants were also observed under competitive (intra- and interspecific) conditions in the greenhouse (Linhart, 1988). Maintenance of differences when plants are grown in uniform greenhouse conditions suggests that the observed variation is genetically based, at least in part. Therefore, differences in the observed traits reflect differences in the genetic structure of center and periphery plants.

This differentiation occurred over just a few meters, a distance over which gene flow by seed and pollen undoubtedly takes place. The data suggest that the homogenizing effects of gene flow can be overcome by the effect of strong disruptive selection. By contrast, similar differentiation was not observed in *V. peregrina* from a disturbed (plowed) pool, where seed mixing was probably extensive. Micro-differentiation within continuous populations of vernal pool annuals was also detected in *Lasthenia fremontii*, *Downingia concolor* and *Boisduvalia glabella* (= *Epilobium pygmaeum*). The most pronounced differentiation was associated with reduced movement of seeds (as above in the undisturbed pool) and with primarily self-pollinated species (i.e. *Veronica peregrina* and *Boisduvalia glabella*) (Linhart, 1976).

## IMPLICATIONS FOR CONSERVATION

### *Level of Genetic Variation*

Using population genetic theory, we can identify circumstances in which populations may be genetically depauperate (i.e. have low levels of genetic diversity). These include situations when 1) populations are continually small in size (ideally measured as effective population size,  $N_e$ ), 2) populations fluctuate in size over time (i.e. undergo population bottlenecks), 3) the taxon is highly or predominantly selfing or otherwise highly inbreeding, 4) gene flow from genetically richer sources is absent, 5) gene flow from genetically depauperate sources is substantial and/or 6) strong selection reduces local variation. These are not strict rules; they are *expectations*. In fact, the situation in natural populations may be extremely complex. Other factors may substantially influence levels of genetic variation within populations including the presence of seed banks. In addition, interactions among the various evolutionary factors discussed

influence expectations about population genetic structure. For example, although disruptive selection within populations may cause micro-differentiation, this selection may be overwhelmed by high levels of gene flow as in the disturbed *Veronica peregrina* population studied by Linhart (1974; 1988). The only way to be certain that a population is genetically depauperate (or genetically diverse) is to estimate levels of genetic variation empirically using allozymes, PCR (polymerase chain reaction)-based molecular markers or measures of quantitative genetic variation under uniform environmental conditions. Most often, obtaining such estimates will not be feasible.

Knowledge of levels of genetic variation within populations may be important in conservation efforts if levels of genetic diversity influence current or future persistence of populations. Reduced levels of variation may decrease the potential for persistence in the face of long-term biotic or abiotic environmental change (e.g. Soulé, 1980; Frankel and Soulé, 1981) or short-term impacts such as herbivores or pathogens (Huenneke, 1991). In addition, decreased levels of variation may have implications for population persistence in the short-term if heterozygosity per se has a direct impact on fitness (Frankel and Soulé, 1981; Mitton and Grant, 1984 and references therein). If populations with higher levels of genetic diversity are more persistent in the face of biotic and abiotic challenges, they may be especially important foci of conservation efforts. Even if genetic diversity is not highly associated with population viability, conserving populations with high levels of genetic variation may be important in ensuring representation of species level variation. However, genetically depauperate populations may also be important in maintaining species level variation if they contain high frequencies of rare alleles (as could be caused by genetic drift in small populations).

### *Distribution of Genetic Variation*

Population genetic theory allows us to identify circumstances in which there may be high levels of genetic differentiation among populations. These include situations when 1) populations are continually small in size (ideally measured as effective population size,  $N_e$ ), 2) populations fluctuate in size over time (i.e. undergo population bottlenecks), 3) the taxon is highly or predominantly selfing or otherwise highly inbreeding, 4) the level of gene flow among populations is very low (hence populations are very isolated) and/or 5) selection causes development of geographic races. Again, these are only expectations. The situation in nature is likely to involve complex, site-specific interactions among factors. The only way to be certain that substantial among-population differentiation exists (or does not) is to estimate it empirically.

If vernal pool plant taxa have high levels of among-population genetic variation (i.e. relatively high  $G_{ST}$ -values) as some data suggest (above) and as is very likely in pools occupying differ-

ent soil types (e.g. Linhart and Grant, 1996), then more populations need to be preserved to ensure adequate representation of allelic and genotypic diversity within the taxon. This consideration is especially important for habitually or obligately selfing taxa because evidence suggests that these taxa may have particularly high levels of among-population differentiation (e.g. Hamrick and Godt, 1990). Similarly, if vernal pool plant taxa form geographic races, as suggested for *Downingia cuspidata* (Bauder, 1993), then maintenance of populations from a variety of geographic locations will be necessary to preserve species level genetic variation.

### *Pool Construction and Restoration*

If populations of vernal pool plant taxa are isolated and highly differentiated, then we ought to be cognizant of whether and how natural patterns of genetic variation might be disrupted by construction of artificial pools. Will construction of pools disrupt natural patterns of gene movement by altering pollinator behavior? Will construction in a given area increase gene flow above historic levels? Is any increase in gene flow likely to disrupt patterns of local adaptation in neighboring natural populations or cause reductions in fitness of plants in neighboring natural populations through outbreeding depression? How will utilization of non-native soils change natural patterns of variation? In addition, local adaptation ought to be considered in restoration or reseeded projects. Attempts to introduce seed or individuals from populations in other areas (e.g. with different soil, moisture, pollinator, pathogen, herbivore conditions) are likely to fail if local adaptation to moisture regimes, soils, pollinators and other factors is important in structuring genetic variation in vernal pool plant taxa. Introduction of seed from other areas also has the potential to disrupt patterns of genetic variation and adaptation of local, natural populations through gene flow. For this reason it is to be discouraged. Finally, it is difficult to imagine that restoration and construction efforts will consider the fine-scale genetic differentiation within populations that has been documented for several vernal pool plants (e.g. Linhart, 1976). Yet, this complexity is a striking reminder of the intricate and specialized nature of the system these efforts are meant to replicate.

### *Unstudied Vernal Pool Plants*

Because population genetic data on vernal pool plant taxa are few, most conservation efforts will deal with plants for which we have no data. Unfortunately, there is no easy prescription in these situations. Thinking carefully about the taxon of concern may be helpful. Appropriate questions to ask include: What population sizes are observed? Do population sizes fluctuate from year to year? What is the taxon's breeding system? If outcrossed, what are the pollinators and their reproductive habits? What is known about pollen and seed dispersal? Are populations geographically isolated? Do we know anything of the evo-

lutionary history of the taxon and populations of concern? Is there evidence of a substantial seed bank? Answers to these sorts of questions may be useful along with population genetic theory in making preliminary guesses about expected population genetic structure.

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