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Genetics of *Lupinus*

IV. Colonization and Genetic Variability in *Lupinus succulentus*[1]

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Summary. Preliminary surveys indicate that most populations of *Lupinus succulentus* are genetically monomorphic for three flower color loci, viz., *BB PP DD*. In one small geographic area, a number of populations were polymorphic for the *D/d* locus. In this case, clinal variation and seasonal variation were found. The *S/s* locus, affecting seed coat pattern, was polymorphic in a large majority of populations and genetic variation at this locus is present in all but a very few colonies. When subdivisions of five large populations were studied, genetic differentiation was found within two of these populations. Gene frequencies tended toward the limits of zero or one in populations from relatively undisturbed sites but tended toward intermediate values in the more disturbed ruderal populations presumed to be recently colonized. It is concluded that recently colonized populations are not always genetically uniform.

Genetic variability has been studied in the recently introduced species, *Bromus mollis* (Knowles, 1943), *Ricinus communis* (Harland, 1947), *Trifolium subterraneum* (Morley, 1958), *Avena fatua* (Imam and Allard, 1965), and *Avena fatua* and *A. barbata* (Jain and Marshall, 1967). Genetic variability has also been studied in the relatively non-colonizing species, *Linanthus parryae* (Epling and Dobzhansky, 1942), *Collinsia heterophylla* (Weil and Allard, 1965) and *Festuca microstachys* (Kannenberg and Allard, 1967). These studies do not support the general theory that colonization is accompanied by a marked loss of genetic variability.

Some California natives have become weedy agrestals after the agricultural revolution drastically changed the ecology of the Central Valley of California (Stebbins, 1965). Others have become roadside weeds (ruderals) throughout the foothills and mountains of California. *Lupinus succulentus* Dougl. (Fabaceae) is an annual California native which has become ruderal in many areas. This species may have been preadapted for colonization since natural populations tend to occur in habitats with a considerable degree of disturbance. In the present study surveys of genotypic frequencies in natural and ruderal populations were made over a large geographic area. This paper reports the results of an investigation of genetic variability associated with four loci in *L. succulentus*.

Genetics

The papilionaceous flowers of *L. succulentus* are predominantly dark blue except for the white sulcus of the banner. Rarely, there occurs an entirely white flowered variant. Crosses between wild type (blue) and white have given acceptable fits to Mendelian expectations based on the assumption that this color difference is conditioned by a single locus with two alleles, blue dominant to white. Pink-flowered plants occur rarely and when crossed to wild type also fit single-locus expectations. This is in accord with the inheritance of white and pink in *Lupinus nanus* Dougl. (Harding and Mankinen, 1967); hence analogous genetic symbols will be assigned, viz. *bb* for white and *pp* for pink. Light-blue flowered plants are observed more frequently and crosses to wild type also fit single locus Mendelian ratios; the light-blue allele is designated *d*. The absence of a band of dark pigmentation across the seed coat was found to segregate as a Mendelian recessive and is designated *ss* (Fig. 1).

Since some populations are polymorphic for *D/d* and *S/s*, the linkage relationship was tested. The F_2 coupling phase dihybrid ratio was 85:4:0:26 for *D-S-*: *D-ss*: *ddS-*: *ddss*, which deviates significantly

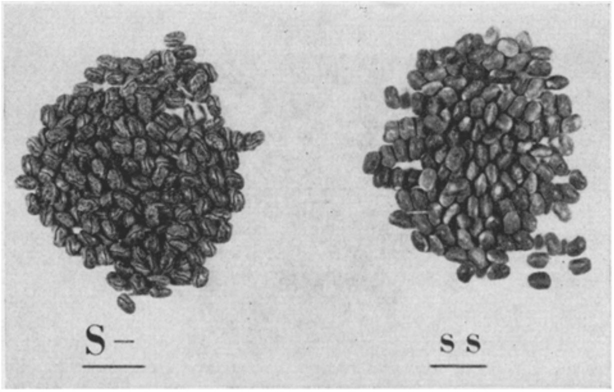

Fig. 1. Seed from dominant (*S*-) and recessive (*ss*) plants

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from the expected 9:3:3:1. The maximum likelihood estimate of the recombination fraction, r, is given by the solution of

$$85\left(\frac{2r-2}{r^2-2r+3}\right)+4\left(\frac{2r-2}{r^2-2r}\right)+26\left(\frac{2}{r-1}\right)=0\,.$$

Use of Allard's tables (Allard, 1956) results in an estimate of $\hat{r} = .04$ with a standard error of .02. Thus, D/d and S/s are very closely linked.

Geography

The distribution of *L. succulentus* is disjunct with the largest concentration along the Pacific Coast of California. This *coastal* group is represented by many populations from Mendocino County, California, to northernmost Baja California, Mexico (Munz, 1959). These populations occur in the foothills of the North Coast, South Coast, Transverse, and Peninsular Ranges and on the off-shore islands of San Clemente (Raven, 1963), Santa Catalina (Thorne, 1967), Anacapa, San Miguel, Santa Cruz and Santa Rosa (Philbrick and Emery, pers. comm.). The *interior* group occurs in the Pinal and Mazatzal Mountains (Gila and Maricopa Counties) of Arizona (Kearney and Peebles, 1951). Since differences between these groups are not yet understood, the loose terms coastal taxon and interior taxon will be used to avoid problems regarding possible sub-specific epithets. The present study is based on the coastal group, including populations from the North and South Coast Ranges, the Transverse and Peninsular Ranges, and the Central Valley. Collection areas are given by the letters A through F in Fig. 2 and by the letters G through P in Fig. 3.

Genotypic Frequencies

The surveys indicate that nearly all populations are fixed for $BBPP$. There were three questionable exceptions: there were numerous bb plants in a small colony in Cowell Valley (Area C), there were three bb plants in Davis-1 (Area D), and there were several pp plants in a small colony near Winters (Area D). The D/d locus is monomorphic for DD in nearly all populations; notable exceptions are the Lower Putah Populations (Table 1) which have varying frequencies of dark and light blue flowered individuals. The relatively infrequent occurrence of polymorphisms for loci affecting flower color may result from natural selection favoring the dark-blue color through pollinator preference. In contrast, the S/s locus is polymorphic in nearly all populations sampled (Tables 1

Fig. 2. Northern half of California showing collection Areas A through G. Area A includes the north end of the Sacramento Valley. Area B is northwest of Lake Berryessa between Putah Creek and Pope Creek. Area C includes Putah Canyon from Monticello Dam to the valley floor and the foothills between Putah Canyon and Cache Canyon. The valley floor in the civinity of Davis is designated Area D. Area E includes the foothills in the vicinity of Vacaville and Vallejo. Area F includes the coastal fogbelt along the Pacific Ocean between San Francisco and Santa Cruz

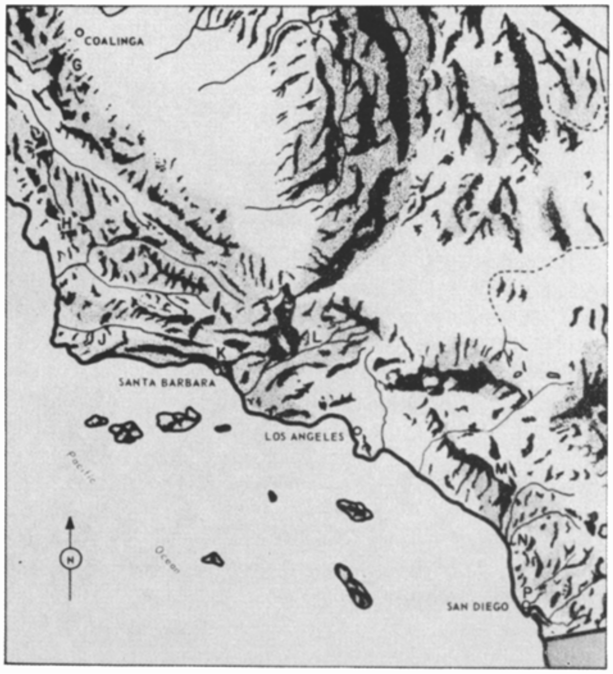

Fig. 3. Southern half of California showing collection areas G through P. The dry foothills of the South Coast Ranges from Coalinga to Taft are designated Area G. Area H includes the coastal area from Atascadero to Santa Maria. In the Transverse Ranges, Area J includes the northwestern half of the Santa Ynez Range. Area L includes the interior Ranges between Newhall and the Tejon Pass. In the Peninsular Ranges, Area M includes the area from Lake Elsinore to Fallbrook. Area N includes the coastal strip between Del Mar and San Clemente. The southernmost populations, including the San Diego and Tijuana areas are designated Area P

Table 1. *Frequencies and binomial standard errors for dominants at the S/s and D/d loci from 1962, 1963, 1966 and 1967 collections. These populations were monomorphic BBPP*

Population		*Frequency*			*Population*		*Frequency*		
Area B		*Year*	*S-*	*D-*	Area C		*Year*	*S-*	*D-*
Upper Putah	−1	1967	.26 ± .0.36	Mono *DD*	Lower Putah	− 1	1962	.38 ± 0.69	Poly *D/d*
	−2	1967	.10 ± .025	Mono *DD*		− 2	1962	.46 ± .094	Poly *D/d*
	−3	1967	.41 ± .042	Mono *DD*		− 1	1963	.76 ± .051	.03 ± .025
	−4	1967	.70 ± .019	Mono *DD*		− 2	1963	.36 ± .063	.40 ± .036
Area D						− 2	1966	.42 ± .047	.49 ± .058
El Macero	−1	1962	.05 ± .026	Mono *DD*		− 3	1966	.14 ± .035	.95 ± .022
	−3	1962	.02 ± .016	Mono *DD*		− 4	1966	.14 ± .031	.87 ± .030
Area E						− 5	1966	.10 ± .028	.95 ± .021
Vacaville	−1	1963	.33 ± .023	Mono *DD*		− 2	1967	.42 ± .046	.30 ± .040
	−2	1963	.03 ± .016	Mono *DD*		− 3	1967	.18 ± 0.39	.95 ± 0.26
Area F						− 4	1967	.14 ± 0.34	.91 ± .025
San Gregorio	−1	1967	Mono *ss*	Mono *DD*		− 5	1967	.14 ± 0.26	.94 ± .013
Area G					Mace	−11	1962	Mono *ss*	Mono *DD*
Tar Canyon	−2	1967	.81 ± .038	Mono *DD*		−30	1967	.01 ± .008	Mono *DD*

Table 2. *Frequencies and binomial standard errors for S/s locus from 1968 collections*

Population Area A		*Frequency S-*	*Population* Area D		*Frequency S-*
Red Bluff	−1	.47 ± .043	Davis	−1	.03 ± .019
Tehema	−1	.79 ± .035	Area K		
Corning	−1	.13 ± .031	Santa Barbara	−1	.62 ± .056
Orland	−1	.77 ± .037		−2	.70 ± .069
	−2	.77 ± .062	Area L		
Area G			Castaic	−1	.36 ± 0.55
Reef City	−1	.17 ± .059		−2	.70 ± 0.44
Area H			Area M		
San Luis Obispo	−1	.27 ± .036	Murrieta	−1	.52 ± 0.96
Area J			Area N		
Lompoc	−1	.85 ± .068	Vista	−1	.32 ± 0.39
	−2	.46 ± .040	Area P		
Solvang	−1	.71 ± .099	MissionBay	−1	Mono *ss*

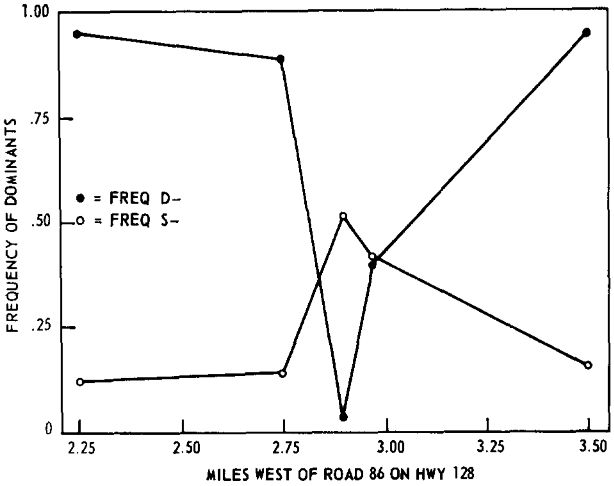

Fig. 4. Frequency of *D*- and *S*- in Lower Putah Canyon Populations, plotted against distance in miles from an arbitrary point near the mouth of the canyon

and 2). The only exceptions are Mace-11, San Gregorio-1 (based on a small sample of 34 individuals, i. e. 68 *s* alleles) and Mission Bay-1. Populations, therefore, are generally homozygous *BBPPDD* and polymorphic for the alleles *S* and *s*. The main exceptions are the Lower Putah Populations which are polymorphic for *D* and *d*. These populations were chosen for study because they were polymorphic for *D/d*. However, seed coat variation for *S/s* was not observed until after the selection of populations to be sampled was made. Consequently, the populations studied are a random sample of *L. succulentus* populations with respect to the *S/s* locus and the observed ubiquity of this polymorphism is not biased by the choice of populations.

Clinical Variation

In the Lower Putah Populations the frequency of light blue flowered plants is low at the base of the canyon and increases in frequency as the canyon is ascended. After reaching a frequency of more than 50 per cent in the middle of the canyon, the frequency of *dd* falls off rapidly to low frequencies. The five Lower Putah Populations were selected at more or less equally spaced intervals. The frequencies, plotted in Fig. 4, represent means for each population taken over the years sampled (see also Table 1). The miles are measured from a road intersection. Fig. 4 shows the predominance of *D-ss* at both ends of the area with sharp increases of *dd* and *S-* in a segment less than one-half mile in length. The associated increase of *d* and *S* may result from their close linkage. This change occurs over a very short distance, well within the radius of activity of the predominant pollinating agent *Apis mellifera* L.

Seasonal Variation

Gene frequencies in Putah Canyon populations appeared to be fairly stable over the period 1962 to 1970. Those populations which were sampled in more than one year are summarized in Fig. 5. The populations could not be sampled in 1964 and 1968

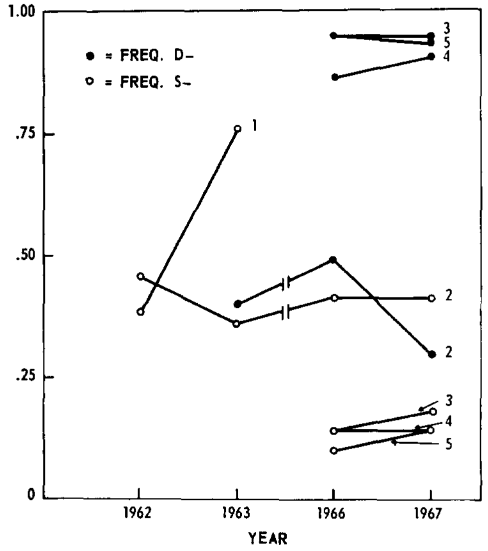

Fig. 5. Frequency of *D*- and *S*- in Lower Putah Canyon Populations for the years sampled 1964, 1965 and 1968 were not sampled (see text)

because there were very few plants, presumably due to very low rainfall in those years. Their immediate return to large numbers in 1965 suggests that many seeds remain hard and *overwinter* during years of low winter rainfall. Considering the seasonal fluctuations in gene frequency which may be expected in plant populations (e. g. Allard and Workman, 1963) the fluctuations are not great with the exception of *S/s* in Lower Putah-1 in 1962 and 1963. This population occurred on such a highly disturbed slope that it was abandoned after 1963.

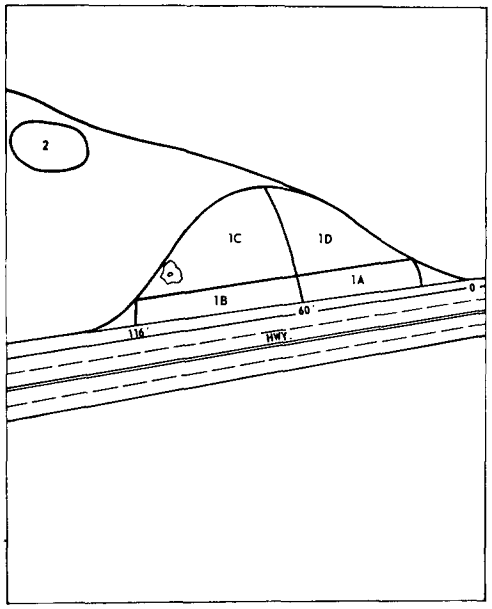

Fig. 6. Schematic representation of the sub-division of Vacaville -1 and -2 in 1963. The highway was U.S. Highway 40, now Interstate 80

Intra-Population Variation

The Vacaville-1 Population occupied a very large road-cut, 116 feet across, with population size more than 3,000 individuals in 1963. For convenience of collection the population was arbitrarily subdivided into four sub-populations according to the schematic representation in Fig. 6. Although the frequency of *S*- in the population as a whole was .33, the frequencies in the sub-populations were

1C- .47 ± .047, 1D- .18 ± .039,
1B- .50 ± .050, and 1A- .17 ± .036

The frequencies and standard errors show clearly that 1A and 1D can be taken as one group and 1B and 1C as another group. Topographically, the difference is between the two sides of the population. The possibility that further vertical subdivision might have revealed a continuous gradation could not be investigated because the population was subsequently destroyed by highway construction.

Another large population, Upper Putah-4, was chosen for further studies on intra-population differentiation. This population occupies a large highly-disturbed hillside site of approximately two acres; variation in topography and population density are very large. The size of the population was estimated to be more than 30,000 individuals. The site was subdivided into four parts according to gulleys and density gradients, but these subdivisions are considered fairly arbitrary because the population approached one large continuum. The frequency of *S*- in the population was .70 and the frequencies in the sub-populations were

4A- .79 ± .033, 4C- .74 ± .036,
4B- .75 ± .034, and 4D- .53 ± .040.

The three sub-populations, 4A, 4B and 4C, are not significantly different, but sub-population 4D differs significantly from the rest. The frequency of *S*- is high in all sub-populations compared to the frequencies from other populations within Geographic Area-B, *viz.* .26, .10 and .41 (see Table 1).

Population differentiation was not observed in all cases. Frequency estimates for the three subdivisions of Lower Putah-5 were .10, .13, and .16 for banded individuals (*S*-). Estimates for the four subdivisions of Mace-30 were 0, 0, .02 and .04, and estimates for the two subdivisions of Castaic-2 were .72 and .69. The extent of population differentiation cannot be determined from these samples. However, intrapopulational differences for the *S/s* locus were found in two of five populations studied.

Discussion

The populations under study all occupy habitats with varying degrees of disturbance. Attempts were made to locate populations in habitats with a minimum of disturbance. The populations with the least apparent disturbance were the Mace Populations, Vacaville-2, San Gregorio-1 and Tar Canyon-2. The

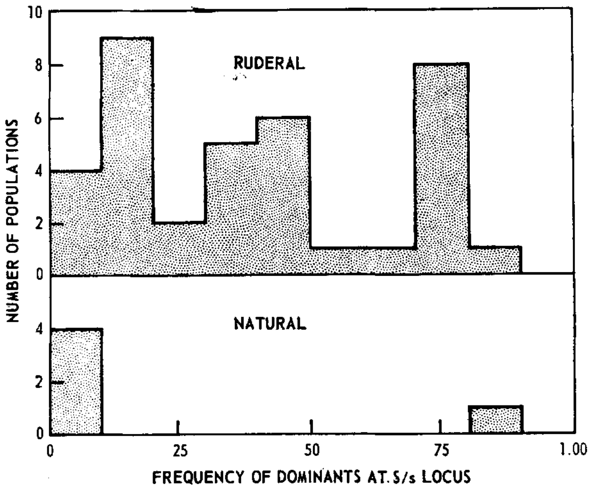

Fig. 7. Frequency of *S*- in all populations sampled, divided into those appearing natural and those ruderals appearing to be recently colonized

remaining populations occupy sites which were probably colonized recently. This classification of populations as to relatively disturbed vs. relatively undisturbed is necessarily subjective and based on limited numbers of observations. The differences (Fig. 7) suggest, however, that polymorphisms at the *S/s* locus are more frequent in disturbed roadside habitats than in less disturbed sites. Furthermore the *D/d* polymorphisms and the possible *B/b* and *P/p* polymorphisms were found only in disturbed sites and these are not included in Fig. 7.

Theory has held that as a new colony is founded by a limited number of immigrants, genetic drift is expected to reduce the genetic variance to an extent inversely proportional to the number of founders and subsequent effective population size. However, genetic variance appears to have increased with colonization in the populations of the present study. This increased genetic variation in recently colonized populations may result from several causes. First, as new environments are encountered, new selective forces may also be encountered. Unoccupied habitats present a much less competitive situation than natural habitats. For alleles at any particular locus this could result in a change in the direction of selection. In addition, observation suggests that the more disturbed habitats often exhibit more environmental variation than undisturbed habitats. This would be expected to increase the extent of intrapopulation differentiation.

In the case of the Vacaville Population it appears that Vacaville-1, on a highly disturbed cut, was founded by immigrants from Vacaville-2 (see Fig. 6). The road cut is deep into relatively undecomposed parent material, presumably low in nitrogen. The nitrogen fixing legume, *L. succulentus*, was apparently suited to colonize such an environment relatively free from the competition of grasses, at least in the early stages of colonization. With the colonization of Vacaville-1 the frequency of *S*- increased. If this were a purely random process, the majority of ruderal populations would be *ss* with occasional populations predominantly *S*-. But, as this is not the case, it appears that selection must have been involved. These hypotheses clearly need testing *in situ* during the early stages of colonizing episodes and such episodes are presently being sought.

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