AN ELECTROPHORETIC TEST OF THE GENETIC INDEPENDENCE OF A NEWLY DISCOVERED POPULATION OF *CLARKIA FRANCISCANA*

L. D. GOTTLIEB
Department of Genetics, University of California,
Davis, CA 95616

S. W. EDWARDS
Regional Parks Botanic Garden, 11500 Skyline Blvd.,
Oakland, CA 94619

ABSTRACT

Clarkia franciscana, originally known from a single population in San Francisco, is a California State-listed rare and endangered species that has figured importantly in our understanding of plant speciation. A second population was recently discovered in the Oakland Hills. To determine whether the Oakland Hills population was genetically distinct from the San Francisco one, we carried out an electrophoretic analysis of isozymes. The two populations are fixed for different alleles at five of the 31 genes examined. This result strongly suggests that the Oakland Hills population did not originate by seed transfer from San Francisco, and that it must be regarded as indigenous to its present locality. We discuss certain implications of this finding for plant conservation policy.

Many plant species designated rare and endangered by federal or state agencies are known from only single populations. The discovery of a second population is important because it will likely influence conservation and recovery programs. However, since range extensions of listed plants are unexpected, it is critical to determine if the second population is indigenous to its site.

Such a concern is relevant to *Clarkia franciscana* (Onagraceae), a slender annual herb originally known and described from a single serpentine outcrop just south of the Golden Gate in the Presidio of San Francisco, California (Lewis and Raven 1958a, b). *Clarkia franciscana* is listed as rare and endangered by the State of California and, as a consequence, its protection is mandated.

A second population has recently been discovered on a serpentine site across the Bay in the hills above Oakland. The possibility that *C. franciscana* seeds had been taken from the Presidio and sown in the Oakland Hills has to be considered because the site is located in the East Bay Regional Park District and the former director of the District's Botanic Garden had grown the species for a number of years at the garden from seeds collected in the Presidio (Roof 1972).

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Clarkia franciscana is important to biologists interested in mechanisms of speciation and genetic divergence. Along with *C. lingulata,* it provided the model that led Professor Harlan Lewis and his colleagues to propose an elegant theory of speciation, termed catastrophic selection, that suggested that plant species often originate in geographically peripheral populations that are subject to severe reductions in population size (Lewis 1962, 1966, 1973).

Clarkia franciscana with its derived self-pollinating breeding system was thought to have originated, perhaps recently, from the survivors of a drought-stricken population of *C. rubicunda,* a morphologically similar species that grows nearby (Lewis and Raven 1958a). The extensive difference between the species in chromosome structural arrangement was considered an indirect consequence of forced inbreeding among the surviving individuals.

However, electrophoretic analysis of isozymes revealed that *C. franciscana* was fixed for a large number of genes that were not present in *C. rubicunda* (Gottlieb 1973). It was later shown that *C. franciscana* also had a duplicated gene for alcohol dehydrogenase that further distinguished the two species (Gottlieb 1974). Such marked genetic divergence suggested that their phylogenetic separation occurred much longer ago than presumed and made the proposed direct mode of origin of *C. franciscana* from *C. rubicunda* unlikely because, if it had originated recently, most of its genes would still be present in its parent (Gottlieb 1973).

The electrophoretic study revealed that the Presidio population of *C. franciscana* was monomorphic at all but one gene, consistent with its predominant self-pollination. This genetic homogeneity simplifies an electrophoretic comparison of the Presidio population with the newly discovered Oakland Hills population since it is sufficient to test, for each sampled gene, whether both populations have the same allele or not. If the Presidio population had been outcrossing and highly polymorphic it might have been difficult to interpret likely differences in allelic frequencies.

Here we test the similarity of the two populations by an electrophoretic analysis of isozymes. If the Oakland Hills population has different alleles than the Presidio population at many genes, it probably is indigenous and represents a significant range extension. However, if the two populations are indistinguishable, it will not be possible to reject the contention that the Oakland Hills population was merely transplanted from the Presidio.

Materials and Methods

The Presidio population of *C. franciscana* is located on a serpentine slope at the east end of the Presidio, San Francisco (Lewis and Raven 1958b). The Oakland Hills population is located on the Skyline Serpentinite Prairie, Redwood Regional Park, E of Skyline Bou-

levard, Alameda Co., California. A cultivated population has been grown for many years in the Regional Parks Botanic Garden in Tilden Park from seeds originally collected at the Presidio site.

We studied the Oakland Hills population, the Botanic Garden population, and two lines, 8–13 and 37–1, that were originally collected as seeds from the Presidio population on 7 August 1971. These two lines had been included in the previous electrophoretic study (Gottlieb 1973), and had since been maintained by occasional self-pollination in a greenhouse at UC–Davis.

We did not sample the population of *C. franciscana* now growing at the Presidio because all genes possibly present there must be a subset of those now in the Botanic Garden selection or in LDG's lines. We have evidence that Roof collected seeds at the Presidio in 1964 (Roof 1972; and letter to LDG dated 13 January 1973), subsequently grew out plants yearly at the Botanic Garden, and then returned seeds to the Presidio location in 1972. (Note that the return of seeds was after LDG's collection in August 1971.)

The Oakland Hills population was estimated by a field count on 2 June 1990 to contain about 5000 plants. Since they were distributed in a large number of patches, sampling was done by collecting seeds from each of ten plants growing in each of ten widely spaced sites throughout the population. In addition, seeds were collected from 25 plants growing in the Botanic Garden.

Sixteen enzyme systems were studied by horizontal starch gel electrophoresis: acid phosphatase (APH; EC 3.1.3.2); alcohol dehydrogenase (ADH; EC 1.1.1.1); catalase (CAT; EC 1.11.1.6); glutamate dehydrogenase (GDH; EC 1.4.1.2); glutamate oxaloacetate transaminase (GOT; EC 2.6.1.1); leucine aminopeptidase (LAP; EC 3.4.11.1); malate dehydrogenase (MDH; EC 1.1.1.37); phosphoglucose isomerase (PGI; EC 5.3.1.9); esterase (EST; EC 3.1.1.–); isocitric dehydrogenase (IDH; EC 1.1.1.41); malic enzyme (ME; EC 1.1.1.40); mannose-6-phosphate isomerase (MPI; EC 5.3.1.8); phosphoglucomutase (PGM; EC 5.4.2.2); 6-phosphogluconate dehydrogenase (6PGD; EC 1.1.1.44); skikimic dehydrogenase (SKD; EC 1.1.1.25); and triose phosphate isomerase (TPI; EC 5.3.1.1).

The first eight of these systems were previously examined (Gottlieb 1973). Three gel electrode buffer combinations were utilized: 6PGD was examined in a pH 6.5 morpholine system (Odryzkoski and Gottlieb 1984), IDH, ME, MDH and SKD in a pH 7.0 histidine–HCI system (System IV, Gottlieb 1981), and all the others in a pH 8.3 tris-citric system (System I, Gottlieb 1981). The enzymes were extracted from leaves of 3–5 week old seedlings in a cold extraction buffer as previously described (Gottlieb 1981). Standard assays of enzyme activity were used (Wendel and Weeden 1989).

Twenty extracts were run on each gel. To facilitate direct visual comparison of the electrophoretic mobility of each isozyme, extracts

from individuals of the two populations were run side by side in different combinations on each gel.

Results

The present study of *C. franciscana* included all eight enzymes previously reported (Gottlieb 1973) and eight additional ones. The improved assay for catalase made it possible to detect two isozymes whereas only a single one had previously been found. Two additional isozymes of acid phosphatase were also detected in the present study.

The 16 enzymes examined appear to be encoded by 31 genes. Depending on the enzyme, 34 to 66 families were analyzed from the Oakland Hills population and 17 to 20 families from the cultivated Botanic Garden population. We also studied two additional lines from the Presidio that had been previously examined (Gottlieb 1973). A family is defined as individuals, usually one or two, grown from seeds collected on different plants in nature.

All plants from the Oakland Hills population proved to have identical electrophoretic mobility for each isozyme; there was no observed polymorphism. Likewise, all plants from the Presidio (the Botanic Garden collection and LDG's lines) also exhibited identical electrophoretic mobility for each isozyme. Thus, for this data set, each population is represented by any single individual.

The Oakland Hills population differed from the Presidio population at five genes or 16% of those sampled. These included *Aphl, Est, Lap, 6Pgd4* and *Tpi2* (Table 1). In addition, at *Got2,* the Oakland Hills population exhibited the "slow" allele and the Presidio population the "fast" allele. Both *Got2* alleles had previously been detected in the Presidio population and this gene was the only one shown to be polymorphic in the species (Gottlieb 1973). Thus, for *Got2,* the Oakland Hills population appears to be monomorphic for one of the two alleles segregating in the Presidio population.

Discussion

The finding that the Oakland Hills population of *Clarkia franciscana* contains at least five genes encoding enzymes that are absent from the Presidio population strongly suggests that it did not originate by seed transfer from the Presidio. Consequently, it must be regarded as indigenous to its present serpentine locality.

This conclusion is further strengthened by the finding that the plants grown in the Botanic Garden are identical to two lines collected at the Presidio about 20 years ago. These lines were fully representative at that time (with the exception that they possessed only one of the two *Got2* alleles).

The Oakland Hills population may be as much as five times larger than the Presidio population and because it is located in a regional

TABLE 1. RELATIVE ELECTROPHORETIC MOBILITIES OF ISOZYMES EXTRACTED FROM PLANTS FROM THE PRESIDIO AND OAKLAND HILLS POPULATIONS. The mobilities are calculated relative to the migration of the bromphenol blue front at 100 mm. [1] Multiple activity bands for ADH reflect duplication of coding gene. [2] Multiple activity bands present; number coding genes uncertain.

	Relative electrophoretic mobilities of isozymes		
Enzyme	Presidio (Botanic Garden)	Presidio (lines 8–13 and 37–1)	Oakland Hills
ADH	50/48/46[1]	50/48/46[1]	50/48/46[1]
APH–1	74	74	71
APH–2	66	66	66
APH–3	41	41	41
CAT–1	41	41	41
CAT–2	28	28	28
EST	80	80	90/78[2]
GDH	31/28/25[2]	31/28/25[2]	31/28/25[2]
GOT–1	56	56	56
GOT–2	51	51	44
IDH	35/29[2]	35/29[2]	35/29[2]
LAP	58	58	60
MDH–1	31	31	31
MDH–2	28	28	28
MDH–3	9	9	9
ME–1	35	35	35
ME–2	22	22	22
MPI	62	62	62
6PGD–1	30	30	30
6PGD–2	27	27	27
6PGD–3	26	26	26
6PGD–4	15	15	12
PGI–1	63	63	63
PGI–2	45	45	45
PGM–1	56	56	56
PGM-2	49	49	49
PGM-3	45	45	45
SKD	35	35	35
TPI-1	76	76	76
TPI-2	68	68	60
TPI-3	46	46	46

park it will be easier and less expensive to protect and conserve. The Skyline site is noteworthy in that as many as 15 species of native perennial grasses as well as a remarkable diversity of wildflowers are also found there, making it one of the richest such areas in the San Francisco Bay Region.

Although neither the Oakland Hills or Presidio populations have much, if any, genetic variability, at least judging from the electrophoretic evidence, the substantial differences between them indicate that *C. franciscana* has a reasonable level of variability and this may

prove useful for future management decisions. Although we now know the species is less rare than previously thought, it still requires protection. Its prospects for persistence are much enhanced by the new discovery.

In addition to the Regional Park site, a small number of plants of the species was discovered on a serpentine outcrop next to the Oakland Hills Tennis Club. The owner of the club was required by the local Planning Commission to hire a botanist to protect them during construction work on his property (Oakland Tribune, 3 November 1989). These plants are almost certainly an historical part of the main population now in the Regional Park, which is uphill and less than a mile away.

It is worth noting that if our study had failed to show that the Oakland Hills population was distinct from the one in the Presidio, leaving the impression that it had originated by human agency, such a transfer would not necessarily have been misguided or the new site unworthy of protection. Plant conservation will almost certainly require deliberate movement of plants and/or propagules to new sites and the problem will be to identify appropriate ones. Obviously this is a difficult task because short term success in a new site does not necessarily predict long term establishment.

Finally, we point out that Roof's replacement of *C. franciscana* seeds in the Presidio in 1972 was done because he could not find the species there in 1969 and 1970 and believed it extinct (letter to LDG previously cited). This was in fact not the case because LDG collected seeds from numerous plants there in 1971. Roof's action almost certainly had little or no genetic consequence because *C. franciscana* is nearly monomorphic and probably has been for some time. However, more generally, seed augmentation of outcrossing plants has the potential to change allelic frequencies. Roof's concern for conservation should probably be viewed favorably. It may be that the botanical community and our native plant societies should debate such practical measures to conserve the California plant heritage.

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ANNOUNCEMENT

THE 1991 JESSE M. GREENMAN AWARD

The 1991 Jesse M. Greenman Award has been won by Scott Zona for his publication "A monograph of *Sabal* (Arecaceae: Coryphoideae)", published in Aliso 12:583–666, 1990. This monographic study is part of a Ph.D. dissertation from Claremont Graduate School, Claremont, California, under the direction of Dr. Sherwin Carlquist.

The Greenman Award, a certificate and a cash prize of $500, is presented each year by the Missouri Botanical Garden. It recognizes the paper judged best in vascular plant or bryophyte systematics based on a doctoral dissertation published during the previous year. Papers published during 1991 are now being accepted for the 24th annual award, which will be presented in the summer of 1992. Reprints of such papers should be sent to Dr. P. Mick Richardson, Greenman Award Committee, Missouri Botanical Garden, P.O. Box 299, St. Louis, MO 63166-0299, U.S.A. In order to be considered for the 1992 award, reprints must be received by 1 June 1992.