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Allozyme Divergence in *Coreopsis cyclocarpa* (Compositae)

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ABSTRACT. *Coreopsis cyclocarpa* is a diploid, suffrutescent perennial occurring in the grasslands of high plateau regions of Mexico. The species consists of two allopatric varieties, var. *pinnatisecta* and the typical one. They are very similar morphologically and appear to differ only by a feature of the leaves. The two infraspecific taxa are highly interfertile. Electrophoresis was utilized to ascertain the amount of divergence between the varieties at 20 genetic loci coding for soluble enzymes. There is high genetic identity among populations of the same variety, with mean values of 0.98 for populations of var. *cyclocarpa* and 0.95 for var. *pinnatisecta*. When comparing mean identities for populations of the two varieties, the value is 0.75, indicating genetic divergence between the taxa. It is suggested that genetic differentiation between the two varieties is a reflection of the time they have been isolated.

In an earlier study one of us (Crawford 1970) reported on the morphology, flavonoid chemistry, chromosome number, and artificial hybridizations involving *Coreopsis cyclocarpa* Blake and *C. pinnatisecta* Blake. The two taxa are perennial suffructescent plants growing in the mountains of Mexico with the latter found primarily in the states of Oaxaca and Guerrero and the former in Jalisco. Both taxa flower at the same time of year and occur in similar open grassy areas of high plateau regions (Crawford 1970). The present known geographic ranges of the two taxa indicate a disjunction of about 700 km, although additional collecting in Guerrero and Michoacan might narrow this hiatus. The only reliable morphological character for distinguishing them is leaf morphology, with *C. cyclocarpa* having simple linear leaves whereas those of *C. pinnatisecta* are pinnatisect with linear segments (Crawford 1970). Both taxa are diploid with a chromosome number of $2n = 24$, and artificial crosses in the greenhouse produced fully fertile F_1, F_2, and backcross hybrids. Meiosis was regular and seed set was not reduced (Crawford 1970). In addition, the leaf characters used to separate the two taxa segregate in the F_2 generation. On the basis of these data, the two taxa were reduced to varietal status. The purpose of the present study was to measure genetic divergence between the two varieties of *Coreopsis cyclocarpa* utilizing data from the electrophoretic separation of allozymes. The advantages of enzyme electrophoresis for addressing systematic questions in plants were discussed by Gottlieb (1977a, 1981a), who also pointed out that most electrophoretic studies comparing different taxa have been done with annuals, there being a paucity of data for perennial angiosperms. The present study of allozymes in woody, perennial, interfertile, morphologically similar plants will provide data for the comparison with results from annual plants.

Materials and Methods

Achenes gathered from plants in natural populations were germinated, raised in the growth chamber, and used as the source of enzymes (table 1). Leaves from near the stem apices were used, although the same banding patterns were obtained whether seedlings or mature leaves from older plants served as the source of enzymes. Younger leaves from established plants were used because they gave superior resolution compared to older plants; seedlings were not routinely employed to avoid sacrificing individuals.

The extracting buffer was 0.1 M tris-HCl, pH 7.5, 14 mM 2-mercaptoethanol, 1 mM EDTA (tetrasodium salt), 10 mM KCl, and 10 mM $MgCl_2$ (Gottlieb 1981b). The buffer was made up in 40% sucrose instead of distilled water to provide the necessary density for loading the acrylamide gels (Crawford and Smith in press). Enzymes were resolved either on 12.5% starch or 5% acrylamide (running gel) with a 3% stacking gel. Glutamate-oxaloacetate transaminase (GOT) was run on acrylamide using the system described by Crawford and Wilson (1977). Alcohol dehydrogenase (ADH), glutamate dehydrogenase (GDH), leucine aminopeptidase (LAP), superoxide dismutase (SOD), and phosphoglucoisomerase (PGI) were resolved on starch using a gel buffer of nine parts tris-citrate (0.05 M tris, 0.007 M citric acid $\cdot H_2O$, pH 8.3) and one part lithium borate (0.038 M lithium hydroxide, 0.188 M boric acid, pH 8.3). The electrode buffer consisted only of lithium borate. Malate dehydrogenase (MDH), phosphoglucomutase (PGM), shikimate dehydrogenase (SKDH), 6-phosphogluconate dehydrogenase (6-PGDH), and acid phosphatase (ACP) were separated on starch with a gel buffer of 0.02 M histidine-HCl, brought to pH 7.0 with NaOH and an electrode buffer of 0.4 M sodium citrate titrated to pH 7.0 with HCl (Gottlieb 1981b).

As in an earlier study of *Coreopsis* allozymes (Crawford and Smith in press), genes are designated according to relative mobilities for which they code. If more than one locus is present for any enzyme, those coding for the faster migrating bands are given the lower number. Letters given as superscripts indicate relative mobilities of bands with "a" representing the fastest enzyme coded by an allele of a gene, "b" the next fastest, etc.

Eleven enzymes apparently coded by 20 genes were scored for 96 individuals from three populations of var. *cyclocarpa*; 160 plants from five populations of var. *pinnatisecta* were examined (table 1). Assignment of number of genes is based on the known active subunit composition of the enzymes, their patterns of segregation within populations, and analogy to known genetic bases of enzymes in two other diploid species of *Coreopsis* (Crawford and Smith in press). Allelic frequencies were determined for each gene locus in each population (table 2).

Genetic identities (table 3) were calculated for each pair-wise comparison of the eight populations using the methods of Nei (1972). Mear genetic identities were determined for all pair-wise comparisons of pop

TABLE 1. Population designations, number of plants examined (in parentheses), and source data for populations of *Coreopsis cyclocarpa* var. *cyclocarpa* and var. *pinnatisecta* serving as sources for allozymes. Collection numbers refer to D. J. Crawford et al.; vouchers are deposited in OS.

C. cyclocarpa var. *cyclocarpa*. Jalisco: 1(54), ca. 14 mi E of El Arenal; *1395*. 2(26), ca. 13.5 mi E of El Arenal, *1395-1*; 3(16), ca. 13.2 mi E of El Arenal, *1395-2*.

C. cyclocarpa var. *pinnatisecta*. Oaxaca: 4(18), 14 mi SE of Tlacolula, *1281*; 5(20), 13.6 mi SE of Tlacolula, *1282*; 6(26), 39.4 mi NW of Cd. Oaxaca, *1285*; 7(71), 44.3 mi NW of Cd. Oaxaca, *1294*. Guerrero: 8(25), 4.3 mi W of Chilpancingo, *1324*.

ulations of each variety as well as comparisons between the varieties. The proportion of loci polymorphic was determined for each variety, a polymorphic gene being here defined as one in which the most common allele is present in a frequency of 0.99 or less. The mean number of alleles per polymorphic gene and proportion of loci heterozygous were calculated for each variety.

RESULTS

Eight gene loci, *Adh-1, Mdh-1, Mdh-2, Mdh-3, Mdh-4, Pgi-1, Sod-1,* and *Sod-2* were invariant in all plants examined. Allelic frequencies at variable loci in each population are presented in table 2. Genetic identities for all pair-wise comparisons of populations are presented in table 3. The mean genetic identity for pair-wise comparison of populations of var. *cyclocarpa* is 0.98 and the mean value is 0.95 for populations of var. *pinnatisecta*. The mean genetic identity for pair-wise comparisons of populations of the two varieties is 0.75.

Four alleles (*Lap*^{a}, *6-Pgdh*^{a}, *Skdh*^{d}, and *Skdh*^{e}) were detected in var. *cyclocarpa* but were not found in var. *pinnatisecta*; a total of fourteen alleles detected in var. *pinnatisecta* were not found in var. *cyclocarpa* (table 2). Seven of these alleles (*Acp*^{c}, *Adh-2*^{a}, *Adh-2*^{c}, *Got-3*^{a}, *Got-3*^{d}, *Pgm-1*^{a}, *Skdh*^{a}) are rare in var. *pinnatisecta* whereas the other seven, namely *Got-2*^{c}, *Got-2*^{d}, *6-Pgdh*^{b}, *Pgm-2*^{a}, *Pgm-2*^{b}, *Skdh*^{b}, and *Skdh*^{c}, are at loci at which the two varieties are highly divergent (table 2).

For several enzymes, one or more additional genes were present, but resolution of banding patterns was not adequate for confidence in interpretation.

In var. *cyclocarpa* 45% of the gene loci are polymorphic whereas the value is 55% for var. *pinnatisecta*. Variety *cyclocarpa* has a mean of 2.56 alleles per polymorphic gene whereas var. *pinnatisecta* averages 3.36 alleles per polymorphic locus. With respect to proportion of loci heterozygous, the value is 0.079 for var. *cyclocarpa* and 0.075 for var. *pinnatisecta*.

DISCUSSION

Although allozymes have been examined in perennial plants (e.g., Schaal and Levin 1976; Mitton et al. 1977; Rudin and Ekberg 1978;

TABLE 2. Allelic frequencies at twelve genes in *Coreopsis cyclocarpa*. Population numbers refer to table 1.

		var. *cyclocarpa*			var. *pinnatisecta*				
Gene	Allele	1	2	3	4	5	6	7	8
Acp	a	0.18						0.50	
	b	0.82	1.00	1.00	1.00	1.00	1.00	0.28	1.00
	c							0.22	
Adh-2	a						0.05	0.03	0.36
	b	1.00	1.00	1.00	1.00	1.00	0.95	0.94	0.64
	c							0.03	
Got-1	a	0.09					0.08		0.08
	b	0.84	1.00	1.00	1.00	1.00	0.92	1.00	0.88
	c	0.07							0.04
Got-2	a	0.08		0.44				0.02	
	b	0.92	1.00	0.56				0.11	0.10
	c				0.89	0.83	1.00	0.86	0.84
	d				0.11	0.17		0.01	0.06
Got-3	a							0.01	
	b				0.58	0.50	0.65	0.35	0.26
	c	1.00	1.00	1.00	0.42	0.50	0.31	0.63	0.68
	d						0.04	0.01	0.06
Lap	a	0.06	0.36	0.16					
	b	0.57	0.54	0.68	0.17		0.13	0.15	0.08
	c	0.31	0.10	0.16	0.83	1.00	0.87	0.83	0.92
	d	0.06						0.02	
Gdh	a	0.03			0.17		0.12	0.08	0.07
	b	0.79	0.77	1.00	0.66	1.00	0.88	0.84	0.93
	c	0.18	0.23		0.17			0.08	
Pgm-1	a								0.37
	b	0.94	1.00	1.00	1.00	1.00	0.75	1.00	0.60
	c	0.06					0.25		0.03
Pgm-2	a				0.45	0.58	0.50	0.41	0.14
	b				0.55	0.42	0.50	0.53	0.02
	c	0.54	0.50	0.38				0.06	0.60
	d	0.46	0.50	0.62					0.24
Pgi-2	a	0.01						0.01	0.46
	b	0.15	0.21	0.56		0.25	0.04	0.13	0.44
	c	0.03	0.21		1.00	0.75	0.86	0.77	0.08
	d	0.81	0.58	0.44			0.10	0.09	0.02
6-Pgdh	a	1.00	1.00	1.00					
	b				1.00	1.00	1.00	1.00	1.00
Skdh	a						0.10		0.32
	b				0.75	0.60	0.80	0.25	0.58
	c				0.25	0.40	0.10	0.75	0.10
	d	0.25	0.21	0.38					
	e	0.75	0.79	0.62					

TABLE 3. Genetic identities for all pair-wise comparisons of eight populations (cf. table 1) of *Coreopsis cyclocarpa*. Determined by method of Nei (1972).

Population	1	2	3	4	5	6	7	8
1	X							
2	0.98	X						
3	0.99	0.98	X					
4	0.75	0.69	0.70	X				
5	0.76	0.76	0.78	0.99	X			
6	0.74	0.73	0.75	0.99	0.98	X		
7	0.76	0.70	0.77	0.96	0.96	0.95	X	
8	0.77	0.79	0.82	0.91	0.93	0.93	0.90	X

Guries and Ledig 1978), the major focus of the studies has been on inheritance of the enzymes or their variation within populations. Most electrophoretic studies of congeneric species or subspecific entities have dealt with annual plants, and the rather sparse data (ca. 50 total taxa) were summarized by Gottlieb (1981a). Mean genetic identity for conspecific populations is about 0.95 whereas this value is reduced to about 0.67 when comparing congeneric species (Gottlieb 1977a, 1981a). The only exceptions to the pattern observed for congeneric species involve species pairs that appear to be little diverged and in some instances may represent a progenitor-derivative situation (Gottlieb 1973, 1974; Gottlieb and Pilz 1976; Crawford and Wilson 1979; Crawford and Smith in press). In several instances it appears that chromosomal repatterning in annuals has caused rapid reproductive isolation and speciation (e.g., Gottlieb 1974; Smith 1974; Crawford and Smith in press). In such cases, speciation can occur with little genetic divergence and this is reflected in extreme morphological similarity as well as high genetic identities at enzyme loci.

The two varieties of *Coreopsis cyclocarpa* examined in the present study appear as morphologically similar as the pairs of species of annual plants shown to exhibit high genetic identities, i.e., except for the "key" leaf character they seemingly are indistinguishable (Crawford 1970). The possibility cannot be dismissed, however, that detailed quantitative studies of growth rates, ratio characters, or other features usually not studied by taxonomists would reveal differences of the nature noted by Gottlieb (1977b, 1978) for two very similar species of *Stephanomeria* (Compositae). In addition to morphological similarity, the two varieties of *C. cyclocarpa* are as interfertile with each other as they are within themselves. That is, seed set in F_2 and backcross plants involving the two varieties is 90–100%, which is the same as crosses involving different populations of the same variety (Crawford 1970, unpublished). Examination of meiosis in F_1, F_2, and first generation backcross plants revealed twelve bivalents at diakinesis and metaphase I (Crawford 1970, unpublished). Thus, there is no evidence that structural differences of the magnitude noted by Smith

(1974) for two species of annual *Coreopsis* characterize var. *cyclocarpa* and var. *pinnatisecta.*

Given their morphological similarity and interfertility, it is perhaps unexpected that the mean genetic identity of populations of the two varieties is so much lower (0.75) than populations of each variety, i.e., 0.98 and 0.95 for vars. *cyclocarpa* and *pinnatisecta,* respectively. Thus, populations of each variety are just as similar at genes coding for isozymes as are conspecific populations in annual plants whereas the varieties are less similar than we anticipated based on results from annuals. It is also of interest to compare the results for the varieties of *Coreopsis cyclocarpa* to data reported for infraspecific taxa of annual *Phlox* (Levin 1978) and varieties of the annual weed *Chenopodium incanum* (Crawford 1979). In *Phlox,* Levin (1978) reported a genetic distance of 0.006 among subspecies, this translating to the very high genetic identity of above 0.99. Among the three varieties of *Chenopodium incanum,* genetic distances varied from 0.104 to 0.139, which is 0.90 to 0.87 in terms of genetic identity. Thus, the varieties of *Coreopsis cyclocarpa* are more divergent at isozyme loci than are infraspecific taxa of annual plants for which data are available.

The genetic divergence between the two varieties of *C. cyclocarpa* contrasts sharply with the lack of divergence (mean genetic identity of 0.97) between populations of the two reproductively isolated annual species *Coreopsis nuecensis* and *C. nuecensoides* (Crawford and Smith in press). These two species are very similar morphologically and reproductive isolation has occurred, conceivably rather rapidly, by chromosomal restructuring (for details, see Smith 1974; Crawford and Smith in press). The greater divergence found between the two varieties of *Coreopsis cyclocarpa* as compared both to that measured between the two species of annual *Coreopsis* and infraspecific entities in *Phlox* and *Chenopodium* may well be a reflection of their greater time of divergence. In other words, allelic differences at the isozyme loci accumulate with time, and perhaps populations of the two varieties of this perennial suffrutescent species have been separated for a considerably longer time than have the annual species of *Coreopsis.* It is also apparent that neither gross morphological nor chromosomal changes have accompanied isozyme divergence between the two varieties of *C. cyclocarpa* (Crawford 1970, unpublished).

Gottlieb (1977a) suggested that electrophoretic data might eventually be useful for the development of procedures for standardizing certain taxonomic decisions within genera. Comparative data for *Coreopsis* suggest that enzyme divergence may be related to life history parameters and mechanisms of speciation. As such, the degree of divergence at enzyme loci may not always aid in making taxonomic decisions per se but rather will provide valuable information for making evolutionary and systematic inferences when taken together with other data.

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