Aliso: A Journal of Systematic and Floristic Botany

Volume 12 | Issue 3 Article 8

1990

Morphological Stasis abd Molecular Divergence in the Intercontinental Disjunct Genus Datisca (Datiscaceae)

Aaron Liston
Rancho Santa Ana Botanic Garden

Loren H. Rieseberg
Rancho Santa Ana Botanic Garden

Thomas S. Elias
Rancho Santa Ana Botanic Gardem

ALISO
12(3), 1989, pp. 525–542

MORPHOLOGICAL STASIS AND MOLECULAR DIVERGENCE IN THE INTERCONTINENTAL DISJUNCT GENUS *DATISCA* (DATISCACEAE)

AARON LISTON, LOREN H. RIESEBERG, AND THOMAS S. ELIAS

Rancho Santa Ana Botanic Garden, 1500 N. College Avenue
Claremont, California 91711-3101

ABSTRACT

The genus *Datisca* comprises two species and has an intercontinentally disjunct distribution: *D. cannabina* is native to southwest and central Asia, whereas *D. glomerata* is distributed from northern California to northern Baja California. In 1975, Axelrod proposed a geohistorical scenario to account for such "Madrean-Tethyan links," suggesting that these disjunctions resulted from migration across the mid-Atlantic from the Paleogene up to the Neogene, approximately 23 to 65 m.y.a.

The two species are quite similar in most phenotypic traits which have been studied to date. The major difference between the two involves their breeding system: *D. cannabina* is dioecious while *D. glomerata* is apparently androdioecious. Despite these similarities, Nei's mean genetic identity between the two species is $I = 0.142$. This is one of the lowest values yet reported for congeneric flowering plants and provides evidence for an ancient origin of the disjunction. Furthermore, the fact that the western populations of *D. cannabina* have a much higher genetic identity value with *D. glomerata* than does the eastern population supports the idea that dispersal occurred across the Atlantic. In addition, the population genetic structure of *D. glomerata* is consistent with an androdioecious breeding system.

Key words: *Datisca,* Datiscaceae, biogeography, intercontinental disjuncts, enzyme electrophoresis, genetic divergence, morphological stasis, androdioecy.

INTRODUCTION

One of the primary goals of biogeography is the interpretation of disjunct distributions (Brown and Gibson 1983). To date, most biogeographical studies of disjuncts have relied on morphological comparisons. The results of such studies, however, may be ambiguous, since rates of morphological change are not readily correlated with time of divergence. Comparative molecular studies provide new data sets which can contribute to our understanding of biogeographical problems.

For example, molecular data has the potential to resolve the most basic question in the analysis of a particular disjunct distribution (Carlquist 1983): is it of recent origin (presumably through long distance dispersal); or is it attributable to geohistorical processes? A low degree of genetic divergence will favor the first alternative, whereas a high degree will favor the second.

Disjuncts can also serve as a model for evolutionary biologists who are interested in comparative rates of evolution. All discrete populations are, by definition, disjunctly distributed (Brown and Gibson 1983). Intercontinental disjunctions simply present the most extreme manifestation of this. As such they allow us to observe evolution in the absence of gene flow, and thereby provide an ideal situation for the study of evolutionary rates.

Molecular data have been successfully applied to biogeographical questions in animals such as the Old and New World monkeys (Sarich and Cronin 1980), Pacific island-dwelling mosquitoes (Pashley, Rai, and Pashley 1985), and within the *Drosophila melanogaster* species complex (Lachaise, Cariou, David, Lemeunier, Tsacas, and Ashburner 1988).

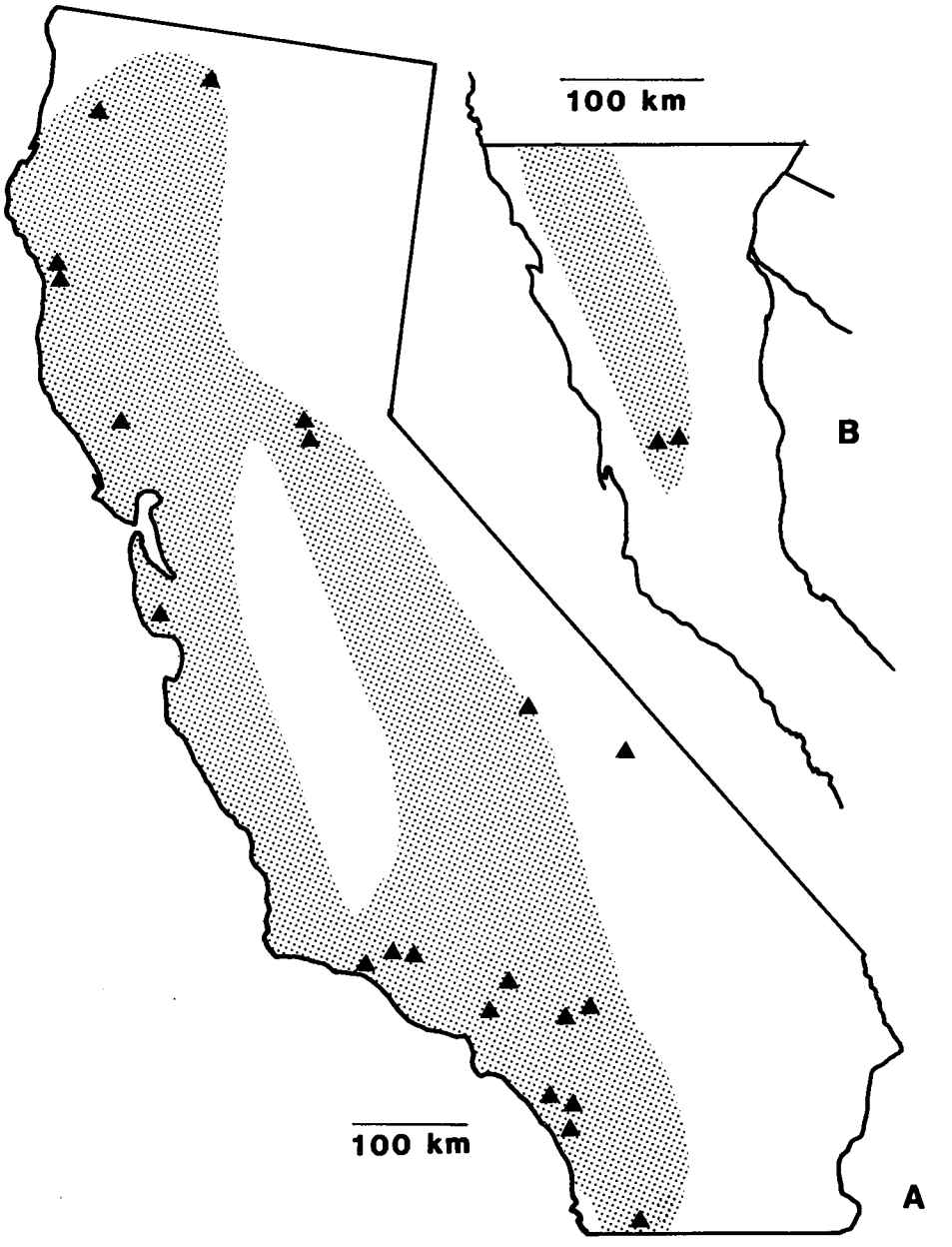

Fig. 1. Map showing collection localities (triangles) and approximate distribution (modified from Davidson [1973]) of *Datisca glomerata*. A = California. B = Baja California.

In plants, isozyme electrophoresis has been used extensively to quantify genetic variation; but only limited use has been made of these data in examining intercontinental disjunctions (Vogelmann and Gastony 1987; Parks and Shan-An 1988; Hoey and Parks 1988; Liston, Rieseberg, and Elias 1989). This is unfortunate, for these data make it possible to evaluate different biogeographical models and to characterize the types and evolution of the genetic systems involved in these

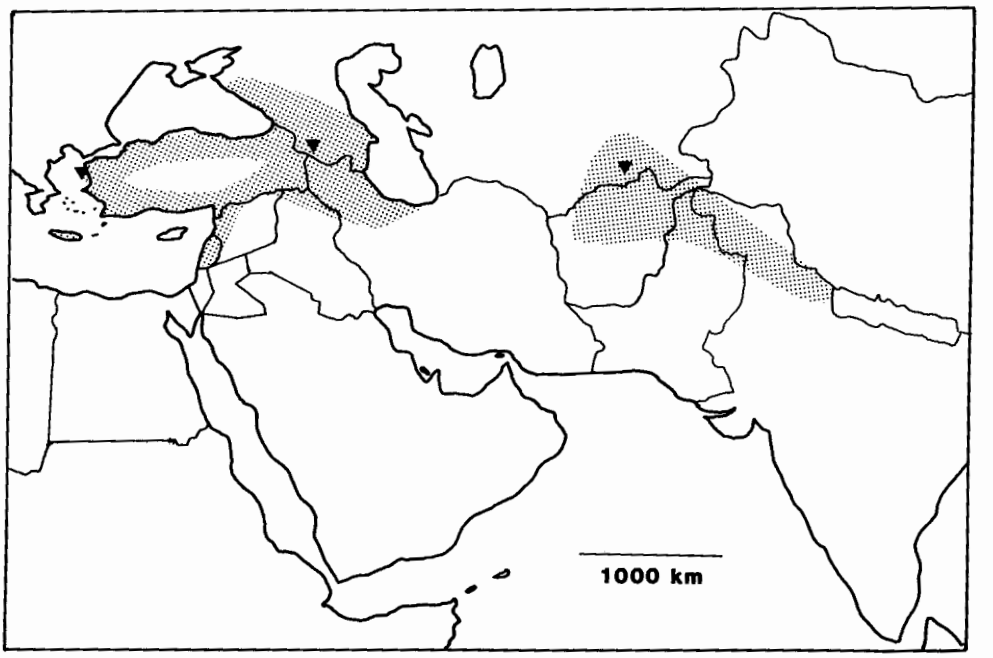

Fig. 2. Map showing collection localities (inverted triangles) and approximate distribution (modified from Davidson [1973]) of *Datisca cannabina.*

disjunctions. Here we use electrophoretic evidence to examine an intercontinental disjunct in the genus *Datisca* (Datiscaceae).

Datisca is a genus of two species. *Datisca glomerata* (Presl.) Baill. is distributed from Siskiyou County in northern California to the Sierra San Pedro Martír in northern Baja California, Mexico (Fig. 1). *Datisca cannabina* L. is distributed from the island of Crete in the east Mediterranean to the foothills of the Himalayas in northwestern India (Fig. 2) but is absent from an approximately 1000-km stretch of desert from east of the Caspian Sea to northwestern Afghanistan (Rechinger 1966).

Both species are long-lived, tall perennial herbs of riparian habitats and are morphologically and anatomically quite similar (Davidson 1973, 1976; Boesewinkel 1984). Bohm (1988) has recently shown that the two species have identical flavonoid profiles. In addition, both species possess root nodules containing the nitrogen-fixing microsymbiont *Frankia* (Chaudhary 1979; Akkermans, Roelofsen, Blom, Huss-Danell, and Harkink 1983) and are apparently diploids with a gametic chromosome number $n = 11$ (Sinotô 1929; Snow 1959). Both taxa also show a high degree of intraspecific uniformity. For example, no intraspecific flavonoid variation was found among 12 populations of *D. glomerata* from throughout California (Bohm 1987).

The only apparent difference between the two species is that *D. cannabina* is strictly dioecious, while *D. glomerata* is apparently androdioecious, possessing hermaphrodite and male individuals. Mature leaves of *D. cannabina* are pinnatisect, while those of *D. glomerata* are incised-serrate, sometimes deeply divided near the base but never to the midrib. However, seedlings and young leaves of

the two species are virtually indistinguishable. Likewise, plants of *D. cannabina* grown in reduced light produce *glomerata*-like leaves (Fig. 3).

Thorne (1972) has summarized the major disjunct ranges of vascular plants. He classifies *Datisca* and approximately 35 other genera with similar disjunctions as Mediterranean-American disjuncts. Axelrod (1975) refers to these disjuncts as Madrean-Tethyan links and has presented a vicariance hypothesis explaining their present distribution in the two regions. Summarizing the fossil data, he concludes that during the Paleogene a sclerophyllous flora adapted to subhumid climate inhabited lower-middle latitudes along the shores of the Tethyan region and across southern North America (Axelrod 1975, fig. 1). Extant links are relicts of effective migration across the middle Atlantic which was then narrower (Phillips and Forsyth 1972; Dietz and Holden 1970). At the beginning of the Oligocene, sea level fell, and it is probable that discontinuous parts of the Mid-Atlantic rise were emergent (Tarling 1980). Volcanic islands along the Mid-Atlantic Rise and its flanks could have provided additional "stepping stones" facilitating dispersal (Axelrod 1975; Tarling 1980). In addition, the lower latitude of the east coast of North America and the ENE orientation of the Appalachian axis (Phillips and Forsyth 1972; Walper and Rowett 1972) would have provided numerous xeric sites for migration, particularly on granite-gneiss domes, "shale barrens," and other igneous substrates found in the Piedmont (Axelrod 1975).

In the present study, we have attempted to answer the following questions for the intercontinental disjunct species pair of *Datisca cannabina* and *D. glomerata*: 1) does the amount of genetic divergence conform with hypotheses concerning the age of the disjunction? 2) what is the relationship between the genetic and morphological divergence of the two species? and 3) is the population genetic structure of the two species consistent with their putative breeding systems?

MATERIALS AND METHODS

Plants

Twenty-three populations of *Datisca glomerata* encompassing the entire geographic range of the species were sampled for electrophoretically detectable genetic variation (Table 1, Fig. 1). Twenty-one of the populations were sampled by field collections of leaves from individual plants. Since most populations were very small (1–30 individuals) all plants could be examined. Two larger populations (DG1 and DG3) of over 100 individuals were sampled at random. Two additional populations were grown up from bulk seed collections (DG18 and DG19). Leaves were either used immediately or stored at −70 C for later analysis.

Three collections of *D. cannabina* were available for study (Table 1, Fig. 2). All were derived from bulk seed collections. Although the sampling of populations was not as complete as for *D. glomerata,* the sites of *D. cannabina* represented the eastern, central and western parts of the species' range.

Enzyme Electrophoresis

Sample preparation, electrophoresis, and staining of enzymes followed the general methodology of Soltis, Haufler, Darrow, and Gastony (1983). The following enzymes were examined: aspartate aminotransferase (AAT), acid phosphatase (AcPH), fructose 1,6-diphosphatase (FDP), glutamate dehydrogenase (GDH),

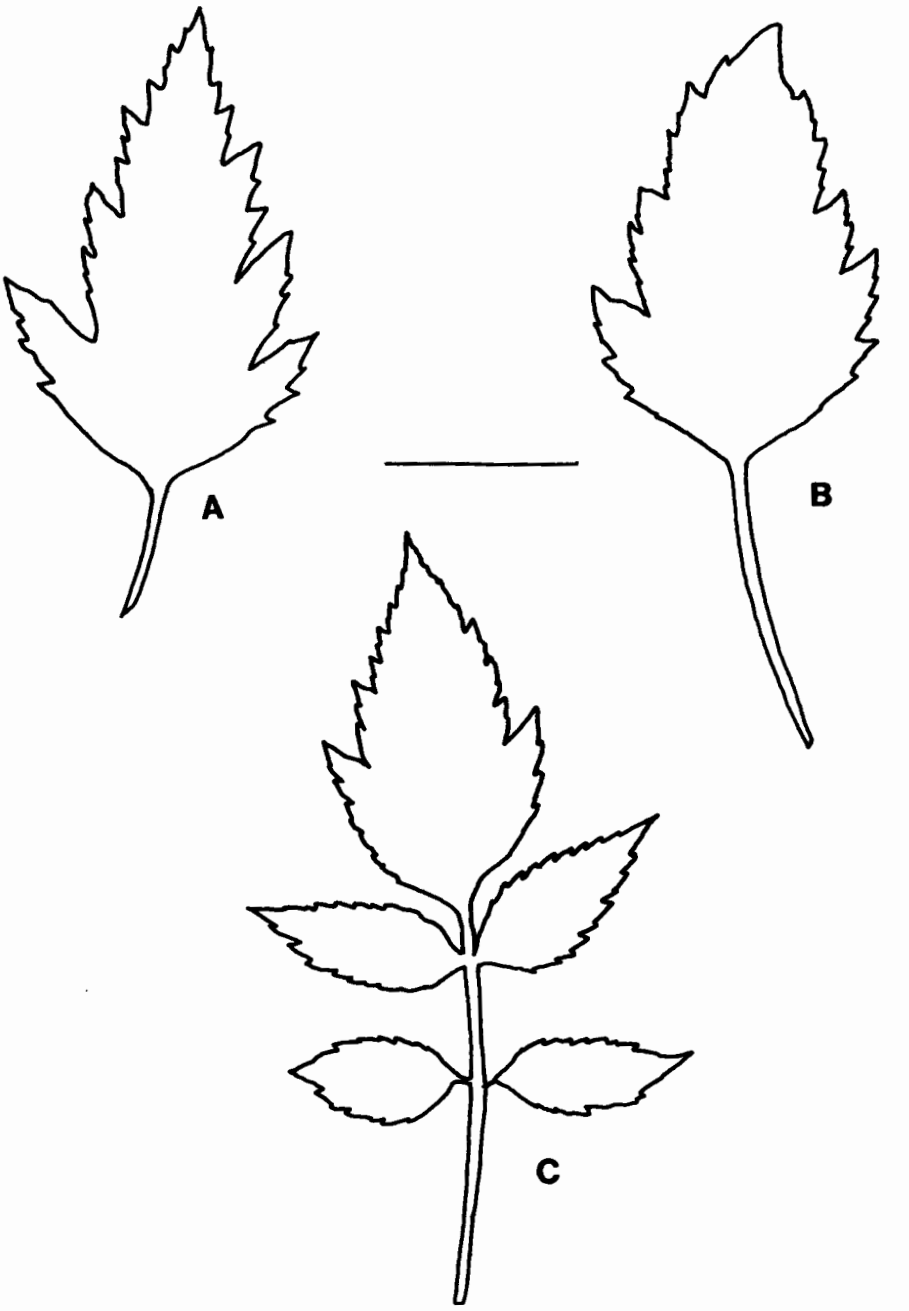

Fig. 3. Leaf outlines of *Datisca*.—A = Leaf of *D. glomerata* from a mature plant.—B = Leaf of *D. cannabina* from a plant grown under low light intensity.—C = Leaf of *D. cannabina* from a mature plant. (Scale bar = 3 cm.)

Table 1. *Datisca* populations examined by enzyme electrophoresis. All individuals in a population were sampled, unless noted otherwise.

Pop.	(N)	Location	Collection
cannabina			
DC1	(41)*	USSR: Tadzhik SSR, 48 km N of Dushanbe.	*Elias 10019*
DC2	(64)*	USSR: Transcaucasus.	*Rabinovich 423*
DC3	(30)*	Greece: Lesvos, southern part of island.	*Strid 26171*
glomerata			
DG1	(13)	USA: CA, Siskiyou Co. 12 km E of Montague.	*Rieseberg 1083*
DG2	(30)**	USA: CA, Humboldt Co. 1.5 km N of Hoopa.	*Rieseberg 1082*
DG3	(39)**	USA: CA, Mendocino Co. Standish-Hickey park.	*Rieseberg 1081*
DG4	(12)	USA: CA, Sonoma Co. 9 km E of Healdsburg.	*Rieseberg 1080*
DG5	(16)	USA: CA, Mendocino Co. Standish-Hickey park.	*Liston 748*
DG6	(8)	USA: CA, El Dorado Co. 3 km E of Auburn.	*Liston 776*
DG7	(4)	USA: CA, El Dorado Co. 11 km NE of Folsom.	*Liston 777*
DG8	(12)	USA: CA, Santa Cruz Co. 25 km NE of Santa Cruz.	*Liston 757*
DG9	(8)	USA: CA, Inyo Co. 7 km SW of Independence.	*Liston 759*
DG10	(4)	USA: CA, Inyo Co. 23 km SW of Stovepipe Wells.	*Liston 762*
DG11	(9)	USA: CA, Santa Barbara Co. Jun Cal Campground.	*Elias 12260*
DG12	(6)	USA: CA, Ventura Co. Rose Valley Campground.	*Elias 12259*
DG13	(4)	USA: CA, Ventura Co. 11 km N of Ojai.	*Elias 12258*
DG14	(7)	USA: CA, Los Angeles Co. 5 km WSW of Cloudburst.	*Rieseberg 1073*
DG15	(1)	USA: CA, Los Angeles Co. 8 km NE of La Crescenta.	*Liston 778*
DG16	(5)	USA: CA, San Bernardino Co. 7 km SW of Devore.	*Liston 779*
DG17	(7)	USA: CA, San Bernardino Co. 5 km N of Cedar Springs Dam.	*Rieseberg 1108*
DG18	(42)*	USA: CA, Orange Co. Lower San Juan Picnic Area.	*Wisura 15402*
DG19	(50)*	USA: CA, Riverside Co. Santa Rosa Plateau.	*Elias 10154*
DG20	(17)	USA: CA, San Diego Co. 1 km NW of De Luz.	*Liston 768*
DG21	(6)	USA: CA, San Diego Co. 5 km W of Dulzura.	*Liston 767*
DG22	(10)	MEX: Baja Calif. 2.5 km W of entrance to Sierra San Pedro Martír Nat. Park.	*Liston 773*
DG23	(4)	MEX: Baja Calif. 5 km E of Rancho Melling.	*Liston 775*

* Derived from bulk seed collections.
** Random sampling of larger populations.

NAD-dependent glyceraldehyde 3-phosphate dehydrogenase ([NAD]GPDH), NADP-dependent glyceraldehyde 3-phosphate dehydrogenase ([NADP]GPDH), isocitrate dehydrogenase (IDH), malate dehydrogenase (MDH), malic enzyme (ME), phosphoglucoisomerase (PGI), phosphoglucomutase (PGM), 6-phosphogluconate dehydrogenase (PGD), superoxide dismutase (SOD), and triosephosphate isomerase (TPI). All enzymes were resolved on 12.0% starch gels. Gel and electrode buffer system 1 of Soltis et al. (1983) was used to resolve AcPH, IDH, and [NADP]GPDH. Gel and electrode buffer system 2 was used to resolve FDP, [NAD]GPDH, and PGD. Gel and electrode buffer system 9 was used to resolve GDH, MDH, ME, and PGM. A modification of gel and electrode buffer system 8 (Rieseberg and Soltis 1987) was used to resolve AAT, PGI, and TPI. SOD was visualized on the agarose overlay used for staining TPI. Additional enzymes were also examined but either were not resolvable or could not be scored consistently including β-galactosidase, menadione reductase and shikimate dehydrogenase.

Cytosolic and plastid isozymes were distinguished for TPI by the enrichment of intact chloroplasts following the methodology of Mills and Joy (1980). The enriched chloroplast pellet was lysed in one drop of extraction buffer, absorbed into a paper wick, and used for enzyme electrophoresis.

Genotype frequencies were inferred directly from isozyme phenotypes based on the knowledge of the generally conserved enzyme substructure, compartmentalization, and isozyme number in higher plants (Gottlieb 1981, 1982). The lack of excessive variation within populations also aided in the genetic interpretation of isozyme patterns.

If more than one locus was present for an enzyme, loci were numbered sequentially with the most anodally migrating locus designated 1. Likewise, enzyme variants at individual loci were given letters with the fastest allozyme designated a.

Data Analysis

Nei's (1972) mean genetic distance (D) and identity (I) were computed for all pairwise population comparisons both within and between species using the GENESTAT program (Lewis and Whitkus 1988). The proportion of polymorphic loci, mean number of alleles per locus, and mean heterozygosity were also computed for each taxon.

F, the inbreeding coefficient or fixation index (Wright 1965, 1969), which measures deviation of heterozygote proportions from Hardy-Weinberg expectations, was determined for all polymorphic loci in each population and for both species. F can range from -1.0 to 1.0, with the former indicating an excess and the latter a deficiency of heterozygotes, respectively, when compared to Hardy-Weinberg expectations. Significance of deviations at each locus was tested by the χ^2 test against Hardy-Weinberg expectations.

The distribution of genetic variation within and among populations of the two species was analyzed using gene diversity statistics (Nei 1975), as calculated by GENESTAT (Lewis and Whitkus 1988). Total gene diversity (H_T) is subdivided into gene diversity within populations (H_S) and gene diversity among populations (D_{ST}) where

$$H_T = H_S + D_{ST}.$$

Differentiation among populations is calculated as

$$G_{ST} = D_{ST}/H_T,$$

where G_{ST} can vary between 0 ($H_S = H_T$) and 1 ($H_S = 0$), i.e., populations are fixed for different alleles.

Quantitative estimates of interpopulational gene flow were calculated using the model of Slatkin (1985). This method provides an indirect estimate of Nm, the amount of interpopulational gene flow, using a linear relationship between the logarithm of Nm and the logarithm of the average frequency of alleles restricted to a single population (private alleles). This relationship is given as

$$\ln[\bar{p}(1)] = a\ln(Nm) + b,$$

where $\bar{p}(1)$ is the average frequency of private alleles, N is the effective population

size, m is the migration rate among populations, $a = -0.505$, and $b = -2.440$. Estimates of Nm less than 1.0 indicate relatively little gene flow, while Nm values of 1.0 or greater suggest high levels of gene flow (Slatkin 1985).

RESULTS

Loci

The following 21 enzyme loci were interpreted: *Aat-1, Acph, Fdp-2, Gdh, [NAD]Gpdh-1, [NADP]Gpdh-2, Idh-1,2, Mdh-1,2, Me-1, Pgi-1,2, Pgm-1,2, Pgd-1,2, Sod,* and *Tpi-1,2,3* (Fig. 4–7). All scored loci migrated anodally. *Tpi-3* was determined to be compartmentalized in the plastid (Fig. 8–9). Among the non-plastid loci, *Tpi-1* behaved as a monomeric enzyme, while *Tpi-2* behaved as a dimer. The dimeric condition is typical of vascular plants (Gottlieb 1981). However, a similar "monomeric" TPI locus has been recently reported in *Isoëtes* (Hickey, Guttman, and Eshbaugh 1989) and evidently results from a post-translational modification of the TPI enzyme. Activity for *Tpi-1* was not observed in the absence of the substrate dihydroxyacetone phosphate, indicating that it was not a nonspecific protein.

The loci *Sod* and *Tpi-1* were not expressed in young tissue, and therefore were not scored in populations DC2 and DC3 of *Datisca cannabina.* Both PGD loci and *Pgm-2* were inconsistently expressed in frozen material and thus data are missing at these loci for some *D. glomerata* populations.

Interspecific Allozyme Variation

Allele frequencies at the 21 interpreted loci were totalled for each species (Table 2). Allele frequencies for each population are available from the authors upon request. No locus was monomorphic over all populations of both species. Nei's mean genetic identity (Nei 1972) between *D. cannabina* and *D. glomerata* was 0.142 ($D = 1.96$). This is one of the lowest values recorded to date for congeneric plant species. All populations of *D. glomerata* had a higher genetic identity value with the two western populations of *D. cannabina* (DC2 and DC3) than with the central Asian population (Table 3).

Three populations of *D. glomerata* (DG8, DG14 and DG23) had no alleles in common with the central Asian population of *D. cannabina* (DC1) and a genetic identity of zero (Table 3). Only three alleles (*Me-1b, Pgd-2b,* and *Tpi-3c*) were shared between interspecific populations. Nineteen populations of *D. glomerata* possessed one or two of these alleles. One population (DG15) shared all three alleles with *D. cannabina.*

Intraspecific Allozyme Variation

The mean genetic identity for intraspecific population comparisons within *D. glomerata* was $I = 0.847$. Genetic identity values ranged from 0.617 to 1.000 (Table 3). Populations in close geographic proximity had the highest genetic identities, but some widely separated populations were also quite close. For example, population DG3 from Mendocino Co. had high genetic identity values with DG22 and DG23 from Baja California ($I = 0.937$ and 0.935, respectively). The genetically most divergent populations within *D. glomerata* were DG9 and DG10.

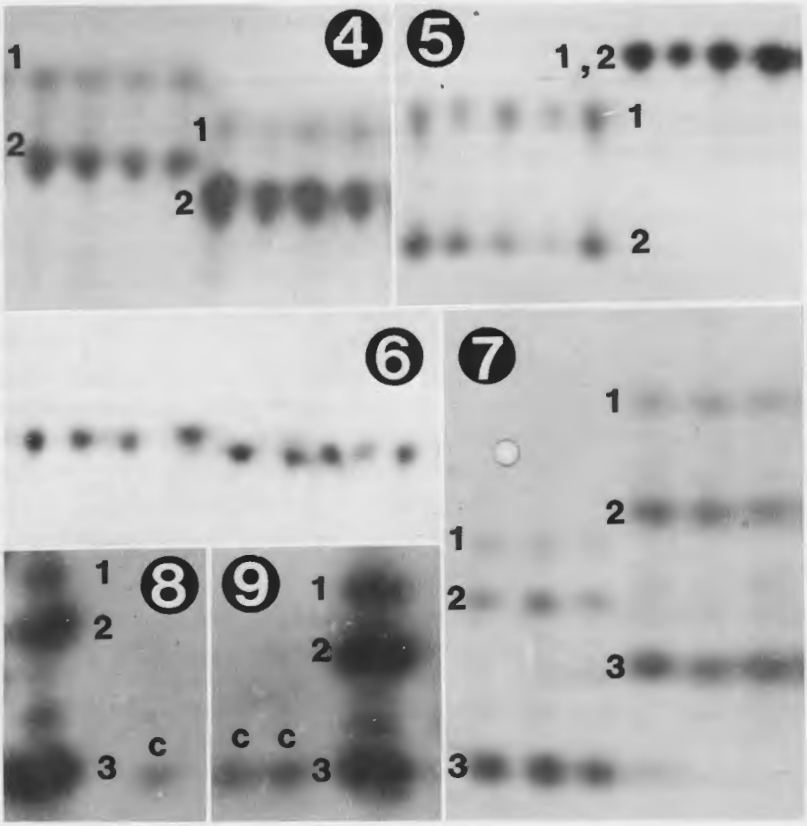

Fig. 4–9. Electrophoretic patterns demonstrating allozyme divergence between *Datisca glomerata* and *D. cannabina*.—4. Interspecific variation at *Fdp-1* and *Fdp-2*. *Datisca cannabina* (DC1) is at the left and *D. glomerata* (DG19) is at the right. Due to inconsistent staining *Fdp-1* was not used in this study.—5. Interspecific variation at *Pgi-1* and *Pgi-2*. *Datisca glomerata* (DG19) is at the left and *D. cannabina* (DC1) is at the right. The two loci overlap in *D. cannabina* and a more intensely stained band is observed.—6. Interspecific variation at *[NADP]Gpdh-2*. *Datisca cannabina* (DC1) is at the left and *D. glomerata* (DG19) is at the right.—7. Interspecific variation at *Tpi-1*, *Tpi-2*, and *Tpi-3*. *Datisca cannabina* (DC1) is at the left and *D. glomerata* (DG6) is at the right.—8. Localization of the locus *Tpi-3* in *Datisca cannabina*. The enriched plastid isozyme is labeled c. The unlabeled band between *Tpi-2* and *Tpi-3* is a nonspecific protein.—9. Localization of the locus *Tpi-3* in *Datisca glomerata*. The enriched plastid isozyme is labeled c. The unlabeled band between *Tpi-2* and *Tpi-3* is a nonspecific protein.

These two populations are isolated in Owen's Valley (DG9) and Death Valley (DG10) and had mean genetic identity values of $I = 0.748 \pm 0.064$ and 0.755 ± 0.060 with all other *D. glomerata* populations.

Among the three populations of *D. cannabina* sampled, the two western populations from the island of Lesvos (DC3) and the Transcaucasus mountain range (DC2) shared a high genetic identity value ($I = 0.893$). Both of these populations had a very low genetic identity value with the Central Asian population DC1 (Table 3).

Genetic variability within the two species of *Datisca* was characterized by several standard methods (Table 4). The mean percentage of polymorphic loci

Table 2. Cumulative allele frequencies for twenty-one loci in three populations of *Datisca cannabina* and twenty-three populations of *D. glomerata*.

Locus-Allele	*cannabina*	*glomerata*	Locus-Allele	*cannabina*	*glomerata*
	N	N		N	N
Aat-3	135	309	B	0.000	0.035
A	1.000	0.000	C	0.000	0.871
B	0.000	1.000	D	0.000	0.094
Acph-1	34	312	*Pgd-2*	135	171
A	0.000	1.000	A	0.000	0.649
B	0.500	0.000	B	1.000	0.351
C	0.500	0.000	*Pgi-1*	135	317
Fdp-2	83	310	A	0.526	0.000
A	0.494	0.000	B	0.474	0.000
B	0.506	0.971	C	0.000	1.000
C	0.000	0.029	*Pgi-2*	135	317
Gdh	135	290	A	0.526	0.000
A	1.000	0.000	B	0.474	0.000
B	0.000	1.000	C	0.000	1.000
[NAD]Gpdh-1	129	317	*Pgm-1*	46	193
A	1.000	0.000	A	0.000	0.161
B	0.000	1.000	B	0.848	0.000
[NADP]Gpdh-2	135	317	C	0.000	0.533
A	0.304	0.000	D	0.152	0.000
B	0.696	1.000	E	0.000	0.304
Idh-1	108	307	*Pgm-2*	63	214
A	0.620	0.000	A	0.619	0.000
B	0.000	1.000	B	0.374	0.000
C	0.380	0.000	C	0.000	0.734
Idh-2	135	307	D	0.007	0.260
A	0.696	0.000	E	0.000	0.007
B	0.304	0.000	*Sod*	29	56
C	0.000	0.782	A	1.000	0.000
D	0.000	0.218	B	0.000	1.000
Me	135	297	*Tpi-1*	41	301
A	0.009	0.000	A	0.000	0.507
B	0.991	0.835	B	0.000	0.493
C	0.000	0.165	C	1.000	0.000
Mdh-1	135	285	*Tpi-2*	134	304
A	0.000	1.000	A	0.644	0.000
B	1.000	0.000	B	0.000	1.000
Mdh-2	135	272	C	0.057	0.000
A	0.000	0.022	D	0.299	0.000
B	0.991	0.000	*Tpi-3*	135	305
C	0.000	0.971	A	0.000	0.254
D	0.000	0.007	B	0.000	0.705
E	0.009	0.000	C	1.000	0.041
Pgd-1	135	171			
A	1.000	0.000			

(P) was similar in *D. glomerata* and *D. cannabina* (7.0% and 5.4%, respectively). However, in *D. cannabina* only population DC2 had any polymorphic loci (P = 21.0%).

The mean number of alleles per locus (A) was also similar in both species (1.05 in *D. glomerata* and 1.06 in *D. cannabina*). No more than two alleles per population were found at any polymorphic locus.

Consistent with the low levels of allelic variation in *Datisca*, values of observed (H_o) and expected (H_e) heterozygosity were also low (Table 4) in both species. The total value of F, the fixation index (Wright 1969), for polymorphic loci in *D. glomerata* reflected a highly significant heterozygote deficiency (Table 4). The comparable value of F for *D. cannabina* reflected a frequency of heterozygotes not significantly different from Hardy-Weinberg expectations.

The apportionment of genetic diversity as measured with the gene diversity statistics of Nei (1975) is similar in *D. cannabina* and *D. glomerata* (Table 4). Both species show low levels of total genetic diversity (H_T), and most genetic diversity is found among populations (D_{ST}) rather than within populations (H_S).

Estimates of Gene Flow

Only two private alleles, *Mdh-2d* and *Pgm-2e* were restricted to single populations of *D. glomerata*. Interpopulational gene flow in this species was estimated as $Nm = 6.4$, when adjusted for sample size (Slatkin 1985), indicating extensive gene flow among populations. Twelve private alleles were found in the three populations of *D. cannabina* and ten of these were fixed in the population. Thus a value of $\bar{p}(1) = 0.845$ is obtained. For this large a value of $\bar{p}(1)$ the relationship between $\ln[\bar{p}(1)]$ and $\ln(Nm)$ is no longer linear and a quantitative estimate of gene flow could not be made (Slatkin 1985). The same is true for the two western populations (DC2 and DC3) where $\bar{p}(1) = 0.535$. These results indicate that the three populations of *D. cannabina* sampled are effectively isolated from each other.

DISCUSSION

Molecular Divergence

The interspecific mean genetic identity value of $I = 0.142$ for the two species of the genus *Datisca* is lower than any of the comparable values summarized in reviews of isozyme data in flowering plants (Gottlieb 1981; Crawford 1983). However, a lower value ($I = 0.087$) has been recently reported for congeneric species of *Ceratophyllum* (Les 1989). Three populations of *D. glomerata* have no alleles in common with the central Asian population of *D. cannabina*. These populations have diverged to such an extent that genetic comparison using allozyme data is no longer feasible ($D = \infty$). The limit of allozyme resolution of genetic divergence usually corresponds to the genus level in animals (Avise 1975). Thorpe (1982) has summarized intergeneric genetic identity values for 160 populations in animals (primarily vertebrates) and has found a mean I value of 0.273 $\pm$ 0.107. In plants, intergeneric divergence is sometimes resolvable using allozymes, for example in the Asteraceae (Witter and Carr 1988) and Saxifragaceae (Soltis and Soltis 1986; Rieseberg and Soltis 1987). Thus the two species of *Datisca*

Table 3. Nei genetic identity (above) and distance (below) values between populations of *Datisca cannabina* and *D. glomerata*.

Pop.	DC1	DC2	DC3	DG1	DG2	DG3	DG4	DG5	DG6	DG7	DG8	DG9	DG10
DC1		0.476	0.579	0.056	0.056	0.057	0.059	0.106	0.053	0.050	0.000	0.095	0.100
DC2	0.743		0.893	0.169	0.172	0.177	0.189	0.238	0.178	0.158	0.106	0.212	0.212
DC3	0.547	0.113		0.169	0.170	0.176	0.188	0.238	0.176	0.158	0.105	0.211	0.211
DG1	2.883	1.775	1.778		0.944	0.829	0.880	0.933	0.887	0.887	0.775	0.775	0.831
DG2	2.882	1.760	1.770	0.058		0.883	0.940	0.934	0.943	0.831	0.719	0.719	0.775
DG3	2.869	1.730	1.739	0.187	0.125		0.821	1.000	0.939	0.944	0.830	0.660	0.773
DG4	2.833	1.665	1.674	0.127	0.062	0.197		0.867	0.882	0.765	0.765	0.765	0.706
DG5	2.243	1.434	1.437	0.069	0.068	0.000	0.143		0.998	0.942	0.839	0.733	0.774
DG6	2.944	1.725	1.735	0.120	0.059	0.063	0.125	0.002		0.889	0.789	0.684	0.722
DG7	2.996	1.844	1.846	0.120	0.185	0.058	0.268	0.059	0.118		0.900	0.650	0.750
DG8	*	2.242	2.251	0.255	0.330	0.186	0.268	0.175	0.236	0.105		0.762	0.750
DG9	2.351	1.549	1.558	0.255	0.330	0.416	0.268	0.311	0.379	0.431	0.272		0.900
DG10	2.303	1.549	1.558	0.185	0.255	0.257	0.348	0.256	0.325	0.288	0.288	0.105	
DG11	2.282	1.542	1.546	0.131	0.198	0.067	0.255	0.009	0.128	0.059	0.150	0.323	0.215
DG12	2.331	1.546	1.550	0.134	0.202	0.070	0.224	0.011	0.124	0.061	0.120	0.288	0.228
DG13	2.328	1.543	1.547	0.132	0.200	0.069	0.230	0.066	0.122	0.116	0.181	0.226	0.160
DG14	*	2.242	2.251	0.185	0.255	0.186	0.348	0.121	0.182	0.105	0.105	0.288	0.288
DG15	2.054	1.409	1.413	0.123	0.191	0.124	0.277	0.002	0.121	0.108	0.229	0.229	0.229
DG16	2.332	1.525	1.529	0.148	0.148	0.085	0.234	0.016	0.078	0.129	0.232	0.267	0.267
DG17	2.341	1.543	1.546	0.192	0.263	0.188	0.194	0.058	0.178	0.169	0.160	0.218	0.366
DG18	3.442	1.929	1.933	0.215	0.290	0.197	0.226	0.150	0.199	0.111	0.067	0.235	0.348
DG19	2.987	1.834	1.836	0.234	0.308	0.233	0.246	0.169	0.231	0.142	0.142	0.282	0.418
DG20	2.876	1.717	1.720	0.199	0.271	0.201	0.209	0.080	0.196	0.130	0.130	0.168	0.311
DG21	2.890	1.725	1.735	0.185	0.120	0.125	0.061	0.067	0.057	0.182	0.182	0.325	0.405
DG22	3.210	1.896	1.906	0.123	0.061	0.066	0.129	0.124	0.002	0.166	0.266	0.486	0.407
DG23	*	2.178	2.197	0.197	0.127	0.067	0.208	0.186	0.061	0.172	0.172	0.379	0.305

* Denotes distance value of infinity.

Table 3. Continued.

Pop.	DG11	DG12	DG13	DG14	DG15	DG16	DG17	DG18	DG19	DG20	DG21	DG22	DG23
DC1	0.102	0.097	0.097	0.000	0.128	0.097	0.096	0.032	0.050	0.056	0.056	0.040	0.000
DC2	0.214	0.213	0.214	0.106	0.244	0.218	0.214	0.145	0.160	0.180	0.178	0.150	0.113
DC3	0.213	0.212	0.213	0.105	0.243	0.217	0.213	0.145	0.159	0.179	0.176	0.149	0.111
DG1	0.878	0.875	0.876	0.831	0.884	0.862	0.825	0.807	0.791	0.820	0.831	0.884	0.821
DG2	0.820	0.817	0.819	0.775	0.826	0.862	0.769	0.748	0.735	0.763	0.887	0.941	0.880
DG3	0.935	0.932	0.934	0.830	0.883	0.919	0.829	0.821	0.793	0.818	0.882	0.937	0.935
DG4	0.775	0.799	0.795	0.706	0.758	0.792	0.824	0.798	0.782	0.811	0.941	0.879	0.812
DG5	0.991	0.989	0.936	0.886	0.998	0.984	0.944	0.861	0.845	0.924	0.935	0.883	0.830
DG6	0.880	0.883	0.885	0.833	0.886	0.925	0.837	0.820	0.794	0.822	0.944	0.998	0.941
DG7	0.943	0.940	0.891	0.900	0.898	0.879	0.845	0.895	0.868	0.878	0.833	0.847	0.842
DG8	0.860	0.887	0.835	0.900	0.795	0.793	0.852	0.935	0.868	0.878	0.833	0.766	0.842
DG9	0.724	0.749	0.798	0.750	0.795	0.766	0.804	0.791	0.754	0.845	0.722	0.615	0.684
DG10	0.807	0.796	0.852	0.750	0.795	0.766	0.693	0.706	0.658	0.732	0.667	0.666	0.737
DG11		0.999	0.950	0.841	0.950	0.943	0.907	0.876	0.856	0.906	0.844	0.786	0.779
DG12	0.001		0.955	0.838	0.943	0.931	0.931	0.892	0.863	0.922	0.868	0.784	0.776
DG13	0.051	0.047		0.788	0.894	0.882	0.879	0.843	0.814	0.922	0.863	0.785	0.777
DG14	0.173	0.176	0.238		0.898	0.901	0.845	0.902	0.863	0.878	0.778	0.817	0.895
DG15	0.051	0.059	0.112	0.108		1.000	0.945	0.879	0.881	0.945	0.829	0.792	0.784
DG16	0.059	0.071	0.126	0.104	0.000		0.928	0.884	0.885	0.939	0.865	0.826	0.838
DG17	0.098	0.071	0.130	0.169	0.057	0.074		0.917	0.909	0.987	0.884	0.740	0.730
DG18	0.133	0.114	0.171	0.103	0.129	0.123	0.087		0.989	0.986	0.867	0.781	0.794
DG19	0.156	0.148	0.206	0.148	0.126	0.122	0.096	0.011		0.989	0.850	0.761	0.750
DG20	0.099	0.081	0.082	0.130	0.057	0.063	0.013	0.014	0.011		0.878	0.818	0.811
DG21	0.169	0.142	0.147	0.251	0.188	0.145	0.124	0.143	0.162	0.130		0.942	0.882
DG22	0.240	0.244	0.243	0.202	0.234	0.191	0.301	0.248	0.274	0.200	0.060		0.967
DG23	0.250	0.254	0.253	0.111	0.243	0.177	0.315	0.231	0.288	0.209	0.125	0.034	

Table 4. Percentage of polymorphic loci (P), mean number of alleles per locus (A), mean observed heterozygosity (H_o), mean expected heterozygosity (H_e), fixation index (F), and gene diversity statistics (H_T, H_S, D_{ST}, and G_{ST}) for the species *Datisca cannabina* and *D. glomerata*.

Species								
P	A	H_o	H_e	F	H_T	H_S	D_{ST}	G_{ST}
cannabina								
7.0	1.06	0.005	0.005	−0.005	0.216	0.005	0.210	0.975
glomerata								
5.4	1.05	0.008	0.021	0.617*	0.180	0.019	0.161	0.896

* $P < 0.001$.

have diverged at genes coding for soluble enzymes as much as typical animal genera and more than some plant genera.

No other plant species pairs with disjunct intercontinental ranges possess genetic identities as low as that found in *Datisca*. Published values of I range from 0.40 to 0.43 in *Liriodendron* (Parks and Shan-An 1988), 0.46 in *Liquidambar* (Hoey and Parks 1988) and from 0.51 to 0.75 in *Agastache* (Vogelmann and Gastony 1987).

There are two basic processes by which divergence can occur between populations at isozyme loci: 1) changes in frequency can occur between populations by drift and other processes or 2) new alleles can develop via mutation. The latter is a slower process and becomes important in more distantly related taxa (Baverstock, Cole, Richardson, and Watts 1979). In *Datisca* unique alleles occur at nearly every locus in each species, indicating that mutations most likely account for the divergence (D. Crawford, pers. comm.). This represents strong evidence for an ancient divergence of the taxa.

The time since the divergence of two species can be roughly estimated using genetic distance values derived from allozyme data (Nei 1987; Sarich 1977; Thorpe 1982). However, when the time since divergence is large, the relationship between D and time is no longer linear (Nei 1987). Furthermore, when D is greater than or equal to one (as in *Datisca*) its variance becomes very large and the reliability of the dating declines even more (Nei 1987). Nei (1975, 1987) calculates that a D value of 1 is equivalent to about five million years. Other estimates of time, given the same value of D, are three to four times larger (Sarich 1977; Thorpe 1982). Using these minimum and maximum values, we can estimate that 10 to 40 million years have passed since the two species of *Datisca* began to diverge.

Despite the high degree of uncertainty inherent in these estimates, the postulated age of Madrean-Tethyan disjuncts (Axelrod 1975) partly overlaps these broad limits. Axelrod considers that migration across the mid-Atlantic was possible from the Paleogene up to the Neogene, approximately 23 to 65 m.y.a. Additional evidence in favor of the migration of *Datisca* across the Atlantic is found in the higher genetic identity between *D. glomerata* and the two western populations of *D. cannabina* than with the central Asian population.

Morphological Similarity

The phenotypic similarity of the two species is well documented (Davidson 1973, 1976; Boesewinkel 1984; Bohm 1988). Nevertheless, detailed morpholog-

ical studies may reveal the presence of additional characters distinguishing the two taxa. The only consistent morphological distinction between the two species is the presence of hermaphrodite individuals in *D. glomerata* and female individuals in *D. cannabina.* Both species have male individuals.

In plants, the best known case of extremely high genetic divergence with high morphological similarity involves the sibling species *Clarkia franciscana* and *C. rubicunda* (Gottlieb 1973). This divergence has been attributed to the prezygotic karyologic isolation of the two species in conjunction with the likely history of sharp fluctuations in population size occurring during the evolutionary history of *C. franciscana* and subsequent founder events (Gottlieb 1981). Further research has, however, revealed additional morphological differences between the two species (Gottlieb 1981).

Comparable examples of morphological similarity accompanied by molecular divergence are best known in animals. Most cases involve sibling species isolated by strong ecological and/or behavioral differences (Ayala, Tracey, Hedgecock, and Richmond 1974; Johnson and Selander 1971; Nanney 1982; Nixon and Taylor 1977; Webster, Selander, and Yang 1972). The initial isolating mechanism may also be major karyotypic differences as in *Drosophila* (Ayala et al. 1974) and bats (Greenbaum and Baker 1976). Extensive molecular divergence can also reveal that morphological similarity is in fact due to convergence (Maxson and Wilson 1974). The example which seems most comparable to *Datisca* has been reported from sea urchins which are allopatric yet continue to occupy similar environments (Lessios 1981).

Evolutionary Stasis

How can we account for the pattern of morphological stability over long periods of evolutionary time as reported here in *Datisca*? Evolutionary stasis has been considered to result from either external stabilizing selection or inherent genotypic constraints (Williamson 1987). Van Valen (1982) has discussed additional possibilities, but he himself dismisses these. Although Williamson (1987) considers that both stabilizing and constraining factors act in concert to maintain species integrity, our data suggest that strong stabilizing selection is the cause of phenotypic stability in *Datisca,* for if genetic constraint were involved we would not expect the high levels of genetic divergence. In this case we need to know how selective forces have remained so constant over evolutionary time (Charlesworth, Lande, and Slatkin 1982).

Genetic Structure

Populations of *Datisca glomerata* are usually of small size, show extreme heterozygote deficiency ($F = 0.617$; $P < 0.001$), and little intrapopulational genetic variation. These traits characterize the species as predominantly inbreeding (Hamrick, Linhart, and Mitton 1979; Loveless and Hamrick 1984). The high level of inbreeding suggests that *D. glomerata* is truly androdioecious, and not functionally dioecious, as has been found in other cases of morphological androdioecy (Charlesworth 1984).

In apparent contradiction, the value of $Nm = 6.4$ suggests a high degree of interpopulational gene flow (Slatkin 1985). However, Slatkin's model assumes that populations are in genetic equilibrium, and most likely this condition is not

met in *D. glomerata.* For example, the populations examined could be remnants of a once wider distribution in California. The present populations simply contain different subsets of the ancestral gene pool. Interesting in this context is the fact that the two most genetically divergent populations, isolated in Owens Valley and Death Valley, do not possess any private alleles. In isolation, these populations have not evolved new alleles, but rather have only preserved a rare subset of the genetic variation present in the species.

In contrast to *D. glomerata,* the populations of *D. cannabina* examined do not show deviations from random mating ($F = -0.005$) and more intrapopulational variation. These findings are in agreement with the fact that this species is dioecious and thus an obligate outcrosser. However we do have effective isolation between the populations sampled as demonstrated by gene flow estimates. Again, historical factors can be invoked to explain this discrepancy. The 1000 kilometers of desert between the eastern and western populations of *D. cannabina* must act as a very effective barrier to gene flow, which, considering the degree of genetic differentiation, has been maintained over long periods of evolutionary time. Interestingly, the two western populations, which are separated by 1500 kilometers, show a lesser degree of genetic divergence, comparable to the level found among populations of *D. glomerata.* The northernmost and southernmost populations of *glomerata* are also separated by approximately 1500 kilometers. In each of these cases, apparently no phenotypic differentiation has occurred, mirroring the process described for the intercontinental disjunct congeners.

ACKNOWLEDGMENTS

Research was supported by the Rancho Santa Ana Botanic Garden and a Sigma Xi award to A. L. Portions of this study were carried out as part of the Bilateral Agreement on Environmental Protection between the United States and the Soviet Union. The help of the International Affairs Staff of the Fish and Wildlife Service is gratefully acknowledged. We thank Dr. A. Rabinovich (Moscow) and Dr. A. Strid (Copenhagen) for supplying seeds of *Datisca cannabina,* and L. Aberbom and W. Wisura for the collection and propagation of *Datisca glomerata.* Daniel Crawford, Christopher Davidson, David Thompson, and Scott Zona provided helpful suggestions to earlier versions of the manuscript.

LITERATURE CITED

Akkermans, A. D. L., W. Roelofsen, J. Blom, K. Huss-Danell, and R. Harkink. 1983. Utilization of carbon and nitrogen compounds by *Frankia* in synthetic media and in root nodules of *Alnus glutinosa, Hippophaë rhamnoides,* and *Datisca cannabina.* Canad. J. Bot. 61:2793–2800.

Avise, J. C. 1975. Systematic value of electrophoretic data. Syst. Zool. 23:465–481.

Axelrod, D. I. 1975. Evolution and biogeography of Madrean-Tethyan sclerophyll vegetation. Ann. Missouri Bot. Gard. 62:280–334.

Ayala, F. J., M. L. Tracey, D. Hedgecock, and R. C. Richmond. 1974. Genetic differentiation during the speciation process in *Drosophila.* Evolution 28:576–592.

Baverstock, P. R., S. R. Cole, B. J. Richardson, and C. H. S. Watts. 1979. Electrophoresis and cladistics. Syst. Zool. 28:214–219.

Boesewinkel, F. D. 1984. Ovule and seed structure in Datiscaceae. Acta. Bot. Neerl. 33:419–430.

Bohm, B. A. 1987. Intraspecific flavonoid variation. Bot. Rev. 53:197–280.

———. 1988. Flavonoid systematics of the Datiscaceae. Biochem. Syst. Ecol. 16:151–155.

Brown, J. H., and A. C. Gibson. 1983. Biogeography. C. V. Mosby Co., St. Louis. 643 p.

Carlquist, S. 1983. Intercontinental dispersal. Sonderb. naturwiss. Ver. Hamburg 7:37–47.

Charlesworth, B., R. Lande, and M. Slatkin. 1982. A neo-Darwinian commentary on macroevolution. Evolution 36:474–498.

Charlesworth, D. 1984. Androdioecy and the evolution of dioecy. Biol. J. Linnean Soc. 23:333–348.

Chaudhary, A. H. 1979. Nitrogen-fixing root nodules in *Datisca cannabina* L. Pl. & Soil 51:163–165.

Crawford, D. J. 1983. Phylogenetic and systematic inferences from electrophoretic studies, pp. 257–287. *In* S. D. Tanksley and T. J. Orton [eds.], Isozymes in plant genetics and breeding. Amsterdam.

Davidson, C. 1973. An anatomical and morphological study of Datiscaceae. Aliso 8:49–110.

———. 1976. Anatomy of xylem and phloem of the Datiscaceae. Sci. Contr. Nat. Hist. Mus. Los Angeles County 280:1–28.

Dietz, R. S., and J. C. Holden. 1970. Reconstruction of Pangaea: breakup and dispersion of continents, Permian to present. J. Geophys. Res. 75:4939–4956.

Gottlieb, L. D. 1973. Enzyme differentiation and phylogeny in *Clarkia franciscana, C. rubicunda* and *C. amoena*. Evolution 27:205–214.

———. 1981. Electrophoretic evidence and plant populations. Progr. Phytochem. 7:1–46.

———. 1982. Conservation and duplication of isozymes in plants. Science 216:373–380.

Greenbaum, I. F., and R. J. Baker. 1976. Evolutionary relationships in *Macrotus* (Mammalia: Chiroptera): biochemical variation and karyology. Syst. Zool. 25:15–25.

Hamrick, J. L., L. B. Linhart, and J. B. Mitton. 1979. Relationships between life history characteristics and electrophoretically detectable variation in plants. Annual Rev. Ecol. Syst. 10:173–200.

Hickey, R. J., S. I. Guttman, and W. H. Eshbaugh. 1989. Evidence for post-translational modification of triose phosphate isomerase (TPI) in *Isoëtes* (Isoëtaceae). Amer. J. Bot. 76:215–221.

Hoey, M. T., and C. R. Parks. 1988. Genetic divergence between *Liquidambar styraciflua* and *L. formosana* (Hamamelidaceae). Amer. J. Bot. 75:99. (*Abstract.*)

Johnson, W. E., and R. K. Selander. 1971. Protein variation and systematics in kangaroo rats (genus *Dipodomys*). Syst. Zool. 20:377–405.

Lachaise, D., M.-L. Cariou, J. R. David, F. Lemeunier, L. Tsacas, and M. Ashburner. 1988. Historical biogeography of the *Drosophila melanogaster* species subgroup. Evol. Biol. 22:159–225.

Les, D. H. 1989. Population genetics of *Ceratophyllum demersum* and *C. echinatum*, two primitive hydrophilous angiosperms. Amer. J. Bot. (Supplement) 76:254 (*Abstract.*)

Lessios, H. A. 1981. Divergence in allopatry: molecular and morphological differentiation between sea urchins separated by the Isthmus of Panama. Evolution 35:618–634.

Lewis, P., and R. Whitkus. 1988. Genestat-PC. Ohio State Univ.

Liston, A., L. H. Rieseberg, and T. S. Elias. 1989. Genetic similarity is high between intercontinental disjunct species of *Senecio*. Amer. J. Bot. 76:383–388.

Loveless, M. D., and J. L. Hamrick. 1984. Ecological determinants of genetic structure in plants. Annual Rev. Ecol. Syst. 15:65–95.

Maxson, L. R., and A. C. Wilson. 1974. Convergent morphological evolution detected by studying the proteins of the tree frogs of the *Hyla eximia* species group. Science 185:66–68.

Mills, W. R., and K. W. Joy. 1980. Rapid isolation and amino acid content of pea chloroplasts. Planta 148:75–83.

Nanney, D. L. 1982. Genes and phenes in *Tetrahymena*. BioScience 32:783–788.

Nei, M. 1972. Genetic distance between populations. Amer. Naturalist 106:283–292.

———. 1975. Molecular population genetics and evolution. Elsevier, New York. 288 p.

———. 1987. Molecular evolutionary genetics. Columbia Univ. Press, New York. 512 p.

Nixon, S. E., and R. J. Taylor. 1977. Large genetic distances associated with little morphological variation in *Polycelis coronata* and *Dugesia tignina* (Planaria). Syst. Zool. 26:152–164.

Parks, C. R., and H. Shan-An. 1988. Patterns of genetic divergence in the genus *Liriodendron* (Magnoliaceae). Amer. J. Bot. 75:100–101. (*Abstract.*)

Pashley, D. P., K. S. Rai, and D. N. Pashley. 1985. Patterns of allozyme relationships compared with morphology, hybridization, and geological history in allopatric island-dwelling mosquitoes. Evolution 39:985–996.

Phillips, J. D., and D. Forsyth. 1972. Plate tectonics, paleomagnetism, and the opening of the Atlantic. Bull. Geol. Soc. Amer. 83:1579–1600.

Rechinger, K. H. 1966. Datiscaceae, p. 1. *In* K. H. Rechinger [ed.], Flora Iranica, Vol. 29. Akademische Druck, Graz, Austria.

Rieseberg, L. H., and D. E. Soltis. 1987. Allozymic differentiation between *Tolmiea menziesii* and *Tellima grandiflora* (Saxifragaceae). Syst. Bot. 12:154–161.

Sarich, V. M. 1977. Rates, sample sizes, and the neutrality hypothesis for electrophoresis in evolutionary studies. Nature 265:24–28.

———, and J. E. Cronin. 1980. South American mammal molecular systematics, evolutionary clocks, and continental drift, pp. 399–421. *In* R. L. Ciochon and A. B. Chiarelli [eds.], Evolutionary biology of the New World monkeys and continental drift. Plenum, N.Y.

Sinotô, Y. 1929. Chromosome studies in some dioecious plants, with special reference to allosomes. Cytologia 1:109–191.

Slatkin, M. 1985. Rare alleles as indicators of gene flow. Evolution 39:53–65.

Snow, R. 1959. Chromosome numbers of California plants, with notes on some cases of cytological interest. Madroño15:81–89.

Soltis, D. E., C. H. Haufler, D. C. Darrow, and G. J. Gastony. 1983. Starch gel electrophoresis of ferns: a compilation of grinding buffers, gel and electrode buffers, and staining schedules. Amer. Fern J. 73:9–27.

———, and P. S. Soltis. 1986. Intergeneric hybridization between *Conimitella williamsii* and *Mitella stauropetala* (Saxifragaceae). Syst. Bot. 11:293–297.

Tarling, D. H. 1980. The geological evolution of South America with special reference to the last million years, pp. 1–41. *In* R. L. Ciochon and A. B. Chiarelli [eds.], Evolutionary biology of the New World monkeys and continental drift. Plenum, N.Y.

Thorne, R. F. 1972. Major disjunctions in the geographic ranges of seed plants. Quart. Rev. Biol. 47:365–411.

Thorpe, J. P. 1982. The molecular clock hypothesis: biochemical evolution, genetic differentiation and systematics. Annual Rev. Ecol. Syst. 13:139–168.

Van Valen, L. M. 1982. Integration of species: stasis and biogeography. Evol. Theory 6:99–112.

Vogelmann, J. E., and G. J. Gastony. 1987. Electrophoretic enzyme analysis of North American and eastern Asian populations of *Agastache* sect. *Agastache* (Labiatae). Amer. J. Bot. 74:385–393.

Walper, J. L., and C. L. Rowett. 1972. Plate tectonics and the origin of the Caribbean Sea and the Gulf of Mexico. Trans. Gulf Coast Assoc. Geol. Soc. 22:105–116.

Webster, T. P., R. K. Selander, and S. W. Yang. 1972. Genetic variability and similarity in the *Anolis* lizards of Bimini. Evolution 26:523–535.

Williamson, P. G. 1987. Selection or constraint?: a proposal on the mechanism for stasis, pp. 129–142. *In* K. S. W. Campbell and M. F. Day [eds.], Rates of evolution. Allen and Unwin, London.

Witter, M. S., and G. D. Carr. 1988. Adaptive radiation and genetic differentiation in the Hawaiian silversword alliance (Compositae: Madiinae). Evolution 42:1278–1287.

Wright, S. 1965. The interpretation of population structure by *F* statistics with special regard to systems of mating. Evolution 19:395–420.

———. 1969. Evolution and the genetics of populations, Vol. 2. The theory of gene frequencies. Univ. Chicago Press, Chicago. 511 p.