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Geographic partitioning of chloroplast DNA variation in the genus *Datisca* (*Datiscaceae*)

A. Liston, L. H. Rieseberg, and M. A. Hanson

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Abstract: *Datisca* (*Datiscaceae*) is a ditypic genus with an intercontinentally disjunct distribution. Chloroplast DNA restriction site data was obtained from 23 populations and four 10–20 year old herbarium specimens of *D. glomerata* and three populations of *D. cannabina* from throughout their geographic ranges in western North America and southwest-central Asia, respectively. In *D. glomerata*, plastome diversity is partitioned geographically. All populations from southern California have a common plastome, while most populations north of this region share a relatively divergent plastome (0.49% sequence divergence). Likewise, these plastomes are highly divergent (0.87% mean sequence divergence) from those found in *D. cannabina*. Biogeographic processes dating to the Pleistocene and Late Miocene may be responsible for these intra- and interspecific patterns of chloroplast DNA divergence.

Over 50 examples of intraspecific chloroplast DNA (cpDNA) variation have been reported in plants (reviewed in Soltis & al. 1991 a). However, relatively few studies have demonstrated geographic partitioning of this variation (Soltis & al. 1989, 1991 b). When patterns of variation in the chloroplast genome (plastome) of a species have a geographic basis, inferences can be made concerning its biogeographic history. In a similar manner, the degree of molecular divergence between vicariant species can provide an important source of evidence for the biogeographic processes responsible for their current distribution (Vogelmann & Gastony 1987; Liston & al. 1989 a, b; Parks & Wendel 1990; Wendel & Percival 1990; Crawford & al. 1991; Haraldsen & al. 1991; Hoey & Parks 1991; Sytsma & al. 1991). In the present study we describe the intra- and interspecific patterns of plastome variation in a genus and propose biogeographic hypotheses to explain these patterns.

The genus *Datisca* L. is one of three genera in the small family *Datiscaceae*. Phylogenetic analysis of cpDNA variation in the family suggests that *Datisca* is monophyletic and that it diverged slightly before the monotypic *Octomeles* Miq. and *Tetrameles* R. Br. became reproductively isolated (Rieseberg & al. 1992). *Datisca* consists of two species: *D. glomerata* (Presl) Baill. and *D. cannabina* L. These are herbaceous perennials occupying riparian habitats in winter-rainfall re-

gions of western North America and southwestern Asia, respectively. Populations of *Datisca cannabina* are dioecious while populations of *D. glomerata* are androdioecious, consisting of both male and hermaphrodite individuals (LISTON & al. 1990). Otherwise, both species are morphologically and anatomically similar (DAVIDSON 1973, 1976; BOESEWINKEL 1984), are diploids with the same chromosome number (SINOTÔ 1929, SNOW 1959), and possess identical flavonoid profiles (BOHM 1988). Despite these similarities, the mean genetic identity (NEI 1972) among populations of the two species for 21 isozyme loci is $I = 0.142$ (LISTON & al. 1989 b).

Datisca glomerata also shows a high degree of intraspecific uniformity. For example, no flavonoid variation was found among 12 populations of this species from throughout its range (BOHM 1987). The mean genetic identity among 23 populations of *D. glomerata* is $I = 0.847$ with no apparent geographic partitioning of isozyme variation. The mean genetic identity among the three sampled populations of *D. cannabina* is relatively lower at $I = 0.649$ (LISTON & al. 1989 b).

The goals of the present study were to (1) document levels of divergence in the plastome among populations of the species *D. glomerata* from throughout its geographic range and (2) quantify cpDNA divergence between this species and the intercontinentally disjunct *D. cannabina*.

Material and methods

Fresh leaves were collected from 23 populations of *Datisca glomerata* from throughout its geographic range in California, U.S.A. and northern Baja California, Mexico (Table 1, Fig. 1). Leaves from three representative samples of *D. cannabina* derived from bulk seed collections were also obtained (Table 1). DNA was isolated by two methods: In the initial survey of restriction site variation, an enriched chloroplast fraction was prepared from 10–30 grams of fresh tissue by centrifugation at low speed as described by PALMER (1982). The resulting pellet was washed and DNA was isolated using the CTAB method of DOYLE & DOYLE (1987). Sufficiently pure cpDNA was obtained from 19 populations of the two species (Table 1) for direct visualization with ethidium bromide on 0.8% agarose gels. For mapping of the restriction site mutations within *Datisca*, DNA samples were cleaved with 23 restriction endonucleases (Table 2), electrophoresed on 0.8% agarose gels, and blotted to Bio Trace (Gelman) nylon filters following standard protocols (PALMER 1986). Filter hybridization to digoxigenin-labelled, heterologous *Petunia* cpDNA probes (described in SYTSMA & GOTTLIEB 1986) was performed as described in BECKSTROM-STERNBERG & al. (1991). The cpDNA of *Datisca* is colinear with *Petunia* (RIESEBERG, unpubl. data). Probes were kindly provided by JEFF PALMER.

In subsequent analyses, total DNA was isolated from tissue stored at − 80 °C and from approximately 30 mg of leaves from dried herbarium specimens (Table 1) using the CTAB method (DOYLE & DOYLE 1987) as modified by RIESEBERG & al. (1992). Samples of DNA from eight previously surveyed populations, seven new populations, and four herbarium specimens were amplified via the polymerase chain reaction (PCR). The primers used were homologous to conserved portions of the gene ribulose-1,5-bisphosphate carboxylase (*rbc*L) and open reading frame 106 (ORF106):
5′-ATGTCACCACAAACAGAAACTAAAGCAAGT-3′ and
5′-ACTACAGATCTCATACTACCCC-3′, respectively (ARNOLD & al. 1991). The first primer is homologous to positions 57586-57615 in the tobacco chloroplast sequence (SHINOZAKI & al. 1986) and the second to positions 60860-60839. Positions 689-710 in the nucleotide sequence of the putative zinc finger protein *zfp*A described from pea plastids (SASAKI & al. 1989) are homologous to the ORF106 primer. These primers flank an intergenic spacer region or "hot spot" which exhibits numerous length mutations and

Table 1. *Datisca* populations examined for chloroplast DNA variation

Species	Location[a]	Collection[b]	Plastome[c]	Evidence[d]
D. cannabina				
	U.S.S.R.: Tadzhik SSR.	ELIAS 10019	DC-1	c p
	U.S.S.R.: Transcaucasus.	RABINOVICH 423	DC-2	c
	Greece: Lesbos.	STRID 26171	DC-3	c
D. glomerata				
North Coast Ranges				
	U.S.A.: CA, Humboldt Co.	RIESEBERG 1082	DG-N	c
	U.S.A.: CA, Mendocino Co.	RIESEBERG 1081	DG-N	c
	U.S.A.: CA, Mendocino Co.	LISTON 748	DG-N′	c
	U.S.A.: CA, Sonoma Co.	RIESEBERG 1080	DG-N	c
Cascades and Sierra Nevada Ranges				
	U.S.A.: CA, Siskiyou Co.	RIESEBERG 1083	DG-N	c
	U.S.A.: CA, El Dorado Co.	LISTON 776	DG-N	c
	U.S.A.: CA, El Dorado Co.	LISTON 777	DG-N	c
	U.S.A.: CA, Tuolomne Co.	MARTIN 20661	DG-N	h (1971)
	U.S.A.: CA, Mariposa Co.	DAVIDSON 1001	DG-N	h (1969)
	U.S.A.: CA, Tulare Co.	THORNE 53701	DG-N	h (1979)
Owens and Death Valleys				
	U.S.A.: CA, Inyo Co.	LISTON 759	DG-N	c
	U.S.A.: CA, Inyo Co.	LISTON 762	DG-N	c p
Central Coast Ranges				
	U.S.A.: CA, Santa Cruz Co.	LISTON 757	DG-S	c p
	U.S.A.: CA, Monterey Co.	TWISSELMAN 16625	DG-S	h (1970)
	U.S.A.: CA, Santa Barbara Co.	ELIAS 12260	DG-N	p
	U.S.A.: CA, Ventura Co.	ELIAS 12259	DG-N	p
	U.S.A.: CA, Ventura Co.	ELIAS 12258	DG-N	p
Transverse Ranges				
	U.S.A.: CA, Los Angeles Co.	RIESEBERG 1073	DG-N	p
	U.S.A.: CA, Los Angeles Co.	LISTON 778	DG-N	p
	U.S.A.: CA, San Bernardino Co.	LISTON 779	DG-N	p
	U.S.A.: CA, San Bernardino Co.	RIESEBERG 1108	DG-N	p
Peninsular Ranges				
	U.S.A.: CA, Orange Co.	WISURA 15402	DG-S	c p
	U.S.A.: CA, Riverside Co.	ELIAS 10154	DG-S	c
	U.S.A.: CA, San Diego Co.	LISTON 768	DG-S	c p
	U.S.A.: CA, San Diego Co.	LISTON 767	DG-S	c p
	Mexico: Baja California	LISTON 773	DG-S	c p
	Mexico: Baja California	LISTON 775	DG-S	c p

[a] See LISTON & al. (1989 a) for more detailed locality information.

[b] All collections are vouchered at RSA.

[c] The three *D. cannabina* populations have unique plastomes (DC-1, DC-2, DC-3). All populations of *D. glomerata* have either the northern (DG-N) or southern (DG-S) plastome. One population (LISTON 748) has the northern plastome with two additional restriction site changes (DG-N′).

[d] Restriction site analysis over the entire plastome, c; PCR analysis of *rbc*L-ORF106 with DNA extracted from a herbarium specimen, h; PCR analysis of *rbc*L-ORF106 with DNA extracted from tissue stored at − 80 °C, p. Dates following the herbarium specimens are the year of collection.

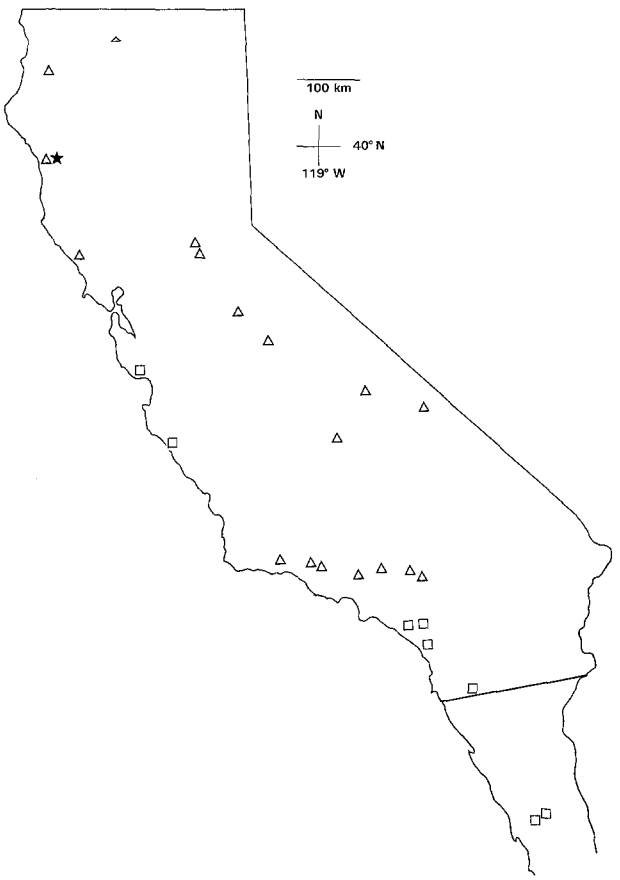

Fig. 1. The distribution of populations surveyed for plastome variation in *Datisca glomerata*. The DG-S plastome is indicated by squares, the DG-N plastome is indicated by triangles, and DG-N′ is indicated by a star. See LISTON & al. (1989 a) for a distribution map of the *D. cannabina* populations

nucleotide substitutions among closely related species of *Triticum* and *Aegilops* (OGIHARA & al. 1991).

Amplification reactions followed RIESEBERG & al. (1992). The PCR products were cleaved with the endonucleases *Alu*I, *Bsa*JI; *Bst*UI, *Dde*I, *Hha*I, *Hinf*I, *Mbo*I, *Msp*I, *Rsa*I, *Sau*96I, and *Scr*FI. All have 4 base pair recognition sites. Restriction fragments were separated by electrophoresis in 1.4% agarose gels and stained with ethidium bromide.

Sequence divergence values were estimated following NEI & TAJIMA (1983). These values were used to produce an unrooted tree using the distance algorithm of FITCH & MARGOLIASH (1967) with the program PHYLIP vers. 3.4 (FELSENSTEIN 1991). In addition, phylogenetic analyses were performed with PAUP vers. 3.0r (SWOFFORD 1991) using Wagner and Dollo parsimony. Restriction site and length mutations were both scored for a total of 39 characters. A branch-and-bound algorithm was used to derive the most parsimonious tree. The resulting tree was unrooted.

Results

The survey of cpDNA variation over the entire plastome in 19 populations revealed a total of 34 restriction site mutations and five insertion/deletions in *Datisca glomerata* and *D. cannabina* (Tables 2, 3). This analysis allowed the division of the *D. glomerata* populations into two major groups based on the distribution of cpDNA mutations (Table 1). All populations of *D. glomerata* from south of the Los Angeles basin (the Peninsular Ranges) share a single plastome type designated DG-S. Most of the populations from north of the Los Angeles basin share a different plastome

Table 2. Chloroplast DNA restriction site mutations in *Datisca*. Mutations are listed with either the ancestral fragments or with the more common fragment(s) first, followed by derived or less common fragment(s). Square brackets indicate where a small fragments was not seen but inferred since length mutations were not seen with other endonucleases. Populations sharing derived or less common mutations are indicated by the plastome abbreviation. * Mutation polarized relative to *Datisca cannabina*. Probes are *Petunia* cpDNA fragments cloned by J. D. PALMER and described by SYTSMA & GOTTLIEB (1986). Fragment sizes are in kilobases

Restriction endonuclease[a]	Mutation	Probe	Distribution of mutations[b]
*Apa*I	15.0 = 12.0 + 3.0	P6	DC-1
*Bam*HI	4.5 + [0.2] = 4.7*	P19, P20	DC-2
	17.4 = 13.0 + 4.4	S6, S8	DC-3
*Bgl*I	23.3 + 4.7 = 28*	S6, S8	DG-N, DG-S
*Bss*HI	8.3 = 7.1 + 1.2	P12, P14	DC-3
*Bst*EII	8.2 = 4.1 + 4.1*	NA	DG-N′
*Eco*RV	11.5 + [0.4] = 11.9	P1, P4	DC-3
	25.5 = 20.6 + 4.9*	NA	DG-N′
*Hind*III	1.2 + [0.2] = 1.4	P1, P4	DC-3
	12.0 = 10.5 + 1.5*	S6, S8	DG-N, DG-S
*Hpa*I	5.1 + 10.0 = 15.1*	P6	DC-2, DG-S
*Kpn*I	24.0 = 17.0 + 7.0	P1, P4	DC-2
*Nde*I	14.2 = 7.1 + 7.1	P19, P20	DC-1
	12.5 + [0.5] = 13	P3, P16	DC-2
*Nhe*I	26.0 + 14.8 = 40.8	P19, P20	DC-2
*Pst*I	25.4 = 12.7 + 12.7*	P1, P4	DG-N
	25.4 = 20.3 + 5.1	P1, P4	DC-3
	24.2 = 15 + 9.3	S6, S8	DC-2
*Pvu*II	20.3 + 9.4 = 29.9*	S6, S8	DG-N, DG-S
	15.2 + 3.9 = 19.1	P8, P10	DC-2
*Sac*I	6.9 = 6.5 + [0.4]*	P8, P10	DG-N, DG-S
*Sal*I	30.5 = 20.0 + 10.5*	P1, P4	DG-N
	18.5 + 4.0 = 22.5*	P12, P14	DG-N
*Sma*I	31.0 = 22.0 + 9.0*	P19, P20	DG-N
*Spe*I	17.5 + 5.2 = 22.7*	S6, S8	DG-N, DG-S
	1.4 = 1.2 + [0.2]	S6, S8	DC-1, DC-3
*Xba*I	25.6 = 21.0 + 4.6*	P1, P4	DG-N
	3.7 = 3.5 + [0.2]	P19, P20	DC-2
	30.0 = 27.0 + 3.0*	P6	DG-S
*Xho*I	12.0 + 5.6 = 17.6*	S6, S8	DG-N
	12.0 + 10.0 = 22.0*	P6	DG-N, DG-S
*Xmn*I	8.3 = 7.6 + 0.7*	S6, S8	DG-N, DG-S
	1.8 + [0.4] = 2.2*	P3, P16	DG-N, DG-S
	4.0 + 2.4 = 6.4*	P3, P16	DG-N

[a] The endonucleases *Pvu*I, *Sac*II, and *Tth*111I revealed no polymorphisms.

[b] Includes only those populations surveyed for cpDNA variation over the entire plastome as indicated in Table 1.

Table 3. Length mutations in *Datisca*. Mutations in *D. glomerata* are polarized relative to *D. cannabina* and vice versa. Does not include the two length variants detected in the *rbc*L-ORF106 PCR-amplified product

Mutation	Probe	Distribution
200 bp deletion	P19, P20	DG-N
300 bp deletion	P6	DC-3
300 bp deletion	P6	DG-N
500 bp deletion	P3, P16	DG-S
50 bp deletion	P3, P16	DC-1, DC-3

type (DG-N). However a population from Santa Cruz county (LISTON 757) in the Central Coast Ranges had the DG-S plastome. Also a single population from Mendocino county (LISTON 748) had all of the DG-N mutations as well as two unique mutations (DG-N').

In order to expand the geographic coverage of the initial survey, an attempt was made to find diagnostic cpDNA differences between DG-N and DG-S that could be analyzed in populations for which small amounts of frozen tissue were available and in DNA isolated from herbarium specimens. These additional samples provided data from the Transverse, Central Coast, and Sierra Nevada Ranges (Table 1). DNA was amplified from four herbarium specimens collected between 1969–1979, but could not be amplified from older specimens (LISTON, unpubl. data). A survey of the *rbc*L – ORF106 PCR-amplified products in northern and southern California DNA samples with 11 endonucleases revealed two insertion/deletions of approximately 10 bp each (Fig. 2). These fragment size differences were consistently found in several endonuclease digestions, indicating that they are indeed length mutations. The insertion/deletions map to the region between the *rbc*L and ORF106 genes (LISTON, unpubl. data). Small length mutations of 1–10 bp in size are common in noncoding regions of cpDNA (PALMER 1991) and appear to be responsible for the fragment differences observed here.

The two length mutations observed in the PCR-amplified product were consistently correlated with the DG-N and DG-S plastomes in seven samples where both cpDNA restriction site mapping and PCR analysis were conducted (Table 1). Thus populations that were previously identified as DG-S, all shared two 10 bp deletions in the *rbc*L – ORF106 region relative to DG-N (Fig. 2). These results indicate that the diagnostic mutations found in the PCR-amplified product could be used to assign unknown samples to a plastome type. On this basis, plastome assignments were made for herbarium specimens and other populations for which only small amounts of tissue were available (Table 1). For the most part, the geographic partitioning of plastome variation between northern and southern California was again found. However, an additional sample from the Central Coast Range in Monterey county (TWISSELMAN 16625) had the DG-S plastome (Fig. 2).

The percentage of cpDNA sequence divergence between the DG-S and DG-N plastome is 0.49% (Table 4). This value is much higher than those commonly reported for conspecific populations (reviewed in SOLTIS & al. 1991 a). However it

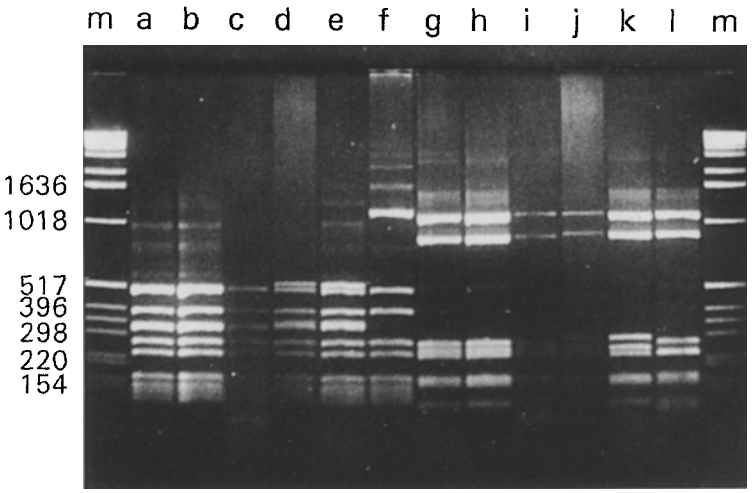

Fig. 2. Restriction digests of a 3280 base pair (bp) amplified fragment in *Datisca* corresponding to *rbc*L and ORF106. Lanes a–f. *Hin*fI digests. Note the approximately 520 and 350 bp bands in lanes d and e. Lanes g–l. D*de*I digests. Note the approximately 300 and 880 bp bands in lanes j and k. Lanes a–c and g–i are DG-S; lanes d–e and j–k are DG-N. Lanes f and l are from *Datisca cannabina* (DC-1). Samples: Santa Cruz Co. (LISTON 757) a, g; Orange Co. (WISURA 15402) b, h; Monterey Co. (TWISSELMAN 16625) c, i; Tuolumne Co. (MARTIN 20661) d; Tulare Co. (THORNE 53701) j; San Bernardino Co. (LISTON 779) e, k; *D. cannabina* (ELIAS 10019) f, l. Sizes of molecular weight standards (m) are given in base pairs

Table 4. Numbers of restriction site mutations (upper right-hand section of matrix) and percentages of cpDNA sequence divergence (lower left-hand portion of matrix) in *Datisca*. Approximately 380 restriction sites were examined for each population. Sequence divergence values were calculated following NEI & TAJIMA (1983)

Plastome	DG-N	DG-N′	DG-S	DC-1	DC-2	DC-3
DG-N	–	2	9	18	23	21
DG-N′	0.09	–	11	20	25	23
DG-S	0.40	0.49	–	13	16	16
DC-1	0.81	0.90	0.58	–	11	7
DC-2	1.04	1.13	0.71	0.49	–	14
DC-3	0.94	1.04	0.71	0.31	0.62	–

is comparable to the divergence values found among the three *D. cannabina* populations which range from 0.31–0.62% (Table 4).

Divergence values between populations of the two *Datisca* species range from 0.58%–1.13% with a mean of 0.87% ± 0.17%. This is slightly lower than the value of 1.10% reported by RIESEBERG & al. (1992) for the same comparison using restriction site variation in a *rbc*L–ORF106 PCR-amplified fragment. The dis-

crepency probably results from the fact that only a single, relatively rapidly evolving region of the plastome was analyzed in the RIESEBERG & al. (1992) study.

Of the 39 cpDNA restriction site and length mutations (Tables 2, 3), 21 were present in more than one taxon, and thus were of potential phylogenetic information. Phylogenetic analysis using Wagner and Dollo parsimony resulted in a single 40-step tree (Fig. 3). A single convergent mutation is hypothesized, resulting in a homoplasy index of only 0.025 (consistency index = 0.975). Removal of length mutations from the data set did not effect the resulting tree topology. Likewise, the unrooted tree generated from sequence divergence values (FITCH & MARGOLIASH 1967) had the same topology as the parsimony tree (Fig. 4).

Discussion

Intraspecific comparisons. Both the high degree of divergence and geographic division between the DG-S and DG-N plastomes in *D. glomerata* require explanations. Hybridization and introgression between congeners are often invoked to explain intraspecific plastome variation (reviewed in RIESEBERG & SOLTIS 1991, RIESEBERG & BRUNSFELD 1991). However in the case of *D. glomerata* there is no North American candidate for the plastid donor and no other evidence for potential introgression in the species.

The following biogeographical scenario could be responsible for the current pattern of plastome divergence in the species. Assuming the DG-S plastome is ancestral (consistent with the hypothesis of phylogenetic relationships) it may have once been widespread throughout the geographic range of the species. During the climatic cooling associated with Pleistocene glaciation, the range of the species may have contracted to the region south of the Transverse Ranges of southern California. However some populations could have survived north of the Transverse Ranges, including the extant ones in Santa Cruz and Monterey counties. Both populations are located in regions of local endemism (RAVEN & AXELROD 1978) and in habitats with high numbers of apparently relictual taxa such as the conifers *Sequoia sempervirens* (D. DON) ENDL. and *Abies bracteata* D. DON.

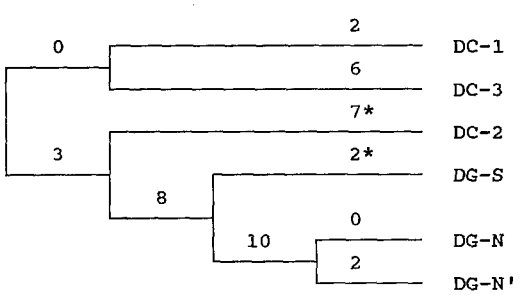

Fig. 3. Single, most parsimonious, unrooted 40-step tree for plastomes of *Datisca*. The number of character state changes per branch is indicated. The asterisk indicates the convergent mutation

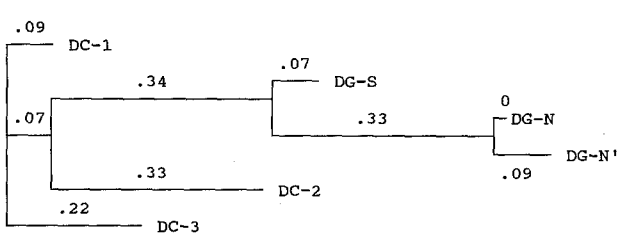

Fig. 4. Shortest unrooted genetic distance tree (FITCH & MARGOLIASH 1967) for *Datisca* based on sequence divergence values (Table 4). Sequence divergence estimates for each branch are shown

In this scenario, a surviving population isolated in northern California that became reduced in size could have become fixed for a number of unique cpDNA mutations. The effective population size of organelles is approximately one-quarter that of nuclear genes when sex ratios are equal (BIRKY & al. 1983). This leads to an increase in the rate of gene fixation by drift and a decrease in expected diversity (BIRKY 1991, RIESEBERG & SOLTIS 1991). The presence of varying ratios of males to hermaphrodites in extant *D. glomerata* populations (LISTON & al. 1990) makes it difficult to estimate the actual reduction in effective population size. BIRKY (1991: 122) has suggested that if a population endures a severe bottleneck of short duration, "organelle diversity might go to zero while nuclear diversity remains substantial". Such a process could explain the establishment of a unique plastome in a *Datisca* population without accompanying nuclear divergence.

This population would have been the source of the DG-N plastome and responsible for the "recolonization" of the northern California range of *D. glomerata.* Although most examined populations of *D. glomerata* are uniform for one of only two plastome types, the presence of two unique mutations in the Mendocino county population LISTON 747 suggests at least the potential for rapid cpDNA sequence evolution. This population is located less than 5 km from RIESEBERG 1081 which has a typical DG-N plastome. Alternatively, the differentiation between the two plastome types may have occurred several million years ago (see below) and be independent of the Pleistocene migrations suggested by the distribution of relictual populations.

Similar examples of north-south disjunctions in plastome type have been reported in *Tellima grandiflora* (PURSH) DOUGL. and *Tolmiea menziesii* (PURSH) T. & G. (SOLTIS & al. 1989, 1991 b) of the *Saxifragaceae*. Like *Datisca*, both species are morphologically and allozymically uniform throughout their geographic ranges (SOLTIS & al. 1991 b). These authors attributed the observed geographic partitioning of plastid variation to plant migration in response to Pleistocene glaciation and "cytoplasmic bottlenecks". In these two species the disjunction occurs in C. Oregon, which is consistent with their more northern geographic distribution. In contrast to *Datisca*, both *Tellima* and *Tolmiea* have higher cpDNA diversity in the southern portion of their geographic ranges.

The degree of cpDNA divergence between the three populations of *D. cannabina* is comparable to that observed between DG-S and DG-N (Table 4). More extensive sampling in SW. and C. Asia is necessary to determine if this differentiation has a consistent geographic basis, and if similar historical processes may be responsible for this pattern.

Interspecific comparisons. The mean cpDNA sequence divergence observed in this study between *D. glomerata* and *D. cannabina* (0.87% ± 0.17%) is greater than that observed for intraspecific comparisons in the genus (Table 4). However this value is less than expected considering the very high degree of allozyme divergence reported for the two species (LISTON & al. 1989 a). This value is also lower than the 1.24% ± 0.145% sequence divergence reported for cpDNA comparisons between species of *Liriodendron* (*Magnoliaceae*) with an Asian–North American disjunct distribution (PARKS & WENDEL 1990).

Assuming a substitution rate of approximately 10^{-9} nucleotides per site per

year for cpDNA (Zurawski & Clegg 1987, Wolfe & al. 1987), a divergence time of 8 700 000 ± 1 700 000 years can be estimated between the two species. However the assumptions of constant rates of molecular evolution over time and between lineages implicit in this estimate may be inaccurate (Scherer 1990). The cpDNA mutation rate can vary widely even among closely related species (reviewed in Palmer & al. 1988) and may be variable in *Datisca* itself. For these reasons, the estimates of divergence times should be considered as approximations.

With these caveats in mind, the divergence of the two *Datisca* species on the order of 10 mya would place this event in the late Miocene. This is the latest period when potential contact between temperate, deciduous forests spanned the northern hemisphere (reviewed in Parks & Wendel 1990). The *D. cannabina* population DC-2 (Transcaucasus) clusters with the populations of *D. glomerata* in both the distance and parsimony analyses (Figs. 3, 4). Considering the large genetic distances among *D. cannabina* populations and the sparse geographic sampling, it would be premature to assume that populations from the central part of the species range were ancestral to *D. glomerata.*

Conclusions. This study has used restriction site mapping of the chloroplast genome in concert with restriction analysis of an amplified PCR product representing a small part of the plastome. The combination of the two approaches has allowed the elucidation of both within and between species patterns of cpDNA variation. The use of the polymerase chain reaction allowed the incorporation of additional populations and 10–20 year old herbarium specimens, broadening the geographic coverage of the plastome variation survey. These samples could not have been assayed with standard methods of cpDNA analysis. The results indicate that substantial cpDNA divergence can exist in an otherwise uniform species, and that the geographic partitioning of this variation may be the product of biogeographic processes. Likewise, the pattern of divergence between two intercontinentally disjunct species can provide important evidence for reconstructing the biogeographic history of these taxa.

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Addresses of the authors: AARON LISTON, Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331-2902, U.S.A. – LOREN H. RIESEBERG and MICHAEL A. HANSON, Rancho Santa Ana Botanic Garden Graduate Program in Botany, Claremont, CA 91711-3101, U.S.A.

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