CHLOROPLAST AND MITOCHONDRIAL DNA VARIATION IN *HORDEUM VULGARE* AND *HORDEUM SPONTANEUM*

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ABSTRACT

A survey of restriction fragment polymorphism in *Hordeum vulgare* and *Hordeum spontaneum* was made using 17 and 16 hexanucleotide restriction endonucleases on chloroplast (cp) and mitochondrial (mt) DNA, respectively. The plant accessions originated from various places throughout the Fertile Cresent and Mediterranean. The types of changes in cpDNA consisted of nucleotide substitutions and insertions and deletions on the order of 100 base pairs. In contrast, mtDNA has most likely undergone larger insertions and deletions of up to 20 kilobase pairs in addition to rearrangements. Grouping of mtDNA fragment data showed that in some cases geographical affinities existed between the two species, whereas in others there were no clear affinities. Nucleotide diversity estimates derived from the restriction fragment data were used in a number of comparisons of variability. Comparisons of overall mtDNA variability (nucleotide diversity = 9.68×10^{-4}) with cpDNA variability (nucleotide diversity = 6.38×10^{-4}) indicated that the former are somewhat more variable. Furthermore, there was no indication that the wild *H. spontaneum* (cpDNA diversity = 5.57×10^{-4}; mtDNA diversity = 6.04×10^{-4}) was more variable than the land races of *H. vulgare* (cpDNA diversity = 5.88×10^{-4}; mtDNA diversity = 9.79×10^{-4}). In fact, on the basis of mtDNA diversity, *H. vulgare* was the more variable species. Comparison of organelle nucleotide diversity estimates with an estimate of nuclear nucleotide diversity derived from existing isozyme data provided evidence that both organelle genomes are evolving at a slower rate than the nuclear genome.

BARLEY (*Hordeum vulgare* L.) is among the oldest of domesticated plant species still in production today. Despite its long history and widespread cultivation, there is no clear understanding of the evolutionary genetic aspects of this important species (HARLAN and ZOHARY 1966). Population genetic studies in cultivated barley and its immediate progenitor species, *H. spontaneum* K. (wild barley), have focused mainly on electrophoretically discernible isozyme variability [see BROWN (1983) for a review]. These studies have shown that considerable variation exists in natural populations, and differentiation with respect to nuclear genes may occur frequently in local populations (BROWN *et al.* 1978; NEVO *et al.* 1979; BROWN and MUNDAY 1982).

Although the vast majority of genetic information is contained in the nucleus, there are important functions encoded by DNA in chloroplasts and mitochondria. These organelles are central to the production and utilization of energy and, accordingly, affect the performance of a plant. Since organelle genomes are much less complex than nuclear genomes, restriction endonuclease digestion of the former generally yields fragments that are easily resolved and thus more amenable to the study of variation. Definitive studies of genetic variation can only be made at the primary sequence level in regions of known genetic function; however, technical limitations seriously limit sample size in such endeavors. Restriction enzyme analysis, although lacking the same degree of resolution, is simpler to perform and larger sample sizes can be analyzed. Furthermore, statistical methods are available to estimate nucleotide divergence between individuals based on restriction fragment data (UPHOLT 1977; GOTOH *et al.* 1979; NEI and LI 1979; NEI and TAJIMA 1983).

There exists one previous study of organelle DNA variation in Hordeum (CLEGG, BROWN and WHITFELD 1984). Chloroplast DNAs from nine accessions of *H. vulgare* and 11 accessions of *H. spontanem* were compared using ten restriction endonucleases. In general, it was found that chloroplast DNA was highly conserved in both species, although *H. spontaneum* was shown to be more variable than *H. vulgare.* It was suggested that cytoplasmic diversity may have been restricted during the domestication of cultivated barley. This conclusion is still subject to scrutiny on two points: (1) mitochondrial DNA was not examined, and (2) the sample was limited in geographic range.

The present study constitutes an analysis of both chloroplast and mitochondrial DNA variability as revealed by 17 and 16 restriction endonucleases, respectively. A survey of both wild and cultivated land races of barley that extends across the primary center of diversity of both the cultivated and wild forms is presented. Estimates of nucleotide diversity using the shared fragment method of NEI and LI (1979) and grouping of mitochondrial DNA types revealed a number of features about the evolution of organelle genomes in these species that could not be seen by chloroplast DNA analysis alone. Although both organelle genomes are conserved relative to the nuclear genome (based on estimates from published isozyme data) the mitochondrial genome is about twice as variable as the chloroplast genome. *H. vulgare* and *H. spontaneum* exhibit the same amount of variation for both chloroplast and mitochondrial DNA, and furthermore, there were no features of the fragments that could be used to distinguish the species.

MATERIALS AND METHODS

Origin and treatment of plant accessions: Plant accessions used in this study were obtained from 24 sites that extend across the Fertile Cresent, Greece (Crete) and Cyprus (Table 1). A single random plant was chosen from each site, with the exception of three sites from which two plants were used (see Table 1). The geographic distribution of the sites is shown in Figure 1. All accessions of *H. vulgare*, excluding entry 28 which is a modern cultivar, are endemic (land races) to the collection areas, as are all accessions of *H. spontaneum*. The Canadian *H. vulgare* cultivar Bonanza was used as a standard for comparison between different gels. No data from this cultivar were used in the

TABLE 1

Origins of *H. vulgare* and *H. spontaneum* accessions

Entry no.	Country	Species	Accession no.[a]	Location
1	Greece	H.v.[b]	83-C5A-1	3 km N of Maronas (Rethimnon district)
2	Greece	H.v.	83-C21-71	Near Potamies (12 km NE of Iraklion)
3	Greece	H.sp.[c]	83-C6-11	Maronas (Rethimnon district)
4	Greece	H.sp.	83-C29-2	Zenia (14 km W of Ayios Nikolaos)
5	Cyprus	H.v.	82-4	Governous Beach (55 km SW of Nicosia)
6	Cyprus	H.v.	82-2	Governous Beach (55 km SW of Nicosia)
7	Cyprus	H.sp.	82-45	Akhera (45 km W of Nicosia)
8	Cyprus	H.sp.	82-12	Governous Beach (55 km SW of Nicosia)
9	Turkey	H.v.	CHC 631-1	40 km SE of Diyarbakir, Diyarbakir province
10	Turkey	H.v.	CHC 616-1	53 km SE of Diyarbakir, Diyarbakir province
11	Turkey	H.v.	CHC 619-1	4 km SE of Sultankoy, Mardin province
12	Turkey	H.sp.	CHC 18	3 km W of Kemalpasa, Izmir province
13	Turkey	H.sp.	CHC 64	Asklepiyon near Bergama, Izmir province
14	Turkey	H.sp.	CHC 16	3 km W of Kemalpasa, Izmir province
15	Iraq	H.v.	CN00005295	Hafriya, (30 km N of Bagdad)
16	Iran	H.v.	CHC 1010	Germi
17	Iran	H.v.	CHC 819	28 km E of Borujerd
18	Iran	H.v.	CHC 1104	11 km SE of Quchan
19	Syria	H.v.	82-12	40 km S of Risafe
20	Syria	H.v.	82-24	40 km S of Risafe
21	Syria	H.sp.	78-Ia-29	76 km SE of Aleppo
22	Jordan	H.v.	84-102-17	3 km SE of Yarqua, (15 km NW of Amman)
23	Jordan	H.sp.	84-91-11	Dibben, (35 km NW of Amman)
24	Jordan	H.sp.	84-80-4	Ashrafia, (12 km SW of Irbid)
25	Jordan	H.sp.	84-80-28	Ashrafia, (12 km SW of Irbid)
26	Israel	H.sp.	ISR 137-80A-52	Bet Gurvin, (Udan Foothills)
27	Israel	H.sp.	ISR 50-161-30	Caesarea, (Coastal Plain)
28	Canada	H.v.		cv. Bonanza

[a] Accessions beginning with CHC or CN are from the Canadian Hordeum Collection, Agriculture Canada, Research Station, Ottawa, Ontario, Canada K1A OC6. Others are from the collections of S. JANA.

[b] H.v. = *Hordeum vulgare.*

[c] H.sp. = *Hordeum spontaneum.*

analysis. Entries 1, 17 and 23 are missing from the analysis of chloroplast DNA variation, as are entries 6 and 18 from that of mitochondrial DNA variation, because DNA samples were insufficient. Seeds from individual plants were washed extensively and germinated in the presence of 50 ppm gibberellic acid (GA_3). Seedlings were grown individually in pots containing accrilite ("Turface," ICM) with a small amount of slow-release balanced fertilizer. Plants were watered daily along with treatments of full strength Hoagland's solution every 2–3 days. Under these conditions, the plants grew vigorously with profuse tillering. This allowed single plants to be used in the isolation of sufficient chloroplast and mitochondrial DNAs to perform all restriction analyses.

Chloroplast DNA isolation: Chloroplast DNA was isolated from leaf tissue by a procedure modified from BOOKJANS, STUMMANN and HENNINGSEN (1984) and PALMER (1982). All steps were carried out at 4° unless stated otherwise. Sixty-five grams of leaves were homogenized in 250 ml of isolation buffer (50 mM Tris-HCl, pH 8.0, 25 mM EDTA, 10 mM 2-mercaptoethanol, 1.25 M NaCl, 0.1% BSA) using four high-speed pulses of 3 sec in a 1-liter Waring blender. After passage through four layers of cheese-

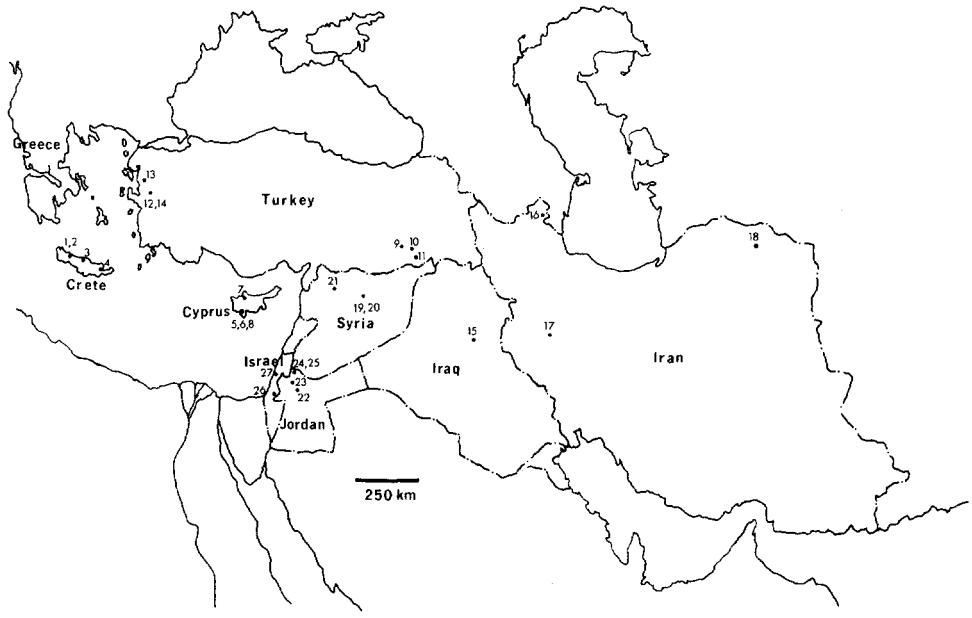

FIGURE 1.—Geographic origins of *H. vulgare* and *H. spontaneum* accessions. Precise locations for each of the sites are given in Table 1.

cloth and four layers of miracloth, the homogenate was centrifuged twice at 1500 × *g* for 6 min in a swinging-bucket rotor. The chloroplast pellets were resuspended in 2 ml of 50 mM Tris-HCl, pH 8.0, 25 mM EDTA using a small sterile brush. The chloroplast suspension was made to 1 mg/ml Pronase (Calbiochem) and incubated at room temperature for 20 min. One-fifth volume of lysis buffer (5% sodium lauryl sarcosinate, 50 mM Tris-HCl, pH 8.0, 25 mM EDTA) was added, and the mixture was incubated at 65° for 10 min. Plastid DNA was purified by equilibrium-centrifugation in CsCl and ethidium bromide by the addition of four volumes of 0.9 g/ml CsCl in 50 mM Tris-HCl, pH 8.0, 25 mM EDTA containing 300 μg/ml ethidium bromide and by centrifugation in a Beckman Ti70.1 rotor at 60,000 rpm and 18° for 20 hr. The resulting single band was removed by side puncture and extracted free of ethidium bromide with isoamyl alcohol before precipitation with ethanol.

Mitochondrial DNA isolation: Mitochondrial DNA was prepared by a modification of the methods described by KEMBLE, MANNS and FLAVELL (1980) and MCNAY, CHOUREY and PRING (1984), whereas the lysis method was modified from PALMER (1982). Unless indicated otherwise, all operations were carried out at 4°. Fifty to 80 grams of leaf material was homogenized in 250 ml of homogenization buffer (0.5% sucrose, 50 mM Tris-HCl, pH 7.5, 5 mM EDTA, 5 mM 2-mercaptoethanol, 0.1% BSA) using a Waring blender. After passage through four layers of cheesecloth and four layers of Miracloth, the filtrate was centrifuged at 1500 × *g* for 10 min to sediment nuclei and chloroplasts. The resulting supernatant was centrifuged at 16,000 × *g* for 10 min in a Beckman JA-14 rotor to sediment mitochondria. The pellet was resuspended in 20 ml of 0.3 M sucrose, 50 mM Tris-HCl, pH 7.5, and was centrifuged at 1500 × *g* for 10 min. The supernatant was then made 10 mM in $MgCl_2$ and 20 μg/ml DNase I (Sigma, crude) added, followed by incubation at 4° for 60 min. This mixture was underlayered with 0.6 M sucrose, 50 mM Tris-HCl, pH 7.5, 20 mM EDTA and was centrifuged at 12,000 × *g* for 20 min. The pellet was washed once in the same underlayering buffer, and mitochondrial DNA was isolated from the final pellet as described above for chloroplasts.

Restriction endonuclease digestion and T4 DNA polymerase-mediated end-labeling: Organellar DNAs (20–50 ng) were digested with individual restriction endonucleases under conditions suggested by the manufacturer, using 20-fold excess of enzyme. The enzymes used were as follows: *Bam*HI, *Bgl*II, *Dra*I, *Eco*RI, *Eco*RV, *Hpa*I (chloroplast DNA only) *Hin*dIII, *Kpn*I, *Nru*I, *Nsi*I, *Pst*I, *Pvu*II, *Sal*I, *Sma*I, *Sst*I, *Xba*I and *Xho*I. Reactions in a total volume of 20 μl were stopped by addition of EDTA followed by extraction with phenol/chloroform and precipitation with ethanol. The restriction fragments were radioactively labeled using deoxyadenosine 5′-(α-[^{35}S]thio)triphosphate by replacement synthesis using T4 DNA-polymerase. The pellet was dissolved in 10 μl of T4 polymerase buffer (MANIATIS, FRITSCH and SAMBROOK 1982) containing 100 μg/ml BSA and 0.1–0.2 units of T4 polymerase. This was incubated for 7–10 min at 37°, followed by the addition of 1 μl of [^{35}S]dATP (500 Ci/mmol, New England Nuclear) and 1 μl of 2 mM dTTP, 2 mM dGTP, 2 mM dCTP. The reaction was incubated at 37° for 15 min, chased by addition of 1 μl of 2 mM dATP, and then further incubated for 60 min. The reaction was stopped by addition of EDTA, extracted with phenol/chloroform followed by precipitation and washing of the labeled DNA with ethanol.

Gel electrophoresis and autoradiography: Radioactively labeled organelle DNA restriction fragments were size fractionated by electrophoresis through 1.0% agarose gels in 1 × TBE buffer (89 mM Tris, 89 mM boric acid, 2.5 mM EDTA, pH 8.3) (MANIATIS, FRITSCH and SAMBROOK 1982). Gels were run for 14–18 hr at 40 V in a water-cooled 20 × 20 cm horizontal apparatus using lambda *Hin*dIII digests as molecular size standards.

After electrophoresis, the gels were stained with ethidium bromide to locate size standards, placed on several layers of 3MM (Whatman) filter paper and covered with plastic wrap. The gel was then held between two metal plates by means of book clamps and was dried overnight in an oven at 55°. This slow drying procedure prevented the distortion of bands that occurs when relatively thick agarose gels are dried directly on a vacuum gel dryer. Gels thus dried were not suitably flat for making autoradiograms and, hence, were removed from the filter paper backing by soaking in water, followed by drying to a new piece of 3MM filter paper using a vacuum gel drier. Vacuum-dried gels were exposed to X-ray film (Kodak XAR-5) from 48 hr to 3 wk.

Cloning of chloroplast *Pst*I 5.2-kb fragment: This fragment was cloned for use as probe to show small insertion/deletions in *Bam*HI and *Hpa*I fragments corresponding to the same regions of the chloroplast genome. *H. vulgare* cv. Bonanza chloroplast DNA digested with *Pst*I was ligated into the *Pst*I site of pUC18 (YANISCH-PERRON, VIEIERA and MESSING 1985). *Escherichia coli* strain HB101 was transformed directly with the products of the ligation reaction, and colonies containing cloned chloroplast DNA fragments were identified by colony hybridization to purified *H. vulgare* chloroplast DNA previously labeled with deoxyadenosine 5′-[α-^{32}P]triphosphate by nick translation to about 10^7 cpm/μg. A clone of the *Pst*I 5.2-kb fragment was tentatively identified by electrophoretic analysis of *Pst*I digests of plasmid preparations from recombinant colonies. The identity of a clone was confirmed by using the same as a ^{32}P-labeled probe (about 4×10^7 cpm/μg) against a chloroplast *Pst*I digest and showing it exclusively hybridized to the chloroplast 5.2-kb *Pst*I fragment. Details of these techniques are described in MANIATIS, FRITSCH and SAMBROOK (1982).

Southern blotting and hybridization to nucleic acid probes: DNA fragments (a total of 1 μg for chloroplast DNAs and 0.5 μg for plasmids) separated by agarose gel electrophoresis were transferred to nylon hybridization membranes (Magna, MSI) by the denaturation and renaturation method of WAHL, STERN and STARK (1979) and were blotted according to SOUTHERN (1975). Hybridization and washing conditions were similar to those of SCHWARZ-SOMMER *et al.* (1984), except that 2 μg/ml of herring sperm DNA was used as heterologous DNA in prehybridization and hybridization solutions.

Analysis of restriction fragment data: Since the majority of restriction fragments for both chloroplast and mitochondrial DNA were similar between the accessions, it

was more convenient to tabulate only variant fragments. The variant fragment data were used to construct a matrix consisting of the number of unshared fragments for all possible pairs of accessions. This matrix was used to group the data according to a chosen number of unshared fragments. The groups were constructed at various levels of stringency: stringency was defined as the allowed number of unshared fragments between any two members of a group. The highest stringency allowed no unshared fragments, with decreasing stringency allowing 1, 2 . . . , etc., unshared fragments. Several different overlapping groups resulted, and they were combined. Combining overlapping groups created a situation in which there were pairs of accessions within a group that had a greater number of unshared fragments than were initially allowed; however, there existed at least one other accession that satisfied the allowed maximum of unshared fragments within that group. The matrix of unshared fragments was further manipulated to generate estimates of intra- and interpopulation nucleotide diversity (π) using the formulas of NEI and LI (1979).

RESULTS

Chloroplast DNA variation: We examined variability by the digestion of chloroplast DNA using 17 different type-II restriction endonucleases. Visual examination of the chloroplast DNA digests indicated that there was little variation between accessions that originated from sites in Crete (Greece), Cyprus and the Fertile Crescent. The restriction endonucleases produced 380 fragments in total, and of those, 13 were variant. Variability was scored as either small motility changes or the presence and absence of particular restriction fragments. It is noteworthy that the method employed here for end-labeling incorporates radiolabeled nucleotides independent of fragment size, allowing detection of smaller fragments with greater sensitivity than ethidium bromide staining. Furthermore, all DNAs examined with the different restriction enzymes originated from the same individual plants. This ruled out any possibility of heterogeneous DNA samples due to mixtures of plants. There were no detectable differences in chloroplast DNA digested with *Bgl*II, *Eco*RV, *Kpn*I, *Nru*I, *Pvu*II, *Sal*I, *Sst*I, *Xba*I and *Xho*I. A typical set of chloroplast DNA samples digested with *Nru*I is shown in Figure 2.

Closer examination of the 13 variant fragments revealed that these represented less than 13 independent events. The *Bam*HI 3.5-kb, *Hpa*I 6.2-kb and *Pst*I 5.2-kb fragments showed similar mobility changes suggesting that they represented the same portion of the chloroplast genome and had thus undergone the same insertions and deletions (Figure 3A; Table 2). Further evidence of this was provided by the cross-hybridization of the *Pst*I 5.2-kb clone to the *Bam*HI 3.5-kb and *Hpa*I 6.2-kb fragments, as shown in Figure 3B. A *Bam*HI 1.7-kb fragment was also discriminated by the probe, but did not show any mobility differences. It most likely represents a *Bam*HI fragment directly flanking the 3.5-kb *Bam*HI fragment and overlapped by the larger *Pst*I probe. Although this is not proof that these fragments have undergone the same insertions and deletions, it seems highly unlikely that they would have simultaneously gained or lost three independent restriction sites about 100 base pairs (bp) from either of their termini. With the exception of a Syrian wild barley sample (entry 21), the gain of an insertion in the respective *Bam*HI, *Hpa*I and *Pst*I fragments was correlated with the simultaneous appearance of

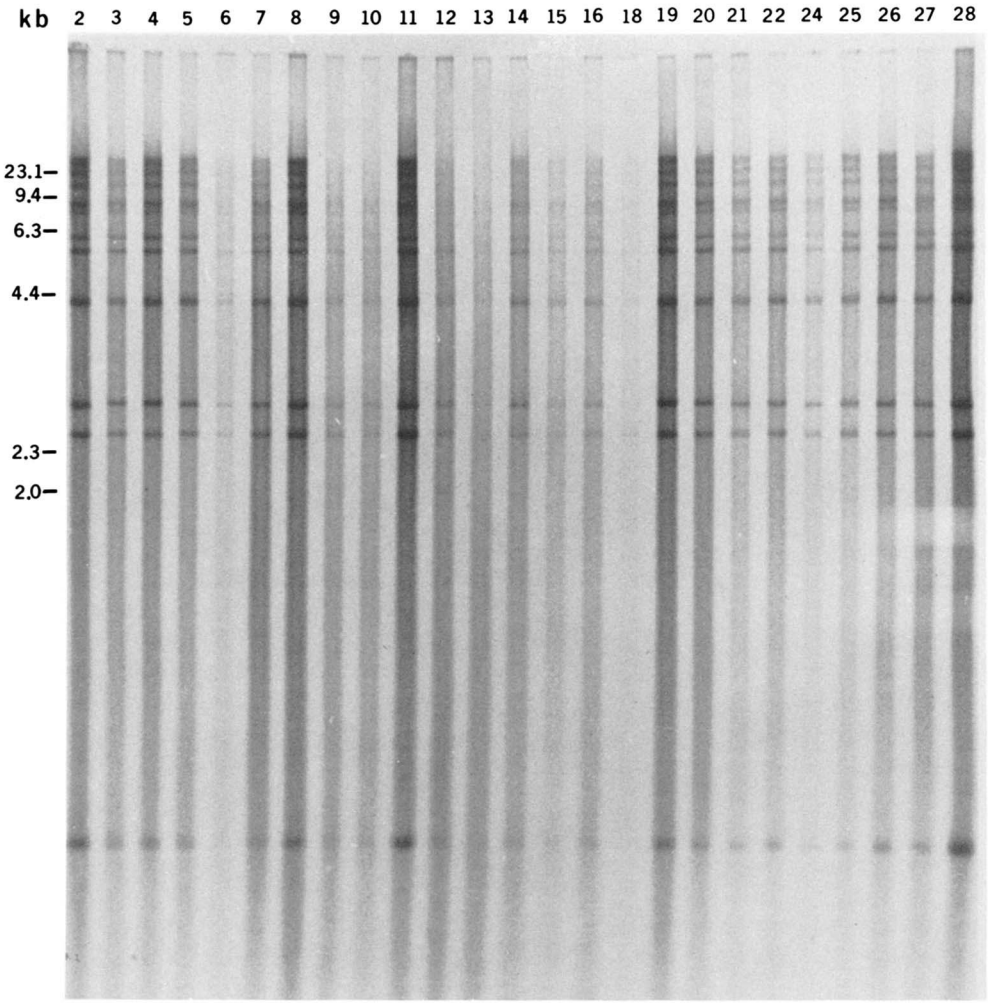

FIGURE 2.—Chloroplast DNA from *H. vulgare* and *H. spontaneum* digested with *Nru*I. The numbers at the top of each lane indicate the origin of each sample as detailed in Table 1.

an *Nsi*I 2.4-kb fragment. It is possible that the insertion contained a unique *Nsi*I site. The Syrian wild barley (entry 21) showed evidence of a larger insertion and, in contrast to the other insertions, absence of the *Nsi*I 2.4-kb fragment, suggesting that this insertion was not only different in size but also did not possess or create an extra *Nsi*I site. A definitive explanation of these possible events will require sequence information from this region. The *Hpa*I 6.2-kb and *Pst*I 5.2-kb fragments correspond to the left side of the small single-copy region on barley chloroplast maps of DAY and ELLIS (1985) and POULSEN (1983). Such small insertions and deletions would not be detected in the larger fragments produced by *Pvu*II (17.2 kb), *Sal*I (13.1 kb), *Sst*I (11.4) and *Xho*I (14.0 kb) that define the same region of the map of DAY and ELLIS (1985).

There was a different series of correlated insertions and deletions apparent

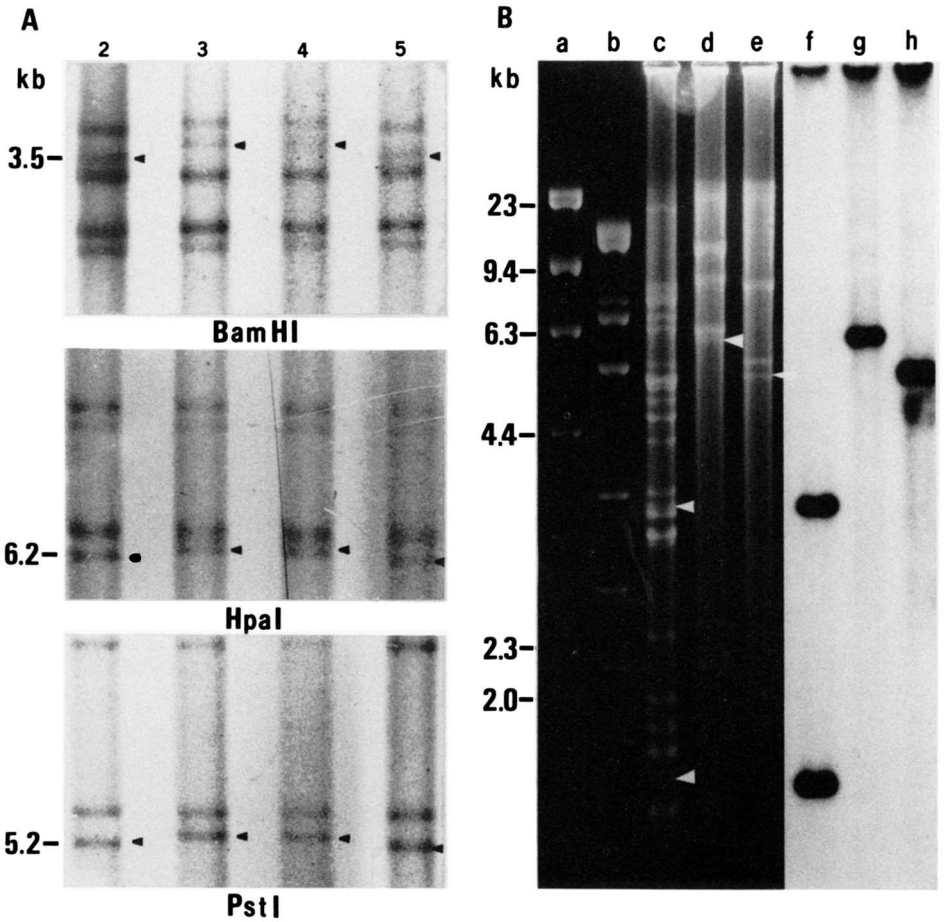

FIGURE 3.—Small insertion and deletions in *H. vulgare* and *H. spontaneum* chloroplast DNA. A, Enlargements, from top to bottom, of the *Bam*HI 3.5-kb, the *Hpa*I 6.2-kb and the *Pst*I 5.2-kb regions of the entries indicated at the top of each lane. Arrowheads to the right of each lane indicate the location of the bands undergoing insertions and deletions. B, The results of hybridization of the *Pst*I 5.2-kb probe to cpDNA digested with *Bam*HI (f), *Hpa*I (g) and *Pst*I (h) are shown. The agarose gel stained with ethidium bromide is to the left and includes lambda DNA digested with *Hin*dIII (a) and *Acc*I (b), in addition to cpDNA digested with *Bam*HI (c), *Hpa*I (d) and *Pst*I (e). Arrowheads indicate the bands that hybridized to the probe.

in *Dra*I 2.4-kb and *Nsi*I 5.1-kb fragments; both fragments gained or lost an insertion of about 100 bp in size in a concerted manner. The homology of these fragments was not tested, but it seems likely that they correspond to the same region of the chloroplast genome in a manner similar to that found for insertions and deletions in the *Bam*HI, *Hpa*I and *Pst*I fragments previously discussed. Furthermore, the absence of this insertion was connected with the presence of a *Dra*I 1.5-kb band, and vice versa (Table 2). It is possible that a *Dra*I site was abolished by the insertion event.

Other types of changes, not ascribable to small insertions and deletions, were apparent in the chloroplast digests. In *Hin*dIII digests there was the presence

TABLE 2

Variant fragments in chloroplast DNA restriction digests from *H. vulgare* and *H. spontaneum*

Country	Entry no.	*Bam*HI	*Dra*I		*Eco*RI	*Hind*III		*Hpa*I	*Nsi*I		*Pst*I	*Sma*I		
		3.5[a]	2.4[a]	1.5	2.0	7.2	5.5	6.2[a]	5.1[a]	2.4	5.2[a]	5.25	3.65	1.5
Hordeum vulgare														
Greece	2	−	−	+	+	+	−	−	−	−	−	+	−	−
Cyprus	5	−	−	+	+	+	−	−	−	−	−	+	−	−
Cyprus	6	−	−	+	+	+	−	−	−	−	−	+	−	−
Turkey	9	−	−	+	+	+	−	−	nd	nd	−	+	−	−
Turkey	10	+	−	+	+	+	−	+	−	+	+	+	−	−
Turkey	11	+	−	+	−	−	+	+	−	+	+	+	−	−
Iraq	15	+	−	+	+	+	−	+	−	+	+	+	−	−
Iran	16	−	−	+	+	+	−	−	−	−	−	+	−	−
Iran	18	−	−	+	+	+	−	−	−	−	−	+	−	−
Syria	19	+	+	−	+	+	−	+	+	+	+	−	+	+
Syria	20	+	+	−	+	+	−	+	+	+	+	−	+	+
Jordan	22	−	−	+	+	+	−	−	−	−	−	+	−	−
Hordeum spontaneum														
Greece	3	+	+	−	+	+	−	+	+	+	+	+	−	−
Greece	4	+	+	−	+	+	−	+	+	+	+	+	−	−
Cyprus	7	+	−	+	+	−	+	+	−	+	+	+	−	−
Cyprus	8	+	−	+	−	−	+	+	−	+	+	+	−	−
Turkey	12	+	−	+	+	+	−	+	−	+	+	+	−	−
Turkey	13	−	nd	nd	+	+	−	−	−	−	−	+	−	−
Turkey	14	−	−	+	+	+	−	−	−	−	−	+	−	−
Syria	21	++	−	+	−	−	+	++	−	−	++	+	−	−
Jordan	24	+	−	+	+	+	−	+	−	+	+	+	−	−
Jordan	25	+	−	+	+	+	−	+	−	+	+	+	−	−
Israel	26	+	−	+	−	−	+	+	−	+	+	+	−	−
Israel	27	+	−	+	−	−	+	+	−	+	+	+	−	−

The molecular sizes of the variant fragments are given at the top of each column in kilobase pairs. Signs indicate the presence (+) or absence (−) of a particular variant fragment.

[a] These columns indicate small (+) or larger (++) insertions or deletions (−). nd = no data.

of a 5.5-kb fragment coincident with the absence of a 7.2-kb fragment, and vice versa (Table 2). These events were not due to the gain of a single *Hin*dIII site within the 7.2-kb fragment, because the presence of the 5.5-kb fragment was not accompanied by a 1.7-kb fragment (*i.e.*, 5.5 + 1.7 = 7.2 kb). It is difficult to craft a simple explanation for these changes without detailed restriction maps or sequence data. The only strong evidence of a single restriction site change was found in *Sma*I digests. In *Sma*I digests the absence of a 5.25-kb band was consistently accompanied by the presence of extra bands at 3.65 and 1.5 kb (Table 2). The gain or loss of single restriction sites in large fragments (greater than 20 kb) could account for the unassociated presence and absence of an *Eco*RI 2.0-kb fragment in some digests. In summary, only five apparently independent changes were detected by cleavage of *H. vulgare* and *H. spontaneum* cpDNA with 17 different restriction endonucleases.

Mitochondrial DNA variation: It was evident from visual examination of the mtDNA restriction digests that there exists a low level of variation between

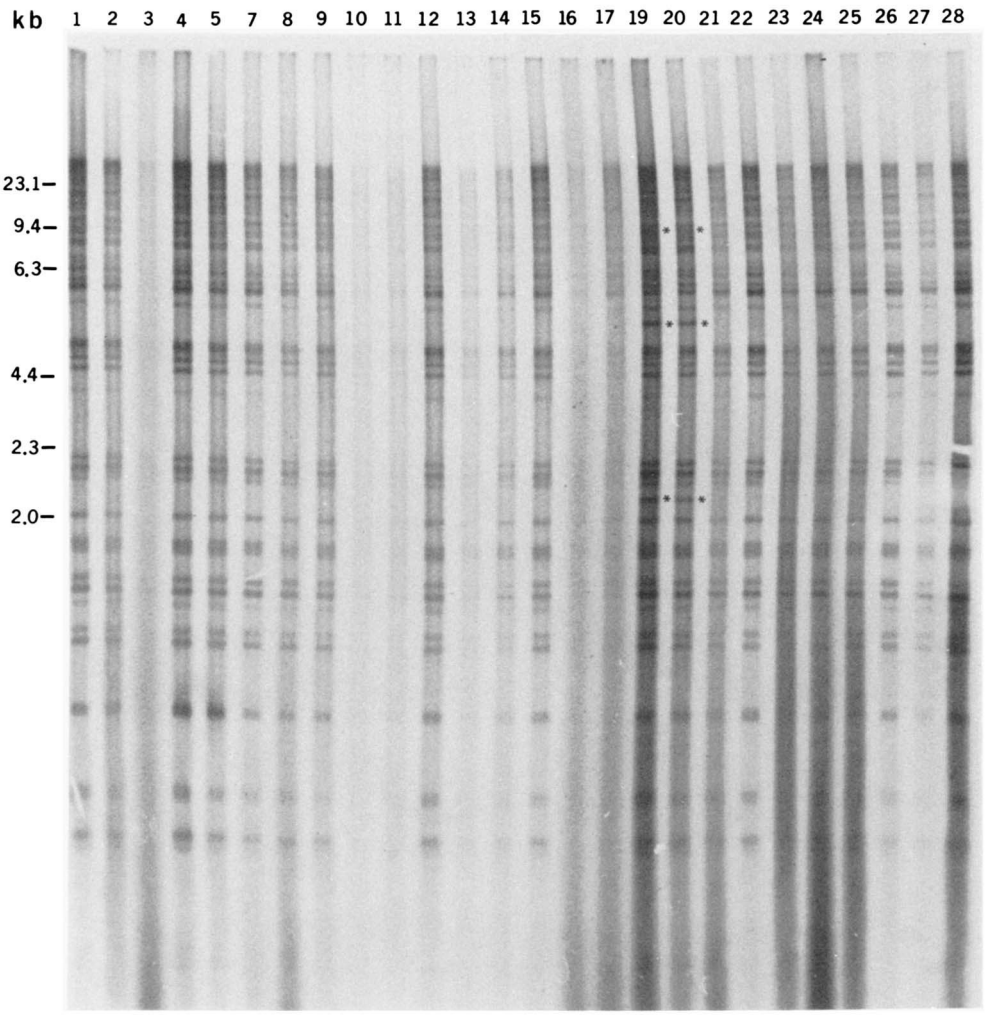

FIGURE 4.—Mitochondrial DNA from *H. vulgare* and *H. spontaneum* digested with *Pst*I. Numbers at the top of each lane indicate the accession as detailed in Table 1. Asterisks to the right of the lanes indicate the position of variant fragments as compiled in Table 3.

the accessions in a fashion similar to that found for cpDNA. However, the mtDNA digests were more complex than the cpDNA digests. Summation of the bands, making allowances for intensity differences, gave a size estimate of approximately 420 kb for the mitochondrial genome. This is about three times the size of the chloroplast genome, which is 132 kb (DAY and ELLIS 1985; POULSEN 1983). A typical set of mitochondrial DNA samples digested with *Pst*I is shown in Figure 4. The 16 restriction enzymes produced approximately 860 fragments, and out of these, 50 were variant (Table 3). It is notable that two accessions of barley from Syria (entries 19 and 20) were singularly responsible for 20 of the variant fragments. Since these Syrian accessions were collected from the same site, it was not unexpected that they should display similarities.

The occurrence of 50 cases of variant fragments did not correspond to the same number of independent changes in the mitochondrial genome. As mentioned above, the correlated presence/absence of 20 variant fragments (the presence of *Eco*RI 4.8 kb; *Eco*RV 7.3 kb; *Hin*dIII 5.2 kb; *Kpn*I 5.3 kb; *Nsi*I 2.15 kb, 3.0 kb; *Pst*I 9.8 kb, 4.5 kb, 2.45 kb; *Sst*I 6.3 kb; *Xba*I 7.0 kb; *Xho*I 8.3 kb, 5.3 kb, 5.0 kb, 4.6 kb associated with the absence of *Dra*I 1.6 kb; *Eco*RI 9.3 kb, 3.9 kb; *Nsi*I 3.1 kb; *Xho*I 2.8 kb, and vice versa) was associated with two accessions of Syrian barley (Table 3). A different sort of change caused either a complete presence or a complete absence of 12 restriction fragments: all of *Bam*HI 5.0 kb, 2.28 kb, 1.4 kb, 1.1 kb; *Bgl*II 15 kb, 5.35 kb, 5.05 kb; *Sma*I 4.4 kb, 2.0 kb, 0.7 kb; *Xho*I 2.75 kb, 2.4 kb were present or absent. In a similar fashion, three other sets of fragments were either entirely present or absent. These sets were the *Nru*I 7.0-kb, 5.3-kb, 4.15-kb, 4.0-kb and *Bam*HI 20-kb fragments, the *Sal*I 6.8-kb and 4.55-kb fragments and the *Xba*I 11.5-kb and 3.4-kb fragments. The concerted presence and absence of more than one fragment could be caused by large insertions and deletions. Other variant fragments not mentioned thus far were not recognizably associated together or with any other fragments. There were no clear indications that any variant mtDNA fragments were caused by single restriction site changes. It is conceivable that some of the smaller fragments (under 3 kb in size) could have been generated by restriction site gains in larger fragments. A loss of under 3 kb from a fragment in excess of 20 kb in size would probably have gone undetected. In summary, the 50 variant fragments detected in the mtDNA digests were produced by 14 apparently independent changes in the mitochondrial genome.

Assignment of groups and nucleotide diversity: The data in Tables 2 and 3 were used separately to produce matrices consisting of the number of unshared fragments for all pairwise comparisons. These matrices were used directly to group the accessions according to the number of unshared fragments. This approach failed to yield any recognizable groups for the cpDNA data, except for the Syrian barley samples (entries 19 and 20). However, sorting of mtDNA fragment data yielded four fairly well-defined groups, as summarized in Table 4. These groups were resolved by the condition that, for any member of a group, there existed at least one other member with no more than five unshared fragments. Although this approach is empirical, the estimates of nucleotide diversity within and between groups provide some justification of the groups (see Table 5). It is evident that there exists some affinity between *H. vulgare* and *H. spontaneum* accessions collected from similar geographic areas (Table 4). For example, group 2 contained all accessions for both species from Crete, as did group 3 for all accessions from Turkey. In contrast to this, different species were assigned to different groups for some accessions from Syria, Cyprus and Jordan. Unexpected affinities were seen in group 2, which contained accessions from Greece, Cyprus, Jordan, Iran and Iraq. It was not readily apparent what underlying reasons were responsible for these affinities. However, comparison of nucleotide diversity within groups and between groups indicated that the within group values were about ten times lower.

TABLE 3

Variant fragments in mitochondrial DNA restriction digests from *H. vulgare* and *H. spontaneum*

		*Bam*HI					*Bgl*II			*Dra*I			*Eco*RI			*Eco*RV		*Hin*dIII	*Kpn*I			*Nru*I			
		20	5.0	2.28	1.4	1.1	15	5.35	5.05	2.85	2.4	1.6	9.3	4.8	3.9	7.3	4.4	5.2	7.5	5.3	1.95	7.0	5.3	4.15	4.0
Hordeum vulgare																									
Greece	1	+	+	+	+	+	+	+	+	−	+	+	+	−	+	−	+	−	−	−	+	+	+	+	+
Greece	2	+	+	+	+	+	+	+	+	−	+	+	+	−	+	−	+	−	−	−	+	+	+	+	+
Cyprus	5	+	+	+	+	+	+	+	+	−	+	+	+	−	+	−	+	−	+	−	+	+	+	+	+
Turkey	9	−	−	−	−	−	nd	nd	nd	−	+	+	+	−	+	−	−	−	−	−	−	−	−	−	−
Turkey	10	−	−	−	−	−	nd	nd	nd	−	nd	nd	+	−	+	nd	nd	−	−	−	−	−	−	−	−
Turkey	11	−	−	−	−	−	nd	nd	nd	−	nd	nd	+	−	+	nd	nd	−	−	−	−	−	−	−	−
Iraq	15	+	+	+	+	+	+	+	+	−	−	+	+	−	+	−	+	−	+	−	+	+	+	+	+
Iran	16	+	+	+	+	+	+	+	+	−	−	+	+	−	+	−	−	−	+	−	+	+	+	+	+
Iran	17	+	+	+	+	+	+	+	+	−	+	+	+	−	+	−	−	−	+	−	+	+	+	+	+
Syria	19	+	+	+	+	+	+	+	+	−	+	−	−	+	−	+	−	+	−	+	+	+	+	+	+
Syria	20	+	+	+	+	+	+	+	+	−	+	−	−	+	−	+	−	+	+	+	+	+	+	+	+
Jordan	22	+	+	+	+	+	+	+	+	−	+	+	+	−	+	−	−	−	+	−	+	+	+	+	+
Hordeum spontaneum																									
Greece	3	+	+	+	+	+	nd	nd	nd	−	+	+	+	−	+	nd	nd	−	−	−	−	nd	nd	nd	nd
Greece	4	+	+	+	+	+	+	+	+	−	+	+	+	−	+	−	+	−	+	−	+	+	+	+	+
Cyprus	7	−	−	−	−	−	−	−	−	+	−	+	+	−	+	−	−	−	−	−	−	−	−	−	−
Cyprus	8	−	−	−	−	−	−	−	−	+	−	+	+	−	+	−	−	−	−	−	−	−	−	−	−
Turkey	12	+	−	−	−	−	nd	nd	nd	−	−	+	+	−	+	−	−	−	−	−	−	−	−	−	−
Turkey	13	−	−	−	−	−	nd	nd	nd	−	−	+	+	−	+	nd	nd	−	−	−	−	−	−	−	−
Turkey	14	−	−	−	−	−	nd	nd	nd	−	+	+	+	−	+	nd	nd	−	−	−	−	−	−	−	−
Syria	21	+	−	−	−	−	−	−	−	+	+	+	+	−	+	−	−	−	−	−	+	+	+	+	+
Jordan	23	+	+	+	+	+	+	+	+	−	+	+	+	−	+	−	+	−	+	−	+	+	+	+	+
Jordan	24	+	−	−	−	−	−	−	−	−	+	+	+	−	+	−	−	−	−	−	−	nd	nd	nd	nd
Jordan	25	+	−	−	−	−	−	−	−	−	+	+	+	−	+	−	−	−	−	−	−	+	+	+	+
Israel	26	+	−	−	−	−	−	−	−	+	−	+	+	−	+	−	−	−	−	−	+	+	+	+	+
Israel	27	+	−	−	−	−	−	−	−	+	−	+	+	−	+	−	−	−	−	−	+	+	+	+	+

		*Nsi*I			*Pst*I			*Pvu*II	*Sal*I					*Sma*I			*Sst*I	*Xba*I			*Xho*I						
		3.1	3.0	2.15	9.8	4.5	2.45	6.0	6.8	4.55	1.75	1.4	1.25	4.4	2.0	0.7	6.3	11.5	7.0	3.4	8.3	5.3	5.0	4.6	2.8	2.75	2.4
Hordeum vulgare																											
Greece	1	+	−	−	−	−	−	+	+	+	−	−	+	+	+	+	−	+	−	+	−	−	−	−	+	+	+
Greece	2	+	−	−	−	−	−	+	+	+	−	−	+	+	+	+	−	+	−	+	−	−	−	−	+	+	+
Cyprus	5	+	−	−	−	−	−	+	+	+	−	−	+	+	+	+	−	+	−	+	−	−	−	−	+	+	+
Turkey	9	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−
Turkey	10	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−
Turkey	11	+	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−
Iraq	15	+	−	−	−	−	−	+	+	+	−	−	+	+	+	+	−	+	−	+	−	−	−	−	+	+	+
Iran	16	+	−	−	−	−	−	+	+	+	−	−	+	+	+	+	−	+	−	+	−	−	−	−	+	+	+
Iran	17	+	−	−	−	−	−	+	+	+	−	−	+	+	+	+	−	+	−	+	−	−	−	−	+	+	+
Syria	19	−	+	+	+	+	+	−	+	+	−	−	+	+	+	+	+	−	+	−	+	+	+	+	−	+	+
Syria	20	−	+	+	+	+	+	−	+	+	−	−	+	+	+	+	+	−	+	−	+	+	+	+	−	+	+
Jordan	22	+	−	−	−	−	−	+	+	+	−	−	+	+	+	+	−	−	−	−	−	−	−	−	+	+	+
Hordeum spontaneum																											
Greece	3	+	−	−	−	−	−	−	+	+	−	−	+	nd	nd	nd	−	nd	nd	nd	−	−	−	−	+	−	−
Greece	4	+	−	−	−	−	−	+	+	+	−	−	+	+	+	+	−	+	−	+	−	−	−	−	+	+	+
Cyprus	7	+	−	−	−	−	−	−	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	+	−	−
Cyprus	8	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−
Turkey	12	+	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−
Turkey	13	+	−	−	−	−	−	nd	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−
Turkey	14	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−
Syria	21	+	−	−	−	−	−	−	+	+	−	−	+	−	−	−	−	+	−	+	−	−	−	−	+	−	−
Jordan	23	+	−	−	−	−	−	+	+	+	−	−	+	−	−	−	−	−	−	−	−	−	−	−	+	+	+
Jordan	24	+	−	−	−	−	−	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−
Jordan	25	+	−	−	−	−	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−
Israel	26	+	−	−	−	−	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−
Israel	27	+	−	−	−	−	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−

Accessions followed by their entry number (see Table 1) are given in the first column. The molecular sizes in kilobase pairs of variant fragments are indicated at the top of each column. All signs indicate the presence (+) or absence (−) of particular fragments.

nd = no data.

TABLE 4

***H. vulgare* and *H. spontaneum* groups based on mitochondrial DNA affinities**

Group 1	Group 2	Group 3	Group 4
Jordan H.sp 24[a]	Jordan H.sp 23	Turkey H.v 9	Syria H.v 19
Jordan H.sp 25	Jordan H.v 22	Turkey H.v 10	Syria H.v 20
Israel H.sp 26	Greece H.v 1	Turkey H.v 11	
Israel H.sp 27	Greece H.v 2	Turkey H.sp 12	
Syria H.sp 21	Greece H.sp 3	Turkey H.sp 13	
	Greece H.sp 4	Turkey H.sp 14	
	Iran H.v 16	Cyprus H.sp 7	
	Iran H.v 17	Cyprus H.sp 8	
	Iraq H.v 15		
	Cyprus H.v 5		

[a] Entries are listed as follows: country, species (H.sp = *H. spontaneum*, H.v = *H. vulgare*) and entry number (see Table 1).

Estimates of nucleotide diversity (π) were derived by a series of operations on the matrices of unshared fragments using the formulas of NEI and LI (1979). Confidence intervals for these estimates are not given, as the formulas for such apply strictly to shared restriction site data. However, the values presented here (Table 5) represent reasonable estimates since a large number of fragments were scored. The formulas of NEI and LI (1979) assume that all changes in fragment patterns are due to nucleotide substitutions. Although some of the fragment changes in both cpDNA and mtDNA were clearly not caused by nucleotide substitutions, all data were treated as such. Notwithstanding, the calculation of nucleotide diversity provided some measure of variability that can be used for comparison to other data.

These calculations show that mtDNA is somewhat more variable than cpDNA. However, it should be pointed out that much of the increased magnitude of the combined species and *H. vulgare* nucleotide diversity for mtDNA was contributed by the rather distinct Syrian barley accessions (entries 19 and 20). This is evident upon interspecific comparison of the respective genomes. Within *H. spontaneum*, nucleotide diversities were 5.57×10^{-4} and 6.04×10^{-4} for cpDNA and mtDNA, respectively, whereas the values within *H. vulgare* were 5.88×10^{-4} and 9.79×10^{-4} for cpDNA and mtDNA, respectively. It is clear that the nucleotide diversity for *H. vulgare* mtDNA was inflated by inclusion of the Syrian barley entries. Interspecific comparisons showed that, for cpDNA, both species have about the same level of variation, whereas mtDNA nucleotide diversity estimates suggest that *H. vulgare* is the more variable species. This result cannot be considered conclusive since accessions of both species were not available from every site.

An estimate for nucleotide diversity is given for nuclear DNA in Table 5. This value was derived from studies of isozyme variation on *H. vulgare* from Iran (BROWN and MUNDAY 1982) and *H. spontaneum* from Israel (BROWN *et al.* 1978; NEVO *et al.* 1979). An average gene diversity (NEI 1973) was calculated by taking the mean over both species of overall gene diversity from the isozyme studies. The nuclear DNA nucleotide diversity was derived using the

TABLE 5

Nucleotide diversity estimates for *H. vulgare* and *H. spontaneum* chloroplast and mitochondrial genomes

Chloroplast DNA	
H. vulgare and *H. spontaneum* combined	6.38×10^{-4}
H. vulgare	5.88×10^{-4}
H. spontaneum	5.57×10^{-4}
Mitochondrial DNA	
H. vulgare and *H. spontaneum* combined	9.68×10^{-4}
H. vulgare	9.79×10^{-4}
H. spontaneum	6.04×10^{-4}
Within groups	
Group 1	1.67×10^{-4}
Group 2	1.85×10^{-4}
Group 3	1.38×10^{-4}
Group 4	3.23×10^{-5}
Between groups	
Group 1 and group 2	1.97×10^{-3}
Group 1 and group 3	1.01×10^{-3}
Group 1 and group 4	4.57×10^{-3}
Group 2 and group 3	2.66×10^{-3}
Group 2 and group 4	3.04×10^{-3}
Group 3 and group 4	5.16×10^{-3}
Nuclear DNA[a]	
H. vulgare and *H. spontaneum* combined	1.96×10^{-3}

[a] Estimated from isozyme data of BROWN *et al.* (1978), NEVO *et al.* (1979) and BROWN and MUNDAY (1982).

assumptions that the average nuclear gene is about 1 kb in length and isozyme variation detects about one of ten nucleotide substitutions. Comparison of the nuclear nucleotide diversity to the combined estimates for organelle genomes indicates that the chloroplast genome is about one-quarter as diverse, whereas the mitochondrial genome is about one-quarter to one-half as diverse as the nuclear genome.

DISCUSSION

The aim of this study was to assess the type and magnitude of organelle DNA variation in *H. spontaneum* and *H. vulgare* from a region that is known to harbor great genetic diversity in both species. The Fertile Cresent in Southwestern Asia is believed to be the primary center of diversity for both cultivated and wild barley (HARLAN and ZOHARY 1966). A total of 24 widely different sites from this region, including parts of Crete (Greece) and Cyprus, were included. Seventeen different hexanucleotide recognizing restriction endonucleases were used to analyze chloroplast DNA (cpDNA), whereas 16 enzymes were used to examine mitochondrial DNA (mtDNA) variability. The 27 plant accessions originate from a wide range of habitats and as such would be

expected to have acquired an adaptive genotype through mutation and selection. The genotype of an individual is considered as the sum total of genetic information, including organelle genomes. The present study reveals evidence that organelle genomes are conserved relative to the nucleus.

The most prevalent feature of the cpDNA digests was the lack of variation between samples from widely different geographic localities. Cleavage of cpDNA with 17 enzymes produced a total of 380 fragments corresponding to (17 × 380 =) 2280 bp. Based on a size of 132 kb for the barley chloroplast genome (POULSEN 1983; DAY and ELLIS 1985) this represents a pseudorandom sampling of 1.7% of the chloroplast genome. In spite of the effort to use several enzymes, only 13 variant cpDNA fragments were revealed which apparently corresponded to only five independent changes. The low level of cpDNA diversity found here is not novel, because the conservative nature of chloroplast genomes has been reported at both the intraspecific and interspecific levels in other angiosperms (BAATOUT *et al.*, 1985; BANKS and BIRKY 1985; PALMER, JORGENSEN and THOMPSON 1985; CLEGG, BROWN and WHITFELD 1984; CLEGG, RAWSON and THOMAS 1984; DEBONTE, MATTHEWS and WILSON 1984; BOWMAN, BONNARD and DYER 1983; PALMER and ZAMIR 1982; TIMOTHY *et al.* 1979).

These results generally support the previous findings of CLEGG, BROWN and WHITFELD (1984) that chloroplast genomes of *H. vulgare* and *H. spontaneum* are highly conserved. However, their conclusion that variability in *H. spontaneum* is greater than *H. vulgare* is in contrast to the present finding that both species are equally diverse. This discrepancy may be due to the more limited sample of *H. vulgare* used in the previous study. CLEGG, BROWN and WHITFELD (1984) used four modern cultivars of diverse origin and five primative land races of *H. vulgare* from Iran, whereas the present study examined more diverse accessions and used a greater number of restriction enzymes. The highly conservative nature of barley chloroplast DNA was also previously shown by the lack of any nucleotide substitutions in limited sequence comparisons, both coding and noncoding, between *H. vulgare* and *H. spontaneum* (ZURAWSKI, CLEGG and BROWN 1984; ZURAWSKI and CLEGG 1984).

There were two different types of variation seen for cpDNA. One was nucleotide substitution as evidenced by restriction site changes, and the other was small insertions and deletions. A combination of these types of changes has been documented for other plant species (BANKS and BIRKY 1985; PALMER, JORGENSEN and THOMPSON 1985; BOWMAN, BONNARD and DYER 1983; PALMER and ZAMIR 1982) and, as such, is probably common to the evolution of angiosperm chloroplast genomes.

In a similar fashion to cpDNA, the mitochondrial genomes of *H. vulgare* and *H. spontaneum* also showed limited variation. Digestion of mtDNA produced an average of 860 fragments corresponding to 5160 bp or about 1.2% of the mitochondrial genome. The number of variant fragments represented a smaller number of apparent independent changes. In this case the 50 variant fragments probably represented 14 independent changes in mtDNA, although some of these are rather complex. Conservation of angiosperm mtDNA has

also been found in Lycopersicon (McCLEAN and HANSON 1986), Hedysarum (BAATOUT *et al.* 1985) and commercial lines of *Zea mays* (LEVINGS and PRING 1977).

The results given here suggest that evolutionary changes in mtDNA are of a different nature than those in cpDNA. The concerted presence or absence of several restriction fragments is consistent with insertions or deletions, respectively. If this is indeed the case, these insertions are, in some instances, on the order of 20 kb in size compared with those in cpDNA of about 100 bp. This premise is not at variance with the known size variations of angiosperm mtDNA (WARD, ANDERSON and BENDICH 1981), except that the present results suggest that substantial size variation may occur at the intraspecific level. These conjectures should be treated with caution in the absence of restriction site data. Existing mtDNA restriction maps of *Brassica campestris* (PALMER and SHIELDS 1984), *Spinacia oleracea* (STERN and PALMER 1986) and *Z. mays* (LONSDALE, HODGE and FAURON 1984) clearly indicate that these genomes undergo intramolecular recombinations to generate subgenomic circular molecules. It is possible that such rearrangements occur in barley mtDNA and, if so, could account for some of the results given here. Intramolecular recombination and amplification of particular subgenomic molecules could also be causal to the extensive changes observed in the Syrian barley samples (entries 19 and 20). Evidence of extensive rearrangements has been found in maize mtDNA, and it has been suggested that such changes are important to the evolution of plant mitochondrial genomes (SEDEROFF *et al.* 1981). Although direct evidence of nucleotide substitutions was not seen here, this does not preclude its contributing role in the evolution of mtDNA. There were several cases of the independent presence or absence of particular restriction fragments, and these may have originated from the gains or losses of restriction sites near the termini of larger fragments resulting in small undetectable mobility changes in the latter.

The grouping of the accessions on the basis of numbers of shared restriction fragments showed that mtDNA types can be geographically widespread (Table 4). The classifications seem justified in that the nucleotide diversity between these groups was about an order of magnitude larger than that within groups (Table 5). There are indications that, in some cases, an affinity exists between land races of *H. vulgare* and *H. spontaneum* collected from similar localities. Land races of *H. vulgare* are primitive cultivars that are generally grown locally. It is possible that they were originally selected for cultivation from locally occurring natural populations of wild *H. spontaneum* as mutants with desirable agronomic features. This could possibly account for some of the cases where affinity existed. However, in cases where no clear affinities exist, it is possible that such land races were artificially introduced from other areas. After all, barley has been cultivated and traded to varying degrees for several millenia. It should also be considered that *H. vulgare* and *H. spontaneum* are interfertile, and this represents another mechanism for the establishment of affinities. The adaptive significance of mtDNA types cannot be investigated without detailed surveys of populations from diverse habitats. More extensive sampling will be

necessary to define with greater precision what relationships exist between land races of *H. vulgare* and wild *H. spontaneum*, and how they were established.

The wide occurrence of mtDNA types is in contrast to the apparent differentiation seen for allozyme variation for nuclear genes over smaller ranges of habitat in *H. vulgare* (BROWN and MUNDAY 1982) and *H. spontaneum* (BROWN *et al.* 1978; NEVO *et al.* 1979). It is reasonable to conclude that organellar genes may not be subject to the same selective pressures that are operative on nuclear genes.

Comparison of nucleotide diversity estimates indicates that mitochondrial genome is somewhat more variable than the chloroplast genome. This may simply be due to the larger size of the former and hence more nonfunctional sequences available to undergo change. However, comparisons to the isozyme-derived estimate of nucleotide diversity indicate that both organelle genomes are conserved relative to the nuclear genome. The assumptions used in deriving a nucleotide diversity from isozyme data can be reasonably justified when compared to one derived from actual sequence data. KREITMAN (1983) compiled sequences of *Drosophila melanogaster alcohol dehydrogenase* genes (*Adh*) from 11 diverse individuals. In that study, only one of 43 nucleotide substitutions resulted in an amino acid change defining the two common electrophoretic variants of *Adh*. Direct calculation of nucleotide diversity from these sequences yields a value of 5.30×10^{-3} (compared with 1.96×10^{-3} from barley isozyme data). This may be an underestimate because it does not include the six small insertions and deletions ranging in size from 1 to 37 bp found throughout the *Adh* sequences surveyed. Since analogous data for barley nuclear genes are not available, the conclusion that organelle genomes are less variable than the nuclear genome remains tentative.

The estimates of nucleotide diversity for cpDNA and mtDNA should be treated with caution since the requirement that fragment polymorphism be due solely to nucleotide substitutions (NEI and LI 1979) is not met. In the case of cpDNA, the small insertions and deletions were simply counted as site changes. This tends to inflate diversity values since differences in the numbers of share fragments are counted more than once. A similar situation is apparent for mtDNA. Despite the uncertainties associated with such estimates, they do serve as a starting point for comparison until definitive sequence data are available. The intraspecific value of nucleotide diversity for *L. esculentum* mtDNA was given as 3.7×10^{-3} by MCCLEAN and HANSON (1986). This value is about 3.8 times that found here (*cf.*, 9.685×10^{-4}, *H. vulgare/spontaneum* combined), but was derived by using different formulas from UPHOLT (1977) as modified by GOTOH *et al.* (1979). In general, the maximum likelihood-derived formulas of GOTOH *et al.* (1979) estimate diversity values about three times the size of those estimated using the probability-derived formulas of NEI and LI (1979). Taking this into account the estimates of nucleotide diversity for *H. vulgare/spontaneum* are quite similar to those for *L. esculentum*.

In summary, chloroplast genomes of *H. vulgare* and *H. spontaneum* are evolving at a slower rate than mitochondrial genomes. The fashion in which cpDNA evolves is by a combination of small insertions and deletions and nucleotide

substitutions, whereas the mtDNA probably evolves by mechanisms involving much larger insertions and deletions in addition to rearrangements. There were no features of the organelle genomes that distinguished either of these species in terms of peculiar restriction fragments. Variability was about equal in cpDNA for both species, and if anything, mtDNA was more variable in *H. vulgare*. Finally, nuclear genes are most likely evolving faster than both organelle genomes. Nuclear genes are afforded greater opportunity to recombine through syngamy and meiosis with other variants in natural populations. Such recombinations in organelle DNA are precluded by maternal inheritance of these organelles and probably account, in part, for the slower rate at which these organelle genomes evolve.

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