Genetic differentiation among Turkish chestnut (*Castanea sativa* Mill.) populations

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Genetic variability in Turkish chestnut (*Castanea sativa* Mill.) was measured by means of horizontal starch gel electrophoresis of 16 isoenzymatic systems in 13 populations. The results were compared with existing data on Italian chestnut populations. Thirty-one out of 43 alleles showed significant heterogeneity in frequency among Turkish populations. Most enzymatic polymorphisms show a gradual or sharp difference between Eastern and Western Turkish populations. Western Turkish demes seem genetically to be more closely related to Italian populations than to Eastern demes. These results are discussed in the light of historical and palinological records of the domestication of this species.

Introduction

Chestnut (*Castanea*) belongs to the family Fagaceae, which also includes beech (*Fagus*) and oak (*Quercus*). Chestnut probably originated in the Orient, perhaps in the Chinese region, where today *C. mollissima* Blume is found. It is believed that the westward extension of the genus in Tertiary times gave rise to *C. sativa* in the Mediterranean region, although according to Zohary & Hopf (1988) our knowledge of the place of origin and time of domestication of *C. sativa* is still inadequate.

Palinological data indicate that *C. sativa* disappeared from Southern Europe during the Würm glaciation and apparently survived only in South-West Asian refugia. Chestnut pollen appears in quantity in Anatolia and Greece only around 1500–1300 BC. A similar increase in pollen frequency is found in Italy and other western Mediterranean sites, but only at the start of classical times. This strongly suggests that *C. sativa* did not arrive in Western Turkey, Greece and the western Mediterranean countries as a wild element but was introduced by man, and also points to north-east Turkey and the Caucasus as the most probable areas for the initial chestnut domestication.

The aim of this work is to assess genetic variability and differentiation within Turkish chestnut, and, in particular, to contrast Eastern populations (the supposed main centre of origin) with Western popula-

tions (the supposed reintroduction after glaciation and the start of domestication). We further compare Turkish demes with previous results from Italian populations, among the most domesticated in the Mediterranean area (Pigliucci *et al.*, 1990; Villani *et al.*, 1990).

Materials and methods

A total of 378 specimens were sampled from 13 Turkish populations of *Castanea sativa* (Table 1). Demes are representative of the distribution of chestnut in Turkey, which is limited to the Black Sea coast and to the Mediterranean area of the country.

Allele frequencies at 16 enzyme loci were obtained by means of horizontal starch gel electrophoresis on frozen wintering buds. The loci studied, for which the genetic determination is known from crossing experiments, are: alcohol dehydrogenase (*ADH*); diaphorase (*DIA*) 1 and 2; shikimate dehydrogenase (*SKDH*); isocitric dehydrogenase (*IDH*) 1 and 2; glutammic oxaloacetate transaminase (*GOT*) 2; phosphogluco-mutase (*PGM*); leucino amino peptidase (*LAP*) 1; esterase (*EST*) 1 and 2; glucose phosphate isomerase (*GPI*) 1 and 2; 6-phospho glucose dehydrogenase (*6PGD*); peroxidase (*PRX*); and superoxide dismutase (*SOD*). Details are given for all these systems in Villani *et al.* (1990) except *SOD* for which we used a Poulik discontinuous buffer system (Poulik, 1957) and a staining solution according to Harris & Hopkinson (1977). The locus which specifies the most anodally migrating

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allozyme was designated as 1, the next 2, and so on. In the following pages, allele's superscripts indicate the relative migration distance on the gel, taking the most frequent Italian allele as 100 (see Villani *et al.*, 1990).

Statistical analysis

Genotypic and allelic frequencies were calculated from gel phenograms for each of the 16 loci scored in the 13 Turkish populations. Allele frequencies for the same loci in 18 Italian populations are known from previous work (Pigliucci *et al.*, 1990; Villani *et al.*, 1990). G-tests for heterogeneity of allele frequencies among populations were carried out according to Sokal & Rohlf (1981). The data were also used to calculate observed heterozygosity, percentage of polymorphic loci, effective number of alleles, total genetic variance (H_t), within populations genetic variance (H_s), among populations genetic variance (D_{st}), and the coefficient of gene differentiation among populations (G_{st}) (see Nei, 1987 for details and comments on these indices).

Standard genetic distances (D) (Nei, 1978) were calculated for each pair of populations and among four groups of demes: (a) Italian populations; (b) total Turkey populations; (c) east Turkish populations; and (d) west Turkish populations. The matrix of genetic distances between the whole dataset (Italian and Turkish demes) was illustrated by means of a UPGMA dendrogram (Sneath & Sokal, 1973).

Results

Figure 1 shows the locations of the 13 Turkish demes. Table 1 gives sample sizes, gene frequencies for 43 alleles belonging to 16 isoenzyme systems, and G-tests for heterogeneity of allele frequencies among populations; 31 alleles showed significant heterogeneity. Some regular patterns emerge from a visual inspection of gene frequencies. Alleles $dia1^{100}$, $dia2^{96}$, $skdh^{97}$, $idh1^{108}$, pqm^{97}, $gpi2^{108}$, and sod^{114} show a much higher frequency in eastern than in western Turkish demes. A West–East cline is clearly shown by allele lap^{98}. Allele $gpi2^{113}$ is present almost exclusively in the more Eastern populations.

Table 2 shows observed heterozygosity, percentage of polymorphic loci, and effective number of alleles for each Turkish deme and also for the groups defined above. On the whole, the Turkish region is more genetically variable than the Italian. Observed heterozygosities are 0.27 and 0.23 for the two groups respectively. The percentages of polymorphic loci are 83 per cent versus 62 per cent, and the numbers of effective alleles are 1.51 and 1.40. A closer inspection of Table 2, however, shows a marked difference between east and west

Table 1 Gene frequencies of Turkish populations of chestnut. Sample sizes in brackets. *G*-values for frequency heterogeneity below allele labels

	adh^{95} 22.7*	adh^{100} 21.8*	adh^{92} 2.9 ns	$dia1^{100}$ 96.6*	$dia1^{110}$ 96.6*	$dia2^{100}$ 83.7*	$dia2^{96}$ 83.7*	$skdh^{97}$ 148.1*	$skdh^{100}$ 174*	$skdh^{94}$ 23.5*	$idh1^{100}$ 52.6*	$idh1^{108}$ 77.7*	$idh1^{105}$ 17.7 ns	$idh1^{96}$ 14.3 ns
meryemana (39)	0.17	0.83	0.00	0.64	0.36	0.74	0.26	0.84	0.02	0.14	0.66	0.18	0.15	0.01
hopa (26)	0.20	0.80	0.00	0.92	0.08	0.61	0.39	1.00	0.00	0.00	0.60	0.30	0.06	0.04
giresun (35)	0.11	0.89	0.00	0.89	0.11	0.28	0.72	0.98	0.00	0.01	0.40	0.53	0.07	0.00
sinop (38)	0.08	0.92	0.00	0.88	0.12	0.54	0.46	0.96	0.04	0.00	0.42	0.46	0.09	0.03
unye (12)	0.10	0.90	0.00	0.92	0.08	0.45	0.55	1.00	0.00	0.00	0.58	0.33	0.04	0.04
ayancik (21)	0.07	0.91	0.02	0.93	0.07	0.43	0.57	0.67	0.00	0.00	0.26	0.45	0.29	0.00
akcakoka (30)	0.07	0.93	0.00	0.95	0.05	0.57	0.43	0.95	0.05	0.00	0.37	0.57	0.05	0.02
inegol (22)	0.18	0.82	0.00	0.55	0.46	0.98	0.02	0.20	0.77	0.02	0.89	0.00	0.00	0.11
inebolu (39)	0.18	0.82	0.00	0.96	0.04	0.60	0.40	0.93	0.07	0.00	0.40	0.44	0.15	0.01
bursa (40)	0.35	0.65	0.00	0.45	0.55	0.99	0.01	0.27	0.73	0.00	0.84	0.00	0.06	0.10
bartin (33)	0.32	0.68	0.00	1.00	0.00	0.44	0.56	0.92	0.03	0.04	0.53	0.33	0.11	0.03
eregli (23)	0.30	0.70	0.00	1.00	0.00	0.59	0.41	0.61	0.39	0.00	0.61	0.37	0.02	0.00
istanbul (20)	0.13	0.88	0.00	0.47	0.53	0.82	0.17	0.68	0.33	0.00	0.72	0.13	0.07	0.07

Table 1. Continued

	$idh2^{100}$ 17.7 ns	$idh2^{105}$ 17.6 ns	$got2^{100}$ 17.6 ns	$got2^{96}$ 17.6 ns	pgm^{100} 26.2*	pgm^{97} 26.2*	lap^{98} 173.8*	lap^{100} 51.4*	lap^{102} 8.2 ns	lap^{97} 70.4*	$est1^{100}$ 50.7*	$est1^{103}$ 42.8*	$est1^{97}$ 47.2*	$est2^{90}$ 43.9*
meryemana (39)	1.00	0.00	0.91	0.09	0.64	0.36	0.01	0.64	0.04	0.30	0.59	0.11	0.30	0.00
hopa (26)	1.00	0.00	0.96	0.04	0.76	0.24	0.00	0.75	0.00	0.25	0.94	0.00	0.06	0.00
giresun (35)	1.00	0.00	0.90	0.10	0.71	0.29	0.00	0.59	0.00	0.41	0.94	0.00	0.06	0.00
sinop (38)	0.90	0.10	0.90	0.10	0.63	0.37	0.07	0.72	0.00	0.21	0.90	0.04	0.07	0.03
unye (12)	1.00	0.00	0.92	0.08	0.44	0.56	0.14	0.36	0.00	0.50	0.83	0.08	0.08	0.00
ayancik (21)	1.00	0.00	0.74	0.26	0.74	0.26	0.19	0.64	0.02	0.17	1.00	0.00	0.00	0.02
akcakoka (30)	0.97	0.03	0.85	0.15	0.88	0.12	0.65	0.33	0.00	0.00	0.92	0.08	0.00	0.00
inegol (22)	0.98	0.02	1.00	0.00	0.89	0.11	0.75	0.25	0.00	0.00	0.68	0.32	0.00	0.29
ineboltu (39)	1.00	0.00	0.94	0.06	0.72	0.28	0.18	0.69	0.00	0.13	0.94	0.05	0.01	0.01
bursa (40)	1.00	0.00	0.99	0.01	0.82	0.18	0.76	0.24	0.00	0.00	0.80	0.20	0.00	0.13
bartin (33)	1.00	0.00	0.91	0.09	0.58	0.42	0.38	0.50	0.00	0.12	1.00	0.00	0.00	0.00
eregli (23)	0.98	0.02	0.94	0.07	0.67	0.33	0.56	0.43	0.00	0.00	0.96	0.04	0.00	0.00
istanbul (20)	1.00	0.00	0.97	0.03	0.93	0.07	0.65	0.30	0.00	0.05	0.75	0.25	0.00	0.17

Table 1. Continued.

	$est2^{100}$ 26.6*	$est2^{105}$ 40.3*	$gpi1^{100}$ 0.0 ns	$gpi2^{100}$ 183.4*	$gpi2^{105}$ 39.9*	$gpi2^{108}$ 87.8*	$gpi2^{110}$ 10.5 ns	$gpi2^{113}$ 40.3*	$6pgd^{100}$ 23.8*	$6pgd^{104}$ 23.8*	prx^{100} 20.8 ns	prx^{104} 6.6 ns	prx^{106} 77.0*	sod^{100} 42.8*	sod^{114} 42.8*
meryemana (39)	0.89	0.11	1.00	0.01	0.16	0.72	0.03	0.08	0.97	0.03	0.63	0.34	0.03	0.69	0.31
hopa (26)	0.69	0.31	1.00	0.02	0.02	0.69	0.08	0.19	0.83	0.17	0.61	0.14	0.25	0.58	0.42
giresun (35)	0.70	0.30	1.00	0.00	0.01	0.80	0.01	0.17	0.83	0.17	0.61	0.39	0.00	0.80	0.20
sinop (38)	0.76	0.21	1.00	0.33	0.10	0.55	0.01	0.00	0.96	0.04	0.55	0.43	0.01	0.71	0.29
unye (12)	0.96	0.04	1.00	0.00	0.04	0.92	0.00	0.04	1.00	0.00	0.88	0.13	0.00	0.82	0.18
ayancik (21)	0.48	0.50	1.00	0.02	0.12	0.86	0.00	0.00	1.00	0.00	0.76	0.19	0.05	0.81	0.19
akcakoka (30)	0.82	0.18	1.00	0.08	0.18	0.73	0.00	0.00	1.00	0.00	0.64	0.29	0.07	0.62	0.38
inegol (22)	0.70	0.00	1.00	0.75	0.00	0.25	0.00	0.00	1.00	0.00	0.59	0.00	0.41	1.00	0.00
ineboltu (39)	0.85	0.14	1.00	0.06	0.26	0.68	0.00	0.00	0.92	0.08	0.63	0.36	0.01	0.83	0.17
bursa (40)	0.85	0.03	1.00	0.81	0.01	0.17	0.00	0.00	0.97	0.03	0.68	0.04	0.29	0.90	0.10
bartin (33)	0.88	0.12	1.00	0.08	0.32	0.61	0.00	0.00	0.95	0.04	0.44	0.45	0.11	0.62	0.38
eregli (23)	0.89	0.11	1.00	0.22	0.11	0.67	0.00	0.00	0.96	0.04	0.34	0.43	0.23	0.67	0.33
istanbul (20)	0.65	0.17	1.00	0.68	0.20	0.13	0.00	0.00	1.00	0.00	0.42	0.03	0.55	1.00	0.00

Asterisks mark significant Gs ($P < 0.05$);
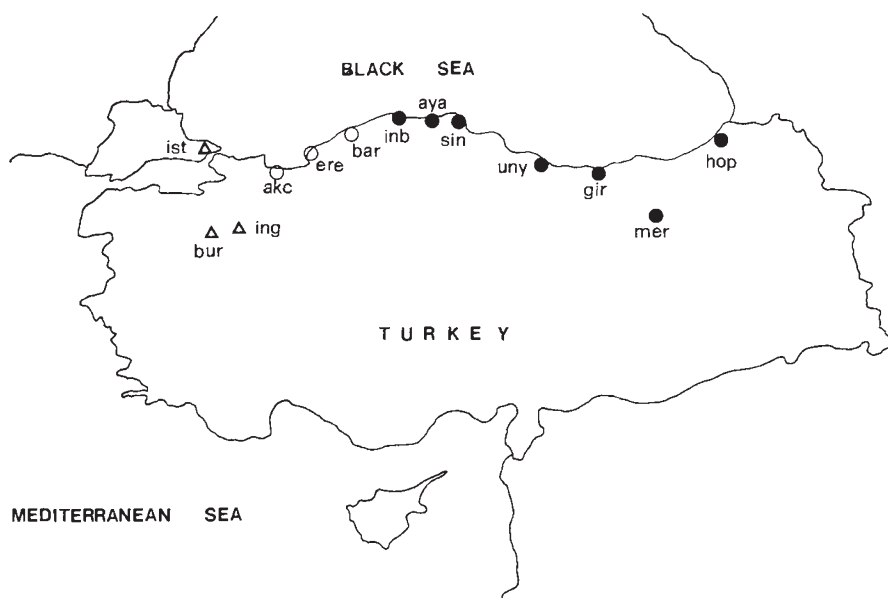ns = not significant.

Fig. 1 Provenances of the Turkish populations of chestnut studied.
($\triangle$) Western demes, ($\bigcirc$) eastern demes, ($\bullet$) far eastern demes.
mer = Meryemana, hop = Hopa, gir = Giresun, sin = Sinop, uny = Unye, aya = Ayancik, akc = Akcakoka, ing = Inegol, inb = Inebolu, bur = Bursa, bar = Bartin, ere = Eregli, ist = Istanbul.

Table 2 Genetic variability of *C. sativa* populations from Turkey. Averages for all Turkish populations and for west and east are reported. Values for Italian populations (Pigliucci *et al.*, 1990) are shown for comparison

Population	Observed heterozygotes	Polymorphic loci (%)	Effective n alleles
MER	0.3200	87.50	1.5854
HOP	0.2570	81.25	1.5174
GIR	0.2864	87.50	1.4642
SIN	0.2862	93.75	1.5683
UNY	0.2763	75.00	1.4621
AYA	0.2887	75.00	1.5494
AKC	0.2857	87.50	1.4567
ING	0.2273	75.00	1.3865
INB	0.2269	87.50	1.4866
BUR	0.2356	87.50	1.3934
BAR	0.2722	75.00	1.6108
ERE	0.3060	87.50	1.5916
IST	0.2656	75.00	1.5073
Turkey	0.2718	82.69	1.5061
East Turkey	0.2805	83.75	1.5293
West Turkey	0.2428	79.17	1.4291
Italy	0.2300	62.15	1.4000

Table 3 Average values of genetic diversity and structure in *C. sativa* populations from Turkey (west and east regions) and Italy

Region	H_t	H_s	D_{st}	G_{st}
Turkey	0.3368	0.2807	0.0561	0.1665
East Turkey	0.3133	0.2891	0.0242	0.0774
West Turkey	0.2611	0.2500	0.0111	0.0425
Italy	0.2522	0.2283	0.0238	0.0945

much higher in east Turkey than in west Turkey and Italy (0.31 vs. 0.26 and 0.25, respectively) and the genetic variance within populations follows the same trend (0.29 vs. 0.25 and 0.23). On the contrary, the two measures of genetic differentiation among populations (D_{st} and G_{st}) show a higher separation of demes within east Turkey and within Italy, compared with those in west Turkey, however, this discrepancy could be

Table 4 Mean standard genetic distance values (D) among populations of *C. sativa* from Turkey (west and east) and Italy. Values in brackets are mean genetic distances within each region

	Turkey	East Turkey	West Turkey	Italy
Turkey	(0.091)			
East Turkey	—	(0.038)		
West Turkey	—	0.176	(0.025)	
Italy	0.145	0.167	0.072	(0.033)

Turkey. The first region is even more variable than Turkey as a whole, while the second region is characterized by variability values close to those of Italian demes (except for the percentage of polymorphic loci, which is higher).

The same pattern is discernible in Table 3, which reports H_t, H_s, D_{st} and G_{st}. The total genetic variance is
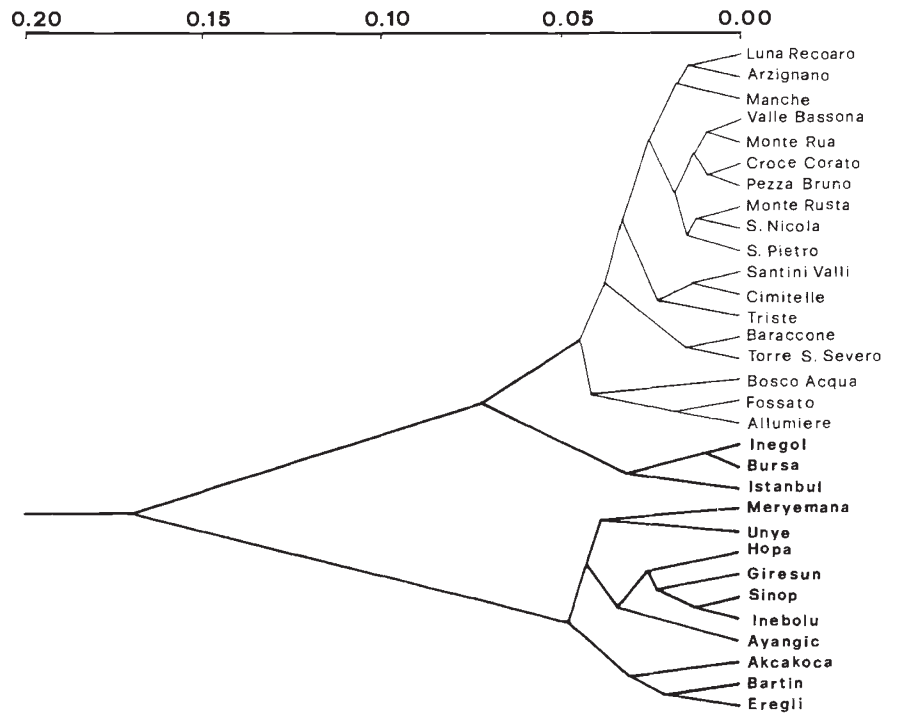
Fig. 2 UPGMA dendrogram of Turkish and Italian populations of chestnut. Italian demes are labelled according to Pigliucci *et al.* (1990).

accounted for by the lower number of demes studied in western Turkey.

This picture is confirmed by Table 4, which reports the mean standard genetic distance values among the four groups. The main diagonal shows the mean distances among populations within a given group. The smallest genetic distance is found between west Turkey and Italy and is comparable to the within-group distances. West Turkish chestnuts are more distantly related to east Turkish ones than they are to Italian demes. Figure 2 clearly visualizes the above mentioned relationships by means of a UPGMA dendrogram.

Discussion

The above results seem to point out a surprising affinity between west Turkish and Italian demes of *Castanea sativa*, versus a major differentiation of east Turkish ones.

Most of the significantly heterogeneous enzymatic polymorphisms show a gradual or sharp difference between the three Western demes and all the other populations. Such differences among Turkish demes cannot simply be explained by sampling errors or geographical isolation because the 13 populations are well scattered on the map.

In our opinion, palinological and genetic data support the following historical scenario: (i) an initial expansion of post-glacial refugia from eastern Turkey

to West regions, characterized by slow long-range gene flow with admixture (from 40,000 years ago to 1500 BC); (ii) a man-driven diffusion in west Turkey, Anatolia and Greece (from 1500 BC to II Century, BC); (iii) a second man-driven expansion to Italy and the rest of the Mediterranean basin during I–II Century AD.

In fact, the values of genetic variability indices (i.e. observed heterozygosities, percentages of polymorphic loci, and effective numbers of alleles) are higher in the region we assumed to be less influenced by the action of man; on the other hand, the degree of genetic variation in west Tukey is only slightly higher than that of Italy, according to our hypothesis of two temporally close diffusion events due to man. In addition, the amount of total genetic variance (H_t) supports the sketched relationships: from east Turkey to west Turkey it drops about 5 per cent, while from west Turkey to Italy it decreases only another 1 per cent.

The distinction between eastern and western Turkish demes is clearly evident from the dendrogram (Fig. 2) and from the values of genetic distances reported in Table 4. Furthermore, G_{st} (i.e. the degree of genetic differentiation between populations) in Turkey as a whole is about twice the value for Italy; but, if the two supposed groups of genetic isolates (i.e. east and west Turkey) are taken to be distinct, G_{st}s of the three subgroups are roughly comparable.

Such conclusions are of interest to us, because eastern Turkey is one of the supposed main centres of

origin of *Castanea sativa* (Zohary & Hopf, 1988); therefore, by studying Turkey chestnut and comparing it to the Italian, we gained a deeper understanding of its origin and natural evolution before man's influence on this species.

Acknowledgements

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