Genet. Res. Camb. (1994), **63**, *pp.* 109–116 *With 3 text-figures*

Evolution of *Castanea sativa* Mill. in Turkey and Europe

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(*Received 4 December 1993*)

Summary

The evolution of sweet chestnut (*Castanea sativa* Mill.) appears to be a complex mixture of long-range gene flow, natural and artificial selection, and local effects of isolation by distance. In this paper we present the most complete analysis to date on the genetic structure and variability of 52 populations of chestnut spanning the entire European area of distribution. The study is based on the use of isozyme data. Our samples came from four major zones, possibly representing relevant steps in the evolution and spread of sweet chestnut in Europe: (i) eastern Turkey, the supposed center of origin of the species; (ii) western Turkey, the area in which human domestication started; (iii) Italy, where domesticated chestnut was first introduced to the rest of Europe by the Romans; and (iv) France, representing the latest phases of the expansion, close to the northern limit of the taxon.

As previous studies based only on Italian and some Turkish populations suggested, the electrophoretic data are consistent with a series of episodes of west- and north-ward migration. The early expansion from the center of origin was probably slow, resulting from natural diffusion of the species. Most of the original genetic variation has been conserved during this phase. Successive episodes of colonization of western Turkey and then of the rest of Europe were probably the more rapid result of human activity. These later stages were associated with genetic drift that reduced the overall heterozygosity of the extant populations. No evidence for selection could be found at the large geographical scale of this study, although previous regional works have shown spatial patterns of allelic frequencies at a few loci and phenotypic differentiation consistent with the action of past selective pressures.

1. Introduction

Sweet chestnut (*Castanea sativa* Mill.) is the only native species of the genus in Europe and it is currently widespread throughout Europe and south-western Asia. Its center of origin is hypothesized to be in east Turkey or Caucasus (Zohary & Hopf, 1988). According to palynological data, it was present in Europe during the Tertiary, but it disappeared from north-western Europe during the Pleistocene glaciations, surviving only as a relict in a few localities in south-eastern Europe and Turkey (Huntley & Birks, 1983). Such dynamics have also been observed in many other plants and in a number of sedentary animal species of the same region. These species occupied a nearly continuous area in the whole Palearctic, Mediterranean and Sub-Mediterranean regions during Tertiary times. They were partially destroyed or driven back into refuges as a result of the progressive climatic changes that accompanied the approach of the Quaternary (Davis, 1965; Kosswig, 1965). Chestnut pollen is not recorded in Europe from the end of the Pleistocene glaciations to 9000 B.P. (years before present), when it appeared in Greece and possibly in Spain. By 5000 B.P. it was widespread in Greece and present in Southern Italy, and it reached the Alps about 3000 B.P. At about 2000 B.P. chestnut invaded Spain and southern France, and it was also present in Germany. Finally in *c.* 1000 B.P., it more or less attained the current distribution, with the exception of England, which was colonized later.

According to Huntley & Birks (1983) *C. sativa* underwent two periods of rapid expansion in Europe and south-western Asia: one after 5000 B.P., in the Neolithic period of forest clearance, and the other after 2000 B.P., during the expansion of Roman power

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in the Mediterranean. Although both events are anthropogenic, it is unlikely that the Neolithic nomad populations would have cultivated a tree with a life cycle of 20 years. It is more probable that the expansion of chestnut at that time was due to decreased competition resulting from deforestation. The second movement, however, was certainly associated with domestication and with the active spread of chestnut by the Romans.

Sweet chestnut is economically very important in Europe for its timber and fruits, but its survival is threatened by the chestnut blight, *Endothia parasitica* (Murr.) And., which largely destroyed the American chestnut (*Castanea dentata* (Marsh) Bork.) in little more than half a century (Anagnostakis, 1982). Information on the genetic variation of parasite and host will be important in the conservation of sweet chestnut. Besides economic considerations, however, the relatively rich fossil and historical records available for *C. sativa* make it a suitable candidate for the study of long term evolution and population genetics of forest trees. Previous studies of allozyme diversity in Italian chestnut indicated geographic variation in gene frequencies and an east–west partitioning of the genetic variation, roughly parallel to the Appennini mountains (Pigliucci, Benedettelli & Villani, 1990*a*). The spatial structure of genetic markers in populations from eastern and western Turkey seems to be largely due to a process of long-range gene flow with partial admixture and isolation by distance. Evidence for natural selection and association with climatic variables was also found for a few alleles (Pigliucci, Villani & Benedettelli, 1990*b*). A phenetic analysis of genetic distances pointed to a dichotomy between north-eastern Turkish populations on one side, and north-western Turkish and Italian populations on the other. This suggests some kind of differentiation event occurring at the boundary between eastern and western Turkey in relatively recent times (Villani *et al.* 1991). The same pattern was found to hold for a series of morphological characters of the fruit and for physiological traits related to CO_2 exchange (Villani *et al.* 1992).

The aims of the present work are: (a) to expand the number of populations sampled to include more sites from the crucial region in western Turkey, as well as from France, which is close to the northern European limit of current chestnut distribution; (b) to describe the large scale spatial structure of allozyme polymorphisms from eastern Turkey to western Europe; (c) to test the low marginal variance theory, according to which the within-population genetic variance should be lower in sites that are geographically peripheral to the species' range (see Wilson *et al.* 1991 for a recent attempt); (d) to derive a tentative intraspecific phylogeny for *Castanea sativa*, based on allozyme data; and (e) to discuss the relative importance of isolation by distance and of natural and artificial selection in shaping the current pattern of genetic variation of chestnut in Europe and south-western Asia.

2. Materials and methods

Fifty-two populations of *Castanea sativa* were analysed for genetic variability at 13 enzyme loci. Four main regions within the current range of the species were represented: 9 populations from France (western Europe), 18 populations from Italy (southern Europe), 15 populations from western Turkey, and 10 populations from eastern Turkey. Fig. 1 shows a map of the sampled localities.

The genetic markers used in this survey were: diaphorase (DIA) 1 and 2; shikimate dehydrogenase (SKDH); isocitric dehydrogenase (IDH) 1 and 2; glutamic oxaloacetate transaminase (GOT) 2; leucine amino peptidase (LAP); esterase (EST) 1 and 2; glucose phosphate isomerase (GPI) 2; 6-phosphoglucose dehydrogenase (6-PGD); peroxidase (PRX) 1; and superoxide dismutase (SOD). Allele frequencies were obtained after starch gel electrophoresis. Details of the techniques are reported in Villani *et al.* (1990), except for SOD, for which we used a Poulik discontinuous buffer system (Poulik, 1957) and a staining solution according to Harris & Hopkinson (1977). Denomination of loci and alleles is consistent with Pigliucci *et al.* (1990*a*) and Villani *et al.* (1991).

Allele frequencies and standard population genetics statistics of gene diversity were obtained using the computer package BIOSYS-1 (Swofford and Selander, 1989). Mean number of alleles per locus, percentage of polymorphic loci, observed and expected heterozygosities (under Hardy–Weinberg equilibrium) were computed, together with Nei's unbiased genetic distances among all pairwise comparisons of populations (Nei 1978, 1987). Cavalli-Sforza and Edwards' chord distances (Cavalli-Sforza & Edwards, 1967) were also computed to be used in the phylogenetic analysis, which was attempted using the Distance Wagner procedure (Farris, 1972) after optimization and rooting using the midpoint method. Nei's distances could not be used for this purpose because they violate the triangle inequality; nonetheless, they were computed to allow comparisons with previously published data.

The spatial structure of allelic frequencies was investigated using spatial autocorrelation analysis (Sokal & Oden 1978*a*, *b*; Heywood, 1991; Sokal & Jacquez, 1991). Moran's I autocorrelation coefficient was preferred over alternative methods because its range of variation is comparable to that of a standard correlation coefficient ($-1 <= I <= +1$). Spatial autocorrelograms were obtained by dividing the populations into five equally spaced distance classes (computer package SAAP v. 4.2, Wartenberg, 1989); the correlograms were then compared and grouped according to a hierarchical clustering algorithm

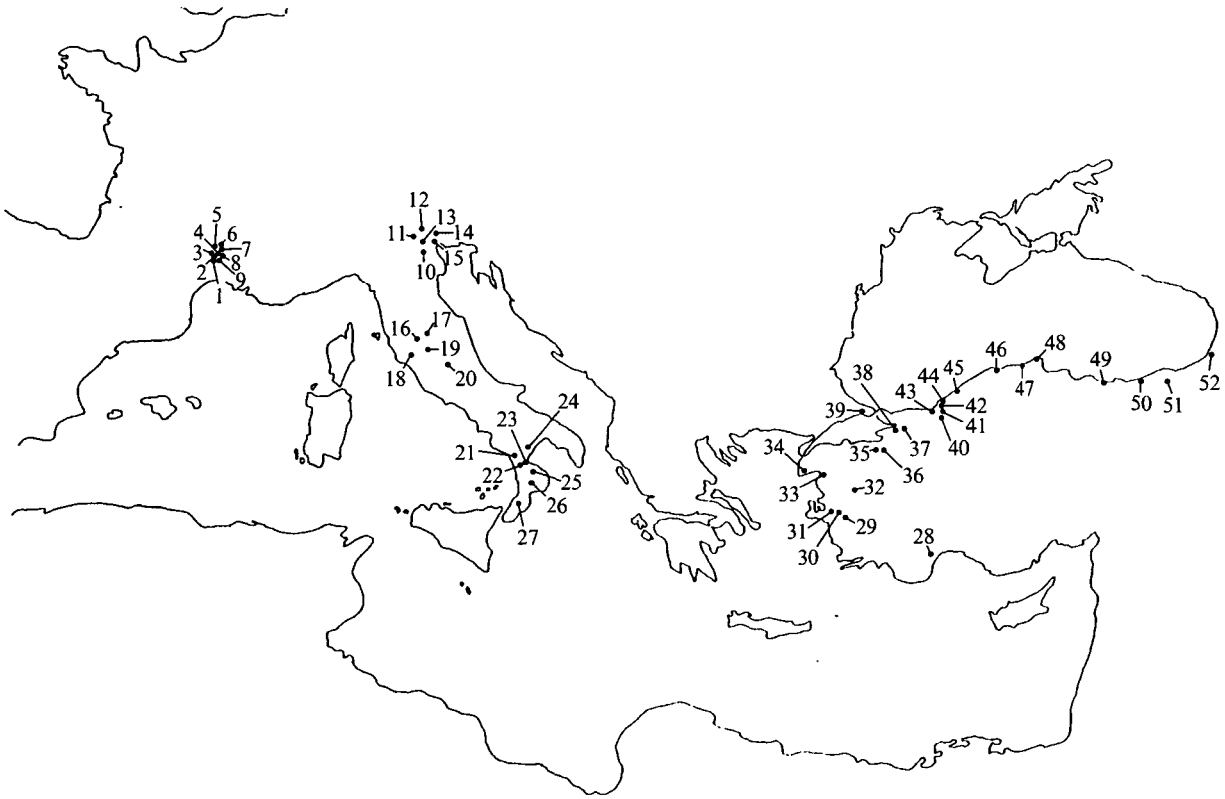

Fig. 1. Map of the sampled populations (sample sizes in parentheses). **France**: 1, Colognac (17); 2, Le Plantiers (20); 3, Tourgueville (24); 4, Mas de Ranc (19); 5, Château de Miral (19); 6, Nojaret (25); 7, Valmale (20); 8, Jouvernagues (20); 9, S. Felix (19); **Italy**: 10, Monte Rusta (12); 11, Luna Recoaro (17); 12, Santini Valli (15); 13, Monte Faldo (15); 14, Monte Rua (19); 15, Valle Bassona (19); 16, Bocca Porco (12); 17, San Pietro (18); 18, Allumiere (24); 19, Triste (20); 20, Cimitelle (15); 21, S. Nicola (18); 22, Bosco Acqua (12); 23, Baraccone (10); 24, Croce Corato (12); 25, Pezza Bruno (16); 26, Manche (19); 27, Fossato (13); **Turkey**: 28, Oluk (27); 29, Beydagi (27); 30, Ovagic (26); 31, Kemalpasa (23); 32, Demirci (25); 33, Edremit (29); 34, Bairamic (27); 35, Bursa (39); 36, Inegol (22); 37, Sapanca (21); 38, Golcuk (30); 39, Istanbul (20); 40, Kainasly (24); 41, Kurtusu (20); 42, Komacan (20); 43, Akcakoka (30); 44, Eregli (23); 45, Bartin (34); 46, Inebolu (39); 47, Ayangic (21); 48, Sinop (38); 49, Unye (12); 50, Giresun (35); 51, Meryemana (38); 52, Hopa (25).

Table 1. *Matrix of Nei unbiased genetic distance coefficients averaged by geographic area*

AREA	Number of populations	France	Italy	E. Turkey	W. Turkey
France	9	0·013			
Italy	18	0·028	0·022		
East Turkey	13	0·122	0·160	0·032	
West Turkey	12	0·115	0·082	0·228	0·055

(UPGMA, Sneath & Sokal, 1973). The resulting dendrogram visualizes the phenetic similarity among correlograms (computer package NTSYS-pc, Rohlf, 1987).

3. Results

We did not find significant deviations between observed and expected heterozygosities (i.e. no evidence of Wahlund effect) in any of the 52 populations (data not shown). The rank of geographical areas for their average percentage of polymorphic loci is: Italy (58·3%) < France (65·8%) < western Turkey (74·7%) < eastern Turkey (82·9%). The average heterozygosity ranks areas in the following way: France (0·22) = Italy (0·22) < western Turkey (0·24) < eastern Turkey (0·28). Overall, there seems to be a cline of decreasing genetic variation from Turkey to Europe.

We analysed the average genetic distances, both within and between geographical areas. Table 1 reports Nei's unbiased genetic distances; average distances within areas are on the diagonal, distances between major areas are below the diagonal. European populations tend to be similar within regions, with French populations more closely related to each other

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Table 2. *Spatial autocorrelation summary statistics (Moran's* I*) for all alleles (if only two alleles are present at a locus, only data for one of them are reported). Significance levels of single coefficients and of the overall correlogram are reported. Significance levels*: * = P < *0·05*; ** = P < *0·01*

	Distance class					
Allele	1	2	3	4	5	Cumulative probability
Dia1-100	+0·29**	−0·25**	−0·10	−0·07	+0·03	0·000
Dia2-100	+0·60**	+0·05	−0·24**	−0·49**	−0·02	0·000
Skdh-97	+0·41**	+0·04	−0·22**	−0·31**	−0·02	0·000
Skdh-100	+0·42**	+0·04	−0·23**	−0·31**	−0·02	0·000
Skdh-94	+0·01	−0·03	−0·05	−0·01	−0·01	0·732
Idh1-100	+0·41**	−0·32**	−0·08	−0·07	−0·04	0·000
Idh1-108	+0·30**	−0·34**	−0·09	+0·06	−0·02	0·000
Idh1-105	+0·31**	−0·04	−0·11*	−0·23**	−0·02	0·000
Idh1-96	+0·28**	+0·14**	−0·35**	−0·19**	+0·03	0·000
Idh2-100	+0·02	−0·10*	+0·08*	−0·07	−0·02	0·093
Got2-100	+0·50**	+0·14**	−0·24**	−0·53**	+0·03	0·000
Lap-98	+0·71**	−0·24**	−0·49**	−0·37**	+0·28**	0·000
Lap-100	+0·71**	+0·16**	−0·69**	−0·50**	+0·22**	0·000
Lap-102	+0·38**	+0·10**	−0·03	−0·46**	−0·09	0·000
Lap-97	+0·40**	−0·12*	−0·14*	−0·15**	−0·09	0·000
Est1-100	+0·29**	−0·02	+0·01	−0·34*	−0·04	0·000
Est1-103	+0·35**	+0·03	−0·08	−0·36**	−0·03	0·000
Est1-97	+0·15**	−0·17**	−0·08	+0·01	−0·01	0·000
Est2-90	+0·40**	−0·16**	−0·29**	−0·04	−0·01	0·000
Est2-100	+0·25**	−0·15**	−0·23**	+0·09	−0·05	0·000
Est2-105	+0·37**	+0·06*	−0·17**	−0·29**	−0·07	0·000
Gpi2-100	+0·45**	−0·35**	−0·18**	−0·06	+0·01	0·000
Gpi2-105	+0·24**	−0·17**	−0·12*	−0·16**	+0·11**	0·000
Gpi2-108	+0·40**	−0·38**	−0·13*	−0·01	+0·02	0·000
Gpi2-110	+0·12**	−0·02	−0·09	−0·04	−0·07	0·001
Gpi2-113	+0·24**	−0·24**	−0·05	0·00	−0·05	0·000
6Pgd-100	+0·34**	+0·23**	−0·05	−0·16**	−0·46**	0·000
Prx1-100	+0·65**	+0·25**	−0·62**	−0·60**	+0·22**	0·000
Prx1-104	+0·26**	−0·31**	+0·16**	−0·12*	−0·08	0·000
Prx1-106	+0·73**	−0·21**	−0·50**	−0·30**	+0·18**	0·000
Prx1-107	+0·02*	−0·04	−0·05	−0·01	−0·01	0·242
Sod-100	+0·46**	+0·28**	−0·27**	−0·58**	0·02	0·000

than Italian ones. Turkish populations show a relatively high average distance within regions, with western Turkey characterized by higher distances than eastern Turkey. Below the diagonal, the highest genetic distance is between eastern and western Turkey, in agreement with the findings of Villani *et al.* (1991). The magnitude of D (0·228) indicates a higher genetic divergence than is typically seen among conspecific populations. The most closely related groups of populations are the French and the Italian (D = 0·028), both of which are less distant from western than from eastern Turkey.

Previous studies suggested the presence of spatial structure for enzyme markers in chestnut, on a smaller geographic scale (within Italy, Pigliucci *et al.* 1990*a*; and within Turkey, Pigliucci *et al.* 1990*b*). Moran's I autocorrelation coefficients were used to investigate this aspect of our data set (Table 2). Five equally spaced distance classes were used in the analysis, the highest inter-population distance being between eastern Turkish and French demes, and the smallest grouping populations well within the four major geographic areas. Instead of relying on visual inspection to group the correlograms according to the particular pattern shown, we used a clustering algorithm to establish similarities in the spatial structure of single loci (see Barbujani & Pigliucci, 1989). A matrix of Manhattan distances (Sneath & Sokal, 1973) was computed among correlograms, using the five autocorrelation coefficients as variables.

A UPGMA clustering algorithm allowed us to group the loci hierarchically according to the similarity in their spatial structures. The resulting phenetic dendrogram (Fig. 2) presents the relative similarities among 32 of the 38 alleles. The second allele at loci with only two allelic forms was dropped to eliminate redundancy; the spatial structure of such an allele is exactly the same as that of the alternative form at that locus. Only four alleles (cluster 2 in the figure), associated with the loci SOD, PRX-1, GPI-2 and

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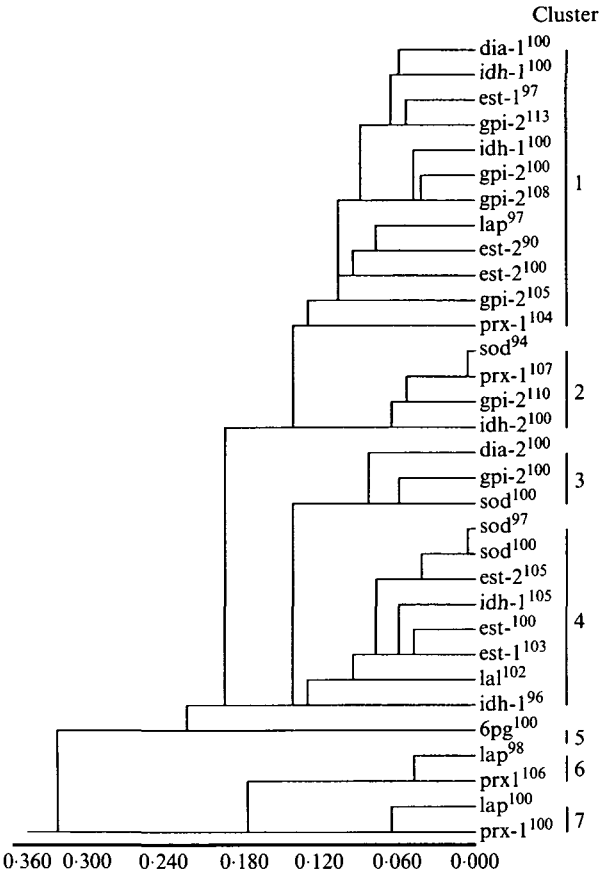

Fig. 2. UPGMA clustering of spatial autocorrelograms. Based on Manhattan distances between Moran's *I* coefficients.

IDH-2, show no significant structure at all (i.e. flat correlograms, with no coefficients significantly different from zero: a 'crazy quilt' in Sokal & Oden's, 1978*a*, *b* terminology). Three clusters (numbers 1, 3 and 4 in the figure), including the majority of the alleles, show basically the same spatial pattern. The first distance class is characterized by a significantly positive value of Moran's *I*, the second (in cluster 1) or the fourth class (in clusters 3 and 4) shows a significant negative autocorrelation coefficient, and all the other classes are not significantly different from zero. The only difference between clusters 3 and 4 is that the last one shows the pattern in a less strong fashion. This pattern has been termed 'double-cline' (Sokal & Oden, 1978*a*, *b*). Finally, there are three remaining small clusters in the dendrogram (numbers 5, 6 and 7 in the figure). Cluster 5 is made up of only one allele, 6PGD^{100}; cluster 6 includes only LAP^{98} and PRX-1^{106}; and cluster 7 is constituted by LAP^{100} and PRX-1^{100}. The correlogram for 6PGD^{100} shows a monotonically decreasing function of the autocorrelation coefficient with distance: the first class is characterized by a very high positive value of *I*, the last class by a very negative value. This pattern is referred to in Sokal & Oden (1978*a*, *b*) as a 'cline'. The remaining four alleles (clusters 6 and 7) show the same pattern, which is stronger in LAP^{98} and PRX-1^{106} than in LAP^{100} and PRX-1^{100}: an initially positive *I* goes toward zero, becomes negative in correspondence with the third distance class, goes through zero again, and then becomes positive and significant again in the last distance class. This pattern has been referred to as a 'circular cline' by Sokal & Oden (1978*a*, *b*). It can be considered as an extension of the double cline shown by clusters 3 and 4 (except that here the extreme populations become similar).

We attempted to obtain a phylogenetic hypothesis for the intra-specific differentiation in *Castanea sativa*, based on the available allozyme data. We constructed a phylogram based on genetic distances, using Distance Wagner procedure (Farris, 1972). Since this procedure assumes that the type of distance coefficient used is metric, we used Cavalli-Sforza's distances (Cavalli-Sforza & Edwards, 1967), instead of Nei's, We allowed the tree to be rooted, placing the root at the midpoint of the longest branch. The tree shown in Fig. 3 has also been treated for branch length optimization (Swofford, 1981). According to the Distance Wagner phylogram, eastern Turkish populations are basal to the entire tree, all of them being grouped in the cluster closer to the root. Within these, Eregli and Kainasly seem to be the least derived, and the subgroup with Meryemana, Hopa, Giresun and Unye to be the most derived. Interestingly, a set of western Turkish populations are basal to the rest of western Turkey, Italy and France (namely, Sapanca, Golcuk, Istanbul, Bursa and Inegol). Oluk and Edremit are basal to the rest of the western Turkish group, the most derived members of which are Bairamic, Kemalpasa, Ovagic, Demirci and Beydagi. From the western Turkish cluster stems the European group, with Italian populations basal to the French ones. There is not much resolution within the two European groups, so it is impossible to establish which populations are ancestral to the French group. The most derived French populations are Nojaret, Jouvernagues and Mas de Ranc. The cophenetic correlation for this cladogram was 0·934, suggesting little distortion of the input matrix, at least for the main clusters. The total length of the tree was 3·534.

4. Discussion

Castanea sativa Mill. is characterized by a very high degree of geographical differentiation across its range in Europe and the Near East. Comparisons of the four major geographic areas (France, Italy, western and eastern Turkey), give some *D* values so high as to suggest inter-specific differentiation (especially between eastern Turkey and the other areas), as previously suggested by Villani *et al.* (1992). The percentages of polymorphic loci, the levels of heterozygosity, and the within-area genetic distances all indicate strong geographical variation. Within-area values of *D* tend to decrease going from Turkey to Europe, as does the mean observed heterozygosity.

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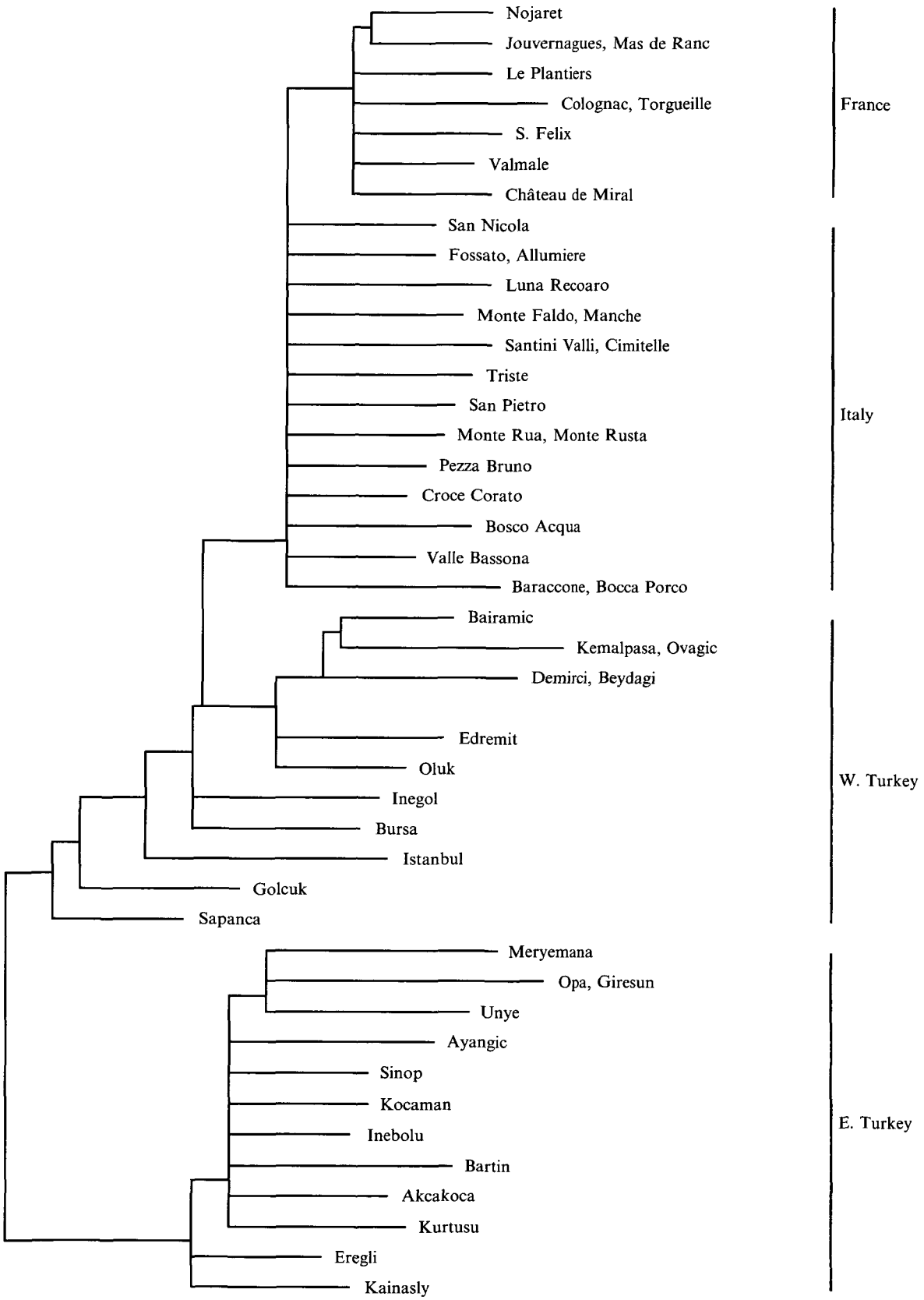

Fig. 3. Wagner tree produced by rooting at midpoint of longest path (after optimization). Cophenetic correlation = 0·934. Total length of the tree = 3·534.

The greatest intra-area genetic distances and heterozygosities in Turkey support the general contention that this area is closest to the center of origin of the species, and has maintained the highest level of gene diversity. Genetic variability probably decreased during expansion of the range due to successive episodes

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of genetic drift and relatively limited long-range gene flow.

Most of our spatial autocorrelograms show either clines or double clines: these structures can be interpreted as the result of either (a) selection forces, or (b) long range gene flow with admixture (as opposed to a simple model of isolation-by-distance, Barbujani, 1987). The long range gene flow with admixture is consistent with the relatively low efficiency of pollen dispersal in chestnut, and with the palynological record of post-glacial expansion of the range, starting from isolated refugia (Huntley & Birks, 1983). Admittedly, the reconstructed phylogeny based on genetic distances probably cannot be trusted in its finer details. Nevertheless, the general scheme of eastern Turkish demes as representatives of the founder stock after glaciation, with successive waves of colonization of new areas from western Turkey to Italy and to France, is in very good agreement with the palynological record. The selection scenario is much less likely because of the high number of genetic polymorphisms showing very similar spatial patterns: given that genotypes, not single loci, are inherited, concerted movements due to gene flow are a more parsimonious explanation than massive and parallel selection episodes. However, some selection on a few enzyme loci and on morpho-physiological characters almost certainly occurred, and it is detectable by studies conducted at smaller spatial scales (Pigliucci *et al.* 1990*b*; Villani *et al.* 1992).

The present data set can be used to test the so-called Low Marginal Variance (LMV) theory (da Cuhna, Burla & Dobzhansky, 1950; White, 1951), according to which the level of heterozygosity should be lower in the peripheral portion of the species range. Some evidence for the LMV has been published by Prakash, Lewontin & Hubby (1969) for allozyme data. A recent test by Wilson *et al.* (1991) for morphological data showed supporting evidence for only one out of six traits studied. Our data on geographical structure of allozyme variation in chestnut, coupled with evidence from the fossil record, fit in with the LMV theory. However, very different explanations for such a pattern can be envisaged: Mayr (1959) proposed severe selection in marginal environments, and ever since a number of authors have proposed ecologically related explanations for low marginal heterozygosity. On the other hand, as discussed earlier, a decrease in the level of within-population genetic variance can simply be due to successive migration events coupled with low-efficiency gene flow, resulting in genetic drift and a spatial pattern of isolation by distance. Our data on chestnut are more consistent with a non-selective explanation for the LMV pattern.

The observed relatively abrupt differentiation between eastern Turkish demes and other populations reported in Villani *et al.* (1991) and confirmed by the new data now available, is not a phenomenon restricted to chestnut. A similar pattern has been observed for several groups of animals and plants (Davis, 1965; Kosswig, 1965). The general explanation for these observations could be similar to that outlined here for chestnut. An initial differentiation was caused by past and present barriers (i.e. mountain systems in northern, western and southern Anatolia). These combined their effects with periodical isolation during glacial periods. However, limited communication was possible during the interglacial periods between the Black Sea and the Mediterranean, through the Sakarya Bosphorus of Pfarmestiel (Kosswig, 1965). A slow recolonization process followed after the last glaciation. Therefore, there was probably a relatively rapid differentiation in a new environment, with possible artificial or natural selection. These could have taken the form of either direct artificial selection (in the case of chestnut), or of new selective forces due to deforestation activities which dramatically altered the environment in a relatively short span of time.

We are grateful to Guido Barbujani for discussions on spatial autocorrelation techniques and for general comments about our work, and to Roberta Mason and Jack Sullivan for a critique of an earlier version of this manuscript. We thank O. Taskin, O. Sun and the technical staff of the Forest Research Institute of Ankara for their assistance during collection of some Turkish populations, and F. Romane (Centre d'Ecologie Functionelle of CNRS, Montpellier) for assistance during the French collection. Finally, we are grateful to F. Cannata for his continuous encouragement.

References

Anagnostakis, S. L. (1982). Biological control of chestnut blight. *Science* **215**, 466–471.

Barbujani, G. & Pigliucci, M. (1989). Geographical patterns of karyotype polymorphism in Italian populations of *Ornithogalum montanum* (Liliaceae). *Heredity* **62**, 67–75.

Barbujani, G. (1987). Autocorrelation of gene frequencies under isolation by distance. *Genetics* **117**, 777–782.

Cavalli-Sforza, L. L. & Edwards, A. W. F. (1967). Phylogenetic analysis: models and estimation procedures. *Evolution* **21**, 550–570.

da Cuhna, A. B., Burla, H. & Dobzhansky, Th. (1950). Adaptive chromosomal polymorphism in *Drosophila willistoni*. *Evolution* **4**, 212–235.

Davis, P. H. (1965). *Flora of Turkey*, vol. 1, pp. 1–25. Edinburgh: Edinburgh University Press.

Farris, J. S. (1972). Estimating phylogenetic trees from distance matrices. *The American Naturalist* **106**, 645–668.

Harris, H. & Hopkinson, D. A. (1977). *Handbook of Enzyme Electrophoresis in Human Genetics*. Amsterdam: North Holland Publ. Co.

Heywood, J. S. (1991). Spatial analysis of genetic variation in plant populations. *Annual Review of Ecology and Systematics* **22**, 335–355.

Huntley, B. & Birks, H. J. B. (1983). *An Atlas of Past and Present Pollen Maps for Europe: 0–13000 Years Ago*. Cambridge: Cambridge University Press.

Kosswig, C. (1965). Zoogeography of the Near East. *Systematic Zoology* **4**, 50–73.

Mayr, E. (1959). Where are we? *Cold Spring Harbor Symposium on Quantitative Biology* **24**, 1–14.

Nei, M. (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89**, 583–590.

Nei, M. (1987). *Molecular Evolutionary Genetics*. New York: Columbia University Press.

Pigliucci, M., Benedettelli, S. & Villani, F. (1990*a*). Spatial patterns of genetic variability in Italian chestnut (*Castanea sativa*). *Canadian Journal of Botany* **68**, 1962–1967.

Pigliucci, M., Villani, F. & Benedettelli, S. (1990*b*). Geographic and climatic factors associated with the spatial structure of gene frequencies in *Castanea sativa* Mill. from Turkey. *Journal of Genetics* **69**, 141–149.

Poulik, M. D. (1957). Starch gel electrophoresis in a discontinuous system of buffers. *Nature* **180**, 1477.

Prakash, S., Lewontin, R. C. & Hubby, J. L. (1969). A molecular approach to the study of genic heterozygosity in natural populations. IV. Patterns of genic variation in central, marginal and isolated populations of *Drosophila pseudoobscura*. *Genetics* **61**, 841–858.

Rohlf, F. J. (1987). *NTSYS-pc*. Setauket, NY: Applied Biostatistics Inc.

Sneath, P. H. A. & Sokal, R. R. (1973). *Numerical taxonomy*. San Francisco: Freeman and Co.

Sokal, R. R. & Jacquez, G. M. (1991). Testing inferences about microevolutionary process by means of spatial autocorrelation analysis. *Evolution* **45**, 152–168.

Sokal, R. R. & Oden, N. L. (1978*a*). Spatial autocorrelation in biology. I. Methodology. *Biological Journal of the Linnean Society* **10**, 199–228.

Sokal, R. R. & Oden, N. L. (1978*b*). Spatial autocorrelation in biology. II. Some biological implications and four applications of evolutionary and ecological interest. *Biological Journal of the Linnean Society* **10**, 229–249.

Swofford, D. L. (1981). On the utility of the distance Wagner procedure. In *Advances in Cladistics: Proceedings of the First Meeting of the Willie Hennig Society* (ed. V. A. Funk and D. R. Brooks), pp. 25–43. New York Botanical Garden, Bronx, New York.

Swofford, D. L. & Selander, R. B. (1989). *BIOSYS-1*. Illinois Natural History Survey.

Villani, F., Benedettelli, S., Paciucci, M., Cherubini, M. & Pigliucci, M. (1990). Genetic variation and differentiation between natural populations of chestnut (*Castanea sativa* Mill.) from Italy. In *Biochemical Markers in the Population Genetics of Forest Trees* (ed. H. H. Hattemer and S. Fineschi), pp. 91–103. SPB Academic Publishing: The Hague, The Netherlands.

Villani, F., Pigliucci, M., Benedettelli, S. & Cherubini, M. (1991). Genetic differentiation among Turkish chestnut (*Castanea sativa* Mill.) populations. *Heredity* **66**, 131–136.

Villani, F., Pigliucci, M., Lauteri, M. & Cherubini, M. (1992). Congruence between genetic, morphometric, and physiological data on differentiation of Turkish chestnut (*Castanea sativa*). *Genome* **35**, 251–256.

Wartenberg, D. (1989). *SAAP v. 4.2*. Setauket, NY: Exeter Software.

White, M. J. D. (1951). Structural heterozygosity in natural populations of the grasshopper *Trimerotropis sparsa*. *Evolution* **5**, 376–394.

Wilson, J. B., Ronghua, Y., Mark, A. F. & Agnew, A. D. Q. (1991). A test of the low marginal variance (LMV) theory in *Leptospermum scoparium* (Myrtaceae). *Evolution* **45**, 780–784.

Zohary, D. & Hopf, M. (1988). *Domestication of Plants in the Old Word*. Clarendon Press: Oxford.