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Einkorn wheat domestication site mapped by DNA fingerprinting*

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ABSTRACT – *Wild relatives of the 'founder' crops of the European agriculture, chickpea, lentil, pea, barley, Emmer and Einkorn wheats, bitter vetch (Zohary and Hopf 1993) continue to grow in the Fertile Crescent. The study of the genetic relationships between cultivated types occuring outside their natural habitat and their wild relatives clarifies important aspects of plant domestication. For example, by comparing – based on DNA fingerprinting – cultivated lines with wild relatives collected in defined areas, we have been able to pinpoint precisely the place of origin of Einkorn wheat within the Fertile Crescent (Heun et al. 1997), a puzzle which archaeology alone has been unable to solve. Similar studies of other Fertile Crescent crops might answer whether the Neolithic revolution in this part of the world had a common origin, or whether the above mentioned other crops were domesticated independently. DNA analyses can contribute to archaeology; more interaction is needed.*

POVZETEK – *Divji sorodniki prvotnih pridelkov evropskega poljedelstva (čičerka, leča, grah, ječmen, žiti Emmer in Einkorn, grenka grašica (Zohary in Hopf 1993) še danes uspevajo v Rodovitnem polmesecu. Raziskave genskih povezav med gojenimi tipi, ki se pojavljajo izven njihovega naravnega okolja, in njihovimi divjimi sorodniki pojasnjujejo pomembne vidike udomačitve rastlin. Na primer, na osnovi primerjave prstnih odtisov DNK gojenih vrst in divjih sorodnikov, ki smo jih nabrali na znanih območjih, smo lahko natančno določili izvor žita Einkorn znotraj Rodovitnega polmeseca (Heun et al. 1997), in tako rešili uganko, ki je sama arheologija ni mogla razrešiti. Podobne raziskave drugih pridelkov z Rodovitnega polmeseca bodo morda odgovorile na vprašanje, ali ima neolitska revolucija v tem delu sveta skupni izvor ali pa so bili zgoraj omenjeni pridelki udomačeni neodvisno drug od drugega. DNK analize lahko prispevajo k arheologiji; potrebno je večje sodelovanje.*

INTRODUCTION

DNA techniques provide powerful tools for studying evolution and domestication. However, use of DNA techniques is limited when only small amounts of high quality DNA can be extracted, as is the case with ancient samples. Although this limitation can be overcome to some extent (*Brown et al. 1994*), an alternative approach to addressing questions about the domestication of plants is to use modern seed samples. Einkorn wheat is a forgotten crop, to which no modern breeding has been applied, and has been cultivated for several thousand years outside its natural habitat.

Wild Einkorns still occur in nature (*Zohary and Hopf 1993*), and large samples of these wild lines are stored in gene banks around the world. Therefore, a representative collection of cultivated Einkorns, geographically well isolated from their wild relatives, can be used to identify the closest wild relative in a defined geographic area. As a result, the possible Einkorn wheat domestication site was pinpointed within the Fertile Crescent (*Heun et al. 1997*).

THE PLANT MATERIAL

Einkorn wheats are diploid, self-pollinating plants (2n = 2x = 14), belonging to the family Poaceae and carrying the A genome. *Triticum monococcum ssp. monococcum* (*T. monococcum*) and *Triticum monococcum ssp. boeoticum* (*T. boeoticum*) are the respective Latin names of the domesticated and the

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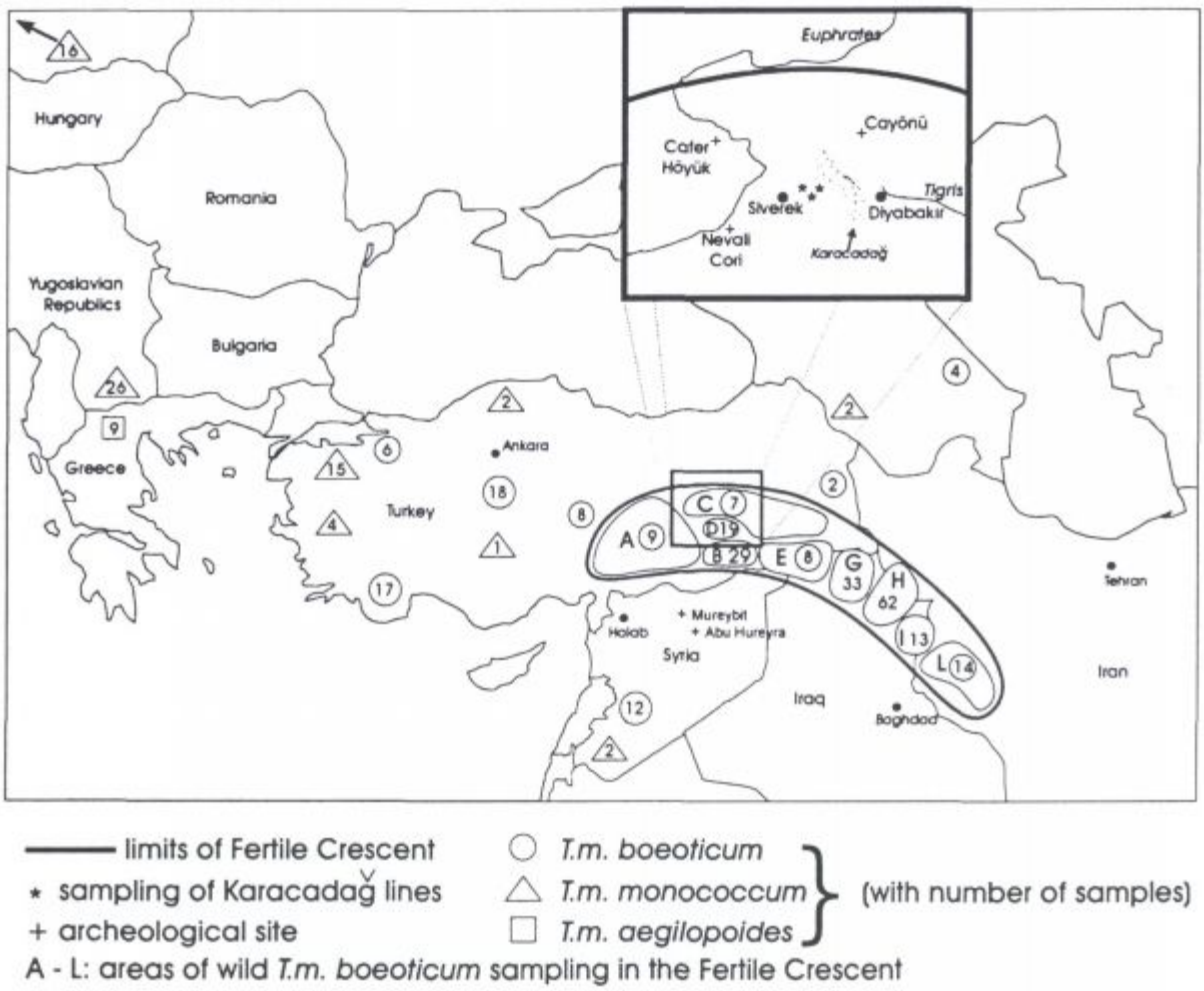

Fig. 1. Sampling sites of 338 Einkorn wheats. Insert: the Karacadağ region. For the area of the Fertile Crescent, where Einkorn occurs in primary habitats, nine groups were formed (see Heun et al. 1997 for details). Reprinted with permission from Science, 14 November 1997, Volume 278, p. 1313, Fig. 1. © 1998 American Association for the Advancement of Science.

wild Einkorn wheat. *Triticum monococcum ssp. aegilopoides (T. aegilopoides)* is another Einkorn wheat which is fully fertile with the two other Einkorns. *T. aegilopoides* occurs in the wild mainly in the Balkans, and is of interest because it shows domestication traits similar to those of *T. monococcum*. Ten gene banks world-wide (see *Heun et al. 1997* for details) provided Einkorn wheat samples. In total we obtained 1362 lines, then verified their taxonomic assignment and evaluated their agronomic performance. The collection sites for about 900 of the samples were provided by some gene banks. For the Fertile Crescent samples, as well as for most of the samples from Turkey, only lines for which the collection site was known within ± 5 km were considered. Outside the primary habitat of wild Einkorn, most lines are frequently known only by their country of origin. Moreover, since agriculture led to the spread of cultivated types, consideration of their sites of collection could be misleading. The geographic distribution of the *T. boeoticum* and *T. aegilopoides* lines present in our collection is in agreement with the distribution of wild Einkorn as published in Harlan and Zohary (*1966*). In their Fig. 3, the primary habitats of *T. boeoticum* are shown to include the Taurus-Zagros region from South-eastern Turkey through North-eastern Iraq into Western Iran (i.e., the Eastern half of the Fertile Crescent). *T. aegilopoides* grows wild mainly in the Balkans and Western Anatolia, where it occupies marginal habitats. In Central Anatolia and Transcaucasia the two wild Einkorns occur in marginal habitats together with cultivated Einkorns (*Zohary and Harlan 1966*). West of the Balkans, only cultivated Einkorns occur.

FORMING GROUPS

The *T. boeoticum* samples collected in the Fertile Crescent were divided into nine geographic groups

(A, B, C, D, E, G, H, I and L). All *T. aegilopoides* samples were included in the 'Aegi' group and the cultivated Einkorn in the 'Mono' group. To test for the monophyletic origin of the cultivated types, this last group was also separated into four subgroups based on their geographic origins (Central Europe, the Balkans, Mediterranean countries and Turkey). Figure 1 (from *Heun et al. 1997*) shows the sampling sites of the 338 Einkorns used for DNA fingerprinting. To reduce our collection to 338, samples were randomly chosen within the above mentioned 11 groups.

DNA FINGERPRINTING DATA

Amplified fragment length polymorphism (AFLP) markers were generated (*Vos et al. 1995*) for all 338 lines. A total of 288 stable and reliably readable AFLPs were scored for presence vs. absence. Different genetic distance estimates were used to construct several phylogenetic trees based on neighbor-joining and restricted maximum likelihood estimation methods. Almost identical topologies were detected by all methods employed. Finally, a consensus tree based on ten different tree-building procedures was obtained (see *Heun et al. 1997* for details).

WILD ANCESTORS OF CULTIVATED EINKORN

Figure 2A shows that the nine geographic groups of *T. boeoticum* collected in the Fertile Crescent can be distinguished genetically. Group D, originating from the Karacadağ Mountains in Southeast Turkey, is the most distant group. By adding the cultivated Einkorns (Mono) and the wild Einkorns from the Balkans (Aegi) to these nine groups, we obtained the results in Figure 2B. Cultivated Einkorn appears closely related to *T. aegilopoides*. Group D links 'Mono' and 'Aegi' with the remaining eight groups. This result is a major achievement, since for the first time cultivated Einkorns can be traced back to a group of wild Einkorns showing all the characteristics of a wild species, whereas the lines that grow wild in the Balkans show clear signs of domestication. It is concluded that *both T. monococcum* and *T. aegilopoides* are derived from group D wheats. Figure 2C clearly demonstrates the monophyletic origin of the cultivated Einkorn and strongly suggests that *T. aegilopoides* is a derivative of the cultivated forms. Group D is again positioned between *T. monococcum* and all other *T. boeoticum* forms. The second major result that emerges from our studies is that all group D lines were collected from a relatively small area on the slopes of the Karacadağ Mountains. A gradient ranging from high to very high relationships within the 19 representatives of group D is evident (Fig. 2F).

CONNECTING DNA STUDIES WITH ARCHAEOLOGY

The localisation of the origin of cultivated Einkorn to the Karacadağ Mountains stimulates questions concerning the human community which achieved this domestication: are there neighboring human settlements with signs of early Einkorn cultivation? It is known that Cafer Höyük, Nevali Cori and Cayönü are all located in the vicinity of these mountains. These are among the oldest settlements at which palaeontologists have found wild and domesticated Einkorn seeds in different horizons. In Table 2 of Nesbitt and Samuel (*1996*) all archaeological data relevant to the origin of agriculture are summarised. From these it becomes evident that the cultivation of Einkorn began between 7800 and 7500 BC in the settlements cited. At the excavated sites in the Jordan Valley mentioned by Jones et al. (*1998*), no decisive (concerning general identification problems see *Hillman et al. 1993*) earlier remains of cultivated Einkorn have been found (*Heun et al. 1998, Nesbitt 1998; Nesbitt and Samuel 1998*), emphasising the importance of the Northern Fertile Crescent in Einkorn domestication. In the case of other excavated sites, such as Abu Hureyra and Mureybit in Northern Syria, wild seeds of *T. boeoticum* seem to have not been collected locally (*Zeist and Casparie 1968; Zeist and Bakker-Heeres 1984*).

SUMMARY

Wild ancestors of cultivated Einkorn have been localized in the Karacadağ Mountains of Turkey. The archaeological evidence from neighboring excavations implies that Einkorn domestication was initiated there about 9500 years ago. The genetic data also indicate that the domestication event was monophyletic (see also *Zohary in press*) and that the cultivated lines differentiated to a limited extent (quickly achieved by domestication, *Hillman and Davies 1990*) during the spread of agriculture to Western Europe. *T. aegilopoides* is probably a feral form of the cultivated types which reached the Balkans as a result of the spread of agriculture.

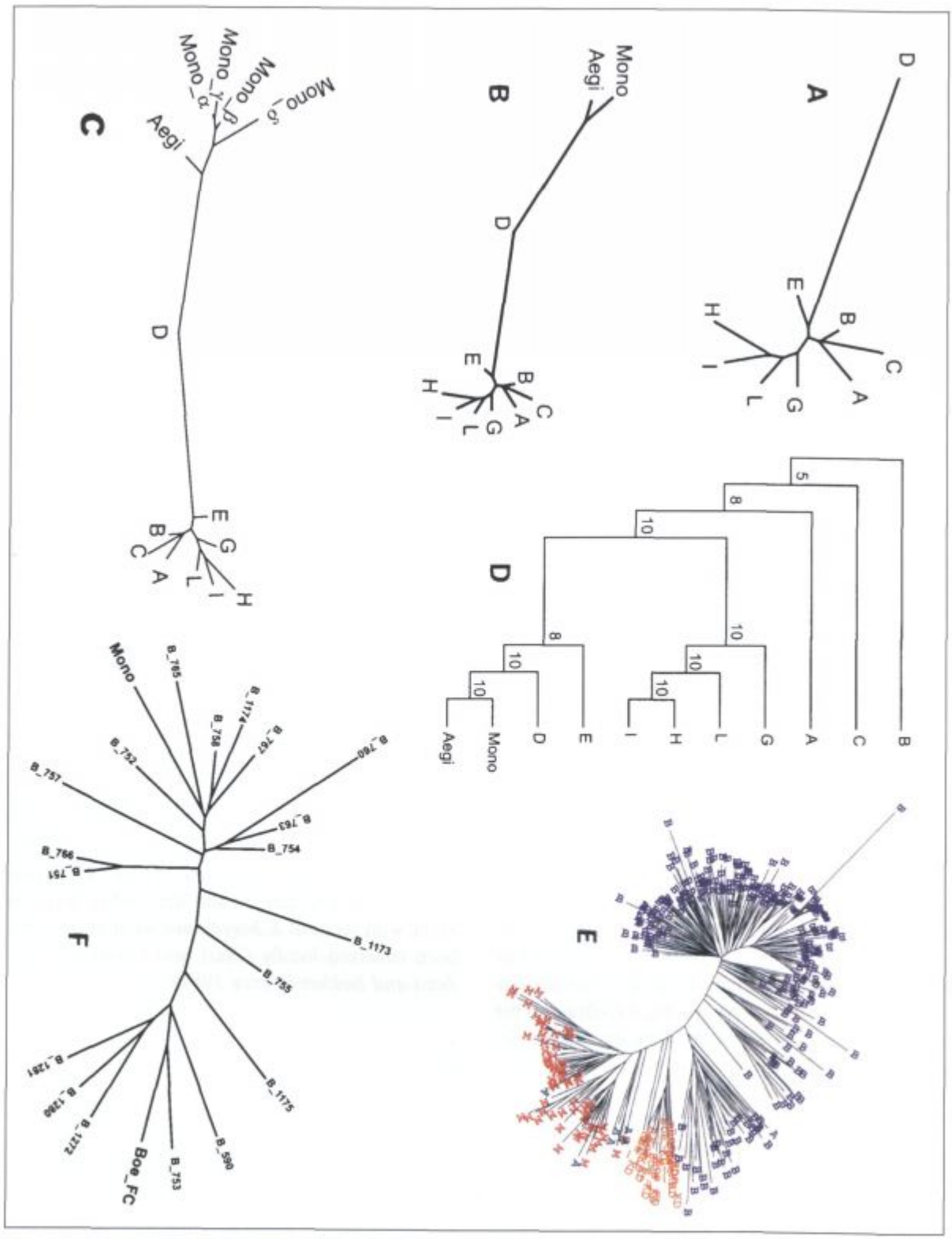

Fig. 2 A, B and C: Unrooted trees with the nine T. boeoticum *groups alone, with the same nine groups plus* T. monococcum *(Mono) and* T. aegilopoides *(Aegi) and the tree resulting from splitting up the Mono group into four distinct subgroups. D: Consensus tree summarising the results with the nine* T. boeoticum *groups and the groups Mono and Aegi. E: Unrooted tree with all fingerprinted lines. red: cultivated Einkorns, green:* T. aegilopoides, *orange:* T. boeoticum *from the Karacadağ, blue: remaining* T. boeoticum. *F: Unrooted tree for the 19 Karacadağ lines aligned to one consensus genotype of the remaining* T. boeoticum *and one consensus genotype of the cultivated Einkorn. For details on the tree building procedures see Heun et al. (1997). Reprinted with permission from Science, 14 November 1997, Volume 278, p. 1314, Fig. 2. © 1998 American Association for the Advancement of Science.*

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