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Site of Einkorn Wheat Domestication Identified by DNA Fingerprinting

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The emergence of agriculture in the Near East also involved the domestication of einkorn wheat. Phylogenetic analysis that was based on the allelic frequency at 288 amplified fragment length polymorphism molecular marker loci indicates that a wild group of *Triticum monococcum boeoticum* lines from the Karacadağ mountains (southeast Turkey) is the likely progenitor of cultivated einkorn varieties. Evidence from archeological excavations of early agricultural settlements nearby supports the conclusion that domestication of einkorn wheat began near the Karacadağ mountains.

Wild einkorn wheat, *Triticum monococcum* subsp. *boeoticum*, is the wild relative of the domesticated einkorn wheat *T. m. monococcum* (*1–3*). In the Near East, primary habitats of *T. m. boeoticum* occur in the northern and eastern parts of the Fertile Crescent (*2*). Archeological evidence points to this region as the area of einkorn domestication (*4*); however, it has been impossible to pinpoint the site of domestication (*3*). We have addressed this question by making two assumptions. The first is that genetic distances within a species can be evaluated by multiple, dominant DNA markers—in our study, amplified fragment length polymorphism (AFLP) fingerprinting (*5, 6*). The second assumption is that the progenitors of crop plants have not undergone significant genetic changes during the past 10,000 years (*4*). In the case of wild einkorn wheat, moreover, the available information indicates for the same period a geographical stability of its primary habitat (*3, 4, 7–12*). In addition, the domesticated einkorns cultivated in marginal areas (*13*) have been left untouched by modern plant breeding. Complications were anticipated, however, because wild einkorn has colonized secondary habitats (*2*) and because a weedy einkorn form (*T. m. aegilopoides*) occurs in the Balkans (*14*).

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In this study we characterized 1362 lines of Einkorn wheats for their agronomic and taxonomic traits. The areas of origin were known for 954 lines. Of these, 338 lines were chosen so as to ensure an even distribution in the area shown in Fig. 1. The 68 *T. m. monococcum* lines were from several countries, and the 9 *T. m. aegilopoides* lines were from the Balkans. The collection sites of the 194 *T. m. boeoticum* lines originating from the Fertile Crescent were known to within ±5 km. The 67 *T. m. boeoticum* lines collected outside the Fertile Crescent were from Turkey, the Caucasus mountains, and Lebanon. DNA from these 338 lines was fingerprinted on the basis of the presence versus the absence of 288 AFLP bands (*15*).

To identify the area where einkorn was domesticated, we assigned 194 lines of *T. m. boeoticum* to nine groups sampled in defined geographical areas of the Fertile Crescent (groups A, B, C, D, E, G, H, I, and L; see Fig. 1). The AFLP results were used to calculate genetic distances among the nine groups, and phylogenetic trees were constructed with different tree-building methods (*16*) and distance measures (*17*). All trees had almost identical topologies (*18*), as exemplified by the tree shown in Fig. 2A. The outcome of this analysis allows two conclusions. The first is that lines sampled within the same area are genetically more closely related than lines sampled in different locations. Indeed, the average genetic distance between lines of the same group is 23.4% smaller than that between the nine *T. m. boeoticum* groups of the Fertile Crescent. The second conclusion is that the group D (originating from the volcanic Karacadağ mountains, southeast Turkey, Diyarbakır district, and consisting of 19 *T. m. boeoticum* lines) is distinctly separated from the remaining groups.

The clustering approach was repeated considering 68 cultivated einkorns and 9 *T. m. aegilopoides* lines. The cultivated lines were from Mediterranean countries (group α), Central Europe (β), the Balkans (γ), and Turkey (δ). The trees obtained were similar to those shown in Fig. 2, B and C. The cultivated einkorns are closely related among themselves and to *T. m. aegilopoides*. Most importantly, both *T. m. monococcum* and *T. m. aegilopoides* show a close phylogenetic similarity to the *T. m. boeoticum* lines from the Karacadağ region. This finding is supported by the majority rule consensus tree shown in Fig. 2D. This result raises the question whether the Karacadağ lines of *T. m. boeoticum* should be considered the closest relatives of the wild progenitors that gave rise to cultivated einkorn about 10,000 years ago.

Before considering this possibility, we again tested if cultivated einkorn is monophyletic. The phylogenesis of the 388 lines studied (Fig. 2E) indicates that cultivated einkorn is indeed monophyletic. To deter-

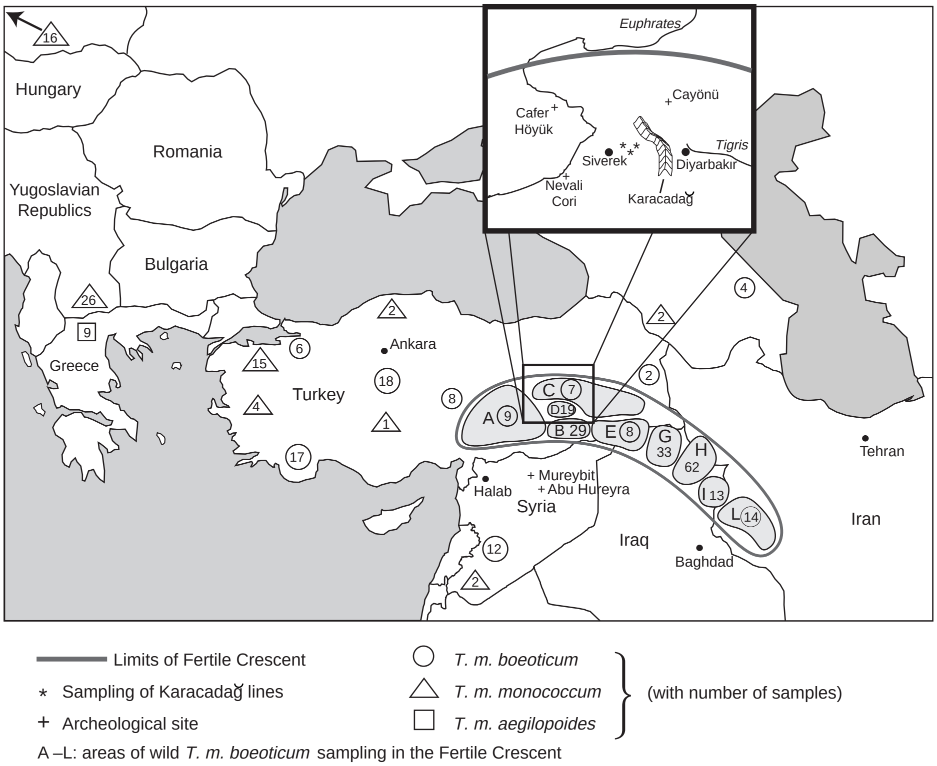

Fig. 1. Sampling sites of 338 einkorn wheat lines. Inset: the Karacadağ region. A, sites west of Lake Atatürk (Turkey); B, sites in the vicinity (±10 km) of the road Sanliurfa-Mardin (Turkey); C, mountainous area east of Elâziĝ and west of Siirt (Turkey); D, special site(s) described in Harlan and Zohary (*2*) corresponding to the Karacadağ mountains (Turkey); E, sites in the vicinity (±20 km) of the 160-km Mardin-Cizre road (Turkey and Syria); G, around Dahuk (±15 km) in Iraq; H, 20 to 30 km northeast of Arbī (Iraq); I, sites in Iraq around As-Sulaymānīyah (±10 km); L, sites in Iran from 20 km west to 90 km southeast of Bakhtarân.

mine whether other *T. m. boeoticum* lines from outside the Fertile Crescent are also closely related to *T. m. monococcum*, we fingerprinted 67 wild einkorns collected in secondary habitats (*19*). All lines (*18, 20*) could be associated with one of the Fertile Crescent groups described in Fig. 2A, but not with the Karacadağ cluster. In addition, the 19 Karacadağ lines were individually subjected to phylogenetic analysis (*16, 17*). As shown in Fig. 2F, 11 of these lines appear to be very closely related to *T. m. monococcum*, whereas the other eight are only moderately related with the remaining *T. m. boeoticum* lines [see also (*18*)].

The 11 Karacadağ *T. m. boeoticum* lines most closely related to cultivated einkorns show clear wild characteristics (Table 1). Although *T. m. aegilopoides* also shows a high degree of DNA relatedness with cultivated einkorns, it has evident signs of domestication (Table 1). Therefore, we conclude that *T. m. aegilopoides* is an intermediate form between *T. m. boeoticum* and *T. m. monococcum*. In contrast, the Karacadağ lines, although closely related to cultivated einkorn at the DNA level, show all traits of a wild einkorn progenitor.

We define the 11 Karacadağ lines as *T. m.* subsp. *boeoticum* form Karacadağ (*21*). These lines were collected in the Fertile Crescent in an area discussed by Harlan and Zohary (*2*). Close to the Karacadağ mountains (see inset in Fig. 1) are several archeological sites: Cafer Höyük [wild and cultivated seeds dated 7600 to 6200 bc (*7, 8*)], Cayönü [wild and cultivated seeds dated 7500 to 6700 bc (*9*)], and Nevali Cori [cultivated specimens dated 7200 bc (*10*)] are among the earliest agricultural settlements in the Near East. Also, the excavations at Abu Hureyra (*11, 12*), like those of Cafer Höyük, Cayönü, and Nevali Cori, show that farming of domesticated einkorn was being practiced in this region by 7800 to 7500 bc (*3, 4*). The data concerning *T. m. boeoticum* at the excavated Syrian sites of Abu Hureyra and Mureybit lead to the hypothesis (8) that wild seeds were gathered some distance away at Turkish Fertile Crescent sites. Hillman (*22*), however, has suggested that late Pleistocene climate may have supported the presence of wild *T. m. boeoticum* much nearer Mureybit and Abu Hureyra. The putative present contraction of the wild einkorn habitats should not, in any case, affect our conclusions.

In summary, the Karacadağ mountains are very probably the site of einkorn domestication. Localization of the precise domestication site of one primary crop does not necessarily imply that the human population living there at the end of the Paleolithic played a role in establishing agriculture in the Near East. Nevertheless, it has been hypothesized (*23*) that one single human group may have domesticated all primary crops of the region.

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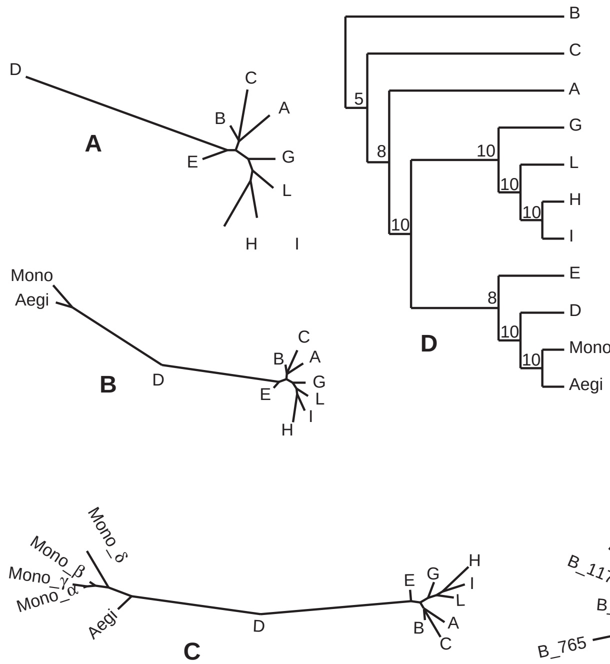
Table 1. Mean values (± standard error) of morphological traits of *T. m. boeoticum*, *T. m. monococcum*, and *T. m. aegilopoides.* Mean values for the same characters have been calculated for 11 lines of *T. m. boeoticum* originating in the Karacadağ region. NS, number of spikelets per spike. The rachis values indicate the following: 0 = tough; 1 = brittle; 2 = very brittle. AS, awns per spikelet: 0 = awnless; 1 = 1 awn, well developed; 2 = 1 awn, developed, and a short one on the second floret; 3 = 2 awns, equally well developed. SS, spikelets with two seeds: 0 = 75 to 100% ; 1 = 55 to 70%; 2 = 25 to 50%; 3 = 0 to 20%. SA I, characters measured in 1993 and 1994 in Sant'Angelo, Italy (SA II, 1994 measurement only); K, data from 1994, Cologne, Germany.

Species	No. of lines	NS	Seed weight (mg)		Rachis		AS	SS
		SA II	SA II	K	SA II	K	SA I	K
T. m. boeoticum	250	21.1 ± 0.2	21.1 ± 0.2	20.3 ± 0.2	1.60 ± 0.05	1.90 ± 0.02	2.50 ± 0.05	0.2 ± 0.03
T. m. monococcum	68	30.9 ± 0.6	30.2 ± 0.7	32.3 ± 4.8	0.03 ± 0.03	0.30 ± 0.05	1.00 ± 0.04	1.8 ± 1.21
T. m. aegilopoides	9	31.6 ± 1.7	22.9 ± 0.7	25.0 ± 1.2	0	1.14 ± 0.29	1.10 ± 0.14	0.7 ± 1.25
T. m. boeoticum form Karacadağ	11	20.8 ± 0.8	21.5 ± 0.9	19.8 ± 1.2	2.00 ± 0	2.00 ± 0	2.33 ± 0.14	0



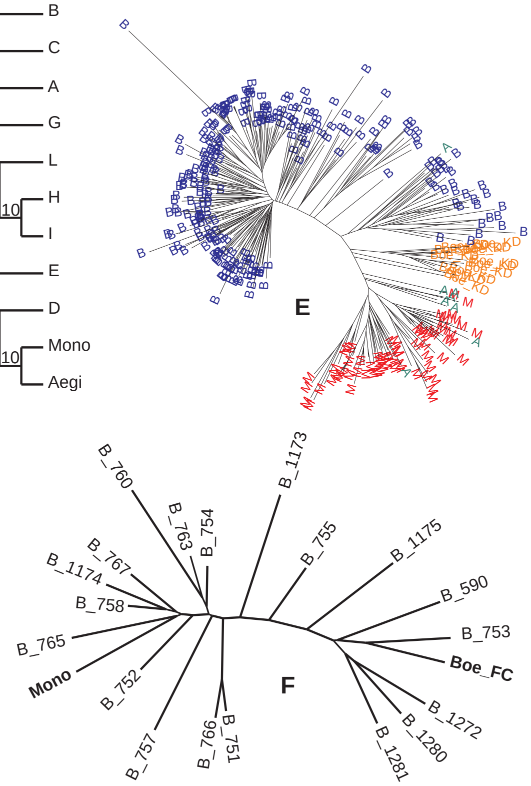


Fig. 2. (**A**) Unrooted tree based on Roger-W genetic distance (*27*) and the Fitch and Margoliash method available in the PHYLIP package (*25*) of *T. m. boeoticum* groups A, B, C, D, E, G, H, I, and L. (**B**) Unrooted tree calculated as in (A). To the nine *T. m. boeoticum* wild groups, two were added: Mono, 68 cultivated einkorns, and Aegi, 9 *T. m. aegilopoides* lines. (**C**) As in (B), but with the 68 cultivated einkorns divided into groups α, β, γ, and δ [based on NEI 72 (*26*) and Fitch and Margoliash (*25*)]. (**D**) Consensus tree summarizing the relative positions in 10 different phylogenetic trees (*16, 17*) of the *T. m. boeoticum* groups A, B, C, D, E, G, H, I, and L and of *T. m. monococcum* (Mono) and *T. m. aegilopoides* (Aegi). The numbers at the forks indicate the number of times that the assemblage, consisting of the groups that are to the right of that fork, occurred among the 10 trees considered. The tree was computed with the PHYLIP program according to Margush and McMorris (*29*). (**E**) Unrooted tree based on the data of all lines studied [DICE genetic distance (*30*) and NJ method (*24*)]. Red, cultivated einkorns; green, *T. m. aegilopoides*; orange, *T. m. boeoticum* form Karacadağ; blue, *T. m. boeoticum* not from Karacadağ. (**F**) Unrooted tree based on the DICE genetic distance (*30*) and the FITCH tree-building method (*25*). The tree considers 19 *T. m. boeoticum* lines sampled in the Karacadağ mountains (D in Fig. 1) and one consensus genotype each for all *T. m. monococcum* lines (Mono) and for *T. m. boeoticum* lines of the Fertile Crescent (Boe-FC; group D excluded). The two consensus genotypes were obtained by scoring as 0 gene frequencies smaller than 0.5 and the remainder as 1.

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16. Phylogenetic trees were created by neighbor-joining (NJ) (*24*), Fitch and Margoliash (FITCH) (*25*), and restricted maximum likelihood estimation (REML) methods [J. Felsenstein, *Evolution* **35**, 1229 (1981)].
17. Genetic distance NEI 72 was from (*26*); NEI-UB from M. Nei, *Genetics* **89**, 583 (1978); and Rogers-W from (*27*). Other distances are given in (*28*). The computer program NTSYS was used to calculate genetic distances (*28*). Trees (*16*) were generated as follows: NEI 72, NEI-UB, and Rogers-W with NJ and FITCH; average, euclidean, and euclidean squared distances with FITCH. PHYLIP (Phylogeny Inference Package) was used in computing trees and for the REML clustering method.
18. Additional results can be found at the Web site www.mpiz-koeln.mpg.de/~salamini/salamini.html. On the basis of the Wagner-Parsimony method, 100 phylogenetic trees were calculated for the lines of the Fertile Crescent. The output was used to determine the average mutation rate for each fragment. The data for those 10 or 20% of fragments having the highest mutability were eliminated and new trees computed. The results were almost identical to those discussed.
19. Nesbitt and Samuel (*3*) reported that in the Konya plain of Turkey, a wild *T. m. boeoticum* population exists outside of the Fertile Crescent. Two *T. m. boeoticum* lines sampled at a distance of 50 km from this site were not closely related to cultivated einkorn. Southern Lebanon *T. m. boeoticum* lines were also fingerprinted and did not show close relations with cultivated einkorns.
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21. The 11 Karacadağ lines were collected as follows: ID 754 (PI 427622), 757 (PI 427626), 758 (PI 427627), 760 (PI 427629), 763 (PI 427632), 765 (PI 427634), 766 (PI 427635), 767 (PI 427636), and 1174 (PI 538540), 52.2 to 52.5 km west of Diyarbakır at 1400 m elevation; and ID 751 (PI 427619) and 752 (PI 427620), 16 km east of Siverek at 1050 m elevation. Lines were collected by B. L. Johnson, University of California, Riverside, and independently by R. J. Metzger, U.S. Department of Agriculture (USDA). PI = plant introduction number, USDA, Agricultural Research Service, Aberdeen, ID, USA.
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