Molecular Diversity at 18 Loci in 321 Wild and 92 Domesticate Lines Reveal No Reduction of Nucleotide Diversity during *Triticum monococcum* (Einkorn) Domestication: Implications for the Origin of Agriculture

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The diploid wheat *Triticum monococcum* L. (einkorn) was among the first crops domesticated by humans in the Fertile Crescent 10,000 years ago. During the last 5,000 years, it was replaced by tetraploid and hexaploid wheats and largely forgotten by modern breeders. Einkorn germplasm is thus devoid of breeding bottlenecks and has therefore preserved in unfiltered form the full spectrum of genetic variation that was present during its domestication. We investigated haplotype variation among >12 million nucleotides sequenced at 18 loci across 321 wild and 92 domesticate *T. monococcum* lines. In contrast to previous studies of cereal domestication, we sampled hundreds of wild lines, rather than a few dozen. Unexpectedly, our broad sample of wild lines reveals that wild einkorn underwent a process of natural genetic differentiation, most likely an incipient speciation, prior to domestication. That natural differentiation was previously overlooked within wild einkorn, but it bears heavily upon inferences concerning the domestication process because it brought forth 3 genetically, and to some extent morphologically, distinct wild einkorn races that we designate here as α, β, and γ. Only one of those natural races, β, was exploited by humans for domestication. Nucleotide diversity and haplotype diversity in domesticate einkorn is higher than in its wild sister group, the einkorn β race, indicating that einkorn underwent no reduction of diversity during domestication. This is in contrast to findings from previous studies of domestication history among more intensely bred crop species. Taken together with archaeological findings from the Fertile Crescent, the data indicate that a specific wild einkorn race that arose without human intervention was subjected to multiple independent domestication events.

Introduction

Archaeological evidence indicates that western agriculture began in the Near East with the remains of founder crops preserved at several excavated sites throughout the region known as the Fertile Crescent (Heun et al. 1997; Moore et al. 2000; Zohary and Hopf 2000; Gopher et al. 2001; Salamini et al. 2002). Beginning about 12,000 years ago, the size and morphology of archaeologically preserved einkorn seeds (*Triticum monococcum*, genome AA with $2n = 14$) changed, with the smaller seeds of wild einkorn (*Triticum monococcum* ssp. *boeoticum*, *T.m.b.*) gradually being replaced at excavated sites by the larger seeds of its domesticate form (*Triticum monococcum* ssp. *monococcum*, *T.m.m.*) (Zohary and Hopf 2000) and genetic data for einkorn are consistent with that view (Heun et al. 1997; Abbo et al. 2006). In crop grasses of the Fertile Crescent—wheats, barley, and rye—domestication is currently thought to mainly involve allele frequency changes at loci governing seed size, rachis stiffness, and bract morphology (Salamini et al. 2002), but the mechanisms through which humans evoked those morphological changes are still debated.

Evidence from archaeological excavation sites indicates that the process of crop domestication spanned up to 1,000 years and entailed multiple domestication events (Hillman and Davies 1990; Willcox 1999; Kislev 2002; Salamini et al. 2002; Tanno and Willcox 2006). Such evidence stands contrary to molecular studies that have suggested a single domestication of each of the Fertile Crescent founder crops (Heun et al. 1997; Badr et al. 2000; Zohary and Hopf 2000). A third view of domestication suggests that superior varieties emerged in a "core area" and were then dispersed throughout the region, displacing local genotypes (Lev-Yadun et al. 2000; Salamini et al. 2002; Abbo et al. 2006). All of these models predict a reduction of genetic diversity in domesticate forms relative to the wild progenitors through a domestication bottleneck—a reduction in genetic diversity stemming from human selection upon domestication traits—as reported in various domesticated species (Pozzi et al. 2004; Doebley et al. 2006; Kilian et al. 2006). However, distinguishing between reduction of genetic diversity through a domestication bottleneck introduced over 10,000 years ago and reduction through intensive breeding bottlenecks during the last few hundred years is extremely problematic (Kilian et al. 2006), and domestication genetic studies of Old World crops that escaped intense breeding are lacking.

Einkorn is unique in this respect because it was of limited agricultural use during the last 5,000 years: the crop was largely abandoned as a food source starting in the Bronze Age and intensive breeding was never undertaken (Salamini et al. 2002). As a consequence, domesticate einkorn germplasm sampled in remote mountain areas across Europe and the Near East (Perrino et al. 1996) should harbor a representative sample of Neolithic genetic variation that was present during domestication in the Fertile Crescent and that was later dispersed by migrating farmers (Nesbitt and Samuel 1996). Given the absence of breeding bottlenecks in einkorn, we reasoned that extensive sampling of genetic diversity among wild and domesticate

Key words: evolution, molecular diversity, haplotypes, einkorn wheat, Fertile Crescent.

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Mol. Biol. Evol. 24(12):2657–2668. 2007
doi:10.1093/molbev/msm192
Advance Access publication September 26, 2007

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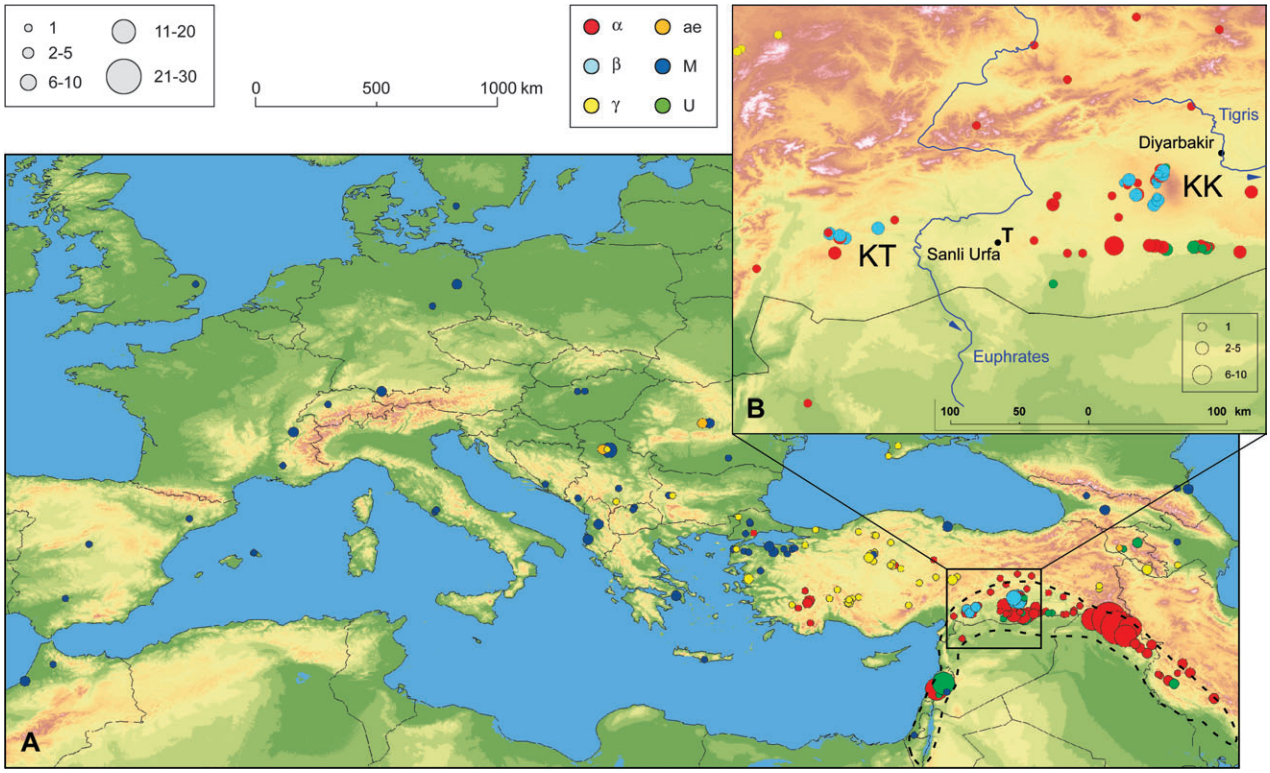


FIG. 1.—Natural distribution and region of einkorn domestication. (*A*) Geographical information system–based overview of the collection sites for accessions sequenced in the present study (supplementary table S2, Supplementary Material online). Race assignments from genetic analyses are color coded: red, *Triticum monococcum* ssp. *boeoticum* (*T.m.b.*) race α; light blue, *T.m.b.* race β; yellow, *T.m.b.* race γ; orange, feral form *Triticum monococcum* ssp. *aegilopoides* (*T.m.ae.*) (ae); blue, domesticate *Triticum monococcum* ssp. *monococcum* (*T.m.m.*) (M); and green, *Triticum urartu* (U). The Fertile Crescent is indicated with a dotted line. Numbers of individuals collected at specific sites are indicated by the key at upper left. (*B*) Enlargement of the region where *T.m.b.* race β occurs. Colors as in (*A*). KK, Karacadag; KT, Kartal–Karadag; T, Göbekli Tepe archaeological site (Schmidt 2001). For 55 lines (3 *T.m.b.* race α, 7 *T.m.ae.*, 39 *T.m.m.*, and 6 *T. urartu*) obtained from seed banks, the precise collection site is unknown, and the capital city of the source country was arbitrarily chosen as collection site.

accessions should discriminate between competing hypotheses for cereal domestication. Here, we report nucleotide variation at 18 loci for 92 domesticate einkorn lines in comparison to 321 lines from wild populations and a genetic view of crop domestication that is unbiased by green revolution breeding.

Materials and Methods

Plant Material

Details of collection sites, taxonomic designations, seed sources, and seed bank accession numbers for the 603 lines used for amplified fragment length polymorphism (AFLP) analysis in figure 2*A* are given in supplementary table S1 (Supplementary Material online). The same information for the 452 lines used for amplification and sequencing at 18 loci are given in supplementary table S2 (Supplementary Material online).

AFLP Analysis

DNA was isolated from freeze-dried or silica-dried leaves using the Qiagen (Hilden, Germany) DNeasy Kit and amplified as described by Zabeau and Vos (1993) using the following primer combinations described in Heun et al. (1997): E_{ACC}/M_{ACC} (E36/M36), E_{ACG}/M_{AGC} (E37/M40), E_{AGC}/M_{ACT} (E40/M38), E_{AGC}/M_{AGC} (E40/M40), E_{AGT}/M_{AAC} (E42/M32), E_{AGT}/M_{AAG} (E42/M33), and E_{AGT}/M_{ACT} (E42/M38). The AFLP bands were scored as binary data. NeighborNet planar graphs of AFLP Dice distances (Dice 1945) between individuals were constructed with SplitsTree 4.6 (Huson and Bryant 2006).

Multilocus Genotype Analysis

DNA was prepared as for AFLP analysis. Polymerase chain reaction (PCR) amplifications were performed in 25 μl containing ~100 ng of single-leaf DNA, 0.4 μM of each primer, 125 μM of each deoxynucleoside triphosphate (AB gene, Surrey, United Kingdom), 3 mM $MgCl_2$, 4% dimethyl sulfoxide , and 1 unit of *Taq* DNA polymerase incubated in a PTC-225 Tetrad Thermal Cycler (MJ Research). The loci are described in supplementary table S3 (Supplementary Material online). Primers and specific amplification conditions for each locus are given in supplementary table S4 (Supplementary Material online). PCR products were sequenced on both strands. Sequence data were processed with Applied Biosystems DNA Sequencing Analysis Software 5.1.1 and manually edited with BioEdit version 7.0.5.3 (Hall 1999). Alignments were generated

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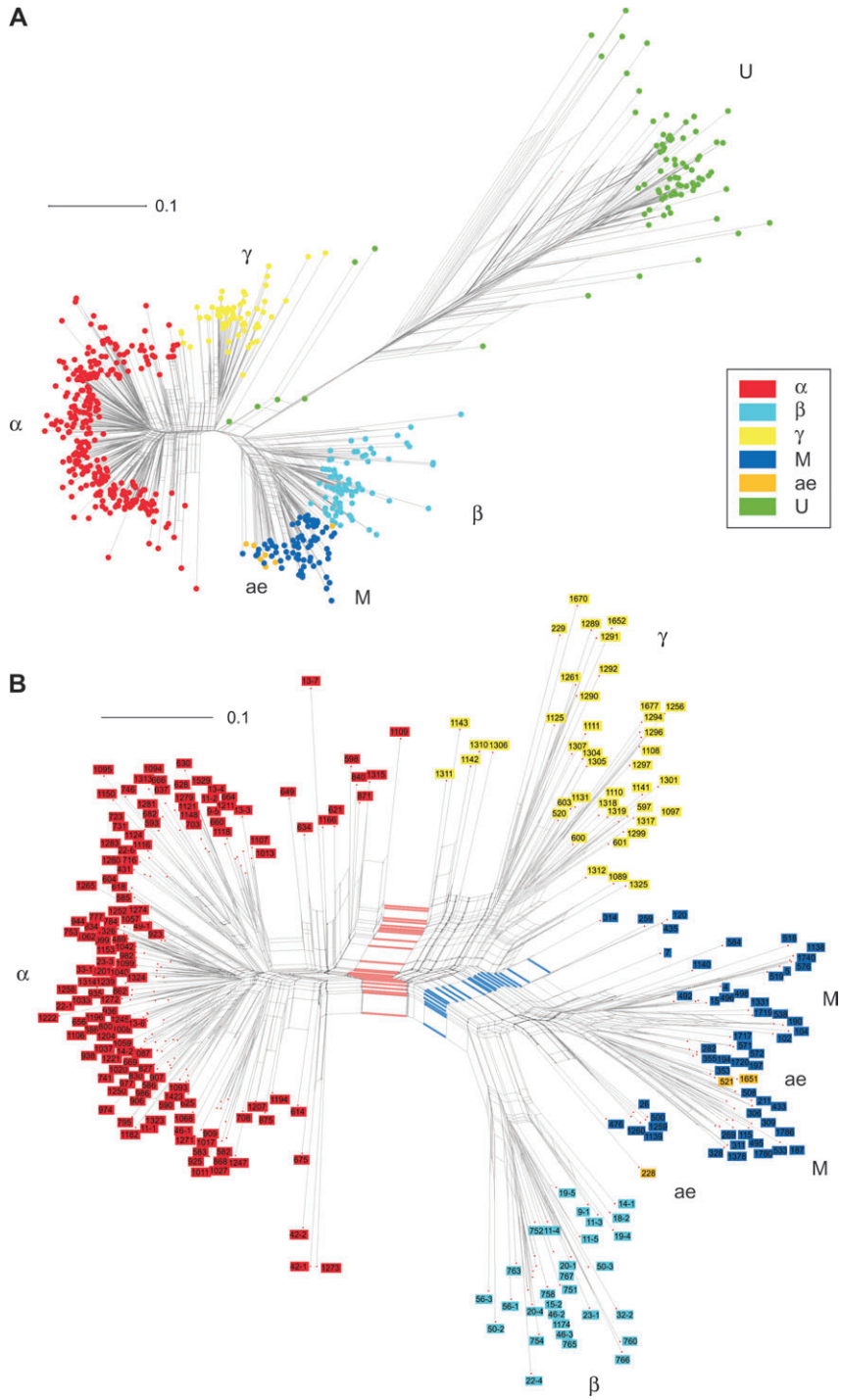


FIG. 2.—Genetic diversity among einkorn wheats and its sister species, *Triticum urartu*. (*A*) Phylogenetic network of 603 wheat individuals based on 151 AFLP chromosome markers. Details of the 603 *Triticum* lines used in this study are listed in supplementary table S1 (Supplementary Material online). The AFLP data circumscribe 436 wild *Triticum monococcum* ssp. *boeoticum* (*T.m.b.*) lines (races α, β, and γ), 70 domesticated *Triticum monococcum* ssp. *monococcum* (*T.m.m.*) (M) lines, 7 *Triticum monococcum* ssp. *aegilopoides* (ae) lines, and 90 *T. urartu* (U) lines. (*B*) NeighborNet of 267 nonredundant multilocus genotypes among 452 individuals (supplementary table S2, Supplementary Material online) based on haplotypes at 16 sequenced nuclear loci (without *Lr10*) and the chloroplast locus *ndhF* that is monomorphic in *T. monococcum*. Splits separating the α and β races from γ are highlighted in red and blue, respectively. The position of *T.m.m.* accession 314 groups stably within the β/M race in (*A*), but lies outside the split uniting race β because it carries a rare allele (haplotype X) at the *pinB* locus that is otherwise characteristic for the race α (see also fig. 5*E*).

with ClustalW, and haplotypes were scored manually. Haplotypes were coded as discrete character data. NeighborNet planar graphs (Huson and Bryant 2006) of Hamming distances between 267 nonredundant multilocus genotypes among 452 individuals were constructed based on haplotypes at 16 sequenced nuclear loci (without *Lr10*) and the chloroplast locus *ndhF*. GenBank accession numbers for the haplotypes determined at all 18 loci are reported

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Table 1
Overview of Haplotypes Found at 18 Loci among 452 Lines Sequenced

SNPs in						Number of genotype-specific haplotypes						
Locus	Exon[a]	Intron[a]	Exon[b]	Intron[c]	Indels[d]	*N*[e]	α	γ	ß	M	ae	U
BAMY1	255	214	4	9	2 (10)	11	1	3				3
GAPDH	300	261	0	3	0 (0)	4	1					1
ACC1	411	393	2	5	4 (55)	7	2	1				1
PGK1	441	275	6	15	1 (9)	5			2			2
AGPL	454	555	16	48	9 (15)	15	4	3	2	1		4
CesA	554	215	2	1	0 (0)	4			1			3
6SFT	87	787	1	34	5 (51)	18	6	3	1	1	1	2
BADH	177	369	6	21	3 (16)	5		2	1			4
PEPC	446	348	1	0	0 (0)	2	1					1
G6PDH	387	362	0	1	1 (1)	3						1
GPT	593	80	1	0	0 (0)	2		1				2
pinB	436	162[g]	11	4	0 (0)	12				1		1
ndhF[f]	719	0	0	0	0 (0)	1						1
GPX	308	356	7	7	2 (4)	7	1	1				2
Lr10[h]	0	709	0	23	3 (21)	11	1	1		1		3
RGA2	600	0	41	0	0 (0)	16	2	2		1		1
Q	228	688	0	12	3 (5)	9	2	1				4
VRN1	189	432	3	2	2 (7)	9	2		3			1
Total	6585	6206	101	185	35 (194)	141	23	18	10	5	1	37

[a] Number of nucleotide positions in exons and introns in the alignment of 452 lines including *Triticum urartu*.
[b] Single nucleotide differences within the exon regions only, excluding *T. urartu*.
[c] Single nucleotide differences within the intron regions only, excluding *T. urartu*.
[d] Number of insertions/deletions with number of bp involved in parentheses.
[e] Number of einkorn haplotypes, gapped sites considered but excluding haplotypes specific to *T. urartu*.
[f] Chloroplast locus.
[g] 5′ untranslated regions region rather than intron.
[h] The *Lr10* locus is empty in over 296 lines sampled.

in supplementary table S5 (Supplementary Material online). Nucleotide diversity (π), haplotype diversity (Hd), and Watterson (θ) (Watterson 1975, eq. 1.4a, but on base pair basis; Nei 1987, eq. 10.3) were calculated using DnaSP version 4.10.9 (Rozas et al. 2003).

Topographic Map

Void-filled seamless Shuttle Radar Topographic Mission (SRTM) data V1, 2004 (International Centre for Tropical Agriculture), available from the CGIAR-CSI SRTM 90 m Database (http://srtm.csi.cgiar.org) were used to draw the topographic map. GPS coordinates for collected and seed bank material are given in supplementary tables S1 and S2 (Supplementary Material online).

Median-Joining Networks

Median-Joining (MJ) networks (Bandelt et al. 1999) were constructed with the Network 4.2.0.1 program (Fluxus Technology Ltd., Clare, Suffolk, UK).

Morphological Character Analysis

Heading date, stem plus ear length excluding awns, and leaf sheath-to-ear tip distance for 341 lines (α [204 lines], β [11], γ [45], ae [6], M [70], and U [5]) were determined for plants grown in Cologne, Germany, and San Angelo Lodigiano, Italy.

Statistical Analysis

Because none of the morphological characters fitted a normal distribution (using Kolmogorov–Smirnov test for normality), we were restricted to use nonparametric statistical tests in our analysis. The distributions of the morphological characters were compared among the haplotypes by using the Wilcoxon nonparametric test (Zar 1999). In addition, we used the Bonferroni correction for multiple comparisons, that is, for 3 characters we used $\alpha = 0.05/3 = 0.0167$ for the 95% significance level. Because nucleotide diversity also did not follow a normal distribution, we used the Friedman nonparametric test (Friedman 1937) to compare its distribution within loci among the different haplotypes.

Results and Discussion

Natural Diversification and Distinct Wild Races

We first investigated at 151 AFLP loci 603 diploid *Triticum* lines including 436 wild *T.m.b.* lines that cover the full range of wild einkorn dispersal (fig. 1), plus 70 domesticate *T.m.m.* lines, 7 *Triticum monococcum* ssp. *aegilopoides* (*T.m.ae.*) lines (*T.m.ae.* feral forms of *T.m.m.*), and 90 lines of *Triticum urartu* (the closest outgroup to *T. monococcum* within the genus). That coarse-grained, but genome-wide, survey uncovered an unexpected level of natural genetic differentiation among morphologically wild einkorn (fig. 2*A*). Genetic differentiation among these wild einkorn races, which we designate here as α, β, and γ,

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is not readily attributable to geographical separation (allopatry): although race α predominates in the Fertile Crescent and γ predominates in western and northwestern Turkey, the wild races have overlapping ranges (fig. 1*A*), with race β occurring only in restricted areas of the Karacadag (KK) and Kartal–Karadag (KT) mountains but together with race α (fig. 1*B*). At these 151 AFLP loci, race β is more closely related to domesticate einkorn (*T.m.m.*) and to its feral form (*T.m.ae.*) from the Balkan Peninsula than the other wild races are (fig. 2*A*).

To get a more detailed picture, we investigate haplotypes for 321 wild *T.m.b.* lines representative for genome diversity at AFLP loci, 84 domesticate lines (European, Asian, and Northern African collections), 8 *T.m.ae.* lines from Southeast Europe, and 39 lines of *T. urartu*. For each line, we sequenced 17 nuclear loci and 1 chloroplast locus (table 1), uncovering 415 single nucleotide polymorphisms (SNPs) and between 2 (*ndhF*) to 20 (*6SFT*) distinct haplotypes per locus within all lines sampled (including *T. urartu*). Einkorn is a typical inbreeder, but occasional outcrossing is observed (Zohary and Hopf 2000), also in the present data. Among 452 lines, 425 were homozygous at all loci sampled, only 4 were heterozygous at more than 2 loci, and 99.4% of all loci sampled were homozygous. In total, we surveyed >15,500 alleles amounting to >12 Mb of sequence data. Among the 452 lines, 322 contained unique haplotype combinations. The wild races α, β, and γ are distinct and are characterized by 23, 10, and 18 race-specific haplotypes, respectively (table 1). *Triticum urartu*, which can be morphologically distinguished from einkorn only in anther length, flowering time, and leaf trichomes (Morrison 1993), harbors an even more distinct haplotype collection. These distinctions are reflected in the network of multilocus genotype differences shown in figure 2*B*.

The somewhat intermediate position of race γ between races α and β in the AFLP and multilocus genotype networks of figure 2 might tend to suggest that it could be an α–β hybrid. However, the presence in race γ of 18 haplotypes that are specific to race γ and that are absent in races α and β (table 1) indicates the contrary. The distributions of 3 phenotypic characters (heading date, stem plus ear length excluding awns, and leaf sheath-to-ear tip distance) also distinguish race γ from α and β at $P << 0.01$ using the Wilcoxon nonparametric test with Bonferroni correction for multiple comparisons (fig. 3). We also found higher levels of haplotype and nucleotide diversity across loci in race γ than in race α (table 2). Race γ is thus the genetically most diverse of the 3 wild races. It is distinct from α and β, and it is morphologically wild, in line with the view that the natural dispersal range of *T.m.b.* includes western Turkey (Harlan and Zohary 1966; Johnson and Dahliwal 1976; Zohary and Hopf 2000) as indicated in figure 1. *Triticum urartu* has far lower nucleotide diversity than einkorn wheat (table 2).

Taken together, the AFLP and haplotype data indicate that the wild α, β, and γ *T.m.b.* races are undergoing natural genetic diversification and that the process of genetic isolation has gone nearly to completion in the case *T. urartu*. The significance of this natural diversification is that it appears to have brought forth the β race upon which einkorn

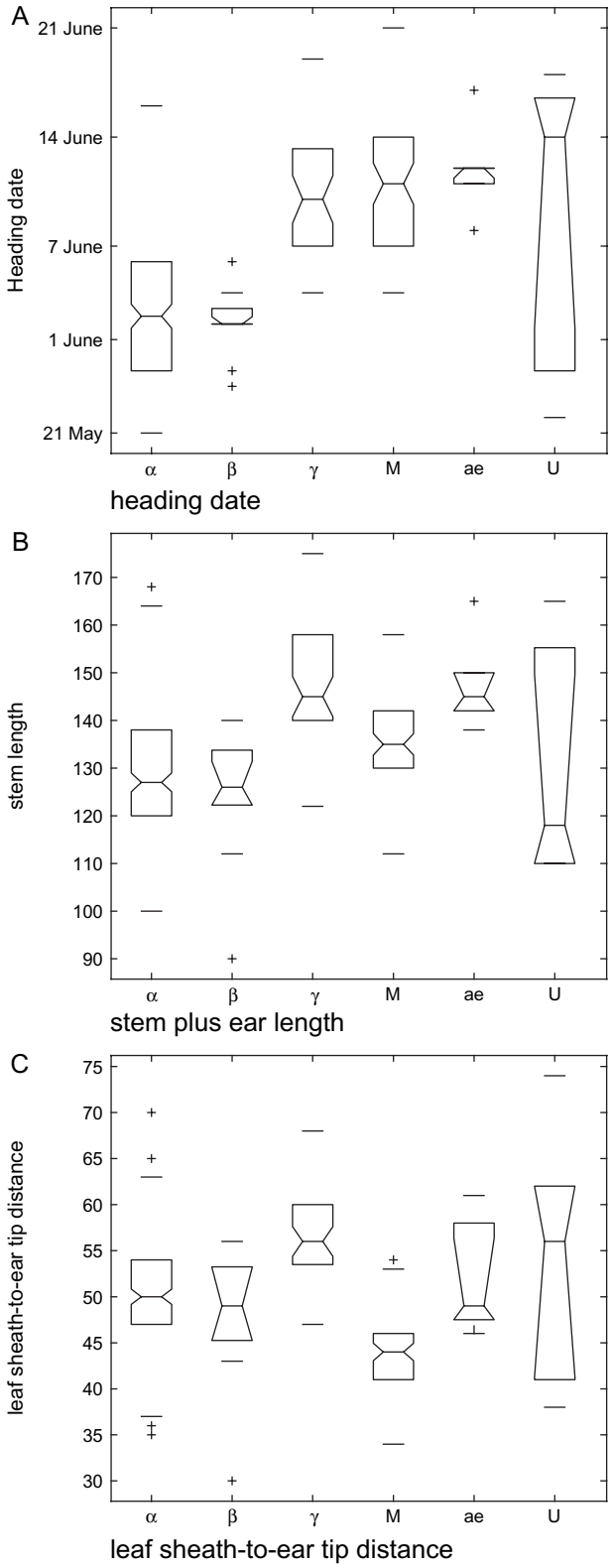


FIG. 3.—Distribution of 3 phenotypic characters that distinguish genotype γ from α and β at $P << 0.01$ using the Wilcoxon nonparametric test with Bonferroni correction for multiple comparisons (Zar 1999) (*A*) heading date, (*B*) stem plus ear length excluding awns, and (*C*) leaf sheath-to-ear tip distance. The boxes have lines at the lower quartile, median, and the upper quartile of the data. The whiskers show the extent of the rest of the data. Outliers are marked with a "plus."

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Table 2
Haplotype and Nucleotide Diversity in Wild and Domesticate Lines

Species/ssp/race	n^a	H^b	Hd[c]	π_{tot}[d]	π_{sil}[d]	θ_{tot}[d]	θ_{sil}[d]
Triticum monococcum ssp. *boeoticum* (*T.m.b.*)	321	114	0.380	4.73	8.51	3.45	4.29
T.m.b.–α	230	77	0.242	3.48	6.37	2.72	3.23
T.m.b.–γ	49	75	0.362	4.59	7.81	4.20	5.27
T.m.b.–β	42	43	0.260	1.56	2.70	2.05	2.15
Triticum monococcum ssp. *monococcum*	84	61	0.278	2.97	5.44	2.45	2.89
Triticum monococcum ssp. *aegilopoides*	8	35	0.353	3.75	6.35	3.45	3.42
Triticum urartu	39	35	0.276	0.53	0.92	0.66	0.96

[a] Number of lines.
[b] Number of haplotypes found (gapped sites and the *Lr10* locus excluded).
[c] Nei's (1987) unbiassed estimate of haplotype diversity for inbreeding species.
[d] Values of nucleotide diversity Pi (π) and Waterson's estimator (θ) are given ($\times 10^3$); Hd, Haplotype diversity for selfing species (Nei 1987); π_{tot}, average number of nucleotide differences per site between 2 sequences calculated on the total number of polymorphic sites; π_{sil}—average number of nucleotide differences per site between 2 sequences calculated on the silent sites (synonymous and noncoding positions); θ_{tot}—Waterson's estimator per site calculated on the total number of polymorphic sites; θ_{sil}—Waterson's estimator per site calculated at silent sites.

domestication at the origin of agriculture in the Fertile Crescent was built.

Nucleotide diversity, π, varies by orders of magnitude and in a locus-specific manner in the present einkorn data (fig. 4). This is in contrast to the observations in maize (Tenaillon et al. 2004), which is an outbreeder, but consistent with observations in another selfing species, *Arabidopsis thaliana* (Schmid et al. 2005). As seen in MJ networks (fig. 5), for loci with low variability, 1 (*VRN1* and *BADH*, fig. 5*K* and *P*) or 2 (*ACC1* and *CesA*, fig. 5*C* and *H*) major haplotypes predominate, with a divergent haplotype present in *T. urartu*. For more variable loci, such as *pinB*, haplotypes differing by 10–20 nt differences or more are distributed across all races, with *T. urartu* remaining distinct (fig. 5*I*). For *RGA2*, the most variable locus, haplotypes differing by more than 40 nt differences are dispersed across wild and domesticate einkorn, with *T. urartu* remaining nearly monomorphic (fig. 5*R*). Taken together, the haplotype data indicate that these inbreeding wild einkorn races have sequestered haplotype diversity present in their common ancestor that this diversity has been vertically preserved in the modern forms and that a severe population bottleneck (of irrelevant nature in the present context) has eliminated allelic diversity in the wild outgroup *T. urartu*. In *T. urartu*, genetic diversity is primarily determined by the fixation of newly arisen mutations at ancestrally monomorphic alleles. By contrast, the α, β, and γ einkorn races are distinct by virtue of unique haplotype combinations.

The Wild Sister of Domesticate Einkorn

The relationship between *T.m.b.* race β and domesticate einkorn is important for understanding einkorn domestication. In principle, there are 3 possibilities among which to discriminate: a sister-group relationship, a β-progenitor/*T.m.m.*-descendant relationship, and a *T.m.m.*-progenitor/β-descendant relationship (in which case β could potentially constitute a feral form). In the AFLP and multilocus genotype networks, *T.m.m.* clusters are adjacent to but not within wild race β (fig. 2). This suggests a sister-group relationship, consistent with the finding that 5 haplotypes are specific to domesticate einkorn, whereas no haplotypes are uniquely shared by race β and *T.m.m.* (table 1). In a β-progenitor/*T.m.m.*-descendant relationship, *T.m.m.* should cluster within race β, both in AFLP analysis and at the level of haplotypes; were race β a feral form of domesticate einkorn, then the former should cluster within the latter, as is

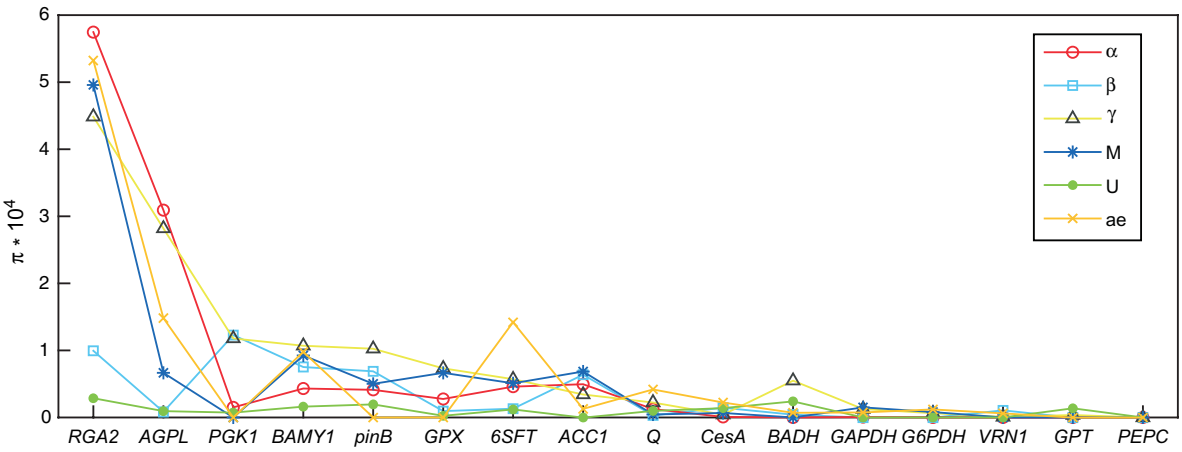


FIG. 4.—Nucleotide diversity at silent sites across sampled loci (except *Lr10*) from *Triticum monococcum* ssp. *boeoticum*. α, β, and γ races are *Triticum monococcum* ssp. *aegilopoides*, *Triticum monococcum* ssp. *monococcum*, and *Triticum urartu*, respectively. Taxon designations as in figures 1 and 2. Note the low diversity in *T. urartu* (U) and lack thereof in domesticate einkorn (M).

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observed in the case of the known feral form, *T.m.ae.* (fig. 2). But neither of those patterns is observed. Furthermore, despite extensive sampling of wild habitats, race β was so far found only in the KK and KT mountain ranges (fig. 1), a region within or at the border of the core area defined by Lev-Yadun et al. (2000) in the upper part of the Tigris–Euphrates valleys. The absence of *T.m.ae.*-like lines in the KK and KT regions, the distinctly wild phenotype of race β (as opposed to the recognizably feral phenotype of *T.m.ae.*), the sister relationship of race β and domesticated forms in figure 2, and the presence of 10 haplotypes specific to race β (table 1), together indicate that race β is not a feral form of *T.m.m.* and not the direct progenitor of *T.m.m.* Rather, the wild race β is the sister of domesticate einkorn. It currently possesses a very restricted dispersal range within the KK and KT regions (fig. 1), which are characterized by basaltic soils—a habitat factor that might figure into einkorn domestication, given that wild *T. urartu* is known to prefer basaltic soils (Zohary and Hopf 2000).

No Reduction of Genetic Diversity in Domesticate Einkorn

Previous studies of crop domestication among various species have reported evidence for reduction of genetic diversity attributed to domestication (domestication bottlenecks) (Buckler et al. 2001; Wright et al. 2005; Doebley et al. 2006). Although we do observe a bottleneck effect in the wild *T. urartu* outgroup, we find no reduction of genetic diversity in domesticate einkorn. On the contrary, nucleotide diversity within *T.m.m.* is greater than within its wild sister, race β. Across the 17 nuclear loci present in nearly all lines sampled—excluding *Lr10*, which is an empty locus in 296 lines (Isidore et al. 2005)—silent-site nucleotide diversity, π, varies in a locus-dependent, rather than in a race- or lineage-dependent manner (fig. 4). Using the Friedman nonparametric test (Friedman 1937) for comparison of medians while accounting for locus-dependency, the distributions of π for wild races and domesticate einkorn are not different ($P = 0.129$). There is thus no significant reduction of polymorphism in domesticated einkorn in comparison to its wild relatives, including race β. The same is reflected at the level of haplotype diversity detected (table 2). Nucleotide diversity across loci (π_{tot}) also uncovers no reduction of diversity, because π_{tot} in domesticate einkorn is higher than that in race β (table 2), but π_{tot} primarily reflects π at the most polymorphic loci only.

We calculated the loss of diversity, $L_\pi = 1 - (\pi_{domest}/\pi_{wild})$ (Tenaillon et al. 2004), at individual loci for domesticate versus wild einkorn accessions (and *T. urartu* for comparison); the results (table 3) show the lack of diversity loss in domesticate einkorn. In the comparison of *T.m.m.* to *T.m.b.* race β, π underwent no reduction during domestication. Rather, it is higher in *T.m.m.* than in the wild sister at 10 of the 18 loci sampled (indicated by negative values of L_π in table 3). At only 2 loci (*PGK1* and *VRN1*) is a reduction of π in *T.m.m.* observed in comparison to all 3 wild races but in both cases owing to a monomorphic *T.m.m.* locus. By comparison, 4, 2, and 6 loci are monomorphic among the α, γ, and β races, respectively. In 31/54 comparisons of *T.m.b.* races to *T.m.m.*, either no loss of diversity or an increase in *T.m.m.* is observed.

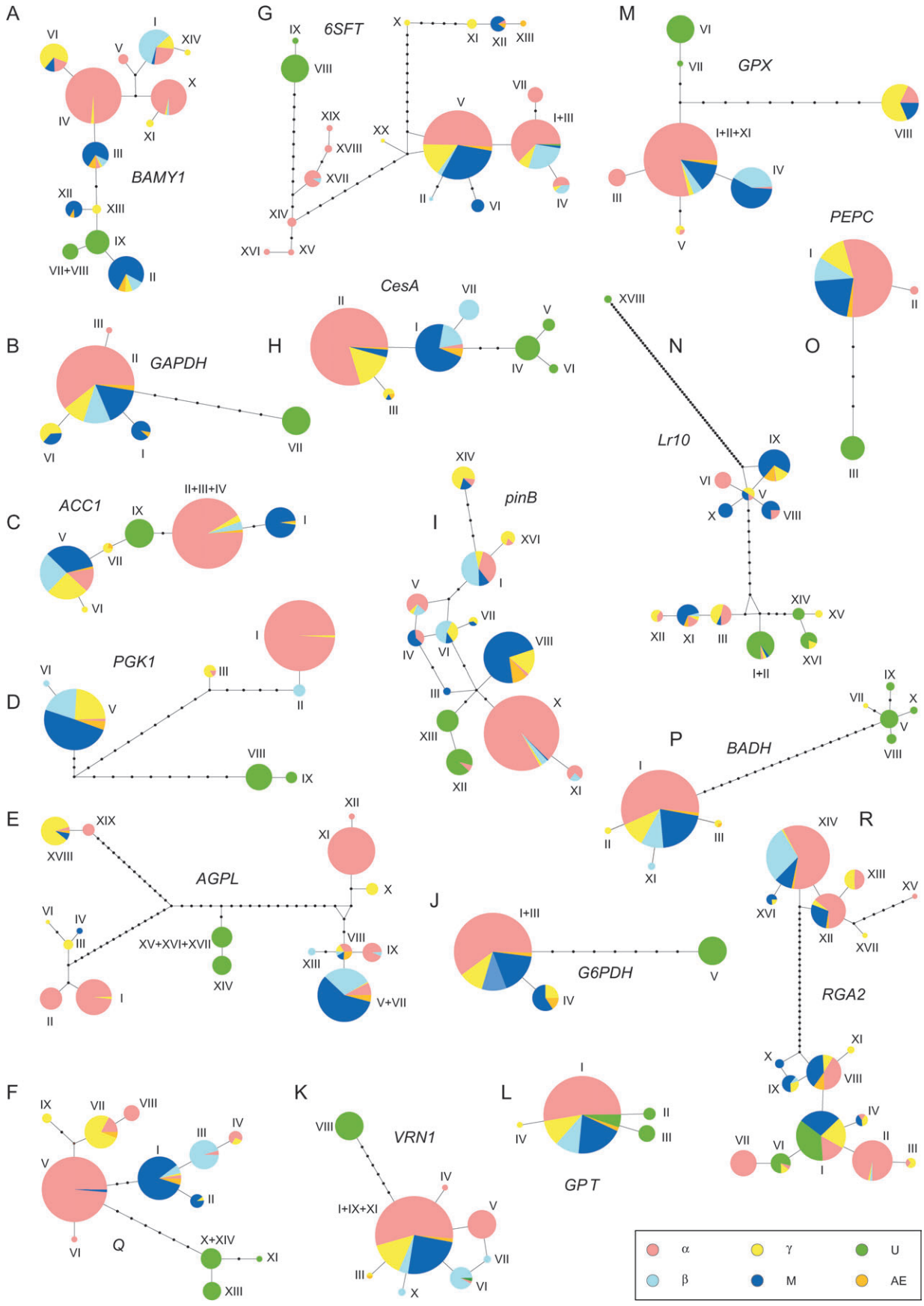
Had we mistakenly grouped the α, β, and γ races together as a single uniform *T.m.b.* "boe" gene pool for the calculation of diversity loss, we might have gained the impression that a loss of diversity had occurred at some loci during domestication (table 3). But through sampling hundreds, rather than dozens, of wild lines, the data reveal that einkorn domestication did not involve random sampling of wild diversity, rather it entailed a specific and naturally preexisting race instead. Table 4 summarizes several previous studies of plant domestication where evidence for domestication bottlenecks was reported, but in most cases, comparatively few wild lines were investigated, with rice (Londo et al. 2006) being a notable exception. It remains to be seen whether larger wild samples of other crop species might uncover similar levels of unexpected natural genetic differentiation as is found for einkorn. Similarly, it remains to be seen whether continued sampling of lines and loci in einkorn confirm the present trends, in particular, with respect to the high diversity of race γ and the narrow geographical distribution of race β.

In einkorn, we observe low diversity in some loci relative to others, but that reduction is found both in the wild races and in domesticated forms. The data for 452 lines uncover no loci that are significantly more polymorphic in wild than in domesticate lines. Because the monomorphic loci of domesticates are often monomorphic in the wild lines as well (fig. 4; table 3), there is no observed reduction of diversity at any locus that could readily be attributed to the domestication process. The only evidence that we see for a loss of diversity in the present data is in the outgroup, *T. urartu* (fig. 4; table 3).

It remains possible that selection underlies some differences in polymorphisms that we observe across loci, but if so, then the same selection is operating in both wild and domesticate populations and can thus hardly be ascribed to domestication, even for loci such as *Lr10* or *Q* that are known to be important for modern breeding (Salamini et al. 2002; Isidore et al. 2005). We clearly see evidence for recombination in the present data because we find a low frequency of heterozygous loci among otherwise homozygous individuals. With the exception of the feral form *T.m.ae.*, we did not observe any obvious hybrids of wild and domesticate einkorn, although wild and domesticate einkorn are interfertile (Zohary and Hopf 2000), and the existence of some introgression cannot be excluded, for example, in the case of the otherwise race α-specific *pinB* locus in domesticate accession number 314 (see legend to fig. 2).

The present findings appear to be unique among haplotype-based investigations of crop domestication genetics to date in 2 respects. First, a natural race, *T.m.b.* β, has been identified both by haplotype and by AFLP data that is genetically more similar to the domesticate form than other naturally existing races of the wild species are. Second, einkorn is one of the few domesticate crop species investigated to date that escaped breeding bottlenecks during the green revolution. Notably, there are other examples known of domesticate plants for which no reduction in genetic diversity in

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Table 3
Changes in Nucleotide Diversity per Locus among Wild and Domesticate Lines

	Loss of diversity[a] (%) in comparisons					Races with $\pi_{tot} = 0$[c]				
Locus	M/α	M/γ	M/β	M/boe[b]	U/boe					
BAMY1	−85[d]	11	−27	−40	79					
GAPDH	−2833	−17	−*[c]	−42	100			β		U
ACC1	−441	−101	−135	−84	100					U
PGK1	100	100	100	100	96				M	
AGPL	79	77	−706	80	97					
CesA	−933	−55	54	38	−28					
6SFT	−9	12	−217	−11	74					
BADH	0*	100	100	100	−162	α			M	
PEPC	100	0*	0*	*	100		γ	β	M	U
G6PDH	−*	−21	−*	−571	100	α		β		U
GPT	0*	100	0*	100	−5550	α		β	M	
pinB	−35	62	28	31	78					
ndhF	0*	0*	0*	0*	0*	α	γ	β	M	U
GPX	−142	9	−606	9	97					
Lr10[e]	25	33	−*	30	−22			β		
RGA2	9	−10	−407	9	97					
Q	62	79	−48	84	66					
VRN1	100	100	100	100	100				M	U

[a] Loss of diversity was calculated as $L_\pi = 1 - (\pi_{domest}/\pi_{wild})$ (Tenaillon et al. 2004) for the wild races indicated with gapped positions excluded.

[b] All wild *Triticum monococcum* ssp. *boeoticum* accessions (α + γ + β) were pooled for calculation of π_{wild}.

[c] L_π could not be calculated when the wild accessions were monomorphic for the locus ($\pi = 0$) as indicated by an asterisk. The value −* indicates that the wild race was monomorphic but domesticate was not; hence, higher diversity exists in the domesticate form. The value 0* indicates that both wild and domesticate were monomorphic at the locus, hence no loss of diversity is observed, even though L_π could not be calculated. Races monomorphic for the locus are shown in the right-hand columns.

[d] Negative values result when π is greater in domesticate than in wild accessions.

[e] The *Lr10* locus is empty in over 296 accessions sampled here.

the comparison of wild and domesticate forms was found, including chicory (Van Cutsem et al. 2003), bell pepper (Hernandez-Verdugo et al. 2001), and pepino (Blanca et al. 2007). Inferences about domestication bottlenecks from investigation of intensely bred domesticate germplasm (Salamini et al. 2004; Ozkan et al. 2005; Willcox, 2005; Abbo et al. 2006; Doebley et al. 2006; Zhu et al. 2007) are best considered in this light, prompting the following brief reconsideration of current views on einkorn domestication in the Fertile Crescent, based upon archaeological and genetic data.

A Dispersed-Specific Model of Einkorn Domestication

Over the last decade, a consensus has been reached on the existence of a core area of agricultural development in southeastern Turkey (Nesbitt and Samuel 1996; Lev-Yadun et al. 2000; Bar-Yosef 2002; Schmidt 2006; Lichter 2007), where the closest wild relatives of einkorn, emmer, barley, rye, chickpea, and lentil still grow (Ladizinsky 1985; Salamini et al. 2002; Ozkan et al. 2005; Abbo et al. 2006). Similar wild populations were necessarily the starting material at the origin of agriculture in the Fertile Crescent. Detailed archaeological reports by Hillman (2000) and Willcox (2005) and the newer report of Weiss et al. (2006) describe how the predomestication cultivation of (wild) cereals lasted even for centuries in the region and how it was followed by gradual (Kislev 2002) and multiple (Gebel 2004) appearance of domesticate phenotypes. The genetic and cultural mechanisms underlying the origin of those phenotypes are the issue (Diamond and Belwood 2003).

If geographically distinct domestication events each entailed random sampling from local genotypes, domesticate lines should trace to different localities across the range of the wild progenitor (Jones 2004). This is not observed for einkorn: race β is the sister to domesticate einkorn, but there is no evident reduction of genetic variation. This can be accommodated by a domestication model that we designate as dispersed specific (fig. 6). In essence, this would entail scenario in which a sedentary Natufian society (Bar-Yosef 2002) first harvested, then cultivated the wild β race of *T.m.b.* in the core area, but in a later phase of agricultural expansion, the β race was transferred to other locations, possibly in a process of nascent domestication. Transport could have involved migrating farmers (Nadel 2002; Renfrew 2002) or exchange of seeds against other goods as not all soils of the Fertile Crescent were adapted to cereal cultivation (Willcox 2005). Given the evidence that wild cereal seeds were harvested at the Ohalo camp (in the Palestine corridor) 21,000 years ago (Nadel 2002), it follows that cereal seeds, particularly hulled forms (Nesbitt and Samuel 1996), were produced and moved across the Fertile Crescent (Willcox 2005). Weedy cereals grown

←

FIG. 5.—MJ networks for 17 loci except the chloroplast locus *ndhF* where only 2 haplotypes among all lines were found (monomorphic for all *Triticum monococcum* lines and one other haplotype in all *Triticum urartu* lines). Haplotypes differing in indel polymorphisms only were grouped together in this analysis such that one haplotype can be designated by more than one Roman numeral. (*A*) *BAMY1*, (*B*) *GAPDH*, (*C*) *ACC1*, (*D*) *PGK1*, (*E*) *AGPL*, (*F*) *Q*, (*G*) *6SFT*, (*H*) *CesA*, (*I*) *pinB*, (*J*) *G6PDH*, (*K*) *VRN1*, (*L*) *GPT*, (*M*) *GPX*, (*N*) *Lr10*, (*O*) *PEPC*, (*P*) *BADH* and, (*R*) *RGA2*.

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Table 4
Selected Studies Reporting Evidence for Diversity Loss during Domesticated Plants (based on sequence information)

	Wild	n_{wild}	Domesticated	n_{dom}	Loci	bp	Reference
Fabaceae							
alfalfa	*Medicago sativa* ssp. *sativa*	19	*M. s. sativa*	31	2	60.500	Muller et al. 2006
soybean	*Glycine soja*	26	*Glycine max*	94	102	~6.300.000	Hyten et al. (2006)
Asteraceae							
sunflower	*Helianthus annuus*	16	*H. annuus*	16	9	262.624	Liu and Burke 2006
Poaceae							
rice	*Oryza rufipogon*/ *Oryza nivara*	161[a]	*Oryza sativa*	203[a]	3	1.636.040	Londo et al. (2006)
		30		30	10	484.740	Zhu et al. (2007)
	O. rufipogon and 5 other AA species	44	*O. sativa*/*Oryza glaberrima*	275	3	675961	Kawakami et al. (2007)
pearl millet	*Pennisetum glaucum*	6	*P. glaucum*	10	1	31.760	Gaut and Glegg 1993
sorghum	*Sorghum bicolor* ssp. *verticilliflorum*	3	*Sorghum bicolor* ssp. *bicolor*	24	95	788.022	Hamblin et al. (2006)
maize	*Zea mays* ssp. *parviglumis*	16	*Zea mays* ssp. *mays*	25	12	358.007	Tenaillon et al. (2004)
		14		14	774	~6.400.000	Wright et al. (2005)
barley	*Hordeum spontaneum*	8	*Hordeum vulgare*	16	23	65.328	Russell et al. (2004)
		25		20	7	252.720	Kilian et al. (2006)
		34		97	5	936.650	Caldwell et al. (2006)
		25[b]		32	7	624.820	Morrell and Clegg (2007)
wheat	*Triticum dicoccoides*	28	*Triticum dicoccum*	12	21	868.800	Haudry et al. (2007)
	Triticum boeoticum/ *Triticum urartu*	360	*Triticum monococcum* ssp. *monococcum*/*Triticum monococcum* ssp. *aegilopoides*	92	18	~12.000.000	This study[c]

NOTE.—bp, estimated amount of base pairs (bp) sequenced; Loci, number of loci investigated; n_{wild}, number of wild lines studied; and n_{dom}, number of domesticate lines studied.

[a] Populations, different numbers of individuals studied per locus.

[b] Three loci with additional wild lines sequenced.

[c] No domestication bottleneck found.

on cultivated fields increased at Abu Hureyra starting about 11,200 years ago (Hillman 2000). Gradually, in several areas, variants of the β race emerged with common domesticated traits.

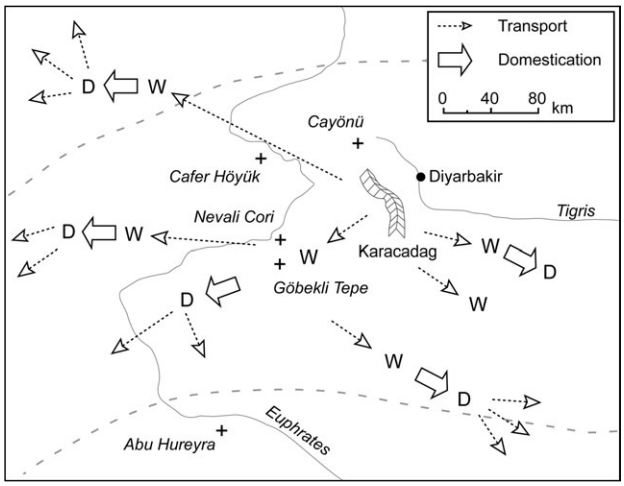


FIG. 6.—The dispersed-specific model for einkorn domestication. Only the Karacadag area in present day Southeast Turkey is shown here. Archaeological sites are indicated in italic. The Fertile Crescent is indicated with a dotted line. W is wild einkorn; D is domesticated einkorn. Note that domestication experiments were not always successful. Topography and environmental conditions determined cereal transport directions. See text for further explanations.

Across cereal species, domestication was unquestionably a convergent genetic event: the same genes for the same traits underlie domestication in different crops (Salamini et al. 2002; Pozzi et al. 2004). Thus, the sequestering of the same traits and genes independently within a given crop can have posed no more of a technological barrier than the same feat across different crops. Through spread of a common technology, domesticate lines could have emerged independently in different places from local samples of the β race. In this process, a genetic bottleneck would have occurred at each domesticating human settlement, but domestication events at numerous villages would have allowed the newly domesticated lines to integrate a full arsenal of wild haplotypes: many independent domestication bottlenecks would result in no domestication bottleneck for the domesticate lines as a whole.

This hypothesis accounts for our molecular data and accommodates the results of archaeological excavations: tools for grinding seeds are present in the majority of Fertile Crescent sites well before the large seed remains of domesticated einkorn wheat (Bar-Yosef 2002), supporting the view that humans in the region were familiar with the harvest of wild seeds both in natural habitats and in cultivated fields, as also new archaeological data underscore (Weiss et al. 2006; Lichter 2007).

Although the einkorn β race is so far only found in the KK and KT mountains, it might have been more widely dispersed throughout the Fertile Crescent and southeast

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Turkey 12,000 years ago, such that KK and KT harbor only relic populations. Although still consistent with our dispersed-specific model, that would allow the possibility that only domestication technology, not race β seeds, was exported from the core area. Finally, it has been proposed that harvesting wild seeds with a basket (Harlan 1989) accumulates disarticulating wild spikelets, whereas mutant plants with stiff rachis will not be harvested, such that their mutant frequency will increase in a given field with time (Hillman and Davies 1990). Unintentional selection, accompanied by repeated sowings of wild material, might have thus increased rachis stiffness and seed size, thereby completing the domestication process for einkorn.

Supplementary Material

Supplementary tables S1–S5 are available at *Molecular Biology and Evolution* online (http://www.mbe.oxfordjournals.org). Sequence data from this article are deposited in GenBank Data library under accession numbers provided in supplementary table S5 (Supplementary Material online).

Acknowledgments

We thank Sigi Effgen, Charlotte Bulich, Isabell Fuchs, Jutta Schütze for excellent technical assistance, Nahal Ahamadinejad for a program to calculate Dice distances from binary data, Manfred Heun, Klaus Schmidt, Andrea Brandolini, László Holly, Brandon Gaut for discussions, and Maarten Koornneef for laboratory facilities. This work was supported by grants from the Bundesministerium für Bildung und Forschung Ontoverse Project to J.K. and grants from the Deutsche Forschungsgemeinschaft to B.K.

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Arndt von Haeseler, Associate Editor

Accepted September 6, 2007

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