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Molecular Phylogeography of Domesticated Barley Traces Expansion of Agriculture in the Old World

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ABSTRACT

Barley (*Hordeum vulgare* ssp. *vulgare*) was first cultivated 10,500 years ago in the Fertile Crescent and is one of the founder crops of Eurasian agriculture. Phylogeographic analysis of five nuclear loci and morphological assessment of two traits in >250 domesticated barley accessions reveal that landraces found in South and East Asia are genetically distinct from those in Europe and North Africa. A Bayesian population structure assessment method indicates that barley accessions are subdivided into six clusters and that barley landraces from 10 different geographical regions of Eurasia and North Africa show distinct patterns of distribution across these clusters. Using haplotype frequency data, it appears that the Europe/North Africa landraces are most similar to the Near East population ($F_{ST} = 0.15$) as well as to wild barley ($F_{ST} = 0.11$) and are strongly differentiated from all other Asian populations ($F_{ST} = 0.34$–0.74). A neighbor-joining analysis using these F_{ST} estimates also supports a division between European, North African, and Near East barley types from more easterly Asian accessions. There is also differentiation in the presence of a naked caryopsis and spikelet row number between eastern and western barley accessions. The data support the differential migration of barley from two domestication events that led to the origin of barley—one in the Fertile Crescent and another farther east, possibly at the eastern edge of the Iranian Plateau—with European and North African barley largely originating from the former and much of Asian barley arising from the latter. This suggests that cultural diffusion or independent innovation is responsible for the expansion of agriculture to areas of South and East Asia during the Neolithic revolution.

THE origin of agriculture is one of the seminal events in human culture (Smith 1998; Diamond 2002). The development of domestic animals and crops from wild species laid the foundation for the Neolithic revolution 10,000–12,000 years ago and resulted in the transition of hunter–gatherer groups to sedentary pastoral and farming societies (Smith 1998; Diamond 2002). Agriculture in the Old World appears to have arisen in several key centers, including the Fertile Crescent in the Near East, the middle Yangtze River Valley in China, and peninsular Southeast Asia (Diamond 2002; Doebley *et al.* 2006), and there has been long-standing interest in the mechanisms by which agriculture expanded from these sites of origin (Ammerman and Cavalli-Sforza 1973; Zvelebil and Rowley-Conwy 1986; Diamond 1997, 2002). Tracing the genetic lineages of crop species as they were established and dispersed allows us to understand the evolutionary origins and histories of domestication and provide clues on the patterns of cultural exchange during human prehistory.

Among the centers of crop origins in the Old World, the Near Eastern cultures of the Neolithic were responsible for developing several of the key founder crops and pastoral animals of Eurasia, including domestic animals such as goats and sheep and plant crop species such as barley, wheat, flax, chickpeas, and lentils (Diamond 2002). Barley (*Hordeum vulgare* ssp. *vulgare*) is believed to be among the oldest cereal crop species in the world and was domesticated from the large-seeded wild barley (*H. vulgare* ssp. *spontaneum*) (Harlan 1995; Salamini *et al.* 2002; von Bothmer *et al.* 2003a). The endemic range of wild barley extends from Turkey, Syria, and the Jordan Valley (the Fertile Crescent) to the east toward Southwest Asian locales in Pakistan and Afghanistan (Zohary and Hopf 2000; von Bothmer *et al.* 2003b). Prehistoric sites in Ohalo II in the Jordan Valley (Kislev *et al.* 1992) suggest collection of wild barley by hunter–gatherer groups as early as the late Paleolithic, 19,000 years before present (BP) (Zohary and Hopf 2000; von Bothmer *et al.* 2003b), while more recent archaeological remains in several sites in the Fertile Crescent indicate that domestication of barley

Sequence data from this article have been deposited with the DDBJ/EMBL/GenBank Data Libraries under accession nos. AB297553–AB297626.

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and its widespread cultivation began ~10,500 years ago (Zohary and Hopf 2000).

The number of origins of barley, whether it was domesticated once or multiple times, has been the subject of intense debate (Zohary 1999; von Bothmer *et al.* 2003b). Previous molecular evidence suggests that barley was domesticated once, from populations in the Fertile Crescent in the western part of the range of its wild progenitor (Badr *et al.* 2000; Salamini *et al.* 2002), subsequently expanding west into Europe and North Africa and east into Asia ~8000 years ago (von Bothmer *et al.* 2003b). Recent evidence indicates that a second domestication event may have occurred in this cereal crop species, possibly in Central Asia at the eastern edge of the Iranian Plateau, and that this separate origin may have been the progenitor of present-day barleys found in East and South Asia (Morrell and Clegg 2007). It has been recognized for some time that East Asian barleys differ from European and North African landraces in the incidence of naked caryopsis and the prevalence of two-rowed *vs.* six-rowed forms (Vavilov 1926; Knüpffer *et al.* 2003) and the distribution of allozyme alleles (Konishi 1995; Graner *et al.* 2003). Moreover, there is evidence for independent loci underlying the genetic basis for nonshattering in European and Asian barleys (Takahashi 1955; Zohary 1999).

Characterizing the origins of domesticated barley and its spread in the Old World is crucial to our understanding of the expansion of agriculture. Together with pastoral animals such as goats (Luikart *et al.* 2001), sheep (Bruford and Townsend 2006), and cattle (Loftus *et al.* 1994; Mannen *et al.* 1998), the movement of cultivated barley across Eurasia and Africa may have been one of the key events in a global adoption of agriculture among Neolithic societies. Resolution of the debate on the origins and spread of barley, however, is hampered by the paucity of East and South Asian barleys in the samples used in previous molecular studies. The focus of attention on largely European and North African cultivated barley may have skewed our picture of the origin and spread of this key founder crop.

We report on a phylogeographic analysis of five genes in a large, worldwide sample of cultivated barley landraces as well as several wild barley accessions. Unlike previous studies, our sampling of domesticated barley drew heavily from cultivars in East and South Asia, with 177 landraces from China, India, the Himalayas, Korea, and Japan. Analysis of haplotypes indicates that an area of genetic discontinuity exists in the region between the Near East and Southwest Asia and that different genetic lineages appear to predominate westward into Europe and Africa, eastward to East Asia, the Indian subcontinent and the Himalayas, and southward into Ethiopia. The data are consistent with a recent molecular study that suggests a separate domestication event (Morrell and Clegg 2007). We show that one of these domestication events possibly gave rise to barley found in much of East and South Asia and together with work in other domesticated species provides clues as to the dynamics of expansion of agriculture across Eurasia.

MATERIALS AND METHODS

Samples: A panel of 263 *H. vulgare* ssp. *vulgare* accessions was chosen for this study to encompass the geographic spread of diversity within the species across Asia as well as representatives from Europe, North Africa, and Ethiopia (supplemental Table S1 at http://www.genetics.org/supplemental/). All are classified into landraces. The Near East accessions represent an area that encompasses the Fertile Crescent, including Syria, Turkey, and Iraq, while Southwest Asia includes accessions from Iran, Afghanistan, Pakistan, and adjacent Central Asian countries. Nineteen accessions of the wild progenitor of barley, *H. vulgare* ssp. *spontaneum*, were also included in the panel; these were distributed around the Fertile Crescent, including Turkey, Syria, and Jordan, as well as accessions from Afghanistan and Pakistan (supplemental Table S1). Seeds from these accessions were preserved in the Barley Germplasm Center, Research Institute for Bioresources, Okayama University. Passport data for the accessions, found at BARLEY DB (http://www.shigen.nig.ac.jp/barley/), were used to score for the presence of naked caryopsis and two- *vs.* six-rowed barley.

PCR and DNA sequencing: DNA was extracted from single plants using automated genomic DNA isolation system NA-2000 (Kurabo Industries, Osaka, Japan). A total of five ~400- to 600-bp gene regions across the barley genome were chosen from a set to serve as molecular markers for a phylogeographic analysis of domesticated and wild barley (supplemental Table S2 at http://www.genetics.org/supplemental/). Three of these genes had been previously used in other studies on the patterns of nucleotide diversity in these species (*GSP,* Caldwell *et al.* 2006; *G3PDH* and *Waxy*, Morrell *et al.* 2003). We selected two other genes, *Bmy1* and *bah45n12*, to serve as molecular markers. *Bmy1*, which encodes the β-amylase enzyme, shows geographic differentiation between western and eastern barleys (Zhang *et al.* 2004). The *bah45n12* encodes an EST that was randomly selected from the barley EST collection and maps to chromosome 3H. Primers were designed from the sequence available from GenBank using the program Primer3 (supplemental Table S2) (Rozen and Skaletsky 2000). Primers were designed in exons, and attempts were made to include both exon and intron sequence within each fragment. All PCR and sequencing was carried out by Cogenics (New Haven, CT) as described in Olsen *et al.* (2006).

Diversity analyses: Base pair calls, quality score assignment, and construction of contigs were carried out using the Phred and Phrap programs (Codon Code, Dedham, MA). Sequence alignment and editing were carried out with BioLign version 4.0.6.2 (Tom Hall, North Carolina State University) and BioEdit version 7.0.5.3 (Hall 1999). Most molecular population genetic analyses, including mismatch distributions of haplotype clusters (Watterson 1975; Slatkin and Hudson 1991; Rogers and Harpending 1992), were conducted using DnaSP 4.10.9 (Rozas *et al.* 2003). Levels of nucleotide diversity per silent site were estimated as π (Nei 1987). DnaSP 4.10.9 was also used in coalescent simulations of expected haplotype diversity under a neutral model, using segregating sites with 1000 runs. Since barley is predominantly selfing, we used a model of no recombination in these simulations.

Haplotype trees were constructed using a maximum-parsimony analysis (branch and bound search, stepwise addition) in PAUP* (Swofford 2000), with 500 bootstrap replicates

TABLE 1

Silent site of nucleotide diversity (π) of five loci in domesticated and wild barley

	Locus					
Geographic regions/species	*Bmy1*	*GSP*	*G3PDH*	*Waxy*	*bah45n12*	Mean
Europe/North Africa	0.0098	0.0124	0.0144	0.0100	0.0086	0.0111
Ethiopia	0.0005	0.0058	0.0123	0.0033	0.0035	0.0051
Near East	0.0032	0.0063	0.0199	0.0103	0.0119	0.0103
SW Asia	0.0084	0.0037	0.0153	0.0109	0.0073	0.0091
India	0.0065	0.0041	0.0025	0.0108	0.0065	0.0061
Himalaya	0.0041	0.0041	0.0005	0.0095	0.0017	0.0040
W China	0.0027	0.0032	0.0021	0.0069	0.0016	0.0033
C/S China	0.0013	0.0000	0.0000	0.0085	0.0000	0.0020
NE China/Mongolia	0.0090	0.0054	0.0032	0.0100	0.0036	0.0063
Korea/Japan	0.0093	0.0022	0.0000	0.0117	0.0041	0.0055
Wild barley	0.0127	0.0057	0.0088	0.0135	0.0123	0.0106
Domesticated barley	0.0087	0.0056	0.0099	0.0115	0.0066	0.0085

of the data. Insertion/deletion polymorphisms (indels) were included in the parsimony analyses, with each indel block treated as a single character. Long mononucleotide repeats were excluded from the analysis. The network topology was verified with TCS (Clement *et al.* 2000), which uses statistical parsimony to generate intraspecific phylogeny by assigning sequences into haplotypes and calculating the frequencies of each haplotype (Clement *et al.* 2000). The connection limit for the TCS analysis was 95% and gaps were treated as a fifth state. The consistency index (CI), which indicates the extent of homoplasy, was calculated by PAUP* (Swofford 2000).

Population structure analyses: Genetic differentiation was evaluated by *F* statistics (Weir and Cockerham 1984). Genotypes are assigned on the basis of membership to each haplotype cluster for the five loci, and the frequencies are calculated from these genotype assignments. Pairwise F_{ST} values between geographic regions were calculated in ARLEQUIN 3.1 using the frequency data based on these haplotype cluster assignments for these five loci (Excoffier *et al.* 2005), and 10,000 permutations were used to determine significance. An unrooted phylogenetic tree based on F_{ST} distance was constructed using the neighbor-joining algorithm in MEGA 3.1 (Kumar *et al.* 2004).

Genetic differentiation was also investigated using the model-based clustering method STRUCTURE 2.1 (Pritchard *et al.* 2000; Falush *et al.* 2003). The number of populations (*K*) in the model was systematically varied from 1 to 11, with 10 runs performed for each *K* value. Burn-in time and replication number were set to 20,000 and 50,000 for each run, respectively. The median likelihood of each *K* value was estimated from the 10 runs. We used both the ΔK method (Evanno *et al.* 2005) and the highest median *K* value representing the highest median likelihood values to assign barley accessions. For calculating ΔK, we used median rather than mean likelihoods to minimize effects of outlier runs. For the chosen *K* value, the run that had the highest likelihood estimate was adopted to assign individuals to clusters.

RESULTS AND DISCUSSION

Nucleotide variation in barley: We sequenced five loci in a large collection of domesticated barley, which included 177 accessions from areas east of Southwest Asia that had been underrepresented in previous studies. The sequenced region for each gene is located within the transcriptional unit and encompasses between 488 and 570 bp of sequence that includes both exons and introns. Together, we sequenced ~2.6 kb of sequence per accession.

One hundred fifteen SNPs were observed across all the genes in our analysis. The level of nucleotide diversity of silent sites, π, is 0.0085 for domesticated barley and 0.0106 for wild barley (Table 1). Three of the five loci sequenced in this study (*GSP*, *G3PDH*, and *Waxy*) were previously used in studies of molecular diversity in wild and/or cultivated barley accessions (Morrell *et al.* 2005; Caldwell *et al.* 2006; Kilian *et al.* 2006). The mean nucleotide diversity estimates for these three genes (mean $\pi = 0.0093$ for wild and $\pi = 0.009$ for domesticated barley) are not substantially different from those in these previous studies (mean $\pi = 0.0095$ for wild barley and $\pi = 0.0075$ for domesticated barley).

Three of the genes used in this study (*GSP*, *Waxy*, and *Bmy1*) are involved in grain quality traits and there is a possibility that they may not be evolving neutrally. We used coalescence simulations to determine whether there was significant deviation in levels of haplotype diversity from a neutral model with no recombination. The levels of haplotype diversity for three genes (*Bmy1*, *Waxy*, and *bah45n12*) did not significantly deviate from neutrality ($P > 0.05$) while two genes (*GSP* and *G3PDH*) were marginally significant ($P < 0.025$). The latter two genes, however, have been shown by other studies to behave neutrally (Morrell *et al.* 2005; Caldwell *et al.* 2006; Kilian *et al.* 2006). Moreover, the Tajima's *D* values for these genes did not show a significant deviation from neutrality (data not shown).

We assigned the domesticated barley to 10 Eurasian and African geographic regions: Europe/North Africa, Ethiopia, the Near East (which includes Turkey and the Caucasus), Southwest (SW) Asia (which includes Kashmir), India, the Himalayas, western (W) China, central/south (C/S) China, northeast (NE) China/Mongolia, and Korea/Japan (supplemental Table S1 at

http://www.genetics.org/supplemental/). Our analysis indicates that Europe/North Africa and the Near East have the highest levels of nucleotide diversity across the Old World. The mean nucleotide diversity level in these regions is ~0.011, which is more than two times higher than the mean of ~0.005 for the rest of Eurasia and Ethiopia.

Haplotype networks of barley genes: There are between 5 and 23 haplotypes for each gene in our sample, and we reconstructed the haplotype networks for each of these loci. Unrooted haplotype genealogies were estimated from the substitution polymorphisms observed at each of the five genes (Figure 1), and each analysis yielded one parsimonious arrangement (consistency index = 0.85–1.00). Between zero and two recombination events were detected for each locus, and trees for genes with inferred recombinants (which also have a consistency index <1) differ in the placement of one to two homoplasious polymorphisms that minimally affects tree topology.

All five genes had haplotypes that were structured into two to three haplotype clusters. These clusters were defined as (i) differentiated by a long internal branch and/or (ii) separating extant haplotypes that are ancestral to more than two alleles. All the haplotype clusters observed in domesticated barley are also found in the wild subspecies (Table 2). A maximum-parsimony analysis with 500 bootstrap replicates indicates that each of these clusters represents clades with bootstrap supports ranging from 56 to 100%. The exception is *G3PDH*, for which the analysis does not provide a bootstrap support for the diverse *B* cluster separated by a group of identical haplotypes that constitutes the *A* cluster; however, these two clusters are clearly separated and delineated by a very long branch (Figure 1).

Geographic patterns in haplotype distribution indicate a genetic divide in domesticated barley: There is a clear phylogeographic pattern in the distribution of domesticated barley gene haplotypes, which provides clues as to the spread of barley across Eurasia and North Africa (Figure 1). For all the five genes studied, there is a boundary of genetic differentiation at or between the Near East and Southwest Asia, which divides the domesticated barleys into western and eastern types. This is a region that spans Turkey, Syria, and Iraq and the Caucasus in the west and north and Iran, Afghanistan, Pakistan, and Kashmir in the east. Interestingly, this area is also the endemic range for wild barley and encompasses the regions where barley was domesticated during the Neolithic (Zohary and Hopf 2000; von Bothmer *et al.* 2003b).

For three genes, *Bmy1*, *G3PDH*, and *GSP*, we find two haplotype clusters that show an asymmetric pattern of distribution for cultivated barley across the Old World. For the *Bmy1* gene, for example, haplotype cluster *B* is found at low frequencies in the Near East ($n = 34$, *Bmy1 B* frequency ~9%) and at moderate frequency in Europe/North Africa ($n = 14$, *Bmy1 B* frequency ~23%). This haplotype cluster, however, dominates in the rest of Asia, where its frequency ranges from 71 to 100% (Figure 1). A similar distribution pattern is observed for the *GSP* and *G3PDH* loci, although the pattern is somewhat weaker for the latter gene. The levels of haplotype diversity for these two genes are significantly lower than predicted by a neutral model (see above), although the significance level is marginal; moreover, the patterns observed in these two genes are concordant with those observed in the three other loci.

For the *Waxy* and *bah45n12* genes, we are able to identify three haplotype clusters, and we also find that the haplotype cluster composition across the Old World differs between geographic regions (Figure 1). For the *bah45n12* locus, members of all three haplotype clusters (*A*–*C*) are found at equivalent frequencies in the Near East (Figure 1). In Europe and North Africa, haplotype cluster *A* predominates ($n = 14$, *bah45n12 A* frequency ~71%), while haplotype cluster *C* is the majority cluster found in the rest of Asia, with frequencies ranging from ~65% in India ($n = 19$) to 100% in central and south China ($n = 29$).

The Ethiopian barley landraces appear to differ genetically from either the European/North African or the Asian barleys; for each of the five genes, the barley sample from Ethiopia has a distinct haplotype composition that is unlike that found anywhere else (Figure 1). This confirms previous reports that also suggested that Ethiopian barley was differentiated from other barley groups (Vavilov 1926; Knüpffer *et al.* 2003).

Population structuring of domesticated barley: We determined the level of geographic differentiation in domesticated barley by estimating pairwise F_{ST} between regions using the frequencies of assignments to each haplotype cluster for the different genes (Table 3). The Europe/North Africa landraces are most similar to the Near East population ($F_{ST} = 0.15$) as well as the wild barley *H. vulgare* ssp. *spontaneum* ($F_{ST} = 0.11$) and are strongly differentiated from all other Asian populations ($F_{ST} = 0.34$–0.74). Geographic regions east and south of SW Asia show less differentiation between each other ($F_{ST} = 0.03$–0.32, with 15 of 21 pairwise comparisons having $F_{ST} < 0.15$), indicating strong genetic similarities between Asian groups. Interestingly, the Ethiopian landraces are strongly differentiated from all other groups ($F_{ST} = 0.37$–0.86). A neighbor-joining analysis of relationships based on F_{ST} values indicates that the East and South Asian barley landraces form one group together with SW Asia accessions, while the Near East, Europe/North Africa, and Ethiopia landraces form another distinct cluster (Figure 2).

We also used the STRUCTURE program (Pritchard *et al.* 2000; Falush *et al.* 2003), which implements a Bayesian method to estimate population structure. Since we examined only five genes, our analysis will not reveal fine-level population structuring, but should

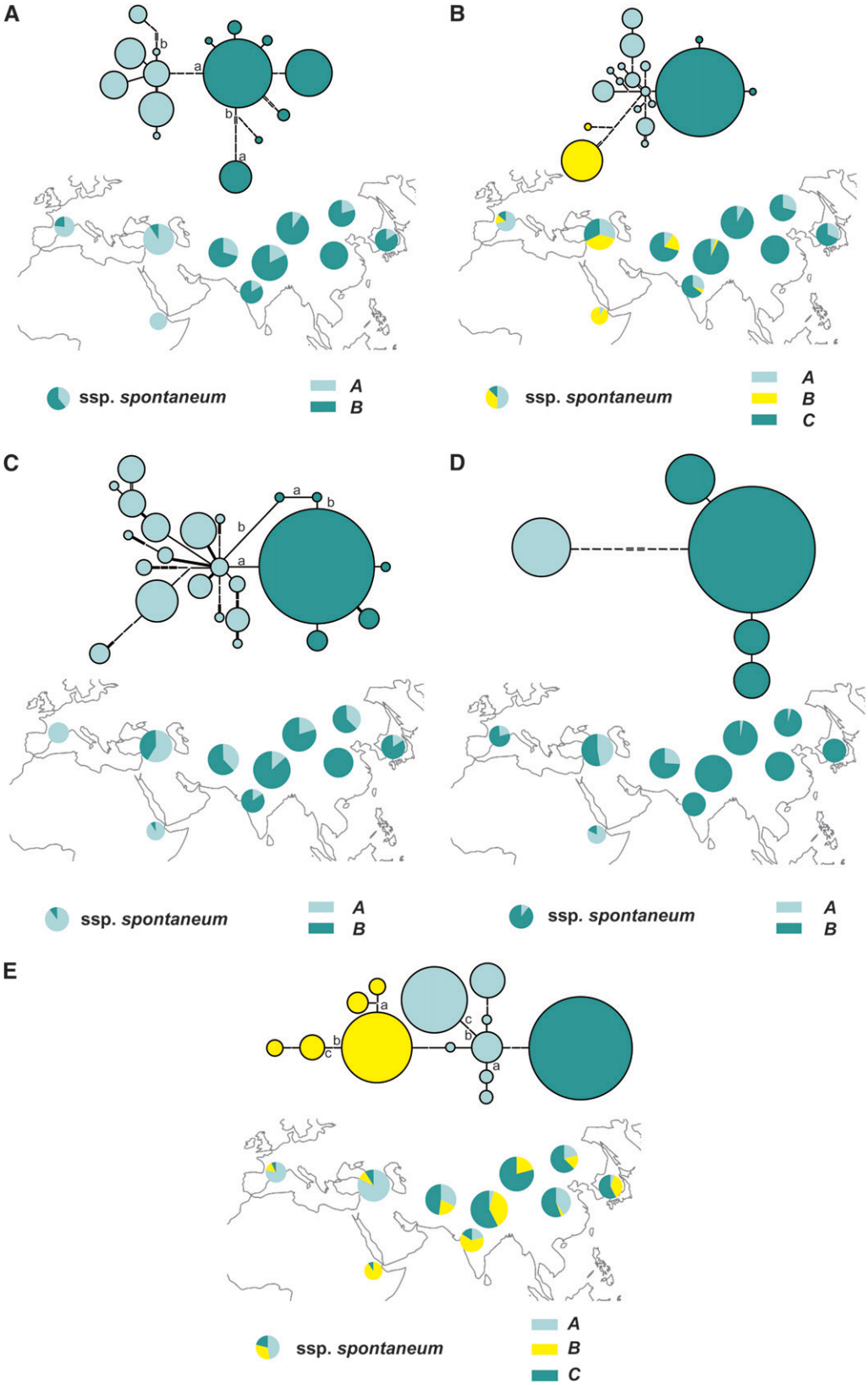

FIGURE 1.—Haplotype networks of five nuclear loci and geographic distributions of the haplotype clusters. The haplotype networks for the (A) *Bmy1*, (B) *bah45n12*, (C) *GSP*, (D) *G3PDH*, and (E) *Waxy* genes are shown. Haplotypes observed in domesticated and wild barley are indicated by circles. Each haplotype tree represents two or three clusters in all the barley accessions. Different colors delineate specific haplotype clusters. Each line of the haplotype networks represents a single mutational step. Silent-site SNPs are indicated by thin lines and nonsynonymous mutations by thick lines. Indel mutations are shown by double lines. Homoplasious mutations are marked by lowercase letters. The sizes of the circles in the haplotype networks and maps correspond to numbers of accessions in a given haplotype or in each region. The haplotype frequencies in wild barley are given below the distribution maps for each gene. The consistency indexes (CI) are as follows: *Bmy1*, CI = 0.93; *bah45n12*, CI = 1.00; *GSP*, CI = 0.95; *G3PDH*, CI = 1.00; and *Waxy*, CI = 0.85.

TABLE 2

Summary of geographic distribution for each haplotype cluster in the five genes

Gene	Haplotype cluster[a]	Geographic region: Europe/North Africa	Ethiopia	Near East	SW Asia	India	Himalaya	W China	C/S China	NE China/ Mongolia	Korea/ Japan	ssp. *spontaneum*
Bmy1	*A*	10	10	30	9	3	8	4		5	3	7
	B	3		3	22	15	37	35	27	19	16	11
	ND	1	1	1		1						1
GSP	*A*	14	10	20	11	3	6	8		9	3	17
	B		1	14	18	16	39	31	27	15	16	2
	ND				2							
G3PDH	*A*	3	9	16	8			1		1		2
	B	11	2	18	23	19	45	38	27	23	19	17
Waxy	*A*	11		28	10	4	2		11	5	1	9
	B	2	10	3	6	12	17	8	1	4	7	6
	C	1	1	3	15	3	26	30	15	15	11	4
	ND							1				
bah45n12	*A*	10	1	10	3	5	2	3		7	6	8
	B	2	10	13	6	1	1					6
	C	2		11	22	11	41	35	27	17	13	2
	ND					2	1	1				3
Total		14	11	34	31	19	45	39	27	24	19	19

[a] Haplotype clusters were designated on the basis of the haplotype network of each gene in Figure 1. ND, no sequence data.

partition strongly differentiated groups. The median maximum likelihood (ML) of the data given a model estimate of $K = 1$–11 populations is shown in Figure 3. The ΔK method (Evanno *et al.* 2005) indicates that a $K = 2$ model best fits the data ($\Delta K = 45.93$ for $K = 2$, $\Delta K < 0.4$ for all other K values), given the large increase in likelihood values from $K = 1$ to $K = 2$ (Figure 3). The analysis, however, indicates that there is a clear peak of likelihood at the $K = 6$ value (median ln $L = -1628$), suggesting that the sample can be divided into six clusters (Figure 3). To remain conservative, we examine the geographic distribution using both the $K = 2$ and the $K = 6$ models.

Given the inferred clusters, the probability of an accession belonging to a particular cluster can be determined. Accessions with membership probabilities >0.50 to a specific cluster were assigned to that cluster; accessions with probabilities <0.50 for all clusters were classified into a "mixed" group (Figure 4). From these, it is evident that barley landraces from each geographical region show distinct patterns of distribution across all the clusters. In the $K = 2$ model, landraces from

TABLE 3

Pairwise F_{ST} among geographic regions (below diagonal) and the significance level (above diagonal)

Geographic regions	Europe/North Africa	Ethiopia	Near East	SW Asia	India	Himalaya	W China	C/S China	NE China/ Mongolia	Korea/ Japan	ssp. *spontaneum*
Europe/North Africa	—	***	***	***	***	***	***	***	***	***	***
Ethiopia	0.5481	—	***	***	***	***	***	***	***	***	***
Near East	0.1491	0.3670	—	***	***	***	***	***	***	***	***
SW Asia	0.3429	0.4895	0.2495	—	***	***	***	***	*	***	***
India	0.5039	0.6574	0.4049	0.1182	—	***	***	***	***	*	***
Himalaya	0.6201	0.7231	0.5102	0.1163	0.1281	—	*	***	***	*	***
W China	0.6470	0.7596	0.5373	0.1244	0.2306	0.0288	—	***	**	*	***
C/S China	0.7369	0.8624	0.5694	0.1938	0.3188	0.1490	0.1689	—	***	***	***
NE China/Mongolia	0.4174	0.6190	0.3621	0.0310	0.1196	0.0818	0.0632	0.1935	—		***
Korea/Japan	0.5305	0.6836	0.4397	0.0868	0.0617	0.0299	0.0526	0.2072	0.0255	—	***
ssp. *spontaneum*	0.1101	0.4588	0.2122	0.1971	0.3203	0.4630	0.4836	0.5883	0.2486	0.3518	—

Significance levels: *$0.05 > P > 0.01$, **$0.01 > P > 0.001$, ***$P < 0.001$.

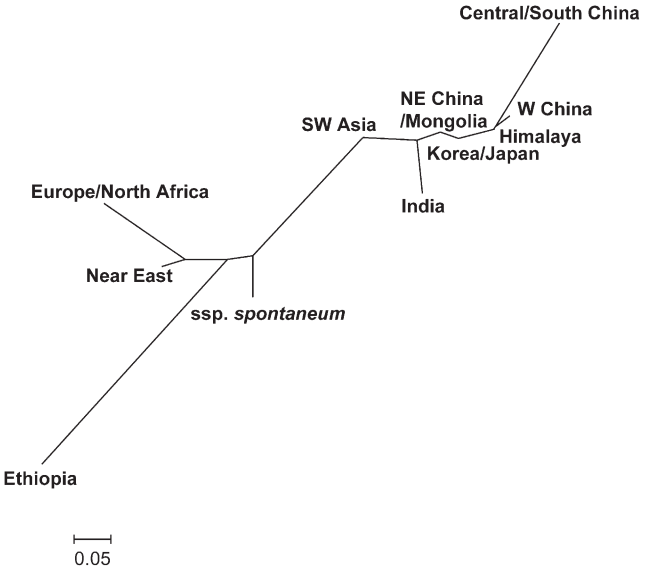

FIGURE 2.—Phylogenetic relationships of geographic regions in barley accessions. Unrooted genetic distance trees of barley populations are generated by the neighbor-joining method on the basis of pairwise F_{ST} values (Table 3).

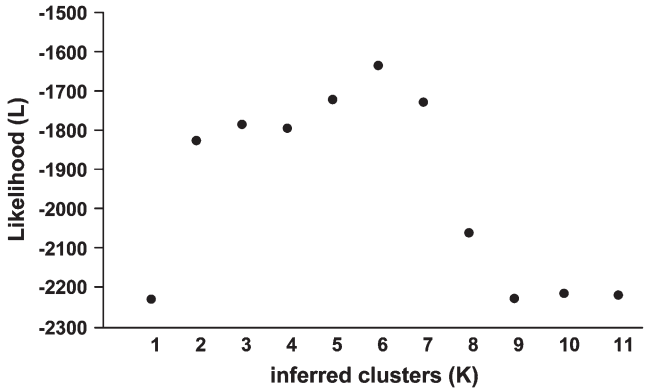

FIGURE 3.—Likelihood plot for STRUCTURE analysis. The median likelihoods for 11 runs for each *K* estimate are shown.

Europe/North Africa, Ethiopia, and the Near East are found primarily in cluster 2, as are the accessions of wild barley *H. vulgare* ssp. *spontaneum* (Figure 4). Barley accessions across the rest of Asia are found primarily in cluster 1. These results mirror those observed using haplotype cluster frequencies (Figure 1) and the neighbor-joining analysis (Figure 2).

The $K = 6$ model shows a more complex geographic patterning, but also shows a clear division of East and South Asian barley from the Near East and Europe/North Africa accessions (Figure 4). Only landraces from SW Asia are found in all six clusters. All of our wild barley samples are found in cluster 1, which is where nearly half of the Europe/North Africa landraces are also found. The second cluster has the majority of India accessions, as well as one-fourth of the Himalaya landraces. Almost all Ethiopia landraces and nearly half of the Near East accessions are classified in cluster 3, which also includes a moderate representation of SW Asian landraces. The highest numbers of NE China/Mongolia landraces are found in cluster 4, which also contains a large fraction of SW Asia and a majority of Himalaya and W China cultivars. The fifth cluster has half of the Europe/North Africa accessions, with strong membership from the Near East, NE China/Mongolia, and Korea/Japan landraces. The latter two may represent recent movement of cultivars from Europe to these Asian regions. The NE China/Mongolia landraces are also found at moderate frequency in cluster 6, which also has a moderate representation of SW Asian landraces and the majority of C/S China and Korea/Japan accessions. Like the F_{ST} results, this analysis indicates that most landraces in East and South Asia (including SW Asia) are more closely related to each other than to the Near East, Europe/North Africa, or Ethiopia accessions.

Morphological variation of domesticated barley across the Old World mirrors its genetic structure: The differentiation in haplotype distribution for our five study genes across the Old World is reflected in the patterns of morphological variation for our domesticated barley sample. We scored each of our accessions for two morphological traits, (i) naked caryposis and (ii) two *vs.* six rows in the spikelet, and examined the frequency of occurrence for these phenotypes across Eurasia and North Africa.

Barleys with naked caryopsis are found at only very low frequencies in the Near East, European/North African, and Ethiopian landraces, while at moderate-to-high frequencies (10–77%) in the rest of Asia (Figure 5). Two-rowed and six-rowed barleys are at near equal frequencies in the Near East, Europe/North Africa, and Ethiopia, while the six-rowed barleys are at high frequencies (>88%) in the rest of Asia (Figure 5). Like the observed haplotype distribution patterns for our study genes, the boundary for morphological types in barley (at least for these two traits) occurs somewhere in the Near East and Southwest Asia (Figure 5).

Mismatch distribution and timing of expansion of haplotype clusters: Relative timing of the expansion of haplotype clusters can be gleaned from a perusal of the mismatch distributions for various genes. For three of the five genes (*Bmy1*, *Waxy*, and *G3PDH*), there was no clear difference in the mismatch distributions of various gene clusters, indicating contemporaneous, recent expansions of haplotypes found in various geographical regions (Figure 6). For *GSP* and *bah45n12*, however, the haplotype cluster associated with East and South Asia appears to have expanded later than the haplotypes that predominate farther west, suggesting a more recent expansion of the eastern alleles (Figure 6).

The origins and spread of barley and other domesticated species: Domesticated barley originated ~10,500 years ago and was cultivated in the Fertile Crescent as one of several crops and animals that represent some of

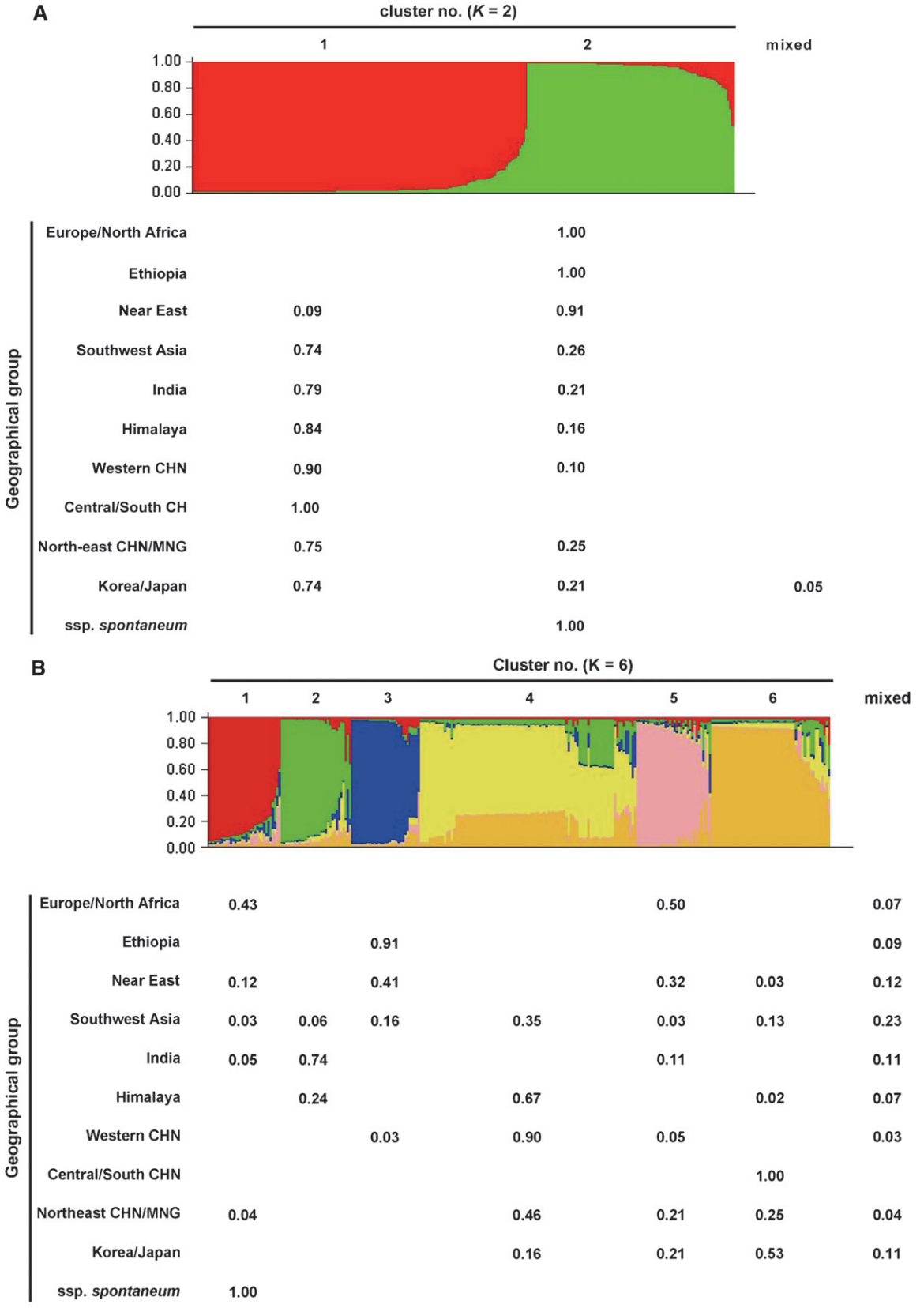

FIGURE 4.—Population subdivision and the frequency distribution of barley populations in each inferred cluster. Results from both the $K = 2$ (A) and $K = 6$ (B) models are shown. Each accession is shown by a thin vertical line that is partitioned into two or six colored segments. The frequency of a cluster group within each geographic region is tabulated. The accessions in which membership probability is <50% are classified into a "mixed" group.

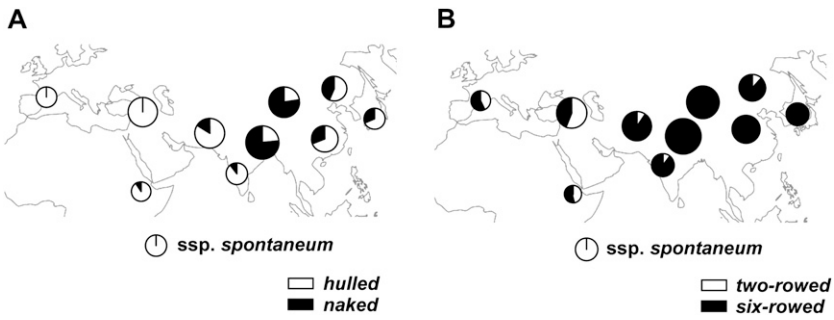

Figure 5.—Geographic differences in two morphological traits in domesticated barley accessions across the Old World. (A) Geographic distribution of hulled (open sections of circles) and naked (solid sections of circles) caryopsis in 10 geographic regions and in wild barley. All of the *H. vulgare* ssp. *spontaneum* accessions have a hulled caryopsis. (B) Geographic distribution of two-rowed (open sections of circles) and six-rowed (solid sections of circles) barley. All wild barleys have two-rowed caryopsis. The phenotype frequencies in wild barley are given below the distribution maps for each trait.

the founder species for Old World agriculture (Smith 1998; Diamond 2002). Sometime 8000 years BP, these domesticated species began to emerge from the Near East and spread across Europe and North Africa (Smith 1998; von Bothmer *et al.* 2003b); archaeological sites in the Mediterranean and other parts of Europe document the movement of farmers westward, bringing with them the crops and pastoral animals of the Fertile Crescent and catalyzing the "Neolithization" of Europe (Zvelebil 2002). Barley was also cultivated to the east in southern Central Asia, in Southwest Asia, and in the Indus Valley, and it was previously believed that this was also the result of dispersal from the Near East (Zohary and Hopf 2000; Salamini *et al.* 2002; Fuller 2006, 2007).

Molecular analyses have documented close genetic relationships between wild barley from the Fertile Crescent and its domesticated form in this region, as well as the cultivated landraces in Europe and North Africa (Badr *et al.* 2000; Morrell *et al.* 2003; Kilian *et al.* 2006). These studies have contributed to the widespread acceptance of a scenario of a single origin for domesticated barley in the Near East, followed by its spread to other parts of the Old World. Other recent molecular evidence, however, supports a putative second domestication (Morrell and Clegg 2007) that eventually gave rise to barley landraces east of the Fertile Crescent, although this conclusion was based on only a fairly small sample of 11 eastern barley accessions.

Our comprehensive study of barley phylogeography, with a more robust sampling from Asia, demonstrates that the domesticated barley found in China, India, and the Himalayan regions is genetically and in some cases morphologically distinct from that found to the west. The mean F_{ST} between European/North African barley and that found in Asia outside of the Near East is 0.5427, which suggests strong genetic isolation between western and eastern accessions. The haplotype distribution of the genes may be due in part to selection, especially for two loci (*GSP* and *G3PDH*) that show marginally significant deviations from neutral evolution. The patterns of distribution of the morphological traits may also be due to selection for different traits in different parts of the range (D. Q. Fuller, personal communication). For these genes, however, the pattern of distribution is consistent with those of three other neutral genes. This indicates that even if directional or diversifying selection was operating on these genes, it does not appear to result in a distinctive geographical distribution pattern among loci.

These results suggest that the origin and diffusion of barley may be more complex than previously thought. The observed genetic and morphological patterns are that *H. vulgare* ssp. *vulgare* are consistent with a previous study that has identified two origins for this crop species, one in the Fertile Crescent and another much farther east, possibly in the southern areas of Central Asia, in the eastern portion of the Iranian Plateau, or at the edges of the Indian subcontinent (Morrell and Clegg 2007).

On the basis of the nucleotide mismatch distributions of Asian haplotypes in two genes (*GSP* and *bah45n12*), the expansion eastward occurred more recently than the establishment of the Fertile Crescent and European barley populations, in accord with archaeological data. The mean F_{ST} within Asia east of the Fertile Crescent is fairly low (mean $F_{ST} = 0.1205$), while the mean nucleotide diversity in these regions is also twofold lower (mean $\pi = 0.0052$) than that for the Europe/North Africa and Near East accessions ($\pi = 0.0107$); both of these may reflect, in part, a more recent origin and expansion of barley eastward into Asia.

The pattern of genetic differentiation between barley landraces across Eurasia is not unique and is shared by several domesticated livestock species of the Old World. There have been extensive molecular studies on the origin of Eurasian domestic animals such as goats, sheep, and cattle (Machugh and Bradley 2001; Bradley 2006), all of which putatively originated from the Fertile Crescent. Mitochondrial DNA data for both sheep (Bruford and Townsend 2006) and cattle (Loftus *et al.* 1994; Mannen *et al.* 1998), however, suggest the existence of two highly divergent lineages that distinguish European and Asian types, indicating a second independent evolution of these livestock species outside the Near East. The data on goats are more complex, but demonstrate the presence of a distinctive Asian mitochondrial haplotype found in eastern and southern Asia and also suggest multiple origins of this

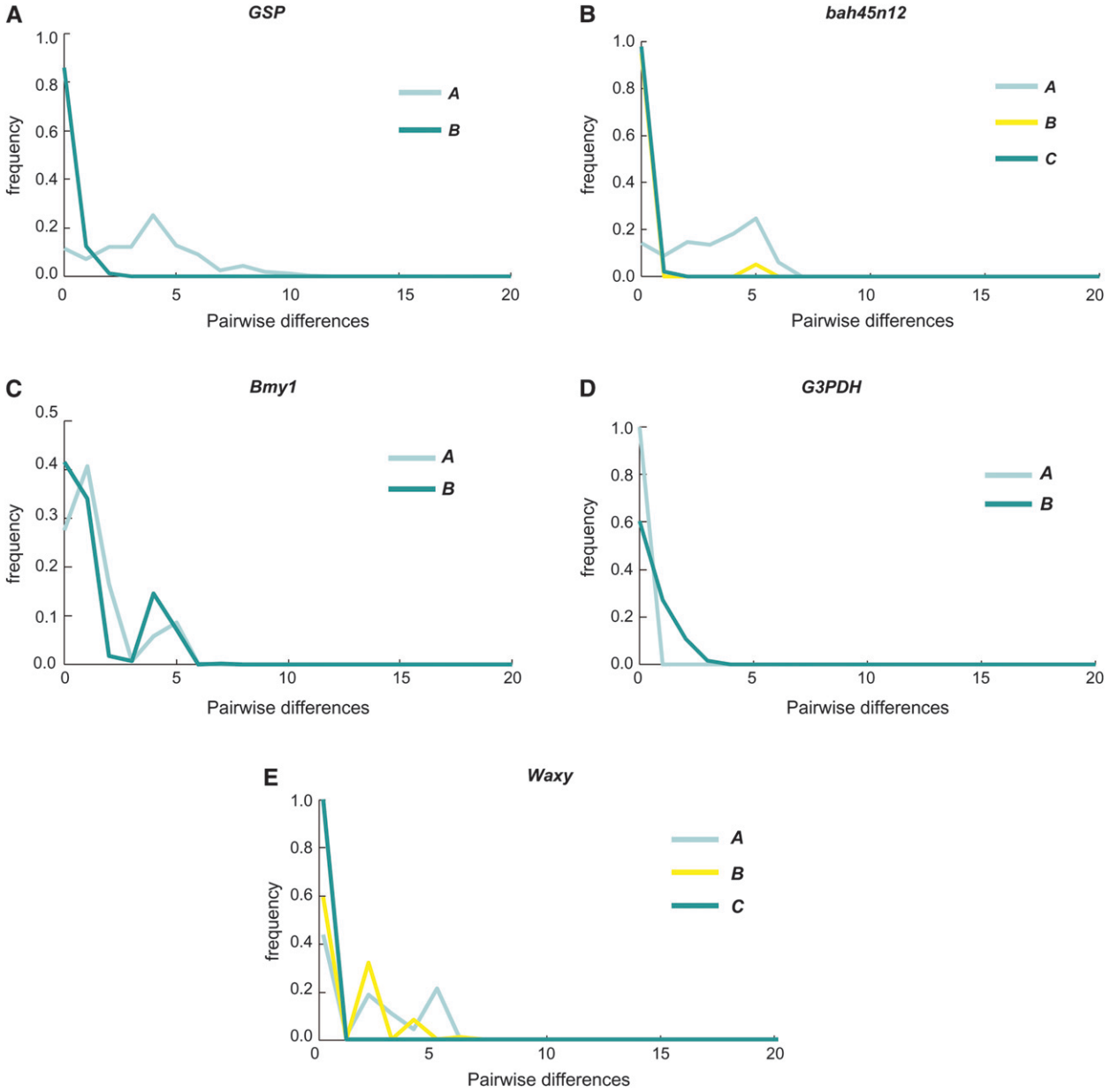

FIGURE 6.—Mismatch distributions for each haplotype cluster in domesticated and wild barley. The distributions for all five study genes are indicated (A–E). The color of each of the lines corresponds to specific haplotype clusters shown in Figure 1.

domesticated animal (LUIKART *et al.* 2001). There is now a widespread consensus that these pastoral livestock species may have had an independent second origin east of the Fertile Crescent, in the same region where barley was also secondarily domesticated.

Naked, six-rowed barley was the dominant crop 8000 years ago in Megrah, a Neolithic site at the boundary of the Iranian Plateau and the Indus Valley (MEADOW 1996), as well as 7000 years ago at the Jeitun cultures at the northeastern border of the Iranian Plateau and southern Turkmenistan (HARRIS and GOSDEN 1996). Bones of domesticated sheep, goats, and cattle have also been found in these sites as part of the larger Southwest Asian Neolithic agricultural and pastoral economy (HARRIS and GOSDEN 1996). These findings indicate that Neolithic farmers in this region redeveloped several key domesticated plant and animal species that had been previously established in the Fertile Crescent 2000–3000 years earlier. There have been intense debates on the relative roles of migration of farmers/pastoralists, cultural diffusion of knowledge, and independent innovation (AMMERMAN and CAVALLI-SFORZA 1973; ZVELEBIL and ROWLEY-CONWY 1986; DIAMOND 2002) in the spread of agriculture from its center of origin in Eurasia. Our finding that genetically distinct barley landraces populated much of East and South Asia, coupled with previous work on the origins of domesticated livestock species, indicates that knowledge

transfer or independent innovation, rather than the spread of domestic species, may have driven the initial expansion of agriculture outside the Near East toward Southwest Asia and the Indus Valley.

The barley that was domesticated at this second, eastern site, as well as the associated domestic animals, eventually expanded into India, China, Tibet, Korea, and Japan. The eastward spread of barley may have occurred fairly late, as the earliest recorded evidence of this grain in East Asia is in a Korean archaeological site 2200 years BP (Crawford 1992). This is consistent with the mismatch distribution of the various barley gene haplotypes, which indicates a more recent expansion for at least two haplotype clusters that predominate in East Asia (Figure 6). The movement of barley eastward possibly followed the trade paths between Southwest Asia and China that are referred to as the Silk Road, which are known to have been in use for at least 2500 years (Fernandez-Armesto 2006). The genetic evidence also suggests a westward movement of this second barley type, since haplotypes that predominate in East and South Asia are also observed at lower frequencies in and west of the Near East and are consistent with archaeological records that show the presence of naked, six-rowed grains at sites in Syria, Turkey, and Greece (Zohary and Hopf 2000).

Many crop and livestock species appear to have multiple evolutionary origins, including rice in Asia (Londo *et al.* 2006), grapes in the Mediterranean (Arroyo-Garcia *et al.* 2006), and cucurbits in the Americas (Sanjur *et al.* 2002). It remains unclear why different cultures sought to reinvent these domesticated species several times rather than simply obtain them through diffusion from other farming societies. Detailed phylogeographic analysis in these and other crop plants may provide greater insights into the dynamics of the spread of agriculture and the roles that migrating human populations and cultural contact played in the expansion of farming societies in the world. Together, these molecular studies allow us to understand the trajectory of the Neolithic revolution that eventually led to the development of the modern world.

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