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D. L. Hyten
Soybean Genomics and Improvement Laboratory, USDA Agricultural Research Service, Beltsville, Maryland, david.hyten@unl.edu

Qijian Song
University of Maryland, qijian.song@ars.usda.gov

Youlin Zhu
University of Maryland

Ik-Young Choi
University of Maryland

Randall L. Nelson
University of Illinois, rlnelson@illinois.edu

See next page for additional authors

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David L. Hyten*†, Qijian Song*†, Youlin Zhu*‡, Ik-Young Choi*, Randall L. Nelson§, Jose M. Costa†, James E. Specht¶, Randy C. Shoemaker‖, and Perry B. Cregan*,**

*Soybean Genomics and Improvement Laboratory, U.S. Department of Agriculture, Agricultural Research Service, Beltsville, MD 20705; †Department of Natural Resource Sciences and Landscape Architecture, University of Maryland, College Park, MD 20742; §Soybean/Maize Germplasm, Pathology, and Genetics Research Unit and Department of Crop Sciences, U.S. Department of Agriculture, Agricultural Research Service, University of Illinois, Urbana, IL 61801; ¶Department of Agronomy and Horticulture, University of Nebraska, Lincoln, NE 68583; and ‖Department of Agronomy, U.S. Department of Agriculture, Agricultural Research Service, Iowa State University, Ames, IA 50011

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Soybean has undergone several genetic bottlenecks. These include domestication in Asia to produce numerous Asian landraces, introduction of relatively few landraces to North America, and then selective breeding over the past 75 years. It is presumed that these three human-mediated events have reduced genetic diversity. We sequenced 111 fragments from 102 genes in four soybean populations representing the populations before and after genetic bottlenecks. We show that soybean has lost many rare sequence variants and has undergone numerous allele frequency changes throughout its history. Although soybean genetic diversity has been eroded by human selection after domestication, it is notable that modern cultivars have retained 72% of the sequence diversity present in the Asian landraces but lost 79% of rare alleles (frequency ≤0.10) found in the Asian landraces. Simulations indicated that the diversity lost through the genetic bottlenecks of introduction and plant breeding was mostly due to the small number of Asian introductions and not the artificial selection subsequently imposed by selective breeding. The bottleneck with the most impact was domestication; when the low sequence diversity present in the wild species was halved, 81% of the rare alleles were lost, and 60% of the genes exhibited evidence of significant allele frequency changes.

artificial selection | crop domestication | genetic diversity | SNPs

The world's food supply depends on a small number of crop species. Because high-yielding cultivars dominate production but are relatively few in number and are genetically similar, genetic diversity in these crops is presumed to have declined to alarmingly low levels (1, 2). The reduction of genetic diversity does not bode well for future genetic gains in crop productivity and could result in broad susceptibility to newly emerging diseases or insect pests, thereby threatening long-term food and feed security (1, 3). The North American soybean crop accounts for 47% of world production (4) and may now be at a critically low level of diversity because of a series of genetic bottlenecks and intensive selection for enhanced agronomic performance. The perception that modern soybean cultivars are exceptionally uniform is supported by data based on coefficient of parentage analyses and surveys of germplasm for differences in genetic marker alleles (5).

Like many of the world's most important crops, soybean is an autogamous species. Inbreeding is predicted to decrease diversity, because purging of deleterious mutations also results in the loss of nondeleterious alleles at linked loci. In addition, evolutionary events such as domestication, founding events, and selection can affect the level of sequence variation within a crop. Domestication occurs when humans exert artificial selection on a wild species. Such selection, both positive and negative, over hundreds of generations results in the creation of a cultivated species. Founding events occur in crops when only a few individuals are used to introduce a crop into a new region or when breeders use only a few cultivars for all subsequent crop improvement. Domestication and founding events create genetic bottlenecks that can decrease genetic diversity, change allele frequencies, increase linkage disequilibrium (LD), and eliminate rare alleles in the resulting population (6). The magnitude of these effects will depend on the number of individuals involved, the selection intensity, and the duration of the genetic bottleneck.

Current evidence indicates that the cultivated soybean was domesticated from its annual wild relative [*Glycine soja* (Sieb. and Zucc.)] in China ≈5,000 years ago (5). The fraction of *G. soja* diversity retained through the domestication bottleneck is undefined. Domestication resulted in a multitude of localized *Glycine max* landraces. An estimated 45,000 of these unique Asian landraces have been collected and are maintained in *G. max* germplasm collections around the world. Despite this seemingly vast reservoir of genetic diversity, just 80 (<0.02%) of those landraces account for 99% of the collective parentage of North American soybean cultivars released between 1947 and 1988 (5). Even then, the contribution of each landrace is unequal, because just 17 of these 80 account for 86% of the collective parentage, with the remaining 63 landraces contributing <1% each (7). The process by which a few landraces, introduced from Asia to North America during the first half of the last century, became the genetic base of North American cultivars is often described as a diversity-reducing introduction bottleneck. As has occurred in other crop species, the intensive selection applied to this founding stock and to its descendants to create the elite soybean cultivars that growers use today is presumed to have led to additional losses in genetic diversity (1, 3).

Thus, the report of relatively low sequence diversity in cultivated soybean (8) relative to that in cultivated maize (an allogamous species) is not unexpected. A fundamental underlying question in the case of the autogamous soybean is the degree to which genetic diversity throughout the genome has been impacted by the domestication and introduction bottlenecks and by the subsequent intensive selection imposed by plant breeding. To answer this question, we evaluated DNA sequence variation within and among four populations of genotypes: elite North American soybean cultivars, Asian landrace founders of those elite cultivars, Asian landraces (with no known relation-

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Abbreviation: LD, linkage disequilibrium.

‡Present address: Department of Bioscience and Biotechnology, Nanchang University, Nanchang 330047, People's Republic of China.

**To whom correspondence should be addressed. E-mail: creganp@ba.ars.usda.gov.

Table 1. Nucleotide diversity per base pair $\times 10^3$ in coding and noncoding regions within the four soybean populations

	Coding sequence diversity						Noncoding sequence diversity							
	Synonymous		Nonsynonymous		Total coding		UTR		Intron		Total noncoding		Total	
Population	π	θ	π	θ	π	θ	π	θ	π	θ	π	θ	π	θ
G. soja	4.73a*	3.15a	0.96a	1.20a	1.05a	1.63a	3.18a	3.24a	2.34a	2.65a	2.76a	3.06a	2.17a	2.35a
Landraces	1.84b	1.18b	0.74ab	0.72b	0.70b	0.81b	2.02b	1.43b	1.55b	1.35b	1.77b	1.36b	1.43b	1.15b
N. Am. Ancestors	1.21b	1.29b	0.56b	0.58b	0.60b	0.73b	1.28b	1.07b	1.14c	1.07bc	1.36b	1.16bc	1.14c	1.00bc
Elite Cultivars	1.22b	0.77b	0.60b	0.54b	0.61b	0.59b	1.10b	0.86b	0.96c	0.76c	1.22c	0.92c	1.11c	0.83c

The UTR sequence includes 5′ and 3′ UTR. N. Am., North American.
*Values within a column followed by the same letter are not significantly different based on Duncan's multiple range test ($P > 0.05$).

ship to the founding stock), and accessions of the wild progenitor species *G. soja*. Our objective was to assess how genetic diversity in annual *Glycine*, as measured by DNA sequence variation, was altered by the human activities of domestication and subsequent founding and intensive breeding over the past 5,000 years. Through an understanding of DNA diversity in these four distinct populations, we were able to assess how soybean genome diversity was impacted by its transit through three genetic bottlenecks, domestication, introduction, and 75 years of intense breeding effort from a wild species to the elite cultivated crop species now grown widely in North America.

Results

Sequence Diversity in Wild and Cultivated Soybean. We sequenced a total of 6.3 Mbp of DNA, which consisted of 111 fragments from 102 randomly selected genes (Table 3, which is published as supporting information on the PNAS web site) in 120 soybean genotypes. These genotypes were representative members of four distinct populations: (*i*) 25 diverse *G. max* cultivars developed in the 1980s, hereafter termed Elite Cultivars; (*ii*) 17 *G. max* Asian accessions that were the primary founders of the North American cultivars, hereafter termed North American Ancestors; (*iii*) 52 diverse *G. max* Asian accessions representing descendant products of domestication, hereafter termed Landraces; and (*iv*) 26 diverse accessions of *G. soja* (Table 4, which is published as supporting information on the PNAS web site). The sequence data set was mostly complete, with only 0.5% missing data (Data Set 1, which is published as supporting information on the PNAS web site). The amount of aligned sequence in these 120 soybean genotypes included 22 kb of coding sequence, 11 kb of 5′ and 3′ UTR sequence, 18 kb of intron sequence, and 2 kb of perigenic genomic sequence, totaling 53 kb (Data Set 2, which is published as supporting information on the PNAS web site). A total of 438 single base changes plus 58 single or multiple base insertion-deletions, all collectively referred to as SNPs, were identified. Of the 496 total SNPs, 84 were nonsynonymous (i.e., the encoded amino acid was altered by the SNP), whereas 59 were synonymous (i.e., the encoded amino acid was not altered); the other 353 SNPs occurred in noncoding DNA (Data Set 1).

Effect of Selection on Diversity. Two common measures used to describe sequence variation or nucleotide diversity are π (pi), the expected heterozygosity per nucleotide site (9); and θ (theta), the number of polymorphic sites in a genotypic sample corrected for sample size (10). The Elite Cultivars had a π value of 0.00111 and a θ of 0.00083 (Table 1), which are very similar to earlier estimates of soybean diversity (8). When compared with other organisms, soybean nucleotide diversity is similar to the θ = 0.00053–0.00083 values reported for humans (11, 12) but lower than the θ = 0.0023 reported for *Sorghum bicolor* (13) and ≈1 order of magnitude lower than the θ = 0.00627 reported for modern maize inbred lines (14). It is generally presumed that intensive artificial selection imposed in modern plant-breeding programs over the last half century has reduced genetic diversity from that present in the founding stock. A comparison of the Elite Cultivars with the founding North American Ancestors indicated no significant reduction ($P > 0.05$) in nucleotide diversity. Indeed, the Elite Cultivars retained 97% (π) and 83% (θ) of the diversity present in the North American Ancestors. The F_{st} value between the North American Ancestors and the Elite Cultivars was 0.005, further supporting the lack of appreciable divergence between the two populations.

We also developed an alternative statistical means of evaluating this apparent lack of reduction in diversity and population divergence. Extensive pedigree information is available for nearly all North American soybean cultivar releases (15). It is thus possible to compute the mathematical contribution of each of the 17 North American Ancestors to the parentage of the 25 Elite Cultivars. Using the pedigree-based contribution data, plus the SNP allele data observed in the 17 North American Ancestors, a theoretical Elite Cultivar sample was created to simulate, with no selection after the founding event, the Elite Cultivar sample. The π and θ of the simulated Elite Cultivar sample were 0.00107 and 0.00091, respectively, which were very similar and not significantly different ($P > 0.05$) from the π and θ of the actual Elite Cultivar sample.

Tajima's *D* is often used to determine allele frequency changes by comparing two populations before and after a genetic bottleneck (14). However, the large number of monomorphic fragments for which Tajima's *D* cannot be calculated makes comparisons difficult and hard to interpret in our populations. However, we did develop a permutation test where alleles were randomly assigned to the two populations based on the combined population allele frequency to determine whether the allele frequency change between populations was significant. Only seven of the genes containing one or more SNPs exhibited a significant allele frequency change ($P < 0.05$) between the North American Ancestors and the Elite Cultivars (Data Set 3, which is published as supporting information on the PNAS web site). Although increased LD is another hallmark of a severe genetic bottleneck, the extensive LD reported previously over a 2- to 3-cM region in soybean (8) makes LD information on a per gene basis unlikely to be informative. In fact, we found complete LD, as indicated by $D' = 1$, within all but one gene in the North American Ancestors and the Elite Cultivars, two genes in the Landraces, and three genes in the *G. soja* (data not shown).

Founding Effect of Soybean Introduction to North America. Although we have shown that long-term selective breeding in soybean after the establishment of a founder population has slightly decreased sequence diversity in the Elite Cultivars and changed only a few allele frequencies, the relatively few North American Ancestors found in the pedigrees of the Elite Cultivars represent a very

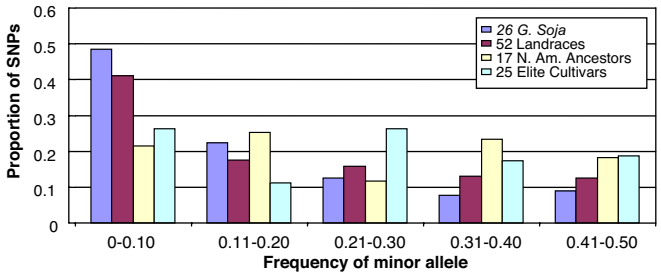

Fig. 1. Distribution of the SNP minor allele frequencies for each of the four soybean populations.

limited sampling of the Asian landraces from which they derive (1, 3). Using only a limited number of introductions from the center of origin would be expected to impose an introduction bottleneck, thereby restricting the genetic variation available for the subsequent creation of elite North American cultivars.

Overall, the founding stock of North American Ancestors retained 80% (π) and 87% (θ) of the nucleotide diversity of the Landraces (Table 1). These reductions in nucleotide diversity were not statistically significant ($P > 0.05$). Still, it is common for low-frequency alleles to be eliminated during a founding event. The proportion of SNPs with a minor allele frequency of ≤ 0.10 in the North American Ancestors was about half that of the Asian Landraces (Fig. 1). Of the 98 low-frequency SNP alleles in the Landrace population, 76 were not present in the North American Ancestors (Data Set 3). Thus, the impact of the limited number of founder genotypes was a 78% loss of the low-frequency alleles detected in the Landraces. Haplotype is a term used to designate a specific combination of linked alleles within a contiguous segment of DNA, and thus haplotype diversity (16) provides another measure of genetic diversity. Mean haplotype diversity in the 102 genes was 0.30 in the North American Ancestors, which was 94% of, and not significantly different ($P > 0.05$) from, the haplotype diversity in the Landraces. A total of 39 gene fragments were monomorphic in the North American Ancestors, whereas 14 of these 39 gene fragments were polymorphic in the Landraces (Table 2).

The Landraces had nucleotide diversity values of $\pi = 0.00143$ and $\theta = 0.00115$ (Table 1). The Landraces were somewhat, but not significantly, more diverse than the North American Ancestors, and the latter were slightly, but not significantly, more diverse than the Elite Cultivars. However, the cumulative effect of both bottlenecks was consequential, in that the genetic diversity of the Landraces was significantly greater ($P < 0.05$) than that of the Elite Cultivars. The Elite Cultivars retained 78% (π) and 72% (θ) of the diversity present in the Landraces. This is surprisingly close to the 77% diversity that maize elite inbred lines retained across 21 loci relative to the diversity found in maize Landraces (17). Low-frequency variants were only minimally impacted by the improvement bottleneck. Although only 22 of the 98 low-frequency SNPs present in the Landraces were present in the North American Ancestors, 21 remained in the Elite Cultivar population.

The cumulative effect of the genetic bottleneck of introduction to North America and subsequent selective breeding also had a significant effect on allele frequency changes. In 28 genes containing at least one SNP, a significant ($P < 0.05$) allele frequency change was observed (Data Set 1). A total of 15 gene fragments, variable in the Landraces, were fixed in the Elite Cultivars, because of selection or genetic drift (Table 2). However, the haplotype diversity for the Elite Cultivars (0.28), the North American Ancestors (0.30), and the Landraces (0.32) were not significantly different ($P > 0.05$).

Table 2. Number of loci fixed within the four soybean populations

	G. soja	Landraces	N. Am. Ancestors	Elite Cultivars
No. loci fixed	7	25	39	40
Percent loci fixed	6.8	24.5	38.2	39.2

N. Am., North American.

Domestication Bottleneck. The domestication bottleneck is the most time-distant genetic constraint in the history of a crop and represents the first occurrence of human selection. We found that the Landraces retained 66% (π) and 49% (θ) of the nucleotide diversity found in *G. soja* (Table 1). The smallest reduction occurred in nonsynonymous sites, with the Landraces retaining 77% (π) of the diversity present in *G. soja*. The greatest number of allele frequency changes also occurred as a result of domestication with 61 genes having one or more SNPs with a significant ($P < 0.05$) allele frequency change (Data Set 3). Haplotype diversity was significantly lower in the Landraces (0.32) than in the wild soybean progenitor (0.51; $P < 0.0001$), which was consistent with the reduction in nucleotide diversity. The Landraces retained $\approx$63% of the haplotype diversity of *G. soja*. A total of 18 gene fragments that were variable in *G. soja* were fixed in the three *G. max* soybean populations (Table 5, which is published as supporting information on the PNAS web site). It is worth noting that in the comparison of unique SNPs among the four soybean populations, *G. soja* had the largest reservoir of unique sequence variants, with a total of 237 SNPs (Fig. 2). *G. soja* contained a total of 215 SNPs with a minor allele frequency ≤ 0.10, of which 175 were unique to *G. soja* and not found in any of the *G. max* populations (Data Set 3). The elimination of 81% of the low-frequency sequence variants in *G. soja* is consistent with the anticipated effects of a genetic bottleneck such as domestication.

Discussion

It has been well documented that selection targeted at individual loci will reduce genetic diversity within and around the selected loci (6). Conversely, it is assumed that selection in modern breeding programs acts simultaneously upon many loci controlling a variety of traits under selection. A logical conclusion would be that such selection would greatly reduce diversity throughout the genome, as has been predicted (3). This would be true for beneficial alleles present before the imposition of selection or *de novo* variation created during domestication and modern plant breeding. However, the lack of a statistically significant reduction in DNA sequence diversity and the lack of allele frequency changes between the Elite Cultivars vs. their North American Ancestors do not support this conclusion. Our data indicate that modern soybean breeding has minimally affected allelic structure of the genome compared with the other historical genetic bottlenecks. The only other major effect could be a significant increase of LD, which would be difficult to assess in the North American Ancestors, due to the small number of individuals in this group, which would inflate estimates of D'.

There are several factors that could be responsible for what seems to be a minimal amount of "genetic erosion" after the North American founding event. One explanation is that selection in modern soybean-breeding programs acts on only a small proportion of the genome. This selection would likely reduce the diversity and change allele frequencies in the sequence of DNA surrounding the loci that are targets of selection. Depending on how much LD is increased surrounding these loci, the effects of such selection might not extend far enough to affect overall genome diversity. Diversity loss also could have been mitigated by balancing selection and epistasis, given that the North Amer-

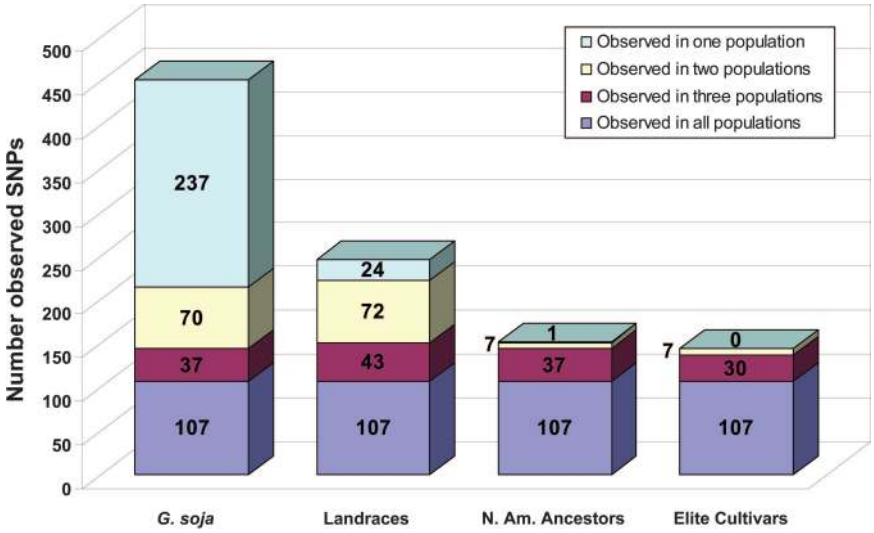

Fig. 2. Comparison of the number of unique and shared SNPs among four soybean populations.

ican cultivated germplasm is comprised of 12 subpopulations (i.e., Maturity Groups 000–IX) adapted to a latitudinal gradient in photoperiod. Soybean breeders have had to develop cultivars for the specific photoperiod and production conditions encountered from Canada to Florida (5), and this would have maintained diversity for photoperiod response as well as combinations of numerous other region-specific biotic and abiotic stress resistance factors.

Besides intensive selection by modern plant breeding, the narrow genetic base is often cited as a contributing factor to low soybean diversity (1, 3). With only 17 North American Ancestors, of the many thousands that were available, contributing 86% of the parentage of modern cultivars, one would presume that only a small amount of diversity could have passed through the introduction bottleneck. Although the conventional population genetic measures of diversity (π and θ) suggested that the 17 North American Ancestors have almost as much diversity as the Asian Landraces, the 78% loss of rare alleles as a result of the introduction bottleneck agrees with theory that genetic bottlenecks can have little effect on diversity but still result in the loss of many rare alleles.

We found that 79% of the low-frequency sequence variants in the Landraces were not present in the Elite Cultivars. Thus, although there was a significant but relatively modest loss of diversity, as measured by θ, there was an extensive loss of rare sequence variants seen in the introduction bottleneck to North America. This suggests that the Elite Cultivars contain most of the common variation of the Asian Landrace collection and that variation useful for genetic improvement not present in the Elite Cultivars will be found at low frequency and require careful screening of the Asian Landrace collection. This conclusion is supported by the results of numerous attempts to discover traits of interest in the exotic soybean germplasm collection. For example, only 45 of the 9,153 genotypes screened for resistance to soybean cyst nematode (*Heterodera glycine* Ichinohe) race 3 possessed even moderate resistance (18). Van Duyn *et al.* (19) screened a large number of accessions in the U.S. Department of Agriculture, Agricultural Research Station National Soybean Germplasm Collection for resistance to foliar feeding insects, and found only three resistant genotypes. Chamberlain and Bernard (20) screened 2,060 Landrace accessions for brown stem rot (*Phialophora gregata*) resistance and found only one with resistance. Given the low frequency of useful variants for a given trait, it is unlikely that randomly adding even 100 new Asian Landraces (by recurrent introgressive matings) to the Elite germplasm pool would increase useful diversity beyond what is already present in the North American Elite Cultivars. Indeed, it is simply more effective to operate on a per need basis by screening the Asian Landrace germplasm to identify the few accessions that posses the desired variants, followed by the introgression of those variants into the Elite germplasm pool.

Overall, the effects of the domestication and introduction bottlenecks, combined with subsequent intensive selection in soybean, have resulted in sequence diversity losses in the Elite Cultivars vs. *G. soja* of 65, 49, and by 44%, as measured by θ, π, and haplotype diversity, respectively. Indeed, no allelic diversity was detected among the Elite Cultivars for ≈40% of the genes analyzed (Table 2). These bottlenecks have also significantly altered the allele frequencies of the genes we sampled. Wright *et al.* (14) sequenced 774 genes in a sampling of teosinte and modern maize inbred lines to determine the effects of domestication and modern breeding on diversity. They found that modern inbred maize lines have retained 57% of the diversity in teosinte. This reduction of 43% was due to the reduction in population size and selection during domestication and modern breeding, although the germplasm studied did not allow the authors to separate the effects of these two bottlenecks. Multiple studies have shown that >60% of the diversity is maintained after domestication of a number of grass species including: *Zea mays*, *Sorghum bicolor*, *Orzya sativa*, etc. (21). Buckler *et al.* (21) suggested that the large proportion of diversity maintained through the domestication bottleneck was due to the use of these crops as a basis for subsistence. This led to large quantities of these grass grains being grown during early cultivation, thereby maintaining large amounts of diversity. In soybean, the domestication bottleneck appears to have been somewhat more severe than the domestication bottlenecks of grasses. It is not known how many domestication events occurred in soybean (5). Our data do not reveal whether there was one or multiple domestications, but overall, the domestication bottleneck was responsible for a 50% reduction in diversity, the elimination of 81% of rare alleles present in *G. soja*, and a significant change in allele frequency in 60% of the genes analyzed.

Our data also indicate that *G. soja*, from which soybean was domesticated, has unusually low levels of sequence diversity for a wild crop species (π = 0.00217, θ = 0.00235). Loblolly pine (θ = 0.0041; ref. 22), *Arabidopsis* (θ = 0.0071; ref. 23), wild barley (θ = 0.0081; ref. 24), and teosinte (θ = 0.0109; ref. 14) have 2- to 5-fold

greater nucleotide diversity than *G. soja*. Several factors may contribute to the lack of genetic diversity in *G. soja*, including effective population size, demography and autogamy (25).

The widely held assumption that intensive modern crop breeding, when applied to the descendants of a small number of founder introductions collected from the center of crop origin, has resulted in a drastic reduction of genome diversity (1, 3) does not appear to be valid in soybean. Instead, it appears that the low nucleotide diversity in modern elite soybean cultivars is mainly due to an unusually low level of genetic variability in the wild progenitor, *G. soja*, followed by a 50% loss of diversity during the domestication bottleneck. The most significant loss of diversity occurred during domestication and the introduction bottleneck where there was a large loss of rare alleles present in *G. soja* and the Asian Landraces. These rare alleles are likely to benefit future soybean improvement. Expansion of the currently low number of *G. soja* accessions available to North American soybean geneticists and breeders should be considered a high priority, given the great amount of diversity in terms of the presence of rare and unique alleles not found in the available *G. max* germplasm collections and the Elite cultivars.

Materials and Methods

Plant Materials. The plant material included genotypes listed in Table 4. The first population consisted of 26 *G. soja* plant introductions from China, Korea, Taiwan, Russia, and Japan collected from 23–50°N and 106–140°E. This population of accessions was selected to sample all of the geographical areas within the range of *G. soja*. Origin and maturity group of accessions were the primary selection criteria. The population of Landraces consisted of 52 Asian plant introductions from China, Korea, and Japan collected from 22–50°N and 104–140°E. More accessions were included from China, where domesticated soybean originated. Cluster analysis has previously determined that Landraces from Japan and Korea are similar but less diverse than and distinct from those originating in China (26). In addition, it has been shown that there was more diversity between Landraces from different Chinese provinces than among Landraces from the same province. Similar diversity differences were not apparent among Landraces from different Korean or Japanese provinces (26). To adequately represent this diversity, at least two Landraces were selected from each Chinese province in which soybean was grown before scientific plant breeding. Landraces within provinces were selected for extremes in maturity groups available to include Landraces that represent diverse geographical regions and/or cropping systems within provinces and for phenotypic differences. Landraces from Korea and Japan were selected to represent the range of diversity in maturity groups and phenotypic descriptors. The 17 North American Ancestors are *G. max* accessions from Asia that are estimated to contribute at least 86% of the genes present in the gene pool of North American soybean cultivars (7). The Elite Cultivars consisted of 25 North American cultivars publicly released between 1977 and 1990, selected to maximize diversity based upon an analysis of coefficient of parentage by Gizlice *et al.* (27). Pure line seeds of all accessions were obtained from the U.S. Department of Agriculture Soybean Germplasm Collection (U.S. Department of Agriculture, Agriculture Research Station, University of Illinois, Urbana, IL). DNA was extracted from bulked leaf tissue of 8–10 *G. soja* plants or 30–50 *G. max* plants, as described by Keim *et al.* (28).

PCR and Sequencing. PCR primers were originally designed by Zhu *et al.* (8) to 178 randomly selected genes and cDNAs for which there was no prior information on sequence diversity. Zhu *et al.* (8) successfully obtained sequence data from 116 of the 178 genes and cDNAs. We screened all 116 genes in the four populations and obtained sequence data for all or most of the 120 *G. soja* and *G. max* genotypes for 102 genes from 111 PCR fragments with sequence lengths from 400 to 600 bp listed in Table 3. Subsequently, 37 of the 102 genes and cDNAs have been genetically mapped with the populations described by Song *et al.* (29) and are distributed throughout 15 of the 20 linkage groups in soybean (Table 5). PCR primers and amplification conditions were described by Zhu *et al.* (8). Forward and reverse sequencing reactions were performed on an ABI 3700 or ABI 3730 using ABI Prism BigDye Terminator (Version 3.1) cycle sequencing (Applied Biosystems, Foster City, CA). Sequence data from each amplicon were aligned and analyzed with the standard DNA analysis software Phred/Phrap, and SNP detection was carried out with a machine learning algorithm based on PolyBayes SNP discovery software (30, 31). The resulting alignments and SNP predictions were visually verified by using the Consed viewer (32). Fragments were resequenced if there was any ambiguity as to which allele was present.

Sequence Analysis. Small insertions and deletions were recorded as a single SNP and included in all SNP sequence analysis. Nucleotide diversity estimates for π (9) and θ (10) were calculated for each of the 102 genes within each population. Each π and θ matrix consists of $n_G \times n_P$ observations, where n_G is the number of genes, and n_P is the number of populations. The total variation in the matrix was partitioned by PROC ANOVA (SAS Institute, Cary, NC) into population, gene, and population $\times$ gene sources of variation. The population $\times$ gene mean square was used to test differences among populations.

The number of synonymous and nonsynonymous sites was measured by using DnaSP sequence polymorphism software (Version 3.5) (33). F_{st} was calculated as described by Hudson *et al.* (34). Tajima's D was calculated without an outgroup as described by Tajima (35). Haplotype diversity was calculated as described by Weir (16) as $1-\Sigma P^2_{ij}$, where ΣP_{ij} is the frequency of the jth haplotype for ith locus summed across all haplotypes in the locus.

Simulation Procedures. The percentage of unique contribution of each North American Ancestor to the Elite Cultivars was obtained from Carter *et al.* (15). The percentage of unique contribution was converted to the number of contributed loci (NCL; or fragments) to each Elite Cultivar based on a total of 102 loci. For example, if ancestor A has a percentage of unique contribution of 50% to Elite Cultivar 1, then ancestor A's NCL would be equal to 51 loci. In some instances, the total contribution from the 17 North American Ancestors to each Elite Cultivar was <102, because other North American Ancestors aside from the 17 included in this study were present in the pedigree. In these cases, one or more of the 52 Landraces was randomly chosen to represent the ancestral contribution not accounted for by the 17 North American Ancestors (i.e., the number of Landraces randomly selected for any given Elite Cultivar was equal to 102 loci minus the sum of loci contributed to that cultivar by one or more of the 17 North American Ancestors).

The genotype of each Elite Cultivar was simulated based upon the calculated number of contributed loci from each North American Ancestor (or Landrace). The SNP genotypes of Elite Cultivars were then extracted randomly from the corresponding contributors where all 102 alleles were represented only once within each simulated Elite Cultivar. For each permutation, the program generated a SNP genotype matrix with 102 unique loci $\times$ 25 Elite Cultivars. π and θ were calculated for each locus at each permutation. A total of 5,000 permutations were performed; the means for each locus, together with the observed π and θ, were used for ANOVA and to test whether there was a significant difference between the observed and simulated values of π and θ of the Elite Cultivars.

Allele frequency differences of each SNP were calculated between *G. soja* and Landraces, Landraces and North American Ancestors, North American Ancestors and Elite Cultivars, and Landraces and Elite Cultivar populations. The frequency difference was defined as $p_{12i} = x_{1i}/n_{1i} - x_{2i}/n_{2i}$, where x_{1i} and x_{2i} are the number of accessions with a given allele at a SNP locus i in populations 1 and 2, respectively; and n_{1i} and n_{2i} are the number of accessions in populations 1 and 2, respectively. The significance of the observed frequency differences was tested by permutation. First, the total number of accessions with the given SNP allele in the two populations being compared was counted ($n_{12} = x_{1i} + x_{2i}$); a total of n_{12} accessions in the two populations were randomly assigned the first allele, and the remaining accessions were assigned the second allele; and the permutated frequency difference under the assumption of no frequency difference between populations was calculated and compared with the observed frequency difference. The process was repeated 10,000 times for each locus. The measure of significance (p) is given by the ratio (N/10,000), where N is the number of times the expected absolute frequency difference between the populations was exceeded by the observed absolute frequency difference.

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Supplementary Material. Tables 3, 4, and 5.

Table 3. GenBank accession nos and genes, cDNAs, and description of genes and cDNAs to which primers were designed and sequence data obtained and analyzed for sequence diversity

GenBank accession no.	Description	GenBank accession no.	Description
AB003680	A3B4 Glycinin	L20310	Nodulin (nod-20)
AB003908	Phosphoenolpyruvate carboxylase	L27265	Phosphatidylinositol 3-kinase
AB004062	A5A4B3 glycinin	L27417	GTP binding protein (STGA1)
AB007127	Acidic chitinase	L28831	Ribosomal protein S11
AB018378	Early nodulin	L29770	Phosphatidylinositol 3-kinase
AB025102	Protoporphyrinogen IX oxidase	L34842	Chloroplast phytochrome A (phyA)
AB029159	GmMYB29A1	L42814	Acetyl coA carboxylase (ACCase-A)
AB030491	Thiamin biosynthetic enzyme	M10594	Uricase I I
AB030493	Thiamin biosynthetic enzyme	M10595	Peribacteroid membrane protein
AB040040	Nonclathrin coat protein	M11317	Low MW heat shock protein
AF005030	2S albumin pre-propeptide	M13759	Alpha'-type beta conglycinin storage protein
AF007211	Peroxidase precursor (GMIPER1)	M16772	Urease
AF022462	Cytochrome P450 monooxygenase	M16884	Cytochrome oxidase subunit I

AF055369	Nitrate reductase (nr2)	M21296	Beta-tubulin (S-beta-1)
AF061564	Glyceraldehyde-3-phosphate dehydrogenase 1	M64267	Iron superoxide dismutase (FeSOD)
AF079058	Alcohol dehydrogenase Adh-1	M76980	Vegetative storage protein (vspB)
AF083880	Alternative oxidase precursor (Aox 1)	M76981	vspA
AF089850	Urate-degrading peroxidase (PP1)	M80664	Late embryogenesis abundant (LEA) protein
AF105199	Glutathione reductase (GR-5)	M94012	Maturation-associated protein (MAT9)
AF117885	Seed maturation protein PM31 (PM31)	M97285	Seed maturation protein
AF124148	Trehalase 1 GMTRE1	M98871	Chalcone synthase (chs7)
AF127110	GO8 ripening related protein	U12150	Protease inhibitor
AF128443	SNF-1-like serine/threonine protein kinase	U26457	Lipoxygenase (vlxC)
AF141602	Cystathionine-gamma-synthase precursor	U31648	Ferritin
AF162283	Acetyl-CoA carboxylase (accB-1)	U32185	Guanine nucleotide regulalory protein
AF167556	Dihydroflavonol-4-reductase DFR1	U41323	Beta-1,3-glucanase (SGN1)
AF195819	Isoflavone synthase 2 (ifs2)	U47143	Nonsymbiotic hemoglobin
AJ223037	Leginsulin	U60500	Actin (Soy57)

AJ239127	Major latex protein homolog	U63726	Gamma glutamyl hydrolase
AJ276407	Pre-pro-subtilisin	U66836	RecA/Rad51/DMC1-like protein
D13505	Early nodulin	U82810	Early light induced protein
D13949	Lipoxygenase -2 (lox2)	U87999	Phosphoribosylpyrophosphate amidotransferase
D16107	Basic 7s globulin	X05024	Nodulin 22
D16248	Ubiquitin	X07675	NADH dehydrogenase and rps7
D26092	Ubiquitin	X16875	Ngm-75
D31700	Cysteine proteinase inhibitor	X52863	Glycinin
D50866	Beta-amylase	X60043	Stress-induced gene (SAM22)
D64115	Cysteine proteinase inhibitor	X63198	Low MW heat shock protein
D78510	Beta-glucan-elicitor receptor	X63565	Seed maturation polypeptide
E00532	Heat-shock protein	X67304	Lipoxygenase 1
E01433	Leghemoglobine c3	X68702	Alternative oxidase
E03629	Lipoxygenase	X68707	Proteinase inhibitor D-II
E13668	DNA-binding protein	X69639	Auxin down regulated gene (ADR6)
J01297	Actin 3 (Sac 3)	X71083	Coproporphyrinogen oxidase
J02746	Proline-rich protein	X78547	Epoxide hydrolase
K00821	Lectin (Le1)	X78548	Epoxide hydrolase
L00921	Maturation protein (MAT 1)	Z11980	Cytochrome oxidase subunit 2
L01433	Calmodulin (SCaM-4)	Z32795	Cysteine endopeptidase

L01447	G-box binding factor (GBF1)	Z46951	Heat shock transcription factor 29
L10292	Ascorbate peroxidase	Z46953	Heat shock transcription factor 34
L19359	Calmodulin (ScaM-5)	Z46954	Heat shock transcription factor 33

Table 4. Soybean germplasm used in this study

Type	Strain designation	Province or state	Country	Cultivar	Maturity group
Elite	A3127	Michigan	USA	A3127	III
cultivars	Burlison	Illinois	USA	Burlison	II
	Century	Indiana	USA	Century	II
	Conrad	Iowa	USA	Conrad	II
	Dassel	Minnesota	USA	Dassel	0
	Dawson	Minnesota	USA	Dawson	0
	Glenwood	Minnesota	USA	Glenwood	0
	Gordon	Georgia	USA	Gordon	VII
	Hoyt	Ohio	USA	Hoyt	II
	Hutcheson	Virginia	USA	Hutcheson	V
	Kershaw	South Carolina	USA	Kershaw	VI
	Lloyd	Arkansas	USA	Lloyd	VI
	Maple Glen	Ontario (Ottawa)	Canada	Maple Glen	00
	OAC Libra	Ontario (Guelph)	Canada	OAC Libra	0
	OAC Musca	Ontario (Guelph)	Canada	OAC Musca	0
	Pennyrile	Kentucky	USA	Pennyrile	IV
	Perrin	South Carolina	USA	Perrin	VIII
	Pershing	Missouri	USA	Pershing	IV
	Preston	Iowa	USA	Preston	II
	Ripley	Ohio	USA	Ripley	IV
	Sprite	Ohio	USA	Sprite	III

Elite cultivars	Thomas	Georgia	USA	Thomas	VII
	Weber	Iowa	USA	Weber	I
	Young	North Carolina	USA	Young	VI
	Zane	Ohio	USA	Zane	III
N. Am. Ancestors	PI548362	Unknown	Unknown	Lincoln	III
	PI 548379	Heilongjiang	China	Mandarin (Ottawa)	0
	PI 548445	Jiangsu	China	CNS	VII
	PI 548406	Jilin	China	Richland	II
	PI 548488	Missouri	USA	S-100	V
	PI 548477	Tennessee	USA	Ogden	VI
	PI 548298	Unknown	China	AK [Harrow]	III
	PI 548318	Jilin	China	Dunfield	III
	PI 548391	Liaoning	China	Mukden	II
	PI 548657	North Carolina	USA	Jackson	VII
	PI 548348	Unknown	China	Illini	III
	PI 548485	Jiangsu	China	Roanoke	VII
	PI 548311	Ontario	Canada	Capital	0
	PI 548603	Indiana	USA	Perry	IV
	PI 548382	Liaoning	China	Manitoba Brown	00
	PI 548456	Pyongyang	Korea, North	Haberlandt	VI
	FC 33243	Unknown	Unknown	Anderson	IV
Landraces	PI059845	Akita	Japan	Sohgetsu	V
	PI081775	Akita	Japan		I
	PI089138	Hamgyong Puk	Korea, North	Zontanoruk-on	II

	PI097094	Hwanghae Puk	Korea, North		VII
	PI398296	Kyonggi	Korea, South		II
	PI399043	Cheju	Korea, South		III
	PI407801	Kyonggi	Korea, South		VI
	PI407849	Cholla Puk	Korea, South		III
	PI408342	Cheju	Korea, South		VI
	PI423954	Kumamoto	Japan	Shirome	0
	PI423967	Kumamoto	Japan	Nabeshima	IX
	PI424391	Cholla Puk	Korea, South		VI
	PI567258	Jiangxi	China	He pi dou	II
	PI567293	Gansu	China	Ben di huang dou	II
	PI567298	Gansu	China	Chan yao dou	V
	PI567364	Ningxia	China	Ping luo huang da dou	II
	PI567368	Ningxia	China	Xi he huang dou	IV
	PI567395	Shaanxi	China	Lai wa dou	IV
Landraces	PI567481	Hebei	China	Bao ding huang dou	II
	PI567503	Hebei	China	Niu mao huang	IV
	PI567525	Shandong	China	Cao qing huang dou	II
	PI567700	Anhui	China	Fu yang (19)	III

	PI587552	Jiangsu	China	Nan jing da ping ding huang yi 1	VII
	PI587666	Anhui	China	Er dao zao	VI
	PI587752	Hubei	China	Xian ning dong huang dou jia	V
	PI587799	Hubei	China	Wu chang zao huang dou	VIII
	PI587906	Zhejiang	China	Huang dou	IX
	PI587946	Fujian	China	Ping nan qiu da dou	X
	PI588000	Sichuan	China	Shi yue huang	X
	PI588047	Guangdong	China	Huang ke wu dou	IX
	PI588053A	Guangdong	China	Xiao li huang	VI
	PI594451	Sichuan	China	Liu yue bao	III
	PI594554	Jiangxi	China	Huang pi tian dou	IX
	PI594579	Hunan	China	Zhong he tian cheng dou	V
	PI594597	Hunan	China	Ning yuan ba yue huang	IX
	PI594615	Guizhou	China	Liu yue zao	IV
	PI594629	Guizhou	China	Xiao hua lian	VI
Landraces	PI594770A	Guangxi	China	Fu sui chang ping hei dou	VI
	PI594773	Guangxi	China	Fu sui qu li dou	IX
	PI594777	Yunnan	China	Liu yue huang	IV
	PI594788	Yunnan	China	Da zao dou	IX
	PI602991	Shandong	China	Niu jiao qi da hei dou	V
	PI603318	Heilongjiang	China		0

	PI603336	Heilongjiang	China	II
	PI603357	Jilin	China	I
	PI603384	Jilin	China	III
	PI603420	Nei Monggol	China	II
	PI603424A	Nei Monggol	China	0
	PI603516	Shaanxi	China	VI
	PI603596	Fujian	China	III
	PI603675	Jiangsu	China	III
	PI603756	Zhejiang	China	II
	PI339871A	Cheju	Korea	V
Glycine soja	PI366120	Akita	Japan	IV
	PI393551	Taiwan	Taiwan	X
	PI407027	Akita	Japan	V
	PI407131	Kumamoto	Japan	VI
	PI407140	Kumamoto	Japan	VII
	PI407170	Kyonggi	Korea, South	V
	PI407275	Kyonggi	Korea, South	IV
	PI407282	Cheju	Korea, South	VI
	PI407288	Jilin	China	II
G. soja	PI407301	Jiangsu	China	V
	PI447004	Jilin	China	III
	PI458536	Heilongjiang	China	0
	PI458538	Heilongjiang	China	000
	PI464935	Jiangsu	China	VI
	PI468400A	Ningxia	China	IV

PI483464A	Ningxia	China	III
PI483465	Shaanxi	China	V
PI518282	Unknown	Taiwan	VI
PI549046	Shaanxi	China	III
PI562559	Cholla Puk	Korea, South	V
PI562565	Cholla Puk	Korea, South	IV
PI597459D	Shandong	China	III
PI597461A	Shandong	China	IV
PI326582A	Primorye	Russia	II
PI468916	Liaoning	China	III

Table 5. Nucleotide polymorphism per base pair × 10^3 in wild and domesticated soybean

GenBank accession no.	Composite map location (linkage group, cM)	*G. soja*(π/θ)	Landraces (π/θ)	North American Ancestors (π/θ)	Elite Cultivars (π/θ)	Tajima's *DG. soja*	Tajima's *D* Landraces	Tajima's *D* North American Ancestors	Tajima's *D* Elite Cultivars
AB003680		4.18/8.16	1.79/0.98	0.00/0.00	0.00/0.00	-1.50	1.08		
AB003908	H:66.0	2.26/3.65	1.78/1.54	1.07/1.03	1.16/0.92	-1.02	0.27	0.09	0.43
AB004062		5.03/6.50	1.02/1.83	0.00/0.00	0.00/0.00	-0.67	-0.78		
AB007127	K:52.9	9.39/6.24	2.99/5.27	2.63/3.52	0.00/0.00	1.49	-1.09	-0.72	
AB018378		0.00/0.00	0.00/0.00	0.00/0.00	0.00/0.00				
AB025102		0.74/2.51	1.46/2.12	2.46/1.42	2.54/1.27	-1.51	-0.55	1.43	1.66
AB029159		1.20/1.06	0.97/0.59	0.65/0.40	0.69/0.36	0.34	1.11	1.24	1.56
AB030491	H:64.5	4.62/3.96	2.68/3.34	3.88/2.23	2.87/2.00	0.36	-0.35	1.43	0.72
AB030493		2.87/3.03	0.27/1.53	0.00/0.00	0.00/0.00	-0.15	-1.69		
AB040040	M:4.7	8.34/6.30	7.70/5.32	9.36/7.11	8.05/4.77	0.87	1.01	0.97	1.71
AF005030		1.87/4.35	0.00/0.00	0.00/0.00	0.00/0.00	-1.22			
AF007211		0.00/0.00	0.00/0.00	0.00/0.00	0.00/0.00				
AF022462	F:70.7	28.30/20.15	22.58/10.21	23.75/13.65	25.12/12.22	1.15		2.10*	2.62*
AF055369		1.40/4.79	0.00/0.00	0.00/0.00	0.00/0.00	-1.16			
AF061564		0.80/2.71	0.00/0.00	0.00/0.00	0.00/0.00	-1.16			
AF079058	B2:70.4	1.31/0.84	1.04/0.71	1.56/0.95	1.63/0.85	0.92	0.61	1.24	1.51
AF083880		5.47/8.66	3.95/2.74	4.01/2.44	4.21/2.19	-1.16	0.90	1.60	2.01*
AF089850	M:98.2	6.36/7.49	3.82/2.11	5.04/2.82	4.83/2.52	-0.37	1.08	1.53	1.51
AF105199		12.32/12.41	14.10/8.38	12.11/11.20	11.42/10.03	-0.02	1.54	0.25	0.38
AF117885	D1b:29.2	9.75/9.83	6.14/2.77	1.84/3.70	5.33/3.31	-0.02	2.15*	-1.26	1.33

AF124148		18.29/9.74	15.55/8.22	14.21/10.99	16.85/9.84	1.88	1.58	0.73	1.54
AF127110		3.66/6.49	0.00/0.00	0.00/0.00	0.00/0.00	-0.71			
AF128443		0.00/0.00	0.00/0.00	0.00/0.00	0.00/0.00				
AF141602		0.67/2.27	0.00/0.00	0.00/0.00	0.00/0.00	-1.73			
AF162283	G:84.2	5.14/6.32	3.79/2.13	3.44/2.14	3.28/1.92	-0.61	1.74	1.72	1.77
AF167556	B2:35.7	29.49/40.00	50.60/33.77	35.01/27.09	38.46/24.25	-0.74	1.20	0.83	1.46
AF195819		2.42/1.29	1.17/1.09	0.58/1.45	0.75/1.30	1.44	0.10	-1.16	-0.70
AJ223037		50.65/48.24	30.46/34.92	34.42/31.12	32.43/34.82	0.15	-0.32	0.32	-0.20
AJ239127		0.28/0.95	0.00/0.00	0.00/0.00	0.00/0.00	-1.16			
AJ276407		10.83/8.95	0.26/1.51	0.00/0.00	0.27/0.90	0.69	-1.46		-1.16
D13505	G:5.8	2.05/2.50	1.42/1.69	1.35/1.13	0.93/1.01	-0.50	-0.36	0.48	-0.18
D13949		0.58/2.06	0.00/0.00	0.00/0.00	0.00/0.00	-1.54			
D16107		1.89/4.40	3.65/1.86	4.32/2.49	4.37/2.23	-1.22	1.28	1.43	1.60
D16248		1.54/1.85	2.06/1.25	0.00/0.00	0.00/0.00	-0.47	1.45		
D26092		2.38/2.98	1.69/0.97	2.21/1.55	1.54/1.39	-0.69	1.79	1.43	0.32
D31700	E:49.0	6.93/7.64	5.67/6.46	7.04/5.18	8.38/4.64	-0.27	-0.29	1.02	2.01*
D50866		4.82/4.08	4.49/2.58	3.78/2.30	3.73/2.06	0.49	1.51	1.60	1.76
D64115	E:49.0	4.89/5.22	3.99/4.41	4.39/3.54	6.03/3.17	-0.18	-0.23	0.69	2.25*
D78510		3.84/4.33	1.77/1.83	1.76/2.44	1.38/1.09	-0.30	-0.06	-0.70	0.43
E00532	A2:38.0	1.76/2.54	0.62/0.31	0.61/0.41	0.81/0.37	-0.94	1.37	0.95	2.02*
E01433	O:94.7	4.95/5.57	3.28/3.52	1.25/3.14	2.02/1.41	-0.30	-0.14	-1.50	0.72
E03629		0.23/0.53	0.08/0.22	0.00/0.00	0.00/0.00	-1.22	-0.88		
E13668		5.70/5.45	0.00/0.00	0.00/0.00	0.00/0.00	0.10			
J01297		0.00/0.00	0.00/0.00	0.00/0.00	0.00/0.00				
J02746	K:48.0	4.58/3.48	9.48/4.90	7.95/6.55	8.76/5.87	0.77	2.24*	0.69	1.41
K00821		7.11/7.34	0.00/0.00	0.00/0.00	0.00/0.00	-0.08			
L00921	M:49.0	8.50/8.52	11.42/5.40	10.76/7.21	8.21/6.46	-0.01	2.82*	1.65	0.81

L01433		2.92/4.19	2.52/1.18	1.18/1.58	0.43/1.41	-0.74	1.51	-0.49	-1.16
L01447		1.49/1.33	0.25/0.45	0.00/0.00	0.00/0.00	0.34	-0.78		
L10292		3.30/4.15	2.12/1.17	0.62/1.56	0.00/0.00	-0.50	1.08	-1.16	
L19359		3.09/3.13	1.89/1.32	3.16/1.77	0.92/1.58	-0.03	0.57	1.53	-0.70
L20310		15.51/15.10	3.15/7.85	0.00/0.00	0.00/0.00	0.09	-1.62		
L27265		0.76/0.60	0.37/0.26	0.25/0.34	0.59/0.31	0.54	0.57	-0.49	1.56
L27417		0.72/1.06	0.34/0.36	0.52/0.48	0.45/0.43	-0.90	-0.11	0.23	0.10
L28831	F:70.9	6.56/7.02	6.99/5.93	7.49/4.75	6.04/4.26	-0.19	0.43	1.63	1.04
L29770		2.61/3.01	0.68/0.64	1.01/2.55	0.00/0.00	-0.36	0.10	-1.71	
L34842	O:56.8	5.20/4.92	1.76/4.16	1.16/1.11	1.70/0.99	0.16	-1.39	0.09	1.18
L42814		0.76/2.58	0.00/0.00	0.00/0.00	0.00/0.00	-1.51			
M10594	I:37.6	7.17/5.94	2.01/2.51	1.33/3.35	2.49/3.00	0.55	-0.35	-1.50	-0.37
M10595		11.71/10.45	11.46/8.82	8.75/8.42	6.91/6.03	0.37	0.78	0.13	0.39
M11317		9.25/10.63	5.75/5.99	10.27/6.67	9.41/8.36	-0.42	-0.10	1.75	0.39
M13759		3.91/5.25	2.09/1.11	1.10/1.48	1.10/1.33	-0.68	1.19	-0.49	-0.28
M16772	B1:123.6	5.44/2.78	5.37/2.35	4.06/3.14	4.81/2.81	2.05*	2.27*	0.73	1.54
M16884		0.00/0.00	0.00/0.00	0.00/0.00	0.00/0.00				
M21296		0.20/0.68	0.00/0.00	0.00/0.00	0.00/0.00	-1.16			
M64267	I:95.7	3.99/4.68	4.48/2.97	2.69/2.64	1.88/1.78	-0.47	1.29	0.06	0.14
M76980		0.26/0.46	0.03/0.19	0.00/0.00	0.00/0.00	-0.93	-1.10		
M76981	M:7.2	2.12/3.69	1.25/1.56	3.10/4.16	0.56/1.86	-0.91	-0.26	-0.63	-1.16
M80664		1.54/2.02	0.00/0.00	0.00/0.00	0.00/0.00	-0.51			
M94012		6.80/6.51	1.82/1.57	1.88/1.05	1.69/0.94	0.14	0.28	1.53	1.33
M97285		13.69/9.78	5.42/4.96	6.15/6.62	6.12/5.93	1.13	0.19	-0.20	0.08
M98871	D1a:115.0	3.00/8.87	2.79/2.50	1.74/1.67	2.56/1.49	-1.96*	0.21	0.09	1.18
U12150		0.00/0.00	0.00/0.00	0.00/0.00	0.00/0.00				
U26457		0.37/0.86	0.00/0.00	0.00/0.00	0.00/0.00	-1.22			

U31648	M:74.3	8.09/6.66	7.30/4.68	3.92/3.76	4.09/4.48	0.64	1.34	0.12	-0.24
U32185	K:5.4	0.98/1.16	2.85/1.97	0.69/0.66	1.10/1.76	-0.33	1.01	0.09	-0.94
U41323	L:46.3	3.18/8.94	6.61/4.31	5.30/4.32	5.91/3.87	-1.97*	1.20	0.64	1.31
U47143		13.39/10.22	14.30/8.63	5.88/11.53	1.85/1.29	0.98	1.78	-1.73	0.72
U60500	E:14.4	9.96/8.27	1.04/5.99	0.53/1.33	0.69/1.19	0.63	-2.09*	-1.16	-0.70
U63726	F:95.1	7.24/5.54	7.18/4.68	4.04/4.69	2.52/1.40	0.82	1.20	-0.39	1.33
U66836		0.99/0.95	0.29/0.27	0.00/0.00	0.18/0.32	0.10	0.10		-0.70
U82810		0.64/2.16	0.00/0.00	0.00/0.00	0.00/0.00	-1.16			
U87999	K:93.9	9.79/14.02	0.98/0.91	0.48/1.22	0.33/1.09	-1.03	0.10	-1.16	-1.16
X05024	E:15.1	0.87/1.49	0.13/0.25	0.00/0.00	0.00/0.00	-1.18	-0.66		
X07675		0.00/0.00	0.00/0.00	0.00/0.00	0.00/0.00				
X16875	A2:57.0	0.84/2.85	5.54/2.41	5.61/3.22	7.33/5.77	-1.16	1.73	1.43	0.59
X52863		2.49/2.70	1.91/1.71	0.00/0.00	0.21/0.68	-0.21	0.24		-1.16
X60043		11.81/9.48	13.34/6.94	1.95/2.14	2.37/1.92	0.85	2.75*	-0.25	0.59
X63198	I:108.0	4.12/2.65	2.12/1.12	2.68/1.50	2.63/1.34	1.74	2.01*	2.42*	2.60*
X63565		4.99/5.88	0.00/0.00	0.00/0.00	0.00/0.00	-0.43			
X67304		0.79/2.69	0.77/2.27	0.00/0.00	0.00/0.00	-1.16	-0.88		
X68702	C1:72.4	2.74/1.64	1.67/1.38	2.62/1.85	2.75/1.65	1.44	0.37	1.04	1.43
X68707		5.53/3.90	5.40/5.49	5.18/5.87	6.35/3.94	1.02	-0.04	-0.36	1.52
X69639		2.76/9.41	0.00/0.00	0.00/0.00	0.00/0.00	-2.22*			
X71083		1.44/1.51	0.58/0.77	0.27/0.68	0.28/0.92	-0.14	-0.51	-1.50	-1.73
X78547		1.35/2.39	0.00/0.00	0.00/0.00	0.00/0.00	-0.71			
X78548	L:102.5	10.68/11.37	4.81/4.80	6.70/3.85	6.37/3.45	-0.20	0.00	2.10*	2.10*
Z11980		4.33/5.47	0.65/2.31	0.00/0.00	0.00/0.00	-0.62	-1.47		
Z32795		3.55/3.05	4.09/3.43	3.19/2.29	3.54/4.10	0.41	0.43	0.98	-0.37
Z46951	D1a:107.8	2.76/6.19	0.00/0.00	0.00/0.00	0.00/0.00	-1.57			
Z46953	D1a:83.3	1.80/2.71	1.30/2.29	0.61/1.53	0.00/0.00	-0.72	-0.76	-1.16	

Z46954	22.13/23.08	4.16/6.50	4.53/4.34	5.82/3.89	-0.12	-0.64	0.09	0.83

* Tajima's *D* value significantly different from 0 ($p < 0.05$)