A single domestication for maize shown by multilocus microsatellite genotyping

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There exists extraordinary morphological and genetic diversity among the maize landraces that have been developed by pre-Columbian cultivators. To explain this high level of diversity in maize, several authors have proposed that maize landraces were the products of multiple independent domestications from their wild relative (teosinte). We present phylogenetic analyses based on 264 individual plants, each genotyped at 99 microsatellites, that challenge the multiple-origins hypothesis. Instead, our results indicate that all maize arose from a single domestication in southern Mexico about 9,000 years ago. Our analyses also indicate that the oldest surviving maize types are those of the Mexican highlands with maize spreading from this region over the Americas along two major paths. Our phylogenetic work is consistent with a model based on the archaeological record suggesting that maize diversified in the highlands of Mexico before spreading to the lowlands. We also found only modest evidence for postdomestication gene flow from teosinte into maize.

Most domesticated plant and animal species originated during a brief period in human history between 5,000 and 10,000 years ago. During this time, many crops and animals were domesticated multiple times independently, including rice (1), common bean (2), millet (3), cotton (4), squash (5), cattle, sheep, and goats (6). Like these, maize (*Zea mays* ssp. *mays*) has been considered to be the product of multiple independent domestications from its wild progenitor (teosinte) because of the remarkable morphological and genetic diversity that exists within it. For example, based in part on the diversity of ear shapes in maize, Galinat (7) concluded that distinct ancestral types of annual teosinte in different regions of Mexico were the starting points for at least two independent domestications of maize. Similarly, based on the diversity of chromosome knob patterns among annual teosinte and maize races, Kato (8) inferred that multiple domestications had occurred independently in several regions of Mexico.

Although the remarkable diversity found within maize is consistent with multiple domestications, it is equally consistent with a single domestication and subsequent diversification. Distinguishing between these two models requires phylogenetic analyses that incorporate comprehensive samples of maize and its progenitor, teosinte. In this article, we report the first such comprehensive phylogenetic analyses for maize and teosinte by using 99 microsatellite loci that provide broad coverage of the maize genome and a sample of 264 maize and teosinte plants.

Materials and Methods

Plant Materials. We sampled 193 maize accessions (one plant each) representing the entire pre-Columbian range of maize from eastern Canada to northern Chile (Fig. 1). This sample includes maize adapted to the short growing season of eastern North America, the deserts of Arizona, the highlands and lowlands of Mexico and Guatemala, the Caribbean Islands, the rainforest of the Amazon Basin, and regions of the Andes Mountains that exceed 3,500 m in elevation. We also sampled 67 Mexican annual teosinte (*Z. mays* ssp. *parviglumis* and ssp. *mexicana*) accessions (one plant each) that represent the full geographic range of ssp. *mexicana* (33 accessions) and ssp. *parviglumis* (34 accessions) (Fig. 1). We included four plants of a more distantly related teosinte (*Z. mays* ssp. *huehuetenangensis*) from Guatemala as an outgroup for rooting the phylogenies. Three other forms of teosinte (*Zea diploperennis*, *Zea perennis*, and *Zea luxurians*) were not included in this study because they are all separate species and it is well-established that they were not involved in the origins of maize (9). The complete passport data for the plant materials, including landrace designations, germplasm bank accession numbers, and geographical coordinates, have been published as supporting information on the PNAS web site, www.pnas.org.

Simple Sequence Repeat Genotyping. The plants were genotyped at Celera AgGen (Davis, CA). The details of the genotyping have been published elsewhere (10). Briefly, DNA was extracted from individual plants by the cTAB method, and the microsatellite regions were amplified by PCR with florescent-labeled primers. PCR products were size-separated on Applied Biosystems automated sequencers equipped with GENESCAN software and then classified to specific alleles or bins by GENESCAN and GENOTYPER software programs (10). We used 99 microsatellite loci that are evenly distributed throughout the genome. A list of the microsatellite loci with their chromosomal locations has been published as supporting information. Primer sequences are available at the MaizeDB (www.agron.missouri.edu/ssr.html).

Phylogenetic Analysis. Obtaining a reliable phylogeny for outcrossing taxa requires one to construct an average tree of the genome by using a large number of loci. For this reason, we used large-scale microsatellite genotyping with 99 microsatellite loci and a genetic distance measure that fits the pattern of mutation displayed by the microsatellites. This approach has been successfully applied in humans (11) and horses (12) by using many fewer microsatellite loci (30 and 15, respectively). Because many microsatellites of maize and other species do not evolve in a stepwise manner (13), they violate the assumptions for the genetic distance measures that are based on the stepwise mutation model. This feature makes the use of these distance measures inappropriate. Therefore, we used the proportion of shared alleles distance that is free of the stepwise assumption, enjoys low variance (14), and is widely used with multilocus microsatellite data (11, 12, 15). We used the FITCH program in the PHYLIP package (16) with the log-transformed proportion of shared alleles distance as implemented in the computer program MICROSAT (17) to construct phylogenetic trees. In FITCH, the J

Abbreviation: PCA, principal component analysis.

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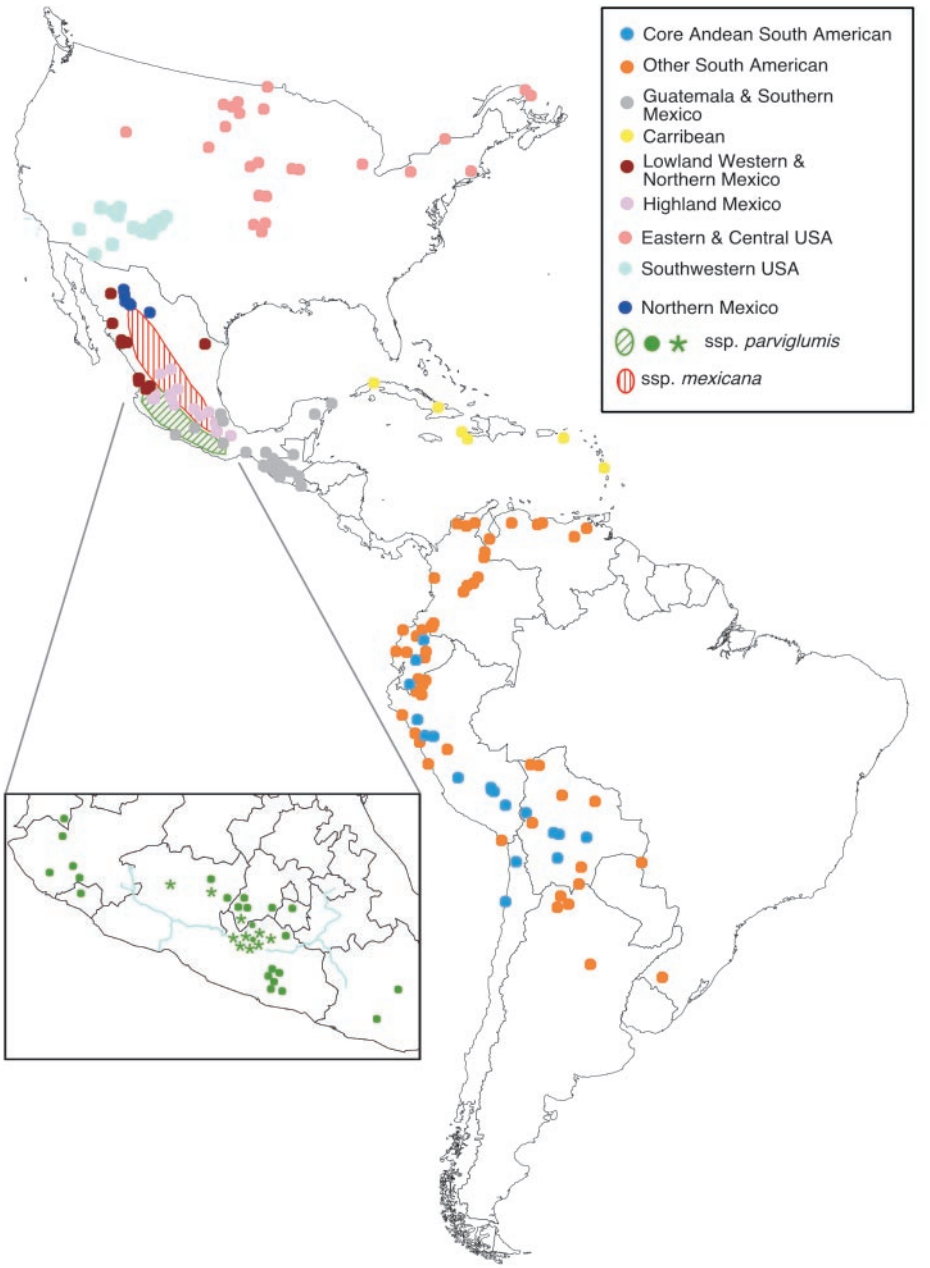

Fig. 1. Geographic distribution of maize and teosinte used in this study. Core Andean maize characterized by hand-grenade-shaped ears (22 samples), other South American maize (47), Guatemalan and southern Mexican maize (31), Caribbean maize (6), lowland western and northern Mexican maize (15), highland Mexican maize (20), eastern and central U.S. maize (24), southwestern U.S. maize (22), northern Mexican maize (6), ssp. *parviglumis* (34), and ssp. *mexicana* (33). *Inset* shows the distribution of the 34 populations of ssp. *parviglumis* in southern Mexico with the populations that are basal to maize in Fig. 2 (represented as asterisks). The blue line is the Balsas River and its major tributaries.

option was used to randomize the input order of samples. To determine the degree of statistical support for different branch points in the phylogenies, we evaluated 1,000 bootstrap samples of the data (16).

Principal Component Analysis (PCA). PCA was performed with the among sample variance-covariance matrix of allele frequencies with SAS software version 6.12 (SAS Institute, Cary, NC). The principal component scores for each plant have been published as supporting information.

Dating the Domestication Event. To estimate the time of divergence of maize and its ancestral teosinte, we used 33 loci with dinucleotide repeats for which fewer than 10% of the alleles did not fit a stepwise distribution. Nonstepwise alleles were treated as missing data. We calculated the mean $(\delta\mu)^2$ distance (18) over the 33 loci between Mexican maize and ssp. *parviglumis*. The number of generations (τ) after a population splits into two fully isolated populations was estimated with the following equation:

$$E[(\delta\mu)^2] = 2\omega\tau, \qquad [1]$$

where ω is the effective mutation rate, that is, the product of the mutation rate (μ) and the second moment of mutational change in the number of repeats (σ_m^2) (19). We estimated μ and σ_m^2 by using 86 recombinant inbreds. The details will be published

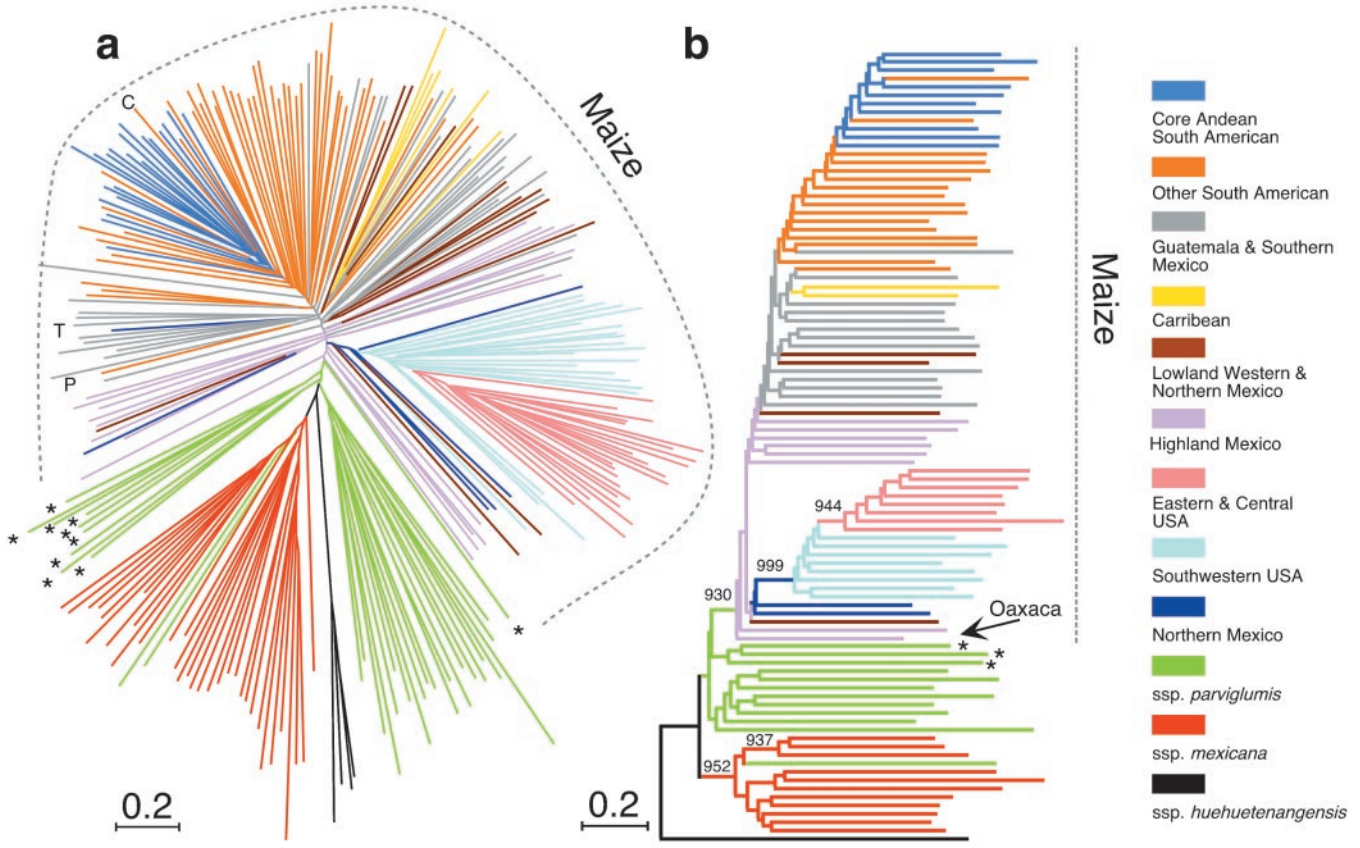

Fig. 2. Phylogenies of maize and teosinte rooted with ssp. *huehuetenangensis* based on 99 microsatellites. Dashed gray line circumscribes the monophyletic maize lineage. Asterisks identify those populations of ssp. *parviglumis* basal to maize, all of which are from the central Balsas River drainage. (*a*) Individual plant tree based on 193 maize and 71 teosinte. (*b*) Tree based on 95 ecogeographically defined groups. The numbers on the branches indicate the number of times a clade appeared among 1,000 bootstrap samples. Only bootstrap values greater than 900 are shown. The arrow indicates the position of Oaxacan highland maize that is basal to all of the other maize.

elsewhere. In short, the recombinant inbreds were selfed for 11 generations on average and then genotyped with their parents as described above. Thirteen novel alleles were found for the 33 loci and confirmed by sequencing. The estimates of μ and σ_m^2 were 4.28×10^{-4} and 2.08, respectively. The estimated number of generations (τ) was converted into years by assuming 1 year equals 1 generation. Ten thousand bootstrap samples for $(\delta\mu)^2$ were computed to estimate 95% confidence limits for the time of divergence.

Population Structure Analysis. To assess gene flow and population structure, we used a model-based clustering method that infers the number of clusters (populations) and the frequency of each allele in different clusters (20). For each individual plant, the method estimates the proportion of its genome derived from the different clusters. The analyses were preformed with the computer program STRUCTURE (www.pritch.bsd.uchicago.edu/software.html), using 10^6 iterations and a burn-in period of 30,000. In the different simulations, no prior information was used to define the clusters. Because these analyses require codominant alleles and are sensitive to missing data, only 78 microsatellites with fewer than 11% missing data or null alleles were used. When assessing the population structure for maize, a plant was assigned to a cluster if an arbitrary value of 75% of its genome is estimated to belong to that cluster.

Results and Discussion

Single Domestication for Maize. The microsatellite-based phylogeny for our sample of 264 maize and teosinte plants shows all maize in a single monophyletic lineage that is derived from within ssp. *parviglumis*, thus supporting a single domestication for maize (Fig. 2*a*). We pooled the individual plant samples into 95 ecogeographically defined groups. Each ecogeographic group consists of two to four plants of similar latitude, longitude, and altitude as well as neighboring positions in Fig. 2*a*. The composition of these groups can be found in the supporting information. With this smaller number of taxonomic units, it was possible to perform statistical testing via bootstrap resampling. The phylogeny for the pooled samples shows maize to be monophyletic in 930 of 1,000 bootstrap samples (Fig. 2*b*), indicating that a single origin for maize is far more likely than multiple independent origins as proposed (7, 8). Our results stand in contrast to those for crops such as rice (1), beans (2), and cotton (4) in which different cultivated forms cluster with distinct wild relatives in phylogenetic trees, supporting multiple origins for these crops. For maize, our data clearly favor a single domestication event.

To explore further the relationship between maize and teosinte with the microsatellite data, we performed a PCA that was free of the assumption of tree-based methods that evolution has been predominantly divergent (Fig. 3). The pattern of relationships revealed by the PCA closely corresponds to that seen in Fig. 2. Subspecies *mexicana* is separated from all maize samples, whereas samples of ssp. *parviglumis* overlap those of maize, documenting the close relationship between ssp. *parviglumis* and maize and supporting the phylogenetic result that the latter subspecies was the sole progenitor of maize.

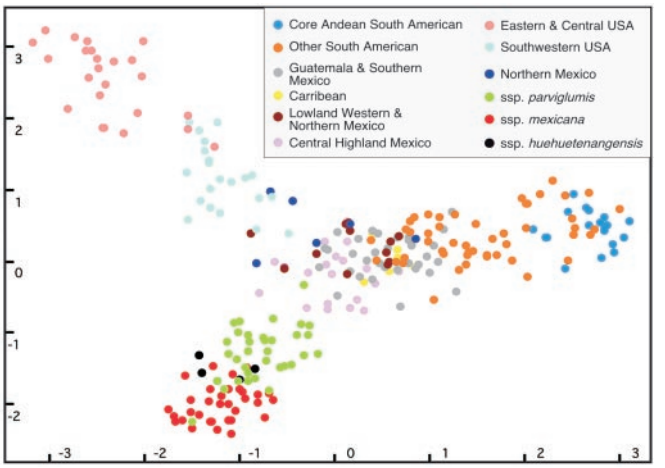

Fig. 3. Graph of the first two axes from a principal component analysis of 193 maize and 71 teosinte individual plants. The first component explains 3.5% and the second 2.6% of the total variation.

Available isozyme and chromosome knob data could also be used in theory to infer the number of domestications for maize. However, the available isozyme data have principally been used to examine relationships among maize races and only two studies (9, 21) have used isozyme data to make inferences about the origin of maize. Like our simple sequence repeat (SSR) phylogeny, the results of the two isozyme studies suggest a single domestication for maize; however, these two studies did not include a comprehensive sample of maize germplasm and thus could not authoritatively differentiate between the single and multiple domestication hypotheses. The chromosome knob data (8) has never been examined by formal phylogenetic analyses with respect to the origin of maize, and thus cannot be directly compared with our SSR phylogeny. Most importantly, chromosome knob data may not be appropriate for phylogenetic studies because chromosome knob frequencies can change in a concerted and nonneutral fashion as a result of meiotic drive (22).

Cradle of Maize Domestication. If maize is the product of a single domestication event as our results indicate, then its origin can be pinpointed to a specific geographic locality. The region harboring those teosinte populations that are phylogenetically most closely allied with maize can be considered a candidate for the region in which maize was domesticated. Previously, populations of ssp. *parviglumis* from the central region of the Balsas River drainage were identified as those most similar to maize by using allozyme data (9). In our microsatellite-based phylogenies, these same populations are basal to maize (populations with asterisks in Fig. 2), supporting this earlier report and identifying the central Balsas River drainage (Fig. 1 *Inset*) as a candidate for the cradle of maize domestication. This region should be considered only a candidate because new teosinte populations that are even more closely related to maize may yet be discovered and the modern distribution of teosinte populations may differ from their distribution during the domestication period. One example is teosinte recovered from a archaeological site in Tamaulipas where teosinte is unknown today (23).

Date of the Domestication Event. Because our phylogenies reveal the origin of maize as a single event, it is possible to estimate the date of this event with the microsatellite data. When microsatellites adhere to the stepwise mutation model, they can provide an estimate of the time of separation of two populations. Of the 99 microsatellites, 33 follow a stepwise model and have a known mutation rate. With this set of microsatellites, ssp. *parviglumis* and Mexican maize have a divergence time of 9,188 B.P. (95% confidence limits of 5,689–13,093 B.P.). This date represents an upper limit on the time of maize domestication because our sample of ssp. *parviglumis* may not contain descendants of the exact population that was ancestral to maize. For this reason, the time of divergence between maize and the specific ancestral population of ssp. *parviglumis* will likely have a somewhat younger date. Our molecular date is consistent with the date of 6,250 B.P. for the oldest known fossil maize (24) and with archaeological estimates that crop domestication in Mexico did not likely precede 10,000 B.P. (25).

Impact of Gene Flow from Teosinte. Our phylogenetic analyses (Fig. 2) provide an estimate of the ancestral–descendent relationships averaged over the entire genome and they indicate that all types of maize were derived from within ssp. *parviglumis* via a single domestication event. After this initial event, introgression from other teosinte types may have contributed to the maize gene pool and thereby helps explain the remarkable phenotypic and genetic diversity in maize. Our data allow us to make an assessment of the importance of gene flow from ssp. *mexicana* as a factor contributing to maize diversity. This subspecies grows as a weed in many maize fields of the highlands (above 1,700 m) of central and northern Mexico where it forms frequent hybrids with maize, whereas ssp. *parviglumis* often grows as part of native vegetation at lower elevations (below 1,800 m) and rarely hybridizes with maize (26). We performed population structure analysis (20) with ssp. *mexicana* and Mexican maize to estimate the proportion of the Mexican maize gene pool that can be attributed to gene flow from ssp. *mexicana*. Estimates from this analysis indicated that the genomes of our sample of Mexican maize from elevations at which ssp. *mexicana* grows are composed of about 2.3% ssp. *mexicana* germplasm (range 0.2–12%), whereas Mexican maize from lower elevations contains only 0.4% ssp. *mexicana* (range 0.2–2%). These numbers suggest a measurable but modest overall contribution of gene flow from ssp. *mexicana* into highland maize. However, the ssp. *mexicana* contribution to some highland maize races is apparently larger. Our samples of maize races Cacahuacintle, Palomero de Jalisco, and Palomero Toluqueño are estimated to have 11, 9, and 12%, respectively, of ssp. *mexicana* germplasm. Thus, gene flow from ssp. *mexicana* may have contributed appreciably to some races of the Mexican highlands as suggested by field observations (26).

Early Diversification. In addition to the single maize domestication from ssp. *parviglumis*, the phylogenies and PCA reveal the geographic diversification of the native landraces of maize. The basal maize types in both phylogenies (Fig. 2) are those from the Mexican highlands, and it is these types that overlap with ssp. *parviglumis* in the PCA (Fig. 3). This result places the early diversification of maize in the highlands between the states of Oaxaca and Jalisco. In this regard, it is striking that the oldest known archaeological maize is from the highlands of Oaxaca (24) and remarkably the basal-most maize in Fig. 2*b* is from Oaxaca. This result presents an enticing correspondence between genetic and archaeological evidence, and calls for further botanical and archaeological exploration in this region. Among archaeologists, there have been two models for the early diversification of maize. According to one, because the oldest directly dated fossil maize comes from the Mexican highlands, then the early diversification of maize occurred in the highlands with maize spreading to the lowlands at a later date (25, 27). The second model interprets maize phytoliths from the lowlands as the oldest maize, and accordingly places the early diversification of maize in the lowlands (28). Our data suggest that maize diversified in the highlands before it spread to the lowlands.

Spread of Maize over the Americas. From an early diversification in the Mexican highlands, the phylogenies and PCA suggest two lineages or paths of dispersal. One path traces through western and northern Mexico into the southwestern U.S. and then into the eastern U.S. and Canada. A second path leads out of the highlands to the western and southern lowlands of Mexico into Guatemala, the Caribbean Islands, the lowlands of South America, and finally the Andes Mountains.

These relationships offer several phylogenetic hypotheses: maize of the eastern U.S. with its long, slender ears was derived from that of the southwestern U.S., which in turn came from northern Mexico. A scenario much like this has been proposed based on morphology and archaeology (29). The maize of the Andes Mountains with its distinctive hand-grenade-shaped ears was derived from the maize of lowland South America, which in turn came from maize of the lowlands of Guatemala and southern Mexico. Consistent with a dispersal out of Mexico along two paths, population structure analysis (20) divides our sample of the maize gene pool into three clusters: (*i*) an Andean group that includes the hand-grenade-shaped ear types and some other Andean maize (35 plants); (*ii*) all other South American and Mexican maize (80 plants); and (*iii*) U.S. maize (40 plants), plus 38 plants whose genomes are intermediate between or admixtures of two of these three clusters.

Although a high degree of ecogeographic patterning is seen in the phylogenies and PCA, there are several exceptions, some of which have clear explanations. For example, the Amazonian race Coroico (C in Fig. 2*a*), which clusters with Andean maize, was thought to be related to Andean maize (30). The northern Mexican race Tuxpeño Norteño (T in Fig. 2*a*), which clusters with southern Mexican maize, was thought to be closely related to race Tuxpeño of southern Mexico (31). The Venezuelan race Puya Grande (P in Fig. 2*a*), which groups with southern Mexican races, is thought to have some parentage from race Tuxpeño of southern Mexico (32). These exceptions present examples of recent movement of maize races. There are also two "misplaced" samples of ssp. *parviglumis* that group with ssp. *mexicana*. Our results from population structure analysis and morphological observations suggest that one of these (J. Sanchez G. 374) is a *parviglumis–mexicana* hybrid and that the other (J. Sanchez G. 159) was misclassified in the germplasm collections (data not shown).

Perspective

There remains an untold chapter in the origin and early diversification of maize. Microsatellite data identify ssp. *parviglumis* of the Balsas River drainage below 1,800 m in elevation as the ancestor of maize. However, the microsatellite data and some archaeological evidence suggest maize from the highlands (above 1,800 m) as the basal or most primitive form of maize. Thus, there is a geographic gap between the present-day location of the progenitor and the location of the basal maize and earliest fossil maize cobs (27). This paradox raises several questions. Was ssp. *parviglumis* first transported to the highlands where it was then domesticated? Did the distribution of ssp. *parviglumis* 9,000 years ago differ from that today? Do even older archaeological maize fossils remain to be discovered at lower elevations in the Balsas Valley? To answer these questions will require additional archaeological and botanical exploration, more powerful molecular analyses, and perhaps DNA analysis of archaeological materials, which could place archaeological specimens in phylogenetic trees such as those presented here.

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