Title: Multi-proxy evidence highlights a complex evolutionary legacy of maize in South America

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Abstract: Domesticated maize evolved from wild teosinte under human influences in Mexico beginning around 9,000 BP, traversed Central America by ~7,500 BP, and spread into South America by ~6,500 BP. By analyzing landrace and archaeological maize genomes from South America, we demonstrate that the ancestral population to South American maize was brought out of the domestication center in Mexico as a partial domesticate, becoming isolated from the wild teosinte gene pool before the domestication syndrome was fixed. Deeply structured lineages with a stable domestication syndrome then evolved within South America out of the partially domesticated progenitor population. Based on genomic, linguistic, archaeological, and paleoecological data, we suggest the southwestern Amazon was a likely secondary improvement center for partially domesticated maize. Multiple waves of subsequent human-mediated dispersal

are responsible for the diversity and biogeography of modern South American maize.

One Sentence Summary: New evidence reveals a complex history of maize domestication and a secondary improvement center of partial domestication in the Southwest Amazon.

Main Text:

Maize (*Zea mays* ssp. *mays*) evolved from wild Balsas teosinte (*Zea mays* ssp. *parviglumis*, hereafter *parviglumis*) in modern-day lowland Mexico beginning around 9,000 years before the present (*1*), and spread to dominate food production systems throughout much of the Americas by the time of European colonization. Archaeological genetic data have highlighted many aspects of maize natural history alongside human cultivators (*2–6*), including the evolution of domestication traits and the adaptation to diverse new environments. Archaeological macro-remains establish that maize was brought to the southwestern US and the Colorado Plateau by ~4,000 BP (*7*), while archaeobotanical remains demonstrate that maize was traversing Panama by ~7,500 BP (*8*, *9*) to arrive in Coastal Peru (*10*), the Andes (*11*, *12*), and lowland Bolivian Amazon (*13*) between ~6,500 and 6,300 BP (Figure 1; Table S1). Today, maize is a paramount staple food, produced at over 1 billion metric tons per year and directly yielding over 6% of all food calories for humans, plus more in livestock feed and processed foods (FAOref).

Conventionally, maize domestication is thought to have occurred once, with very little subsequent gene flow from *parviglumis* (*14*, *15*). However, maize was only partially domesticated in Mexico at ~5,300 BP (*2*, *3*), well after it became established in South America, raising the question of how South American maize came to possess the full complement of fixed domestication traits. Here, we aim to reconcile archaeobotanical and genomic data concerning the domestication and dispersal history of maize in South America. We sequenced maize genomes from forty indigenous landraces and nine archaeological samples from South America (Figure 1; Tables S2, S3), and analyzed them alongside published modern (n=68) (*16*, *17*) and ancient (n=2) (*2*, *3*) maize and teosinte genomes.

Model-based clustering highlights extensive admixture and population overlap between maize populations, but we observe several robust lineages (Figure 1): i) The Andes and the Pacific coast of South America, ii) lowland South America, including the Amazon and Brazilian Savanna, iii) North America north of the domestication center, and iv) highland Mexico and Central America, previously observed to contain introgression from wild *Z. mays* ssp. *mexicana* (*15*, *17*). Finally, we observe a widespread 'pan-American' lineage spanning from northern Mexico into lowland South America. In previous multi-locus analyses, maize formed a monophyletic subset of teosinte with South American lineages at the most derived position of a microsatellite tree (*14*). This pattern has been interpreted as evidence for a single episode of domestication followed by dispersal culminating in the Andes after becoming established throughout the rest of the range of cultivation (*14*). However, given some of the earliest archaeological evidence for persistent maize cultivation in numerous locations throughout South America by ~6500-4000 BP regionally, this model seems unlikely. Instead, we propose that South American maize was carried away from the domestication center soon after initial domestication (*8*, *9*), and may have been one of several partially domesticated maize lineages that independently fissioned from the primary gene pool following the onset of domestication (Figure 2).

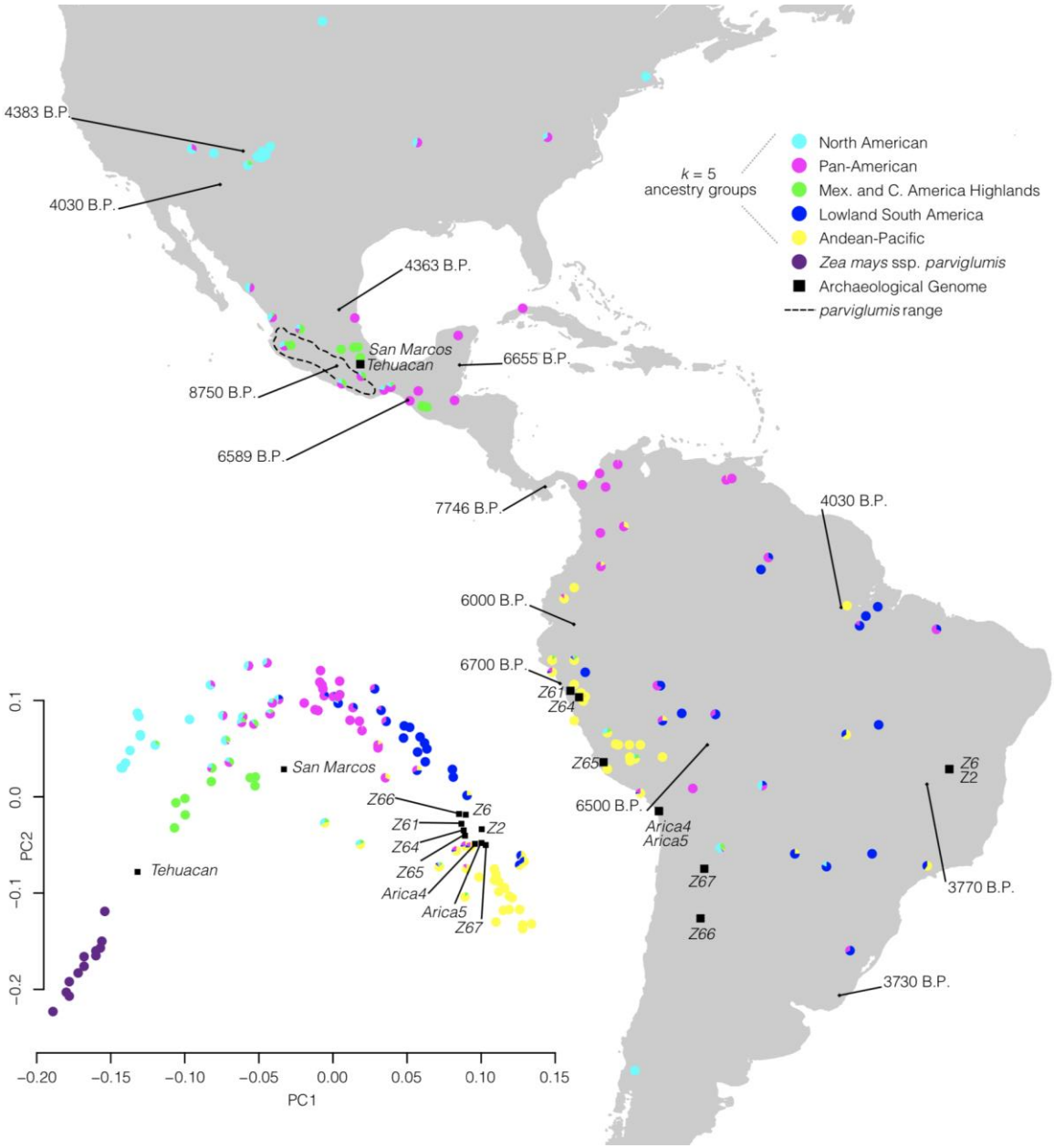
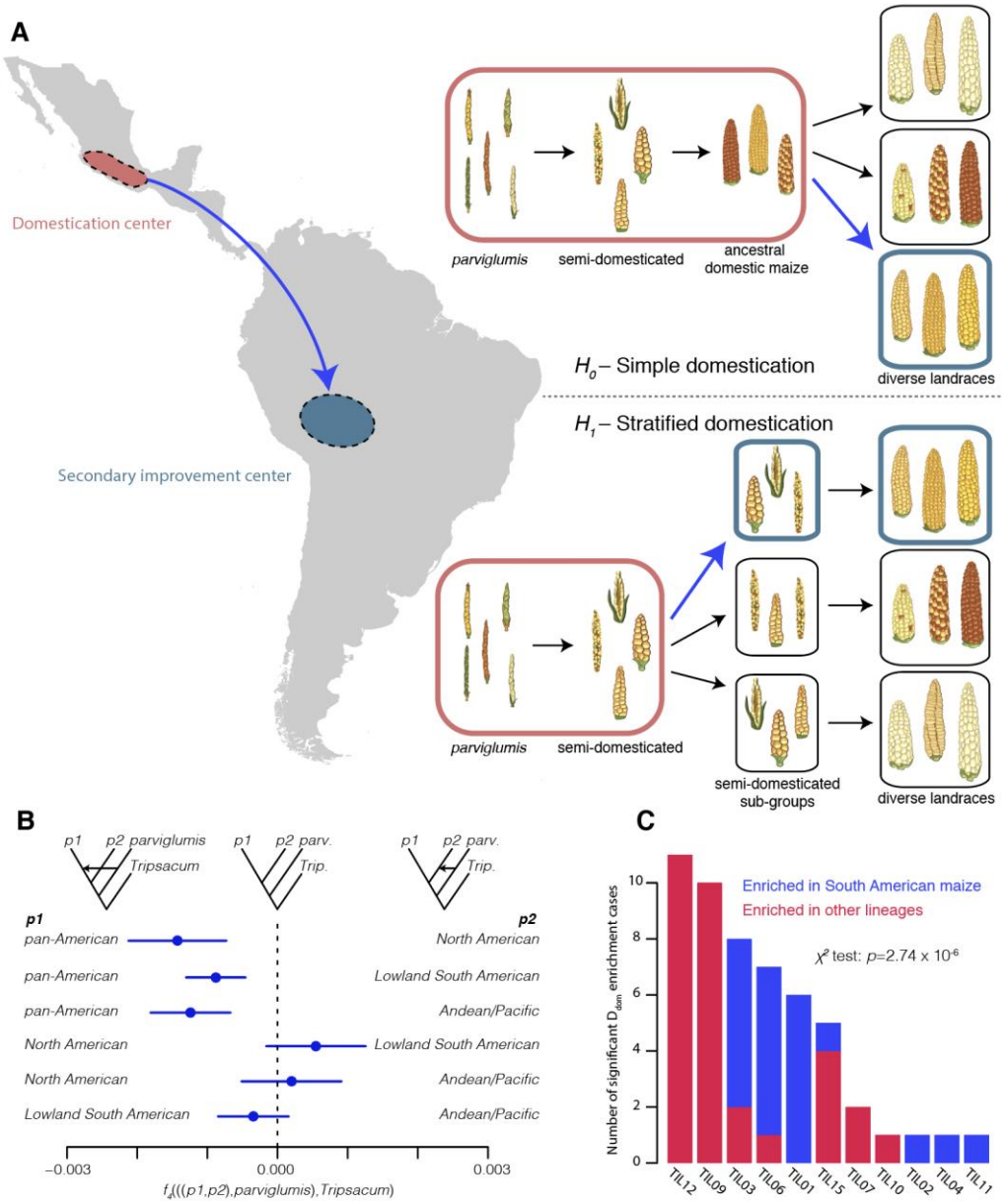


Figure 1 – Distribution and ancestry of maize genomes, and principal components analysis of maize and *parviglumis* genomes. Pie colors reflect ancestry proportions estimated at *k*=5 via model-based clustering of modern maize genomes (SOM). Archaeological genomes were projected onto the PCA using a procrustes transformation to mitigate degradation biases (SOM). Map dates reflect early regional maize archaeobotanical remains (Table S1 and Figure S1 for full details).

Using f_4 statistics (*18*), we observe extensive asymmetry in *parviglumis* ancestry among modern maize populations (Figure 2). This reveals that maize-*parviglumis* gene flow was ongoing in some lineages after others became reproductively isolated. Whereas later gene flow from *mexicana* is well documented in some maize (*6*, *15*, *17*), this finding contradicts the assumption of the current domestication model that dispersal and diversification throughout the Americas



happened only

Figure 2 – A stratified domestication model for maize. **A)** A schematic comparing the conventional domestication model under which maize became fully domesticated and then dispersed throughout the Americas, vs. a stratified domestication model in which partially domesticated sub-populations became reproductively isolated before the fixation of the domestication syndrome. **B)** f_4 statistics demonstrating excess allele sharing between the pan-American lineage and wild *parviglumis* compared with other maize, revealing non-uniform crop-wild gene flow after initial domestication. Bars are three standard errors computed using a block jackknife (SOM Methods). **C)** Barplot of enriched *parviglumis* contributions to ancestry near domestication genes, where each bar is a *parviglumis* genome contributing to South American maize (blue) or other maize (red) D_{dom} enrichment.

after the severance of gene flow from *parviglumis*, the progenitor (*14*, *15*). Thus, while South American maize became reproductively isolated from the wild progenitor when it was carried south out of the domestication center in Mexico, other maize lineages remaining behind underwent continued crop-wild gene flow before diversifying into numerous extant landraces over subsequent millennia. The pan-American lineage shows significant shared ancestry with

parviglumis relative to all other major groups (Figure 2), suggesting that this group emerged from the domestication center and dispersed widely after other maize had become regionally established. Because the pan-American lineage carries excess *parviglumis* ancestry relative to the strictly South American lineages, it therefore appears to represent a second major episode of maize dispersal from Mesoamerica, reinforcing previous molecular evidence for two major waves of maize movement into South America (*5*).

Two maize genomes from the Tehuacan Valley of Mexico at ~5300 BP recently revealed a state of partial domestication—a mixture of maize-like and *parviglumis*-like alleles at known loci involved in domestication (*2*, *3*). This is puzzling, given sustained use of domesticated maize from ~6500 BP onward in South America (Figure 1; Table S1) (*13*, *19*). However, principal components analysis and f_3 statistics reveals substantial genomic distance between these two Mesoamerican archaeogenomes (Figure 1; Figure S2), and f_3 statistics confirm that the SM10 genome (*3*) is more maize-like while the Tehuacan162 genome (*2*) is more *parviglumis*-like (Figure S2). In total, the two genomes are from the same region and time period, and both are partially domesticated, but otherwise they appear to represent independent samples out of a diverse semi-domesticated population with a wide array of domestic and wild-type alleles in circulation.

Given the partial domestication state of the Tehuacan and San Marcos genomes, the early South American lineage emerging from their ancestral population would likely also have been a partially domesticated form of early maize with an assortment of wild and domestic alleles. This population would have harbored the building blocks for fully domesticated maize, but lacked the allelic fixation and linkage needed for a stable domesticated crop. Loci under selection would have therefore been continually de-coupled from their chromosomal neighborhood through recombination ameliorating any suppression of selection by linkage (*20*, *21*), and we expect *parviglumis* components specific to the migrating lineage to have left an enriched ancestry trace near domestication genes compared with genome-wide.

We compared *D*-statistics (*22*) across the whole genome (D_{WG}) and within 10kb of 186 known domestication loci ($D_{dom,}$) (*23*) to test for asymmetrical *parviglumis* contributions between pairs of South American and non-South American maize around domestication genes (SOM). We found that *parviglumis* enrichment associated with domestication is highly patterned according to major ancestry groups, with several *parviglumis* genomes contributing exclusively to either South American or non-South American D_{dom} enrichment, and a strongly significant association with ancestry overall (Figure 2; chi-square test p=2.74 x 10^{-6}). That is, we observe that *parviglumis* ancestry is enriched near domestication genes in a pattern demonstrating that domestication-associated selection was still ongoing after the stratification of the major extant lineages out of the common ancestor of all maize. This pattern validates a model where ancestral population in South America was itself only partially domesticated during its dispersal away from the domestication center. This model is also consistent with archaeobotanical evidence from Panama, where phytolith morphologies from the earliest *Zea*-containing strata suggest that wild alleles of *tga1*—the gene conferring open fruitcases now fixed in domesticated maize—may still have existed in this migrating population (*24*).

The earliest sustained evidence in lowland South America places maize in the southwestern

Amazon by ~6,500 BP (*13*), and this region interfaces the lowland and Andean/Pacific genetic lineages (Figure 1). We hypothesize that the southwestern Amazon may have been a secondary improvement center for the partially domesticated crop before the divergence of the two South American groups. When maize arrived, southwestern Amazonia was an active domestication hotspot (*25*), so partially domesticated maize could be expected to thrive in an anthropogenic ecosystem with behaviors supporting domestication. Additionally, microfossil assemblages (*13*) reveal the presence of polyculture (mixed cropping) from ~6,500 cal BP onward, where a new crop species could be integrated into existing food production systems.

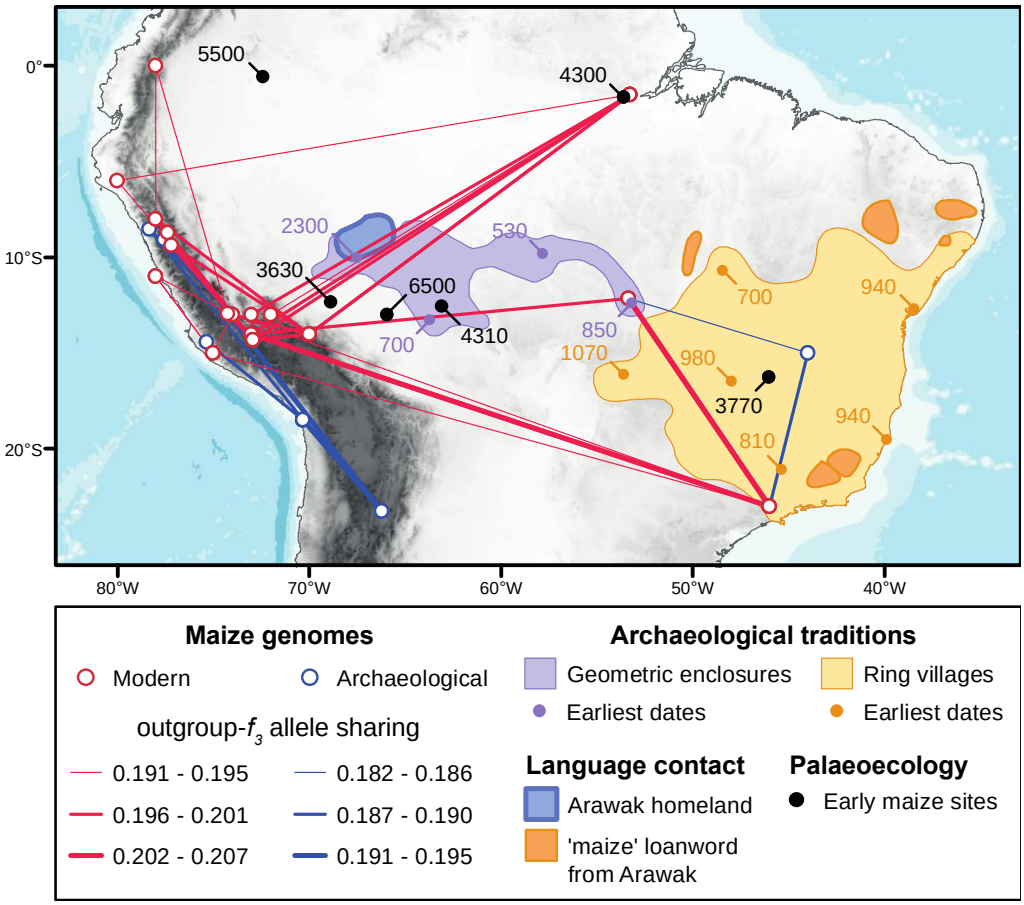


Figure 3 – Genomic relatedness overlapping linguistic and archaeological patterns in lowland South America. Maize genomes with ≥50% Andean/Pacific ancestry and ≥99% South American ancestry are connected by lines with the two other genomes with which it shares the highest outgroup-f_3 value. Geometric enclosures (geoglyphs, ring ditches and others) and mound ring villages of southern Amazonia broadly coincide with the expansion of Arawak languages, whereas the Uru and Aratu ring villages coincide with the distribution of Macro-Jê languages (see SOM Text, Figure S3, Figure S4). Only the earliest regional dates for each archaeological tradition are shown (see Table S3). Macro-Jê languages that borrowed an Arawak loanword for 'maize' based on (*26*). Arawak homeland is shown approximately in the modern location of Apurinã following (*27*).

Pollen and phytolith data demonstrate a west-to-east pattern of maize expansion across the Amazon, and show that maize was consistently present from ~4,300 BP onward in the eastern Amazon (*19*). Initially, maize in the eastern Amazon was part of a polyculture agroforestry

system combining annual crop cultivation with extensive wild resource use and low-level management through burning (*19*). Maize cultivation proceeded alongside the progressive enrichment of edible forest species and subsequent waves of new crop arrivals, including sweet potato (~3,200 BP), manioc (~2,250 BP) and squash (~600 BP). The development of anthropogenically enriched Amazonian Dark Earth soils ~2,000 BP (*28*) enabled the expansion and intensification of maize cultivation, likely increasing carrying capacity to sustain growing populations in the eastern Amazon (*19*). The extant endemic maize lineage in lowland South America likely originated with this long-term process involving millennia of evolving land use practices.

Several landraces and two archaeogenomes (~700 BP) in eastern Brazil also show strong genetic links to Andean maize near the southwestern Amazon (Figure 3). This pattern closely mirrors linguistic patterns linking Andean, Amazonian, and eastern Brazilian maize cultivation, and suggests a second major west-to-east cultural expansion of maize traditions. The proto-Arawak word for maize is rooted in Quechua (*29*), while the Arawak homeland is located in southwest Amazonia (*27*) (SOM). A loanword for maize was transmitted from Amazonian Arawak languages into Macro-Jê stock languages in the Brazilian savanna and Atlantic coast (*26*) (SOM; Figure S3). Archaeological evidence suggests this expansion occurred ~1200-1000 BP with the spread of a cultural horizon of geometric enclosures and mound ring villages throughout southern Amazonia, and ring villages in the central Brazilian savannas and the Atlantic coast (Figure 3; Figure S4; SOM) (*30–32*). This process is roughly contemporaneous with archaeological Andean-admixed genomes in the area, suggesting the arrival of Andean maize germplasm at this time. Thus, Arawak speakers likely brought non-local Andean/Pacific maize lineages into a landscape where maize was already an established component of a land management and food production strategies.

Finally, we observe that both South American lineages carry a significantly higher mutation load than other maize. Mutation load increases linearly with distance from the domestication center and is closely linked with ancestry, the Andean/Pacific group carrying an especially large burden of potentially deleterious variants (Figure 4; SOM). The high mutation load in the Andes has been attributed to selection associated with high-altitude adaptations (*17*), but the elevated mutation load in lowland maize also suggests a history of shared selection and drift effects even prior to highland adaptation in Andean maize. These processes would likely have included a founder episode as maize was carried into South America, and the persistent selection pressures for regional adaptation and the latter stages of domestication after isolation from the large wild gene pool. We also find that ancient Andean and Pacific maize sampled from ~1000 BP into the early colonial period has an unexpectedly low mutation load compared with its modern Andean/Pacific counterparts (Wilcoxon p=0.002477; SOM; Figure 4), though still significantly elevated compared with non-South American lineages. It is possible that Andean maize experienced a wave of deleterious allele accumulation as human and crop populations were massively disrupted by the social upheaval brought by Europeans (*33*). Alternatively, the increasing mutation load in modern crops could represent the ongoing effects of burdensome allele accumulation in the ninth millennium of human interventions in the gene pool.

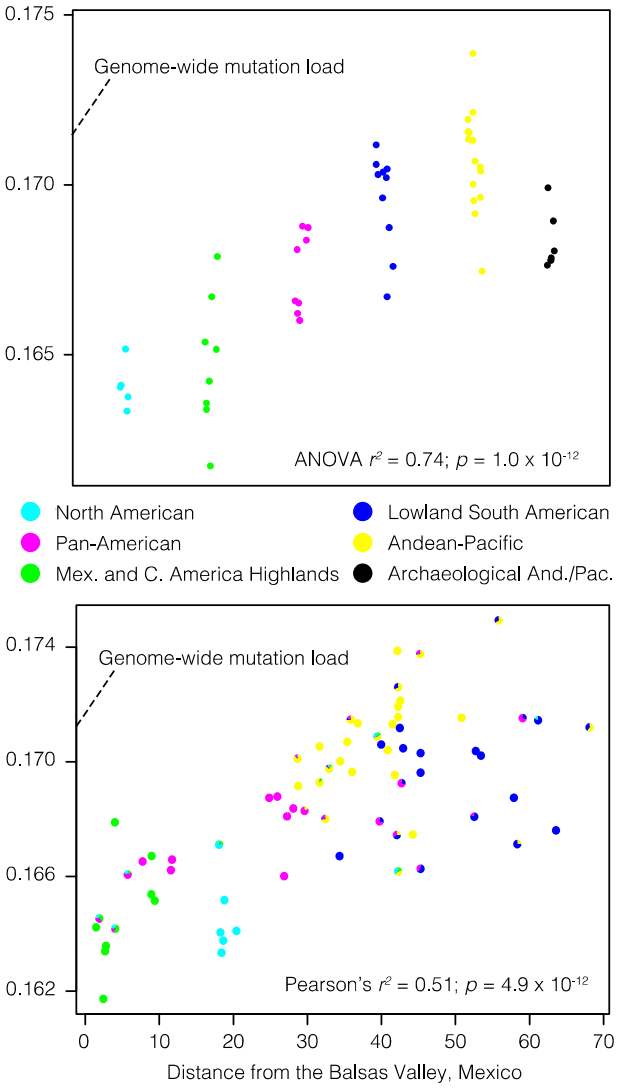


Figure 4 – Genome-wide mutation load across major ancestry groups (non-admixed samples only in top panel), and load compared with distance to the domestication center. Mutation load is calculated as a proportion of the theoretical maximum load over observed SNPs, and ancient load scores are re-scaled for missingness using a Procrustes transform (SOM). Simple Euclidean distance in degrees to the Balsas River Valley is shown.

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Sequence Read Archive accession SRP152500. In-house scripts and other materials, Dryad Digital Repository doi:TBD.

Supplementary Materials:

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External Databases S1

References (*34-125*)

Supplementary Materials for

Multi-proxy evidence highlights a complex evolutionary legacy of maize in South America

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This PDF file includes:

Materials and Methods
Supplementary Text
Figs. S1 to S4
Tables S1 to S3
Captions for Data S1

Other Supplementary Materials for this manuscript include the following:

Data S1

Materials and Methods

Materials

Modern maize landrace accessions originally collected in traditional farming contexts were sampled from the Embrapa germplasm collection in Brasilia. Landrace details are provided in Dataset S1. Archaeological sample details are provided in Table S1.

/mnt/data/logan/maize/S_am/ancient/bwa/fullBams/mapDamage

Methods

Modern DNA isolation and library preparation
We attempted seed germination from two seeds of each modern landraces and harvested ~100mg of first-leaf tissue from successful seedlings for DNA extraction (n=30). For 10 landraces where germination failed, we isolated DNA directly from seed tissue. DNA isolation followed a conventional CTAB protocol: Tissue was ground in liquid nitrogen with a sterile mortar and pestle and incubated for 24 hours with gentle agitation in lysis buffer (2% w/v CTAB; 100mM Tris pH 8; 20mM EDTA; 1.4M NaCl; 2% w/v PVP; 0.5% v/v β-Mercaptoethanol for seeds only), then subjected to two rounds of chloroform purification. The recovered aqueous fraction was mixed with 1.5 volumes Qiagen Buffer AW1 and bound to a silica spin column. The column was washed twice with Qiagen Buffer AW2 and once with acetone, air-dried for 5 minutes, and eluted in 100uL TE buffer. DNA was quantified by Qubit, and 1µg DNA was sheared in a 100uL volume using a bioruptor ultrasonicator to a target size of 350bp. We used a 2-stage SPRI bead size selection on sheared DNA to target 250bp-450bp fragments following the KAPA Hyper Prep Library protocol size selection guidelines (KR0961, v. 4.15) with AMPure XP beads (Beckman Coulter A63880). We then used NEBNext Ultra II kits (E7645) to prepare PCR-free Illumina-compatible libraries following the manufacturer's protocol, using Illumina TruSeq Nano DNA LT single indexed adapters diluted to a 1:4 ratio (from Illumina FC-121-4001 and FC-121-4002). Final libraries were purified using 1.2 volumes of SPRI beads, quantified by qPCR using the NEB Quant kit (E7630), pooled in equimolar ratios, and sequenced across six lanes of a HiSeq X10 instrument.

Ancient DNA isolation and library preparation
All ancient DNA handling up to the point of library pooling (no PCR was used) was carried out in dedicated ancient DNA clean lab facilities at the University of Warwick and University of Copenhagen, with strict observation of established protocols to prevent and detect contamination (*34*), including sequencing and analysis of control negative libraries.

We isolated DNA from 16 ancient maize samples at Warwick using the above protocol with the following modifications: Lysis incubation was extended to 72 hours, 5 volumes of binding buffer were used, and elution was in a final volume of 60µL. Additionally, DNA was freshly extracted from two ancient maize samples from the Arica site at the University of Copenhagen following the protocol described in ref (*35*), and shipped to Warwick for library preparation. We prepared PCR-free sequencing libraries from ancient DNA isolates as follows: We treated 53.5 µL DNA with 2 µL NEB FFPE DNA repair mix and 6.5 µL FFPE DNA Repair Buffer (M6630). We purified the enzyme reaction using Qiagen MinElute kits (28004) with an elution volume of 50 µL, and carried out NEB Ultra ii PCR-free library preparation as above, with modifications: Ligation time was extended from 15 to 30 minutes, and 1.5 volumes of SPRI beads were used for final purification. 18 ancient maize libraries and a negative control library prepared in parallel were pooled in equimolar ratios and sequenced on one MiSeq v3 150 cycle flowcell to assess endogenous DNA content. On the basis of a preliminary screen for endogenous DNA content, we selected nine libraries suitable for whole genome sequencing, and pooled them to sequence across six lanes of a HiSeq X10 instrument. Additional tissue from these nine samples was also sent for AMS radiocarbon dating at Beta Analytic (Table S1).

Read processing and alignment

Paired modern reads underwent adapter trimming and 3' quality trimming following the first base quality score below 20, if present, using Flexbar (*36*). For ancient samples, forward and reverse reads were merged with a

minimum base quality of 20 following (*37*), and only merged reads were carried forward into analysis.

We used BWA to map all reads directly to the soft-masked maize reference genome (*Zea mays* B73 RefGen_v4 (*38*)), requiring a minimum mapping quality of 20. For modern reads, we used bwa-mem (*39*) with default settings, and for ancient datasets consisting of shorter reads, we used bwa-backtrack (bwa *aln* (*40*)) with seed disabled for improved mapping in the presence of terminal mismatches introduced through base misincorporation (). Although we used PCR-free libraries, we observed some redundancy from exclusion amplification duplicates on the HiSeq-X patterned flowcells. Migration of molecules during exclusion amplification creates localized duplicates, so that 96 separate flowcell tiles can be expected to yield largely independent sets of starting molecules. Therefore, we removed possible flowcell duplicates by separating read alignments by tile, independently removing duplicates with the samtools (*41*) *rmdup* command, and re-combining the resulting duplicate-removed files into a single read alignment, thereby avoiding removal of independent starting molecules with identical genomic coordinates by chance (Dryad: /scripts/rmdup_by_tile.pl). We used GATK (*42*) to locally realign reads around short indels.

We acquired maize HapMap2 data for maize landraces and wild *Zea mays* ssp. *parviglumis* (*16*) and additional published landrace data (*17*) from the NCBI sequence read archive, and processed reads exactly as above for modern paired reads, except we used the samtools *rmdup* function as normal for duplicate removal.

Given the extreme repetitive content of the maize genome (*43*), we assessed sitewise mappability of short reads based on the method described in (*2*): We used Jellyfish (*44*) to summarize all 35mers in the soft-masked maize reference genome (Zea mays B73 RefGen_v4 (*38*)) and mapped unique 35mers back to the genome using bwa *aln* (*40*). We filtered the resulting bam file to remove reads with one or more suboptimal mapping locations differing from the source location by only one mismatch. We used samtools *mpileup* (*41*) to summarize positional depth of coverage, so that any position covered by 18 reads satisfied the requirement that the majority of position-containing 35mers are uniquely mappable at the 1-mismatch level. This method estimated 21% of the maize reference assembly to be uniquely mappable at the majority (18 read) level, consistent with previous estimates (*2*). However, we opted for a more conservative approach requiring all 35mers covering a position to be uniquely mappable at the 1-mismatch level with a 20nt buffer masked around non-unique positions, retaining 10.4% of the reference assembly (mappability bed file available on Dryad: /misc/ZeaV4.map35.plus20.bed).

For all ancient DNA datasets, we used mapDamage 2.0 (*45*) to assess DNA degradation characteristics in read alignments. Treatment with FFPE enzyme mix includes uracil removal, so the recovered damage profiles are thought to primarily derive from 5-methyl-cytosine deamination to thymine rather than cytosine-to-uracil deamination recovered through an adenine template (*46*). As such, damage is less prominent than may be expected for libraries without pre-treatment (*47*), but is unambiguously prominent in all ancient samples (all mapDamage outputs available in Dryad: /mapDamage/).

SNP selection

We analyzed the LLD set of high-quality linkage-validated SNPs from hapmap3 (*48*). We also carried out *de novo* SNP discovery using ANGSD (*49*) over our modern South American samples and the hapmap2 (*16*) panel using the command "angsd -bam [bamList.txt] -GL 1 -out [outfileStem] -doMaf 2 -doMajorMinor 1 -SNP_pval 1e-2 -sites [mappableRegions.angsd] -doCounts 1 -doGeno 12 -doPost 1", and we filtered the output to require coverage by a minimum of 20 samples to consider a position. Because of the high repetitive element content of the maize genome during to transposable elements and recent whole genome duplication (*43*), we discovered SNPs only in the uniquely mappable fraction as above, and strictly filtered the highest-coverage SNPs likely to originate in non single-copy regions as follows: We excluded all sites in the top .5% of coverage from any single sample, the top 1% of coverage shared between any 2 independent samples, and sites in the top 5% of coverage in 5 or more samples. Before culling on this basis, coverage at some sites was extreme even in the hapmap3 LLD set, whereas this pruning strategy substantially curtailed SNP coverage toward the poissonian expectation. We worked only with bi-allelic SNPs, and for all analysis except mutation load (see below), we only analyzed SNPs with a minor allele frequency $\geq$0.02 and at least half of all samples called. The complete SNP set (n=17,672,809 sites) is included on Dryad in PLINK format: "/snpCalls".

SNP calling

We analyzed SNPs using a pseudohaplotype approach so as to include the inbred lines from hapmap2 without biases with regards to heterozygosity. For each sample, we used samtools *mpileup* (*41*) to summarize positional support at

SNP sites from curated bam files, and selected a base at random supporting an allele with at least two independent reads. We used PLINK 1.9 (*50*) to prune SNPs for linkage disequilibrium using the option '--indep 5kb 5 2' for analyses assuming linkage independence (PCA and model-based clustering). SNP pseudohaplotype calls used for analysis are available on dryad in plink format: "/snpCalls".

Ancestry estimation using model-based clustering
We ran Admixture (*51*) over the SNP set of modern maize pruned for LD with *k* range from 2-10 for each of 10 replicates with independent starting seeds, and visualized the results from each replicate with the highest log-likelihood. Given extensive overlap between substructured populations, we selected *k=5* as a level for further analysis based on observed geographic structure and consistency among replicate runs.

Principal components analysis
To perform a principal components analysis of genomic variation including variably degraded archaeological genomes (144,757 to 7,365,319 SNPs called), we first carried out a PCA using only modern genomes, and used Procrustes-based projection to add ancient genomes in turn, after ref (*52*). Using the LD-pruned and maf-filtered SNP set (above) in modern genomes, we performed a reference PCA using PLINK 1.9 (*50*). We then independently repeated the procedure adding one ancient genome in each case, and used the MCMCpack (*53*) R package to carry out a Procrustes transformation to estimate the best fit to the reference panel based on the first three principal components. The dilation, translation, and rotation values from the Procrustes transformation were then applied to the complete ancient sample-containing eigenvector matrix, and the ancient sample was projected accordingly onto the reference PCA.

Mutation load estimation
We used last (*54*) to align 27 repeat-masked plant genomes independently to the unmasked maize v4 reference genome, then used the sequence of *maf-convert*, *axtChain*, *chainMergeSort*, *chainPreNet*, *chainNet*, *netToAxt*, *axtToMaf*, and *MultiZ* to make multi-alignment with 20nt minimum aligned blocks (http://genome.ucsc.edu/) (*55*). We used *splitMaf* to make single-chromosome mafs and *maf2fasta* to make fasta multi-alignments by chromosome. We used a perl script (Dryad: /scripts/collapseFA.pl) to collapse indels to match the frame of the maize reference genome, and a perl script (Dryad: /scripts/matchMasking.pl) to lift masking from the repeat-masked maize genome onto all aligned genomes. We then excluded the maize genome from all model fitting and sitewise calculations. We extracted fourfold-degenerate sites from the chromosome 10 alignment using a perl script (Dryad: /scripts/quickDegen.pl) to fit a neutral model for calculating evolutionary constraint, and fit model the neutral model using *phyloFit* (*56*). The resulting neutral model tree is on Dryad: /GERP/chr10.4degen.tre. We then used GERP++ *gerpcol* (*57*) to calculate rejected substitution (RS) scores at all genomic sites with ≥3 aligned non-maize genomes.

For estimating genome-wide mutation load, we considered all SNP sites with ≥ 4 expected substitutions and an RS score ≥ 2, signifying a significant level of constraint (*58*). We polarized derived and ancestral alleles according to *Sorghum bicolor* and, if *Sorghum* was not represented, *Setaria italica.* Because the expected substitutions value, and therefore the RS score, are variable according to number of genomes present at the position, we summarized the mutation load as:

(sum of RS scores at all sites with a derived allele) / (sum of RS scores at all sites with a base called)

This value gives the proportion of the theoretical maximum number of rejected substitutions for a panel of potentially informative SNPs. Genome-wide expected substitutions and RS scores are on Dryad: /GERP/Zea_mays.allChr.rates.gz. We did not estimate mutation load in the inbred accessions from HapMap2, given that recessive deleterious alleles may be purged during inbreeding.

Because of sequence-based and genomic biases in ancient DNA degradation (*59*), including specifically in maize (*2*), we re-scaled ancient mutation loads to correct for potential biases introduced by non-random missing data. For each ancient genome overlapping at least 10,000 scorable SNPs, we recalculated genome-wide mutation loads for all modern samples using only SNPs present in the ancient sample, as well as the load score for the ancient sample, and culled the top and bottom 20% of modern estimates to conservatively remove outliers. We then performed a procrustes transform using the R package MCMCpack (*53*, *60*) to refit the mutation load panel from the SNP-restricted modern datasets to the reference set of complete-genome mutation load estimates. We then added the

ancient sample to the SNP-restricted dataset, applied the dilation and translation values from the procrustes transform to the complete set, and used the resulting re-scaled value as the corrected mutation load score for ancient samples.

Domestication gene ancestry enrichment
We used a perl script (Dryad: /code/dStat.pl) for estimating *D*-statistics and standard error using an unweighted jackknife procedure in 5Mb blocks from SNP data in PLINK format. We
first estimated genome-wide D-statistics in the form *D*(((X, Y), *parviglumis*), *Tripsacum*) for all pairs of South American (X; Andean/Pacific plus Lowland ancestry ≥99%) vs. non-South American (Y; Andean/Pacific plus Lowland ancestry <1%) maize genomes compared with 11 *parviglumis* genomes (D_{WG}). We then estimated D-statistics on a subset of the genome within 10kb of 186 genes previously documented as having been involved during the evolution of domestication (*23*) (D_{dom}), and identified sets of individuals whose genome-wide and domestication-gene *D*-statistics differed significantly—non-overlapping at the level of 2 standard errors in each test estimated using an unweighted block jacknife. Incongruent D_{dom} and D_{WG} statistics signify an enrichment of teosinte ancestry associated with domestication genes in either genome X ($D_{dom} < D_{WG}$) or genome Y ($D_{dom} > D_{WG}$).

f_3 and f_4 estimation
We used an in-house perl script (Dryad: /scripts/plink2freq.pl) to estimate sitewise allele frequency for input populations, and two other in-house perl scripts (Dryad: /scripts/f3.pl ; /scripts/f4.pl) to estimate f_3 and f_4 statistics and standard error using an unweighted jackknife procedure in 5Mb blocks from SNP data in plink format, following the equations in (*18*).

Supplementary Text

Archaeological context in lowland South America

The southern rim of the Amazon was densely settled by archaeological cultures characterized by the construction of earthworks (*30*). Despite the diversity in ceramic traditions, they all shared the practice of enclosing their settlements with roughly circular ditches. Prototypes appeared in the Brazilian state of Acre and surroundings ~2500 cal BP (*61*). Popularly known as *geoglyphs*, these sites combined perfectly symmetrical ditched enclosures with circular, rectangular, octagonal and other shapes, often combined in complex arrangements (*61–63*). *Geoglyphs* lack evidence of occupation, most finds being restricted to the sites' ditches or banks, including structured deposits of fine ceramics (*64*). For those reasons, they are considered ceremonial centers for periodic feasting. After ~700 cal BP, the construction of ditched enclosures expanded throughout southern Amazonia. In the Llanos de Moxos, Bolivia, the sites are known as *zanjas* and are associated with hydraulic earthworks, causeways, canals and fishweirs (*65–67*). Unlike the *geoglyphs*, they have irregular contours, mostly roughly circular or elliptical. They contain clear habitation strata with house floors, burial urns and domestic debris, confirming their function as enclosed settlements (*68, 69*). The Bolivian *zanjas* started to be built ~700 cal BP (*68*) and have historical counterparts in the palisaded villages described in colonial accounts(*70*). In the Upper Xingu, state of Mato Grosso, Brazil, dozens of settlements connected by a networks of roads and containing anthropogenic dark earth (ADE), multiple surrounding ditches, and enclosed central plazas have been documented (*31, 71*). Upper Xingu fortifications were mostly built after ~700 cal BP, with one earlier example dated ~850 cal BP, and the settlement system shows clear historical continuity with modern indigenous communities in the same region (*72*). Finally, in the Tapajós headwaters, Mato Grosso, the discovery of ditched enclosures provided a spatial link between the earthworks found to the west and east (*30*). Sites contain ADE and ceramic debris on the surface, suggesting a function as fortified settlements similar to the *zanjas* or the Xinguano sites. A single site was dated ~530 cal BP (*30*). The Mato Grosso enclosures may be the archaeological counterpart of large villages connected by straight roads described in historical accounts about the Upper Tapajós region (*73*).

At the same time that geometric enclosures expanded, and partly coinciding with their distribution, archaeological sites with platform mounds arranged in one or more circles around a central plaza appeared in different parts of southern Amazonia. Mound villages are found from Acre, where they are often built on top of or near earlier *geoglyphs* (*74*), to Mato Grosso, where they are also found in proximity to or inside ditched enclosures (*30*). Archaeological sites resemble present-day villages where houses are arranged in one or more concentric circles

around a public/ceremonial plaza. This type of settlement is common among Arawak peoples, many of which were distributed along the southern rim of the Amazon, and is still in use among modern groups in the Upper Xingu (*31*, *75*). Macro-botanical charred remains of maize have been recovered from one site (*76*). Archaeological mound villages expanded ~700 cal BP with one earlier exception dated ~850 cal BP (Neves et al., 2016; Saunaluoma, 2010; Saunaluoma et al., 2018) (*74*, *76*, *77*). In summary, the southern periphery of the Amazon was occupied by Formative supra-regional systems (Heckenberger, 2008) with networks of fortified circular settlements and plaza villages, most probably correlated with the belt of Arawak-speaking groups found in the same region in historical times.

A related and contemporary phenomenon is represented by the expansion of plaza villages in Central and Eastern Brazil. Two different archaeological traditions, Uru and Aratu, characterized by distinct ceramic complexes, built settlements with houses arranged in circles around a central plaza (*78*). Unlike the southern Amazonian examples, Uru and Aratu villages do not contain platforms, with house locations being identified solely by the distribution of artefacts on the surface. Such archaeological sites are predecessors of ethnographic plaza villages that were a defining feature of Macro-Jê and related peoples of the Central Brazilian *cerrado* (savannas) (*79*, *80*). Archaeological plaza villages were much larger than present-day ones, with up to three concentric rings of houses and deep strata of ADE, suggesting long occupations (*78*, *81*). Particularly in the case of the Aratu Tradition, material culture, settlement plans and funerary practices are remarkably similar across a large territory from the Brazilian *cerrado* to the Atlantic coast. Secondary burials in urns (reutilized oversized jars) are ubiquitous (*82–84*). Oversized ceramic vessels resembling those of the archaeological Aratu Tradition persisted among historical Macro-Jê peoples of the Brazilian coast who used them for fermenting maize beverages during ceremonial feasts (*85–87*). Plaza villages are dated ~1050-1000 cal BP in Central Brazil and in the Atlantic coast, pointing to a rapid expansion. The maize samples preserved in caves of the Peruaçu region, dated ~850-600 cal BP (*88*), most likely belong to this cultural horizon.

Similarities between the ceramic complexes found in Amazonian ring villages and the Uru Tradition, together with the resemblance in settlement layouts, suggested a possible role of diffusion from Amazonia ~1200 cal BP in the genesis of Central Brazilian plaza villages (*89–91*). Here, we hypothesize that the expansion of fortified circular settlements (geometric enclosures) and plaza villages ~1000-700 cal BP occurred in the midst of a continental interaction sphere involving mainly Arawak and Macro-Jê peoples. Contact is attested by ethnographic evidence, such as the sharing of certain rituals between the Arawak groups of the Upper Xingu and Eastern Brazilian Macro-Jê (*92*). We hypothesize that the diffusion of maize with significant proportion of Andean/Pacific ancestry occurred as part of this interaction sphere. Further support is provided by linguistic evidence for a ‘maize’ loanword of Arawak origin in Eastern Macro-Jê languages (*26*).

Linguistic patterns reflecting maize biogeography

The mapping of loanwords provides valuable evidence to reconstruct ancient routes and directions of diffusion of cultural traits (*93*). A case in point is provided by Eastern Brazilian languages of the Macro-Jê stock which borrowed their word for ‘maize’ from an Arawak source (*26*). Macro-Jê languages are mostly distributed outside of Amazonia, covering a broad territory in Central and Eastern Brazil, although some families are located in the southern fringe of the Amazon and in Bolivia (*94*, *95*). The Macro-Jê dispersal is of considerable antiquity, with estimates of ~6000-5000 BP (*96*).

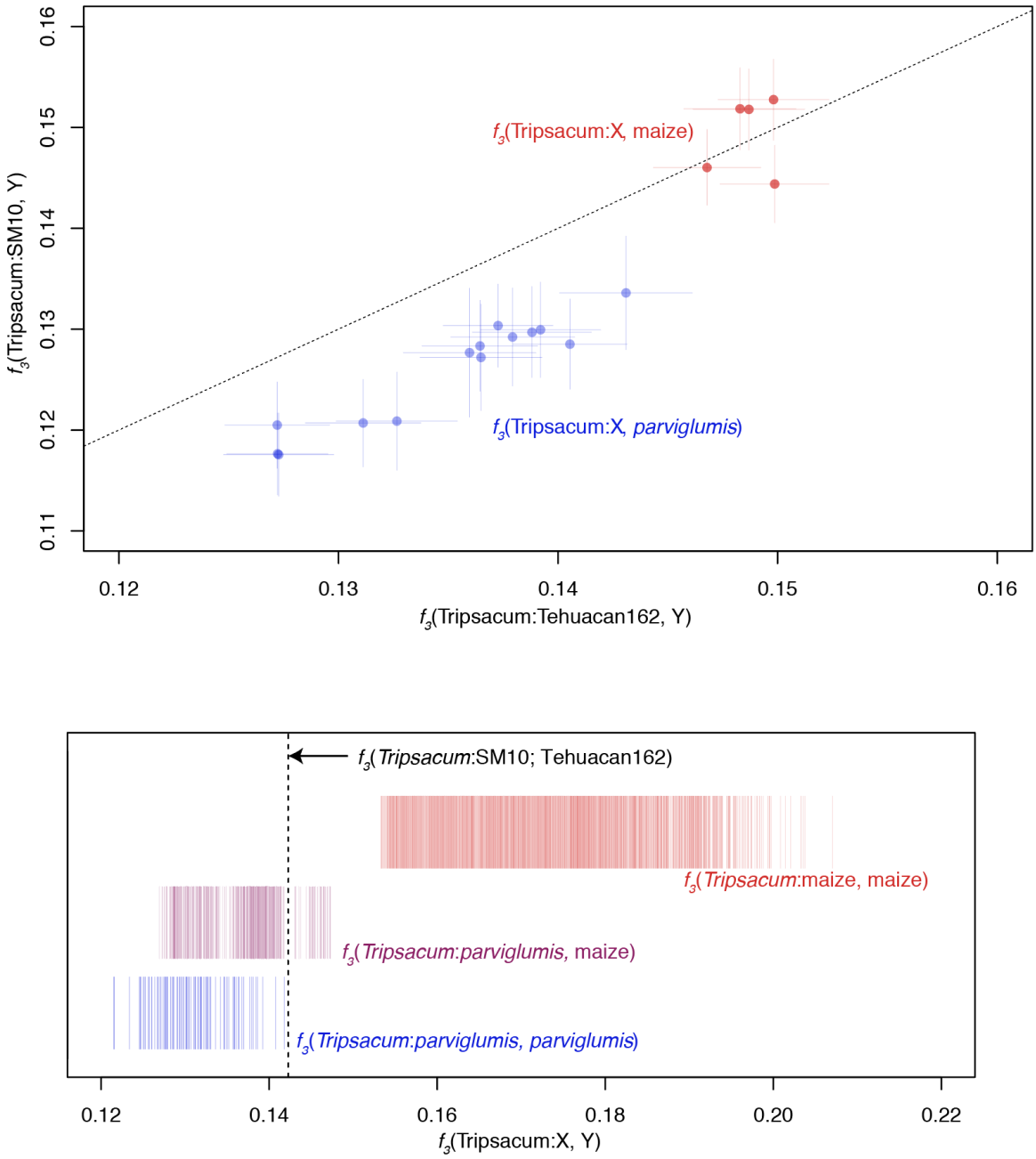
The Arawak languages are even more widely spread, being found throughout Amazonia and extending outside of South America, into the Caribbean (*97*). Their expansion is estimated to have started ~3000 BP (*96*). The mechanism of expansion of the Arawak languages has been debated, with some suggesting that it did not involve substantial migrations but rather diffusion through trade networks (*98*). The proto-Arawak homeland has also been a matter of contention, with recent Bayesian phylogenetic approaches suggesting a dispersal from southwestern Amazonia (*27*). In any case, the dispersal of the Arawak languages coincides in time and space with the adoption throughout Amazonia of similar ceramic complexes (the Barrancoid series), more sedentary settlements, formation of ADEs and polyculture agroforestry (*19*, *99*, *100*). In fact, unlike the case of the Macro-Jê stock, a word for ‘maize’ (***mariki**) can be reconstructed for proto-Arawak (*101*, *102*), suggesting that maize cultivation was practiced by its speakers even before their expansion. The proto-Arawak root itself may derive from proto-Quechua ***maʎki** ‘cultivated plant’, reinforcing a homeland near the Andean slopes and pointing to highland-lowland contacts already by ~3000 BP (*29*).

The Arawak word for ‘maize’ was borrowed by a number of Macro-Jê languages in Central and Eastern Brazil. It is present in Karajá *maki* (Karajá family), Kipeá *masiki* and Dzubukuá *madiqui* (Kariri family), Iatê *maltʃi* (Iatê family), Coroado *maheky* and Puri *maki* (Puri family) (*26*). These languages were isolated from each other in modern times and, with the exception of Karajá, were far removed from any Arawak neighbours, suggesting diffusion through long-range connections or intermediate groups that were later displaced or disappeared. Combining linguistic, archaeological and genetic evidence, we propose that the most parsimonious explanation for the ‘maize’ loanword distribution is that it was after ~1000 cal BP, together with maize with a significant proportion of Andean/Pacific ancestry, as part of the same Arawak-Macro-Jê interaction sphere that led to the adoption of plaza villages along the southern rim of the Amazon and in Central/Eastern Brazil.

Fig. S1.

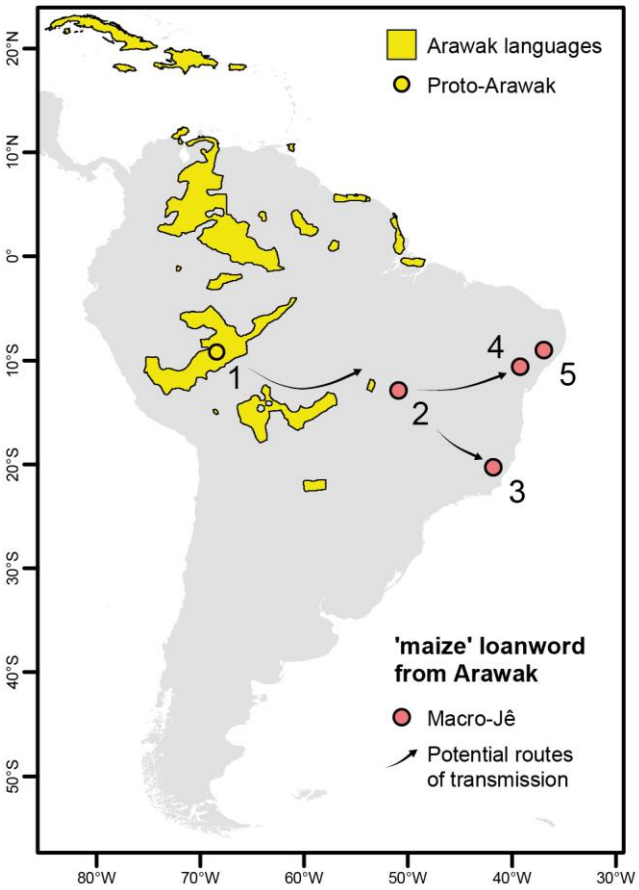
Early regional archaeological maize remains. Named sites correspond with dates on Figure 1, and additional information including type of remains and full references on Table S1.

Fig. S2.



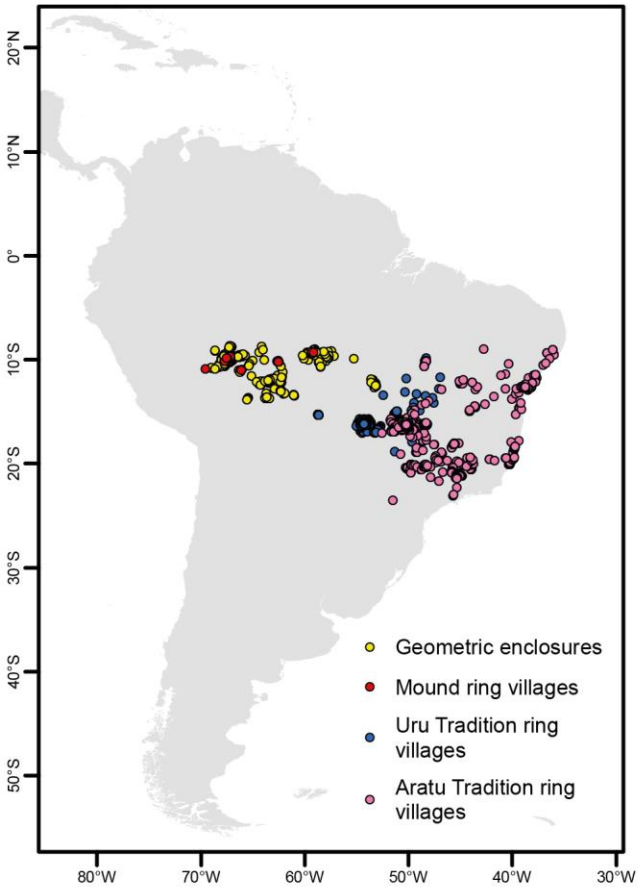
Outgroup-f_3 comparison of two ancient partially domesticated maize samples from the Tehuacan Valley—SM10 (*3*) and Tehuacan162 (*2*). Above, Tehuacan162 is significantly more *parviglumis*-like than SM10. Error bars are three standard errors estimated using an unweighted block jackknife. Below, vertical bars are all vs. all maize and teosinte comparisons organized as pairs of maize, maize and *parviglumis*, and pairs of *parviglumis*. SM10 and Tehuacan162 show allele sharing typical of a maize-*parviglumis* comparison.

Fig. S3.



Distribution of languages of the Arawak family and ‘maize’ loanwords into other families. 1) Proto-Arawak ***mariki** (*102*); 2) Karajá **maki**; 3) Puri ***maky*** and Coroado ***maheky***; 4) Kipeá **masiki** and Dzubukuá **madiki**; 5) Yatê **máltʃi**. Arawak homeland as per (*27*).

Fig. S4.



Distribution of archaeological traditions discussed in the main text and Supplementary Text, data from [JONAS].

Table S1.

Site Name	Latitude	Longitude	cal B.P.	Reference	Evidence
Lake San Pablo	0.22	-78.22	4900	(*103*)	pollen
Lake Ayauch	-3.04	-78.03	6000	(*12*)	pollen
Lake Sauce	-6.7	-76.21	6320	(*11*)	pollen
Abeja	-0.57	-72.4	5500	(*104*)	pollen
Huaypo	-13.4	-72.13	2800	(*105*)	pollen
Lake Gentry	-12.33	-68.87	3630	(*106*)	pollen
Lake Rogaguado	-13	-65.93	6500	(*13*)	pollen
Parmana	7.86	-65.77	1600	(*107*)	macro
Monte Castelo	-12.55	-63.09	4310	(*108*)	phytoliths
Geral	-1.64	-53.59	4030	(*109*)	pollen
Gentio Cave	-16.25	-46.03	3770	(*110*)	macro

Los Ajos	-33.7	-53.96	3730	(*111*)	phytoliths
Waynuna	-15.27	-72.75	4000	(*112*)	phytoliths and starch
Paredones/ Huaca Prieta	-7.93	-79.29	6700	(*10*)	macro, phyotoliths and starch
Chavin	-9.59	-77.18	2800	(*113*)	macro, stable isotopes
Real Alto	-2.37	-80.72	4750	(*114*)	phytoliths and starch
Xihuatoxtla	18.32	-99.53	8750	(*1*)	phytoliths and starch
Caye Coco	18.4	-88.39	6655	(*115*)	starch
Laguna Martínez	10.6	-85.35	5512	(*116*)	pollen
Aguadulce	8.33	-80.64	7746	(*24*)	phytoliths
Pijijiapan	15.49	-93.09	6589	(*117*)	phytoliths and pollen
Lake Yojoa	14.94	-88.02	5464	(*118*)	pollen
Romero and Valenzuela Caves	22.97	-99.32	4363	(*119*)	macro
Chaco Canyon	36.08	-108.01	4383	(*120*)	pollen
McEuen Cave	33.31	-110.1	4030	(*7*)	macro

Regionally early maize remains shown in Figure 1, Figure 3, and Figure S1.

Table S2.

Reference number	Altitude (m above sea level)	Collection site	AGE (C14 BP)	Calibrated Date BP (95.4%)	Latitude	Longitude
Z2	700	Peruaçu Valley - Januaria - Boquete Cave	570 +- 60	650-490	-15.00	-44.00
Z6	700	Peruaçu Valley - Januaria - Lapa da Hora Cave	630+- 60	660-515	-15.00	-44.00
Z61	200	Site EC-11, lower Santa valley, Ancash, Peru	800 +/- 30	730-660	-8.53	-78.34
Z64	3990	Site Cho9 Machay D, Chorrillos, Ancash, Peru	630 +/- 30	646-587 (58.7%) 573-535 (36.7%)	-9.06	-77.56
Z65	220	Site H13, lower Ica valley, Ica, Peru	970 +/- 30	920-770	-14.43	-75.34
Z66	2465	Argentina	1010 +/- 30	929-798	-27.34	-66.55
Z67	3700	Site Chayal, Susques, Jujuy, Argentina	100 +/- 30	253-225 (11.8%) 143- present (83.6%)	-23.24	-66.21
Arica4	N/A coastal	Arica, Chile	990 +/- 30	925-790	-18.47	-70.32
Arica5	N/A coastal	Arica, Chile	780 +/- 30	725-655	-18.47	-70.32

Archaeological maize genomes sequenced and analyzed in this study. Date calibration was done in OxCal (*121*) using the SHCal13 calibration curve (*122*).

Table S3.

Type or paste caption here. Create a page break and paste in the Table above the caption.

Site	cal BP	Lat	Long	Type	Location	Reference
Severino Calazans	2350-2160	-10.03	-67.51	Geometric enclosure (*geoglyph*)	SW Amazon (Acre, Brazil)	(*61*)
Tumichucua	2295-1870	-11.15	-66.16	Geometric enclosure	SW Amazon (Riberalta, Bolivia)	(*77*)
MT-FX-13	930-740	-12.38	-53.19	Geometric enclosure (*Xinguano fortification*)	S Amazon (Mato Grosso, Brazil)	(*31*)
Bella Vista	735-675	-13.26	-63.71	Geometric enclosure (*ring ditch*)	SW Amazon (Baures, Bolivia)	(*68*)
Mt04	625-505	-9.81	-57.82	Geometric enclosure	S Amazon (Mato Grosso, Brazil)	(*30*)
Sol de Campinas	930-795	-10.06	-67.31	Mound ring village	SW Amazon (Acre, Brazil)	(*76*)
MT-SL-29	1240-930	-16.12	-53.60	Ring village (*Uru*)	Cerrado (Mato Grosso, Brazil)	(*123*)
GO-CP-02	1275-920	-16.96	-51.58	Ring village (*Aratu*)	Cerrado (Goiás, Brazil)	(*124*)
Guipe	1175-760	-12.70	-38.45	Ring village (*Aratu*)	Atlantic coast (Bahia, Brazil)	(*82*)

Monsarás	1050-810	-19.52	-39.88	Ring village (*Aratu*)	Atlantic coast (Espírito Santo, Brazil)	(*125*)
GO-NI-06	1225-785	-16.47	-47.98	Ring village (*Aratu*)	Cerrado (Goiás, Brazil)	(*126*)
Sapucaí Phase	955-670	-21.08	-45.36	Ring village (*Aratu*)	Cerrado (Minas Gerais, Brazil)	(*127*)
Estiva 2	890-560	-10.68	-48.44	Ring village (*Aratu*)	Cerrado (Tocantins, Brazil)	(*128*)

Data S1. (separate file)

Details of modern landraces newly sequenced in this study.