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Ancient genomes reveal early Andean farmers selected common beans while preserving diversity

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1 LETTER

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4 **Ancient genomes reveal early Andean farmers selected common beans while preserving diversity**

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36

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42 **Introductory paragraph**

43 All crops are the product of a domestication process that started less than 12,000 years ago from  
44 one or more wild populations [1, 2]. Farmers selected desirable phenotypic traits, such as  
45 improved energy accumulation, palatability of seeds and reduced natural shattering [3], while  
46 leading domesticated populations through several more or less gradual demographic contractions  
47 [2, 4]. As a consequence, erosion of wild genetic variation [5] is typical of modern cultivars making  
48 them highly susceptible to pathogens, pests and environmental change [6,7]. The loss of genetic  
49 diversity hampers further crop improvement programs to increase food production in a changing  
50 world, posing serious threats to food security [8,9]. Using both ancient and modern seeds, we  
51 analyzed the temporal dynamic of genetic variation and selection during the domestication  
52 process of the common bean (*Phaseolus vulgaris*) that occurred in the southern Andes. Here we  
53 show that most domestic traits were selected for prior to 2,500 years ago, with no or only minor  
54 loss of whole-genome heterozygosity. In fact, i) the majority of changes at coding genes and linked  
55 regions that differentiate wild and domestic genomes are already present in the ancient genomes  
56 analyzed here; ii) all ancient domestic genomes dated between 600 and 2,500 years ago are highly  
57 variable - at least as variable as modern genomes from the wild, and single seeds from modern  
58 cultivars show reduced variation when compared to ancient seeds, indicating that intensive  
59 selection within cultivars in the last centuries likely partitioned ancestral variation within different  
60 genetically homogenous cultivars. When cultivars from different Andean regions are pooled,  
61 genomic variation of the pool is higher than that observed in the pool of ancient seeds from north  
62 and central western Argentina. Considering that most desirable phenotypic traits are likely  
63 controlled by multiple polymorphic genes [10], a plausible explanation of this decoupling of  
64 selection and genetic erosion is that early farmers applied a relatively weak selection pressure [2]  
65 by using many phenotypically similar but genetically diverse individuals as parents. Our results  
66 imply that selection strategies during the last few centuries, as compared to earlier times, more  
67 intensively reduced genetic variation within cultivars, and produced further improvements  
68 focusing on few plants carrying the traits of interest at the cost of marked genetic erosion within  
69 Andean landraces.

70

71 **Main text**

72 The onset of domestication by early farmers has been suggested as the period during which the  
73 most intense genetic bottleneck affecting genome-wide diversity occurred [4,11]. Yet, artificial  
74 selection, possibly at different rates in different times, was likely a continuous process that  
75 produced both landraces and, more recently under modern breeding programs, high-yielding and  
76 more resistant elite cultivars [2,12,13]. Understanding when the majority of genetic diversity was  
77 lost, and how such loss is related with the intensity of the selection process, is not only relevant  
78 from an evolutionary perspective but it will also help planning more sustainable breeding options  
79 to revert or mitigate crop genetic erosion [14,15]. We directly address these questions by analyzing  
80 both modern and ancient genomes of common bean from South America.

81

82 Common bean constitutes one of the major sources of vegetable proteins worldwide. The wild  
83 progenitor of common bean originated in Mesoamerica and colonized South America along the  
84 Andes [16,17]. This natural colonization process, dated by whole genome analysis between  
85 146,000 and 184,000 years ago [17], was accompanied by an intense and long bottleneck, as  
86 supported by the higher genetic diversity observed in the Mesoamerican as compared to the  
87 Andean gene pool [18]. Domestication of common bean occurred independently more or less  
88 simultaneously in both Mesoamerica and the Andes ca. 8,000 years ago, followed by divergence  
89 into distinct landraces due to drift, local selection, and adaptation [16].

90

91 We here obtained the first whole genome *shotgun* data from 30 ancient common bean seeds  
92 representing nine archaeological sites in north and central western Argentina (Supplementary  
93 Information S1-2,4, Supplementary Figure S1, Supplementary Table S1). All 30 seeds were dated  
94 between 2500 and 600 years before present (yrs BP) by AMS radiocarbon dating (Supplementary  
95 Information S3). Initial screening showed remarkable DNA preservation, with on average 44% (SD =  
96 12%) endogenous DNA mapped to the common bean reference genome [17] (Supplementary  
97 Table S1). Unimodal length distributions with short fragment lengths (average = 65, SD = 22 bp)  
98 and purines enrichment upstream of break points were consistent with those expected for  
99 degraded DNA (Supplementary Table S1, Supplementary Figures S3 and S4). The presence of  
100 deamination patterns at the end of the reads varied from 0.76% to 32.75% C to T and G to A  
101 misincorporations, with half of the seeds showing percentages below 10% (Supplementary Table  
102 S1, Supplementary Figure S5). Damage patterns were partially explained by the location of the  
103 archaeological sites: all seeds from sites at >2,500 meters above sea level showed less than 10%  
104 base misincorporations (with many lower than 5%), whereas the majority of seeds from lower  
105 altitude exhibited levels above 15% (Supplementary Figure S6). The observed pattern shows that  
106 both favorable environmental conditions and the structure of the seed (*e.g.*, presence of an  
107 external cuticle) likely resulted in good DNA preservation, as previously suggested for quinoa seeds  
108 from the same sites [19]. A subset of the specimens (Figure 1a,b) was selected for further  
109 sequencing based on DNA preservation and representation of age and locations, resulting in a final  
110 dataset of 15 ancient bean genomes with coverage from 4.2X to 23.2X in the non-repeated regions  
111 (Supplementary Table S1). Whole-genome data from modern common bean accessions  
112 (domesticated landraces/cultivars: 9 Andean, 3 Mesoamerican; wild: 1 Andean, 1 Mesoamerican)  
113 and a closely related species (*P. hintonii*, 1 accession) were also included in the analyses  
114 (Supplementary Information S6; Supplementary Table S3). All statistical analyses (excluding the  
115 selection test where only mutations observed in all ancient seeds are considered) were performed  
116 on transversions to avoid the bias introduced by post-mortem deamination [*e.g.*, 20].

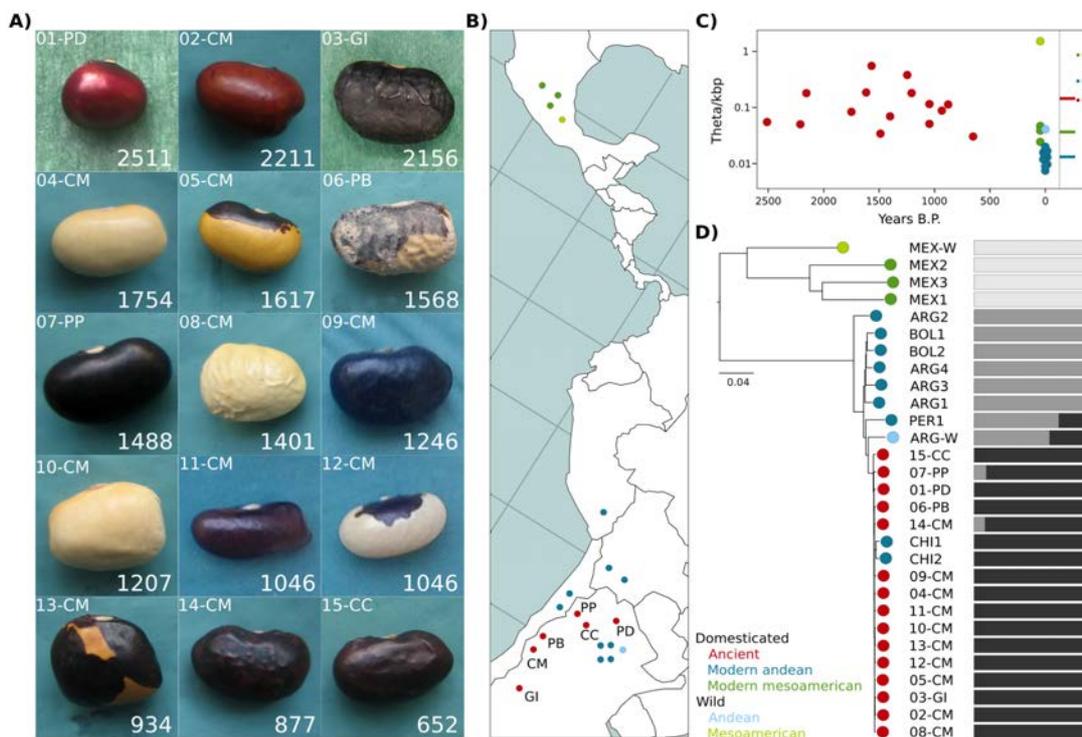
117  
118 Individual heterozygosity (probabilistically estimated as the variation parameter  $\theta$  every thousand  
119 nucleotides; Supplementary Information S7) is higher in all ancient seeds than in each of nine  
120 modern landraces/cultivars from the Andean gene pool (Figure 1c, Supplementary Table S4), and  
121 the average heterozygosity is more than ten times higher in ancient than in modern seeds (0.144  
122 vs. 0.013  $\theta$ /kbp, respectively, Mann-Whitney U,  $P < 0.001$ ). This difference remains high (0.110 vs.  
123 0.013  $\theta$ /kbp) and highly significant when the ancient seeds with the lowest coverage are excluded  
124 (see Supplementary Information 7). The effect of decreased heterozygosity due to the selfing  
125 procedure applied to modern accessions in seed banks was tested in two ways: by pooling pairs of  
126 modern seeds and estimating  $\theta$ /kbp in different seeds pairs, and by increasing the heterozygosity  
127 values in modern seeds as a function of the number of selfing cycles to which they were subjected  
128 (Supplementary Information S8). As expected, variation in modern pairs increased compared to  
129 single seeds, but it is on average still significantly smaller (at least 2.5 times) than that observed in  
130 ancient seeds (Mann-Whitney U test,  $P < 0.005$ , Supplementary Figure S9; Supplementary Table S5).  
131 It is possible that our pairing strategy did not entirely recover the heterozygosity just before the  
132 implementation of the seed bank protocol. A more exact estimate cannot be obtained since the  
133 detailed protocols applied to each modern Andean cultivar in seed banks are not known, and some  
134 level of outcrossing during this procedure cannot be excluded. Furthermore, the average  $\theta$ /kbp is  
135 likely increased in modern seed pairs because our paired seeds belong to different landraces (*i.e.*,  
136 the increase in the seed pairs is inflated by the genetic structure among landraces). When single  
137 seed heterozygosity was conservatively doubled or quadrupled in modern seeds subjected to one  
138 or two cycles of selfing, respectively, average variation remained significantly higher in the ancient

139 seeds (see Supplementary Information S8). We therefore conclude that single modern seeds  
140 representative of modern landraces have lower genetic variation than single ancient seeds.

141  
142 The modern genome available from a wild Andean seed [21], and two publicly released but  
143 unpublished genomes from wild Argentinean seeds (Supplementary Information S7), have a lower  
144 heterozygosity than the wild Mesoamerican seed in our dataset but comparable to that observed  
145 in the ancient Argentinean seeds (Figure 1c, Supplementary Figure S7). This result is a) consistent  
146 with the bottleneck that occurred during the natural colonization of the Andes from Mesoamerica  
147 [18], b) it indirectly supports the finding that modern Andean domestic seeds have lower diversity  
148 than ancient domestic seeds, because otherwise we should exclude any decrease of heterozygosity  
149 throughout the whole domestication process up to the modern cultivars, and c) it appears  
150 compatible with a minor effect of the initial Andean domestication on bean genetic diversity. With  
151 the current dataset, it cannot be excluded that heterozygosity was initially reduced following  
152 domestication, but subsequently restored by mutation. Such an effect would, however, require a  
153 ten times higher mutation rate than that estimated on the basis of the divergence time between *P.*  
154 *vulgaris* and *P. hintonii* (Supplementary Information S10. These findings challenge the conventional  
155 view of the domestication bottleneck resulting in severe genetic erosion [3] and is consistent with  
156 recent observations in maize, sorghum and barley [22].

157  
158 The lack of a temporal trend across ancient seeds of different age (Figure 1c) indicates that  
159 genome-wide diversity was largely maintained between 2,500 and 600 years ago by agricultural  
160 practices of Andean societies. Therefore, loss of diversity within each cultivar occurred more  
161 recently, certainly less than 600 years ago and likely in the last century [13]. A similar loss of  
162 diversity characterizes the recent domestication history of horses [20]. The absence of data on  
163 seeds from the last 600 years currently hinders a direct reconstruction of the temporal dynamic of  
164 genomic diversity during the last six centuries.

165



166  
167  
168 **Figure 1. Diversity in ancient and modern common bean.** A) Pictures of the 15 common bean seeds sequenced for whole-genome  
169 analyses: sample ID with two letters referring to the archaeological site of origin as shown in panel B is reported in the top-left corner  
170 of the pictures (see Supplementary Information S1); AMS radiocarbon age in yrs BP is reported in the bottom-right corner. B) Map

171 showing the locations of the archaeological sites where the seeds were retrieved (red) and the country of origin of the modern  
172 domesticated cultivars from South and Central America (blue and green, respectively) and the wild specimens (pale blue, pale green).  
173 Modern seed locations are only indicative of the country of origin **C**) Whole-genome diversity estimated as  $\theta$  kbp<sup>-1</sup> in individual seeds  
174 (solid circles), average of individual estimates per group (solid line), and in groups of seeds (dashed line) using only transversions in  
175 callable regions (see Supplementary Information S5 for details); only domesticated cultivars are included in the group estimates. **D**)  
176 Neighbor-Joining tree based on genetic distances and Admixture analysis both based on transversions only (one SNPs every 50 kb was  
177 used in the Admixture analysis) in callable regions; note that modern seeds from Chile cluster together with the ancient seeds.

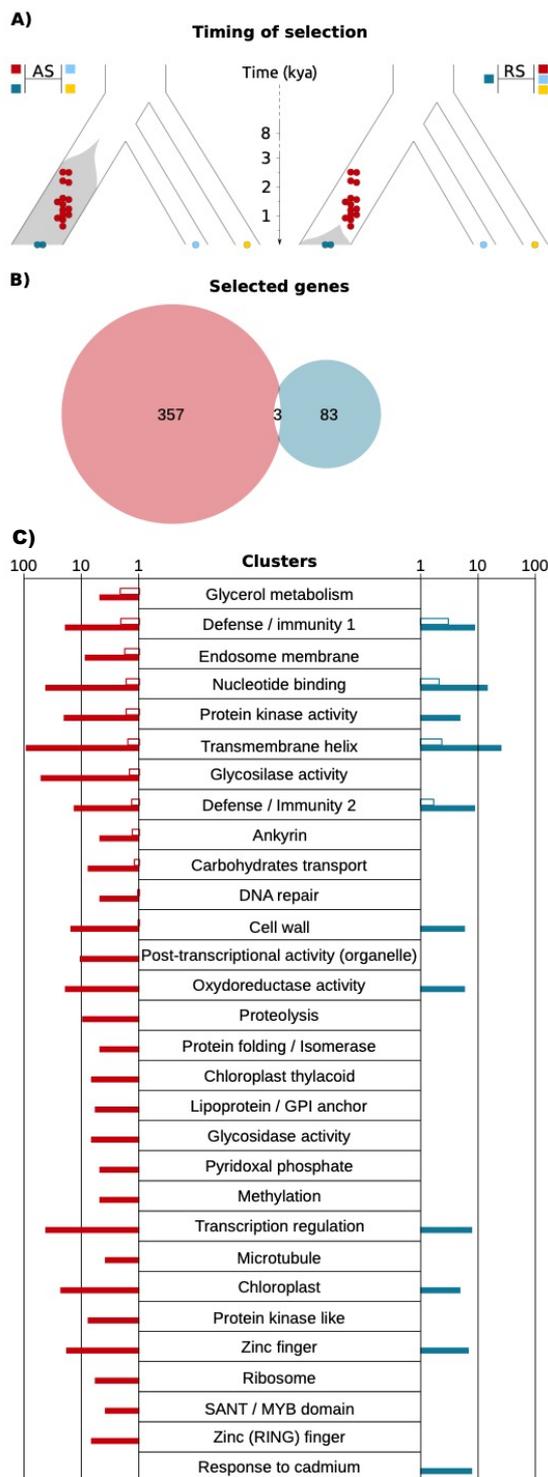
178  
179 When estimating genetic variation in groups of modern Andean (0.294  $\theta$ /kbp) or Mesoamerican  
180 seeds (0.860  $\theta$ /kbp), we observe a substantial increase compared to the average individual  
181 estimates (Figure 1c), indicating that modern genomic diversity is highly structured in different,  
182 highly homogeneous, cultivars. The abandonment and extinction of any modern cultivar would  
183 therefore result in the significant loss of its private fraction of the whole crop diversity [8,23]. On  
184 the contrary, the estimate of genetic diversity obtained by pooling all ancient individuals is very  
185 similar to the average of the 15 individual estimates (0.134  $\theta$ /kbp; Figure 1c), implying that these  
186 ancient genomes represent a single genomic pool. Ancient seeds, as a group, do not reach the  
187 level of variation observed in the pooled modern cultivars. This is not unexpected considering that  
188 a) the latter have a long, complex and partially independent history of recent landrace  
189 establishment, and b) the former (the ancient domestic seeds) were used during the Holocene by  
190 human populations with shared subsistence practices, which included the use of wild beans [24]. A  
191 large fraction of the ancient seeds could individually recover most of the ancient crop diversity in  
192 that area, while modern seeds never reach individual heterozygosity values close to the pooled  
193 group from Chile, Bolivia, Argentina and Peru, or the groups obtained by separately pooling the  
194 Chilean (0.045  $\theta$ /kbp), the Bolivian (0.102  $\theta$ /kbp), or the Argentinean (0.198  $\theta$ /kbp) landraces. A  
195 more widespread sample of ancient beans might also reveal that ancient populations harbored  
196 more genetic diversity than pooled cultivars.

197  
198 Three main genetic groups were revealed by different clustering methods (Supplementary  
199 Information S9) and a whole-genome NJ tree (Figure 1b; Supplementary Figures S10-S11). The  
200 largest divergence is observed between Mesoamerican and Andean genomes, while the Andean  
201 seeds are further partitioned into two less differentiated groups. All ancient seeds belong to the  
202 same genomic clade, indicating that the same or similar ancient landraces were in use in Argentina  
203 for about 2,000 years. - This clade also includes modern landraces used today on the other side of  
204 the Andes, in Chile. The so-called Chilean race [25] therefore appears to be the direct descendants  
205 of the seeds used in Argentina before the Incaic conquest of the area occurred at the end of the  
206 15<sup>th</sup> century [26]. We interpret this result as a consequence of the trade of goods, including crop,  
207 between the eastern (in Argentina) and western (in Chile) slopes of the Andes, with early farmers  
208 relying on Llama caravans for regular-long distance exchanges [27]. Modern Argentinian cultivars,  
209 instead, do not trace their ancestry back to the local ancient cultivar but were likely introduced in  
210 the area some time during the last 600 years.

211  
212 A gene-by-gene selection scan (Figure 2a) was performed to discriminate early (>2,500 years ago)  
213 from late (<600 years ago) selection targets, by taking into account the different levels of genetic  
214 drift expected in the two time periods of different length (Supplementary Information S11). We  
215 found that: *i*) a selection signature is present in 443 out of 27,000 genes tested (FDR<0.001); *ii*)  
216 selection affected many more genes (ca. 4.2 times more) in the early compared to the late phase  
217 (Fig. 2b), suggesting that recent improvement of cultivars strongly affected few genes, but the  
218 main genomic turnover occurred several thousand years ago; if adjacent genes are merged in  
219 "sweep blocks", thus considering that genes under selection may produce selection signals also in  
220 adjacent genes, the ratio between ancient and recent sweeps remains larger than 3 (see  
221 Supplementary Information S11); *iii*) genes selected in the early phase belong to functional clusters

222 mainly related to glycerol metabolism, carbohydrate and sugar transport and metabolism,  
223 intracellular transport (endosome membrane), regulatory elements (nucleotide binding),  
224 modification of proteins and glycosylation, whereas recent selection primarily targeted traits  
225 involved in immunity and defense, regulatory elements and transmembrane transport (Figure 2c;  
226 Supplementary Table S7). Evidence for selection on sugar biosynthesis genes at the early stages of  
227 domestication has also been found in ancient maize from 2,000 and 750 years ago [28]. One of the  
228 functional clusters identified in the early selected genes includes the MYB-domain genes that are  
229 important regulatory elements of development, metabolism and responses to biotic and abiotic  
230 stresses [29], and have been suggested as causative of different phenotypic changes associated to  
231 the shattering trait in common bean [30], maize and rice [4,31].

232  
233 Genomic studies of ancient DNA are much less numerous in plants compared to animals remains  
234 [32-33], but have already provided fundamental insights into the process of domestication [28,34-  
235 38]. Our results show that common bean seeds, and likely legume seeds in general, can be an  
236 excellent source of high quality ancient DNA, especially if seeds are preserved in favorable climatic  
237 conditions (*i.e.*, cold and dry environments). The dynamic of domestication is still debated [22],  
238 but, as recently summarized [2], the consensus is that domestication was slow and gradual, with  
239 selected traits emerging in association with population decline and loss of genetic variation. Here,  
240 we contribute to this debate with the first study on ancient bean genomics, and provide an  
241 alternative example in which selection and loss of variation are decoupled during the process of  
242 domestication. Our data reveals that in common bean early agriculture in the Andes genomic  
243 diversity was preserved within single seeds, whereas more recent breeding practices produced  
244 structured landraces that are internally more homogenous. At the same time, however, assuming  
245 that the genetic changes we infer correspond at least in part to phenotypic changes, early  
246 agriculture was also very efficient in selecting most of the desirable traits now typical of this crop.  
247 We hypothesize that these patterns are the result of the larger number of seeds used by early  
248 farmers as founders in each generation, all likely displaying the selected trait(s) but heterogeneous  
249 in the rest of the genome. Initial improvement of the common bean was therefore based on serial  
250 soft sweeps, as has also been suggested for the domestication of maize [10]. Encompassing several  
251 thousand years, and likely assisted by cultivar exchanges and hybridization with wild plants  
252 [2,25,39-40], such breeding practice allowed many traits to be selected for, without the significant  
253 loss of genomic variation that has likely occurred in more recent times within landraces and  
254 cultivars.



**Figure 2. Timing of selection in common bean from South America.** All genes (27,000) in the common bean genome were tested for a significant enrichment of fixed alternative alleles according to the topologies described below, taking into account the different level of drift expected in periods of time of different length (see Supplementary Information S11 for details). **A)** Alternative topologies for a SNP: on the left (AS: ancient selection) the SNP is fixed for an alternative variant in both the ancient seeds from Argentina (red circles) and the modern cultivars from Chile (blue circles) as compared with the wild Andean *P. vulgaris* seed (pale blue circle) and the outgroup (*Phaseolus hintonii*, yellow circle); on the right (RS: recent selection) the SNP is fixed for an alternative variant in the modern cultivars from Chile only as compared with the ancient seeds, the wild Andean and the outgroup; the gray shade represents the temporal fixation of an alternative allele; ancient seeds age as well as the putative divergence time between Andean domesticated and wild *P. vulgaris* is reported. Using the genome of the wild Mesoamerican *P. vulgaris* as outgroup instead of *P. hintonii* produced largely similar results (see Supplementary Table S7). **B)** Number of genes significantly enriched with SNPs in RS (blue) and/or AS (red) topology (FDR<0.001, top 1% for the proportion of SNPs within gene  $\pm$  1kb, and with an *Arabidopsis thaliana* ortholog). **C)** Functional gene clusters (see Supplementary Information S11) in RS (blue) or AS (red) enriched groups (the complete list of genes can be found in Supplementary Table S7). Filled bars: max gene count in the cluster; empty bars: enrichment score of the cluster (only scores >1 are reported).

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262 archaeo-botanical specimens and studying the archaeological sites.

263

## **Data accessibility**

265 Sequenced raw reads from ancient samples are publicly available as NCBI Bioproject (ID:  
266 PRJNA574560). Unpublished modern sample genomic data are available with the following NCBI  
267 Accession Codes: SRR10161640; SRR10161629; SRR10161767; SRR10161647; SRR10161646;  
268 SRR10161645; SRR10161601; SRR10161592; SRR10161584; SRR10161684; SRR10161683;  
269 SRR10161697; SRR10161690; SRR10161688.

270

## **Competing Interests statement**

272 The authors declare no competing interests.

273

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355 **SUPPLEMENTARY METHODS AND RESULTS**

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396 file)

397

398

399 **S1. Ancient beans collections and archaeological context**

400

401 Ancient bean seeds were selected from five museum collections in Argentina: Museo de La Plata (La Plata,  
402 Buenos Aires, Argentina); Museo de Historia Natural de San Rafael (Parque Mariano Moreno, San Rafael,  
403 Argentina); Instituto de Investigaciones y Museo Arqueológico "Prof. Mariano Gambier", Universidad  
404 Nacional de San Juan (San Juan, Argentina); Instituto de Arqueología y Museo, Universidad Nacional de  
405 Tucumán (Tucumán, Argentina); Museo Arqueológico Pio Pablo Díaz (Cachi, Salta, Argentina).

406

407 Seeds were originally collected from nine archaeological sites located in different geographical regions of  
408 north and central-western Argentina (Figure 1a, main text). A brief description of each site is reported  
409 below. Codes for each seed, referring to Figure 1b (main text) and Figure S1, are reported in Table S1.

410

411 ***Puente del Diablo***

412 Puente del Diablo (SSallap20) archaeological site is a cave situated in the northern sector of the Calchaqui  
413 Valleys in a pre-puna landscape (Salta province). It was excavated in the 1970s and includes both domestic  
414 and funerary contexts. This is a multicomponent site with burials dating back to 10,000 yrs BP, remains  
415 corresponding to burials and temporal occupations of hunter gatherers of ca. 3,000 yrs BP and also from ca.  
416 2,000 yrs BP corresponding to the first agro-pastoralist societies in the area. From these last occupations,  
417 remains of *Cucurbita maxima*, *Prosopis* spp. and cactaceae were recovered together with remains of  
418 rodents (*Lagidium* sp.), *Cervidae* sp. and camelids (*Lama* sp.) (Lema, 2009, 2015).

419

420 ***Gruta del Indio***

421 Gruta del Indio is an archaeological site located in Central Western Argentina, close to San Rafael city, in  
422 Monte phytogeographic province, at 700 m asl. Gruta del Indio was occupied by hunter-gatherers since the  
423 Pleistocene-Holocene transition ca. 10,500 years ago (Semper and Lagiglia 1968, Long et al 1998). Hunter-  
424 gatherers occupied the cave until at least ca. 1,900 yrs BP, when a strong domestic plant record appears in  
425 the archaeological record. This domestic archaeobotanical record include *Zea mays*, *Chenopodium quinoa*,  
426 *Cucurbita*, and *Phaseolus vulgaris* seeds, and *Zea mays* remains, such as canes and starches (Semper and  
427 Lagiglia 1968, Lagiglia 1999). This domestic plant context (the oldest in the region) is isolated in time and  
428 space, and is associated to human bone remains, indicating the use of the cave as a cemetery. This  
429 archaeological context was called "Atuel II culture" (Semper and Lagiglia 1968) and all radiocarbon dates are  
430 between 2,100 and 1,900 yrs BP (Gil et al 2014). Stable isotopes analyses from human bone suggest that  
431 the *Zea mays* was probably part of the diet but not as major component (Gil et al 2010). The *Phaseolus*  
432 *vulgaris* beans were found in a grass container holding ca. 500 grs. of seeds. Another grass container was  
433 full of *Chenopodium quinoa* seeds in the same archaeological mortuary context. After Atuel II, there is little  
434 evidence of human occupation and temporally close to the arrival of the Spanish (ca. 500 yrs BP).

435

436 ***Los Morrillos, Río Salado, Cerro Calvario and Punta del Barro (San Juan sites)***

437 The San Juan sites are in the two western Departments of the Province of San Juan, Iglesia (to the north) and  
438 Calingasta (to the south). They are in a southern Andean area that extends between 28°25'S and 32°33'S on  
439 the arid eastern slope of the Andes. This territory includes (from west to east) the "Cordillera de los Andes",  
440 two wide valleys between 1,900 and 1,600 m asl and another system of high altitude but of older age than  
441 the mountain range called "Precordillera of La Rioja, San Juan and Mendoza"; its maximum summits  
442 constitute the eastern limit of these Departments.

443

444 **Los Morrillos (caves 2 and 3) and Río Salado cave**

445 The first evidence of agricultural and breeding (*Lama glama*) activity in the eastern slope of the Andes in San  
446 Juan province did not originate in the area, but were introduced together with the domesticated species. The  
447 human groups that introduced and developed agriculture were located in favorable small sites corresponding  
448 to the exit of the cordilleran streams in the great plain of the high foothills (approximately 2,900 m asl). Such  
449 human groups cultivated in summer, gathered wild fruits and ñandu eggs, and entered the high Andean  
450 valleys for the hunt of the guanaco (*Lama guanicoe*). They mostly inhabited natural caves in the area.  
451 Examples of such caves are Los Morrillos (caves 2 and 3). In the Los Morrillos caves, the thick layer with  
452 agriculture evidence overlapped with an older layer of hunter gatherers. By contrast, in the Río Salado cave

453 there is only a thick level with agricultural remains (Gambier 1977, 1988, 2000, Roig 1977). Los Morrillos caves  
454 were excavated in seven long seasons between 1969 and 1977, while the Río Salado cave was excavated in  
455 1972. The three caves correspond to habitation sites without specific areas of activity. The botanical remains  
456 were found among the anthropic sediments with diverse remains: lithic, ceramic, artifacts in wood and bone,  
457 textiles, etc.).

458

#### 459 Cerro Calvario sites (Calingasta Valley) and Punta del Barro site (Iglesia Valley)

460 The same agricultural and breeder groups described previously, with some foreign influences, later  
461 descended from the “Cordillera de Los Andes” towards the valleys at lower altitude and settled in open areas  
462 with the possibility of irrigation from small springs or streams. This is the case for the Cerro Calvario sites in  
463 the Calingasta Valley and Punta del Barro in the north of the Iglesia Valley (Basurero Norte, Basurero 3)  
464 (Gambier 1988, 2000; Michieli 2016).

465

#### 466 Punta del Barro “1º canal” site

467 Around 1200 AD the local groups settled in the valleys of the Eastern slope of Los Andes in the current  
468 province of San Juan developed a large-scale agricultural system based on irrigation by means of large  
469 channels connected to the rivers. The agricultural sites of Punta del Barro “1º canal” represent a residential  
470 area, excavated in 2011, which included an occupational floor almost at surface level with storage and  
471 combustion wells. Various cultivated botanical remains were found in one of the storage wells: corn, squash,  
472 quinoa and beans. It was related to a large hydraulic channel and three tombs with excellent preservation of  
473 both the bodies and the woven garments that enveloped them and other items (ceramics, pumpkin  
474 containers, wooden objects, etc.) (Michieli 2015).

475

#### 476 **Punta de la Peña**

477 Punta de la Peña 9 is a residential site with stone-walled structures, blocky shelters, bedrock mortars,  
478 caches, open air activity areas, and rock art that was continuously occupied by agro-pastoralist farmers  
479 between ca. 2,000 to 400 yrs BP -post-Hispanic time (Babot et al 2006, López Campeny et al 2017, Somonte  
480 and Cohen 2006). It comprises processing, discard, workshop, funerary and ritual areas spatially segregated.  
481 The site is located on the southern margin of the Las Pitás River at an elevation of 3,665 m asl in the Salty  
482 Puna desert (Catamarca province). Subsistence was based on llama (*Lama glama*) herding, vicuña (*Vicugna*  
483 *vicugna*) hunting, small-scale cultivation, and plant gathering and exchange. Wild, domesticated and weed  
484 plant macro and micro-remains belong to a number of native and foreign taxa as *Chenopodium quinoa*,  
485 *Solanum tuberosum*, *Oxalis tuberosa*, *Phaseolus vulgaris*, *Prosopis* sp., *Geoffroea decorticans*, *Zea mays*,  
486 *Amaranthus caudatus*/A. *mantegazzianus*, *Lagenaria siceraria*, *Cucurbita* sp., *Canna edulis* and Cyperace  
487 and Cactaceae species (Babot 2009, Rodríguez 2013). Alero 1 is a blocky shelter rich in well preserved plant  
488 remains from the first millennium DC, placed in the sector I of the Punta de la Peña 9 site (Babot et al 2013).

489

#### 490 **Pampa Grande**

491 Pampa Grande is an archaeological locality including seven caves explored in the 1970s. It is located in the  
492 Las Pirguas mountain range (Salta province), between 2,500 and 3,000 m asl in a montane grassland and  
493 forest landscape corresponding to the highest zone of the Yunga biogeographical province. Ancient remains  
494 are mainly related to funerary contexts and few occupational areas, corresponding to Candelaria groups.  
495 The age of these occupations range between 400 – 3,000 yrs BP and corresponds to the first agro-  
496 pastoralist societies in the area. Remains related with subsistence include domestic, wild and hybrid plant  
497 species of *Cucurbita maxima* and *Phaseolus vulgaris*. *Phaseolus lunatus*, *Lagenaria siceraria*, *Zea mays*,  
498 *Arachis hypogaea*, *Prosopis* spp., *Geoffroea decorticans*, tubers, *Capsicum* spp. and other wild and weed  
499 forms were recovered. Remains of wild and domestic camelids were also recovered together with fish,  
500 rodent and bird bones (Baldini et al 2003, Lema 2010).

501

#### 502 **Cueva de los Corrales**

503 Cueva de Los Corrales 1 is an archaeological site located in the Quebrada de Los Corrales (El Infiernillo,  
504 Tucumán) at 3,000 m asl in a dry shrub / grassland landscape, corresponding to the highest zone of the  
505 Yunga biogeographical province (Oliszewski et al 2008). It is a cave that was occupied at different times  
506 throughout 2,400 years (ca. 3,000-600 yrs BP), from transitional time between hunter-gatherer groups to

507 agro-pastoralist groups until purely agro-pastoralist time (Oliszewski et al. 2018). Remains related to  
508 subsistence include domestic camelids and dasiphodids; wild plants: *Prosopis nigra*, *Geoffroea decorticans*,  
509 *Celtis tala* and *Trichocereus* sp.; cultivated plants: *Phaseolus vulgaris*, *Zea mays* and *Chenopodium quinoa*  
510 and wild *Cucurbita maxima* (Oliszewski and Arreguez 2015, Oliszewski and Babot 2015, Lema 2017).

511 **S2. Seeds selection**

512

513 Thirty ancient bean seeds (Figure S1) were selected for preliminary screening based on the site locations  
514 and archaeological context. All seeds had a clear domestic phenotype. After visual inspection, we selected  
515 seeds with different morphological features (e.g. coat color and dimension) and good preservation (e.g.,  
516 less visible cracks or damages in the outer coat).  
517



518

519

520 **Figure S1.** Pictures of the 30 seeds sequenced at low coverage for preliminary screening of aDNA content and preservation.  
521 Archaeological site information for each seed is reported in Supplementary Table S1.

522

523 **S3. AMS radiocarbon dating**

524

525 A fragment of ca.  $\frac{1}{4}$  of the cotyledon was removed from each seed and used for AMS radiocarbon dating at  
526 CEDAD, Center of Applied Physics, Dating and Diagnostics, Department of Mathematics and Physics "Ennio  
527 De Giorgi", University of Salento. Conventional radiocarbon ages and calibrated ranges for 30 seeds are  
528 reported in Table S1. Calibration was performed using OxCal Ver. 3.10 based on atmospheric data (Reimer et  
529 al 2009). Distribution curves of the calibrated age for each seed, and additional details on the dating  
530 procedure are available on request.

531

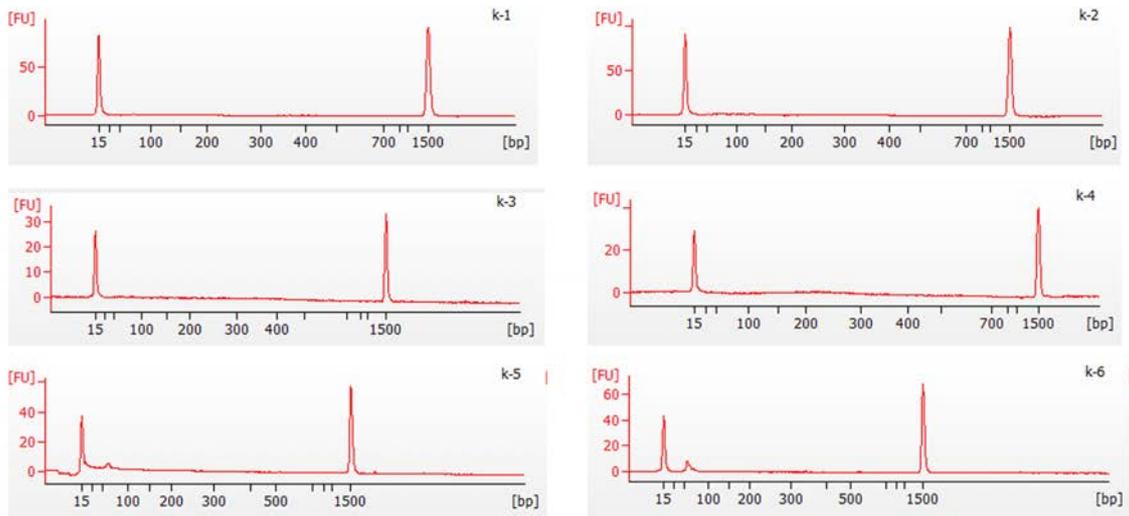
532

533 **S4. Ancient DNA extraction and sequencing**

534

535 DNA extraction and library preparation were performed at the Molecular Anthropology and Paleogenetic  
536 Laboratory of the Department of Biology, University of Florence, using facilities that are exclusively  
537 dedicated to processing ancient DNA and following all the necessary precautions to minimize contamination  
538 from exogenous DNA. One sample (O9-CM) was extracted and a library built at the ancient DNA laboratory  
539 of the University of Oslo. Negative controls were included in each step of the molecular analysis. All seeds  
540 were photographed prior to sampling (Figure S1). After removing the cuticle, each seed was UV irradiated in  
541 a crosslinker for 45 minutes. The embryo and a portion of cotyledon were then pulverized by hand with  
542 mortar and pestle that had been decontaminated with bleach and UV. Half of the powder, was used for DNA  
543 extraction following a high-salt CTAB extraction protocol according to the Small Fragment Protocol of  
544 Qiagen DNeasy *mericon* Food Handbook. The amount of powder used in the DNA extraction ranged  
545 between 48 and 240 mg, depending on the size of the seed. Food Lysis Buffer was substituted with a  
546 homemade CTAB high salt extraction buffer according to the following recipe: 100mM Tris-HCl pH 8, 25mM  
547 EDTA pH 8, 4M NaCl, 2% CTAB, 0.3%  $\beta$ -mercaptoethanol. An aliquot of each extract was converted into a  
548 sequencing library with 7 bp P7 indexes according to Meyer & Kircher 2010. No UDG treatment was  
549 performed. The fill-in reaction was split in 4 aliquots that were amplified separately in 100 $\mu$ l reaction mixes  
550 with 0.25mM dNTPs mix (New England Biolabs), 0.30 mg/ml BSA (New England Biolabs), 2.5U Agilent Pfu  
551 Turbo Polymerase (Agilent Technologies) and 0.4 $\mu$ M of each primer. The following indexing PCR profile was  
552 performed by default on each library: 95°C 2', [95°C 30'', 58°C 30'', 72°C 1'] x 14, 72°C 10'. DNA  
553 quantification and quality control were performed on a Agilent 2100 Bioanalyzer using the Agilent DNA  
554 1000 kit, following manufacturer's instructions. All libraries and extraction blanks showed flat BioAnalyzer  
555 profiles (see Figure S2), but were included in the pool of the first sequencing round (see below). The  
556 libraries were sequenced at the Norwegian Sequencing Centre, University of Oslo, Norway, where all  
557 samples were pooled in equimolar ratios and paired-end sequenced on the Illumina HiSeq 2500. First,  
558 samples were screened for DNA preservation by low-coverage sequencing. Based on the results of this first  
559 sequencing run and on sample characteristics (age, location, phenotype, mapping quality, age-related  
560 damage patterns), a subset of 18 samples was re-pooled and sequenced further on four Hiseq2500 lanes.  
561 Additionally, one sample (O1-PD) was sequenced on one lane each on the HiSeq 4000 platform. One sample  
562 (O9-CM) was sequenced on the Hiseq 2500 in a different pool from all other beans. Based on the final  
563 results we further selected 15 samples with coverage higher than 4X for downstream population genomic  
564 analyses (Table S1).

565



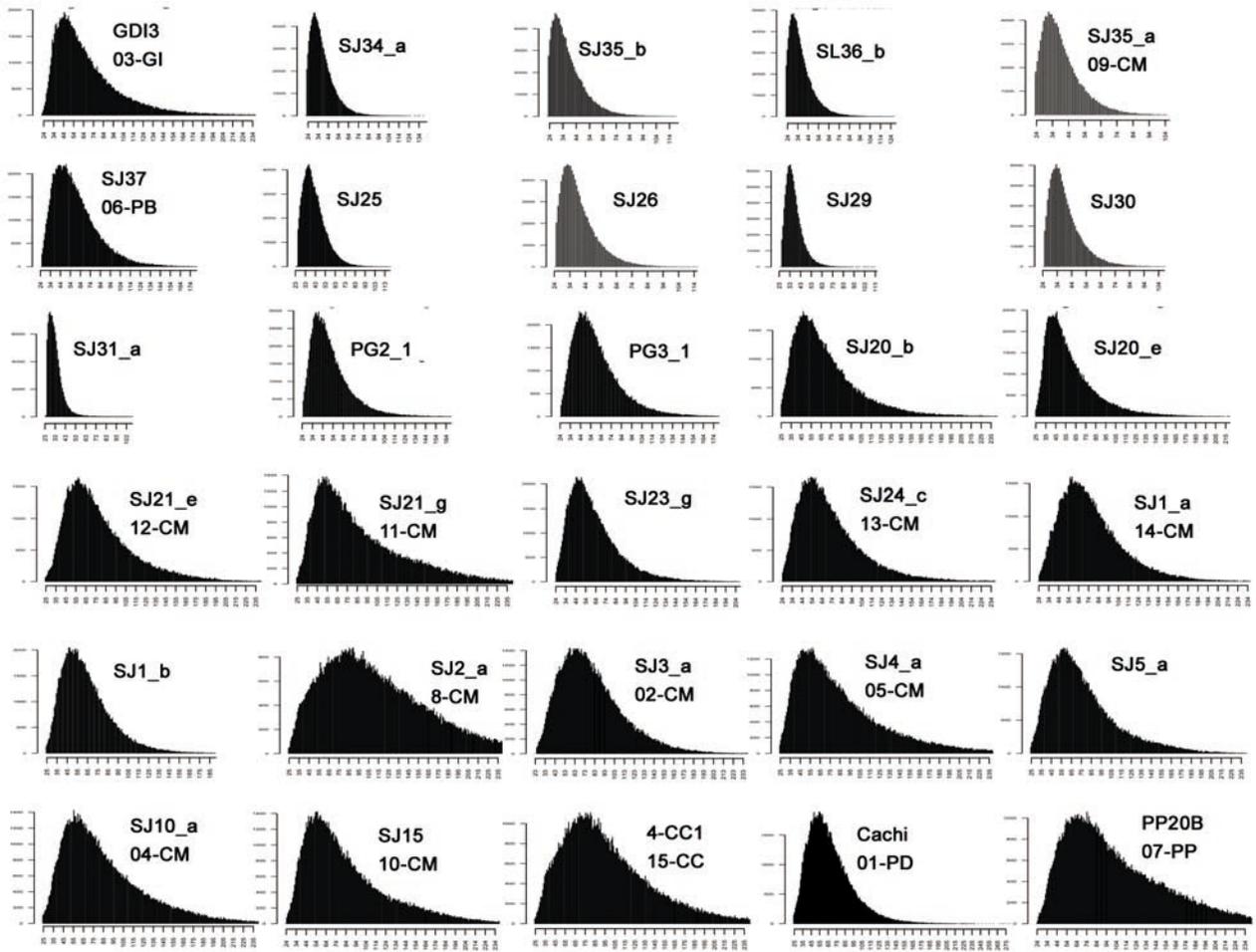
566  
567 **Figure S2.** BioAnalyzer profiles of library (k-1, k-3, k-5) and extraction (k-2, k-4, k-6) blanks.  
568  
569

570 **S5. DNA damage assessment and mapping to the reference genome**  
571

572 Paleomix v.1.2.12 (Schubert et al 2014), a pipeline specifically designed for aDNA, was employed for the  
573 filtering and mapping of aDNA data. Adapter trimming, collapsing of overlapping mate-pairs, trimming of  
574 low-quality bases and removal of reads shorter than 25 bp was performed with AdapterRemoval (Lindgreen  
575 et al 2012). Filtered reads were aligned to the *Phaseolus vulgaris* reference genome v2.1 (Schmutz et al  
576 2014), downloaded from Phytozome (<https://phytozome.jgi.doe.gov/pz/portal.html>) using bwa aln (Li and  
577 Durbin 2009) and retaining reads with mapping quality  $\geq 25$ . PCR duplicates were filtered using the  
578 Paleomix rmdup\_duplicates option. Fragment length distributions, break point and misincorporation  
579 patterns were explored in each sample (Figures S3, S4, S5) using MapDamage2.0 (Jónsson et al 2013)..  
580 Indel-realigned bam files were generated using the GATK Indel realigner (McKenna et al 2010). Realigned and  
581 filtered bam files were then indexed and sorted with Samtools-0.1.19 (Li et al 2009). The percentage  
582 endogenous DNA and coverage were determined from these realigned and filtered bam files.  
583

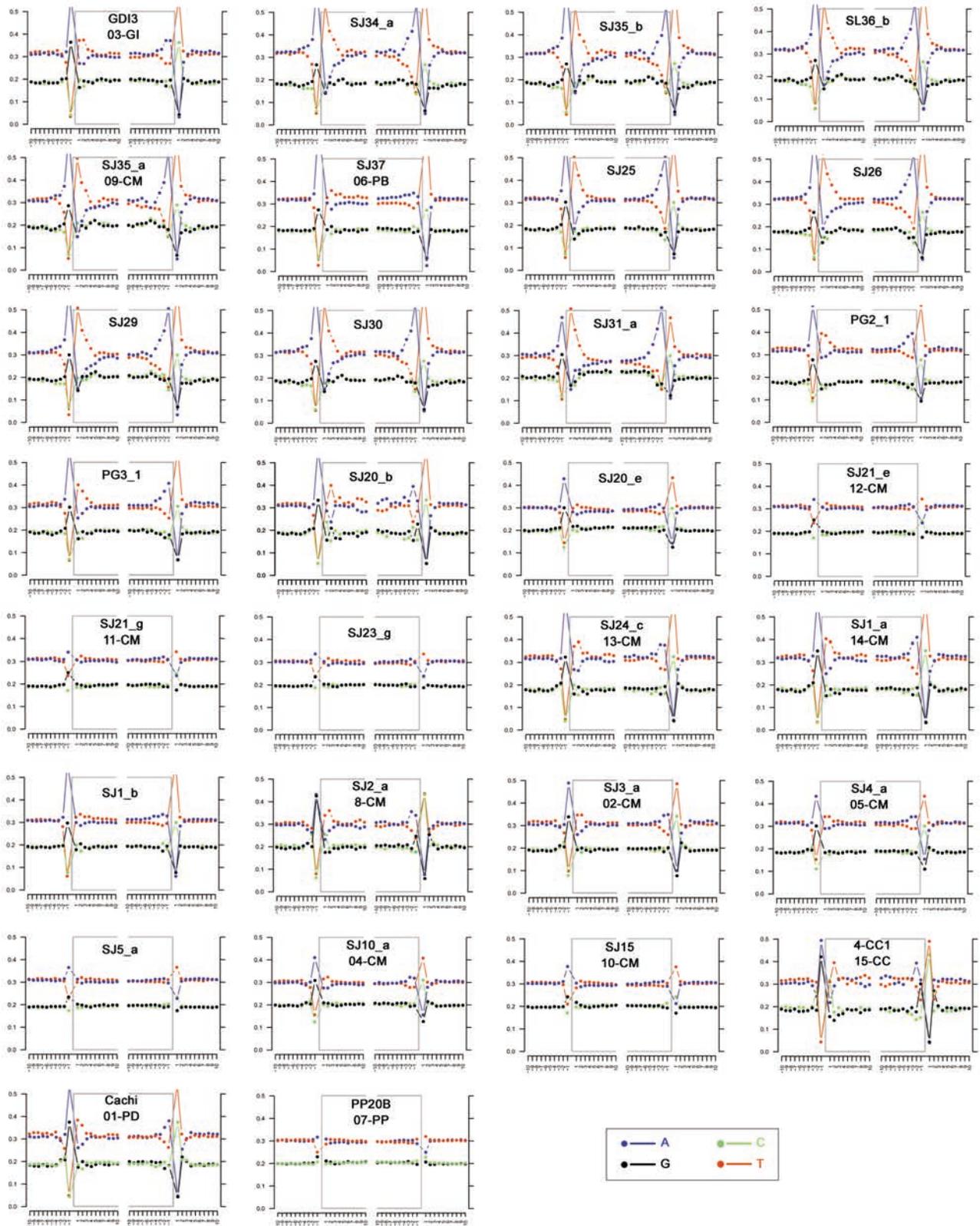
584 An evaluation of the DNA preservation (percentage of endogenous DNA and deamination pattern) in the  
585 ancient bean seeds as a function of sample age and site location is reported in Figure S6. Altitude, and not  
586 age, seems to be a good predictor of deamination patterns.  
587

588 Almost all of the following analyses including both ancient and modern (see section “Modern accessions  
589 genomic data) samples were based on transversions, since transitions are more affected by post-mortem  
590 damages than transversion and could reflect DNA damage rather than true DNA variation (e.g., Hofreiter et  
591 al 2012). Only the selection test (Supplementary Information S11) considered both transitions and  
592 transversions because in this test only changes observed in all the 15 ancient seeds are used. We estimate  
593 that these simultaneous changes are very unlikely related to post-mortem damages. The following regions  
594 were analyzed separately for the Admixture and Neighbor-Joining analyses (see below): *i) callable* regions,  
595 defined as the whole genome excluding repeated regions; *ii) neutral* regions defined as callable regions  
596 excluding genes (both exons and introns) and 10kb upstream and downstream each gene and *iii) exons*. The  
597 estimation of Wattersons Theta ( $\theta_w$ ) and the FineStructure analysis were performed on *callable* regions only  
598 (see below).  
599



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**Figure S3.** Fragment length (bp) distributions for the 30 ancient seeds. Labels present the original sample ID and the manuscript ID (see Table S1). Plots were generated using MapDamage 2.0.



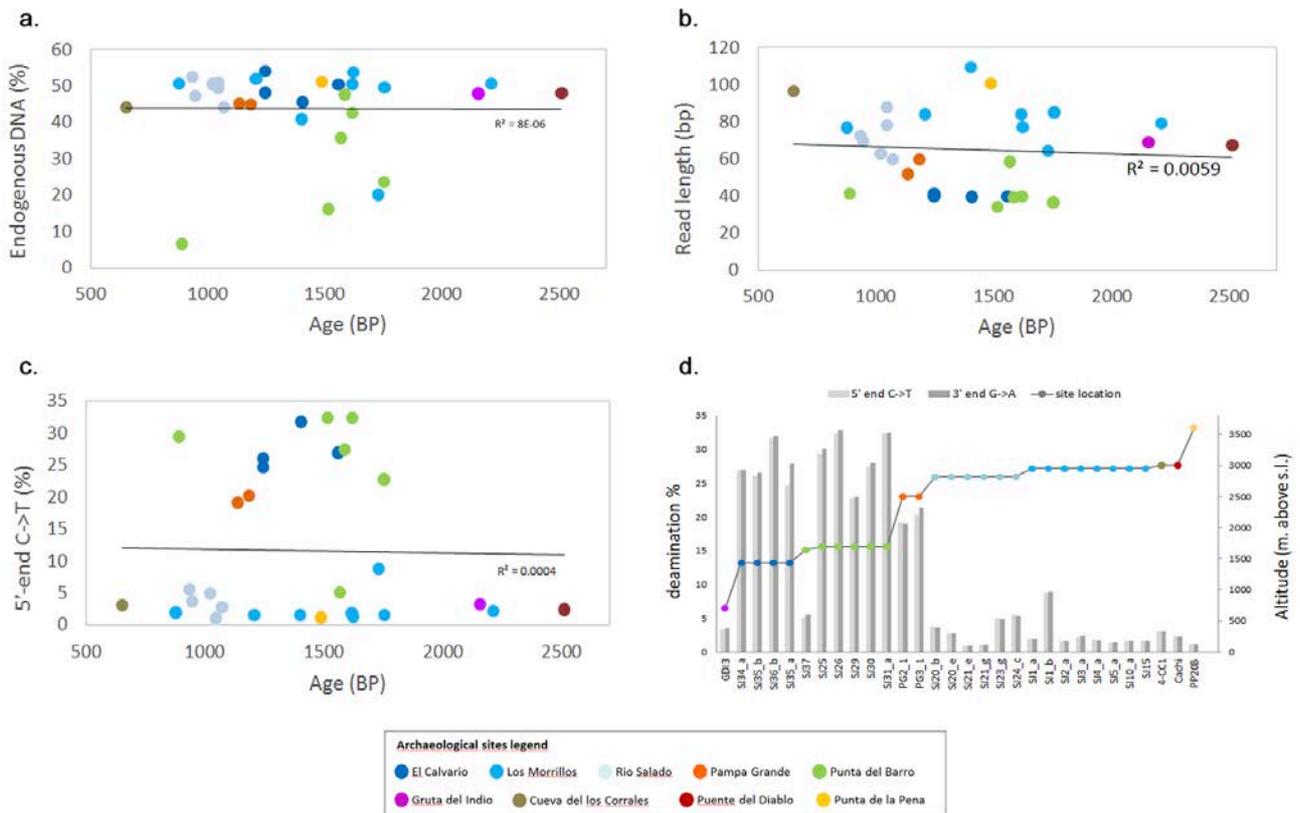
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**Figure S4.** aDNA fragmentation plots for ancient bean seeds. Labels present the original sample ID and the manuscript ID (see Table S1). Plots were generated using MapDamage 2.



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**Figure S5.** Frequency of C to T (red) and G to A (blue) misincorporation at 5' and 3' ends for the 30 ancient seeds. Plots were generated using MapDamage 2.0. Labels present the original sample ID and the manuscript ID (see Table S1).



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**Figure S6.** Endogenous DNA preservation in the 30 ancient bean seeds of the preliminary screening. a) Proportion of reads mapping to the common bean reference genome vs. age of the samples; b) Average read length vs. age of the samples; c) Misincorporation pattern at 5' end vs. age of the samples; d) Misincorporation pattern at 5' and 3' ends vs. site locations.

The fragments lengths and the fragmentation and deamination patterns of our data concur with what could be expected from these kinds of samples and we are confident about the authenticity of our ancient genomes. To further support the absence of contamination in our ancient DNA data, we estimated the heterozygosity level in the chloroplast calls. Contamination can introduce additional chloroplast haplotypes in the original library resulting in the presence of heterozygous sites. We performed a SNP calling analysis in chloroplast reads extracted from 14 ancient seeds (one seed was excluded because of low chloroplast coverage). The complete chloroplast sequence from the Negro Jamapa cultivar (accession: NC\_009259, Guo et al, 2007) was added to the nuclear reference, and the bioinformatic approach described above was used to align the sequencing data from ancient samples to this new reference genome. Each resulting bam file was processed using ANGSD v0.916 (Korneliussen et al 2014) and the chloroplast consensus sequences were called for each seed setting the “-doFasta 2” option. The analysis was restricted to reads having a mapping quality score higher than 20 and to nucleotides showing a minimum base quality score of 20. The mean coverage ranges from 69X to 633X, and approximately 40% of the chloroplast genome was recovered in each sample (Table S2). This fraction is expected considering that several short chloroplast fragments (as those obtained from our samples), have multiple mapping position on the nuclear genome and were therefore excluded from further analyses. Each bam file was independently analysed with the mpileup/call commands from BCFtools, calling both monomorphic and polymorphic sites of the chloroplast, setting the ploidy of the region to 2. The following flags were added: a minimum base quality score of 20 (-Q 20), a minimum mapping quality score of 30 (-q 30) and the mapping quality of reads with two mismatches compared to the reference was adjusted (-C 50). Indels were not called. The resulting vcf files were filtered and only sites having a QUAL field > 30 were retained. The number of called heterozygous seeds was very small: 0 in 11 seeds, 1 in two seeds (13-CM and 03-GI), and 2 in one seed (02-CM). The total number of high quality/mapping base pairs used varied in different seeds between 45448 and 61699. The three ancient seeds with one or two heterozygous sites in the chloroplast genome do not show any significant pattern of increased nuclear heterozygosity when compared to other seeds.

644 Table S2. Chloroplast genomes coverage.

645

MANUSCRIPT ID	MEAN COVERAGE	FRACTION OF CALLED (NON-MISSING) BASE PAIRS
15-CC	167.7	0.42
01-PD	633.1	0.40
03-GI	151.4	0.37
07-PP	475.1	0.43
04_CM	290.6	0.38
10-CM	153.7	0.37
14-CM	102.4	0.35
12-CM	511.9	0.41
11-CM	752.4	0.43
13-CM	369.3	0.42
08-CM	450.3	0.41
06-PB	79.5	0.38
02-CM	627.7	0.42
05-CM	336.8	0.43

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650 **S6. Modern accessions genomic data**

651

652 Genomic data from modern domesticated and wild common bean samples were included in our analyses  
653 (Table S3). In particular, we included: *i*) 12 modern domestic common bean genomes from both Andean and  
654 Mesoamerican domestication gene pools, sequenced by the ERA-CAPS funded project “BeanAdapt: The  
655 Genomics of Adaptation during Crop Expansion in Bean” (unpublished; provided by the coordinator of the  
656 project, Prof. Roberto Papa, Marche Polytechnic University); *ii*) two published genomes from wild *P. vulgaris*  
657 (G19901 from Argentina and G24594 from Chiapas, Mexico; Rendón-Anaya et al 2017); *iii*) one published  
658 genome of the wild relative *P. hintonii* to be used as outgroup in our selection scan (Rendón-Anaya et al  
659 2017). Trimmomatic v0.36 (Bolger et al 2014) was used to remove poor-quality regions from downloaded  
660 Illumina reads, discarding nucleotides having an average base quality score less than 20 across a 5bp  
661 window. Reads longer than 35bp were mapped to the *P. vulgaris* reference genome v2.1 in the paired-end  
662 mode using bwa mem (Li and Durbin 2009) with the parameter “-M”. The quality of bam alignments was  
663 improved removing PCR duplicates with Picard (software.broadinstitute.org) and performing the Indel  
664 realignment procedure, as described above for ancient samples. Depth of coverage was estimated in each  
665 sample using Samtools depth. Two publicly available but unpublished genomes from Argentinean wild  
666 seeds, G16798 and G18705 in the SRA archive and obtained from 2x150 bp libraries sequenced on an  
667 Illumina Hiseq 3000 platform, were used to confirm that the global pattern of variation of ancient seeds is  
668 compatible with the pattern of variation observed in modern seeds from the same geographic area. The  
669 mean coverage of these genomes was estimated at 9.9X and 8.8X, respectively.

670

671 **Table S3.** Modern genomes included in our analyses. Gene pool indicates to which gene pool the sample belongs; AD: Andean  
672 domesticated, MD: Mesoamerican domesticated, AW: Andean wild, MW: Mesoamerican wild. Sequencing mode was 2x125 bp for all  
673 Bean Adapt samples and 2x100 for the other samples.

674

Sample ID	Manuscript ID	Species	Wild/ Domestic	Gene pool	Country/Sampling site	Mean coverage (genome / callable regions)	Source
ECa005	CHI1	<i>P. vulgaris</i>	D	AD	Chile	11.6 / 13.0	Bean Adapt Project
ECa006	BOL1	<i>P. vulgaris</i>	D	AD	Bolivia	24.9 / 27.7	Bean Adapt Project
ECa016	CHI2	<i>P. vulgaris</i>	D	AD	Chile	9.1 / 9.8	Bean Adapt Project
ECa038	PER1	<i>P. vulgaris</i>	D	AD	Perú	8.9 / 9.9	Bean Adapt Project
ECa040	ARG1	<i>P. vulgaris</i>	D	AD	Argentina	16.6 / 19.5	Bean Adapt Project
ECa041	ARG2	<i>P. vulgaris</i>	D	AD	Argentina	6.1 / 6.7	Bean Adapt Project

ECa086	MEX1	<i>P. vulgaris</i>	D	MD	México	10.5 / 11.6	Bean Adapt Project
ECa095	MEX2	<i>P. vulgaris</i>	D	MD	México	6.2 / 6.7	Bean Adapt Project
ECa104	MEX3	<i>P. vulgaris</i>	D	MD	México	10.1 / 11.2	Bean Adapt Project
ECa332	ARG3	<i>P. vulgaris</i>	D	AD	Argentina	5.3 / 5.8	Bean Adapt Project
ECa330	ARG4	<i>P. vulgaris</i>	D	AD	Argentina	13.9 / 13.9	Bean Adapt Project
ECa333	BOL2	<i>P. vulgaris</i>	D	AD	Bolivia	7.9 / 8.9	Bean Adapt Project
G24594	MEX-W	<i>P. vulgaris</i>	W	MW	México	19.6 / 22.5	Rendón-Anaya et al 2017
G19901	ARG-W	<i>P. vulgaris</i>	W	AW	Argentina	16.6 / 15.5	Rendón-Anaya et al 2017
<i>P. hintonii</i>	-	<i>P. hintonii</i>	W	-	México	9.5 / 10.6	Rendón-Anaya et al 2017

675

676

677 **S7. Analysis of genomic diversity**

678

679 To test whether there has been a decrease in genetic diversity due to the history of domestication, we  
680 compared levels of diversity between ancient and modern common bean seeds both as individual- and as  
681 population-level diversity. Genetic diversity was measured (using only transversions) estimating Watterson's  
682 Theta ( $\theta_w$ ) in non-overlapping windows of 10kb, taking into account per-individual inbreeding coefficients  
683 and hence accounting for possible deviations from Hardy-Weinberg equilibrium, as proposed by Vieira et al  
684 2013. This is a two-step procedure: first, ANGSD v0.916 (Korneliussen et al 2014) was used to call a high  
685 confidence SNP set based on GATK genotype likelihoods (-GL 2) including only alignment positions having an  
686 associated base and mapping quality score equal or higher than 20 and 30, respectively. To increase the  
687 reliability of the called set, we set the baq computation (-baq 1), adjusted the mapping quality in case of  
688 excessive mismatches (-C 50), and only retained positions having a high probability to be polymorphic (-  
689 SNP\_pvalue 1e-6), without any missing data. From this set, we randomly sampled 2,000 SNPs without  
690 replacement and we used ANGSD to recompute the genotype likelihoods at these positions (-doGlf 3)  
691 applying the same quality filters. The program ngsF (Vieira et al 2013) was then used to estimate the  
692 individual genome-wide inbreeding coefficient for each accession using default parameters, except for the -  
693 min\_epsilon option that was decreased to  $10^{-9}$ . The SNP sampling and the estimation of inbreeding  
694 processes were repeated five times in order to obtain five independent estimates of the inbreeding  
695 coefficient for each individual. The average across replicates was then used as point estimate of each  
696 individual inbreeding coefficient.

697

698 We then estimated a site frequency spectrum, using the command -doSaf 2 in ANGSD, to calculate the per-  
699 site posterior probabilities of the site allele frequencies based on individual genotype likelihoods accounting  
700 for the previously estimated inbreeding coefficients. Genotype likelihoods were calculated with the GATK  
701 method (-GL 2 option), and a folded spectrum was used (*i.e.*, unknown ancestral state, option -fold 1).  
702 Options -minQ 20 -minMapQ 30 -setMaxDepth 50 were applied to filter low quality bases and positions  
703 showing excessive coverage, while the option -sites was set to filter for *callable* regions. The option -noTrans  
704 1 was applied to all the above steps to systematically remove transitions from the analysis. The output of  
705 this command line was then used as input for the calculation of per-site thetas, using the command -  
706 doThetas 1. Window-based theta estimates were calculated using the command thetaStat do\_stat.  
707 Windows having a proportion of usable sites less than 50% were discarded.

708

709 **Table S4.** Estimates of diversity in ancient and modern accession of common bean from Central (Meso) and South (Andean) America.  
710 The number of 10kb-windows with at least 50% of the region covered is reported.

711

Manuscript ID	Gene pool	Country	10kb-windows	$\theta_w$ mean	$\theta_w$ median
CHI1	Andean	Chile	24486	1.29E-05	9.62E-08
BOL1	Andean	Bolivia	24686	1.56E-05	0
CHI2	Andean	Chile	24493	1.18E-05	1.73E-07
PER1	Andean	Perú	24597	9.62E-06	8.87E-08
ARG1	Andean	Argentina	24636	1.51E-05	1.18E-08

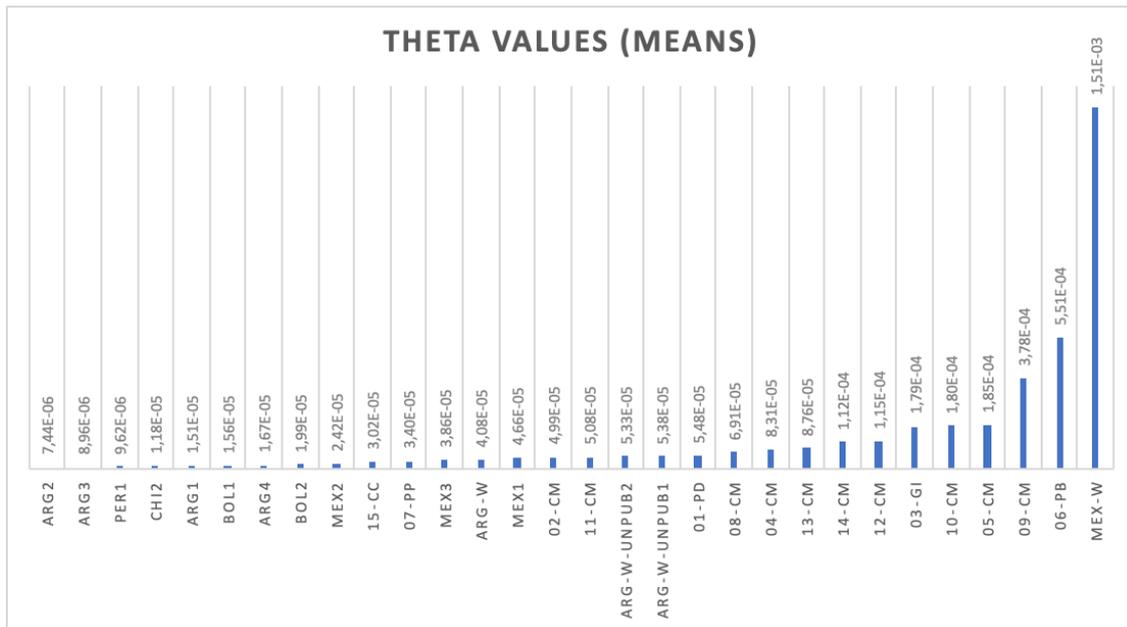
ARG2	Andean	Argentina	23827	7.44E-06	2.98E-07
MEX1	Meso	México	21749	4.66E-05	9.05E-07
MEX2	Meso	México	20071	2.42E-05	1.06E-06
MEX3	Meso	México	20965	3.86E-05	5.76E-07
ARG3	Andean	Argentina	23674	8.96E-06	1.40E-06
ARG4	Andean	Argentina	24272	1.67E-05	2.35E-06
BOL2	Andean	Bolivia	24491	1.99E-05	4.43E-07
15-CC	Andean	Argentina	15865	3.02E-05	1.72E-05
09-CM	Andean	Argentina	13284	3.78E-04	3.44E-04
01-PD	Andean	Argentina	20897	5.48E-05	5.41E-07
03-GI	Andean	Argentina	15241	1.79E-04	1.50E-04
07-PP	Andean	Argentina	15303	3.40E-05	1.03E-05
14-CM	Andean	Argentina	9372	1.12E-04	9.15E-05
04-CM	Andean	Argentina	9892	8.31E-05	6.10E-05
10-CM	Andean	Argentina	8422	1.80E-04	1.50E-04
08-CM	Andean	Argentina	13130	6.91E-05	4.27E-05
12-CM	Andean	Argentina	16859	1.15E-04	9.23E-05
11-CM	Andean	Argentina	18346	5.08E-05	1.25E-05
13-CM	Andean	Argentina	22600	8.76E-05	3.10E-05
02-CM	Andean	Argentina	21224	4.99E-05	8.47E-06
06-PB	Andean	Argentina	19426	5.51E-04	5.17E-04
05-CM	Andean	Argentina	17519	1.85E-04	1.58E-04
ARG-W	Andean wild	Argentina	24255	4.08E-05	1.93E-06
MEX-W	Meso wild	México	22583	1.51E-03	9.25E-04
G16798 (unpublished)	Andean wild	Argentina	23982	5,38E-05	1,91E-05
G18705 (unpublished)	Andean wild	Argentina	23960	5,33E-05	2,00E-05

712

713 The genomic variation in ancient beans is compatible with the variation observed in modern wild Argentinean  
714 seeds (see also Figure 1b in the main text), larger than that observed in modern domestic seeds with Andean  
715 gene pool, and smaller than that found in the Meso-American wild seed (Figure S7).

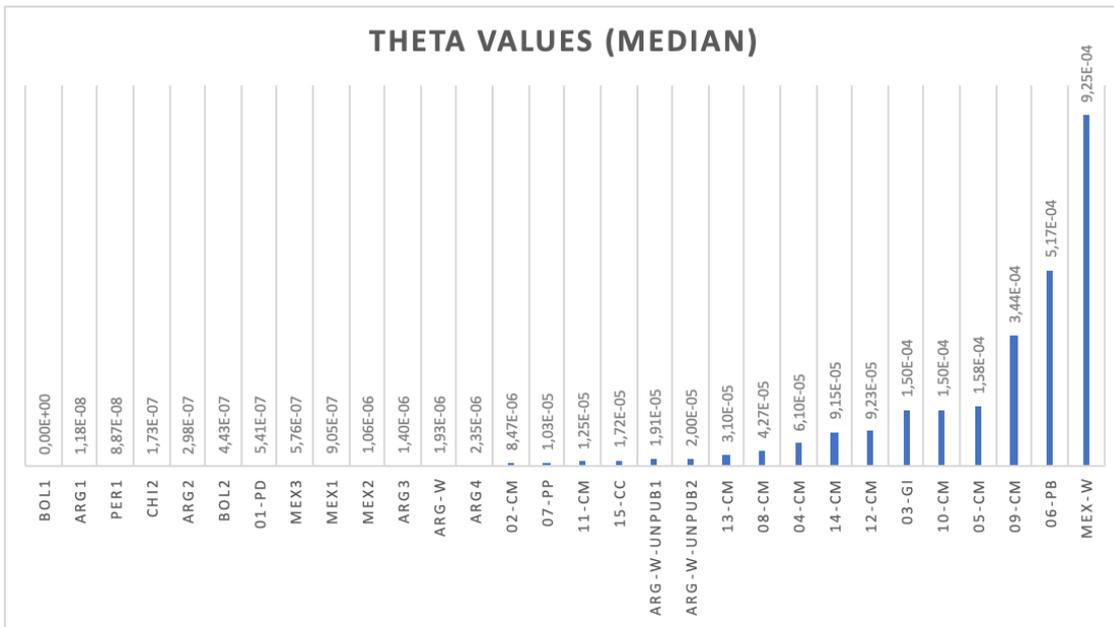
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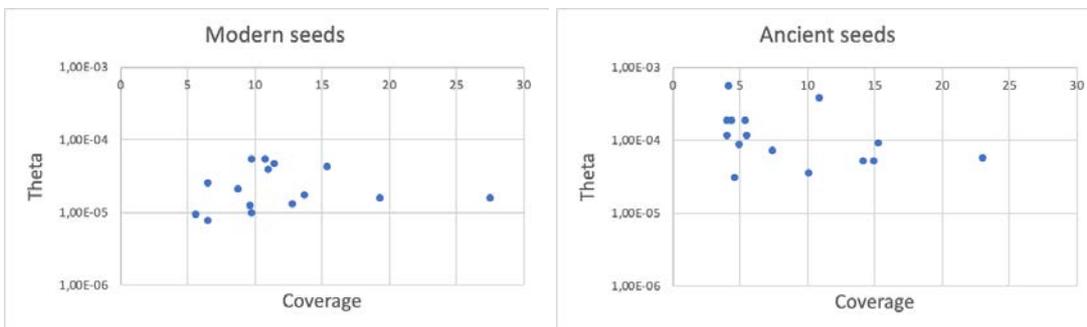
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Figure S7. Mean (upper panel) and median (lower panel) values of Theta estimates for modern and ancient seeds, in ascending order. Seed codes as in Table S4.

The impact of coverage (in callable regions) on heterozygosity estimates cannot be excluded. However, we note that a) the range of coverage in these regions is very similar in the ancient (4.2X – 23.2X; average: 9.0X) as compared to modern (5.8X – 27.7X, average: 12.7X) genomes; b) in the modern genomes data set, excluding the wild Mexican sample (typed at high coverage, and where genomic variation is expected to be much higher than the Andean samples for biological reasons), the increase of coverage from the smaller value (which is >5) does not seem to affect genetic variation in different cultivars (Figure S8, left panel); b) in the ancient genomes data set, 6 seeds with coverage >10 have, on average, a heterozygosity that is one third smaller than the remaining seed at coverage <10 (Figure S8, right panel). There might be therefore a small bias, probably due to the overestimation of theta by the probabilistic ANGSD approach when the coverage is low. Nevertheless, using only the 6 ancient seeds at coverage >10, or only the 10 ancient seeds at coverage >5, the average theta results equal to 1.10E-4 and 1.09E-4, respectively, which is only 23% smaller than the global (15 seeds) average reported in the main text. Most importantly, this value of theta does not change the main pattern identified in our study: 1.1E-4 (or 1.09E-4) is still 8.5 times larger than the average variation in the modern Andean cultivars (1.3E-5), and these differences are highly significant (Mann-Whitney, U<0.001).



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Figure S8. Plots of Theta estimated in modern (left panel) and ancient (right panel) seeds, as a function of the coverage in callable regions.

**S8. Re-calibrating estimates of modern genomic diversity**

748 Genomic diversity of each modern accession was likely reduced by the protocols commonly used before  
 749 whole-genome sequencing, which included a selfing protocol after collecting the seeds from the seed bank.  
 750 In addition, previous maintenance practices applied at each seed bank could have also affected genome-  
 751 wide diversity. To test whether such very recent events could be the cause of the observed pattern of lower  
 752 diversity in modern seeds as compared to ancient seeds, we re-estimated whole-genome diversity in pairs  
 753 of seeds from closely related cultivars. The rationale behind this test is the following. Diversity loss caused  
 754 by recent seed bank or sequencing protocols occurred at random along the genome in each cultivar, so that  
 755 each cultivar should have lost a different fraction of its diversity. Taking together two (or more) closely  
 756 related cultivars should restore most of the variation prior to the stocking into the seed banks. Of course,  
 757 this estimate is also affected by the possible genetic divergence between the cultivars used in each pair.

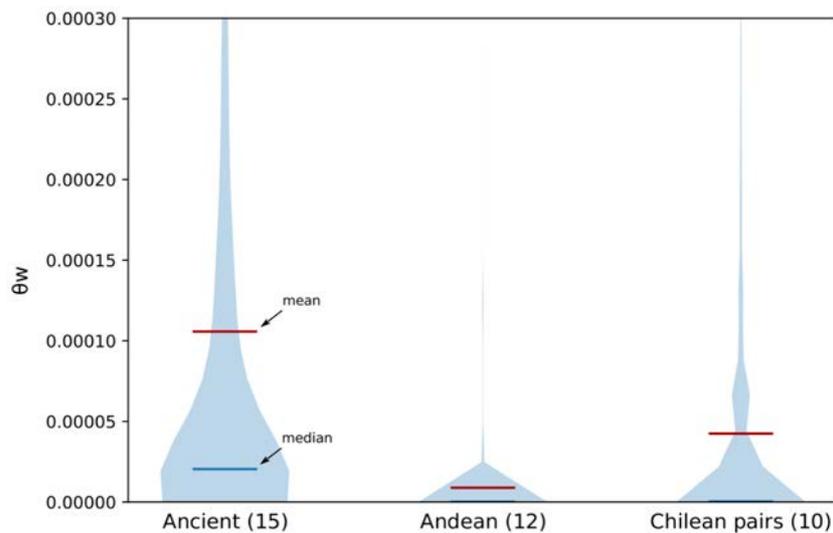
758  
 759 Estimates of diversity ( $\theta_w$ ) in pairs of cultivars should better represent the single cultivar variation before  
 760 seed bank and sequencing protocols (or even over-estimate, due to cultivar genetic divergence). We  
 761 selected the two modern Chilean cultivars, already included in all our analyses (see Table S3) and added  
 762 three cultivars from the same country sequenced within the ERA-CAPS funded project “BeanAdapt: The  
 763 Genomics of Adaptation during Crop Expansion in Bean” (unpublished; provided by the coordinator of the  
 764 project, Prof. Roberto Papa, Marche Polytechnic University). We estimated  $\theta_w$  in ten random pairs of seeds.  
 765 Specifically, we compared average  $\theta_w$  estimated in three groups of accessions: 10 pairs of Chilean  
 766 accessions, 12 single modern accessions from South America, and in 15 ancient seeds. Alongside individual  
 767 variation, we also compared the distribution of  $\theta_w$  across all 10kb-windows from each group.

768  
 769 As expected, genome wide diversity in pairs of seeds is higher than in single seeds (Table S5). However,  
 770 average modern seed pairs diversity is still more than three times lower (Mann-Whitney U test,  $P = 0.003$ )  
 771 than that estimated in ancient seeds (Table S4). The same pattern appears when considering the  
 772 summarized diversity across all 10kb-windows in each group (Figure S5).

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 776 **Table S5.** Estimates of diversity in five accessions from Chile (two of them were also used in the main analyses) and in the ten pairs of  
 777 Chilean samples. The number of 10kb-windows with at least 50% of the region covered is reported.

Manuscript ID	10kb-windows	$\theta_w$ mean	$\theta_w$ median
CHI1 (ECa005)	24486	1.29E-05	0.96E-07
CHI2 (ECa016)	24493	1.18E-05	1.73E-07
CHI3 (ECa004)	24524	1.37E-05	0.62E-07
CHI4 (ECa018)	24609	1.57E-05	0.33E-07
CHI5 (ECa020)	24541	1.47E-05	0.94E-07
CHI3.CHI1	27870	4.33E-05	2.89E-07
CHI3.CHI2	27875	4.24E-05	4.43E-07
CHI3.CHI4	27912	3.96E-05	1.95E-07
CHI3.CHI5	27891	4.63E-05	3.46E-07
CHI1.CHI2	27856	4.47E-05	4.89E-07
CHI1.CHI4	27903	5.32E-05	3.14E-07
CHI1.CHI5	27889	4.32E-05	3.69E-07
CHI2.CHI4	27916	4.57E-05	4.07E-07
CHI2.CHI5	27888	3.59E-05	4.49E-07
CHI4.CHI5	27930	5.61E-05	4.56E-07

779  
 780



781

782 **Figure S9.** Estimates of whole-genome diversity in 15 ancient seeds (Ancient), in 12 modern accession from South America (Andean),  
 783 and in 10 pairs of cultivars from the same region (Chile). Distribution of  $\theta_w$  are based on all 10kb-windows across all samples in each  
 784 group.

785

786 In order to exclude major effects of selfing before sequencing, we also applied a conservative procedure and  
 787 increased the heterozygosity values of modern seeds as a function of the number of selfing cycles. We note  
 788 that our modern cultivar samples are a subsample of the cultivars sequenced during the ERA-CAPS project  
 789 “BeanAdapt” (see Data accessibility in the main text), used here for comparison. Notably, not all the modern  
 790 seeds followed in that project the same selfing protocol: some of them were sequenced directly after the  
 791 collection at the CIAT seed bank, where one cycle of selfing was performed, whereas the other seeds were  
 792 subjected to two cycles of selfing. More specifically, some modern Andean seeds in our sample (ARG3, ARG4,  
 793 and BOL2) were subjected to one cycle of selfing, and others (BOL1, CHI1, CHI2, PER1, ARG1, and ARG2) were  
 794 subjected to two cycles of selfing. Two additional Andean seeds sequenced for the BeanAdapt project (two  
 795 cultivars from Argentina) were selfed only once, and we included them in this analysis (ARG5 and ARG6).  
 796 Considering only modern seeds with Andean ancestry, we have therefore 15 ancient seeds (no selfing before  
 797 sequencing) and 11 modern seeds (5 and 6 with one and two cycles of selfing, respectively).  
 798

799

800 Four out of five modern seeds subjected to one cycle of selfing have a mean  $\theta_w$  higher than all the modern  
 801 seeds subjected to two cycles of selfing, and five out five modern seeds subjected to one cycle of selfing have  
 802 a median  $\theta_w$  higher than all the modern seeds subjected to two cycles of selfing. This is consistent with the  
 803 expectation that selfing is reducing heterozygosity as a function of selfing cycles.

804

805 The original  $\theta_w$  values for the modern seeds were then doubled or quadrupled (depending on the number of  
 806 selfing cycles) to conservatively (some level of outcrossing during the procedure cannot be excluded) account  
 807 for the reduction of variation before sequencing, allowing for a more robust comparison between ancient  
 808 and modern seeds. Considering the means, 10 out of 15 ancient seeds have a  $\theta_w$  value larger than the 11  
 809 modern seeds. This produces a significant difference between  $\theta_w$  values ( $p < 0.01$ , Mann-Whitney U test).  
 810 Considering the medians, 14 out of 15 ancient seeds have a  $\theta_w$  value larger than the 11 modern seeds. This  
 811 produces a significant difference between  $\theta_w$  values ( $p < 0.01$ , Mann-Whitney U test).  
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### 813 **S9. Structure of the genomic diversity in ancient beans**

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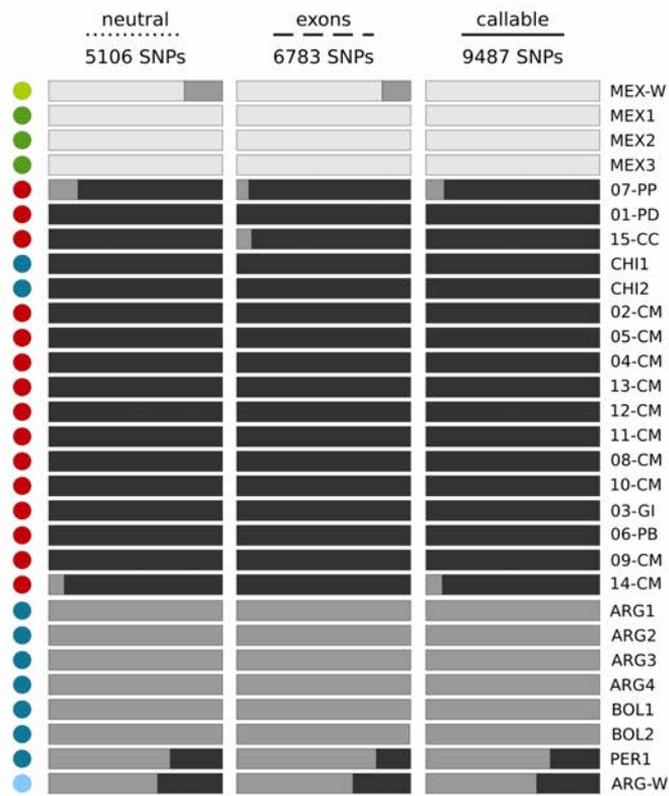
815 A haploid calling on all samples (15 ancients, 14 modern) was performed on each chromosome, separately,  
 816 using ANGSD 0.916 (Korneliussen et al 2014). We set -minQ 20 and -minMapQ 30 to exclude low quality  
 817 bases and low mapping quality bases; -only\_proper\_pairs 0 to use all reads (not only reads with both  
 818 mapping pairs); -dohaplocall 2 to select the most frequent base in the haploid calling (*i.e.*, not a random  
 819 one); -doCounts 1 to count the bases at each sites after filters; -minMinor 2 to exclude singletons and -

820 noTrans 1 to exclude transitions. The output of the haploid calling was then masked according to the three  
 821 genomic regions as defined above (*callable*, *neutral* and *exons*) using *Bedtools intersect* (Quinlan and Hall  
 822 2010). The fraction of missing calls (after haploid calling) and the total number on variable sites is reported  
 823 in Table S6. Higher values in ancient compared to modern genomes can be attributed to the shorted read  
 824 length.

825  
 826 TableS6. Proportion of missing calls after haploid calling in neutral, exons and callable regions. The total number of variable  
 827 positions across samples is indicated within brackets.  
 828

Manuscript ID	Neutral (500,580)	Exons (335,124)	Callable (3,517,565)
15-CC	0.29	0.09	0.29
09-CM	0.41	0.13	0.33
03-GI	0.30	0.10	0.30
10-CM	0.45	0.13	0.45
02-CM	0.17	0.07	0.15
05-CM	0.27	0.10	0.26
04-CM	0.42	0.11	0.41
14-CM	0.41	0.14	0.41
08-CM	0.33	0.09	0.34
01-PD	0.14	0.06	0.12
07-PP	0.29	0.07	0.30
06-PB	0.25	0.11	0.21
12-CM	0.29	0.09	0.27
11-CM	0.24	0.06	0.23
13-CM	0.13	0.07	0.11
CHI1	0.04	0.03	0.03
BOL1	0.03	0.02	0.02
CHI2	0.05	0.04	0.03
PER1	0.04	0.03	0.02
ARG1	0.04	0.03	0.02
ARG2	0.06	0.04	0.04
MEX1	0.09	0.04	0.05
MEX2	0.11	0.06	0.07
MEX3	0.09	0.03	0.05
ARG4	0.04	0.02	0.02
ARG3	0.07	0.06	0.05
BOL2	0.05	0.04	0.03
ARG-W	0.04	0.03	0.03
MEX-W	0.07	0.03	0.04

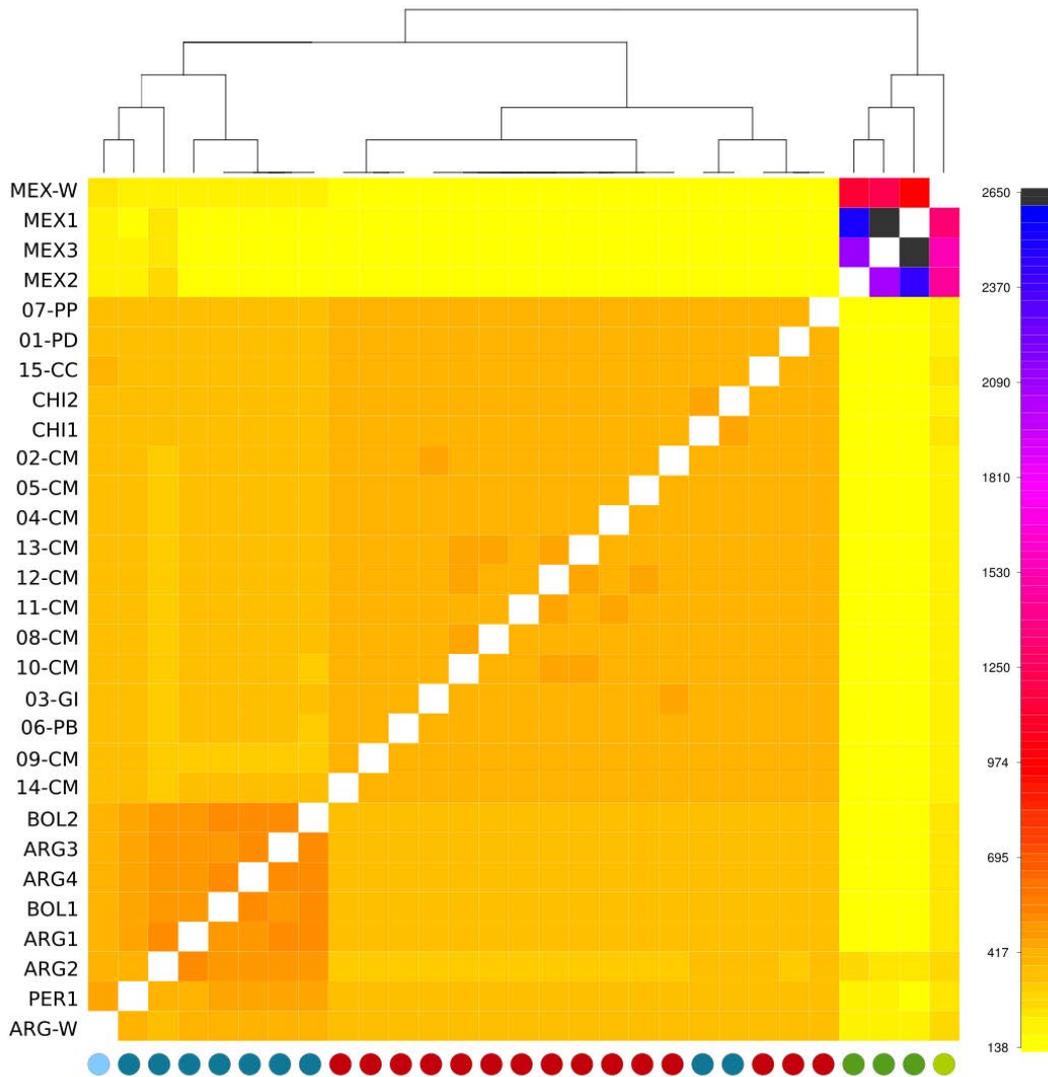
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 831 The main components of the genetic diversity and the eventual admixture proportion in all samples were  
 832 inferred by *Admixture* analyses (Alexander and Novembre 2009; Supplementary Figure S10). This analysis  
 833 was performed using the haploid data after combining all chromosomes in one file. As *Admixture* input is  
 834 binary *Plink* (.bed) or ordinary *Plink* (.ped), the haploid data was converted in tped format (*Plink* transposed  
 835 text genotype table), using the haploToPlink function implemented in *ANGSD*. Non biallelic sites were  
 836 excluded. This file was then converted to bed format, using `-make-bed` option in *plink-1.90* (Chang et al  
 837 2015). The option `-bp-space 50000` was added in order to select one SNP every 50kb (thinning) and  
 838 minimize the effect of linkage disequilibrium in the *Admixture* analysis. We tested *k* values from 1 to 15, and  
 839 cross validation error (Alexander and Lange 2011) was calculated for each *k* value, adding `--cv` flag to the  
 840 command line. Cross validation plots and admixture plots (for each *K* value) were created in *R*  
 841 (Supplementary Figure S10).  
 842



843  
 844 **Figure S10.** Structure of the genetic diversity and cross-validation error inferred using three different genomic regions (*callable*: solid  
 845 line, *exons*: large dashed line, *neutral*: small dashed line). For all genomic regions  $k = 3$  represented the best fitting model. Ancient  
 846 domesticated Andean (red), modern domesticated Andean (blue) and Mesoamerican (green), wild Andean (pale blue) and  
 847 Mesoamerican (pale green).

848  
 849 The output of the haploid calling masked according to the three genomic regions was also used to calculate  
 850 per chromosome pairwise genetic distances across all modern and ancient individuals using a custom *awk*  
 851 script (available at <https://github.com/anbena/ancient-beans>). Per-chromosome distances were then  
 852 combined into a single genome-wide distance matrix, which was used to reconstruct a Neighbor-Joining  
 853 tree using the *ape* package in *R* (Paradis et al 2004). Trees were visualized with FigTree  
 854 (<http://tree.bio.ed.ac.uk/software/figtree>). As in the Admixture analysis, the three genomic regions  
 855 produced almost identical results (the Neighbor-Joining tree based on callable region only is presented in  
 856 Figure 1d in the main text).

857  
 858 Population structure was also inferred using the method implemented in FineStructure (Lawson et al 2012;  
 859 Figure S11). The pattern of similarity and dissimilarity between haploidized individuals can be visualized by  
 860 a distance matrix, and discrete populations can be separated by a cladogram. This method is able to capture  
 861 at least as much information as that captured by other exploratory analyses such as PCA and Structure, but  
 862 it also has the advantage to help identifying the pattern of population structure and to combine the  
 863 information at linked markers. The latter advantage is of course reduced, or absent, in our haploidized data  
 864 set. We used Plink to filter the dataset in order to exclude any variable position with missing data (not  
 865 allowed in FineStructure) and the *plink2chromopainter.pl* conversion script to prepare the input file to run  
 866 the FineStructure pipeline. In the first step of the pipeline, haplotype blocks are “painted” (Li and Stephens  
 867 2003) according to the nearest (in terms of genetic distance) individual in the sample (chromosome painting  
 868 step). Painting results are then employed to build a distance matrix summarizing the blocks similarity  
 869 between all pairs of individuals. The distance matrix is, in turn, the basis of a Bayesian clustering approach  
 870 aiming at partitioning the sample into  $K$  homogeneous groups (Supplementary Figure S11).



871  
 872 **Figure S11.** Finestruure analysis based on callable genomic region (483885 SNPs). Ancient domesticated Andean (red), modern  
 873 domesticated Andean (blue) and Mesoamerican (green), wild Andean (pale blue) and Mesoamerican (pale green).  
 874

875 **S10. Loss and recovery of variation after domestication?**

876 Here we briefly support the view that the heterozygosity in the ancient seeds is comparable to that observed  
 877 in the wild seeds because of a smaller or negligible drift effect during the initial phases of domestication, and  
 878 not as a consequence of a strong bottleneck during domestication and subsequent recovery by mutation.  
 879 Let's assume an individual heterozygosity of 1/10000 in an ancient seed at 2500 BP (the order of magnitude  
 880 we observe). If a severe bottleneck occurred around the initial phases of domestication (say 8000 BP),  
 881 followed by expansion, the coalescence tree of two homologous bases in the ancient seed would likely  
 882 coalesce at 8000 BP, i.e, 5500 year earlier. Then, if we equate individual heterozygosity with the expected  
 883 difference between the two bases ( $10^{-4}$ ), the transversion rate per base pair per year should take a value of  
 884 almost  $10^{-8}$ /base/year ( $10^{-4}/(2 \times 5500) = 0.91 \times 10^{-8}$ ). This rate is more than one order of magnitude larger  
 885 than what can be estimated by, for example, comparing *P. vulgaris* with *P. hintonii* (separated 5MYA, Delgado-  
 886 Salinas et al., 2006), which we estimated at  $0.72 \times 10^{-9}$ /base/year using only transversions in a pairwise  
 887 comparison between *P. hintonii* and the Mexican reference genome for *P. vulgaris*). In other words, it seems  
 888 very unlikely that if a bottleneck occurred, a significant fraction of the variation was recovered after 5500  
 889 years, unless a much higher transversion rate than currently estimated is assumed.

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### **S11. Gene-focused scan for signature of ancient and/or recent selection**

A gene-by-gene selection scan was performed to identify genes targeted by artificial selection during early and/or late stages of domestication. A gene underlying a desirable trait is expected to have undergone one or more selective sweeps targeting specific alleles coupled with recurrent hitchhiking at linked neutral sites. Selected genes will then accumulate variants which are either directly advantageous or got fixed because of locally low effective population size due to linked selection (Slotte 2014, Renaut and Rieseberg 2015, Beissinger et al 2016). We then expect to find an excess of fixed differences at genes under selection when samples of pre (*i.e.*, wild) and post domestication populations are compared.

Leveraging our ancient data, we tested each of the 27,000 genes for a significant enrichment of fixed alternative alleles in two possible configurations. The first configuration is AS (Ancient Selection), indicated by the topology in the left panel in Figure 2A (main text), where all the ancient + modern Chilean samples have the same allele, and the wild samples (one wild Andean *P. vulgaris* and one *P. hintonii* as wild outgroup) have the alternative allele. We expect AS to be frequent within genes that experienced changes before 2,500 years ago. The second configuration is RS (Recent Selection), indicated by the topology in the right panel in Figure 2B (main text), where all modern Chilean samples have the same allele, and all the other samples (ancient + wild samples) have the alternative alleles. We expect RS to be frequent within genes that experienced changes in last 600 years, *i.e.*, more recently than the estimated age interval for the ancient seeds.

Clearly, the enrichment of AS or RS allelic configurations (corresponding to the different topologies based on fixed differences) depends on many factors beside the selection dynamic, such as the sample sizes and the drift intensity in different time intervals. We therefore used a randomization procedure that keeps constant the groups, their sample sizes, and the total number of SNPs, but randomly assigns the SNPs to the genes. The relative frequency of SNPs with fixed differences in AS and RS topologies, computed after this randomization, becomes our null hypothesis, which is used to test if the observed frequencies of such topologies in each gene are higher than expected by chance.

In particular, we applied the following protocol: 1) for 27,000 genomic region each identified by a gene and 10 kb before and after it, we counted the proportion of fixed differences in the AS and the RS configuration; 2) we randomly re-assigned each SNP found in the real dataset (including the modern seeds from Chile, the ancient seeds from Argentina, the wild seed and the outgroup) to the 27,000 regions; 3) we estimated from different random assignment runs the proportion of AS and RS configurations expected by chance in a "average" genomic region; 4) we used the proportion estimated in point 3 as the null hypothesis, and applied a binomial test to each proportion observed in the real genes (point 1) to compute a *p*-value of significant enrichment of that particular configuration; 5) we converted the *p*-values in *q*-values (False Discovery Rate, FDR) using the *python* function `qvalue.py` (<https://github.com/nfusi/qvalue/blob/master/qvalue/qvalue.py>) based on (Storey and Tibshirani, 2003); 6) only genomic regions with a  $FDR < 0.001$  were initially considered as genes significantly enriched in a particular topology (see Supplementary Figure S12); 7) finally, we applied an additional conservative criterion to filter each list of AS and RS enriched genes, and retained only those genes with numbers of fixed differences within the coding region and a 1kb region upstream and downstream the gene higher than a threshold defined by the 99-percentile of all the 27,000 genes.

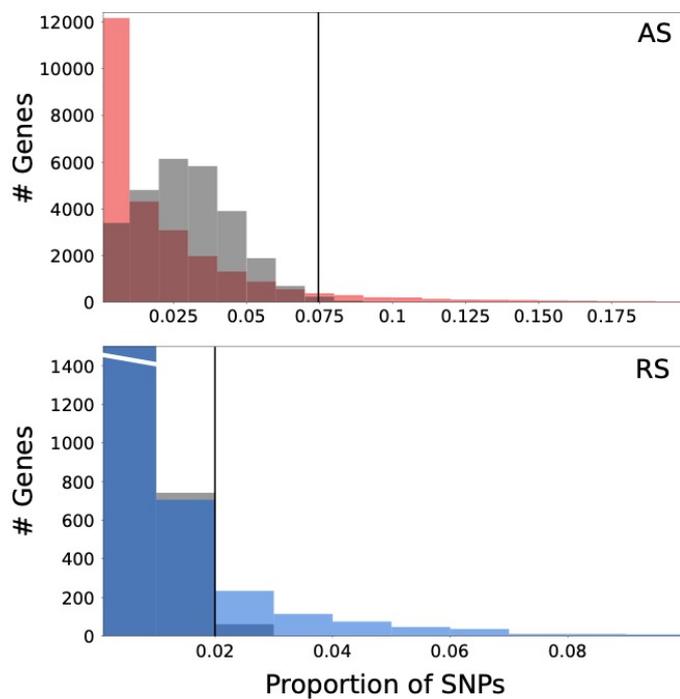
The procedure we applied is highly conservative, and we likely missed genes where the selection process may have modified the allelic compositions. It allows however to significantly reduce the rate of false positives, and to correctly estimate the relative proportion of genes with relevant modifications before and after the time interval defined by the ages of the seeds we analyzed, respectively.

947 We found 360 and 86 genes with a significant enrichment of SNPs in the AS and RS topology, respectively,  
948 with a AS/RS ratio = 4.2. We also estimated the ratio of ancient vs. modern sweeps considering the  
949 hypothesis that different genes on the same linkage block may produce a signal of selection without being  
950 effectively selected. Partially following the approach used by Schmutz et al (2014), we merged all AS or RS  
951 genes in our study if they were separated by less than 40kb, and we considered these extended regions as  
952 anciently selected regions (ASR, i.e., ancient sweeps) or recently selected regions (RSR, i.e., recent sweeps).  
953 The number of ASR and RSR is of course smaller than AS and RS, but the ratio between ancient and recent  
954 sweeps is very similar: 161 vs. 46 for ASR and RSR, respectively, with a ratio of 3.5. If the maximum distance  
955 allowed to merge genes was increased from 40kb to up to 150kp, the ratio ASR/RSR remains larger than 3.1.  
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957 Considering the ancient seeds as a single population, we estimated Tajima's D (Tajima, 1989) along the  
958 genome and compared it with the position of ancient selection signals (AS) identified by our previous test.  
959 Using the same pipeline applied in section S7 to estimate genomic diversity in groups of seeds (i.e.,  
960 command `thetaStat do_stat` in ANGSD 0.916), we computed Tajima's D in non-overlapping 10kb genomic  
961 windows. This is a one-sample test (i.e., it is not based on the divergence among groups), and negative  
962 values from this test can be therefore considered as additional evidence of selection which is independent  
963 from the results of the ad hoc test we developed. Using a threshold of -1.25 to identify Tajima's D values  
964 supporting a sweep (this threshold was chosen so to have a comparable numbers of candidate  
965 windows/genes in both tests), we found that 66 out of 360 (18.3%) genes (including 50Kb before and after  
966 the gene) show both a signature of ancient selection in our test and a Tajima's D score smaller than -1.25.  
967 As a comparison, 73 out of 678 (10.8%) Andean domestication genes identified in Schmutz et al (2014)  
968 show also a Tajima's D score smaller than -1.25. When blocks around AS genes are defined including 50 kb  
969 upstream and downstream of the gene, and the same is done for the genes identified in Schmutz et al  
970 (2014), 25 blocks are shared among studies and also overlap with the windows with reduced Tajima's D  
971 (<1.25).

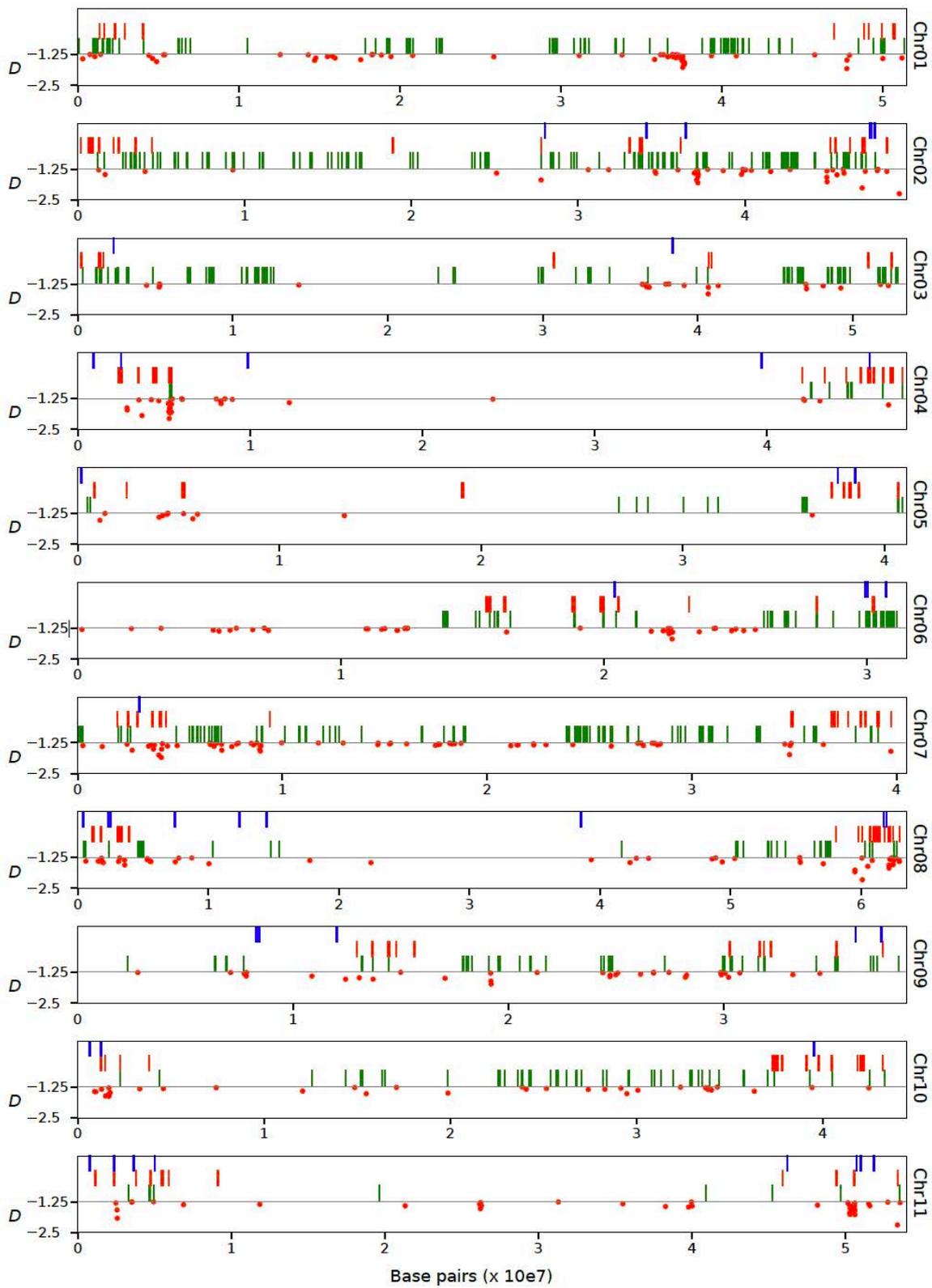
972 The chromosomal position of the selective sweeps that may result from domestication found in our data  
973 was visually compared in Supplementary Figure S13 with those inferred in Schmutz et al, (2014) and with  
974 the Tajima's D candidate windows. Several chromosomal regions (e.g, beginning of chromosome 1; between  
975 46 and 48 Mb in chromosome 2; between 36 and 38 Mb in chromosome 7; end and beginning of  
976 chromosome 8) show co-localization of the selection signals, pointing to regions that deserve specific  
977 attention in further studies. The ASR/RSR ratio considering only the blocks that overlap with the sweeps  
978 inferred in Schmutz et al, (2014) is 12.5 (25/2) and 5.9 (41/7) applying a strict overlap criterion or extending  
979 the blocks to 50kb upstream and downstream, respectively.

980 The two final lists of genes showing signature of AS and/or RS were grouped into functional clusters using  
981 the web-based software David (Huang et al 2009). For this analysis, we could only use genes with a known  
982 ortholog in *A. thaliana* (317 and 72 for the ancient and the recent selection targets, respectively, excluding  
983 multiple matches with the same *A. thaliana* orthologs). We used the functional enrichment score estimated  
984 by David only to rank the functional clusters found in the two lists of genes without excluding any cluster.  
985 Analyses were also replicated using the genome of a wild Mesoamerican *P. vulgaris* as outgroup instead of  
986 *P. hintonii* (Table S7), with 65% and 68% of the genes in the first set (with *P. hintonii* as outgroup) found as  
987 AS and RS in the second set (using Mesoamerican *P. vulgaris* as outgroup). In this case, the ratios AS/RS and  
988 ASR/RSR reduced to 2.3 and 2.1 respectively, still indicating that at least twice as many selection events  
989 occurred before the time period covered by the ancient seeds. The reduction of this ratio is almost entirely  
990 due to an increase of genes classified as RS (147) or RSR (75), whereas AS genes and ASR regions remain  
991 approximately constant (333 and 160, respectively). If RS (and RSR) topologies with the *P. hintonii* outgroup  
992 are likely related to real changes occurring in genes in recent centuries (because modern seeds are  
993 genetically differentiated from genes conserved in wild and ancient beans, which are still similar to the  
994 distantly related outgroup *P. hintonii*), RS (and RSR) topologies may increase when a wild Mesoamerican *P.*  
995 *vulgaris* is used as outgroup because of incomplete lineage sorting within the *P. vulgaris* species. When the  
996 wild Mesoamerican *P.vulgaris* is used as outgroup, the ASR/RSR ratio for the blocks that overlap with  
997 sweeps inferred in Schmutz et al, (2014) becomes 6.5 (26/4) and 4.1 (45/11) applying a strict overlap  
998 criterion or extending the blocks to 50kb upstream and downstream, respectively.  
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**Figure S12.** Observed (in color) and null (gray) distribution of proportion of SNPs per gene in AS (red, top panel) or RS (blue, bottom panel) topology used as summary statistics in the selection scan. The lowest fraction of AS or RS topologies in the selected genes (after using the FDR threshold of  $q < 0.001$ , and before the implementation of the additional conservative filter based on the absolute number of SNPs, see above) is indicated as a solid line in each panel.



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Figure S13. Candidate genes as selected during ancient (AS, red) and recent (RS, blue) domestication stages identified by our test, and genes suggested as candidates for selection during domestication in the Andes (Schmutz et al 2014, dark green). Genomic windows with a Tajima's D smaller than -1.25 are also shown (red dots)

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