Genomic and phenotypic evidence for an incomplete domestication of South American grain amaranth (*Amaranthus caudatus*)

Markus G. Stetter, Thomas Müller and Karl J. Schmid

Institute of Plant Breeding, Seed Science and Population Genetics, University of Hohenheim, Fruwirthstr. 21, 70599 Stuttgart / Germany

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Corresponding Author

Name:	Karl Schmid, Prof. Dr.
Address:	Institute of Plant Breeding, Seed Science and Population Genetics (350)
	University of Hohenheim
	Fruwirthstraße 21
	D-70599 Stuttgart / Germany
Tel:	+49 711 459 23487
Fax:	+49 711 459 24458
E-Mail address:	karl.schmid@uni-hohenheim.de

Running title: Incomplete domestication of *A. caudatus*

1 Abstract

2 The process of domestication leads to major morphological and genetic changes, which in
3 combination are known as domestication syndrome that differentiates crops from their wild an-
4 cestors. We characterized the genomic and phenotypic diversity of the South American grain
5 amaranth *Amaranthus caudatus*, which has been cultivated for thousands of years and is one
6 of the three native grain amaranths of South and Central America. Previously, several models
7 of domestication were proposed including a domestication from the close relatives and putative
8 ancestors *A. hybridus* or *A. quitensis*. To investigate the evolutionary relationship of *A. caudatus*
9 and its two close relatives, we genotyped 119 amaranth accessions of the three species from
10 the Andean region using genotyping-by-sequencing (GBS) and compared phenotypic variation
11 in two domestication-related traits, seed size and seed color. The analysis of 9,485 SNPs re-
12 vealed a strong genetic differentiation of cultivated *A. caudatus* from the relatives *A. hybridus*
13 and *A. quitensis*. The two relatives did not cluster according to the species assigment but
14 formed mixed groups according to their geographic origin in Ecuador and Peru, respectively.
15 *A. caudatus* had a higher genetic diversity than its close relatives and shared a high proportion
16 of polymorphisms with their wild relatives consistent with the absence of a strong bottleneck
17 or a high level of recent gene flow. Genome sizes and seed sizes were not significantly differ-
18 ent between *A. caudatus* and its relatives, although a genetically distinct group of *A. caudatus*
19 from Bolivia had significantly larger seeds. We conclude that despite a long history of human
20 cultivation and selection for white grain color, *A. caudatus* shows a weak genomic and pheno-
21 typic domestication syndrome and is an incompletely domesticated species because of weak
22 selection or high levels of gene flow from its sympatric close undomesticated relatives that
23 counteracted the fixation of key domestication traits.

Introduction

Research on the domestication of crop plants revealed that numerous traits can be affected by domestication, which results in so-called domestication syndromes. The type and extent of domestication syndromes depends on the life history and uses of crop plants (Meyer *et al*, 2012), although crops from distantly related plant families frequently show similar domestication phenotypes. For example, the 'classical' domestication syndrome, which includes larger seeds, loss of seed shattering, reduced branching, loss of seed dormancy and decreased photoperiod sensitivity, is observed in legumes and grasses (Abbo *et al*, 2014; Hake & Ross-Ibarra, 2015). Similar to phenotypic diversity, crops show variable genomic signatures of domestication because of differences in their biology and utilization by humans (Meyer *et al*, 2012). In particular, domestication affects the level and structure of genetic diversity in crops because selection and genetic drift contributed to strong genetic bottlenecks (Doebley *et al*, 2006; Olsen & Wendel, 2013; Sang & Li, 2013; Nabholz *et al*, 2014). The geographic expansion of domesticated crops provided the opportunity for gene flow with new crop wild relatives, which further contributed to genetic differentiation from wild ancestors. Such a diversity of phenotypic and genomic changes associated with domestication suggest that the classical model of a single domestication event in a short time span within a small geographic region may not apply to numerous crop plants like barley, apple and olive trees (Besnard & Rubio de Casas, 2015; Cornille *et al*, 2012; Poets *et al*, 2015). The motivation of the present study was to investigate both the phenotypic and genomic consequences of amaranth cultivation in the light of these concepts.

The genus *Amarantus* L. comprises between 50 and 75 species and is distributed worldwide (Sauer, 1967; Costea & DeMason, 2001). Four species are cultivated as grain amaranths or leaf vegetables (Sauer, 1967; Brenner *et al*, 2010). The grain amaranths *Amaranthus caudatus*, *Amaranthus cruentus* and *Amaranthus hypochondriacus* originated from South and Central America while *A. tricolor* is used as leafy vegetable in Africa. Amaranth is an ancient crop because archaeological evidence in Northern Argentina suggested that wild amaranth seeds were collected and used for human consumption during the initial mid-Holocene (8,000 - 7,000 BP; Arreguez *et al*, 2013). In the Aztec empire, amaranth was a highly valued crop and tributes were collected from the farmers that were nearly as high as for maize (Sauer, 1967). Currently,

53 amaranth is promoted as a healthy food because of its favorable composition of essential amino

54 acids and high micronutrient content.

55 The three grain amaranth species differ in their geographical distribution. *A. cruentus* and

56 *A. hypochondriacus* are most common in Central America, whereas *A. caudatus* is cultivated

57 mainly in South America. In the Andean region, *A. caudatus* grows in close proximity to the

58 two related (i.e., wild) species *A. hybridus* and *A. quitensis*, which are considered as potential

59 ancestors (Sauer, 1967). *A. hybridus* has the widest distribution range from Central to South

60 America while *A. quitensis* is restricted to the central part of South America. *A. quitensis* was

61 tolerated and possibly cultivated in Andean home gardens and used for coloring in historical

62 times.

63 The history of amaranth cultivation and extent of its domestication are still under discussion

64 (Figure 1). Sauer (1967) proposed two domestication scenarios based on the morphology and

65 geographic distribution of the different species. One scenario postulates three independent

66 domestication events from three different wild ancestors, and another scenario proposes the

67 domestication of *A. cruentus* from *A. hybridus* followed by a migration and intercrossing of *A.*

68 *cruentus* with *A. powellii* in Central America and an intercrossing of *A. cruentus* with *A. quiten-*

69 *sis* resulting in *A. caudatus* in South America. A third scenario was based on genetic markers

70 and suggested that all three cultivated amaranths evolved from *Amaranthus hybridus*, but at

71 multiple locations (Maughan *et al*, 2011). Most recently, Kietlinski *et al* (2014) proposed a

72 single domestication of *A. hybridus* in the Andes or in Mesoamerica and a subsequent spatial

73 separation of two lineages leading to *A. caudatus* and *A. hypochondriacus*, or two indepen-

74 dent domestication events of *A. hypochondriacus* and *A. caudatus* from a single *A. hybridus*

75 lineage in Central and South America (Figure 1C and D). A more recent analysis based on the

76 phylogeny of the whole Amaranthus genus supports independent domestication of the South

77 American *A. caudatus* and the two Central American grain amaranths from two different, geo-

78 graphically separated lineages of *A. hybridus* as shown in Figure 1D (Stetter & Schmid, 2016).

79 Despite its long history of cultivation and the self-pollinating breeding system, the domestication

80 syndrome of cultivated amaranth is remarkably indistinct because it still shows strong photope-

81 riod sensitivity and has very small shattering seeds (Sauer, 1967; Brenner *et al*, 2010). Other

82 crops like maize that were cultivated at a similar time period in the same region exhibit the
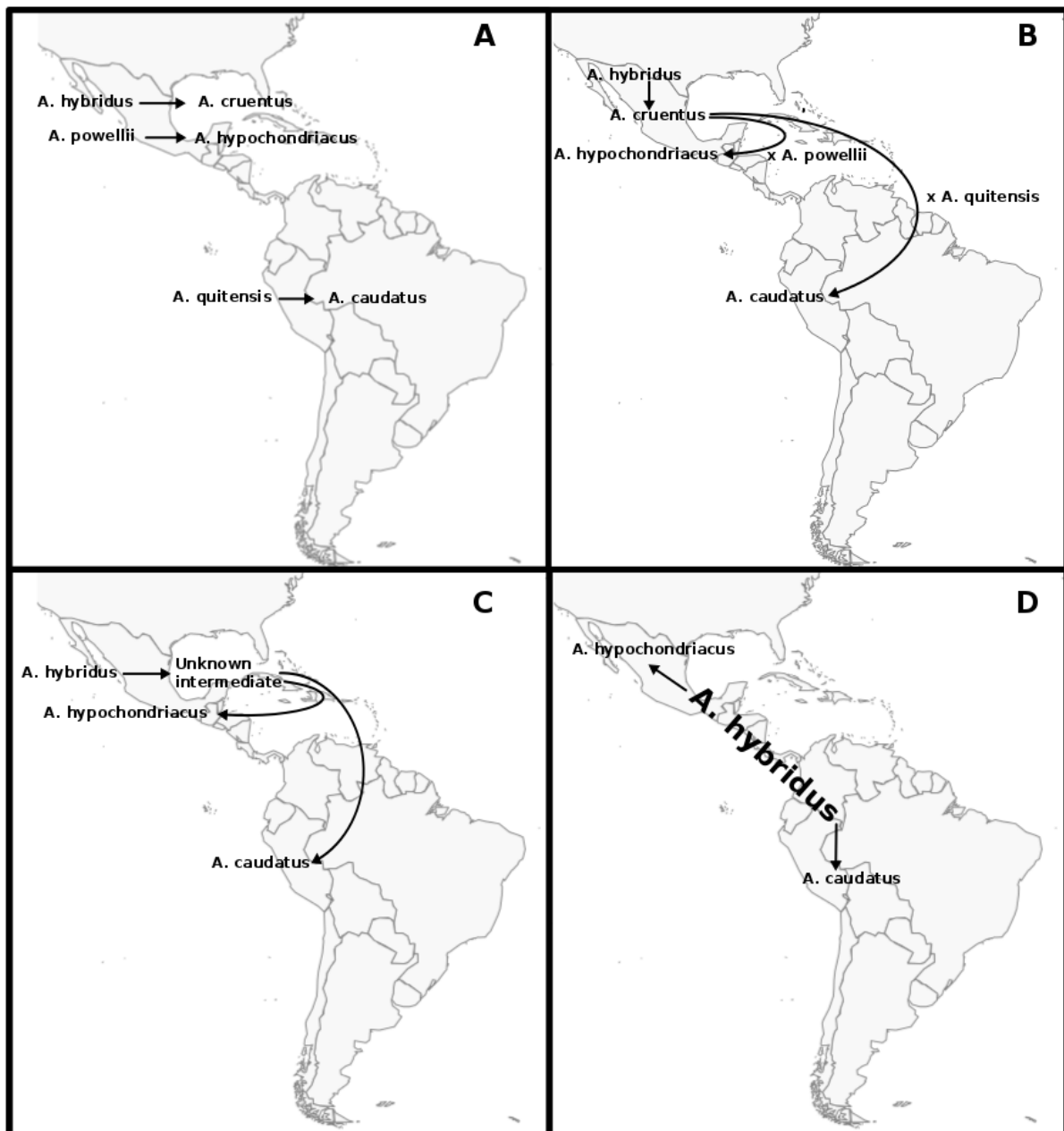
Figure 1: Models of amaranth domestication. (A) Independent domestication of three grain amaranths from different close relatives (Sauer, 1967). (B) Initial domestication from *A. hybridus* and subsequent migration and hybridization with additional close relatives (Sauer, 1967). (C) Single domestication in the Andes or in Mesoamerica and subsequent spatial separation of two lineages leading to *A. caudatus* and *A. hypochondriacus* (Kietlinski *et al*, 2014). (D) Two domestication events from a single *A. hybridus* lineage spanning Central and South America (Kietlinski *et al*, 2014).

83 classical domestication syndrome (Sang & Li, 2013; Lenser & Theißen, 2013). This raises the

84 question whether amaranth is domesticated at all or has a different domestication syndrome,

85 and if the latter is true whether genetic constraints, a lack of genetic variation or (agri-)cultural

⁸⁶ reasons determined its domestication syndrome. The phenotypic analysis of amaranth do-
⁸⁷ mestication is complicated by the taxonomic uncertainty of cultivated amaranth species and
⁸⁸ their close relatives. Although *A. quitensis* was suggested to be the ancestor of *A. caudatus*,
⁸⁹ the state of *A. quitensis* as a separate species is under debate. Sauer (1967) classified it as
⁹⁰ species, but later it was argued that it is the same species as *A. hybridus* (Coons, 1978; Bren-
⁹¹ ner *et al*, 2010). However, until today *A. quitensis* is treated as separate species and since
⁹² genetic evidence for the status of *A. quitensis* as a separate species is based on few studies
⁹³ with limited numbers of markers, this topic is still unresolved (Mallory *et al*, 2008; Kietlinski
⁹⁴ *et al*, 2014).

⁹⁵ The rapid development of sequencing technologies facilitates the large-scale investigation of
⁹⁶ the genetic history of crops and their close relatives. Among available methods, reduced repre-
⁹⁷ sentation sequencing approaches such as genotyping-by-sequencing (GBS) allow a genome-
⁹⁸ wide and cost-efficient marker detection compared to whole genome sequencing (Elshire *et al*,
⁹⁹ 2011; Poland *et al*, 2012). Despite some biases associated with reduced representation se-
¹⁰⁰ quencing, GBS and related methods are suitable and powerful approaches for studying inter-
¹⁰¹ specific phylogenetic relationships (Cruaud *et al*, 2014) and intraspecific patterns of genetic
¹⁰² variation in crop plants (Morris *et al*, 2013).

¹⁰³ We used GBS and genome size measurements to characterize the genetic diversity and rela-
¹⁰⁴ tionship of cultivated *A. caudatus* and its possible ancestors *A. quitensis* and *A. hybridus*, and
¹⁰⁵ compared patterns of genetic structure with two domestication-related phenotypic traits (seed
¹⁰⁶ color and hundred seed weight). For this study, we focussed on the South American amaranth
¹⁰⁷ species, because *A. caudatus*, *A. quitensis* and South American accessions of *A. hybridus* form
¹⁰⁸ a clade that is strongly separated from the two Central American grain amaranths in a phylo-
¹⁰⁹ genetic analysis of the whole genus (Stetter & Schmid, 2016). For this reason, we reasoned
¹¹⁰ that the domestication of *A. caudatus*, which is native to South America, and its relationship
¹¹¹ to the sympatric relatives, *A. hybridus* and *A. quitensis* can be conducted independently of the
¹¹² Central American amaranth species. Our analysis includes a comparison of genetic diversity
¹¹³ and seed-related traits like size and color between cultivated and wild amaranths and analyses
¹¹⁴ the taxonomic relationship and gene flow among species. Our results indicate that *A. caudatus*
¹¹⁵ has a history of domestication that may be considered as incomplete.

Material and Methods

Plant material

A total of 119 amaranth accessions of three *Amaranthus* species originating from South America were obtained from the USDA genebank (http://www.ars-grin.gov/npgs/searchgrin.html). Of these accessions, 89 were classified as *A. caudatus*, 17 as *A. hybridus*, seven as *A. quitensis* and six as interspecific hybrids according to the passport information (Table S5). We selected *A. caudatus* accessions based on the altitude of the collection site and focused on high-altitude regions (2,200 to 3,700 m) where amaranth has been cultivated for thousands of years and survived until today since it fell into disuse after the Spanish conquest (Kauffman & Weber, 1990). Therefore, high-altitude accessions may represent a large proportion of the species-wide genetic diversity. The smaller sample sizes of *A. hybridus* and *A. quitensis* accessions reflect that fewer accessions of these species than of *A. caudatus* are available from the USDA genebank. However, the geographic origin of the two wild relatives covers the Andean highlands, which is the distribution range of *A. caudatus*, and we compared the population structure of the sample derived from the genomic data with the passport information to test for consistency between the population structure and geographic origin. Accessions were planted in a field in Nürtingen (Germany), and one young leaf of one representative plant per accession was sampled to avoid the sampling of potential seed cross-contamination. We sampled and sequenced three plants each of 12 accessions independently for quality control.

Genome size

To compare genome sizes among the three diploid *Amaranthus* species, we measured the genome size of 22 *A. caudatus*, 8 *A. hybridus* and 4 *A. quitensis* accessions. Genome size differences of individuals within species are expected to be low, and we therefore estimated species-specific genome sizes using 25% the total sample of *A. caudatus* and 50% of *A. hybridus* and *A. quitensis* accessions, respectively. Plants were grown for four weeks in the greenhouse before one young leaf was collected for cell extraction. A tomato cultivar (*Solanum lycopersicum* cv Stupicke) was used as internal standard because it has a comparable genome

size that has been measured with high accuracy (DNA content = 1.96 pg; Dolezel *et al*, 1992).
Fresh leaves were cut up with a razor blade and cells were extracted with CyStain PI Absolute P (Partec, Muenster/Germany). Approximately 0.5 cm^2 of the sample leaf was extracted together with similar area of tomato leaf in 0.5 ml of extraction buffer. The DNA content was determined with CyFlow Space (Partec, Muenster/Germany) flow cytometer and analyzed with FlowMax software (Partec, Muenster/Germany). For each sample, 10,000 particles were measured each time. Two different plants were measured for each accession. The DNA content was calculated as:

$$\text{DNA content 2C [pg]} = \text{genome size tomato} \times \frac{\text{fluorescence amaranth}}{\text{fluorescence tomato}}$$

and the genome size (in Mbp) was calculated as:

$$\text{genome size 1C [Mbp]} = (0.978 * 10^3) \times \frac{\text{DNA content 2C [pg]}}{2}$$

The conversion from pg to bp was calculated with 1pg DNA = 0.978×10^9 bp (Dolezel *et al*, 2003). Means were calculated using R software (Team, 2014) and an ANOVA was performed to infer differences in genome size for the species.

DNA extraction and library preparation

Genomic DNA was extracted using a modified CTAB protocol (Saghai-Maroof *et al*, 1984). The DNA was dried and dissolved in 50-100 µl TE and diluted to 100 ng/μl for further usage. Two-enzyme GBS libraries were constructed with a modified protocol from the previously described two-enzyme GBS protocol (Poland *et al*, 2012). DNA was digested with a mix of 2 μl DNA, 2 μl NEB Buffer 2 (NEB, Frankfurt/Germany), 1 μl ApeKI (4U/μl, NEB), 1 μl HindIII (20U/μl, NEB) and 14 μl ddH$_2$O for 2 hours at 37℃ before incubating for 2 hours at 75℃. Adapters were ligated with 20 μl of digested DNA 5 μl ligase buffer (NEB), T$_4$- DNA ligase (NEB), 4 μl ddH$_2$O and 20 μl of adapter mix containing 10μl barcode adapter (0.3 ng/μl) and 10 μl common adapter (0.3ng/μl). Samples were incubated at 22℃ for 60 minutes before deactivating ligase at 65℃ for 30 minutes. Subsequently, samples were cooled down to 4℃. For each sequencing lane, 5μl of 48 samples with different barcodes were pooled after adapter ligation. Samples of the different species were randomized over the 3 pools and different barcode lengths. The 12 replicated samples were added to each pool. The pooled samples were purified with QIAquick

171 PCR purification kit (Qiagen, Hilden/Germany) and eluted in 50 μl elution buffer before PCR

172 amplification of the pools. The PCR was performed with 10 μl of pooled DNA, 25 μl 2x Taq

173 Master Mix (NEB), 2 μl PCR primer mix (25pmol/μl of each primer) and 13 μl ddH$_2$O for 5 min

174 at 72°C and 30 sec at 98°C before 18 cycles of 10 sec at 98°C, 30 sec at 65°C and 30 sec at

175 72°C after the 18 cycles 5 min of 72°C were applied and samples were cooled down to 4°C.

176 Samples were purified again with QIAquick PCR purification kit (Qiagen) and eluted in 30μl

177 elution buffer. Three lanes with 48 samples per lane were sequenced on an Illumina HighScan

178 SQ with single end and 105 cycles on the same flow cell (see supporting data).

Data preparation

180 Raw sequence data were filtered with the following steps. First, reads were divided into sepa-

181 rate files according to the different barcodes using Python scripts. Read quality was assessed

182 with fastQC (`http://www.bioinformatics.babraham.ac.uk/projects/fastqc/`). Due to lower

183 read quality towards the end of the reads, they were trimmed to 90 bp. Low quality reads were

184 excluded if they contained at least one N (undefined base) or if the quality score after trimming

185 was below 20 in more than 10% of the bases. Data from technical replicates were combined

186 and individuals with less than 10,000 reads were excluded from further analysis (Table S5).

187 The 12 replicated samples were used to detect a lane effect with an analysis of variance.

SNP calling and filtering

189 Since no high quality reference genome for *Amaranthus* sp. was available for read mapping,

190 we used `Stacks 1.19`, for the *de novo* identification of SNPs in GBS data (Catchen *et al*,

191 2011, 2013). The SNP calling pipeline provided by Stacks `denovo_map.pl` was used to call

192 SNPs from the processed data. Highly repetitive GBS reads were removed in the `ustacks`

193 program with option `-t`. Additionally, the minimum number of identical raw reads required to

194 create a stack was set to three (`m=3`) and the number of mismatches allowed between loci

195 when processing a single individual was two (`M=2`). Four mismatches were allowed between

196 loci when building the catalog (`n=4`). The catalog is a set of non redundant loci representing all

197 loci in the accessions and used as reference for SNP calling. SNPs were called with the Stacks

198 tool `populations 1.19` without filtering for missing data using option `-r 0`. One individual, PI

199 511754, was excluded from further analysis because it appeared to be misclassified. According

200 to its passport information it belonged to *A. hybridus*, but with all clustering methods it was

201 placed into a separate cluster consisting only of this individual, which suggested it belongs to

202 a different species. Therefore, we repeated the SNP calling without this individual. The SNPs

203 were filtered over the whole sample for missing data with vcftools (Danecek *et al*, 2011) by

204 allowing a maximum of 60% missing values per SNP position. Given the stringent filtering

205 criteria for SNP calling and the restricted number of *A. quitensis* individuals, we did not filter

206 SNPs by their minor allele frequency for further analysis.

Inference of genetic diversity and population structure

208 Nucleotide diversity (π) weighted by coverage was calculated with a Python script that imple-

209 ments the formula of Begun *et al* (2007) which corrects for different sampling depths of SNPs in

210 sequencing data. The confidence interval of π was calculated by bootstrapping the calculation

211 10,000 times. To account for the difference in sampling between wild and cultivated amaranths,

212 we sub-sampled *A. caudatus* 100 times with the the same number of individuals (23) as used

213 for wild amaranth. The pairwise difference in π between *A. caudatus* and the close relatives was

214 calculated for each site. Mean expected (H_{exp}) and observed (H_{obs}) heterozygosities based on

215 SNPs were calculated with the R package `adegenet 1.4-2` (Jombart & Ahmed, 2011). The

216 inbreeding coefficient (F) was calculated as:

217
$$\frac{H_{\text{exp}} - H_{\text{obs}}}{H_{\text{exp}}}$$

218 Weir and Cockerham weighted F_{ST} estimates were calculated with vcftools (Weir & Cocker-

219 ham, 1984; Danecek *et al*, 2011). To infer the population structure, we used ADMIXTURE for a

220 model-based clustering (Alexander *et al*, 2009) and conducted the analysis with different num-

221 bers of predefined populations ranging from $K = 1$ to $K = 9$ to find the value of K that was most

222 consistent with the data using a cross-validation procedure described in the ADMIXTURE man-

223 ual. To avoid convergence effects we ran ADMIXTURE 10 times with different random seeds

224 for each value of K. As a multivariate clustering method, we applied discriminant analysis of

225 principal components (DAPC) implemented in the R-package adegenet (Jombart *et al*, 2010;

226 Jombart & Ahmed, 2011) and determined the number of principal components (PCs) used

227 in DAPC with the `optim.a.score` method. To investigate the phylogenetic relationship of the

228 species, we calculated an uncorrected neighbor joining network using the algorithm Neighbor-

229 Net (Bryant & Moulton, 2004) as implemented in the `SplitsTree4` program (Huson & Bryant,

230 2006). The Euclidean distance was calculated from the genetic data to construct a neighbor

231 joining tree, which was bootstrapped 1,000 times with the `pegas` R-package (Paradis *et al*,

232 2004). The migration between genetic groups was modeled with TreeMix (Pickrell & Pritchard,

233 2012). For the TreeMix analysis we used the groups that were identified by ADMIXTURE (K

234 = 5) without an outgroup, and allowed four migration events, as preliminary runs indicates four

235 migration events to be the highest number. The tree was bootstrapped 1,000 times.

Seed color and hundred seed weight

237 For each accession we calculated the hundred seed weight (HSW) by weighting three samples

238 of 200 seeds. Seed color was determined from digital images taken with a binocular (at 6.5x

239 magnification) and by visual comparison to the GRIN descriptors for amaranth (http://www.

240 ars-grin.gov/cgi-bin/npgs/html/desclist.pl?159). There were three colors present in

241 the set of accessions, white, pink, which also indicates a white seed coat and dark brown.

242 To infer how the species, assigned genetic groups or seed color influenced seed size, we

243 conducted an ANOVA. Differences were tested with a LSD test implemented in the R package

244 agricolae (http://tarwi.lamolina.edu.pe/~fmendiburu/).

Results

Genome size measurements

247 Although the genomic history of amaranth species still is largely unknown, genome sizes and

248 chromosome numbers are highly variable within the genus *Amaranthus* (http://data.kew.

249 org/cvalues/). We therefore tested whether a change in genome size by polyploidization or

250 large-scale insertions or deletions played a role in the speciation history of *A. caudatus* and the

251 two relatives *A. quitensis* and *A. hybridus* by measuring the genome size of multiple individuals

252 from all three species with flow cytometry. The mean genome size of *A. caudatus* was 501.93

253 Mbp, and the two relatives did not differ significantly from this value (Table 1) indicating that

254 all measured individuals are diploid and that polyploidization did not play a role in the recent

255 evolution of cultivated amaranth.

Table 1: Genome size of representative group of individuals for each species. There are no significant differences between genome sizes ($p \leq 0.05$). The number of individuals per population is N and SD is the standard deviation for each parameter.

	N	DNA content (pg)	SD	genome size (Mbp)	SD
A. caudatus	22	1.026	0.026	501.93	12.74
A. hybridus	8	1.029	0.025	502.96	12.20
A. quitensis	4	1.021	0.016	499.07	7.91

SNP identification by GBS

257 To investigate genome-wide patterns of genetic diversity in *A. caudatus* and its two closest

258 relatives, we genotyped a diverse panel of 119 amaranth accessions from the three species

259 that were initially collected in the Andean region and then obtained from the USDA genebank.

260 The sequencing data generated with a two-enzyme GBS protocol consisted of 210 Mio. raw

261 reads with an average of 1.5 Mio. reads per accession (Supporting information S2). We tested

262 for a lane effect of the Illumina flow cell by sequencing the same 12 individuals on each of the

263 three lanes used for sequencing of the whole sample. An ANOVA of the read number did not

264 show a lane effect (Table S1). Since a high-quality reference genome of an amaranth species

265 was not available, we aligned reads *de novo* within the dataset to unique tags using Stacks

266 (Catchen *et al*, 2011). The total length of the aligned reads was 16.6 Mb, which corresponds

267 to approximately 3.3 % of the *A. caudatus* genome. For SNP calling, reads of each individual

268 were mapped to the aligned tags. SNPs were called with parameters described in Materials

269 and Methods, which resulted in 63,956 SNPs and a mean read depth of 40.28 per site. Since

270 GBS data are characterized by a high proportion of missing values, we removed SNPs with

271 more than 60% of missing values. After this filtering step, we obtained 9,485 biallelic SNPs

272 with an average of 35.3 % missing data for subsequent analyses (Figure S1). The folded site

273 frequency spectrum showed an expected distribution but *A. quitensis* had more sites with low

274 frequency due to the restricted number of individuals (Figure S2)

Inference of population structure

276 To infer the genetic relationship and population structure of the three amaranth species, we

277 used three different methods that included ADMIXTURE, Discriminant Analysis of Principal

278 Components (DAPC) and phylogenetic reconstruction with an uncorrected neighbor-joining

279 network. The ADMIXTURE analysis with three predefined groups ($K = 3$) that corresponds

280 to the number of *Amaranthus* species included in the study did not cluster accessions by their

281 species, but combined the two relatives *A. hybridus* and *A. quitensis* into a single cluster and

282 grouped the *A. caudatus* accessions into two distinct clusters. Higher values of K did not lead

283 to subdivision of the two close relatives into separate groups that correspond to the species

284 assignment (Figure 2), however, the they were split according to their geographic origin. Cross-

285 validation showed that $K = 5$ was most consistent with the data (Figure S3), which produced

286 three different groups of *A. caudatus* accessions that included a few accessions from the close

287 relatives, and two clusters that both consist of *A. hybridus* and *A. quitensis* accessions. These

288 two clusters are not separated by the species assignment but by the geographic origin of ac-

289 cessions because the clusters consist of *A. hybridus* and *A. quitensis* accessions from Peru

290 and Ecuador, respectively, which indicates a strong geographic differentiation among the close

291 relatives.

292 The groups of *A. caudatus* accessions also showed a clear geographic differentiation. The first

293 cluster consisted of individuals from Bolivia (Figures 2 and 3; $K = 5$, red color). *A. caudatus*

294 accessions from Peru were split into two clusters of which one predominantly represents a

295 region from North Peru (Huari Province; Figures 2 and 3; $K = 5$, yellow color), whereas the

296 second cluster contains individuals distributed over a wide geographic range that extends from

297 North to South Peru ($K = 5$, green color). Ten *A. caudatus* accessions from the Cuzco region

298 clustered with the three accessions of the close relatives from Peru ($K = 5$, blue color). These

299 ten accessions showed admixture with the other cluster of *A. hybridus/A. quitensis* and with

300 a Peruvian cluster of *A. caudatus*. Accessions that were labeled as 'hybrids' in their passport

301 data, because they express a set of phenotypic traits of different species, clustered with different

302 groups. 'Hybrids' from Bolivia were highly admixed, whereas 'hybrids' from Peru clustered with
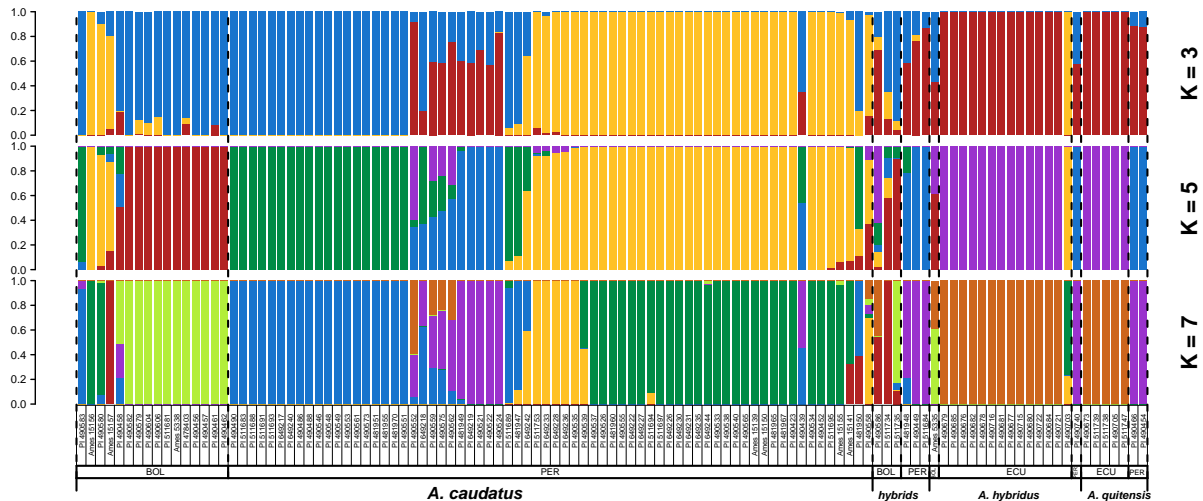
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Figure 2: Model based clustering analysis with different numbers of clusters (K=3, 5, 7) with ADMIXTURE. The clusters reflect the number of species in the study (K=3), the number of single populations (species per country of origin, K=7) and the optimal number as determined by cross validation (K=5). Individuals are sorted by species and country of origin (BOL=Bolivia, PER = Peru and ECU = Ecuador) as given by their passport data.

303 the close relatives from Peru (Figure 2). Taken together, the population structure inference

304 with ADMIXTURE identified a clear separation between the cultivated *A. caudatus* and its to

305 close relatives, and a high level of genetic differentiation among cultivated amaranths with some

306 evidence for gene flow between groups.

307 The inference of population structure with a discriminant analysis of principal components

308 (DAPC) and Neighbor-Joining network produced very similar results as ADMIXTURE. The first

309 principal component of the DAPC analysis which we used to cluster accessions based on their

310 species explained 96% of the variation and separated the cultivated *A. caudatus* from its two

311 relatives (Figure S4A). In a second DAPC analysis that was based on information on species

312 and country of origin (Figure S4B) the first principal component explained 55% of the variation

313 and separated most cultivated amaranth accessions from the close relatives. The second prin-

314 cipal component explained 35% of the variation and separated the Peruvian from the Bolivian

315 *A. caudatus* accessions.

316 The phylogenetic network outlines the relationships between the different clusters (Figure 4).

317 It shows two distinct groups of mainly Peruvian *A. caudatus* accessions and a group of acces-

318 sions with a wide geographic distribution (Figure 3; green color). The latter is more closely
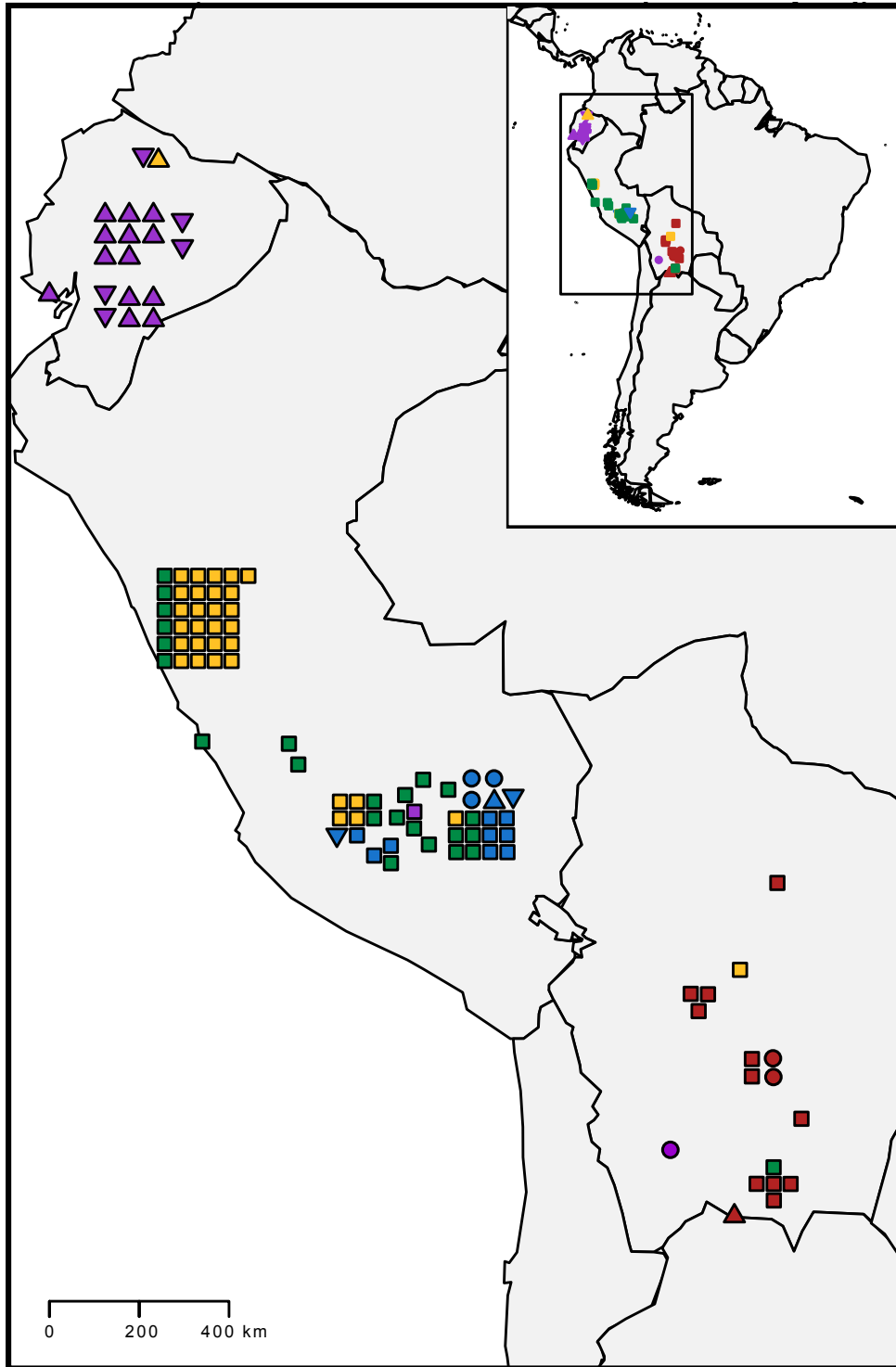
Figure 3: Geographic distribution of accessions for which data was available from passport information. Locations are not exact geographic locations because location data was given as country province . Colors are given by ADMIXTURE with K=5 (Figure 2). Species are indicated by shapes. *A. caudatus* (□), *A. hybridus* (△), *A. quitensis* (▽) and hybrids between species (○)

319 related to the Bolivian *A. caudatus* and the close relatives. The strong network structure be-

320 tween these three groups suggests a high proportion of shared ancestral polymorphisms or
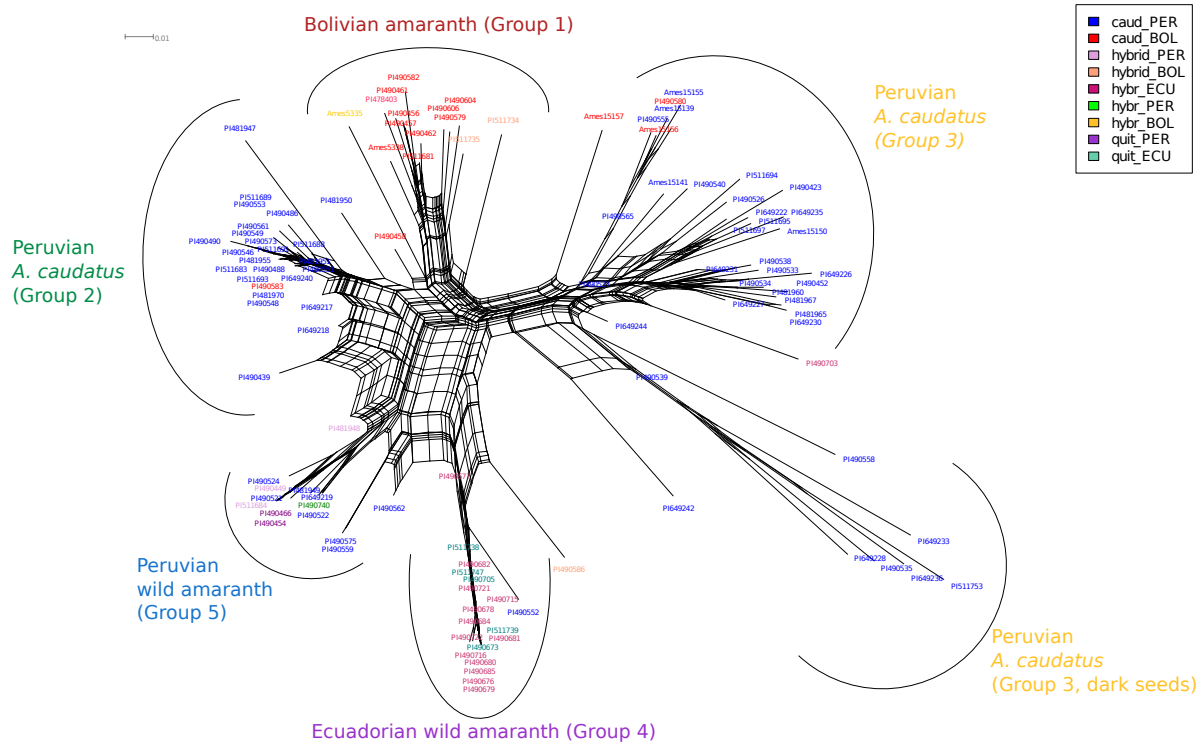
Figure 4: Neighbor-joining network of 113 amaranth accessions from six potential populations. Different colors indicate the species and origin according to gene bank information. *A. caudatus* from Peru (blue) and from Bolivia (red), *A. hybridus* from Ecuador (magenta), from Peru (green) and Bolivia (yellow), *A. quitensis* from Ecuador (turquoise) and Peru (purple) and hybrids between species from Peru (salmon) and Bolivia (light orange). Arches show genetic clusters as inferred with ADMIXTURE ($K = 5$).

321 a high level of recent gene flow. In contrast, *A. caudatus* accessions from Northern Peru are

322 more strongly separated from the other groups (Figure 3; yellow color) and are split into two

323 subgroups, of which the smaller one includes only accessions with dark seeds. In a bifurcat-

324 ing phylogenetic tree, ten cultivated amaranth accessions clustered within the same clade as

325 the close relatives *A. quitensis* and *A. caudatus* (Figure S5). The same clustering was also

326 obtained with ADMIXTURE and $K = 7$ (Figure 2).

327 To quantify the level of genetic differentiation between the species and groups within *A. cau-*

328 *datus*, we estimated weighted F$_{ST}$ values using the method of Weir and Cockerham (Weir &

329 Cockerham, 1984). F$_{ST}$ values between *A. caudatus* and *A. hybridus* and *A. quitensis* species

330 were 0.31 and 0.32, respectively (Table 2), and 0.041 between *A. hybridus* and *A. quitensis*

331 based on the taxonomic assignment. The latter reflects the high genetic similarity of the acces-

332 sions from both species observed above. Within *A. caudatus* subpopulations, the F_{ST} between

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Table 2: Weir and Cockerham weighted F_{ST} estimates between populations based on the taxonomic assignment of their passport data. The group of close relatives are *A. hybridus* and *A. quitensis* taken together.

	F_{ST}
A. caudatus x *A. hybridus*	0.319
A. caudatus x *A. quitensis*	0.274
A. caudatus x close relatives	0.322
A. hybridus x *A. quitensis*	0.041
A. caudatus (PER) x *A. caudatus* (BOL)	0.132

333 *A. caudatus* populations from Peru and Bolivia was 0.132, three times higher than between *A.*

334 *hybridus* and *A. quitensis*. The above analyses suggested that some individuals may be mis-

335 classified in the passport information, and we therefore calculated F_{ST} values of population sets

336 defined by ADMIXTURE. Although such F_{ST} values are upward biased, they allow to evaluate

337 the relative level of differentiation between groups defined by their genotypes. The comparison

338 of F_{ST} values showed that the three *A. caudatus* groups (groups 1-3) are less distant to the

339 group of *A.quitensis/A.hybridus* accessions from Peru (group 5) than from Ecuador (group 4;

340 Table S2). A TreeMix analysis, which is based on allele frequencies within groups (Figure 5),

341 suggests gene flow from the Peruvian *A. caudatus* (group 2) to *A. quitensis* and *A. hybridus*

342 amaranths from Peru (group 5) and, with a lower confidence level, from *A. quitensis* and *A.*

343 *hybridus* from Ecuador (group 4) into Bolivian *A. caudatus* (group 1), as well as from Bolivian

344 *A. caudatus* to Peruvian *A. caudatus* (Group 2).

Analysis of genetic diversity

346 We further investigated whether domestication reduced genetic diversity in cultivated *A. cau-*

347 *datus* (Table 3). All measures of diversity were higher for *A. caudatus* than its relatives. For

348 example, nucleotide diversity (π) was about two times higher in *A. caudatus* than in the two

349 relatives combined. The diversity values of the accessions classified as 'hybrids' showed in-

350 termediate values between cultivated amaranth and its relatives supporting their hybrid nature.

351 The inbreeding coefficient, F, was highest in the cultivated amaranth but did not differ from the

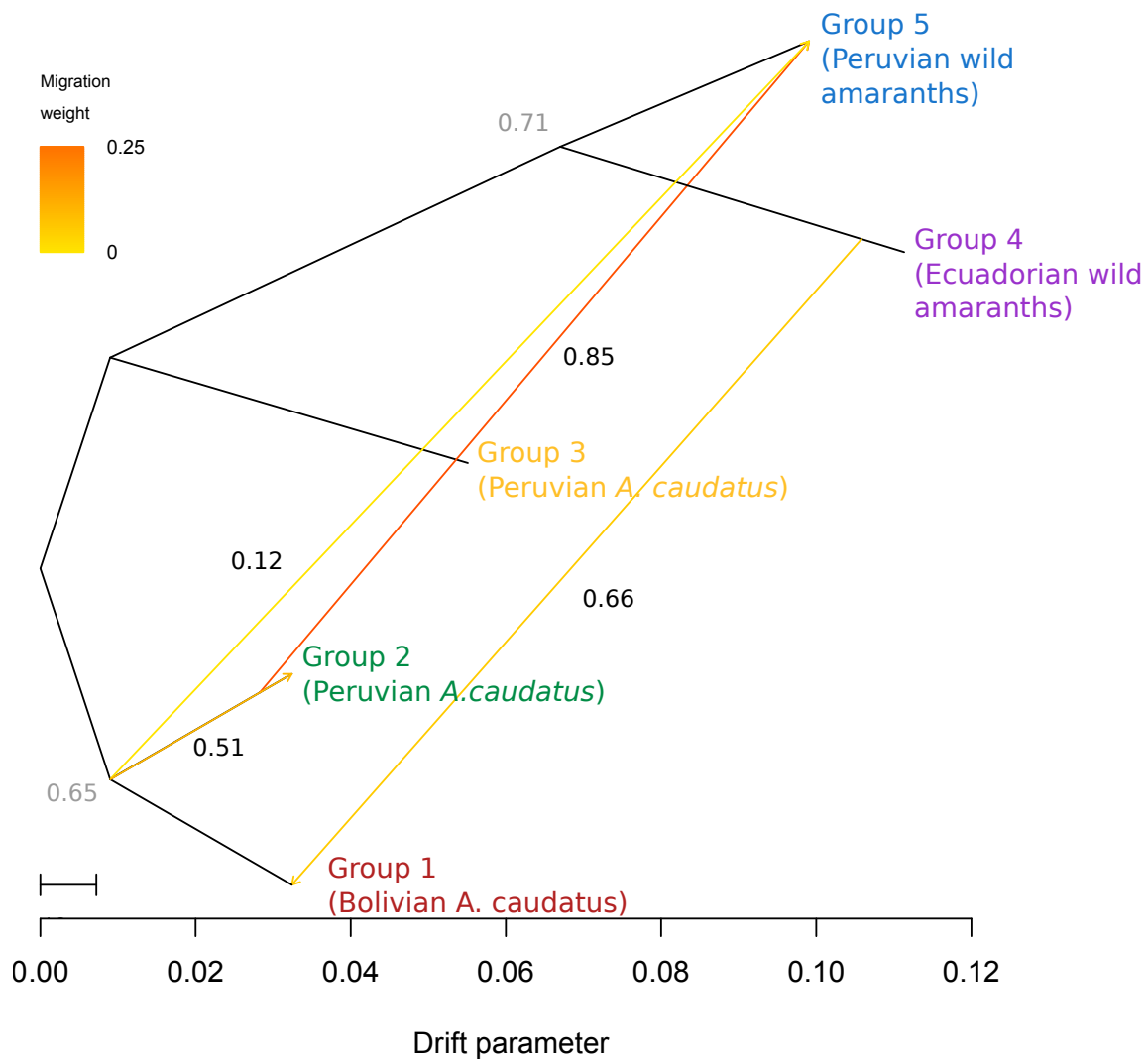352 two close relatives if they are combined. In contrast, accessions classified as 'hybrids' and *A.*

Figure 5: Tree of five genetic clusters of South American amaranths inferred with TreeMix. The genetic clusters which were used to calculate the tree were inferred with ADMIXTURE. Groups 1 to 3 represent *A. caudatus* clusters from Peru and Bolivia, group 4 represents accessions of *A. quitensis* and *A. hybridus* from Ecuador and group 5 wild amaranth from Peru, respectively. The migration events are colored according to their weight. Numbers at branching points and on the migration arrow represent bootstrapping results based on 1,000 runs.

353 *quitensis* had lower inbreeding coefficients. Within the groups of accessions defined by AD-

354 MIXTURE, genetic diversity differed substantially. The close relatives from Ecuador had the

355 lowest ($\pi = 0.00031$) while the group from northern Peru showed the highest level of nucleotide

356 diversity ($\pi = 0.00111$; Table S3). Figure 6 shows that even though the overall diversity of *A.*

357 *caudatus* was higher, a substantial proportion of sites were more diverse in the close relatives

358 ($\pi_{caud} - \pi_{hyb/quit} < 0$; Figure 6).

Table 3: Genetic diversity parameters for the cultivated *A. caudatus* and the close relatives *A. hybridus* and *A. quitensis*. π is the nucleotide diversity over all sites, CI_π is the 95% confidence interval of π, H_{exp} the mean expected heterozygosity for the variant sites and SD_{He} its standard deviation, H_{obs} the mean observed herterozygosity and SD_{Ho} its standard deviation. F is the inbreeding coeficient and SD_F its standard deviation.

| Population	N	π	CI_π	H_{exp}	SD_{He}	H_{obs}	SD_{Ho}	F	SD_F	θ_w
A. caudatus	84	0.00117	± 0.00002	0.175	0.167	0.049	0.140	0.688	0.462	0.00123
A. hybridus	16	0.00061	± 0.00001	0.085	0.135	0.041	0.170	0.679	0.608	0.00073
A. quitensis	7	0.00059	± 0.00001	0.076	0.169	0.040	0.170	0.451	0.763	0.00048
Close relatives combined combined	23	0.00062	± 0.00002	0.090	0.140	0.041	0.166	0.681	0.591	0.00070
Hybrids	6	0.00091	± 0.00001	0.112	0.179	0.060	0.173	0.436	0.645	0.00107
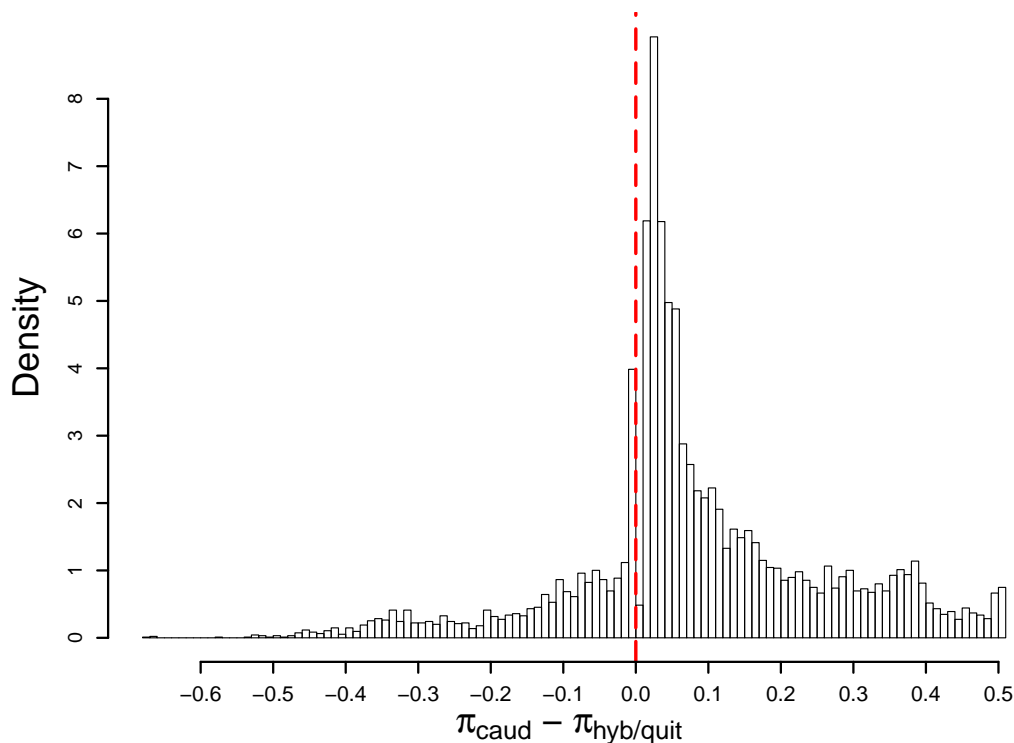
Figure 6: Per site difference in nucleotide diversity (π) between cultivated amaranth (*A. caudatus*) and the close relatives *A. hybridus* and *A. quitensis*.

Seed color and seed size as potential domestication traits.

In grain crops, grain size and seed color are important traits for selection and likely played a central role in domestication of numerous plants (Abbo *et al*, 2014; Hake & Ross-Ibarra,

362   2015). To investigate whether these two traits are part of the domestication syndrome in grain

363   amaranth, we compared the predominant seed color of the different groups of accessions and

364   measured their seed size. The seeds could be classified into three colors, white, pink and

365   brown. The white and pink types have both a white seed coat, but the latter has red cotyledons

366   that are visible through the translucent seed coat. A substantial number of seed samples (19)

367   from the genebank contained seeds of other color up to a proportion of 20%. One *A. caudatus*

368   accession from Peru (PI 649244) consisted of 65% dark seeds and 35% white seeds in the

369   sample. No accession from the two close relatives *A. hybridus* and *A. quitensis*, or from the

370   hybrid accessions had white seeds, whereas the majority (74%) of *A. caudatus* accessions

371   had white (70%) or pink (4%) seeds, and the remaining (26%) brown seeds (Figure 7 A). We

372   also compared the seed color of groups defined by ADMIXTURE ($K = 5$; Figure 2), which

373   reflect their genetic relationship and may correct for mislabeling of accessions (Figure 7 B).

374   No group had only white seeds, but clusters consisting mainly of *A. hybridus* and *A. quitensis*

375   had no white seeds at all. In contrast to seed color, the hundred seed weight (HSW) of the

376   different *Amaranthus* species did not significantly differ between cultivated *A. caudatus* and

377   the two relatives. The mean HSW of *A. caudatus* was 0.056 g and slightly higher than the

378   HWS of *A. hybridus* (0.051 g) and *A. quitensis* (0.050 g; Figure 7 C and Table S4). Among

379   the groups identified by ADMIXTURE ($K = 5$), one group showed a significantly higher HSW

380   than the other groups, while the other four groups did not differ in their seed size. The group

381   with the higher HSW consisted mainly of Bolivian *A. caudatus* accessions and had a 21 % and

382   35 % larger HSW than the two groups consisting mainly of Peruvian *A. caudatus* accessions,

383   respectively (Figure 7 D). An ANOVA also revealed that seed color has an effect on seed size

384   because white seeds are larger than dark seeds (Table 4).

Table 4: Analysis of variance for the hundred seed weight in dependence of the seed color and population as determined by ADMIXTURE

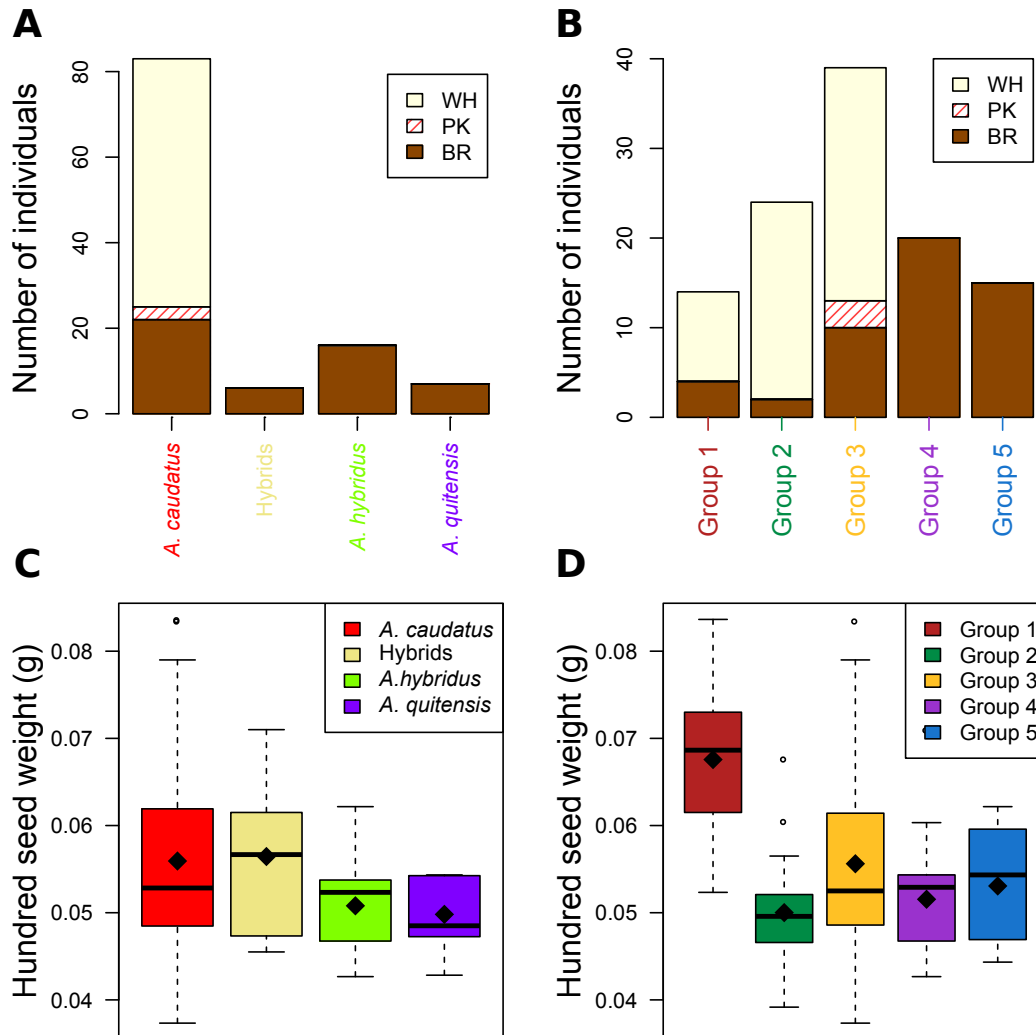|	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Seed color	2	0.000657	0.0003285	4.657	0.0116 *
Group	4	0.003151	0.0007877	11.165	1.46e-07 ***
Seed color:Group	2	0.000042	0.0000209	0.297	0.7440
Residuals	103	0.007266	0.0000705		

Figure 7: Predominant seed color (**A,B**) and hundred seed weight (**C,D**) by *Amaranthus* species (**A,C**) and groups identified with ADMIXTURE for $K = 5$ (**B,D**) where group 1 (red) resembles *A. caudatus* from Bolivia, group 2 (green) and 3 (yellow) *A. caudatus* from Peru, group 4 (purple) represents the close relatives *A. quitensis* and *A. hybridus* from Ecuador and group 5 (blue) from Peru, respectively. Seed colors were white (WH), pink (PK) and dark brown (BR). While there were no significant differences in seed size between the species, group 1 had significantly higher hundred seed weight ($p \leq 0.05$) than the other groups.

Discussion

Genotyping-by-sequencing of amaranth species

The genotyping of cultivated amaranth *A. caudatus* and two close relatives *A. quitensis* and *A. hybridus* revealed a strong genetic differentiation between both groups and a high level of genetic differentiation within cultivated *A. caudatus*. We based our sequence assembly and SNP calling on a *de novo* assembly of GBS data with Stacks because currently no high quality reference sequence of these species is available. Stacks allows SNP calling without a reference genome by constructing a reference catalog from the data and includes all reads in the analysis (Catchen *et al*, 2011). *De novo* assembled fragments without a reference are unsorted and can not be used to investigate genetic differentiation along along the genomic regions, but they are suitable for analysing genetic diversity and population structure (Catchen *et al*, 2013). GBS produces a large number of SNPs (Poland *et al*, 2012; Huang *et al*, 2014), albeit with a substantial proportion of missing values. Missing data lead to biased estimators of population parameters such as π and θ_w (Arnold *et al*, 2013) and need to be accounted for if different studies are compared. Additionally, variable error rates in different GBS data sets can inflate differentiation estimates (Mastretta-Yanes *et al*, 2015). The comparison of accessions and groups within a study is possible, however, if all individuals were treated with the same experimental protocol. We filtered out sites with high levels of missing values to obtain a robust dataset for subsequent population genomic analysis. The SNPs were called based on the total sample without accounting for the species which should not bias diversity estimates. Since a smaller sample from the close relatives may underestimate their diversity compared to cultivated *A. caudatus*, we compared diversity estimates by repeated random sampling of 23 out of 84 *A. caudatus* accessions and calculating π from the smaller sample. Diversity estimates of the smaller *A. caudatus* did not differ from the full sample and estimates were in all cases higher than in the close relatives (Figure S6). We conclude that the different sample sizes of the two groups do not introduce a bias on diversity estimates.

Genetic relationship of *A. quitensis* and *A. hybridus*

Coons (1978) suggested that *A. quitensis* is the same species as *A. hybridus*, but in the genebank passport data *A. quitensis* is still considered as a separate species. The taxonomic differentiation between the two species rests on two minor morphological trait, namely the shape of the tepals and the short utricles, which are very small and prone to misidentification (Sauer, 1967; Adhikary & Pratt, 2015). The high phenotypic similarity of *A. quitensis* and *A. hybridus* is supported by the GBS data because accessions from the two species are closely related. They are not separated by their species assigment but cluster into two groups that both consist of accessions from the two species and reflect their geographic origin from Peru and Ecuador, respectively. The F_{ST} value between *A. quitensis* and *A. hybridus* was lower than between the two *A. caudatus* groups from Peru and Bolivia (Tables 2 and S2). The highly similar genome sizes of all three diploid species is consistent with genetic relationship inferred from the GBS data and indicates that large-scale genomic changes like polyploidization events did not occur in the recent history of these species. For comparison, other species in the genus *Amaranthus* have very different genome sizes due to variation in chromosome numbers and ploidy levels (Baohua & Xuejie, 2002; Rayburn *et al*, 2005).

In contrast to our analysis, Kietlinski *et al* (2014) found stronger evidence for a genetic differentiation between *A. hybridus* and *A. quitensis* based on the 11 SSR markers. However, their data also show that both species are distinct groups that do not cluster their species assignment but by geographic origin. These differences may result from the different marker types (SNPs vs. SSRs) and a different sample composition because our sample consists of accessions from the Andean region, whereas Kietlinski et al. included putative wild amaranth accessions with little geographic overlap between the two species. The groups of *A. hybridus* and *A. quitensis* accessions from Peru and Ecuador show a high level of differentiation ($F_{ST} = 0.579$; Table S2), which is similar to the differentiation between one of two Peruvian *A. caudatus* groups and the *A. hybridus/A.quitensis* accessions from Peru ($F_{ST} = 0.553$). Although the sample size of *A. quitensis* and *A. hybridus* is small, genetic differentiation between species should be stronger than between individuals within species in the ADMIXTURE and phylogenetic analyses. In summary, our analysis and the work of Kietlinski *et al* (2014) show that *A. quitensis* and *A. hybridus* do not have a simple genetic relationship that follows species assignment. The high level

of intraspecific differentiation in both cultivated amaranth and their relatives is relevant for investigating domestication because the genetic distance between groups of cultivated amaranth is related to the geographic distance of the putative wild ancestors. Therefore, future studies of these two close relatives of the grain amaranths should include large number of accessions from the whole species range to model genetic differentiation within the two species as well as the relationship between species.

Diversity of South American amaranth

In numerous crops, domestication was associated with a decrease in genome-wide levels of diversity due to bottleneck effects and strong artificial selection of domestication traits (Gepts, 2014). Under the assumption that the cultivated grain amaranth *A. caudatus* was domesticated, genetic diversity should be reduced compared to the two close relatives. In contrast, the overall genetic diversity in our sample of cultivated amaranths was higher than in the two close relatives. The distribution of diversity between the GBS fragments includes genomic regions with reduced diversity in *A. caudatus*, which may reflect selection in some genomic regions (Figure 6). Without a reference genome it is not possible to position reads on a map to identify genomic regions that harbor putative targets of selection based on an inference of the demographic history. Despite the indirect phenotypic evidence for selection, the higher genetic diversity of cultivated grain amaranth may result from a strong gene flow between cultivated amaranths and its relatives. Gene flow between different amaranth species has been observed before (Trucco *et al*, 2005) and is also consistent with the observation of six highly admixed accessions classified as 'hybrids' in the passport data and which appear to be interspecific hybrids (Figure 2 and Table 3). Gene flow between *A. caudatus* and the relatives *A. quitensis* and *A. hybridus* in different areas of the distribution range, not only from populations included in this study, could explain a higher genetic diversity in cultivated amaranth. This is also consistent with the strong network structure (Figure 4) and the TreeMix analysis (Figure 5). In summary, cultivated *A. caudatus* is unusual in its higher overall genetic diversity compared to populations of its putative wild ancestors originating from the same geographic region. The high genetic diversity of *A. caudatus* is in contrast to other domesticated plants and suggests that a domestication bottleneck in its cultivation history absent (i.e., no domestication), very weak or masked

470 by recurrent gene flow. We consider these results to be robust, because in comparison to

471 previous work (Maughan *et al*, 2009, 2011; Khaing *et al*, 2013; Jimenez *et al*, 2013; Kietlinski

472 *et al*, 2014), our study includes a larger number of accessions and more genetic markers. This

473 allowed us to assess the genetic diversity and population structure of South American ama-

474 ranth on a genome-wide basis, but we note that a more complete geographic sampling of all

475 cultivated amaranths and their relatives is required for a complete understanding of amaranth

476 history.

Evidence for a weak domestication syndrome

478 Despite their long history of cultivation, diverse uses for food and feed and a high importance

479 for the agriculture of ancient cultures (i.e., *A. hypochondriacus* during the Aztec period), grain

480 amaranths do not display the classical domestication syndrome as strongly as other crops

481 (Sauer, 1967). Cultivated amaranth shows morphological differentiation from putative wild an-

482 cestors like larger and more compact inflorescences (Sauer, 1967) and a level of genetic dif-

483 ferentiation (Table 2) which is comparable to other domesticated crops and their wild relatives

484 (Sunflower: F_{ST}=0.22 (Mandel *et al*, 2011); common bean: 0.1-0.4 (Papa *et al*, 2005), pi-

485 geonpea: 0.57-0.82 (Kassa *et al*, 2012)). However, the numerous amaranth flowers mature

486 asynchronously and produce very small seeds that are shattered (Brenner *et al*, 2010). All

487 putative wild amaranths have dark brown seeds, whereas the predominant seed color of cul-

488 tivated grain amaranth is white, which suggests that selection for seed color played a role in

489 the history of the latter. Dark-seeded accessions are present in all three groups of *A. caudatus*

490 defined by the genotypic data, which indicates that white seed color is not a fixed trait. Seed

491 sizes between cultivated amaranth and its relatives are not significantly different with the excep-

492 tion of white-seeded *A. caudatus* accessions from Bolivia (Figure 7), which have larger seeds.

493 The larger seeds in this group and of white seeds in general (Table 4) indicates past selection

494 for domestication-related traits, but only in specific geographic regions or in certain types of

495 amaranth, and not in the whole cultivated crop species.

496 These findings suggest that some selection occurred in the history of amaranth cultivation that

497 may reflect domestication. Possible explanations for the incomplete fixation of domestication

498 traits in South American grain amaranth include weak selection, genetic constraints or ongoing

gene flow. First, weak selection of putative domestication traits indicate that they were not essential for cultivation. Although white seeds are predominant in cultivated amaranth and most likely a selected trait, other seed colors may have been preferred for different uses. Since amaranths are also an important leaf vegetable in Mexico, the grain use of *A. caudatus* may not have been a main target of selection during domestication, thereby allowing a diversity of seed traits due to weak or incomplete selection. Second, genetic constraints may limit phenotypic variation in domestication traits. In contrast to genes with strong pleiotropic effects or epistatic interactions, domestication genes that are part of simple molecular pathways, have minimal pleiotropic effects, and show standing functional genetic variation have a higher chance of fixation by selection (Doebley *et al*, 2006; Lenser & Theißen, 2013). Numerous genes with these characteristics were cloned and characterized in major crops like rice, barley and maize. They contribute to the distinct domestication syndrome such as a loss of seed shattering, larger seed size and compact plant architecture. The molecular genetics of amaranth domestication traits remains unknown, but the absence a strong domestication syndrome may reflect genetic constraints despite a long period of cultivation. A third explanation is ongoing gene flow between cultivated amaranth and its relatives that may prevent or delay the formation of a distinct domestication syndrome and contributes to the high genetic diversity (Table 3), similar seed size (Figure 7 C), and the presence of dark seeds (Figure 7) in cultivated amaranth. Both historical and ongoing gene flow are likely because amaranth has an outcrossing rate between 5% and 30% (Jain *et al*, 1982; Stetter *et al*, 2016). In South America, cultivated amaranth and its relatives are sympatric over wide areas and the latter were tolerated in the fields and home gardens with *A. caudatus* (Sauer, 1967), where they may have intercrossed. Gene flow between wild and domesticated plants has also been observed in maize and teosinte in the Mexican highlands, but did not have a major influence on the maize domestication syndrome (Hufford *et al*, 2013). Further support for ongoing gene flow in amaranth is given by the presence of hybrids and admixed accessions in our sample with evidence for genetic admixture and dark seeds that demonstrate the phenotypic effects of introgression. Since the dark seed color is dominant over white color (Kulakow *et al*, 1985) and *A. caudatus* is predominantly self-pollinating, dark seeds could have efficiently removed by selection despite gene flow. Additionally, amaranth was grown in small plots in the Andean highlands, which favors fixation of traits (Kauffman & Weber, 1990). Thus, gene flow is a plausible explanation for the absence of a distinct domesti-

530 cation syndrome.

531 Although our sample does not include *A. hypochondriacus* or *A. cruentus* accessions, our data
532 are consistent with the model by Kietlinski *et al* (2014) who proposed the domestication of
533 *A. caudatus* and *A. hypochondriacus* from different *A. hybridus* lineages in Central and South
534 America (Figure 1D). Gene flow between *A. caudatus* and its close relative *A. quitensis* in the
535 Southern distribution range (Peru and Bolivia) may explain the higher genetic diversity of the
536 latter despite a strong genetic differentiation.

Conclusions

538 The genotypic and phenotypic analysis of cultivated South American grain amaranth and its
539 close relatives suggests that *A. caudatus* is an incompletely domesticated crop species. Key
540 domestication traits such as the shape of inflorescences, seed shattering and seed size are
541 rather similar between cultivated amaranths and their close relatives and there is strong evi-
542 dence of ongoing gene flow between these species despite selection for domestication-related
543 traits like white seeds. Grain amaranth is an ancient crop of the Americas but genomic and
544 phenotypic signatures of domestication differ from other, highly domesticated crops that orig-
545 inated from single domestication events like maize (Hake & Ross-Ibarra, 2015). In contrast,
546 the history of cultivated amaranth may include multiregional, multiple and incomplete domes-
547 tication events with frequent and ongoing gene flow from sympatric relatives, which is more
548 similar to the history of species like rice, apple or barley (Londo *et al*, 2006; Cornille *et al*,
549 2012; Poets *et al*, 2015). The classical model of a single domestication in a well-defined center
550 of domestication may not sufficiently reflect the history of numerous ancient crops. Our study
551 further highlights the importance of a comprehensive sampling to study the domestication of
552 amaranth. The three cultivated amaranths and all close relatives should be included in further
553 studies for a full understanding of amaranth domestication and its broader implications for crop
554 plant domestication.

Acknowledgments

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Data Accessibility

The original genomic data are available on the European Nucleic Archive (ENA) under the study accession number PRJEB13013. Scripts and phenotypic raw data are available under `http://dx.doi.org/10.5061/dryad.m5kk3` on Dryad (http://datadryad.org/).

Author Contributions

M.G.S. and K.J.S. designed research; M.G.S. and K.J.S. performed research; T.M. contributed analytic tools; M.G.S. analyzed data; and M.G.S. and K.J.S. wrote the paper.

Conflict of interest

The authors declare no conflict of interest.