Incomplete domestication of South American grain amaranth (*Amaranthus caudatus*) from its wild relatives

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

Keywords

Amaranthus, genotyping-by-sequencing, genetic diversity, domestication, flow cytometry, orphan crop

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*

Abstract

Grain amaranth is a pseudo-cereal and an ancient crop of Central and South America. Of the three species of grain amaranth, *Amaranthus caudatus* is mainly grown in the Andean region. Several models of domestication were proposed including a domestication from the wild relatives *A. hybridus* or *A. quitensis*. To investigate the domestication history of *A. caudatus* and its relationship to the two wild relatives, we used genotyping-by-sequencing (GBS) to genotype 119 amaranth accessions from the Andean region. We determined the genome sizes of the three species and compared phenotypic variation in two domestication-related traits, seed size and seed color. We show that the population genetic analysis based on 9,485 SNPs revealed very little genetic differentiation between the two wild species, suggesting they are the same species, but a strong differentiation between wild and domesticated amaranths. *A. caudatus* has a higher genetic diversity than its wild relatives and about 10% of accessions showed a strong admixture between the wild and cultivated species suggesting recent gene flow. Genome sizes and seed sizes were not significantly different between wild and domesticated amaranths, although a genetically distinct cluster of Bolivian accessions had significantly larger seeds. Taken together our analysis suggests that grain amaranth is an incompletely domesticated species, either because it was not strongly selected or because high levels of gene flow from its sympatric wild relatives counteract the fixation of key domestication traits in the domesticated *A. caudatus*.

Introduction

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, 2000). The grain amaranths *Amaranthus caudatus*, *Amaranthus cruentus* and *Amaranthus hypochondriacus* originated from South and Central America. Amaranth is an ancient crop, 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, amaranth is promoted as a healthy food because of its favorable composition of essential amino acids and high micronutrient content.

The three grain amaranth species differ in their geographical distribution. *A. cruentus* and *A. hypochondriacus* are most common in Central America, whereas *A. caudatus* is cultivated mainly in South America. In the Andean region, *A. caudatus* grows in close proximity to the two wild *Amaranthus* species *A. hybridus* and *A. quitensis*, which are considered as potential ancestors (Sauer, 1967). Of these, *A. quitensis* was tolerated or cultivated in Andean home gardens and used for coloring in historical times.

Past research on the domestication of major crop plants revealed that crops from different plant families have similar domestication syndromes that include larger seeds, loss of seed shattering, reduced branching, loss of seed dormancy and increased photoperiod insensitivity (Abbo *et al*, 2014; Hake & Ross-Ibarra, 2015). In addition to phenotypic changes, domestication strongly affected the structure of genetic diversity of domesticated plants and created a genetic signature of selection and drift because domestication is frequently associated with a strong genetic bottleneck (Doebley *et al*, 2006; Olsen & Wendel, 2013; Sang & Li, 2013; Nabholz *et al*, 2014). The history of amaranth domestication is still under discussion. Sauer (1967) proposed two scenarios based on the morphology and geographic distribution of the different species. The first model postulates three independent domestication events, in which *A. hypochondriacus* originated from *A. powellii*, *A. cruentus* from *A. hybridus*, and *A. caudatus* from *A. quitensis*. The second model proposes an initial domestication of *A. cruentus* from *A. hybridus* followed

by a migration and intercrossing of *A. cruentus* with *A. powellii* in Central America and an intercrossing of *A. cruentus* with *A. quitensis* resulting in *A. caudatus* in South America. Another model based on SNP markers suggested that all three domesticated amaranths evolved from *Amaranthus hybridus*, but at multiple locations (Maughan *et al*, 2011). Most recently, Kietlinsky et al. (Kietlinski *et al*, 2014) proposed a single domestication *A. hybridus* in the Andes or in Mesoamerica and a subsequent spatial separation of two lineages leading to *A. caudatus* and *A. hypochondriacus* or two independent domestication events of *A. hypochondriacus* and *A. caudatus* from a single *A. hybridus* lineage in Central and South America. Taken together, the diversity of hypotheses indicates either a complex domestication history or insufficient data to strongly support a single model of domestication.

Despite its long history of cultivation, the domestication syndrome of cultivated amaranth is remarkably indistinct because it still shows strong photoperiod sensitivity and has very small shattering seeds (Sauer, 1967; Brenner, 2000). Other crops like maize that were cultivated at a similar time period in the same region exhibit the classical domestication syndrome (Sang & Li, 2013; Lenser & Theißen, 2013). This raises the question whether amaranth has a different domestication syndrome or whether genetic constraints, a lack of genetic variation or (agri)cultural reasons led to a distinct domestication pattern compared to other crops. The phenotypic analysis of amaranth domestication is complicated by the taxonomic uncertainty of wild amaranth species. Although *A. quitensis* was suggested to be the ancestor of *A. caudatus*, the state of *A. quitensis* as a separate species is under debate. Sauer (1967) classified it as species, but later it was argued that it is the same species as *A. hybridus* (Coons, 1978; Brenner, 2000). However, until today *A. quitensis* is treated as separate species and since genetic evidence for the status of *A. quitensis* as a separate species is based on few studies with limited numbers of markers, this topic is still unresolved (Mallory *et al*, 2008; Kietlinski *et al*, 2014).

The rapid development of sequencing technologies facilitates the large-scale investigation of the genetic history of crops and their wild relatives. Among available methods, reduced representation sequencing approaches such as genotyping-by-sequencing (GBS) allow a genome-wide and cost-efficient marker detection compared to whole genome sequencing (Elshire *et al*, 2011; Poland *et al*, 2012). Despite some biases associated with reduced representation sequencing, GBS and related methods are suitable and powerful approaches for studying inter-

specific phylogenetic relationships (Cruaud *et al*, 2014) and intraspecific patterns of genetic variation in crop plants (Morris *et al*, 2013).

We used GBS and genome size measurements to characterize the genetic diversity and relationship of cultivated *A. caudatus* and its putative wild ancestors *A. quitensis* and *A. hybridus*, and compared patterns of genetic structure with two domestication-related phenotypic traits (seed color and hundred seed weight). We tested whether domestication led to a reduction of genetic diversity and larger seed size in domesticated amaranth, and clarified the taxonomic relationship and gene flow with the close relatives. Our results indicated that *A. caudatus* has a history of domestication that may be considered as incomplete and is consistent with models of multiple domestication.

Material and Methods

Plant material

A total of 119 South American amaranth accessions of three *Amaranthus* species were obtained from the USDA gene bank (`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 (Figure S5). We selected the *A. caudatus* accessions based on the altitude of the collection site and focused on high-altitude populations (2,200 to 3,700 m). We further subdivided the species into populations according to their country of origin and included *A. caudatus* from Peru, Bolivia, *A. hybridus* from Peru, Bolivia, Ecuador, *A. quitensis* from Peru and Ecuador as well as hybrids from Peru and Bolivia. Accessions were planted in a field in Nürtingen (Germany), where a single young leaf of one representative plant per accession was sampled. From 12 accessions, three plants were sampled and sequenced individually for quality control.

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_2O 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_2O 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 in each pool. The pooled samples were purified with QIAquick PCR purification kit (Qiagen, Hilden/Germany) and eluted in 50 μl elution buffer before PCR amplification of the pools. The PCR was performed with 10 μl of pooled DNA, 25 μl 2x Taq Master Mix (NEB), 2 μl PCR primer mix (25pmol/μl of each primer) and 13 μl ddH_2O for 5 min at 72℃ and 30 sec at 98℃ before 18 cycles of 10 sec at 98℃, 30 sec at 65℃ and 30 sec at 72℃ after the 18 cycles 5 min of 72℃ were applied and samples were cooled down to 4℃. Samples were purified again with QIAquick PCR purification kit (Qiagen) and eluted in 30μl elution buffer. Three lanes with 48 samples per lane were sequenced on an Illumina HighScan SQ with single end and 105 cycles on the same flow cell (see supporting data).

Data preparation

Raw sequence data were filtered with the following steps. First, reads were divided into separate files according to the different barcodes using Python scripts. Read quality was assessed with fastQC (`http://www.bioinformatics.babraham.ac.uk/projects/fastqc/`). Due to lower read quality towards the end of the reads, they were trimmed to 90 bp. Low quality reads were excluded if they contained at least one N (undefined base) or if the quality score after trimming

was below 20 in more than 10% of the bases. Data from technical replicates were combined and individuals with less than 10,000 reads were excluded from further analysis (Table S5). The 12 replicated samples were used to detect a lane effect with an analysis of variance.

SNP calling and filtering

Since no high quality reference genome for *Amaranthus* sp. was available for read mapping, we used `Stacks 1.19`, for the *de novo* identification of SNPs in GBS data (Catchen *et al*, 2011, 2013). The pipeline provided for Stacks `denovo_map.pl` was used to call SNPs from the processed data. Highly repetitive GBS reads were removed in the `ustacks` program with option `-t`. Additionally, the minimum number of identical raw reads required to create a stack was set to three and the number of mismatches allowed between loci when processing a single individual was two. Four mismatches were allowed between loci when building the catalog. The catalog is a set of non redundant loci representing all loci in the accessions and used as reference for SNP calling. SNPs were called with the Stacks tool `populations 1.19` without filtering for missing data using option `-r 0`. One individual, PI 511754, was excluded from further analysis because it appeared to be misclassified. According to its passport information it belonged to *A. hybridus*, but with all clustering methods it was placed into a separate cluster consisting only of this individual, which suggested it belongs to a different species. Therefore, we repeated the SNP calling without this individual. The SNPs were further filtered with vcftools (Danecek *et al*, 2011), by allowing a maximum of 60% missing values per SNP position.

Inference of genetic diversity and population structure

Nucleotide diversity (π) weighted by coverage was calculated with a Python script implementing the formula of Begun *et al* (2007) which corrects for different sampling depths of SNPs in sequencing data. The confidence interval of π was calculated by bootstrapping the calculation 10,000 times. Mean expected (H_{exp}) and observed (H_{obs}) heterozygosities based on SNPs were calculated with the R package `adegenet 1.4-2` (Jombart & Ahmed, 2011). The inbreeding coefficient (F) was calculated as:

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

Weir and Cockerham weighted F_{ST} estimates were calculated with vcftools (Weir & Cockerham, 1984; Danecek *et al*, 2011). To infer the population structure, we used ADMIXTURE for a model-based clustering (Alexander *et al*, 2009) and conducted the analysis with different numbers of predefined populations ranging from $K = 1$ to $K = 9$ to find the value of K that was most consistent with the data using a cross-validation procedure described in the ADMIXTURE manual. To avoid convergence effects we ran ADMIXTURE 10 times with different random seeds for each value of K. As a multivariate clustering method, we applied discriminant analysis of principal components (DAPC) implemented in the R-package adegenet (Jombart *et al*, 2010; Jombart & Ahmed, 2011) and determined the number of principal components (PCs) used in DAPC with the `optim.a.score` method. To investigate the phylogenetic relationship of the species, we calculated an uncorrected neighbor joining network using the algorithm Neighbor-Net (Bryant & Moulton, 2004) as implemented in the `SplitsTree4` program (Huson & Bryant, 2006). The Euclidean distance was calculated from the genetic data to construct a neighbor joining tree, which was bootstrapped 1,000 times with the `pegas` R-package (Paradis *et al*, 2004). The migration between genetic groups was modeled with TreeMix (Pickrell & Pritchard, 2012). For the TreeMix analysis we used the groups that were identified by ADMIXTURE (K = 5) without an outgroup, and allowed 4 migration events, as preliminary runs indicates 4 migration events to be the highest number. The tree was bootstrapped 1,000 times.

Genome size

To compare genome sizes between *Amaranthus* species, we measured the genome size of 22 *A. caudatus*, 8 *A. hybridus* and 4 *A. quitensis* accessions. 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 followed:

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

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

Seed color and hundred seed weight

For each accession we calculated the hundred seed weight (HSW) by weighting three samples of 200 seeds. Seed color was determined from digital images taken with a binocular (at 6.5x magnification) and by visual comparison to the GRIN descriptors for amaranth (`http://www.ars-grin.gov/cgi-bin/npgs/html/desclist.pl?159`). There were three colors present in the set of accessions, white, pink, which also indicates a white seed coat and dark brown. To infer how the species, assigned genetic groups or seed color influenced seed size, we conducted an ANOVA. Differences were tested with a LSD test implemented in the R package `agricolae` (`http://tarwi.lamolina.edu.pe/~fmendiburu/`)

Results

SNP identification by GBS

To investigate genome-wide patterns of genetic diversity in cultivated amaranth and two putative ancestors, we genotyped a diverse panel of 119 amaranth accessions from the Andean region that we obtained from the USDA genebank. The sequencing data generated with a two-enzyme GBS protocol, consisted of 210 Mio. raw reads with an average of 1.5 Mio. reads per accession (Supporting information S2). We tested for a lane effect of the Illumina flow cell, by sequencing

12 individuals on each of the three lanes used to sequence all accessions. A subsequent analysis of variance (ANOVA) of the read number did not show a lane effect (Table S1). Since a high-quality reference genome of an amaranth species was not available, we aligned reads *de novo* within the dataset to unique tags using Stacks (Catchen *et al*, 2011). The total length of the aligned reads was 16.6 Mb, which corresponds to approximately 3.3 % of the *A. caudatus* genome. For SNP calling, reads of each individual were mapped to the aligned tags. SNPs were called with parameters described in Materials and Methods, which resulted in 63,956 SNPs. Since GBS data are characterized by a high proportion of missing values, we removed SNPs with more than 60% of missing values. After this filtering step, we obtained 9,485 biallelic SNPs with an average of 35.3 % missing data for subsequent analyses (Figure S1).

Inference of population structure

To infer the genetic relationship and population structure of *A. cauduatus* and its putative ancestors, we used three different methods, ADMIXTURE, Discriminant Analysis of Principal Components (DAPC) and phylogenetic reconstruction with an uncorrected neighbor-joining network. The ADMIXTURE analysis with three predefined groups ($K = 3$), which corresponds to the number of *Amaranthus* species included in the study, did not cluster accessions by their species origin, but grouped the *A. caudatus* accessions into two distinct clusters and combined the two wild accessions *A. hybridus* and *A. quitensis* into a single cluster. This analysis indicates a clear separation between domesticated and the wild Amaranths, but the two wild amaranths appeared to be a single genetic group because with higher values of K did not lead to subdivision of the two wild species into separate clusters that corresponds to the species assignment (Figure 1). Cross-validation showed that $K = 5$ was most consistent with the data (Supplementary Figure S2), which produced three different groups of *A. caudatus* accessions that included a few wild amaranth accessions, and two wild amaranth clusters that both consist of *A. hybridus* and *A. quitensis* accessions. The two wild amaranth clusters differ by the geographic origin because one cluster contains both *A. hybridus* and *A. quitensis* accessions from Peru and the other cluster from Ecuador. This indicates a strong strong geographic differentiation among wild ancestors.

The *A. caudatus* accessions clustered into three groups that also showed geographic differenti-

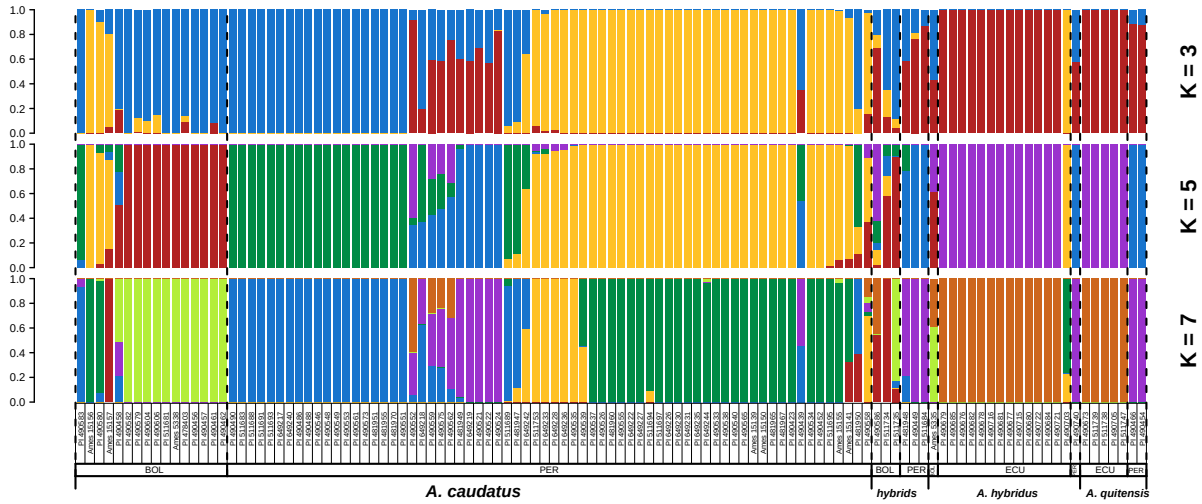

Figure 1: Model based clustering analysis with different numbers of clusters (K=3, 5, 7) with ADMIXTURE. The clusters reflect the number of species in the study (K=3), the number of single populations (species per country of origin, K=7) and the optimal number as determined by cross validation (K=5). Individuals are sorted by species and country of origin (BOL=Bolivia, PER = Peru and ECU = Ecuador) as given by their passport data.

ation. The first cluster consisted of individuals from Bolivia (Figures 2 and 1; $K = 5$, red color). *A. caudatus* accessions from Peru were split into two clusters of which one predominantly represents a region from North Peru (Huari Province; Figures 2 and 1; $K = 5$, yellow color), whereas the second cluster contains individuals distributed over a wide geographic range that extending from North to South Peru ($K = 5$, green color). Ten *A. caudatus* accessions from the Cuzco region clustered with the three accessions of wild amaranths from Peru ($K = 5$, blue color). These ten accessions showed admixture with the other cluster of wild amaranths and with a Peruvian cluster. Accessions that were labeled as 'hybrids' in their passport data, because they express a set of phenotypic traits of different species, clustered with different groups. 'Hybrids' from Bolivia were highly admixed, whereas 'hybrids' from Peru clustered with the Peruvian wild amaranths (Figure 1). Taken together, the population structure inference with ADMIXTURE identified a clear separation between the wild and domesticated amaranth species and genetic differentiation among domesticated amaranths but also gene flow between populations.

The inference of population structure with a discriminant analysis of principal components (DAPC) and Neighbor-Joining network produced very similar results as ADMIXTURE. The first

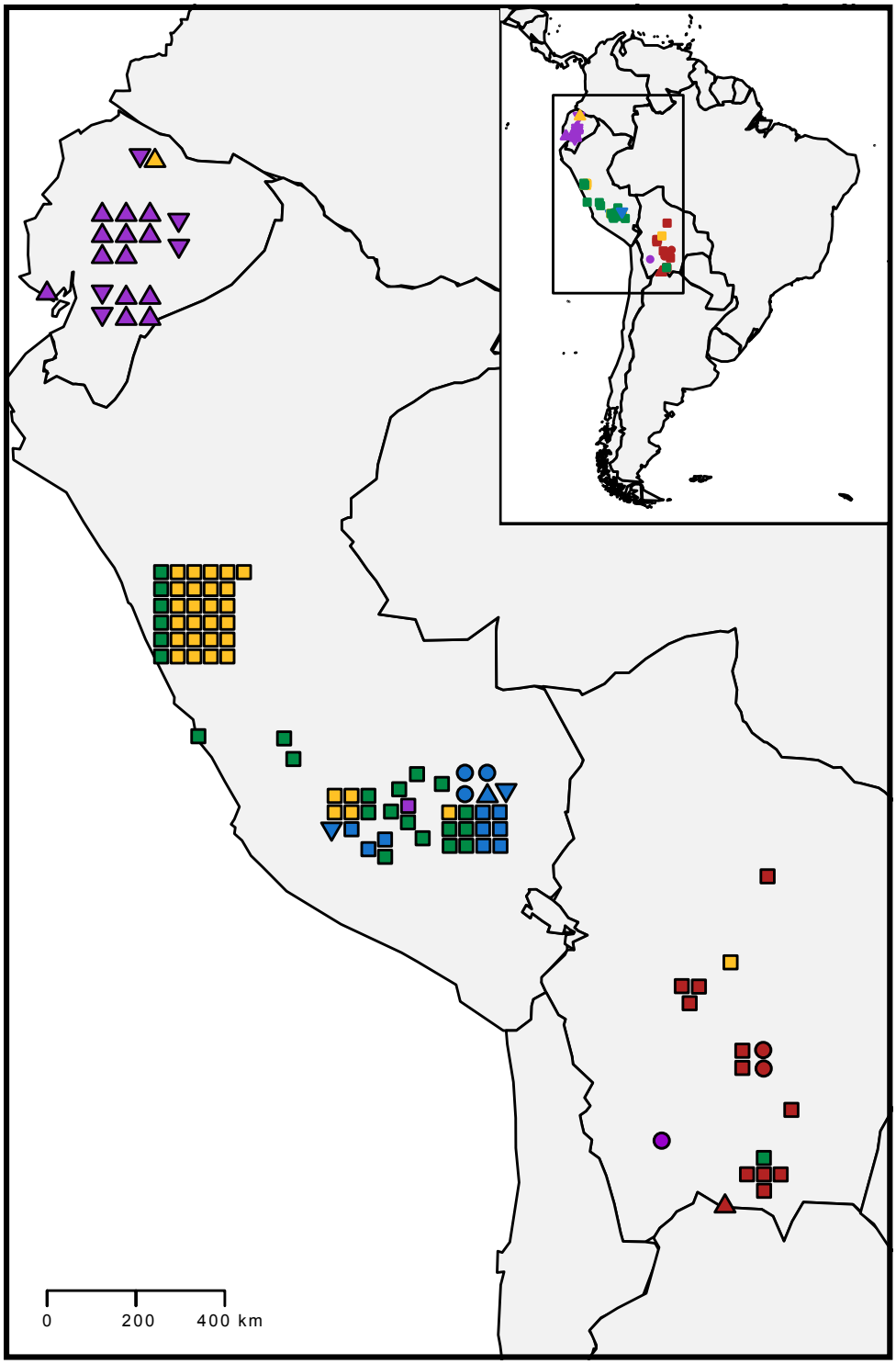

Figure 2: Geographic distribution of accessions for which data was available from passport information. Locations are not exact geographic locations because location data was given as country province . Colors are given by ADMIXTURE with K=5 (Figure 1). Species are indicated by shapes. *A. caudatus* (□), *A. hybridus* (△), *A. quitensis* (▽) and hybrids between species (○)

principal component of the DAPC analysis which we used to cluster accessions based on their species explained 96% of the variation and separated the two wild species from the domesti-

cated *A. caudatus* (Figure S3A). In a second DAPC analysis that was based on information on species and country of origin (Figure S3B) the first principal component explained 55% of the variation and separated most of the wild from the domesticated amaranths. The second principal component explained 35% of the variation and separated the Peruvian from the Bolivian *A. caudatus* accessions.

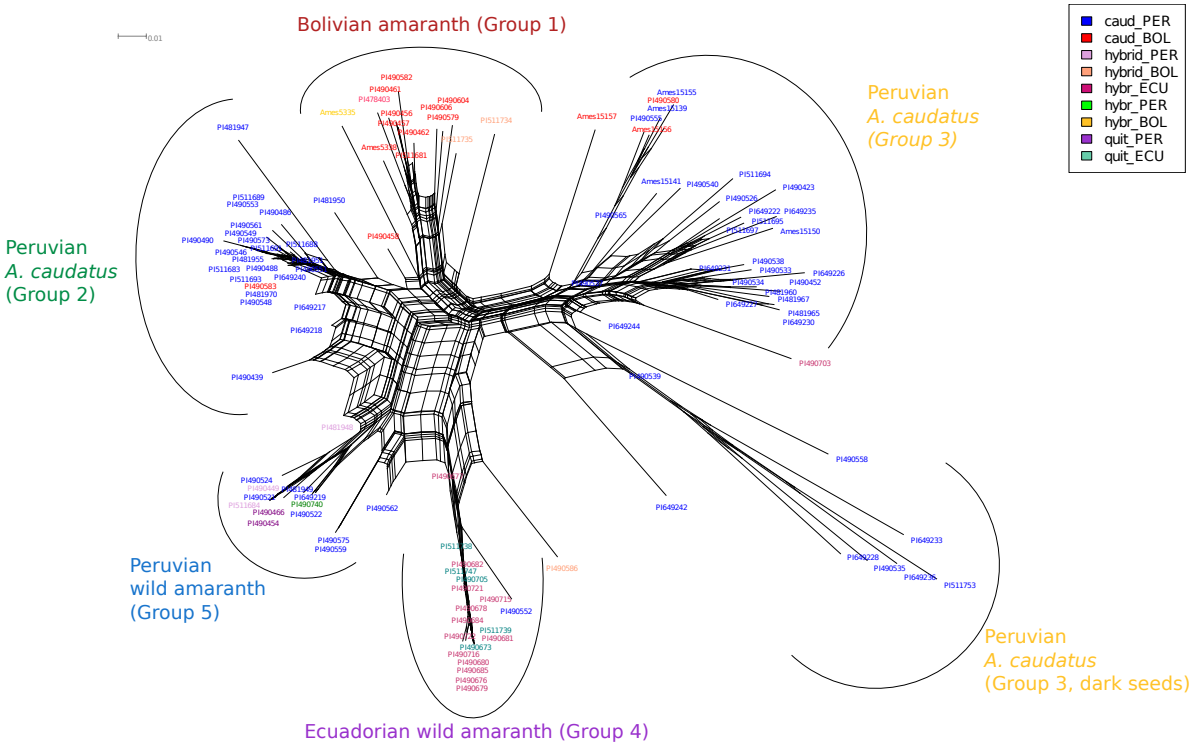

Figure 3: Neighbor-joining network of 113 amaranth accessions from six potential populations. Different colors indicate the species and origin according to gene bank information. *A. caudatus* from Peru (blue) and from Bolivia (red), *A. hybridus* from Ecuador (magenta), from Peru (green) and Bolivia (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).

The phylogenetic network outlines the relationships between the different clusters (Figure 3). It shows two distinct groups of mainly Peruvian *A. caudatus* accessions and a group of accessions with a wide geographic distribution (Figure 2; green color). The latter is more closely related to the Bolivian *A. caudatus* and the wild relatives. The strong network structure between these three groups suggests a high proportion of shared polymorphisms or a high level of recent gene flow. In contrast, the clade with *A. caudatus* accessions from Northern Peru are more separated from the other clades which indicates a larger evolutionary distance, less ongoing

gene flow with the wild ancestors or stronger selection (Figure 2; yellow color). They are split into two groups, of which the smaller includes only accessions with dark seeds. In a bifurcating phylogenetic tree, ten domesticated amaranth accessions clustered within the same clade as the wild species (Figure S4). The same clustering was also obtained with ADMIXTURE and $K = 7$ (Figure 1).

Table 1: Weir and Cockerham weighted F_{ST} estimates between populations based on the taxonomic assignment of their passport data. The group of wild amaranth 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 wild amaranth	0.322
A. hybridus x *A. quitensis*	0.041
A. caudatus (PER) x *A. caudatus* (BOL)	0.132

To quantify the level of genetic differentiation between the species and groups within *A. caudatus*, we estimated weighted F_{ST} values using the method of Weir and Cockerham (Weir & Cockerham, 1984). F_{ST} values between *A. caudatus* and the wild *A. hybridus* and *A. quitensis* species were 0.31 and 0.32, respectively (Table 1), and 0.041 between *A. hybridus* and *A. quitensis* based on the taxonomic assignment. The latter reflects the high genetic similarity of the accessions from both species observed above. Within *A. caudatus* subpopulations, the F_{ST} between *A. caudatus* populations from Peru and Bolivia was 0.132, three times higher than between *A. hybridus* and *A. quitensis*. The above analyses suggested that some individuals may be misclassified in the passport information, and we therefore calculated F_{ST} values of population sets defined by ADMIXTURE. Although such F_{ST} values are upward biased, they allow to evaluate the relative level of differentiation between groups defined by their genotypes. The comparison of F_{ST} values showed that the three *A. caudatus* groups (groups 1-3) are less distant to the Peruvian (group 5) than to the Ecuadorian wild amaranths (group 4; Table S2). A tree constructed with TreeMix, which is based on allele frequencies within groups (Figure 4), suggests gene flow from the Peruvian *A. caudatus* (group 2) to Peruvian wild amaranth (group 5) and, with a lower confidence level, between wild amaranths from Ecuador (group 4) into Bolivian *A. caudatus* (group 1), as well as from Bolivian *A. caudatus* to Peruvian *A. caudatus*

(Group 2).

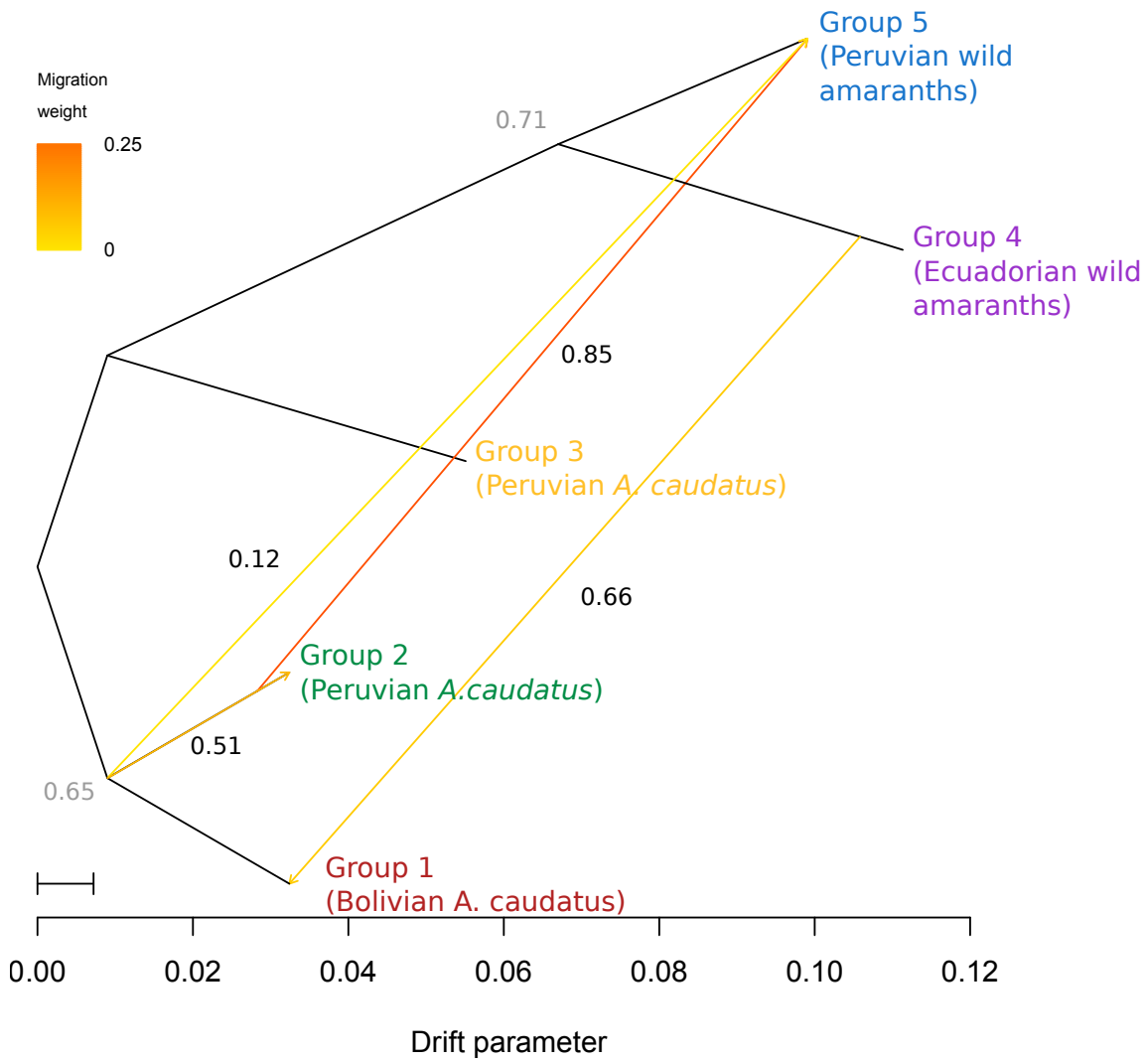

Figure 4: Tree of five genetic clusters of South American amaranths inferred with TreeMix. The genetic clusters which were used to calculate the tree were inferred with ADMIXTURE. Groups 1 to 3 represent *A. caudatus* clusters from Peru and Bolivia, group 4 represents wild amaranth form Ecuador and group 5 wild amaranth from Peru. 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.

Analysis of genetic diversity

We further investigated whether domestication reduced genetic diversity in *A. caudatus* compared to wild amaranths (Table 2). All measures of diversity were higher for the cultivated than the wild amaranths. For example, nucleotide diversity (π) was about two times higher in

A. caudatus than in the two wild species combined. The diversity values of the accessions classified as hybrids showed intermediate values between wild and domesticated populations supporting their hybrid nature. The inbreeding coefficient, F, was highest in the domesticated amaranth but did not differ from the wild amaranths combined. In contrast accessions classified as 'hybrids' and *A. quitensis* showed lower inbreeding coefficients. Within the groups of accessions defined by ADMIXTURE, genetic diversity differed substantially. The wild amaranths from Ecuador had the lowest ($\pi = 0.00031$) while the group from northern Peru showed the highest level of nucleotide diversity ($\pi = 0.00111$; Table S3). Figure 5 shows that even though the overall diversity of *A. caudatus* was higher a substantial proportion of sites were more diverse in the wild amaranths ($\pi_{caud} - \pi_{wild} < 0$; Figure 5).

Table 2: Genetic diversity parameters for the three putative *Amaranthus* species and the wild amaranth (*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	π	CI_π	H_{exp}	SD_{He}	H_{obs}	SD_{Ho}	F	SD_F	θ_w
A. caudatus	0.00117	$\pm$ 0.00002	0.175	0.167	0.049	0.140	0.688	0.462	0.00123
A. hybridus	0.00061	$\pm$ 0.00001	0.085	0.135	0.041	0.170	0.679	0.608	0.00073
A. quitensis	0.00059	$\pm$ 0.00001	0.076	0.169	0.040	0.170	0.451	0.763	0.00048
Wild amaranth	0.00062	$\pm$ 0.00002	0.090	0.140	0.041	0.166	0.681	0.591	0.00070
Hybrids	0.00091	$\pm$ 0.00001	0.112	0.179	0.060	0.173	0.436	0.645	0.00107

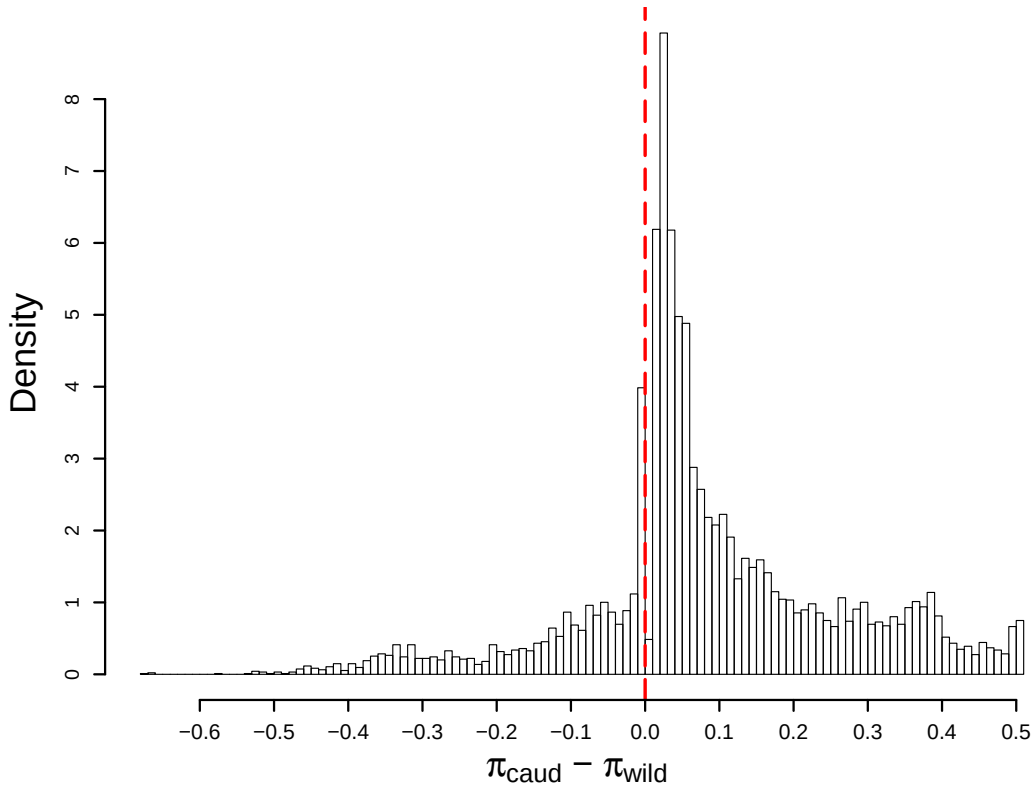

Figure 5: Per site difference in nucleotide diversity (π) between domesticated amaranth (*A. caudatus*) and wild amaranth (*A. hybridus* and *A. quitensis*)

.

Genome size in wild and cultivated amaranth

Although the genomic history of amaranth species still is largely unknown, genome sizes and chromosome numbers are highly variable within the genus *Amaranthus* (`http://data.kew.org/cvalues/`). This raises the possibility that the domestication of *A. caudatus* was accompanied by polyploidization events as observed in other crops. We therefore tested whether a change in genome size played a role in the context of domestication by measuring the genome size of multiple individuals from all three species with flow cytometry. The mean genome size of *A. caudatus* was 501.93 Mbp, and the two wild ancestors did not differ significantly from this value (Table 3) indicating that polyploidization did not play a role in the recent evolution of domesticated amaranth.

Table 3: 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

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, 2015). To investigate whether these two traits are part of the domestication syndrome in grain amaranth, we compared the predominant seed color of the different groups of accessions and measured their seed size. The seeds could be classified into three colors, white, pink and brown. The white and pink types have both a white seed coat, but the latter has red cotyledons that are visible through the translucent seed coat. A substantial number of seed samples (19) from the genebank contained seeds of other color up to a proportion of 20%. One *A. caudatus* accession from Peru (PI 649244) consisted of 65% dark seeds and 35% white seeds in the sample. No accession from the two wild species or hybrid accessions had white seeds, whereas the majority (74%) of *A. caudatus* accessions had white (70%) or pink (4%) seeds, and the remaining (26%) brown seeds (Figure 6 A). We also compared the seed color of groups defined by ADMIXTURE ($K = 5$; Figure 1), which reflect genetic relationship and may correct for mislabeling of accessions (Figure 6 B). None of these groups had only white seeds, but clusters that mainly consist of accessions from the wild relatives had no white seeds at all. In contrast to seed color, the hundred seed weight (HSW) of the different *Amaranthus* species did not significantly differ between wild and cultivated amaranths. The mean HSW of *A. caudatus* was 0.056 g and slightly higher than the HWS of *A. hybridus* (0.051 g) and *A. quitensis* (0.050 g; Figure 6 C and Table S4). Among the groups identified by ADMIXTURE ($K = 5$), one group showed a significantly higher HSW than the other groups, while the other four groups did not differ in their seed size. The group with the higher HSW consisted mainly of Bolivian *A. caudatus* accessions and had a 21 % and 35 % larger HSW than the two groups consisting

mainly of Peruvian *A. caudatus* accessions, respectively (Figure 6 D). An ANOVA also revealed that seed color has an effect on seed size because white seeds are larger than dark seeds (Table 4).

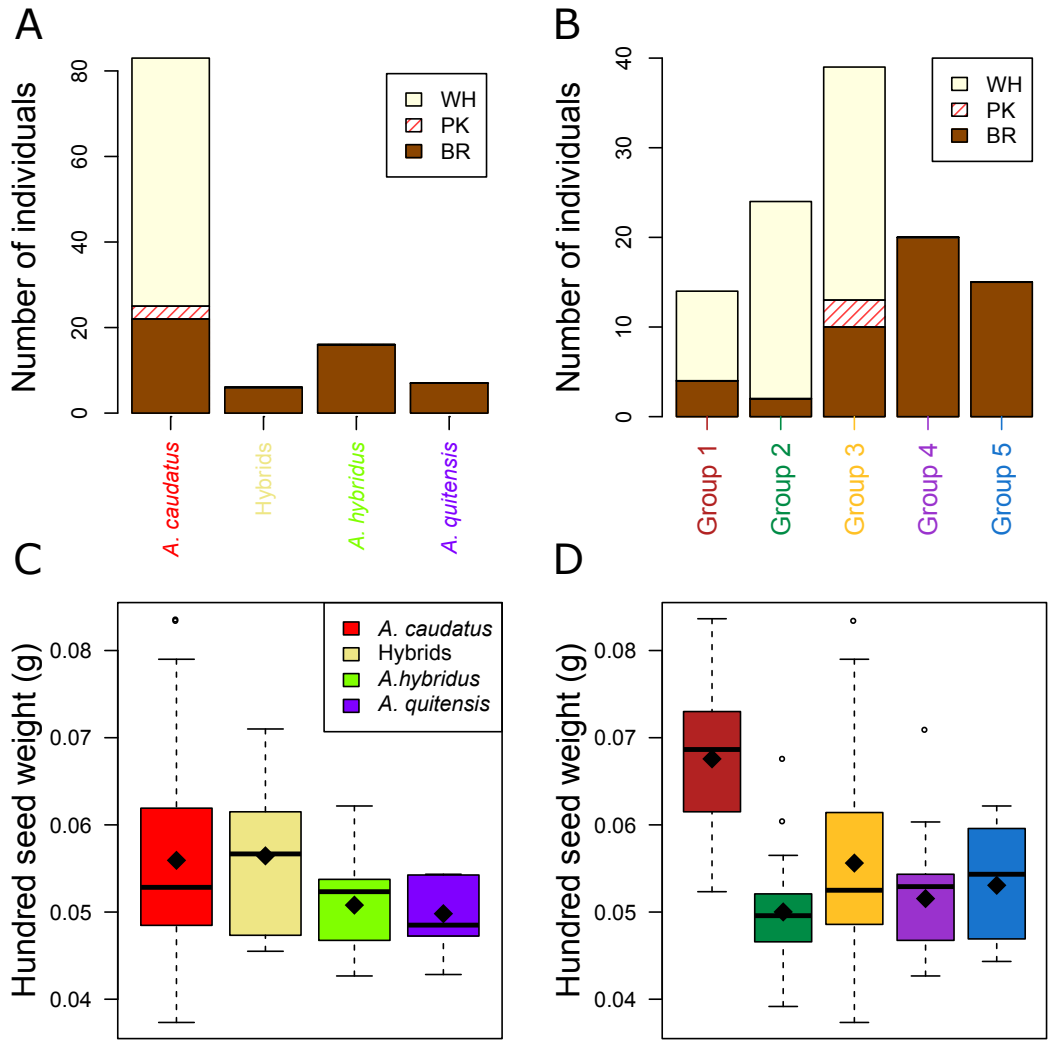

Figure 6: Hundred seed weight (**A,B**) and predominant seed color (**C,D**) by *Amaranthus* species (**A,C**) and groups identified with ADMIXTURE for K=5 (**B,D**) where group 1 (red) resembles *A. caudatus* from Bolivia, group 2 (green) and 3 (yellow) *A. caudatus* from Peru, group 4 (purple) represents wild amaranth form Ecuador and group 5 (blue) wild amaranth from Peru. 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.

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		

Discussion

Genotyping-by-sequencing of amaranth species

The genotyping of wild and cultivated amaranth accessions revealed a strong genetic differentiation between wild and cultivated amaranths and a high level of genetic differentiation within domesticated *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 an amaranth 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). Since *de novo* assembled fragments are not mapped to a reference, they are unsorted and do not allow to investigate differentiation along genomic regions but the data are suitable for the analysis of 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. The comparison of accessions and groups within a study is possible, however, because 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. Compared to previous studies on amaranth genetic diversity (Maughan *et al*, 2009, 2011; Khaing *et al*, 2013; Jimenez *et al*, 2013; Kietlinski *et al*, 2014), our study combines a larger number of accessions and more genetic markers, which allowed us to asses the genetic diversity and population structure on a genome-wide basis.

***A. quitensis* and *A. hybridus* are not different species**

The two wild relatives *A. quitensis* and *A. hybridus* do not appear to be separate species in our analyses but form two distinct subgroups of Peruvian and Ecuadorian wild amaranths that both consist of accessions from the two species. It was suggested before that *A. quitensis* is the same species as *A. hybridus* (Coons, 1978), but the passport information regarding the species of genebank accessions was not changed and *A. quitensis* is still considered as a separate species in these records. The taxonomic differentiation between the two species rests on a single morphological trait, namely the shape of the tepals, which are very small and prone to misidentification (Sauer, 1967). The high phenotypic similarity of *A. quitensis* and *A. hybridus* is supported by our analyses which showed that they are very closely related and mainly separated by their geographic origin, from Peru and Ecuador. The F_{ST} value between the two wild species was lower than between the two *A. caudatus* groups from Peru and Bolivia (Tables 1 and S2). A close relationship is also supported by the highly similar genome sizes of all three species, although the genus *Amaranthus* harbors species with very different genome sizes due to variation in chromosome numbers and ploidy levels (Baohua & Xuejie, 2002; Rayburn *et al*, 2005). In contrast to our results, a recent study found evidence for a genetic differentiation between *A. hybridus* and *A. quitensis* (Kietlinski *et al*, 2014). Thia discrepancy may result from the different composition of samples because our sample consists of accessions of both species from the Andean region whereas Kietlinski et al. (2014) used *A. hybridus* and *A. quitensis* accessions with little geographic overlap between the two species. Our F_{ST} values also indicate that Peruvian and Ecuadorian wild amaranths 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 wild amaranths from Peru ($F_{ST} = 0.553$). In summary, under the assumption that the passport information of the wild amaranths is correct, we propose that *A. quitensis* and *A. hybridus* are a single species. The high level of intraspecific differentiation in both wild and cultivated amaranth is relevant for investigating domestication because the genetic distance between groups of cultivated amaranth is related to the geographic distance of the wild ancestors.

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). In contrast, the overall genetic diversity in our sample of domesticated amaranths was higher than in the two wild 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 5). 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 domesticated grain amaranth may result from a strong gene flow between wild and domesticated amaranths. 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 1 and Table 2). Gene flow between *A. caudatus* and other *Amaranthus* species in different areas of the distribution range could explain a higher genetic diversity in the domesticated amaranth, which is also consistent with the strong network structure (Figure 3) and the TreeMix analysis (Figure 4). Taken together, cultivated *A. caudatus* is unusual in its higher overall genetic diversity compared to its putative wild ancestors, which is uncommon in domesticated crops.

Amaranth domestication syndrome

Despite its long history of cultivation, diverse uses for food and feed and its high importance during the Aztec period, grain amaranth does not display the classical domestication syndrome as strongly as other crops (Sauer, 1967). On one hand, domesticated amaranth shows morphological differentiation from wild amaranths like larger and more compact inflorescences (Sauer, 1967) and a level of genetic differentiation (Table 1) which is comparable to the level of differentiation of other domesticated crops and their wild relatives (Sunflower: 0.22 (Mandel *et al*, 2011); common bean: 0.1-0.4 (Papa *et al*, 2005), pigeonpea: 0.57-0.82 (Kassa *et al*, 2012)). On the other hand, the individual flowers of a plant do not mature synchronously and produce

very small seeds that are shattered (Brenner, 2000). In contrast to wild amaranths, which all have dark brown seeds, the predominant seed color of cultivated grain amaranth is white, which suggests that selection for seed color played a role in the history of *A. caudatus*. However, dark-seeded accessions are present in all three groups of *A. caudatus* defined by the genotypic data, which indicates that white seed color is not a fixed trait. Similarly, seed sizes between wild and domesticated amaranths are not significantly different with the exception of *A. caudatus* accessions with white seeds from Bolivia (Figure 6), which have larger seeds. The increased seed size in this group and in white seeds in general indicates past selection for domestication-related traits, but only in specific geographic regions or in certain types of amaranth, and not in the whole domesticated crop species.

Possible explanations for the incomplete fixation of domestication traits in South American grain amaranth include weak selection, genetic constraints or ongoing gene flow. First, weak selection of putative domestication traits may reflect that they were not essential for domestication. Although white seeds are predominant in cultivated amaranthe and unambigously a domestication-related trait under selection, other seed colors may have been preferred for different uses with the consequence that genes for white seed color were not fixed. Second, domestication traits may experience genetic constraints that limit phenotypic variation. Genes controlling domestication traits that are part of simple molecular pathways, have minimal pleiotropic effects, and show standing functional genetic variation have a higher chance of fixation than genes with high pleiotropic or epistatic interactions (Doebley *et al*, 2006; Lenser & Theißen, 2013). Numerous genes with these characteristics were cloned and characterized in major crops like rice, barley and maize and shown to contribute to the distinct domestication syndrome such as a loss of seed shattering, larger seed size and compact plant architecture. Since the molecular genetic basis of domestication traits in amaranth is unknown, the lack of a strong domestication syndrome and a lack of fixation of putative domestication traits despite a long period of cultivation may result from genetic constraints which limited the origin and selection of domestication phenotypes. A third explanation is ongoing gene flow between wild and domesticated amaranth that may prevent or delay the formation of a distinct domestication syndrome and contributes to the high genetic diversity (Table 2), similar seed size (Figure 6 C), and the presence of dark seeds (Figure 6) 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). In South America, wild and domesticated amaranths are sympatric over wide areas and the wild *A. hybridus* and *A. quitensis* 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 a have 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), dark seeds could have efficiently removed by selection despite gene flow. Therefore, gene flow likely is not the only explanation for the lack of a distinct domestication syndrome.

Our data are consistent with the model by Kietlinski et al. (2014) who proposed a single domestication of *A. caudatus* and *A. hypochondriacus* in Central America followed by migration of *A. caudatus* to South America. Gene flow between wild amaranths and *A. caudatus* in the Southern distribution range (Peru and Bolivia) may explain the higher genetic diversity of the latter despite a strong genetic differentiation. The second model of Kietlinski et al. of two independent domestication events from a single *A. hybridus* lineage that ranged from Central America to the Andes is supported under the assumption that domestication occurred in South Peru because of the strong differentiation between Ecuadorian and Peruvian wild amaranths (Table S2). Since the Peruvian group of wild amaranths inferred with ADMIXTURE comprises *A. quitensis* and *A. hybridus*, but also *A. caudatus* accessions, the latter may represent accessions from the center of domestication.

Conclusions

The genotypic and phenotypic analysis of wild and domesticated South American grain amaranths suggest that *A. caudatus* is an incompletely domesticated crop species. Key domestication traits such as the shape of inflorescences, seed shattering and seed size are rather similar between wild and cultivated amaranths and there is strong evidence of ongoing gene flow from

its wild ancestor despite selection for domestication traits like white seeds. Although grain amaranth is an ancient crop of the Americas, genomic and phenotypic signatures of domestication differ from other, highly domesticated crops that originated from single domestication events like maize (Hake & Ross-Ibarra, 2015). In contrast, the history of cultivated amaranth may include multiregional, multiple and incomplete domestication events with frequent and ongoing gene flow from sympatric wild relatives, which is more similar to the history of species like rice, apple or barley (Londo *et al*, 2006; Cornille *et al*, 2012; Poets *et al*, 2015). The classical model of a single domestication in a well-defined center of domestication may not sufficiently reflect the history of numerous ancient crops. Our study further highlights the importance of a comprehensive sampling to study the domestication of amaranth. All three domesticated amaranths, *A. caudatus*, *A. cruentus* and *A. hypochondriacus*, as well as all wild relatives throughout the whole distribution range should be included in further studies to fully understand and model the domestication history of Central and South American amaranth.

Acknowledgments

We thank David Brenner (USDA-ARS) and Julie Jacquemin for discussions and Elisabeth Kokai-Kota for support with the GBS library preparation and sequencing. The work was funded by an endowment of the Stifterverband für die Deutsche Wissenschaft to K. J. S.

References

Abbo S, van Oss RP, Gopher A, Saranga Y, Ofner I, Peleg Z (2014) Plant domestication versus crop evolution: a conceptual framework for cereals and grain legumes. *Trends in Plant Science*, **19**, 351 – 360.

Alexander DH, Novembre J, Lange K (2009) Fast model-based estimation of ancestry in unrelated individuals. *Genome Research*, **19**, 1655–64.

Arnold B, Corbett-Detig RB, Hartl D, Bomblies K (2013) RADseq underestimates diversity and introduces genealogical biases due to nonrandom haplotype sampling. *Molecular Ecology*, **22**, 3179–90.

Arreguez GA, Martínez JG, Ponessa G (2013) *Amaranthus hybridus* L. ssp. *hybridus* in an archaeological site from the initial mid-Holocene in the Southern Argentinian Puna. *Quaternary International*, **307**, 81–85.

Baohua S, Xuejie Z (2002) Chromosome numbers of 14 species in *Amaranthus* from China. *Acta Phytotaxonomica Sinica*, **40**, 428–432.

Begun DJ, Holloway AK, Stevens K, *et al* (2007) Population genomics: whole-genome analysis of polymorphism and divergence in *Drosophila simulans*. *PLoS Biology*, **5**, e310.

Brenner D (2000) Genetic resources and breeding of *Amaranthus*. *Plant Breeding Reviews*, **19**, 228–285.

Bryant D, Moulton V (2004) Neighbor-net: an agglomerative method for the construction of phylogenetic networks. *Molecular Biology and Evolution*, **21**, 255–65.

Catchen J, Hohenlohe PA, Bassham S, Amores A, Cresko WA (2013) Stacks: an analysis tool set for population genomics. *Molecular Ecology*, **22**, 3124–40.

Catchen JM, Amores A, Hohenlohe P, Cresko W, Postlethwait JH (2011) Stacks: building and genotyping loci *de novo* from short-read sequences. *G3 (Bethesda, Md.)*, **1**, 171–82.

Coons MP (1978) The status of *Amaranthus hybridus* L. in South America. *Cienc. Nat.*, **18**.

Cornille A, Gladieux P, Smulders MJM, *et al* (2012) New insight into the history of domesticated apple: Secondary contribution of the european wild apple to the genome of cultivated varieties. *PLoS Genetics*, **8**, e1002703.

Costea M, DeMason D (2001) Stem morphology and anatomy in *Amaranthus* L. (*Amaranthaceae*), taxonomic significance. *Journal of the Torrey Botanical Society*, **128**, 254–281.

Cruaud A, Gautier M, Galan M, *et al* (2014) Empirical assessment of RAD sequencing for interspecific phylogeny. *Molecular Biology and Evolution*, **31**, 1272–4.

Danecek P, Auton A, Abecasis G, *et al* (2011) The variant call format and VCFtools. *Bioinformatics (Oxford, England)*, **27**, 2156–8.

Doebley JF, Gaut BS, Smith BD (2006) The molecular genetics of crop domestication. *Cell*, **127**, 1309–21.

Dolezel J, Bartos J, Voglmayr H, Greilhuber J (2003) Nuclear DNA content and genome size of trout and human. *Cytometry. Part A : The Journal of the International Society for Analytical Cytology*, **51**, 127–8; author reply 129.

533 Dolezel J, Sgorbati S, Lucretti S (1992) Comparison of three DNA fliioroclirome for flow cyto-
534 metric estimatioe of nuclear DNA conteet in plants. *Physiologia Plantarum*, **85**, 625–631.

535 Elshire RJ, Glaubitz JC, Sun Q, *et al* (2011) A robust, simple genotyping-by-sequencing (gbs)
536 approach for high diversity species. *PloS ONE*, **6**, e19379.

537 Gepts P (2014) The contribution of genetic and genomic approaches to plant domestication
538 studies. *Current Opinion in Plant Biology*, **18**, 51–9.

539 Hake S, Ross-Ibarra J (2015) Genetic, evolutionary and plant breeding insights from the do-
540 mestication of maize. *eLife*, **4**, 1–8.

541 Huang YF, Poland JA, Wight CP, Jackson EW, Tinker NA (2014) Using genotyping-by-
542 sequencing (GBS) for genomic discovery in cultivated oat. *PloS ONE*, **9**, e102448.

543 Hufford MB, Lubinksy P, Pyhäjärvi T, Devengenzo MT, Ellstrand NC, Ross-Ibarra J (2013) The
544 genomic signature of crop-wild introgression in maize. *PLoS Genetics*, **9**, e1003477.

545 Huson DH, Bryant D (2006) Application of phylogenetic networks in evolutionary studies.
546 *Molecular Biology and Evolution*, **23**, 254–67.

547 Jain S, Hauptil H, Vaidya K (1982) Outcrossing rate in grain amaranths. *Journal of Heredity*,
548 **73**, 71–72.

549 Jimenez FR, Maughan PJ, Alvarez A, *et al* (2013) Assessment of genetic diversity in Peru-
550 vian amaranth (*Amaranthus caudatus* and *A. hybridus*) germplasm using single nucleotide
551 polymorphism markers. *Crop Science*, **53**, 532.

552 Jombart T, Ahmed I (2011) adegenet 1.3-1: new tools for the analysis of genome-wide SNP
553 data. *Bioinformatics (Oxford, England)*, **27**, 3070–1.

554 Jombart T, Devillard S, Balloux F (2010) Discriminant analysis of principal components: a new
555 method for the analysis of genetically structured populations. *BMC Genetics*, **11**, 94.

556 Kassa MT, Penmetsa RV, Carrasquilla-Garcia N, *et al* (2012) Genetic patterns of domestication
557 in pigeonpea (*Cajanus cajan* L. millsp.) and wild cajanus relatives. *PLoS ONE*, **7**.

558 Khaing AA, Moe KT, Chung JW, Baek HJ, Park YJ (2013) Genetic diversity and population
559 structure of the selected core set in *Amaranthus* using SSR markers. *Plant Breeding*, **132**,
560 165–173.

561 Kietlinski KD, Jimenez F, Jellen EN, Maughan PJ, Smith SM, Pratt DB (2014) Relationships
562 between the weedy (*Amaranthaceae*) and the grain amaranths. *Crop Science*, **54**, 220.

563 Kulakow P, Hauptli H, Jain S (1985) Genetics of grain amaranths I. mendelian analysis of six
564 color characteristics. *Journal of Heredity*, **76**, 27–30.

565 Lenser T, Theißen G (2013) Molecular mechanisms involved in convergent crop domestication.
566 *Trends in Plant Science*, pp. 1–11.

567 Londo JP, Chiang YC, Hung KH, Chiang TY, Schaal BA (2006) Phylogeography of asian wild
568 rice, *Oryza rufipogon*, reveals multiple independent domestications of cultivated rice, *Oryza*
569 *sativa*. *Proceedings of the National Academy of Sciences*, **103**, 9578–9583.

570 Mallory MA, Hall RV, McNabb AR, Pratt DB, Jellen EN, Maughan PJ (2008) Development and
571 characterization of microsatellite markers for the grain amaranths. *Crop Science*, **48**, 1098.

572 Mandel JR, Dechaine JM, Marek LF, Burke JM (2011) Genetic diversity and population struc-
573 ture in cultivated sunflower and a comparison to its wild progenitor, *Helianthus annuus* L.
574 *Theoretical and Applied Genetics*, **123**, 693–704.

575 Maughan P, Smith S, Fairbanks D, Jellen E (2011) Development, characterization, and linkage
576 mapping of single nucleotide polymorphisms in the grain amaranths (*Amaranthus sp.*). *The*
577 *Plant Genome Journal*, **4**, 92.

578 Maughan PJ, Yourstone SM, Jellen EN, Udall Ja (2009) SNP discovery via genomic reduction,
579 barcoding, and 454-pyrosequencing in amaranth. *The Plant Genome Journal*, **2**, 260.

580 Morris GP, Ramu P, Deshpande SP, *et al* (2013) Population genomic and genome-wide asso-
581 ciation studies of agroclimatic traits in sorghum. *Proceedings of the National Academy of*
582 *Sciences*, **110**, 453–458.

583 Nabholz B, Sarah G, Sabot F, *et al* (2014) Transcriptome population genomics reveals severe
584 bottleneck and domestication cost in the african rice (*Oryza glaberrima*). *Molecular Ecology*,
585 **23**, 2210–27.

586 Olsen KM, Wendel JF (2013) A bountiful harvest: genomic insights into crop domestication
587 phenotypes. *Annual Review of Plant Biology*, **64**, 47–70.

588 Papa R, Acosta J, Delgado-Salinas a, Gepts P (2005) A genome-wide analysis of differentiation
589 between wild and domesticated *Phaseolus vulgaris* from Mesoamerica. *Theoretical and*
590 *Applied Genetics*, **111**, 1147–1158.

591 Paradis E, Claude J, Strimmer K (2004) APE: Analyses of phylogenetics and evolution in R
592 language. *Bioinformatics*, **20**, 289–290.

593 Pickrell JK, Pritchard JK (2012) Inference of population splits and mixtures from genome-wide
594 allele frequency data. *PLoS Genetics*, **8**.

595 Poets AM, Fang Z, Clegg MT, Morrell PL (2015) Barley landraces are characterized by geo-
596 graphically heterogeneous genomic origins. *Genome Biology*, **16**, 173.

597 Poland JA, Brown PJ, Sorrells ME, Jannink JL (2012) Development of high-density genetic
598 maps for barley and wheat using a novel two-enzyme genotyping-by-sequencing approach.
599 *PloS ONE*, **7**, e32253.

600 Rayburn AL, McCloskey R, Tatum TC, Bollero GA, Jeschke MR, Tranel PJ (2005) Genome size
601 analysis of weedy species. *Crop Science*, **45**, 2557.

602 Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard RW (1984) Ribosomal DNA spacer-
603 length polymorphisms in barley: mendelian inheritance, chromosomal location, and popu-
604 lation dynamics. *Proceedings of the National Academy of Sciences of the United States of*
605 *America*, **81**, 8014–8018.

606 Sang T, Li J (2013) Molecular genetic basis of the domestication syndrome in cereals. In
607 *Cereal Genomics II* (edited by PK Gupta, RK Varshney), pp. 319–340. Springer Netherlands,
608 Dordrecht.

609 Sauer J (1967) The grain amaranths and their relatives: a revised taxonomic and geographic
610 survey. *Annals of the Missouri Botanical Garden*, **54**, 103–137.

Team RC (2014) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Trucco F, Jeschke MR, Rayburn AL, Tranel PJ (2005) *Amaranthus hybridus* can be pollinated frequently by *A. tuberculatus* under field conditions. *Heredity*, **94**, 64–70.

Weir B, Cockerham C (1984) Estimating F-statistics for the analysis of population structure. *Evolution*, **38**, 1358–1370.

Data Accessibility

The original genomic data will be available on the European Nucleic Archive (ENA). Scripts and phenotypic raw data are available under 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.