

## Original Paper

## Open Access

## Swiss maize (*Zea mays* L) landraces. Their genetic diversity and distinctiveness in a global comparison

Niclas Freitag<sup>1</sup>, David Schneider<sup>1</sup>, Céline Mir<sup>2</sup>, Peter Stamp<sup>1</sup>, Andreas Hund<sup>1</sup>, Rainer Messmer<sup>1\*</sup>

<sup>1</sup>ETH Zurich, Institute of Agricultural Sciences, Universitaetstrasse 2, 8092 Zurich, Switzerland

<sup>2</sup>INRA-CNRS-UPS-INAPG, Station de Génétique Végétale, Ferme du Moulon, 91190 Gif sur Yvette, France

\*Corresponding author: E-mail: [rainer.messmer@usys.ethz.ch](mailto:rainer.messmer@usys.ethz.ch)

### Abstract

Swiss maize landraces are expected to be genetically diverse, as they have been cultivated in different climatic regions of Switzerland for almost 500 years. A core collection of 35 Swiss maize landraces was recently defined. This core collection was analyzed in the present study, with the objectives (i) to resolve genetic diversity and phylogeny of the core collection, (ii) to relate these results to those obtained in a worldwide collection of maize landraces, thereby (iii) analyzing separation and admixture and (iv) to identify unique alleles that were detected only in Swiss maize landraces (Swiss alleles). A high diversity ( $H_r = 0.61$ ) in an international comparison and many Swiss alleles pointed at the value of this core collection as a plant genetic resource. The genetic differentiation within the core collection was in very good accordance with the geographic separation caused by the Swiss Alps. The accessions grouped into three major clusters, two northern and a southern one. Additionally, landraces from Valais built an intermediate cluster, which is probably the result of hybridization between different European germplasm. Continuous maize cultivation in remote areas may have favored genetic drift and intentional selection by farmers and may have led to this particular cluster. In the international comparison, northern Swiss accessions were related to European and American Northern Flints, whereas southern Swiss accessions were closely related to southern European Flints (e.g. Italian Orange Flints). Some northern Swiss accession combined high diversity with many Swiss alleles, which may be valuable for broadening the European Flint pool.

**Keywords:** maize landraces (*Zea mays* L), genetic diversity, phylogeny, Swiss alleles, genetic resources

### Introduction

Several thousands of maize accessions (*Zea mays* L) are currently stored in gene banks worldwide (Warburton et al, 2002; Labate et al, 2003). These genetic resources often remain untapped due to the expense of phenotyping (Ortiz et al, 2008; Hoisington et al, 1999) or because effective methods for identifying important alleles are lacking (Bhullar et al, 2009). However, these accessions are genetically diverse and may become essential if new breeding goals emerge due to changing agronomic, climatic, or socio-economic conditions (Goodman 1990). Although breeders intend to maintain allelic variation, continuous selection of favorable alleles may have led to a genome-wide loss of diversity and to a genetic shift at selected loci (Wright et al, 2005; Rafalski and Ananiev 2009). Furthermore, domestication (Doebly 2004; Tenaillon et al, 2004) and the transition from landraces to hybrids were probably important bottlenecks in the history of cultivated maize (Buckler et al, 2006; Reif et al, 2005a; Yamasaki et al, 2007).

Edwards and Leng (1965) were among the first to assess the necessity to preserve ancient local varieties as genetic resources and potential pools for prospective breeding programs. Later, Smith et al (2004) suggested using genetic resources to broaden

the genetic basis of pre-breeding programs. However, a detailed characterization is required in order to harness these resources. Large scale characterizations of national and international germplasm aimed at identifying diverse and representative subsets. As a result, a representative European core collection comprising 96 accessions with maximized allelic richness and a high phenotypic variation was defined and assessed for their potential use in breeding programs (see Gouesnard et al, 2005 for review). Further studies on American and European maize landraces analyzed the relationship between molecular and morphological variation (Rebourg et al, 2001), their genetic diversity and phylogeny (Gauthier et al, 2002) and revealed that multiple introductions of maize into Europe led to the fast dispersion of maize throughout Europe (Rebourg et al, 2003; Dubreuil et al, 2006).

A high genetic diversity in Swiss maize landraces can be assumed, as there were multiple introductions of maize into Switzerland (Koblet, 1965) with continuous cultivation for almost 500 years (Matthioli 1571; for a review see Dubreuil et al, 2006). Switzerland is located in central Europe, and presents, due to its geography, different climatic (almost mediterranean in the south, moderate in the north, and continental in

the central part) and sociocultural conditions (e.g. different languages and eating preferences), factors that probably contributed to the diversification of Swiss maize landraces over time.

Between 1930 and 1960, approximately 180 Swiss maize landraces were collected and stored in the National Gene Bank at Agroscope Changins-Wädenswil ACW (Changins, Switzerland). Based on the molecular data of 10 simple sequence repeat (SSR) markers, tested on agarose gels, [Eschholz et al \(2010\)](#) identified a northern and a southern group, which were seemingly related to northern European Flints and Italian Orange Flints, respectively ([Eschholz et al, 2010](#)). From this set, a core collection was defined, containing only 34 maize landraces (accessions) but covering 95% of the genetic diversity of the entire core collection ([Eschholz et al, 2008](#)). This core collection was analyzed thoroughly in the present study, the objectives of which were i) to assess the phylogenetic relationship and genetic diversity within the core collection with molecular markers that were optimized for diversity studies, ii) to estimate the

extent of admixture among Swiss landraces, iii) to investigate the relationship between Swiss accessions and a global set of maize accessions in view of the known routes of migration of maize, and iv) to identify particularly diverse accessions carrying unique Swiss alleles that may be valuable for pre-breeding purposes.

## Materials and Methods

### Plant material

The Swiss core collection, defined by [Eschholz et al \(2008\)](#), was complemented by an accession from the Anterior Rhine valley (AR138) to ensure that all geographic regions, from which accessions had been collected, are represented by at least one accession ([Table 1](#)). Twenty accessions of the core collection originate from north of the Alps, namely from the Anterior Rhine valley (AR), the Posterior Rhine valley (PR), the Rhine valley (RV), and the Linth valley (LV). The remaining 14 accessions originate from southern or central alpine regions, namely from the

**Table 1** - Accessions of the Swiss core collection, their regions of origin (and locations), genetic diversity ( $H_s$ ), average number of alleles per accession ( $A_N$ ), accession-specific  $F_{ST}$  values and cluster affiliation in [Figure 1](#).

Accession	Region of origin (location)	$H_s$	$A_N$	$F_{ST}$	Cluster
AR138	Anterior Rhine valley (Trins)	0.45	3.1	0.23	B
LV054	Linth valley (Schänis)	0.37	2.6	0.38	C
LV059	Linth valley (Eschenbach)	0.37	2.5	0.37	C
LV082	Linth valley (Mels)	0.32	2.3	0.45	C
PR002	Posterior Rhine valley (Sils)	0.23	1.9	0.62	B
PR003	Posterior Rhine valley (Rodels)	0.39	2.5	0.34	C
PR007	Posterior Rhine valley (Tartar)	0.38	2.7	0.36	C
PR014	Posterior Rhine valley (Realta)	0.36	2.6	0.39	C
PR015	Posterior Rhine valley (Scharans)	0.27	1.9	0.54	D
PR132	Posterior Rhine valley (Scharans)	0.43	2.8	0.27	B
RV018	Rhine valley (Pfäfers)	0.37	2.5	0.37	D
RV094	Rhine valley (Buchs)	0.38	2.9	0.36	C
RV098	Rhine valley (Eichberg)	0.35	2.5	0.41	C
RV103	Rhine valley (Au)	0.39	2.5	0.33	B
RV104	Rhine valley (Au)	0.42	2.7	0.30	B
RV141	Rhine valley (Tamins)	0.44	3.0	0.25	B
RV142	Rhine valley (Trimmis)	0.46	2.9	0.23	B
RV149	Rhine valley (Igis)	0.38	2.6	0.35	B
RV160	Rhine valley (Diepoldsau)	0.40	2.7	0.33	B
RV176	Rhine valley (Balgach)	0.38	2.7	0.36	B
RV179	Rhine valley (Mels)	0.42	2.9	0.29	B
PV125	Poschiavo valley (Brusio)	0.40	3.0	0.32	A
TM073	Ticino (Gerra)	0.32	2.3	0.45	A
TM074	Ticino (Preonzo)	0.34	2.6	0.42	A
TM079	Ticino (N.A)	0.40	2.8	0.32	A
TM116	Ticino (Mesocco)	0.33	2.6	0.44	A
TM119	Ticino (Camorino)	0.43	2.9	0.27	A
TM121	Ticino (Bodio)	0.34	2.4	0.43	A
TM139	Ticino (Aquila)	0.40	3.0	0.33	A
TM198	Ticino (Ludiano)	0.45	3.0	0.23	A
VS025	Valais (Drone)	0.40	2.7	0.33	D
VS028	Valais (Eyholz)	0.36	2.6	0.39	D
VS030	Valais (Niedergesteln)	0.32	2.1	0.46	D
VS113	Valais (Sion)	0.32	2.3	0.45	D
VS114	Valais (Sion)	0.29	2.1	0.51	D
Magister	Hybrid	0.38	2.6	0.38	D

Poschiavo valley (PV), Ticino (TM), and Valais (VS). A list of the accessions of the core collection can be found at the Swiss National Database (<http://www.bdn.ch/lists/261/content/>). The geographic regions from which accessions were sampled were illustrated by Eschholz et al (2008). The hybrid variety Magister (Syngenta, Basel, Switzerland) was evaluated together with the core collection as a control. In addition to the Swiss accessions, 131 European and 144 American landraces that had been analyzed by Dubreuil et al (2006) were also considered in this study. These accessions (passport data are available on request from A. Charcosset, [charcos@moulon.inra.fr](mailto:charcos@moulon.inra.fr)) represent European as well as northern, central and southern American maize landraces. These international accessions assured the comparison between the Swiss core collection and an international set of accessions with regards to their phylogeny and genetic variability.

#### Molecular analyses and data processing

For each accession, leaf discs of 15 plants were pierced at the 4-leaf stage, bulked, frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$ . The DNA was extracted from the bulked leaf discs according to a modified CTAB extraction protocol (CIMMYT, 2005). All Swiss accessions were genotyped at 35 SSR loci (Table 2), selected among 53 that provided a good discrimination between different germplasm and showed a high repeatability and applicability without preferential amplification of alleles (Warburton et al, 2002; Dubreuil et al, 2006). The primers were fluorescently tagged and amplified by PCR using a MJ Research PTC 225 cyclor (GMI Inc, Ramsey, MN, USA). The PCR reaction was done using 10 ng of DNA per accession. The annealing temperatures were chosen according to Warburton et al (2002). The PCR products were denaturated at  $95^{\circ}\text{C}$  for 5 min and put on ice before separating them in a capillary sequencer (ABI 3100, Applied Biosystems, Foster City, CA, USA). SSRs with different ranges of alleles and different fluorescence tags were combined for electrophoretic separation; 0.3  $\mu\text{l}$  internal lane standard (Gene Scan 350 or 500, Applied Biosystems, Foster City, CA, USA) was used as a size control. Four control populations and two inbred lines were used as allele standards for genotyping. Twelve loci were comparably scored in both, the present study (Table 2) and the study by Dubreuil et al (2006). These 12 loci were used to integrate the Swiss data set into an international context. The dataset comprising Swiss, American and European maize landraces is referred to as "combined data set". Since the data of all accessions were analyzed in the same way, Swiss alleles could be identified.

The allelic data were exported from the sequencer with the GeneScan software (v3.7) and analyzed with the Genotyper software (v3.7). The main allele peaks were often accompanied by a minor allele peak, which differed in size by less than one base pair. These false positive alleles were excluded from the

**Table 2** - SSR markers used to genotype the core collection of Swiss maize landraces, their polymorphism information content (PIC), locus specific  $F_{ST}$  value and the number of alleles per locus ( $A_N$ ).

Marker	PIC	$F_{ST} (\theta)$	$A_N$
phi008	0.53	0.51	9
phi029 <sup>#,§</sup>	0.58	0.27	9
phi031 <sup>#,§</sup>	0.68	0.46	11
phi034	0.79	0.21	13
phi041 <sup>#,§</sup>	0.52	0.43	6
phi046 <sup>#,§</sup>	0.48	0.32	6
phi056 <sup>#,§</sup>	0.51	0.42	9
phi062 <sup>#</sup>	0.66	0.46	6
phi063	0.57	0.30	9
phi065	0.78	0.24	10
phi075	0.63	0.58	9
phi076	0.64	0.54	6
phi079	0.60	0.28	5
phi083 <sup>#,§</sup>	0.69	0.47	9
phi085	0.44	0.58	5
phi102228	0.54	0.36	5
phi109188	0.27	0.48	7
phi109275	0.65	0.42	10
phi112 <sup>#</sup>	0.61	0.27	10
phi114	0.67	0.51	5
phi123	0.64	0.40	10
phi127	0.56	0.13	9
phi227562 <sup>#</sup>	0.69	0.46	9
phi308707 <sup>#,§</sup>	0.64	0.41	9
phi331888 <sup>#,§</sup>	0.66	0.11	9
phi96100 <sup>#</sup>	0.56	0.18	7
umc1161	0.65	0.40	12
umc1266	0.42	0.51	5
umc1304	0.28	0.21	7
umc1367	0.36	0.24	6
umc1447	0.47	0.63	7
umc1545	0.52	0.10	5
umc1917	0.53	0.41	8
umc2047	0.64	0.26	10
umc2250	0.32	0.41	8
Mean	0.56	0.37	8.0

Markers that were used in the combined analysis are marked with #; those amplifying Swiss alleles were additionally marked with §.

analysis. For each accession, allele frequencies were then calculated from the height of the allele peaks using the FREQS.R program (Dubreuil et al, 2006) in R (R Development Core Team, 2008). Based on these allele frequencies, 15 dummy genotypes were simulated for each accession using the FtOL.R program (Dubreuil et al, 2006; Franco et al, 2005) in R.

#### Phylogenetic relationships

The phylogenetic relationship among accessions was drawn as an unrooted phylogenetic tree based on Modified Rogers' Distances, estimated from allele frequencies per accession and calculated as described by Reif et al (2005c). Visualization was done with the DISTDIV.R software (J. Franco, personal communication, February, 2008), which makes use of the R packages ape (Paradis et al, 2004) and cluster (Maechler et al, 2005). The phylogenetic tree of the

**Table 3** - Total diversity of different subsets of the Swiss core collection, number of alleles ( $A_N$ ), relative differentiation among clusters ( $F_{ST}$ ) and Analysis of Molecular Variance (AMOVA).

Cluster	Genetic diversity	$A_N$	$F_{ST}$	AMOVA
A	0.57 ± 0.03	5.9	0.06	0.08
B	0.55 ± 0.03	6.0	0.10	0.08
C	0.51 ± 0.02	5.1	0.15	0.06
D	0.56 ± 0.03	5.0	0.07	0.08
Northern accessions	0.55 ± 0.03	6.7	0.10	0.14
Southern accessions	0.57 ± 0.03	5.9	0.07	0.08
Entire core collection	0.61 ± 0.02	8.1	0.34	0.09

Standard deviations of genetic diversity were obtained by bootstrapping.

combined data set was simplified by means of the option `use.edge.length`, which shortens the branches and equalizes the angles between accessions.

#### Diversity indices

PowerMarker was used to analyze both data sets at different hierarchical levels. At the level of the marker loci, the classical (Theta) and population-specific F statistic as well as the information about the total gene diversity (expected heterozygosity,  $H_T$ ) across all accessions and their geographic region of origin (northern Switzerland,  $H_{T,N}$ ; southern Switzerland,  $H_{T,S}$ ) was deduced with 1,000 bootstrap replications. At the population level, the within-population diversity ( $H_S$ ), analysis of molecular variance (AMOVA) and the relative differentiation of a population with respect to the entire population ( $F_{ST}$ ) were estimated. The number of alleles ( $A_N$ ) was estimated per accession (Table 1), at a given locus (Table 2) or for geographic regions (Table 3). Parameters were calculated as described by Berg and Hamrick (1997). Finally, the relative differentiation between populations of the same cluster and among clusters ( $G_{ST}$ ) was calculated according to Nei (1973), describing the proportion of diversity that is due to differences between clusters (Reif et al, 2004). Inbreeding coefficients could not be estimated because of bulked DNA.

#### Genetic structure

The simulated data of the dummy individuals were analyzed by STRUCTURE (Pritchard et al, 2000) in order to infer population structure and to assign individuals to populations and clusters (Pritchard et al, 2000). Thereby, admixture, genetic uniformity, distinctness or redundancy (duplicates) within and among accessions could be detected. The F model, allowing for correlation of allele frequencies among populations, was used for the analysis. It assumes that all populations descended from a common ancestor but experienced different amounts of genetic drift. The burn-in length was set at 10,000 and the Markov chain Monte Carlo length at 25,000 with four independent runs per assumed value of K clusters (Pritchard et al, 2000). The number of K clusters was simulated from 1 to 10 for the Swiss set and from 5 to 25 for the combined data set. The most likely value of K was chosen according to the procedure described by Evanno et al (2005).

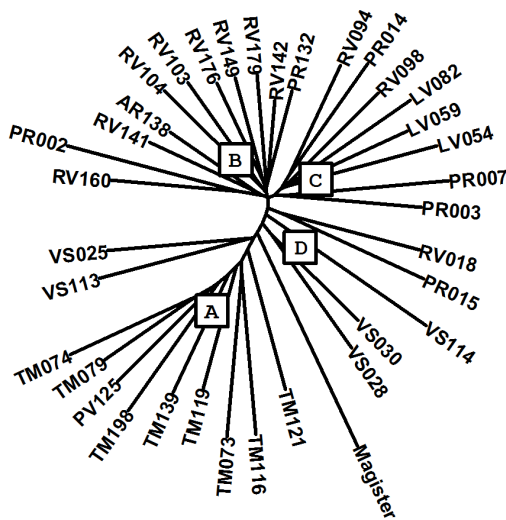
## Results

### Allelic variation and Swiss alleles

The 35 markers revealed 280 alleles in the Swiss core collection, corresponding to an average of 8.0 alleles per marker (Table 2). The number of alleles per marker ranged from 5 to 13. The polymorphism information content (PIC) varied from 0.27 to 0.79, with an average of 0.56; 6.6% of the data points were missing. Between 1.9 (PR002 and PR015) and 3.1 (AR138) alleles were amplified per individual accession (Table 1), 2.62 on average. The largest number of alleles was preserved within the group of northern Swiss accessions forming cluster B, followed by the southern cluster A (Figure 1, Table 3).

For the combined data set, the 12 SSR markers revealed a total of 139 alleles with an average of 11.6 alleles per locus (between 6 and 15) and an average PIC of 0.62 (between 0.38 and 0.82) (data not shown). Eighteen of these 139 alleles were found exclusively in Swiss accessions. Among these 18 Swiss alleles, 7 were found exclusively in northern Swiss accessions. Five of these northern accessions contained up to five Swiss alleles. In addition, there were five Swiss alleles that were found exclusively in southern Swiss accessions. In general, northern Swiss accessions contained more than twice as many Swiss alleles as southern Swiss accessions on average (2.4 vs 1.1 alleles per accession, respectively).

The overall genetic diversity of the Swiss core collection was 0.61 (Table 3). The northern groups contained the most extreme accessions in terms of genetic diversity. The diversity of a single accession ( $H_S$ ) ranged from 0.23 for PR002 to 0.46 for RV142 (Table 1), with a total diversity of northern Swiss accessions of 0.55. The genetic diversity of southern Swiss accessions was slightly higher compared to the diversity of northern Swiss accessions; however, the difference was not significant. The low  $F_{ST}$  values of the clusters A and D, which coincided with elevated diversity, suggested panmixis and an elevated amount of molecular variation. The northern cluster C, in contrast, was characterized by higher  $F_{ST}$  value, slightly lower diversity and lower molecular variation than southern accessions (Table 3). Genetic differentiation among the below-mentioned geographical clusters ( $G_{ST} = 0.34$ ) indicated that 66% of the genetic diversity resided within clusters. The population-



**Figure 1** - Genetic relationship among accessions of the Swiss core collection, based on Ward's clustering on the pairwise Modified Rogers' distance matrix. Capital letters indicate the different geographical clusters. A: southern, B and C: northern and D: central- alpine cluster.

specific  $F_{ST}$  values ranged from 0.25 (RV142) to 0.63 (PR002); the accessions PR002, PR015 and VS114 had the highest  $F_{ST}$  values ( $> 0.5$ ). High  $F_{ST}$  values generally coincided with low  $H_s$  and vice versa (Table 1). The locus-specific  $F_{ST}$  values ranged from 0.10 to 0.63 (Table 2).

The diversity of the combined data set ( $H_T = 0.67 \pm 0.03$ ) did not differ significantly from either the diversity found within the European populations ( $H_T = 0.64 \pm 0.03$ ) or the set of European and American populations together ( $H_T = 0.66 \pm 0.04$ ), both excluding Swiss accessions. Still, the tendency towards higher genetic diversity of the combined data set, including the Swiss accessions, is remarkable, given the small size of the Swiss core collection and the broadness of the international collection. Eight accessions of Swiss maize landraces were found among the most diverse 25% of all 293 accessions of the combined data set. Five of them originate from northern Switzerland (AR138, PR132, RV141, RV142, RV179), two from Valais (VS025 and VS028) and one from southern Switzerland (TM119). The latter one had the highest within-population diversity of all Swiss maize landraces in the combined data set ( $H_s = 0.55$ ). Its diversity was almost comparable to that of some Mexican germplasm in the combined analysis ( $H_s = 0.57$ ). Besides the Mexican accessions, only some northeastern European accessions exhibited greater diversity than the accessions from northern Switzerland. However, nine accessions in the Swiss core collection were found among the least diverse 25% of the combined data set, four of them originating from northern Switzerland. Accessions VS114 and PR002 exhibited particularly low diversity ( $H_s =$

0.23 and 0.18, respectively).

#### *The differentiation among Swiss accessions resembles the global differentiation between northern and southern germplasm*

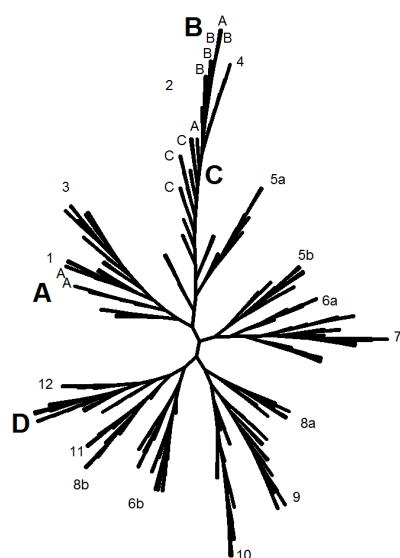
The phylogenetic tree of Swiss accessions revealed three main clusters (A, B, and C) and an intermediate cluster D (Figure 1). Cluster A contained accessions from southern Switzerland (TM and PV), and clusters B and C contained accessions from northern Switzerland (AR, LV, RV, and PR). Accessions from the central-alpine region Valais, as well as the northern accession RV018, PR015, and the hybrid variety Magister, were assigned to the intermediate cluster D (Figure 1). There were 12, 13, 6, and 10 group-specific alleles differentiating between clusters A, B, C, and D, respectively.

Although the number of markers for the combined data set was relatively low, the phylogenetic tree of the combined data set revealed five major genetic clades, differentiating between 12 clusters (Figure 2). All of them contained accessions that originate from different geographic areas. The combined analysis revealed the position of Swiss maize landraces in an international context and showed that accessions of the northern Swiss clusters B and C, as identified in Figure 1, were closely related to northern European and North American Flints (clusters 1 to 4 in Figure 2). The Swiss cluster B consisted of the same accessions in both the Swiss and the combined analysis. Four accessions that were assigned to cluster C in Figure 1 were grouped between clusters B and D in Figure 2. Accessions of the Swiss cluster D (Figure 1) were distributed among northeastern and Balkan Flints in Figure 2 (cluster 2). VS113, however, grouped together with TM198 and LV054 among accessions of the French and Spanish Pyrenees (sub-cluster 8a). Southern Swiss maize landraces of the cluster A, as identified in Figure 1, were closely related to southern European germplasm, in particular to germplasm from Italy and the Balkan (clusters 11 and 12 in Figure 2).

Given the fact that relatively few Swiss accessions from a comparably small geographic area were investigated, it is remarkable that northern Swiss accessions showed a separation that was similar to that found between Northern Flints of Europe and Northern America (clusters 1 to 4 in Figure 2).

#### *Refining the genetic grouping of northern, central and southern Swiss maize landraces*

Accessions originating from northern, southern and central Switzerland were assigned into separate STRUCTURE clusters, thereby confirming the phylogenetic analysis as displayed in Figure 1. However, the log-likelihood procedure revealed twice the number of clusters compared to the phylogenetic analysis (eight vs. four clusters, Figures 3 and 1, respectively). These eight clusters included two (6 and 7), which the hybrid variety Magister belonged to, two clusters (namely clusters 5 and 8) of northern, two



**Figure 2** - Phylogenetic tree revealing the genetic relationship among American and European (including Swiss) maize accessions, based on Ward's clustering on the pairwise Modified Rogers' distance matrix. Big, bold capitals (A to D) refer to the clusters in [Figure 1](#), small, plain letters indicate the exact position of at least two accessions belonging to the Swiss clusters A, B, C or D. Numbers 1 to 12 refer to the geographic origin of the population; 1: German Flints, 2: North Eastern European Flints including Balkan Flints, 3: North American Flints, 4: North American and German Flints (all Northern Flints), 5a/b: Central American accessions, 6a/b: Caribbean accessions, 7: South American accessions, 8a/b: Pyrenean accessions, 9: Southern and Corn Belt Dents, 10: French and Spanish Flints, 11: Italian Orange Flints, and 12: accessions from the Balkan Peninsula.

(1 and 2) of southern and one cluster (3) of mainly central Swiss accessions ([Figure 3](#)). Additionally, one cluster (4) was identified that comprised PR015 and VS114, even though their names suggested distinct regions of origin. Their close genetic relationship towards each other and the admixture with RV179 ([Figure 3](#)) revealed a separate grouping and confirmed the intermediate position between accessions of northern and central-alpine Switzerland in the phylogenetic tree ([Figure 1](#)). The analysis in STRUCTURE, in contrast, identified RV018 as more closely related to accessions from northern than to those from central-alpine Switzerland ([Figure 3](#)). Moreover, the close phylogenetic relationship between RV018 and PR015 was refined by assigning PR015 into a separate cluster (cluster 4, [Figure 3](#)). Since both accessions were sampled in close geographic proximity, the assignment into different clusters indicated a more distant relationship than expected.

Even though the sampling site identified TM121 as a southern Swiss accession, this accession shared more alleles with accessions of the central-alpine cluster D than with those of the southern Swiss cluster A. However, several northern Swiss accessions

(especially PR132) were found to be more admixed compared to southern accessions ([Figure 3](#)). Seed exchange and admixture between the regions TM and VS, on the one hand, and between PR and RV, on the other hand, were most probably the reasons for these hybridizations.

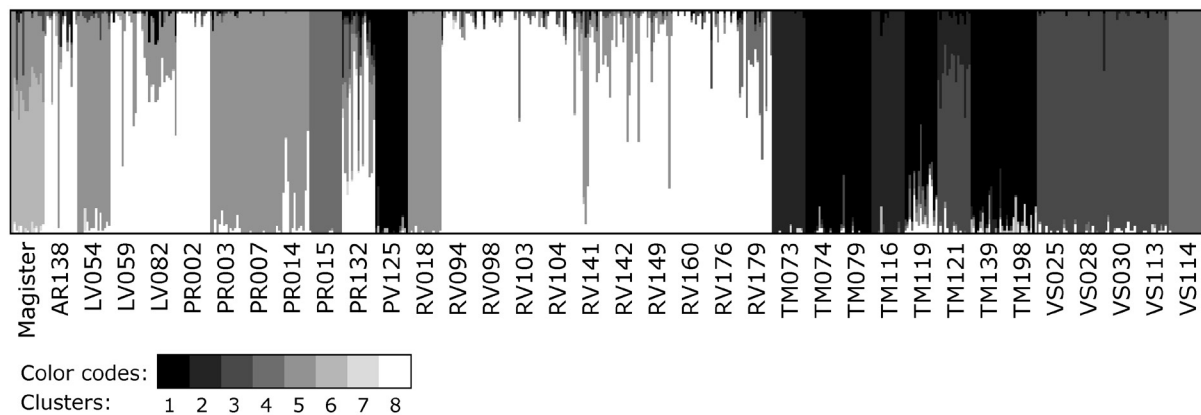
For the combined data set, log-likelihood procedure revealed an optimal value of  $K = 17$  different clusters (data not shown). The subsequent STRUCTURE analysis confirmed the genetic separation as shown in [Figure 2](#). Northern Swiss accessions grouped with accessions of central-alpine Switzerland (i.e. Valais), forming a discrete cluster. However, there was no admixture between this cluster and any other cluster of northeastern European or North American accessions. Thus, accessions of northern and central alpine Switzerland seemed to be a distinct and unique group of maize landraces. By contrast, the high admixture between accessions of southern Switzerland and Italian Orange Flints indicated a close genetic relationship between southern Swiss and southern European Flints, which is explainable by geography and socio-cultural proximity.

## Discussion

The identification of a southern (A), two northern (B and C) and an intermediate (D) cluster refined the distinction between northern and southern Swiss maize landraces as described by [Eschholz et al \(2008, 2010\)](#). The attribution of accessions from VS into a separate cluster (D), the diversity within Swiss clusters, as well as the comparison to international germplasm clearly improved previous findings on Swiss maize landraces. The used methodology thereby resulted in a better accordance between the phylogeny of Swiss maize landraces and their geographic origin.

### *Improved methodology caused comparable and representative estimates of phylogeny*

The comparison of genetic diversity and phylogeny among different studies is feasible, as [Pejic et al \(1998\)](#) showed a high correlation between results gained by different molecular marker systems. Thus, present results can be related towards other national and international analyses on maize landraces, especially when using a common set of markers that had already proven its reliability and repeatability in diversity studies in maize ([Warburton et al, 2002](#); [Reif et al, 2003](#); [Dubreuil et al, 2006](#)). Previous analyses on Swiss maize landraces included di- and oligonucleotide markers that were separated on agarose gels ([Eschholz et al, 2008, 2010](#)). Since dinucleotide repeats tend to overestimate genetic diversity and the number of alleles per locus ([Liu et al, 2003](#)), these estimates of genetic relationship and diversity may have been biased due to a higher mutation rate compared to oligonucleotide repeats ([Bachtrog et al, 2000](#)). This explains why [Eschholz et al \(2010\)](#) found considerably higher diversity ( $H_T = 0.78$ ) in the entire collection compared to a previous study in which a



**Figure 3** - Population structure of the Swiss core collection of maize landraces and the hybrid variety Magister. Each accession is represented by 15 vertical lines, revealing the estimated membership to eight genetic clusters that were defined based on log-likelihood estimation in STRUCTURE.

subset with maximized diversity was analyzed ( $H_T = 0.44$ ) (Eschholz et al, 2008). Since the methodology used for the present analysis accounted for these constraints, it enabled a more accurate and representative estimation of phylogeny and diversity among Swiss maize landraces.

#### **Central Switzerland displayed a contact zone for different germplasm.**

The genetic structure provides additional information, which the assignment of single accessions into different genetic clusters can be facilitated with. Intermediate and admixed accessions originate from central Switzerland. As central Switzerland is connected with northern and southern Switzerland by multiple mountain passes and trading routes, this region may have served as a contact zone in which maize cultivation favored seed admixture, migration and hybridization between germplasm of different regions. Accordingly, intermediate accessions (cluster 4 in Figure 3) may represent germplasm that resulted from such a contact zone, neither grouping with northern, nor with southern Swiss germplasm. Thus, labeling of accessions has to be reconsidered as it does not provide reliable means to distinguish among accessions of different origin. Subsequent cultivation in more remote valleys of the Valais may have led to genetic drift that caused a distinct group of maize landraces (cluster D, Figure 1), which is characterized by little admixture with other accessions (Figure 3). Genetic drift is most likely, since intentional selection by farmers prior to sampling may have favored the appearance of a distinct and less diverse group of accessions. The combination of phylogenetic data, genetic structure and the geographic proximity of sampling sites facilitated both, the detection of hybridization between germplasm of different origin and the separation between apparently related accessions.

In the combined analysis, accessions of cluster D grouped separately to those of clusters A, B and C. They were found to be closely related to accessions belonging to the Balkan Peninsula. Since Pyrenees-

Galicía Flints resulted from the hybridization between gene pools of North America (Northern Flints) and the Caribbean (Rebourg et al, 2003; Dubreuil et al, 2006), the joint position of VS113 and those of the French and Spanish Pyrenean Flint (Figure 2) indicated that a similar hybridization between different gene pools may have occurred in Switzerland as well. Finally, the co-location of southern European and southern Swiss accessions demonstrated a close relationship between TM and the Italian Orange Flints, as suggested by Eschholz et al (2010).

#### **Swiss maize displays a rich source of genetic diversity**

The greatest diversity of maize is undoubtedly found in populations from the highlands and lowlands of Mexico ( $H_T = 0.69$ , Matsuoka et al, 2002). Vigouroux et al (2005) found great diversity in pre-Columbian maize landraces ( $H_T = 0.64$ ) and teosinte ( $H_T = 0.74$ ). Reif et al (2004) reported  $H_T$  values of 0.56, 0.61, and 0.61 for tropical, subtropical, and temperate maize populations, respectively; Reif et al (2006) 0.61 for Mexican landraces. Thus, the genetic diversity of both the combined data set and the set of Swiss maize landraces of the present study are in agreement with literature. Also the number of alleles that were preserved within each phylogenetic cluster of the Swiss core collection was comparable to the number of alleles that were preserved within native Mexican maize landraces (Matsuoka et al, 2002). Besides, it was in agreement to what was previously described by Eschholz et al (2008, 2010) for a larger set of Swiss maize landraces.

The genetic diversity of the Swiss core collection was higher compared to that found by Labate et al (2003) for open-pollinating Corn Belt Dents ( $H_T = 0.53$ ), Northern Flints ( $H_T = 0.42$ ) and Southern Dents ( $H_T = 0.54$ ) and almost as high as that found in Mexican maize landraces ( $H_T = 0.67$ ) (Matsuoka et al, 2002). Twice as many Swiss alleles were maintained within northern compared to southern Swiss maize landraces. Especially northern Swiss accessions AR138,

PR132, RV141, RV142 and RV179 (all contained in cluster B) combined a high diversity with many Swiss alleles. These findings are quite remarkable, given the small size of the Swiss core collection. The diversity of northern Swiss accessions was higher than that found in some European flint maize populations ( $H_T = 0.53$ ) (Reif et al, 2005b) and northeastern European populations ( $H_T = 0.50$ ) (Rebourg et al, 2001). Labate et al (2003), Rebourg et al (2003) and Gauthier et al (2002) reported higher diversity for southern European accessions than for northern ones. Rebourg et al (2003) suggested selective adaptation as a genetic bottleneck during the introduction of maize into Europe, explaining the reduced diversity of northern European populations compared to American ones. The southern Swiss accessions analyzed herein, in contrast, did not have significantly higher diversity than the northern ones. Considering the lower number of southern Swiss accessions, compared to the number of northern Swiss accessions, and the relatively small size of the core collection, it is, however, not possible to finally disprove that the southern Swiss accessions are genetically more diverse than northern ones. However, present results differed from the limited diversity of an accession from the Rhine valley ( $H_T = 0.16$ ) that was reported by Reif et al (2005b) and of the Swiss 'Rheintaler' PPS 94 analyzed by Rebourg et al (2001) ( $H_T = 0.37$ ).

***Differentiation between northern Swiss accessions coincides with those of European and North American Flints.***

The total genetic differentiation among clusters of the Swiss core collection ( $G_{ST} = 0.34$ ) was comparable to the differentiation that was found among European populations ( $G_{ST} = 0.35$ ) (Rebourg et al, 2003) but higher than that found among 47 Corn Belt Dents ( $G_{ST} = 0.15$ ) (Labate et al, 2003). Since the diversity within the Swiss data set resulted from fewer accessions compared to the two above mentioned studies, the amount of diversity within the Swiss collection resulted rather from variation within clusters and regions than from variation between clusters and regions. Thus, random mating and allogamy within clusters and regions were the driving forces that created the amount of preserved diversity.

The phylogeny of the Swiss core collection reflected the genetic differentiation found in large-scale studies including many European and American maize populations. This is remarkable, given the small area of Switzerland and the small size of the Swiss core collection. The phylogenetic analysis of the combined dataset revealed a close relationship between accessions of northern and central Switzerland and Northern Flints of Europe and northern America. The separation within the Northern Flint populations into clusters 1 and 3, on the one hand, and into clusters 2 and 4, on the other hand, is in agreement with Rebourg et al (2001) and Gauthier et al (2002). Swiss accessions in cluster B (Figure 1) tended to co-locate

with accessions belonging to European Flint (clade 2 and 4, Figure 2), whereas cluster C was more closely related to German Flints as described by Rebourg et al (2001, 2003). The Swiss 'Rheintaler', as analyzed by Rebourg et al (2001, 2003), co-located with 'Jaune de Bade' ('Gelber Badischer Landmais') in cluster 4 (Figure 2), which also consisted of North American Flints.

***Swiss maize landraces may be used to broaden European Flint breeding pools.***

Relatively few European open-pollinating varieties were used for the development of European Flint first-cycle inbred lines (Messmer et al, 1992) and only few first-cycle inbred lines were developed from Swiss maize landraces (M. Menzi, personal communication, 2009). Since there were few alleles in common between northern and central Swiss clusters on the one side and Northern Flints of Europe and America on the other side (data not shown), accessions from northern and central Switzerland display an underexplored source of unique, Northern-Flint related germplasm with many Swiss alleles. Since Swiss maize landraces are underrepresented in European collections (cf., Gauthier et al, 2002), there is a high probability that beneficial alleles were successfully preserved within this collection. Additional allelic variation, already adapted to temperate climates, is highly desirable as it may contribute to enlarging the genetic base of the Northern Flints, counteracting the bottlenecks caused by adaptation to temperate climate and dispersion of maize across Europe (Rebourg et al, 2003; Dubreuil et al, 2006). In particular, accessions of cluster B (Table 1, Figure 1) display a promising start to harness Swiss germplasm as a plant genetic resource. These accessions should be considered for allele-mining projects in order to detect allelic variation for known genes (see Bhullar et al, 2009 with reference to wheat). Thus, the present core collection is a potential source of untapped allelic variation that may be useful to comply with future breeding goals.

***Conclusions***

The phylogenetic analysis revealed four clusters, each containing accessions that were sampled in different geographic regions in Switzerland. The detection of admixture among accessions of an intermediate cluster was remarkable, as it suggested hybridization between different gene pools in Switzerland. Genetic drift and intentional selection prior to the collection of germplasm may have been additional causes for an intermediate cluster. The analysis of an internationally representative set of maize landraces demonstrated a close relationship among Flints from northern Switzerland, northern Europe and North America. Furthermore, the combined analysis proved that some Swiss maize landraces are highly diverse in an international comparison and that the Swiss core collection contains unique Swiss alleles. Thus, our results clearly improved and complemented earlier



results of studies on Swiss maize landraces. Swiss maize landraces harbor allelic diversity that should be preserved and used to broaden the genetic base of Flint breeding pools. Particular alleles may become essential for future crop improvement, especially if they provide the variation that is required to confer certain traits (e.g. the tolerance against biotic and abiotic stresses). Genome sequencing of the core collection would allow breeders and geneticists to mine such alleles and to study their effect under field and lab conditions. New allelic variation may thereafter be introduced into breeding programs using a pre-breeding approach via double haploids production and marker assisted backcrossing.

### Acknowledgements

The authors would like to thank the Swiss Federal Office for Agriculture (FOAG, Bern, Switzerland) for funding this project within the framework of the National Action Plan for the Conservation and Sustainable Use of Plant Genetic Resources for Food and Agriculture (NAP-PGREL). Special thanks appertain to A Charcosset for providing data of the global set of maize landraces, as well as to ML Warburton and J Franco for assistance in molecular and statistical analyses.

### References

- Bachtrog D, Agis M, Imhof M, Schlötterer C, 2000. Microsatellite Variability Differs Between Dinucleotide Repeat Motifs - Evidence from *Drosophila melanogaster*. *Mol Biol Evol* 17(9): 1277-1285
- Berg EE, Hamrick JL, 1997. Quantification of genetic diversity at allozyme loci. *Can J For Res* 27: 415-424
- Bhullar NK, Street K, Mackay M, Yahiaoui N, Keller B, 2009. Unlocking wheat genetic resources for the molecular identification of previously undescribed functional alleles at the *Pm3* resistance locus. *PNAS USA* 106: 9519-9524
- Buckler ES, Gaut BS, McMullen MD, 2006. Molecular and functional diversity of maize. *Curr Opin Plant Biol* 9: 172-176
- CIMMYT, 2005. Laboratory Protocols: CIMMYT Applied Molecular Genetics Laboratory. Third Edition. Mexico, DF: CIMMYT. ISBN: 968-6923-30-6
- Doebley JF, 2004. The Genetics of Maize Evolution. *Annu Rev Genet* 38: 37-59
- Dubreuil P, Warburton M, Chastanet M, Hoisington D, Charcosset A, 2006. More on the introduction of temperate maize into Europe: large-scale bulk SSR genotyping and new historical elements. *Maydica* 51: 281-291
- Edwards RJ, Leng ER, 1965. Classification of some indigenous maize collections from southern and southeastern Europe. *Euphytica* 14: 161-169
- Eschholz TW, Peter R, Stamp P, Hund A, 2008. Genetic diversity of Swiss maize (*Zea mays* L ssp *mays*) assessed with individuals and bulks on agarose gels. *Genet Resour Crop Ev* 55: 971-983
- Eschholz TW, Stamp P, Peter R, Leipner J, Hund A, 2010. Genetic structure and history of Swiss maize (*Zea mays* L ssp *mays*) landraces. *Genet Resour Crop Ev*. doi: 10.1007/s10722-009-9452-0
- Evanno G, Regnaut S, Goudet J, 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Mol Ecol* 14: 2611-2620
- Franco J, Warburton M, Dubreuil P, Dreisigacker S, 2005. User's manual for the FREQS-R program for estimating allele frequencies for fingerprinting and genetic diversity studies using bulked heterogeneous populations. CIMMYT, Mexico
- Gauthier P, Gouesnard B, Dallard J, Redaelli R, Rebourg C, Charcosset, Boyat A, 2002. RFLP diversity and relationships among traditional European maize populations. *Theor Appl Genet* 105: 91-99
- Goodman MM, 1990. Genetic and Germplasm Stocks Worth Conserving. *J Hered* 81: 1-16
- Gouesnard B, Dallard J, Bertin P, Boyat A, Charcosset A, 2005. European maize landraces: Genetic diversity, core collection definition and methodology of use. *Maydica* 50: 225-234
- Hoisington D, Khairallah M, Reeves T, Ribaut JM, Skovmand B, Taba S, Warburton M, 1999. Plant genetic resources: What can they contribute toward increased crop productivity? *Proc Natl Acad Sci USA* 96: 5937-5943
- Koblet, R, 1965. *Der landwirtschaftliche Pflanzenbau, unter besonderer Berücksichtigung der schweizerischen Verhältnisse*. Birkhäuser Verlag, Basel
- Labate A J, Lamkey K R, Mitchell S E, Kresovich S, Sullivan H, Smith J S C, 2003. Molecular and Historical Aspects of Corn Belt Dent Diversity. *Crop Sci* 43: 80-91
- Liu K, Goodman M, Muse S, Smith JS, Buckler E, Doebley J, 2003. Genetic structure and diversity among maize inbred lines as inferred from DNA microsatellites. *Genetics* 165: 2117-2128
- Maechler M, Rousseu P, Struyf A, Hubert M, 2005. Cluster Analysis Basics and Extensions. Maintained by [maechler@stat.math.ethz.ch](mailto:maechler@stat.math.ethz.ch)
- Matsuoka Y, Vigouroux Y, Goodman MM, Sanchez Garcia J, Buckler E, Doebley J, 2002. A single domestication for maize shown by multilocus microsatellite genotyping. *PNAS* 99: 6080-6084
- Matthioli PA, 1571. *Compendium de plantis omnibus*. In: *Officina Valgrisiana*, Venice
- Messmer MM, Melchinger AE, Boppenmaier J, Brunklaus-Jung E, Herrmann RG, 1992. Relationships among early European maize inbreds: I. Genetic diversity among flint and dent lines revealed by RFLPs. *Crop Sci* 32: 1301-1309
- Nei M, 1973. The theory and estimation of genetic distance, pp45-54. In: *Genetic Structure of Populations*. Morton NE eds. University Press of Ha-

- waii, Honolulu
- Ortiz R, Crossa J, Sevilla R, 2008. Minimum resources for phenotyping morphological traits of maize (*Zea mays* L) genetic resources. *Plant Genetic Resources: Characterization and Utilization* 6(3): 195-200 doi:10.1017/S1479262108994168
- Paradis E, Claude J, Strimmer K, 2004. APE: Analysis of phylogenetics and evolution in R language. *Bioinformatics* 20: 289-290
- Pejic I, Ajmone-Marsan P, Morgante M, Kozumplick V, Castiglioni P, Taramino G, Motto M, 1998. Comparative analysis of genetic similarity among maize inbred lines detected by RFLPs, RAPDs, SSRs, and AFLPs. *Theor Appl Genet* 97: 1248-1255
- Pritchard JK, Stephens M, Donnelly P, 2000. Inference of population structure using multilocus genotype data. *Genetics* 155: 945-959
- Rafalski A, Ananiev E, 2009. Genetic diversity, linkage disequilibrium and association mapping, pp 201-219. In: *Handbook of maize: genetics and genomics*. Bennetzen JL, Hake SC eds. Springer, New York
- R Development Core Team, 2008. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org/>
- Rebourg C, Gouesnard B, Charcosset A, 2001. Large scale molecular analysis of traditional European maize populations. Relationships with morphological variation. *Heredity* 86: 574-587
- Rebourg C, Gouesnard B, Welcker C, Dubreuil P, Chastanet M, Charcosset A, 2003. Maize introduction into Europe: the history reviewed in the light of molecular data. *Theor Appl Genet* 106: 895-903
- Reif JC, Melchinger AE, Xia XC, Warburton ML, Hoisington DA, Vasal SK, Beck D, Bohn M, Frisch M, 2003. Use of SSRs for establishing heterotic groups in subtropical maize. *Theor Appl Genet* 107: 947-957
- Reif JC, Xia XC, Melchinger AE, Warburton ML, Hoisington DA, Beck D, Bohn M, Frisch M, 2004. Genetic diversity determined within and among CIMMYT maize populations of tropical, subtropical, and temperate germplasm by SSR markers. *Crop Sci* 44: 326-334
- Reif JC, Hamrit S, Heckenberger M, Schipprack W, Maurer H, Bohn M, Melchinger A, 2005a. Trends in genetic diversity among European maize cultivars and their parental components during the past 50 years. *Theor Appl Genet* 111: 838-845
- Reif JC, Hamrit S, Heckenberger M, Schipprack W, Maurer PH, Bohn M, Melchinger AE, 2005b. Genetic structure and diversity of European flint maize populations determined with SSR analysis of individuals and bulks. *Theor Appl Genet* 111: 906-913
- Reif JC, Melchinger AE, Frisch M, 2005c. Genetical and mathematical properties of similarity and dissimilarity coefficients applied in plant breeding and seed bank management. *Crop Sci* 45: 1-7
- Reif JC, Warburton ML, Taba S, Hoisington D, Crossa J, Franco J, Xia X, Muminovic J, Bohn M, Frisch M, Melchinger AE, 2006. Grouping of accessions of Mexican landraces of maize revisited with SSR markers. *Theor Appl Genet* 113: 177-185
- Smith JSC, Duvick DN, Smith OS, Cooper M, Feng L, 2004. Changes in Pedigree Backgrounds of Pioneer Brand Maize Hybrids Widely Grown from 1930 to 1999. *Crop Sci* 44: 1935-1946
- Tenaillon MI, U'Ren J, Tenaillon O, Gaut BS, 2004. Selection versus demography: a multilocus investigation of the domestication process in maize. *Mol Biol Evol* 21: 1214-1225
- Vigouroux Y, Mitchell S, Matsuoka Y, Hamblin M, Kresovich S, Smith JSC, Jaqueth J, Smith OS, Doebley J, 2005. An analysis of genetic diversity across the maize genome using microsatellites. *Genetics* 169: 1617-1630
- Warburton ML, Xianchun X, Crossa J, Franco J, Melchinger AE, Frisch M, Bohn M, Hoisington D, 2002. Genetic Characterization of CIMMYT Inbred Maize Lines and Open Pollinated Populations Using Large Scale Fingerprinting Methods. *Crop Sci* 42: 1832-1840
- Wright SI, Vroh Bi I, Schroeder SG, Yamasaki M, Doebley JF, McMullen MD, Gaut BS, 2005. The Effects of Artificial Selection on the Maize Genome. *Science* 308: 1310-1314
- Yamasaki M, Wright SI, McMullen MD, 2007. Genomic Screening for Artificial Selection during Domestication and Improvement in Maize. *Ann Bot-London* 100: 967-973