Analysis of the genus *Zea* (Poaceae) using polymorphic chloroplast simple sequence repeats

Jim Provan¹, Pat Lawrence¹, George Young¹, Frank Wright², Robert Bird³, GianPaolo Paglia⁴, Federica Cattonaro⁴, Michele Morgante⁴, and Wayne Powell¹

¹Department of Cell and Molecular Genetics, Scottish Crop Research Institute, Invergowrie, Scotland, UK
²Biomathematics and Statistics Scotland, Scottish Crop Research Institute, Invergowrie, Scotland, UK
³IICD, Raleigh, NC, USA
⁴Dipartimento di Produzione Vegetale e Tecnologie Agrarie, Università di Udine, Udine, Italy

Received May 20, 1998
Accepted August 29, 1998

**Abstract.** We have used polymorphic chloroplast simple sequence repeats (cpSSRs) to analyse levels of diversity and relationships within the genus *Zea*. Between two and nine alleles were found at 15 polymorphic loci and combining the data from these loci gave 32 haplotypes in the 37 accessions studied. Genetic differentiation between the two sections within the genus was calculated using the $\rho_{ST}$ statistic which showed that ~70% of the total variation was found to exist between the sections. A phylogenetic analysis based on the $\delta^{2}$ distance metric showed a large split between the two sections and suggested multiple origins of modern cultivated maize *Zea mays* subsp. *mays*. The agreement of the phylogenetic tree with other molecular, morphological and karyological studies suggests that cpSSRs may have value in phylogenetic studies in plants.

**Key words:** Poaceae, *Zea* chloroplast, simple sequence repeats, diversity.

The genus *Zea*, which includes maize and its wild relatives, is probably the most diverse of all crops. It is divided into sections *Zea* and *Luxuriantes*, the former containing *Zea mays* and the latter comprising *Z. perennis* and *Z. diploperennis*. Of the American members of the Maydeae tribe, maize (*Z. mays* subsp. *mays*) and teosinte (*Z. mays* subspp. *mexicana*, *parviglumis* and *huehuetenangensis*) share the same chromosome number (2$n = 20$) and many morphological features, but differ greatly in the structure of their female inflorescences and in their chromosome knob patterns (McClintock et al. 1981).

Five hypotheses concerning the evolution of maize and teosinte are still considered worth investigating. The first involves the descent of both maize and teosinte from a common ancestor before the domestication of maize (Weatherwax 1954). A second, more widely held hypothesis (de Wet et al. 1971, Galinat 1971, Doebley 1990), suggests that maize evolved monophyletically from teosinte. This is largely based on the considerable cytogenetic, isozyemic and morphological (male tassel and plant) similarity of maize to Mexican annual teosinte (MAT). Whilst maize and teosinte share many cytogenetic and some morphological features, other morphological features clearly differentiate the two and
Mangelsdorf (1974) has highlighted the fact that there is no archaeological evidence to support this theory and that teosinte is much more similar to modern maize than it is to the oldest maize samples from both Central and South America. Consequently, Mangelsdorf suggested a third hypothesis: that teosinte is descended from maize. Wilkes (1977) has suggested that a small early domesticate hybridised with a member of genus *Zea* section *Luxuriantes*, forming a highly variable hybrid swarm from which modern maize and MAT emerged, followed by continued introgression. This theory, which combines elements of the first three hypotheses, would appear to be supported by the fact that the early samples from South and Central America are purely maize in form, whilst also explaining the similarities between maize and MAT. Proponents of the fifth model (Kato 1976) argue for more than one domestication of maize, with the ancestors being teosinte or maize in form, largely because the high variability of maize often resolves into a bipolar pattern.

Molecular characterisation of maize accessions has been carried out using RFLPs (Dudley et al. 1991, Melchinger et al. 1991, Mumm and Dudley 1994) and RAPDs (Hahn et al. 1995, Novak et al. 1996, Zhang et al. 1996, Lanza et al. 1997). Kantety et al. (1995) measured levels of diversity in popcorn using inter-simple sequence repeat (ISSR) amplification, while several studies have been carried out using sequence-tagged SSRs to characterise maize (Senior and Heun 1993, Chin et al. 1996, Phelps et al. 1996, Taramino and Tingey 1996, Smith et al. 1997). DNA sequencing studies have revealed that there is enormous variation not only within the genus but within many of the species and subspecies (Gaut and Clegg 1991, 1993). Length polymorphism at chloroplast simple sequence repeat (cpSSR) loci provides a high-resolution assay for population, genotyping and systematics studies (Powell et al. 1995a, b; 1996). We have previously used cpSSRs to study the relationships between wild and cultivated rice (*Oryza* spp.) and to infer the origins of polyploid complexes within the genus (Provan et al. 1996, 1997).

In this study, polymorphism detected at 15 polymorphic cpSSR loci was used to analyse diversity in maize and to study the relationships between species and subspecies within the genus *Zea*.

### Materials and methods

#### Plant material

Accessions representing 37 maize and teosinte races were obtained from the CIMMYT maize germplasm bank. DNA was extracted from two-week-old maize seedlings using the Nucleon DNA extraction kit (Scotlab). The accessions studied, along with their CIMMYT accession numbers and information on geographical origin are given in Table 1.

#### Primer design and polymerase chain reaction

**Plant material.** Accesions representing 37 maize and teosinte races were obtained from the CIMMYT maize germplasm bank. DNA was extracted from two-week-old maize seedlings using the Nucleon DNA extraction kit (Scotlab). The accessions studied, along with their CIMMYT accession numbers and information on geographical origin are given in Table 1.

**Primer design and polymerase chain reaction.** Primers were designed to amplify 39 SSRs within the completely sequenced maize chloroplast genome (Maier et al. 1995) using the computer program PRIMER (V0.5). SSR loci were designated as regions of at least ten mononucleotides or at least five di- or trinucleotides and information on maize cpSSR loci and primer sequences is given in Table 2. Fifteen of these were used for the analysis and are listed in Table 1.

For primers ZMCP6359, ZMCP12968, ZMCP18704 and ZMCP20597, PCR was carried out in a total volume of 10 μl containing 1 × PCR buffer (10 mM Tris-HCl, 1.5 mM MgCl₂, 50 mM KCl, pH 8.3), 200 μM dNTPs, 10 pmol [³²P]-ATP end-labelled forward primer, 10 pmol reverse primer, 0.5 U *Taq* polymerase (Promega) and 50 ng genomic DNA. Reactions were carried out on a MJ Research PTC 200 DNA Engine thermal cycler using the following parameters: (a) initial denaturation at 94 °C for 3 min; (b) 30 cycles of denaturation at 94 °C for 15 s, annealing at [Tₐ] for 15 s and extension at 72 °C for 60 s; (c) final extension at 72 °C for 5 min, for [Tₐ] values see Table 2. After addition of 10 μl loading buffer (95% formamide), products were resolved on 6% denaturing polyacrylamide gels containing 1 × TBE buffer and 8 M urea at 80 W constant power for 2–4 h. Gels were transferred onto 3 MM blotting paper (Whatman), dried and exposed to