

Genetic changes over breeding generations of *Festulolium*

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Introduction *Festulolium* hybrids are a valuable breeding source for tolerance to abiotic stress and to make grass more persistent under drought and in cold environments. In 2004, the EU Commission enlarged the definition of *Festulolium* which may now include all hybrids between *Lolium* sp. and *Festuca* sp. and not only those between *L. multiflorum* and *F. pratensis*. We here report allele frequencies at two unlinked PCR-based marker loci in populations derived from tetraploid ($2n=4x=28$) *L. multiflorum* x *F. glaucescens* hybrids where breeding history enables us to test the effects of selection vs that of genetic drift.

Material and methods The two markers used to monitor allele frequency were an STS-marker, OSRB (Lem & Lallemand, 2003), and an SSR-marker, BIB6 (Lauvergeat *et al.*, 2005). The locus OSRB was mapped on the corresponding *Triticeae* linkage group 5 (Jones *et al.*, 2002) and the BIB6 locus, on group 1 (Ghesquière, unpublished results). Two of the populations used were derived from an original population of 50 tetraploid primary hybrids between *L. multiflorum* and *F. glaucescens*. Another three populations were obtained following one generation of backcrossing (BC1) onto *L. multiflorum*. From 162 to 177 individuals per population were genotyped at both loci. In addition 22 individuals of *F. glaucescens*, 26 individuals of *L. multiflorum* and a sample of 26 primary hybrids were genotyped at the OSRB locus where allele species-specificity was not known. No assumption was made about allele dosage in banding patterns so that allele frequency was estimated directly from band frequency. Pedigree and polycross size at each generation of the breeding process enabled us to estimate the effective size (N_e) of all populations, assuming tetrasomic inheritance (Gallais, 2003), and to compute confidence interval of allele frequencies.

Results Seven and six segregating alleles were found at locus OSRB and BIB6 respectively. Allele differentiation between parent species was much lower at OSRB than at BIB6 where only one allele was found in *F. glaucescens* and none in *L. multiflorum*. Due to a low sampling error, all pairs of populations differed significantly in the frequency of at least one allele at one or other locus. However, in no instance was it possible to reject the null hypothesis that gene changes only resulted from genetic drift based on the confidence interval for N_e . In particular, it was striking to find no significant discrepancy in the BC1 populations although *Festuca* chromosomes had no homologous counterpart and, consequently, the frequency of *Festuca* alleles was expected to decrease.

Conclusions *Festulolium* populations are subject to many antagonistic evolutionary forces after primary hybridization. These include chromosome structural rearrangements, modification of gene expression from genome conflicts as well as indirect selection for life-history traits or fertility. In a breeding programme, there are genome effects on gene frequency as well as the effects of selection by the breeder for desirable traits and genetic drift due to a narrowing of effective population size at each generation. However, the allele frequencies presented here are consistent with changes only through genetic drift. It appears that either or both loci are essentially neutral with respect to selection, or that breeding for *Festuca* traits (such as persistency) may have almost balanced exactly any counter-selection of the *Festuca* genome due to lower fertility or a lower transmission rate of chromosomes. Thus, *Festulolium* populations provide interesting material for surveying gene changes in recent interspecific hybrid populations and a better understanding of polyploidization that so frequently occurred in the evolution of grasses. However, we conclude that to detect the effects of selection will require populations of large N_e and/or extensive genome coverage by markers or use of the GISH procedure.

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