

RELATIONSHIPS AMONG SOME *LOLIUM* AND *FESTUCA* SPECIES

G. Charmet, F. Balfourier and C. Ravel

INRA, station d'amélioration des plantes, F63039 Clermont-Ferrand cedex, France

ABSTRACT

Molecular markers were used to investigate phylogenetic relationships among the 8 species of ryegrass (*Lolium*) and 11 species of fescue (*Festuca*). Nine sequences of non-coding chloroplastic or mitochondrial DNA were amplified through PCR, then digested by 20 restriction enzymes. Restriction sites data were used to draw an UPGMA tree. The main features are: 1) a clear-cut distinction between fine-leaved fescues (subg. *Festuca*) and broad-leaved fescues (subg. *Schedonorus*), which include the ryegrasses. 2) among the broad-leaved fescues, meadow fescue and tall fescue are very closely related, which may indicate that they share a common maternal ancestor, while giant fescue is the most differentiated. 3) in the ryegrass group, the endemic *L. canariense* and perennial ryegrass seem to have diverged first from their common ancestor with meadow fescue, while all the annual ryegrasses, both outbreeders or inbreeders, have a recent origin.

KEYWORDS

Lolium, *Festuca*, phylogeny, chloroplast DNA, genetic distance, evolution.

INTRODUCTION

The genus *Festuca* L. is one of the largest in the grass family with more than 400 species (Clayton and Renvoize, 1986). Several species of important agronomic use in temperate countries are found in two main subgenera: broad-leaved fescues (subg. *Schedonorus*) meadow fescue (*F. pratensis*) and tall fescue (*F. arundinacea*) in natural and sown grassland, fine-leaved fescues (subg. *Festuca*) red fescue (*F. rubra*) and *F. ovina* group in natural meadows and turf lawns. The related genus *Lolium* includes two worldwide used forage species: perennial ryegrass (*L. perenne*) for grazing and turf and Italian ryegrass (*L. multiflorum*) for grazing or conservation. Other uses such as soil preservation or nitrate capture are also developing. All the *Lolium* species are diploid with $2n=14$ chromosomes while the genus *Festuca* is a polyploid complex with $2n$ ranging from 14 to 70 (Borrill *et al.*, 1971). A better knowledge of phylogenetic relationships may be very useful for the collection, conservation and use of wild species related to cultivated ones as genetic resources. This paper describes the use of restriction site analysis of non-coding organelle DNA for inferring phylogenetic trees among some *Lolium* and *Festuca* species.

MATERIAL AND METHODS

Twenty nine natural populations belonging to the 8 species of *Lolium* and to 11 species of *Festuca*, including those of importance in agriculture, were used in this study (Table 1). In addition, one species of *Vulpia* and one of *Poa* were added as « outgroup ». A bulk of at least 50 plantlets was used to extract total DNA by the CTAB method (Murray and Thompson, 1984). Nine pairs of 20-mers primers were used to specifically amplify non-coding regions of chloroplastic or mitochondrial DNA (Ogihara *et al.*, 1991, Taberlet *et al.*, 1991, Demesure *et al.*, 1995). PCR was performed using a Perkin-Elmer thermocycler, according to the protocol described by Demesure *et al.* (1995). Amplified fragments were digested overnight using 20 restriction enzymes (10 units/10 μ l of amplification product): 12 with six-base recognition sites: BamHI, BglII, ClaI, DraI, EcoRI, EcoRV, HindIII, KpnI, PstI, StyI, XbaI, XhoI; and 8 with four-base recognition sites: AluI, CfoI, DdeI, HaeIII, HinfI, MspI, RsaI, TaqI. Digestion products were analysed by electrophoresis in 2% agarose gels run for 4-5 hours at 2.5 Vcm⁻¹. Gels were stained with 0.5mg/ml ethidium bromide and photographed at 313nm UV light.

Presence/absence of restriction sites was binary encoded for each

population. To estimate the proportion p of nucleotide substitution from the proportion of shared restriction sites, we used the maximum likelihood formula of Nei and Tajima (1983), which takes into account the length of recognition sites for each class of enzyme. The genetic distance of Jukes and Cantor, which estimates the expected number of nucleotide substitution per site, is given by $d = -3/4 \text{ Log} (1 - 4/3 p)$. The pairwise distance matrix was used to draw a dendrogram using the classical UPGMA method of hierarchical clustering.

RESULTS AND DISCUSSION

On a whole, 369 restriction sites were observed on the 9 fragments, among which 87 are common to all species and 282 are polymorphic (191 when considering only *Festuca* and *Lolium*). This represents, if sites do not overlap, a surveyed sequence of 1690 nucleotides, which was obtained at a cheaper cost than direct sequencing.

The UPGMA dendrogram is displayed in Figure 1. Assuming a molecular clock hypothesis, which seems reasonable for non-coding organelle DNA, it can be seen as a phylogenetic tree. While *Poa trivialis* L. clearly lies outside the *Festuca-Lolium* complex, which could thus be considered as a monophyletic group, *Vulpia myuros* shows some affinities with the fine fescues of subgenus *Festuca*. This agrees with an old classification of Hackel (cited in Darbyshire and Warwick, 1992), who proposed *Vulpia* as a subgenus of *Festuca*. A clear-cut partition appears between fine-leaved fescues (subgenus *Festuca*) and broad-leaved fescues (subg. *Schedonorus*), which is consistent with previous results from morphological and cytogenetic studies (Borrill *et al.*, 1977, biochemical (Butkute and Konarev, 1982; Bulinska-Radomska and Lester, 1988) or molecular (Lehvaslaiho *et al.*, 1987; Darbyshire and Warwick, 1992; Xu and Sleper, 1994; Stammers *et al.*, 1995) markers. Within the subgenus *Schedonorus*, tall fescue and meadow fescue are closely related to each other and to the *Lolium* species, which suggests that they inherit their cytoplasm from a common ancestor, while *F. arundinacea* var *glaucescens* and all the North African polyploid species derived from *F. mairei* form a distinct group which diverged earlier from the phylum of *F. arundinacea* and *F. pratensis*. This suggests that *F. pratensis*, which contributed one genome of the allohexaploid *F. arundinacea* (*F. arundinacea* var *glaucescens* to the other two, Humphreys *et al.*, 1995), also contributed the cytoplasm (female parent of the cross). Giant fescue is the most differentiated species of broad-leaved fescues. The eight *Lolium* species appear to be of recent origin, but contrarily to the results of Stammers *et al.* (1995), we found that, if we except the endemic *L. canariense*, *L. perenne* diverged first from the common ancestor, and the self-fertilizing species last. This history agrees with that hypothesized by Thomas (1981) from C-banding karyotypes.

REFERENCES

- Borrill, M., B.F. Tyler and M. Lloyd-Jones. 1971. Studies in *Festuca*. 1. A chromosome atlas of Bovinae and Scariosae. New Phytol. **71**: 523-
 Borrill, M.B., M. Kirby and W.G. Morgan. 1977. Studies in *Festuca*. 11. Interrelationships of some diploid ancestors of the ployploid broad-leaved fescues. New Phytol **78**:661-674.
 Bulinska-Radomska, Z. and R.N. Lester. 1988. Intergeneric relationships of *Lolium*, *Festuca* and *Vulpia* (Poaceae) and their phylogeny. Plant Syst. Evol. **159**:217-227.
 Butkute, B.L. and A.V. Konarev. 1982. Studies of the seed proteins in the genera *Lolium* and *Festuca* (Poaceae) in connection with their phylogeny. [in Russian] Bot. Zh.(Leningr.) **67**:812-819.
 Clayton, W.D. and S.A. Renvoize. 1986. *Genera Graminum*. Grasses of the world. Kew Bull. Addit. Ser. 13.

Darbyshire, S.J. and S.L. Warwick. 1992. Phylogeny of North American *Festuca* (Poaceae) and related genera using chloroplast DNA restriction site variation. *Can. J. Bot.* **70**: 2415-2429.

Demesure, B., N. Sodji and R.J. Petit. 1995. A set of universal primers for amplification of polymorphic non-coding regions of mitochondrial and chloroplast DNA in plants. *Mol. Ecol.* **4**:129-131.

Humphreys, M.W., H.M. Thomas, W.G. Morgan, M.R. Meredith, J.A. Harper, H. Thomas, Z. Zwierzykowski and M. Ghesquiere. 1995. Discriminating the ancestral progenitors of hexaploid *Festuca arundinacea* using genomic in situ hybridization. *Heredity* **75**:171-174.

Leväslaiho, H., A. Saura, and J. Lokki. 1987. Chloroplast DNA variation in the grass tribe Festuceae. *Theor. Appl. Genet.* **74**:298-302.

Murray, M.G. and W.F. Thompson. 1980. Rapid isolation of high molecular weight plant DNA. *Nucl. Acids Res.* **8**:4321.

Nei, M. and F. Tajima. 1983. Maximum likelihood estimation of

the number of nucleotide substitutions from restriction sites data. *Genetics* **105**:207-217.

Ogihara, Y., T. Terachi and T. Sasakuma. 1991. Molecular analysis of the hot spot region related to length mutations in wheat chloroplast DNAs. I. nucleotide divergence of genes and intergenic spacer regions located in the hot spot region. *Genetics* **129**:873-884.

Stammers, M., J. Harris, G.M. Evans, M.D. Hayward and J.W. Forster. 1995. Use of random PCR (RAPD) technology to analyse phylogenetic relationships in the *Lolium/Festuca* complex. *Heredity* **74**:19-27.

Taberlet, P., L. Gielly, G. Pautou and J. Bouvet. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Mol. Biol.* **17**:1105-1109.

Thomas, H.M. 1981. The Giemsa C-band karyotypes of six *Lolium* species. *Heredity* **42**:263-267.

Xu, W.W., and D.A. Sleper. Phylogeny of tall fescue and related species using RFLPs. *Theor. Appl. Genet.* **88**:685-690.

Table 1
Description of the genetic material used in this study

code	genus	subgenus	species	ploidy	genomes	origin
11118	<i>Lolium</i>		perenne	2x	L	France
210007	<i>Lolium</i>		perenne	2x	L	Bulgaria
30018	<i>Lolium</i>		multiflorum	2x	L	Portugal
40032	<i>Lolium</i>		multiflorum	2	L	Italy
120028	<i>Lolium</i>		multiflorum	2x	L	Germany
11460	<i>Lolium</i>		rigidum	2x	L	Corsica
40126	<i>Lolium</i>		rigidum	2x	L	Italy
330009	<i>Lolium</i>		rigidum	2x	L	Tunisia
610008	<i>Lolium</i>		temulentum	2x	L	Tunisia
620001	<i>Lolium</i>		remotum	2x	L	France
630001	<i>Lolium</i>		persicum	2x	L	Iran
640001	<i>Lolium</i>		subulatum	2x	L	Greece
20501	<i>Lolium</i>		canariense	2x	L	Tenerife
20516	<i>Lolium</i>		canariense	2x	L	Gomera
650005	<i>Festuca</i>	Schedonorus	pratensis	2x	P	France
650008	<i>Festuca</i>	Schedonorus	pratensis	2x	P	G Britain
680001	<i>Festuca</i>	Schedonorus	glaucescens	4x	G1 G2	France
660001	<i>Festuca</i>	Schedonorus	arundinacea	6x	P G1 G2	France
660008	<i>Festuca</i>	Schedonorus	arundinacea	6x	P G1 G2	France
660010	<i>Festuca</i>	Schedonorus	mairei	4x	M1 M2	Marocco
660011	<i>Festuca</i>	Schedonorus	atlantigena	8x	G1 G2 M1 M2	Marocco
660012	<i>Festuca</i>	Schedonorus	letourneuxiana	10x	Q G1 G2 M1 M2	Marocco
690006	<i>Festuca</i>	Schedonorus	gigantea	6x		France
670002	<i>Festuca</i>	<i>Festuca</i>	rubra	6x		France
670010	<i>Festuca</i>	<i>Festuca</i>	heterophilla	4x		France
670011	<i>Festuca</i>	<i>Festuca</i>	ovina	6x		France
670012	<i>Festuca</i>	<i>Festuca</i>	filiformis	2x		France
720001	<i>Vulpia</i>		myuros	6x		France
700002	<i>Poa</i>		trivialis	2x		France

Figure 1
UPGMA tree from Nei and Tajima genetic distance

