The use of extended gene pools comprising related species to improve the environmental adaptability of temperate forage grasses

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Abstract

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The evidence obtained from the study of two polyploid complexes namely *Dactylis* and *Lolium/Festuca* shows that it is possible to enrich the gene pools of polyploids in these grasses by exchange of genes through crossing (even by intergeneric crosses), and to extend their adaptability to grow over a wider range of climatic conditions.

Temperate grass polyploid complexes

The grasslands of Europe are dominated by species belonging to two major polyploid complexes, *Dactylis* and *Lolium/Festuca*. Tetraploids are the most common and widely distributed species in the *Dactylis glomerata* L. group, of which *D. glomerata* subsp. *glomerata* is the most important for agriculture. However this subspecies only accounts for about one third of the genetic variation of the *Dactylis glomerata* L. complex (Lumaret 1993). Enclaves of diploid *Dactylis* species, including putative ancestors of tetraploids, occur in specific localities (often forest zones) in southern Europe and hexaploid species are found in N. Africa.

In evolutionary terms, the *Lolium/Festuca* complex is probably older than *Dactylis*, as few ancestral diploid species still exist (Borrill 1976). However, some diploids within the complex, such as *Lolium multiflorum, L. perenne* and *Festuca pratensis*, are very important in European livestock farming. Useful tetraploids include *F. pratensis* var. *apennina* and *F. arundinacea* var. *glaucescens*, while *F. arundinacea* and *F. gigantea* are valuable hexaploid species. Both hexaploid and octoploid forms of *F. rubra* are used for agricultural and amenity purposes.

Species distribution in Europe

Within species complexes, polyploids generally have a higher genetic diversity and are distributed over a wider range of habitats than related diploids, often predominating at the edges of geographical distributions. In *Dactylis*, it appears that considerable adaptive radiation occurred among diploid species in the varied topography and climate of the Mediterranean basin and central southern Europe (Borrill 1976, Lumaret 1988). Subsequent hybridisation and polyploidisation events involving genetically differentiated diploids resulted in versatile tetraploids which extended the range of *Dactylis* further to the north, west and east of Europe. The centre of origin of diploid *Lolium* species (*L. perenne* and *L. multiflorum*) also appears to be in the Mediterranean Basin from where they spread to north and west Europe following the development of more intensive grazing agriculture. The distribution of diploid species is more restricted than related polyploid fescues. Hexaploid *F. rubra* extends well beyond the northern limit of diploid *F. pratensis* in Scandinavia. In the south of Europe, tall fescue extends beyond the range of diploid ryegrasses and fescues through the Mediterranean Basin to N. Africa where higher polyploid forms are also found (Tyler 1988). At a more local level, the tetraploid form of *F. pratensis* var. *apennina* is found at altitudes above 1800 m in the Swiss and Italian Alps where the diploid form is absent (Tyler 1988).

Ploidy level and adaptation

Early theories concerning direct and simple causal connections between ploidy level and tolerance to environmental factors are now largely discounted. However, there is increasing evidence that changes in the amounts of DNA caused by differences in ploidy level or by other means (e.g. DNA amplification in heterochromatin) can have adaptive significance (Bennett 1987). DNA C-value is positively correlated with cell size and minimum cell doubling time which interact to determine growth rate and minimum generation time. Superior spring growth at low temperatures has been found in species with high DNA content and a positive correlation between DNA content and altitude has been found in a number of species. Differences in genome size among populations of hexaploid *F. arundinacea* in Italy (Ceccarelli & al. 1992) were correlated positively with mean temperature during the year and the temperature of the coldest month at sites of origin which varied in latitude. DNA C-values of the *F. arundinacea* populations also correlated negatively with the germination power of seeds and growth rate during early plant development and positively with generation time (Ceccarelli & al. 1993). In *Dactylis glomerata* from the Galician mountains DNA C-value was negatively correlated with altitude of origin (Creber & al. 1994). Thus variation in DNA C-value appears to have a role in improving the fitness of plants subjected to environmental differences in climatic factors such as temperature.

Although polyploidy *per se* may not be essential for environmental adaptability, increased gene copy number in polyploids provides opportunities for selection to test new gene interactions and assimilate new mutations into genomes which may increase adaptive response. In natural populations of *D. glomerata*, individuals which showed maximal allelic diversity at an isozyme locus (GOT 1) possessed an advantage in transitional climatic areas where there was high and unpredictable variation in temperature and water availability (Lumaret 1984). A significant positive correlation was found between multilocus heterozygosity and both leaf weight and panicle number (Tomekpe & Lumaret 1991).

Thus although polyploids have been successful in extending the environmental range of species complexes, they appear to have achieved this through the accumulation of new mutations including repetitive DNA sequences which increase DNA C-value. Therefore genetic adaptability should be transferable between species including those at different ploidy levels.

Species' relationships

Comparisons between diploid and tetraploid *Dactylis* species suggests that hybridisation has occurred repeatedly in different localities (Borrill 1976, Lumaret 1988) and that tetraploids can be regularly generated in diploid populations of *Dactylis glomerata* L. by sexual polyploidisation (De Haan & al. 1992). In *Festuca*, strong evidence that *F. arundinacea* has one genome in common with *F. pratensis* and two genomes in common with tetraploid *F. arundinacea* var. *glaucescens* was obtained using genome specific RFLP probes (Xu & al. 1991). This was confirmed by using genomic *in situ* hybridisation (GISH) to visualise chromosome identities (Humphreys & al. 1995). It is also clear from restriction fragment length polymorphism (RFLP) data (Xu & Sleper 1994) and the use of polymerase chain reaction (PCR) technology for random amplification of polymorphic DNA (RAPD) in the *Lolium/Festuca* complex (Stammers & al. 1995), that the genomes of *Festuca pratensis* and *Lolium perenne* are very closely related together with *Lolium multiflorum*. It has also proved possible to construct a phylogenetic tree involving a wide range of other *Lolium* and *Festuca* species (Stammers & al. 1995) which is in good overall agreement with classical taxonomy but with some novel features. Hybridisation between related species, which may increase heterozygosity and create novel gene combinations, has probably played a large part in extending the adaptive range of European grasses during their evolution (Lumaret 1988). Increasing knowledge concerning relationships between species and the potential for recombination between their component genomes based on phylogenetic information, indicates that interspecific hybridisation and introgression of genes between *Lolium* and *Festuca* species has great potential for interspecific gene association and transfer in breeding programmes to increase environmental adaptability.

Hybrids between species/subspecies

Grass breeders can exploit the potential of allopolyploids to increase adaptive range by creating novel hybrids for new agronomic niches. Successful tetraploid hybrids have been produced between *L. perenne* and *L. multiflorum*, that show a very flexible response to cutting and grazing managements (Jones & Humphreys 1993). Tetraploid hybrids between *F. pratensis* and the two ryegrass species have extended the ability of grasses with good early growth and high nutritive value to cope with extremes of temperature and moisture (Thomas & Humphreys 1991, Joks & al. 1994). Good agronomic potential has also been demonstrated in tetraploid hybrids between *L. multiflorum* and *F. arundinacea* var. *glaucescens* (Jadas-Hecart & al. 1992) and octoploid hybrids between *L. multiflorum* and

F. gigantea have potential to improve summer growth (Humphreys & al. 1989). In *Dactylis*, good yield was combined with high digestibility in hybrids between tetraploid *D. glomerata* subsp. *glomerata* and *D. glomerata* subsp. *marina* (Borrill & al. 1974).

Genetic traits for improved adaptation

Summer growth and drought survival

Recent interspecific grass breeding has concentrated on the transfer of specific adaptive traits between species rather than combinations of complete genomes in amphiploid hybrids. Success in this approach depends on identification of relevant and relatively simple genetic traits as well as on good knowledge of species relationships governing the potential for recombination between genomes. Identification of useful genes depends on good physiological understanding of adaptive traits. Rates of water use, photosynthesis, leaf conductance, leaf growth and epidermal and stomatal cell characteristics were found to differ between *D. glomerata* subsp. *glomerata* and *D. glomerata* subsp. *marina* (Wilson & al. 1980). Better water conservation in subsp. *marina* than in subsp. *glomerata* was associated with the presence of large epidermal papilla cells which was negatively associated with adaxial leaf conductance. Epidermal cell height was found to be a heritable character in hybrids between subsp. *glomerata* and subsp. *marina* and therefore it should be possible to improve the water use efficiency of subsp. *glomerata* by incorporating the large papilla cell trait from subsp. *marina*.

Both shoot and root characteristics have been shown to have a role in water use efficiency and summer survival in ryegrasses and fescues. Differences in leaf orientation, rolling and adaxial ridging among fescue species, have significance in adaptation to reduced soil moisture and high temperature (Silcock & Wilson 1981). There are also large differences in root growth among fescue and ryegrass species with *F. arundinacea* giving significantly more root than ryegrasses at depths below 20 cm (Humphreys 1993). Better developed root systems in ryegrasses, which can improve adaptation to drought conditions (Veronesi 1990), may be achieved through the incorporation of fescue genes (Thomas & al. 1995). Improved root growth also aids efficient use of plant nutrients and improves uptake of minerals such as magnesium (Humphreys 1993) which can help reduce inputs into grassland farming.

Winter hardiness

Winter hardiness is a complex trait involving resistance to frost and ice/water cover, resistance to parasitic low-temperature fungi and desiccation due to frozen soils and drying winds. The distribution of assimilates between shoots and roots also affects persistency of grasses during winter. *D. glomerata* populations from Norway and Portugal showed differences in seasonal growth, photosynthetic activity and assimilate distribution (Eagles 1971). *F. pratensis* has generally good winter hardiness which is also expressed in hybrids with ryegrasses (Thomas & Humphreys 1991, Humphreys & Honne 1995). Asymmetric somatic hybridisation is being used to transfer snow mould resistance and rhizomatous growth from *F. rubra* into ryegrasses (Takamizo & Spangenberg 1994).

Introgression between species/subspecies

It is evident that the range of environmental adaptation within species could be greatly enhanced by the transfer of physiological traits between species. Possibilities are increasing for utilising the gene pool available throughout species complexes such as *Dactylis* and *Lolium*/*Festuca*. In *Dactylis*, naturally occurring and artificially produced triploids demonstrate the feasibility of interploidy hybridisation (Zohary & Nur 1959, Jones & Borrill 1962). *D. glomerata* may be improved this way (Casler & Hugessen 1988) and varieties have been released which contain genes for improved seasonal growth derived from the diploid subspecies *lusitanica* (Borrill & al. 1972, Rumball 1982). Model schemes for controlled introgression between ryegrasses and fescues, which involve intermediate triploid and pentaploid 'genetic bridges', have been developed using isozymes as genetic markers (Thomas & al. 1988, Humphreys 1989, Thomas & Humphreys 1991, Thomas & al. 1992). Breeding programmes based on these schemes have been successful in transferring good summer growth from *F. pratensis* into *L. perenne* (Humphreys 1993) and drought tolerance from tall fescue into Italian ryegrass (Humphreys & al. 1993, Humphreys & Thomas 1993, Thomas & al. 1995). Relative yields, under rain-out shelters at Aberystwyth (UK) and under natural drought conditions in Lusignan (France), of Italian ryegrass selections containing genes from tall fescue compared to ordinary Italian ryegrass cultivars are shown in Table 1. Work is also in progress to transfer genes for improved winter hardiness from *F. pratensis* into ryegrasses (Humphreys & Honne 1995).

Table 1. Relative yields, under rain-out shelters at Aberystwyth (UK) and under natural drought conditions in Lusignan (France) of Italian ryegrass selections containing genes from tall fescue compared to ordinary Italian ryegrass cultivars.

Relative dry matter yields (± s.e. of means) of ryegrass selections compared to trial means		
	Aberystwyth	Lusignan
Italian ryegrass without fescue genes (mean of 4 cultivars)	0.95 ± 0.05	1.07 ± 0.04
Italian ryegrass with fescue genes (mean of 8 selections)	1.26 ± 0.01	1.25 ± 0.02

Table 2 shows the percentage tiller survival (after two winters in Norway) of perennial ryegrass selections containing genes from meadow fescue compared to meadow fescue × perennial ryegrass tetraploid hybrids, diploid perennial ryegrass cultivars and a Norwegian meadow fescue cultivar. Work is also in progress to improve nitrogen use efficiency in ryegrass using fescue genes and to improve the nutritive value of tall fescue through gene transfer from ryegrasses.

Genetic maps, QTL's and in situ hybridisation

Work on gene transfer between species will be aided considerably by recent developments in the construction of genetic maps, the identification of quantitative trait loci (QTL's) and use of *in situ* hybridisation to differentially label chromosomes.

Table 2. The percentage tiller survival (after two winters in Norway) of perennial ryegrass selections containing genes from meadow fescue compared to meadow fescue × perennial regrass tetraploid hybrids, diploid perennial ryegrass cultivars and a Norwegian meadow fescue cultivar.

Percent tiller survival of spaced plants after 2 winters in Norway (± s.e. of means)	
Meadow fescue (1 Norwegian cultivar)	85
Meadow fescue × perennial ryegrass hybrids (tetraploid) (mean of 3 hybrids)	63 ± 2.0
Perennial ryegrass containing meadow fescue genes (mean of 4 selections)	40 ± 2.0
Diploid perennial ryegrass (mean of 3 cultivars)	30.2 ± 1.8

In *L. perenne*, associations with isozyme loci have been identified for a number of traits such as dark respiration rate (Rainey & al. 1990), water soluble carbohydrate content (Humphreys 1992) and yield and flowering time (Hayward & McAdam 1988). RFLP's and PCR techniques greatly increase the range of genetic markers available to allow genetic maps to have more detailed and extended coverage of grass genomes (Xu & al. 1991, Evans & al. 1991, Hayward & al. 1994, Stammers & al. 1995). This also increases precision in QTL detection (Hayward & al. 1994). Nine QTLs concerned with different aspects of the flowering process in ryegrass have been identified of which 3 control inflorescence emergence (Humphreys & al. 1995). Careful choice of QTLs should minimise undesirable correlated selection responses in marker assisted selection. For example in Linkage Group (LG)1 and LG7 of perennial ryegrass, QTLs for heading date are closely associated with head number, whereas there are separate QTLs for heading date on LG2 and head number on LG4.

Development of *in situ* hybridisation techniques (Humphreys & al. 1995, Thomas & al. 1995) can help to locate QTL's on chromosomes and allow direct visual monitoring of introgression between species. For example in an introgressed line of *L. multiflorum*, derived from a cross between *L. multiflorum* and *F. pratensis*, chromosome segments carrying the *F. pratensis* derived *sid* (senescence induced degradation) allele could be identified (Thomas & al. 1994).

Similarly introgressed segments of *F. arundinacea* have been identified in drought resistant lines of *L. multiflorum* derived by backcrossing a *L. multiflorum* × *F: arundinacea* hybrid into *L. multiflorum* (Thomas & al. 1995). It is of interest that this segment is in a similar position to a QTL which has been shown to affect aftermath heading (Humphreys & al. 1995) and that reduced aftermath heading is one of the features of the drought resistant lines of *L. multiflorum* (Thomas & al. 1995).

Conclusions

It is clear that increasing opportunities to transfer genetic traits between species comprising polyploid complexes greatly extends the gene pool available to grass breeders. Many products of genetic diversification arising from the natural selection and evolution of grasses can now be harnessed to meet the future demands of European agriculture which include more consistent and efficient grassland production and an ability to cope with possible effects of climate change. There are reports that viable hybrids can be produced between *Dactylis* and *Lolium*/*Festuca* species which unites the gene pools of the two major polyploid complexes found in temperate grasslands (C. Oertel, personal communication). Also hybrids with some fertility have been produced between *Lolium* and *Secale* (M. K. Pavlova, personal communication). Such links between forage grass species and cereals, together with recent information on syntenic relationships between Graminaceous species (Moore & al. 1995) and the ability to use genetic transformation techniques on an increasing number of Graminaceous species (S. Dalton, personal communication), increasingly widens the gene pool available to breeders to meet the demands of a changing world.

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