Interspecies Hybrids and Polyploidy

E. L. Breese, E. J. Lewis and G. M. Evans

Phil. Trans. R. Soc. Lond. B 1981 **292**, 487-497
doi: 10.1098/rstb.1981.0043

Phil. Trans. R. Soc. Lond. B **292**, 487–497 (1981)
Printed in Great Britain

Interspecies hybrids and polyploidy

By E. L. Breese†, E. J. Lewis† and G. M. Evans‡

† *Welsh Plant Breeding Station, Plas Gogerddan,*
Aberystwyth, Dyfed, SY23 3EB, U.K.
‡ *Department of Agricultural Botany, University College of Wales,*
Aberystwyth, Dyfed, SY23 3DD, U.K.

Polyploidy has featured strongly in plant evolution as a means of conserving favoured hybrid combinations during sexual reproduction. Combining different genomes is likely to extend adaptation rather than increase yield *per se*. Subsequent stabilization by polyploidy depends on the degree of differentiation of the genomes and on the breeding system, but success is mediated by low chromosome numbers while reduced fertilities may be offset where the crop is harvested primarily for vegetative parts and/or is perennial.

In old established grain crops like the cereals, raw synthetic polyploids are not likely to offer immediate advantages but may be subsequently improved by arduous selection in an extended gene pool (e.g. triticale). The relatively undeveloped herbage grasses offer unique opportunities. Flexibility in grass swards is presently sought through unstable mixtures of races and species. It is the breeders' aim to combine genetically the complementary features of these in stable varieties. Agronomically useful hybrids between diploid *Lolium* species have been stabilized at the tetraploid level through tetrasomic inheritance reinforced by a degree of preferential pairing. This preferential pairing may be genetically enhanced, thus raising the possibility of producing a new agriculturally useful amphiploid ryegrass species. Prospects for developing useful amphiploid hybrids between less closely related *Lolium*/*Festuca* species is considered and related to more limited objectives of transferring desirable genes or gene complexes.

Introduction

Induced polyploidy has two major consequences. The first is in the development of the plant, chiefly through an increased cell size and related pleiotropic effects including larger but fewer plant parts (the gigas effect). The second is on the genetic system, particularly in preserving hybrid combinations over sexual generations through genome duplication. Almost without exception it is the latter property that has featured in natural plant evolution in the development of race and species hybrids with wider adaptability and a greater pioneering potential than their parents (Stebbins 1950). Lewis (1967) developed this theme to point out that there is little evidence that natural polyploids display what he terms exophenotypic effects (= gigas characteristics) and considers that the diploid size must be retained or regained during evolution. He further points out that since plants can, and usually do, achieve polyploidy in certain somatic cells through controlled endoreplication, the significance of polyploidy in evolution must be in the qualitative rather than quantitative aspects of the gene complement. Schwanitz (1953) also points to the fact that many 'gigas' characteristics associated with polyploidy can be achieved through selection at the diploid level. It is therefore logical to consider that the greatest plant breeding benefits will derive from the use of polyploidy in developing race and species hybrids (allopolyploidy) rather than from autopolyploidy *per se*.

Application of polyploidy to plant breeding

The discovery of the chromosome-doubling properties of colchicine more than 40 years ago raised great expectations of the use of induced polyploidy in genetic manipulation. Its use is attempted in three ways: (i) induced autopolyploidy in an attempt to improve yield or quality (or appearance in the case of ornamentals) by capitalizing on the developmental aspects of large cells and large parts; (ii) to restore fertility to sterile species hybrids with a view to stabilizing them genetically (amphiploidy or allopolyploidy); (iii) to serve as a genetic bridge between species in the transfer of gene complexes (Stebbins 1956; Dewey 1980).

A number of factors predispose success. First, the two near-universal consequences of induced polyploidy are increased cell size and decreased fertility (Eigsti & Dustin 1955). Thus crops that are harvested chiefly for their vegetative parts may enjoy advantages that are less likely to be outweighed by reduced fertilities than seed crops. This advantage also applies to crops which are perennial and/or asexually propagated (e.g. potatoes, herbage legumes and grasses).

Secondly, experience has shown the importance of chromosome number and present ploidy state. Many species have achieved optimal numbers beyond which growth and development is inhibited. Similarly, most taxa have an optimal ploidy level and further additions produce disharmonious growth or are difficult to stabilize. Amphiploid hybrids above this critical level often lose chromosomes or whole genomes in later generations. In many crop plants this optimal ploidy level appears to be hexaploid (Stebbins 1950). Thus for instance, in triticale, breeding has been more successful with tetraploid wheats ($2n = 28$), which give hexaploid ($2n = 42$) hybrids in crosses with diploid rye ($2n = 14$) rather than with hexaploid bread wheat (*Triticum aestivum*) ($2n = 42$), which yield octoploid ($2n = 56$) hybrids (Larter 1976). Other examples are frequent in the literature and some are cited by Dewey (1980). The conclusion is that beyond a certain ploidy level we may be forced to seek improvement through the controlled introgression of genes or gene complexes rather than through genome incorporation.

Thirdly, the breeding system is important. Experience has shown that in polyploid breeding it is important to assemble a large number of raw polyploids that can be intercrossed to form a large gene pool followed by selection for improved performance. This is true for autopolyploid and allopolyploid breeding, and success in most polyploid breeding programmes has undoubtedly been achieved by these means. Cross-fertilizing species are at an advantage in securing this extended gene pool but there are important differences in the consequences of inbreeding and outbreeding with respect to polyploidy which will emerge later.

Principles determining breeding strategies in allopolyploid breeding

Principles that govern our approach to a breeding programme are of two sorts, agronomic and genetic.

From the agronomic standpoint it has to be borne in mind that wide hybrids and their allopolyploid derivatives are usually successful in colonizing new habitats distinctly different from those to which their progenitors have become adapted. The more radical the change in genetic constitution the more radical is the change required in the environment (Stebbins 1956; Tigerstedt 1978). For well adapted, highly selected and intensively managed crops like the cereals, wide polyploids are unlikely to offer immediate advantages, and improvement is likely to derive from the controlled introgression of genes and gene complexes. There are exceptions, particularly in spreading the region of adaptation (e.g. triticale). At the other extreme in herbage plants, where intensive grassland utilization is relatively recent, management systems are now evolving to provide the new environments that novel allopolyploids might exploit.

From the genetic point of view we have to consider first gene expression, particularly dominance and epistatic relations, which determine the value of the hybrid, and second, gene transmission during reproduction, which affects fertility and stabilty. In sexual allopolyploids, the segregational properties of the genes depend on the 'pairing' relations of the homologous and homoeologous chromosomes. In outbreeders, useful hybrid combinations can be preserved even where the chromosome pairing is random through the effects of tetrasomic inheritance (Stebbins 1953; Breese & Thomas 1978), though this will not obtain under inbreeding (Lewis 1967). Further stability is achieved through preferential pairing of homologous chromosomes. This may arise even between closely related, relatively undifferentiated genomes, through genetic control mechanisms (Riley & Chapman 1958).

These general considerations only achieve real significance when related to a specific crop. Above all they indicate the need for an intimate knowledge of the crop and its requirements, and of the biology and genetic relations of the target species. Here allopolyploidy and its potential will be considered in relation to the herbage grasses with special reference to *Lolium* and *Festuca* spp.

Breeding for agronomic flexibility in grasses

The *Lolium*/*Festuca* complex of species is adapted to a wide range of conditions, and the four major agricultural species, *L. perenne*, *L. multiflorum*, *F. pratensis* and *F. arundinacea*, have complementary characteristics, which are recognized as useful agronomic attributes. At present the complementation of these attributes is possible only through the use of mechanical seeds mixtures. These mixtures are difficult to manage because of competitive interactions.

The four species are related to an extent that will allow hybridization, and thus genetic exchange, and it is a breeding aim to combine genetically their attributes through hybrids or their derivatives. We would thereby achieve sward flexibility through genotypic versatility rather than genetic heterogeneity.

Tetraploid *Lolium* hybrids

The two ryegrasses, *L. perenne* and *L. multiflorum*, which are diploid ($2n = 14$), are the most important grassland species in British agriculture. Together they offer a most useful complementation of characters and it has long been the aim to incorporate these characters in stable hybrids. The species cross easily to produce fertile diploid F_1s but in F_2 and later generations there is transgressive segregation leading to rapid genetic deterioration, including a degree of sterility (Naylor 1960).

Male-sterile clones have been used to produce commercial quantities of diploid F_1 seed (J. Joordens, personal communication). An allotetraploid programme was started as an alternative method, on the assumption that tetrasomic inheritance reinforced by a degree of preferential pairing would arrest genetic deterioration during seed multiplication. The programme was further advocated by the existence of valuable synthetic autotetraploid varieties of these species whose success derives from the larger cell size, bringing about reduced fibre and thus improved feed quality. We may thus hope to exploit favourable physiological (exophenotypic) effects of polyploidy together with stabilizing (endophenotypic) effects, giving wider adaptation through hybridity.

Genetic assumptions and breeding tactics

The consequences of tetrasomy and partial preferential pairing in terms of gene segregation have to be followed over enough generations (four or more) to achieve sufficient seed multiplication. First, consider a single gene locus with two alleles (*A*:*B*) in outbreeding populations, assuming random chromosome assortment (no double reduction) at the tetraploid level and that simplex, duplex and triplex (i.e. *AAAB*; *AABB*; *ABBB*) individuals confer the hybrid phenotype. In the diploid, equilibrium is achieved in the F_2 with 50 % hybridity. In tetraploids, equilibrium is achieved exponentially after four or five generations. Then 87.5 % of the population are hybrid with tetrasomic inheritance, increasing to 100 % with increasing levels of preferential pairing (Breese & Thomas 1978). If we consider more loci, the disparity between the different inheritance patterns becomes more striking. Figure 1 gives the situation for seven unlinked loci. At the diploid level at equilibrium, only a small fraction of less than

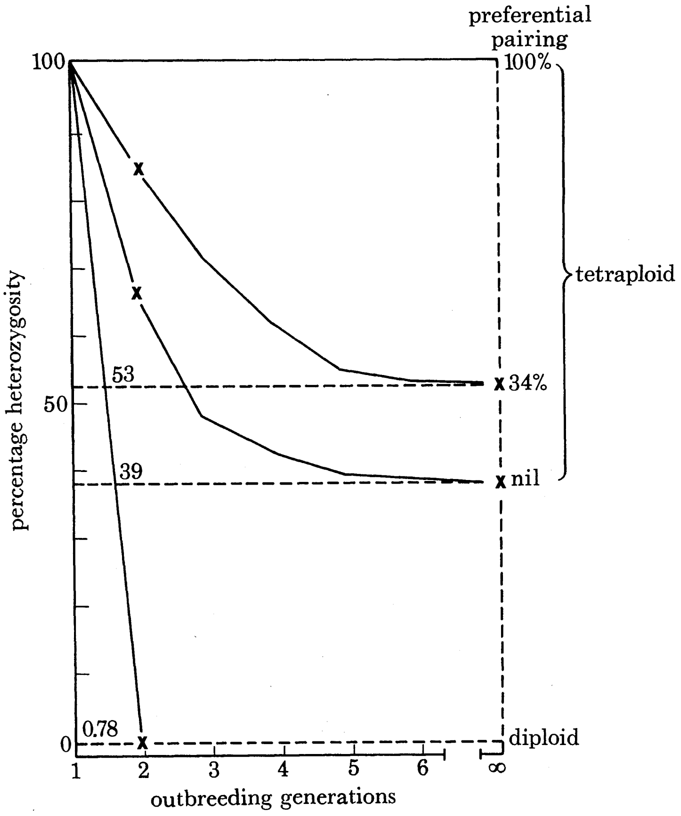

FIGURE 1. Gene segregation in diploid and tetraploid hybrids over successive outbreeding generations: percentage of population that are fully hybrid at seven loci, with two alleles (*A*:*B*), assuming no double reduction and that simplex, duplex and triplex (i.e. *AAAB*, *AABB*, *ABBB*) genotypes give the hybrid phenotype.

1% are hybrid at all seven loci. In tetraploids, on the other hand, more than 40% of the population retain hybridity for all these loci, assuming tetrasomic inheritance, while this is increased to over 50% with the moderate degree of preferential pairing assumed. Breese (1981) has advanced comparisons to take into account combinations of these loci held in the hybrid state, and shows that up to 80% of the populations will retain hybridity for at least six loci by tetrasomic inheritance alone. Of course, amphiploidy will retain 100% hybridity at the duplex level.

If we equate the seven loci with the seven centromeres of the seven chromosomes of the haploid *L. multiflorum* and *L. perenne* genomes, we can see the potentially high capacity of the tetrasomic state alone to preserve hybrid combinations and how this can be considerably reinforced by preferential pairing. Of course, the situation is grossly oversimplified. For instance, we have to consider the gene linkages and recombination frequencies between the homoeologous chromosomes. All this can only be determined by experiment and experience.

The above considerations emphasize the need to select specific F_1 hybrid combinations for stabilization through tetrasomy. The breeding tactics were therefore the controlled paired crossing of individual genotypes (clones) from existing tetraploid varieties or newly induced tetraploid populations, followed by the selection of individual F_1s for fertility, stability and agronomic performance.

Fertility, uniformity and stability

In selected hybrids, relatively good fertilities were achieved despite fairly high levels of aneuploidy. In common with other 'raw' tetraploids, aneuploid gametes arise in the tetraploid hybrids from the irregular disjunction of quadrivalents and the presence of trivalents and univalents. In herbage crops a proportion can be tolerated since the less vigorous tend to be crowded out.

For individual plant characters the hybrids vary considerably in their uniformity, but usually they appear much more conservative of genetic variability than the diploid hybrids. The differences are less easy to quantify for individual characters in statistical terms because of the high errors attached to variances. Also the variances of the tetraploid families may be inflated by aneuploidy effects.

The stability of mean performance over generations also varies. For selected families, the change from F_2 to F_5 (equilibrium) is less than would be expected from tetrasomic inheritance and bespeaks a degree of preferential pairing as considered later.

Hybrid performance

Differences between F_1 families in performance as well as uniformity are marked (Breese *et al.* 1975). It will be appreciated that the method is largely empirical and has not so far involved critical experiment to determine the precise dominance and interactive properties at the tetraploid level of the genes controlling the different characters. Nevertheless, generalized comparisons of dominance relations can be made with respect to parental varieties of the different species. Thus in some of the hybrids the dominance of the Italian ryegrass parent is apparent up to the heading phase, when there is a switch to partial dominance of the perennial parent during recovery growth. This switch affects the canopy structure of the sward, which leads to more stable yields over frequent and infrequent defoliation than either parent and offers a possible greater flexibility for conservation and grazing managements.

Commercial varieties have now been produced that offer good conservation potential coupled with early and late grazing. They are highly palatable and have digestibility and intake characters that match or exceed the high quality of Italian ryegrass. Good winter hardiness and tolerance of drought and some diseases have been incorporated.

Future prospects

The hybrids thus show that it is possible to combine a plastic response to controllable (management) variables with low sensitivity to uncontrollable (environmental) variables. As pointed out, these first (primary) hybrids are single crosses between selected pairs of clones of the two parental species. Thus although hybridity is maximized between homoeologous chromosomes it is minimal between homologous chromosomes. Clearly the technique offers scope for the production of a wide range of such hybrids that are still better adapted to conservation and for grazing conditions. It allows rapid selection at the diploid level for complementary genotypes and uniting these in relatively stable tetraploids. But in fully exploiting this we need to know more of the dominance and epistatic relations of the genes controlling the various characters at both the diploid and tetraploid level so that we may reduce still further the empiricism currently involved in selecting parents and hybrids. But the tetraploid state offers the possibility of a second dimension of hybridity, that between homologues of the same genome, and thus affords further opportunities for enhancing heterosis and extending adaptability. This course, however, requires a fundamental reappraisal of the relations between chromosomes and the consequent segregational properties when more than two alleles are involved at a locus. For tetrasomy it can be shown that only 37 % of a population at equilibrium is heterozygous for all four possible alleles at a single locus and only a very small proportion of the population is simultaneously fully hybrid for even three loci. It can also be shown that maximum hybridity cannot be advanced beyond 44 % even for a single locus.

Apart from the inability to maximize hybridity with more than two alleles, tetrasomic segregation will also of course hamper the selection and stabilizing of desirable gene combinations in new varieties. Secondary hybridity will no doubt add to the genetic heterogeneity of the population, and by intercrossing our primary hybrid we can effectively create a new hybrid ryegrass species of the status of 4*x* *Dactylis glomerata* (cocksfoot). However, to continue to add more accurately to genotypic flexibility we require a more precise control of the chromosome pairing. We thus need to enquire more into the basis of stability and uniformity of the primary hybrids.

CHROMOSOME AFFINITIES IN *LOLIUM* HYBRIDS

Pairing preferences

The diploid hybrids between *L. multiflorum* and *L. perenne* regularly form bivalents and thus there is no indication of marked structural differences between the genomes. Nevertheless, the allotetraploid hybrids show on average an increased number of bivalents and a consequent reduction in multivalents compared with autotetraploids within the parental species. This suggests, but does not prove, a degree of preferential pairing.

More precise information in pairing preferences is now being obtained through the segregational patterns of enzyme variants (isozymes), which can be used as a means of genetically marking the different chromosomes. Using variants of the enzyme phosphoglucoisomerase (PGI/2), Breese & Thomas (1978) have estimated preferential pairing of the order of 34% among basic F_1 plants of the tetraploid hybrid cv. Sabrina. It would thus appear that the selection of this hybrid on the basis of its performance and stability had indeed involved a degree of preferential pairing, at least in the initial F_1 generation. To what extent this control is maintained in later generations is difficult to determine in this hybrid from the use of the PGI/2 locus. This is because we cannot completely identify parental chromosomes with isozyme allele for all basic plants. However, an analysis was carried out on samples from nine different seed lots of F_5/F_6 generations produced commercially in various parts of the country (E. W. Bean, unpublished). As expected, the data were too insensitive to detect differences between complete tetrasomic inheritance and partial preferential pairing, but they demonstrated that this level was significantly short of complete. The results were valuable in showing that the gene frequencies of the original F_1 plants had been consistently preserved over all seed lots, thus indicating that there had been no differential loss of parental genomes, for this marker at least, by selection or through aneuploidy. At the same time, through their genotypic frequencies, they demonstrated the need to increase preferential pairing before secondary hybridity could be usefully exploited.

Prospects for improving preferential pairing

Cytological evidence shows that the degree of bivalent formation varies markedly for different F_1s and would thus appear to be under genetic control. Most importantly, isozyme and other genetic markers have now been used to demonstrate that the degree of preferential pairing varies widely between different *L. multiflorum* × *L. perenne* hybrids for at least two loci (table 1).

More precise evidence of genes controlling variation in the degree of association of homoeologous chromosomes is available from hybrids between other species in the *Lolium* genus. Thus in an experimental cross between *L. temulentum* and *L. perenne* it was established not only that B chromosomes suppressed pairing in diploid hybrids and promoted bivalent pairing in the tetraploids, but that there were genes on the A chromosomes of *L. perenne* that had a similar and supplementary effect (Evans & Macefield 1972; Taylor & Evans 1977). Recent work by G. M. Evans & E. W. Davies (unpublished) with isoenzyme marker genes confirms that where bivalent association is enhanced in tetraploid hybrids it is between homologous chromosomes. These results are summarized briefly in table 2.

The usefulness of B chromosomes in breeding work is questionable owing to their non-mendelian inheritance and their dispensible nature. However, any variation on the A chromosomes is potentially useful and could possibly be utilized in breeding programmes.

There is thus evidence to suggest that useful genetic variation in terms of chromosome pairing control at the hybrid level exists within the parental species. A selection programme is now under way to use these genes in enhancing the degree of pairing in allotetraploids. This programme may also throw light on the nature, and hence the selective value, of such genes in the diploid parents since their most obvious effects are concerned with polyploid hybrids. Waines (1976) has advanced the argument that they may function as an isolating mechanism at the species level by reducing chromosome pairing and consequently the fertility level between diploid interspecific hybrids.

Incorporating pairing genes from other species

The stability and high fertility of triticale is dependent to a large degree on the action of the Ph gene from wheat. Not only does it suppress pairing between homoeologous wheat chromosomes but it also suppresses the synapsis of wheat and rye chromosomes. It is very likely that diploidizing mechanisms similar to the *Ph* locus in wheat occur in many other polyploid taxa. Although there are no known instances of natural polyploidy in the genus *Lolium* itself, they abound in the closely related *Festuca*. Although a pairing control mechanism has not been clearly identified in these natural allopolyploids, there are strong indications of its presence (Adams & Allard 1977; Lewis *et al.* 1980; Jauhar 1975). As a long-term commitment, therefore, the identification and incorporation of such a mechanism into synthetic hybrids is indicated.

TABLE 1. GENOTYPIC VARIATION IN LEVEL OF PREFERENTIAL CHROMOSOME PAIRING IN TETRAPLOID *L. PERENNE* × *L. MULTIFLORUM* CALCULATED FROM GENETIC SEGREGATION AT TWO LOCI

locus	range in percentage preferential pairing random chromosome assort.	 random chromatid assort.
anthocyanin 'c'	0–21.85	0–39.22
PGI/2	0–44.10	13.79–56.54

TABLE 2. CHROMOSOME PAIRING IN TETRAPLOID *L. TEMULENTUM* × *L. PERENNE* HYBRIDS

hybrids	bivalents per cell	preferential† pairing (%)
L. temulentum (Ba 6902) × *L. perenne* (clone A)		
0B	12.4	38.5
4B	13.0	84.4
L. temulentum (Ba 6902) × *L. perenne* (clone B)		
0B	11.5	0
4B	12.5	48.3

Note: (*a*) Ba 6902 is a homozygous line; (*b*) both *L. perenne* parents contained identical B chromosomes.

† Based on segregation at three unlinked isozyme loci.

Either by selection within the species, or by incorporation of specific genes controlling homoeologous pairing, we thus have good prospects of producing a new amphidiploid ryegrass species with greater agronomic flexibility. It would then provide new opportunities for widening adaptability still further through secondary hybridity of homologous chromosomes. At this stage the incorporation of a third diploid genome from related *Festuca* spp. could be considered.

LOLIUM/FESTUCA HYBRIDS

Hybrids between Lolium *spp. and* F. pratensis

Meadow fescue (*F. pratensis*, $2n = 14$) is agriculturally far less important than the ryegrasses in the United Kingdom and complements these species in but a few characters. Nevertheless, the combination of these characters has been attempted by numerous workers (e.g. Jenkin 1933; Reusch 1959; Wit 1959; Hertzsch 1966), encouraged by the fact that natural hybrids that are very competitive are frequently found in certain habitats.

These *Festulolium* diploid hybrids, both natural and artificial, are highly sterile, which has been considered to be genic (Reusch 1960) or chromosomal (Ahloowalia 1965). Induced polyploidy restores fertility to a fairly high degree (Lewis 1972), and the formation of bivalents is higher than in the *Lolium* hybrids (table 3), suggesting a higher degree of preferential pairing. However, as shown in table 3, the level of bivalent association varies with the genotype, again indicating scope for improvement by selection (Osborne *et al.* 1977). A number of potential varieties of both amphiploids (i.e. *F. pratensis* hybridized with both *Lolium* species) show some complementation for late summer growth and good drought and winter hardiness (Lewis *et al.* 1973). Although markedly superior in yield to meadow fescue, these hybrids have not so far matched the ryegrass parents or ryegrass hybrids under intensive U.K. conditions. Their tolerance of environmental stresses may, however, widen their adaptability into newer grassland areas. The amphiploids are also being backcrossed to the ryegrass parents with the aim of transferring the more desirable gene combinations of the fescue.

Table 3. Chromosome pairing in autotetraploids and allotetraploids in *Lolium–Festuca*

	mean frequency	
	II	IV
L. perenne (4*x*)	6.5 (5.2–7.6)	3.4 (3.0–3.6)
L. perenne × *L. multiflorum*	9.0 (6.6–10.9)	2.1 (1.2–3.6)
L. perenne × *F. pratensis*	10.4 (8.8–11.8)	1.1 (0.6–1.6)
L. multiflorum × *F. pratensis*	10.8 (9.6–12.6)	0.8 (0.2–1.6)

Hybrids between F. arundinacea *and* Lolium *spp.*

Next to the ryegrass hybrids, the combination of tall fescue with Italian ryegrass (*F. arundinacea* × *L. multiflorum*) would appear to offer the greatest complementation in characteristics, particularly for an increased, persistent conservation model. As such, it has received perhaps wider attention than most other interspecific hybrids (Terrell 1966). The hybrid with perennial ryegrass also has potentially high agronomic value.

F. arundinacea is an outbreeding hexaploid species ($2n = 42$) considered to be an amphidiploid, involving three basic diploid genomes (Lewis *et al.* 1980), with a close relationship to the *Lolium* spp. and *F. pratensis*. F_1 hybrids with the *Lolium* species are sterile, but fertility is restored in chromosome-doubled forms ($2n = 56$) (Buckner *et al.* 1961; Lewis 1966). The initial amphiploids have a high level of bivalent pairing, which is assumed to be preferential since the 28 chromosome undoubled hybrid shows a high number of multivalent associations and hence homoeologous pairings. It would thus appear that the genes controlling pairing in hexaploid tall fescue extend their influence to control pairing to some extent in the octoploid hybrid. However, the control is not absolute; a proportion of multivalents and univalents are formed, leading to chromosome loss and subsequent genetic instability (Lewis 1966). There is some parallel here with the instability of octoploid triticale considered earlier; this must raise the question of the hexaploid as an optimum number in these crop plants. In the Festuceae generally, however, octoploid and higher ploidy levels are not uncommon and this encourages the hope of developing more stable higher ploidy hybrids.

Although stably combining genomes of *Lolium* species with *F. arundinacea* has proved an elusive goal, polyploidy can be used as a genetic bridge for the transfer of genes and gene combinations between the parent species. Thus certain quality characteristics have been transferred from *L. multiflorum* into *F. arundinacea* (Buckner *et al.* 1977). In the United Kingdom we are attempting the transfer of valuable genes from the tall fescue to the ryegrass parent. The scope for this form of genetic transfer is enormous in the wide range of related *Lolium–Festuca* species.

In polyploid breeding, whether for combining genomes or genetic transfer, the limits are initially set by the numbers of hybrids that can be obtained. The crossability of the species varies markedly between different genotypes of donor species as well as between species. The development of tissue culture techniques for embryo culture and ultimately somatic hybridization (Cocking 1977) offers hopes of overcoming these obstacles (Dale 1976).

Conclusions

In this paper we have chiefly considered polyploidy as a means of genetically stabilizing interspecific hybrids in grasses. A number of features of the crop advocate such an allopolyploid breeding programme. First, there are unique opportunities for producing new and novel hybrids and their derivatives that are better adapted to intensive grassland systems than existing species. Secondly, since seed fertility and seed yield are not at a premium and a degree of non-uniformity may be tolerated, a range of polyploid techniques may be used. It is clear that polyploidy can be a valuable tool in combining genomes or facilitating genetic exchange, but its effective use depends on (i) defining realistic objectives in relation to the requirements and biology of the crop, (ii) a knowledge of the dominance/epistatic relations of the genes in the polyploid state, and (iii) an understanding of the nature and genetic control of homoeologous (intergenomic) chromosome pairing.

References (Breese *et al.*)

Adams, W. T. & Allard, R. W. 1977 Effect of polyploidy on phosphoglucose-isomerase diversity in *Festuca microstachys*. *Proc. natn. Acad. Sci. U.S.A.* **74**, 1652–1656.

Ahloowalia, B. S. 1965 Cytogenetic studies on natural hybrids between ryegrass and meadow fescue. *Z. Vererb-Lehre* **97**, 226.

Breese, E. L. 1981 Exploitation of genetic resources through breeding: *Lolium* species. In *Proc. Symp. on Genetic Resources of Forage Plants* (ed. R. A. Bray & J. G. McIvor). Melbourne: C.S.I.R.O. (In the press.)

Breese, E. L., Stephens, D. E. & Thomas, A. C. 1975 Tetraploid ryegrasses: allopolyploidy in action. In *Ploidy in fodder plants* (ed. B. Nuesch), pp. 46–49. Zurich: Eucarpia Report.

Breese, E. L. & Thomas, A. C. 1978 Uniformity and stability of *Lolium multiflorum* × *Lolium perenne* allotetraploids. In *Interspecific hybridization in plant breeding* (ed. E. Sanchez-Monge & F. Garcia-Olmedo) (Proc. 8th Eucarpia Congress, Madrid), pp. 155–160. Madrid: Ciudad Universitaria.

Buckner, R. C., Hill, H. D. & Burrus, P. B. 1961 Some characteristics of perennial and annual ryegrass × tall fescue hybrids and the amphiploid progenies of annual reygrass × tall fescue. *Crop Sci.* **1**, 75–80.

Buckner, R. C., Burrus, P. B. & Bush, L. P. 1977 Registration of 'Kenhy' tall fescue. *Crop. Sci.* **17**, 672–673.

Cocking, E. C. 1977 Plant protoplast fusion: progress and prospects for agriculture. In *Recombinant molecules: impact on science and society* (ed. R. F. Beers, Jr. & E. G. Bassett), pp. 195–208. New York: Raven Press.

Dale, P. J. 1976 Tissue culture in plant breeding. *Rep. Welsh Pl. Breed. Stn 1975*, pp. 101–115.

Dewey, D. R. 1980 Some application and misapplication of induced polyploidy to plant breeding. *In Polyploidy: biological relevance* (ed. W. H. Lewis), pp. 445–466. New York: Plenum.

Eigsti, O. J. & Dustin, A. P. 1955 *Colchicine in agriculture, medicine, biology and chemistry*. (470 pages.) Ames: Iowa State College Press.

Evans, G. M. & Macefield, A. J. 1972 Suppression of homoeologous pairing by B chromosomes in a *Lolium* species hybrid. *Nature, new Biol.* **236**, 110–111.

Hertzsch, W. 1966 Interspecific and intergeneric hybrids between *Lolium* and *Festuca*. In *Proc 10th Int. Grassl. Congr.*, Helsinki (ed. A. G. G. Hill), pp. 683–685. Helsinki: Finnish Grassland Association.

Jauhar, P. P. 1975 Genetic control of diploid-like meiosis in hexaploid tall fescue. *Nature, Lond.* **25**, 595–597.

Jenkin, T. J. 1933 Interspecific and intergeneric hybrids in herbage grasses. Initial crosses. *J. Genet.* **28**, 205–264.

Larter, E. N. 1976 Triticale. In *Evolution of crop plants* (ed. N. W. Simmonds), pp. 117–120. London: Longman.

Lewis, E. J. 1966 The production and manipulation of new breeding material in *Lolium–Festuca*. In *Proc. 10th Int. Grassl. Cong.*, Helsinki (ed. A. G. G. Hill), pp. 688–693. Helsinki: Finnish Grassland Association.

Lewis, E. J. 1972 Production of *Festuca/Lolium* hybrids. *Rep. Welsh Pl. Breed. Stn* 1971, p. 20.

Lewis, E. J., Humphreys, M. W. & Caton, M. P. 1980 Disomic inheritance in *Festuca arundinacea Schreb. Z. PflZücht.* **84**, 335–341.

Lewis, E. J., Tyler, B. F. & Chorlton, K. H. 1973 Development of *Lolium/Festuca* hybrids. *Rep. Welsh Pl. Breed. Stn 1972*, pp. 34–37.

Lewis, K. R. 1967 Polyploidy and plant improvement. *Nucleus* **10**, 99–110.

Naylor, B. 1960 Species differentiation in the genus *Lolium*. *Heredity, Lond.* **15**, 219–233.

Osborne, R. T., Thomas, H. & Lewis, E. J. 1977 *Lolium/Festuca* amphiploids. *Rep. Welsh Pl. Breed. Stn 1976*, p. 106.

Reusch, J. D. H. 1959 The nature of the genetic differentiation between *Lolium perenne* and *Festuca pratensis*. *S. Afr. J. Agric. Sci.* **2**, 271–283.

Reusch, J. D. H. 1960 The effects of gamma radiation on crosses between *Lolium perenne* and *Festuca pratensis*. *Heredity, Lond.* **14**, 51.

Riley, R. & Chapman, V. 1958 Genetic control of cytologically diploid behaviour of hexaploid wheat. *Nature, Lond.* **182**, 713–715.

Schwanitz, F. 1953 Die Zellgrösse als Grundelement in Phylogenese und Ontogenese. *Züchter* **23**, 17–44.

Stebbins, G. L. 1950 *Variation and evolution in plants*. New York: Columbia University Press.

Stebbins, G. L. 1953 Species hybrids in grasses. In *Proc. 6th Int. Grassl. Congr.* (ed. R. E. Wagner, W. M. Myers & S. H. Gaines), pp. 247–253. Washington: National Publishing Company.

Stebbins, G. L. 1956 Artificial polyploidy as a tool in plant breeding. In *Genetics in plant breeding* (*Brookhaven Symposia in Biology*, vol. 9), pp. 37–52. New York: Brookhaven National Laboratory.

Taylor, I. B. & Evans, G. M. 1977 The genetic control of homoeologous chromosome association in *Lolium temulentum* × *Lolium perenne* hybrids. *Chromosoma* **62**, 57–67.

Terrell, E. E. 1966 Taxonomic implications of genetics in ryegrasses (*Lolium*). *Bot. Rev.* **32**, 138–164.

Tigerstedt, P. M. A. 1978 Ecological aspects of adaptation in hybrid swarms of plants. In *Interspecific hybridization in plant breeding* (ed. E. Sanchez-Monge & F. Garcia-Olmedo) (*Proc. 8th Eucarpia Congr.*, Madrid), pp. 31–40. Madrid: Ciudad Universitaria.

Waines, J. G. 1976 A model for the origin of diploidizing mechanisms in polyploid species. *Am. Nat.* **110**, 415–430.

Wit, F. 1959 Hybrids of ryegrasses and meadow fescue and their value for grass breeding. *Euphytica* **8**, 1–12.