PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

The Control of Recombination

R. Riley, C. N. Law and V. Chapman

Phil. Trans. R. Soc. Lond. B 1981 **292**, 529-534
doi: 10.1098/rstb.1981.0047

Phil. Trans. R. Soc. Lond. B **292**, 529–534 (1981)
Printed in Great Britain

The control of recombination

By R. Riley, F.R.S., C. N. Law and V. Chapman
Plant Breeding Institute, Maris Lane, Trumpington, Cambridge CB2 2LQ, U.K.

Recombination results in the release of variation upon which selection is practised in plant breeding. However, according to the objectives of the programme it may be necessary to reduce recombination to limit the disturbance of arrangements of genes already well suited to agricultural needs or to increase recombination to maximize the likelihood of recombination's giving rise to transgressive segregation. Appropriate breeding manipulations are discussed. In addition, descriptions are provided of the induction of recombination between chromosomes that are distantly related in evolution and between which meiotic pairing and recombination does not normally take place.

Since most plant breeding is dependent upon the release of variation as a consequence of recombination and segregation, a good deal of attention has been directed in plant breeding research to the management of this variation and to the control of its release. From the outset it is important to establish that the efficiency of the breeding process may be improved on some occasions by increased, and on other occasions by reduced, recombination. The more usual condition is that genetic advance can only be achieved by maximizing the range of variation in the population concerned, to provide transgressive segregation among the extremes of which selection can be practised. Alternatively, genetic advance may be of the most practical use when already well adjusted genotypes are disturbed to the minimum extent necessary to incorporate new, perhaps simply inherited, beneficial characters. It was to satisfy the latter objective that the backcross system of breeding was developed. It is appropriate to make this point since many of the systems of breeding used in variety production, like backcrossing, have the purpose of adjusting the consequence of recombination. In the present discussion, by contrast, attention will be concentrated on the direct control of recombination rather than on adjusting its effects. More attention has been paid to the control of recombination in wheat than in other genera, so that most of our examples will be drawn from the wheat crop group.

Relation of recombination to hybridity

The conventional processes of variety production in wheat (*Triticum aestivum*) are of classical pedigree selection from intervarietal hybrids, or some variant of this involving bulk selection, or the isolation of individual lines from heterogeneous populations generated from intervarietal hybrids. In addition, backcrossing is important where characters of complex inheritance, such as grain quality, must be preserved as the first requirement of successful varieties. Curiously, chiasma frequency – as represented by the frequency of bivalent chromosomes at the first metaphase of meiosis – is lower in intervarietal F_1s than in their varietal parents. By diallel cross analysis, using data obtained by Watanabe (1962), Riley & Law (1965) showed that this negative heterosis for chromosome pairing was largely the product of non-allelic gene interaction. Person (1956) showed in the backcross derivatives of intervarietal wheat hybrids that there was a higher frequency of chromosome pairing with an increase in the expected homozygosity. However, the return to the parental level of chiasma frequency was delayed relative to the return to homozygosity.

This example of the control of chromosome pairing, which shows a coarse association with the extent of heterozygosity, implies that the maintenance of linkage and of synteny may be high in intervarietal wheat hybrids and that, if a high frequency of recombination is sought, it may be necessary to prolong the period of maximum heterozygosity by intercrossing early generation segregants or by top-crossing. However, intercrossing F_2 plants derived from hybrids between two west European winter wheat varieties led to no greater release of variation because of the insignificance of linkage for the characters studied, which included height and grain yield (Snape 1979). The delayed return, following heterozygosity, to the maximum level of chromosome pairing may cause the production of aneuploids precisely at that stage at which new varieties are being multiplied for release, when genetic instability often creates problems for breeders. If a high standard of stabilization is demanded, there is no resolution to this problem that does not either delay the availability of improved genotypes to agriculture or diminish, by the introduction of backcrosses, the range of genetic variability among which the breeder can select.

So far reference has been made to aspects of the genetic control of chromosome pairing, and hence recombination, that must inevitably be considered by the wheat breeder and which can be manipulated according to the objective of the programme. We shall now describe breeding manipulation that can be used to alter recombination.

Limitation of recombination

Haploids

In inbreeding crop species, such as wheat and barley, the continuation of recombination and segregation in a number of the generations derived from intervarietal hybrids delays the fixation in the homozygous state of new potential varieties. Ways of circumventing this delay and of reducing the extent of recombination are provided by haploid breeding methods. In their simplest form these consist of deriving haploid sporophytes directly from the gametophytes produced by F_1 hybrids. Such sporophytes can be treated with substances, such as colchicine, which inhibit spindle formation and so have the chromosome numbers of some of their cells returned to the diploid condition. In these diploid sectors and in the lines derived from them the first products of recombination and segregation from the F_1 are fixed and, in inbreeding crops, each is potentially a new variety.

Much attention has been paid to the feasibility of culturing anthers or pollen grains in nutrient media and stimulating the development of haploid sporophytes from the pollen grains. This is a procedure that has proved particularly effective in the Solanaceae (Nitzsche & Wenzel 1977) but less so in the Leguminosae and the Gramineae. However, in the latter, for barley and possibly for wheat, there are alternative opportunities for haploid production. These arise because in F_1 hybrids between *Hordeum vulgare* (cultivated barley) and *Hordeum bulbosum* (a wild perennial) the chromosomes of *H. bulbosum* are frequently eliminated in the first few cell divisions in the embryo, leaving a sporophyte with only the haploid set of chromosomes of *H. vulgare* (Kasha & Kao 1970). The phenomenon of chromosome elimination permits the use of haploid breeding methods and the fixation of the first products of F_1 meiosis in barley breeding and thus the limitation of the breeding cycle to one occasion for recombination.

Subsequently, Barclay (1975) discovered that F_1 hybrids from the cross of *T. aestivum* × *H. bulbosum* developed into wheat haploids. Unfortunately the crossability of wheat with *H. bulbosum* proved to be confined, probably because of the activity of alleles at two loci, to some of varieties of Asian and Australian origin. There is as yet, therefore, no generally available methodology for the limitation of recombination in the derivatives of intervarietal F_1s in wheat.

Recombination confined to a single chromosome pair

Cytogenetic methods in wheat by which recombination could be confined to a single chromosome pair were first used by Law (1966). The procedure depends upon the initial construction of chromosome substitution lines in which a single unmodified pair of chromosomes from a donor variety replaces its homologous pair in a recipient variety. The background chromosomes in such a substitution line are those of the recipient variety, which are unchanged.

Hybridization between the recipient variety and the substitution line produces a hybrid in which there is heterozygosity for genes only in the pair with a substituted chromosome, while the background chromosomes are entirely homozygous. When this single chromosome heterozygote is used to pollinate a plant which is monosomic for the heterozygous chromosome, monosomic derivatives can be selected. In this case, the monosomic chromosome is that which was heterozygous in the pollen parent, so the first products of recombination and segregation are fixed in the hemizygous condition. Selfing of this recombinant-chromosome hemizygote will lead to the fixation of the chromosome in the disomic condition in euploid plants. Opportunities are thus offered of selecting for precise recombination in a single chromosome and thus for unusually 'fine tuning' of a plant genotype.

Control of homoeologous recombination

In euploids of *T. aestivum* ($2n = 6x = 42$) genetically corresponding (homoeologous) chromosomes of the different sets assembled at each advance in polyploidy do not pair at meiosis, principally because of the activity of the allele at the *ph* locus on chromosome 5B (for key to references see Sears 1977). In mutants of *ph* or when chromosome 5B is deficient, or when alien dominant or epistatic alleles are present, homoeologous chromosomes will pair and recombine (for references, see Riley (1974)).

In hybrids between *T. aestivum* and many of its relatives, in the genera *Aegilops*, *Secale*, *Agropyron* and *Haynaldia*, there is little or no pairing at meiosis between the chromosomes of the parental species. Consequently there is little or no recombination between wheat chromosomes and those of such relatives so that, in breeding, their potentially useful genes cannot be transferred to wheat chromosomes. The relation between the chromosomes of wheat and those of such relatives is one of homoeology, so that removal of pairing suppression by the *ph* activity results in pairing and recombination. Experimental interference with the *ph* activity in interspecific hybrids thus provides access to a new range of genetic variation.

The first example of the transference of alien genetic variation to wheat by induced homoeologous recombination involved the incorporation in *T. aestivum* of rust resistance from *Aegilops comosa* (Riley *et al.* 1968). In this work, the single chromosome (2M) of *Ae. comosa* that determines stripe rust resistance, and which would not normally pair with its wheat homoeologues, was isolated, by backcrossing, from all other *Ae. comosa* chromosomes in plants that also had the full complement of wheat chromosomes. Such monosomic 2M addition plants were then pollinated by *Aegilops speltoides* to produce hybrids in which, because *Ae. speltoides* has a genetic activity dominant or epistatic to *ph*, there was homoeologous chromosome pairing at meiosis and chromosome 2M could pair and recombine with its wheat homoeologues. Backcrossing to wheat followed and this resulted in the isolation of phenotypically normal euploid forms of *T. aestivum* with the rust resistance of *Ae. comosa* in which recombination had occurred between chromosome 2M and 2D. The recombinant chromosome possessed the short arm, the centromere and the proximal part of the long arm of 2M and the distal part of the right arm of 2D.

Subsequently a line was isolated with the segment of chromosome 2M determining rust resistance incorporated by recombination into chromosome 2A. This line arose in the same segregating population in which 2M–2D recombination had occurred.

Recombination between 2A and 2M was also obtained by using plants that were simultaneously nullisomic for 5B and tetrasomic for 5D. Because 5B is absent there is homoeologous meiotic chromosome pairing, but other effects of the deficiency of 5B are largely corrected by the compensating effects of tetrasomy for 5D. A crossing programme was followed that created a line nullisomic for 5B, tetrasomic for 5D and monosomic for 2M. Homoeologous pairing at meiosis enabled recombination to occur between 2M and its wheat homoeologues. After this cytogenetic condition had been maintained for one or more meiotic cycles, the nulli-5B, tetra-5D, mono-2M plants were pollinated by wheat plants tetrasomic for chromosome 5B. The derivatives of the cross had chromosome 5B returned to the disomic state, were trisomic for 5D and may or may not have carried chromosome 2M. In the next generation, which was obtained by selfing, selection was practised for 42-chromosome euploid plants with rust resistance. These segregants had undergone homoeologous recombination. The structure of the 2A/2M recombinant chromosome was analogous to that of the 2D/2M chromosome derived by the *Ae. speltoides* *ph*-suppression procedure. Methods corresponding to these were also used by Sears (1973) to induce homoeologous recombination between wheat and *Agropyron* chromosomes to incorporate alien leaf rust resistance in wheat.

Promising work is being carried out by P. Harris of the Plant Breeding Institute, on the transference from chromosome $1C^u$ of *Ae. umbellulata* to wheat of genes controlling high molecular mass storage glutenin proteins of the grain, which may be important in bread-making. The process that has been used to induce homoeologous recombination is based on the creation of nullisomy for 5B and tetrasomy for 5D in the presence of $1C^u$ but, since $1C^u$ was introduced from a line in which it was substituted for wheat chromosome 1A, it may be present as a monosomic addition or a monosomic substitution. It is suspected that 42-chromosome homoeologous recombinants have been isolated after the restoration of 5B disomy by the cross with tetrasomic 5B, because some plants possess the high molecular mass glutenins that mark chromosome $1C^u$ but not the gliadin bands that are also determined by genes on the same chromosome.

There is consequently ample evidence of manipulation of the *ph* system leading to the ready incorporation in wheat by recombination of simply inherited genetic variation of related species especially in the genera *Aegilops* and *Agropyron*. A surprising anomaly is that the frequency of meiotic pairing and recombination is very low between wheat and rye chromosomes, even when the *ph* activity does not occur. This is so even though there is much evidence to indicate the close genetic relationship between wheat and rye, such as the ability of rye chromosomes to compensate genetically for their wheat homoeologues (Riley 1965) and the high frequency of DNA segments with closely comparable nucleotide sequences (Flavell *et al.* 1977). There is some evidence of meiotic pairing between wheat and rye chromosomes (Bielig & Driscoll 1970; Mettin *et al.* 1976), but it apparently takes place with great rarity and there is no clear evidence of crossing-over.

Something of the nature of the genetic control of homoeologous meiotic pairing in rye was described by Riley *et al.* (1973). A genetic activity of the short arm of rye chromosome 5R is responsible for the increase of homoeologous pairing in wheat–rye hybrids. However, it is uncertain whether in plants with the highest level of pairing which had the haploid set of 21 chromosomes of *T. aestivum* (namely the ABD genomes) and two complete genomes of rye (RR) and additionally the short arm of $5R^s$, had any wheat–rye recombination. Probably this could only be detected by breeding experiments, and such have yet to be successfully undertaken with this highly infertile material.

Nevertheless, the derivation of recombinants between wheat and rye, which enables the association of the beneficial characters of both crops without the inadequacies of triticale, remains an important objective in the exploitation of the control of recombination. It is tempting to speculate that the failure of wheat–rye pairing is related to the presence of telomeric heterochromatin that occurs in the chromosomes of *S. cereale* and not in those of wheat and which may represent up to 18% of the nuclear DNA content of rye (Bedbrook *et al.* 1980). If meiotic synapsis is initiated terminally or the positioning of chromosomes before synapsis depends upon the composition of telomeric DNA, then modification of the distinctive structure of rye chromosomes may be a necessary preliminary to the occurrence of recombination between homoeologous chromosomes of wheat and rye.

Conclusions

The genetic control of recombination will influence the course of plant breeding programmes whether or not breeders attempt to intervene in the process. Clearly the structure of any programme will make allowance for this as one of the factors bearing upon the likelihood of success and the time course of the work. It is also apparent that the breeder can intervene in the control of recombination and obtain recombinants that would not have occurred without such intervention. These possibilities have arisen as a result of the availability of knowledge of the genetic and cytogenetic systems through which control is exercised. As further knowledge is provided of these systems, and particularly of such components of them as may be represented by telomeric structure in rye, intervention in the control of recombination will have greater precision than is now possible.

References (Riley *et al.*)

Barclay, I. R. 1975 High frequencies of haploid production in wheat (*Triticum aestivum*) by chromosome elimination. *Nature, Lond.* **256**, 410–411.

Bedbrook, J. R., Jones, J., O'Dell, M., Thompson, R. D. & Flavell, R. B. 1980 A molecular description of telomeric heterochromatin in *Secale* species. *Cell* **19**, 545–560.

Bielig, L. & Driscoll, C. J. 1970 Substitution of rye chromosome $5R^n$ for chromosome 5B of wheat and its effect on chromosome pairing. *Genetics, Princeton* **65**, 241–247.

Flavell, R. B., Rimpau, J. & Smith, D. B. 1977 Repeated sequence DNA relationships in four cereal genomes. *Chromosoma* **63**, 205–222.

Kasha, K. J. & Kao, K. N. 1970 High frequency haploid production in barley (*Hordeum vulgare* L.) *Nature, Lond.* **225**, 874–876.

Law, C. N. 1966 The location of genetic factors affecting a quantitative character in wheat. *Genetics, Princeton* **53**, 487–498.

Mettin, D., Schlegel, R., Blüthner, W. D. & Weinrich, M. 1976 Giemsa-banding von M1-Chromosomen bei Weizen-Roggen-Bastarden. *Biol. Zbl.* **95**, 35–41.

Nitzsche, W. & Wenzel, C. 1977 Haploids in plant breeding. *Fortschr. PflZücht.* **8**, 1–101.

Person, C. 1956 Some aspects of monosomic wheat breeding. *Can. J. Bot.* **34**, 60–70.

Riley, R. 1965 Cytogenetics and wheat breeding. In *Genetics Today, Proceedings of the 11th International Congress of Genetics* (ed. S. J. Geerts), **3**, 681–688. Oxford: Pergamon Press.

Riley, R. 1974 Cytogenetics of chromosome pairing in wheat. *Genetics, Princeton* **78**, 193–203.

Riley, R., Chapman, V. & Johnson, R. 1968 The incorporation of alien disease resistance in wheat by genetic interference with the regulation of meiotic chromosome synapsis. *Genet. Res.* **12**, 199–219.

Riley, R., Chapman, V. & Miller, T. E. 1973 The determination of meiotic chromosome pairing In *Proceedings of the 4th International Wheat Genetics Symposium*, Columbia, Missouri (ed. E. R. Sears & L. M. S. Sears), pp. 731–732. Agricultural Experiment Station, University of Missouri.

Riley, R. & Law, C. N. 1965 Genetic variation in chromosome pairing. *Adv. Genet.* **13**, 57–114.

Sears, E. R. 1973 *Agropyron*–wheat transfers induced by homoeologous pairing. In *Proceedings of the 4th International Wheat Genetics Symposium*, Columbia, Missouri (ed. E. R. Sears & L. M. S. Sears), pp. 191–199. Agricultural Experiment Station, University of Missouri.

Sears, E. R. 1977 An induced mutant with homoeologous pairing in common wheat. *Can. J. Genet. Cytol.* **19**, 585–593.

Snape, J. W. 1979 Detection of linkage for quantitative characters in wheat. In *Proceedings of 5th International Wheat Genetics Symposium*, New Delhi (ed. S. Ramanujam), pp. 591–596. New Delhi: Indian Society of Genetics and Plant Breeding.

Watanabe, Y. 1962 Meiotic irregularities in intervarietal hybrids of common wheat. *Wheat Inform. Serv.* **14**, 5–7.

Discussion

R. Johnson (*Plant Breeding Institute, Cambridge, U.K.*). Dr Riley showed a graph at the Meeting, indicating that pairing of chromosomes was reduced in F_1 hybrids and gradually reverted to normal over subsequent generations. However, restoration of full pairing appeared to lag behind the return to full homozygosity. Has chromosome pairing been examined in doubled haploids produced from F_1 hybrids or from segregating material in subsequent generations?

R. Riley. As far as I am aware, no detailed study of chromosome pairing of derived double haploids obtained from F_1 wheat hybrids has been carried out. Undoubtedly such an investigation would be of interest and might provide useful information about the relations between particular homozygotes, chromosome pairing and stability.