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Review

Genetic manipulation in plant breeding: somatic versus generative

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Summary. A comparison is made between molecular/in vitro/somatic and plant-level/generative approaches in the reconstruction of genotypes and reproductive systems. Although classical methods will remain the basis of plant breeding, a number of new somatic as well as generative genetic manipulation techniques are definitely applicable in several special situations. The first are technically more demanding, the latter are often conceptually more difficult, and both are laborious. Choice of approach is determined by the plant species, the stage of development of the techniques, the amount of background genetic information and the genetic diversity available, and the capacity of the institution involved. In the final stages of the program traditional selection and testing procedures remain indispensable. Whether any particular breeding program will profit from the incorporation of sophisticated genetic manipulation techniques must be carefully analysed. This discussion is intended to provide a basis for this analysis.

Key words: Genetic manipulation – Molecular/in vitro/somatic and plant level/generative approaches – Plant breeding

Introduction

1 Somatic vs. generative

The introduction and manipulation of genetic variation are the principal means of plant breeders in realizing their objectives. These objectives are the construction of genotypes (or collections of genotypes) that meet specified requirements, combined with reproductive systems ensuring the faithful reproduction or the repeated production of these (or equivalent) genotypes. Until recently, by far the majority of all plant breeding depended on the generative cycle, both for the indroduction (by hybridization) and for the manipulation (by recombination followed by selection) of genetic variation. Spontaneous and induced mutation, including the doubling of chromosome numbers, usually involved the somatic phase, but further possibilities of exploiting this phase were very limited.

With the development of molecular and in vitro techniques the prospects of exploiting the somatic phase have improved dramatically with respect to both the manipulation and the analysis (for collecting information) of the genetic content of plants. The application of these techniques to the generative phase have lagged behind, and as a consequence there appears to be, at present, an interrelation between molecular, in vitro and somatic approaches on the one hand and an interrelation between morphological, plant-level and generative approaches on the other. These relations may gradually become less pronounced, but necessarily a full exploitation of the possibilities of the somatic phase will continue to require a stronger molecular and in vitro input than that of the generative phase. It is to be expected, therefore, that in the future too, the question will remain relevant for the plant breeder, whether to invest considerably in molecular and in vitro techniques which open up (but will not remain restricted to) the somatic phase, or to rely on "old-fashioned" plant breeding through the generative phase. The answer to this question remains difficult not only because at present the development of costs and assets of the new techniques is difficult to predict, but also because certain developments of meiotic cytogenetics promise results which cannot yet be fully appreciated. Neither approach offers simple and cheap

solutions, and a careful analysis is required before a good choice can be made in any particular case. The following is an attempt to provide a basis on which such an analysis can be carried out. The format is different from the reviews of Barton and Brill (1983); Rees et al. (1981); Thomas et al. (1979) and others.

2 *Genotype and reproductive system*

Broadly speaking, plant breeding implies the adjustment of the genotype to meet specific requirements at the plant level, i.e. to adapt it to the "given" environment. Ideally, this results in a specific reproducible genotype or collection of genotypes. These genotypes are not necessarily simply the combination of desired alleles as may be realized (to a considerable extent) in a diploid self-fertilizing species. For maximal results interallelic interactions (heterosis) and gene dose effects (duplications, polyploidy) can play important roles.

The reproductive system is under control of the genotype and as such can be a breeding objective. Although the reproductive system of a plant species is usually taken for granted, there are notable exceptions. These derive in part from the conflict between the production and the maintainance of a specific genotype. The best known examples are hybrid varieties which cannot normally reproduce true to type, but there are others, which will be considered below. Most of such unusual reproductive systems are intended for the systematic repeated production of specific genotypes which do not breed true in the regular generative cycle, or which suffer from reduced fertility.

3 *Techniques of manipulation and their feasibility*

Somatic genetic manipulations can take place at three levels: plants, cells or cell aggregates in culture and isolated molecules. Induction of somatic mutation and somatic segregation can be carried out at the plant level without reference to the cells in which they occur.

Micro injection into cells as part of a differentiated plant is in principle possible although insufficiently worked out. It can be used to transfer cell organelles, nuclei, isolated chromosomes and DNA (usually in a carrier such as a Ti plasmid) for transformation. Application of isolated DNA to differentiated tissues has repeatedly been attempted and has not been proven to be principally impossible. Cells in culture can be treated the same way, but the separation of protoplasts from cell walls has until now been considered a more attractive substrate for manipulation than complete cells.

Protoplasts can be fused to produce hybrids or cybrids (a nucleus in an alien plasma) at different levels of ploidy and taxonomic distance. They can also be made to incorporate isolated chromosomes or DNA for nuclear transformation. Isolated DNA can be biochemically altered in various ways or entirely synthesized artificially and transferred to the nucleus of a cell to be integrated in the autochtonous DNA, or to be incorporated as extra chromosomal element in the nucleus or in a cell organelle, integrated or as extra element.

Manipulations through the *generative* phase involve the production of hybrids, again at different levels of ploidy and taxonomic distance. At the segregational end of the reproductive cycle (meiosis) segregation after recombination is the main means of manipulation. The products are usually isolated after fertilization but can also be isolated directly (as "haploids") by in vitro somatic techniques or otherwise. Recombination and segregation can be exploited further by employing special chromosomal constructions resulting from meiotic instability of special karyological combinations. In addition to manipulation of the genotype, manipulation of the reproductive system can be effected by special chromosomal and genetic constructions. Constructions made by somatic manipulations can be manipulated further through the generative cycle and vice versa. For some purposes the two approaches can be considered competitive, i.e. either one or the other can be chosen to realize a defined objective, in other cases they are complementary.

The various techniques of somatic and generative genetic manipulation are at very different stages of development. There is also great variation in applicability to different species and for different objectives. It is very difficult, therefore, to make a general comparison with respect to feasibility and prospects. In tobacco, for instance, in vitro and molecular techniques necessary for successful genetic manipulation have been developed to an extent where theoretically no barrier seems to exist for the transfer of any gene and the production of any somatic hybrid or cybrid. True practical application has not been realized yet, and in most other plant species and genotypes technical difficulties still seem almost unsurmountable. However, the range of genotypes which can be successfully manipulated is broadening. In general, one can conclude that when much effort is spent on a species, in the long run openings are found to achieve a goal by a special variant of a technique which is more widely applicable in "easy" species.

For approaches requiring meiotic cytogenetic techniques something similar is true: when complex systems are to be used, success can only be expected in species or genera where much basic research has already been carried out. One can state then, that whenever a commercial plant species is, or is expected to become, of great pratical importance, it pays to have theoretical geneticists "play" with it.

4 The collection of information

Information, on a particular species or genus, both of a general genetic (including feasibility for manipulation) and agronomic nature as well as specific information on individual plants is, therefore, essential. When not available at the onset of the program, this information is collected with the aid of several auxilliary sciences: (cyto)genetics, phytopathology, physiology, biochemistry, molecular biology, etc. The release of genetic variation usually required for such investigations is generally realized through the generative phase.

Although for the collection of information use is made of techniques related to those used for manipulation, they are not the same. In addition, molecular techniques for collecting information may be useful in programs mainly based on generative manipulation and vice versa. For instance, in situ hybridization to detect presence and localization of specific genes is not the same as introducing the gene into a genome but does require molecular probes related to the gene DNA. These can be used for detecting genes introduced by somatic or by generative techniques. The analysis of generative transmission of somatically manipulated chromosomes is best done by meiotic cytogenetic techniques. Assessing the chance of successful transfer of alien genes by generative methods requires specialized theoretical genetic knowledge. Clearly, the availability of special techniques for collecting information is as important as techniques for manipulation. This subject will not be considered in detail.

5 Phases in a plant breeding program

In a plant breeding program different steps or phases can be distinguished, carried out in sequence, and with some feedback interaction between them. One (very simple) example of such feedback is the adjustment of the objectives when the material appears to have properties not anticipated at the start of the program. There are, of course, more subtle interactions. The phases do not all have the same character: there are decision, design and operations phases. In Table 1, the sequence and a few feedback lines are shown, and in addition the character of the phases. It also shows where the need for information appears.

Choice and implementation of approach in the construction of genotype and reproductive system

The first phase of a plant breeding program (formulation of breeding objectives, Table 1) provides the basis of all further phases. It may be necessary, during the course of the program, to adjust the objectives, but they must be formulated at the start with as much precision as possible. The choice of approach in the construction of genotype and reproductive system is the first step towards the realization of the objectives. In relatively simple programs it hardly plays a role: the reproductive system of the species at hand is left unchanged and the desired genotype is the result of selection after within-species hybridization, possibly involving a series of backcrosses. For more ambitious programs a range of options is available from which a choice must be made. Some involve typical somatic manipulations, other manipulations through the generative phase. Many are presently still at the experimental stage and may turn out to be applicable only in a limited number of situations. The present analysis centers around this choice. An outline is given first with chapter headings of the main text indicated.

1 Construction of genotype

There are four general options, which need not always be separated.

1.1 Large scale segregation after recombination in progenies of hybrids between selected parents. It results in overall new genotypes and is the basis of conventional plant breeding with recombination occurring in the generative phase (1.1.1). Somatic segregation may play a modest but increasing role in the future (1.1.2).

1.2 Introduction of specific new genes, single or in low numbers, without other (major) changes in the genotype. These can be obtained a) by mutation of existing alleles, b) by replacing existing genes via introduction and recombination, or c) by addition, either integrated in the chromosomes of the recipient genome or in an additional chromosome.

1.3 Gene dose effects. Accumulation of genes in doses higher than two can be desirable for various reasons, including biochemical, physiological, morphological and segregational effects. Four forms will be distinguished:

a) Single gene multiplication
b) Duplication of short chromosome segments
c) Addition of entire chromosomes, if desired after size reduction and modification
d) Multiplication of entire genomes, identical (autopolyploids) or different to various degrees (allopolyploids)

1.4 Heterosis involving only a few, or numerous genes

2 Reproductive system

There is a conflict between the two functions of the reproductive system: at one stage it must permit introduction and manipulation (by recombination and selection) of genetic variation, and at another stage it must guarantee faithful reproduction or repeated production of the optimal genotype once it has been obtained. Natural systems have ways to cope with this dilemma, but at the expense of the quality of the end result. In plant breeding ways have been sought to maximize both, which has led to the development of a number of artificial reproductive systems. Non-sexual reproduction would be the best solution as it avoids segregation. Since vegetative reproduction has serious disadvantages (low reproductive rate; storage, transport and phytosanitary problems) except in cases where in vitro reproduction is satisfactory, apomictic seed production would seem the best solution. It circumvents most forms of sterility caused by instability of the generative cycle. Meiotically unstable transformants and directly applicable but sterile wide hybrids may become of increasing importance with the advent of somatic in vitro tech-

Table 1. The phases of a plant breeding program

Examples of feedback lines	Phase	Type			Requirements		
		Decision	Design	Operations	Information		Techniques
					general	specific	
	I						
	a. formulation of objectives	×			×		
	b. choice of approach in construction of genotype and reproductive system	×			×		
	c. program design		×		×		
	II						
	a. collection of material			×	×	×	×
	b. testing and preselection of material	×		×		×	×
	c. induction and checking of additional genetic variation			×		×	×
	d. design and construction of reproductive system		×	×	×	×	×
	e. combination (hybridization, etc.)			×	×		×
	f. recombination			×	×		×
	III						
	a. selection	×		×		×	×
	b. testing			×		×	×
	IV						
	a. propagation/maintenance			×	×		×

The present discussion concentrates on phase *I* b.
In most breeding programs emphasis is on phase *III*

niques. These will be entirely dependent on vegetative reproduction, and only apomixis will be satisfactory in seed reproduced plants. Since in most cases meiotic recombination or any other aspect of generative reproduction will remain indespensable at some stage of a breeding program, a major condition for success of apomixis is that it should be facultative and controlled. Apomixis as such is not easily introduced in crop plants and controlled facultative apomixis is as yet unknown. Therefore, on one hand it must be concluded that this should be one of the major subjects of basic plant breeding research, but on the other hand, it will remain necessary for a long time to rely on the development and exploitation of alternatives, even when these are difficult to realize, cumbersome to apply and only partly successful.

The following breeding- and reproductive systems will be considered:

2.1 Normal sexual reproduction

2.1.1 Disomic inheritance, diploids and allopolyploids
a) Self-fertilizers
b) Cross-fertilizers

2.1.2 Polysomic inheritance
a) Autopolyploids
b) Segmental- and auto-allopolyploids

2.2 Controlled or limited sexual reproduction

2.2.1 Hybrid varieties, including different ploidy levels
a) Hand emasculation and pollination
b) Cytoplasmic male sterility, hand or open pollination
c) Genic male sterility, hand or open pollination
d) Controlled incompatibility

e) Dioecy
f) Gametocides

2.2.2 Special systems
a) Permanent heterozygosity for chromosomal rearrangements
b) In vitro propagation
c) Apomictic seed formation
The choice will be determined partly by considerations specific to the material and partly by techniques available.

1 Construction of a genotype

1.1 Selection after (large scale) segregation

1.1.1 Generative phase. Heterozygous starting material from which segregating progeny is to be obtained is usually produced by generative hybridization. Although there are no principal objections against using somatic hybrids, there are technical reasons to avoid them: haploid parental cultures are not easy to obtain; somatic hybridization and regeneration are not standard technique yet for most crop plants; the in vitro phase is likely to be unstable. Usually the type of hybrid required is easily made by classical methods if necessary supplemented by in vitro culture of embryos. There is no reason why normal meiotic recombination in hybrids within species or between related species, followed by segregation and selection, could not remain the basis of most plant breeding programs (Borlaug 1983).

Recombination, especially within chromosomes, is usually neglected in plant breeding because of lack of time (Stam 1978). Effective recombination requires the maintenance of heterozygosity over several generations. This would cause an unacceptable delay in the release of marketable varieties. Probably the best way to make use of extensive recombination would be in the production of basic stocks of high quality, not prepared directly for the release of varieties but as parental material for future breeding programs.

The transfer of limited numbers of specific genes by recombination from one species to another will be considered in 1.2.

The possibilities of regulating segregation by modifying meiotic recombination have not been fully exploited. Within a species, recombination is regulated by two systems: overall level control and restriction in distribution. Number of points of crossing over and number of chromosomes determine overall level. Grouping of genes into linkage groups in chromosomes, and localization of crossing-over and interference determine distribution. Raising the overall level of recombination is possible only to a limited extent by external agents (Ihrke and Kronstad 1975) or special genotypes (Rose and Baillie 1979). This is not yet very effective, and the same result is obtained by selecting from a larger population. Lifting an overall restriction pattern, resulting in random instead of localized crossing-over, can have more drastic effects and several cases are known in which chiasma localization patterns have been thoroughly altered, usually by genetic means: hybrids between related but different species, inbreeding, and mutants (Jones 1967, 1974).

This approach offers possibilities which again have not even been explored satisfactorily. The disadvantage is the randomness of the effect, resulting in undesired in addition to desired recombination. The gene combination which enabled the recombination should be replaced ultimately. More promising but even less explored are external agents disturbing normal restriction patterns. High temperature shock may be a candidate, but usually mainly reduces recombination.

Specific breakage of linkage may be attempted by induced translocation, but this approach is not very promising: the chances of success are slight and the resulting translocation is usually not a desired new trait. Favourable exceptions are translocations between chromosomes of different species, made with the purpose of introducing chromosome segments from one species into another. These will be discussed in 1.2.

Translocation within species between non-homologous chromosomes are a form of alteration of chromosome recombination which can be used extremely well to restrict recombination where it is not desired by placing genes together in one chromosome, with a chromosomal break point in between. This seems more promising than breaking linkage, but is limited to special applications (2.2)

Although not yet known to be applicable in plants, in other organisms two forms of (drastically) altering recombination patterns in *specific* chromosome segments are known. In *Drosophila,* inversions in one chromosome resulting in practical elimination of crossing-over in the segments involved may induce very greatly increased crossing-over in specific segments in other chromosomes (Green and Green 1949) but this does not seem to occur in plants. From recombination pattern studies in lower eukaryotes (Simchen and Stamberg 1969) it has appeared that crossing-over in chromosomal regions is regulated by genes specific for that region but situated elsewhere. By selecting or introducing regulating genes increasing crossing-over in specific segments, breakage of undesired linkage can be envisaged. It is not certain that this approach will be easier then other methods of replacing one gene or allele by another (1.2).

Recombination barriers in hybrids between genetically differentiated forms require special approaches. There may be insufficient homology, or chromosomal rearrangements preventing crossing-over. The intricate systems developed to overcome such barriers in the generative phase will be discussed in 1.2.

There is one form of application of generative segregation which makes use of in vitro somatic techniques: raising plants from cells in the haplophase. It has been quite successful for recovering homozygous diploids after chromosome doubling, but for exploiting large scale segregation the technique has not been developed far enough, especially in monocotyledons. For self fertilizing species and for making inbred lines it is potentially an excellent technique which deserves further development. Spontaneous haploids have been used for this purpose for a long time (Chase 1952) and methods of selection have been developed (Hermsen and Verdenius 1973). Their frequency is genetically determined, occasionally rather simply (haploid-inducing genes) and can be greatly increased by pollinating with foreign pollen.

1.1.2 Somatic segregation is not normally considered to be of much practical importance in plants. In a sense sectorially appearing mutations (spontaneous as sports, or artificially induced) can be considered the product of somatic segregation but are not relevant in the present context (1.2). Somatic segregation possibly resulting from somatic crossing over was reported long ago (Jones 1939) and continues to be observed. Its use is limited, except when it can uncover recessive mutations. This may be of special interest in in vitro mutagenesis and certainly deserves more attention than it has had. Potentially more interesting is somatic segregation as first described by Franzke and Ross (Ross 1965) for a special genotype of sorghum. The application of colchicine to seedlings of the variety 'Experimental III' led to the segregation of homozygous diploid sectors in heterozygotes, arisen by doubling after somatic chromosome reduction. Colchicine and other spindle disturbing substances can induce "reductional groupings" in most plants, but only in a few genotypes will this lead to balanced genomes (Sybenga 1955). Chloramphenicol (Yoshida and Yamaguchi 1973) is also able to effect chromosome reduction. Parafluorophenylalanine, known to induce haploidization in diploid strains of *Aspergillus nidulans,* however, does not seem to be effective in plants, its main effect being restricted to the induction of occasional aneuploidy (Nitzsche 1980). With the present increased knowledge of spindle behaviour and genome compartmentalisation in the nucleus (Bennett 1982; Avivi et al. 1982), it should be possible to direct this process much more effectively than formerly considered practical and to apply it to in vitro cell cultures which permit a large and homogeneous population of cells to be treated and selected. A special form of somatic segregation at the plant level occurs in some unstable hybrids, where the genome of one species is eliminated. It is best known in the hybrid between *Hordeum vulgare* (barley) and *H. bulbosum* where the entire genome of the latter can be lost, resulting in haploid barley. It is presently the most effective way of producing haploids in barley, which can be doubled to completely homozygous diploids in one generation (Kasha and Reinbergs 1979). The use of the same technique in another important self fertilizing grain crop, wheat, is sufficiently far developed to be practically used on a limited scale. In other plant species, the in vitro culture of the male gametophyte seems to be the best approach to large scale utilization of haploidy for the production of homozygous diploids. Somatic segregation of chromosomes and of spontaneously mutated genes in cultured tissues is one of the factors contributing to undesired genetic instability for which such cultures are notorious. The practical use of this instability, which includes gene mutations and chromosome structural rearrangements in addition to numerical abnormalities resulting from irregular chromosome segregation (somaclonal variation, Larkin and Scowcroft 1981) has been suggested (Shepard et al. 1980; Shepard 1982). Although some variants in potato have been found which surpass the parent variety in performance in limited ecological situations, there are reasons not to be very optimistic. Especially in polyploids like the potato, gross chromosomal and gene abnormalities will be tolerated because of the buffering capacity of the polyploid condition, and would be eliminated rapidly in a diploid. They have a dominant phenotypic expression but will be overall-deleterious in the great majority of cases. Improved local adaptation outside the main production area may be found but in highly bred cultivars the risk of exclusive negative results is great.

A special form of somatic segregation of considerable importance is the segregation of cell organelles with their own genetic control system: mitochondria and plastids. There are three situations in which segregation is to be expected:

1. Mutation or transformation of some (not all) of the organelles.
2. Somatic hybrids between genetically different forms.
3. Cybrids in which the nucleus of one form is brought into the cytoplasm of another, carrying some organelles with it. Usually, either by drift or selection, perhaps even actively, all except one type of the organelle are eliminated. In mitochrondria some form of fusion or contact is possible, leading to recombination between the circular DNA main chains or to exchange of plasmid-like DNA. As yet it does not seem possible to direct the segregation process, but the phenomenon is of enough importance to be paid attention to.

1.1.3 Conclusion. Segregation after recombination in the generative phase is expected to remain the basis of most plant breeding programs. There are possibilities of increasing its effectiveness, mainly by altering natural systems of restricting recombination. These possibilities deserve further development and must be complemented by the use of external agents. Their importance resides in the occasional breakage of undesired very

close linkage or in the repatterning of gene blocks of an inspecific nature. The application of in vitro culture of the haploid gametophyte after generative segregation should be extended to include in vitro selection of rare recombinants. For several characters, however, the limitation will remain the lack of success of selection (low heritability) rather than lack of genetic variation.

Control of artificial somatic (in vitro) segregation, not based on instability of the mitotic process alone, can and should be further worked out. It results in chromosome recombination practically without crossing-over and permits the handling of massive numbers of segregants, provided the proper selection techniques are available. As always with somatic genetics, this will remain a serious limitation.

Organelle segregation is of sufficient importance to be controlled artificially, one reason being that this is required for artificial transfer of cytoplasmic male sterility and other organelle DNA controlled characters. The generative and somatic approaches to the exploitation of segregation complement rather than compete with each other.

1.2 Introduction of specific new genes

1.2.1 Mutation. With hardly any exception, mutations are induced in the somatic phase in the plant or in vitro, permitted to segregate somatically and selected either directly in the plant or in vitro when (co)dominant, or after segregation in the generative phase when recessive or when the somatic phase is not considered suitable for selection. Although mutations are not induced randomly and each group of agents has its own mutation spectrum, in practice all desired mutations tend to be accompanied by undesired mutations when the dose is not very low. The inability to strike a balance between the necessity of extensive selection programs necessary with low doses and the disadvantage of negative side effects of higher doses is a major reason of the limited practical success of mutation programs involving a generative segregation phase. Good solutions are available (Dellaert 1979) but usually neglected by mutation breeders. The use of dominant somatic plant level mutations on the other hand has been very successful in ornamental plant breeding (Broertjes and van Harten 1978). In vitro somatic mutations appear to be induced readily by some, but hardly by other agents effective in the plant. On the condition that good selection criteria are available (a general bottleneck in in vitro breeding) the system is quite promising because low mutagenic doses can be used, or none at all, since the selection of a single mutant in millions of unmutated cells is in principle possible. Low doses tend to avoid the negative effects of simultaneously induced negative mutants. A problem is the usually high level of spontaneous mutation in the callus phase (genetic instability) for which somehow a solution must be found in all in vitro breeding programs. Short callus phases and immediate formation of embryoids from protoplasts isolated from differentiated tissue may be the best solution but is not yet possible in the majority of cases.

A second problem is the exclusive recovery of dominant or codominant mutations when diploid or polyploid somatic cells are used. This is the reason that haploid cells are preferably employed in in vitro mutation induction programs. These are of direct use after chromosome doubling in self-fertilizers and as inbred lines, and as such quite valuable. On the other hand, dominant mutations in themselves are a very important class of mutations and neglected in programs involving a generative segregation phase, because of their extreme scarcity compared to recessive mutations, which have received all attention. In vitro selection permitting very large scale selection is a good way to rediscover the importance of dominant mutations (Chalef 1983). Some of great promise have already been recovered or are expected to be found soon: disease (toxin) resistance (Gengenbach et al. 1977); stress tolerance (heat, cold, salt, heavy metals, Chalef and Parsons 1978; Colijn et al. 1983). Co-dominant factors (iso-enzymes, seed proteins) may follow, and several novel useful mutants may appear as experience accumulates. Some tolerance mutants appear to be based on gene amplification, and will be discussed in 1.3.

1.2.2 Introduction into the genome from outside. Entirely new genes which cannot be obtained by mutation of an existing allele can be introduced into the genome from outside. Depending on dominance and epistasis relations of genes already present in the genome and coding for existing functions incompatible with that of the new gene, the original gene may have to be removed by deletion, inactivated by mutation or replaced by homologous recombination. Since the generative cycle permits the use of several subsequent rounds or recombination, it has been widely used in classical plant breeding in the form of repeated backcrossing to replace undesired genes by desired alleles. When the allele to be introduced is recessive, intermittent generations of selfing have to be inserted to ensure that the recessive allele is carried over to the next generation. Even after extensive recombination it is practically impossible to transfer a gene without carrying along other genes (hitch-hiking). When the transfer is between related forms of the same species, this may be acceptable. More difficulties are encountered with the transfer of genes between different species. As long as there is some recombination, the program may still succeed, but the removal of undesired co-trans-

ferred genes may become difficult. It may then be necessary to depart from the original objectives and accept the necessity to adjust the genotype to the effects of incorporation of sets of alien genes, rather than single genes, i.e. create a genetic background in which the action of the alien genes is acceptable. An alternative is not to transfer the specific gene from a donor into a recipient species but to combine the two into an allopolyploid (1.3.4). Namai et al. (1980) showed that in *Brassica campestris* and *B. oleracea* both approaches may work and that the choice between the two is legitimate.

A number of intricate programs of introducing foreign genes (often for dominant disease resistance) through the generative cycle have been developed, particularly for allopolyploids in which the necessary steps of addition and substitution of entire chromosomes are carried out much more readily than in diploids. As early as 1956, Sears published a report on the transfer of leaf rust resistance from diploid *Aegilops umbellulata* to allohexaploid wheat via the addition of the *Ae. umbellulata* chromosome carrying the resistance gene, followed by radiation induced interchange with a wheat chromosome. This means that part of a wheat chromosome was lost and a segment of an alien chromosome was introduced. Although in an allopolyploid the effects of such irregularities are rather limited, the transfer (after addition) by induced homoeologous recombination (Riley et al. 1968: stripe rust resistance from *Ae. comosa* to wheat) is more elegant, as the loss of a chromosome segment is compensated by the gain of a homoeologous segment. It is also more favourable as it permits, in principle, the transfer of recessive alleles, the dominant allele being removed in the process. In allopolyploids, however, it may be necessary to remove dominant homoeologous or epistatic alleles in other chromosomes, for instance by mutation. Numerous genes have been transferred by such methods, especially in wheat, but the interest has decreased especially since resistance due to single genes is often easily broken and the techniques are laborious. In wheat homoeologous recombination can be increased by genetic methods: removal of the pairing restriction gene in chromosome 5B or by hybridizing with one of a number of related species resulting in loss of the pairing restriction system. In other species this is less simple and external agents with the same effect are not known.

The interest in remodeling genomes by transferring groups of less specific genes from one species to another often also including a gene for a special character, has increased after it appeared that several successful bread wheat varieties carried rye chromosomes replacing homoeologous chromosomes. Also, hexaploid triticales of AABBRR constitution sometimes have particular rye chromosomes replaced by homoeologous D genome wheat chromosomes. Some-times half chromosomes (single arms or even parts thereof) are involved. The development of staining methods permitting the cytological recognition of specific chromosome segments has greatly facilitated the selection of transferred segments. The relatively easy production of translocations with breaks at the centromere by using univalents at meiosis greatly increases the possibilities of transfer of specified parts of chromosomes (Lukaszewsky and Gustafson 1983). Allopolyploids other than wheat lag behind, and in diploids comparable techniques are not yet available, although experiments with alien trisomics are promising.

In the somatic cycle only in vitro techniques are available to transfer single genes or small groups of genes. These suffer from the same problems as other in vitro methods: frequent technical failure at some important step in the process and genetic instability in the callus phase. Yet the molecular techniques presently available are so promising that the plant breeders cannot afford to neglect them. The principal aim is to introduce alien DNA into the recipient genome (nuclear, mitochondrial, plastid) by some form of transformation. Many methods have been attempted: naked DNA, liposome encapsulated DNA, bacterial plasmids and phages and their derivations, viral vectors. Of the latter, only cauliflower mosaic virus is a DNA element which has been suggested; as yet without success, although considerable effort is being spent on it. Tissues, cells and protoplasts have been used as target material. Several reviews of the techniques involved and their evaluation are available (Cocking et al. 1981). Isolated bulk DNA applied as such has been reported to be successful as transformant, but the reports are insufficiently convincing. Fertilization of an egg cell by an irradiated, desintegrating male nucleus appears to result in transformation of the egg by integration of DNA in a random manner (Pandey 1978). The only really successful vectors for transfer of specific DNA, are the *Agrobacterium* Ti plasmids. Modified Ti plasmids lacking the undesired oncogenic properties but still carrying the sequences necessary for integration into plant nuclear DNA can be provided with any DNA segment of reasonable length, and made to integrate stablely into the nuclear DNA of the host (Hoekema et al. 1983). Expression is variable. Of several genes transferred, only resistance genes have been found to be expressed until now (Schell 1983). Since DNA of any segment of any plant species can be isolated, cloned pure and stored (in a library), in principle all genes of any plant and even genes from other sources are available for introduction into the genome of a selected host. A number of restrictions, however, must be made.

– The specification of a desired trait in terms of genes to be isolated is only infrequently possible: only a few recognizable single genes can be characterized as "desirable" genes.
– The isolation of the donor DNA sequences covering the entire gene with all introns necessary for normal functioning, still requires very much specialized labour.
– Insertion of the new gene into a segment of the genome where it can come to expression in the plant is not guaranteed. Even with its own regulation system expression may be insufficient.
– Since the insertion process is one of transposition rather than homologous recombination, any dominant or epistatic genes present in the host genome will not be automatically removed.
– Many species, including all monocotelydons, among which the most important grain crops, are nonhosts to *Agrobacterium.*

On the other hand, the testing of the effect of numerous alien DNA sequences in plant genomes will reveal so much about the functioning of such sequences, that sooner or later the information will become available on the basis of which more directed, effective gene transfers will become possible, including sequences affecting quantitative characters. In several years to come, this will be the most important consequence of the application of recombinant DNA techniques to plants. DNA libraries of many plant species and cultivars, including complete sequences of specified genes, will be built up in many places and, even when (partly) patented, will become available for the scientist and plant breeder on request and when paid for.

Gradually new techniques will develop: transformation by homologous recombination with cloned DNA without vector, applied by micro-injection directly into nuclei, even in tissues, eggs or young embryos, as has been so successful in mammals (Palmiter et al. 1982). This will circumvent the problems of in vitro culture. Already it is possible in a number of cases to avoid a callus phase and to regenerate plants via embryoids from single protoplasts, cells or small cell clusters (Pierson et al. 1983), even in monocotyledons (Vasil and Vasil 1980).

In addition to the introduction into a plant cell or protoplast of DNA fragments, entire chromosomes can also be taken up. This is considered in more detail in 1.3.4. For the present section two applications of chromosome uptake are important: 1. alien chromosomes as a source of alien genes, subsequently to be incorporated in the host genome by some form of recombination; 2. minichromosomes as permanent vehicle of a single gene or a cluster of genes.

1.2.3 Conclusions. There is a modest but legitimate place for mutation breeding at the plant level, with emphasis on recessive mutations in generatively reproduced species and dominant and recessive mutations in vegetatively reproduced species. In vitro mutation breeding is promising, especially for scarce dominant mutations and for recessive mutations when the generative cycle is difficult to handle and haploid cultures are available. The bottleneck is the selection system. In additions to toxin-, drugs- and some forms of stress resistance, new systems will gradually be developed which may ultimately give rise to some very important new dominant mutations in addition to recessive mutations.

Transformation using Ti plasmids will be with us in the near future. However, there are still so many technical difficulties with somatic manipulation that it pays to continue to develop generative techniques of gene transfer. Manipulation of homoeologous recombination and the necessary preceeding steps (chromosome addition and heterozygous substitution possibly after somatic hybridization) for the introduction of alien genes or groups of genes deserve further study in spite of decreasing interest in favour of somatic techniques. When the pendulum swings too far in the somatic direction, the critical mass of cytogeneticists working on generative genetic problems may not be available and high level research at a sufficient scale will then become impossible.

1.3 Gene dose effects

1.3.1 Multiple copies. It is quite common in eukaryotes to find that genes or gene clusters occur in *multiple copies,* usually in tandem (histone, r-RNA, seed protein genes), or spread over the genome (5S-r-RNA- and t-RNA genes). This is necessary for the production of large amounts of the primary gene product which in these cases is also the end product. Numerous non-transcribed sequences occur in multiple copies, millions occasionally (repetitive DNA), but these are of limited importance in the present context. It may be expected that multiplication of specific genes can be of interest in plant breeding for the production of specific proteins which may be useful as such or for producing certain effects such as stress tolerance. In principle, errors during meiotic recombination may produce duplication of single genes, but these are scarce and hard to detect. Probably the best analysed example of large scale gene amplification in the somatic phase is methotrexate resistance in animal cells (Cowell 1982). The gene for dehydrofoliase, which is an alternative target for methotrexate, amplifies continuously under a methotrexate regime. It can do so in tandem, which leads to relatively stable mega chromosomes, or in the form of minichromosomes without centromers, which occur in large numbers but disappear after removal of metho-

trexate from the medium. Amplification involves the large gene itself with sizeable introns and large flanking sequences including an autonomous origin of replication and a regulator. There must also be a sequence regulating the amplification. Amplification of chromosome segments resulting in mega chromosomes without any selective agents has been observed in *Nicotiana* hybrids by Gerstel and Burns (1966). Sequence amplification is quite common during evolution and speciation in many plant genera in heterochromatin. Even large blocks can occasionally be observed to be lost or doubled (Gustafson et al. 1983). It is also not impossible that gene amplification may be involved in drug or toxin resistance after in vitro selection in plants. In principle the mechanism might also be exploited to amplify other genetically important sequences, but the regulation of amplification is as yet insufficiently understood to be manipulated. Although application will be limited, it is a development expected to be successful in the not too far-off future.

Multiple gene copies can also be introduced by repeated or large scale transformation by non-homologous integration, or by the addition of numerous minichromosomes made artificially. Although transposon integration appears to show interference in the sense that there is a minimum distance between subsequent integrations, the eukaryote genome is large enough to accomodate several copies of a gene. When all have to be active each may have to carry its own system of regulation. This is doubtlessly a possibility, and in fact, with high doses of DNA offered, multiple integration may be a disadvantage rather than an asset for genes for which a single dose is the optimum.

1.3.2 Duplication of small chromosome segments may be an alternative when only one or a few additional copies are required. The presence of excess random groups of genes is usually a disadvantage, but their negative effect can often be neutralized by specific genetic backgrounds. Because of their often negative effects, large duplications are difficult to make homozygous in diploids. Random duplications carried along when specific genes are duplicated as parts of larger segments can be considered to constitute a genetic load in the sense that they resistrict the tolerated variation in genetic background, leaving a reduced potential to select from for other puposes. Yet duplications, including relatively large ones, have been important in evolution (Ohno 1970) and have been demonstrated to occur on a considerable scale in numerous diploid organisms. They can be introduced in the somatic phase by agents causing chromosomal rearrangements. The simplest origin is symmetric interchange between homologous chromosomes, but there are several more. This must be followed by some form of segregation, usually in the generative phase (Sybenga 1975). Duplications can also be the consequence of aberrant meiotic segregation of other types of chromosomal rearrangements. By selection of the proper rearrangement, duplications of specific segments can be realized (Hagberg 1965; Patterson 1973; Sybenga and Verhaar 1980). The method of combining specific translocations to produce the duplication of a chromosomal segment containing a specific gene has been applied in barley to duplicate the α-amylase gene which is important for the brewing properties of the grain (Hagberg 1965). The method is applicable only in crops where a large stock of rearrangements is available, as it can hardly be expected to pay to introduce these for the purpose of producing a specific duplication alone. Even in the case of barley, where hundreds of translocations are available, the results are far from impressive. The dose effect of the extra copy of the gene is disappointing and the negative effects of the duplication of accompanying genes is not negligible.

In addition to a dose effect, there is the interesting possibility of allelic interactions, ranging from general heterotic effects to interactions between specific alleles. Since duplications do not usually recombine, a specific combination is simply maintained even in self-fertilizing species. Very little is known about the real role of such effects in the establishment and maintenance of duplications in nature, but it is almost certain that such effects exist (Ramanna, personal communication).

1.3.3 Addition of entire chromosomes (polysomy). Extra chromosomes can be found in all conceivable forms and sizes. Besides (1) functioning as a large duplication, there are two more applications: (2) as intermediate in a process of transferring specific genes carried by that chromosome from one species or form to another, (1.2.2) and (3) as an independent but permanent vehicle of a single gene or small specific gene cluster (1.2.3). It is not difficult to produce trisomics, but these are not stable in normal meiosis unless provided with a balancing system which usually works at the expense of reproductive efficiency. Some such systems are promising for producing very specific reproductive systems (2.2.1), but as a means of effecting practically applicable duplication of genes they tend to fail. One can say that when a separate chromosome is large enough to function at meiosis, it is too large to be tolerated as a duplication. There are two possible exceptions:

a) Compensating trisomics in which two rearranged chromosomes replace one normal chromosome, together carrying all the information of this chromosome in addition to segments of other chromosomes. Some such compensating trisomics have reasonably stable meiotic behaviour and may carry relatively small additional segments (Khush 1973; de Vries 1983).

b) Translocated B-chromosomes produced by interchange between an A-chromosome and a B-chromo-

some, followed by meiotic segregation yielding a B-chromosome as an extra chromosome, but carrying an extra fragment of a normal chromosome (Roman 1947; Beckett 1982). When the accumulation mechanism of the B-chromosomes is not impaired by the translocation, muliple copies can be obtained but their generative reproductive capacity may be low. A B-chromosome with a segment added to it containing little more than a single gene with major effect, and without the accumulation system, is in principal an excellent vehicle for single genes to be added to an existing genome, if necessary after the homologous undesired alleles have been removed.

Both types (compensating trisomics and translocated B) can be produced by meiotic segregation after induction in the somatic phase. The desired effect can be again a dose effect or an allelic interaction or a heterotic effect. No practical application has yet been realized. The reason is simply that duplication of a specific small segment (less than one or a few percent of the genome) is in practice very difficult and laborious – almost no plant breeding groups are actively engaged in this type of work.

Somatic approaches to transferring (molecularly modified) B-chromosomes may in principle be developed in the future, but need not be much simpler to apply than meiotic approaches.

In vitro transfer of entire chromosomes from one plant to another is a real possibility. Isolation only succeeds practically in the form of condensed mitotic chromosomes. In order to obtain sufficient numbers in relation to the debris from which living chromosomes are hard to separate, large numbers of synchronized cells blocked in mitosis are the best source. Rapidly growing tissues must thus be used, and when in vitro cultures of the donor species or donor genotype are slow in growth, root tips of plants can be a good source. The chromosomes can be applied in bulk or after sorting in a flow cytometer, marked with a vital fluorescent stain (de Laat 1983). Other sorting methods may become available but have not yet been developed so far (electrophoresis, gravitational methods, fluctuating electric fields). Incorporation of free chromosomes into host cells by simple Ca^{++} precipitation on mono layers of cells, as may be successful with mammalian material, does not seem practical yet with plant cells. Fusion with protoplasts using polyethylene glycol is the simplest approach (Griesbach et al. 1982; Szabados et al. 1981). One prerequisite is that the host cell is at mitosis, or nearly so, as the condensation pattern of host nucleus and the chromosomes to be introduced must correspond. If not, too rapid adjustment processes may damage the chromosome structure. In some cases, this may exactly be one of the purposes of the project, but the effect is random. Mitosis is also the best stage as free chromosomes may not be readily incorporated into a "closed" nucleus at interphase, and separate condensed chromosomes do not survive long enough in the cytoplasm to be included in the next cycle. This prerequisite implies that only a fraction of the recipient protoplast population is available, and that slowly growing cultures will be hardly accessible to whole chromosome transfer in vitro. In such cases micro-injection into selected cells at the right stage may someday become the solution and then not necessarily protoplasts but even cells in differentiating meristems can perhaps be treated. When a selective system on a specific gene in the transferred chromosome is available, this is sufficient. In other cases the small number of treated cells must be sustained by co-culture in an auxotrophic (Hein et al. 1983) or a temperature sensitive feeder line which is inhibited later at the appropriate stage.

Additional whole chromosomes may be useful as such as a duplication, when of the proper size and composition, perhaps in addition to having a heterotic effect. A more probable application of somatically introduced chromosomes is to function as an intermediate for the transfer of one of its genes to the host, by recombination or translocation, probably through one or more generative cycles (1.2.2). It may replace time consuming or even impossible hybridization followed by a complex procedure to eliminate all other chromosomes introduced in the process. A third application is as a permanent vehicle of one or of a cluster of very specific genes, with the exclusion of any other genes which are potentially deletereous when occurring additionally to homologous genes elsewhere in the genome. Translocations between A and B-chromosomes are potentially of interest as they can be made large enough to be functional in mitosis and meiosis and yet can be made genetically empty, except for the genes to be added. It is expected that such chromosomes can be constructed from isolated, size reduced B-chromosomes transformed in vitro with DNA from other sources. It may be too optimistic to expect that especially reconstructed cereal B-chromosomes containing all genes required for nitrogen fixation and their promoters, would soon become available for incorporation in any cereal species but the principle is not just imagination.

Another suggested approach is to start with yeast minichromosomes containing centromere sequences and having genes, ARS, promotor and other necessary sequences built onto it. Although it may not be impossible to construct a complex which is properly replicated and transcribed, it may be expected that mitotic transmission in plant cells requires more than an active centromere sequence. Meiotic transmission is almost certainly excluded because the small size prevents proper association by chiasmata or otherwise with a

pairing partner, which is necessary for orientation and segregation. Unpaired eukaryote sex chromosomes can orientate properly when in addition to a major centromere, a weaker (neo)centromere is present. Other univalents can behave similarly (Sybenga 1981). Dicentric yeast minichromosomes, however, do not function (Mann and Davis 1983) as is the case with normal dicentrics with well separated centromeres in eukaryotes. Only when the secondary centromere is much weaker than the main one can proper but still random meiotic segregation be expected.

1.3.4 Genome multiplication. Many important crop species are allopolyploids, some are autopolyploids, yet it appears difficult to produce artificial polyploids good enough to compete with the diploid progenitors. The main effects of *autopolyploids* are: gene dosage effects (specific and general: gigas characteristics, lateness), special interallelic interactions and, in outbreeders, which autopolyploids usually are, heterosis of a complex nature. The genetic system is tetrasomic which implies retarded release of genotype-based phenotypic variation. This may be a positive aspect in some instances, but implies difficulties with selection. The effects mentioned are usually nonspecific and may be partly negative. As a consequence, only a limited number of genotypes permit the full use of autopolyploidy. Fertility and meiotic stability especially are weak points and in most successful autopolyploid crops, natural and artificial, the seed is usually not the major product. Induction is most successful in the somatic phase by the simple doubling of chromosome numbers using colchicine. The resulting partial homozygosity is undesired, and the generative alternative – (spontaneous) doubling at meiosis resulting in a higher degree of heterozygosity – is to be preferred. Meiotic doubling is normally scarce, but to start with a somatically produced tetraploid, which is subsequently used to pollinate a diploid with a few unreduced gametes, is a practical approach. Very often the triploid majority of the progeny is not viable at early embryonic stages or at least is sterile when grown. The few fertile tetraploids are readily selected against this background. Depending on when in meiosis the process of reduction fails, the unreduced gametes will have more or less lost the original heterozygosity of the parent (Hermsen and Ramanna 1981; Skiebe et al. 1963). Certain genotypes of *Solanum, Brassica, Medicago* (McCoy 1982) have relatively high frequencies of unreduced gametes. The use of unreduced gametes of a diploid even results in higher levels of heterozygosity in the tetraploid progeny than can be realized by using reduced gametes of a fully heterozygous tetraploid.

The contribution of somatic in vitro techniques to the production of autotetraploids goes along two lines. Some cell populations or calluses are unstable and produce doubled subclones. This can be exploited when colchicine treatment of plant parts is ineffective, and it has been applied in potato (Hermsen et al. 1981) and tomato. The result is not better than colchicine treatment as simple doubling is involved. Much more promising is somatic hybridization by protoplast fusion of selected, genetically different strains. This results in autotetraploids with optimal levels of heterozygosity. In a number of in vitro programms with potato, a natural autopolyploid, this is presently being applied.

Allopolyploids also are constructed because of expected favourable gene dose effects and complex allelic interactions. The main purpose, however, usually is the stable combination of different genotypes, either because of specific character combinations or heterosis of a general nature, or both. Since the genetic system is essentially disomic, the combinations are maintained during generative reproduction. Although many important established crop plants are allopolyploids, including species grown primarily for seed, it appears to be quite difficult to produce successful artificial allopolyploids. The main reason is meiotic instability and other sterility causing factors. These are partly due to general negative gene dose effects, as in autopolyploids, and partly to interactions between the genomes, which may even act at the diploid somatic level, resulting in hybrid instability. Again, only a limited number of genotypes will finally be satisfactory at the allopolyploid level, and this is a serious restriction on any breeding program with artificial allopolyploidy. Allopolyploids are often produced by somatic doubling of species hybrids. As with autopolyploids, meiotic doubling is superior but again usually is preceded by somatic doubling. The major contribution of in vitro cell manipulation to this field is the direct production of heterozygous allopolyploids by somatic hybridization. In a limited number of cases this may succeed where generative hybridization is not successful. Wide species crosses, not possible in the generative phase (even when aided by modern techniques), may succeed in vitro, but there is no great chance that the resulting allopolyploid will be more than a curiosity, containing a combination of genomes which is not useful from a practical point of view. Such combinations, however, may be an acceptable starting point of gene transfer between species. There is a continuous progress in the development of techniques of producing somatic hybrids (Shepard et al. 1983). In vitro selection of hybrids is one field which requires improvement. The technique of isolating fusion products by micromanipulation, followed by growing them in an auxotrophic feeder medium which is ultimately removed (Hein et al. 1983) is promising. It is also the best approach for growing isolated cells after cell micromanipulation.

Improvement of regeneration is another important aspect. A number of interesting allopolyploids have been produced, mainly in families which in general are favourable for in vitro culture (Solanaceae: tomato-potato hybrids, Melchers et al. 1978; Cruciferae: *Arabidopsis-Brassica,* Gleba and Hoffmann 1979). The Gramineae are more resistant, also because protoplasts are not easily made and fused. For use as allopolyploids, less exotic combinations will be more promising. Some may soon be expected to be made on a scale sufficient for including the extensive genetic variation necessary for genetic adjustment to the polyploid character and the hybrid combination.

1.3.5 Conclusion. As soon as molecular transformation becomes widely adopted the usefulness of introducing multiple copies of single genes will become clearer. There will be possibilities for raising production levels of specific substances and for resistance to stress and certain diseases. Autonomous amplification systems are still unsufficiently understood to be applied. Duplication of small chromosome segments and entire chromosomes does not seem to be sufficiently interesting to justify extensive effort except when very specific segments can be duplicated. There is still insufficient knowledge of the reaction of most genotypes to such duplications – which are not rare in nature. The main application of additional chromosomes is probably in the form of chromosomes which are genetically empty except for specific (clusters of) genes and which are large enough to be stable in mitosis and meiosis (manipulated B-chromosomes). A combination of generative and somatic approaches, presently still at their infancy, is potentially promising.

Polyploidy remains important and somatic hybridization is potentially useful even for closely related species in which somatic techniques do not meet with great difficulties. Both autopolyploidy and allopolyploidy still require considerable generative cytogenetic study before sufficient knowledge is available for regulating meiotic stability and fertility.

1.4 Heterosis

Heterosis implies complementary interactions of specific alleles of different genes, occasionally interactions between different alleles of the same genes. The two mechanisms are not readily distinguished experimentally, and this is not necessary when heterosis has a general character ("hybrid vigour") and can be realized by combining two or more different specific genomes. Complementary specific alleles of different genes can, in principle, be combined in one genome by recombination, but for complex characters of an unspecific nature this is difficult. Different alleles of the same genes can be combined in one "genome" in allopolyploids, and in some duplications in diploids. This can, at the same time, involve specific allele combinations of different genes, and these are then reproductively stabilized. More artificial forms of reproductive stabilization of heterosis have a different basis, but also depend on the reproductive (including the genetic) system, and will, therefore, be considered in the next section.

2 *Reproductive systems*

The reproductive system must fulfill two conflicting duties: it must permit the introduction, recombination and removal of genes and it must subsequently faithfully reproduce the new combinations.

2.1 Natural sexual reproduction

2.1.1 Disomic inheritance: self- and crossfertilizing diploids and allopolyploids. When the existing reproductive system is maintained, the introduction of genes from closely related taxa does not present problems. Genes from less related forms present special problems, as discussed earlier. Recessive and dominant alleles are readily expressed in diploid self-fertilizers, and there is no problem with selection except in unusual situations involving gene interactions. Undesired recessive alleles are not readily removed from cross fertilizing populations. In allopolyploids, expression of recessive alleles can be problematic by dominance of alleles in homoeologous chromosomes. Highly diploidized allopolyploids suffer less from this drawback. Artificial resynthesis of an allopolyploid from its supposed parental diploids can be used to introduce certain new alleles, but in practice is not really very simple.

The change from one breeding system to another is not readily accomplished. In order to exploit the possibilities of heterosis in self fertilizers it is usually ineffective to try to turn it into a cross-pollinator. Thus, an artificial systems of constructing hybrid varieties, or some other system (2.2), is much more attractive as it ensures a completely stable and optimal expression of heterosis without segregation. A few successful attempts have been made to produce productive self pollinating inbred lines from naturally cross breeding horticultural crop plants, especially in facultative cross breeders. However, if anything artificial with respect to the breeding system is planned, it usually pays to try a system which fully exploits heterosis. Even in allopolyploids, which already have a great heterotic potential, and many of which are (predominantly) self pollinators, such artificial systems have a promise. In general, when a change in the reproductive and genetic systems is considered, it will be towards an artificial rather than a different natural system.

2.1.2 Polysomic inheritance. Except for very few natural and experimental exceptions, polysomic inheritance is found only at the (auto)polyploid, predominantly the tetraploid level. It is not probable that any of these cases have developed because of the advantages of the genetic system. The main reason will have been gene dose effects and specific allele interactions. In natural autopolyploid species, the genetic differentiating effect of autopolyploidy may have played a role in speciation. Yet, polysomic inheritance as such may also have its advantages: with low levels of outbreeding a considerable level of heterozygosity is maintained.

There are two major disadvantages:

a) Although heterozygosity is maintained longer than in diploids under inbreeding, it is not stable, as it is in allopolyploids.

b) In most instances polysomy is accompained by multivalent formation and reduced chiasma frequencies which lead to unequal segregation as a result of aberrant multivalent behaviour and univalent formation. This is a serious cause of reduced fertility, in addition to gene balance related causes. The solution is complex and can be sought in a reduction of multivalent frequencies or regular orientation of multivalents. Usually, merely selection for fertility is practiced, irrespective of the causal mechanisms. It should be possible to select on different fertility factors more selectively. It is improbable that enough genes can be specified, identified and isolated for somatic transformation to really improve fertility of artificial autopolyploids by somatic genetic manipulation.

Although artificial allopolyploids have their own problems with respect to fertility (1.3): they do not form multivalents and at least part of their heterosis is fixed. For both reasons there have been several attempts to change the polysomic type of inheritance of autopolyploids into a disomic system. It is not enough to introduce a gene such as the *Ph* gene in wheat which prevents pairing between closely related homoeologues in allopolyploids. There must be a basic differentiation upon which such a gene can act. Attempts have been made to use chromosomal rearrangements as a differentiating agent (Bender and Gaul 1966). Suggestions have also been forwarded of using genetic intra-species differentiation systems in addition to such rearrangements but results so far have been disappointing as the principle must be applied to all chromosomes to a suffucient extent (Sybenga 1973). Nevertheless, more recently new attempts have been made using rearrangements to allopolyploidize autotetraploid barley (Scholz and Künzel 1981, personal communication; Meister and Brettschneider 1977). It may perhaps be feasible to make use of artificial differentiation between a limited number of chromosomes which are of particular importance for heterotic effects. Such differentiation can be reinforced by an overall acting gene, such as the wheat *Ph* gene, effecting compartmentalization of nuclei (1.1.2). The result would be segmental allopolyploidy. The opposite is also available: an allopolyploid (hexaploid wheat) in which two homoeologous pairs have been replaced by four identical chromosomes (Sears 1966), but practical application is not obvious. It is clear that this type of genomic manipulation offers certain possibilities but requires considerably more research than has been spent on it. It is a typically meiotic approach, perhaps to be aided by the somatic introduction of specific genes which, however, have not yet been isolated.

A special variant is autoallopolyploidy (AAAABB), which is suspected to occur in nature, but which has not received serious consideration in plant breeding except in a transient stae in programs to transfer genes (Kleijer, 1982: Festuca-Lolium) or to study meiotic processes.

2.2 Controlled or limited sexual reproduction

2.2.1 Hybrid varieties. By far the most important reason to manipulate the reproductive (primarily the breeding) system of a commercial plant species is to ensure the full exploitation of heterosis. At the same time uniformity can be maintained. This had led to the development of a number of systems to produce hybrid varieties. Most operate at the diploid level, some at the allopolyploid level, some are inter-ploidy hybrids (triploid hybrid sugarbeets, for instance, resulting from systematic crossing of diploid and tetraploid strains). Autopolyploid hybrid varieties are not yet commercially available, but seed-reproduced tetraploid potato seems to be the best candidate for the near future.

There are several possibilities for producing hybrids between inbred lines or other homogeneous stocks. These can be simply maintained separately and then systematically hybridized to make hybrid seed. In the most important crop where hybrid seed is used on very large scale, maize, often two rounds of hybridization are applied to decrease cost of seed, but this is somewhat at the expense of productivity and uniformity (double cross).

There are several different systems for maintaining the basic stocks and for producing the hybrids. Most are based on the elimination of the male gametes from monoecious plants, leaving essentially female stands to be fertilized by interspersed pollinators. There has been an extensive search for chemical gametocides which so far has been really successful only in wheat. The substance is strictly monopolized by the producer. In the self pollinator, wheat, the gain by heterosis is marginal and the parental strains are sufficiently productive as to compete with the hybrid without requiring a costly seed production program. Obviously, the main reason is to prevent the distribution of excellent seed stocks which

can easily be reproduced by others. As long as generally applicable gametocides are not on the market, other techniques of eliminating male gametes and even completely different approaches will have to be used.

2.2.1.1 Hand emasculation, followed by hand pollination, as carried out with some horticultural crops, is economical only when the price of the seed is extremely high. Variations including hand emasculation combined with open pollination in isolation, or using hand selected male sterile segregants, hand or open pollinated, may be somewhat less expensive but are still restricted to a limited number of commercially grown plant species. Only in maize, which has an exceptionally favourable plant structure, is large scale mechanical or hand emasculation possible.

2.2.1.2 Cytoplasmic male sterility, followed by open pollination, is the most successful generally applicable large scale technique for producing hybrid varieties. Male sterility is maternally inherited, and fertility can be restored by specific genes in the pollinator parent. The present approach is to introduce the nuclear genotype of one parent into a sterile cytoplasm and the restorer gene into the other parent, both by backcrossing. The male sterile line is maintained by pollination with a fertile isogenic line. The entire system is based on the presence of two genes in the material (one mitochondrial, one chromosomal), and it is understandable that several attempts are being made to transfer these genes by somatic manipulation. Isolation of restorer genes does not yet seem to have been successful, but the observation that cytoplasmic male sterility is, in a number of cases, determined by the mitochondrial genome – sometimes a mitochondrial minicircle or other plasmid – has opened up very favourable ways of transferring this trait from one variety and even one species, to another. This would not only make large series of backcrosses unnecessary, but also create possibilities for transferring male sterility to species where it was not previously available in the desired form. It is assumed then that somatic transfer of mitochondria or their components is easier than transfer by generative hybridization. This is not an unreasonable assumption, but insufficient data are available as yet for stating that this is a solved problem. A further advantage of somatic transfer is that it may be done at a level where molecular modifications of the DNA involved are possible.

This may prove important as some excellent male sterility inducing mitochondrial mutants have appeared to produce side-effects which may be disastrous (*Helminthosporium* susceptibility in 'Texas' cytoplasm in maize). It may be expected that in species which are favourable for somatic cell genetics (Solanaceae and Cruciferae, for instance) within a few years cytoplasmic male sterility will have been transferred. The role of restorer genes in the control of the proliferation of mitochondrial plasmids is being elucidated (Palmer et al. 1983). At present, the characterization and isolation of restorer genes are not far enough advanced to seriously think of transfer by DNA transformation. This will take longer in the Gramineae, which are less easy to work with, e.g. in maize, which is the most important crop plant in which hybrid varieties are used. The attractive prospects will no doubt serve as a stimulus in solving the special problems of somatic in vitro genetics with the Gramineae: difficulties with the production of good protoplasts, genetic instability and problems with regeneration to plants. These same promising prospects, however, will retard the development of alternative (meiotic) approaches and even the optimal application of established traditional techniques. It is not certain that this is at all favourable.

2.2.1.3 Genetic male sterility with open pollination in isolation has been proposed as an alternative to cytoplasmic male sterility when good sterile plasmas are not available or appear to be accompanied by undesirable side effects. Although restorers are not required (the fertile dominant allele is automatically introduced by the pollinator parent), the large scale maintenance of male sterile lines is so complicated that in spite of some seemingly successful attempts, no full scale commercial application is known presently. In some horticultural crops it pays to select the 25% male sterile plants from a segregating F_2 or 50% in a back cross, but this is not applicable to field crops. Here, a self regulating system is required. The system first proposed (Ramage 1965) is based on balanced trisomics. A line homozygous for the recessive male sterility allele and a (conditional) lethal marker is provided with an extra chromosome with the two dominant alleles. Recombination with the normal chromosomes either by replacing it or by crossing-over, must be prevented. This is usually done by introducing a chromosomal rearrangement, often a translocation, so that the plant is a tertiary trisomic (Ramage 1965) or a compensating trisomic (Sybenga 1982), but it may take other forms too. Driscoll (1981) designed a system for wheat and Paterson (1973) has employed a duplication for the same purpose in maize. The result in all these cases is an apparently normal, fertile plant. The extra chromosome must be large enough not to be transmitted by the pollen, which consequently only contributes the normal chromosomes to the progeny. These have the recessive alleles of the male sterility and the marker gene. Used as a pollinator of a male sterile female, the result is completely male sterile progeny. The trisomic fertile plant upon selfing produces trisomics only since the marker is (conditionally) lethal and plants lacking the extra chromo-

some die. The marker is also transferred to the sterile partner, and 50% lethality must be accepted. If the selective marker is a conditional lethal, there is no lethality as long as the killing agent is not applied.

In genetically well studied material, all necessary markers and rearrangements are either available or can be induced in a moderately extensive program. The problem is mainly that all components together function only in a limited number of genotypes. These are not only rather hard to find, but the limitation they impose on the genetic variation available for selection on production factors may be of importance. The system works in the self fertilizer barley, although the tolerance to extra chromosomal material is sufficient to permit some undesired pollen transmission. The second bottleneck is that sufficient pollen for cross pollination is not always available. In maize other problems have appeared. In rye, balanced systems can be readily produced as long as inbreeding has not gone far. Trisomics in inbred material, however, are usually insufficiently fertile. However, genotypes can be found which apparently function reasonably well, and sometimes only a limited number of genes seem to be responsible for proper functioning. A useful variant may be the introduction of a gametocidal alien chromosome (Endo 1982). There also appear to be single genes with reduced pollen transmission which make the use of extra chromosomal material unnecessary.

This should be developed further. The introduction by molecular genetic engineering of the dominant alleles of a) a pollen killing gene, b) a conditional selective marker and c) a male sterility gene, together into an otherwise empty but meiotically stable extra chromosome, transmissible by somatic engineering, would be a worthwhile goal to achieve. At present nuclear gene male sterility is mainly a subject for generative cytogenetics, also in the exploratory phases. Once developed, the system is better manageable than it seems at first sight and in species where the proper cytoplasmic male sterility system is not available it may be wise to attempt the construction of a nuclear system. The prospects are reasonably positive, but progress may be expected only with considerable investment.

2.2.1.4 Conditional self-incompatibility in principle offers excellent possibilities for managing heterosis. When two lines can be selfed under one regime and are self-incompatible under hybrid seed production conditions, they can be made to intercross and produce hybrid seed exclusively. Although in nature established self-incompatibility systems will readily generate and maintain new incompatibility alleles, it is not yet possible to produce incompatibility genes in species where they do not exist already. Although temporarily breaking of self-incompatibility is successful in some horticultural species, mainly *Brassicas*, application to other crops, although in principle interesting, seems remote as yet. It is a field which deserves more attention, but the question is open as regards the approach which will finally appear to be the best. It is an aspect of the generative reproductive system, but somatic methods of genetic manipulation may contribute to the analysis of the nature of the genes involved and may some day produce a genetic incompatibility system which can be controlled.

2.2.1.5 Dioecy, although an exception, is found in a number of important commercial plant species: hemp, spinach, asparagus and others. Since usually one of the sexes is preferred above the other for reasons of productivity, sometimes for other reasons (hemp), there is a tendency to restrict cultivation to that sex and turn it into a genetic hermaphrodite (monoecy). In asparagus the hybrid between female XX and artificial male YY gives uniformly the most productive XY. Since in principle dioecy is an excellent basis for heterosis breeding, it will pay to look for ways to link sexual dimorphism in reproduction to a selective system which eliminates one of the two sexes in the production phase. It is known that chemical treatments can transform one sex into a hermaphrodite (Mohan Ram and Sett 1982). There are also possibilities for cytogenetic systems related to the balanced trisomy systems, which result in the production of only one sex in certain progenies. Although such systems are not known to have been developed yet, their possibilities should be explored further.

2.2.2 Special systems of sexual reproduction with maintenance of stable heterosis have their best examples in *Oenothera* spp. and *Rhoeo*. The basis is a balanced system of translocation heterozygosity. In some species of *Drosophila*, notably *D. pseudoobscura*, inversion heterozygosity is a well known and very effective means to preserve recombination free gene blocks with pronounced heterotic effects. The phenomenon has certain characteristics enhancing its functionality which cannot be described here. Translocation heterozygosity has similar properties, but is more abundant in plants, where inversions are scarce. Single translocations have been found "floating" in populations of numerous plant species, among which several Onagraceae, including the genus *Oenothera*. They appear to be especially favourable for maintaining moderately specific heterosis involving limited chromosome segments under conditions of periodical inbreeding. For practical purposes they are not sufficiently stable to be effective. In some species of *Oenothera* such translocations have means of preventing the formation or survival of homozygotes of either type. Other related species combine more than one translocation in a complex with similar properties. The most perfect system is that of *Oenothera lamarckiana*, where 12 chromosomes are involved in one large complex which forms a ring or chain at meiosis with practically perfect alternate segregation. The result is that at anaphase one genome moves to one pole and the other genome to the other

pole with recombination only by crossing-over near the ends of the chromosome. The two genomes together form a heterotic combination and there are principically two ways of preventing homozygotes from forming or surviving:

1. Recessive zygote lethality, resulting in 50% seed abortion compensated by a great abundance of seeds.
2. "Renner-effect", with non-functionality of one genome in the pollen and of the other genome in embryo sac formation. In the latter, the non-functioning genome, if by chance situated in the position from where the embryo sac is normally formed, is replaced by a functional genome in the other position. This is the system best fitted for a practical application as there is no waste of seed.

It has been possible to combine a series of translocations in barley, including all chromosomes (Sisodia and Shebesky 1965). However, sterility was almost complete, one reason being that the original translocations had been detected by their semisterility. Watanabe (1962) induced translocations somatically in a sequence in a clone of *Tradescantia palludosa* (2n = 12), a species related to *Rhoeo,* which has a very effective system of complex translocation heterozygosity. Although individual translocations in *Tradescantia* tend to segregate quite favourably, the complex failed to do so. Also, multiple interchanges in pearl millet were not functional (Brar and Minocha 1982). Apparently, predominant alternate orientation is not sufficient, it must be accompanied by sequential orientation combined with reorientation. It may be possible to construct a satisfactory system in a crop plant after considerable additional research on centromere reorientation has been carried out. Genes affecting pollen functioning (2.2.1.3) are known to exist but genes regulating embryo sac formation have not been reported outside *Oenothera* yet. As a first step, a system involving only a limited number of translocations may be developed.

2.2.3 Asexual reproduction. For practical purposes three forms can be distinguished:

a) Natural vegetative reproduction: stolons, runners, bulbs, etc.
b) In vitro propagation
c) Apomictic seed formation

2.2.3.1 Vegetative reproduction by stolons, runners, bulbs, etc. is common in numerous economical plant species, especially in horticulture. There are several advantages: propagation of reproductively sterile cultivars; maintenance of the same genotype over generations, also when highly heterozygous; lack of a juvenile stage (except some bulbils). There are also disadvantages: easy transmission of several (especially virus) diseases, low reproduction rate, poor keepability, etc. There is a certain interest in extending the possibilities of plant level vegetative reproduction, but in general, the following method appears to be more interesting.

2.2.3.2 In vitro propagation is becoming of increasing commercial interest and its use ranges from potted flower plants to forest trees. For typical field crops it is important in rapidly propagating special genotypes (sugarbeets) in plant breeding programs, but as yet unsuited for large scale field use.

2.2.3.3 Apomictic seed production would probably be the best method of reproduction in cultivars where juvenile stages are not important, provided it is controlled or can be induced after the appropriate genotype has been established. It has all the advantages of vegetative reproduction and lacks most of the disadvantages. Apomixis is very rare among commercial plant species. The grass *Poa pratensis* is an exception. Here apomixis is not strict and irregular segregation occurs. It can certainly not be controlled yet. There are several modes of apomixis, some maintaining a form of meiosis. In a number of *Solanum* species (Mok and Peloquin 1975) and some *Brassicas* and other Cruciferae, suppression of the first or second meiotic division or restitution by spindle fusion is rather common and can be seen as a first step towards the development of apomixis. Especially in hybrids and amphidiploids, tendencies toward apomixis after meiotic breakdown can be observed (Ellerström and Zagorcheva 1977; Mujeeb-Kazi 1981). Restitution after the first meiotic division results in practically unrecombined progeny. After the second meiotic division, however, it permits some recombination, and inbreeding upon selfing. When pollinated with foreign pollen, pseudogamous parthenogenesis will result in the development of diploid, maternal type embryos and seeds. When pollinated with the same species, reduced gametes may be fertilized and used for further breeding. Forms with a high frequency of first division restitution combined with male sterility or self-incompatibility can be interplanted with pollinator plants of a related species or of a special genotype of the same species. It may not be impossible to identify and isolate specific genes responsible for first division suppression. When such genes would be found and could be manipulated molecularly an important avenue would open up. For the development of parthenogenetic varieties, however, more will be needed than the introduction at the right stage of a single gene. An interesting combination is asynapsis followed by restitution (Ramanna personal communication).

There are other, relatively simple modifications of meiosis which result in the absence of reduction and recombination, and where pollination is necessary only for stimulation of embryo growth and fertilization of the secondary pole nucleus. One is found in a number of tetraploid *Allium* species (Gohil and Kaul 1981) and consists of an extra cycle of DNA synthesis, followed by endoreduplication in the last premeiotic interphase, similar to what has been found in a number of parthenogenetic insects. The original sister chromatids instead of the homologous chromosomes form pairs

and meiosis follows normally with reduction from the doubled to the somatic number without segregation. Its genetics is not clear, but there is a good chance that only one or a few genes are involved.

Apomixis without meiosis (apospory) is common in numerous wild species and many different types are known, some of which have been genetically analysed (Rutishauer 1967). A rather common variant is the development of a nucellar cell into an embryo (nucellar embryony) followed by normal seed formation. In facultative apomixis many factors affect the degree of sexual reproduction: genetic factors, day length, temperature, etc. In view of the importance of the subject, it is very important that the basic possibilities of inducing apomixis in economically important plant species and the ways of modifying it are thoroughly studied and that an attempt be made to identify and molecularly isolate genes with a major effect on apomictic reproduction. Somatic introduction by any form of transformation of such genes should be a major goal of molecular plant breeding.

2.3 Conclusion

There seems to be little promise in changing one natural reproductive system into another. If anything is worth attempting, it is an artificial system. By far the best is controlled apomixis, but so far only the first steps have been taken. It is a subject of sufficient interest to deserve intensive study. In the distant future, vectors (DNA segments of the proper composition or small chromosomes with apomixis inducing genes) may become available which can be introduced into and if necessary removed again from selected genotypes at will after the genotype has been constructed.

As long as this has not been realized other systems must be relied upon for (re)producing segregationally unstable genotypes. Heterosis breeding will continue to use male sterility. Chemical gametocides applied to susceptible lines intersown with resistant pollinators will become available for some crops. For crops where this appears to be unsatisfactory, cytoplasmatic male sterility will be increasingly important. There seem to be possibilities for somatic transfer of the mitochondrial *ms* gene into species where such genes are not known. Typical meiotic systems involving complex chromosome behaviour and chromosomally located male sterility genes are in principle promising and even operative but in practice still disappointing. Although insight into these problems is accumulating, it is uncertain if sufficient scientific man power will be available to develop these and other (e.g. permanent translocation heterozygosity) techniques far enough for practical use. In self pollinators one reason of increasing importance for heterosis breeding will be the possibility to monopolize special genetic stocks. In cross-pollinatoes the heterotic effect is of primary importance, but monopolization is not negligible.

Information

The choice of an approach in constructing a genotype with specified properties, and of a reproductive system implies that the techniques required must be, or become, available. This includes the techniques necessary to collect specific information on the initial material, on the operations carried out, and on the results of such operations. These techniques belong to several biological disciplines: phytopathology, plant physiology, biochemistry etc. and several genetic subdisciplines. A number of aspects of the operations (level of recombination, presence and functioning of special meiotic systems, etc.) and of the reproductive system, can be studied in meiosis by techniques which are not especially difficult, and can be carried out at most plant breeding institutions. Perhaps electron microscopic observations on synaptonemal complexes and microtubule behaviour at meiotic anaphase are not readily accessible to smaller institutions, but the majority of the techniques are simple. The bottleneck, rather, is the conceptual complexity of the meiotic process involving special chromosomal constructions, and consequently the difficulty of correctly interpreting the observations, for which expertise is generally lacking. This is true even for seemingly simple gene transfer by recombination.

For the collection of information on the presence or functioning of genes in plants, the complexity of the cytological, physiological, phytopathological and biochemical techniques required is steadily increasing. Nevertheless, there is a tendency for plant breeding institutions to keep up with it, since industry offers excellent equipment.

In conjunction with the development of techniques which are so promising for genetic engineering at the somatic phase, techniques have been developed which make it possible to carry out analyses at the cellular and molecular level. In vitro selection methods have been mentioned, which can be used to check the presence of certain genes. There are also cytochemical techniques useful for analysing specific gene functions. The use of molecular probes in the exact localization of genes on chromosomes (Szabo and Ward 1982) can be applied for their detection after introduction by any of the methods described. Although not yet far enough developed to detect differences between alleles, their use can be quite important. It may not be expected that a practical plant breeding station will readily develop new molecular probes. The application of probes with fluorescent markers ordered from the shelf or tailor

made may be expected in the near future to such an extent that their use can be considered an extension of regular, somewhat sophisticated, cytological techniques.

General conclusions

Returning to the starting point, we can again broadly distinguish between in vitro/molecular/somatic and plant-level/generative approaches. It appears then, that the latter are conceptually more difficult but technically less demanding than the first, although in total amount of work required for a positive end result, the two may be comparable. The stage of applicability of the generative approaches is presently further advanced than that of the somatic techniques and very much so of course when traditional programs are included. The expected development in the near and intermediate future is the reverse: somatic, in vitro and molecular techniques will continue to be developed at a high rate, and the meiotic developments will come slowly. The reason is partly one of fashion and the fact that the developments in biotechnology, medicine and plant breeding run parallel; a very important aspect. There is also the attractive possibility of patenting molecular techniques. The conceptual complexity of meiotic phenomena and the laborious programs are a clear bottleneck for the development of generative approaches. Although short cuts using in vitro somatic methods at some stages are possible, this will solve only part of the problem.

Now, evaluating possibilities for the near and moderately far future, a number of predictions can be made.

In the choice of an approach in the construction of a genotype with specified characteristics, expectations with regard to feasibility play a significant role. It is obvious that generative segregation will remain important: it is a proven approach to plant breeding and by far not exhausted. There are possibilities of modifying existing generative recombination patterns by choosing gene combinations which disturb regulation of chiasma localization. This is important when a genotype has to be grossly repatterned in order to fulfill new requirements. Although there are a few general rules, each species makes its own demands with respect to the manipulation of recombination. There is every reason for the plant breeder to pay attention to this subject, but in a normal breeding program there is insufficient time. One solution is to produce several high quality base stocks using extensive recombination and draw from these for practical breeding programs.

In vitro culture of haploid meiotic segregants (immature pollen) will become of increasing importance when new techniques develop. In how many species this will ultimately appear to be applicable is hard to predict, probably many in the long run. Preconditioned, free cells in a feeder culture with a conditional dominant lethality may be a promising approach. Combination with selection on specific recombinants perhaps even on induced mutants, as yet an unexplored field, will doubtlessly develop with the improvement of in vitro selection techniques in general. The techniques will not be too difficult for application in an average laboratory, as existing in many institutions, but not in simple set-ups.

Somatic plant level chromosome segregation is possible only for few genotypes. It is a special form of limited segregation of existing variation. Special forms of hybrid instability are already standard in a few cases (barley haploids). In vitro, the possibilities are greater but as yet unpredictable and must be developed further before application is possible. Its use is most apparent in conjunction with other in vitro work. The plant breeder must keep track of the possibilities but has no reasons yet to seriously contemplate incorporating somatic segregation as such in any program.

Cell organelle segregation is not interesting as such, but only as part of programs of organelle manipulation for special purposes.

In the field of segregation, somatic and generative approaches are complementary rather than competitive, with a strong emphasis on the generative phase.

The introduction of specific new genetic variation by plant-level mutations followed by large scale selection may be more interesting for generatively reproduced crop plants than is often believed. Strict adherence to the protocols is generally neglected but is essential. There are no serious technical difficulties. In vitro somatic mutagenesis and selection requires more technical specialization but has important potentialities, especially with respect to dominant mutations. The necessity of working in the haplophase to recover recessive mutations is a serious draw-back. The average medium sized plant breeding institutions would be advised to postpone embarking on this field until more positive results have became available. Field testing of all mutated segregants is a possibility for vegetatively reproduced crops for which the in vitro phase then is merely a reproduction phase. Spontaneous genetic aberrations arising in vitro are not necessarily positive.

The transmission of existing specific genes from one form to another remains possible along the generative way but is laborious, imperfect and, with genes from remote relatives sometimes practically impossible in spite of some very sophisticated techniques. It still is the only practical method available. Some help in intermediate stages may be received from somatic in vitro techniques (such as somatic hybridization). The knowledge necessary to control all steps required for long

distance transmission accumulates only slowly, particularly with respect to recombination and non-homologous exchange. There is definite progress, enough to apply the techniques available with some confidence, but not enough for wide application. The programs will always require very careful preparation and a large scale of operations which limits their practical use. As long as the alternative in vitro approach has been insufficiently worked out to handle transmission of complexes of numerous interacting genes, there is every reason to invest more in research on generative transmission of alien genes and gene complexes than is presently practiced. There is a host of meiotic techniques available, but most require further development. Meiotic techniques of exchange (homologous, homoeologous and directed non-homologous) will remain indispensable for the transfer of large gene blocks, both for analytic and practical purposes.

The in vitro molecular transformation systems have not yet yielded practical results but will soon do so. It will not be long before ready-made vectors with a specific gene, a regulator and an autonomous replication sequence, integrated into Ti plasmid derivatives, will be available for sale or to be applied under licence. Perhaps mini-chromosomes with similar properties, probably containing entire gene complexes (N-fixation?), may follow later. Reasonably well equipped laboratories will be able to perform the transmission independently, others will prefer to buy stocks with the gene already incorporated and some will order some specific vehicles to be made for them. The Solanaceae and Cruciferae will come first and other genera will follow. Mitochondrial (male sterile) and plastid genes will within reach at a somewhat later stage. The costs are high, and specifying and isolating genes, and somatic handling of the recipient genotype are still difficult. Therefore, application will remain limited until much more is known about the functioning of the genotype, in particular as regards the interaction of recognizable genes in the expression of quantitative characters. The ways to grow plants from protoplasts or single cells at will, maintenance of genetic integrety, etc., will remain bottlenecks for some time. Alternative target cells, possibly in differentiated plant organs will come into use.

For special purposes where dominance and epistasis are no bottlenecks, a meiotically stable, modified B chromosome with specific gene complexes added by molecular manipulation may become of practical significance.

Regulated, specific gene amplification for dose effect purposes may result from multiple integrating of transforming vehicles. Directed amplification of genes already present is not impossible, but does not seem to be close to application. In fact, it has not been established what the practical use really is. Simple specific duplications produced by somatic rearrangement and segregation are scarce and too random to be applicable. Duplications produced through directed meiotic processes are better but of limited effect and usually accompanied by many deleterious side effects. The same is true for modified B-chromosomes with large segments added, and for compensating trisomics.

Multiplication of entire genomes in autopolyploids and allopolyploids will continue to be of interest although the information necessary for making them successful is accummulating only slowly. There is an important task for cytogeneticists in increasing our understanding of the problems underlying the difficulties encountered and in finding a solution. The production of auto- and allopolyploids through the generative phase by the occasional failure of meiotic reduction, starting from some somatically doubled material deserves preference over other techniques. Somatic hybridization serves the same purpose and will be applicable after the technique has become really simple. This limits its use to only a few commercial species but some will certainly be successful. Very wide hybrids are not promising as such, but they may be starting material for gene transfer, perhaps in combination with improved somatic segregation methods.

With respect to the reproductive system, there is little reason to expect that natural systems will be replaced by others except by very artificial systems which primarily serve to conserve special allele combinations and heterosis. The most important is controlled apomixis. There will be little progress in the near future towards its development in any crop. It would be very valuable if some day for all important economical plant species a transmissable apomixis inducing vector would be available, even when it would be patented and expensive. Before this is realized, several intricate alternative systems to preserve heterosis will evolve and be improved. If it will indeed be possible, as is expected for a number of species, to transfer cytoplasmatic male sterility by somatic genetic manipulation, hybridization systems based on this form of sterility will expand. Plant breeders will probably buy ready-made stocks rather than develop them independently. Genic sterility is an alternative with considerable potential, and in some cases somatic in vitro techniques may help to introduce the components necessary to make it work. Gametocides may in the end replace genetic systems for maintaining male sterile stocks but their application is not without problems. Genetic systems, once developed, work automatically and can be transferred. In several instances the major purpose of making hybrids will be the possibility to strictly monopolize valuable genetic material. Whether or not any of the approaches available will really be applied depends on the number

of qualified theoretical scientists sufficiently interested to use them as a playing ground. The same is true for other special systems, including the Eunothera type of permanent heterozygosity, allopolyploidization of autopolyploids, and dioecy. When the pendulum will swing back again somewhat from somatic to meiotic genetics, the time will come.

In vitro somatic genetic manipulation includes a number of techniques which can rather simply be adopted by medium sized laboratories of plant breeding institutions (in vitro multiplication, embryo culture, haploid culture from young pollen). Others require more sophistication (somatic hybridization by protoplast fusion, in vitro selection after mutagenesis) and some techniques will be available only to specialized laboratories (transformation with DNA with or without vector, isolation of specific genes, in vitro transfer of chromosomes, cell micromanipulation etc.). The same is true for the collection of information at any stage of the program. There will be a change towards simplification, but the most complex techniques will be available only to large specialized institutions. There are three kinds: 1. Government institutions and universities which will make some techniques and materials available without charge, others at a moderate price. 2. Specialized research laboratories mainly interested in patents, available under licence or sold for the best price they can get. 3. Large (often multinational) companies with their own R and D laboratories and plant breeding departments who will patent and partly monopolize their techniques, genes and plant stocks.

Although techniques based on the generative reproductive phase will occasionally be patented, there is much less interest in doing so, and in addition, the major patentable techniques have been published already. In respect to molecular biochemical and other techniques of collecting information, there is also little interest in patenting except for the apparatus required.

A small plant breeder would do well not to bother about these sophisticated developments as such, but to keep track of any interesting material available from whatever source, adopt new techniques only when really simple to apply and concentrate on a small sector of the market where he is specialized, and which is too small to be of interest for or even detected by large companies.

The moderately sized plant breeding companies and institutions will have reason to worry. They will not be able to contribute to the new developments and when not bought up by the large companies, they will depend on others, who will charge high prices for genetic manipulation material or will not even sell it. It will take many years before this will become really serious and in the mean time the medium sized plant breeders must try to find a solution. One is to stimulate unpatented alternatives, the other is to associate with public or small private research institutions in a way acceptable to both. A relatively small investment will open up channels to information and expertise on one hand and a possibility to influence new developments on the other. For public research institutes there is the duty to contribute directly to practical applications and make the results available at a moderate price. The sophisticated meiotic techniques should certainly receive more attention, if possible balanced or combined with somatic approaches (transfer of genes between species, auto- and allopolyploidy, allopolyploidization, genic male sterility based hybrids, complex translocation heterozygosity). If as much effort would be spent on these as on somatic genetic manipulation techniques, the results would be striking.

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Attempts to make a complete literature review have not been made. Only a limited number of representative references has been included.

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