

NOTES AND COMMENTS

CHROMOSOME ELIMINATION IN INTER-SPECIFIC HYBRIDS

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THE production of interspecific hybrid embryos between certain plant species is followed by the selective elimination of the chromosomes of one of the parents. A well-analysed example of this has been observed in crosses of *Hordeum vulgare* and *H. bulbosum* (see Lange (1971) and Subrahmanyam and Kasha (1973) for references). In crosses at the diploid level and irrespective of the species used as the maternal parent the production of hybrid embryos is followed by a complete loss of *H. bulbosum* chromosomes. The same process of elimination occurs when both parents are tetraploid, or when tetraploid *H. vulgare* is crossed with diploid *H. bulbosum*. The main exception to this chromosome loss occurs when triploid embryos are produced as a result of crossing tetraploid *H. bulbosum* with diploid *H. vulgare*. The general rule therefore is for all hybrid cells other than those which have the ratio of genomes of 1V:2B (V = *H. vulgare*, B = *H. bulbosum*) to lose the B chromosomes. The only other exception is that approximately 1 per cent of diploid and a few tetraploid Kasha (personal communication) hybrid embryos remain as such. The loss of chromosomes occurs within the first few days of embryonic development, and abnormalities such as chromosome fragments, micronuclei and degraded chromatin with a granular appearance are observed (Lange, 1971; Subrahmanyam and Kasha, 1973). An analogous process may well occur in other inter-specific crosses in the genus *Hordeum* (Hamilton *et al.*, 1955; Cauderon and Cauderon, 1956). Another example of somatic chromosome elimination is provided in the genus *Nicotiana*, where the production of inter-specific hybrids between *N. tabacum* and *N. plumbaginifolia* is followed by the systematic loss of the *N. plumbaginifolia* chromosomes (Ar-rushdi, 1957).

If the phenomenon was due to an incompatibility of cytoplasm and alien chromosomes, then a different chromosome complement would be eliminated in reciprocal crosses; however, no reciprocal differences are observed, and this interpretation has to be rejected. An alternative interpretation proposed by Gupta (1969) and Subrahmanyam and Kasha (1973) involves an assumed asynchrony of mitotic cycle times and phases between the two species. As a result, DNA replication and other cell processes would be uncoordinated in the hybrid and chromosome elimination would occur. There is, however, no definitive evidence to support this concept either in the *Hordeum* or *Nicotiana* hybrids (Collins, 1968; Gupta, 1969). A more general explanation of these results would simply assume an incompatibility of the synthetic and metabolic apparatus in two species which have evolved independently. But the *Hordeum* and *Nicotiana* data imply a more specific process than this; it is inevitably the *H. bulbosum* and *N. plumbaginifolia* chromosomes that are lost and it is not easy to interpret this on the basis of a general incompatibility of genomes.

Another possible interpretation for these observations is suggested here, primarily as a basis for further experimentation; the *Hordeum* data will be used to illustrate it. It is based on the fact that the elimination of one set of chromosomes is controlled by the chromosomes of the other genome and not by the general cytoplasmic background; more specifically the products of the *H. vulgare* genome are assumed to recognise and lead to a "degradation" of the *H. bulbosum* chromosomes. This interpretation is based in the first instance on an analogy with the host-restriction and modification systems of bacteria. The restriction phenomenon involves the ability of a given bacterial cell to recognise and degrade an alien genome by endonuclease attack; the modification involves the non-heritable modification of DNA, possibly by methylation, such that it is resistant to the restriction enzyme. Recently Sager and Ramanis (1973) have suggested that a restriction-modification mechanism could account for the preferential maintenance of chloroplast genes transmitted from the plus mating type parent, and the loss of those from the minus parent following sexual fusion in *Chlamydomonas reinhardi*. They also suggest that the chromosome loss that often occurs following somatic fusion of mammalian cells might be due to a similar process. There are problems involved in interpreting the plant data in terms of endonuclease attack, as chromosome elimination occurs over a period involving many cell cycles.

If an analogy is made between the process of genome elimination in prokaryotes and that in the plant hybrids then the exceptions that occur have also to be interpreted in these terms; there are the few true diploid and tetraploid hybrids (VB and VVBB) and the stable VBB triploids in *Hordeum*. The assumption made is that a product (endonuclease?) of the *H. vulgare* chromosome causes the loss of the chromosomes of *H. bulbosum*. There is evidence of genetic variability for this activity (Kasha *et al.*, 1970) and more specific evidence that genes on chromosomes 2 and 3 of *H. vulgare* regulate it (Barclay *et al.*, 1972; Ho, 1972). The fact that the two genomes involved in these inter-specific hybrids are not isolated in space must mean that there are recognition sites which allow a distinction to be made between them. To protect the *H. vulgare* genome from degrading its own chromosomes it must either lack those sites which are susceptible to breakage or it has the capacity to modify such sites. The few true hybrids seen in any cross are unlikely to have arisen as a result of a genetic heterogeneity in the homozygous *H. vulgare*; they could be due to the heterozygosity of the *H. bulbosum* parent, in which case the *H. bulbosum* chromosomes must also carry information determining their susceptibility to attack (see also Subrahmanyam and Kasha, 1973). The survival of the triploid hybrids which have two doses of *H. bulbosum* chromosomes could then be related to the presence of two copies of this genetic information. Alternatively, the few true hybrids may be due not to a genetic heterogeneity but to a modification of the B as well as of the V chromosomes in these rare instances, *i.e.* the sites on the *H. bulbosum* chromosomes would also be phenotypically altered such that they are protected. More detailed consideration of possible mechanisms is premature as are more detailed analogies with known molecular mechanisms involved in the bacterial restriction modification system (Boyer, 1971).

In the genus *Nicotiana* also there is evidence of genetic variation for susceptibility to damage by *N. tabacum* chromosomes. *N. plumbaginifolia* chromosomes are lost in inter-specific hybrids with *N. tabacum*, whereas

N. longiflora chromosomes are not eliminated by *N. tabacum*. Yet, *N. plumbaginifolia* and *N. longiflora* are entirely similar morphologically and their hybrids form 10 bivalents at meiosis.

The primary aim in suggesting this new interpretation is to consider whether it opens new possibilities for an experimental evaluation of the basis of the process of somatic chromosome elimination. There are at least three possible kinds of experiments which could be undertaken. In the first place if the few diploid and tetraploid hybrids mentioned earlier are due to a chance phenotypic modification of the B chromosomes then this could be tested; if progeny could be produced from these hybrids then the B chromosomes should once more be susceptible to elimination by V chromosomes in the next generation.* A second series of experiments centres around the possibility of influencing the process of chromosome elimination. There are means of affecting restriction modification in bacteria, and if, as seems likely a similar mechanism operates in the *Chlamydomonas* chloroplast system, then Sager's work (Sager and Ramanis, 1967, 1973) has shown how chemical and U.V. radiation treatments can influence the process. It would be relatively easy for example to examine the effect of U.V. irradiation of the V pollen on the elimination of B chromosomes from a diploid or tetraploid interspecific hybrid of *Hordeum*. Thirdly, it may be possible to assay *in vitro* for the presence of species- or genotype-specific endonucleases which can attack other species' DNA.

The extent to which such mechanisms may operate in other inter-specific crosses is entirely unknown. If attempts had not been made in the *Hordeum* crosses to maintain the hybrids by embryo culture techniques, the process of chromosome elimination is unlikely to have been observed. Furthermore, only detailed studies of the *Hordeum* hybrids have indicated the precise mechanism involved, and few comparable analyses of embryos have been undertaken elsewhere. There may thus be other instances in the plant kingdom where mechanisms of this kind exist as a barrier to inter-specific or inter-generic hybridisation; their recognition is not easy and often depends on the ability of a haploid plant to survive following chromosome elimination. Further work is necessary not only to test this hypothesis but to examine the generality of the phenomenon.

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* Kasha (personal communication) has suggested that the results of Schooler support this expectation.

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