Interspecific and Intergeneric Hybridization

H. H. Hadley
S. J. Openshaw
University of Illinois
Urbana

Most plant breeders correctly assume that choice of parental materials is a most important step in their breeding programs. They choose parents of a cross only after careful consideration of program objectives and available parental materials. At least one parent likely will be a relatively successful cultivar of the crop (cultigen) to be improved, particularly if much plant breeding effort already has been invested in the cultigen.

A majority of plant breeders prefer that the other parent be from the same biological species, because representatives of the same species usually cross readily to produce fertile hybrids and present little or no hindrance to genetic recombination. Circumstances may indicate, however, that a certain plant breeding problem can be solved only by using a wide cross, one involving representatives of different species or genera. Often such a cross is difficult to make and, even if accomplished, produces a hybrid that is inviable or sterile. Either situation could hinder or even block the transfer of genes from one parental population to the other. The breeder contemplating use of wide crosses, therefore, should make them for reasons that warrant the extra effort they require.

Briggs and Knowles (1967) have listed four reasons for making interspecific hybrids: (i) to transfer one or a few genes from one species to another, (ii) to achieve new character expression not found in either parent, (iii) to produce new alloplloid species, and (iv) to determine the relationship...
of one species to another. The latter two reasons obviously require interspecific hybridization. The first and, perhaps to a lesser degree, the second, correspond to reasons for making intraspecific hybridizations. Breeders making crosses for these reasons, therefore, should thoroughly explore the easier intraspecific path to their goals before committing themselves to the more difficult interspecific route.

Many breeders apparently have concluded that the interspecific path is necessary for achieving their goals, because the literature has become voluminous on the subject of wide crosses in plant breeding programs. A common justification for this choice is that wild relatives and progenitors of our crops can be tapped for "genes carrying special attributes not apparently in the cultivated forms" (Bates and Deyoe, 1973) or that, "...an exchange of genes between cultivated and feral or semi-feral species...would open a vast potential resource of variability for exploitation by breeders..." (Deakin et al., 1971).

Plant evolutionists recently have urged breeders to collect wild relatives of crop plants and to exploit useful genes they might find in the collections. Zohary et al. (1969) have pointed to the existence of wild diploid progenitors of wheat that constitute large gene pools thus far unexplored and untapped by wheat breeders. The authors deplored the fact that the world wheat collections were extremely deficient in these forms and hoped that wheat breeders would soon collect, screen, and exploit them in their breeding programs.

In a recent discussion of genetic resources in wild relatives of crops, Harlan (1976) suggested that the extent of use of wild relatives in breeding programs depends on: (i) how wild the crop is, (ii) how desperate the situation is, (iii) how great are the pressures to turn out new cultivars, (iv) how available are the materials, (v) how difficult are the materials to use, and (vi) how are the characters of plant breeders or the institutions in which they work. In Harlan's opinion, the use of wild relatives is accelerating rapidly. As more useful results of incorporating genes from these forms into cultivars are reported, more workers will be inclined to use wild genetic resources. "In the future, the need for genetic variability and sources of resistance shall drive us to a much fuller exploitation of all the genetic resources that we can assemble" (Harlan, 1976).

The opinions of plant breeders vary concerning the value of interspecific hybridization, but undoubtedly this procedure has taken and will probably maintain a significant position among the plant breeder's tools. The purpose of this chapter is to consider the difficulties associated with the generation of mature fertile or partially fertile interspecific and intergeneric hybrids and the techniques now available to generate them. We shall assume that the main difference between attempts to cross two species from the same genus and two from different genera is degree of difficulty; therefore, the term interspecific will be used to encompass both types of crosses. In presenting our discussion, we shall not attempt to cover exhaustively the extensive literature on the subject. We will cite examples taken mostly from recent publications to illustrate the points made in our discussion. Chapters in the book dealing with specific crops contain additional examples.
I. REPRODUCTIVE ISOLATION BARRIERS

The essential problem in using interspecific hybridization in plant breeding programs is the low probability of obtaining in one individual the desired combination of genes from the parental species. Though one might protest that obtaining a desired genetic combination is the one basic and universal problem of all plant breeding programs, its severity is increased significantly when wide crosses are used. The process of speciation leads to the development of reproductive isolation barriers that maintain the integrity of species by restricting the flow of genes from one to another. The breeder who wishes to temporarily break down those barriers should at least become aware of what they are to improve the chances of success.

Stebbins (1950) proposed a classification of the several types of barriers to genetic interchange between related taxa. His outline will be the basis of our present discussion (Fig. 1).

Sections I and II-A of Fig. 1 correspond to what some persons call prefertilization reproductive barriers, whereas section II-B corresponds to postfertilization barriers. Sections I and II-A1-3 have also been referred to as external barriers; II-A4 and all of II-B as internal barriers. The latter barriers reside in the plant tissues themselves rather than in the outside environment.

A. External Barriers

External barriers to genetic interchange between related populations prevent pollen of plants in one population from falling on stigmas of plants in the other. These barriers are "physical separations in time, space, environment, and specific ecological niches" (Bates and DeYoe, 1973). Combinations of barriers, such as geographical and ecological or ecological and seasonal (flowering time), are more common than individual barriers and tend to reinforce discontinuity between populations.

---

I. Spatial isolation
II. Physiological isolation
   A. Barriers between the parental species
      1. Ecological isolation
      2. Temporal and seasonal isolation
      3. Mechanical isolation
      4. Prevention of fertilization
   B. Barriers in the hybrids
      1. Hybrid inviability or weakness
      2. Failure of flowering in the hybrids
      3. Hybrid sterility
      4. Inviability and weakness of F1 and later generation segregates

Fig. 1—Types of reproductive isolation mechanisms (Adapted from Stebbins, 1950).
The barrier of spatial isolation is frequently an easy one to overcome. If it is the sole barrier, individuals from the separated populations can be brought together and crossed to produce fertile hybrids which will allow the free flow of genes from one population to the other. Stebbins (1958) has described this type of behavior and Wright (1976) has summarized numerous examples of such interspecific hybridizations that have succeeded in forest tree improvement programs. When this type of behavior occurs, the two populations should not be considered distinct species according to the precise definition of biological species. The breeder may continue to refer to the populations as species for the sake of convenience, but would be more accurate to label them as subspecies, races, or some other intraspecific category.

Another form of external barrier is ecological isolation. Two populations, even in the same geographical area, may become separated by adaptation to different habitats or ecological niches. Such separation may be incomplete because the habitats may meet in certain areas within the ranges of both populations, thus affording them the opportunity to cross. At these points of contact, F₁ hybrid and later generation hybrid swarms may arise and allow the introgression of genes from one population to the other. The presence of such swarms would indicate to the plant breeder that he could make artificial crosses between plants from the two populations. The absence of such swarms, on the other hand, does not mean he could not obtain hybrids between plants from the different populations. Interpopulation F₁ hybrids and their F₂ and later generation derivatives might not be adapted to either habitat which favors one or the other parent and thus would not persist.

If the related populations we have been discussing are both wild species, their relationships may be of little interest to a plant breeder unless both are closely related to a cultigen. However, if one is a crop species and the other a wild relative, the situation could be of considerable and direct importance. An example of this situation involves sorghum, a cultigen which has many wild relatives. In Africa, a wild race is widely distributed in the tropical forests of the Guinea coast and the Congo, occupying stream banks, alluvial soil, or the edges of paths in the jungle (de Wet et al., 1976). But a wild race is also found around villages and cultivated fields where it hybridizes with cultivated sorghum to produce troublesome weeds. Other wild races in similar ways cross with cultivated races even though adapted to different habitats. Hybrid swarms are not often found, probably because nature is selecting for genotypes adapted to wild habitats and man is selecting, perhaps indirectly and unconsciously, for genotypes adapted to cultivated fields. His selections may become weed companions of the crop and facilitate the transfer of genes from the wild to the cultivated races.

Data on controlled crosses among sorghums suggest that all races (cultivated and wild) hybridize freely in any combination to produce fertile F₁'s. Wild races of *Sorghum bicolor* make up an extensive gene pool from which the sorghum breeder should be able to transfer genes to the cultivated races. One can transfer genes with little difficulty because the isolation mechanisms between taxa in this species are generally external and easy to circumvent. The senior author, on the basis of knowledge about crossability
between wild and cultivated sorghums, has succeeded without difficulty in transferring two genes for seed shedding from a wild race (virgatum) to a cultivated sorghum (broomcorn).

Two populations occupying the same geographical area may be genetically isolated by differences in flowering times. This is another external barrier that may be incomplete, with enough overlapping in flowering periods to allow some crossing. At least the breeder, with an acceptable amount of inconvenience, should be able to modify the flowering periods of plants from one or both of the populations sufficiently to obtain functional flowers from each at the same time.

Stebbins (1958) mentioned an external barrier which he referred to as mechanical isolation. Examples of this barrier are plant species whose flowers have become adapted to insect pollination. Some plant species have evolved jointly with bee species so that one species of plant is pollinated by only one species of bee which, in turn, is attracted only to flowers of that species of plant. Thus, two plant species, each with its own exclusive insect vector, will not hybridize in nature because no insect will visit flowers of both species. Some insects, e.g., honeybees, may even work the flowers of two or more plant species, but never visit flowers of more than one species during the same foraging flight. Interspecific hybridization will not occur in nature, but the plant breeder might make hybrids if no internal isolation mechanism has developed. Possible dependence upon highly discriminating insect vectors potentially interests the breeder who hopes to use such insects to make crosses in a breeding program or in commercial fields for hybrid seed production. Insect vectors with a too highly developed sense of discrimination will probably be useless to the breeder in these situations.

B. Internal Barriers

Internal barriers to genetic interchange between related populations operate through disharmonies between physiological or cytological systems of plants from the different populations. They may: (i) prevent the production of $F_1$ zygotes, even if pollen from flowers in one population falls on stigmas of flowers in the other, (ii) produce $F_1$ hybrids that are inviable, weak, or sterile, or (iii) cause hybrid breakdown in $F_1$ or later generations.

1. Prevention of Fertilization

The prevention of $F_1$ zygote formation, referred to as cross incompatibility, is caused by disharmony between reproductive tissues of plants from different parental populations. Pollen does not germinate on the stigma; the pollen tube does not completely traverse the style; or the male gamete does not combine with the egg, even though the pollen tube reaches the ovary. These expressions of failure to achieve fertilization are similar to those found within self-incompatible species. One hypothesis to explain cross-incompatibility between species assumes that genes at the S-locus have two functions, preventing (i) self-fertilization within a species and (ii) cross-
fertilization between two species. How these two functions can be accomplished apparently is still unexplained, but when one species is self-compatible and the other is self-incompatible, the cross (self-compatible × self-incompatible) often results in fertilization, whereas the reciprocal cross does not. This phenomenon is known as unilateral incompatibility. Other evidence, however, has shown that unilateral incompatibility occurs also in some crosses where both parents are self-incompatible species or both are self-compatible.

Elaborate modifications of the hypothesis based on the S-locus have included other loci to explain the incompatibility reactions in all types of crosses (Abdalla and Hermsen, 1972). Hogenboom (1972), working with interspecific crosses between *Lycopersicon peruvianum* (L.) Mill. and *L. esculentum* Mill., concluded that, in his material, self-incompatibility and interspecific incompatibility were two distinct phenomena based on different genetic systems. He indicated that the inhibition of *L. esculentum* pollen in styles of *L. peruvianum* was governed by a number of independent dominant genes, each governing a distinct process, in the unilateral inhibition of pollen tubes. De Nettancourt et al. (1974) observed the same interspecific cross and concluded that unilateral incompatibility in *L. esculentum* pollen is governed by one gametophyte factor which is either linked or allelic to the S-locus.

Although incompatibility itself appears to be a formidable barrier to interspecific hybridization, Stebbins (1958) observed that failure of pollen growth and fertilization is rarely the primary cause of reproductive isolation between closely related species. Disharmonies within the hybrid zygote and developing plant or in F₁ segregates from fertile F₁ plants are more likely to be the major barriers. Stebbins has discussed these in detail and Allard (1960) has already considered them from a plant breeding standpoint. The reader who wishes to pursue the subject further may consult these references, which are largely the basis for our following brief comments.

### 2. Hybrid Weakness or Inviability

Some species can be crossed to produce hybrid zygotes, but the F₁’s are either inviable or too weak to be of use to the plant breeder. The causes of this hybrid weakness or inviability can be grouped into three categories: (i) disharmonies between genomes of the parental species, (ii) disharmonies between the genome of one species and the cytoplasm of the other, and (iii) disharmonies between the genotype of the F₁ zygote and the genotypes of endosperm or the maternal tissue with which the developing F₁ embryo is associated.

Interactions between the parental genomes probably have a polygenic basis and are difficult to analyze. In a few cases, severe disability or lethality of the F₁ has been determined by one or only a few pairs of genes, but no such case appears to account for an effective reproductive barrier between species (Stebbins, 1958). Other barriers always seem to be involved. A wide sampling of possible parents within two species usually reveals combinations that do not give the lethal or deleterious effect.
Hybrid weakness probably results from the evolution of differences between species in timing of critical processes, such as cell division, organization and differentiation of meristems, and germination of seeds. The combination of genetic messages from the two genomes in the F₁ zygote may be too incoherent to direct a viable pattern of development in the hybrid embryo. Such a situation would constitute a barrier that is difficult or impossible to overcome.

Disharmonies between the genes of one species and the cytoplasm of another are not so difficult to avoid and are much less effective as reproductive barriers. Unless other barriers are present, when the transfer of genes from one species to the other cannot be accomplished through the cross A × B, it may sometimes be accomplished through the cross B × A. A common type of cytoplasmic genome interaction that is deleterious or even lethal involves development of abnormal chloroplasts. This phenomenon has been well illustrated in genetics textbooks by description of Renner's classical work with interspecific hybrids in *Oenothera*. Other deleterious interactions between the cytoplasm of one species and the genome of another have been discussed by Grun (1976).

Successful development of an embryo depends upon the accompanying development of endosperm tissue capable of nourishing the embryo and of a harmonious interaction of related embryo, endosperm, and maternal tissues. To illustrate the effect of different genomes on these tissues, we can assume the genomic contents in species I to be AA for embryo, AAA for endosperm, and AA for maternal tissue, and in species II the embryo is BB, endosperm BBB and maternal tissue BB. A proper balance in dosage effect of genes as they act in these three tissues will have been achieved in both species I and II, although the particular loci involved and individual allelic effects probably differ in the two species. In the interspecific hybrid I × II, genomic contents for the embryo will be AB, endosperm AAB, and maternal tissue AA, whereas in the hybrid II × I they will be AB, ABB, and BB. Possible difference in dosage effects are easy to imagine in these genomic combinations and could account for the observed fact that some interspecific crosses are more successful when made in one direction than if made in the other, while in other cases reciprocal crosses are equally successful or unsuccessful.

In some crosses, serious disharmony does not occur in the embryo (AB), but does in the endosperm (AAB or ABB). Possibly the combination of two genomes from the female parent and one very different genome from the male may produce unfavorable dosage effects. Where the parental species differ in chromosome number, disharmony may result from different ratios of chromosome numbers in endosperm and embryo. To illustrate, if genome A from species I has 10 chromosomes and B from species II has five, the ratios of chromosome numbers in endosperm and embryo tissues would be 30 to 20 (1.5:1) in species I, 15 to 10 (1.5:1) in species II, but 25 to 15 (1.67:1) in the cross I × II and 20 to 15 (1.33:1) in II × I. Less disharmony seems to occur with a ratio of 1.67:1 than with 1.33:1, because, in general, crosses between species differing in chromosome number are more successful when the species with the greater number is used as the female parent. Abnormal endosperm development may be characterized by a low
but steady rate of cell division or by an early rapid rate associated with lack of cell wall formation followed by a sudden cessation of mitosis and degeneration of existing endosperm tissue. The former situation may make embryo development difficult, but the latter causes the embryo to starve to death.

Another serious disharmony, termed somatoplastic sterility by Cooper and Brink (1940), sometimes exists in certain combinations of endosperm and maternal tissue. Excessive growth of maternal tissue impairs the capacity for endosperm development and leads to starvation and collapse of the embryo. The term somatoplastic sterility has been applied also to deleterious interactions between endosperm and embryo (Rieger et al., 1968).

3. Hybrid Sterility

Attempts to cross two species may succeed in producing a viable, even vigorous, F₁, but disharmonies between the parental genomes or between the genome of one parent and the cytoplasm of the other may cause the F₁ to be sterile. Such an expression has been called chromosomal sterility (chromosomal hybrid sterility) if caused by structural differences between the parental chromosomes that interfere with their pairing and disjunction at meiosis (Rieger et al., 1968). If the sterility is caused by specific gene complexes, it is known as genic sterility (genic hybrid sterility). Genic sterility usually is due to the genotype of the organism as expressed in the sporophytic phase, but may also include disharmonious genetic combinations in the gametophytic phase of the life cycle.

In wide crosses, sterility of the F₁ often is associated with failure of chromosome pairing (asynapsis) during late prophase and the first metaphase. Asynapsis may indicate lack of homology between chromosomes from the different parents or a lack of synchronization of metabolic processes taking place in early meiosis. One way of distinguishing between the two is to double the chromosome number of the sterile diploid hybrid. In the resulting tetraploid hybrid, each chromosome should have a complete homologue and should associate with it as a pair (bivalent) from late prophase through the first metaphase. If such pairing does not occur in the tetraploid F₁, sterility is probably genic. If pairing is prevalent in the tetraploid F₁, sterility of the diploid F₁ is probably chromosomal.

Whether sterility of an interspecific hybrid is genic or chromosomal may seem of little consequence to the breeder because both lead to reproductive isolation. However, the difference is important to the breeder who wishes to produce a useful alloploid from the hybrid. An alloploid is an individual with two or more different chromosome sets (genomes). An alloploid obtained by interspecific hybridization has genomes from each species in the cross. If sterility is genic, the alloploid likely will be just as sterile as the original F₁; if it is chromosomal, the alloploid will likely be at least partially fertile and of some usefulness to the breeder.

When chromosome pairing is absent in interspecific hybrids, sterility results from abnormal distribution to the gametes of different numbers and combinations of chromosomes from the parental genomes. Only non-re-
duction gametes or gametes which, by chance, include all or almost all of
the chromosomes from one parental genome and none or almost none from
the other genome will function. In some interspecific hybrids, however, the
chromosomes seem to pair and disjoin normally, yet the hybrid is sterile.
Chromosomes of the genomes involved in these cases may still differ struc-
turally by very small inversions, transpositions, etc. Recombination of
chromosome segments during meiosis in the F₁ hybrid involving small struc-
tural differences can lead to gametes with small, but significant deficiencies
and duplications which may render the gametes inviable. Recombination
could also produce abnormal F₂ or later generation sporophytes, if the ab-
normal gametes escape elimination in the gametophytic phase of the life
cycle. The former situation would be observed as F₁ hybrid sterility; the
latter as F₂ hybrid breakdown.

Intergenomic interaction can result in the elimination of chromosomes
from one of the parental genomes in the interspecific F₁ zygote during early
mitoses in the developing embryo. The resulting mature plant is highly
sterile, but is a haploid rather than a hybrid. Kasha (1974) has used this
phenomenon to obtain haploids on a large scale by crossing *Hordeum
vulgare* L. × *H. bulbosum* L. In both this cross and its reciprocal, the
*bulbosum* chromosomes are eliminated. This behavior indicates that
chromosome elimination is not caused by alien cytoplasm. Kasha, however,
suggests that some other reported cases of chromosome elimination in inter-
specific crosses could be caused by incompatibility between a genome and
an alien cytoplasm.

Sterility is another expression induced by some combinations of cyto-
plasm of one species and genomes of another. The cytoplasmic-genetic type
of male sterility is a phenomenon that has been recorded for numerous
interspecific crosses by plant breeders attempting to exploit hybrid vigor in
many crops, including cotton (Meyer, 1969) and wheat (Sage, 1976). Dif-
fferences in reciprocal interspecific crosses might not always reveal simply
inherited male-sterile systems, but nevertheless show a cytoplasmic effect.
Palmer and Hadley (1968) found that F₁ hybrids of *Glycine tomentosa*
(tomentella Hayata) × *G. tabacina* (Labill.) Beuth. produced pollen with
lower stainability and anthers that usually failed to dehisce compared to the
reciprocal cross which produced more highly stainable pollen which was
shed abundantly. The average number of seed per plant on the former F₁
was 12 while that of the latter was 29.

4. Hybrid Breakdown

Some interspecific F₁ hybrids are both vigorous and fertile, yet give rise
to F₂ plants that are weak or sterile. Such a situation has been referred to as
hybrid breakdown or genetic disability. An illustration of this phenomenon
occurs in the F₂ generation from crosses between the cultivated cotton tetra-
ploids, American Upland (*Gossypium hirsutum*) and Sea Island (*G. barba-
dens*) (Stephens, 1950). The F₁ hybrids between these cottons are easy to
make, quite vigorous, and apparently normal in fertility. Some breeders, in
fact, are interested in the possible exploitation of the F₁’s as commercial hy-
brids because of the considerable heterosis they exhibit (Marani, 1967). In
the F₂ generation, however, most individuals are weak, sterile, or generally
unfit agronomically. This result would not interfere with commercial use of
F₁ hybrids, but could cause inconvenience in breeding programs with the
goal of obtaining inbred lines with combinations of genes from both
species. Two explanations have been offered for this type of behavior. One
suggests small structural differences between chromosomes of the genomes
in the two species (Section 1B3). The other assumes complementary genetic
systems, such that genotype AABB has been selected for in the one species
and aabb in the other. In the F₂ generation, genetic recombination will pro-
duce unfavorable genotypes. If many loci are involved in such systems, it is
easy to see how few F₂ segregates would be phenotypically successful.

Further discussion of this important barrier to the flow of genes from
one species to another would describe the use of interspecific hybrids in
breeding programs, a topic that is beyond the scope of this book. Informa-
tion regarding the severity of hybrid breakdown in F₂ or later hybrid
generations from particular crosses, however, might influence the breeder’s
choice of parental combinations.

II. OVERCOMING REPRODUCTIVE BARRIERS

Man has developed ways of overcoming or circumventing barriers that
restrict or prevent genetic interchange between related plant populations.
We shall now consider some of the techniques available to the breeder who
wishes to cross representatives from populations that have become spatially
or genetically separated from one another.

A. Prefertilization Barriers

Plant breeders may increase their chances of obtaining interspecific hy-
brid zygotes by making judicious decisions and by exploiting special tech-
niques in the following four steps of a crossing program: (i) choice of par-
tenal populations, (ii) choice of lines or even individual plants within each
parental population, (iii) manipulation or modification of parental plants
during or before crossing, and (iv) choice of emasculation and pollination
procedures.

1. Choice of Parental Populations

The first opportunity for a breeder to affect the probability of success
with wide crosses lies in the choice of parental populations (taxa). The ex-
tent of freedom in this choice will vary according to the plant breeding ob-
jectives and the phenotypic characteristics of potential parental materials.
Breeders make wide crosses for different reasons ranging from a search for haploids to commercial production of F₁ hybrids. Some reproductive isolation barriers may be tolerable, even favorable, for certain objectives, while others are not. For purposes of discussion, we will assume an objective of transferring a desirable characteristic, like disease resistance, to a cultigen from a wild relative. We will be concerned with the whole range of possible barriers.

The breeder first needs to screen potential parental populations for expression of the desired character. If a choice is possible, individuals should be selected from populations that are most closely related to the cultigen.

Zohary (1973) recently emphasized this point while describing different types of germplasm resources (gene pools) available to the plant breeder. He classified these pools as:

1. cultivated—cultivars of the crop concerned
2. wild—representatives of the wild progenitor stock and companion weeds
3. alien—other species in the genus represented by the crop.

He advised that alien gene pools should be exploited only when possibilities of the cultivated and wild pools apparently have been exhausted.

Harlan and de Wet (1971) proposed three categories of genetic resources that differ somewhat from Zohary’s in respect to placement of wild races in the same species that includes the cultigen. Their categories were primary, secondary, and tertiary gene pools. The primary gene pool corresponds to the traditional concept of a biological species, and almost always includes spontaneous (wild and weedy), as well as cultivated races. Crossing is easy among forms in this gene pool, hybrids are fertile, and gene transfer unhindered. The secondary gene pool includes all biological species that will cross with the crop. Gene transfer is possible between the cultigen and members of this pool, but is hindered by weakness, sterility, or inviability of the hybrids. Still the pool is available to the breeder willing to invest some hard work. The tertiary gene pool is rather ill-defined and perhaps most useful in describing the outermost limits of the gene pool of the crop. It includes relatives that will cross with the crop, but which produce anomalous, sterile, or lethal hybrids that require special techniques (embryo culture, bridging crosses, etc.) to obtain or to use (production of allopolyploids, etc.). The authors did not rank the pools in order of preference, but comparisons of difficulties in gene transfer associated with each emphasizes an order of primary, secondary, and tertiary.

The breeder cannot always determine closeness of relationship between a cultigen and its relatives. Taxonomic classifications based on morphological characteristics may be the best available help, but often do not accurately reflect genetic relationships. Data from crossing experiments are more useful. Biosystematic studies made by cytotaxonomists with an appreciation of plant breeding problems probably serve best. Many data of this type are available for some crops, but meager for others. Breeders working with the latter will have to invest time in attempts to obtain different hybrid combinations. In doing so, they should view taxonomic classifications with a somewhat skeptical eye.
2. Sampling the Parental Populations

After deciding which wild relative or relatives to cross with the cultigen, the breeder should sample adequately both the cultigen and the wild relatives. Choice of specific individual plants or even subgroups within the parental populations can influence: (i) the probability of obtaining a hybrid, (ii) the degree of sterility in hybrids that are obtained, and (iii) the worth of backcross, F₁, or later generations. Considerable genetic variation exists in some cultigens, as well as in their wild relatives. Restriction of crossing attempts to a combination of only a few individuals from the wild species and a few from the cultigen may provide too small a sample for making sound conclusions regarding crossability of the two populations. The results of several recent experiments with wide crosses will illustrate this fact.

Pittarelli and Stavely (1975) crossed autotetraploid Nicotiana repanda Willd. ex. Lehm (2n = 4X = 96) with three cultivars of N. tabacum L. (2n = 2X = 48). All three crosses produced capsules and seeds, but only the one with cultivar ‘SC72’ produced F₁ hybrid plants. Hoven (1962) used two accessions from Italy and three from Turkey to represent Trifolium nigrescens Viv. (ball clover) as a male parent in crosses with T. repens L. (ladio white clover). The accessions from Italy were more cross compatible than those from Turkey. Lambert and Leng (1965) crossed corn inbred HY2 by five strains of teosinte (Zea mexicana Schrad, ex. Reeves and Mangelsdorf) and found that the strains differed markedly in their response to backcrosses to HY2. Harlan and de Wet (1977) tested dozens of clones of Tripsacum dactyloides (L.) L. in crosses with corn for effectiveness in transferring genetic material, and found that only one clone could be used effectively. Other illustrations of the importance of adequately sampling potential parental populations have been presented in plant breeding texts by Allard (1960) and by Briggs and Knowles (1967).

3. Manipulation and Modification of Parental Plants

Any breeder attempting to make interspecific hybrids should consider making reciprocal crosses, particularly if no knowledge is available for the parental combination. The breeder might prefer crossing attempts in only one direction, like A × B, because A has flowers that are easier to emasculate or because flowers of B produce more pollen. Or perhaps A has a greater chromosome number and the breeder is aware of the general rule that when two species differ in chromosome number, it is better to use the species with the greater number as the female parent. Nevertheless, breeders should try the cross in both directions, even though one is more difficult than the other. For example, Crowder (1953) crossed Lolium perenne L. (2n = 14) with Festuca arundinacea Schreb. (2n = 42) and found that matings with L. perenne as the female parent were much more successful than those
with the reciprocal, even though *L. perenne* had the lower chromosome number.

If one obtains seed from both reciprocal crosses, indicating no serious prefertilization barriers, incompatibilities between endosperm and embryo, or somatoplastic sterility, one still should grow and observe the reciprocal F₁ plants for possible post-fertilization barriers caused by cytoplasmic-genomic disharmonies. The cross A × B may be inviable or sterile, but the reciprocal, B × A, may not be. It also may be useful to observe at least a small sample of F₁ individuals from each of the reciprocal crosses. The F₂ segregates derived from cross A × B might be of greater plant breeding value than those from cross B × A, even though the reciprocal F₁ plants seemed to be similar. One example of this type of behavior is male sterility that is based on interactions between the cytoplasm of one species and nuclear genes contributed by the other species. In one cytoplasm the F₂ could show segregation; in the reciprocal, all F₁ plants could be male fertile.

Increasing chromosome numbers (ploidy levels) can affect crossability between two species. Olsson (1963) reported that attempts to cross *Brassica campestris* L. (2n = 2X = 20) × *B. oleracea* L. (2n = 2X = 18) resulted in only 16 F₁ plants from 10,395 pollinations, whereas the same cross on the tetraploid level yielded 133 F₁ plants from 22,884 pollinations. The reciprocal cross (*B. oleracea* × *B. campestris*) failed completely on the diploid level, but on the tetraploid level gave 130 F₁ plants from 18,874 pollinations. Greater success of crosses on the tetraploid level may result from greater gene dosages that break down incompatibility systems.

Chromosome doubling has been particularly successful in crosses between species that differ in number of genomes, as well as total chromosome numbers. Wernesman et al. (1965) obtained no seed of the cross *Lotus tenuis* Wald. et Kit. (2n = 2X = 12) by *L. corniculatus* L. (2n = 4X = 24); however, seed set in reciprocal crosses between an advanced generation tetraploid *L. tenuis* (2n = 4X = 24) and *L. corniculatus* averaged more than eight seed per pod. Crosses between species that differ in number of genomes may fail because of incompatibility between pollen tubes and stylar tissue. Perhaps 2X pollen tubes from 4X sporophytes are too thick to grow down 2X stylar tissue, whereas pollen tubes from 2X sporophytes have no such difficulty in 4X stylar tissue. This could account for cases where reciprocal crosses between untreated parents give different degrees of success.

The ability to double chromosome numbers is an especially useful tool for the breeder working with wide crosses. Numerous techniques that can be used to induce chromosome doubling include temperature shock, wounding, exploitation of certain mutant genes, such as *el* for elongate chromosomes in corn (Alexander, 1957), and the application of various chemicals, such as nitrous oxide and colchicine. The most commonly used technique involves the application of colchicine. This alkaloid can be applied in many ways to produce its chromosome doubling effect.

Colchicine is used most often in an aqueous solution. Glycerine is sometimes added to the solution or the solution is mixed with lanolin to form an emulsion (paste) when a more prolonged treatment is desired and immersion of plant parts in an aqueous solution is not practicable. Strength
of solution, duration of treatment, or both usually must be great enough to kill some of the treated tissue in order for treatment to be effective. Treatment must be applied to meristematic tissue. Tissue that develops after treatment will usually be mixaploid, i.e., containing some cells with the original chromosome number and others with an increased number. Selection will occur naturally within the plant, and cells with the original chromosome number usually will have an advantage. The breeder counteracts this advantage by selecting for tissue that appears to be polyploid.

Many variations have been reported in strength of solution, manner of application, kinds of accompanying chemicals or agents, and environmental regimes under which treated plants or seeds are grown. Those interested in pursuing the subject of colchicine and its uses should consult Eigsti and Dustin (1955) for many phases of colchicine research done prior to 1954 and Elliott (1958) who describes the use of this agent and polyploidy in plant breeding. Other leads into literature on the subject can be found in references from this chapter and others on specific crops. We shall present a few examples of successful treatment procedures.

Yermanos and Gill (1967) tested six concentrations of colchicine (0.0125 to 0.4% in water) for induction of polyploidy in *Linum* species. Length of treatment was 3, 6, 12, and 24 hours. Two days after germination, seedlings were immersed in the solution from the crown upward. The roots were not dipped into the solution, but were kept moist with water. The most effective treatment usually was 0.1% colchicine for 6 hours. Treatment of 4-week-old plants was about half as effective as treatment of seedlings. Stems of these plants were trimmed at the top and capped for 24 hours with 3 × 35 mm vials filled with the colchicine solution. Effectiveness of treatment varied with different species.

Sebastianpillai and Jones (1976) compared four colchicine treatments applied to species of *Fragaria*. The most successful, which they designated as the dropper method, consisted of the application of a 0.01 cm³ drop of colchicine solution (0.5 to 3.0% in water) between the cotyledons to cover the shoot apex. The seedlings were grown in 3.7 cm diam pots, and had emerged from the soil long enough for the apex and cotyledons to turn green. The treated seedlings were placed in a desiccator containing water for 24 hours. The relative humidity was 98 ± 2% and the cotyledons remained moist throughout the treatment period.

A method of possible general use for grasses has been described by Morgan (1976). Young tillers are isolated from a plant to be treated. The leaves of each tiller are trimmed and the roots are removed completely. A sharp scalpel is used to make a triangular incision above the stem apex and the section is removed, leaving a cavity through the leaf bases enclosing the apex. Groups of tillers are placed on a Perspex tray in which holes have been drilled to accommodate 30 bunches of tillers. The tray is placed in a dish containing 150 cm³ of freshly prepared 0.2% colchicine + 2% dimethyl-sulfoxide for 7 to 8 hours. Following treatment, the tray is transferred to a washing tank and the tillers thoroughly washed in running tap water for at least 4 hours. The tillers are transplanted in shallow boxes containing potting compost. The treated tillers do not survive, but new ones emerge from their bases. When the treated tillers each have produced five
new tillers, the latter are subdivided into individual tillers for chromosome counting. Each tiller, with roots removed, is placed for 2 to 4 days in a culture tank containing aerated culture solution until new roots are formed. These roots are used for chromosome counts and tillers with polyploid sectors are isolated and transplanted into pots containing potting compost.

4. Bridging Crosses

The breeder who cannot cross two species, in spite of skillful use of available techniques for overcoming reproductive isolation barriers, may try to circumvent the situation by an indirect approach. Such an approach may consist of a series of crosses involving the cultigen and two or three related species. Although the cultigen and a particular cross-incompatible species are never directly crossed, chromosomes of their genomes are brought together in the same organism.

An experiment by Burk (1967) with tobacco will illustrate. Commercial tobacco, *Nicotiana tabacum* (TT), is susceptible to tobacco mosaic virus, but a wild relative, *N. repanda* (RR), is resistant. These species apparently will not cross, but a third species, *N. sylvestris* Speg. and Comes. (SS), will cross with both. Burk crossed *N. repanda* with *N. sylvestris* to produce a highly sterile F₁ (RS) which he treated with colchicine. The resulting alloplloid (RRSS) was highly stable and crossed readily with *N. sylvestris* to produce a sesquidiploid RSS. Subsequent backcrosses to *N. sylvestris* resulted in the loss of R chromosomes, except for the one carrying the gene for resistance to tobacco mosaic virus. It was maintained by selecting virus-free segregates as parents of succeeding backcrosses. After two backcrosses, some plants were enough like *N. sylvestris* to cross with tobacco. Resistant F₁ hybrids from this cross were genomically ST, plus one or more R chromosomes. Although these hybrids were highly sterile, they did set a few seed when pollinated by tobacco. Subsequent backcrosses to tobacco resulted in elimination of R chromosomes, but selection of resistant plants maintained the presence of the desired R chromosome. Burk’s original plan was to first produce an alloplloid containing some RR pairs of chromosomes before backcrossing to tobacco. Occasional production of nonreduced eggs by the F₁, with ST + R chromosomes, however, accomplished the same purpose as that of an alloplloid.

Although the F₁ allotoid represents success in so far as the scope of this chapter is concerned, the breeder’s problem is not yet solved at this stage. Complete elimination of R chromosomes and incorporation of a small R chromosomal segment with the gene for tobacco mosaic virus resistance into the T genome by naturally occurring or induced translocation are still required. Use of resistant genes in a T genome or one closely enough related to T to allow pairing of chromosomes from the two genomes would greatly facilitate transfer of the gene for resistance. Consideration of this factor should be made in the choice of the donor parent, if a choice is possible. The gene for resistance, however, may be found only in a genome not closely related enough to T to permit pairing. Radiation of the chromosome addition line (T genomes plus the R chromosome with the gene for resist-
ance) could induce translocation of a short segment of the chromosome with the gene for resistance to a chromosome of the T genome. The classical example of this procedure was reported by Sears (1956) who transferred a short segment of an *Aegilops umbellulata* Zhuk. chromosome with a gene for rust resistance into a chromosome of hexaploid wheat.

An example in which similar genomes have been brought together involves the transfer of genes from the A genome of Asiatic commercial diploid cottons *Gossypium herbacum* L. or *G. arboreum* L. (both 2A) to the A subgenome of American Upland cotton 2 (AD). The 2A species do not cross readily with American Upland, but can be crossed with American wild relatives (2D) to produce a highly sterile AD F₁. Colchicine treatment of this combination yields alloplody synthetic Upland cotton 2(AD) that can be crossed with normal Upland cotton to produce what has become known as a trispecies hybrid. In this hybrid, gene transfer from genome A to subgenome A of the (AD) *hirsutum* genome is relatively easy. The American wild relative (2D) could be considered a bridging species, although the situation differs somewhat from that described by Burk (1967).

Numerous other combinations of crosses and induced ploidy may aid in obtaining forms that include genomes the breeder wishes to bring together. For example, another form of trispecies hybrid might be described by the formula AC (AD). In cotton, Meyer (1974) used a trispecies hybrid, *(G. anomalum* Wawra and Peyr. 2(−) × *G. thurberi* Tod. 2D) × *G. hirsutum* 2 (AD), to obtain Upland types with cytoplasmic male sterility. Sometimes crosses between a diploid species and a tetraploid produce triploid hybrids that are fertile enough to cross back with the tetraploid. Such a tetraploid F₁ also has been referred to as a bridge. In some cases, triploid F₁ plants are highly sterile, but can be treated with colchicine to produce hexaploids that are fertile enough to cross back to the tetraploid or even to diploid forms.

The use of bridging species in a series of crosses may not be easy. It may require many crossing attempts and the screening of large segregating populations to maintain the desired genes under transfer. Our last example of such a procedure illustrates how complicated and difficult it can be. Hermens and Rammanna (1973) attempted to transfer disease resistance from *Solanum bulbocastanum* Dun., a diploid wild species, to the cultivated potato, *S. tuberosum* L., a tetraploid. These species would not cross on either the diploid or the tetraploid level. They managed to get the genomes of the two in the same organism by using two additional wild species, *S. acaule* Bitt. (a tetraploid) and *S. phureja* Juz. and Buk. (a diploid). They crossed *S. bulbocastanum* and *S. acaule* to produce a triploid F₁. The chromosome number of the sterile 3X F₁ was doubled to give a fertile hexaploid that was crossed with *S. phureja*. The resulting triple-cross hybrid (tetraploid) was crossed with potato cultivars to give quadruple hybrids, most of which could be backcrossed to the cultivars. As involved as this scheme may seem on paper, it was even more complex to carry out in practice. For instance, 20,000 pollinations were attempted to cross the triple-cross hybrid with potato cultivars, but only 40 quadruple hybrids {((*S. acaule* × *S. bulbocastanum*) × 2) × *S. phureja*) × *S. tuberosum*} were obtained.
5. Choice of Emasculation and Pollination Procedures

A necessity in making any cross is to have receptive stigmas of one parent at the same time pollen is available from the other. This procedure may be more inconvenient for interspecific than for intraspecific hybridization programs. The crop and its relative may have been separated by adaptation to different habitats and may require different environmental regimes to flower or even to grow with vegetative vigor. Techniques used to match flowering dates are discussed in Chapter 1.

Internal prefertilization barriers might be overcome in some cases by attempting a great number of crosses and in other cases by modifying procedures of preparing flowers of the female parent, by improving pollinating techniques, or both. Harlan et al. (1970) took the former approach in making a biosystematic study of the genus *Cynodon* L. C. Rich. They made "tens of thousands" of emasculations and pollinations over a 3-year span to obtain some interspecific or intervarietal combinations. Out of 56 combinations attempted, 18 failed, but 20 yielded from one to five hybrids per 100 attempts. The remaining 18 were somewhat easier to make. Any method of bulk emasculation or pollination that eliminates working with individual florets or flowers would greatly reduce the tedious labor, such as these workers experienced. A hot water emasculation technique, for example, was used successfully by Hadley (1958) to emasculate grain sorghum (*Sorghum bicolor*) heads which were then bagged together with detached heads of johnsongrass to produce several interspecific hybrids.

Methods for eliminating emasculation and hand pollination increase the number of crosses that can be attempted. If marker genes are available in the parental populations, for example, the breeder can cross-pollinate without emasculation, harvest seed from the female seed parent in bulk, and screen the progeny to separate hybrids from offspring resulting from sib crosses or selfs. The wild relative may differ so much from the crop that no marker gene is required because hybrids can be distinguished by their general morphological characteristics.

Examples of such procedures for obtaining interspecific crosses are numerous. Starling (1961) obtained hybrids between *Phalaris arundinacea* L. and *P. tuberosa* var. *stenoptera* (Hack.) Hitch. by bagging together unemasculated panicles of each species. Dewey and Pendse (1968) used a similar technique in obtaining hybrids between *Agropyron desertorum* (Fisch. ex Link) Schult. and *A. cristatum* (L.) Gaertn., except that panicles from the male parent were detached and supported in a carton of water inside the bag. In both examples, seeds from the female parent were planted and the seedlings were screened to identify hybrids. This procedure of producing hybrids is particularly effective if selfing is absent, as in self-incompatible cultivars or wild relatives. In such material, all offspring should be hybrids.

Some form of male sterility is now available in many crops and can be used in much the same way as self-incompatibility for crossing on a large scale. Either genetic or cytoplasmic-genetic male sterility, or even cyto-
genetically abnormal forms such as aneuploids, may suffice as long as they are female fertile. Both cytoplasmic-genetic male sterility and an unknown type of sterility were used by Bingham (1968) to obtain hybrids between tetraploid *Medicago sativa* L. and diploid *M. falcata* L. Hadley (1958) obtained 15 hybrids between *Sorghum bicolor* (vulgare) and *S. halepense* by bagging together heads of a genetic male sterile (*ms* 1, *ms* 2) of Texas Blackbull kafir and detached heads of Johnsongrass. He also obtained 13 hybrids by using heads of a cytoplasmic-genetic male sterile of Combine Kafir 60 and heads of johnsongrass in a similar manner.

If plant material is naturally cross-fertilized or is self-fertilized, but modified by male sterility to enforce cross-fertilization, controlled pollination on an individual flower or inflorescence basis may be eliminated. The cultigen and its wild relative may be interplanted in an isolation block where insects or wind can function as pollen vectors. The basic principles for making open-pollinated crosses are discussed in Chapter 5. We shall mention only a few examples of success in making interspecific hybrids in this way. Blowflies were used successfully by Mackay (1973) as pollen vectors in cages to obtain crosses between *Brassica compestris* (turnip) cultivars and *B. napus* L. (rape). Ashri and Rudich (1965) were able to obtain crosses between *Carthamus panaestinus* Eig. and *C. tinctorius* L. (safflower) by growing them in pots near an apiary. Buckner et al. (1976) crossed tall and giant fescue in the greenhouse by using fans to move pollen. Natural wind pollination was used by Porter and Tuleen (1972) to obtain crosses between male sterile wheat and rye, an intergeneric cross with a low percentage of success.

Techniques for preparing female flowers and pollinations are for the most part the same for wide crosses as for intraspecific crosses. These techniques are summarized in Chapter 6 and are illustrated in chapters on specific crops. We shall consider here only a few techniques particularly useful in making wide crosses. They relate primarily to incompatibilities that prevent fertilization.

One technique used to overcome cross-incompatibilities between species is to graft the embryos from germinating seeds of one species onto the endosperms of germinating seeds of the other species. Hall (1954) found that mature wheat plants that developed from wheat embryos grafted to rye endosperms and pollinated with rye pollen produced five times the number of hybrids as ungrafted controls.

Mixing incompatible pollen with compatible pollen has been successful in overcoming certain incompatible reactions. Working with poplars, in which certain interspecific matings are characterized by failure of germinated pollen to penetrate the stigma, Knox et al. (1972a, b) mixed inviable, compatible pollen with viable, incompatible pollen. They killed the compatible pollen by gamma irradiation, by freezing and thawing, or by exposure to methanol. The treated pollen, which they termed recognition pollen, was used to obtain interspecific hybrids. Recognizing that pollen grains release proteins when moistened, they hypothesized that non-enzymatic proteins may be concerned with compatibility reactions. A mixture of incompatible pollen with a protein extract obtained from compatible pollen enabled them to obtain hybrids from normally incompatible matings.
Willing and Pryor (1976) found that in poplar, incompatibility between pollen grain and foreign stigmatic surfaces could be broken by application of organic solvents to the stigma, or treatment of incompatible pollen with organic solvents would render the pollen compatible. The lipoidal mixture removed from the pollen by this procedure was designated tryphine. Pollen coated with tryphine from another poplar species carried the incompatibility response of the coated tryphine. They hypothesized that incompatibility was controlled by a two-factor system, one factor in the stigma and one in the pollen. The absence of either would disrupt the incompatibility process.

The biological interactions occurring between pollen and pistil are discussed in Chapter 2. The failure of pollen from one species to germinate on stigmas of another is common. Martin (1970) attempted 254 different interspecific combinations out of a possible 552 among 24 species from 16 families. Pollen germination occurred in only 11 cases. Some workers have circumvented this reproductive barrier by physically removing the stigma or disrupting its surface. Swaminathan (1955) overcame failures of certain Solanum interspecific crosses by removing the stigma and replacing it with a drop of an agar-sucrose gelatin medium known to support pollen tube growth in many Solanum species.

If pollen does germinate on a foreign stigma, the resulting pollen tubes may grow so slowly through the style that the egg degenerates or the flower drops before fertilization can take place. This problem may be solved by application of growth regulators that promote pollen tube growth or fruit development. Larter and Chaubey (1965) found that gibberellic acid applied to culms of autotetraploid barley increased pollen tube growth when pollinated by rye. The application of growth regulators could overcome a pre-fertilization barrier by promoting more rapid growth of pollen tubes or by increasing longevity of the pistil so that pollen tubes have time to grow farther at the same rate. Such regulators also can be used to increase embryo survival after fertilization has occurred.

Surgical procedures have been used to shorten styles of one species, so that pollen tubes from the other could reach the egg. Mangelsdorf and Reeves (1939) severely cut back the silks of corn before dusting them with pollen from the short-styled tripsacum parent. Kanta and Maheshwari (1963) overcame incompatibility by injecting pollen suspensions into surgically opened ovaries in four different species. They also succeeded in culturing excised pistils and pollen on the same medium and obtained crossed seed. Although we are not aware of such techniques being used to make interspecific hybrids, it would seem to be useful for species in which the surgery is feasible.

Protoplast fusion may be a potential method of achieving hybrid combinations that are otherwise unobtainable. This technique has been used to produce interspecific hybrids in Nicotiana (Carlson et al., 1972; Smith et al., 1976) and in Petunia (Power et al., 1976). In both cases, however, hybrids also can be obtained by sexual fertilization. Prerequisites that must be met before protoplast fusion can become a useful hybridization technique include: (i) culture of parental and hybrid protoplasts, (ii) fusions of nuclei and of cytoplasms, (iii) selection of hybrid cells, and (iv) regeneration of hybrid plants (Bhojwani et al., 1977). Protoplast fusion has interesting possi-
ilities and may become potentially useful to plant breeders. At present, however, we know of no examples where it has made possible a wide cross that could not be obtained by more conventional methods (see Snowcroft, 1977).

B. Postfertilization Barriers

Thus far we have considered primarily methods for overcoming pre-
fertilization barriers to wide crossing. The remaining techniques we shall 
consider are related chiefly to postfertilization barriers and include (i) application of flower and fruit setting hormones, (ii) use of mixed pollinations, 
(iii) embryo culture, (iv) grafting, and (v) production of alloplloid \( F_1 \)\'s.

1. Growth Promoting Substances

One difficulty in obtaining interspecific hybrids in plants that have 
multilocular ovaries may be that seed set is too low to prevent abscission of 
flowers and young fruits. Thus the few \( F_1 \) hybrid zygotes or young developing 
embryos that do result from a successful pollination are lost. Some 
breeders have succeeded in retaining flowers and fruits by applying growth 
regulators immediately after pollination. In some cases, even though fruit 
set is achieved by such application, the embryos fail to develop (Deakin et 
\textit{et al.}, 1971). In other cases, fruit set is maintained long enough for embryos to 
grow sufficiently large to be cultured. Al-Yasiri and Coyne (1964) were able 
to maintain a high percentage of developing pods for 30 days from inter-
specific crosses in \textit{Phaseolus} compared to abortion in the control 15 days 
after pollination. Thirty days was long enough for embryos to become large 
enough for culturing. Payan and Martin (1975) tested gibberellic acid, al-
phanaphthalene acetamide, and indole butyric acid for their effectiveness in 
preventing abscission of flowers and fruit in \textit{Passiflora} (passion fruit). Each 
growth regulator was mixed with lanolin in 1% and 0.1% concentrations; the 
resulting pastes were applied directly to ovaries following pollination. An application of 1% gibberellic acid was judged most effective in stimulating 
fruit set.

2. Mixed Pollinations

Mixed pollinations have been used to accomplish essentially the same 
objective as the application of growth regulators. Beasley (1940) obtained 
interspecific crosses in \textit{Gossypium} (cotton) by mixing 6 to 12 pollen grains 
of the female parent with an excess of pollen from the male parent. Bolls 
resulting from pollinations with the mixed pollen contained some large 
seeds of the female parent (selfs), plus some small seeds that were hybrids. 
Payan and Martin (1975) made interspecific hybrids in \textit{Passiflora}, a genus 
with three stigmas per flower, by pollinating two of the stigmas with pollen 
from a different species and one with pollen from a compatible plant in the 
same species.
3. Embryo Culture

Where a hybrid embryo is formed but fails to develop normally due to the inability of the endosperm to nourish it, the embryo can sometimes be cultured and germinated on an artificial medium. Laibach (1929) used such a technique to obtain hybrids from *Linum austriacum* × *L. perenne* L., a cross which would otherwise have produced shrivelled, inviable seeds.

Although various methods and media have been employed for embryo culture, the general features of the techniques are quite similar. The embryo should be excised at the time it has reached its maximum development on the female plant and before it begins to disintegrate. Dissection and transfer operations should be performed under sterile conditions to avoid contamination. The particulars of the culture medium may vary depending upon the species of concern, but in general, media are liquid or partly solidified with agar and contain mineral salts, a carbohydrate source, vitamins, hormones, and sometimes an organic nitrogen source. Immature embryos may also require addition of coconut milk (itself a liquid endosperm) or malt extract (Rappaport, 1954). Nutritional requirements of embryos and osmotic potential of the medium may depend upon their stage of development (Mauney, 1961; Pecket and Selim, 1965; Wilmar and Hellendoorn, 1968). Younger embryos generally require higher osmotic values to continue development, with lower osmotic values promoting germination.

Culturing of barley embryos will be used to illustrate some of the general principles. Barley inflorescences are collected, wiped with a cloth dampened in 10% Chlorox solution, placed in polyethylene bags, and stored in a refrigerator until use (Norstog, 1965). Embryos should be excised and cultured on the day of collection for best results.

Hands and working area are cleansed with ethanol. The lemma and palea are stripped from a floret and the exposed caryopsis is placed on a flamed slide under a dissection microscope at 25 to 50× magnification. Watchmaker’s forceps and bacteriological inoculating needles that have been ground to a fine point are used to excise embryos and transfer them to the medium. For embryos smaller than 0.2 mm, the portion of the ovule containing the embryo can be excised and transferred to a small drop of sterile paraffin oil where the embryo can be teased away. This procedure prevents drying of the smaller embryos. A transferred embryo should be positioned with the scutellum in contact with the medium.

Cultures can be kept in petri dishes or small vials containing a suitable medium. Norstog (1973) reported that 0.2 mm embryos cultured on Barley Medium II produced plantlets in 2 to 3 weeks. Cultures may be maintained at 20 to 30°C in darkness or light, although light tends to inhibit germination. Embryos that fail to germinate after cessation of growth can be induced to do so by transferring them to a new spot on the medium (Norstog, 1965). If necessary, embryos can be transferred to new cultures. After germination, hybrid plants with one or more roots and two to three leaves can be transplanted to a sterile soil consisting of 1/2 sand:1/2 sphagnum to which liquid inorganic fertilizer is applied. Plants should be protected from strong sunlight for the first few days (Kruse, 1974).
Various modifications of the embryo culture technique have been successfully employed. Ziebur and Brink (1951) reported that entire barley endosperms when placed on the agar culture medium promoted the growth of nearby barley, Raphanus, and Capsella embryos. Kruse (1973, 1974) transplanted excised hybrid embryos onto barley endosperms that had been previously cultured on the agar medium. He used this method to recover hybrids from intergeneric crosses involving Hordeum vulgare as a female parent crossed with Secale, Triticum, and Agropyrum. Harberd (1969) successfully cultured Brassica embryos by placing halved ovules in a liquid medium under constant agitation, where many embryos were washed out and subsequently grew. This method eliminated the technical skill normally required in dissecting the embryo away from the endosperm, as well as the need for stringent sterile conditions. McLean (1946) used embryo culture in obtaining interspecific Datura hybrids. She found that seedlings that developed only shoots could be grafted onto young D. stramonium plants. In addition to the culture of immature embryos, artificial media have been used to induce mature embryos or entire seeds to germinate in instances where the hybrid embryo survived to maturity, but failed to germinate when sown (Lammerts, 1942; North and Wills, 1969; Skirm, 1942; Skovsted, 1935).

4. Grafting

One of the most obvious expressions of hybrid inviability is abnormal chlorophyll development. If this expression results from unfavorable interaction between the cytoplasm of one parent and the genome of the other, use of the reciprocal cross may solve the problem. If the reciprocal cross fails because of other barriers, such as unilateral incompatibility or deleterious interactions between genomes, the breeder might resort to grafting the F₁ seedlings onto normal plants of either parent. Smith (1943) succeeded in maintaining F₁ tissue from crosses between Melilotus alba and M. dentata in this manner. Another expression of hybrid inviability is the failure of F₁ seedlings to develop a root system adequate for survival. Coe (1954) solved this problem with interspecific hybrids in sugarbeet by grafting the F₁ seedlings onto sugarbeet seedlings. The good root system of the sugarbeet stock allowed the F₁ scions to produce flowers for crossing.

Examples of grafting are rather difficult to find for field crops, perhaps because workers do not usually think such crops are capable of this type of propagation. While some crops such as the monocots are difficult or impossible to graft, other crops can be grafted, at least on the small scale required for providing functional flowers of interspecific hybrids.

5. Producing Alloploids

Numerous interspecific hybrids are highly sterile. If sterility is genic, the breeder may be able to do little except to try backcrossing the F₁ as the female parent to the cultigen which is to be the recipient of the desirable
genes. Since it is difficult to determine whether sterility is complete or just very high, one should make as many attempts as practicable before abandoning the project. Sterility may be chromosomal and doubling the chromosome number of the F1 may be used to produce an alloploid. If the F1 is a cross between two distantly related diploid species, the alloploid form will be an amphidiploid where each chromosome has one complete homologue. Pairing should be normal and the plant should be at least partially fertile.

If the interspecific F1, came from a cross between a diploid and a tetraploid species, the F1 would be a triploid either of the form ABC or AAB depending upon whether the parents had one genome in common or not. In either case, however, the F1 would be highly sterile. Alloploids can be produced from the sterile F1's by colchicine treatment. Alloploid sectors in inflorescences of treated plants are often quite easy to detect by their fertility which stands out sharply against the contrasting background of sterility in the rest of the inflorescence. Doubled F1's from the two types of triploids would differ somewhat in cytological behavior in that one would be 2(ABC), an allohexaploid, while the other would be AAAABB, an autoallohexaploid. Both types are likely to be more fertile than the F1's from which they came, and both have been synthesized and used in plant breeding programs featuring interspecific crosses.

In summary, one or more of the following procedures may be used by the breeder to obtain interspecific hybrids of potential value in a breeding program:

1. Assemble a collection of cultivars, related cultivars, and wild relatives for use as potential parents.
2. Provide an adequate environmental regime to induce flowering of potential parents.
3. Try many parental combinations of cultivars and wild relatives.
4. Make reciprocal crosses.
5. Change ploidy levels of one or both parents before crossing.
6. Use combinations of three or more parents in bridging crosses.
7. Increase the number of possible crossing attempts by use of efficient emasculation and pollination procedures.
8. Apply growth regulators to the female parent to allow or promote pollen germination and pollen tube growth, and to prevent flower or fruit abscission.
10. Make grafts to obtain a hybrid zygote, to save an inviable F1, or both.
11. Perform surgery on the pistil.
12. Use protoplast fusion.
13. Culture F1 embryos.
14. Backcross partially sterile or male sterile F1 plants as the female parent to the cultivar.
15. Produce alloploid F1 plants.
REFERENCES


INTERSPECIFIC AND INTERGENERIC HYBRIDIZATION


Rieger, R., A. Michaelis, and M. M. Green. 1968. A glossary of genetics and cyto-genetics. Springer-Verlag, N.Y.


