**Breeding cross-pollinated species**

**Concept of population improvement**

As stated in the introduction, the methods of selection (discussed in Chapter 16) for improving self-pollinated species tend to focus on improving individual plants. The methods of improving cross-fertilized species, on the other hand, tend to focus on improving a population of plants. A population is a large group of interbreeding individuals. The application of the principles and concepts of population genetics are made to effect changes in the genetic structure of a population of plants. Overall, breeders seek to change the gene frequency such that desirable genotypes predominate in the population. Also, in the process of changing gene frequencies, new genotypes (that did not exist in the initial population) will arise.

It is important for breeders to maintain genetic variability in these populations so that further improvements of the population may be achieved in the future. To improve the population, breeders generally assemble germplasm, evaluate selected selfed plants, cross the progenies of the selected selfed plants in all possible combinations, and bulk and develop inbred lines from the populations. In cross-pollinated species, a cyclical selection approach, called recurrent selection, is often used for intermating.

The cyclical selection was developed for increasing the frequency of favorable genes for quantitative traits. Various methods of recurrent selection are used for producing progenies for evaluation as will be discussed next. The procedures for population improvement may be classified in several ways, such as according to the unit of selection – either individual plants or family of plants. Also, the method may be grouped according to the populations undergoing selection as either intrapopulation or interpopulation.

**Intrapopulation improvement**.

Selection is practiced within a specific population for its improvement for specific purposes. Intrapopulation improvement is suitable for:

(a) Improving populations where the end product will be a population or synthetic cultivar.

(b) Developing elite pure lines for hybrid production.

(c) Developing mixed genotype cultivars (in selffertilized species).

**Interpopulation improvement**.

Methods of interpopulation improvement entail selection on the basis of the performance of a cross between two populations. This approach is suitable for use when the final product will be a hybrid cultivar. Interpopulation heterosis is exploited.

**Concept of recurrent selection**

The concept of **recurrent selection** was introduced in Chapter 16 as a cyclical and systematic technique in which desirable individuals are selected from a population and mated to form a new population; the cycle is then repeated. The purpose of a recurrent selection in a plant breeding program is to improve the performance of a population with respect to one or more traits of interest, such that the new population is superior to the original population in mean performance and in the performance of the best individuals within it (Figure 17.1).



The source material may be random mating populations, synthetic cultivars, single cross, or double cross plants. The improved population may be released as a new cultivar or used as a breeding material (parent) in other breeding programs. The most desirable outcome of recurrent selection is that the improved population is produced without reduction in genetic variability. This way, the population can respond to future improvement The success of a recurrent selection program rests on the genetic nature of the base population. Several key factors should be considered in the development of the base population. First, the parents should have high performance regarding the traits of interest and should not be closely related. This would increase the chance of maximizing genetic diversity in the population. It is also recommended to include as many parents as possible in the initial crossing to increase genetic diversity. Crossing provides opportunity for recombination of genes to increase genetic diversity of the population. More rounds of mating will increase the opportunity for recombination, but it increases the duration of the breeding program. The breeder should decide on the number of generations of intermating that is appropriate for a breeding program.

**Key features**

A recurrent selection cycle consists of three main phases:

1. Individual families are created for evaluation. Parents are crossed in all possible combinations.
2. The plants or families are evaluated and a new set of parents selected.
3. The selected parents are intermated to produce the population for the next cycle of selection.

This pattern or cycle is repeated several times (3–5 times). The first (original) cycle is labeled C0, and is called the base population. The subsequent cycles are named consecutively as C1, C2 . . . C*n* (Figure 17.1). It is possible, in theory, to assemble all the favorable genes in a population in a single generation if plant breeders could handle a population of infinite size. However, in practice, as J. K. Frey pointed out, the technique of recurrent selection is applied to breeding with the hope that desirable genes will be gradually accumulated until there is a reasonable probability of obtaining the ultimate genotype in a finite sample.

**Applications**

Recurrent selection may be used to establish a broad genetic base in a breeding program. Because of multiple opportunities for intermating, the breeder may add new germplasm during the procedure when the genetic base of the population rapidly narrows after selection cycles. Research has indicated that recurrent selection is superior to classic breeding when linkage disequilibrium exists. In fact, the procedure is even more effective when epistatic interactions enhance the selective advantage of new recombinants. Recurrent selection is applied to legumes (e.g., peanut, soybean) as well as grasses (e.g., barley, oats).

**Genetic basis of recurrent selection**

Various recurrent selection schemes are available. They exploit additive partial dominance to dominance and overdominance types of gene action. However, without the use of testers (as in simple recurrent selection) the scheme is effective only for traits of high heritability. Hence, only additive gene action is exploited in the selection for the trait. Where testers are used, selection for general combining ability (GCA) and specific combining ability (SCA) are applicable, permitting the exploitation of other gene effects. Recurrent selection for GCA is more effective than other schemes when additive gene effects are more important; recurrent selection for SCA is more effective than other selection schemes when overdominance gene effects are more important. Reciprocal recurrent selection is more effective than others when both additive and overdominance gene effects are more important. All three schemes are equally effective when additive with partial to complete dominance effects prevail.

**Types of recurrent selection**

There are four basic recurrent selection schemes, based on how plants with the desired traits are identified:

1. **Simple recurrent selection:** This is similar to mass selection with 1 or 2 years per cycle. The procedure does not involve the use of a tester. Selection is based on phenotypic scores. This procedure is also called phenotypic recurrent selection.
2. **Recurrent selection for general combining ability:** This is a half-sib progeny test procedure in which a wide genetic-based cultivar is used as a tester. The testcross performance is evaluated in replicated trials prior to selection.
3. **Recurrent selection for specific combining ability:** This scheme uses an inbred line (narrow genetic base) for a tester. The testcross performance is evaluated in replicated trails before selection.
4. **Reciprocal recurrent selection:** This scheme is capable of exploiting both general and specific combining ability. It entails two heterozygous populations, each serving as a tester for the other.

**Intrapopulation improvement methods**

Common intrapopulation improvement methods in use include mass selection, ear-to-row selection, and recurrent selection. Intrapopulation methods may be based on single plants as the unit of selection (e.g., as in mass selection), or family selection (e.g., as in various recurrent selection methods).

**Individual plant selection methods**

Mass selection for line development is different from mass selection for population improvement.

Mass selection for population improvement aims at improving the general population performance by selecting and bulking superior genotypes that already exist in the population.

***Key features***

The selection units are individual plants. Selection is solely on phenotypic performance. Seed from selected plants (pollinated by the population at large) are bulked to start the next generation. No crosses are made, but a progeny test is conducted. The process is repeated until a desirable level of improvement is observed.

***Procedure***

* **Year 1:** Plant the source population (local variety, synthetic variety, bulk population, etc.). Rogue out undesirable plants before flowering, and then select several hundreds of plants based on the phenotype. Harvest and bulk.
* **Year 2:** Repeat year 1. Grow selected bulk in a preliminary yield trial, including a check. The check is the unselected population (original), if the goal of the mass selection is to improve the population.
* **Year 3:** Repeat year 2 for as long as progress is made.
* **Year 4:** Conduct advanced yield trials

**Family selection methods**

Family selection methods are characterized by three general steps:

1. Creation of a family structure.
2. Evaluation of families and selection of superior ones by progeny testing.
3. Recombination of selected families or plants within families to create a new base population for the next cycle of selection. Generally, the duration of each step is one generation, but variations exist.

***Half-sib family selection methods***

The basic feature of this group of methods is that half-sib families are created for evaluation and recombination, both steps occurring in one generation. The populations are created by random pollination of selected female plants in generation 1. The seed from generation 1 families are evaluated in replicated trials and in different environments for selection. There are different kinds of half-sib family selection methods including the following.

***Ear-to-row selection***

This is the simplest scheme of half-sib selection applicable to cross-pollinated species (Figure 17.2).

***Applications***

Half-sib selection is widely used for breeding perennial forage grasses and legumes. A polycross

mating system is used to generate the half-sib families from selected vegetatively maintained clones. The families are evaluated in replicated rows for 2–3 years. Selecting traits of high heritability (e.g., oil and protein content of maize) is effective.

******

***Procedure***

**Season 1:** Grow the source population (heterozygous population) and select desirable plants (S0) based on the phenotype according to the traits of interest. Harvest plants individually. Keep remnant seed of each plant.

**Season 2:** Grow replicated half-sib progenies (S0 × tester) from selected individuals in one environment (yield trial). Select best progenies and bulk to create progenies for the next cycle. The bulk is grown in isolation (crossing block) and random mated.

**Season 3:** The seed is harvested and used to grow the next cycle. Alternatively, the breeder may bulk the remnant seed of S0 plants whose progeny have been selected, and use that to initiate the next cycle.

***Half-sib selection with progeny test***

**Half-sib** or **half-sib family selection** is so-called because only one parent in the cross is known. In 1899, C. G. Hopkins first used this procedure to alter the chemical composition of corn by growing progeny rows from corn ears picked from desirable plants. Superior rows were harvested and increased as a new cultivar. The method as applied to corn is called **ear-to-row breeding**.

***Key features***

There are various half-sib progeny tests, such as the topcross progeny test, open-pollinated progeny test, and polycross progeny test. A half sib is a plant (or family of plants) with a common parent or pollen source. Individuals in a half-sib selection are evaluated based on their half-sib progeny. Unlike mass selection, in which individuals are selected solely on phenotypic basis, the half sibs are selected based on the performance of their progenies. The specific identity of the pollen sources is not known.

***Applications***

Recurrent half-sib selection has been used to improve agronomic traits as well as seed composition traits in corn. It is suited for improving traits with high heritability, and in species that can produce sufficient seed per plant to grow a yield trial. Species with self-incompatibility (no self-fertilization) or some other constraint of sexual biology (e.g., male-sterile) are also suited to this method of breeding.

***Procedure***

A typical cycle of half-sib selection entails three activities – crossing the plants to be evaluated to a common tester, evaluating the half-sib progeny from each plant, and intercrossing the selected individuals to form a new population. In the second season, each separate seed pack is used to plant a progeny row in an isolated area (Figure 17.5). The remnant seed is saved. In season 3, 5–10 superior progenies are selected, and the seed is harvested and composited; alternatively, the same is done with the remnant seed. The composites are grown in an isolation block for open-pollination. Seed is harvested as a new open-pollinated cultivar, or used to start a new population.

******

***Genetic issues***

Like mass selection, half-sib selection is based on maternal plant selection without pollen control.

Consequently, heritability estimates are reduced by 50%. Half-sib selection is hence less effective for changing traits with low heritability.

***Advantages and disadvantages***

The major advantages and disadvantages of half-sib selection include the following.

***Advantages***

1. The procedure is rapid to conduct.
2. Progeny testing increases the success of selection, especially if quantitative gene action occurs or heritability is low.

***Disadvantages***

1. The trait of interest should have high heritability for success.
2. It is not readily applicable to species that cannot produce enough seed per plant to conduct a yield trial.
3. Lack of pollen control reduces heritability by half.

***Full-sib family selection***

Full sibs are generated from biparental crosses using parents from the base population. The families are evaluated in a replicated trial to identify and select superior full-sib families, which are then recombined to initiate the next cycle.

***Applications***

Full-sib family selection has been used for maize improvement. A selection response per cycle of about 3.3% has been recorded in maize.

***Procedure:***

***cycle 0***

* **Season 1** Select random pairs of plants from the base population and intermate, pollinating one with the other (reciprocal pollination). Make between 100 and 200 biparental crosses. Save the remnant seed of each full-sib cross (Figure 17.3).
* **Season 2** Evaluate full-sib progenies in multiple location replicated trails. Select the promising half sibs (20–30).
* **Season 3** Recombine the selected full sibs.

***Cycle 1***

This is the same as for cycle C0.

****

**Development of synthetic cultivars**

**Synthetic cultivars versus germplasm composites**

There are two basic types of open-pollinated populations of crops – those produced by population improvement, and synthetics. As previously discussed, population improvement methods can be categorized into two – those that depend on purely phenotypic selection (mass selection), and those that involve selection with progeny testing. A **synthetic cultivar** may be defined as an advanced generation of cross-fertilized (random mating in all combinations) seed mixture of parents that may be strains, clones, or hybrids. The parents are selected based on GCA. The primary distinction between the basic types of population mentioned in this section is that population improvement cultivars can be propagated indefinitely as such. However, a synthetic cultivar is propagated for only a limited number of generations and then must be reconstituted from the parental stock. A synthetic population differs from a natural population by consisting of breeder-selected parental stocks.

**Germplasm composite** is a broad term used to refer to the mixing together of breeding materials on the basis of some agronomic trait (e.g., yield potential, maturity, disease resistance), followed by random mating. There are many ways to put a composite together. Germplasm composites are by nature genetically broad based and very complex. They can be used as for commercial cultivation over a broad range of agroecological environments. However, they can also be used as reservoirs of useful genes for use in breeding programs.

**Desirable features of a synthetic cultivar**

K. J. Frey summarized three major desirable features of synthetic cultivars:

1. Yield reduction in advanced generations is less than with a single or double cross. For example, in maize, an estimated 15–30% reduction occurs between F1 and F2, as compared to a reduction of only 5–15% from *syn-1* to *syn-2*. This slow rate of reduction in yield makes it unnecessary for producers to obtain new seed of the cultivar for planting in each season.
2. A synthetic cultivar may become better adapted to the local production environment over time, as it is produced in successive generations in the region.
3. A synthetic cultivar is genetically heterogeneous, a population structure that makes it perform stably over changing environmental conditions. Further, because of this heterogeneity, both natural and artificial selection can modify the genotypic structure of synthetic cultivars. That is, a breeder may achieve gain in performance by practicing selection in *syn-2* and subsequent generations.

**Key features**

There are three primary steps in the development of a synthetic:

1. Assembly of parents.
2. Assessment of general combining ability.
3. Random mating to produce synthetic cultivars.

The parents used in synthetics may be clones (e.g., forage species) or inbred lines (e.g., corn, sugar beet). Whereas forages can be increased indefinitely by clonal propagation, inbred lines are needed to perpetuate the genotypes used in hybrid production. The parental materials are reproducible and may be substituted with new genotypes as they become available, for some improvement in the synthetic cultivar. The parents are selected after progeny testing or GCA analysis using a testcross or topcross, but most frequently a polycross, for evaluation.

**Applications**

The synthetic method of breeding is suitable for improving cross-fertilized crops. It is widely used to breed forage species. Successful synthetic cultivars have been bred for corn, sugar beet, and other species. The suitability of forage species for this method of breeding stems from several biological factors. Forages have perfect flowers, making it difficult to produce hybrid seed for commercial use. The use of male sterility may facilitate controlled cross-pollination, which is difficult to achieve in most forage species. In order to test individual plants for use in producing commercial seed, it is essential to obtain sufficient seed from these plants.

The amount of seed obtained from single plants of these species is often inadequate for a progeny test. Furthermore, forage species often exhibit self-incompatibility, a condition that inhibits the production of selfed seed. Synthetic cultivars are also used as gene pools in breeding progeny. Synthetic cultivars are advantageous in agricultural production systems where farmers routinely save seed for planting. One of the well known and widely used synthetics is the Iowa stiff-stalk

synthetic (BSSS) of maize.

**Procedure**

A procedure for crops in which selections are clonally propagable is as follows.

1. **Year 1: The source nursery**.

The source population consists of clones. The source nursery is established by planting several thousands (5,000–10,000) of plants assembled from many sources to provide a broad genetic base of the clonal lines for selection. The germplasm in the nursery is screened and evaluated to identify superior individuals according to the breeding objectives.

1. **Year 2: Clonal lines**.

The breeder first selects 100–200 superior plants on a phenotypic basis to multiply clonally to produce clonal lines. A clonal line nursery is established, each line consisting of about 20–25 plants derived from the same parental line. The breeder may impose various biotic and abiotic selective pressures (e.g., drought, specific disease epidemic, severe clipping) to aid in identifying about the 25–50 most desirable clones.

1. **Year 3: Polycross nursery**.

The selected clonal lines are planted in a polycross nursery to generate seed for progeny testing. Ideally, the layout of the polycross in the field should allow each clone to be pollinated by a random sample of pollen from all the other entries. A method of layout to achieve this objective is a square plot (e.g., 12 × 12) in which every clone occurs once in every row. Covering with a fine mesh tent or separating the plots by an adequate distance isolates pollination period is over. A large number of replications (10 or more) of the single randomized clones should be used to achieve a highly mixed pollination. Seed from each clone is harvested separately. The polycross test is valid if the layout ensures random interpollination. Alternative methods of evaluating clones for quantitatively inherited traits are available. Selffertilization may be used but it often yields only a small amount of seed. A diallel cross is cumbersome to conduct, especially for large entries. A topcross evaluates SCA. The polycross is used because it evaluates GCA.

1. **Year 4: Polycross progeny test**.

Seed is harvested from the replicated clones and bulked for planting progeny rows for performance evaluation. The progeny test evaluates yield and other traits, according to the breeding objective. The top performing 5–10 clones are selected for inclusion in the synthetic cultivar.

1. **Year 5: *Syn-0* generation**.

The selected clones are vegetatively propagated and randomly transplanted into an isolated field for cross-fertilization to produce *syn-0* seed. Leguminous species may be isolated in an insect-proof cage and crossfertilized by using insects.

1. **Year 6: *Syn-1* generation**.

The *syn-0* seed is increased by planting in isolation. Equal amounts of seed are obtained from each parent and mixed to ensure random mating in the field. Bulk seed is harvested from seed increased in the *syn-1* generation, which may be released as a commercial cultivar provided sufficient seed is produced.

1. **Year 7: Subsequent *syn* generations**.

Frequently, the *syn-1* seed is not sufficient to release to farmers. Consequently, a more practical synthetic breeding scheme is to produce a *syn-2* generation by open-pollinated increase of seed from *syn-1*. The *syn-2* seed may be likened to a breeder seed. It is further increased to produce *syn-3* (foundation seed) and *syn-4* (certified seed). Commercial seed classes are discussed in detail in Chapter 24. The pattern of loss in vigor, progressively with the advancement of generations from *syn-1*, *syn-2*, to*syn-n*, is similar to that which occurs when hybrids are progressively selfed from F1, F2, to F*n* generations. It is important to maintain the original clones so that the synthetic can be reconstituted as needed. The steps described are only generalized and can be adapted and modified according to the species and the objectives of the breeder.

**Factors affecting the performance of synthetic cultivars**

Three factors are key in determining the performance of a synthetic cultivar.

1. **Number of parental lines used**.

Synthetic cultivars are maintained by open-pollination. Consequently, the F2 (*syn-2*) yield should be high to make it a successful cultivar. Hardy–Weinberg equilibrium is reached in *syn-2* for each individual locus and hence should remain unchanged in subsequent generations. It follows then that the F3 (*syn-3*) should produce as well as *syn-2*. Some researchers have even shown that F3 and F4 generations yield as much or slightly better than F2 generations, provided the number of lines included in the synthetic cultivar is not small. With *n* = 2, the reduction in performance will be equal to 50% of the heterosis. Consequently, *n* has to be increased to an optimum number without sacrificing high GCA. When *n* is small, the yields of *syn-1* are high, but so is the decline in *syn-2* yields. On the other hand increasing *n* decreases *syn-1* and *syn-2* yields. A balance needs to be struck between the two effects. Some researchers estimate the optimum number of lines to include in a synthetic to be five or six.

1. **Mean performance of the parental lines (in *syn-0*)**.

The lines used in synthetics should have high performance. A high value of parental lines reduces the reduction in performance of *syn-2* over *syn-1*. Preferably, the parents should be non-inbreds or have minimum inbreeding (e.g., S0 or S1).

1. **3 Mean *syn-1***.

In theory, the highest value of *syn-1* is produced by a single cross. However, alone, it will suffer from a higher reduction in performance. It is important for the mean F1 (*syn-1*) yield of all the component F1 crosses is high enough such that the *syn-2* yield remains high in spite of some decline.

**Advantages and disadvantages**

The major advantages and disadvantages of the synthetic cultivar method include the following.

***Advantages***

1. The method is relatively easy to implement.
2. It can be used to produce variability for hybrid breeding programs.
3. Advanced genotypes of synthetics show little yield reduction from *syn-1*, making it possible for farmers to save and use seed from the current season to plant in the next season.

***Disadvantages***

1. Because inadequate seed is often produced in *syn-1*, the method fails to exploit to the maximum the effects of heterosis, as is the case in conventional F1 hybrid breeding. The method of synthetics is hence a compromise to the conventional means of exploiting heterosis.
2. Natural selection changes the genotypic composition of synthetics, which may be undesirable.