Teaching Recurrent Selection in the Classroom with Wisconsin Fast Plants

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SUMMARY. Plant breeding is a process that is difficult to compress into laboratory exercises for the classroom. At the heart of plant breeding is the act of selection, a process whereby differential reproduction and survival leads to changes in gene frequency. Given the relatively short span of an academic semester, it has been difficult for students to gain experience with the practice of selection using plant materials. Nearly 15 years ago, P.H. Williams developed Wisconsin Fast Plants, a model system for teaching plant biology in a classroom setting. Wisconsin fast plants are rapid-cycling versions of various *Brassica* species amenable to a variety of genetic studies due to their short life cycle and ease of handling. This paper describes the development of a model system using *Brassica rapa* L. fast plants for teaching the cyclical selection process known as recurrent selection in the context of a course on plant breeding. The system allows for up to three cycles of recurrent selection during a single 15-week semester and enables students to gain experience in planting, selection, pollination, and seed harvest during each cycle. With appropriate trait choice, phenotypic changes resulting from selection can be visualized after just three cycles. Using the Fast Plant model, recurrent selection can be practiced successfully by students in the classroom.

Plant breeding is a process. Unlike many experimental sciences, classroom exercises in plant breeding do not fit conveniently into laboratory time blocks or even into 15-week semesters. Instead, at a minimum, the process of plant breeding requires generations of hybridization and selection, a simple requirement that can take years to complete. Teachers of plant breeding have long struggled with laboratory exercises because it is difficult to compress a meaningful part of the breeding process into a short period. In a first course in plant breeding, students should be exposed to the full range of activities involved in the breeding process, such as germplasm resources, parent selection, hybridization, data collection, selection criteria, seed harvest, and the like. However, it is even more compelling to introduce students to the process of selection through a hands-on experiment. After all, the fundamental nature of the selection process is perhaps the most important concept taught in introductory plant breeding courses. But lacking a model organism such as the fruit fly *Drosophila melanogaster*, teachers of plant breeding have not steered their students toward model systems for the purpose of selection.

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Instead, they have relied upon data from selection experiments and comparisons of selected cycles from an experiment to communicate the effects of selection. While these data can be extremely useful in teaching how gain from selection is evaluated, students do not have a stake in data collection, hybridization, or the selection process itself.

In my own undergraduate education, one particular model does stand out as an example of how a classroom exercise can be used to communicate the power of selection. At the University of Illinois at Urbana-Champaign, R.J. Lambert used an isolated, open-pollinated population of maize (*Zea mays* L.) as a tool for teaching about selection. Professor Lambert's course was taught in the fall semester, which typically ran from mid-August to mid-December. During the middle of the semester, Lambert brought students to an isolated corn field, handed them empty mesh bags and a ruler, and assigned them rows of corn to evaluate. Students were instructed to select the longest ears from a proportion of the ears in a row. Harvested ears were then brought back to a barn where they were sorted, shelled, and bagged for the next cycle. Data from ear measurements were compiled and distributed to class members, who analyzed gain from selection for their cycle based on the data collected from previous classes. When I took this course in the early 1980s, some 20 previous classes of students had preceded me in this selection experiment; thus I had a wealth of data against which to compare performance of our cycle. Furthermore, my classmates and I could easily see we were part of a long-term recurrent selection experiment and we thereby took a small part of ownership in the experiment. This exercise in selection became one of the most compelling plant breeding concepts I learned during my undergraduate years. When I began to teach plant breeding, I recalled this experience in Lambert's course and sought a way to replicate it in my own environment.

Origin of recurrent selection

Recurrent selection as a scientific plant breeding method was not practiced in a systematic fashion until the middle of the 20th century. The process of recurrent selection arose from a

practical desire to improve parent line performance in the inbred-hybrid breeding method. During the early days of hybrid maize breeding programs, inbred lines were selected from successful open-pollinated cultivars and used directly as parents of F_1 hybrids. These inbred lines became known as first-cycle lines. As breeding efforts intensified, the relatively low frequency of successful inbred lines extracted directly from open-pollinated cultivars led to greater attention on crosses among inbred lines (Allard, 1960). These second-cycle lines exhibited greater per se performance but did not necessarily make better F_1 hybrids. Improved inbred performance was important to the developing seed industry, since hybrid seed must be produced on productive inbred lines to make hybrids commercially feasible. However, the lack of improvement in hybrid performance was a concern. Breeders began to reconsider certain aspects of the inbred-hybrid method, particularly the relatively high degree of inbreeding required to produce elite inbred lines.

The proportion of superior inbred lines from a source population is contingent upon the frequency of favorable alleles in that source population. The greater the frequency of such alleles, the better the chance that desirable lines will be extracted. The fact that inbred lines derived from previously existing inbred lines did not contribute significantly to hybrid performance suggested the frequency of favorable alleles in the original source populations was lower than desirable. Several workers proposed recurrent selection as a solution to this problem in the 1940s. An example provided by Allard (1960) serves to illustrate the point: assume five genes of equal effect govern characters under selection. Assume q , the frequency of the more favorable of two alleles, is 0.5 for all of the five loci. In this case, only one individual in a population of 1000 will contain the favorable allele at all five loci. If q were increased to 0.95 at each locus through selection, 600 out of 1000 individuals should contain the favorable allele at all five loci. Thus, selection designed to increase the frequency of favorable alleles in breeding populations should theoretically improve the chance of identifying superior lines.

The method proposed for increas-

ing the frequency of favorable alleles in breeding populations became known as recurrent selection and was first formally suggested by Hull (1945). Hull (1952) considered that recurrent selection included reselection generation after generation with interbreeding of selected progeny. Because of the additional recombination provided each generation, new combinations of alleles can arise and offer new genotypic and phenotypic possibilities. In the past 50 years, recurrent selection has been used effectively by a number of plant breeders. Recent reviews of recurrent selection methods and the success of recurrent selection approaches have been presented (Dudley and Lambert, 1992; Hallauer, 1985; Weyhrich et al., 1998).

Recurrent selection typically involves evaluation of plants from a population, selection of a proportion of these plants, intermating of selected individuals, and reevaluation of progeny from these matings. The simplest form of recurrent selection is mass selection, where seed from selected individuals in a given generation is used as the basis of the next cycle of selection. In simple recurrent selection, no family structure is imposed on the selection program and record keeping is greatly simplified by the bulking of seed from selected individuals each generation. The three main phases of recurrent selection: evaluation, selection, and recombination, are compressed into one generation in mass selection, making it ideal for a classroom setting.

The Wisconsin Fast Plant Model

Wisconsin fast plants were developed by P.H. Williams and colleagues in the Department of Plant Pathology at the University of Wisconsin-Madison during the 1970s and 1980s for use as an educational and research tool (Williams and Hill, 1986; Williams, 1989). Fast plants are rapid-cycling derivatives of various *Brassica* species, including *B. rapa* L., *B. oleracea* L., *B. carinata* L., *B. juncea* L., and *B. nigra* L. developed by recurrent selection.

The genus *Brassica* contains many important crop plants, including oilseed rape and mustard (*B. nigra*), turnip and Chinese cabbage (*B. rapa*), broccoli, cauliflower, and kale (*B. oleracea*), and many others. Comparison of conventional and rapid-cycling

derivatives of these crop plants illustrates the power of artificial selection by plant breeders and is often used to communicate the effectiveness of selection to students beginning their work with fast plants. P.H. Williams developed these rapid-cycling plants through a process of recurrent selection for early and concentrated flowering, short stature, ability to grow under continuous fluorescent lighting, absence of seed dormancy, and ability to grow at high density (Williams and Hill, 1986). Since their introduction, fast plants have been adopted by thousands of primary and secondary schools throughout the world for use in teaching biological principles. In addition, fast plants have served a useful role in demonstrating advanced genetic concepts in university courses. The Fast Plants Program is complemented by a wealth of genetic stocks and supporting information, now distributed worldwide through Carolina Biological Supply Co. (Burlington, N.C.) and the Crucifer Genetics Cooperative (Dept. of Plant Pathology, Univ. Wisconsin-Madison).

Among the primary advantages of fast plants for genetic study are their relatively short life cycle and ease of pollination. Some of the fast plants species flower 2 to 3 weeks after sowing and complete their life cycle within 45 to 50 d. This short generation time makes it possible to perform controlled crosses and evaluate segregating populations within the span of a single academic semester, or possibly within a single academic quarter if advanced planning is utilized. In addition to genetics, students working with fast plants gain experience in pollination biology, seed harvest and handling, and plant husbandry.

Recurrent selection with Wisconsin fast plants

I chose *B. rapa* for recurrent selection because of its short life cycle compared to other rapid-cycling populations. In addition, this species exhibits variation for the trait under selection: anthocyanin pigment production. Anthocyanin pigment is produced in various organs of *B. rapa*, including hypocotyl (Fig. 1), leaf margins, and to a very slight degree,

the terminal portions of flower buds. High levels of expression of anthocyanin pigmentation are present in other Brassica species as well, including the familiar red cabbage (*Brassica oleracea*). Like many plant pigments, the inheritance of anthocyanin pigmentation is relatively simple and likely governed by a single major gene. In fact, anthocyaninless and high-anthocyanin stocks of *B. rapa*. Fast plants are available through the Crucifer Genetics Cooperative. On the other hand, pigmentation also behaves like a quantitative character and is amenable to recurrent selection. A similar situation can be found in table beet (*Beta vulgaris* L. ssp. *vulgaris*), where major regulatory genes control the presence or absence of betalain pigment and yet recurrent selection is effective in increasing pigment concentration (Goldman et al., 1996). Once alleles conditioning pigment production are present at these regulatory loci, selection can be used to increase the expression of modifying alleles at additional loci.

The degree of pigmentation can be assessed visually at early stages of plant development. Trait identification at an early developmental stage allows students to focus their attention on differentiation among individual plants in a population during class and does not leave the selection decisions to later, once all the plants

have been cleared away and the data have been examined. On the other hand, visual selection in this way also results in the greatest limitation in using pigmentation as a trait under selection. Because no quantitative information is assessed on individual plants, students do not gain experience collecting and analyzing data from selection. In addition, they cannot calculate gain from selection, which would be a valuable exercise in a plant breeding laboratory. In practice, however, I have found that a relatively simple trait is advantageous in the classroom as it will allow students a maximum amount of time examining plant material. In this way, students gain the greatest degree of familiarity with variability within a population, an important insight into the process of plant breeding.

Using control populations in recurrent selection

Many recurrent selection pro-

Fig. 1 (clockwise from top left). *Brassica rapa* fast plant flower; Stages of development of the *B. rapa* fast plant with number of days from sowing printed on white containers; Variation in anthocyanin pigmentation of *B. rapa* fast plant hypocotyls; Moveable cart used to grow fast plant populations. All photos courtesy of the Wisconsin Fast Plant Program.

grams are carried out with a single population or series of populations under directional selection. Use of only one population in a selection scheme is to be expected because of the practical goals of most breeding programs. Fewer programs make use of bidirectional selection because of the relatively impractical changes in plant populations selected in a direction opposite to that desired agriculturally. The Illinois long-term selection experiment is an important exception to this practice (Dudley and Lambert, 1992). Even fewer studies still make use of control or unselected populations in their experiments. Such populations are atypical in selection experiments because of the tremendous time and cost required to conduct a selection program over many years. On the other hand, control or unselected populations provide a tremendous amount of insight into the selection process for students beginning their study of plant breeding. In the absence of genetic drift, selection, migration, and mutation, plants in an unselected population should not change phenotypically during the course of a selection experiment. This unselected population can therefore be used as a control to compare changes resulting from selection in the experimental population. Commonly referred to as a drift population by the students, this unselected population can be used to detect the effect of variables associated with the physical conditions of the experiment itself on changes in phenotype. In addition, selection in this population is often practiced through random-number generation, allowing for a great contrast to selection in the selected populations, which is most often practiced with great care and decision-making. A recent selection study (Sills and Nienhuis, 1998) using the model plant *Arabidopsis thaliana* L. (Heynh.) revealed significant directional changes at certain molecular marker loci in the control population, suggesting the value of such a population for monitoring the effects of selection.

A recurrent selection model for the classroom

For the past several years, I have made use of the *B. rapa* Fast Plant Model for an ongoing recurrent selection experiment with my upper-division undergraduate and beginning graduate

level course Techniques of Plant Breeding. This 1-credit course, which meets for a 2-h time period 1 day per week, is designed to complement the 3-h, 3-credit lecture course that meets 3 times per week and deals with plant breeding principles. The goal of the laboratory course is to familiarize students with the tools of the plant breeder. Students in the course conduct three cycles of recurrent selection as a backbone to topics presented during the semester. Interspersed around the activities of the recurrent selection experiment are presentations and demonstrations from plant breeders in the private and public sectors. During the 15-week semester, about nine weekly meetings are devoted to the recurrent selection experiment (Table 1).

Plant populations used in this experiment are maintained in plastic flats and grown on a portable metal cart (Fig. 1) with 24 h fluorescent lighting. Irradiance $>200 \text{ mEm}^{-2}\cdot\text{s}^{-1}$ is required for fast plants. Forty-watt fluorescent bulbs, spaced at 5 to 6 cm (≈ 2 inches) apart, are suspended from the shelves of the cart to obtain the desired amount of light. Currently, the Wisconsin Fast Plants Program suggests Sylvania Octron 4100K FO32/741 bulbs for lighting fast plants.

Planting

In 1996, a population of *B. rapa* fast plants designated C 1-33 was obtained from P.H. Williams and D. Lauffer (University of Wisconsin-Madison) and used as a source population for the recurrent selection experiment. Three samples of 400 seeds each were chosen randomly from the source population and sown in Redi-Earth Peat-Lite potting mix (Scotts, Marysville, Ohio) in seedling trays, which were then placed in reservoir trays and filled with one-half strength Hoagland's solution (Hoagland and Arnon, 1950). Each population of 400 plants comprised 2 trays or flats of 200 seedlings each; thus each population of 200 plants was replicated twice. The three populations planted from the three samples of seed were designated H, L, and D, corresponding to high anthocyanin (H), low anthocyanin (L), and drift (D), respectively. Students sowed seeds on day 0 of the experiment (Table 1).

Selection

One week later, on day 7, students initiated selection. Because se-

lection takes place prior to flowering, 100% of the additive genetic variance can be exploited, thus potentially allowing for greater gains in recurrent selection (Falconer and Mackay, 1996). Thirty-two plants from each tray of the H populations were selected visually based on level of pigmentation in the hypocotyl (Fig. 1). In a similar fashion, 32 plants from each tray of the L and D populations were selected, however in the case of the L populations plants were selected for lack of anthocyanin pigmentation in the hypocotyl and D populations were selected randomly. Groups of students were assigned one of the populations to select and were given the freedom to choose the manner in which selection would be practiced. Some groups worked together to identify plants to select while other groups designated a particular portion of the population to group members with the objective of selecting a proportion of the total 32 plants. In most cases, groups working on the D populations chose plants by generating random numbers corresponding to coordinates on a grid. For all three populations, the 32 selected plants were transplanted into two small flats with 16 receptacles each, where each receptacle is ≈ 1.5 cm in diameter. This step was taken to simplify the handling of selected plants for pollination.

Pollination

Selected plants were pollinated beginning on day 14. In this way, activities involving recurrent selection were conducted in three consecutive weeks: sowing on day 0, selection on day 7, and pollination on day 14. In practice, pollination requires ≈ 5 d as not all flowers are open on day 14. Thus, on day 14, students construct bee sticks for use as pollination devices and begin the pollination process. A bee stick is composed of a freeze-dried bee thorax mounted on the end of a toothpick. The bee's thorax is used as a pollen collector and distributor, mimicking the activity of pollen and nectar-foraging bees in nature. Each day, pollen is transferred to open flowers among plants within a replicate. The bee stick is used to collect pollen from open flowers of the selected plants in a replicate, thus no attempt is made to control the male parent in this selection scheme. Pollinations are generally accomplished over 5 d, taking the populations into day 19. At day 19,

Table 1. Timeline of activities during a 15-week (105-d) semester for a recurrent selection experiment with *Brassica rapa* Wisconsin fast plants as conducted by students in Horticulture 502: Techniques of Plant Breeding at the University of Wisconsin–Madison.

Day	Cycle	Activity	Activity duration (h) ^z
0	1	Seed sown	1.5
7	1	Selection	1.5
14	1	Pollination	1 ^y
19	1	Pollination termination	0.2
42	1	Seed harvest	0.5
	2	Seed sown	1.5
49	2	Selection	1.5
56	2	Pollination	1
61	2	Pollination termination	0.2
84	2	Seed harvest	0.5
	3	Seed sown	1.5
91	3	Selection	1.5
98	3	Pollination	1
103	3	Pollination termination	0.2

^zDuration is approximate and based on groups of three students working with three trays of 200 plants each.

^yPollination must be continued daily for ≈5 d.

remaining unpollinated flowers are removed and pollination is terminated. Since seed harvest may generally be expected to occur from day 40 to 42, 3 weeks must elapse before the next cycle can begin.

Harvest

Seed harvest and cleaning can be coupled with sowing the next cycle in order to fit as many cycles of selection as possible during the semester or quarter. At the University of Wisconsin, we are able to have students participate in three cycles of selection during a semester. This is accomplished by sowing seed 1 week prior to the start of the semester and completing the third growth cycle after the semester has ended. Selection coincides with the first day of class, and is followed by 5 additional weeks of the fast plant life cycle (Table 1). At the beginning of week 6 of the semester, students harvest seed and plant the next cycle. This second cycle is terminated at the end of week 12 of the semester. Students then participate in the first aspects of the third cycle, by sowing seeds, selecting, and pollinating. Seed harvest for the third cycle takes place after the semester is over. In this way, students can participate in three rounds of selection and pollination during a semester.

Conceptual issues in teaching recurrent selection

An important aspect of learning

the selection process is the continuity of selection. Students are able to participate in this continuity by initiating their selection on the selected cycles developed by the previous years' students. At seed harvest, remnant seed (seed not planted in the next cycle) is placed in plastic tubes and stored under refrigeration. Saving of seed should allow students to sample plants from any cycle of selection in future years and ask questions about changes in genotype or phenotype in this experiment. An additional suggestion made by students in this course for preserving selection cycles involved freezing remnant bee sticks. Because these bee sticks carry pollen from a particular cycle, this pollen may be preserved and potentially used to pollinate future selection cycles in order to answer particular questions about these materials.

In the past 3 years, students in this course have been able to accomplish nine cycles of selection. Growouts of randomly sampled seeds from any cycle allow students to observe phenotypic changes in these populations as a result of their selection. For example, a growout of the first three cycles revealed significant visual differences in hypocotyl pigmentation between the H and L populations. Selection progress can be assessed at any time during the course of a semester using remnant seed from previous generations. In addition, remnant seed can be used for a variety of experimental

exercises performed in the classroom. Such experiments have spawned a number of independent study projects including techniques to measure pigment nondestructively in fast plant tissue, progeny testing of families from the recurrent selection experiment, and interspecific crosses between *B. rapa* fast plants and other *Brassica* species.

Because selection is practiced in groups, decision making activities with respect to selection criteria are among the most educational parts of the recurrent selection exercise. Students must work together to forge common selection goals and then carry them out efficiently in order to remove 84% of the plants via selection. Even though hypocotyl pigmentation is a relatively simple trait to score, because subjective visual criteria are used, students may be challenged to come to consensus regarding selection choices. In addition, missing plants (empty cells) present an interesting educational opportunity, as some students feel a selection intensity of 16% should be maintained while others simply consider them "missing data." While this aspect of the selection program can lead to excellent discussion, problems may arise in that groups of students may choose slightly different selection criteria from cycle to cycle and from semester to semester. I hope that the anthocyanin pigmentation model is robust enough to handle these variations in students' decision making and still produce populations that respond to selection.

As previously stated, a major limitation to visual selection is the lack of a data collection step in recurrent selection. Without data, students do not have the opportunity to use the recurrent selection experiment as a means for experimenting with concepts such as the genetic gain formula or performing realized heritability calculations. Standard methods for measuring anthocyanin concentration in plant tissues are relatively simple; however they typically require destructive sampling. Because fast plant hypocotyls are small and tender, it would be difficult to sample tissue nondestructively from these plants for measurement of anthocyanin concentration. During the past several years, students have suggested a number of methods for obtaining data nondestructively. These include: use of a color chart for assigning color values, digital photography

and computer imaging, measurement of light reflectance from hypocotyl tissues, and progeny testing with destructive sampling of crossed progeny. All of these suggestions may have merit in modifying the recurrent selection program for anthocyanin pigmentation and improving the use of Wisconsin fast plants for teaching plant breeding concepts.

The Wisconsin Fast Plant Model is an ideal system for teaching and conducting recurrent selection in the classroom. Students have the opportunity to plant, select, pollinate, harvest, and replant selected individuals. By repeating this process two or three times during the semester, students gain valuable experience with the crucial aspects of recurrent selection. Perhaps most importantly, evaluation of several cycles of recurrent selection at the end of the semester will typically reveal significant visual changes in phenotype, allowing students a glimpse into the tremendous power of the artificial selection process.

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