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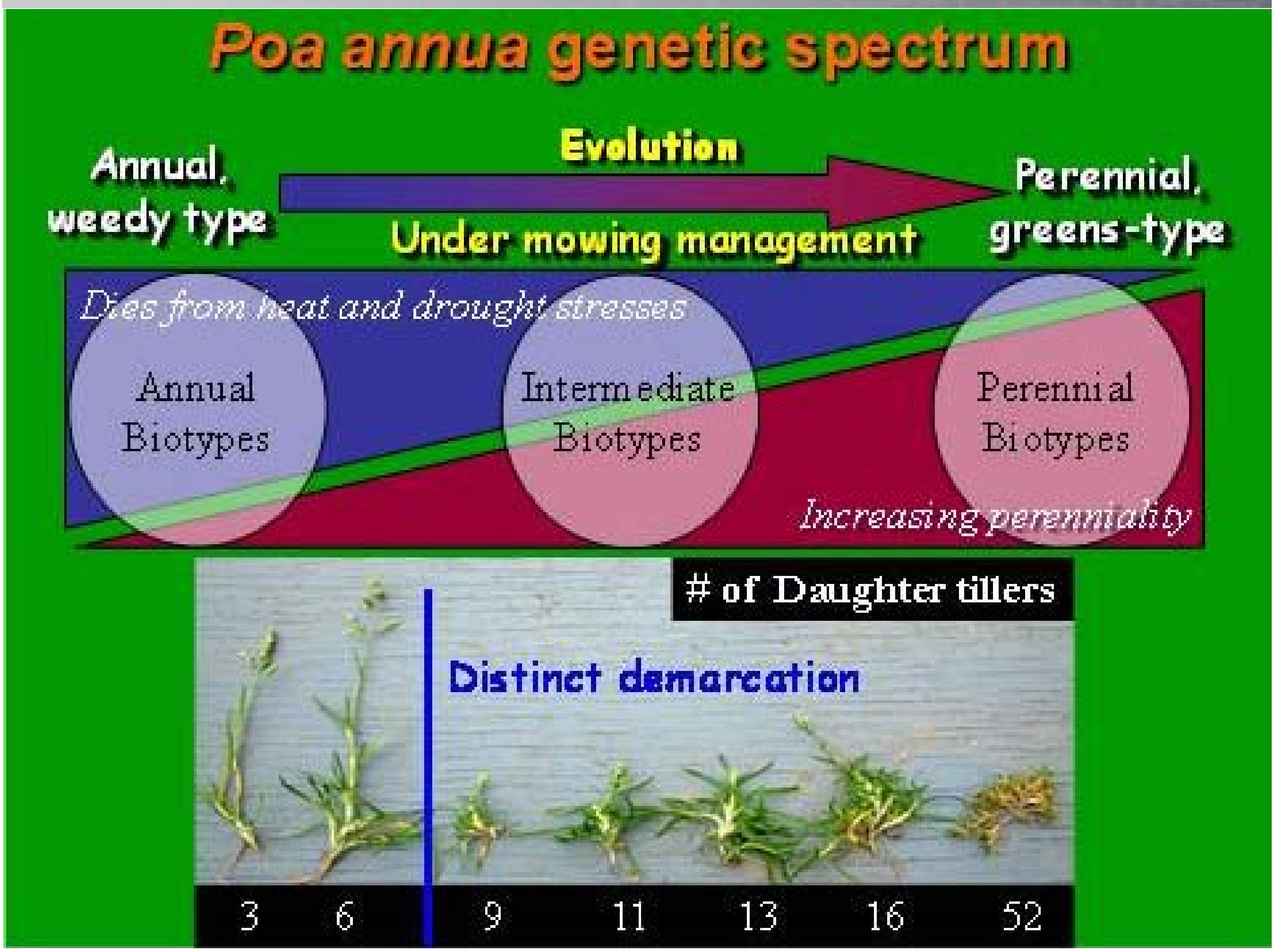

Researchers at Penn State University investigated the genetics of annual bluegrass (*Poa annua* L). They found that *Poa annua* exhibits a spectrum of morphological and life history characteristics, ranging from wild weedy annual-types to high shoot density, perennial greens-types. Under the mowing pressure of golf course putting greens, annual-types are capable of gradually evolving into greens-types with increasing tolerance to biotic and abiotic stress tolerance and an enhanced number of daughter tiller and hence perenniality.

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PURPOSE

The purpose of *USGA Turfgrass and Environmental Research Online* is to effectively communicate the results of research projects funded under USGA's Turfgrass and Environmental Research Program to all who can benefit from such knowledge. Since 1921, the USGA has funded more than $40 million for research at universities. The private, non-profit research program provides funding opportunities to university faculty interested in working on environmental and turf management problems affecting golf courses. The outstanding playing conditions of today's golf courses are a direct result of ***using science to benefit golf***.

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Greens-type *Poa annua* Violates the Laws of Genetics

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SUMMARY

Annual bluegrass (*Poa annua* L.) displays a wide range of range of life history characteristics, from wild weedy annual-types to long-lived perennial types (also known as greens-types), enables it to adapt to a wide range of environmental conditions and diverse habitats world-wide. Researchers at Penn State University investigated the genetics of annual bluegrass (*Poa annual* L.) in an effort to understand this complex species. The study found:

- Greens-type *Poa annua* plants are characterized by single branching inflorescences and reduced culm, tiller, leaf, and panicle lengths compared to annual-type *Poa annua* plants.
- A single recessive genetic mechanism is hypothesized to control the differences between greens-type and annual-type *Poa annua*.
- However, the genetic inheritance of greens-type *Poa annua* phenotype does not follow the Laws of Mendelian inheritance in advanced generations.
- Greens-type *Poa annua* plants were observed to vegetatively revert to annual-types over time when not mowed.
- We conclude that the greens-type *Poa annua* phenotype is likely to be epigenetically controlled and, as such, the greens-type *Poa annua* phenotype is not stable outside of its greens management environment.

Recently, annual bluegrass (*Poa annua* L.) made international news as one of the most prevalent and widespread alien invaders in the pristine Antarctic landscape (1). The researchers found that the scientists, volunteer staff, and visitors to the Antarctic region were bringing seeds of *Poa annua* on their equipment and gear and, now that *Poa annua* has become established, it is beginning to spread and dominant areas far away from the region's main human settlements and into the virgin Antarctic landscape.

Golf courses have long known about the invasive tendencies of *Poa annua*, particularly in their putting greens. In most circumstances, the *Poa* invasion detracts from the overall quality of the putting green because of its annual life habit which lacks tolerance to biotic and abiotic stress. However, there are exceptions within the golf industry where *Poa annua* actually serves as the preferred putting surface because it is capable of evolving a perennial life habit which displays an exceptionally high turf quality and tolerance to several biotic and abiotic stresses (Figure 1).

The ability of *Poa annua* to display such a wide range of range of life history characteristics, from wild weedy annual-types to long-lived perennial types (also known as greens-types), enables it to adapt to a wide range of environmental conditions and diverse habitats world-wide. On golf courses, we have observed that the number of daughter tillers is different among *Poa annua* depending on the playing surface from which the plants are collected. In roughs and fairways, *Poa annua* typical produces as few as 3 and as many as 8 daughter tillers per flowering culm. On putting greens this grass will produce anywhere from 9 to 52 daughter tillers per flowering culm (Figure 1).

We view the production of daughter tillers to be a measure of perenniality and thus, greens-type *Poa annua* not only have a higher shoot density and better turf quality than plants from fairways and roughs, they are also more perennial by virtue of their increased production of more daughter tillers per flowering culm. In addition, greens-type *Poa annua* has been observed to show reasonably good field tolerance to stresses including diseases such as anthracnose and dollar spot (5) and environmental stress like drought and salinity (2). As such, *Poa annua* has historically always been (9), and continues today to be an important component of golf course putting greens and the global golf industry.

Currently, no commercial seed sources exist for cultivars of greens-type *Poa annua*, a fact that limits its use for renovating old, premier golf

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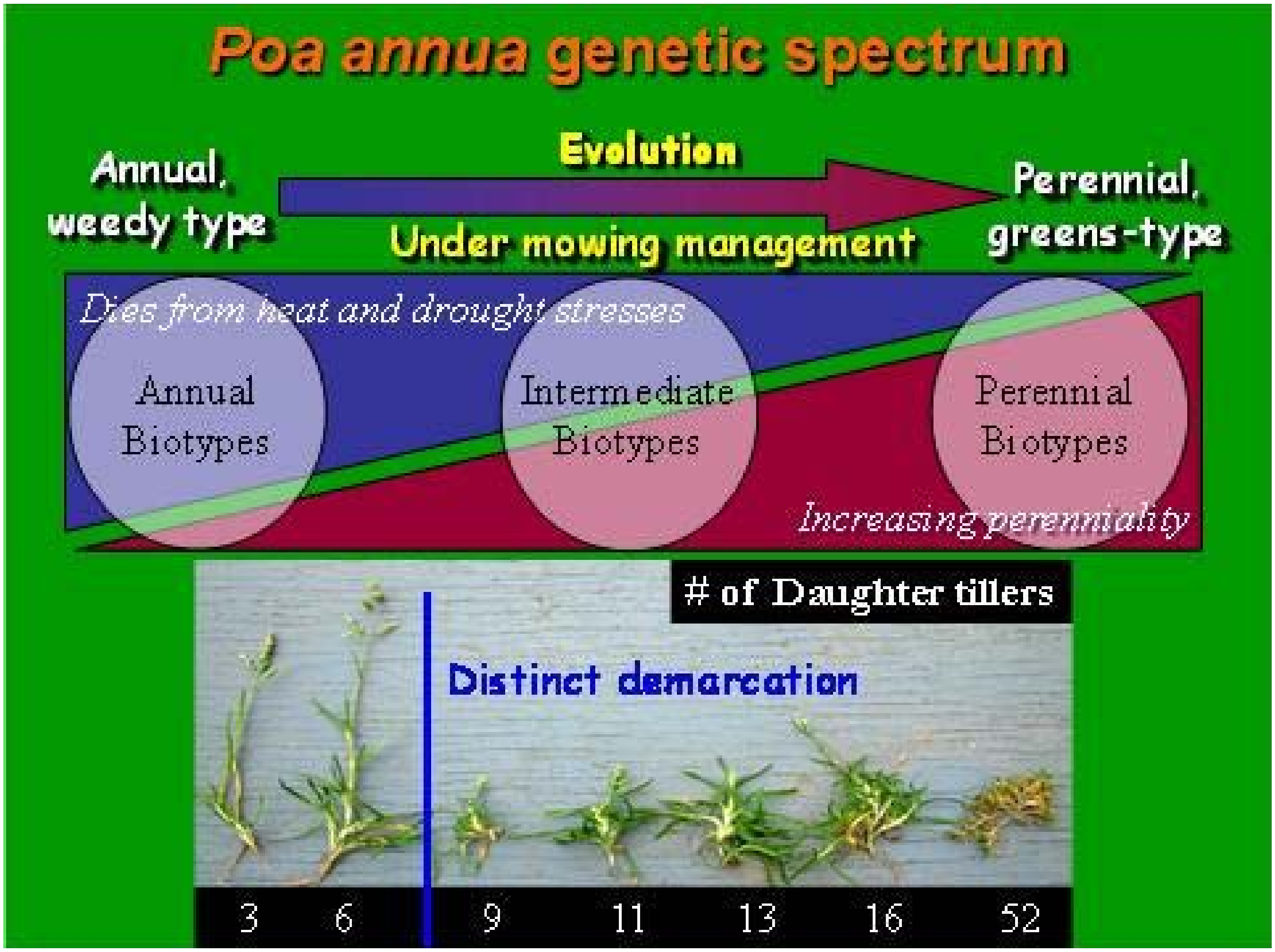

Figure 1. *Poa annua* exhibits a spectrum of morphological and life history characteristics, ranging from wild weedy annual-types to high shoot density, perennial greens-types. Under the mowing pressure of golf course putting greens, annual-types are capable of gradually evolving into greens-types with increasing tolerance to biotic and abiotic stress tolerance and an enhanced number of daughter tiller and hence perenniality. (Figure design by J. Dai).

course where *Poa* greens are common place or for new golf course construction projects. Several attempts to breed improved commercial cultivars of greens-type *Poa annua* have been attempted (3, 4, 6) but to date, none of these programs have been successful.

Similarly, our greens-type *Poa annua* breeding program at Penn State University, initiated in 1994, has also experienced difficulty in developing a commercial cultivar. The main problem we have faced is that the phenotype of the greens-type *Poa annua* tends to be lost after several years of seed production, resulting in seed progeny plants that morphologically resemble wild, weedy annual types.

This reversion of greens-types back to annual-types is very perplexing because according to the Laws of Mendelian genetics, such reversions should not be taking place. Morphological traits such as tiller density, leaf length, inflorescence height, etc. are controlled by the expression of various genes located in the DNA of the plant's cells. The array of genetic information of any particular plant is known as its genotype and is inherited intact, with the exception of genetic recombination, generation after generation. In addition, the outward appearance of any particular genotype, or what it is that we see, is also modified by the environmental growing conditions of the plant. Thus, the morphology of a plant is determined by both its genotype and its environment, resulting in what we call its phenotype, i.e. genotype + environment = phenotype.

When the same genotype is grown under

different environments it is not uncommon to observe different phenotypes. For example, turf-grasses typically look very different when grown under high versus low levels of nitrogen. However, we were observing the loss of the greens-type *Poa annua* phenotype under the uniform environment of the seed production field. The genotype-phenotype relationship is the basis of all breeding programs, from dairy cows to Easter lilies, and so it made no sense to us that *Poa annua* should be an exception to the fundamental principles of basic biology. In order to explain our observations, we have embarked on a quest to determine how and why greens-type *Poa annua* would appear to defy the basic laws of genetics.

From our research, we have learned that greens-type *Poa annua*s all share some distinct morphological differences from the annual-types of *Poa annua* when both are grown under the identical set of environmental conditions (Figure 2). For example, greens-type *Poa annua* tends to have and overall dwarf appearance with shorter leaves, shorter tiller lengths, and shorter culm heights compared to annual-types. In addition, greens-types have only a single branch at the basal node of their inflorescence whereas, annual-types have a double branch basal node. The association of all of these traits and characteristics with the two types of *Poa annua* has led us to hypothesize that there may be only a single genetic difference between the two types.

In order to test this hypothesis, we initiated a series of crossing experiments to examine the resulting Mendelian segregation ratios. We first crossed an annual-type with a greens-type, and the resulting progeny showed an intermediate morphology between the parental extremes with a double branch inflorescence and thus we classified it as an annual-type. The progeny of this cross is genetically referred to as an F_1(first filial generation) and the inheritance of the double branching inflorescent trait in the F_1 indicates that its gene expression is dominant over that of the single branching trait of the greens-type *Poa annua*.

Thus, according to our single gene hypothesis and the dominant nature of the double branching inflorescence trait, we were able to designate the genotypes of the two types of *Poa annua* as AA (pronounced big A, big A) for the annual-type

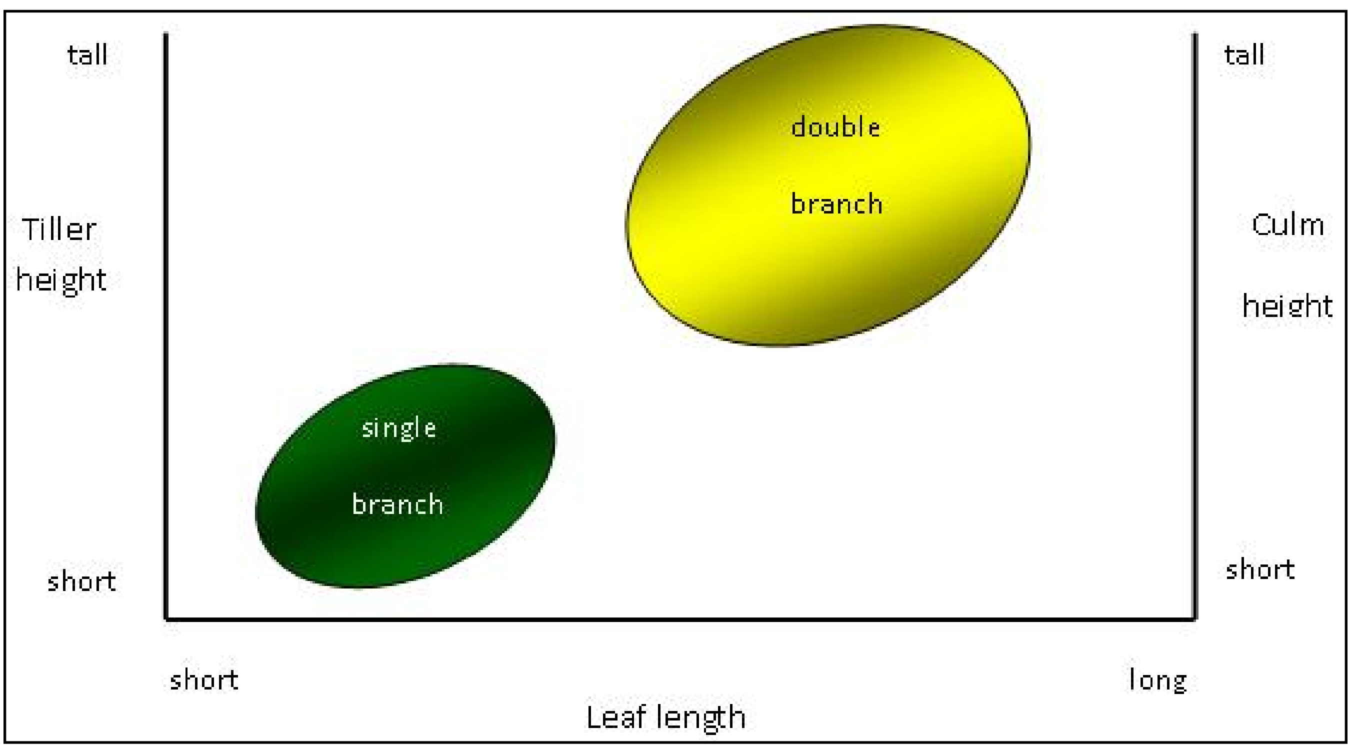

Figure 2. Morphological traits ofannual (yellow) and greens-type (green) *Poa annua*.

		Annual-type Parent (Aa)	
		A	a
Annual-type Parent (Aa)	A	AA (Annual)	Aa (Annual)
	a	Aa (Annual)	aa (Greens)

Figure 3. A Punnett square depicting the segregating progeny from a hypothetical cross between two heterozygous plants of annual-type Poa annua. In this example, the trait of interest (annuality vs. perenniality) is controlled by a single gene with two alleles (capitol A and small a) with A being dominant to a. After the pollen and eggs (each of which can only carry one allele) randomly unite, the progeny segregate into a ratio of 3 annual types : 1 greens-type.

and aa (pronounced little a, little a) for the greens-type. The F_1 progeny were then self-pollinated to yield an F_2 generation (Figure 3). Figure 3 is a summary, known as a Punnett square, of every possible combination of the different forms of the genes (genetically known as alleles) possessed by the two parents.

In our example here, each parent possesses two alleles, a big A and a little a. The pollen and eggs of each parent are capable of carrying only a single allele which randomly unite to form a progeny seed and thereby reconstitutes the genotype of the progeny plant. As typical with F_2 populations, we observed a wide range of segregation, from large robust annual-types to dwarf greens-types in a ratio approximating what genetic theory predicts will occur which is a 3:1 ratio (Figure 3). At this stage of our experiments, Poa annua was following the Mendelian Laws of genetics and appeared to be behaving as a normal organism.

We continued to assess the genetic nature of *Poa annua* by advancing the F_2 population to the subsequent F_3 and F_4 generations. It was in these advanced generations that we began to observe *Poa annua* violate the laws of genetics.

Figure 4. The dwarf, single branch inflorescence greens-type phenotype in Poa annua is observed to revert to a large, double branch inflorescence annual-type over time when left unmowed in the greenhouse.

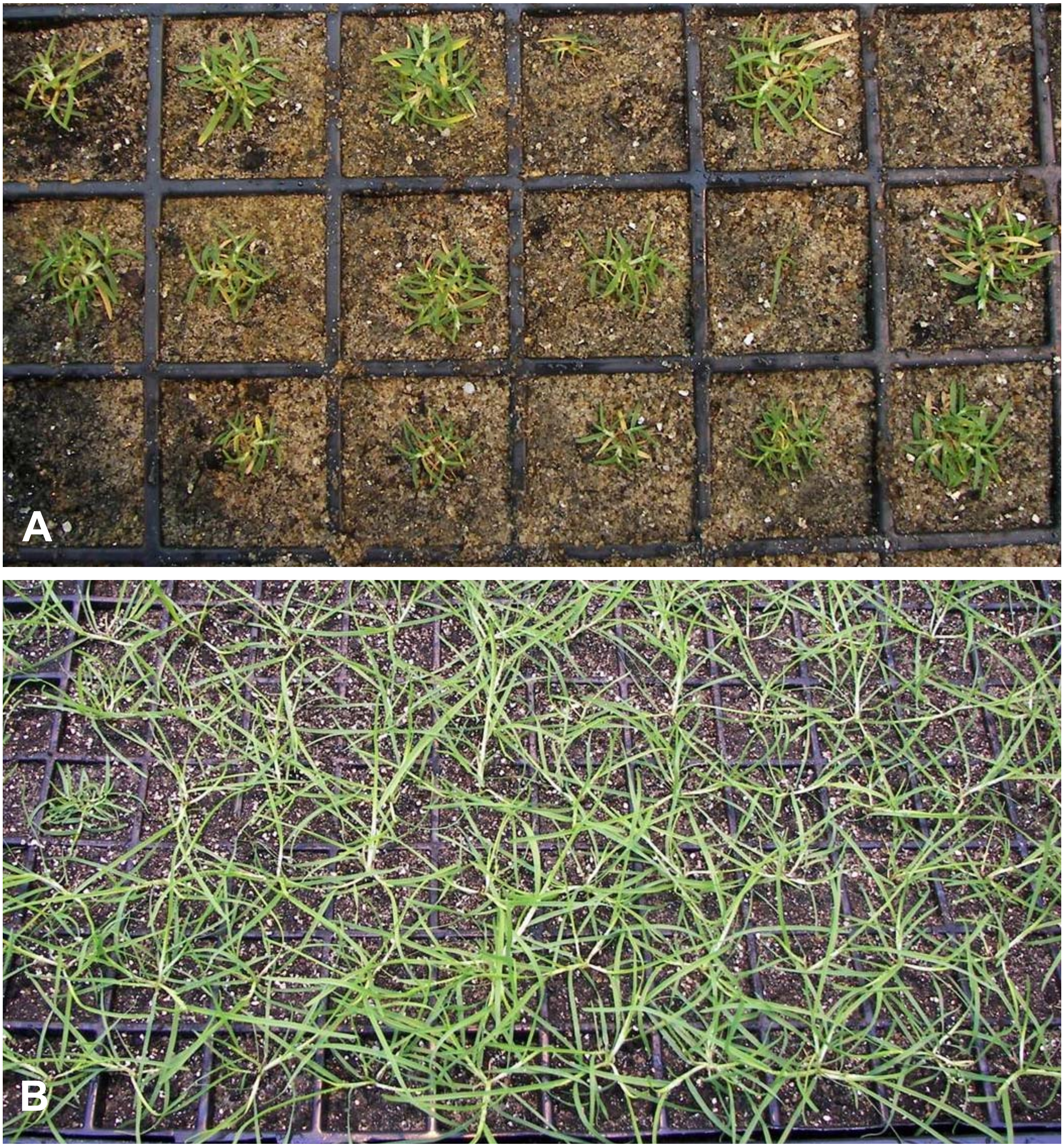

Figure 5. Seedling progenies from an advanced F_8 generation of a greens-type x greens-type *Poa annua* cross treated with either mowing (A) or left unmowed (B). All 24 plants retained the greens-type phenotype when mowed; whereas, only 6 out of 96 plants expressed the greens-type phenotype when unmowed with the remaining 90 unmowed plants expressing the annual-type phenotype.

Greens-type *Poa annua* plants of the F_2 generation, which are genetically homozygous for the recessive allele, and thus should only produce greens-type progeny, began to produce annual-type progeny in the F_3 and F_4 generations in ratios of 65:33, 82:73, 58:65, and 39:44 annual-type to greens-type in four separate crossing experiments, respectively. How was this possible? We repeated our experiments under enhanced isolation conditions to ensure that no foreign pollen was contaminating our experiments and similarly observed that advanced generations of greens-type x greens-type crosses would produce segregation ratios of 72:23 and 58:64 annual-types to greens-types.

If our original hypothesis that the greens-type phenotype was inherited by a single gene with two alleles exhibiting a dominance-recessive relationship was correct, then these advanced generations should only produce greens-type progeny. Therefore, we began to modify our original hypothesis to include additional models of genetic inheritance. We examined our observed results for the presence of multiple genes, multiple alleles, tetrasomic inheritance, quantitative inheritance, and gene complementation. However, none of these additional models of genetic inheritance were capable of explaining our observed results.

During the course of time that it took to repeat our experiments and re-analyze our results, we made an interesting observation in the greenhouse (Figure 4). We found that when left unmowed, the greens-type plants would spontaneously begin to produce annual-type shoots. In other words, the phenotype of the greens-type plants was reverting back to an annual-type phenotype not through the process of sexual recombination, but rather through the mitotic cell division of normal growth and development of the greens-type plant itself. It was this observation that led us to hypothesize that the greens-type phenotype may be the result of an epigenetic mechanism.

Epigenetics is a newly discovered field of genetics that involves very complicated mechanisms of genetic inheritance. Traits that are epigenetically controlled do not follow Mendelian inheritance, but rather involve changes in gene expression controlled by mechanisms other than changes in the underlying DNA nucleotide sequence. Most often, it is the environment itself that is controlling the epigenetic expression of genes. Once the organism is removed from the environment, the expression patterns of its genes may continue for several generations and then revert back to whatever the gene expression patterns were prior to the environment that induced the epigenetic change. Some examples of epigenetic regulation of traits include the cold temperature vernalization requirements for flowering in plants (10) and stress-induced cancers in humans (7).

Epigenetics is currently a hot topic topic among geneticists, but, unfortunately, at this time we will not fully understanding all of its controlling mechanisms nor how each of these mechanisms is induced by the environment or how these mechanisms interact. Nonetheless, it is our conclusion that the greens-type *Poa annua* phenotype is likely to be epigenetically controlled and as such, the greens-type *Poa annua* phenotype is not stable outside of its greens management environment.

To begin to provide some preliminary evidence in support of this conclusion, we treated an advanced generation line of F_8 individual seedlings, initially derived from a greens-type x greens-type cross, to mowing in the greenhouse (Figure 5). We observed that 24 out of 24 individual seedlings retained the greens-type phenotype when mowed (Figure 5A), whereas only 6 out of 96 individual seedlings retained the greens-type phenotype when left unmowed (Figure 5B). This result suggests that it is the action of mowing that is the environmental parameter that induces the epigenetic change in gene expression that results in the greens-type *Poa annua* phenotype. This result also explains why the greens-type phenotype would be apparently lost in the seed progeny over time during the process of seed production in the unmowed seed fields.

Additional research will be required to confirm our new hypothesis involving the epigenetic regulation of the greens-type phenotypes of *Poa annua*. Such additional research will likely yield new insights into the widespread adaptability of *Poa annua* as a species and may provide us with an opportunity to finally achieve the commercial production of greens-type *Poa annua*.

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